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Influence of added of fines to the decanted carrier surfaces used in dry powder formulations

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Abstract
Purpose: Investigation of the effect of adding carrier fines to the wet decanted carriers in addition, the subsequent effects of this change in the carrier surface characteristics on the *in vitro* deposition of salbutamol sulphate.
Methods: The wet decantation process used to remove the surface carrier fines carried out nine time for both lactose and mannitol carriers. Addition of 2%, 4% and 8% lactose fines to decanted lactose. Additionally, mannitol fines of 4%, 8% and 16% added to decanted mannitol. *In vitro* deposition carried out for those treated carriers after mixing with salbutamol sulphate using a Next Generation Impactor (NGI). The treated carriers with fines characterized by scanning electron microscope, Brunauer Emmet and Teller specific surface area determination, differential scanning calorimetry, X-ray powder diffraction and water vapour sorption in comparison with the treated carrier without added fines.
Results: For decanted mannitol and decanted lactose carriers, there were significant increase in fine particle fraction (FPFs). This probably by reducing drug-carrier adhesion and allowing easy detachment of the drugs from the carrier surface. Although this addition of fines could not revert the FPFS to the original value of the untreated carriers, which may indicate that they were not equal to the original fines removed by wet decantation. Added carrier fines exhibit another shape, surface area and energetic charge than the inherent fines.
Conclusions: Addition of micronized carriers to decanted carriers found to increase significantly the dispersion of the drug and subsequently the FPF in the *in vitro* deposition test.

Keywords: dry powder inhalation, decanted lactose, decanted mannitol, carrier fines, salbutamol sulphate

1. Introduction

Drug deposition in the lungs found to be highly dependent on the dry powder formulation in both pressurized and dry powder inhalers [2, 6]. Almost every DPI formulated as an interactive/ordered mixture where the drug particles adsorbed on the surface of coarse lactose as drug carrier. Coarser carrier fractions used mainly to yield a much better dose emission from the inhaler [9, 18, 24]. The carriers for these mixtures mostly have a size range of 90-200µm [2]. The drug-to-carrier interaction forces precisely controlled to ensure efficient drug delivery to the deep lung parts. They have to be strong enough to guarantee good mixture stability during handling and storage, but weak enough to enable the separation forces during inhalation to detach a substantial fraction of the drug dose from the carrier crystals. This requires that the size distributions of the interaction forces (during mixing) and separation forces (during inhalation) must be balanced properly [5]. The particle size, shape, surface morphology and chemical composition of carrier particles can directly influence aerosol dispersion. Increased drug deposition generally observed with smaller carrier size [2], and increased proportion of fine particles at the carrier surface [21]. However, the carrier size did not affect the FPF in some formulations [24]. Fines may be present on the carrier surface from attrition (naturally adhering fines), or by the addition of certain quantities of fine or intermediate sized lactose to coarser lactose carrier fractions [18, 25]. The effect of very fine particles may play an important role in the flow and aerosol dispersion behaviour of particles in the 1-10 µm range [25]. Some literatures [2, 12] showed the effect of added fine carrier particles to the powder mixture improved drug deposition for a number of drugs. The added ternary components examined include magnesium stearate, lactose, L-leucine, PEG 6000, and lecithin [32]. Although the mechanism for improved FPF by ternary components has not been fully characterized, possible explanations, include the saturation of active sites on the carrier, electrostatic interactions, and drug redistribution on the ternary component. Saturation of active sites on the carrier particle by the lactose have proposed as the mechanism for increased drug deposition, whereby the adhesion of micronized excipient onto active (high adhesion) sites leaves passive (low adhesion) sites available for drug adhesion [25]. The reduced adhesion between drug and the carrier particle suggested increasing drug detachment. The use of micronized or fine carriers as ternary agent is preferred than other ternary agents due to the known toxicology profile of sugar carriers. Active sites interpreted; as locations of disturbance on the surface of a crystal were more active, molecular groups presented to the outside. This might be due to simple dislocations in the crystal lattice, or the complete distortion of the molecular order. Such regions might have different depth but in every case present areas of higher surface interaction

compared with the surrounding crystal areas [10]. This study discusses the possibility of adding carrier fines to the carrier materials, which smoothed using the wet decantation process, and the subsequent effect of those added fines on the *in vitro* deposition. This study also deals with the influence of lactose and mannitol fine particles, which can be present in the carrier and resulting in variation in surface and contact area, on the performance of dry powder inhaler formulations.

The percent of naturally adhering carrier fines present on the untreated coarse carrier surface was determined using an air jet sieve [3]. Lactose fines removed by air jet sieving yielded about 4.5%, whilst mannitol was determined to contain 13.7% fines [3]. Accordingly, carrier fines added to the decanted carriers, namely 2%, 4%, 8% to the lactose, and 4%, 8%, 16% to the mannitol carrier in order to find out whether the FPF might be reverted to the original value of the raw carriers before applying the wet decantation process.

Many literatures [12, 17, 25, 27] report that maximum dispersion occurs when about 10%-15% fine particles are present in the mixture. Interactive mixtures consisting of very large proportions of fine lactose particles showed a significantly lower FPF. Zeng *et al.*, 1999, found that, formulations produced by blending the coarse carrier and the carrier fines before adding the drug (thus giving the fines the first opportunity to bind to areas of high adhesion on the carrier) gave greater performance (Figure 8, A) than formulations produced by reverse way of addition. The carriers treated by nine times decantation[3] and the treated carriers with added carrier fines (lactose plus 2%, 4%, 8% lactose fines, mannitol plus 4%, 8%, 16% mannitol fines) were characterized using different techniques.

2. Material and Methods

Roquette GmbH (Lestrem, France) supplied mannitol (Pearlitol160c) kindly; Meggle GmbH (Wasserburg, Germany) supplied lactose (InhaLac120) kindly. Lindopharm (Hilden, Germany) kindly supplied salbutamol sulfate. Absolute ethanol, methanol, and acetonitrile (HPLC grade) purchased from VWR International GmbH (Darmstadt, Germany). Dichloromethane purchased from KMF Laborchemie Handles GmbH (Lohmar, Germany). Acetic acid purchased from Mallinckrodt Baker B.V. (Deventer, Holland).

2.1 Preparation of coarse carrier

The sieved fractions (112-140 μm) of coarse carrier comprising lactose and mannitol was obtained by sieving a quantity of the powder sequentially through test sieves with an aperture width of 112 and 140 μm, using a sieve shaker AS 200 control (Retsch GmbH & Co. KG, Haan, Germany) for 20 minutes at an amplitude of 1.5 mm. The sieved carriers were placed over silica gel in a desiccator until further required.

2.2 Wet decantation process

The carrier sieve fractions were decanted with absolute ethanol for nine times, respectively. Finally, the powders washed with dichloromethane in which the solubility of lactose and mannitol is negligible, in order to prevent solid bridging. Mannitol (60 g; 112-140 μm fraction) washed with absolute ethanol to remove the fine particles. The mixture stirred to make a homogenous suspension and then allowed to settle for 10 minutes at ambient conditions. The cloudy supernatant fluid decanted and replaced by 60 mL dichloromethane (CH_2 Cl_2) in the final washing step. Again, the powder allowed settling for 10 minutes at ambient conditions. The cloudy supernatant was decanted. During the removal of the supernatant, special care taken to ensure minimum disturbance of the lower part of the suspension. The powder sample left for 2-4 days under the fume hood to dry, and sieved through 112 and 140 μm sieves. The same steps repeatedly applied to lactose.

2.3 Micronization of salbutamol sulfate

Salbutamol sulfate milled by using an air jet mill (50AS, Hosokawa Alpine AG, Augsburg, Germany). The injection pressure was set to 3 bar, the milling pressure to 2 bar, and the feeding rate was adjusted to approximately 1 g/min. Micronized salbutamol sulfate was placed over silica gel in desiccators until further required.

2.4 Preparation of ordered mixtures

Blends of salbutamol sulfate (X_{50} = 2.03 ± 0.03 μm) with coarse lactose or coarse mannitol (112-140 μm) were prepared in a ratio of 1:99 (w/w) in 8 g batches in a stainless steel mixing container using a tumbling mixer (Turbula T2C, WA Bachofen AG, Basel, Switzerland) for 90 minutes, a standard mixing speed of 42 rpm was used. Micronized salbutamol sulfate blended with lactose or mannitol in a sandwich method. Briefly, an amount of the carrier, equivalent to about half the total mass of the carrier used to 'sandwich' the drug in the blend. To minimize the effects of tribocharging, a stainless steel container used. The sample was then stored in a vacuum desiccator over silica gel at least 24 hours to allow the electrostatic charge decay.

2.5 Determination of blend uniformity

The blend uniformity was determined by taking 12 samples with a special sample taker. Each sample dissolved in acetate buffer of pH 3 in a 50 mL graduated cylinder. The amount of salbutamol sulfate in each sample analyzed using high-performance liquid chromatography (HPLC). Mean and relative standard deviation (SD) of the samples calculated to assess the homogeneity of the different blends.

2.6 Differential scanning calorimetry

Differential scanning calorimetry (DSC) carried out using a differential scanning calorimeter (DSC30, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) calibrated with indium. Small amounts (3 mg) of the carriers were crimp-sealed in aluminum pans with pierced lids. One pan then placed in the sample chamber and an empty matched aluminum pan used as the reference for all measurements. The experiments performed in the range from 0°C to 300°C under nitrogen flow of 50 mL/min. The scanning rate adjusted to 10°C/min. The onset temperatures and heat of enthalpy (ΔH) for each peak was determined from the normalized DSC thermogram. Each experiment carried out in duplicate.

2.7 X-ray diffractometry

Powder X-ray diffraction patterns of samples were obtained using the Miniflex diffractometer (Rigaku Corporation,

Tokyo, Japan) with a Cu-Kα radiation (λ = 1.5406Å) as the source of radiation. The diffractometer operated at the voltage of 30 kV and the current of 10 mA. Each sample placed in the cavity of an aluminum sample holder. All samples measured in the 2θ angle range between 5° and 40° with a step size of 0.02°. All samples analyzed in triplicate. X-ray diffraction is a sensitive technique and levels of quantification of 1% amorphous content have been reported [4].

2.8 Water vapor sorption

Sorption/desorption profiles were determined using water vapor sorption. The gravimetric studies were undertaken in a temperature and humidity-controlled system (SPS11, Projekt Messtechnik, Ulm, Germany). Approximately 3 g of samples were loaded. The relative humidity (RH) was first set to 0% and then raised in steps of 10% to 90% and one-step further to 95%. Subsequently, the RH decreased from 95% to 90% then to 0% in the same way. This cycle repeated once more. The equilibrium condition was set to 0.01% mass change per 60 minutes, which reached before the program moved to the next humidity step. The temperature was set to 25°C. Samples weighed in time intervals of 6 minutes.

2.9 Laser diffractometry

The particle size distributions of the micronized drug and the excipients were determined with a Sympatec HELOS laser diffraction spectrometer equipped with a RODOS dry powder dispersing system (Sympatec GmbH, D-Clausthal-Zellerfeld, Germany). The samples fed to the dispersing air stream using a funnel connected to the injector of the RODOS. All calculations made using the Fraunhofer theory. All data given represent the average values of at least 10 determinations at the dispersing pressure of 2.5 bar.

2.10 Particle morphology

The particle morphology of lactose, mannitol, and salbutamol sulfate was examined by using scanning electron microscopy (SEM; LEO VP 1430, LEO Electron Microscopy Ltd., Cambridge, England), operated by using an electron beam at an acceleration voltage of 19 kV and a working distance of approximately 18 mm. Samples (≈0.5 mg) were mounted via a graphite tape to an aluminum stub. After stripping off the upper side of the adhesive, a small amount of particles was scattered on the stub and dispersed by tapping lightly on the edge of the stub with a spatula to break agglomerates or by using an air stream. The particles were then coated with ∼15-20 nm of gold with an Agar manual sputter coater (Agar Scientific Ltd., Stansted, Essex, England), using an electrical potential of 1.5 kV and 20 mA. Photomicrographs of several different areas of the powder on each stub randomly taken. Representative areas of stub photographed with different magnification power.

2.11 Surface area measurement

The surface areas of carrier and drug particles measured by nitrogen adsorption. Prior to surface area measurement, known masses of the samples were accurately weighed into sample tubes and outgassed overnight at 40°C using vacuum for mannitol and at 50°C using a continuous N_2 flow for lactose to remove any adsorbed gases from the surfaces of the particles. This difference of preparation temperatures between the two carriers is due to the sensitivity of each carrier and relies on preliminary experiments. Samples were prepared for 24 hours, the sample tubes then connected to a surface area apparatus. The specific surface carrier area (m^2/g) was obtained by Brunauer, Emmett, and Teller (BET) nitrogen adsorption measurements using a Micromeritics Tristar 3000 (Micromeritics GmbH, Moenchengladbach, Germany) using multipoint analysis. Each sample measured in triplicate and the mean and standard deviation (SD) were calculated.

2.12 *In vitro* deposition by Next Generation Impactor

The aerodynamic particle size distribution of aerosolized salbutamol sulfate carried out using a Next Generation Impactor (Copley Scientific Limited, Nottingham, UK). Methodology followed that of the European Pharmacopoeia [8]. The in vitro dose delivery performance was investigated over a range of pressure drops (1-6KPa) considered representative of that range achievable by asthmatic and chronic obstructive pulmonary disease patients [1]. The adhesive mixtures filled into Novolizer® cartridges (Viatris GmbH & Co. KG, Frankfurt, Germany) and dosing performed with the built-in metering system. The aerosolization done at 79.3 L/min. The impactor plates coated with a viscous solution of the emulgator polyoxyethylene-20-cetylether (0.25%) in glycerol anhydride (4.75%) and isopropanol HPLC grade (95%). A specified volume [2 mL for stages 2-7 and 4 mL for stage 1 and the micro-orifice collector (MOC)] of this solution distributed on each collection plate to provide a thick film. The plates were left to dry under ambient conditions for at least 2 hours prior to each analysis. The preseparator coated with 7.5 mL acetate buffer. The impactor assembled and the Novolizer® then fitted into the molded rubber mouthpiece attached to the throat of the impactor. The TPK Copley pump (Copley Scientific Limited), which was connected to the outlet of the apparatus, was switched on and allowed to run for 3 seconds prior to the release of the dose. The pump then allowed to run for another three (3) seconds at 79.3 L/min, 50 doses were released in this way. The impactor dismantled and the individual plates as well as the MOC carefully washed with acetate buffer pH 3. The inhaler mouthpiece, the throat and the preseparator washed into volumetric flasks of 100 mL, and the washing solution was made up to a set volume with the same solvent (acetate buffer, pH 3). The concentration of salbutamol sulfate in each of the samples analyzed by HPLC. The particles of less than 5 μm expected to be deposited in the lung after inhalation. The ratio of the mass of drug particles less than 5 μm and the emitted dose is defined as the FPF, which is used to describe the respirable fraction of DPI formulations.

2.13 HPLC analysis of salbutamol sulfate

Salbutamol sulfate analyzed by HPLC (Shimadzu C-R4AX CHROMATPAC, Kyoto, Japan) employing a 15 cm × 4.6 mm internal diameter reversed phase column packed with 5 μm C-18 Nucleosil HD RP 18 MN 250/4 (Macherey-Nagel GmbH & Co. KG, Dueren, Germany). A mobile phase is a mixture of 50% acetonitrile and 50% acetate buffer (2.5 g glacial acetic acid (100%) in 1000 mL distilled water), adjusted to pH 3.0. The 50:50 ratio mobile phase was running at a flow rate of

0.52 mL/min. The HPLC system consisted of a pump (LC 6A, Shimadzu, Duisburg, Germany), a multiple wavelength detector (SPD-6AV), and an auto sampler (SIL-6B). The UV/VIS detector operated at a wavelength of 276 nm and the injection volume was 10 µL.

2.14 Statistical tests

The *in vitro* deposition data examined for statistically significant differences by using the single variance analysis (ANOVA) test. A *P*-value of <0.05 was considered significant.

3. Results and Discussion

The carrier materials used as it were supplied after sieve fractionation, the treated carrier (carrier treated with decantation process for nine times) and the treated carrier mixed with 2%, 4%, 8% of micronized lactose (InhaLac120) and 4%, 8%, and 16% of micronized mannitol (Pearlitol 160C) in a tumbling mixer for 45 minutes respectively.

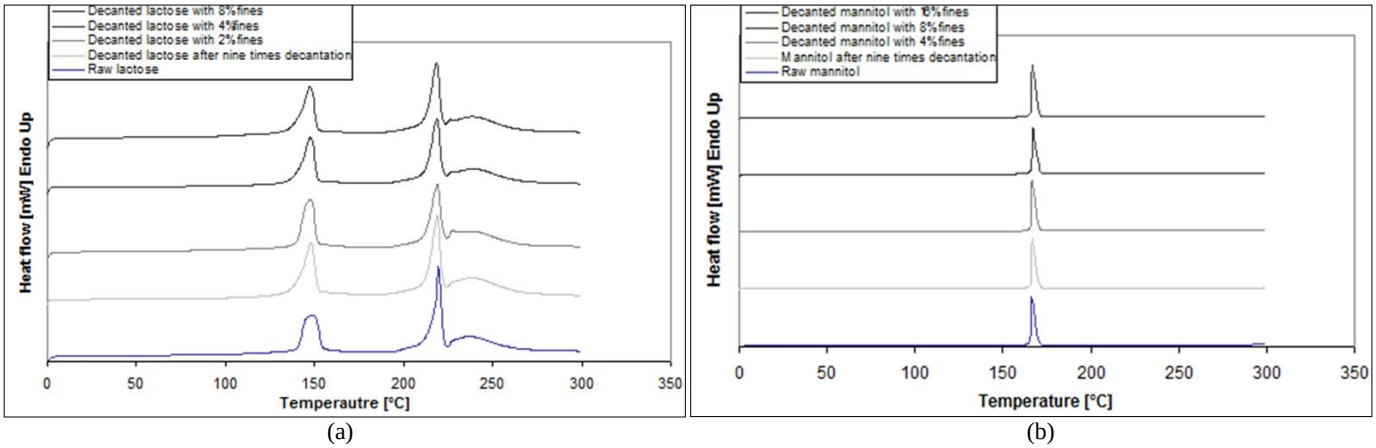
The prepared binary carriers (carriers plus carrier fines) subsequently mixed with salbutamol sulphate for further 45 minutes in a sandwiching manner. The prepared ternary mixtures kept over silica gel for at least 24 hours to allow for electrostatic charge decay. The decanted carriers and the decanted carriers with carrier fines characterized by differential scanning calorimetry (DSC) and X-ray powder diffraction in order to inspect the modification in the crystallinity of the carrier materials. The carrier particle sizes, morphology and specific surface area investigated by laser diffraction, scanning electron microscopy (SEM), Brunauer Emmet and Teller (BET) gas adsorption method and water vapour sorption. Finally, the influence of this rugosity modification to the decanted carriers on the *in vitro* deposition examined.

3.1 Determination of the extent of crystallinity by differential scanning calorimetry

The major influence of the added fines to the formulations is depending on the crystallinity and particle shape of the added smaller-sized lactose and mannitol particles. Micronized lactose expected to be rich in amorphous regions, which is physically softer than the crystalline form. If micronized drug particles bound to these amorphous regions, then deformation of the binding sites would be more likely to occur than the binding sites of the crystalline lactose with increased hardness. The drug particles and coarser lactose carrier expected to bind to the micronized lactose more strongly than to the crystalline lactose [31]. The thermal properties of treated lactose and mannitol before and after addition of carrier fines are similar as shown in Table 1. The change of the percent of fines added to treated lactose and mannitol shows no change on the crystallinity of both carriers. The DSC thermograms (Figure 1) show approximately the similar thermal behaviour of the carriers before and after addition of fines, which suggested no change in the crystallinity of those carriers occurred after addition of carrier fines (micronized lactose and micronized mannitol respectively).

Table 1: Thermal properties of lactose and mannitol samples determined by differential scanning calorimetry using a heating rate of 10°C/min.

Substance	Onset temperature [°C]	Peak temperature [°C]	Heat of fusion [J/g]
Lactose after nine times decantation	158.51 ± 4.384	139.58 ± 0.537	147.74 ± 0.113
Decanted lactose with 2% fines	162.97 ± 1.181	140.35 ± 0.240	147.73 ± 0.071
Decanted lactose with 4% fines	156.15 ± 5.671	140.16 ± 0.212	147.60 ± 0.134
Decanted lactose with 8% fines	157.84 ± 9.588	139.48 ± 0.332	147.59 ± 0.106
Mannitol after nine times decantation	301.69 ± 4.031	166.06 ± 0.007	166.61 ± 0.113
Decanted mannitol with 4% fines	305.25 ± 8.026	165.96 ± 0.021	166.68 ± 0.049
Decanted mannitol with 8% fines	307.38 ± 2.843	165.99 ± 0.106	166.78 ± 0.106
Decanted mannitol with 16% fines	299.20 ± 9.235	166.00 ± 0.106	166.82 ± 0.262

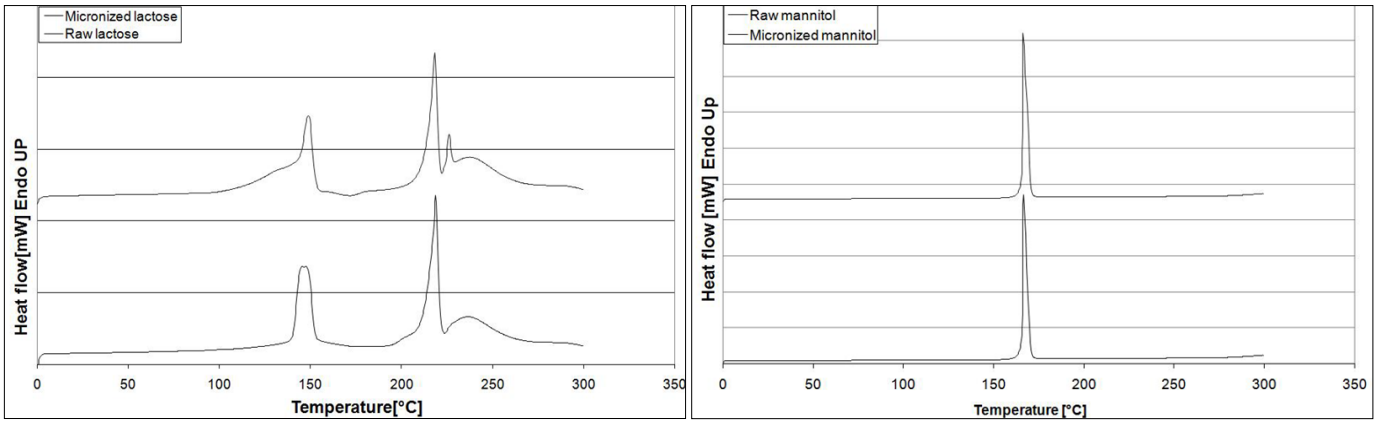


(a) (b)

Fig 1: DSC thermograms of lactose (**a**) and mannitol (**b**) before and after addition of carrier fines.

Moreover, the thermal behavior of lactose after micronization shows a broadening of the first melting peak and small endothermic peak appeared after the second melting peak (Figure 2, a) which could be related to an artefact during

measuring this sample in DSC. Additionally the mannitol DSC thermograms before and after micronization process (Figure 2, b) are approximately the same, which suggest no detectable amorphous part could be attained by using the DSC technique for those micronized carrier materials.

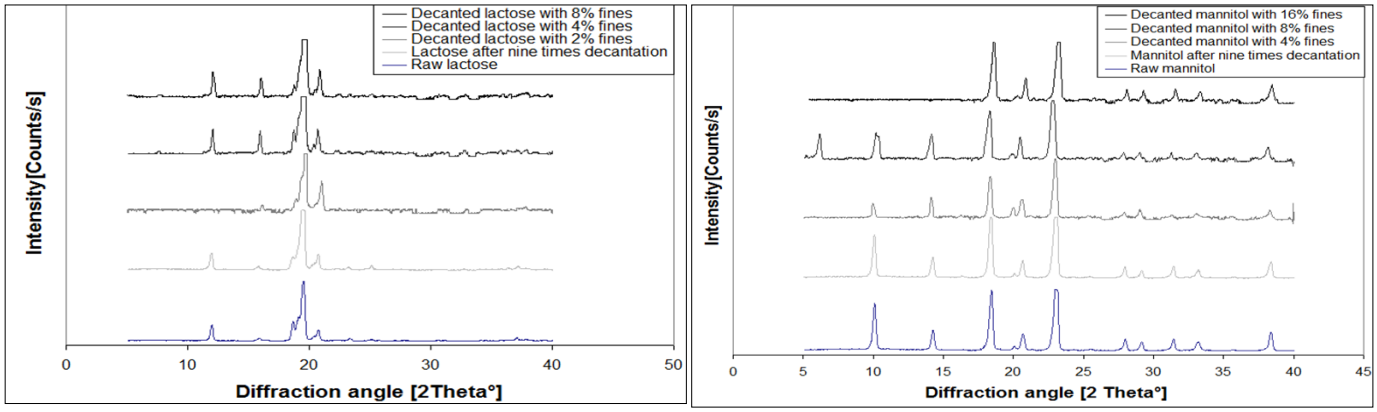


(a) (b)

Fig 2: DSC traces of lactose (a) and mannitol (b) before and after micronization.

3.2 Determination of the extent of crystallinity by X-ray powder diffraction

The XRD diffractograms (Figure 3) are approximately the same for the carriers before and after addition of fines, which suggest no change in the crystallinity of those carriers occurred after addition of carrier fines. This step of characterization suggests the stability of lactose and mannitol carrier materials even after adding different percents of micronized carriers (Although that, milled lactose known to contain an amorphous part).

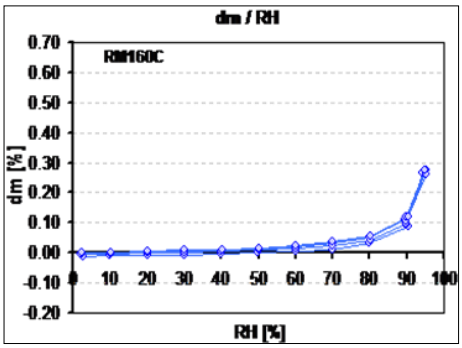


(a) (b)

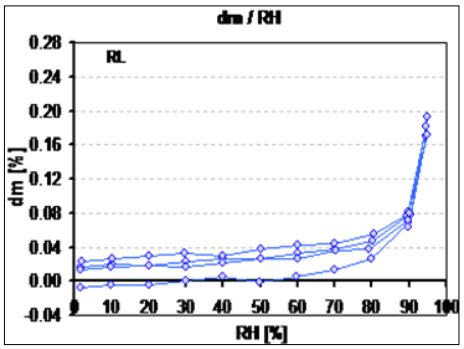
Fig 3: XRD diffractograms of lactose (**a**) and mannitol (**b**) before and after addition of carrier fines.

3.3 Investigation of surface characteristics by water vapour sorption

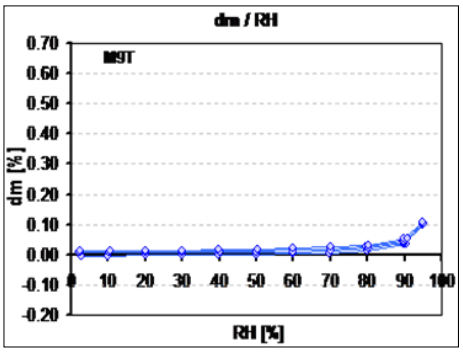
Lactose and mannitol are non-hygroscopic carriers and show an increase of the mass with the increase of relative humidity (Figure 4). The water vapour sorption plots show that the carriers with added fines are taking up more water than, the treated carriers without fines. The lower lines are the adsorption responses and the upper lines are the desorption responses. The adsorption response is due to the building up of few layers of water molecules on the surface. The maximum weight gain for lactose without fines is 0.12% and for mannitol without fines is 0.10%. The water vapour sorption isotherms of lactose and mannitol after addition of fines show an increase in water uptake by the increasing the fines percent in comparison to the lactose and mannitol without fines. This explained by the part of fine carrier particles which has been added to the decanted carrier surface leading to an increase of the surface area and subsequent increase of water sorption. The results of water vapour sorption of lactose are in agreement with the results of the BET measurements, whereas the increase of surface area due to the addition of fines accompanied with an increase of water uptake after addition of 4%, 8% and 16% fines respectively. Nine times treated lactose used in this study show an increase of the mass with the increase of the relative humidity followed by a decrease of the carrier mass with the decrease of the relative humidity in the first sorption cycle. The sorption behaviour of the second cycle is similar. The absence of the mass loss at 60% RH in the first cycle and the similarity of the sorption behaviour in the second cycle assure that there is no amorphous part in this lactose after nine times decantation. Similarly, mannitol shows sorption behaviour of a crystalline carrier with no amorphous content.



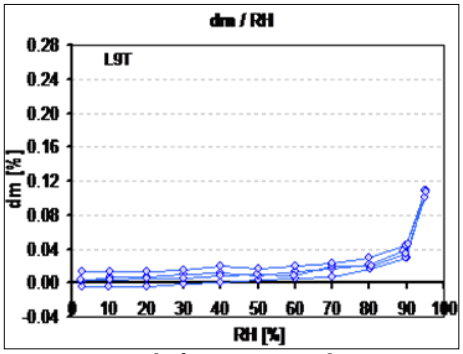
Lactose (112-140µm)



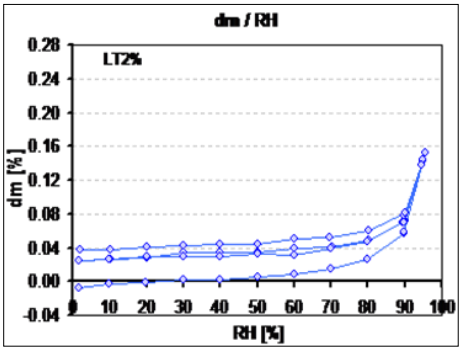
Mannitol (112-140µm)



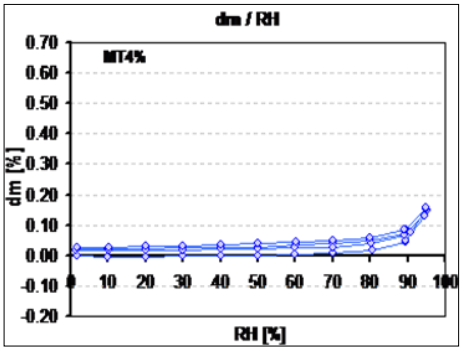
Lactose after nine times decantation



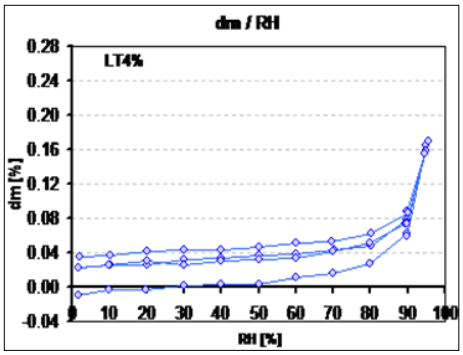
Mannitol after nine times decantation



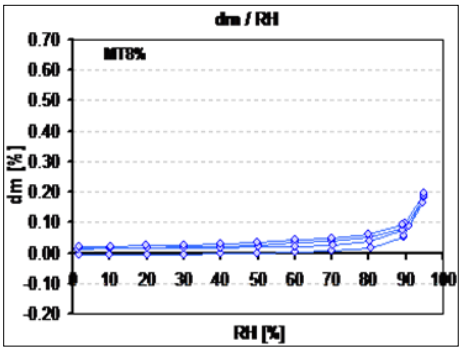
Decanted lactose with 2% fines



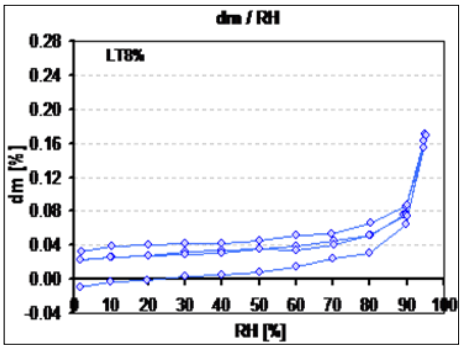
Decanted mannitol with 4% fines



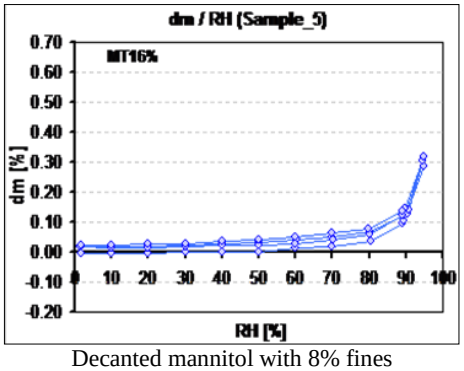
Decanted lactose with 4% fines



Decanted mannitol with 8% fines



Decanted lactose with 4% fines



Decanted mannitol with 8% fines

Fig 4: Water vapour sorption isotherms of lactose and mannitol before and after addition of fines.

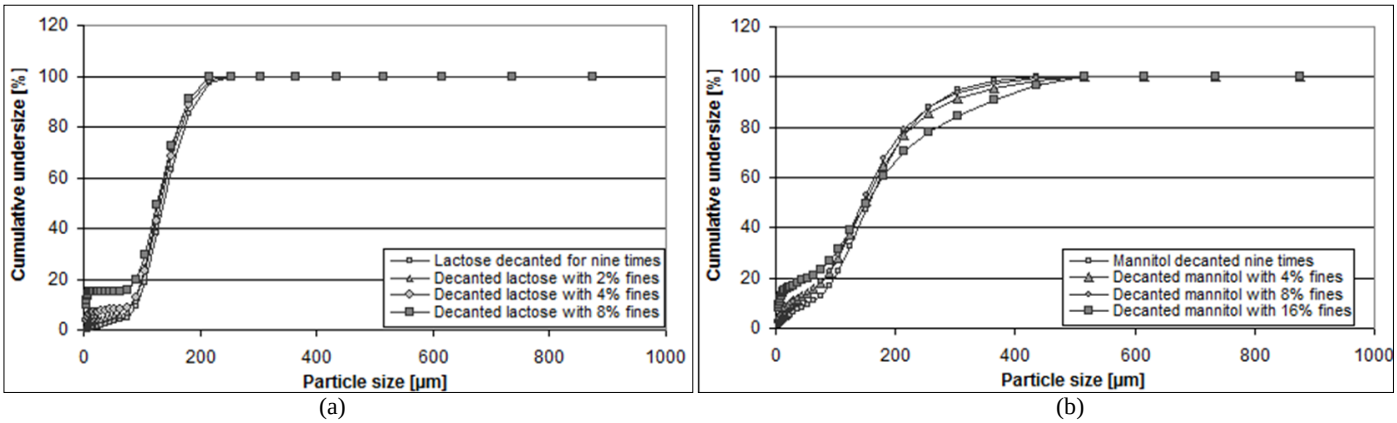
3.4 Particle size distribution

Salbutamol sulphate exhibited a median diameter of 2.03 ± 0.07µm, which show the suitability to use in DPI inhalation formulation (1-5 µm respirable particle range). Fine sugar particles have median diameter of 3.55-5.73µm, suggesting that the fines cannot be directly deposited in a distal lung parts with a large quantity, due to agglomeration tendency of those particles. As shown below in Figure 5 and Table 2, no significant change in the particle size distribution before and after addition of fines. Decanted mannitol with 16% fines showed significant decrease in X_{10} percentile that attributed to formation of fines agglomerates, which have size more than

X_{10} before. Decanted lactose with 8% added fines showed also a small deviation from other decanted lactose carriers with added small percent of fines due to agglomerate formation.

Table 2: Laser diffraction analysis of the carrier particle size distribution before and after addition of fines (n = 3, mean ± SD).

Substance	X_{50} [µm]	X_{10} [µm]	X_{90} [µm]	Span
Lactose after nine times decantation	136.76 ± 1.00	91.42 ± 2.07	193.29 ± 1.15	192.62
Decanted lactose with 2% fines	127.98 ± 0.69	81.80 ± 1.46	177.75 ± 0.45	177.11
Decanted lactose with 4% fines	132.37 ± 0.65	81.11 ± 1.09	185.05 ± 1.22	184.43
Decanted lactose with 8% fines	124.86 ± 2.77	7.035 ±1.78	177.72 ± 2.09	177.16
Mannitol after nine times decantation	155.70 ± 5.23	56.50 ± 4.54	269.48 ± 1.23	269.11
Decanted mannitol with 4% fines	149.86 ± 3.16	29.83 ± 8.04	283.49 ± 11.51	183.29
Decanted mannitol with 8% fines	145.60 ± 2.93	18.01 ± 0.94	278.69 ± 21.59	278.56
Decanted mannitol with 16% fines	148.88 ± 5.50	6.41 ± 1.55	374.59 ± 5.47	374.54

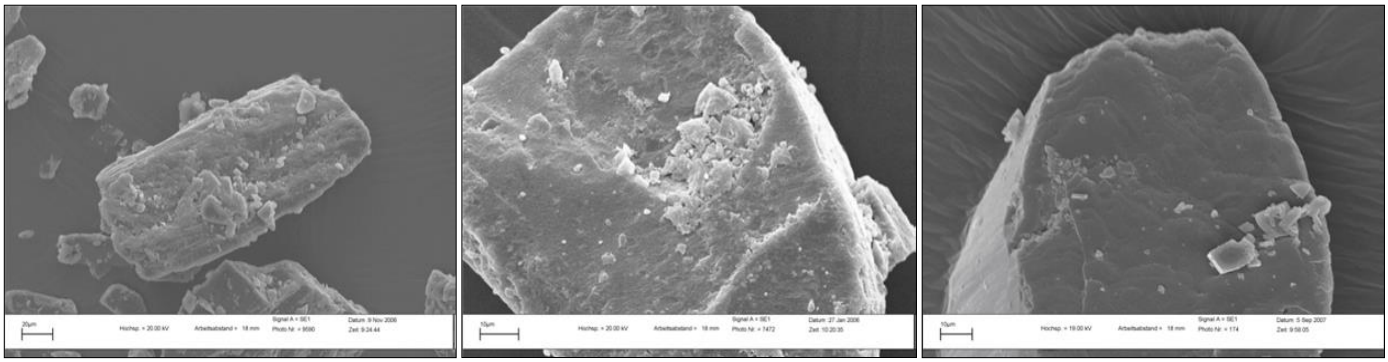


(a) (b)

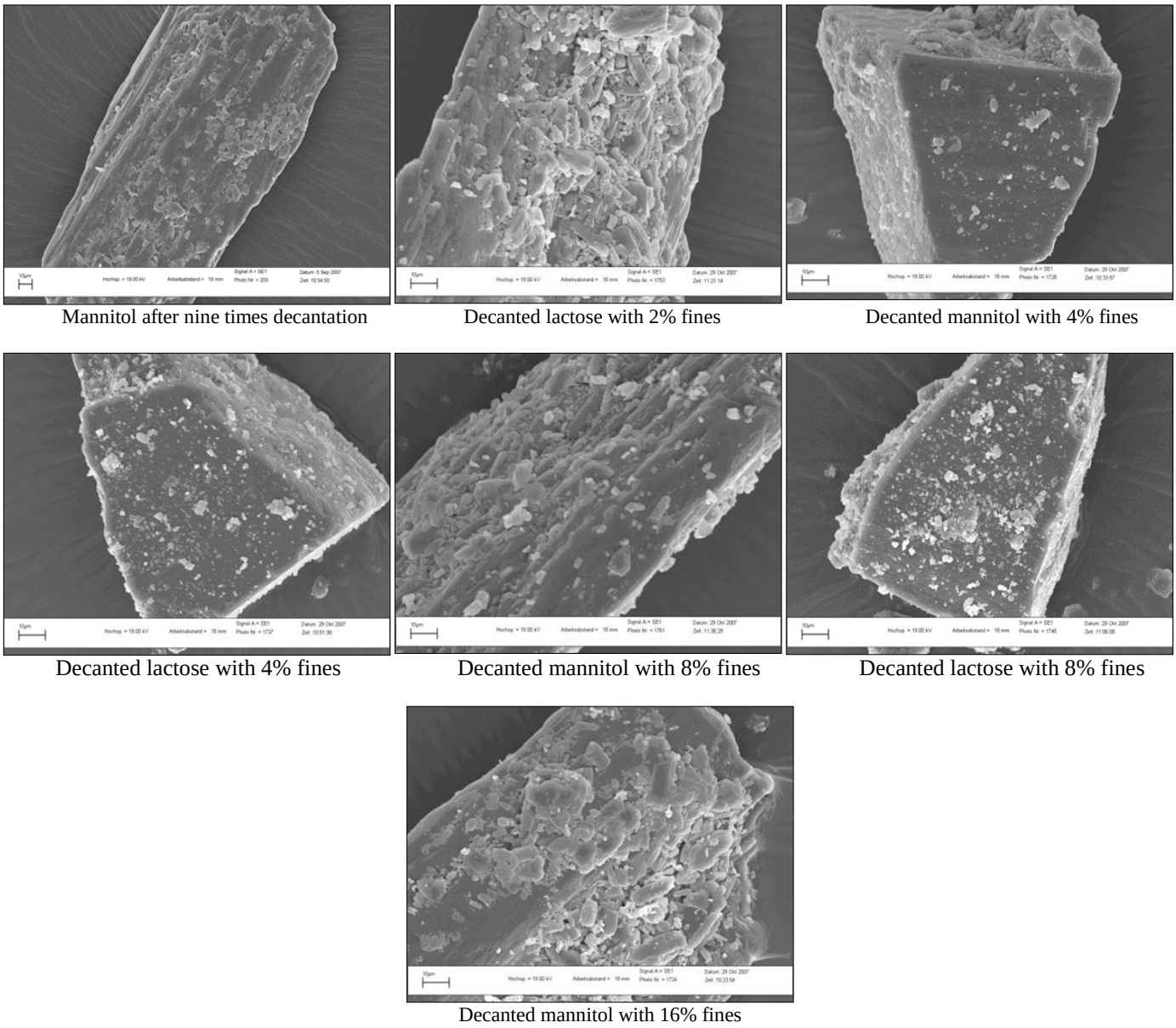
Fig 5: Particle size distribution of lactose (**a**) and mannitol (**b**) carriers before and after addition of carrier fines.

3.5 Particle morphology

Previous investigations reported that the carrier morphology directly affects the aerosolization efficiency from a dry powder inhalation formulation (DPI) [13, 15, 16, 29, 30]. Mannitol shows a very rough and splitted surface with many irregularities where the micronized drug could capture. When this mannitol quality pre-blended with 16% of fine material these irregularities covered by the fine mannitol particles leading to less adhesion of the drug particles. This covering process permits the easiest drug delivery during the inhalation process. Mannitol appears as elongated particles with more surface asperities than the tomahawk shaped lactose crystals. Some of the added fines shown to adhere to the coarse carrier whilst the others dispersed in the powder. The photomicrographs of the ternary carriers (Figure 6) showed the formation of fine particle layers on the treated even carrier surface, whereby the strong active sites were saturated first, and the subsequent particles selectively bind with their own species to form particle layers which decreases the possibility of drug particle adherence to carrier particle after that. This tendency of particle layer formation increased with increased added fine concentration despite the free space (macro-roughness) that was available on the carrier surface.



Lactose (112-140µm) Mannitol (112-140µm) Lactose after nine times decantation



Mannitol after nine times decantation | Decanted lactose with 2% fines | Decanted mannitol with 4% fines

Decanted lactose with 4% fines | Decanted mannitol with 8% fines | Decanted lactose with 8% fines

Decanted mannitol with 16% fines

Fig 6: Scanning electron micrographs of lactose and mannitol before and after addition of carrier fines.

3.6 Determination of surface area by BET measurements

The decanted carrier materials have small surface area due to the removal of fines from these carriers as shown in Figure 7 and Table 3. Apparently, the added fines increase the surface roughness on the smoothed carrier surface, which results in an increase of the specific surface area as expected. This increase in the specific surface area of the treated carriers after addition of carrier fines expected to affect negatively the *in vitro* deposition of the drug using such ternary carriers. Whereby these large surface area could carry higher amount of drug particles, which would held more firmly in the inhaled air stream as suggested by Kawashima *et al.* 1998, since the drug adsorption to the carrier surface seems to be higher at sites of higher rugosity as suggested by most literatures. Podczeck *et al.*, 1998 concluded that this was related to the large increase in the total surface area of the carrier (and thus contact area between drug and excipient) caused by increasing the proportion of fines.

Table 3: The specific surface area of carrier before and after decantation and addition of carrier fines (n = 3 ± SD).

Substance	Specific surface area [m^2/g]
Lactose (112-140µm)	0.2079 ± 0.0170
Lactose after nine times decantation	0.1347 ± 0.0120
Decanted lactose with 2% fines	0.2661 ± 0.0189
Decanted lactose with 4% fines	0.2917 ± 0.0148
Decanted lactose with 8% fines	0.3173 ± 0.0227
Mannitol (112-140µm)	0.3758 ± 0.0690
Mannitol after nine times decantation	0.1843 ± 0.0210
Decanted mannitol with 4% fines	0.2548 ± 0.0287
Decanted mannitol with 8% fines	0.3352 ± 0.0334
Decanted mannitol with 16% fines	0.4678 ± 0.0148

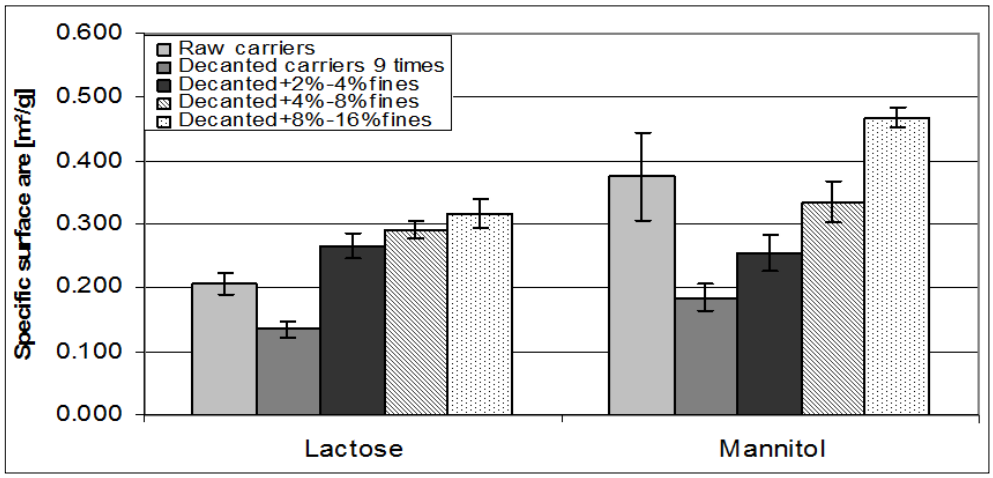


Fig 7: Specific surface area of lactose and mannitol before and after decantation and production of ternary mixtures (n = 3 ± SD).

3.7 *In vitro* deposition

The presence of coarse carrier particles was essential for maximum dispersion in salbutamol sulphate mixture with lactose and mannitol carrier materials. Maximum dispersion occurred when about 10-15% fine particles were present in the mixture as indicated by most literatures. Interactive mixtures consisting of very large proportions of fine lactose particles showed significantly low FPF. Zeng *et al.*, 1999, found that formulations produced by blending the coarse carrier and fines before adding the drug. Thus giving the fines the first opportunity to bind to areas of high adhesion on the carrier gave greater performance (Figure 8, a), than formulations produced by blending the coarse carrier and drug first. Thus giving the drug particles the first opportunity to bind to areas of high adhesion on the carrier. The magnitude of this effect ranged between a 60 and 70% increase in FPF, the other possible blending order (drug and fine excipient particles before addition of coarse carrier) tended to yield an intermediate FPF (Figure 8, b). In this study the first mixing sequence by Zeng *et al.* applied and all mixtures show a good content uniformity with coefficient of variation < 5%. The disadvantage of the fine particles is that they are very adhesive, thus handling them is extremely difficult especially during the addition of fines to the coarse carriers. The percent of carrier fines present on the untreated coarse carrier surface is determined using an air jet sieve. Lactose contains about 4.5% fines; whilst mannitol contains about 13.7% fines[3]. Accordingly, carrier fines added to the decanted carriers, namely 2, 4, 8% to the lactose, and 4, 8, 16% to the mannitol carrier to inspect if the FPF might be reverted to the original value of the raw carriers before subjecting them to the wet decantation process. Increasing the concentration of carrier fines in order to increase the FPF of the drug still further may not be practical, since this may result in poorer flow properties of the powder formulation, which is one of the primary reasons for incorporating coarse carrier particles within the formulation [28]. The aerodynamic studies conducted under ambient conditions of 20.8-22.2°C and 24.7-38.6%RH. The recovery of salbutamol sulphate ranged between 80% and 110%. A summary of the data obtained from NGI impaction presented in Table 4. The removal of carrier fines from the coarse carrier surface by decantation leads to a significant decrease in the FPF as shown in Figure 9. This attributed to the separation of the carrier fines from the carrier surface, which leads to more free active sites on the carrier surface that could occupied with drug particles during the interactive mixture production. This occupation results in higher adhesion forces between the drug and the carrier, leads to a poor drug particle detachment from the carrier particles during the inhalation process.

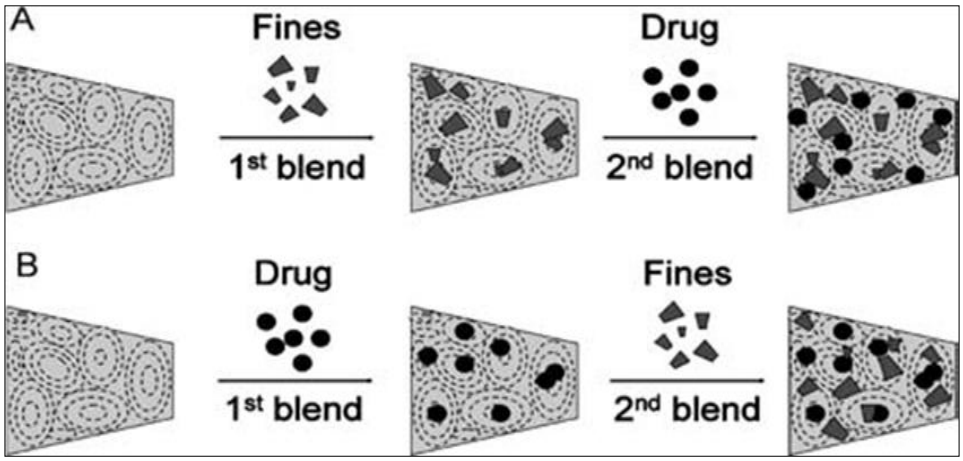


Fig 8: Effect of mixing sequence suggested by Zeng *et al.*, 1999 (Chow *et al.*, 2007).

The results of this study suggest the removal of fines from the coarse carrier surface by wet decantation process, where the reduction of surface micro-roughness leads to produce more surface area available to drug adhesion and subsequently reduced fine particle fraction. Furthermore, the addition of carrier fines after decantation process leads to formation of micro-roughness and decreases the surface area available for drug adhesion. Subsequently, increases the fine particle fraction by quick release of drug particles upon inhalation. These suggestions come in agreement with Iida *et al.*, 2003 who suggest that smoothing of the surface of carrier particles decreased drug particles attached to macro-depressions of the carrier particle surface, and the separation of drug particles from carrier particles may have improved. These results are also in agreement with the results of Kawashima *et al.*, 1998, who reported that lactose particles with larger surface areas could carry a larger amount of drug particles, since they held the drug particles more firmly in the inhaled air-stream. This explained by the increase in the adhesion force between the micronized salbutamol sulphate and the carrier particles, as the macro-roughness of the lactose particle surfaces is increased.

Table 4: The fine particle fraction and recovered dose of the carriers before and after addition of carrier fines (n = 3 ± SD).

Substance	Fine particle fraction [%]	Recoverd dose [%]
Lactose (112-140µm)	33.40 ± 2.88	5.09 ± 0.015
Lactose after nine times Decantation	20.93 ± 1.29	91.28 ± 1.68
Decanted lactose with 2% fines	31.88 ± 0.70	88.25 ± 12.78
Decanted lactose with 4% fines	32.35 ± 3.55	81.77 ± 9.98
Decanted lactose with 8% fines	36.43 ± 3.61	73.36 ± 18.48
Mannitol(112-140µm)	41.14 ± 2.78	76.10 ± 4.42
Mannitol after nine times decantation	30.74 ± 0.72	84.20 ± 11.32
Decanted mannitol with 4% fines	33.37 ± 0.56	77.07 ± 4.79
Decanted mannitol with 8% fines	38.59 ± 1.64	77.64 ± 3.80
Decanted mannitol with 16% fines	39.05 ± 0.72	76.25 ± 4.58

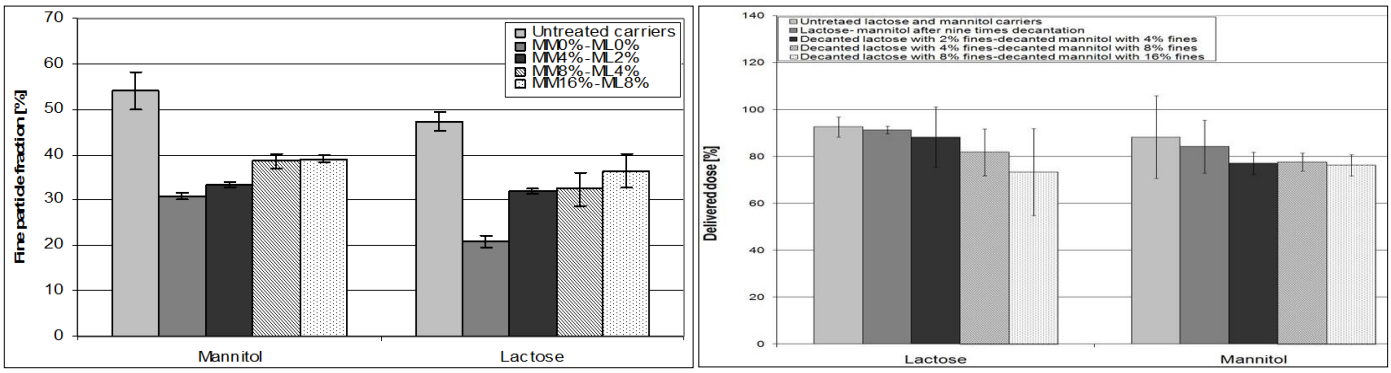


Fig 9: Fine particle fraction and delivered dose of salbutamol sulphate with decanted lactose and decanted mannitol before and after production of ternary mixtures (n = 3 ± SD).

The addition of fine lactose and mannitol to the treated coarse carriers is likely to affect drug dispersion by interacting with some of active sites, which would otherwise adhere to drug strongly. This displaces drug from such sites on the coarse mannitol and hence more of the drug is located at sites where the binding action might be weaker (Staniforth *et al.*, 1995; Zeng *et al.*, 1998). Consequently, the more weakly adhered drug particles dislodged and detached more easily from the surface of carrier particles, and dispersed in the air stream prior to inhalation. Another study that used atomic force microscopy (AFM) to measure the adhesive force between a 10 µm silica sphere and various sites on the surface of a lactose carrier found a log-normal distribution of forces however, suggesting that, the division of a carrier surface into areas of strong and weak adhesion may be too simplistic (Louey *et al.*, 2001). Instead, they speculated that during the blending process, drug particles distributed between the surface of the carrier and multiplets formed by the aggregation of fines and drug particles. The experimental evidence to support this was limited to SEM of the formulations that showed the presence of both drug-coarse carrier adhesion units and fine particle multiplets; although it had described previously for other ternary powder blends by Soebagyo *et al.*, 1985. Lucas *et al.*, 1998 further speculated that upon aerosolization, drug particles were more easily liberated from fine particle multiplets (Figure 10, b) than from the surface of coarse carrier particles (Figure 10, a), as fine lactose was thought to have a smoother surface than coarse lactose, giving a reduced force of adhesion between drug and fines. They also suggested that certain fine particle multiplets might also be small enough to form part of the FPD without detachment of drug particles, a premise supported by the work of Srichana *et al.*, 1998, whose work on the deposition of salbutamol sulphate and lactose found that these two components could travel together to the lower regions of the lung.

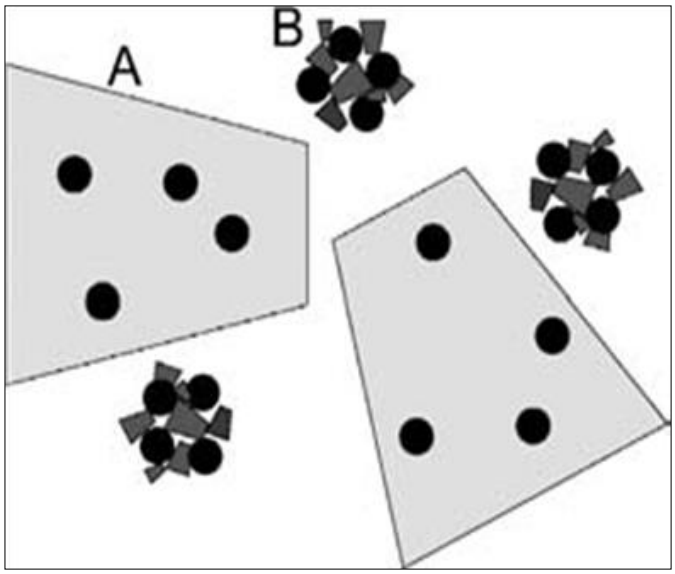


Fig 10: The preference of drug particle attachment to the carrier fines (Chow *et al.*, 2007).

Based on theories concerning the binding between crystals, it expected that the largest crystals of drug would separate first because of having a greater mass. However, Zanen *et al.*, 1992 found that the most readily separated crystals were those of smaller size. An explanation for this found in the experiments reported by Staniforth *et al.* (1981, 1982). Their experimental results demonstrate that in a powder composed of a relatively large vehicle with smaller crystals bound to the surface, there may exist a weakly bound fraction, which increases with increasing concentrations of the smaller bound crystal. In addition, a relationship exists with the diameter of the carrier crystals; separation occurs faster when the carrier crystals are smaller. Zanen *et al.*, 1992 experiment confirms the observations of Staniforth *et al.* and provides additional finding that the non-bound or weakly bound fraction consists of small crystals. Elaborating on this finding one may speculate as to whether the small crystals have bound at all.

4. Conclusion

This study clarifies the important role of the carrier fines on the *in vitro* deposition of the drug particles. Removal of carrier fines by decantation decreases *in vitro* deposition of the drug, which provoke the need of the carrier fines presence to enhance the drug deposition in the peripheral lung parts. Addition of micronized carriers to decanted carriers found to increases significantly the dispersion of the drug probably by reducing drug-carrier adhesion and allowing easy detachment of the drugs from the carrier surface. Although the addition of fines to the treated carriers could not revert the fine particle fraction to the original value of the untreated carriers, which may indicate that the added fines were not equal to the original fines which removed by wet decantation. Added carrier fines exhibit another shape, surface area and energetic charge than the inherent fines. Inherent fines appear to have different size distribution than the carrier fines, which clarifies the difference of the *in vitro* deposition in this study. Future studies would undertake to elucidate this phenomenon.

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