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RESEARCH PAPER

Respirable Low-Density Microparticles Formed *In Situ* from Aerosolized Brittle Matrices

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ABSTRACT

Purpose Inhalation of low-density porous particles enables deep lung delivery with less dependence on device design and patient inspiration. The purpose of this study was to implement Thin Film Freezing (TFF) to investigate a novel approach to dry powder inhalation.

Methods Powders produced by TFF were evaluated for aerodynamic and geometric particle size by cascade impaction and laser light scattering, respectively. Density measurements were conducted according to USP methods and calculated using data from particle size measurements. Excipient inclusion and its effect on moisture sorption was measured by Dynamic Vapor Sorption (DVS).

Results TFF-produced brittle matrix powders were sheared apart into respirable microparticles using a passive DPI device, producing fine particle fractions (FPF) up to 69% and mass median aerodynamic diameters (MMAD) as low as 2.6 μm. Particles had a mean geometric diameter ranging from 25 μm to 50 μm and mass densities of approximately 0.01 g/cm^3. Powders were susceptible to moisture-induced matrix collapse, capillary forces and electrostatic charging; although formulations containing mannitol or no sugar excipient proved to be more robust.

Conclusions Aerosolized brittle matrices produced by TFF may prove to be a useful platform for highly efficient pulmonary delivery of thermally labile, highly potent, and poorly soluble drugs.

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KEY WORDS dry powder inhalation · low-density particles · particle engineering · respirable powder

ABBREVIATIONS

DPI	dry powder inhaler
DVS	dynamic vapor sorption
FPF	fine particle fraction
GSD	geometric standard deviation
MMAD	mass median aerodynamic diameter
NGI	next generation pharmaceutical impactor
SEM	scanning electron microscopy
TAC	tacrolimus
TACLAC	tacrolimus:lactose
TACMAN	tacrolimus:mannitol
TACRAF	tacrolimus:raffinose
TFF	thin film freezing

INTRODUCTION

Dry powder inhalation has been well established as a method for pulmonary delivery and as a viable and efficacious method for lung disease management. Important advancements including multidose capabilities, improved device design, and drug/carrier particle engineering have led to improvements in drug aerosolization efficiency and patient compliance. While setbacks in use of dry powder inhalers (DPI), for systemic drugs have been noted, as in inhaled insulin (1), many academic and industrial researchers continue to investigate the potential of the pulmonary route as a fast, noninvasive means of systemic therapy that bypasses first pass metabolism.

Nearly all currently marketed DPI products rely on carrier-based formulations where micronized drug particles adhere to a coarse carrier, typically lactose. The carrier is used to improve powder flow through ordered mixing and reduce electrostatic and van der Waals interactions between micronized drug particles. Inertial and aerodynamic forces

provided by a combination of patient inspiration and device design subsequently deaggregate drug from carrier, allowing inhaled micronized drug particles to ultimately reach the deep lung. This formulation strategy is inexpensive and easily scalable; however, it can result in high dose variability and low efficiency, making it less than ideal for delivery of expensive biopharmaceuticals or potent drugs with a narrow therapeutic window. Typically, carrier-based DPIs can be expected to deliver only 10 to 35% of the emitted dose to the lower airways (2), while the majority of the dose fails to deaggregate from the carrier particles and deposits in the mouth or on the oropharynx. Interest in delivery of more potent and expensive pharmaceuticals is leading to the investigation of more efficient and precise drug formulations.

Recently, new formulation strategies have been designed for more effective aerosolization and flow-rate independent delivery. Large porous particles for inhalation were introduced by Edwards *et al.*, as a method to enable the delivery of geometrically large particles (20 μm) to the deep lung where sustained release of PLGA encapsulated drugs could occur (3). The fundamental concept behind this delivery strategy relies on the similar aerodynamic behavior of particles in the respirable range (1–5 μm) and large particles (>10 μm) with a low mass density (<0.4 g/cm^3). The relationship between aerodynamic (d_{ae}) and geometric (d_g) diameter is given in Eq. 1.

$$d_{ae} = d_g(\rho_s/X)^{0.5} \quad (1)$$

where ρ_s is the particle mass density and X is the dynamic shape correction factor. The dynamic shape factor is necessary to calculate the equivalent aerodynamic diameter of particles with increased drag due to their nonspherical shape. Typically, dynamic shape factors range from 1 to 2, where 1 represents a perfectly spherical particle with a smooth surface and 2 represents a plate-like particle with a rough or irregular surface. Another aspect of aerodynamic behavior, particle slip, has also been shown to affect the aerosolization of respirable particles, however, this factor need only be considered for particles less that 10 μm in diameter (2).

Several advantages have been proposed and demonstrated in the use of porous particles for lung delivery. In comparison to traditional carrier-based DPIs which require the generation of high flow rates for deaggregation, porous formulations are easily dispersed with less dependence on the air velocity. Increased deaggregation is thought to stem from limited surface contact and reduction of particle cohesion due to van der Waals forces. Additionally, elevated drag produced by high surface area and irregular surface morphology also contributes to improved powder dispersion. Once deposited in the lower airway, insoluble particles with a geometric diameter between 1 and 5 μm are rapidly phagocytized by alveolar macrophages, 10% being cleared from the epithelial fluid within the first hour, and over 80% after 24 h (4). Particle diameters less than 100 nm or more than 10 μm have shown irregular macrophage clearance, enabling the possibility of sustained release drug therapy from the pulmonary epithelium. Porous particles in the deep lung, while too large for phagocytosis, also might be expected to be only partially submerged in the 7–70 nm thick layer of epithelial fluid lining the alveolar space (5), leaving only a portion of the particle available for dissolution. In contrast, nanoparticles can be more easily submerged and wetted in lung fluids after deposition and are available for partitioning into tissues and systemic circulation (6,7). Furthermore, the high surface area and thin boundary layer facilitates rapid dissolution. (8–10)

Because nanoparticles rely on Brownian motion for deposition and are aerodynamically too small for consistent deep lung delivery by sedimentation, they must be delivered in a microparticle carrier. Nanoparticles have been delivered by nebulization to the deep lungs as a dispersion in aqueous media (6,11–15). Trojan particles have also enabled the delivery of nanosized drug through preformed hollow spherical nanoparticle aggregates produced by spray drying (16). Recently, open friable flocs of high aspect ratio drug particles, formed by thin film freezing, have been delivered to the deep lung with pressurized metered dose inhalers (7). The space filling high aspect ratio particles are templated by the hydrofluoroalkane droplets upon actuation to form low-density particles with the proper aerodynamic diameter for deep lung delivery and high dosages.

Formulation technologies such as milling, spray drying, spray-freeze drying, supercritical fluid processing, and controlled aggregation have all been used in production of low-density, dry particles for inhalation (17–19). In this study, a new approach to low-density particle delivery is investigated using a cryogenic particle production method. In thin film freezing (TFF), a frozen solid solution is formed rapidly by dropping a solution directly onto a cryogenically cooled solid substrate. The rapid freezing process prevents segregation and heterogeneity of the solutes (20). Solvent removal by lyophilization limits the mobility of the dissolved solute and results in a low-density matrix. This freezing technique has also been used to produce stable protein particles for inhalation (7) and was found to result in less denaturation than other cryogenic techniques due to the reduction of air exposure (21). TFF also presents a distinct advantage over other particle engineering methods in that it can be scaled-up to produce powders in the final packaging material, increasing yields and eliminating filling procedures. In this study, TFF was applied to an immunosuppressive drug, tacrolimus, which could be used to provide a more targeted therapy to lung transplant recipients.

We hypothesize that highly respirable low-density microparticles can be produced by *in situ* shearing of aerosolized brittle matrices in a passive inhalation device. Powder geometries created by TFF are designed to have a lower mass density (<0.05 g/cm^3) than those measured in previous studies (0.05 to 0.1 g/cm^3). The powders created by TFF are composed of large interwoven matrices rather than discrete particles (3). Additionally, the concept of *in situ* shearing of brittle matrices to produce respirable particles ~50 μm in diameter is novel relative to previous studies in which respirable particles are fully formed during manufacturing. Given that the alveolar diameter has been shown to be 400 μm in human adults, these particles are sufficiently small for deep lung deposition (22). The high surface area of the brittle matrices (composed of sub-500 nm primary structures) produced by TFF enhances the dissolution rate, which favors high absorption and bioavailability (10,20). Furthermore, clearance by alveolar macrophages before dissolution may be reduced due to the large geometric size of deposited particles (3). The effects of formulation and moisture sorption were studied using various *in vitro* characterization techniques to determine the most suitable powder for aerosolization.

MATERIALS AND METHODS

Formulation Preparation

Alpha-lactose monohydrate, mannitol, and raffinose pentahydrate were purchased from Fisher Scientific (Fair Lawn, NJ). Tacrolimus anhydrous was purchased from Haroui Pharma-Chem (Edison, NJ).

TFF technology was employed for the production of dry powders. Briefly, a cosolvent mixture of acetonitrile (ACN) and water was used to dissolve tacrolimus and sugar excipient. Tacrolimus and lactose (TACLAC), tacrolimus and mannitol (TACMAN), tacrolimus and raffinose (TACRAF), and tacrolimus without a sugar excipient (TAC) were dissolved in the cosolvent solution. The ratio of tacrolimus to excipient was 1 to 1 and each solution prepared for TFF had a total solids concentration of 0.75%w/v. The solutions were rapidly frozen on a cryogenically cooled (< −50°C) stainless steel surface and then maintained in the frozen state in liquid nitrogen. A detailed description of the TFF process is given by Overhoff *et al.* (23) and Engstrom *et al.* (21). Solvents were removed by lyophilization using a VirTis Advantage Tray Lyophilizer (VirTis Company Inc., Gardiner, NY), leaving a drug and sugar solid dispersion in dry low-density particles. Lyophilization was performed over 40 h at pressures less than 200 mTorr while the shelf temperature was gradually ramped from −60°C to 25°C. Product was removed from the lyophilizer after dry N_2 was bled into the chamber to equilibrate to atmospheric pressure. Product was quickly covered in order to prevent ambient humidity from affecting the formulation. Powders were stored in a transparent vacuum desiccator at room temperature. Final production yields were approximately 85%.

Powder Density and Compressibility

Tapped density of TFF produced powders was measured according to a method adapted from USP <616> method I using a Varian Tapped Density Tester (Varian, Palo Alto, CA). An adaptation was made due to the limited supply of powder for testing where a 100 mL graduated cylinder was replaced by a 5 mL graduated cylinder. Hausner ratio and Carr's (Compressibility) index were calculated for each formulation based on USP guidelines. Additionally, particle mass densities of dispersed powders were calculated based on measured aerodynamic and geometric diameter for comparison to measured density values. Calculations were performed according to Eq. 1, where the dynamic shape factor (X) was assumed to be 1.5 for all dispersed powders.

Powder Morphology and Homogeneity

For qualitative determination of particle structure and morphology, scanning electron microscopy (SEM) was performed. A Hitachi S-5500 SEM (Hitachi High Technologies America, Inc., Pleasanton, CA) equipped with energy-dispersive X-ray (EDX) was used at 10 kV accelerating voltage after sputter coating the specimen with silver for 30 s at vacuum.

Geometric Particle Size Analysis

Geometric diameter of TFF produced aerosolized and non-aerosolized powder was determined by low angle light scattering using a Malvern Spraytec® (Malvern, UK) outfitted with an inhalation cell and an induction port. A Handihaler® (Boeringher Ingelheim GmbH, Ingelheim am Rhein, Germany) containing a size 3 hypromellose (HPMC) capsule (Capsugel, Peapack, NJ) filled with 3 mg of formulation was secured to the mouth of the induction port by a molded silicone adapter. Aerosolization of powder was achieved at a flow rate of 51 L/min, providing a 4 kPa pressure drop across the device. Data acquisition took place over 4 s and only when laser transmission dropped below 95%. Non-aerosolized powder diameter was measured by adding powders to the opening of the inhalation cell by spatulation without the induction port and without air flow. Geometric sizing by light scatter assumes spherical particles, thus the sizing of the irregularly shaped particles reported in this manuscript may only serve as estimation.

Aerodynamic Particle Size Analysis

A Next Generation Pharmaceutical Impactor (NGI) (MSP Corp., Shoreview, MN) was used to determine the aerodynamic properties of low-density microparticles. A Handihaler® containing size 3 capsules and approximately 3 mg of formulation was attached to the induction port by a molded silicone adapter. All tests, with the exception of those investigating the influence of gelatin capsules on aerodynamic diameter, were conducted with HPMC capsules. Aerosols were produced over 4 s at a flow rate of 51 L/min. Stage cut-off size diameters were calculated as instructed by Marple *et al.* to be 8.8, 4.9, 3.1, 1.8, 1.0, 0.6, 0.4, and 0.2 μm for stages 1 through 7 and micro-orifice collector (MOC), respectively (24). During impaction testing, collection surfaces were coated with 1% Tween 80 in ethanol, which is one of many coating materials recommended by the European Pharmaceutical Aerosol Group (EPAG) (25). Tween solution was applied to each collection surface (approx 1 mL) and allowed to dry for 1 h. After aerosolization, deposited powders were extracted from the capsule, device, adaptor, induction port, and stages 1 – MOC, respectively, using mobile phase. High performance liquid chromatography (HPLC) and a method for tacrolimus detection were used to quantify the collected drug from each rinsing (26). Samples were analyzed using a Waters 515 liquid chromatograph with a Waters 996 Photo Diode Array (Waters Corp., Milford, MA) at 215 nm. A Lichosphere RP C18 column 4 mm × 250 mm, 5 μm (Varian Corp. Lake Forest, CA) was used at 50°C and a flow rate of 1.5 mL/min. Mobile phase was composed of ACN, water, and phosphoric acid at a ratio of 600 to 400 to 1.

Percent delivered dose (%D) of each test was calculated as the dose emitted from the device as a percentage of total dose loaded. FPF and MMAD were calculated using Sigmaplot (Systat Software Inc, San Jose, CA) to fit a 3 parameter logistic curve to the plotted data. MMAD and geometric standard deviation (GSD) were calculated based on drug deposition on stage 1 through MOC, while FPF was calculated as a percentage of delivered dose with an aerodynamic diameter less than 5 μm. Statistical differences in FPF were determined using one way ANOVA followed by a post hoc Tukey test.

Water Sorption

Water sorption profiles were determined for brittle matrix powders manufactured by TFF using Dynamic Vapor Sorption (DVS-1) (Surface Measurement Systems Ltd, London, UK). For each formulation, glass sample cells were filled to capacity (0.5 mL) resulting in weights ranging from 5 to 30 mg, depending on particle density. Samples were dried with nitrogen gas until a baseline was established with less than 0.002% change in dm/dt. Each formulation was run for a complete sorption/desorption cycle between 0 and 90% relative humidity (RH). Humidity was increased/decreased by 5% after equilibrium was reached, as determined by a dm/dt less than 0.002%. Sorption isotherms were calculated and plotted according to percent change in mass minus the initial dry formulation weight. DVS was also used to create a controlled humidity environment for powder dispersion to be tested using laser scatter. Humidities of 90, 50, 20, and 0% were exposed to powder formulations for 30 min in succession. Equilibrium was assumed after 30 min, and an aliquot of powder was removed for testing. All testing began with 90% humidity so that particle mass density changes due to matrix collapse would be applied to subsequent samples taken at 50, 20, and 0% RH.

RESULTS AND DISCUSSION

Influence of Testing Conditions

Prior to characterization of TFF produced brittle matrices, methods of analysis, specifically NGI testing, were evaluated. For determination of aerodynamic diameter, many researchers have noted that NGI testing conditions and parameters can greatly influence results, the extent of which is often dependant on formulation properties (27). For example, liquid aerosols have been shown to experience droplet evaporation during testing, causing a skewing of data toward aerosols with smaller MMADs and larger FPFs. The dry environment of impaction equipment is most likely not predictive of aerosol performance *in vivo* since physiological relative humidity in the airways are near 100%. Adjustments for humidity have been made by cooling NGI equipment prior to use, resulting in a device internal RH of approximately 100% (28). Testing of dry aerosols produced by pMDI and DPI devices may also show bias in some cases due to particle bounce and re-entrainment. This behavior has been addressed by several authors, the majority of which recommend correction by coating of collection surfaces (27, 29). While no guidelines have been adopted by the USP for collection plate coating, the EPAG has recommended coatings of the collection cups with Brij, silicone, Tween, among others, to create a thin film to cause impacted particles to adhere after deposition (25).

The influence of particle bounce on NGI characterization of low-density powders has not been fully investigated, and was therefore considered before evaluation of low-density microparticles. As seen in Table I, a substantial increase in MMAD was seen when a film coating was applied to stages 1–7 and MOC. During investigation of beclomethasone dipropionate aerosols of unit density, Kamiya *et al.* showed an increase in MMAD of 1.06 μm after NGI collection plates were surface coated (30). A more

Table I Investigation of NGI Testing Parameters of Highly Porous Particles Produced by TFF

Formulation	Test conditions		Aerodynamic properties		
	Coating	Pre-sep	FPF (%)	MMAD (μm)	GSD (μm)
TACLAC	no	no	81.5	2.19	2.22
TACLAC	no	yes	51.3	1.85	1.92
TACLAC	yes	no	45.6	5.25	2.41

exaggerated difference was seen in the low-density particles tested in this study showing an MMAD increase of 3.06 μm after coating. Fine particle fraction was also affected by surface coating, decreasing almost 2 fold when tested on coated surfaces (Note: this formulation was exposed to ambient humidity for some time, therefore the aerodynamic properties are more similar to the formulation tested after 50% exposure in Fig. 5). It could be assumed that highly porous particles with low density are not only subject to particle bounce, but also particle fracture and re-entrainment after plate impaction. Similar fracturing effects may also explain reduction in MMAD when a pre-separator was included with no liquid in the cup (Table I). Although addition of a pre-separator would be expected to affect MMAD based on reduced deposition on stage 1, the skewing of deposition toward plates 4 through 6 suggests that particle fracture in the pre-separator occurred (Fig. 1). Given the potential for MMAD biasing and the fact that no coarse carrier particles are included in this DPI formulation, pre-separators should be excluded when characterizing brittle matrices for aerosolization.

Investigation of Formulation Strategy

TFF technology produces low-density pharmaceutical matrices, often containing amorphous drug, stabilized with high T_g excipients (20). In previous reports, TFF has been used as a particle engineering technology to enhance the aqueous solubility of poorly water soluble drugs for oral and pulmonary applications (7,31). Through stabilization of amorphous drug morphologies with glassy excipients, inclusion of hydrophilic materials, and increased surface area, TFF manufactured powders have been shown to offer improvements in wetting, dissolution rates, and solubility, leading ultimately to increased bioavailability (12,32–34). Given the desirable attributes of these powders and the efficiency of low-density powders for deep lung delivery, we hypothesized that these particles would result in improved performance relative to previously researched porous particles made by traditional manufacturing techniques. The structure of aerosolized brittle matrices of tacrolimus and lactose are shown in SEM images of Fig. 2. Figure 2a shows the size/morphology of particles after aerosolization by the device, while Fig. 2b, c, and d, show the primary structures that make up a particle. In this formulation, drug and excipient are present in a one-to-one ratio in a solid dispersion.

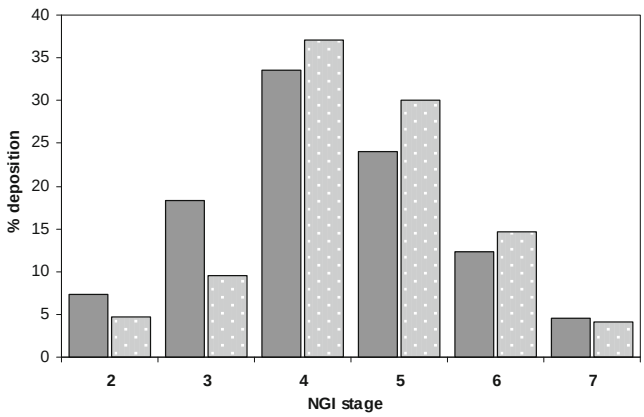

Fig. 1 Deposition of brittle porous powder in a NGI tested with (*light bars*) and without (*dark bars*) a pre-separator. Percent deposition is based only on stages 2 through 7 and does not include particles collect on the pre-separator, stage 1, or MOC.

For effective delivery of respirable low-density microparticles, a passive inhalation device with the ability to produce high shear velocities is required. Fortunately, most device designs already require turbulent, high shear airflow to provide adequate force for the separation of micronized drug from carrier lactose. The Handihaler®, a single dose capsule-based DPI, was chosen for aerosolization of brittle matrices in this study. Through a patient induced pressure drop, contents of a size 3 capsule within the device are released by flow within and around the capsule. Prior to formulation considerations, aerosol performance dependence on capsule type was investigated. HPMC and gelatin capsules were studied for their influence on the aerodynamic performance of emitted powders. While both types of capsules showed emptying efficiencies >95%, other significantly different behavior was observed. Interestingly, Table II shows HPMC capsules produced a significant improvement ($P<0.05$) in FPF over that of gelatin capsules while MMAD was unchanged. We hypothesize that shape and area of the puncture hole created could influence the velocity/turbulence intensity of air entering and leaving the capsule. While capsule water content may have an effect on long term stability and performance of the formulation, these capsules were fired immediately after filling and would likely not be effected by water leaching from the capsule. In previous reports of puncture shape of gelatin and HPMC capsules, it was concluded that more irregularly shaped holes were formed in the less brittle HPMC capsules, relative to gelatin (35). For delivery of this formulation, a smaller, non-spherical puncture may provide a greater shear force than a large spherical opening, allowing for fracture of friable matrices. Other non-aerodynamic advantages of HPMC

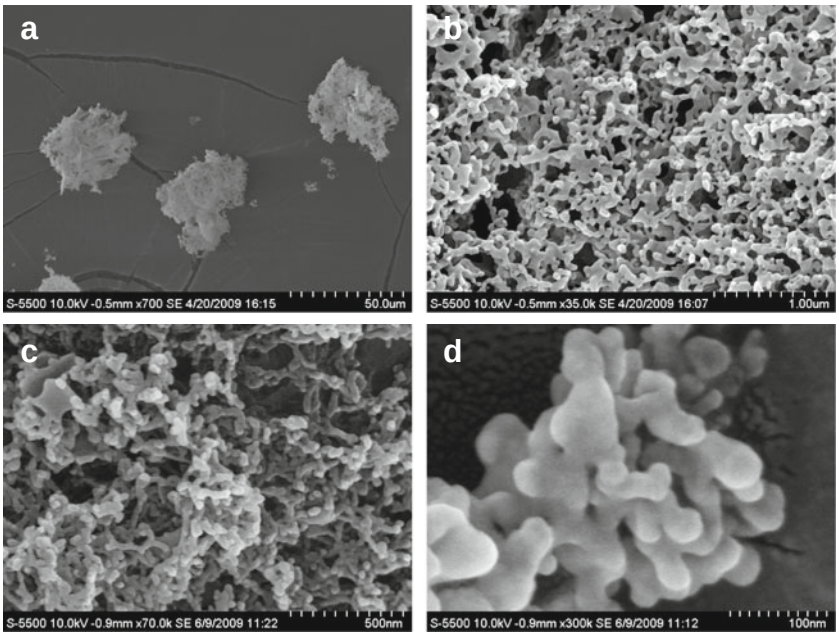

Fig. 2 SEM images of TACLAC (**a**) aerosolized brittle matrix particles collected on NGI stage 3, (**b**, **c**) drug/excipient matrix, and (**d**) primary particle structure.

capsules include low capsule moisture content and increased capsule stability at elevated humidity (36).

Determination of friability of a brittle matrix formulation was performed by comparing geometric particle distribution of low-density particles emitted from the DPI device with that of "bulk" or non-aerosolized matrices. The effect of shearing induced by the Handihaler® was substantial as indicated by the difference between the volume moment mean ($d_{4,3}$) of bulk (502.4 μm) and DPI emitted (62.0 μm) particles (The image in Fig. 3 illustrates the aerosolization concept). The volume moment mean is a numerical representation of the "center of gravity" of a volumetric distribution, also known as the De Brouckere mean diameter (37). Because particle fracture is vital to the aerodynamic performance of these particles, excipient selection focusing on material properties such as strength, brittleness, and hygroscopicity is critical. The ability to fracture the bulk particles with air flow is consistent with the fracture of large open friable flocs of similar particles produced by TFF, in which shear was produced by a hydrofluoralkane in a pMDI (7). In each case, the shear produces particles with proper aerodynamic and geometric diameters to achieve high fine particle fractions. A major difference for the pMDI approach is that TFF particles collapse as the HFA droplets evaporate. An additional requirement to formulation of dry powder for inhalation is that these excipients be nontoxic and non-irritating for delivery to the lungs or otherwise generally recognized as safe (GRAS) by the FDA.

Table II Effect of Capsule Type on the Aerodynamic Performance of Porous Particles Emitted

	FPF (%)	MMAD (μm)	GSD
Gelatin	47.93 ± 2.31	1.84	1.97
HPMC	59.03 ± 2.66	1.83	1.84

Pharmaceutical Sugars in Aerosolized Brittle Matrices

The influence of pharmaceutical sugars on aerosol performance was determined by measurement of both geometric and aerodynamic properties. In this pulmonary delivery platform, the fundamental principle for producing highly respirable microparticles relies on the brittle fracture of ultra low-density matrices to create small diameter particles of the same structure and density. Accordingly, pharmaceutical materials shown to experience brittle fracture under applied stress were chosen, such as those used in direct compression

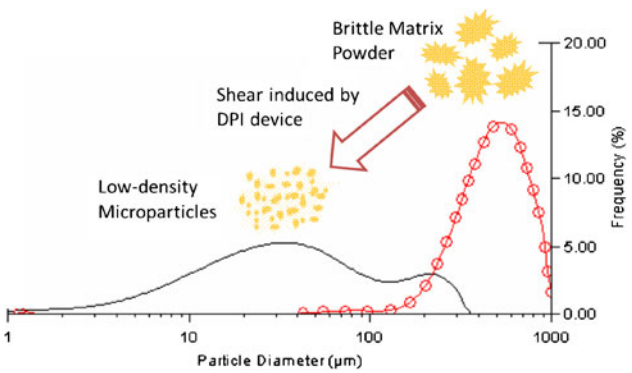

Fig. 3 Geometric particle size distribution of bulk powder (o) and DPI emitted powder (−).

(DC) tabletting. Saccharides used for DC are more likely to experience brittle fracture than ductile cellulose excipients, and are established as being non-irritating in the lungs. Two saccharides, α-lactose and raffinose, were selected based on their brittle properties; however, the ability to induce brittle fracture with a passive DPI device had not been determined. Mannitol, a less hygroscopic sugar alcohol, was also selected for evaluation as an excipient in brittle matrix powders. After production, initial visual observations of unpackaged product showed that the bulk structure of TACMAN and TAC were less susceptible to ambient humidity and matrix collapse than other formulations.

Aerodynamic evaluation of emitted low-density microparticles on a stage coated NGI showed elevated FPF compared to traditional dry powder inhalation formulations (2). Initial testing of newly prepared formulation revealed TACLAC and TACRAF as the most efficiently performing aerosols, with FPF of 68.7% and 69.2%, respectively (Fig. 4). Distribution of deposition within the NGI, shown in Fig. 4 demonstrated a lower stage deposition of TACLAC and TACRAF in comparison with TAC and TACMAN. Assuming that all formulations have similar density, it could be concluded that increased particle fracture of particles containing anhydrous α-lactose and anhydrous raffinose resulted in improved aerodynamic properties. Although some drawbacks of anhydrous materials exist (as will be discussed), complete water removal from saccharides often results in an significant increase in friability and brittleness (38,39). Specifically, anhydrous raffinose is noted for its friability and has been determined to be the "most fragile" pharmaceutical sugar (38). It is interesting to note that the more brittle, anhydrous form of raffinose is also amorphous, contrasting with the general conception that amorphous sugars are more ductile. Differing from raffinose, anhydrous α-lactose is similar to other excipients in that the amorphous form is commonly less brittle than the crystalline form. Low capsule retention for all formulated powders is indicative of the reduced surface cohesion of low-density powders, normally caused by van der Waals, capillary, and electrostatic forces in traditional formulations; although, further analysis shows that these forces may still play a role in particle dispersion.

Effect of RH on the aerosols comprising low-density microparticles was studied to determine sensitivity to water sorption. Figure 5 shows a comparison of NGI tested formulations stored at 50% RH and results from initial tests. Humidity had a detrimental effect on the performance of TACLAC, most likely due to increased plasticity of the brittle matrix. TACMAN proved to benefit from additional moisture, as shown by an increase in delivered dose and FPF, perhaps due to reduction in electrostatic charging. Figure 7d shows a bimodal distribution indicative of electrostatic adhesion of TACMAN at low RH. Water sorption to powder surfaces can both improve and hinder aerosol dispersibility. Previous reports have shown that dry powder formulations stored at approximately 60% RH maximize the drug FPF (40). In general, humidities >60% results in capillary forces predominating, while electrostatic charge remains low. Relative humidities <60% will cause elevated electrostatic adhesion of powders due to the lack of moisture-induced charge dissipation. For brittle matrices, presuming they are amorphous, the plasticizing effect of water must also be considered. Amorphous materials are particularly susceptible to water plasticization, as is the case for anhydrous lactose, which will result in reduced brittle fracture and could lead to increased particle density due to collapse of the matrix structure (39).

Influence of Moisture Sorption

Based on differences found in aerosol characteristics between initially tested and powder tested after storage at

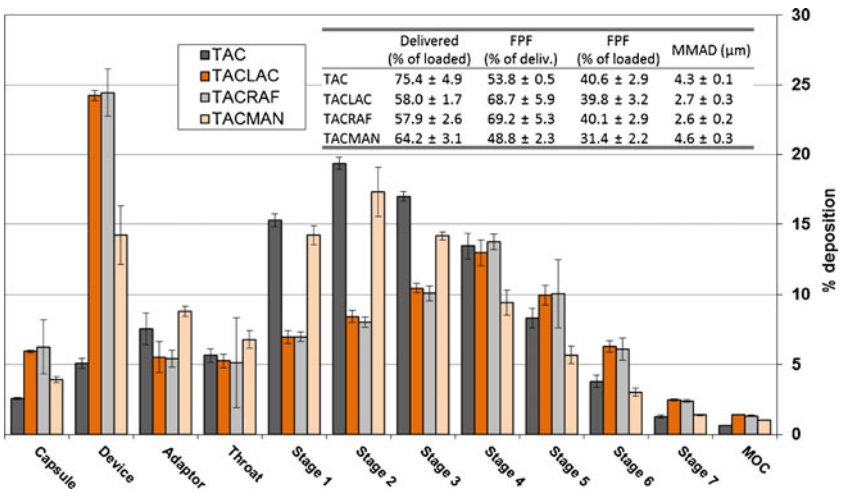

Fig. 4 Aerodynamic diameter distribution of TAC, TACLAC, TACRAF, and TACMAN directly after production determined by a plate coated NGI at 51 L/min.

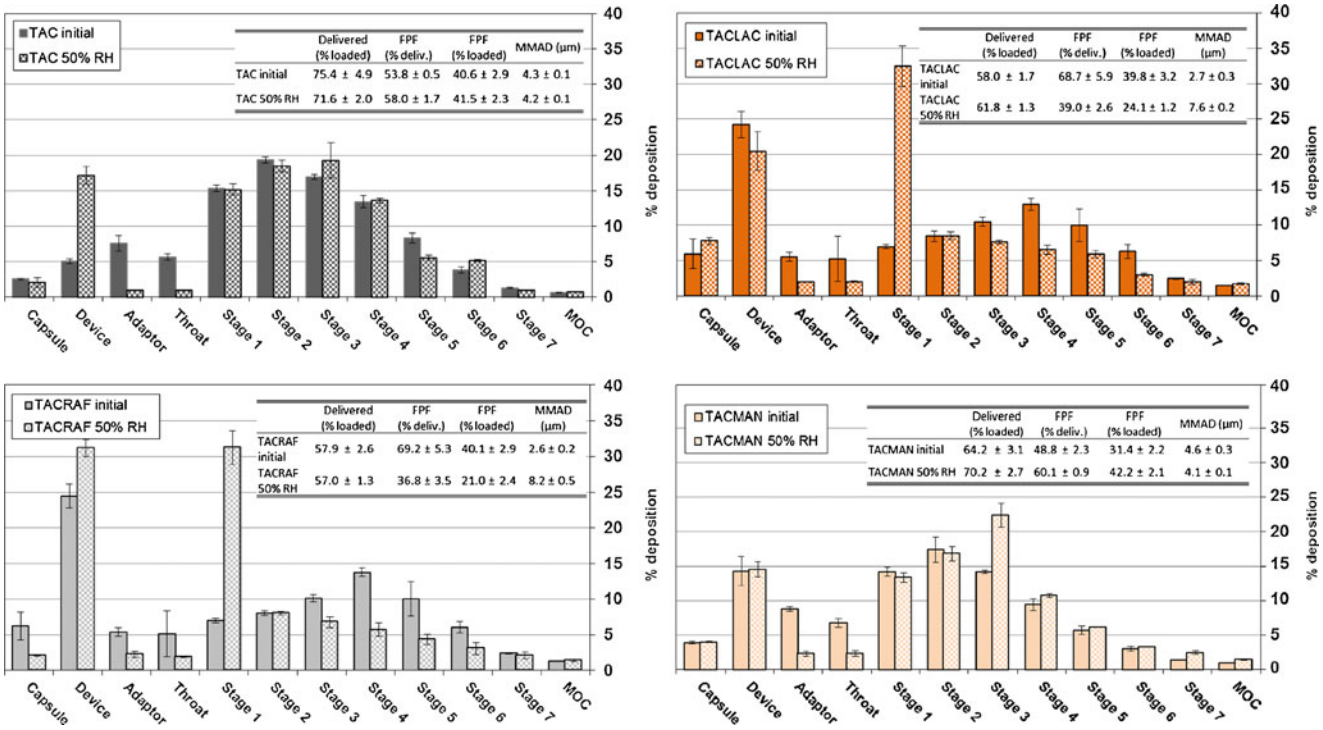

Fig. 5 Comparison of each initial formulation aerosol with the same after exposure to 50% RH.

50% RH conditions, moisture sorption may play a key role in the respiration of low-density microparticles. Moisture sorption of each formulation at RH between 0 and 90% was determined gravimetrically by DVS. Sorption and desorption isotherms were determined after one cycle and shown in Fig. 6. As might be expected, hydrophilic materials lactose and raffinose showed high moisture sorption, particularly at RH above 70%. Previous investigators have determined that raffinose samples with more than 17.9% weight gain due to moisture are capable of forming the pentahydrous form, although no reduction in mass loss due to crystallization is apparent in sorption analysis. Unlike the rapid crystallization seen in lactose, it has been reported that full crystallization of raffinose takes up to 30 h to occur at elevated humidity (41), so crystallization would not be apparent at each step. Slow moisture uptake by raffinose was evident during sorption testing of TACRAF using the method described in this study, since slow water absorption (and corresponding slow baseline stabilization) meant long cycle times. The time required for raffinose to complete sorption/desorption cycle was 75 h, while lactose only required 35 h. This leads to the conclusion that both materials are very hydroscopic; although, anhydrous lactose adsorbs water more readily.

To determine the influence of moisture adsorption on aerosol production, geometric particle size distribution of each formulation at humidities from 0 to 90% were measured. Each sample was exposed to 90% relative humidity before the appropriate humidity was applied so that moisture-induced density changes (matrix collapse) would not cause skewing of volumetric size distributions. Three major forces are likely to influence the geometric particle size distribution (PSD) of these formulations; at low humidity electrostatic forces may predominate, at high humidity capillary forces may cause particle cohesion and increased plasticity may reduce particle friability. All formulations experienced electrostatic charging at 0% RH, resulting in bimodal particle size distribution (Fig. 7). Similar moisture induced bimodal distribution was seen in TACLAC when

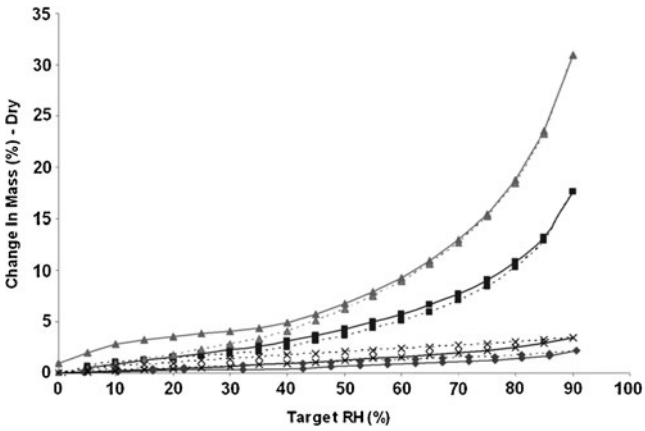

Fig. 6 Isotherms of sorption (—) and desorption (– – –) of (▲) TACRAF, (■) TACLAC, (X) TAC and, (♦) TACMAN produced after one cycle between 0% and 90% RH.

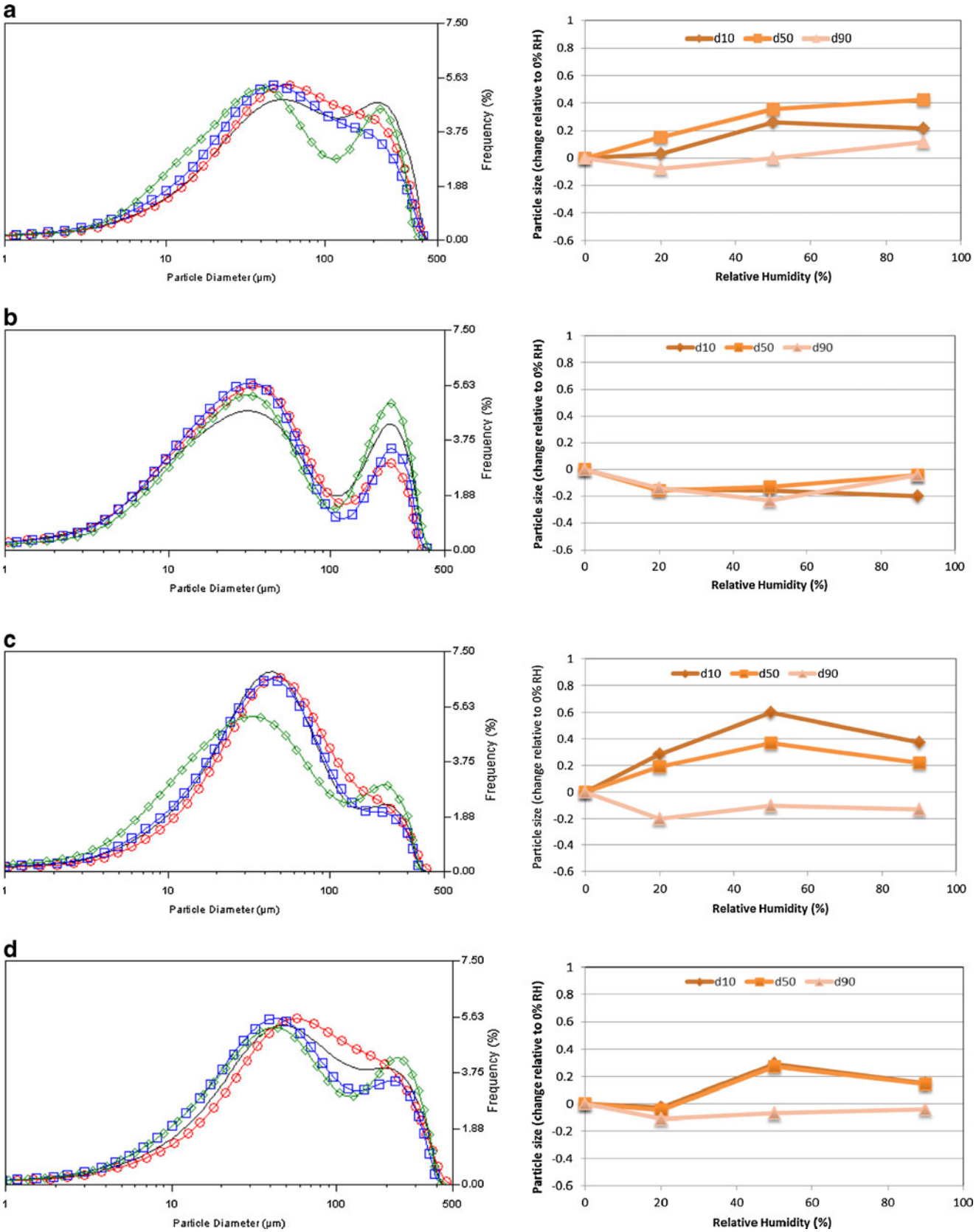

Fig. 7 Influence of (◊) 0%, (□) 20%, (○) 50%, and (−) 90% RH on the volume distribution of (**a**) TAC, (**b**) TACLAC, (**c**) TACRAF, and (**d**) TACMAN dry powder formulations.

stored at 90%RH; although, in this case cohesion could be assumed to be caused by capillary forces of the adsorbed water. TACLAC was the only formulation of the four that improved geometric PSD upon increasing humidity (of course this is after initial matrix collapse was induced), likely because of moisture allowing for crystal formation. Raffinose, being more hygroscopic than lactose, might be expected to show similar cohesion at high humidities; however, raffinose is known to incorporate water in crystal hydrates so much of the water is not present on the particle surface to induce cohesion (41). Increased brittleness resulting in smaller particle diameters of TACRAF is noted in Fig. 7c due to formation of anhydrous/amorphous material at 0% RH. In dry conditions, previous studies have observed that hydrogen bonding between raffinose and water is replaced by bonding between neighboring raffinose molecules, leading to disorder in packing (or amorphous state) (41). Humidity influence on TACMAN (Fig. 7d) and TAC (Fig. 7a) proved to be quite similar, which is a reflection on their similar structural and morphological stability during RH changes. While it seems only electrostatic adhesion had a major impact on the particle distribution of TAC and TACMAN, TAC at 0% RH appears to undergo increased brittle fracture.

Further investigation into anti-cohesive and hydrophobic materials (e.g. leucine, DPPC) may help limit water adsorption, decreasing cohesion at high humidity (42). Added aerodynamic improvements may also be imparted by promoting crystallization of anhydrous lactose without adsorbing moisture, enabling increased brittle fracture and smaller particle diameter. Inclusion of a crystal forming agent, such as mannitol, in the TFF formulation of TACLAC may impart more brittleness to the particles through formation of anhydrous crystals (43).

Formulation Density

Although each formulation was produced with 0.75%w/v solids per cubic centimeter, it cannot be assumed that all powders have identical density. Lyophilized powders, particularly ones containing hygroscopic and low T_g materials, have been shown to be susceptible to collapse, shrinkage, and meltback (44). Interestingly, mannitol is a low T_g; sugar; however, when combined 1:1 with tacrolimus and processed by TFF, resulting powders proved amorphous, as did all other initial formulations in this study. While moisture sorption is normally monitored in lyophilized products to ensure protein or chemical stability, in this study the effect of moisture sorption on powder cohesiveness, brittleness, and particle density are of primary interest.

Bulk and Tap density testing, as defined by the USP, were used to characterize compressibility of each powder formulation (Table IIIa). Carr's index, or compressibility index, is used to describe a ductile material that undergoes plastic deformation or a brittle material that fractures under an applied force (45). Assuming that all changes in powder density were due to brittle fracture, this data provided another indication that TACRAF is the most brittle of the powders investigated, showing a Carr's index of 50.

Correlation between size distribution data produced by cascade impaction (NGI) and laser diffraction (Spraytec®) analysis were also used to determine microparticle density. Knowing both the MMAD and the volumetric median diameter ($D_{[50]}$), Eq. 1 was used to calculate the mass density of the sheared microparticles exiting the DPI. Approximation of the shape factor was necessary due to its effect on aerodynamic diameter, and was assumed to be 1.5. SEM images (Fig. 2) portrayed a jagged and irregular morphology of the aerosolized particles, similar to that of a sand particle, which has a dynamic shape factor of 1.57 (2). Calculation of particle density proved to be quite similar to the theoretical density of 0.0075 g/cm^3. Furthermore, since initial density is constant, prediction of aerodynamic properties is possible using geometric PSD data, and *vice versa* (Fig. 8). Upon exposure to 50% RH, TACMAN and TAC densities were essentially unchanged (no matrix collapse), while TACRAF, and particularly TACLAC, showed increased density. Changes in particle mass densities of lactose, and perhaps raffinose, upon exposure to ambient moisture are due to their tendency to adsorb water and could be explained by two mechanisms. It is likely that adsorbed water effectively plasticizes the fragile matrix causing lowering of the glass transition temperature (T_g) and relaxation of supporting structures, subsequently collapsing the particle. Increased mobility of amorphous material will also lead to formation of a more thermodynamically stable, crystalline form. Powder collapse due to low material T_g has been observed previously in sucrose formulations, where inclusion of Dextran-40 significantly increased the T_g and resulted in improved structural integrity and longer stability (46). By increasing T_g of the matrix material, molecular mobility would be limited resulting in reduction of particle shrinkage and crystal formation. Another possibility exists for particle collapse at high humidity (< 65%) where the material becomes deliquescent, partially dissolving in adsorbed moisture and effecting the integrity of the particle (47). It is doubtful, however, that deliquescent dissolving of lactose is a viable cause for particle collapse since the critical relative humidity needed for this to occur is 99% RH. Mannitol and tacrolimus, being non-hygroscopic and hydrophobic, respectively, do not experience noticeable changes in density over time due to less water adsorption and a higher T_g.

It is clear that hygroscopicity plays a large role in the final mass density of these low-density powders. Even if packaging and storage conditions are maintained at 0%

Table III Density of Formulated Powders Based on (**A**) USP <616> Guidelines for Density Testing and (**B**) Measured Aerodynamic and Geometric Diameters

A

Formulation	Tap density (g/cm^3)	Hausner ratio	Carr's index
TAC	0.0294	1.67	40.0
TACLAC	0.0553	1.58	36.7
TACRAF	0.0423	2.00	50.0
TACMAN	0.0227	1.54	35.0

B

Formulation	Initial testing				Stored at 50% RH			
	d50 (μm)	MMAD (μm)	X	ρ (g/cm^3)	d50 (μm)	MMAD (μm)	X	ρ (g/cm^3)
TAC	51.45	4.25	1.5	0.010	59.61	4.24	1.5	0.008
TACLAC	49.10	2.71	1.5	0.005	30.22	7.59	1.5	0.095
TACRAF	40.71	2.60	1.5	0.006	45.31	8.17	1.5	0.049
TACMAN	64.78	4.62	1.5	0.008	60.30	4.10	1.5	0.007

RH, it is conceivable that highly hygroscopic particles may show substantial changes in density and geometric diameter when exposed to the humidity of the airways; although, reduction of density and geometric diameter might not have any impact on aerodynamic diameter once the aerosolized particles are sheared apart. Non-hygroscopic materials, such as mannitol, may allow for greater control of density through dissolved solids concentration and may allow for the creation of brittle matrices with tailored densities.

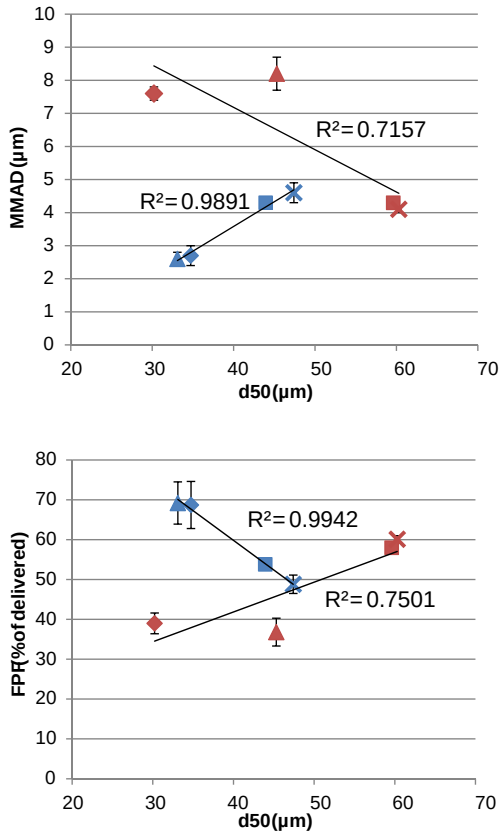

Fig. 8 Linear relationship between all formulation (TAC (□), TACLAC (◊), TACRAF (Δ), TACMAN (X)) geometric and aerodynamic properties before (blue) and after (red) 50% RH exposure.

While aerosolization of brittle saccharides, lactose and raffinose, showed superior performance, selection of appropriate formulation will depend on intended storage conditions and dosing requirements. As hygroscopic materials, both lactose and raffinose are susceptible to ambient moisture and should be packaged in completely dry conditions. Formulation with mannitol proved to be influenced less by humidity than others, however; the 100% potent TAC formulation proved to behave similarly. The only benefit of mannitol in this regard may be in stabilizing the amorphous form of the drug for increased solubility (34). Long term stability studies are still necessary to determine any additional performance variability of these formulations.

CONCLUSION

Inhalation of low-density microparticles formed from brittle matrices with a marketed DPI device is a viable platform for highly efficient deep lung delivery of drugs. Unlike delivery strategies that utilize preformed particles, the brittle matrix TFF powders are sheared into extremely low-density (< 0.01 g/cm^3) microparticles *in situ* by patient inspiration. After incorporation of biocompatible materials such as

pharmaceutical sugars into the formulations, aerosolization of the resulting brittle matrices produced FPF as high as 69.2%.

Additional benefits of this platform for inhalation therapeutics include solubility enhancement, avoidance of macrophage uptake, and the ability to formulate process-sensitive actives. Future studies focusing on dose uniformity, *in vivo* characterization, and process scale up will be investigated to determine the viability of this platform as an alternative to large porous particles and traditional carrier-based formulation.

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