Lactose Surface Modification by Decantation: Are Drug-Fine Lactose Ratios the Key to Better Dispersion of Salmeterol Xinafoate from Lactose-Interactive Mixtures?

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Purpose. The role of fine lactose in the dispersion of salmeterol xinafoate (SX) from lactose mixtures was studied by modifying the fine lactose concentration on the surface of the lactose carriers using wet decantation.

Methods. Fine lactose was removed from lactose carriers by wet decantation using ethanol saturated with lactose. Particle sizing was achieved by laser diffraction. Fine particle fractions (FPFs) were determined by Twin Stage Impinger using a 2.5% SX mixture, and SX was analyzed by a validated high-performance liquid chromatography method. Adhesion forces between probes of SX and silica and the lactose surfaces were determined by atomic force microscopy.

Results. FPFs of SX were related to fine lactose concentration in the mixture for inhalation grade lactose samples. Reductions in FPF (2-tp 4-fold) of Aeroflo 95 and 65 were observed after removing fine lactose by wet decantation; FPFs reverted to original values after addition of micronized lactose to decanted mixtures. FPFs of SX of sieved and decanted fractions of Aeroflo carriers were significantly different ($p < 0.001$). The relationship between FPF and fine lactose concentration was linear. Decanted lactose demonstrated surface modification through increased SX-lactose adhesion forces; however, any surface modification other than removal of fine lactose only slightly influenced FPF.

Conclusions. Fine lactose played a key and dominating role in controlling FPF. SX to fine lactose ratios influenced dispersion of SX with maximum dispersion occurring as the ratio approached unity.

KEY WORDS: dry powder inhalers; salmeterol xinafoate; wet decantation; influence of fine lactose on dispersion; atomic force microscopy.

INTRODUCTION

Most commercially available carrier-based dry powder inhalers (DPIs) have been shown to be relatively inefficient, delivering only about 20–30% of the total dose to the lungs (1). The poor efficiency of DPIs is related not only to the complex physiology of the respiratory trac, but also to the characteristics of the powder formulations for inhalation and their inhalation devices. Dispersion of micronized drug particles in respiratory delivery will be dependent on the drug's cohesive and adhesive properties in the powder formulations. Particulate interactions occur in mixtures of micronized drugs because particle-detachment forces are relatively low as a result of the small mass of the micronized particle and the balance between interactive and detachment forces favors cohesion or adhesion. Thus, micronized particles interact with themselves to produce agglomerates and with other surfaces, including excipients to form interactive units. Effective respiratory delivery requires the dispersion of drug from agglomerates and interactive units using the energy generated by the inhalation device.

A number of studies have reported formulation strategies to improve the delivery of drugs into the lung. Many of these studies have focused on improving dispersion through optimizing the drug–carrier interaction. These studies will not be addresses in detail but include smoothing the carrier surface (2,3), optimizing carrier size (4,5–7), mixing different grades of lactose (8,9), using lactose carriers with different surface morphologies (3,10), using other carriers like mannitol, Avicel®, and sorbitol (11,12), and modifying the surface of hydrophobic drugs with hydrophilic particles (13).

One of the interesting approaches that have been taken to improve respiratory delivery has been the addition of fine excipient, usually micronized lactose, to the inhalation mixtures. Higher respirable fractions of salmeterol xinafoate were obtained when a mixture of lactose carrier with a higher proportion of fine particles was used (14). The fine particle fraction (FPF) of salbutamol sulphate decreased from 11.8% when the original lactose was used to 6.7% for treated lactose where some of the fine lactose was removed by compressed air treatment; however; addition of 1.5% w/w fine lactose to the formulation increased FPF to 14% (15). Inclusion of 2.5% fine lactose (7.0 μm) to dry powder formulations of beclomethasone dipropropionate (4.6 μm) produced significantly higher FPF (3.1–6.1%) than those for binary mixtures (FPF, 0.3–0.4%; Ref. 16). Dispersion of salbutamol sulphate significantly increased with increased concentration (1–10%) of fine lactose (4.0 μm) for a range of lactose carriers and the dispersion of these ternary mixtures was independent of order of mixing of the drug and fine lactose (17). In contrast, a ternary mixture of salbutamol sulphate produced higher FPF when the formulation was prepared by first blending the fine (5.0 μm) and large lactose (90.8 μm) before mixing with drug (18). The presence of coarse lactose particles was essential for maximum dispersion in a salbutamol sulphate mixture with lactose. Maximum dispersion occurred when about 10–15% of fine particles were present in the mixture; interactive mixtures consisting of very large proportions of fine lactose particles showed significantly low FPF (19). When fine particles of a synthesized sugar (0–25%) were mixed with the large carriers of the same sugars, the FPF of salbutamol sulphate was increased from 16.7% to 38.9% (20). Higher FPF of spray dried bovine serum albumin occurred with increasing concentrations of fine lactose (5.4 μm) up to 5%; however, no further increase of FPF was observed at concentration of 7.5 to 10% (21). Improved aerodynamic properties of dry powder inhalations due to higher amount of fine carrier particles were found in the formulations containing salmeterol xinafoate and lactose monohydrate (22). For example, the mass median aerodynamic diameter of Pharmatose® 125 M remained nearly constant for 10 and 20% of fine carrier particle but increased considerably after the addition of 30% of fine lac-

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tose. Addition of other fine particles including mannitol (4.3 μm), sorbitol (6.3 μm; Ref. 12), micronized glucose (4.4 μm; Ref. 17), magnesium stearate (7.6 μm; Refs. 15,23), and L-leucine (24) were found to increase drug dispersion. It has been reported that mannitol or sorbitol may be used as coarse or fine carriers for dry powder aerosol to increase FPF (12). Similar deposition profiles of salbutamol sulphate were observed from the interactive mixture containing fine particles of magnesium stearate (15), micronized glucose (17), and fine lactose. Beclomethasone dipropionate showed significantly higher fine particle doses for the lactose processed with magnesium stearate than for untreated lactose, that is, 102 ± 16 μm compared with 24.2 ± 10.7 μm (23). A significant increase in the fine particle fraction of beclomethasone dipropionate was observed when α-lactose monohydrate was mixed with 0.25% magnesium stearate (25) and a mixture of salbutamol sulphate and 1% L-leucine was found to increase the respirable fraction of salbutamol sulphate (24).

It is clear from the literature that the effect of fine carrier particles in the powder mixture has been to improve drug deposition for a number of drugs; however, it is not clear how fine lactose influences drug dispersion. Saturation of active sites on the carrier particle by the lactose has been proposed as the mechanism for increased drug deposition, whereby the adhesion of micronized excipients onto active (high adhesion) sites leaves passive (low adhesion) sites available for drug adhesion (26,15). The reduced adhesion between drug and carrier particles was suggested to increase drug detachment. In a recent study (17), a hypothesis was proposed to explain the effect of fine excipient on dispersion 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 that could be more easily detached from the carrier surface and dispersed by the DPI.

Therefore, the overall objective of this current study was to further the understanding of the mechanism by which the addition of fine lactose improves dispersion in respiratory delivery. The study involved the investigation of the role of the lactose carrier through surface modification of several inhalation grade lactose samples by removing fine lactose from the carrier surface by a novel wet decantation process allowing the inherent dispersive effect of the lactose surface, free from fine lactose particles, to be determined. The study used a model interactive mixture of salmeterol xinafoate and several commercial inhalation grade lactose samples to explore the relationship between salmeterol xinafoate and fine lactose particles on the carrier surface.

MATERIALS AND METHODS

Materials

Salmeterol xinafoate, micronized (inhalation grade; SX), was obtained from GlaxoSmithKline, Boronia, Victoria, Australia. Aeroflo 95, Aeroflo 65, and Aeroflo 20, inhalation grades of α-lactose monohydrate, were donated by Foremost Farms, Rothschild, WI, USA; other lactose grades of Inhalac 120, Inhalac 230, and Sorbolac 400 were donated by Meggle GmbH, Wasserburg, Germany. Ammonium acetate (Analar, BDH, Victoria), methanol (high-performance liquid chromatography [HPLC] grade, Biolab, Mulgrave, Victoria), and absolute alcohol (HPLC grade, CSR, Victoria) were used as supplied.

Methods

Dry Sieving and Fractionation

About 25 g of Aeroflo 95 and Aeroflo 65 lactose powders were sieved separately using a sieve shaker (Fritsch, Germany) through a series of test sieves (Endecotts, UK) with an aperture width of 45, 63, 90, and 106 μm. Fractions (45–63 μm, 63–90 μm, 90–106 μm, and >106 μm) of Aeroflo 95 and 65 were achieved by dry sieving for 30 min and stored in a desiccator over silica gel.

Decantation and Removal of Fine Lactose

Fine lactose particles were removed from the lactose carrier (Aeroflo 65 and 95) by decantation (27). About 20 g of lactose was dispersed (4–5 min) in 1 L of absolute ethanol presaturated with lactose to make a homogeneous suspension and then allowed to settle for a predetermined time. The supernatant liquid was then decanted and replaced by fresh saturated ethanol, vigorously mixed and the process repeated until the supernatant liquid was clear. During removing the supernatant, special care was taken to ensure minimum disturbance of the lower part of suspension. The coarse particles remaining in the beaker after 15 cycles of decantation were wet sieved by spraying with saturated ethanol 3 times. Finally the samples were dried at room temperature, 21 ± 1°C. For first 3 repeats, 10-min interval was used and then a 5-min interval was applied until supernatant liquid was clear. Although, 15 repeats produce a clear supernatant (28), Heywood estimated that 45 repeats would be necessary to eliminate 99% of particles near to the size of separation (29).

Particle Sizing of Lactose

The particle size of lactose carriers was measured by laser diffraction (Malvern Mastersizer S, Malvern Instruments Ltd., U.K.) using the 300 RF lens and the small volume sample presentation unit (capacity 150 mL). Approximately 500 mg of lactose powder was dispersed in 5 mL of butan-1-ol with the aid of a sonication in a water bath for 3 min. The sonicated sample was added drop-wise into sample cell containing 150 mL of ethanol until an obscuration between 10 and 30% was obtained. Size measurement of each sample was performed using 2000 sweeps and analyzed with the reference refractive index of lactose (1.533) and ethanol (1.36) and estimated imaginary refractive index of lactose is 0.1. The average particle size distribution was measured from five replicates of each sample. The size distributions were characterizing by the 10, 50, and 90%iles ($d_{10\%}$, $d_{50\%}$, and $d_{90\%}$, respectively). The percentage of particles below 5 μm and 10 μm were determined using cumulative frequency distribution (undersized) curve. The residual value was always below 1%.

Preparation of Interactive Mixtures

The interactive powder mixtures were prepared by a hand mixing (30). Binary interactive mixtures of SX (2.5%) and lactose were prepared in 5 g batches (125 mg SX and 4.875 g of lactose). The SX was placed between two layers

of carrier powder in a glass test tube with three ceramic beads of approximately 10 mm in diameter. After stoppering, the test tube was then vigorously shaken by hand for 5 min, where the ceramic beads provided a ball-milling effect for breaking up any drug agglomerates formed during mixing. The powder formulations were loaded (≈20 mg) into hard gelatin capsules (size 3, Fawns and McAllan Pty Ltd., Australia) manually.

Homogeneity Test of Interactive Mixtures

The mean drug content assessed the homogeneity of each powder mixture prepared for this study. Twenty samples, each of around 20 mg weight, were dissolved in an appropriate volume of 40% methanol (HPLC grade) and the amount of SX was determined by UV assay. The acceptable degree of homogeneity was achieved with a mean drug content within 100 ± 3% (mean ± SD) of the theoretical value and coefficient of variation (CV) less than 3%.

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 TSI (Apparatus, A; British Pharmacopoea, 2000; Copley, UK). A solvent of 60% methanol (HPLC grade) was used as the collection liquid, with 7 and 30 mL placed into stage one and stage two of the TSI, respectively. The air flow was drawn through the TSI using a vacuum pump (Model OD5/2, Dynavac Engineering, Australia) and the air flow rate was adjusted to 60 L/min at the mouthpiece before each measurement (Fisher and Porter, Model 10A3567SAX, UK). The temperature (20.0 ± 1.0°C) and relative humidity of the surrounding environment (50 ± 3%) was measured by a thermohygrometer (Shinyei TRH-CZ, Japan). The FPF was defined as the amount of SX particles deposited in the lower stage of the TSI as a percentage of the total dose. The recovered dose (RD) was the total amount of drug collected from inhaler, stage one (S1) and stage two (S2). The emitted dose (ED) was the amount of drug delivered from the inhaler presented as a percentage of RD.

HPLC Analysis of Salmeterol Xinafoate

SX was analyzed by HPLC using a C_{18} column (μBondapak™, 3.9 × 300 mm, Waters, Milford, MA, USA) 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/minute by a HPLC pump (Waters 510, USA). The peak area was recorded by integration (Shimadzu CR6A Chromatopack, Japan). The retention time of SX was 4.2–4.9 min (the variation from batch to batch ranged from 4.2–4.9 min whereas a single batch showed a constant retention time). The calibration plot of standard SX solution 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 were 91.4 ± 2.4%, 100.4 ± 5.3%, 101.3 ± 1.1%, and 99.8 ± 0.6%, respectively (mean ± SD). The precision was tested before each experiment by analyzing 4.0 μg/mL standard solution of SX using five replicates, and the CV was below 2%.

Morphological Properties of Lactose by Scanning Electron Microscopy (SEM)

For surface morphological studies of different lactoses and interactive mixtures, samples were glued onto aluminium stubs. Particles were then gold coated (thickness ≈15–20 nm) with a sputter coater (BAL-TEC SCD 005, Japan) using an electrical potential of 2.0 kV and 25 mA for 3 min. Several photomicrographs were taken at several magnifications with a Scanning Electron Microscope (Hitachi S-570, Japan).

Adhesion Force Measurement

The adhesional properties of different lactose carriers were measured using atomic force microscopy (AFM) with a colloid probe technique (31). Sample were prepared using Araldite Epoxy Resins (A & B; Selleys Chemical Company Pty. Ltd., Australia) that were mixed and spread uniformly over the cleaned surface of a 1 cm^2 section of the silicon wafer. Lactose particles were attached to the resin by sprinkling the lactose particles onto the viscous resin. The samples were dried overnight and the excess particles were removed by nitrogen flow. This sample was adhered onto a stainless steel sample stub by a double-sided adhesive tape (Scotch, 3M Australia Pty Ltd.) and subjected for force measurement. The silica (5 μm) and SX probe were prepared using a micromanipulator (Model M3301R; World Precision Instruments, Inc., Sarasota, FL, USA) and optical microscope. A 5μm silica sphere (Bangs Laboratories Inc., Fishers, IN, USA) and a particle of SX were attached to the apex of V-shaped silicon nitride cantilevers (Nanoprobe™ SPM Tips, Type NP-S, Digital Instruments, Santa Barbara, CA, USA; spring constant, k = 0.33N/m). The colloid probe was examined under an optical microscope to ensure successful attachment on the right position of the cantilever and to measure the radius of the colloid particle.

The adhesion forces between the probe(s) and the surfaces of individual lactose particles were measured by an atomic force microscope (Nanoscope Multimode IIIa, Digital Instruments, USA) in air and ambient humidity. The individual force curves captured by AFM (keeping constant contact time and constant maximum force) were analyzed using AFM software written by Patrick Hartley. The individual force was calculated using Hooke's law, where the cantilever deflection was multiplied by the cantilever spring constant. The spring constant of the cantilevers was determined by the attachment of known masses to the cantilever (32). Individual adhesion forces were measured by the detachment of the silica sphere from the lactose surface. More than 100 individual adhesion force plots were analyzed at 50 random sites on 5 different particles for each sample.

Statistical Analysis

Linear regression analysis (Sigmastat, Jandel Scientific, USA) was used in the calibration of HPLC and UV analyses. Comparison between different groups of FPF was performed by one-way analysis of variance (Sigmastat). Covariance Analysis by Minitab (Minitab Corporation, State College, PA, USA) was undertaken to compare the slopes of regression lines.

RESULTS AND DISCUSSION

Drug Dispersion from Lactose Carriers

Several commercial inhalation-grade lactose carriers were used to prepare interactive mixtures of 2.5% SX with good homogeneity, that is, CVs less than 3%. The lactose carriers had inherently different particle size distributions with volume mean diameters ranging from about 10 to 150 μm (Fig. 1). The dispersion of SX from these mixtures, measured by the twin stage impinger, was dependent on the type of lactose carriers in the interactive mixtures and showed FPFs ranging from 4 to 24%; this type of effect of carrier on drug dispersion in DPIs has been demonstrated previously (17,19,22). Close observation of the lactose carriers by SEM demonstrated the presence of fine lactose associated with the lactose carrier (Fig. 2). The small particles of lactose were mainly adhered on the carrier surface and were present to differing extents in the lactose samples used. The existence of fine lactose also was evident in the particle size distributions in Fig. 1. SX dispersion increased as the concentration of fine lactose particles in the mixture (designated by lactose particles less than 5 μm) increased (Fig. 3). The influence of fine lactose was significant with a 5- to 6-fold difference in SX dispersion for the six interactive mixtures. This observation also was not new and has been supported by investigations in our laboratories (19) and by others using a range of drugs and lactose carriers (15,16,20,21). Increased dispersion by the fine lactose has been explained by theories proposed in these studies and includes the "activated site" theory (15,26) and the "redistribution and agglomeration" hypothesis (17,19). However, the mechanism of drug dispersion in DPI formulations is still unclear. To gain a better understanding of the effect of fine lactose on dispersion, the surface of the lactose carrier was modified using a wet decantation method to remove fine lactose from the carrier surface. The purpose of this study was to determine if there were any carrier specific effects on dispersion when the carrier was stripped of fine lactose particles, i.e., does the carrier's surface characteristics have an effect on dispersion or are the effects that are seen in this study and elsewhere related to the presence of fine lactose powder on the surface of the lactose carrier?

Removal of Fine Particles by Wet Decantation

To study the effect of wet decantation on the efficiency of the lactose carrier to disperse drug, Aeroflo 65 and 95 were chosen for the decantation process because they possessed

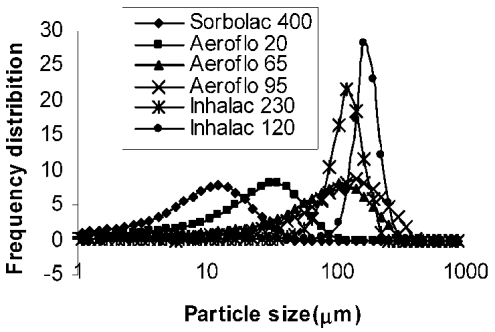

Fig. 1. Particle size distributions of the commercial inhalation grade lactose samples using laser diffraction.

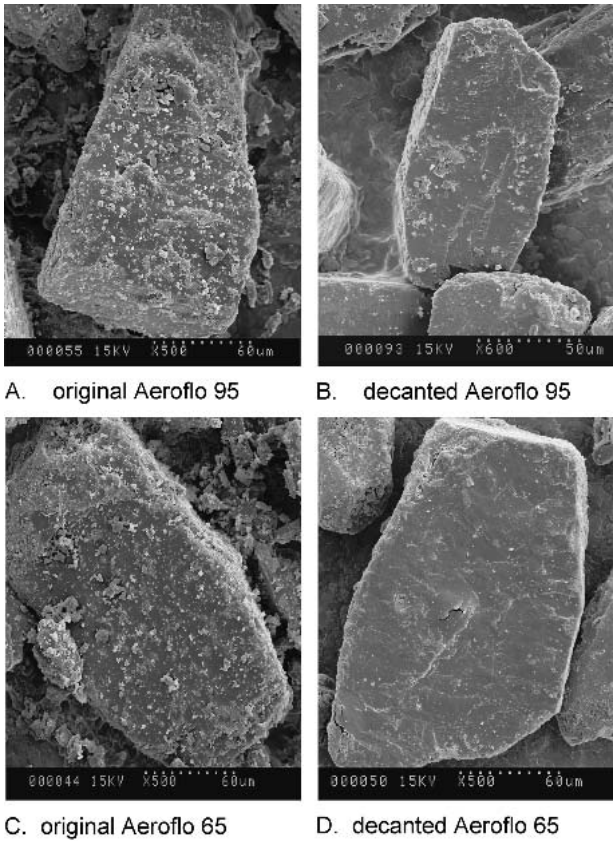

Fig. 2. Scanning electron microscope photographs of Aeroflo 95: A, original; B, decanted using ethanol saturated with lactose, and Aeroflo 65; C, original; D, decanted using ethanol saturated with lactose.

significant concentrations of lactose less than 5 μm, a relatively broad particle size distribution which was more conducive to decantation and sieve classification and a reasonable high FPF of SX. Wet decantation decreased the fine lactose in the Aeroflo 65 and 95 with the decanted fractions showing about 60–70% removal of fine lactose (estimated using a less than 5 μm fraction) after 15 repeat wet decantation cycles (Table I). In addition, the measured volume median diameter (VMD) of the original Aeroflo 65 (83 μm) and Aeroflo 95 (113 μm) were significantly ($p < 0.05$) increased. Such changes were expected with the removal of the fine particles of lactose from the distribution. SEM demonstrated clearly

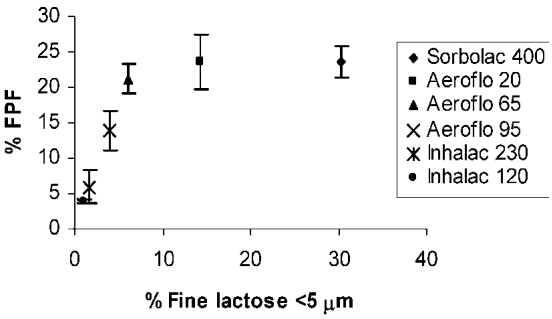

Fig. 3. Relationship between the fine particle fraction (FPF) of salmeterol xinafoate and the concentration of fine lactose (<5 μm) for interactive mixtures of different commercial inhalation grade lactose samples containing 2.5% salmetarol xinafoate.

Table I. Volume Mean Diameter (VMD), Percentage of Fine Lactose <5 μm (%<5 μm), and Fine Particle Fraction (FPF) of Aeroflo 95 and Aeroflo 65 Before and After Decantation

Lactose samples	VMD (μm)	% <5 μm	FPF (%)
Aeroflo 95 original	113	4.0	13.8
Aeroflo 95 decanted	149	1.4	5.9
Aeroflo 65 original	83	6.2	21.2
Aeroflo 65 decanted	113	1.7	4.7
Aeroflo 95 decanted with lactose < 5 μm restored to that of original	–	4.0	12.3
Aeroflo 65 decanted with lactose < 5 μm restored to that of original	–	6.2	18.9

the difference between the original and decanted lactose samples. A large number of fine lactose particles can be seen on the surface of lactose carrier before decantation, whereas decanted carriers showed only a few scattered fine particles on the large carriers (Fig. 2). Fine lactose particles observed on the decanted surface seemed to be an integrated part of large carriers. These fine particles, which were difficult to remove during decantation, could be more firmly bound to the surface through solid bridging.

The FPF of the original Aeroflo 65 was significantly higher than that of Aeroflo 95 ($p < 0.001$). The FPF of Aeroflo 65 and 95 was reduced after decantation (Table I). Tukey's pairwise comparison test of both Aeroflo 65 and Aeroflo 95 found significant differences between FPF of dry sieved and decanted samples ($p < 0.05$). The wet decanting process therefore caused a significant decrease in dispersion of SX. Interestingly, FPFs were found to revert very close to their original values after the addition of micronized lactose (VMD 4.0 μm) to the decanted carriers (Table I). Although numerically the FPFs of ternary components were not the same as the original values, the differences were not statistically significant ($p < 0.05$). Any variation may be associated with the difficulty in adding a fine particle distribution similar to that of the original lactose and the absence of intermediate sized lactose particles in the restored decanted samples; intermediate sized particles have been reported to have significant effect in improving drug FPF (15).

Sieved Fractions of Aeroflo 95 and 65

To gain more information about the influence of decantation on dispersion, sieved fractions of 45–63, 64–90, 91–106, and >106 μm of Aeroflo 95 and 65 were prepared and each fraction underwent the decantation process. Dispersion of SX (2.5%) from mixtures of these dry sieved and decanted lactose carriers was measured by the FPF using the twin stage impinger. Results are shown in Table II.

After decantation, the Aeroflo 95 and 65 fractions contained similar amounts of fine lactose. It was more difficult to remove adhered fines from the smallest fractions of Aeroflo 65 and 95. The 45–63 μm fraction of Aeroflo 65 and 95 contained 2.8 and 3% of particles less than 5 μm, respectively, whereas all other fractions showed more consistent concentrations of fine particles less than 5 μm at about 1–2%. The VMD of sieved fractions, 45–63, 64–90, 91–106,and > 106 μm of Aeroflo 95 and 65 significantly increased after decantation ($p < 0.05$).

The FPF of all SX (2.5%) mixtures with the sieved and decanted fractions of Aeroflo 95 and 65 were determined (Table II). For the Aeroflo 95 mixtures, there was a significant difference between the FPF of the four dry sieved fractions ($p < 0.001$). Pairwise comparison found significant difference in FPF between fraction 45–63 μm vs other three fractions, and between the 63-90 μm vs 90-106 μm & >106 μm fractions, but no difference between the 90 and 106 μm and >106 μm with 0.05 level of significance. The relationship between FPF and concentration of fine lactose (<5 μm) is clearly evident and consistent with the data in Fig. 3 for all the lactose carriers.

After decantation, a significant reduction in FPF of all fractions occurred ($p < 0.001$). Tukey test showed only difference ($p < 0.05$) between 45–63 μm and the 64–90 μm, 91–106 μm, and >106 μm fractions, whereas there was no significance difference ($p < 0.05$) between the 63–90 μm, 91–106 μm, and >106 μm fractions. The reason for the higher FPF for 45–63 μm was probably due to higher concentration of fine lactose (3.0%) present in this fraction, in comparison to similar fine lactose concentrations in the other fractions (i.e., around 1.5%).

Aeroflo 65 mixtures showed similar trends in FPF to those of the Aeroflo 95 mixtures (Table II). Highly significant differences were found between FPF of all corresponding fractions before and after decantation ($p < 0.001$). Multiple comparisons showed significant difference between fraction 45–63 μm and the other fractions ($p < 0.05$). No significant difference ($p < 0.05$) was observed within the 63–90, 91–106, and >106 μm fractions. For both Aeroflo 95 and 65, the FPF was unrelated to the lactose carrier size fraction where the size fractions possessed similar concentrations of fine lactose.

Table II. Volume Mean Diameter (VMD), Percentage of Fine Lactose (FL) <5 μm (%<5 μm), and Fine Particle Fraction (FPF) of Sieved Fractions of Aeroflo 95 and Aeroflo 65 Before (Dry Sieved) and After Decantation (Mean ± SD, n = 5)

	% FL (<5 μm)				VMD (μm)				FPF (%)			
	Dry sieved		Decanted		Dry sieved		Decanted		Dry sieved		Decanted	
Size fraction	Aeroflo 95	Aeroflo 65	Aeroflo 95	Aeroflo 65	Aeroflo 95	Aeroflo 65	Aeroflo 95	Aeroflo 65	Aeroflo 95	Aeroflo 65	Aeroflo 95	Aeroflo 65
45–63	6.9 (0.1)	7.7 (1.0)	3.0 (0.1)	2.7 (0.0)	45.9 (0.1)	38.3 (0.7)	62.7 (0.1)	62.2 (0.6)	18.38 (2.7)	22.2 (2.3)	8.1 (1.3)	7.4 (2.2)
64–90	4.1 (0.1)	5.0 (0.7)	1.9 (0.0)	1.9 (0.0)	82.1 (1.2)	70.2 (0.9)	100.1 (0.3)	90.5 (0.8)	14.61 (1.2)	20.2 (1.7)	5.9 (1.4)	5.4 (1.2)
91–106	2.8 (0.0)	3.5 (0.7)	1.5 (0.0)	1.6 (0.0)	120.7 (0.5)	106.3 (0.9)	131.3 (0.1)	115.0 (0.5)	9.88 (2.6)	13.6 (1.3)	5.13 (0.3)	4.5 (0.9)
>106	1.6 (0.0)	1.5 (0.2)	1.2 (0.1)	1.2 (0.0)	184.4 (0.7)	166.6 (1.6)	189.6 (0.9)	169.2 (0.4)	7.55 (1.63)	6.8 (2.9)	4.9 (1.1)	4.5 (0.5)

Relationship between FPF and Concentration of Fine Lactose

The relationship between FPF of SX and concentration of fine lactose of different dry sieved and decanted fractions of Aeroflo 95 and 65 is shown in Fig. 4a and b. There is both a reasonably good linear relationship and significant regression between FPF of SX and concentration of fine lactose less than 5 μm for the dry sieved fractions of Aeroflo 95 ($r^2 = 0.972$, $p = 0.014$) and Aeroflo 65 ($r^2 = 0.908$, $p = 0.047$). Although two linear regressions for dry fractions are not superimposed, the slopes of the regressions are not significantly different. The behavior of the fine lactose has the same quantitative effect on the two grades of lactose. In addition, the slopes of the regressions are very similar to the slope of the linear portion of the curve in Fig. 3, where the concentration of fine lactose in the different lactose samples does not exceed about 8% and includes Inhalac 120 and 230 in addition to Aeroflo 95 and 65. Therefore, these results further confirm that fine lactose is a key factor of controlling FPF.

The relationship between the FPF of SX and concentration of fine lactose for the decanted fractions of Aeroflo 95 and 65 was linear and significant, although over a much lower fine particle concentration range and due to the slightly higher fine lactose particle concentration of the 45- to 63-μm fractions. The best-fit lines were slightly displaced from the regression lines of the dry sieved fractions; however, the displacement was not significant for the Aeroflo 65 and only marginally significant for the Aeroflo 95. This displacement indicated that the decantation process not only removed fine lactose particles, but also caused some other change to the surface of the lactose carrier that influenced dispersion. The extent of decreased dispersion was small. Although absolute alcohol saturated with lactose was used in the wet decantation, some modification to the lactose surface may have been caused through the recrystallization of amorphous parts of the surface or through a physical smoothing due to lactose dissolution. The surface change was confirmed by measuring the adhesion force between probes of SX and a silica probe and the lactose surface using atomic force microscopy (Table III). Adhesion increased for the interaction with the decanted lactose samples for both carriers. However, since the original FPF of the Aeroflo 95 and 65 could be restored by the addition of fine lactose to the decanted samples (Table II), any change to the surface had little inherent effect and the presence of fine lactose particles was seen to be dominant in controlling the extent of SX dispersion.

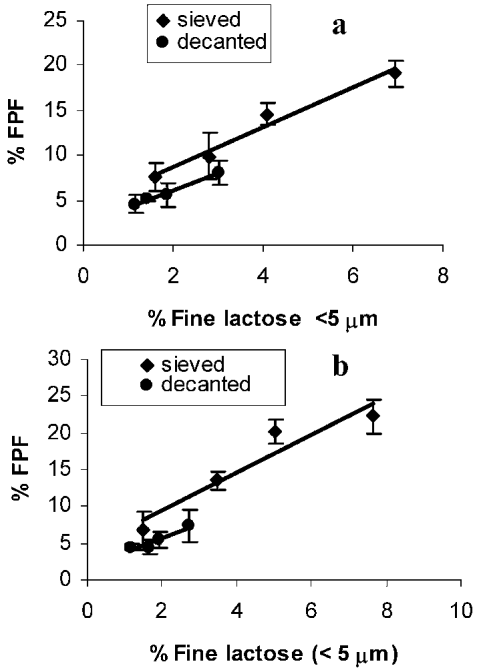

Fig. 4. Relationship between the fine particle fraction (FPF) of salmetarol xinafoate and the concentration of fine lactose less than 5 μm for different dry sieved and decanted size fractions (45–63 μm, 64–90 μm, 91–106 μm, and >106 μm) of (a) Aeroflo 95 and (b) Aeroflo 65. (Mean, n = 5).

Relative Mumber of SX and Fine Lactose Particles

The particle number (i.e, number of particles per unit weight: $n = 6/\pi(d_{vn})^3\rho$, where d_{vn} is the volume number diameter and ρ is the density) can be used to estimate the number of particles of lactose carrier, fine lactose less than 5 μm and SX in a sample of the mixture. Using this information, the number of SX and fine lactose particles associated with the lactose carrier particle can be estimated. These calculations possess a number of assumptions, for example, the particles were spherical and the volume number diameter of fine lactose particles used in the calculation was 4.9 μm. The relationship between FPF and ratio of SX to fine lactose on the lactose carrier surface is shown in Fig. 5. This figure represented data from 14 different lactose samples that included all original lactose carriers from two manufacturers, and dry sieved and decanted fractions. There was remarkable consistency in the pattern of behavior shown in Fig. 5 given the wide range of lactose samples. Optimum dispersion occurred when the ratio of SX to fine lactose approached 1; the dispersion of SX decreased in a sigmoidal manner and reached a minimum FPF when the ratio of SX to fine lactose was about 15–20. The interpretation of the behavior at this stage must be speculative. The pattern of behavior was consistent with a previously proposed hypothesis relating to the mechanism of dispersion of micronized salbutamol sulphate (SS) in mixtures with lactose carriers (17). The hypothesis suggested that dispersion occurred through redistribution of fine SS and lactose particles producing mixed agglomerates of SS and fine lactose in the mixture, probably associated with the lactose carrier surface. Dispersion was proposed to occur from the mixed agglomerates, with the presence of fine lactose enhancing the degree of dispersion. The data in Fig. 5 can be interpreted using the proposed hypothesis. For example, as the mixed

Table III. Adhesion Force Between 5-μm Probes of Salmeterol Xinafoate and Silica and Original and Decanted Aeroflo 95 and 65 Surfaces Using Atomic Force Microscopy (n = 100)

Lactose surface	Adhesion force of 5-μm salmetarol xinafoate probe (nN/m)	Adhesion force of 5-μm silica probe (nN/m)
Aeroflo 95 original	49.3 (26.8)	29.2 (17.5)
Aeroflo 95 decanted	72.5 (44.0)	56.1 (39.3)
Aeroflo 65 original	55.4 (33.0)	38.8 (14.6)
Aeroflo 65 decanted	134.5 (71.4)	120.7 (78.7)

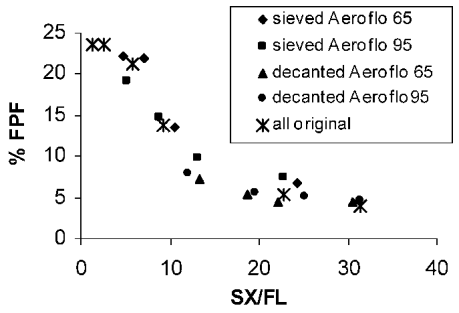

Fig. 5. Relationship between the fine particle fraction (FPF) of salmetarol xinafoate and the calculated ratio of number of particles of salmetarol xinafoate to lactose less than 5 μm in the mixture for all original inhalation grade lactose samples, decanted Aeroflo 95 and 65, and all dry sieved and decanted fractions of Aeroflo 95 and 65.

agglomerate became rich in SX, the degree of dispersion decreased and approached a constant value that might be indicative of pure SX agglomerates held together by strong cohesive interactions. The degree of dispersion was at a maximum level when the mixed agglomerate contained equal numbers of SX and fine lactose particles. The role of the fine lactose in causing dispersion is unknown, but is likely to be associated with the disruption of cohesive interactions within the agglomerate allowing greater dispersion when subjected to dispersion forces in the inhaler. Previous studies have shown using SS that, upon further addition of fine lactose to the mixture to produce agglomerates rich in lactose, the degree of dispersion of SS decreased due to drug dilution in the agglomerate (19); however, this was not able to be investigated with the lactose carriers used in this study.

CONCLUSION

The outcomes of this study showed that the fine particles of lactose play a key role in SX dispersion from lactose mixtures. Wet decantation modified the lactose surface, exemplified by SEM and the different adhesion characteristics between the lactose surfaces and SX and silica probes; however, when the fine lactose, removed by wet decantation, was restored to its original concentration by the addition of micronized lactose, the FPFs were not significantly different from the original lactose samples. Finally, the consistent pattern demonstrated in the plot of FPF vs. SX/fine lactose ratio, indicated that the lactose surface characteristics of different samples of lactose carriers were less important in controlling the dispersion than the critical ratios of drug to fine lactose. The results obtained in this study were obtained from laboratory scale powder mixing processes; extending the interpretations to full scale processing where mixing energies may be different should be undertaken cautiously.

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