Research Article

De-agglomeration Effect of the US Pharmacopeia and Alberta Throats on Carrier-Based Powders in Commercial Inhalation Products

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Abstract. The US pharmacopeia (USP) and Alberta throats were recently reported to cause further de-agglomeration of carrier-free powders emitted from some dry powder inhalers (DPIs). This study assessed if they have similar influences on commercially available carrier-based DPIs. A straight tube, a USP throat, and an Alberta throat (non-coated and coated) were used for cascade impaction testing. Aerosol fine particle fraction (FPF $\leq 5\ \mu\text{m}$) was computed to evaluate throat-induced de-agglomeration. Computational fluid dynamics are employed to simulate airflow patterns and particle trajectories inside the USP and Alberta throats. For all tested products, no significant differences in the *in vitro* aerosol performance were observed between the USP throat and the straight tube. Using fine lactose carriers ($<10\ \mu\text{m}$), Symbicort[®] and Oxis[™] showed minimal impaction inside the Alberta throat and resulted in similar FPF among all induction ports. For products using coarse lactose carriers ($>10\ \mu\text{m}$), impaction frequency and energy inside the Alberta throat were significant. Further de-agglomeration was noted inside the non-coated Alberta throat for Seretide[®] and Spiriva[®], but agglomerates emitted from Relenza[®], Ventolin[®], and Foradil[®] did not further break up into smaller fractions. The coated Alberta throat considerably reduced the FPF values of these products due to the high throat retention, but they generally agreed better with the *in vivo* data. In conclusion, depending on the powder formulation (including carrier particle size), the inhaler, and the induction port, further de-agglomeration could happen ex-inhaler and create differences in the *in vitro* measurements.

KEY WORDS: dry powder inhaler; idealized mouth-throat geometry; lactose carrier; powder aerosols; USP throat.

INTRODUCTION

The US Pharmacopeia (USP) throat is a 90° bend tube commonly used to connect a pharmaceutical inhaler device to a cascade impactor as a standard practice for *in vitro* assessment of aerosol performance, both in the pharmaceutical industry and the research environment. The Alberta throat has been evaluated worldwide by researchers because of its demonstrated capability of mimicking aerosols and flow dynamics in the human mouth-throat (1–6), and it is now commercially available from Copley Scientific. Ex-inhaler de-agglomeration of drug powders upon mechanical impaction

on different throat geometries has been reported (7–9). Our previous work (10) showed the USP and Alberta throats could cause further de-agglomeration of carrier-free powders emitted from dry powder inhalers (DPIs) which have not sufficiently broken up the powder agglomerates into fine particles. An example was disodium cromoglycate powder (Intal[®]) dispersed using a Spinhaler[®]. However, with other less cohesive powder formulations (budesonide in Pulmicort[®] and mannitol in Aridol[™]) and/or higher performance DPIs (Turbuhaler[®] and Osmohaler[™]), there was no significant throat-induced powder de-agglomeration because the drug powders had been efficiently de-agglomerated within the inhalers. This finding has an important implication for both the regulatory and scientific perspectives. It implies that the *in vitro* aerosol performance of DPIs has to be assessed with caution, since the USP and/or Alberta throats has the potential to create artifacts in the results, depending on the powder formulation and the inhaler.

There are three main powder de-agglomeration mechanisms taking place inside a DPI upon patients' inhalation, including (i) impaction of agglomerates onto a wall, (ii) turbulence-induced transient flow acceleration, and (iii) dispersion by mechanical forces such as fluidization, scraping, and vibration (11,12). The relative contributions of the de-agglomeration mechanisms for specific inhalation products

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are dependent upon the inhaler design and powder formulation (11–14). Further de-agglomeration could, therefore, happen ex-inhaler in two ways: (i) artificial de-agglomeration as particles bounce/re-entrain from the surface of an *in vitro* throat and (ii) de-agglomeration caused by the transient flow acceleration at the restricted region inside the Alberta throat which could occur *in vivo* as powder passing the mouth-throat area. While the former is not desirable, the latter can be important for better *in vitro-in vivo* data comparison.

While carrier-free powder agglomerates break up into smaller fragments and/or primary drug particles, drug particles in carrier-based systems have to detach from the carrier surfaces *via* sliding and/or rolling (14). Due to the intrinsic difference in the dispersion mechanisms, predicting the effect of throat-induced de-agglomeration for carrier-based systems on the basis of the carrier-free counterparts may not be valid. The present study extends our previous work (10) to investigate the de-agglomeration effects of the USP and Alberta throats on carrier-based powder systems using commonly available commercial products containing lactose carriers. A straight tube is included to serve as a negative control for throat impaction as it has identical dimensions to the USP throat, except for the 90° bend. Zhou *et al.* (5) highlighted the importance of grease coating the Alberta throat to obtain comparable throat deposition with the *in vivo* data, especially for large agglomerates (>10 µm), as particle bounce/re-entrainment is not expected to occur *in vivo*. While a non-coated Alberta throat allows direct comparison with the pharmacopeia-approved non-coated USP induction port, the use of a coated Alberta throat minimizes particle bounce within the geometry and mimics the *in vivo* condition better. Therefore, the Alberta throat is used with and without a grease coating to directly assess the effects of powder impaction on further de-agglomeration inside the throat.

A Spraytech laser diffraction system was used in our previous work to study the throat-induced de-agglomeration of pure drug formulations (10). However, this technique cannot be adopted in the present study because it is unable to differentiate between carrier and drug particles in a carrier-based formulation. In addition, the laser diffraction signal from the active pharmaceutical ingredients (APIs) may not be high enough for detection due to the low drug contents in most carrier-based formulations (generally less than 1% *w/w*). Therefore, cascade impaction testing was used and the fine particle fraction (FPF) was computed to evaluate the throat-induced de-agglomeration for commercially available carrier-based products. It is noteworthy that the use of a next generation impaction (NGI) could also cause further de-agglomeration due to turbulence through the nozzles and/or impaction on the collection surfaces, which could mask the influence of throat-induced de-agglomeration. The multi-stage liquid impinger (MSLI) was preferable for the present study, because the nozzle diameters (25 and 14 mm) for the first two stages of the MSLI are comparable to the internal diameter of the USP induction port (19 mm). These diameters are generally much greater than the air inlet dimensions of the test inhalers. Therefore, minimal de-agglomeration would be expected as powders passing through the MSLI. In addition, fine particles (≤5 µm) are mostly collected on the third to fifth stages of the impinger. To

minimize particle bouncing within the impinger, all stages were loaded with 20 ml collecting solvent prior to testing. Therefore, any difference in the FPF noted between the tested induction ports should arise from the impactions and turbulences inside them.

Computational fluid dynamic (CFD) simulations were also performed to study the airflow pattern and agglomerate trajectories in the USP and Alberta throats at different flow rates. This allowed further understanding of the potential influence of the throats on further de-agglomeration of drug particles from the carrier surface.

MATERIALS AND METHODS

Materials

Seven commercially available lactose carrier-based powder inhaler systems were examined, including Relenza® Diskhaler® (GlaxoSmithKline, Brentford, England, UK), Ventolin® Rotacaps® Rotahaler® (GlaxoSmithKline, Brentford, England, UK), Spiriva® HandiHaler® (Boehringer Ingelheim Pharmaceuticals, Inc., Connecticut, USA), Seretide® Diskus® (GlaxoSmithKline, Brentford, England, UK), Foradil® Aerolizer® (Novartis Pharmaceuticals Australia Pty Limited, NSW, Australia), Symbicort® Turbuhaler®, and Oxis™ Turbuhaler® (Astra Zeneca Pty Ltd, NSW, Australia). These products were purchased commercially and used prior to its labeled expiry date. Details of the APIs, excipients, inhaler flow resistance, and the experimental dispersion conditions are shown in Table I. The seven investigated DPIs were selected to cover various aspects, including the drug, drug contents, drug to carrier ratios, size of lactose carriers, aerosol dispersion mechanisms, and air resistance. The range of airflow resistance is representative of all commercially available DPIs. Rotahaler® and Aerolizer® are examples of low resistance inhalers and HandiHaler® has the highest resistance, whereas Diskhaler®, Turbuhaler®, and Diskus® fall between these extremes.

Three induction ports were used in this study, including a USP throat (the current pharmaceutical industry standard apparatus), a straight tube having identical dimensions to the USP throat except the 90° bend (as a control for minimum impaction), and an Alberta throat which is a more physiologically realistic throat model (1). A picture of the three induction ports is shown in Fig. 1.

Particle Morphology and Size Distribution

Scanning electron microscopy (SEM) was employed to visualize the particle morphology of the investigated commercial formulations. Powders were scattered onto carbon tape, mounted on a SEM stub, and coated with gold (15-nm thick) using a K550X sputter coater (Quorum Emitech, UK). The images were captured using a Hitachi S4500 FESEM (Hitachi, Japan) at 5 kV.

Particle size distributions of the powder formulations were measured by laser diffraction using a Mastersizer 2000 (Malvern Instruments, UK). As the concentrations of the API are generally less than 1% *w/w* in the drug powders, excluding Relenza® (25% *w/w*), they make a negligible contribution to particle sizing measurements. Therefore, the

Table I. Summary of the Commercial DPIs with Lactose Carrier

DPI	APIs	Lactose carrier (mg)	Airflow resistance (kPa ^{1/2} /(L/min))	Flow rate (L/min)	Number of doses*
Relenza [®] Diskhaler [®]	5 mg zanamivir	20.0	0.0212 (15)	80	1
Spiriva [®] HandiHaler [®]	18 µg tiotropium	5.5	0.0467 (16)	28	3
Seretide [®] Diskus [®]	50 µg salmeterol xinafoate (SX); 500 µg fluticasone propionate (FP)	12.5	0.0208 (15)	80	3
Foradil [®] Aerolizer [®]	12 µg eformoterol fumarate	25.0	0.0176 (15)	100	3
Symbicort [®] Turbuhaler [®]	12 µg eformoterol fumarate (EF); 400 µg budesonide (B)	0.73	0.0270 (17)	70	3
Oxis [™] Turbuhaler [®]	12 µg eformoterol fumarate	0.6	0.0352 (15)	56	3
Ventolin [®] Rotacaps [®]	200 µg salbutamol sulfate	13.5	0.0126 (15)	100	1
Rotahaler [®]					

DPI dry powder inhaler, API active pharmaceutical ingredient

powders were dispersed through the measurement window with compressed air at 4 bar using a Scirocco 2000 dry powder module (Malvern Instruments, UK) to ensure complete breakup of lactose agglomerates. The material and absorption refractive index for lactose were set at 1.53 and 0.1, respectively. The d_{10} (diameter at 10% undersize), d_{50} (diameter at 50% undersize), and d_{90} (diameter at 90% undersize) were reported. All measurements were done in triplicate.

Dispersion Methodology

The USP throat and straight tube were used without a grease coating on the interior surface as is the standard practice (18). Since there is no standard practice for the usage of the Alberta throat, dispersions with both non-coated and grease-coated throats as described in Zhang *et al.* (4) were performed. The airflow rate across each DPI was set to give a pressure drop of 4 kPa as specified by the USP, using a flow meter (TSI Inc, Model 4040, Shoreview, MN, USA) (18). Since the Aerolizer[®] and Rotahaler[®] require a flow rate higher than 100 L/min to generate a 4-kPa pressure drop, the flow rate was set at 100 L/min in accordance with the USP. The duration of suction was set to draw 4 L of air. All dispersion experiments were performed in a controlled temperature (20±3°C) and relative humidity (50±3%) environment. Triplicate measurements were carried out for each product and throat. While a single dose was dispersed for Relenza[®] Diskhaler[®] and Ventolin[®] Rotacaps[®] Rotahaler[®], three doses were dispersed for other formulations to generate sufficient concentrations for chemical assay. Unless specified, the FPF was defined as the mass fraction of

particles ≤5.0 µm with respect to the nominal dose. Mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were determined according to the USP Test chapter <601> (18).

A multi-stage liquid impinger (MSLI, Apparatus 4, USP Test chapter <601>, Copley, UK) was used to investigate the drug deposition for each formulation, except for Rotahaler[®] which will be described below. The cutoff aerodynamic diameters of stages 1–4 at a flow rate of 60 L/min are 13.3, 6.7, 3.2, and 1.7 µm, respectively. The cutoff diameters of the impinger stages at different airflow rates were calculated to be inversely proportional to the square root of the flow rates (19).

For the Rotahaler[®] DPI, the orientation of the inhaler (horizontal with the USP throat and vertical with the straight tube) was found to affect the capsule-emptying efficiency. When it was connected to the USP throat horizontally, the mass retention in the capsule (10.6±1.0%) was less than that in the device (18.5±0.6%). However, the opposite was observed in the vertical orientation using the straight tube (16.3±1.6% in the capsule and 9.0±2.8% in the device). These differences were attributed to how the capsule body dropped onto the device after it was twisted to open the capsule. This different capsule-emptying behavior could complicate the interpretation of the throat-induced de-agglomeration effect on the FPF. Therefore, a next generation impactor (NGI, Apparatus 6, USP Test chapter <601>, Copley, UK) with all plates coated with silicon grease (Slipicone; DC Products, Waverley, VIC, Australia) was used. The induction ports were directly coupled to the NGI, as the use of a pre-separator may cause impaction and de-agglomeration, complicating data interpretation. The NGI was positioned perpendicularly to the bench to ensure that the Rotahaler[®] was horizontally oriented as it would be when it is used by patients. The capsule-emptying behavior and deposition profiles are similar with a FPF of 16.9±0.9% and 17.8±1.3% for the normal and sideways NGI positions, respectively. This confirmed the NGI can be used in the sideways position. The cutoff diameters of stages 1–7 of the NGI at 100 L/min are 6.12, 3.42, 2.18, 1.31, 0.72, 0.40, and 0.24 µm, respectively (20).

Chemical Assay

Drug depositions were determined using a high-performance liquid chromatography (HPLC) system (Model

Fig. 1. Picture of the USP throat, opened Alberta throat and straight tube (from left to right)

LC-20; Shimadzu, Japan). The configuration used consisted of a CBM-20A controller, LC-20AT pump, SPD-20A UV/VIS detector, SIL-20A HT auto-sampler, and LCSolution software. Separation columns and assay conditions are shown in Table II.

Statistical Analysis

One-way analysis of variance (ANOVA) and unpaired two-sample *t* test at a confidence level of 95% were employed to identify any statistically significant differences in mass deposition in the throats and PPFs. A *p* value of less than 0.05 (*p* < 0.05) was considered statistically significant.

CFD Modeling

The flow field inside the USP and Alberta throats under the flow conditions for different products as shown in Table I was simulated using CFD. The Reynolds-averaged Navier-Stokes equations governing the fluid flow were solved by the commercial software Fluent®, with the description of the CFD model detailed in previous papers (21,22). A Reynolds stress model was used for the turbulent flow. The inlets were given an area averaged velocity based on the total airflow rate. A velocity inlet and pressure outlet boundary conditions were used in all simulations. Three grid domains were tested in our preliminary computation. The difference was less than 5% for all variables examined, suggesting that the computed results are independent of the characteristics of the mesh size. The whole computational domains of the USP and Alberta throats were divided by 13041 and 85211 grids, respectively. The computational domains, meshes, and mesh-independent results were provided in the Supplementary materials (S1–S4). Lagrangian particle tracking was also performed as a post-processing operation, in which the lactose carrier particles with a density of 1590 kg/m³ and diameter range of 2–100 µm were tracked through the fluid from the center of the inlet and subjected to drag and turbulent dispersion forces. It is noteworthy that computational data provided in the present study should not be treated quantitatively but illustrated the significant trends in the particle impaction within the throats.

RESULTS

Physicochemical Properties of Powders

Figure 2 shows the SEM images of the seven commercial formulations studied. The lactose carrier particles exhibited irregular angular shapes with sharp edges in all formulations. While coarse lactose particles were used for Relenza®, Ventolin®, Spiriva®, Seretide®, and Foradil®, Symbicort® (Fig. 2e) and Oxis™ (Fig. 2f) formulations contain fine lactose particles of size <10 µm blended with the micronized drugs. In fact, these Turbuhaler® products were loaded with soft agglomerates (~0.5 mm in size) of the micronized drug and lactose particles prepared by spheronisation (23). The addition of fine lactose particles to the Turbuhaler® formulations are not just to increase the powder bulk for filling but also to modulate the aerosol performance of the drug (24). Fig. 2e–f shows that the soft aggregates were broken up into individual primary particles during the sample preparation.

As the drug content of most formulations was less than 1% of the total powder, the scattered light signal was more likely accounted by the lactose carrier. As seen in Fig. 3, the sizes of the lactose carriers obtained from the SEM images matched with the laser diffraction measurements. The *d*₅₀ for the Symbicort® and Oxis™ powders was 2.0±0.1 µm, while all the other formulations were in the range of 40–73 µm.

In Vitro Aerosolization

Drug Recovery

The mass recovered from capsule-based products was within ±15% of the nominal dose. For reservoir devices, the remains in the inhalers were not quantified. The emitted dose for fluticasone propionate and salmeterol xinafoate from the Diskus® ranged from 60.9 to 81.3 and 86 to 115% of the nominal doses, respectively. Both Turbuhaler® products had similar emitted doses of eformoterol fumarate (54.4–88.9%), but the combination product Symbicort® had a slightly lower emitted dose of budesonide (42.6–68.3%).

Table II. Chromatographic Conditions for the Drug Assays

API	Column	Mobile phase	Flow rate (mL/min)	Detection wavelength (nm)	Injection volume (µL)
Zanamivir	Waters NovaPak C18 column (4 µm, 3.9×150 mm; Waters, USA)	Acetonitrile and water (50 : 50)	1	230	100
Tiotropium bromide	Waters NovaPak C18 column (4 µm, 3.9×150 mm; Waters, USA)	Acetonitrile and potassium phosphate buffer (0.1 M, pH 4) (20 : 80)	1	230	100
Salmeterol xinafoate and fluticasone propionate	Intersil ODS2 column (5 µm, 4.6×200 mm; Capital HPLC, UK)	Methanol and 0.6% ammonium acetate buffer (75 : 25)	1	228	100
Eformoterol fumarate and budesonide	LiChrosphere 60® RP-select B column (5 µm, 4×125 mm; Merck, Germany)	Methanol, water and acetic acid (550 : 450 : 1)	1	230	100
Salbutamol sulfate	Waters NovaPak C18 column (4 µm, 3.9×150 mm; Waters, USA)	Methanol and 0.1% sodium lauryl sulfate buffer (60 : 40)	1.5	276	100

API active pharmaceutical ingredient

Fig. 2. Scanning electron microscopy images of particles emitted from **a** Relenza[®] Diskhaler[®], **b** Spiriva[®] HandiHaler[®], **c** Foradil[®] Aerolizer[®], **d** Seretide[®] Diskus[®], **e** Symbicort[®] Turbuhaler[®], **f** Oxis[™] Turbuhaler[®], and **g** Ventolin[®] Rotacaps[®] Rotahaler[®]

Capsule and Device Retention

In capsule-based products, including Relenza[®] Diskhaler[®], Spiriva[®] HandiHaler[®], Foradil[®] Aerolizer[®], and Ventolin[®] Rotacaps[®] Rotahaler[®], it is important to assess throat-induced de-agglomeration effects without influences caused by inconsistent capsule and device emptying. Figure 4 confirms the drug retentions in capsules and devices were comparable among all throats. The capsule retentions were in general less than 20%, except Spiriva[®] Handihaler[®] which had an extremely high capsule retention (~44%). Shur *et al.* (25) also reported a high level of capsule retention (34–45%) of Spiriva[®] Handihaler[®] for flow rates between 20 and 55 L/min. Close examination of the capsules after dispersion revealed that only a thin coating of powder was left on the capsule interior walls, suggesting extensive segregation

between drug and carrier in the Spiriva formulation which caused the high retention of tiotropium bromide in the capsule.

Throat Retentions

Figure 5 shows the drug deposition/retention in the induction ports based on the recovered dose for the seven tested products. The results showed no significant difference between the straight and USP induction ports, despite the wide range of airflow rate (28–100 L/min) used. In regard to Oxis[™] Turbuhaler[®] and Symbicort[®] Turbuhaler[®], comparable drug retention was found in all induction ports. In comparison, drug retentions in the Alberta throat for Relenza[®] Diskhaler[®], Ventolin[®] Rotacaps[®] Rotahaler[®], Foradil[®] Aerolizer[®], and Seretide[®] Diskus[®] were

Fig. 3. Particle size distributions of commercialized lactose carrier-based inhalation powders determined using laser diffraction. Error bars represent one standard deviation ($n=3$)

significantly higher than the other two induction ports ($p<0.05$). These results agree with Zhang *et al.* (4) who reported the deposition of terbutaline was higher in the coated Alberta throat ($67.8\pm2.2\%$) compared with the coated USP throat ($57.3\pm4.5\%$) when it was dispersed *via* a Turbuhaler® at 60 L/min. The coating within the Alberta throat had a significant influence on drug retention, with the amounts of drugs deposited on the coated Alberta throat being double the non-coated throat for these products. Spiriva® HandiHaler® showed similar drug retention in the non-coated Alberta throat as that inside the straight tube and USP throat, but the coated Alberta throat had significantly higher deposition.

Fine Particle Fractions

Figure 6 shows the effects of throat-induced deagglomeration on the FPF determined based on the nominal dose. For all tested products, no significant difference in FPF was observed between dispersions using the USP and straight induction ports. Using the coated Alberta throat, the FPF values were significantly reduced for the tested products, except Symbicort® Turbuhaler® and Oxis™ Turbuhaler®. In contrast, the

responses using the non-coated Alberta throat can be classified into three groups: (i) Foradil® Aerolizer®, Symbicort® Turbuhaler®, and Oxis™ Turbuhaler® showed no significant difference among the three induction ports; (ii) Relenza® Diskhaler® and Ventolin® Rotacaps® Rotahaler® showed ~10% FPF reductions in the non-coated Alberta throat; and (iii) a minor increase in the FPF (~4%) was found for Spiriva® HandiHaler®, while ~6 and ~20% FPF increments were found for fluticasone propionate and salmeterol xinafoate, respectively, in Seretide® Diskus®.

In vivo lung depositions obtained from healthy volunteers for all products (26–29), except Seretide® Diskus® and Symbicort® Turbuhaler® which were obtained from severe asthmatics patients (30), were included in Fig. 6 for comparison. The post throat emitted dose obtained with coated Alberta throat was included because theoretically, when this idealized throat is coated, the emitted dose should reflect the amount of powder entering the lung. However, the values of the post coated Alberta throat emitted dose are significantly higher than the FPFs ($<5\ \mu\text{m}$) except for Relenza®, Spiriva®, and Seretide®. This implies that some large aerosols escaped the coated Alberta throat. Hence, the FPFs of the coated Alberta

Fig. 4. Mean **a** capsule and **b** device retentions of Relenza® Diskhaler®, Spiriva® HandiHaler®, Foradil® Aerolizer®, and Ventolin® Rotacaps® Rotahaler®. Error bars represent one standard deviation ($n=3$)

Fig. 5. Mass deposition of active ingredients on the induction ports. (Single asterisk or double asterisk denotes a statistically significant difference between the indicated and following results, where * is for $p<0.05$ and ** is for $p<0.01$). Error bars represent one standard deviation ($n=3$)

throat agree better than the post coated throat emitted dose to the *in vivo* lung dose across all products. The effect of the coating material and its quantity on the filtration of large particles by the Alberta throat will be worth to investigate for future work. In general, the coated Alberta throat provided the best *in vitro-in vivo* comparison among all induction ports, with slight underestimation of Spiriva[®] HandiHaler[®] and fluticasone propionate from the Seretide[®] Diskus[®]. In fact, all the throats underestimated the lung dose for Spiriva[®] HandiHaler[®], with the non-coated Alberta throat giving the closest estimation. The straight and USP induction ports over-estimated the *in vivo* lung depositions of Ventolin[®] Rotacaps[®] Rotahaler[®] and Foradil[®]

Aerolizer[®] but provided reasonably good agreement for other products.

Table III showed the MMAD and GSD of all tested products dispersed with different throats. As Ventolin[®] Rotacaps[®] Rotahaler[®] was dispersed using a NGI at 100 L/min (the cutoff diameter for stage 1=6.12 μm), much less powder was collected in the upper stages of NGI, with the cumulative mass less than 6.12 μm being only $31.8\pm3.1\%$ (straight tube), $37.9\pm3.1\%$ (USP), $33.1\pm2.6\%$ (non-coated Alberta), and $44.7\pm8.1\%$ (coated Alberta). Therefore, no MMAD was determined for this product. Comparable MMAD was found among all throats for Relenza[®], Symbicort[®], and Oxis[™]. Both non-coated and coated Alberta throats generated smaller MMAD for Spiriva[®], Foradil[®],

Fig. 6. Fine particle fraction (FPF) for all tested products and the corresponding *in vivo* lung dose reported in the literature. The post coated Alberta throat emitted dose was also displayed. (Single asterisk or double asterisk denotes a statistically significant difference between the indicated and the subsequent results, where * is for $p<0.05$ and ** is for $p<0.01$). Error bars represent one standard deviation ($n=3$)

Table III. MMAD and GSD for the Tested Products

DPI	Straight tube		USP throat		Non-coated Alberta throat		Coated Alberta throat	
	MMAD (μm)	GSD	MMAD (μm)	GSD	MMAD (μm)	GSD	MMAD (μm)	GSD
Relenza [®]	2.9 (0.2)	1.8 (0.0)	2.8 (0.2)	1.8 (0.1)	3.1 (0.2)	1.8 (0.2)	2.9 (0.1)	1.8 (0.0)
Ventolin [®]	—	—	—	—	—	—	—	—
Spiriva [®]	6.0 (0.2)	2.1 (0.0)	5.8 (0.1)	2.2 (0.1)	4.8 (0.1)	1.8 (0.0)	3.8 (0.1)	1.6 (0.1)
Foradil [®]	3.4 (0.1)	1.8 (0.0)	3.3 (0.0)	1.8 (0.0)	3.1 (0.1)	1.7 (0.0)	2.9 (0.3)	1.7 (0.2)
Seretide [®] (FP)	3.6 (0.2)	2.0 (0.1)	3.4 (0.1)	2.0 (0.1)	2.6 (0.1)	1.8 (0.0)	2.7 (0.1)	1.6 (0.0)
Seretide [®] (SX)	3.7 (0.2)	2.1 (0.0)	3.6 (0.1)	2.1 (0.2)	2.3 (0.1)	1.7 (0.1)	2.6 (0.1)	1.6 (0.0)
Symbicort [®] (EF)	2.4 (0.1)	1.8 (0.1)	2.6 (0.2)	2.1 (0.1)	2.7 (0.0)	2.2 (0.0)	2.6 (0.2)	1.8 (0.0)
Symbicort [®] (B)	2.3 (0.0)	1.9 (0.1)	2.1 (0.0)	2.1 (0.0)	2.6 (0.1)	2.2 (0.2)	2.7 (0.3)	1.8 (0.1)
Oxis [™]	2.2 (0.1)	1.9 (0.1)	2.2 (0.1)	2.1 (0.0)	2.2 (0.2)	2.2 (0.1)	2.3 (0.1)	1.7 (0.0)

MMAD mass median aerodynamic diameter, GSD geometric standard deviation

and Seretide[®], due to the filtration of large particles and/or further de-agglomeration within the geometry.

Simulated Flow Patterns and Particle Trajectories

In order to illustrate the potential of further de-agglomeration within the throats, CFD simulations were performed to depict the flow pattern and carrier particle trajectories inside the USP and Alberta throats under the flow rates specified for the seven products (Table I). For the same flow condition, pronounce flow acceleration occurs at the outer bend of the USP throat and the constricted region in the middle section of the Alberta throat due to the change in flow direction, with local velocity magnitudes reaching the maximum in those regions (Fig. S5 in Supplementary materials). Comparing the two throats, the velocity magnitudes in the Alberta throat are double than those in the USP throat.

The impaction frequency and kinetic energy for different particle size are tabulated in Table IV. For small particles of size <10 μm , there was no impaction between the carrier

particles and either throat at flow rates ≥ 56 L/min, indicating they follow well with the airflow inside the two throats. Due to the complex internal geometry of the Alberta throat, it had significantly higher frequency of impaction and impact kinetic energy for larger agglomerates (≥ 10 μm) than those of the USP throat. At a low flow rate (28 L/min), almost no impaction occurred inside the USP throat for all agglomerate sizes. In contrast, 6–21 collisions occurred inside the Alberta throat depending on the size of the carrier particles, but the impaction kinetic energy was relatively low when compared with other flow rates used in the present study (Table IV).

DISCUSSION

The powder dispersion mechanisms of carrier-based formulations are complex and depend upon the specific device, drug-to-carrier interaction, level of air turbulence, and impaction (12). This study demonstrated the potential influence of throat-induced de-agglomeration for carrier-based DPI products.

Table IV. Impaction Number Between the Carrier Particles and the Throats, and Total Impact Kinetic Energy (Ke, $1/2 mV^2$)

Throat	Particle size (μm)		Flow rate (L/min)				
			28	56	70	80	100
USP	2	Impact no.	0	0	0	0	0
		Total Ke (nJ)	0	0	0	0	0
	10	Impact no.	0	0	0	0	0
		Total Ke (nJ)	0	0	0	0	0
	50	Impact no.	2	1	2	2	1
		Total Ke (nJ)	0.08	0.31	0.48	0.62	0.99
Alberta	100	Impact no.	2	4	3	3	3
		Total Ke (nJ)	0.88	2.18	4.5	6.66	9.71
	2	Impact no.	6	1	0	0	0
		Total Ke (nJ)	7.78E-06	1.68E-06	0	0	0
	10	Impact no.	21	2	8	2	6
		Total Ke (nJ)	2.01E-04	2.42E-02	1.28E-02	2.81E-02	9.11E-03
	50	Impact no.	10	10	12	10	7
		Total Ke (nJ)	1.12	5.24	4.88	6.18	10.0
	100	Impact no.	7	10	12	12	14
		Total Ke (nJ)	3.45	25.1	22.7	41.6	51.3

USP US pharmacopeia

Upon impaction with the throat, powders may further break up if the inter-particulate cohesion holding the drug particles and the carriers together is overcome by the stress induced by the impaction, resulting in a higher fraction of small particles (7) which is not desirable as particle bounce is not expected *in vivo*. Powder impaction may also result in higher throat retention if the powders are not able to re-entrain back to the airflow after impaction. All seven products showed similar drug deposition in the straight and USP induction ports (Fig. 5). This could be due to the airflow being sufficiently high to remove most of the deposited powder from the throat, and/or the lactose carriers were not particularly adhesive to the metal surface. Also, similar FPF values between these two induction ports were obtained, indicating the emitted powder was not further broken up upon impaction onto the USP throat.

Despite the more complex internal geometry, both the non-coated and coated Alberta throats had comparable drug retention to the straight tube and USP throat for OxisTM and Symbicort[®] (Fig. 5). As the soft aggregates of the micronized drug and lactose particles can be easily break up into individual primary particles ($d_{50}=2.0\pm0.1\ \mu\text{m}$) upon inspiration, they should be able to follow well with the airflow and experience negligible impactions inside the throat at the dispersion flow rates of 56 and 70 L/min. This was confirmed by the simulation data (Table IV). Moreover, ex-inhaler de-agglomeration caused by impaction with the throat was more likely to occur when the powder had not been sufficiently dispersed by the inhaler (10). The reasonably high FPF on the basis of the emitted dose of the two products, OxisTM (FPF_{emitted}=56.7%) and Symbicort[®] (FPF_{emitted} of EF=49.6% and B=45.3%), demonstrated Turbuhaler[®] was an efficient device in dispersing these formulations.

Conversely, products with larger carrier sizes ($d_{50}=40\text{--}73\ \mu\text{m}$) showed significantly higher drug retention in both the non-coated (~2 times higher) and coated (~4 times higher) Alberta throats, compared with the other two induction ports. The velocity is significantly accelerated and substantial impactions occurred in the constricted region of the Alberta throat. These features have been proven to mimic better the aerosols and flow dynamics *in vivo* (1–6). Both the enhanced turbulence and impaction could potential cause further de-agglomeration of powders, with the former being expected *in vivo* and the latter being an artifact for inhaler testing.

Using a non-coated Alberta throat, the above further de-agglomeration mechanisms within the complex internal geometry could take place, leading to different powder dispersion behavior in products containing coarse carriers. Firstly, the FPF values of Relenza[®] and Ventolin[®] were significantly lower than those obtained with the straight tube and USP throat. The difference could be attributed to a combination of the high throat retention and negligible further de-agglomeration because of the strong attachment of the drug particles to the lactose carriers. Secondly, Foradil[®] Aerolizer[®] showed comparable FPF among the three non-coated induction ports. Zhou *et al.* (14) reported that the detachment of drug particles from the lactose carrier surface was mainly achieved inside the inhaler prior to entering the throat. Further de-agglomeration was, therefore, not expected to take place inside the Alberta throat. Thirdly, higher FPF values were found in Spiriva[®] HandiHaler[®] (4%

increment) and Seretide[®] Diskus[®] (a 6 and 19% increment for FP and SX, respectively) (Fig. 6). This may be due to localized high-velocity airjets (turbulence) at the constricted region of the throat and/or increased impaction, leading to de-agglomeration and particle bounce. The increased turbulence and impactions inside the non-coated Alberta throat provided sufficient energy to overcome the inter-particulate cohesion forces which hold the drug and carrier particles together, leading to further powder breakup.

Since particle bounce/re-entrainment was not expected in the upper airway in human, coating the Alberta throat would be more sensible for inhaler testing. When powder agglomerates impact on the Alberta throat, they can be (i) retained and/or (ii) de-agglomerated upon impaction, rebounded and entrained back into the airflow. The chance of these agglomerates being retained in the throat was higher when the throat was coated, and the likelihood further increased with particle size because of the larger momentum. Therefore, Relenza[®], Ventolin[®], Spiriva[®], Foradil[®], and Seretide[®] which used coarse lactose carriers had significantly higher throat retention in the coated Alberta throat, as shown in Fig. 5. Though similar MMAD and GSD were obtained for both non-coated and coated Alberta throat (Table III), the FPF values of these five products obtained from the coated throat were significantly reduced (Fig. 6). Such disparity was attributed to a combined effect of the high throat retention within the coated throat and further de-agglomeration upon impaction inside the non-coated throat in the cases of Spiriva[®] and Seretide[®].

Overall, dispersion using the coated Alberta throat gave better *in vitro-in vivo* comparison in the FPF and lung deposition (except Spiriva[®] HandiHaler[®] of which all *in vitro* data collected in the present study under-predicted the *in vivo* lung dose). Shur *et al.* (25) showed experimentally that the FPF of Spiriva[®] HandiHaler[®] increased from 13 to 23% as the flow rate increased from 20 to 55 L/min. Therefore, the difference between the *in vitro* and *in vivo* results was possibly due to the variations of flow rates achieved by volunteers in the *in vivo* measurements.

CONCLUSION

Ex-inhaler de-agglomeration is governed by the interplay between the throat impaction and the drug-carrier adhesion strength. In all cases, the commercial carrier-based DPI products investigated showed negligible USP throat-induced de-agglomeration. In comparison, the more complex internal geometry of the Alberta throat, when used without a grease coating, could induce further detachment of drug particles from the lactose carrier surface, depending on the powder formulation and the inhaler. However, when the grease coating was present, the Alberta throat had minimal particle bounce and negligible ex-inhaler de-agglomeration.

Negligible differences in the FPF were noted for Symbicort[®] Turbuhaler[®] and OxisTM Turbuhaler[®] regardless of the induction ports used. This was partly because sufficient dispersion was achieved in the devices before the powders were emitted. More importantly, the small carrier size used in these products reduced the likelihood of impactions inside the Alberta throats and contributed to the similar dispersion results among all induction ports.

When a non-coated Alberta throat was used, Spiriva® HandiHaler® and Seretide® Diskus® both showed significant increases in FPF indicating further de-agglomeration had taken place upon throat impaction. However, if the adhesion forces are sufficiently strong, throat impaction may not lead to further de-agglomeration in powders or increased FPF, as shown in Relenza® Diskhaler® and Ventolin® Rotacaps® Rotahaler®. The coated Alberta throat significantly reduced the FPF for Relenza® Diskhaler®, Ventolin® Rotacaps® Rotahaler®, Spiriva® HandiHaler®, Foradil® Aerolizer®, and Seretide® Diskus® compared with dispersions using the straight tube and USP throat. These corresponded to the much higher drug retention within the Alberta throat due to the minimum particle bounce as *in vivo*. The FPF is a better indicator for the lung dose compared to the post coated Alberta throat emitted dose. Four out of seven products tested in our work (Ventolin®, Foradil®, Symbicort® and Oxis™) show significantly higher post throat emitted dose than the FPF values which implies that the coated Alberta throat do not completely filter the large particles. In general, better comparison with the *in vivo* data was achieved when the Alberta throat was coated, except for Spiriva® HandiHaler®.

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