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Research paper

The Cyclops for pulmonary delivery of aminoglycosides; a new member of the Twincer™ family

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ABSTRACT

Patients infected with pathogenic bacteria have to be treated with antibiotics. When the infection is in the lungs, as for instance in cystic fibrosis, bronchiectasis and tuberculosis, inhaled antibiotics have certain advantages over systemically administered antibiotics. In this study, it is shown that re-designing the Twincer™ high dose disposable inhaler into a device named Cyclops enables effective dispersion of up to 50 mg of pure spray dried tobramycin. This proves that spray dried tobramycin powders in the preferred size range for inhalation can be administered without applying complex particle engineering techniques and/or using excipients. Only some coarse sweeper crystals added separately are desired to minimise the inhaler losses to less than 20% at 4 kPa. The fine particle fractions <5 μm of the aerosol obtained from the Cyclops closely resemble the primary particle size distribution of the spray dried tobramycin powder. Moreover, without any further optimisation the Cyclops performs good with other spray dried aminoglycosides such as kanamycin and amikacin too. Therefore, the results of this study show that with an appropriate inhaler design, adapted to the physico-chemical properties of a particular drug or drug class, excellent dispersion can be achieved for high doses of pure (spray dried) drug.

1. Introduction

Patients infected with pathogenic bacteria have to be treated with antibiotics to control the disease or eradicate the infection. When the infection manifests primarily in the lungs, as in tuberculosis (TB), the respiratory airways comprise the main target area for the antibiotic drug(s) [1–3]. Particularly patients with cystic fibrosis (CF) and non-CF bronchiectasis have a high risk of respiratory infections with for instance *Pseudomonas aeruginosa* (*Psa*), and once chronically infected with *Psa* they need long term treatment with inhaled antibiotics to control the infection. Inhaled antibiotics against lung infections have certain advantages over antibiotics administered via the oral or parenteral route. In contrast with injection, inhalation is non-invasive. Administration directly to the site of infection may result in much higher local antibiotic concentrations compared to oral or parenteral administration. In addition, the systemic concentration will remain lower and so will systemic side effects.

Currently the most frequently applied method of administration for inhaled antibiotics is wet nebulisation which is ineffective, laborious and time-consuming. It also bears the risk of patient re-infection and bacterial resistance development within the device when regular cleaning and disinfection of the apparatus is omitted [4,5]. An interesting alternative to wet nebulisation is dry powder inhalation. Dry powder inhalers (DPIs) are effective, easy to use and eliminate the need for cold chain transport and storage, since dry powders are in general more stable than solutions [3]. Recently several dry powder developments for inhaled antibiotics have appeared (e.g. TOBI®, Colobreathe®, Cipro Inhale, Capreomycin DPI) [6–9]. Most of them are PulmoSphere™ developments, except for Colobreathe®, making use of the Turbospin® DPI (also named Podhaler™ for TOBI®). Although the dispersion behaviour of these new dry powder systems is often good, the strategy of using complex (multi-step) particle engineering processes and high excipient fractions makes the products expensive and the inhaled powder doses unnecessarily high. Also the use of capsule based inhalers for the administration of antibiotics is arguable because of the risk of bacterial resistance development in the device for some of the

Abbreviations: CF, cystic fibrosis; DPI, dry powder inhaler; FPF, fine particle fraction; PSD, particle size distribution; *Psa*, *Pseudomonas aeruginosa*; RH, relative humidity; TB, tuberculosis; TNBSA, 2,4,6-Trinitrobenzene Sulfonic Acid.

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antibiotics. Additionally, the hygroscopic nature of many antibiotics in general, and that of aminoglycosides in particular, is a risk for good dispersion when a used DPI is stored inappropriately and powder residues in the inhaler absorb moisture from the air to become sticky or even liquefied.

An alternative approach for pulmonary antibiotic therapy is with the use of high dose disposable DPIs. Previously, we have shown that the Twincer™ DPI can deliver pure micronised colistimethate sodium with a high efficiency to control chronic pulmonary infections with *Psa* in the respiratory tract [10]. It was computed that approximately 55 mg of this antibiotic drug is needed as dry powder from this high dose disposable DPI to achieve the same pharmacokinetic profile as that after 160 mg of wet nebulised colistimethate sodium [11]. In studies with healthy volunteers and CF patients, the dry powder dose from the Twincer™ was well tolerated in a dose of 2 × 12.5 mg, and meanwhile it has been shown that the dispersion efficiency of colistimethate sodium in this DPI remains nearly the same for the entire dose range from 5 to 50 mg [12].

The aminoglycoside antibiotic tobramycin, is also widely used in CF and non-CF bronchiectasis patients colonised with *Psa*. Kanamycin and amikacin are other examples of potentially inhalable aminoglycosides which are used as second line anti-TB drugs and are of particular importance for the treatment of drug resistant TB [13,14]. Delivering these drugs directly to the lungs, could offer an opportunity to overcome drug resistance, since drug resistance development is related to drug concentration at the site of infection. Moreover, with inhaled dry powder antibiotics in addition to standard therapy of TB patients it is likely that the upper airways can be cleared from pathogenic bacteria. This may be a valuable strategy to protect non-infected patients and healthcare workers [15] as TB spreads by small airborne droplets containing *Mycobacterium tuberculosis* that are formed in the upper airways and released into the environment during sneezing, coughing and talking [16].

Aminoglycosides show completely different physico-chemical properties compared to colistimethate sodium however. This has serious consequences for the performance of the inhaler-drug combination when the drug itself is not engineered into a well dispersible powder formulation with the aid of a relatively high concentration of excipients. As explained previously, using pure (micronised or spray dried) active ingredient makes the inhaler performance highly dependent on the physico-chemical drug properties [17]. Hence, the inhaler design has to be adjusted to these properties. Therefore, the aim of this study was to investigate the physico-chemical properties of the aminoglycosides relevant to dispersion and retention in order to decide how the Twincer™, which was developed for colistimethate sodium, needs to be modified to meet the properties of pure aminoglycosides.

2. Materials and methods

2.1. Materials

Tobramycin as free base was purchased from BUFA (The Netherlands). Amikacin sulfate (in this manuscript referred to as amikacin) and L-leucine were obtained from Sigma-Aldrich Chemie B.V. (The Netherlands) whereas kanamycin sulfate (in this manuscript referred to as kanamycin) was supplied by Gold Biotechnology Inc. (USA). Pharmatose 80 M (starting material for the preparation of a 150–200 μm size fraction of sweeper crystals) was granted by DFE Pharma (The Netherlands). For this study we used machined copies of the Cyclops DPI that were manufactured by the research workshop of the University Medical Center Groningen (Fig. 1).

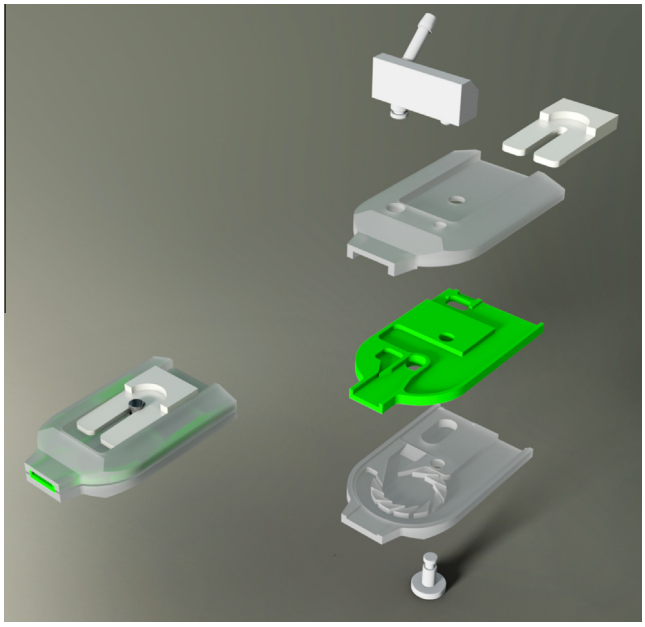

Fig. 1. Presentation of the Cyclops disposable DPI for spray dried aminoglycosides. On the left side the assembled Cyclops that was used for the *in vitro* evaluation programme and on the right side an exploded view of the Cyclops that was used in a pilot study and was modified to be able to measure the pressure drop across the Cyclops.

2.2. Particle preparation

Spray drying of the different aminoglycosides with and without 5% L-leucine into the desired aerodynamic size distributions for inhalation was performed using a Büchi Mini spray dryer B-290 (Büchi Labortechnik, Switzerland). The aminoglycosides were dissolved in demineralised water to a concentration of 50 mg/mL or 47.5 mg/mL (when 5% L-leucine was co-spray dried). The inlet air temperature was set to 130 °C and a nozzle size of 0.7 mm was used. Aspirator setting (100%), nozzle pressure (50 mm) and pump speed (2.5 mL/min) were used to control the particle size distribution (PSD) and the final water content of the powder. Powder handling was performed in a climate box to preserve the moisture content of the powder from spray drying before the dispersion experiments were performed. All powders were left to rest for at least one day after spray drying, before experiments were conducted.

Sweeper crystals in a size fraction of 150–200 μm were obtained from Pharmatose 80 M by 20 min vibratory sieving at an amplitude of 1.5 mm (Retsch AS 200, Germany) followed by 10 min air jet sieving (e200LS, Hosokawa Alpine AG, Germany) on a 150 μm analytical sieve at an underpressure of 2000 Pa to remove adhering lactose fines. Sweeper crystals in classifier based dispersion principles are used to wipe off adhering drug particles from the cylindrical classifier walls. They are not inhaled by the patient as their diameter exceeds the cut-off point of the classifiers at normal inhalation flows [10]. Because they are large crystals and not part of the drug formulation (added to the blister in a second filling step), not inhaled, and contribute negligibly to the powder volume in the blister, even in high mass concentrations, they are not considered as excipients in the formulation.

2.3. The filling of blisters

Aluminium blister cups for the Cyclops were hand filled at ambient conditions (relative humidity: RH < 35%) with the desired

dose weight of spray dried aminoglycoside using a five decimal analytical balance. Subsequently, coarse lactose monohydrate crystals (sieve fraction 150–200 μm) were added (not blended) to the blisters as sweeper crystals to control the inhaler retention (15, 20 or 30 mg added to 15, 30 or 50 mg tobramycin respectively). Because filled blisters were used for dispersion experiments in the Cyclops immediately after filling, they were not sealed.

2.4. Particle characterisation by scanning Electron Microscopy

Scanning electron micrographs of the powders for surface texture examination were obtained with a JSM 6301-F microscope (JEOL, Japan) at an acceleration voltage of 4 kV and probe current 7. Samples were fixed on an aluminium specimen mount by means of double sided adhesive carbon tape. Excess sample was gently tapped from the tape. The powders were sputter coated with 15 nm of a gold-palladium alloy prior to the examination.

2.5. Water content measurements

The water contents of the powders were determined in duplicate by Karl Fischer water titration (831 KF Coulometer, Metrohm Applikon, the Netherlands). Powder sampling into sealed vials was undertaken in a climate box immediately after spray drying to prevent moisture uptake of the powder before the water content was determined.

2.6. Recording of moisture isotherms (hygroscopicity)

Moisture isotherms at 25 °C were recorded with a dynamic vapour sorption apparatus DVS-1000 (SMS, UK). Prior to recording of the isotherms, the samples were dried in the apparatus by exposure to 0% RH. The isotherms were obtained by increasing the relative humidity in steps of 10% and each step was undertaken after the weight increase rate due to moisture absorption was reduced to less 0.0005%/min.

2.7. Particle size distribution analysis of the powders

PSDs of the powders were determined with a HELOS BF laser diffraction apparatus (Sympatec, Germany) equipped with a 100 mm (R3) lens, applying the LD calculation mode based on the Fraunhofer theory. The powders were dispersed into the laser beam with a RODOS system at 3 bar (Sympatec, Germany) after it was checked that this pressure is sufficient for dispersion into primary particles without breaking individual entities. The start of the measurements was triggered on an optical signal of 0.2% on channel 30, and the measurements were stopped either after the signal decreased to a value lower than 0.2% on the same channel for a period of 1 s, or after 3 s of real measurement time. All RODOS measurements were performed at least in duplicate.

2.8. Characterisation of the dispersion performance of the Cyclops

The dispersion efficiency of the Cyclops was determined by comparing the PSDs from RODOS dispersion (3 bar) with the PSDs of the aerosols obtained from dispersion by the Cyclops. For measurement of the PSDs in the aerosols from the Cyclops the same HELOS BF laser diffraction apparatus, lens, trigger conditions and calculation mode were used as for the powder characterisation. The Cyclops was connected to an inhaler adapter (INHALER 2000, Sympatec, Germany) and operated at 2, 4 or 6 kPa (respectively 0.02; 0.04 and 0.06 bar) pressure drop during 3 s. Dispersion measurements in the Cyclops were only performed when the RH of the ambient air was lower than 35%, because of the highly hygroscopic nature of the aminoglycosides. All INHALER 2000 measurements with the Cyclops were performed at least in triplicate.

2.9. Measurement of the delivered doses from the Cyclops

The amount of powder retained in the Cyclops was determined chemically after every single dispersion experiment. In combination with the dose weight measured into the Cyclops this enabled calculation of the delivered dose. To chemically quantify aminoglycosides a 2,4,6-Trinitrobenzene Sulfonic Acid (TNBSA) assay in 0.05 M Borax buffer was used. The Cyclops DPIs were rinsed with 0.05 M Borax buffer. Next, 2 mL of this solution was incubated for 2 h at room temperature with 1 mL of 0.01% (*w*/*v*) TNBSA solution. The reaction was stopped by adding 0.5 mL 1.0 M HCl-solution and followed by VIS spectrophotometry (UnicamUV-500, ThermoSpectronic, UK) at a wavelength of 340 nm.

3. Results and discussion

Preliminary Twincer™ experiments (data not shown) revealed that micronised (crystalline) aminoglycosides are extremely adhesive and cohesive and thus, difficult to disperse, particularly when the dispersion principle (as in the Twincer™) makes use of inertial forces. The powders were compacted against the cylindrical classifier walls and only slowly and incompletely removed by the sweeper crystals. Adapting the classifier based Twincer™ to the physico-chemical properties of the micronised tobramycin powders required a radical change of the Twincer™ geometry and this aspect is further discussed under the heading *Twincer™ modification into the Cyclops.*

3.1. Salt choice and production process

Although we prefer milling over spray drying, because spray dried powders are generally in the amorphous state and therefore, more hygroscopic than micronised powders, which is at the cost of their physical stability. For aminoglycosides the difference in moisture sensitivity between both solid states is small however. Fig. 2 shows this for tobramycin base. When spray drying does not negatively influence the moisture sensitivity of the powder, this technique may even have certain advantages over micronisation, as spray dried particles have frequently a more favourable particle shape (round or raisin shaped) and surface structure for dispersion [18]. Based on the extreme difference in the moisture isotherms in Fig. 2, we also chose tobramycin free base and not the commonly used sulfate salt of tobramycin for the experiments. An additional advantage of this choice is that the sulfate group increases the dose weight considerably compared to the free base due to its higher molecular weight. To emphasize that dispersion enhancing particle engineering is indeed not necessary when an effective inhaler design is used, we compared the dispersion and retention performance of pure spray dried tobramycin in the modified inhaler concept with that of co-spray dried tobramycin with 5% L-leucine. Co-spray drying of this excipient is known to modify the particle structure in a beneficial way for dispersion and retention [19,20]. To study the robustness of the new inhaler concept for aminoglycosides, spray dried batches of kanamycin and amikacin with and without 5% L-leucine were also tested.

3.2. Primary particle size distributions and water content of the powders

The dispersion efficiency of hygroscopic and highly water soluble drugs, such as aminoglycosides, depends on their water content. They can become sticky or even fuse together when they

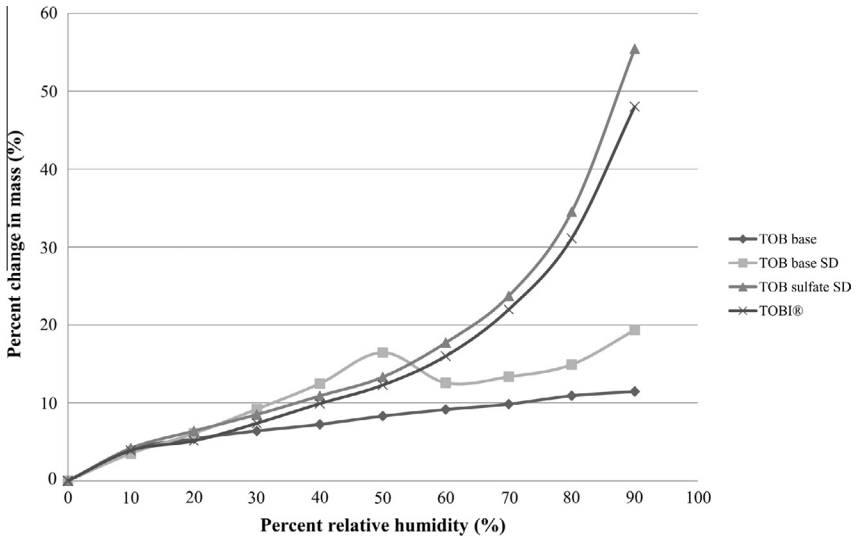

Fig. 2. Moisture isotherms of pure crystalline tobramycin (TOB) free base, spray dried free base (SD), pure spray dried tobramycin sulfate (SD) and tobramycin sulfate in the marketed tobramycin powder for inhalation (TOBI®).

absorb too much water. On the other hand, powders that are almost water free may exhibit tribocharge effects which also affect dispersion negatively. For these reasons, tobramycin powders with different water contents were produced to determine the range of values within which the water content can be varied without deteriorating dispersion and inhaler retention. Table 1 shows the PSDs of the powders from laser diffraction analysis at 3 bar dispersion pressure. These primary PSDs are the reference for our inhaler development measurements for which we use the same technique. By comparing the PSD in the aerosol from the inhaler with the primary PSD of the particles, conclusions can be drawn about the dispersion efficiency of the inhaler. When the difference is negligible, the dispersion is good; when the difference is quite significant, dispersion has to be improved. We are aware of the fact that laser diffraction diameters of inhalation powders may differ from their aerodynamic diameters but for the type of solid particles used in our study the difference is very small. Such powders generally yield very high mass fractions of particles within the most desirable aerodynamic size range between 1 and 5 μm in a cascade impactor when they are dispersed effectively. Ultimately, developed inhalers have to be characterised extensively with cascade impactor technique, but for the concept development phase this is not necessary when the laser diffraction and aerodynamic size distribution are very well comparable. The minor differences between the PSDs of the powders in Table 1 are mainly due to small variations in the spray drying process. The differences in water content are primarily caused by the differences in physicochemical properties of the aminoglycosides. The addition of 5% L-leucine does not seem to have a major effect on the water content and the primary PSD of the powders.

3.3. Particle morphology and the influence of L-leucine on that

Fig. 3 shows scanning electron micrographs of the spray dried tobramycin, kanamycin and amikacin powders with and without 5% L-leucine. The addition of 5% L-leucine, significantly increased the irregular (wrinkled) particle surface structure. It has been shown that this irregular structure may add to improved dispersion behaviour of the powder from classifier based inhalers [18]. All powders showed a fairly broad PSD, as confirmed with laser diffraction technique (Table 1), but the mass fractions of the finest particles were low and all X_{90} values were smaller than 5 μm.

3.4. Twincer™ modification into the Cyclops

It was observed that all produced pure aminoglycoside powders exhibited a much stronger tendency to form soft agglomerates than colistimethate sodium irrespective of the preparation technique used. Such agglomerates appeared to block the narrow classifier inlet passageways for the powder towards the parallel classifiers in the basic Twincer™ concept. In addition, it was observed that aminoglycosides have a much higher tendency to adhere to the cylindrical classifier walls than colistimethate sodium. This resulted in a thick and dense powder coating of the classifier walls caused by the relatively high centrifugal forces upon the particles during circulation in the classifiers. Circulating sweeper crystals in the classifiers could only partly and gradually remove this coating from the classifier walls, but removed fragments of this coating appeared to be too strong to de-agglomerate subsequently into primary particles before leaving the classifiers. Hence, the aerosol emission time from the inhaler was prolonged

Table 1
Primary PSDs of spray dried tobramycin, kanamycin and amikacin powders with and without 5% L-leucine determined by laser diffraction analysis using a HELOS BF laser diffractometer and a RODOS dry powder disperser operated at 3 bar.

Formulation	X_{10} (μm)	X_{50} (μm)	X_{90} (μm)	FPF < 5 μm (%)	Water content (%)
TOB	0.78	2.07	4.56	92.92	3.4
TOB + 5% LL	0.84	2.21	4.60	93.07	7.9
KAN	0.79	2.03	4.26	94.8	9.4
KAN + 5% LL	0.80	2.17	4.82	91.4	10.1
AMK	0.80	2.09	4.36	94.27	5.8
AMK + 5% LL	0.79	2.09	4.60	92.76	5.1

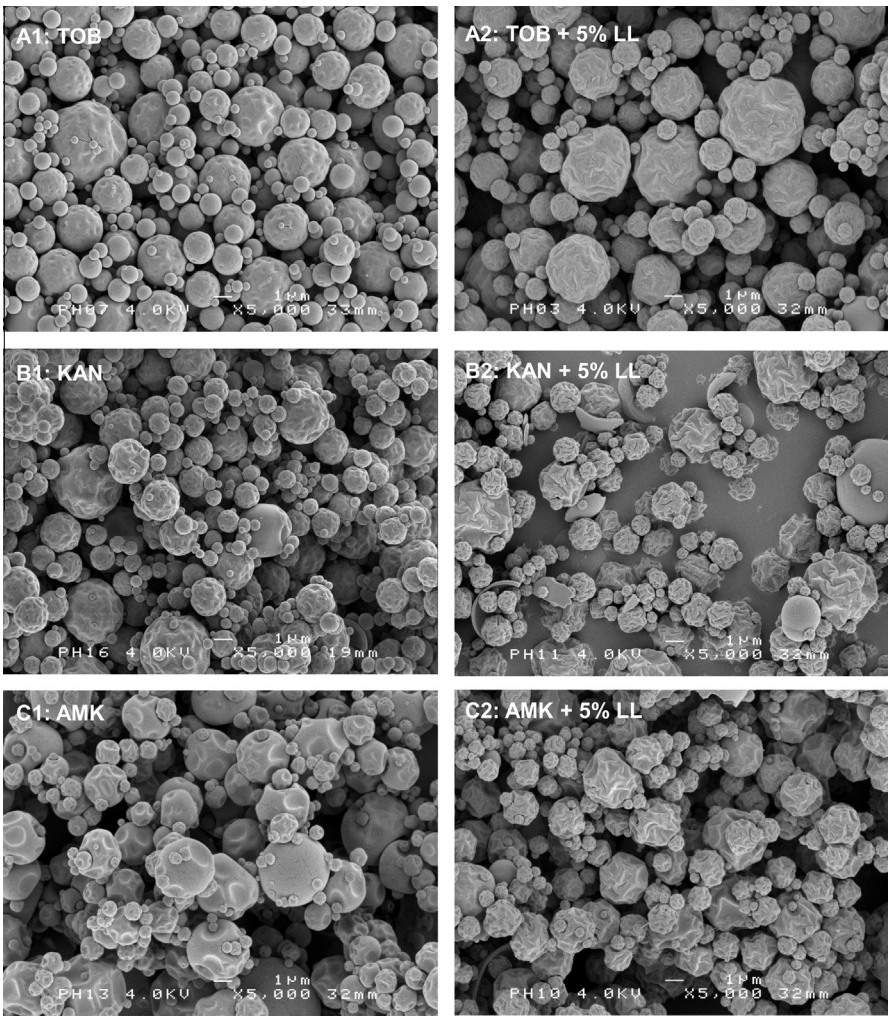

Fig. 3. Scanning electron micrographs of spray dried tobramycin (TOB), kanamycin (KAN) and amikacin (AMK) powders (A1, B1, C1, respectively) and with 5% L-leucine (LL) (A2, B2, C2, respectively).

and the PSD was influenced negatively as a result of the release of these small agglomerates. Although these agglomeration, adhesion and compaction phenomena appeared to a lesser extend with the spray dried powders than with micronised aminoglycosides, insufficient improvement could be obtained from adapting the powder properties of the pure drug (or formulations containing at least 95% drug) alone. Since the addition of larger excipient fractions to the powder formulation for high dose drugs and also the use of advanced particle engineering processes more in general are undesirable, modification of the Twincer™ design was considered the best option to improve the dispersion performance of the inhaler for aminoglycoside antibiotics.

In order to reduce adhesion of the powder to the classifier wall(s) and the formation of non-dispersible powder agglomerates two major design changes were made. First of all, the particle impaction angle of the rotating powder particles in the classifier was reduced to 30°. This change reduces the force of impact and by that, the force of adhesion between drug particles and the classifier wall [21]. Secondly, the number of air supply channels (including the powder channel) towards the classifier was increased from three to eleven. This increased the number of interruptions in the classifier wall and significantly reduced the total contact area with the powder. To enable these design modifications, the classifier diameter had to be increased from 15 to 21 mm. In order to maintain the small dimensions of the Twincer™ and the air flow rate through the classifier, the number of classifiers was reduced from two to one which inspired us to give this new Twincer™ concept the name Cyclops. By keeping the classifier depth the same (2.5 mm), the air velocity remained the same at the same total flow rate. In contrast, the air flow resistance was increased from 0.040 to 0.060 $kPa^{0.5}$ min L_N^{-1}. This is considered beneficial since the lower aerosol velocity will lead to a reduced oropharyngeal deposition. For the Cyclops, 4 kPa corresponds with 34 L/min which classifies the inhaler as a high resistance device [22]. Increasing the diameter of the classifier also enabled widening the powder inlet to the classifier, which eliminates the risk of blockage by large soft agglomerates in the powder formulation. Finally, a small chamber was added adjacent to the mouthpiece channel, having a connection with this channel through a capillary, which enables us to measure the pressure drop across the Cyclops (Fig. 1). This pressure drop measurement facilitates the recording of flow curves during inhalation in clinical trials without changing the inhaler resistance or interfering with the emitted aerosol. The Cyclops is the first step towards the development of a series of DPIs as part of the Twincer™ family that will have the same exterior (especially for the same type of disease), but different classifiers (or other dispersion principles) inside, which are tailored to the physico-chemical properties of the drug to be dispersed.

3.5. Effect of dose weight on dispersion and inhaler retention in the Cyclops

The dispersion efficiency of the Cyclops for tobramycin was determined by comparing the PSDs of the aerosols generated by the Cyclops for different doses of pure spray dried base at 4 kPa (0.04 bar) pressure drop with the PSDs from RODOS dispersion at 3 bar. Fig. 4 shows that the dispersion efficiency of the Cyclops is excellent. Mass fractions of fine particles (FPFs < 5 μm) in the aerosols from the device were approximately 90% of the delivered dose for all dose weights. This is exceptionally high compared to the FPFs < 5 μm for the primary particles (93%) from RODOS dispersion at the much higher pressure of 300 kPa (Table 1). Fig. 4 also shows that there is hardly any effect of dose weight (15, 30 or 50 mg) on the dispersion efficiency of spray dried tobramycin from the Cyclops. The maximum amount of spray dried tobramycin base that can be filled in the currently used blister is 50 mg. Therefore, this is the maximum dose investigated. However, Fig. 4 suggests that even higher doses can be dispersed efficiently in one inhalation with the new Cyclops DPI. The corresponding inhaler retentions were 21%, 14% and 14% of the weighed drug mass in the blister for the 15, 30 and 50 mg doses respectively, showing that the retention does not increase between 30 and 50 mg which facilitates high dose delivery. These results show that well designed single dose disposable DPIs have a high potential for pulmonary delivery of dry powder tobramycin, in contrast to what has recently been concluded from an incorrect evaluation of the Twincer™ with colistimethate sodium [23].

3.6. Effect of the pressure drop on the dispersion efficiency and retention in the Cyclops

Patients may generate different pressure drops because of differences in age and gender, severity of the disease, quality of the inhalation instruction, motivation, et cetera. For that reason, the performance of the Cyclops was tested at three different pressure drops (2, 4 and 6 kPa; Fig. 5A and B). Between 2 and 4 kPa a slight improvement in the dispersion efficiency is observed, however, already at 4 kPa pressure drop the FPF < 5 μm from the aerosol generated by the Cyclops is 90.7% and almost the same as FPF < 5 μm for the primary particles (measured with RODOS). This explains why there is no change in dispersion efficiency between 4 and 6 kPa. The small error bars indicate the high reproducibility of the dispersion efficiency at 4 and 6 kPa. Fig. 5B shows that the retention after dispersion at 4 and 6 kPa is approximately 15%. Only at 2 kPa pressure drop, the retention is about twice as high

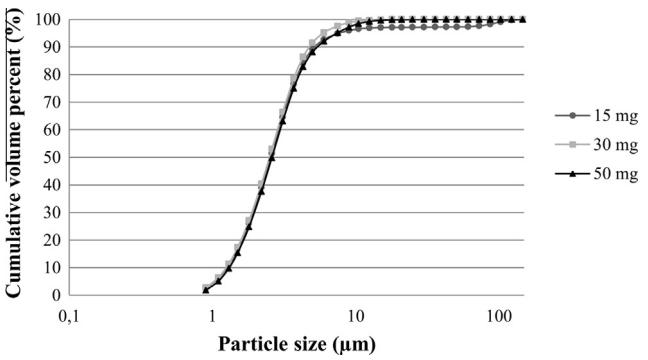

Fig. 4. Effect of the tobramycin dose on the dispersion efficiency of the Cyclops. PSDs in the delivered aerosols from the Cyclops operated at 4 kPa for doses of 15, 30 and 50 mg pure spray dried tobramycin (TOB) measured by laser diffraction analysis using an INHALER 2000 adapter and a HELOS BF laser diffractometer.

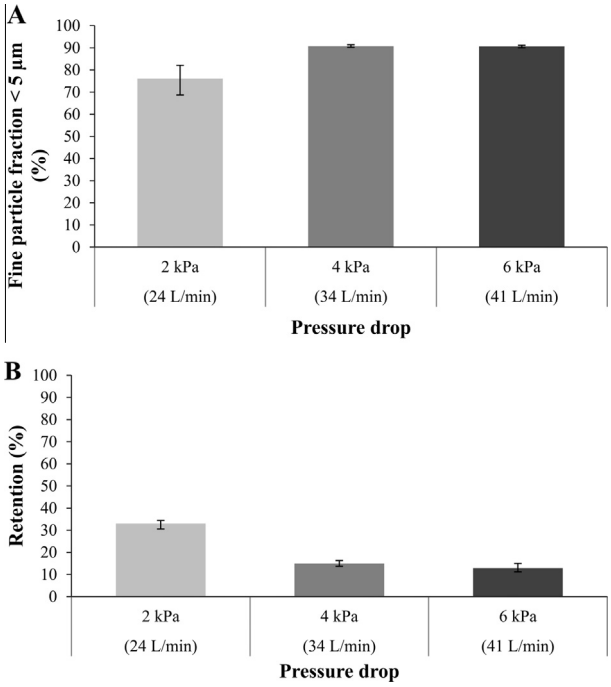

Fig. 5. Effect of the pressure drop on the dispersion (A) and retention (B) of a 30 mg tobramycin dose in the Cyclops. Comparison of the FPF < 5 μm (A) and the retention values (B) for doses of 30 mg pure spray dried tobramycin (TOB) at 2, 4 and 6 kPa in the Cyclops. The error bars indicate the minimum and maximum values found.

(30%). However, considering the relatively high air flow resistance of the Cyclops, it is expected that all patients are able to generate at least 4 kPa [24], which releases the aerosol from the Cyclops at a flow rate of about 34 L/min.

3.7. Effect of the water content on the dispersion efficiency and retention in the Cyclops

For hygroscopic powders, the water content is expected to have a significant effect on the dispersion and retention performance of the DPI. Fig. 6A and B shows the effect of water content for spray dried tobramycin base on the performance of the Cyclops. Fig. 6A compares the ratio of the X_{50} value from RODOS dispersion (3 bar) to the X_{50} value from Cyclops dispersion (for a 30 mg dose at 4 kPa), with this ratio (RODOS to Cyclops) for the FPF < 5 μm as function of the water content in the powder (from Karl Fischer titration). All ratios are close to one and vary within a narrow range between 1.2 and 1.4 for the X_{50} value and from 1.0 to 1.1 for FPF < 5 μm. Evenly important is the conclusion that no clear trend can be observed for both parameters, which suggests that dispersion is unaffected by the water content within the investigated range. This is highly beneficial because it shows the robustness of the inhaler-formulation combination. The ratio of the FPF < 5 μm varying between 1.0 and 1.1 confirms the excellent dispersion efficiency of the Cyclops for tobramycin base. Fig. 6B shows the effect of the water content on the powder retention in the Cyclops. Although the differences are larger than the differences in dispersion efficiency, there is (similar to dispersion efficiency) no clear relationship observed between the inhaler retention and the water content which indicates that particle losses due to adhesion are a rather random phenomenon. Furthermore, nearly all retention values are lower than 20%.

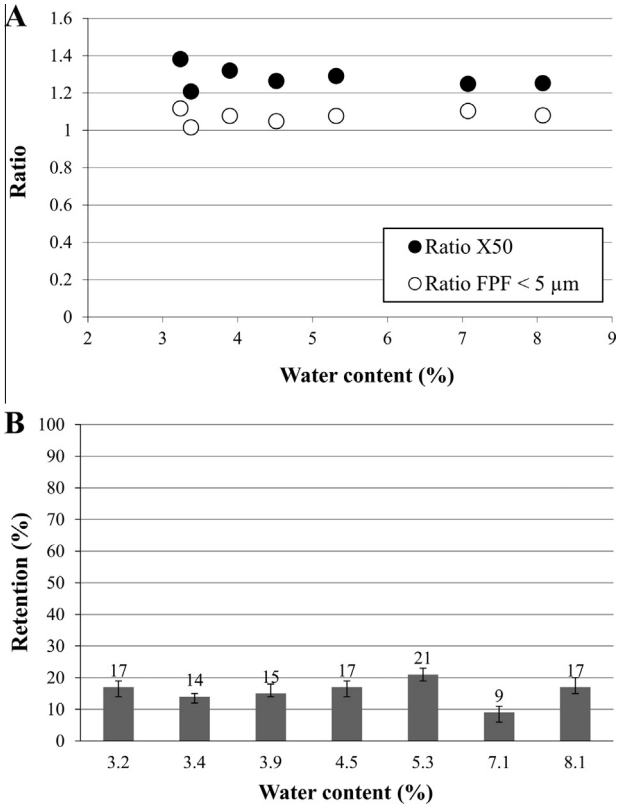

Fig. 6. Effect of the water content on the dispersion efficiency (A) and retention (B) for 30 mg pure spray dried doses dispersed with the Cyclops at 4 kPa. The closed circles in figure A show the ratio of X_{50} for the primary PSD to the X_{50} for the aerosol from the Cyclops and the open circles show this ratio for the FPF < 5 µm as function of the water content in the samples. In figure B for the inhaler retention, the error bars indicate the minimum and maximum values measured.

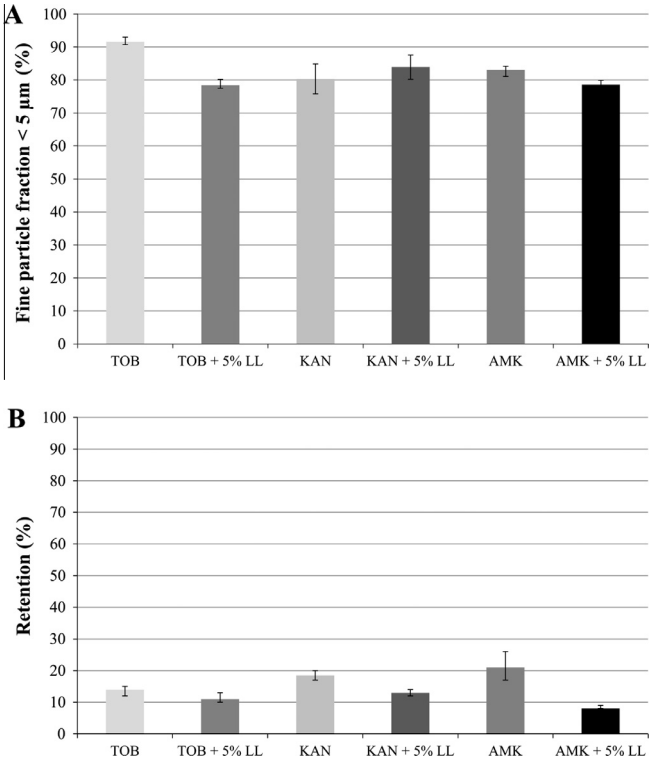

Fig. 7. Comparison of the dispersion (A) and retention (B) of 30 mg tobramycin free base (TOB), kanamycin sulfate (KAN) and amikacin sulfate (AMK) doses with and without 5% L-leucine (LL) in the Cyclops (4 kPa). The error bars indicate the minimum and maximum values found.

3.8. The effect of co-spray drying of 5% L-leucine on dispersion and retention in the Cyclops

Fig. 7A and B shows the dispersion efficiencies and inhaler retentions of different spray dried aminoglycoside (tobramycin, kanamycin, amikacin) powders with and without 5% L-leucine. In contrast with tobramycin base, the sulfate salts of kanamycin and amikacin were used, as only these salts were commercially available. The size of the error bars again demonstrates the high reproducibility of the Cyclops performance for all spray dried powders. Fig. 7A shows that pure spray dried tobramycin is most efficiently dispersed by the Cyclops. This could be expected because the Cyclops was optimised for this drug. However, also the pure spray dried kanamycin and amikacin powders yielded aerosols with FPFs (<5 µm) of more than 80% of the delivered dose. Although it changed the surface texture of the particles noticeably (Fig. 3), co-spray drying with 5% L-leucine did not improve the dispersion efficiency of the three aminoglycosides in the Cyclops. For kanamycin and amikacin the FPFs were not significantly different with and without excipient; for tobramycin, the dispersion was even slightly worse after co-spray drying with L-leucine. We did not investigate this inconsistency in behaviour between the three aminoglycosides tested, because a general improvement from the use of L-leucine did not occur. Therefore, an explanation seems irrelevant, but it could have to do with differences in the rates of classifier loading and discharge which are strongly related to differences in powder bulk behaviour and agglomerate break-up mode (and rate), particularly for the high doses tested (Fig. 7). The inhaler retention is hardly affected by L-leucine for tobramycin and kanamycin (Fig. 7B). Only for amikacin a significant reduction is found. A reduction in inhaler retention may be important as it increases the lung dose.

4. Conclusions

The results of this study show that with an appropriate inhaler design, adapted to the physico-chemical properties of a particular drug or drug class, excellent dispersion can be achieved for high doses of pure (spray dried) drug. This strategy technically enables the inhalation of doses up to at least 50 mg in one inhalation. Delivered FPFs of tobramycin with the Cyclops exceed 90% of the delivered dose at 4 and 6 kPa for tobramycin free base without the addition of excipients and/or using advanced particle engineering techniques. Similar results were obtained with kanamycin and amikacin. These results show that for a specific drug (class) this can be an excellent alternative for the strategy to formulate the drug (class) for an existing inhaler device.

Like the Twincer™, the Cyclops is a disposable DPI which is to be recommended for highly hygroscopic drugs or drug formulations of which inhaler residues can absorb moisture. Moisture absorption may hinder all following inhalations with the same device when this leads to increased stickiness or even liquefying of the retained particles. Furthermore, a disposable inhaler eliminates the risk of bacterial resistance development within the device and subsequent patient re-infection with drug-resistant strains of bacteria. Like the Twincer™ the Cyclops has a very simple design comprising only three plate like parts, without any moving parts and a simple dose compartment for the drug. This makes the Cyclops production cheap, a prerequisite for being disposable.

The Cyclops has a relatively high inhaler resistance, and its high dispersion efficiency and low inhaler retention enable the delivery

of high dose fractions of the metered mass in the size range of 1.0–5.0 μm to the respiratory tract at a flow rate of only 34 L/min at 4 kPa. This prevents the loss of substantial drug fractions by deposition in the oropharynx. Compared to the Podhaler™, delivering 78% of the metered mass at 72 L/min of which nearly 44% was recovered in the oropharynx, oesophagus and stomach of healthy volunteers, a higher lung dose may therefore be expected [25]. Moreover, even with the same delivery efficiency to the lung, the total dose may be strongly reduced by using the free base instead of the sulfate in a formulation with various excipients. Administration of that dose would even be possible in one single inhalation manoeuvre when the size of the blister is slightly increased, or alternatively in two inhalations when the high amount of powder appears to be inconvenient to the patient.

Finally, it has been shown that the Cyclops has a robust performance giving similar results for different spray dried aminoglycosides. Since the various antibiotics from this drug class may be interesting therapeutic options for a variety of diseases, the Cyclops is an interesting inhaler candidate for applications such as the treatment of non-CF bronchiectasis or TB [3]. In a next publication we will show that high doses of tobramycin base from the Cyclops are well tolerated by patients with non-CF bronchiectasis and pharmacokinetic data from this pilot study with tobramycin in the Cyclops will be presented as well.

Conflict of interest

The authors have no conflict of interest to declare.

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