The development of a single-use, capsule-free multi-breath tobramycin dry powder inhaler for the treatment of cystic fibrosis

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Abstract

The aerosol performance and delivery characteristics of tobramycin for the treatment of respiratory infection were evaluated using the Orbital™, a multi-breath, high dose, dry powder inhaler (DPI). Micronised tobramycin was prepared and tested in the Orbital and in the commercially available TOBI Podhaler (Novartis AG). Furthermore, the TOBI Podhaler formulation containing tobramycin as Pulmospheres was tested in both the commercial Podhaler device (T-326) and Orbital for comparison. By varying the puck geometry of the Orbital, it was possible to deliver equivalent doses of micronised tobramycin (114.09 ± 5.86 mg) to that of the Podhaler Pulmosphere product (116.01 ± 2.59 mg) over 4 sequential simulated breaths (60 $l.min^{-1}$ for 4 seconds) without the need for multiple capsules. In general, the aerosol performance of the micronised tobramycin from the Orbital was higher than the T-326 Podhaler device, with fine particle fraction (FPF) of 44.99% ± 1.09% and 37.03% ± 0.86%, respectively. When testing the Pulmosphere powder in the two devices, the T-326 had marginally better performance with a FPF of 68.77% ± 2.10% compared to 61.30% ± 3.45%. This is to be expected since the TOBI Podhaler and Pulmosphere are an optimised powder and device combination. The Orbital was shown to be capable of delivering high efficiency, high dose antibiotic therapy for inhalation without the need for the use of multiple capsules as used in current devices. This approach may pave the way for a number of antibiotic therapies and medicaments where high dose respiratory deposition is required.

1. Introduction

The use of inhaled antibiotics for diseases such as cystic fibrosis (CF), chronic obstructive pulmonary disease and bronchiectasis has been a core research area within the respiratory field (Brodt et al., 2014; Chmiel et al., 2014; Ryan et al., 2011; Traini and Young, 2009), still there are few therapeutic options available. The U.S. Food and Drug Administration (FDA) approved inhaled tobramycin in 1997 (Novartis TOBI®- Tobramycin)(Rose and Neale, 2010) and aztreonam lysine (AZLI, Cayston; Gilead Sciences, Foster City, CA) in 2010, as nebuliser-based therapies for treatment of respiratory infection; however, not specifically for chronic use (McCoy et al., 2008; Retsch-Bogart et al., 2008). Inhaled colistin (Colomycin; Forest Labs, New York, NY) is also used extensively in Europe and the UK as Colobreathe (125mg single capsule Turbospin DPI), for treatment of chronic *P. aeruginosa* infection, but is not FDA approved to-date (Schuster et al., 2013). Furthermore, a number of other antibiotics are in late-stage development (Quon et al., 2014) and will hopefully appear on the market in the near future.

Interestingly, only one antibiotic and one device have been marketed in the US that contains inhalable antibiotic dry powder: the TOBI Podhaler (T326 Inhaler-Novartis)(Geller et al., 2011; Maltz and Paboojian, 2011). The delivery of dry powders is considerably more convenient than nebulization since the devices are portable, have shorter administration times and do not require cold chain storage (Geller, 2005).

However, conventional DPI devices were historically designed to deliver individual micrograms to individual milligrams active drug per dose; orders of magnitude less than the quantity of antibiotics required for respiratory therapy. Thus, innovative devices and particle engineering technology are required to deliver high doses of medication in a convenient

package. In the TOBI Podhaler, Pulmosphere® technology (Inhale Therapeutic Systems; San Carlos, CA), (Duddu et al., 2002a; Geller et al., 2011; Newhouse et al., 2003; Weers et al., 2013) is used to enhance aerosolisation of such a high payload (112 mg) and is incorporated into an optimized device capable of delivering 4 sequential, 28 mg drug loaded capsules (Duddu et al., 2002b; Weers, 2000). This results in a formulation that is delivered over a number of minutes. However, there are some drawbacks to such a formulation. Firstly, the particles have to be engineered from the 'bottom-up' using advanced spray drying methodology, coupled with the use of excipients. Secondly, during patient use, multiple capsule loading, actuation, inhalation manoeuvres followed by reloading is required several times.

Moving forward, if we are to rapidly develop additional antibiotic therapies for a wide range of infections, while simultaneously improving ease of use (and the number of operational steps), we need to develop a new inhaler platform. Furthermore, if this platform is capable of incorporating the active pharmaceutical ingredient produced by both conventional 'top-down' micronisation processes as well as 'bottom-up' engineered particles (such as the TOBI Pulmosphere technology), this would offer a major advantage to respiratory scientists working within the field.

The Orbital DPI device (Figure 1) is a novel inhaler that has been shown to be able to deliver high payload of active ingredients (up to 500 mg), without the use of carriers (Young et al., 2014a; Young et al., 2014b; Young et al., 2015; Zhu et al., Accepted 30th March 2015). High payloads are required for a number of therapies including Bronchitol (400 mg, Pharmaxis Pty Ltd) and in the use of high dose antibiotics as studied here. Briefly, the Orbital has been designed as a single use disposable inhaler (reducing re-infection risk) that delivers high

doses of powder to respiratory tract over a number of inhalation manoeuvres (Young et al., 2014a; Young et al., 2014b). However, the device only requires one actuation step followed by multiple inhalation manoeuvres, significantly reducing usage time and potential patient error, enhancing compliance.

The Orbital device has 4 main components, (1) the mouthpiece, (2) dispersion grid, (3) aerosolisation chamber with tangential air inlets and (4) sample compartment or 'puck'. The puck allows for the delivery of a high dose without the need to 'fill-prime-inhale-dispose-re-fill' typical of current dry powder devices. Previously, work has been presented using this device with spray-dried material, describing its versatility in delivery high doses of engineered inhalation particles (Young et al., 2014a; Young et al., 2014b).

The aim of this investigation was to assess the capability of the Orbital device in delivering conventionally milled tobramycin via multiple breath manoeuvres, while only actuating the device once. We compared the aerosol performance and dosing of a non-optimized powder in the commercial multi-capsule device Podhaler and the gold standard TOBI-Tip Pulmosphere powder in both the Podhaler and Orbital.

2. Materials and Methods

2.1. Materials

Tobramycin base was supplied by Lisapharma (Erba, CO, Italy). Tris (dydroxymethyl) aminomethane (TRIS) was purchased from Bio-Rad Laboratories (Hercules, CA, USA). Dimethylsulfoxide (DMSO), 1-Fluoro-2,4-dinitrobenzene, sulfuric acid (≥ 95%), ethanol (100%) and acetonitrile were of analytical/HPLC grade and used as provided by Sigma-

Aldrich (Sydney, Australia). High purity water was purified by reverse osmosis (MilliQ, Millipore, France).

2.2. Micronisation and physico-chemical characterisation of tobramycin powder

To obtain a powder suitable for inhalation, the tobramycin was micronized using a Labomill jet milling system (Food Pharma System, Italy). A matrix of milling parameters was investigated and the following optimal settings were chosen: injection pressure 2.8 bar and grind pressure 3 bar. The resulting micronized powder size distribution was determined by laser diffraction (Mastersizer 3000, Malvern, Worcestershire, UK) at a refractive index of 1.65. A dry dispersion unit (Aero S) was used at a dispersion pressure of 4 bar, flow rate of 40 L/min, vibration feed rate of 100% and feed height of 0.7 mm.

The size and morphology of the micronised tobramycin particles was further investigated using a Scanning Electron Microscope (SEM) (JMC 6000 JEOL, Japan) operating at 15 keV. Samples were mounted on adhesive black carbon tabs (pre-mounted on aluminium stubs) and coated with gold, using a sputter coater (Smart coater DII-29030SCTR, JEOL, Japan) to a thickness of approximately 40 nm, prior to analysis. The micronized and raw tobramycin was studied in terms of thermal response using differential scanning calorimetry (DSC) (Model DSC-1 Mettler-Toledo, Schwerzenbach, Switzerland) at a heating rate of $10^{\circ}C.min^{-1}$ over a temperature ramp from 25 to 300˚C. 3-5 mg samples were weighed into 40µL aluminium DSC sample pans, which were crimp-sealed and pierced with a 1 mm pinhole to insure constant pressure, prior to analysis.

2.3. Emitted dose and aerosol performance of tobramycin

In order to evaluate the performance of the Orbital device in delivering multiple doses of tobramycin both the emitted dose and aerosol performance were measured using conventional *in vitro* methods as outlined in the British Pharmacopoeia. A prototype Orbital device was used throughout this study with a resistance (ex. Puck) of 0.039 $kPa^{1/2}$ /$L.min^{-1}$.

2.3.1. Choice of Orbital puck geometry

As previously discussed, the Orbital device is a single-use, disposable device containing a sample compartment "puck". The puck rotates within the aerosolisation chamber, during inhalation, releasing the formulation through a precision-engineered sample orifice that acts as a rate-limit step for powder release (Young et al., 2014a; Young et al., 2014b). Thus, prior to aerosol studies, optimum puck geometry must be chosen that controls powder release to chosen specifications.

Based on a dose of 112 mg, a targeted inhalation dose per breath of ca. 30 mg was chosen since it has been shown to be well tolerated in patients clinically (Konstan et al., 2011; Rose and Neale, 2010; Vandevanter and Geller, 2011). Four different pucks, whose characteristics are listed in Table 1, were investigated to establish a relationship between puck orifice geometry and release rate. Pucks were filled with 200 mg of micronized tobramycin and shot weight release profiles were recorded by weighing the mass difference between shots, when the device was actuated into a dose unit collection device (DUSA) at 60 $l.min^{-1}$ for a 4 second period (equivalent to a 4 L inhalation volume). Flow rates and timings were set using a GAST Rotary vein pump (VT 4.25, Becker Pumps, Sydney, Australia) and critical flow controller (WP-CFC-01, Westech Instruments, Bedfordshire, UK), respectively. Flow rates were adjusted to 60 $L.min^{-1}$ using a calibrated flow meter (Model 4040, TSI Instruments, Minnesota, USA) prior to measurements.

2.3.2. Impaction studies and aerodynamic assessment

In vitro aerosolisation performance of the micronized tobramycin was assessed using a Multi-Stage Liquid Impinger (MSLI) (Copley Scientific, Nottingham, UK) fitted with a United States Pharmacopeia (USP) induction port. The first four MSLI stages were filled with 20 mL of MilliQ water, whereas the filter stage was fitted with a 0.2 µm glass filter (Pall Corporation, Surry Hills, Australia). The airflow was set at 60 $L.min^{-1}$ using a GAST rotary vein pump (VT 4.25, Becker Pumps, Sydney, Australia) and critical flow controller (WP-CFC-01, Westech Scientific Instruments, UK) calibrated using a flow meter (mod. 4040 F, TSI Instrument, USA).

After a suitable puck geometry was chosen for the micronized powder (in this case a 0.5 mm x 0.9 mm) the Orbital was tested using the MSLI for four sequential 4 sec inhalation cycles at 60 $L.min^{-1}$ (each simulating one breath at 4L volume), with the device being weighed between each test. This simulated 'breath' number is referred to as 'shot' number hereafter. After completion, each stage of the MSLI, USP induction port and device was washed into suitable volumetric flasks for chemical quantification. Data was processed to produce: emitted dose (ED: cumulative dose across all MSLI stages and USP induction port); fine particle dose (FPD: particles with an aerodynamic diameter ≤ 6.8 µm calculated from cumulative drug deposition on MSLI stage 3-filter), fine particle fraction (FPF_{ED}: FPF/ED x 100); mass aerodynamic diameter (MMAD and geometric standard deviation GSD as outlined in the British Pharmacopeia (2015).

In order to evaluate the Orbital with respect to the current marketed tobramycin DPI (TOBI T326 Podhaler, Novartis AG, Basel, Switzerland) 28 mg aliquots of the micronized

tobramycin was weighed into gelatin capsules (size 2, Capsugel, Sydney, Australia) and tested in the T326 Podhaler. For each experiment, 4 sequential capsules were loaded, pierced and tested using the MSLI operating at 60 l.min^{-1} for 4 seconds per capsule. At the end of each experiment the device, USP induction port and all stages of the MSLI were washed into suitable volumetrics for chemical quantification. Lastly, to complete the study, both the TOBI T326 Podhaler and Orbital were tested using the commercial Pulmosphere Tobramycin. For the Podhaler measurements, 4 sequential capsules were tested as per the patient information leaflet of the commercial product [25], while for the Orbital device studies, the contents of four capsules were emptied into the same geometry puck as used for the micronized powder studies, assembled and tested using the same protocol. All experiments were conducted in triplicate and data represented as mean ± standard deviation

2.3.3. Evaluation of the aerosolisation performance of the micronized powder from the Orbital using laser diffraction

Since the Orbital is a multi-breath device (i.e. actuate once and then take sequential breaths until dosing is complete) the emptying profile and aerosol performance for each breath may play a role on efficacy and tolerance. Thus, to further support the shot weight and cascade impactor-based measurements, in-line laser diffraction measurements were conducted.

A commercially available in-line aerosol laser diffraction apparatus (Spraytec®, Malvern, Worcestershire, UK) was utilized for measurements. All measurements were conducted at 60 L.min^{-1} using the same pump and assembly as in the cascade studies; however, the Spraytec Inhalation cell was assembled between the USP induction port and MSLI. As with the cascade impactor study, the puck of the Orbital device was filled with 200 mg of micronized tobramycin and the device was weighed before and after every actuation to assess the emitted

dose. The device was operated for 4 second intervals and data recorded at a sampling rate of 1000 Hz. Data was processed to produce time-average volume-weighted particle size distributions and temporal obscuration profiles during each sample period. Additionally, the time-average 10^{th} ($d_{0.1}$), 50^{th} ($d_{0.5}$) and 90^{th} ($d_{0.9}$) percentage volume-weighted particle size undersize values and Span were calculated. The experiment was repeated in triplicate and data reported as mean ± standard deviation.

2.4. Chemical analysis of tobramycin

2.4.1 Derivatisation of tobramycin

Tobramycin has five primary amines, one primary hydroxyl group and four secondary hydroxyl groups. Due to the low chromophore capacity in the molecule, direct high performance liquid chromatography (HPLC) for the detection of tobramycin is not straightforward. In order to increase the UV absorptivity and therefore enhance the detection sensitivity, a derivatization method was applied (Pharmacopoeia, 2008). All samples were collected in 100 mL volumetric flasks with 1 mL of H_2SO_4 1N. For the derivatization procedure, 50 µL of each sample were transferred to HPLC vials and 125 µL of 2,4 dinitrofluorobenzene reagent (solution of 2,4 dinitrofluorobenzene in absolute ethanol containing 10 mg/ml) and 125 µL of TRIS reagent (2 mL of a stock solution of Tris(dydroxymethyl)aminomethane in water containing 15 mg/mL with 8 mL of dimethyl sulfodioxide) were added. After vortex mixing, samples were placed in a water bath at 60˚C for 50 minutes. Vials were then removed from the bath and allowed to stand for 10 minutes at ambient temperature. 325 µL of acetonitrile was then added and vortex mixed.

2.4.2 High-performance liquid chromatography

Drug content of the derivatised tobramycin samples and standards were quantified using a Shimadzu high performance liquid chromatography (HPLC) system consisting of a LC20AT pump, SIL20AHT autosampler and SPD-20A UV-VIS detector (Shimadzu, Sydney, NSW, Australia). Tobramycin was analysed at a detection wavelength of 365 nm, using a 3.9 x 150 mm, 5 µm packing, reverse-phase C18 column (Waters Ltd. Milford, MA, USA). The injection volume of each sample was 20 µL. The mobile phase was prepared by dissolving 2 g of Tris(dydroxymethyl)aminomethane in 800 mL of water, adding 20 mL of H_2SO_4 1N, diluting with acetonitrile to obtain 2 L and filtering with a 0.45 µm filter. Flow rate was set at 1 $ml.min^{-1}$ and the retention time was 4.5 min. Standard solutions were prepared daily in H_2SO_4 0.01 N and the linearity was verified between 5 and 500 $\mu g.ml^{-1}$ ($R^2 \geq 0.999$). The HPLC method and derivatisation procedure was modified and validated internally, based on a method reported previously by the authors (Parlati et al., 2009).

2.5. Statistical analysis

Data were subjected to statistical analysis using unpaired T student analysis. Differences were considered statistically significant at a level of $p < 0.05$.

3. Results and Discussion

3.1. Physiochemical characterization of the micronized tobramycin powder

The micronized tobramycin powder was characterized in terms of particle size distribution, morphology and thermal response. The particle size distribution of the raw starting material and micronized tobramycin is shown in Figure 2 (A) and particle size descriptors given in Table 2. The median volume-weighted diameter ($d_{0.5}$) of raw and milled tobramycin was

18.87 ± 0.70 μm and 1.82 ± 0.05 μm, respectively. Results indicated that the milling process also reduced the span (measured as $(d_{0.9}-d_{0.1})/d_{0.5}$)) and the majority of particles had a volume-weighted size of < 5 μm. Although not the aerodynamic size, assuming spherical particles of density ca. 1.5 $g.cm^{-3}$ the particles may be considered suitable for inhalation therapy (Patton and Byron, 2007). A scanning electron microscopy image of the micronised tobramycin is shown in Figure 2 (B). The micronised powder had a size distribution comparable to that measured by laser diffraction and presented an angular shape, indicative of a predominantly crystalline structure. The physico-chemical properties of the micronized tobramicyn were further investigated using DSC and a thermogram of the micronized and raw starting material is shown in Figure 3. In general, the thermal response for both the micronized and raw samples was similar to previously reported data for tobramycin base (Dash and Suryanayanan, 1991). Three endothermic peaks at approximately 115 ˚C, 170 ˚C and ~230 ˚C and one exothermic peak at 190 ˚C were observed. The thermal events in Figure 3 are labelled 1-4 and can be attributed as follows: the first endothermic peak (1) can be attributed to dehydration of tobramycin monohydrate base to a metastable anhydrous polymorph; the second endothermic peak (2) can be attributed to the melting the metastable form of tobramycin; the following exothermic peak (3) can be assigned to crystallisation of the stable anhydrous form before melting (4) (Dash and Suryanayanan, 1991).

3.2. Emitted dose and aerosol performance of tobramycin

3.2.1. Choice of Orbital puck geometry

The cumulative shot-weight, as a function of shot number, for each of the four different puck geometries is shown in Figure 4. In general, all four-puck geometries resulted in a linear release of drug over four shots, as indicated by a coefficient determination of $R^2 \geq 0.990$.

Furthermore the release rate (dose per shot) was dependent on the orifice geometry with increased orifice size (and to a smaller degree orifice number) resulting in increased drug released per shot. The total cumulative metered dose over four shots for each puck geometry and linear regression R^2 values are given in Table 3. The commercially available TOBI Podhaler DPI delivers a nominal dose of 112 mg over 4 capsules. Thus, four sequential inhalation manoeuvres from the Orbital would provide equivalent dosing and be well tolerated. Based on a design space of 112 mg with preliminary upper-lower specifications of ± 25%, (related to Pharmacopeia specifications for inhaled medicines), an emitted dose was considered acceptable, between 84 and 140 mg over the four doses. Consequently puck D (0.5 x0.9 mm) was chosen since its release rate was linear (R^2 = 0.999), producing a dose of 109.4 mg ± 1.2 mg after four shots.

3.2.2. Impaction studies and aerodynamic assessment

Using puck D, the aerosol performance of micronised tobramycin was evaluated using an MSLI. In addition, the micronised material was tested in the TOBI Podhaler and the TOBI Pulmosphere product powder, tested in both the Orbital and Podhaler for comparison. The aerosol performance parameters for all four combinations are shown in Table 4.

The ED of the micronized tobramycin powder from the Orbital was 114.1 mg ± 5.9 mg with a FPD of 51.3 mg ± 2.5 mg. This resulted in an overall FPF_{ED} of 45%, which was significantly higher than when the same powder was used in the Podhaler (where an FPF_{ED} of 37% was observed; *p = 0.0007*). Furthermore, the MMAD of micronised tobramycin emitted from the Orbital was significantly lower (2.9 µm ± 0.1 vs. 3.8 µm ± 0.1 µm; *p= 0.0024*), indicating a finer emitted particle fraction. It is interesting to note that a visibly higher amount of powder is retained/coated on the walls of the puck-aerosolisation chamber in the

Orbital device when compared to the capsule compartment of the Podhaler. Even after exhaustion of the puck, ca. 30% of the micronised material was retained within the device (primarily on the walls of the puck chamber) and was not removed with further shots (data not shown). This retention can be linked to the higher FPF_{ED} and lower MMAD of the Orbital since the puck rotation pushes powder centrifugally toward the device wall, acting as a mill whilst enhancing powder coating inside the puck (Young et al., 2014a; Young et al., 2014b). Overall however, a high-dose, high-efficiency, excipient-free aerosol powder containing tobramycin can be produced over a number of breaths without the need for multiple capsules and actuation steps, as used with the Podhaler.

The commercially available TOBI Podhaler formulation contains Pulmosphere tobramycin powder delivered via 4 capsules. Thus, the standard formulation and Podhaler was evaluated using the MSLI and powder from emptied capsules tested in the Orbital for comparison. It must be noted that emptying the capsules into the Puck may influence the powder performance, since it is no longer contained in a sealed package. To address this, the time from emptying to testing was minimised to reduce impact of the local environment. In general, the performance of the Pulmosphere powder in both the Podhaler and Orbital was higher than when both devices were tested with the micronized powder. Emitted Pulmosphere doses of 100.0 mg ± 2.6 mg for the Orbital and 116.0 mg ± 2.6 mg for the Podhaler indicating better emptying efficiency overall with FPD values of 61.3 mg ± 4.7 mg and 79.8 mg ± 4.1 mg for the Orbital and Podhaler, respectively. This translated to similar FPF_{ED} and MMAD values for the two devices, where the Orbital produced a FPF_{ED} of 61.3% ± 3.5% and MMAD of 2.9 μm ± 0.2 μm while the Podhaler produced a FPF_{ED} of 68.8% ± 2.1% and MMAD of 3.0 μm ± 0.1 μm. This difference was significant for the FPF ($p = 0.0431$) but non-significant for the MMAD between devices ($p = 0.4579$). It is important to

note that, while the Podhaler-Pulmosphere combination outperforms the Orbital, the emitted dose and FPF were similar even though the later device was not optimised for this powder. Another interesting aspect of studying the commercial powder in the Orbital was that the majority of the loaded dose was emitted in the first shot, with much lower retention on the puck chamber walls. For example 90% of the emitted dose was released in the first shot and only 15% of the loaded mass remained in the device after 4 shots. This may be due to increased flowability and reduced density of the Pulmoshere powder, reducing residency time in the puck chamber and reducing the mill/coating effect observed with the micronized material. While this study did not focus on optimising drug delivery of Pulmosphere powder from the Orbital, it is feasible that the emitted mass-per-shot can easily be modified if required by adjusting the puck hole size and geometry. For example, recent work by Zhu et al., (Zhu et al., Accepted 30th March 2015) showed that 400 mg of mannitol could be delivered over 2 shots or over > 10 shots by simply modifying the Orbital's puck parameters.

3.3.3. Evaluation of the aerosolisation performance of the micronized powder from the Orbital using laser diffraction

The temporal release profile of micronized tobramycin from the Orbital device over four consecutive shots, as well as the time-average volume-weighted particle size distributions, is given in Figure 5. Temporal release is measured indirectly, via percentage laser obscuration as the powder is emptied and aerosolised from the device. In general, the release profile for each shot number (Figure 5A) were similar with a ~1s ramp in obscuration followed by a plateau for the remainder of the actuation time. Such observations would be expected since the mass-per-shot remains constant at ~20% per shot (Figure 4) and the ramp is due to the puck spinning faster to release the dose over the 4 s testing period.

Importantly, the particle size distribution during each shot remained constant, as evident from the volume-weighted cumulative particle size distribution plots shown in Figure 5B. In general the volume d0.5 for micronised tobramycin through the Orbital was 3.06 μm, 2.83 μm, 2.88 μm and 2.94 μm for shots 1, 2, 3 and 4, respectively.

4. Conclusions

It has been demonstrated that the Orbital DPI device can be used to deliver high doses of micronised tobramycin, with a size suitable for inhalation therapy. The device has been shown to be capable of delivering an equivalent dose to that of a commercial inhalation tobramycin product over four 'breaths' without the need to use multiple capsules. This single-use, multi-breath approach paves the way for high dose inhalation therapies that will open up opportunities for innovative inhalation therapy and improve patient ease of use and compliance.

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Table 1 Types of Orbital pucks investigated with milled tobramycin

Puck	Orifice/s shape	Number of orifices	Orifice dimension (mm)
A	Circular	Single	Ø 0.6
B	Circular	Double	Ø 0.5
C	Rectangular	Single	0.5 x 0.77
D	Rectangular	Single	0.5 x 0.9

Table 2 Comparison of volume-weighted diameters and span of raw and milled tobramycin measured using laser diffraction (mean ± StDev, n = 3).

Size descriptor	Raw Tobramycin	Milled Tobramycin
$d_{0.1}$ (µm)	0.81 ± 0.01	0.32 ± 0.01
$d_{0.5}$ (µm)	18.87 ± 0.70	1.82 ± 0.05
$d_{0.9}$ (µm)	197.67 ± 8.08	5.85 ± 0.61
Span	10.46 ± 0.46	2.85 ± 0.19

Table 3 Emitted dose over 4 shots (mean ± StDev; n = 3) and coefficient of determination (R^2). *Pucks A and B were not measured in triplicate.

Puck ID.	Emitted dose (mg) in 4 shots	Regression (R^2) of cumulative dose vs. shot no.
Puck A*	28.70	0.990
Puck B*	43.00	0.993
Puck C	58.50 ± 28.49	0.995
Puck D	109.43 ± 1.22	0.999

Table 4. Aerosol performance parameters of Orbital and T-326 Podhaler with micronized and Pulmosphere tobramycin powders, using a MSLI at 60 L.min^{-1}. Data is represented as mean ± STDev). *Indicates weighed powder mass that contains drug and excipients.

Powder	Micronized Tobramycin	Micronized Tobramycin	Pulmosphere powder	Pulmosphere powder
Device	Orbital	T-326	Orbital	T-326
Loaded dose (mg)	200.23 ± 0.21	(28.00 ± 0.02) x 4	196.30 ± 3.16*	(49.08 ± 0.79) x 4
Emitted dose (mg)	114.09 ± 5.86	95.24 ± 2.87	99.96 ± 2.60	116.01 ± 2.59
FPD (mg <6.8 µm)	51.32 ± 2.48	35.28 ± 1.87	61.31 ± 4.68	79.81 ± 4.07
FPF_{ED} (% <6.8 µm)	44.99 ± 1.09	37.03 ± 0.86	61.30 ± 3.45	68.77 ± 2.10
MMAD (µm)	2.86 ± 0.13	3.78 ± 0.05	2.86 ± 0.21	2.98 ± 0.13
GSD	2.34 ± 0.06	2.22 ± 0.03	2.23 ± 0.03	2.21 ± 0.04

Figure 1 Schematic and photograph of Orbital device and puck assembly

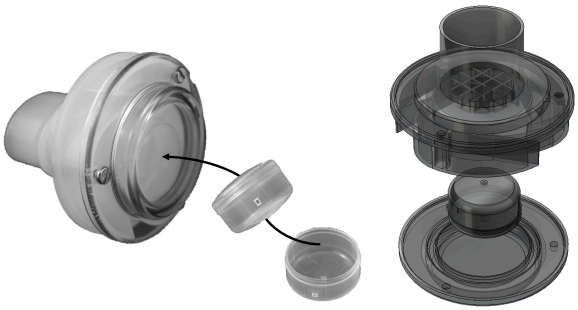

Figure 2 (A) Particle size distribution of raw and milled tobramycin and (B) electron microscopy imaging of micronized tobramycin

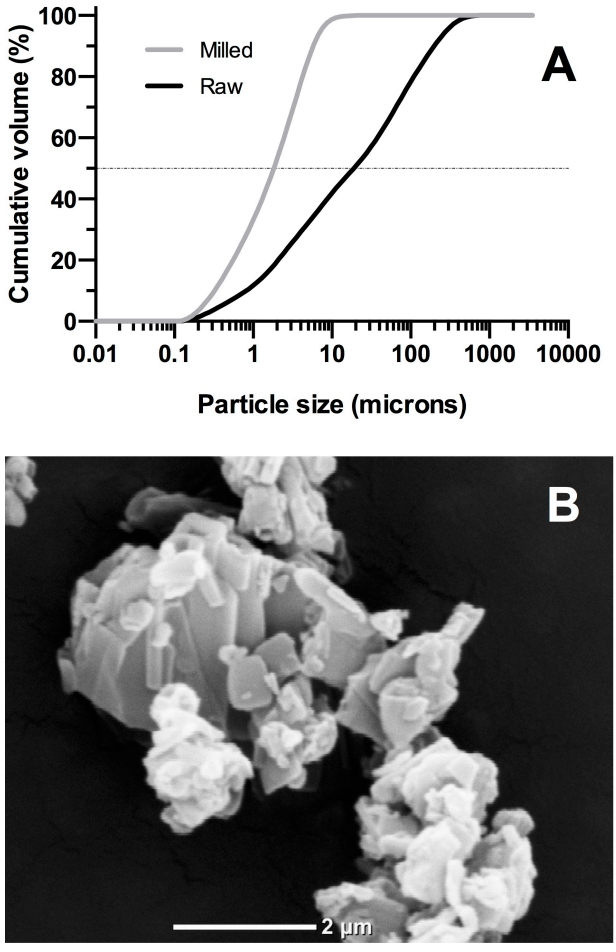

Figure 3 Differential scanning calorimetry thermogram for raw and milled tobramycin

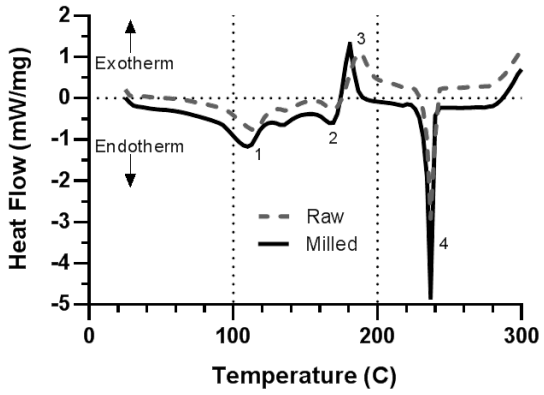

Figure 4 Cumulative shot-weight for 4 different Orbital DPI puck geometries. NB. Puck B contains two 0.5 mm holes.

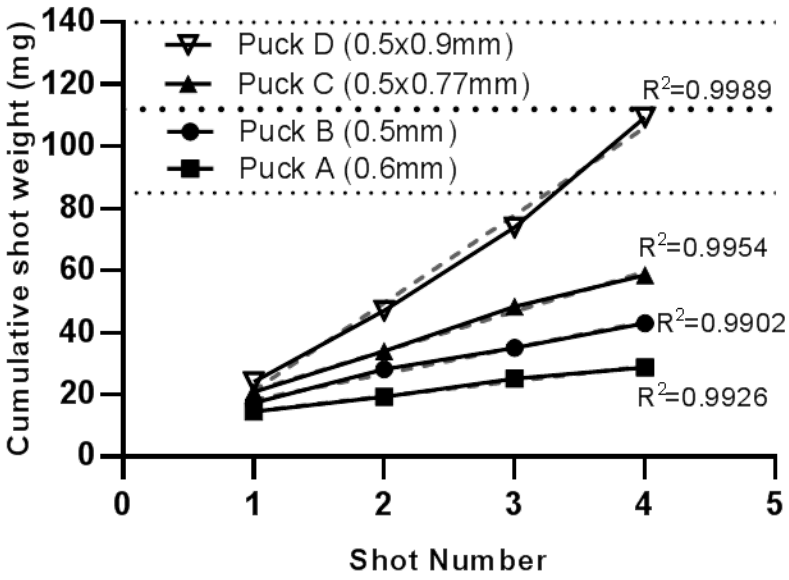

Figure 5 **Laser diffraction analysis of emitted micronised tobramycin from the Orbital as a function of shot number (A), and time-averaged volume-weighted cumulative mass distributions (B) for four shots.**