



Dose administration maneuvers and patient care in tobramycin dry powder inhalation therapy



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ABSTRACT

The purpose of this work was to study a new dry powder inhaler (DPI) of tobramycin capable to simplify the dose administration maneuvers to maximize the cystic fibrosis (CF) patient care in antibiotic inhalation therapy.

For the purpose, tobramycin/sodium stearate powder (TobraPS) having a high drug content, was produced by spray drying, characterized and the aerodynamic behavior was investigated *in vitro* using different RS01 DPI inhalers. The aerosols produced with 28, 56 or 112 mg of tobramycin in TobraPS powder using capsules size #3, #2 or #0 showed that there was *quasi* linear relationship between the amount loaded in the device and the FPD.

An *in vivo* study in healthy human volunteers showed that 3–6 inhalation acts were requested by the volunteers to inhale 120 mg of TobraPS powder loaded in a size #0 capsule aerosolized with a prototype RS01 device, according to their capability to inhale. The amount of powder emitted at 4 kPa pressure drop at constant air flow well correlated with the *in vivo* emission at dynamic flow, when the same volume of air passed through the device.

The novel approach for the administration of 112 mg of tobramycin in one capsule could improve the convenience and adherence of the CF patient to the antibiotic therapy.

1. Introduction

Cystic fibrosis (CF) is a genetic rare disease caused by mutations in the gene coding the CF trans-membrane conductance regulator protein leading to viscous mucus presence in the airways (Moskowitz et al., 2005; Sheppard and Nicholson, 2002). Thus, CF patients are susceptible to pulmonary infections caused mainly by *Pseudomonas aeruginosa* (PA). Three approaches to the management of infections in CF patients are suggested by the European consensus guidelines (Flume et al., 2007): (i) prophylactic therapy for the prevention of infection and colonization; (ii) intravenous therapy for acute pulmonary infections and (iii) maintenance therapy by inhalation to prolong the interval between exacerbations. Thus, inhalation antibiotic therapy is recommended in exacerbation prevention of PA infection (Doring et al., 2000). Despite different antibiotics are available on the market (aztreonam lysine,

colistimethate sodium and levofloxacin) (Buttini et al., 2016; Hewer, 2012), the aminoglycoside tobramycin remains the standard to manage PA infection in order to delay exacerbation infection episodes (Cheer et al., 2003; Ratjen et al., 2009).

Local concentrations of tobramycin in the lung of patients higher than those reached by intravenous administration is the objective of inhalation administration. The antibiotic deposition in the lung does not elevate the plasma drug concentration and, consequently, drug toxicity is limited (Weers, 2015). A tobramycin dry powder inhaler (TOBI™ Podhaler™, Novartis) has been authorized for the use in CF patients. This tobramycin dry powder inhaler introduced a significant advantage for patient compliance and quality of life, compared to nebulization. This method has been demonstrated satisfactory for PA infection management (Geller et al., 2007), since the inhalation of 112 mg of tobramycin was clinically equivalent to 300 mg of drug administered by nebulization.

Abbreviations: CF, cystic fibrosis; ED, emitted dose; FPD, fine particle dose; FPF, fine particle fraction; MMAD, mass median aerodynamic diameter; PA, *Pseudomonas aeruginosa*; PIF, peak inspiratory flow; TobraPS, spray-dried tobramycin powder

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From the technological point of view, the large amount of powder (200 mg), carrying 112 mg of tobramycin, could not be loaded in a single hard capsule reservoir of the device and inhaled in one inhalation act. Consequently, in TOBI™ Podhaler, the powder amount was divided in four hard capsules size #2, each one loading ~50 mg of PulmoSphere™ powder (Galeva et al., 2013). The inhalation maneuvers, i.e., load one capsule into the inhaler, pierce it and inhale twice, have to be repeated four times to administer the entire dose. The therapy has to be done two times per day for 28 days. Despite the advantage for patient over nebulization, the administration procedure significantly affected the patient adherence to the therapy (Boerner et al., 2014).

These facts evidence that there is a need of novel powders and devices for better managing the lung administration of high dose antibiotics.

Regarding the devices, Twincer®, a disposable inhaler containing two air classifier technology, was developed with the aim to release up to 25 mg of unprocessed micronized particles or soft spherical agglomerates without special particle engineering processes (De Boer et al., 2006). Based on the same principle, Cyclops, a single classifier version of Twincer®, was developed to aerosolize up to 50 mg of pure spray-dried amorphous aminoglycosides (Hoppentocht et al., 2015). FPFs (at 34 L/min) of tobramycin, amikacin or kanamycin with the Cyclops ranged between 78–90%. The Orbital device is another single-use, disposable unit containing a “puck” that holds up to 400 mg of drug powder. A key innovative step in the Orbital design is the puck orifice that acts as the rate-limiting step for the amount of released powder. This system proved capable to efficiently release a fixed dose over a series of inhalation acts of both amorphous, crystalline and co-spray dried powder systems (Young et al., 2014a). These included 400 mg of mannitol, 100–400 mg doses of spray-dried ciprofloxacin/mannitol, 200 mg combination co-spray-dried azithromycin/mannitol (Young et al., 2014b), 200 mg tranexamic acid (Haghi et al., 2015) and 200 mg of micronized crystalline tobramycin (Zhu et al., 2016). Finally, the fluidized bed DPI is another novel multi-breath high dose DPI, which consists of a formulation reservoir (dosing sphere) that contains up to 100 mg of powder which is released during the inhalation act via 2–6 dosing holes into a fluidized device bed. Using this design, the FPF of co-spray-dried mannitol and ciprofloxacin reached 93% (Farkas et al., 2015).

On the formulation perspective, a novel tobramycin inhalation powder formulated with a lipophilic adjuvant has been described (Buttini et al., 2010). The tobramycin powder microparticles, constructed by spray drying, using a minimal amount of sodium stearate, exhibited a very small aerodynamic diameter, low density and favorable shape for aerosolization. Sodium stearate, a surface-active substance, molecularly coated the tobramycin microparticles, due to the preferential accumulation at the droplet air interface during drying. Microparticles with sodium stearate on their surface showed a great aerosol performance. Moreover, the excipient provided protection from the environmental humidity determining a superior stability (Parlati et al., 2009), as also recently confirmed (Yu et al., 2018).

The high drug content in tobramycin/sodium stearate microparticulate powder helps in capturing the patient adherence during PA infection management, if the formulation/device combination provides a patient-centric product. The driving hypothesis of this research was that the time for the daily antibiotic administration would be minimized by using a highly respirable and high drug content formulation, provided that a device is available capable to regulate the amount of powder emitted. Hence, the aim of this work was to study a new dry powder inhaler of tobramycin capable to simplify the dose administration maneuvers and to maximize the patient care in antibiotic inhalation therapy. The first objective was to identify the powder amount per capsule of the novel tobramycin inhalation powder equivalent to TOBI™ Podhaler™ dose of tobramycin; then, after selecting the device for aerosolization, to set down the most convenient technique of inhalation. Expected result could be that the number of capsules to inhale

and time required for the daily tobramycin dose administration in CF patients could be reduced. Thus, the *in vitro* and *in vivo* respirability of the product have been tested and correlated.

2. Materials and methods

2.1. Materials

Tobramycin base ($D_{v,0.5} 3.55 \pm 0.67 \mu\text{m}$) was supplied by Teva API B.V (Amsterdam, The Netherlands). Sodium stearate was European Pharmacopoeia grade. Mueller Hinton agar (Biokar Diagnostics, Allonne – Beauvais, France), Mueller Hinton broth (DIFCO, Sparks, USA), calcium chloride dihydrate and magnesium chloride hexahydrate (MERCK, Darmstadt, Germany) were purchased for drug activity test on PA. Water was purified by reverse osmosis (MilliQ, Millipore, Molsheim, France). All chemicals were of analytical grade (Sigma-Aldrich S.r.l., Milan, Italy). TOBI™ Podhaler T-326 inhaler medium resistance (Novartis AG, Basel, Switzerland; batch CP0037) was obtained from the local hospital pharmacy. The three RS01 dry powder inhalers used, namely a size #0 prototype medium resistance, a size #2 prototype low resistance and the commercial size #3 high resistance, were kindly donated by Plastiapa Spa (Osnago, LC, Italy). Hypromellose capsules for dry powder inhaler size #3 (Quali V-I) and size #2 and size #0 (Quali-V) were donated by Qualicaps (Madrid, Spain).

2.2. Tobramycin microparticle and agglomerate manufacturing

Tobramycin dissolved with 1% (w/w) of sodium stearate was spray-dried according to (Parlati et al., 2009). Briefly, 4.95 g of tobramycin was dissolved in 350 ml of purified water at 30 °C, whereas 0.05 g of sodium stearate was dissolved in 150 ml ethanol 95° at 30 °C. The two solutions were mixed and the final solution with a total solid content of 1% (w/v), kept at 30 °C, was spray-dried using a Buchi B-290 (Buchi, Flawil, Switzerland). The spray dryer conditions were: feed rate 3 ml min⁻¹, aspiration rate 100%, air flow rate 600 L h⁻¹, inlet and outlet temperatures 125 °C and 75–78 °C, respectively. The spray-dried powder, coded TobraPS, was used as it was after the production or transformed in soft agglomerates by mechanical vibration as previously described (Belotti et al., 2014). In detail, 5 g of powder were placed on a stack of two sieves of 600 and 106 μm size and vibrated using a sieve shaker for size analysis (Fritsch GmbH, Oberstein Deutschland) at the amplitude 3 for 5 min in a cabinet under nitrogen atmosphere. The agglomerates in the size range 106–610 μm were collected, stored in sealed glass vial at 25 °C-60% RH. The yield of the process was higher than 70%.

2.3. Powder characterization

2.3.1. Assay of tobramycin

The assay of tobramycin was performed by the USP37 HPLC method. System suitability was assessed according to USP37. The method precision (Relative Standard Deviation calculated following six injections of a 0.5 mg/mL standard solution) was 0.85% and the linearity was in the range from 0.1 to 1.5 mg/mL ($R^2 = 0.9945$). LOD and LOQ values were 0.02 mg/ml and 0.06 mg/ml, respectively.

2.3.2. Particle size distribution

Particle size distribution of TobraPS powders was measured using the laser light scattering technique (SprayTec, Malvern Instruments Ltd, Malvern, UK). Approximately 10 mg of the sample was dispersed in 20 ml of a 0.1% (w/v) Span 80 solution in cyclohexane and sonicated for 5 min. The measurement was performed in triplicate with an obscuration threshold of 10%. Data were expressed in terms of median volume diameter and percentiles, $D(v,0.1)$, $D(v,0.5)$, $D(v,0.9)$.

2.3.3. Loss on drying

The residual solvent in the powders was determined as loss on drying with thermo-gravimetric analysis. The instrument used was the Mettler Toledo Thermogravimetric Analyzer (Mettler Toledo, Switzerland). About 5 mg of powder was introduced in 70 μ l alumina pans with a pierced cover and analyzed from 25 °C to 250 °C at a heating rate of 10 °C/min under a nitrogen stream flowing at 80 ml/min. The weight loss was measured in the range between 25 and 125 °C.

2.3.4. Crystallinity

The study the crystal form of tobramycin powders was performed by X-ray Powder Diffraction analysis. The instrument employed was a MiniFlex X-Ray Diffractometer (Rigaku, Japan). About 200 mg of powder were loaded on the sample holder and then analyzed from a start angle of 2θ to an end angle of 35θ with 0.5θ steps.

2.3.5. Scanning electron microscopy

TobraPS powder morphology was assessed by Scanning Electron Microscopy (SEM) (JSM-6400, JEOL Ltd., Japan) at high magnifications (10,000x–30,000x) with a EHT of 1.60 kV. The samples were placed on a double-sided adhesive tape pre-mounted on an aluminium stub and analyzed after a 30 min depressurization.

2.3.6. Dissolution profile

The dissolution rate of tobramycin powders was measured using a Franz-type cell constituted by a donor compartment separated from receptor compartment by a disc of high pure filter paper (Albet LabScience 84 g/m²). The receptor compartment had a sampling port that allowed to withdraw the samples for tobramycin HPLC determination.

The Franz cell receptor was filled with 20 ml of degassed purified water by reverse osmosis and the filter paper disc was wet with 0.5 ml of the same water to create a thin film above it. The Franz cell was thermostated at 37 °C; a stir-bar rotating at 200 rpm was introduced in the receptor compartment and the absence of air bubbles under the membrane was verified. Approximately 10 mg of TobraPS powder accurately weighed, were distributed on the filter paper. In the case of TOBI™ Podhaler™ 10 mg of powder were sampled from the capsule content.

At time zero, through the sampling port, 2 ml of receptor solution were withdrawn with a syringe and analysed by HPLC. After each sampling, the cell was refilled with an equivalent volume of purified degassed water. The sampling was performed at prefixed time of 5, 10, 15 min. Each powder was tested in triplicate.

2.3.7. Activity of tobramycin formulation on *Pseudomonas aeruginosa* strain

Minimal Inhibitory Concentration (MIC) and the Minimal Bactericidal Concentration (MBC) against PA, reference strain ATCC27853, were determined. Antibiotic stock solutions were prepared by dissolving the powders in sterile deionized water at the concentration of 5.12 mg/ml. Stock solutions of antibiotic were diluted ten times with cation-adjusted Mueller Hinton broth to a final volume of 10 ml and then, on base two. Dilutions were 256, 128, 64, 32, 16, 8, 4, 2, 1 and 0.5 μ g/ml. 50 μ l of each dilution were then transferred into separate wells of a microtiter plate. Three to five colonies of PA grown on Mueller Hinton agar plates were inoculated into tubes containing 6 ml of cation-adjusted Mueller Hinton broth. Tubes were incubated at 37 °C on a shaker at 225 rpm for 4 h. The suspension containing approximately 108 CFU/ml was diluted 1:100 by adding 200 μ l thereof to 19.8 ml of cation-adjusted Mueller Hinton broth. Within 30 min, 0.05 ml of this suspension (106 CFU/ml) were inoculated for each well, so that the final concentration of bacteria was approximately 5×10^5 CFU/ml. To verify the correct final inoculum concentration, a 0.01 ml aliquot was removed from the growth control well immediately after inoculation and diluted in 10 ml of 0.9% sterile saline. After

mixing, 0.1 ml of this suspension was spread over Mueller Hinton agar supplemented with Ca²⁺ and Mg²⁺ and incubated overnight at 37 °C. The test was considered valid with a presence of approximately 50 colonies, indicating an inoculum density of 5×10^5 CFU/ml. Plates were read, after incubation at 37 °C for 18–24 h in moistened air, with the aid of a magnifying mirror. The test was considered valid when acceptable growth had occurred in the antibiotic-free growth control well and no contaminant growth was present in the medium sterility control well.

The MIC was defined as the lowest concentration of antibacterial agent at which there is no visible growth of PA ATCC 27853 after overnight incubation. The MBC was defined as the lowest concentration of antibacterial agent that reduced the viability of the initial bacterial inoculum by > 99.9%.

2.4. In vitro aerodynamic drug deposition

The dispensing and dispersion performance assessments were performed using the Next Generation Impactor (NGI) (Copley Scientific, Nottingham, UK). The methodology employed followed USP38 General Chapters, Physical tests and determinations for dry powder inhalers (Apparatus 5). The collection stages were coated with Span 85 in cyclohexane solution (1% w/v) to prevent particle bounce. Powder formulations were aerosolized by the inhaler(s) while attached to the NGI and the tobramycin retained in the capsule, inhaler, and impactor was collected using a H₂SO₄ 0.01N and assayed by HPLC. Furthermore, a fast screening impactor (FSI, Copley Scientific Ltd, Nottingham, UK) was used as an abbreviated impactor to assess the aerodynamic performance of different loaded dose of TobraPS powders. FSI comprises a Coarse Fraction Collector (CFC) that captures particles with an aerodynamic diameter larger than 5 μ m and a Fine Fraction Collector (FFC) that collects particles with an aerodynamic diameter smaller than 5 μ m.

TobraPS powder was studied in combination with the T-326 inhaler (Podhaler) and with different types of the RS01 device, able to load different sizes of HPMC capsules. The flow rate used during each test was adjusted with a Critical Flow Controller TPK (Copley Scientific, Nottingham, UK) in order to produce a pressure drop of 4 kPa across each inhaler used. The flow rate corresponding to these pressures were measured before each experiment (DFM 2000 Flow Meter, Copley Scientific, Nottingham, UK) and the test duration time was adjusted so that a volume of 4 L of air was drawn through each inhaler during each test. The capsules were manually filled with the test powder under nitrogen atmosphere (RH $10 \pm 5\%$, temperature 20 °C) using an analytical balance, precision 0.1 mg.

The DPI devices, capsule size, intrinsic resistance and flow rate adopted are reported in Table 1.

The tobramycin mass deposited inside the impactor and inhaler allows for the calculation of aerodynamic parameters. The Emitted Dose (ED) is the amount, quantified by HPLC, of drug leaving the device and entering the impactor (induction port, stages 1–7 and MOC). The mass median aerodynamic diameter (MMAD) was determined by plotting the cumulative percentage of mass less than the stated aerodynamic diameter for each stage on a probability scale versus aerodynamic diameter of the stage on a logarithmic scale. The Fine Particle Dose (FPD) is the mass of drug < 5 μ m calculated from log-probability plot and the Fine Particle Fraction (FPF) is the ratio of the FPD to the ED. Finally, the emitted amount of powder was checked as well by weighting the device before and after aerosolization.

2.5. In vivo testing for powder emission

The *in vivo* study was performed in 3 healthy volunteers (two male and one female; 72, 42 and 32 years old, respectively) in order to assess the number of inhalation acts required to inhale 120 mg of TobraPS powder loaded in a size #0 capsule and aerosolized by RS01 prototype device. The amount of powder extracted after each inhalation was also

Table 1
Device, capsule size and flow rates.

Formulation	Inhaler/capsule size	Resistance (kPa ^{0.5} /LPM)	Loaded powder /capsule	Powder capsule filling (%) [*]	Flow rate at 4 kPa (L/min)
TOBI™ Podhaler™	T-326 Inhaler size #2	0.025	50	–	78
TobraPS	T-326 Inhaler size #2	0.025	32	48	78
TobraPS	RS01 size #3	0.033	32	60	60
TobraPS	RS01 size #2	0.022	60	94	90
TobraPS	RS01 size #0	0.027	120	88	72

* Calculated as fraction of the capsule body volume.

measured. The work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and a signed informed consent was obtained by each volunteer after careful explanation of the purpose of the study and its execution.

Preliminarily, the capsule was inserted in the device but not pierced, and the volunteers were instructed to inhale twice the capsule with a deep single breath with an inspiratory flow sufficient to hear the capsule spinning. In this way, the subjects become confident with the inspiratory effort required to lift and rotate the capsule inside the device. The minimum air flow suitable for this was experimentally measured *in vitro* to be > 20 L/min. The actual capsule rotation was easy to verify by hearing the noise produced by its spinning.

After the training session, the volunteers were requested to inhale TobraPS powder. An apparatus suitable for the measurement of the air flow rate produced by the inhalation act during dose extraction was developed in-house (Fig. 1). It consisted of a 1.25 L chamber in which the RS01 was tightly inserted, leaving the mouthpiece out. The air to the chamber was supplied through a flow-meter (DFM Flow Meter, Copley Scientific, Nottingham, UK) connected to a PC computer for time and air flow data collection (LabVIEW version 10.0.1, National Instruments, US). Using this equipment, it was possible to measure the inhalation profile and determine the peak inspiratory flow (PIF) and the air volume inhaled. Then, the RS01 prototype device was loaded with a size #0 capsule containing 120 mg of TobraPS agglomerated corresponding to 112 mg of tobramycin. The capsule was pierced and the device was weighed before being introduced in the inhaler and sealed in the chamber connected to flow-meter, leaving the mouthpiece outside the chamber. After the first inhalation act, the amount of powder emitted was measured by weighing the device recovered from the

apparatus. Then, the system was re-assembled and the procedure was repeated until the capsule was emptied. The waiting time between two inhalation acts of the volunteers was not less than 1 min.

2.6. Statistical analysis

Statistical calculations were performed with the software KaleidaGraph (Synergy Software, U.S). For statistically significant differences, unpaired *t*-test analysis was performed. A significance level of 5% was accepted.

3. Results and discussion

3.1. Powder characterization

Using the lab scale spray dryer, several batches up to 10 g of tobramycin solution containing 1% (w/v) of sodium stearate were dried and the process yields were in the range 68–72%, similar to previously (Parlati et al., 2009). The powder recovered (TobraPS) from the spray dryer collector appeared as irregular large clusters of micronized particles; the drug content was of 91% (w/w). The microparticle 10%, 50% and 90% size distribution percentiles, measured as volume diameter (D_v) by laser diffraction, were $1.03 \pm 0.21 \mu\text{m}$, $2.40 \pm 0.36 \mu\text{m}$ and $5.29 \pm 0.91 \mu\text{m}$, respectively. The particle size distribution was log normal.

However, the scanning electron micrographs (Fig. 2) showed many particles larger than $5 \mu\text{m}$, spherical and with smooth surface. Apparently, the particles were hollow as many were inflated and exploded. The corresponding fragments were visible as flat flakes in the particle population. Curiously, frequently smaller particles were observed inside the cavity of the exploded larger ones. Together with large particles, there were small ones with less smooth surface; in general, these particles were grouped together or adherent to the largest ones or to their fragments.

This morphology was recently observed also in spray-dried amikacin powders for inhalation prepared in the same conditions and it was attributed to the amikacin solubility in the water-alcohol mixture used for spray drying. Since the solubility in alcohol of the aminoglycoside is lower than in water, during the drying of sprayed droplet, an early precipitation of the drug took place at droplet surface creating a crust that hindered the solvent evaporation. Thus, the increase of vapor pressure inflated the particle during drying causing its explosion, more frequently when alcohol was present in the drug solution (Belotti et al., 2014; 2015). Morphology and volume diameter of the tobramycin spray-dried microparticles contributed to the powder favorable characteristics for pulmonary deposition.

X-ray diffraction analysis of TobraPS powder evidenced the amorphous state of spray-dried tobramycin powder (see supplementary

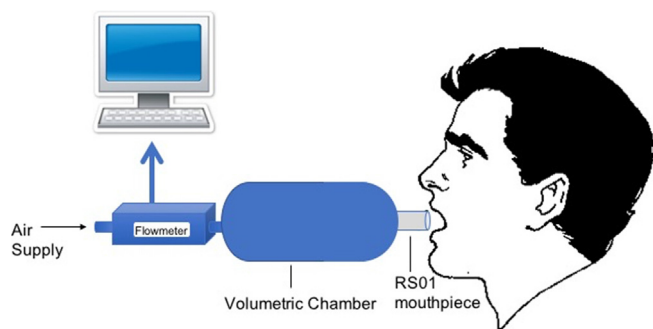


Fig. 1. In-house assembled system capable to register the inhalation profile during powder extraction by the volunteer. TobraPS powder was loaded in a capsule and inserted into the RS01 size #0 device, which was sealed inside a volumetric chamber leaving only the mouthpiece outside. When the volunteer was inhaling the supplied air passed through a flow-meter that recorded the air velocity and transferred the data to a software.

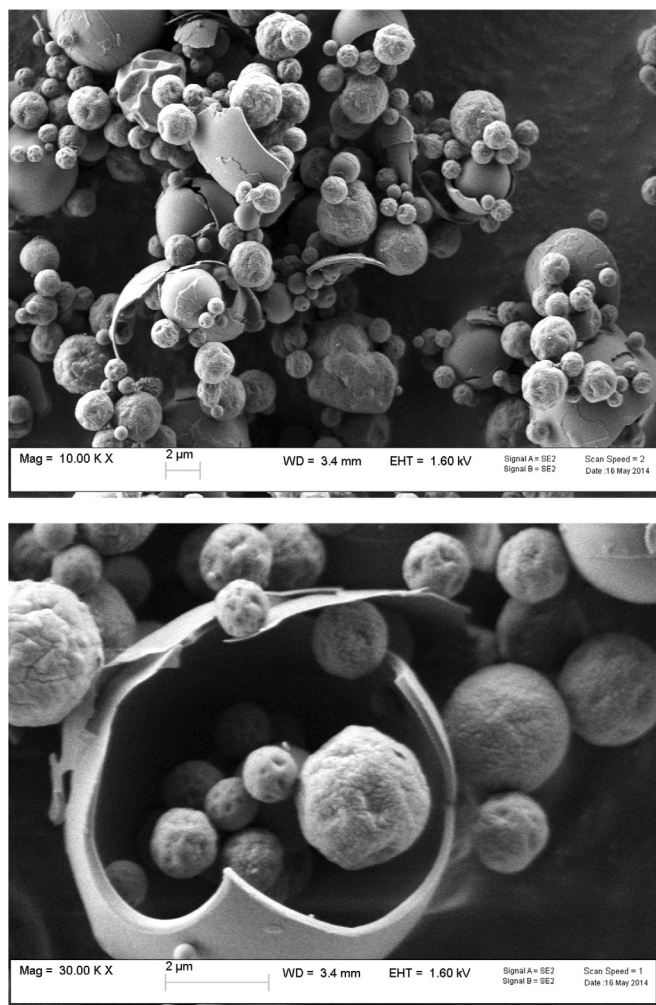


Fig. 2. Tobramycin spray-dried powder (TobraPS) morphology by scanning electron microscopy: (top) magnification 10,000 \times ; (bottom) 30,000 \times .

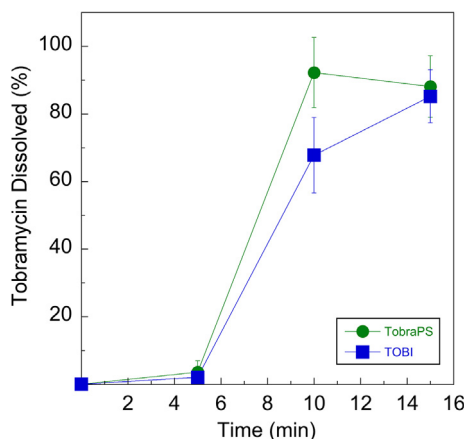


Fig. 3. Dissolution profiles of tobramycin from TobraPS and TOBI™ Podhaler™ powder (n = 3, mean \pm st.dev.).

data). TOBI™ Podhaler™ formulation was described to be in an amorphous state (Geller et al., 2011) and to be stable at low relative humidity storage conditions. TobraPS packed in a sealed glass vial showed good chemical stability as it remained unmodified at 25 °C for 9 months; the physical stability, in particular the aerodynamic performance, showed a significant decrease of 15% during this storage time,

hence requesting controlled air-tight storage conditions. The comparison of the powder DSC traces before and after the 9-month storage showed no signs of recrystallization (see supplementary data).

TobraPS powder batches presented an average loss of weight during TGA analysis of $7.93 \pm 1.35\%$, confirmed as water by Karl Fisher titration.

The administration of antibiotic solid particles requires a quick local availability of dissolved drug molecules for the pharmacological activity. For this purpose, the dissolution profiles of TobraPS powders, compared to the tobramycin formulation of TOBI™ Podhaler™, are shown in Fig. 3.

Both powders dissolved rapidly and more than 85% of drug was in solution in 15 min. However, in the first ten minutes, tobramycin spray-dried powders with sodium stearate reached almost complete dissolution (92.2%), whereas TOBI™ powder dissolution was slightly slower (67.8%). Moreover, in the case of TobraPS, all the powder was dissolved without any visible residue on the diffusion cell membrane where the powder was deposited. In contrast, the TOBI™ powder left a white solid residue at the end of the dissolution. Likely, some excipients from tobramycin Pulmosphere™ remained on the filter of the cell donor compartment. In summary, antibiotic availability at the lung deposition site from TobraPS powder is expected to be prompt and complete.

The microbiological activity of the TobraPS powder was assessed in an experiment in which the powder was dissolved before being put in contact with the microorganisms. The results demonstrated a microbiological activity not different from the non-processed raw material: the MIC value obtained for TobraPS (0.5 $\mu\text{g}/\text{ml}$) was consistent with EUCAST data (Leclercq et al., 2013) and were similar to tobramycin raw material and TOBI™ powder. MBC values were of 1 $\mu\text{g}/\text{ml}$ and similar as well for all the tested powder formulations (tobramycin raw material, TobraPS and TOBI™). Thus, the spray drying process did not affect the antimicrobial activity of tobramycin in the TobraPS formulation. It was reported that the maximum tobramycin concentrations in sputum 30 min after single-dose administration of TOBI™ (4 \times 28 mg tobramycin) were $1048 \pm 1080 \mu\text{g}/\text{g}$ (Geller et al., 2007). This exceeds, by several times, the MIC of tobramycin measured *in vitro* in the vast majority of isolates from infected CF patients (Konstan et al., 2010).

3.2. Aerodynamic *in vitro* deposition and dose definition

The aerodynamic behavior of TobraPS powder was compared to the reference product. The aerodynamic performance of TOBI™ Podhaler™ was investigated with its registered device at flow rate of 78 L/min, equivalent to a 4 kPa pressure drop. Each TOBI™ contained about 50 mg of powder corresponding to 28 mg of tobramycin. The respirable dose (FPD) of TOBI™, i.e., the amount of tobramycin having aerodynamic size lower than 5 μm , was 14.7 mg, corresponding to a FPF of 65.2%. (Table 2). For correctness of comparison, the aerosolization performance of TobraPS formulation was also studied using the T-326™ inhaler loading the same dose of 28 mg of tobramycin in a size #2 capsule. According to its drug content, 32 mg of TobraPS powder were weighed and aerosolized. Comparing the aerodynamic parameters (Table 2), no significant differences in FPD (p = 0.52) nor in MMAD (p = 0.65) were observed. Therefore, the two tobramycin formulations, aerosolized in the same conditions, had similar respirability parameters. However, it has to be noted that the similar deposition of tobramycin was obtained using significantly different mass of powders, since TOBI™ capsule contained 50 mg, whereas TobraPS was loaded with 32 mg of formulation, which is 36% less powder to inhale. In summary, the results demonstrated that using the same device, TobraPS formulation deposits a sensibly lower mass of powder in the lungs for giving a similar antibacterial activity. On the basis of the drug content of TOBI™ (60.6%) and TobraPS (91.1%), 39.4% of the emitted powder from TOBI™ powder were excipients, versus only 8.9% for TobraPS.

It is well known that the reduction of operation steps and maneuvers

Table 2

Aerodynamic parameters of TOBI™ and TobraPS powders using the Podhaler™ T-326 inhaler and capsule size #2 (mean ± st.dev.; n = 3). Each capsule contained 28 mg of tobramycin, but different excipients.

Formulation	Loaded Powder (mg)	Powder Emitted [#] (mg)	Emitted Dose (mg)	FPD (mg)	MMAD (μm)	FPF (%)
TOBI™ Podhaler™	≈ 50	46.7 ± 4.8	22.4 ± 0.7	14.7 ± 0.3	1.61 ± 0.20	65.2 ± 2.3
TobraPS	≈ 32	26.9 ± 4.7	20.6 ± 0.1	15.4 ± 1.9	1.56 ± 0.03	74.9 ± 8.8

[#] determined by weighing.

Table 3

Aerodynamic assessment of TobraPS using RS01 devices and capsules of different size (4 L of air through the device at flow rate corresponding to the pressure drop of 4 kPa).

Loaded TobraPS (as tobramycin) mg	Powder Emitted [#] (mg)	ED (mg)	FPD (mg)	MMAD (μm)	FPF (%)
Capsule size #3 32.0 (28.0)	27.8 ± 2.4	20.1 ± 1.9	16.5 ± 1.9	1.79 ± 0.17	81.8 ± 5.1
Capsule size #2 59.3 (56.0)	54.1 ± 1.2	41.8 ± 1.6	32.0 ± 0.1	1.85 ± 0.02	76.6 ± 3.1
Capsule size #0* 120.0 (112.0)	94.7 ± 0.7	75.4 ± 0.3	57.6 ± 1.6	1.82 ± 0.03	76.3 ± 1.8

* powder agglomerates;

[#] determined by weighing.

decreases the probability of serious therapeutic errors with DPI (Voshaar et al., 2014). Similarly, it was reported that patient adherence to the antibiotic therapy in cystic fibrosis PA management was negatively affected by dividing the dose to administer in fractions: only 52% of 288 patients enrolled in the study declared to inhale all 4 capsules (Boerner et al., 2014). Hence, with the goal to attempt a reduction of the number of capsules constituting the unit dose, TobraPS was loaded in incrementing doses into capsules of progressively bigger size and aerosolized using the appropriate RS01 device.

The set of experiments was started by testing the performance of TobraPS powder with the commercial high resistance (0.033 kPa^{0.5}/LPM) device RS01 using a capsule size #3, loaded with 32 mg of (corresponding to 28 mg of tobramycin, i.e., the same dose of TOBI™). Due to the TobraPS bulk density (0.17 g/ml), this capsule size could easily accommodate the mass of powder. In the RS01 device, the capsule, pierced in correspondence of the head and body extremities, rotated along the main axis inside the device due to the inhalation air flow rate, thus centrifuging out the powder. Conversely, the medium resistance T-326 inhaler (0.025 kPa^{0.5}/LPM) has the same powder emission mechanism of Turbospin® device (PH&T, Milan, Italy), i.e., the capsule is pierced at the bottom, rattles and swirls for powder emission (Martinelli et al., 2015). Thus, Table 3 shows the performance of 32 mg of TobraPS powder aerosolized using the RS01 device with capsule size #3. Again, for comparative purposes, TOBI™ Pulmosphere™ (32 mg) was tested using this device/capsule combination and, as previously demonstrated using the T-326 inhaler, no statistical difference could be assigned to the aerodynamic performance of the two devices and products (data not shown). However, it has to be stressed again that, in the case of the TobraPS, a consistently lower amount of powder has to be aerosolized to deliver the same dose as TOBI™, due to the high drug content.

Aiming at reducing the number of capsules, the amount of powder corresponding to the entire dose (112 mg) had to be increased in the capsule. Capsules #3 could not accommodate an amount of TobraPS powder larger than 40 mg without compacting the powder, which would affect the aerodynamic performance. Hence, the switch to the use of larger capsule was a forced choice. Capsule #2 and #0 were selected and the content aerosolized using novel RS01 inhalers, having

the same emission mechanism, but capable of accommodating the larger capsules.

Thus, 56 mg of formulation, that would correspond to the entire dose divided in two capsules, were loaded in capsule #2. The results of the aerodynamic assessment of this capsule are illustrated in Table 3. The fine particle dose obtained after inhalation of 56 mg tobramycin was approximately doubled and equal to 32.0 mg. These results could allow to propose the halving the entire dose from four to two capsules to be inhaled by the patients.

The therapy could reach a more relevant benefit in term of convenience and adherence for the patient if the dose could be administered employing only one capsule for the total dose delivery. Therefore, a RS01 device capable to accommodate a capsule size 0 or 00 was tested for delivery of high payload formulations (Parumasivam et al., 2017). A similar prototype with a resistance of 0.027 kPa^{0.5}/LPM was employed in this work in order to administer 112 mg of tobramycin (as 120 mg of TobraPS), the equivalent of 4 capsules containing 28 mg each.

It was not possible to load the entire dose of microparticles in one capsule size #0, unless an agglomeration process was applied to increase the powder bulk density. Agglomerates are soft pellets in which the microparticles are held together by weak interaction forces (Russo et al., 2004); they can be destroyed inside the device by the air turbulence produced by patient inhalation. This technology enabled to introduce 120 mg of formulation inside the capsule #0. The agglomerates or pellets are free flowing and the homogeneity of the powder was substantially increased, thus facilitating the loading of the capsule to inhale.

In detail, approximately 120 mg of agglomerated TobraPS were loaded in the capsule #0 and the entire content was aerosolized in one shot of 4 L of air at pressure drop of 4 kPa. The FPD value obtained was 57.6 ± 1.6 mg (Table 3). This value was not far away from the FPD accumulated from four capsules containing 28 mg of TobraPS each aerosolized with RS01 size #3 that was 66 mg (16.5 mg × 4). More interestingly, the value was similar to TOBI™ Podhaler™ FPD that, following the emission of four capsules, was 58.8 mg. The FPD from one capsule with RS01 size #0 corresponded to 97.8% of the expected dose from four capsules with Podhaler™.

The inclusive comparison of the fine particle dose of the aerosols

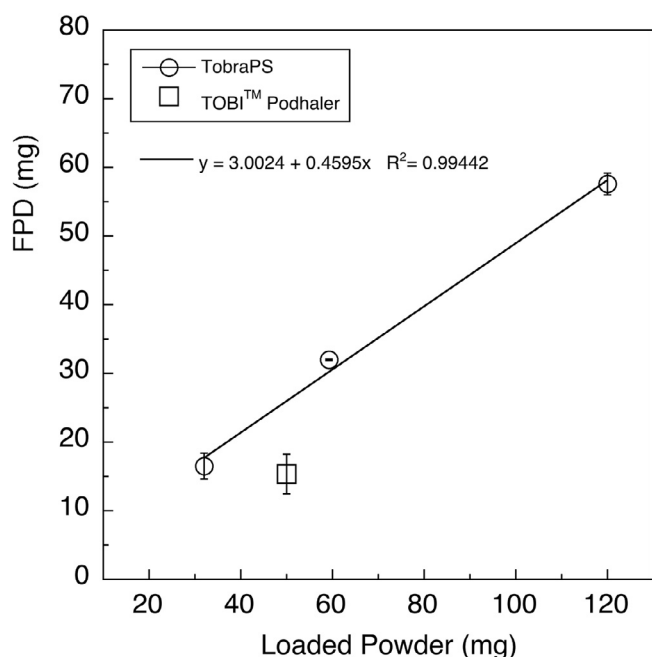


Fig. 4. Fine particle dose versus the amount of loaded powder in the capsule of tobramycin DPIs. TobraPS was aerosolized by RS01 devices and TOBI™ by Podhaler device (n = 3, mean ± st.dev.).

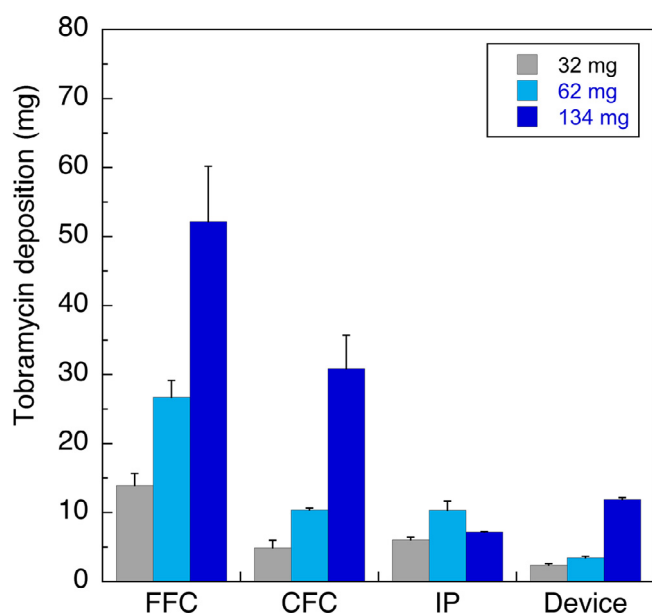


Fig. 5. Fast Screening Impactor deposition of three different doses of TobraPS using RS01 devices (IP = induction port; CFC = coarse fraction collector; FFC = fine fraction collector), (n = 3, mean ± st.dev.).

produced with 28, 56 or 112 mg of tobramycin in TobraPS powder and agglomerates with the RS01 device using capsules size #3, #2 or #0 (Table 3) shows that the FPF at the same 4 kPa pressure drop slightly decreased by increasing the amount of drug in the capsule. The FPD of TobraPS aerosolized with the RS01 devices was plotted versus the different amounts of powder and compared with the FPD of TOBI™ Podhaler™ (Fig. 4). There is a quasi linear relationship ($R^2 = 0.99442$) between the amount loaded in RS01 devices and the FPD. The “position” in the graph of TOBI™ Podhaler™ illustrates the difference existing between this formulation and TobraPS.

The comparison among the three different doses has been tested also by using the Fast Screening Impactor that could allow to determine

the amount of drug remaining in the device, in the IP, emitted as coarse fraction and as fine fraction (less than 5 µm). It was confirmed that the fine particles approximately doubled by doubling the dose, as well as the coarse fraction. The amount of formulation remaining in the device was also correlated with the amount loaded, whereas it was not for the amount deposited in the IP (Fig. 5).

3.3. In vivo powder emission after multiple inhalation acts

TobraPS cumulated in one capsule size #0 could be extracted by the device *in vitro* with one single actuation at 72 L/min per 3.3 s. However, *in vivo* this amount of powder should be delivered to the patient in successive inhalation acts. Recently, the antibiotic colistimethate sodium powder for inhalation at the dose of 125 mg was introduced in a single capsule in order to be extracted by the user in 6–7 successive inhalation acts (Tappenden et al., 2013). The tobramycin deposition data reported here until now have been collected *in vitro*. *In vivo*, with the high tobramycin dose, the extraction of all the powder in one shot would be unfeasible and unsafe. A gradual emission could be done by performing successive inhalation acts through the same loaded RS01 device. Therefore, for the *in vivo* test measurements, the air flow profiles of three healthy volunteers during powder inhalation were recorded, using the device RS01 capsule size #0 loaded with 120 mg of TobraPS. These obtained profiles of healthy volunteers were paralleled to the amount of drug extracted or emitted and could be compared to those of CF patient profiles published by Haynes (Haynes et al., 2016).

Fig. 6 illustrates the air flow rate versus time plots of four inhalation acts successively performed by the three volunteers during powder inhalation. The volunteers repeated the test more times. The flow rate variability of inhalation through the loaded DPI can be appreciated both intra- and inter-individually among the volunteers. In general, the PIF value of the inhalation through the device was in the range of 0.4–1.4 L/s, over a time interval of 1–4 s. Two volunteers were able to perform successive inhalation acts giving consistent air flow profiles. One volunteer inhaled slowly for a longer time, still maintaining a flow rate (> 20 L/min) capable to lift and rotate the capsule. In comparison with the *in vivo* profiles of CF patient (Haynes et al., 2016), these data and, in particular, the PIF values are slightly lower. However, the aim of the *in vivo* test was to relate the amount of powder extracted with the PIF and the total inhaled volume values provided by the volunteers.

Using the set-up described in the experimental section, the measurement of the amount of powder emitted during the volunteer inhalation together with the air flow profile was performed. All the replicated experiments are reproduced in Fig. 7 versus the inhalation act number. Volunteer 1, constantly performing the inhalation acts (Fig. 7, blue symbols), was able to extract the drug dose in three-four acts. Volunteer 2 employed four acts (Fig. 6, red symbols), whereas volunteer 3 (Fig. 7, green symbols), inhaling more slowly due to personal apprehension, required from five to six inhalations in order to extract the entire dose. No evident discomfort was felt by the three volunteers enrolled in the test.

In order to identify the determinant of the dose emission in correspondence on each inhalation act, the emitted dose after inhalation from the different volunteers and successive replicas were plotted versus the PIF value of the inhalation profile (Fig. 8A). For assuring the comparison in the same conditions, only the first inhalation act of each volunteer was considered. There was a quite expected dependence of the emitted amount from PIF, albeit the significance of the linear relationship was low ($R^2 = 0.36857$). Despite the scattering of the values, this result underlines the relevance of PIF for the dose extraction. The PIF initiated the rotation of the capsule in RS01 device, but PIF could not be the only determinant for powder emission from device. The flow profile during powder emission allows for the calculation of the area under the curve, i.e., the volume of air passed through the device during the inhalation act. The measured volumes were plotted versus the emitted dose (Fig. 8B). This plot shows a more significant linear

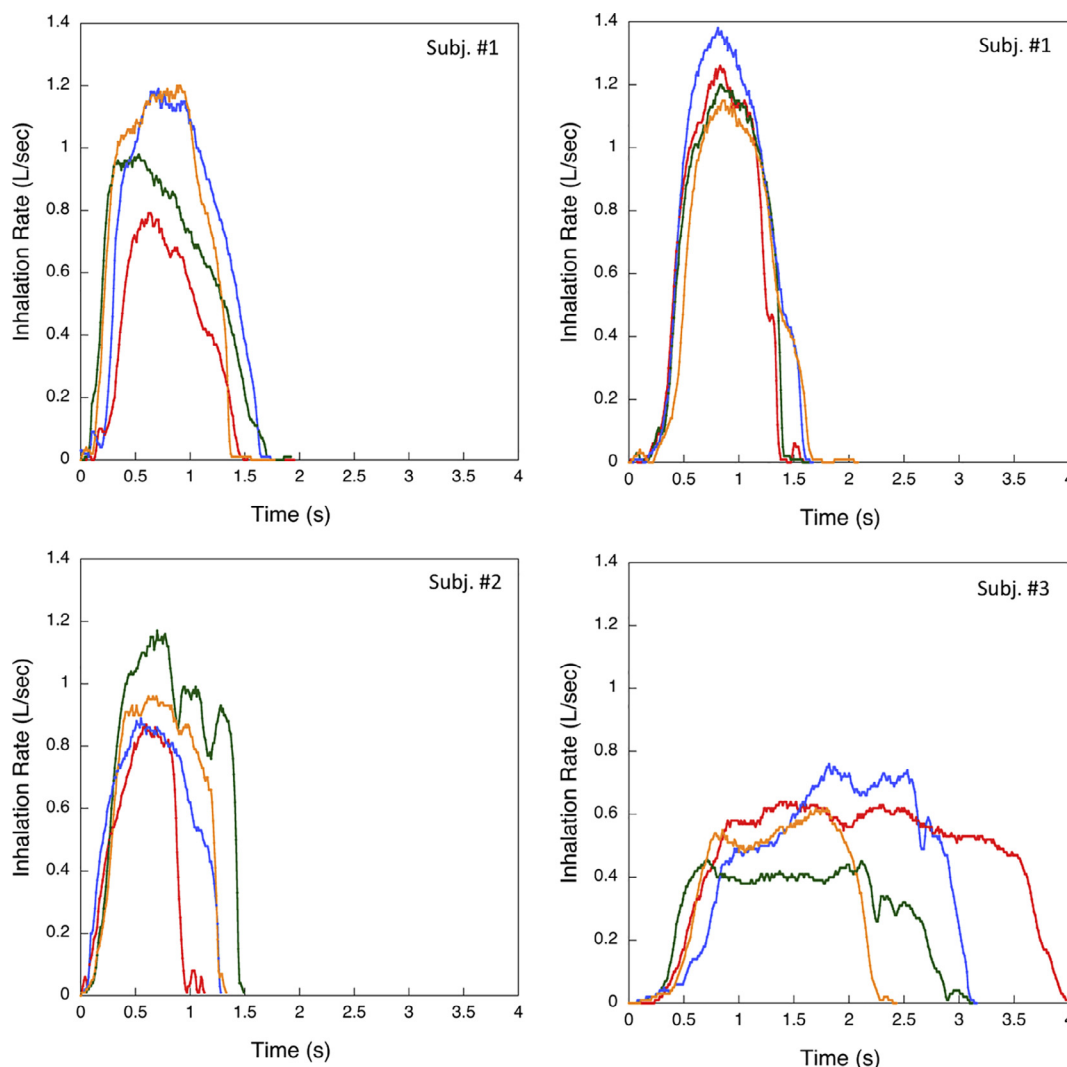


Fig. 6. Air flow profiles of four dynamic inhalation acts successively performed by the three volunteers during TobraPS powder emission. Inhalation sequence progression: 1 red, 2 green, 3 blue, 4 yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

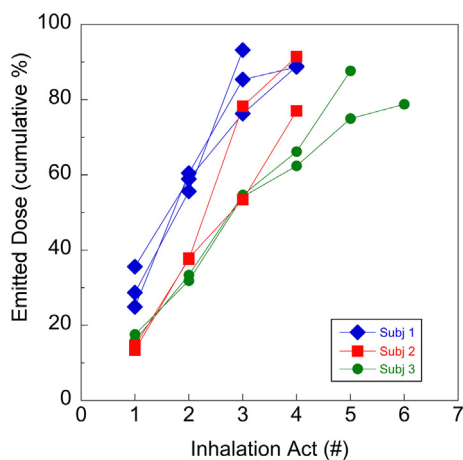


Fig. 7. Cumulative percent of TobraPS dose extracted *in vivo* by three human volunteers in successive inhalation acts. Capsule size #0 filled with 120 mg of powder delivered by RS01 prototype inhaler.

relationship between the powder amount emitted and the volume of air passed through the device, at PIF values higher than 20 L/min. This aspect, noticed by other authors (Sosnowski, 2018; Weers and Clark, 2017), supports the importance of the air volume for the quantitative emission of powder. In fact, the linearity of the relationship between powder emitted at first inhalation versus air volume inhaled, supported the relevant role of air volume, together with the PIF presented.

In summary, using the RS01 device, which has a mechanism of powder emission dictated by the rotation of the pierced capsule in a spinning chamber, the air flow through the device guarantees the lift of the capsule and its rotation at threshold values of flow rate higher than 20 L/min. Increasing the PIF value, the amount emitted increased, but the relationship was less predictable. There was a stronger correlation between the amount emitted and the volume of air passed through the device. This meant that the device emission was not only dependent on the air PIF, but the dose extracted at low PIF depended also by the duration of the inhalation act. Repetition of the inhalation act on the same capsule made more reliable the amount of drug inhaled.

Finally, the amount of powder emitted *in vivo* was compared to the

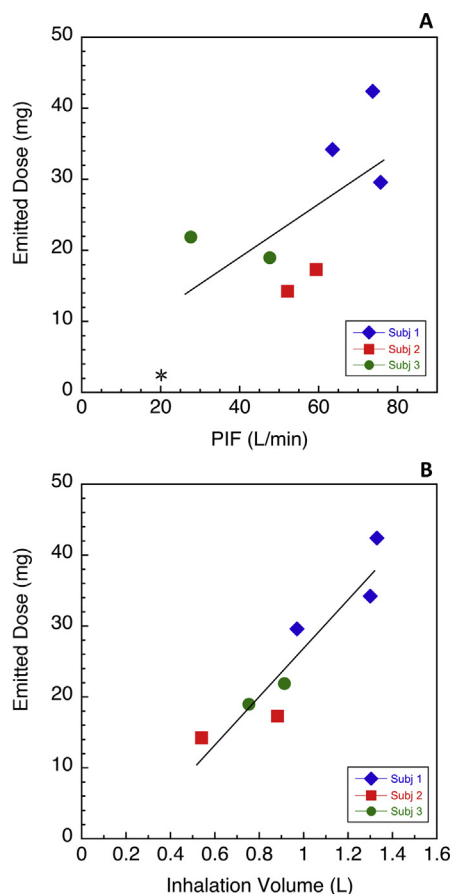


Fig. 8. Relationship between the emitted dose with the first inhalation act and (A) the inspiratory flow (PIF), ($y = 4.4012 + 0.37116x$; $R^2 = 0.36857$) and (B) the inhalation volume, ($y = -6.4252 + 33.514x$; $R^2 = 0.86771$). The star represents the threshold air flow value capable to lift and rotate the capsule.

amount of powder emitted *in vitro* at a flux corresponding to three established pressure drops. The plot shows as well the *in vitro* emitted powder when the inhaler was activated at pressure drop values lower than 4 kPa, i.e., 3.3 and 2 kPa. In these cases, for all the air volumes passed through the device, the *in vitro* emitted powder was lower than the one measured at 4 kPa. The powder amounts extracted at first inhalation act by the three volunteers correlate with the *in vitro* emitted dose values obtained at similar air volume for 4 kPa pressure drop (Fig. 9A). This relationship is limited at a volume of air between 0.5 and 1.5 L, that is the range of air volumes measured in the three volunteers. The *in vitro* values of emitted powder were obtained at constant flow, whereas *in vivo* the air flow was dynamic increasing to PIF and decreasing. This *in vivo-in vitro* relationship indicates that the dynamic inhalation flow achieved *in vivo* through the RS01 DPI produces the emission of powder amount similar to the 4 kPa constant flow at the same volume of air.

Fig. 9B shows the correlation between the *in vitro* emitted powder at different air volumes, obtained at 4 kPa pressure drop, and the amount of powder emitted on the first and all successive inhalation acts by the three volunteers. The *in vivo* emitted powder is linearly correlated with the volume of air used for emission. The straight line describing this relationship, despite the variability of the *in vivo* data, is not significantly different from the straight line describing the relationship between the *in vitro* emission and the volume of air used for extracting the powder from device. Performing the *in vitro* measurements at 4 kPa pressure drop, passing the same air volume through the device, provides a close emulation of the *in vivo* behaviour only in quantitative terms. However, this *in vivo-in vitro* relationship could allow to assess *in*

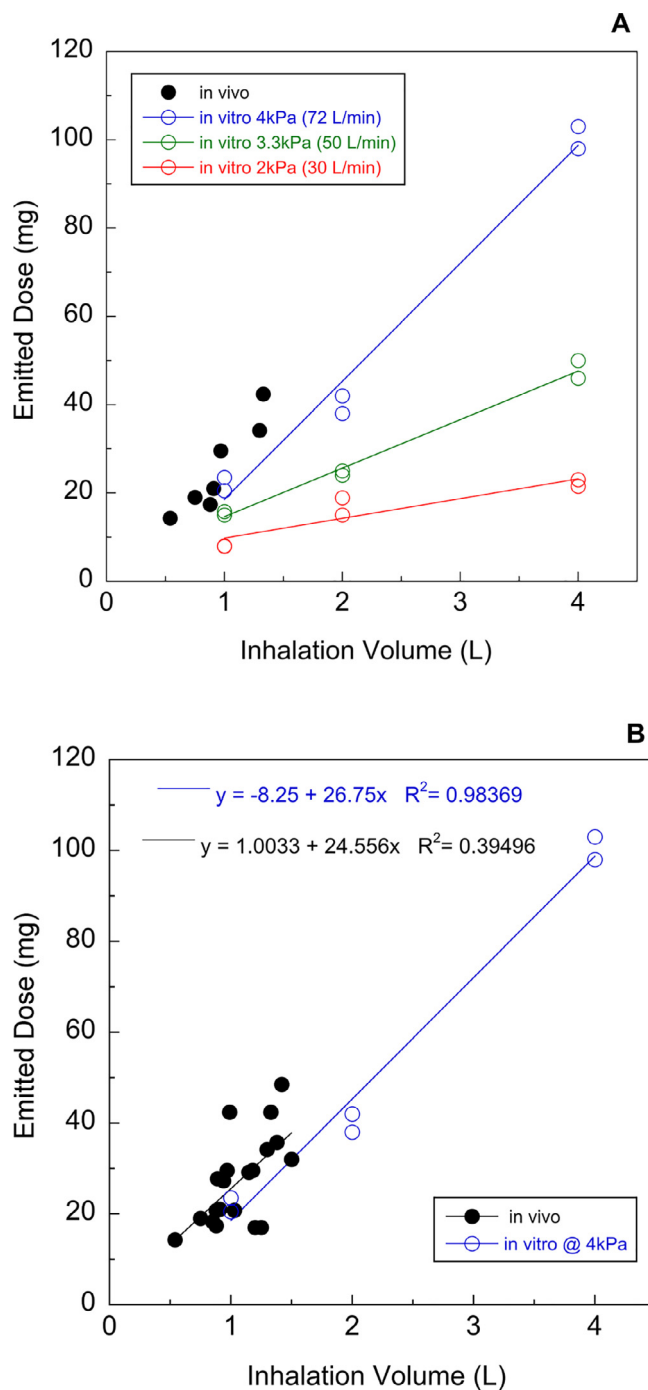


Fig. 9. Emitted dose of TobraPS vs. air inhalation volume. Data *in vivo* (full symbol) and *in vitro* (open symbol) by activation of RS01 size 0 at different pressure drops (4, 3.3 and 2 kPa). (A) first *in vivo* inhalation act and (B) all *in vivo* inhalation acts versus air volumes.

vitro the qualitative performance of the powder in terms of respirable dose during successive inhalation acts.

4. Conclusions

The increase of tobramycin content in TobraPS inhalation dry powder contributed to a significant reduction of the mass of powder entering the lung of the patient for administering the prescribed dose of tobramycin. In the TobraPS tobramycin strength 112 mg/capsule, the amount of formulation to inhale is around 120 mg, approximately 40%

less than the reference approved product (TOBI™ Podhaler™).

The RS01 emission mechanism, based on the spinning of the reservoir capsule, demonstrated to be able to control the amount of powder emitted during the inhalation act. The number of acts to perform in order to inhale the entire high dose of powder is dependent on the capability of the patient to inhale. A minimum air flow rate value of 20 L/min through the inhaler was requested to make the capsule lifting and rotating. The amount of powder emitted at 4 kPa pressure drop, at constant air flow rate, well correlated with the *in vivo* emission in dynamic flow conditions, when the same volume of air passed through the device.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ijpharm.2018.06.006>.

References

- Belotti, S., Rossi, A., Colombo, P., Bettini, R., Rekkas, D., Politis, S., Colombo, G., Balducci, A.G., Buttini, F., 2014. Spray dried amikacin powder for inhalation in cystic fibrosis patients: a quality by design approach for product construction. *Int. J. Pharm.* 471, 507–515. <http://dx.doi.org/10.1016/j.ijpharm.2014.05.055>.
- Belotti, S., Rossi, A., Colombo, P., Bettini, R., Rekkas, D., Politis, S., Colombo, G., Balducci, A.G., Buttini, F., 2015. Spray-dried amikacin sulphate powder for inhalation in cystic fibrosis patients: the role of ethanol in particle formation. *Eur. J. Pharm. Biopharm.* 93, 165–172. <http://dx.doi.org/10.1016/j.ejpb.2015.03.023>.
- Boerner, G., Prante, S., Borella, F., 2014. Some cystic fibrosis patients do not tolerate tobramycin. Abstract of the 37th European Cystic Fibrosis Conference. *J. Cyst. Fibros.* 13 (Suppl. 2), S121.
- Buttini, F., Colombo, P., Parlati, C., 2010. A drug powder for inhalation administration and a process thereof. WO2010003465A3.
- Buttini, F., Rossi, I., Di Cuia, M., Rossi, A., Colombo, G., Elviri, L., Sonvico, F., Balducci, A.G., 2016. Combinations of colistin solutions and nebulisers for lung infection management in cystic fibrosis patients. *Int. J. Pharm.* 502, 242–248. <http://dx.doi.org/10.1016/j.ijpharm.2016.02.005>.
- Cheer, S.M., Waugh, J., Noble, S., 2003. Inhaled tobramycin (TOBI): a review of its use in the management of *Pseudomonas aeruginosa* infections in patients with cystic fibrosis. *Drugs* 63, 2501–2520.
- De Boer, A.H., Hagedoorn, P., Westerman, E.M., Le Brun, P.P.H., Heijerman, H.G.M., Frijlink, H.W., 2006. Design and in vitro performance testing of multiple air classifier technology in a new disposable inhaler concept (Twincer) for high powder doses. *Eur. J. Pharm. Sci.* 28, 171–178. <http://dx.doi.org/10.1016/j.ejps.2005.11.013>.
- Doring, G., Conway, S.P., Heijerman, H.G., Hodson, M.E., Høiby, N., Smyth, A., Touw, D.J., 2000. Antibiotic therapy against *Pseudomonas aeruginosa* in cystic fibrosis: a European consensus. *Eur. Respir. J.* 16, 749–767.
- Farkas, D.R., Hindle, M., Longest, P.W., 2015. Characterization of a new high-dose dry powder inhaler (DPI) based on a fluidized bed design. *Ann. Biomed. Eng.* 43, 2804–2815. <http://dx.doi.org/10.1007/s10439-015-1335-2>.
- Flume, P.A., O'Sullivan, B.P., Robinson, K.A., Goss, C.H., Mogayz Jr., P.J., Willey-Courand, D.B., Bujan, J., FINDER, J., Lester, M., Quittell, L., Rosenblatt, R., Vender, R.L., Hazle, L., Sabadosa, K., Marshall, B., 2007. Cystic fibrosis pulmonary guidelines. *Am. J. Respir. Crit. Care Med.* 176, 957–969. <http://dx.doi.org/10.1164/rccm.200705-664OC>.
- Galeva, I., Konstan, M.W., Higgins, M., Angyalosi, G., Brockhaus, F., Piggott, S., Thomas, K., Chuchalin, A.G., 2013. Tobramycin inhalation powder manufactured by improved process in cystic fibrosis: the randomized EDIT trial. *Curr. Med. Res. Opin.* 29, 947–956. <http://dx.doi.org/10.1185/03007995.2013.805122>.
- Geller, D.E., Konstan, M.W., Smith, J., Noonberg, S.B., Conrad, C., 2007. Novel tobramycin inhalation powder in cystic fibrosis subjects: pharmacokinetics and safety. *Pediatr. Pulmonol.* 42, 307–313. <http://dx.doi.org/10.1002/ppul.20594>.
- Geller, D.E., Weers, J., Heuerding, S., 2011. Development of an inhaled dry-powder formulation of tobramycin using PulmoSphere™ technology. *J. Aerosol. Med. Pulm. Drug Deliv.* 24, 175–182. <http://dx.doi.org/10.1089/jamp.2010.0855>.
- Haghi, M., van den Oetelaar, W., Moir, L.M., Zhu, B., Phillips, G., Crapper, J., Young, P.M., Traini, D., 2015. Inhalable tranexamic acid for haemoptysis treatment. *Eur. J. Pharm. Biopharm.* 93, 311–319. <http://dx.doi.org/10.1016/j.ejpb.2015.04.015>.
- Haynes, A., Geller, D., Weers, J., Ament, B., Pavkov, R., Malcolmson, R., Debonnett, L., Mastoridis, P., Yadao, A., Heuerding, S., 2016. Inhalation of tobramycin using simulated cystic fibrosis patient profiles. *Pediatr. Pulmonol.* 51, 1159–1167. <http://dx.doi.org/10.1002/ppul.23451>.
- Hewer, S.L., 2012. Inhaled antibiotics in cystic fibrosis: what's new? *JRSM* 105, S19–S24. <http://dx.doi.org/10.1258/jrsm.2012.12s004>.
- Hoppentocht, M., Akkerman, O.W., Hagedoorn, P., Frijlink, H.W., De Boer, A.H., 2015. The Cyclops for pulmonary delivery of aminoglycosides; a new member of the Twincer™ family. *Eur. J. Pharm. Biopharm.* 90, 8–15. <http://dx.doi.org/10.1016/j.ejpb.2015.01.012>.
- Konstan, M.W., Geller, D.E., Minić, P., Brockhaus, F., Zhang, J., Angyalosi, G., 2010. Tobramycin inhalation powder for *P. aeruginosa* infection in cystic fibrosis: the EVOLVE trial. *Pediatr. Pulmonol.* 46, 230–238. <http://dx.doi.org/10.1002/ppul.21356>.
- Leclercq, R., Cantón, R., Brown, D.F.J., Giske, C.G., Heisig, P., MacGowan, A.P., Mouton, J.W., Nordmann, P., Rodloff, A.C., Rossolini, G.M., Soussy, C.-J., Steinbakk, M., Winstanley, T.G., Kahlmeter, G., 2013. EUCAST expert rules in antimicrobial susceptibility testing. *Clin. Microbiol. Infect.* 19, 141–160. <http://dx.doi.org/10.1111/j.1469-0691.2011.03703.x>.
- Martinelli, F., Balducci, A.G., Rossi, A., Sonvico, F., Colombo, P., Buttini, F., 2015. “Pierce and inhale” design in capsule based dry powder inhalers: effect of capsule piercing and motion on aerodynamic performance of drugs. *Int. J. Pharm.* 487, 197–204. <http://dx.doi.org/10.1016/j.ijpharm.2015.04.003>.
- Moskowitz, S.M., Gibson, R.L., Effmann, E.L., 2005. Cystic fibrosis lung disease: genetic influences, microbial interactions, and radiological assessment. *Pediatric Radiol.* 35, 739–757. <http://dx.doi.org/10.1007/s00247-005-1445-3>.
- Parlati, C., Colombo, P., Buttini, F., Young, P.M., Adi, H., Ammit, A.J., Traini, D., 2009. Pulmonary spray dried powders of tobramycin containing sodium stearate to improve aerosolization efficiency. *Pharm. Res.* 26, 1084–1092. <http://dx.doi.org/10.1007/s11095-009-9825-2>.
- Parumasivam, T., Leung, S.S.Y., Tang, P., Mauro, C., Britton, W., Chan, H.-K., 2017. The delivery of high-dose dry powder antibiotics by a low-cost generic inhaler. *AAPS J.* 19, 191–202. <http://dx.doi.org/10.1208/s12248-016-9988-9>.
- Ratjen, F., Brockhaus, F., Angyalosi, G., 2009. Aminoglycoside therapy against *Pseudomonas aeruginosa* in cystic fibrosis: a review. *J. Cyst. Fibrosis* 8, 361–369. <http://dx.doi.org/10.1016/j.jcf.2009.08.004>.
- Russo, P., Buttini, F., Sonvico, F., Bettini, R., Massimo, G., Sacchetti, C., Colombo, P., Santi, P., 2004. Chimera agglomerates of microparticles for the administration of caffeine nasal powders. *J. Drug Deliv. Sci. Technol.* 14, 449–454. [http://dx.doi.org/10.1016/S1773-2247\(04\)50083-7](http://dx.doi.org/10.1016/S1773-2247(04)50083-7).
- Sheppard, M.N., Nicholson, A.G., 2002. The pathology of cystic fibrosis. *Curr. Diagn. Pathol.* 8, 50–59. <http://dx.doi.org/10.1054/cdip.2001.0088>.
- Sosnowski, T.R., 2018. Powder particles and technologies for medicine delivery to the respiratory system: challenges and opportunities. *KONA* 35, 122–138. <http://dx.doi.org/10.14356/kona.2018020>.
- Tappenden, P., Harnan, S., Uttley, L., Mildred, M., Carroll, C., Cantrell, A., 2013. Colistimethate sodium powder and tobramycin powder for inhalation for the treatment of chronic *Pseudomonas aeruginosa* lung infection in cystic fibrosis: systematic review and economic model. *Health technology assessment (Winchester, England)* 17, v–181. <http://dx.doi.org/10.3310/hta17560>.
- Voshaar, T., Spinola, M., Linnane, P., Campanini, A., Lock, D., Lafratta, A., Scuri, M., Ronca, B., Melani, A.S., 2014. Comparing usability of NEXThaler® with other inhaled corticosteroid/long-acting β₂-agonist fixed combination dry powder inhalers in asthma patients. *J. Aerosol. Med. Pulm. Drug Deliv.* 27, 363–370. <http://dx.doi.org/10.1089/jamp.2013.1086>.
- Weers, J., 2015. Advanced drug delivery reviews. *Adv. Drug Deliv. Rev.* 85, 24–43. <http://dx.doi.org/10.1016/j.addr.2014.08.013>.
- Weers, J., Clark, A., 2017. The impact of inspiratory flow rate on drug delivery to the lungs with dry powder inhalers. *Pharm. Res.* 34, 507–528. <http://dx.doi.org/10.1007/s11095-016-2050-x>.
- Young, P.M., Crapper, J., Phillips, G., Sharma, K., Chan, H.-K., Traini, D., 2014a. Overcoming dose limitations using the orbital™ multi-breath dry powder inhaler. *J. Aerosol. Med. Pulm. Drug Deliv.* 27, 138–147. <http://dx.doi.org/10.1089/jamp.2013.1080>.
- Young, P.M., Salama, R.O., Zhu, B., Phillips, G., Crapper, J., Chan, H.-K., Traini, D., 2014b. Multi-breath dry powder inhaler for delivery of cohesive powders in the treatment of bronchiectasis. *Drug Dev. Ind. Pharm.* 41 (5), 859–865. <http://dx.doi.org/10.3109/03639045.2014.909841>.
- Yu, J., Romeo, M.-C., Cavallaro, A.A., Chan, H.-K., 2018. Protective effect of sodium stearate on the moisture-induced deterioration of hygroscopic spray-dried powders. *Int. J. Pharm.* 541, 11–18. <http://dx.doi.org/10.1016/j.ijpharm.2018.02.018>.
- Zhu, B., Padroni, M., Colombo, G., Phillips, G., Crapper, J., Young, P.M., Traini, D., 2016. The development of a single-use, capsule-free multi-breath tobramycin dry powder inhaler for the treatment of cystic fibrosis. *Int. J. Pharm.* 514, 392–398. <http://dx.doi.org/10.1016/j.ijpharm.2016.04.009>.