*Acta Pharmaceutica Sciencia*
49: 39-54 (2007)

# The Effect of Different Levels of Fines on DPI Performance *in Vitro*

**Moawia M. Al-Tabakha[1]* and Adi I. Arida[2]**

[1]Faculty of Pharmacy and Health Sciences, Ajman University of Science and Technology Network, P.O. Box 2202, Al-Fujairah, UAE.

[2] Faculty of Pharmacy, Philadelphia University, P.O.Box 1, Postal Code 19392, Jordan.

**Abstract**

The purpose of the current study was to optimise the level of fine particle lactose (FPL) present in carrier-based dry powder aerosols and to examine the effect of the model drug, fluorescein isothiocyanate (FITC)-Dextran (mol. wt. 4,400 daltons), to carrier ratio on the *in vitro* performance when aerosolised from a Spinhaler™. FITC-Dextran with sieved α-lactose monohydrate (75-106 μm) or modified sieved lactose, which contained a range (1-20 % w/w) of added FPL, were mixed in the ratio of 1:25. The formulations were tested for aerosolisation using Andersen cascade impactor (ACI). The different ratios of FITC-Dextran to the optimised carrier (1:25-4:25) were similarly examined. Although flowability was shown to get worse as indicated by compressibility results, the increased addition of FPL showed to improve deaggregation of the emitted FITC-Dextran. The fine particle fraction based on the emitted dose ($FPF_{Emitted}$) was increased from 32.1 % to 43.7 % when the added FPL increased from 0 to 20 % w/w. The optimum level of added FPL was shown to be 5% w/w which showed the highest stepped-increase in the $FPF_{Emitted}$. The higher ratio (4:22) of FITC - Dextran to the optimised carrier reduced device retention from 63.1 % to 49.7 % and the FPFs based on loaded and emitted dose were improved. The model drug to carrier ratio was more influential than the FPL as it affected all the important inhalation parameters.

***Key Words***: FITC-Dextran, fine particle lactose, dry powder, Spinhaler™, Andersen cascade impactor

## Introduction

Dry-powder inhalers (DPIs) offer a suitable alternative to pressurised metered-dose inhalers (pMDIs) for aerosol generation that have yet to meet its full potential (Hickey *et al.*, 1994). Three factors affect the performance of DPI generated aerosols. These are the with an excipient (Telko and Hickey, 2005). The total mass in the dose may vary patient's performance (including the inspiratory effort and the disease condition of the patient), the device performance and the dry powder

*Corresponding author e-mail: sphmaa@hotmail.com

formulation (Zeng *et al.*, 1995; Frijlink and De Boer, 2004). A dry powder formulation for inhalation consists of either the microparticles of drug alone or drug with an excipient (Telko and Hickey, 2005). The total mass in the dose may vary from as low as 0.1 mg when the drug is used alone to several tens of milligrams when the drug is incorporated with excipients (Hugosson *et al.*, 1993). Lactose, glucose and mannitol have been used as carriers.

Spinhaler™ (Fisons, UK; long before becoming part of Rhône-Poulenc Rorer) was the first successful single-dose system to be introduced for the delivery of powder through inhalation. In their experimentation with Spinhaler™, Bell *et al.* (1971) concluded that because of poor flow of finest powder through the inhaler device a coarser flow aid can be used. This would improve flow characteristics of the drug, hence enabling efficient processing and promoting device emptying. Nevertheless, the inefficient detachment of fine drug particles from the carrier surface is a reason why the performance of the carrier-based formulation can be poor (Lucas *et al.*, 1998). Spinhaler™ used in this work is also known to have poor delivery characteristics (Tobyn *et al.*, 2004), but it allows easier optimisation of powder formulations because its operation relies on loading size 2 capsules containing the prepared formulations. In previous years, attention has been given to the role of FPL as a performance modifier (Lucas *et al.*, 1998; Zeng *et al.*, 1998) to improve the redispersion of the drug particles. To investigate this further, α-lactose monohydrate was modified by sieving and blending with known percentages of fine particle lactose prior to blending with the model drug. Since the addition of fines might affect the flowability of the resultant formulation, the flowability of the powders was determined in order to understand any relation with the performance of aerosolised powder formulations.

The water soluble nature of many drugs formulated as a dry powder for inhalation and the increasing use of recombinant DNA technology to produce new class of peptide and protein drugs make FITC-Dextran a suitable choice since it is water soluble and can be obtained in a wide range of molecular weights. Because drugs intended for inhalation in dry powder aerosols are formulated with pre-determined dose, there is a lack of published data regarding changing the drug content in the capsule. Because FPL was shown to play an important role in the redispersion of drug particles, it was considered of interest to investigate the effect of fine particle FITC-Dextran. Therefore the amount of FITC-Dextran present in the formulation was changed. This was done using different ratios of FITC-Dextran to lactose while retaining similar formulation fill weight.

## Materials and methods

*Materials:* α-Lactose monohydrate (batch no. 750707) was obtained from Borculo Whey Products, UK. FITC-Dextran mol wt 4,400 Daltons (lot 77H0362) and sorbitan trioleate (Span 85) were purchased from Sigma, UK. Chloroform (batch no. 9806708059) was obtained from Fisher, UK. Gelatin capsules of size 2 (Farillon Limited, UK) was used with Spinhaler™ from Fisons.

*Direct micronisation of FITC-Dextran (mol wt 4,400 Daltons) and lactose*

Using a fluid jet mill (Glen Creston Ltd., UK) the drug substance was fed into a milling chamber, where two air streams met at high velocity. Micronisation resulted from collision of particles, which acted as combined impaction and attrit ion forces.

Before micronisation, the required fractions of FITC-Dextran and lactose were triturated separately using mortar and pestle to reduce any large particles and to aid in achieving a normal particle size distribution. The differential pressure sets of 70 psig in the feeder side and 100 psig in the opposing side were used to micronise FITC-Dextran and lactose separately.

*Preparation of coarse lactose (75-106 μm)*

Sieving with a mechanical tap sifter (Pascall Engineering, England) was utilised to sieve lactose. The sieving was carried out as described in the US Pharmacopeia 24-NF 18 (2000) using two sieves, one with an aperture of 75 μm and the other of 106 μm (laboratory test sieves, Endecotts Ltd, England).

*Fluorimetric analysis of FITC-Dextran*

Different concentrations of FITC-Dextran solution (0.00-0.42 μg/ml) were prepared in triplicate to prepare a standard calibration curve using a fluorimetric analysis for which the LS-5 luminescence spectrometer (Perkin Elmer, UK) was used. Phosphate buffer medium (pH 8.0 ± 0.05) was prepared according to British Pharmacopoeia (2004) and used as a medium. The temperature of the cuvette in the jacketed slot was maintained at 25°C by circulating water. The scanned excitation wavelength ($\lambda_{ex}$) and emission wavelength ($\lambda_{em}$) by the LS-5 of the solution containing FITC-Dextran were found at 492 and 516 nm respectively. From the equation of the calibration curve, the amounts of FITC-Dextran in any sample can be calculated.

*Preparation of the powder blends*

The carrier system was first prepared by blending a known amount of FPL (to give range of concentration from 0-20 %) with sieved lactose (75-106 μm) by tumbling on a roller (speed of 90 rpm) for one hour. When no FPL was added, the sieved lactose fraction was tumbled alone. Powder blends of FITC-Dextran and the prepared carrier in the ratio of 1:25 were prepared. The fractions of powders were accurately weighed and transferred to a container which in turn tumbled on a roller for 1 hr at a speed of 90 rpm.

After the determination of the level of added FPL to sieved lactose (75-106 μm) required for optimum FITC-Dextran deposition, the same method of mixing was followed to prepare blends of FITC-Dextran and the chosen carrier in the ratios of 2:24 and 4:22.

Uniformity of pre-dispensed dose of powders for inhalation was applied in accordance to European Pharmacopoeia (2004) in order to ensure the homogeneity of the prepared blends. Therefore 10 aliquots of 26 mg each were taken randomly

from each blend. The appropriate dilution with phosphate buffer (pH 8.00 ± 0.05) was made for fluorimetric measurements with LS-5. The average content of FITC-Dextran in the 10 aliquots and the coefficient of variation (CV %) were then calculated for each blend.

***Particle size measurement by laser diffraction method***

Micronised FITC-Dextran, sieved lactose and sieved lactose containing the different fractions of FPL were measured for particle size using Malvern system 2600. The sizing method employs laser diffraction. Size analysis was carried out in liquid suspension using chloroform in which 0.1 % w/v sorbitan trioleate was added. Lens 300 mm was used when sieved lactose (75-106 μm) containing the different levels of FPL was measured. On the other hand micronised FITC-Dextran and lactose were measured using the lens 63 mm. That was in order to cover as much of the particle size distribution as possible. Independent particle size model was used and the obscuration of the measured samples was adjusted between 0.10 and 0.18. Each sample was measured at least in triplicate.

***Particle shape and size characterisation by an electron microscopy***

Specimens from micronised FITC-Dextran, sieved lactose and sieved lactose containing the different levels of added FPL were examined by an electron microscope (Philips XL-20 SEM, Holland). On a clean stub, double-sided adhesive tape was placed and particles from each specimen were then scattered on it. The stub was held in air and turned over to remove unstuck particles. Gold was applied as a coating material to the specimen using a diode sputter coater in order to avoid sample charge when irradiated by the electron beam. The accelerating voltage in electron microscope used was 15-25 kV. Several micrographs were randomly produced at different magnification.

***Investigation of powder flowability***

The carrier system consisting of sieved lactose (75-106 μm) or modified sieved lactose, to which a range of FPL (0-20 % w/w) was added, was tested for flowability by the determination of bulk (fluffy) density and tap density. To carry out this determination 40 to 50 g of the specified powder was used and transferred to a 100 ml measuring cylinder at about a 70° angle through a wide opening funnel. The first reading (before any tapping) was that from which the bulk density (or fluffy density) has been calculated as the ratio of powder weight in grams to the volume reading in millilitres. The powder was then tapped until there was no change in the volume reading of the cylinder for 100 consecutive taps. The percent compressibility of the powder was then calculated according to the following Equation 1:

$$\% \text{ Compressibility} = \frac{D_T - D_B}{D_T} \times 100 \qquad (1)$$

where $D_T$ and $D_B$ is the tap and the bulk (fluffy) densities respectively.

The results obtained of percent compressibility were compared to flowability described by Carr (1965).

***Thermal analysis by a differential scanning calorimeter (DSC) and thermogravimeter (TG)***

The thermal properties of the powders were studies using two types of analysis, the differential scanning calorimetry DSC (Perkin Elmer, UK) and thermogravimetric analysis TGA (Perkin Elmer, UK). Such studies would reveal events like drying (desorption), loss of water of crystallization, melting and decomposition. The scanning rate was at 5°C/min for both the DSC and TGA experiments. The temperature scan ranged from 20°C to 240°C and the sample weight varied from 5 mg to 13 mg.

***Deposition profile of FITC-Dextran using Andersen cascade impactor (ACI)***

Because the deposition profile of hygroscopic drugs can be affected by environmental conditions (Chan, 2006), the storage of powders and the aerosolisation experiments were carried out at controlled temperature and relative humidity laboratory of 18°C and 35-40 %, respectively. ACI was used to assess depositions from the different formulations emitted from a Spinhaler™ at the flow rate of 60 l/min for 4 sec (Taylor and Gustafsson, 2005) that is equivalent to 4 liters through the impactor according to British Pharmacopoeia (2004). It classifies particles according to their inertia. The impactor is widely used (Marple *et al.*, 1998) and is official in both British Pharmacopoeia (2004) and United States Pharmacopoeia (2005). A pre-separator was fitted on top of the impactor to prevent particle bouncing and re-entrainment errors and to reduce overloading of the Andersen stages used. Exactly 26 mg of the formulation containing FITC-Dextran and lactose was filled in the size 2 capsule. The formulation was aerosolised that based on single dose experiments into the Andersen since multi-dose aerosolization results in modification of the impaction plates (Feddah and Davies, 2003). The operation of the Andersen at flow rate of 60 l/min instead of that calibrated at 28.3 l/min required the recalculation of the effective cut-off diameters (ECDs) utilising Stokes' equation in accordance with the works carried out by Van Oort *et al.* in 1996.

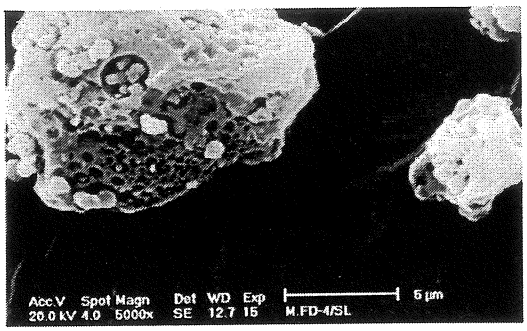
The masses of FITC-Dextran deposited on the various sites of the impactor were washed with phosphate buffer (pH 8.00 ± 0.05) and quantified fluorimetrically. The most important inhalation parameters were calculated to characterise the deposition profile of the FITC-Dextran. These included device retention, fine particle fraction (FPF), based on emitted dose ($FPF_{Emitted}$) and loaded dose ($FPF_{Total}$), and mass median aerodynamic diameter (MMAD).

## Results and Discussion

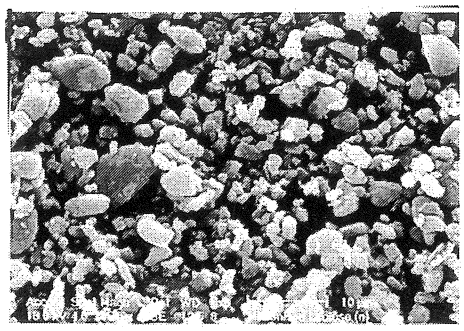
### *Particle size and shape*

Laser sizing of micronised FITC-Dextran revealed that the volume median diameter (VMD) was 6.15 μm with all particles below 22.30 μm. Micrograph of the micronised FITC-Dextran revealed that the particles were asymmetric with rough and porous surfaces as shown in Figure 1 (a). The VMD of micronised lactose was 5.62 μm with all particles below 20.70 μm. Micronised lactose particles were rounded and had smooth surfaces as seen in figure 1 (b).

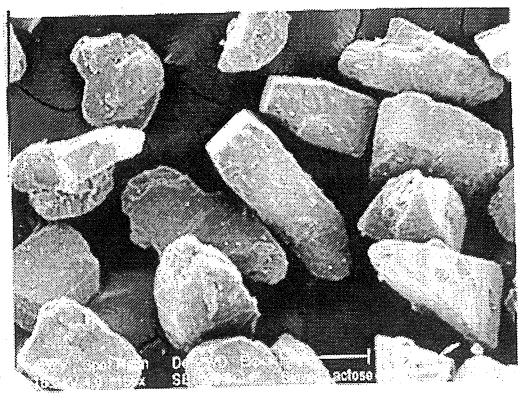
(a) Micronised FITC-Dextran *(bar=5 μm)*




*(b) Micronised lactose (bar=10 μm)*



*(c) Lactose fraction (75-106 μm) prepared by mechanical sifting (bar=100μm)*



*(d) Sieved lactose fraction (75-106 μm) containing 5 % w/w of added fine particle lactose (FPL) (bar=100 μm)*

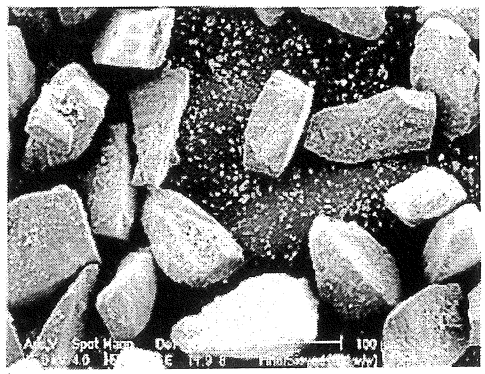


**Figure 1 (a), (b), (c), (d).** Scanning electron micrographs of the FITC-Dextran and lactose powders

Sieved lactose fraction (75-106 μm) had a VMD of 116.39 μm. Lactose particles are not spherical (see Figure 1 (c)) and this may explain why the VMD falls outside the designated sieve fraction (Diffraction reference, 1993). Sieving of lactose to

remove all fine particles was not completely successful as shown by both micrographs of electron microscope and Malvern results. The particle size distribution shown in Figure 2 exhibit a flat tail towards the fine particle size range. This supports the work of Zeng *et al.* (1998) who attempted to remove fine particles from a sieved lactose fraction (63-90 μm) by subjecting it to an air stream with a flow rate of 160 l/minute, but were not successful.

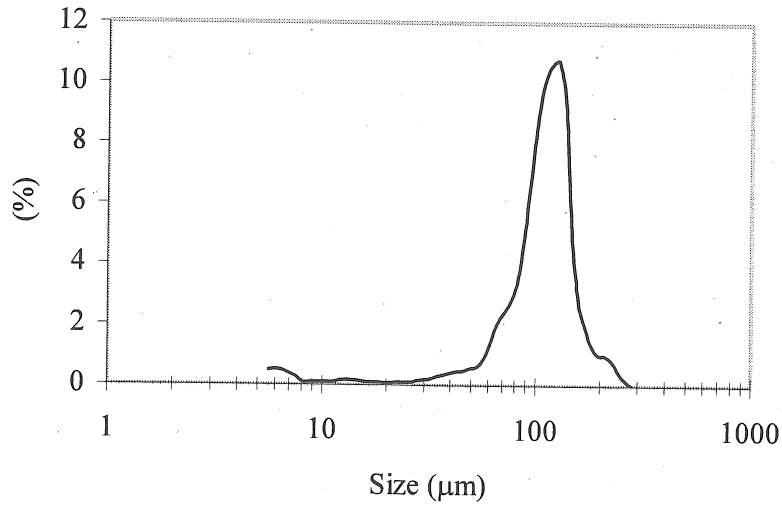



**Figure 2**. The particle size distribution of a sieved lactose fraction (75-106 μm) after sifting by mechanical tapping.

The percentage of micronised lactose in the sieved fraction (75-106 μm) that is required to form a close-packed monolayer onto the coarse lactose particles can be calculated from the following Equation 2 (Jones and Pilpel, 1965):

$$\%\,\text{Fines} = \frac{2\pi r(R+r)^2}{R^3\sqrt{3}} \times 100 \qquad (2)$$

where r is the radius of the adsorbed fines and R is the radius of the coarse particles.

Substituting the corresponding values calculated from Table 2 into Equation 2, the theoretical percentage of micronised lactose required to form an adsorbed monolayer is 19 % w/w assuming 0 % w/w of fines exist in the lactose fraction (75-106 μm). The formation of such a monolayer would be expected to reduce the interparticulate forces between the drug and the coarse lactose and hence to improve detachment of the drug. As such, a range of up to 20 % w/w of micronised lactose was added to the sieved lactose fraction (75-106 μm).

The blending of FPL with the sieved lactose resulted in saturation of active sites even at a low concentration (i.e. 1 %), this would be expected since the carrier

surfaces were not so rough and the active sites were not present all over them. Similarly Zeng *et al.* (1998) noted the presence of such free fines upon the addition of 1.5 % w/w of micronised lactose to a lactose fraction (63-90 μm). At 20 % w/w of added FPL, the formation of fine particle agglomerates became apparent. This was apparent from the free FPL shown by the micrographs. Figure 1 (d) shows the blending at the level of 5 %.

***Flowability of the carrier system***

The results of compressibility % (see Table 1) show that with the increasing percentage of fines in the carrier system, the flowability was adversely affected. Jones and Pilpel (1965) documented that fine particles affected the flowability of powders due to enhancement of the Van der Waals cohesive forces which operate between neighbouring particles. But the presence of these fines provides a way of enhancing deaggregation of drug particles and hence improving $FPF_{Emitted}$. Hence a balance is needed between these two factors.

***Thermal analysis of powders***

The enthalpy change (ΔH) of the endotherm obtained by DSC (165.9 J/g) is the energy needed to remove adsorbed water from micronised FITC-Dextran sample. Evidence for this is the weight loss observed, using TGA, at the corresponding temperature to the endotherms (Barrall and Johnson, 1970; Miller and York, 1985). The moisture content of the micronised FITC-Dextran sample obtained by TGA was 11.4 % w/w.

For lactose (both micronised and sieved) two endotherms were obtained. The first endotherm represents the loss of water of crystallization (one mole of water) and corresponds to a loss of about 5.0 % w/w of sample weight at about 140°C. Lerk *et al.* in 1984 documented that the DSC of α-lactose monohydrate have exhibited an endothermic transition at ~140°C. Saleki-Gerhardt and Zografi (1994) have shown with DSC scans at 10°C/min that lactose undergoes melting with decomposition at 213°C. This corresponds to the second peak occurring just before the decomposition of lactose at about the same temperature (212°C for both micronised and sieved lactose).

While the results of FITC-Dextran indicated the presence of a high percentage of adsorbed moisture, lactose did not show any such presence and therefore such a material is suitable to be incorporated with a hygroscopic material such as FITC-Dextran to reduce vulnerability to high humidity.

**Table 1.** Compressibility % (mean ± standard deviation (SD)) and flow description according to Carr (1965) (n=3)

| Material | Compressibility % and (SD) | Description of flow |
|---|---|---|
| Lactose fraction (75-106 μm) plus 0% added fines. | 16.4 (0.0) | Fair |
| Lactose fraction (75-106 μm) plus 1% added fines. | 17.4 (0.7) | Fair |
| Lactose fraction (75-106 μm) plus 5% added fines. | 24.9 (0.7) | Passable |
| Lactose fraction (75-106 μm) plus 10% added fines. | 33.0 (0.4) | Very poor |
| Lactose fraction (75-106 μm) plus 20% added fines. | 41.3 (0.5) | Extremely poor |

*Content uniformity*

Each analysed dose would contain an equivalent of 1000, 2000 and 4000 μg of FITC-Dextran for a perfect mixture of the blend with lactose in the ratio of 1:25, 2:24 and 4:22 respectively. The average weight of FITC-Dextran in the 10 aliquots for each blend with the coefficient of variation (CV %) is shown in Table 2. All blends passed the test for the uniformity content of pre-dispensed dose described in European Pharmacopoeia (2004).

**Table 2.** The average content (μg) of micronised FITC-Dextran in ten aliquots of different formulation and coefficient of variation (%CV)

| Formulation | Average FITC-Dextran content (μg) and (CV %) |
|---|---|
| Added FPL (0% w/w) with blend ratio 1:25 | 991 (1.8) |
| Added FPL (1% w/w) with blend ratio 1:25 | 1005 (1.5) |
| Added FPL (5% w/w) with blend ratio 1:25 | 1011 (1.4) |
| Added FPL (10% w/w) with blend ratio 1:25 | 1022 (2.4) |
| Added FPL (20% w/w) with blend ratio 1:25 | 1016 (1.0) |
| The optimised level of FPL (i.e. 5% w/w) with blend ratio 2:24 | 2056 (1.8) |
| The optimised level of FPL (i.e. 5% w/w) with blend ratio 4:22 | 4127 (2.9) |

*Effect of FPL on the deposition profile of FITC-Dextran*

Five prepared formulations consisting of FITC-Dextran and lactose in the ratio of 1:25 were tested *in vitro* for their aerosolisation performance. Data collected from five aerosolisation experiments (single-dose experiment) for each formulation were compared using ANOVA ($p<0.05$, $n=5$) and any differing group was identified using the least significant difference test. The results for FITC-Dextran aerosolisation experiments are shown in Table 3.

**Table 3.** FITC-Dextran deposition (mean % ± (SD)) after aerosolisation using the five formulations containing various amounts of fine particle lactose (n=5)

| | (0%) | (1%) | (5%) | (10%) | (20%) |
|---|---|---|---|---|---|
| Device | 61.1 (1.1) | 64.7 (3.0) | 63.1 (3.5) | 67.9 (3.3) | 67.6 (2.1) |
| $FPF_{Total}$ | 12.4 (0.9) | 11.4 (1.3) | 14.3 (1.6) | 12.8 (1.6) | 14.2 (0.8) |
| $FPF_{Emitted}$ | 32.1 (3.0) | 32.2 (2.3) | 38.6 (1.3) | 39.8 (1.2) | 43.7 (2.5) |
| MMAD | 4.24 (0.22) | 4.21 (0.28) | 3.58 (0.08) | 3.50 (0.13) | 3.38 (0.05) |

Small but significant increases in the amount of FITC-Dextran retained in the device occurred when the percentage of fine particle lactose added was increased from 0 to 10 or 20% w/w, which suggests that the flowability of the carrier system may affect the emission of the incorporated drug. The increases were however small (about 6.8 and 6.5 % respectively) and were not consistent with other emission results. In the work of Lucas *et al.* (1998), the effect of the carrier flowability was also not clearly demonstrated in the emission results. Figure 3 shows Carr's index (compressibility %) of the carrier and the retained percentage of FITC-Dextran in the device as a function of added FPL.

A large percentage of FITC-Dextran was retained by the device with different formulations. Although this may be a characteristic of older devices such as the Spinhaler™, adhesion to the walls of the capsule or the presence of surface charge on the FITC-Dextran particles may be other explanation (Chan, 2006).

The presence of an increased amount of fine particle lactose may have made more of the FITC-Dextran available for emission by replacing those particles which adhere to the capsule walls. Bell *et al.* (1971) reported the extensive coating of the internal wall of hard gelatin capsules by fine particle lactose. Also, FPL may have replaced or weakened attachment of FITC-Dextran particles to the large carrier surfaces and therefore performed better in terms of emission and dispersion of the loaded dose. In both cases, these may have counteracted the affect of the flowability.

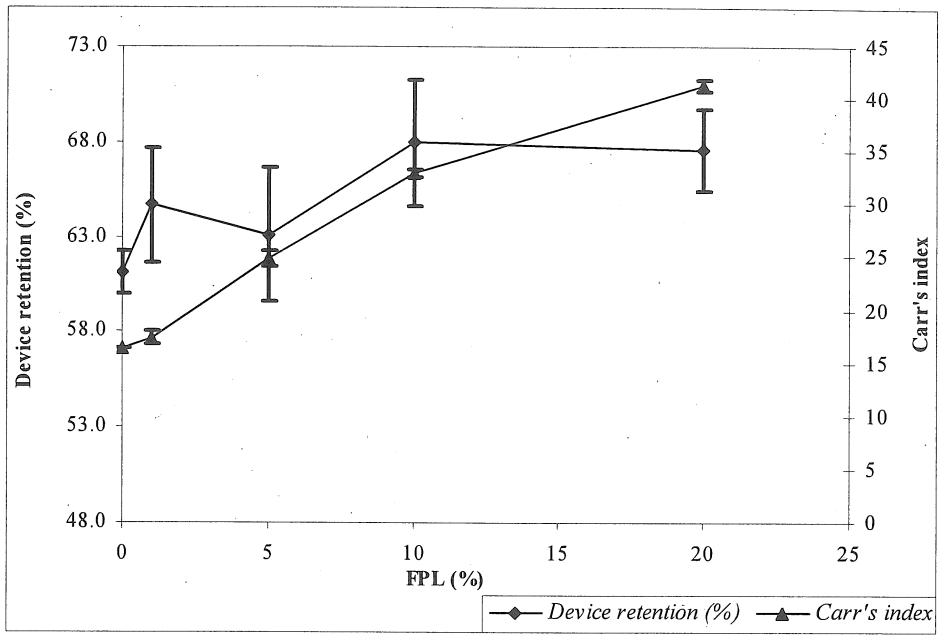



**Figure 3.** The relationship of the carrier Carr's index (compressibility %) and the retained percentages of FITC-Dextran by the device as a function of added fine particle lactose (FPL) (n=3 for compressibility and n=5 for device retention)

As with the device retention results, there was no clear trend of the FPL effect on the $FPF_{Total}$. A significant increase in the fine particle fraction ($FPF_{Total}$) occurred when the added fine particle lactose was increased from 0 to 20 % w/w, which is opposite to what was expected from the emission results. It indicates that such an increase was a result of less FITC-Dextran depositing on the pre-separator up to stage 1, perhaps because larger fraction of loose agglomerates of fine particles are emitted and dispersed in air rather than tenaciously adhering to large carrier particles. $FPF_{Total}$ is calculated from the amount retained from stage 2 to 8 of the impactor (<3.98 μm) relative to the loaded dose (Newman, 1998).

The results of $FPF_{Emitted}$ and MMAD support the work of Lucas *et al.* and Zeng *et al.* in 1998. It was possible to significantly increase the $FPF_{Emitted}$ from 32.1 to 43.7 % and to reduce MMAD from 4.24 to 3.38 μm when the added FPL was increased from 0 to 20% w/w. The presence of a higher percentage of fine particle lactose (FPL) can therefore be beneficial to the extent of deaggregation and redistribution of aerosolised powders, but the greatest stepped-increase in the $FPF_{Emitted}$ was obtained when FPL level used was 5 %. The greatest stepped-decrease in the MMAD was also shown to be at 5 % added FPL level. Figure 4 shows the effect of FPL level on both $FPF_{Emitted}$ and MMAD. When modified lactose contained 5 % added FPL, the flow was described as "passable" compared to "extremely poor" with 20 % added FPL. Therefore, one can not increase the percentage of FPL

indefinitely and the need for better flowability necessitates the use of lowest level of FPL, therefore 5 % level was thought to be the optimum one.

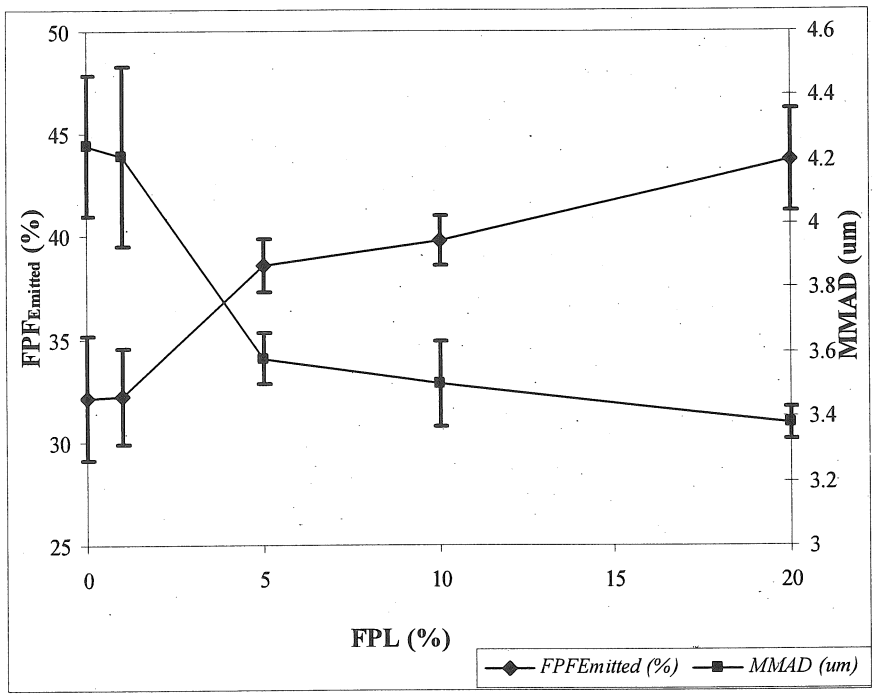



**Figure 4.** The relationship between the concentration of fine particle lactose (FPL) and the fine particle fraction based on delivered dose ($FPF_{Emitted}$) and the mass median aerodynamic diameter (MMAD) (n=5)

Zeng *et al.* (1998) explained such results by stating that "Addition of fine lactose to the powder formulations may result in the formation of mono- or multi-layers of the fine lactose adhered to the coarse lactose. Therefore, fine particles of lactose would be expected to reduce the interparticulate forces between the drug and the coarse lactose and consequently, enhance the detachment of the drug from the carrier thereby improving FPF of the drug". They also suggested that the reduction of electrostatic charge generated on the surface of the larger particles was brought about by the free fines which might have acted as a lubricant between the large particles. This may have lead to a reduction in the electrostatic interactions between the drug and the carrier. On the other hand, Lucas *et al.* (1998) suggested the formation of drug-fine lactose multiplets which were expected to disperse more easily compared to those attached to coarse particles.

***Effect of the ratio of micronised FITC-Dextran to lactose on the deposition of profile FITC-Dextran***

Preparations of different ratios of micronised FITC-Dextran and lactose (2:24 and 4:22) were tested for aerosolisation performance using the Andersen impactor with

the carrier containing the optimum level of FPL (i.e. 5 %). The outcome of the tests was compared with the corresponding formulation (blend ratio 1:25) reported earlier. To avoid differences due to loading the Andersen stages, the same fill weight (26 mg) was tested for the blends and was prepared just before aerosolisation. For statistical analysis, ANOVA was used ($n=5$, $p<0.05$) and the differing groups were identified using the least significant difference test. The results are shown in Table 4.

Increasing the percentage of FITC-Dextran in the formulation led to an increase in its emitted percentage from the device. This is perhaps because more micronised FITC-Dextran particles became available for emission after coating the internal wall of the capsule. Also the presence of agglomerated FITC-Dextran particles rather than being attached tenaciously to large lactose particles may have resulted in better emission and dispersion.

**Table 4.** FITC-Dextran deposition (mean % ± (SD)) after aerosolisation using three formulations (n=5)

| | **1:25** | **2:24** | **4:22** |
|---|---|---|---|
| Device | 63.1 (3.5) | 55.4 (1.0) | 49.7 (1.2) |
| $FPF_{Total}$ | 14.3 (1.6) | 19.3 (0.4) | 23.1 (0.7) |
| $FPF_{Emitted}$ | 38.6 (1.3) | 43.4 (0.8) | 46.0 (1.6) |
| MMAD | 3.58 (0.08) | 3.39 (0.05) | 3.27 (0.07) |

The $FPF_{Total}$ improved from 14.3 % to 23.1 % on increasing the ratio of FITC-Dextran to lactose from 1:25 to 4:22, as a result of an increased percentage of micronised FITC-Dextran emitted from the device. The increase of $FPF_{Emitted}$ from 38.6 % to 46.0 % also suggests that the deaggregation of the emitted powder has improved significantly. This may have resulted from an increase in free FITC-Dextran, i.e. not attached to the large sieved lactose particles. The mass median aerodynamic diameter (MMAD) has decreased indicating better deaggregation of the emitted dose when the ratio of FITC-Dextran to lactose is increased. The greatest stepped-increase in the device emission, $FPF_{Total}$ and $FPF_{Emitted}$ occurs when the FITC-Dextran to lactose ratio increases from 1:25 to 2:24, which is also true for the greatest step-decrease in the MMAD.

## Conclusions

FPL can be used to improve deaggregation of the emitted drug dose from Spinhaler™. The level of FPL added to the coarse lactose (75-106 μm) for optimum drug deposition was found to be 5% w/w for the drug to carrier ratio of 1:25. Saturating the adhesion sites on coarse lactose particles by FPL and the formation of drug-drug or drug-FPL multiplets which were probably easier to disperse when aerosolised provide an explanation. It has to be noted that although lactose is the only excipient that is approved for general use, the inhalation of a considerable proportion of FPL is not intended (Cryan, 2005). Also, one of the main reasons for adding lactose was to improve flow properties of the formulation, but clearly the presence of increased proportion of fines will negate this effect.

A large percentage of FITC-Dextran was retained by the device when the drug to carrier ratio was 1:25. An increase in the emitted proportion was obtained upon the increase in the blend ratio which is probably because more FITC-Dextran was available for emission after coating the internal wall of the capsule and the formation of loose agglomerates of FITC-Dextran. The increase in emission was a key factor to an increase in the $FPF_{Total}$. The deaggregation pattern indicated by $FPF_{Emitted}$ and MMAD has also been improved as in the case with FPL. As the blend ratio of the drug to carrier showed more profound effect compared to the level of FPL, drugs that are less potent (thus allowing greater drug-carrier ratio), may have better performance than the corresponding low drug-carrier ratio. However, one must consider the segregation that could result from increased detachment of drug particles to large lactose carrier particles upon saturation of the active sites. The long term effect on segregation has not been evaluated in this work. An alternative to increasing drug-carrier ratio would probably be the use of surface treated lactose carrier in order to reduce the number of active sites responsible for fine particles adhesion (Zeng *et al.*, 2001; Iida *et al.*, 2003). Nevertheless, this will not solve the problem of possible segregation.

## References


Barrall, E.M. and Johnson, J.F. (1970). Differential scanning calorimetry: Theory and applications. In:- Slade, P.E., Jenkins, L.T. (Eds.), Thermal Characterization Techniques, Marcel Dekker, Inc., New York, pp. 1-39.

Bell, J.H., Hartley, P.S. and Cox, J.S.G. (1971). Dry powder aerosols I: A new powder inhalation device. *J. Pharm. Sci.* 60(10):1559-1563.

British Pharmacopoeia (2004). D. Buffer solutions. Volume II, Appendix 1 D, A131-A136.

British Pharmacopoeia (2004). Apparatus D-'Andersen' sizing sampler. Volume II, Appendix XII F, A270-A272.

Carr, R.L. (1965). Evaluating flow properties of solids. *Chem. Eng.* 72(2):163-168.

Chan, H.K. (2006). Dry powder aerosol delivery systems: current and future research directions. *J. Aerosol Med.* 19(1):21-7.

Cryan, S.A. (2005). Carrier-based strategies for targeting protein and peptide drugs to the lungs. *AAPS Journal* 7(01):E20-E41.

Diffraction reference (1993). Windows Sizer Reference Manual, Malvern Instruments Ltd., Worcs, Manual number 0073, Issue 1.1.

European Pharmacopoeia (2004). Uniformity of content of single-dose preparation. Volume 1, 5th edition, Council of Europe, 67075 Strasbourg Cedex, France, pp. 234.

Feddah, M.R. and Davies, N.M. (2003). Influence of single versus multiple actuations on the particle size distribution of beclometasone dipropionate metered-dose inhalers. *J. Pharm. Pharmacol.* 55(8):1055-1061.

Frijlink, H.W. and De Boer, A.H. (2004). Dry powder inhalers for pulmonary drug delivery. Expert Opin. *Drug Deliv.* 1(1):67-86.

Hickey, A.J., Concessio, N.M., Van Oort, M.M. and Platz, R.M. (1994). Factors influencing the dispersion of dry powders as aerosols. *Pharm. Tech.* 18:58-82.

Hugosson, S., Lindberg, J., Lööf, T. and Olsson, B. (1993). Proposal for standardized testing of powder preparations for inhalation, stimuli to the revision process. *Pharm. Forum* 19(3):5458-5466.

Iida, K., Hayakawa, Y., Okamoto, H., Danjo, K. and Leuenberger, H. (2003). Preparation of dry powder inhalation by surface treatment of lactose carrier particles. *Chem. Pharm. Bull.* 51(1):1-5.

Jones, T.M. and Pilpel, N. (1965). Some physical properties of lactose and magnesia. *J. Pharm. Pharmacol.* 17(7):440-448.

Lerk, C.F., Andreae, A.C. and De Boer, A.H. (1984). Alterations of α-lactose during differential scanning calorimetry. *J. Pharm. Sci.* 73(6):856-857.

Lucas, P., Anderson, K. and Staniforth, J.N. (1998). Protein deposition from dry powder inhalers: fine particle multiplets as performance modifiers. *Pharm. Res.* 15(4):562-569.

Marple, V.A., Olson, B.A. and Miller, N.C. (1998). The role of inertial particle collectors in evaluating pharmaceutical aerosol delivery systems. *J. Aerosol Med.* 11(S1):139-153.

Miller, T.A. and York, P. (1985). Physical and chemical characteristics of some high purity magnesium stearate and palmitate powders. *Int. J. Pharm.* 23:55-67.

Newman, S.P. (1998). How well do in vitro particle size measurements predict drug delivery in vivo? *J. Aerosol Med.* 11(S1):97-104.

Saleki-Gerhardt, A. and Zografi, G. (1994). Non-isothermal and isothermal crystallization of sucrose from the amorphous state. *Pharm. Res.* 11(8):1166-1173.

Telko, M.J. and Hickey, A.J. (2005). Dry powder inhaler formulation. *Respir. Care* 50(9):1209-1227.

Tobyn, M., Staniforth, J.N., Morton, D., Harmer, Q. and Newton, M.E. (2004). Active and intelligent inhaler device development. *Int. J. Pharm.* 277(1-2):31-37.

US Pharmacopeia 24-NF 18 (2000). Chapter <786>, "Particle-Size Distribution Estimation by Analytical Sieving". U.S. Pharmacopeial Convention, Rockville, MD, pp. 1965–1967.

US Pharmacopeia 28-NF 23 (2005). Physical tests and determinations, <601> Particle size: Aerodynamic size distribution. United States Pharmacopeial Convention, Inc., 12601 Twinbrook Parkway, Rockville, MD 20852, pp. 2364-2378.

Van Oort, M., Downey, B. and Roberts, W. (1996). Verification of operating the Andersen cascade impactor at different flow rates. *Pharm. Forum* 22(2):2211-2215.

Zeng, X.M., Martin, G.P., Marriott, C. and Pritchard, J. (2001). Lactose as a carrier in dry powder formulations: the influence of surface characteristics on drug delivery. *J. Pharm. Sci.* 90(9):1424-1434.

Zeng, X.M., Martin, G.P., Tee, S.-K. and Marriott C. (1995). The controlled delivery of drugs to the lung. *Int. J. Pharm.* 124:149-164.

Zeng, X.M., Martin, G.P., Tee, S.K. and Marriott, C. (1998). The role of fine particle lactose on the dispersion and deaggregation of salbutamol sulphate in an air stream in vitro. *Int. J. Pharm.* 176(1):99-110.

*Received: 13.07.2006*

*Accepted: 27.11.2006*