A Study of the Effect of Jet Milling Process with or without Pre-treatment on Aerosolisation Characteristics of FITC-Dextran Particles

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Abstract

In vitro aerosolisation studies from a Spinhaler™ into the Andersen Cascade Impactor (ACI) of fluorescein isothiocyanate (FITC) -Dextran M. Wt. 4,400 (model drug) produced by different techniques were carried out. Three techniques, namely; micronisation, freeze-drying followed by micronisation and spray-drying followed by micronisation, were used to produce FITC-Dextran samples of similar particle sizes (Volume Median Diameter (VMD) =5.7μm) which, yet, differ in their surface characteristic. The measured Specific Surface Areas (SSAs) of FITC-Dextran were different, which indicated different surface characteristics. Wadell's shape factor was calculated to provide measurement for the particles' shape. Device retention after aerosolisation was similar for the three formulations as it was high, which is a characteristic of Spinhaler.™ However, the dispersion of each of the aerosolised blends were distinctly different from the others, as it was noticed that the micronised FITC-Dextran performed better than the remaining samples. The results were explained on the basis that porous particles of the micronised FITC-Dextran, as indicated by the SSA and the use of electron microscope, are light in weight, and therefore would have lower inertia; allowing them by this to remain longer in the airstream before impaction.

Keywords: FITC-Dextran; Dry powders for inhalation; Surface characteristics; Wadell's factor; Spinhaler;™ Andersen Cascade Impactor.

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Introduction

Dry Powder Inhalers (DPIs) were first introduced thirty five years ago by Fisons (long before becoming part of Rhône-Poulenc Rorer) for the drug sodium cromoglycate, which was administered using a device called a Spinhaler.™[1] These systems first gained importance because of problems associated with the pressurised pack such as poor coordination when breathing, [2] and because the metering system of the pressurised Metered Dose Inhalers (pMDIs) limits the amount of delivered drug to a maximum of 1 mg. The suggestion that ozone depletion could be caused by chlorofluorocarbon propellants by

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Molina and Rowland in 1974, [3] and the subsequent discovery of the reduction of ozone layer in the upper atmosphere in 1985 led to what is called the "Montreal Protocol"; which initiated an agreement leading to a ban on the production of all CFCs from the beginning of 1995. [4] This made the industry re-evaluating the potential of DPIs.

For pulmonary delivery, attention currently is given to developing new formulations of macromolecular drugs (i.e. peptides and proteins), [5] such as growth hormone (192 amino acids), insulin (51 amino acids), parathyroid hormone, [6] and interferon. [7] In dry powder formulations for inhalation, the active ingredient is usually mixed with a carrier powder, which acts both as diluent and, at the same time, helps in emitting and dispersing the aggregated drug particles. [8] FITC-Dextran of M. Wt. 4,400 Daltons was used as a model to represent a macromolecular, water-soluble group of drugs. Because FITC-Dextran can be analyzed in low concentrations, that are fractions of a microgram per milliliter, when prepared in phosphate buffer solution (pH 8.00 ± 0.05), this allowed its incorporation into carrier systems in minute quantities in dry powder formulations intended for aerosolisation and conducting a single-dose experiment. The use of a single-dose in the aerosolisation experiment minimizes the modification of the impaction plates in ACI and gives a better estimation of Mass Median Aerodynamic Diameter (MMAD). [9]

The performance of inhalation systems depends not only on the patient inhalation technique used by the patient, but also on the inhaler design and dry powder formulation for inhalation. [10] The aim of the investigation described in this research paper was to demonstrate that, in addition to particle size, factors such as particle density and shape are important factors in determining the aerodynamic diameter of the aerosolised formulations.

The physical properties of fine powders which are critical for improved deaggregation and lung deposition are examined. Also, techniques have been adopted to achieve reduction in the drug particles' sizes to suitable ones. These techiques are: micronisation by jet milling (M), freeze-drying followed by micronisation (MFD) and spray-drying followed by micronisation (MSD). Each technique has an influence on the resultant particle shape of the final product, [11, 12] which is of importance to aerosolisation and deposition. The particle shape was related to a modified Wadell's factor which is calculated from SSAs and obtained by Malvern and NOVA-1000. ACI was used to assess depositions from the different formulations that are emitted from a Spinhaler™ at the flow rate of 60 litres per minute (l/min). This impactor provides a clinically meaningful tool in the development and the quality control of powder formulations. [13] This tool is widely used and is familiar to most companies.

Materials and methods

Inhalation grade lactose was obtained from Borculo Whey Products, UK. FITC-Dextran M. Wt. 4,400 Daltons (lot 106H1180 and 77H0362) was purchased from Sigma, UK. Also, Gelatin capsules of size 2 (Farillon Limited, UK) were used with a Spinhaler™ from Fisons.

Direct Micronisation of FITC-Dextran (M FITC-Dextran) M. Wt. 4,400 Daltons

Before micronisation, about 0.58 g of FITC-Dextran was triturated using mortar and pestle to reduce any large particles and to aid in achieving a normal particle size distribution; as direct micronisation of the powder resulted in a bimodal particle size distribution. Using a fluid jet mill (Glen Creston Ltd., UK) at a differential pressure set of 70 pounds per square inch gauge (psig) in the feeder side and 100 in the opposing side, the triturated FITC-Dextran was micronised.

Micronisation of Freeze-dried FITC-Dextran (MFD FITC-Dextran) M. Wt. 4,400 Daltons

Freeze-drying provides a mean, whereby hardening of materials can be avoided and the product is made light and porous. [14] The ease of comminution depends on the material toughness, [15] and hence, this may affect the resultant particle size and shape. FITC-Dextran solution (0.25% w/v) was prepared by dissolving 0.75 g of FITC-Dextran in 300 ml of distilled water in a round bottom flask. This solution was freeze-dried using Edwards Super Modulyo freeze-dryer (Edwards, UK) and the resultant dry fluffy mass was triturated using a mortar and pestle. The powder was then micronised using an air jet mill at the differential pressure of 70 and 100 psig.

Micronisation of Spray-dried (MFD) FITC-Dextran M. Wt. 4,400 Daltons

For the spray-drying experiment, a 2% w/v solution of FITC-Dextran was prepared by dissolving 1 g of FITC-Dextran in 50 ml of distilled water. The spray-drier was constructed from laboratory tools, and comprised of a drying chamber (FG 25 Quickfit, England) heated by thermal coil to 82 ± 2°C, filtration unit to collect the spray-dried powder, vacuum pump and a nebuliser (NEB-U-MIST,® Hudson Oxygen Therapy Sales Company, USA) connected to an air cylinder to atomize the solution. Although initially the aim was to prepare a spray-dried FITC-Dextran as the final product, the associated aggregation problem with spray-dried FITC-Dextran was a reason for considering the micronisation of the spray-dried FITC-Dextran. Therefore, the resultant spray-dried powder was triturated and micronised by jet milling at the differential pressure of 70 and 100 psig.

Particle Size Measurement by Laser Diffraction Method

Each of the three produced samples of FITC-Dextran was measured for particle size using Malvern system 2600 which employs laser diffraction as a method of analysis. To a small amount (about 10 mg) of each sample, 10 ml of chloroform containing a wetting agent sorbitan trioleate (0.1% w/v) was added. This was then subjected to 30 seconds ultrasonic action to break down any visible aggregates. Independent particle size model was used and the obscuration was adjusted between 0.14 and 0.18. Each of the produced samples was measured in triplicate.

Surface Area Measurement by Nitrogen (N_2) Adsorption Method

Using nitrogen (N_2) as the adsorbate, NOVA-1000 (Quantachrome Corporation, UK) was employed to determine the specific surface area of the FITC-Dextran samples. The equipment utilises BET equation, which best describes type II isotherm in the calculation and is applicable to a range of relative pressure ($P/P_\circ$) between 0.05 and 0.35. [16] Each sample was conditioned at an elevated temperature (40° C) under vacuum (10^{-3} torr) for 18 hours prior to analysis, since the measurement of surface area requires that the solid surface should be free from contaminants such as physically adsorbed gases and moisture. Each of the produced samples was measured in triplicate.

Calculation of Wadell's Shape Factor

Several attempts have been made to define "particle shape", and these have been reviewed by Allen (1975). [17] One of the techniques described by Allen is a definition of sphericity (ψ) used by Wadell as shown in equation (1).

$$\psi = \frac{\text{Surface area of sphere having the same volume as particle}}{\text{surface area of particle}} \qquad \textit{(1)}$$

Wadell's definition can only be applied with precision to individual particles whose surface area can be accurately measured. For example, a cube has ψ of 0.81. However, Wadell's definition can be adapted, with some approximation, to a population of particles.

The NOVA-1000 determines the specific surface area with units m^2 g^{-1}. Using a median particle diameter, the Malvern system 2600 calculates automatically the specific surface area assuming spherical particles. Since the Malvern gives a volume distribution, the surface area calculated by this method has units of m^2 $cc.^{-1}$ Because the true density of the particles could not be accurately measured, it was not possible to recalculate the values so as to have identical units. It is, therefore, proposed to use a modified Wadell factor (ψ′) of units g cc^{-1} as in equation (2):

$$\Psi' = \frac{\text{Specific surface area calculated from Malvern size data}}{\text{Specific surface area from NOVA-1000}} \qquad \textit{(2)}$$

Particle Shape Characterisation by an Electron Microscopy

Each of the FITC-Dextran samples was viewed by an electron microscope (Philips XL-20 SEM, Holland). A clean stub was used when mounting the sample. The sample was kept in position by means of double-sided adhesive tape stuck onto the stub. An appropriate amount of the sample was applied onto the adhesive portion in the stub, and then the stub was held in air and turned over to remove the non-adhering particles. About 20-nm thick coating material (gold) was applied to the specimen using a diode sputter coater, which means that signal to noise ratio is high because of the gold high atomic number. Several video prints micrographs were randomly produced at different magnifications.

Water Content as Determined by a Thermogravimer (TG) and Correlated by a Differential Scanning Calorimeter (DSC)

Two types of thermal analysis were carried out, Differential Scanning Calorimetry DSC (Perkin Elmer, UK) and Thermogravimetric Analysis TGA (Perkin Elmer, UK). The aim was to study the effect of the production techniques on the thermal properties of the generated powders, and to examine the moisture content of FITC-Dextran by desorption (drying). All measurements using DSC and TGA were carried out at a scanning rate of 5° C/min since this can affect the resulting peak resolution. [18, 19] The temperature scan ranged from 25° to 240° C and the sample weight varied from 5 to 9 mg.

Fluorimetric Analysis of FITC-Dextran

The LS-5 luminescence spectrometer (Perkin Elmer, UK) was used for the fluorimetric analysis of FITC-Dextran. The fluorescence of FITC-Dextran was found to be adequately stable in phosphate buffer of pH 8.00. Therefore, Phosphate buffer (pH 8.0 ± 0.05) was prepared in accordance to the BP (2000). [20] The scanned excitation wavelength (λ_{ex}) and emission wavelength (λ_{em}) by the LS-5 of the solution containing FITC-Dextran were found at 492 and 515 nm, respectively. A standard calibration curve of FITC-Dextran fluorescence was prepared from an average of triplicate preparations (0.00-0.36 μg/ml). From the equation of the calibration curve, the amounts of FITC-Dextran deposited on the Andersen stages were calculated.

Preparation of the Powder Blends and Uniformity of Pre-dispensed Dose

Powder blends of FITC-Dextran and inhalation grade lactose in the ratio of 1:25 were prepared respectively. The fractions of powders were accurately weighed and transferred to a small glass vial. This small glass vial was kept in a centre of a larger jar by means of paper filler. Then, the large container was tumbled on a roller for 1 hour at a speed of 90 rpm.

In the European Pharmacopoeia (1997), [21] test B is applied to the uniformity of the pre-dispensed dose of powders for inhalation. That is, 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 doses and the coefficient of variation (% CV) were then calculated for each blend.

Deposition Test of FITC-Dextran Using Andersen Cascade Impactor (ACI)

The storage of powders and the aerosolisation experiments were carried out at a controlled temperature and relative humidity laboratory of 18° C and 35-40 %, respectively, as there has been suggestions that environmental conditions can affect the testing results. [22] Coating of the impactor stages was not carried out. Exactly 26 mg of the formulation containing FITC-Dextran and lactose in the ratio of 1:25 was filled in the size of 2 capsule, which corresponds to 1 mg of FITC-Dextran. The formulation was aerosolised based on single dose experiments into the Andersen impactor via the Spinhaler™ at the flow rate of 60 l/min for 4 s. Andersen impactor consists of 8 impaction plates and a filter stage, which was fitted with glass microfibre filter paper. After that, 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.

The operation of the Andersen at a 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; as shown in the following equation:[23]

$$ECD_{Flow2} = ECD_{28.3l/min}\,(28.3/Flow2)^{1/2} \qquad (3)$$

The ECDs of the Andersen stages calibrated at the flow rate of 28 l/min and that calculated at 60 l/min are shown in Table (1). The masses of FITC-Dextran emitted from the device and deposited in the various sites of the impactor assembly were quantified using fluorimetric analysis. The following inhalation parameters, which are important in assessing the performance of dry powder formulations for inhalation, were calculated:

1. Device Retention (%)

Calculated by subtracting the emitted FITC-Dextran from the loaded. Reducing the device retention will create an opportunity for more particles to be deposited on the Andersen stages. Spinhaler™ is known to have poor delivery characteristics, [24] and it is the aim of this research to test the performance of the aerosolised fraction rather than increase the emitted fraction.

2. Fine Particle Fraction (FPF)

Fine particles are those < 3.98 μm; i.e. from stage 2 up to the filter. The calculation of fine particle fraction was made in two ways. The first way is by dividing the mass of fine particles by the loaded dose (FPF_{Total}). Increase in this fraction means that a larger mass of FITC-Dextran is depositing onto the lower stages of the Andersen impactor; and hence, an improved efficiency can be achieved by allowing the reduction of the loaded dose. This fraction is affected negatively by the high device retention. The other way to calculate the FPF is by dividing the mass of fine particles by the emitted dose, this is denoted by $FPF_{Emitted}$.

This fraction indicates the extent of the emitted dose dispersion [25] which is important to allow particles to deposit onto the lower stages of the impactor. Therefore; $FPF_{Emitted}$ can serve as an important parameter in comparing between the aerosolised fractions of different formulations.

3. Mass Median Aerodynamic Diameter (MMAD)

MMAD (μm) is the most appropriate size parameter to measure since the complicating factors of shape and density are incorporated in this measurement. The mass distribution of FITC-Dextran on the various stages of the impactor was converted to a cumulative percentage under size in order to calculate MMAD. [26] The probit values of the cumulative percentage were plotted as the ordinate; versus the log ECD as the abscissa. Because a good straight line was not obtained for the all points of log ECD versus probit values, four points were selected in order to give more weight to the points close to the cumulative percentage of 50% (probit = 5), which corresponds to the MMAD, [27] that is from 20 to 80% (from probit 4.16 to probit 5.84). From the best fit regression line on the selected points, MMAD was calculated from the slope and intercept.

Table 1: Effective cut-off diameter (μm) calibrated at the flow rate of 28.3 l/min and that calculated at 60 l/min for the Andersen cascade impactor.

Flow rate (l/min)	*28.3*	*60*
Stage		
Pre-separator	*> 10.00*	*> 6.87*
Stage 0	*9.00*	*6.18*
Stage 1	*5.80*	*3.98*
Stage 2	*4.70*	*3.23*
Stage 3	*3.30*	*2.27*
Stage 4	*2.10*	*1.44*
Stage 5	*1.10*	*0.76*
Stage 6	*0.70*	*0.48*
Stage 7	*0.40*	*0.27*

Results and Discussion

Size, Surface Area and Shape of the Generated FITC-Dextran Particles

Table (2) summarises data obtained for the VMD and the width of distribution given by the diameters at which 10% and 90% of the particles' volumes are below i.e. D (v, 0.1) and D (v, 0.9), respectively. FITC-Dextran produced by different techniques showed similar VMD of about 5.7 μm when measured by Malvern. Also, because the width of distribution has also shown to be similar as indicated by D (v, 0.1) and D (v, 0.9), the factor related to volumetric size can be excluded in the comparison. This will facilitate the detection of other physical properties that might affect particle movement in the airstream and hence, MMAD i.e. particle density and shape.

Table (3) shows SSAs (as from Malvern and NOVA-1000) and the calculated shape factor (sphericity). As the size distributions of the samples measured by Malvern were similar, the calculated SSAs based on volume would also be similar. These values were about 1.2 m^2/cc as calculated by Malvern. On the other hand, the results of SSAs based on weight calculated by NOVA-1000 indicate a different surface characteristic; with M FITC-Dextran having the largest SSA (6.24 m^2/g), whilst MFD FITC-Dextran showed the smallest SSA (3.81 m^2/g). Because of these differences, the calculated shape factor was different for the different powders. Wadell's shape factor approaches 1 for spherical particles and is < 1 as the particles depart from sphericity. The different production techniques used resulted in samples with modified Wadell's shape ranging from 0.20 to 0.31 g/cc.

Table 2: Mean Volume Median Diameter (VMD) and the diameters at which 10% and 90% of the particles' volumes are below for FITC-Dextran powders produced by three methods as generated by Malvern ± (standard deviation), (n = 3).

Material	*VMD (µm)*	*D (v, 0.1)[a] µm*	*D (v, 0.9)[b] µm*
M FITC-Dextran	*5.64 (0.10)*	*2.81 (0.02)*	*10.79 (0.67)*
MFD FITC-Dextran	*5.70 (0.14)*	*2.84 (0.06)*	*11.56 (1.07)*
MSD FITC-Dextran	*5.71 (0.10)*	*2.84 (0.05)*	*11.41 (1.07)*

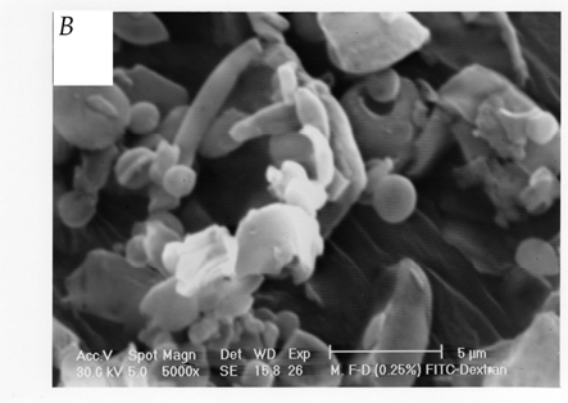
a: diameter at which 10% of the particles' volumes are below.
b: diameter at which 90% of the particles' volumes are below.

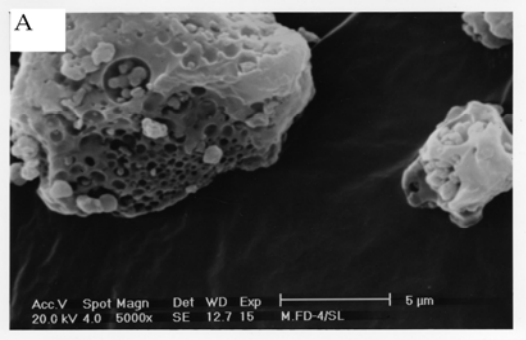
Table 3: Mean Specific Surface Area (SSA) ± (standard deviation), (n = 3) and sphericity for FITC-Dextran powders produced by three methods.

Material	*SSA (m^2/cc) by Malvern*	*SSA (m^2/g) by NOVA-1000*	*Sphericity (ψ)[a] (g/cc)*
M FITC-Dextran	1.22 (0.03)	6.24 (0.09)	0.20
MFD FITC-Dextran	1.19 (0.05)	3.81 (0.07)	0.31
MSD FITC-Dextran	1.20 (0.04)	4.16 (0.04)	0.29

a: Modified Wadell's shape factor.

Scanning electron micrographs of FITC-Dextran samples are shown in Figure (1). The micrographs confirm the results obtained from NOVA-1000 and the calculated sphericity. M FITC-Dextran showed porosity with irregular surfaces compared to less irregular surfaces for MSD FITC-Dextran. On the other hand, MFD FITC-Dextran showed smooth surfaces with no visual pores. Therefore, the followed techniques were practically successful in producing FITC-Dextran particles that have similar particle size yet differ in shape.





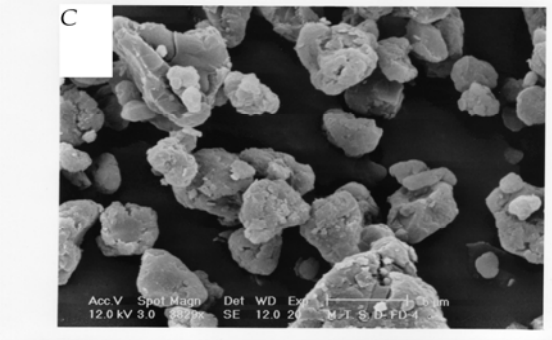


Figure 1. The SE micrographs of FITC-Dextran samples produced by three different techniques. (A) Micronised FITC-Dextran showing asymmetric particles with porous and irregular surfaces (bar = 5 µm). (B) Micronised, freeze-dried FITC-Dextran showing modular particles with smooth surfaces (bar = 5 µm). (C) Micronised, spray-dried FITC-Dextran showing granular particles with less irregular surfaces (bar = 5 µm).

Thermal Analysis of FITC-Dextran Powders by Thermogravimetric Analysis (TGA)and Differential Scanning Calorimetry (DSC)

A summary of the thermal characterisation of FITC-Dextran samples is in Table (4). DSC experiments indicate the presence of an endothermic event with the peak at around 80°C. The endotherms obtained by DSC are the energy needed to remove absorbed water from FITC-Dextran samples. Evidence for this is the weight loss observed, using TGA, at the corresponding temperature to the endotherms. Unlike DSC experiment where the sample is in sealed pan, the sample in the TGA experiment is carried out in open pan; which explains why the temperature of drying starts at lower point with the later. This is because the partial pressure of water in the sealed pan of DSC would be higher than that in the TGA open pan and hence higher temperature will be required to vaporise water. [28] The moisture contents of the samples obtained by TGA were similar at around 11% w/w. Since the moisture contents are similar, this can be excluded as a factor influencing the aerosolisation results. The high moisture content of FITC-Dextran and its hygroscopicity, therefore, justifies the use of lactose carrier to protect it from ambient humidity. The thermal properties of the FITC-Dextran samples produced by different techniques as obtained from the TGA and DSC experiments did not reveal apparent differences.

Content Uniformity

Each analyzed dose would contain an equivalent of 1000 μg of FITC-Dextran for a perfect mixture. The average weight of FITC-Dextran in the 10 aliquots for each blend with the coefficient of variation (% CV) is shown in Table (5). All the three blends passed the test for the uniformity content of the pre-dispensed dose described in EP (1997). [21]

The Deposition Profile of the Three Generated FITC-Dextran Powders from Their Formulation with Lactose

The results from aerosolisation experiments were compared using ANOVA (single factor, n= 5, $p< 0.05$). When the results indicated a significant difference between groups, least significant test was applied to identify the differing group(s). The results of FITC-Dextran aerosolised from blends are shown in Table (6) along with the actual p-values. Because the Malvern results have shown similar particle size distributions of the different FITC-Dextran powders, (see Table (2)) and since the moisture content of the different FITC-Dextran samples were similar (see Table (4)), the differences in the results from the impactor must be attributed to other physical properties. Shape may be particularly important, [29] as the more porous and irregular is the surface of the particles, the lower were the resulting MMAD and greater the FPF based on emitted and loaded dose.

Table 4: Summary (mean ± (standard deviation)) of the thermal events obtained from analysing different FITC-Dextran samples using a Differential Scanning Calorimeter (DSC) and a thermogravimetric analyser (TGA) at the scanning rate of 5° C/min, (n = 3).

Thermal event	*DSC experiment*	*TGA experiment*		
Material	*Endothermic Peak temperature (°C)*	*I[a] (°C)*	*F[b] (°C)*	*(%) weight loss*
M FITC-Dextran	*82.7 (3.3)*	*25.1 (0.2)*	*141.9 (6.20)*	*11.4 (0.9)*
MFD FITC-Dextran	*79.2 (3.3)*	*24.8 (0.1)*	*133.2 (9.0)*	*10.8 (0.5)*
MSD FITC-Dextran	*77.1 (3.3)*	*25.0 (0.1)*	*138.0 (7.7)*	*11.4 (1.0)*

a: Initial temperature reading.
b: Final temperature reading.

Table 5: The average weight content of ten samples containing FITC-Dextran in the blends with lactose and the coefficient of variation (% CV).

Material blended with lactose	*Average weight per dose (μg)*	*(% CV)*
M FITC-Dextran	*987*	*2.4*
MFD FITC-Dextran	*999*	*1.6*
MSD FITC-Dextran	*1024*	*1.4*

Table 6: FITC-Dextran deposition (mean % ± (standard deviation)) after aerosolisation using three different formulations (n = 5) with the actual p-values.

Material blended with lactose	*Device retention (%)*	*FPF_{Total} [a]*	*$FPF_{Emitted}$ [b]*	*MMAD[c] (μm)*
M FITC-Dextran	*59.6 (2.8)*	*13.3 (0.8)*	*33.1 (1.9)*	*3.37 (0.03)*
MFD FITC-Dextran	*65.8 (5.1)*	*6.2 (0.9)*	*18.1 (0.6)*	*4.22 (0.11)*
MSD FITC-Dextran	*60.9 (3.4)*	*8.7 (1.3)*	*22.2 (2.0)*	*3.98 (0.16)*
Actual p-value	*0.063875*	*$3.03 * 10^{-7}$*	*$1.88 * 10^{-8}$*	*$1.7 * 10^{-7}$*

a: Fine particle fraction based on loaded dose.
b: Fine particle fraction based on emitted dose.
c: Mass median aerodynamic diameter.

Device retention did not differ significantly. However, it was notable that a large percentage (about 62%) was retained for all the formulation. This is because much of FITC-Dextran particles adhere to the walls of the capsule. This was confirmed visually by looking into the transparent capsule shells after the emission of the loaded dose. Empty capsule shells that were equilibrated at various relative humidities showed moisture content ranging from 5.6 – 18.0%. [30] In addition, TGA experiment revealed that a considerable proportion of water (11%) was contained in the different FITC-Dextran samples. The high water contents of both capsule shells and FITC-Dextran and the hygroscopity of the later suggest that water interaction is a major influence. The transfer of moisture between the capsule shell and its contents is well documented. [31] Bell et al. (1971) have shown that fine particles < 10 μm extensively coat the internal wall of the hard gelatin capsules used with the Spinhaler.™ [1] They explained that the vibratory motion of the capsule was inadequate to overcome the interparticulate forces between the finer particles and the gelatin. Under condition of low relative humidity, up to 35% van der waals forces play a significant role. [32]

Those results of directly micronised FITC-Dextran indicate that the powder can be easily dispersed into individual particles once emitted, and hence, an increased deposition of FITC-Dextran onto the lower stages of the impactor compared to the other powders. For example, with M FITC-Dextran, the $FPF_{Emitted}$ (33.1%) was significantly higher compared to MFD FITC-Dextran (18.1%) and MSD FITC-Dextran (22.2%). Both ANOVA and t-test showed actual p-values less than <0.0005. This was reflected on the results of MMAD. The MMAD of M FITC-Dextran (3.37 μm) was significantly smaller than that of MFD FITC-Dextran (4.22 μm), $p = 1.58 * 10^{-7}$. It has also shown to be significantly smaller than MSD FITC-Dextran (3.98 μm), $p = 2.97 * 10^{-5}$. An increase was achieved with the FPF_{Total} of M FITC-Dextran (13.3%) compared to MFD FITC-Dextran (6.2%), $p = 1.75 * 10^{-7}$ and MSD FITC-Dextran (8.7%), $p = 2.46 * 10^{-5}$.

Because of higher specific surface area for the M FITC-Dextran (6.24 m^2/g), the points of contact for adhesion should be higher, resulting in increased particle-particle interaction. [33] As such, MFD FITC-Dextran should have achieved the best results. To explain the fact that M FITC-Dextran achieved the best results, it is suggested that M FITC-Dextran particles are less dense because they are porous; and so, they are lighter

in weight as evident from the specific surface area results and the micrographs. Introducing asperities or pores increases the surface area but decreases the effective diameter, reducing by this the interparticulate forces and, therefore, aiding in the dispersion of aerosolised formulations.

The range of sphericity used was narrow (0.20–0.31 g/cc), therefore, it would not be adequately wise to predict that spherical particles will be inferior to the FITC-Dextran used in this work. However, with the FITC-Dextran samples used, the lower was the sphericity the better were the results as shown in Figure (2).

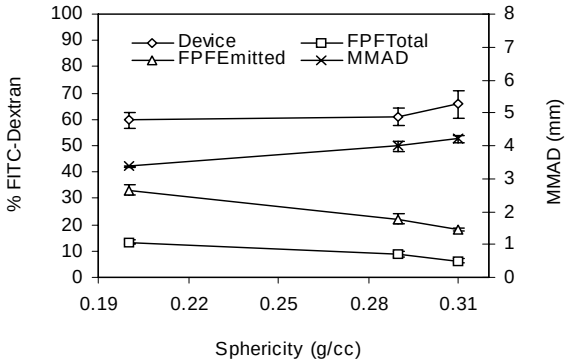


Figure 2. The relation between particle shape (sphericity) versus the % FITC-Dextran retention in the device, the fine particle fraction calculated based on the loaded dose and delivered dose (FPF_{Total} and $FPF_{Emitted}$) and the mass median aerodynamic diameter (MMAD), (n=5).

Conclusion

FITC-Dextran samples of similar volume diameter but that was different in their surface area and shape characteristic were successfully generated using three different techniques. TGA experiment, that is showing similar moisture contents for the different FITC-Dextran samples, allowed their exclusion as a factor affecting aerosolisation results when formulated at a controlled temperature and humidity conditions. The device retention of FITC-Dextran was notably high. This may provide an opportunity to improve FPF_{Total} by increasing emission. One way which might be experimented to this end in the future is to use increasing amounts of FITC-Dextran in the capsules. By doing so, the capsule internal wall will eventually be saturated with FITC-Dextran adhered particles leaving; increasing by this the amounts of FITC-Dextran available for emission. The fact that peptides/proteins require excellent aerosol properties to be delivered in relatively high doses (mg's) to the lungs due to their low bioavailability, [34] necessitates experiments aimed at increasing the loaded and emitted dose. Alternatively, the aerosolisation experiments could be carried out using formulations loaded to hydroxypropyl methylcellulose (HPMC) capsules as they contain lower moisture content compared to gelatine capsules.

Shape, although showed a considerable effect on the FPF and MMAD, could not be applied to predict a wide range of particle shapes because with FITC-Dextran it applied to a narrow range (0.20-0.31 g/cc). The lighter M FITC-Dextran particles may provide an explanation for its superior performance. Therefore, the production of more porous particles may be highly advantageous in dry powder formulations for inhalation.

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