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*Research Paper*

# Agglomerate Strength and Dispersion of Salmeterol Xinafoate from Powder Mixtures for Inhalation

**Handoko Adi,[1] Ian Larson,[1] Herbert Chiou,[2] Paul Young,[2] Daniela Traini,[2] and Peter Stewart[1,3]**

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***Purpose.*** The study investigated the role of agglomeration and the effect of fine lactose size on the dispersion of salmeterol xinafoate (SX) from SX–lactose mixtures for inhalation.
***Methods.*** Particle size distributions were characterised by Malvern Mastersizer S, Aerosizer and Spraytec, and imaging conducted by scanning electron microscopy (SEM). Inter-particulate adhesion was quantified by atomic force microscopy. Deposition of SX was measured using a twin stage impinger. SX was analysed using validated high-performance liquid chromatography method ($r^2$=1.0, CV=0.4–1.0%).
***Results.*** Addition of fine lactose with a volume median diameter (VMD) of 7.9 μm to a SX–lactose carrier and carrier-free mixture resulted in significantly better dispersion (16.8% for 20% added fine lactose) than fractions with VMD of 3.0, 17.7 and 33.3 μm (less than 9.1% for 20% fine lactose). Using the carrier-free mixtures, particle sizing of the aerosol cloud using the Spraytec, coupled with the application of the Aerosizer using differing dispersion energies and SEMs of the samples, indicated that an open packed, agglomerate structure improved SX dispersion. The highest extent of SX dispersion occurred when SX and fine lactose were detached from the surface, usually in the form of loose agglomerates.
***Conclusions.*** The outcomes of this research demonstrated how agglomerate structure influenced dispersion and the key role of fine lactose particle size in SX dispersion from mixtures for inhalation.

**KEY WORDS:** agglomeration; drug dispersion; dry powder inhalation; fine lactose; interactive mixture; salmeterol xinafoate.

## INTRODUCTION

Optimisation of the powder formulation in a dry powder inhaler (DPI) device is required to enable efficient, reproducible drug delivery to the respiratory tract. One of the interesting approaches that have been undertaken to improve respiratory delivery has been the addition of fine excipients, usually fine lactose, to the inhalation mixtures. Fine lactose, either associated with large carriers or added as a ternary component in the formulation, has a significant role in controlling deposition of drug in the airways (1–4). For example, in previous investigations, Mackin *et al*. demonstrated that higher respirable fractions of salmeterol xinafoate were obtained when lactose containing higher proportions of fine particles were used (5). Similarly, in another study, Zeng *et al*., demonstrated the inclusion of 2.5% fine lactose (7.0 μm) to dry powder carrier-formulations of beclomethasone dipropionate produced significantly higher fine particle fraction (FPF, 3.1–6.1%) than those of the binary mixture (FPF, 0.3–0.4%) (6). In a more recent study, Louey and Stewart showed that the dispersion of salbutamol sulphate was significantly increased with increased concentration (1–10%) of fine lactose (4.0 μm) in a range of lactose carriers (7). Furthermore, the dispersion of these ternary mixtures, containing fine lactose, was independent of the order of mixing of the drug and fine lactose (7). When considering binary systems, previous studies have suggested that lactose carriers smaller than 10 μm produced higher respirable fractions than larger carriers (up to 180 μm), at air flow rates of 60–200 l/min using the Rotahaler (8). Such observations were attributed to smaller carriers promoting more extensive drug detachment via intense perturbations in the turbulent air stream. A similar investigation by Braun *et al*. suggested that an increased FPF of disodium chromoglycate (DSCG) were observed with smaller carrier sizes of lactose and glucose (9). Such observations were attributed to the adhesive bonds between the DSCG and carrier particles being weaker than the cohesive bonds between DSCG particles. Decreasing the carrier size enabled more carrier particles to disturb the cohesive bonds between the DSCG particles, which consequently increased de-aggregation in a turbulent air stream (8).

While it is clear that the presence of fine lactose is a key factor in controlling dispersion, the influence of the properties of the fine lactose on drug dispersion is not known. Some studies have reported how the particle size of the 'fine lactose' affects drug dispersion. For example, a higher FPF of salbutamol sulphate (1.5% concentration) occurred from

[1] Department of Pharmaceutics, Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia.
[2] Advanced Drug Delivery Group, Faculty of Pharmacy (A15), University of Sydney, Sydney, NSW 2006, Australia.
[3] To whom correspondence should be addressed. (e-mail: peter.stewart@vcp.monash.edu.au)

interactive mixtures containing micronised lactose (8.6 μm) than those containing Lactochem lactose (20.1 μm) (10). Furthermore, in this study, the effect of intermediate size fine lactose (15.9 μm) in the formulation of the interactive mixtures was studied and increased FPF of salbutamol sulphate occurred with increasing concentration of intermediate size lactose (3). For example, an increase in the dispersion of salbutamol sulphate occurred when 1.5% of intermediate-sized lactose was added and a further 50% increase in FPF was observed when the concentration of intermediate lactose was increased from 1.5 to 9% (3). It is important to note, however, that these studies have not been extensive and have not specifically compared the effects of cohesive and non-cohesive particle distributions.

The mechanisms by which fine lactose improves drug dispersion in mixtures for inhalation have not been conclusively defined. Two mechanisms are believed to dominate interactions in such systems; the 'active site' theory and 'redistribution' theory. The 'active site' theory relies on fine lactose occupying high energy sites on the coarse lactose surface leaving low energy sites available for the drug, thus enabling greater drug detachment (3). The redistribution theory proposes that interaction between the drug and fine lactose produce dispersible agglomerates that are detached from the carrier surface (7). Indeed, both theories may exist in a dynamic equilibrium; however, it is interesting to note, that both require the fine lactose to be adhesive and capable of interaction with other particles. Previous studies have not specifically investigated the particle size effects in the particle size range that is likely to be interactive and pertinent to the above theories. Knowledge of such particle size effects is important not only to the development of more effective mixtures for inhalation, but also to help in understanding the mechanism of drug dispersion in the presence of fine lactose.

The purpose of this current study therefore was to investigate the influence of agglomeration on the dispersion of a model drug, salmeterol xinafoate, in interactive mixtures in order to gain a better understanding of the mechanism of drug dispersion. In particular, the investigation focused on the structure of agglomerates, especially their propensity to break-up during inhalation, in both carrier-based and carrier-free mixtures containing both salmeterol xinafoate and fine lactose fractions. A secondary objective of this study was to determine if the size distribution of the added fine lactose fraction changed the degree of dispersion. Fine lactose particle size fractions included both cohesive and non-cohesive particle distributions. The salmeterol xinafoate and fine lactose concentrations were relatively high and compatible with conditions where particulate agglomeration was likely to occur.

## MATERIALS AND METHODS

### Materials

Micronised salmeterol xinafoate (SX) was supplied by Glaxo SmithKline (NSW, Australia). The SX was of inhalation grade, had a volume median diameter (VMD) of 2.4 μm and was employed as the model drug in the interactive mixtures for inhalation. Inhalac 120 (Meggle, Wasserburg, Germany) and milled lactose (edible 90, New Zealand Milk Products Pty., Kapuni, New Zealand) were used as model coarse lactose carrier and fine lactose excipients, respectively. Ammonium acetate (Analar, BDH, Victoria, Australia), methanol [high-performance liquid chromatography (HPLC)-grade, Biolab, Victoria, Australia], 1-butanol (Chem-supply Pty., Victoria, Australia) and propan-2-ol (Chem-supply Pty. Ltd., Victoria, Australia) were used as supplied.

### Methods

#### *Micronisation and Classification of Fine Lactose*

Lactose edible 90 was micronised using a fluid energy mill (K-tron Soder, NJ, USA). Samples of 200 g lactose were fed into the milling inlet with injector pressure setting of 862 kPa and grinding pressure (690 kPa). Different feed rates (400, 600, 800, 1,200 and 1,600 rpm) were used to produce different size fractions of milled lactose. The micronised lactose was classified using wet sieving with 1-butanol, presaturated with lactose, and test sieves (CSIRO, Victoria, Australia) to the following fractions: <5, 5–10, 10–20 and 20 – 40 μm (11). Approximately 5 g of lactose was dispersed by sonication in the 1-butanol for 5 min, to obtain a homogeneous suspension. Serial sieves were set with the sonicator, positioned 3 mm above the surface of the sieve. The supernatant liquid was removed and replaced by fresh 1-butanol, followed by vigorous mixing. The process was repeated 15 times to obtain narrow particle size distributions. The fine lactose fractions used in this study will be designated as FL and specific fractions defined by a number indicating the VMD. The distributions of the FL fractions (FL with a VMD of 3.0, 7.9, 17.7 and 33.3 μm) are shown in Fig. 1. The FL with a VMD of 3.0 and 7.9 μm were cohesive while the FL with a VMD of 17.7 and 33.3 μm were non-cohesive, free-flowing powders.

#### *Particle Sizing of Lactose*

Using a developed methodology, the particle size distribution of the sieve fraction lactose powders was measured by laser diffraction (Malvern Mastersizer S,

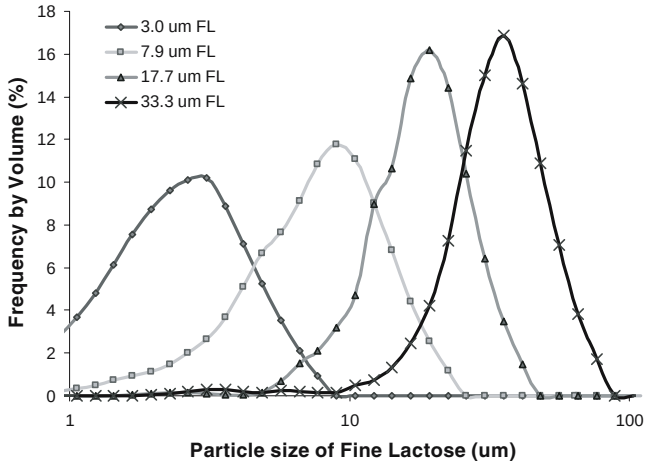


**Fig. 1.** Particle size distributions of different fine lactose size fractions (FL) measured using laser diffraction with propan-2-ol as dispersant.

Malvern Instruments, Worcestershire, UK) using the 300 RF lens equipped with a small volume sample presentation unit (capacity 150 ml) (12). Approximately 500 mg of lactose powder was sonicated in 5 ml of propan-2-ol in a water bath for 3 min prior to sampling. Particle size analysis of each sample was performed using 2,000 sweeps and analysed with the reference refractive index of lactose (1.533) and propan-2-ol (1.378), and an estimated imaginary refractive index for lactose of 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. The residual value was always below 1%.

*Preparation of Interactive Mixtures*

The interactive powder mixtures were prepared by a validated laboratory mixing method. Drug powder and fine lactose were placed between two layers of lactose carrier powders in a glass test tube with two ceramic balls and vigorously shaken for 5 min (2, 13). Two different types of SX formulations, namely carrier-based and carrier-free interactive mixtures were prepared. The binary carrier-based interactive mixture was composed of SX (2.5%) and Inhalac 120 as the carrier. Inhalac 120 contained very small quantities of fine lactose associated with its surface (1.3%, <5 μm); it should be noted that such small amounts of fine lactose were difficult to remove even using decantation techniques (14). Thus, these binary mixtures consisted essentially of SX and carrier lactose; however, it must be recognised that very small quantities of fine lactose existed in these mixtures. The ternary carrier-based interactive mixture was composed of SX (2.5%), Inhalac 120 as the carrier and FL fractions. The carrier-free formulations consisted only of SX and FL fractions in ratios of 1–2 to 1–8. All the mixtures were stored in a desiccator over silica gel until further required.

The homogeneity of each powder mixture prepared was assessed for content uniformity. Twenty random samples (20 mg) were taken and dissolved in 60% water – 40% methanol. The amount of SX in each sample was determined by UV assay at wavelength of 252.5 nm (Cecil CE6600 UV Spectrophotometer, UK). All mixtures were considered to be homogeneous with accuracy ranging from 99.6 to 100.3% and the CV below 1.5%.

*In vitro Aerosol Particle Size Measurement*

Using a Rotahaler as a model inhaler (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 40% methanol (HPLC grade) – 60% water was used as the collection liquid and 7 and 30 ml were placed into stage one and stage two of the TSI, respectively. The airflow was drawn through the TSI using a vacuum pump (Model OD5/2, Dynavac Engineering, VIC, Australia) and the airflow rate was adjusted to 60 l/min at the mouthpiece prior to each measurement (Fisher and Porter, Model 10A3567SAX, UK). Powder blends (20 mg) were loaded into hard gelatin capsules (size 3, Fawn and McAllan, Pty., Victoria, Australia). Single capsule was actuated for 4 s (4 l volume) for each measurement ($n$ = 5). All deposition studies were conducted in an air-conditioned laboratory with the temperature (20.0 ± 1.0°C) and relative humidity of the surrounding environment (50 ± 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 assay described below. The fine particle fraction was defined as the amount of SX deposited in the lower stage (stage two) 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.

*High-Performance Liquid Chromatography*

The SX drug content recovered from TSI studies was analysed by HPLC using a $C_{18}$ column (5 μm, 4.6 × 150 mm, Apollo) and a UV detector (Waters Tunable Absorbance Detector, MA, USA) at a wavelength of 252 nm. The mobile phase consisted of a mixture of methanol and 0.2% ($w/v$) ammonium acetate solution (55:45, pH ~6.9), filtered and degassed prior to use using 0.45-μm membrane filter (Millipore, County Cork, Ireland). A flow rate of 1.0 ml/min by a HPLC pump (Waters 510, MA, USA) was employed at ambient temperature with the injection volume of 15 μl. The retention time of SX was approximately 4.2 min with peaks area being recorded by integration (Shimadzu CR6A Chromatopack, Japan). Linear regression analysis over the SX concentration range of 0.4 to 10 μg/ml using four concentrations and four replicates was performed using Sigmaplot (Jandel Scientific, USA). The precision was tested before each experiment by analysing 1.0 and 4.0 μg standard solutions of SX using five replicates. The accuracy ranged from 99.2 to 104.4% and the precision ranged from 0.4 to 1.0%.

*Scanning Electron Microscopy*

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.

*Atomic Force Microscopy*

The force of adhesion between SX and the various lactose samples was determined using the colloid probe atomic force microscopy (AFM) technique. The methods of preparation and measurement of SX force measurements using this technique is described in more detail elsewhere (14). Briefly, the AFM measurements were preformed using a commercial AFM (TMX 1010, Topometrix, USA). Prior to

force measurement, particles of SX (2- to 3-μm diameter, $n$ = 3) were fixed onto standard V-shaped cantilevers (Topometrix, CA, USA) using an epoxy resin glue (Araldite, Selley Pty., 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.

*Particle Sizing of Aerosol by Spraytec*

An *in situ*, real-time measurement of the particle size distribution of DPI mixtures (via laser diffraction) was performed using a Spraytec particle sizer (Malvern Instruments, Worcestershire, UK) equipped with an inhalation cell attachment. Experiments were conducted using the same formulations and Rotahaler as in the *in vitro* aerosol particle size measurement described above. The flow rate through the Spraytec was maintained at 60 l/min [calibrated using a TSI 4000 series flow meter (TSI, USA)]. All measurements were made on five replicates at room temperature (25°C, 50% RH). Each measurement was performed over 4 s with an arbitrary triggering level of 50, noise level 0 and background level of 100. The particle size distributions of the aerosolised mixtures were analysed using RTsizer software V.5.51 (Malvern Instruments, Worcestershire, UK).

*Aerodynamic Particle Size Analysis by Aerosizer*

The particle size of the powder mixtures were analysed by an Aerosizer (Amherst Process Instruments, USA) using the dry powder dispersion system (aerodisperser). A small amount of powder (about 5 mg) was placed in the sample cup of the aerodisperser. Particle measurements were carried out at medium feed rate and a sample run time of 300 s. Different shear pressures were applied to study the effect of increasing shear pressure on the agglomerate strength of the powder (3.4, 10.3, 20.7 and 27.6 kPa). The particle size of the mixture was analysed using API Aerosizer software (LD Version 7.04). The average particle size distribution was measured from five replicates of each sample.

*Statistical Analysis*

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

## RESULTS AND DISCUSSIONS

### Dispersion of Salmeterol Xinafoate from the Carrier-Based Mixtures

Initially, the extent of drug dispersion and emitted dose of SX were determined for formulations of SX alone and the SX–carrier binary mixtures. The formulation of SX alone and the SX–carrier mixture showed FPF values of 10.8 and 4.0%, respectively, while the ED were 71.3 and 81.8%, respectively.

The use of carriers in DPI formulations is regularly added to act as a diluent and improve flow in a highly cohesive system. Inclusion of Inhalac 120 carrier material in the study conducted here, significantly improved the emitted dose of SX from 71.3 ± 3% for SX alone to 81.8 ± 2.5% ($P$ < 0.05). Interestingly, alongside the improvement of the emitted dose of drug, the FPF of SX from this binary mixture significantly decreased and was about two- and a half-fold lower than the formulation employing SX only. That is to say that, although the addition of carrier material to SX improved flow, the number of particles being liberated from the carrier into the air stream was significantly less than when aerosolising SX alone. SEM observation of SX–carrier binary mixtures demonstrated interaction between SX and carrier (Fig. 2a). Most of the SX particles adhered as agglomerates or single particles on the surface of carrier (Fig. 2b). Atomic force microscopy studies quantified the adhesion force between an SX particle and the coarse lactose as 104 ± 5 nN. Such observations correlate well with the *in vitro* studies, where adhesion of SX to the carrier surface improved SX emission from the device (i.e., the emitted dose); however, the SX–coarse lactose adhesion would most likely reduce SX detachment and cause a decrease in FPF.

The influence of FL on the aerosolisation efficiency (FPF) of SX in coarse lactose-based ternary mixtures was determined using different FL fractions (VMD of 3.0, 7.9, 17.7 and 33.3 μm) at different formulation concentrations (5, 10 and 20%). Results are summarised in Fig. 3. For all FL fractions, the presence of FL in the interactive mixture of SX and Inhalac 120 improved the dispersion; this was particularly evident for the FL fractions of 3.0 and 7.9 μm. The mixtures of SX and Inhalac 120 with FL fractions of 3.0 and 7.9 μm at all concentrations and mixtures of SX and Inhalac 120 with of FL fraction of 17.7 and 33.3 μm at 20% FL were significantly greater than the binary mixture of SX and Inhalac 120 ($P$ < 0.05). These results are consistent with previous studies from our laboratories (7) and with other investigations in the literature (3). The dispersion of SX was dependent on the FL size (Fig. 3a). Both FL with a VMD of 3.0 and 7.9 μm were effective in improving the FPF of SX at low FL concentration (5%); however, at higher concentrations (10–20%), FL with a VMD of 7.9 μm was superior compared to other fractions (Fig. 3a). Further increase in the primary particle size of FL fractions (VMD of 17.7 and 33.3 μm) decreased the FPF of SX compared to FL with a VMD of 7.9 μm ($P$ < 0.05).

When comparing the efficiency of SX removal from the device, the most apparent variation in emitted dose existed at higher FL concentrations (20%) and with smaller particle size FL fractions (i.e., 3 and 7.9 μm).

To further investigate such phenomena, SEM images for the SX mixtures containing all fractions of FL were taken. Representative SEM images of the 10% added FL carrier-based formulations are shown in Fig. 2b–e. In the SX mixture containing FL with a VMD of 3.0 μm, particles were associated with the Inhalac 120 and few particles or agglomerates were detached from the surface (Fig. 2c). Furthermore, examination of the inter-particulate structure

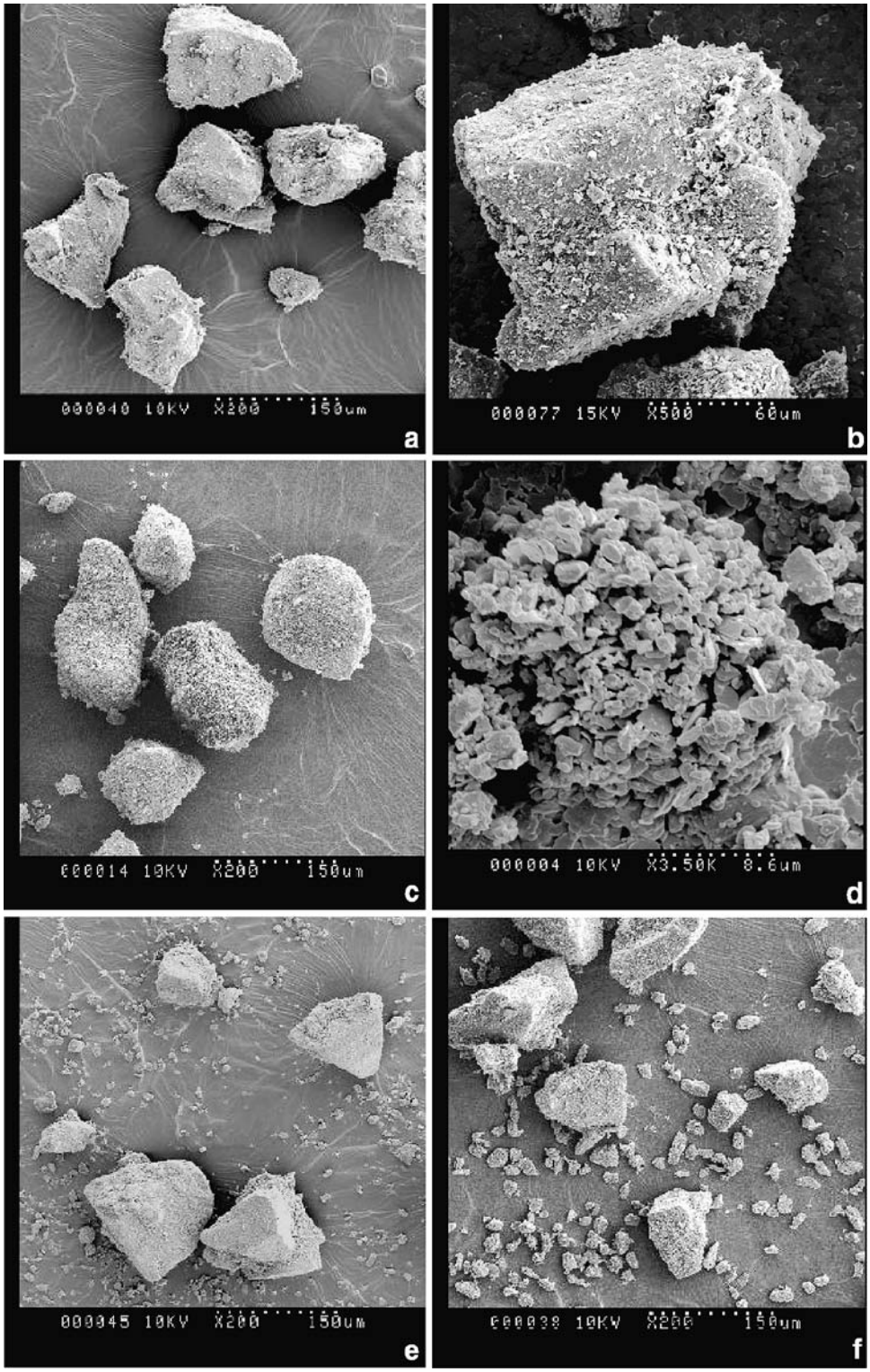


**Fig. 2.** Scanning electron micrographs of carrier-based mixtures (**a**, **c**, **e**, **f**, magnification ×200; **b** and **d**, magnifications are ×500 and ×3.5K, respectively). (a) Mixture of Inhalac 120 and SX, (b) SX adhered on the Inhalac 120 lactose carrier from the binary mixture of Inhalac 120 and SX, (c) mixture of Inhalac 120 and SX and 10% FL VMD of 3.0 μm, (d) agglomerate off the surface from the mixture of Inhalac 120 and SX and 10% FL VMD of 3.0 μm, (e) Mixture of Inhalac 120 and SX and 10% FL VMD of 7.9 μm and (f) mixture of Inhalac 120 and SX and 10% FL VMD of 17.7 μm.

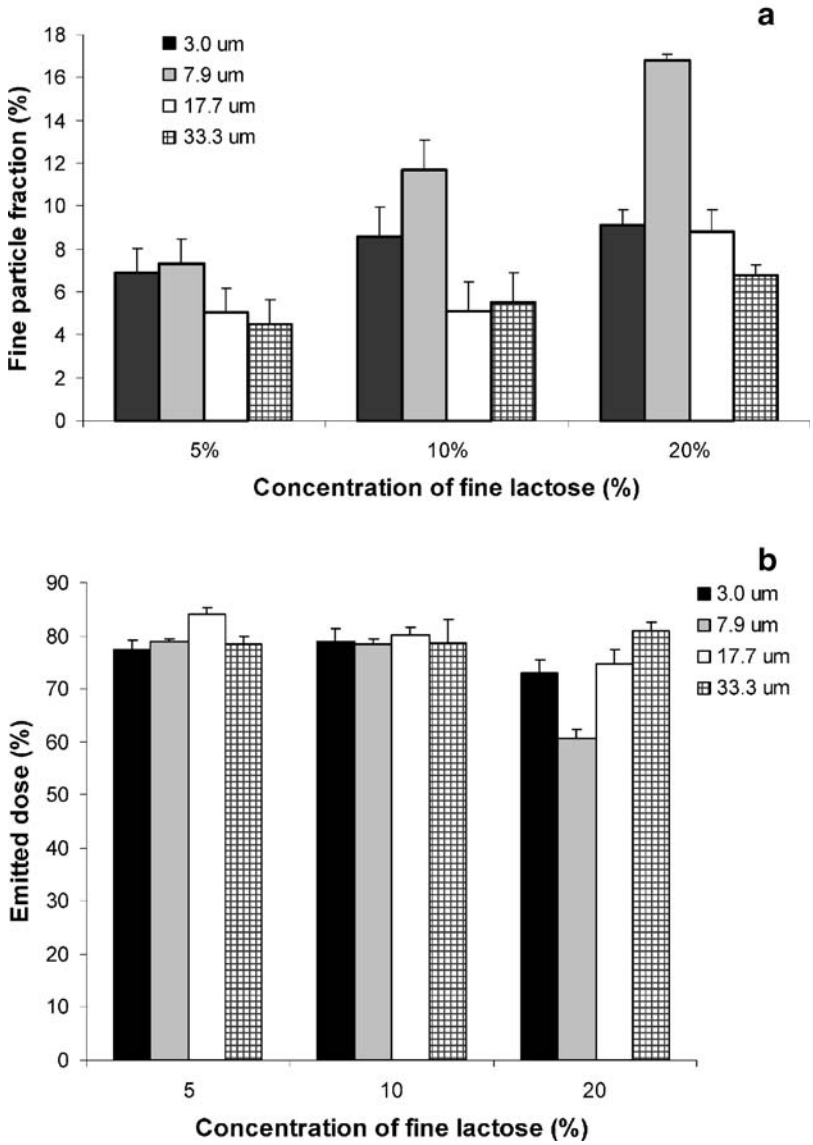


**Fig. 3.** Effect of different fine lactose particle size fractions on the fine particle fraction and emitted dose of salmeterol xinafoate from the carrier-based mixtures containing SX (2.5%), Inhalac 120 and fine lactose. The Rotahaler was used as model inhaler with the flow rate of 60 l/min. (a) Fine particle fraction of SX and (b) emitted dose of SX.

suggested that a dense packing existed in this system (Fig. 2d). In comparison, the SX carrier mixture containing 7.9 μm FL suggested particulate detachment from the surface of the coarse lactose. Further analysis of the 7.9 μm FL-carrier–SX system suggested a loose network structure (or less dense packing) between individual agglomerates (Fig. 2e). In general these results were in a good agreement with both the emitted dose and FPF of SX from the two (3.0 and 7.9 μm) FL carrier systems (Fig. 3b). For example, FL, with a VMD of 3.0 μm, had a relatively similar emitted dose to that of the binary mixture (the emitted dose was reduced slightly from 77.4 to 73.1% at 20% concentration of FL), probably due to strong adhesive forces of FL and SX to carrier lactose and the subsequent good flow properties of this mixture. In addition, SEM images of mixtures containing FL with a VMD of 7.9 μm, showed the presence of detached agglomerates, and these were thought to be the key factor in the improvement of SX dispersion. The role of agglomerates in drug dispersion would be further explored in the next sections.

Interestingly, when FL with VMD of 17.7 and 33.3 μm were added to the SX–Inhalac 120 interactive mixture, the FL acted as a secondary carrier, where adhesion of SX to both the Inhalac 120 and secondary carrier was observed (Fig. 2f). Atomic force microscopy studies quantified the adhesion force between an SX particle and the secondary carriers (FL with a VMD of 17.7 and 33.3 μm) as 160.9 ± 11 and 122.3 ± 11.6 nN, respectively. Formulations using these FL size fractions resulted in low SX dispersion, especially those containing FL with a VMD of 33.3 μm (Fig. 3a), where the presence of this excipient caused SX redistribution and attachment to the secondary carrier surface. Consequently, a high emitted dose of SX was expected even for the mixture containing 20% FL (Fig. 3b).

### Deposition of Salmeterol Xinafoate from SX and FL Mixtures (Carrier-free Mixtures)

Studies in the previous section demonstrated that the coarse lactose carrier interacted with SX to reduce the extent of dispersion and the addition of FL with a VMD of 7.9 μm caused some detachment of particles from the carrier through agglomeration of the SX and FL particles. Although the mechanism of dispersion was not clear, the particle detachment pointed to dispersion through de-agglomeration of possible mixed agglomerates of SX and FL. Furthermore, the role of specific size fractions within the agglomerate seemed to affect the ability of these agglomerates to disperse. However, it is important to note that the possibility of some interaction of the FL with the coarse lactose surface causing SX detachment could not be discounted. In order to further investigate such phenomena, the influence of the FL on agglomeration in mixtures of SX and FL only (VMD of 3.0, 7.9, 17.7 and 33.3 μm) were investigated. The effect of different FL particle size fraction on the FPF of SX are summarised in Fig. 4.

In general, the removal of the coarse lactose carrier from the interactive mixtures produced significantly higher FPF than formulation containing Inhalac 120. For example, addition of 10% FL (7.9 μm) to the SX–Inhalac 120 mixture produced a FPF of 11.7% (compared to 4.0% for the binary carrier blend), while the mixture with the carrier removed produced a FPF of 17.2%. It is envisioned that the high dispersion in SX–FL only formulations occurred since SX was not adhered on the coarse lactose surface and was more available for dispersion. It is interesting to note, that although all SX–FL only blends produced significantly higher FPFs than when blended as a binary carrier system, the formulation containing FL (7.9 μm) produced significantly higher FPF compared with other FL sizes ($P < 0.01$) (Fig. 4). This behaviour was consistent with previous results for the dispersion of the ternary mixtures, where the addition of 7.9 μm FL to the SX–Inhalac 120 mixture produced the greatest FPF (Fig. 3a). Given that SEM images for the ternary SX mixture containing 7.9 μm FL demonstrated particulate detachment from the coarse lactose as agglomerates, the data obtained for the SX–FL mixtures support the fact that dispersion of the particulate networks and agglomerates, not associated with the Inhalac 120, was a major mechanism for dispersion of SX in these mixtures. No significant difference in FPF of SX was observed in the other formulations of SX and FL (VMD of 3.0, 17.7 and 33.3 μm) ($P > 0.05$) (Fig. 4).

To further investigate the influence of FL size on the interactive mixtures of FL–SX only mixtures, the samples were analysed by SEM (Fig. 5). Visual examination by SEM demonstrated agglomeration in mixtures containing SX and FL with a VMD of 3.0 and 7.9 μm, whilst SX was adhered on the surface of the FL with VMD of 17.7 and 33.3 μm, producing a carrier-based interactive mixture. It is interesting to note that the SX mixture with 7.9 μm FL demonstrated a more open packing network of interacting particles, whilst the FL with a VMD of 3.0 μm had a dense, compact packing (Fig. 5b,a, respectively). It is proposed that the different agglomeration behaviour could account for the difference in dispersion behaviour. The mixtures (with and without Inhalac 120), containing 7.9 μm FL, demonstrated the highest FPF and loosest network structure by SEM. The decreased adhesion seen in the SX mixtures with the larger size fractions of FL was attributed to the SX interaction with the secondary carrier and thus decreased availability for dispersion. The extent of interaction with these secondary carriers will be explored using AFM data in a following section.

### Particle Size Distribution of SX and FL Mixtures During Aerosolisation

To further examine the difference of SX dispersion behaviour in the carrier-free mixtures containing FL (VMD

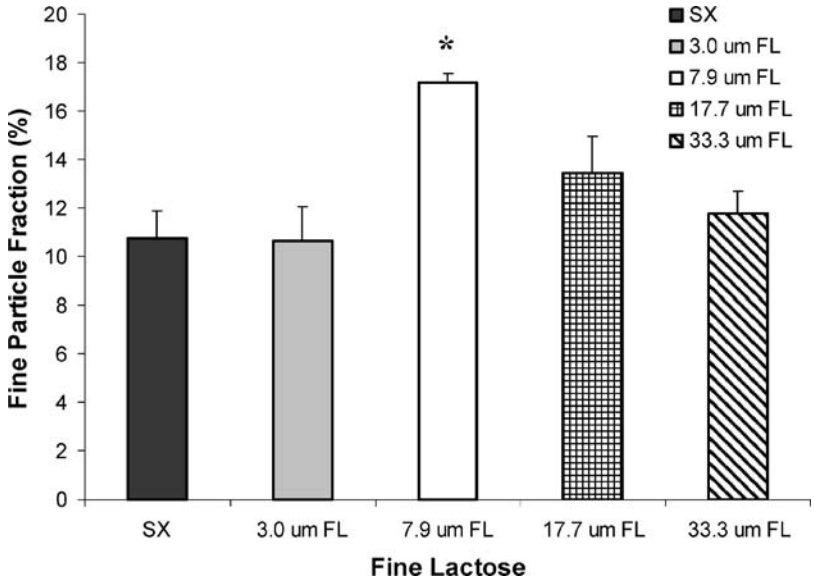


**Fig. 4.** Effect of different fine lactose particle size fractions on the fine particle fraction of salmeterol xinafoate (*SX*) from mixtures containing SX and fine lactose (1:4). The Rotahaler was used as model inhaler with the flow rate of 60 l/min. *Asterisk* represents a significant difference ($P < 0.01$) compared with other FL fractions and SX.

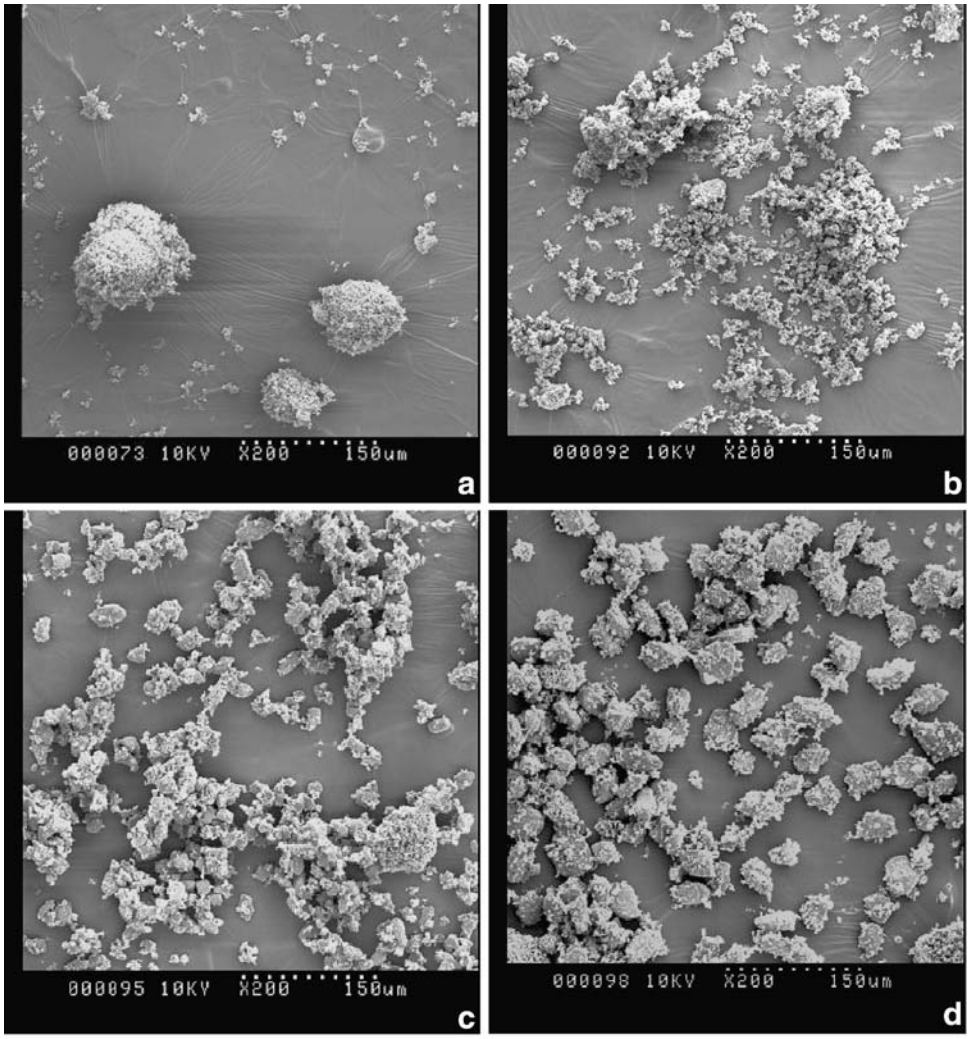


**Fig. 5.** Scanning electron micrographs of salmeterol xinafoate and fine lactose only formulation (1:4) (magnification ×200). (a) FL VMD of 3.0 μm, (b) FL VMD of 7.9 μm, (c) FL VMD of 17.7 μm and (d) FL VMD of 33.3 μm.

of 3.0 and 7.9 μm), the particle size distribution of SX and FL mixtures during aerosolisation was determined using the Spraytec particle sizer (Fig. 6). The carrier-free mixtures containing FL of VMD of 17.7 and 33.3 μm were excluded because the fine lactose fractions were not cohesive and because little useful information could be obtained due to the masking of any potential agglomerate distributions by the larger sized fractions of lactose. The mixture containing 3.0 μm FL resulted in a multimodal distribution; four distinct regions were observed: a particle size distribution of fine particles in the range <10 μm, another distribution between 10 and 50 μm, possibly small agglomerates, a shoulder on a large distribution around 100 μm and a distribution of larger agglomerates about 200 μm. The results obtained were consistent with the SEM data, where mixtures containing 3.0 μm FL showed compact, dense agglomerates, which require high shear energy to de-agglomerate to the primary particle sizes. Analysis of the fine particle concentration in the 3.0 μm FL–SX system suggested poor aerosolisation performance with the percentage of particles (both SX and FL) <5 μm being 6.5%. In comparison, a bimodal distribution was observed in the mixture containing 7.9 μm FL. Furthermore, the bimodal distributions could be represented as dispersed particles <10 μm and small agglomerates between about 10 and 50 μm. In addition, the proportion of fine particle <5 μm was 21.1% compared to 6.5% in the 3.0 μm FL system. Since the SX particle size distribution contained 93.4% <5 μm and the FL with a VMD of 7.9 μm contained only 40% <5 μm, the probability of SX particles being delivered into a respirable aerosol cloud was higher for the SX mixture containing FL with a VMD of 7.9 μm compared with the SX mixture containing FL with a VMD of 3.0 μm.

In general, the results obtained using the Spraytec were in good agreement with the FPF results, where mixture of SX and 7.9 μm FL performed significantly better compared to other mixtures (Fig. 4). As previously discussed, this difference in dispersion capability of the SX mixtures containing FL of 3.0 and 7.9 μm may be due to the different agglomerate structures in the mixtures and their ability to de-agglomerate.

### Analysis of Agglomerate Strength of SX–FL Agglomerates Using the Aerosizer

In order to support the qualitative interpretation from SEM observation (Fig. 5) and the aerosolisation data provided by the Spraytec (Fig. 6), the dispersibility of different agglomerate structures was investigated using the Aerosizer. The influence of shear pressure on the dispersion of mixtures of SX and FL (3.0 and 7.9 μm) was examined using the Aerosizer to investigate agglomerate strength and results are summarised in Fig. 7. Each SX –FL mixture was subjected to the increasing shear pressure from 3.4 to 27.6 kPa. Increasing shear pressure resulted in different de-agglomeration rate profiles for the mixtures containing different FL particle sizes. In general, the mixture using SX and 7.9 μm FL was easily de-agglomerated at low shear pressure. The particle size of the mixture was reduced to 10.1 μm even at 3.4 kPa, and to 7.5 μm at the maximum shear pressure of 20.7 kPa. In contrast, the mixture of SX and 3.0 μm FL required higher shear pressure to de-agglomerate the mixture. The particle size of the agglomerate gradually decreased from 23.3 to 5.2 μm as the shear pressure increased from 3.4 to 27.6 kPa. Higher shear pressure was needed to overcome the strong tensile strength of the dense packed agglomerates. The technique used here has considerable importance in identifying the strength of the agglomerate.

### Interparticulate Interaction within the Powder Mixture

In order to gain more quantitative evidence to support the behaviour seen in the SEM, Spraytec and Aerosizer, the adhesion forces between SX probes and the two smaller fractions of lactose were determined using colloidal probe AFM. The adhesion force between SX and 3.3 μm lactose was 246.8 ± 8.5 nN and between SX and 7.9 μm lactose was 238.0 ± 8.9 nN. No significant difference in adhesion force of SX and FL between 3.3 and 7.9 μm were observed ($P > 0.05$).

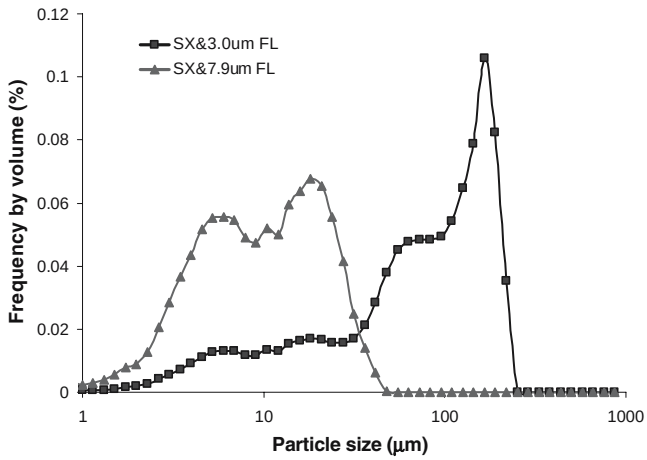


**Fig. 6.** Particle size distribution of salmeterol xinafoate and fine lactose mixtures during inhalation measured using Spraytec at a flow rate of 60 l/min.

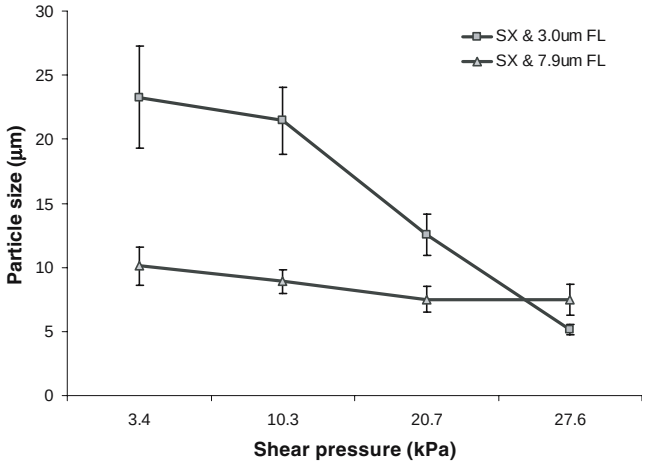


**Fig. 7.** Particle size distribution of salmeterol xinafoate and fine lactose mixtures at different shear pressure measured using the Aerosizer.

A relationship between tensile strength and particle size of interacting particles, packing fraction and work of adhesion is as follows (15):

$$\sigma = \frac{15.6\Phi^4 W}{d} \quad (1)$$

where $\sigma$ is the tensile strength of the agglomerate, $\phi$ is the packing fraction (volume of particles/volume of aggregate), $W$ is the work of adhesion and $d$ is particle diameter.

Thus, for the mixtures containing agglomerates of SX with FL (3.0 and 7.9 μm), the use of Eq. 1 would allow a qualitative comparison of the agglomerates containing FL of both sizes.

The adhesion force between SX and the 3.0 and 7.9 μm FL fractions is about the same and would not have a great influence on tensile strength differences. However, the agglomerates formed with 3.0 μm FL would be expected to have a greater tensile strength, since the particle size is smaller and the packing fraction is likely to be greater. Clearly, such observations are evident in the SEM (Fig. 5a) and in the de-agglomeration rate profiles shown in Fig. 7. The use of Eq. 1 to qualitatively determine the propensity of agglomerates, formed in the SX and FL with a VMD of 3.0 μm, to disperse based on tensile strength was consistent with the SX dispersion data shown in Figs. 3a and 4.

## CONCLUSION

The outcomes of these studies showed that the presence of FL played a significant role in powder dispersion through its role in agglomerate formation. The presence of a coarse lactose carrier, Inhalac 120, significantly reduced the fine particle fraction of SX, while the presence of different size fractions of FL added to the carrier system, generally increased the FPF of SX to values greater than that of the SX alone. SEM images demonstrated that, for FL (3.0 μm), strong adhesion occurred on the surface of the Inhalac 120, with adhesion characterised by multi-layers and agglomerates of SX and FL. In contrast, the FL fraction with a VMD of 7.9 μm caused particulate detachment from the surface of the Inhalac 120, with agglomerates clearly seen in the powder

mixture. However, further increase in the particle size of FL (for example, FL with VMD of 17.7 and 33.3 μm) showed that the added FL fractions were acting as secondary carriers, where the SX was redistributed from the surface of Inhalac 120 to the added "fine lactose" fractions. In addition, for the SX–FL mixtures (1:4), the FPF of SX for the mixture containing FL (7.9 μm) was significantly greater than the other mixtures, indicating that the role of FL in the dispersion of SX was independent of the presence of a carrier when the particle load was high. Visual examination of SX and FL mixtures by SEM further showed that the agglomerates containing FL with a VMD of 7.9 μm had open packing network of interacting particles, whilst FL with a VMD of 3.0 μm showed a dense, compact packing. Since the tensile strength of the agglomerates was dependent on packing fraction, work of adhesion and particle size, the agglomerates containing the 7.9 μm FL were more readily de-agglomerated than the agglomerates containing 3.0 μm FL. It is proposed that this was most probably due to the greater particulate size and the lower packing fraction of the agglomerate, resulting in decreased tensile strength. Maximum SX dispersion occurred when the SX and FL were detached from the carrier surface, usually in the form of loose agglomerates. This finding was further confirmed by the Aerosizer data, where FL with a VMD of 7.9 was more easily de-agglomerated even at low shear pressure. The outcomes of this study demonstrated that the presence of loose agglomerates detached from the Inhalac 120 surface played a significant role in powder dispersion. Particulate detachment was influenced by the particle size and concentration of the FL in the mixture. This investigation reflects the importance of the FL properties in optimising drug dispersion and show consistency with previous findings (7, 14). From the author's knowledge, this is the first detailed study demonstrating the role of agglomeration in drug dispersion from DPI mixtures for inhalation.

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