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# Preparation and characterization of spray-dried tobramycin powders containing nanoparticles for pulmonary delivery

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ABSTRACT

Using high-pressure homogenization and spray-drying techniques, novel formulations were developed for manufacturing dry powder for inhalation, composed of a mixture of micro- and nanoparticles in order to enhance lung deposition. Particle size analysis was performed by laser diffraction. Spray-drying was applied in order to retrieve nanoparticles in dried-powder state from tobramycin nanosuspensions. The aerolization properties of the different formulations were evaluated by a multi-stage liquid impinger. Suspensions of nanoparticles of tobramycin containing Na glycocholate at 2% (w/w) relative to tobramycin content and presenting a mean particle size about 200 nm were produced. The results from the spray-dried powders showed that the presence of nanoparticles in the formulations improved particle dispersion properties during inhalation. The fine particle fraction (percentage of particles below 5 μm) increased from 36% for the raw micronized tobramycin material to about 61% for the most effective formulation. These new nanoparticle-containing tobramycin DPI formulations, based on the use of very low level of excipient and presenting high lung deposition properties, offer very important perspectives for improving the delivery of drugs to the pulmonary tract.



## 1. Introduction

The ability to deliver therapeutic agents to the site of action may allow efficient treatments of cystic fibrosis, asthma, lung cancer, and tuberculosis as well as many other diseases specific to the respiratory tract (Gelperina et al., 2005). The particular use of pulmonary drug delivery may increase patient treatment compliance as it represents a non-invasive means of drug administration (LaVan et al., 2003). Nevertheless, the efficiency of utilizing aerosolized particles depends on their size and density (Pandey and Khuller, 2005). Due to rapid advances in nanotechnology, the use of nanoparticles has become a subject of very active research (Hadinoto et al., 2007a). A wide range of drugs for oral and parenteral delivery, in the form of nanoparticulate suspensions and nanoparticulate composites has been investigated. In vitro and in vivo studies have demonstrated that nanoparticles are promising carrier systems for drug-targeting strategies (Sham et al., 2004). However, much less attention has been paid to the dry powder aerosol delivery of nanoparticulate drugs (Kipp, 2004).

In fact, nanoparticles are most often delivered to the lungs by nebulization of colloidal solutions. However, nanoparticles stored in an aqueous medium will, over time, lead to chemical instability and therefore loss of drug. Solution instability is another concern owing to particle agglomeration and settling, results of the small size and strong particle–particle interactions of nanoparticles, which could lead to poor functionality of nebulizers (Dailey et al., 2003).

The disadvantage of using nano-sized delivery systems for pulmonary dry powder application is that their mass median aerodynamic diameter (MMAD) is not suitable for inhalation delivery (Finlay et al., 1997; Finlay and Gehmlich, 2000). Many nanoparticles are of a size that places them in a transition region where neither diffusion nor sedimentation nor impaction is an effective deposition mechanism (Sham et al., 2004). Consequently, it is expected that a large fraction of the inhaled dose will be exhaled and little particle deposition will take place in the lungs. A second problem is the persistent aggregation of the particles arising from their small size, which makes their physical handling extremely difficult for dry powder inhalation (DPI) applications (Hadinoto et al., 2007a). The percentage of the emitted dose deposited in the lungs is dependent on the powder's dispersibility, which is limited by interparticular cohesive forces. Strong interparticulate forces result in poor powder flow, as well as in poor powder dispersion from passive DPI devices,

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which results in decreased drug deposition in the lung (Weers, 2000).

However, if nanoparticles can be effectively delivered to the lungs, then their unique properties in avoiding mucociliary clearance and in delivering drugs directly to the target tissue or target cells might be utilized for therapeutic treatments of lung-specific diseases (Sham et al., 2004).

To circumvent the above-mentioned problems, novel particulate forms incorporating nanoparticles into micron-scale structures composed of polystyrene, polyacrylate, gelatine or chitosan have been engineered to produce microparticles that are consisted of nanoparticles as carriers for lung delivery (Tsapsis et al., 2002; Sham et al., 2004; Grenha et al., 2005, 2007; Hadinoto et al., 2006, 2007a,b).

This paper describes how, using high-pressure homogenization (HPH) and spray-drying techniques, novel formulations were developed to manufacture dry powder for inhalation composed of a mixture of micro- and nanoparticles.

In HPH, the drug suspension is pressed through a small gap at high pressure, and the cavitation forces are high enough to disrupt the microparticles into nanoparticles. This technique has been extensively described relative to particle size reduction efficiency by Müller et al. with respect to reproducibility and processing of highly concentrated suspensions (Grau et al., 2000; Krause and Müller, 2001). It presents several advantages as it is very simple and time-saving. To retrieve nanoparticles in dried-powder state, spray-drying, which allows control over particle surface, morphology and density, was applied (Hickey et al., 1996).

Therefore, the objective of this study was to develop tobramycin nanosuspensions using high-pressure homogenization and to further characterize the powder obtained after spray-drying in terms of aerolization properties, surface composition and physical state in order to determine which formulations could be the most suitable for pulmonary delivery.

## 2. Materials and methods

### 2.1. Materials

Tobramycin was supplied as micronized powder from Teva (Petah Tiqva, Israel). Phospholipon 90H was donated by Nattermann Phospholipids (Koln, Germany). Sodium glycocholate was purchased from Acros Organics (Geel, Belgium) and sodium taurocholate was purchased from Sigma (Steinheim, Germany). All chemicals used were of analytical grade.

### 2.2. Methods

#### 2.2.1. Preparation of nanosuspensions

Tobramycin powder was poured in a surfactant solution of isopropanol (5% Tobra, w/v, suspension) under magnetic stirring (500 rpm). After dispersion, a first size reduction step using a CAT high speed homogenizer X620 (HSH) (CAT M. Zipperer, Staufen, Germany) at 24,000 rpm (10 min for a 50 ml sample) was conducted on the suspension (in an ice bath to prevent sample temperature increase). Nanosuspensions were then prepared using an EmulsiFlex-C5 high-pressure homogenizer (Avestin Inc., Ottawa, Canada). Pre-milling low-pressure homogenization cycles were first conducted on the tobramycin suspension to further decrease particle size (10 cycles at 12,000 PSI). High-pressure homogenization was then finally applied for 10–20 cycles at 24,000 PSI. Since HPH causes sample temperature increase (increase of 30 °C following 20 cycles at 24,000 PSI), all operations were carried out using a heat exchanger, placed ahead of the homogenizing valve, with sample temperature maintained at $10 \pm 1$ °C. Samples were withdrawn after the different size reduction steps for size distribution analysis.

#### 2.2.2. Spray-drying

Spray-drying using a Büchi Mini Spray Dryer B-191a (Büchi laboratory-Techniques, Switzerland) was applied in order to retrieve nanoparticles in dried-powder state from the nanosuspensions described above. The nanosuspensions were spray dried with constant stirring. The following conditions were used during spray-drying: spraying air flow, 800 l/h; drying air flow, 35 $m^3$/h; suspension feed rate, 3.5 g/min; nozzle size, 0.5 mm. The inlet temperature was set at 80 °C. The resultant powder was blown through the cyclone separator and collected in a container. Powders were stored in a dessicator at ambient temperature.

#### 2.2.3. Particle size analysis

The size and size distribution of the particles in suspension following the different homogenization steps and in dried state, were determined by laser diffraction with a wet sampling system (Mastersizer, Hydro 2000, Malvern instruments, UK). Samples were dispersed in isopropanol, saturated with tobramycin, in order to avoid any particle solubilisation and the diameters reported were calculated using volume distribution (three sets of five measurements). A refractive index for the measurements of 1.54 and 1.39 was used for the drug and the solvent, respectively. The median volume particle size, $D(0.5)$, i.e. the size in microns at which 50% of the sample is smaller and 50% is larger, $D(0.1)$, $D(0.9)$, and $D[4.3]$, the volume mean diameter, were used as characterization parameters.

The second technique used a Malvern Spraytec® (Malvern, UK) diffraction-based device equipped with an inhalation cell, specifically modified for measuring the particle size diameter (PSD) generated from medicinal aerosols, including MDI, DPI and nebulizers. It consists of a Spraytec® unit with a throat held in place by the inhalation cell and a connection for a Multi-Stage Liquid Impinger (MsLi). The entire assembly is a closed system and allows a controlled airflow rate (100 l/min during 2.4 s) in the measurement zone. This allows the size properties of DPIs to be measured under simulated breathing conditions.

#### 2.2.4. Scanning electron microscopy (SEM)

Evaluation of particle size and morphology was achieved by SEM, using a JSM-610 microscope (Jeol, Japan). Samples were scattered onto a thin film of a two-component epoxy resin and then coated with a platinum layer (Balzers SCD 030, Balzers Union Ltd., Liechtenstein). Acceleration during observation was 25 kV.

#### 2.2.5. X-ray powder diffraction

X-ray powder diffraction (XRPD) is a powerful and widely used tool for crystalline state evaluation. Diffraction patterns of the tobramycin formulations were determined using a Siemens Diffractometer D5000 (Siemens, Germany), with a Cu line as the source of radiation (WL1 = 1.5406 A, WL2 = 1.54439 A), and standard runs using a 40 kV voltage, a 40 mA current and a scanning rate of 0.02°/min over a $2\theta$ range of 2–70°.

#### 2.2.6. Bulk density

Bulk and tapped density were measured using a tap density tester (Stampfvolumeter, STAV 2003, Jel, Germany). Bulk density was determined by filling the powder into a 10-ml measuring cylinder and tapped density was measured by tap density measurements following 1000 taps. Bulk and tapped density values allow the determination of the Carr's compressibility index using

**Table 1**
Composition of tobramycin (5%, w/v) suspensions with various surfactants (% w/w relative to tobramycin content)

| | Phospholipon 90H (%) | Na taurocholate (%) | Na glycocholate (%) |
|---|---|---|---|
| T1 | 2 | | |
| T2 | | 2 | |
| T3 | | | 2 |
| T4 | 1 | 1 | |
| T5 | 1 | | 1 |
| T6 | | 1 | 1 |

the formula:

$$\text{Carr's Index}(\%) = 100 \times \frac{\rho_t - \rho_b}{\rho_t}$$

where $\rho_t$ is the tapped density and $\rho_b$ is the bulk density.

*2.2.7. Aerodynamic particle size analysis*

The aerodynamic particle size distribution was determined using a Multi-Stage Liquid Impinger (MsLI). A dry powder inhalation device (Aerolizer®, Novartis, Switzerland) was filled with a No. 3 HPMC capsule (Capsugel, France). HPMC capsules were used because gelatine capsules have a tendency to break during testing and to produce agglomerates during particle size measurements. Moreover, with a hygroscopic material such as tobramycin, gelatine capsules are not recommended because of their higher water content. The flow rate was adjusted to a pressure drop of 4 kPa, as is typical for inspiration by a patient, resulting in a flow rate of 100 l/min for 2.4 s. Three capsules loaded with 15 mg powder were taken for each test. Drug deposition in the device, the throat, the four stages and the filter (stage 5) was determined by high-pressure liquid chromatography (HPLC) analysis. For accuracy, each test was repeated three times. The suitable and validated quantification method has been described previously (Pilcer et al., 2006).

The total dose of particles with aerodynamic diameters smaller than 5 μm was calculated by interpolation from the cumulative mass against cut-off diameter of respective stages and considered as the fine particle dose (FPD) (mg) or fine particle fraction (FPF), expressed as a percentage of the total drug dose and not of the emitted dose.

## 3. Results and discussion

### 3.1. Preparation of the nanosuspensions

*3.1.1. Formulation composition*

The use of surfactants is necessary for the preparation of drug suspensions in order to stabilize the newly formed micro-/nanoparticles, thus preventing agglomeration of these particles following exiting of the homogenization gap. Judicious surfactant selection (type and concentration) and optimization are thus very important factors to take into account. They are listed in Table 1.

The results presented in Fig. 1 show the size of suspensions of tobramycin in isopropanol (5%, w/v) processed using pre-milling low-pressure homogenization cycles (10 cycles at 12,000 PSI) and 20 cycles at 24,000 PSI.

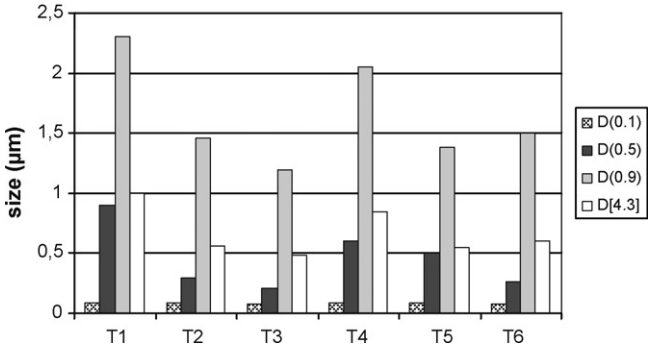


**Fig. 1.** Laser diffraction diameters $D(0.1)$, $D(0.5)$, $D(0.9)$ and $D[4.3]$ after 10 homogenization cycles at 24,000 PSI for formulations T1–T6.

Judging from the results shown in Fig. 1, it is obvious that all formulations containing Phospholipon (T1, T4 and T5) appeared to be less effective, with a $D(0.5)$ over 0.5 μm for the three suspensions. Moreover, the $D(0.9)$ and $D[4.3]$ of formulation T1 was about 2 and 1 μm, respectively, which is closer to the particle size range for microsuspensions than for nanosuspensions. In the formulations with Phospholipon, it appeared that deaggregation of the particles could not be achieved, and the surfactant was not effective in stabilizing the particles. Based on this screening, the most successful surfactant combination for stabilizing tobramycin as a nanosuspension turned out to be formulation T3. The presence of Na glycocholate at 2% (w/w) relative to tobramycin content proved to be most suitable for stabilizing tobramycin as a nanosuspension, with a $D(0.5)$ about 0.21 μm. Moreover, in comparison with T2, which contained Na taurocholate, the $D(0.9)$ was lower, with a value below 1.2 μm. Na glycocholate also presents a relatively high melting point (130 °C) which is useful for further processing such as spray-drying in order to avoid partial melting or softening of the excipient.

The minimum size that can be achieved mainly depends on the hardness of the drug and the homogenization parameters applied (number of cycles and pressure). However, the surfactant mixture is the determining factor for possible aggregation of the ultrafine drug nanoparticles produced during the size reduction step.

*3.1.2. Influence of pre-homogenization and HPH operations*

To investigate the homogenization process in more detail, formulation T3 was chosen and particle size reduction as a function of the homogenization cycles applied was determined. For each drug and application, depending on the size requirements of the application route, the number of cycles has to be optimized (Hecq et al., 2005).

The results given in Table 2 show that the HSH operation (10 min, 24,000 rpm—suspension placed in an ice bath) allowed a reduction that is not significant in tobramycin particle size, with a $D(0.5)$ about 1.1 μm for pre-homogenized tobramycin in comparison with a $D(0.5)$ of 3.6 μm for the micronized tobramycin raw material. However, the low-pressure homogenizing cycles were more efficient regarding particle size reduction than the HSH operation carried

**Table 2**
Laser diffraction results following successive size reduction steps for T3 suspension

| | $D(0.1)$ | $D(0.5)$ | $D(0.9)$ | $D[4.3]$ |
|---|---|---|---|---|
| No operation | 0.54 ± 0.03 | 3.57 ± 0.05 | 6.39 ± 0.05 | 10.44 ± 0.05 |
| HSH | 0.22 ± 0.01 | 1.10 ± 0.09 | 4.62 ± 0.05 | 8.29 ± 0.05 |
| pre-homogenization HPH | 0.111 ± 0.007 | 0.50 ± 0.01 | 3.60 ± 0.02 | 1.44 ± 0.01 |
| HPH 10C 24,000 PSI | 0.080 ± 0.005 | 0.221 ± 0.009 | 1.28 ± 0.01 | 0.51 ± 0.01 |
| HPH 20C 24,000 PSI | 0.079 ± 0.004 | 0.213 ± 0.009 | 1.22 ± 0.01 | 0.48 ± 0.01 |

**Table 3**
Composition of the spray-died suspensions used for the preparation of the tobramycin DPI formulations and Na glycocholate content of the formulations (dried forms)

| | Suspensions tobramycin 5% (w/v) | | Dried form |
|---|---|---|---|
| | Tobramycin (%, w/w) | Nanoparticles Tobra (%, w/w) | Na glycocholate (%)[a] |
| F1 | 95 | 5 | 0.1 |
| F2 | 90 | 10 | 0.2 |
| F3 | 50 | 50 | 1 |
| F4 | | 100 | 1 |
| F5 | | 100 | 2 |
| F6 | | 100 | 5 |

[a] Data expressed in percentage of tobramycin's weight.

out as a particle population characterized by a $D(0.5)$ of 0.5 μm was obtained. From the results obtained, we can clearly see that further processing of the drug suspensions allows for greater particle size reduction with achievement of a population with a $D(0.5)$ around 0.2 μm and a $D(0.9)$ around 1.2 μm. Nevertheless, limitation in particle size reduction can be observed at a homogenizing pressure of 24,000 PSI. Little change in $D(0.5)$ was observed after 10 cycles. $D(0.5)$, $D(0.9)$ and $D[4.3]$ are shown to slightly decrease with the increase in the number of cycles for up to 20 cycles. The particle size reduction was thus limited to 10 homogenizing cycles following low-pressure homogenization size reduction operations, primarily for time-saving purposes.

The particle size distribution curves following the different size reduction also indicate that the low-pressure homogenization cycles are not sufficient for achieving adequate particle size reduction as they only yield a small percentage of submicron-size particles. HPH cycles were found to be necessary in that regards, yielding a nanoparticle population with a $D(0.5)$ around 200 nm and 80% of the population particles below 1 μm. The small fraction of microparticles left after the HPH cycles is responsible for the bimodale size distribution curve. This second population, around the micro-range, is thought to be the consequence of nanoparticle agglomerates or residual microparticles. In fact, laser diffraction size curves represent a volume distribution meaning that even a very few residual microparticles will strongly influence the particle size distribution. No particular attempt was made to remove this second population before spray-drying.

### 3.2. Evaluation of the dry powder formulations

From the nanosuspensions described above, different types of dry powder formulations for inhalation were developed in order to enhance lung deposition (Table 3).

On the one hand, nanoparticles were used to coat micron-size particles in order to modify their surface properties and to decrease the agglomeration tendency of the powder (Fig. 2.1). Different proportions of these nanosuspensions were added to a suspension of micronized tobramycin in isopropanol and homogenized with the HSH and then spray dried (F1, F2 and F3).

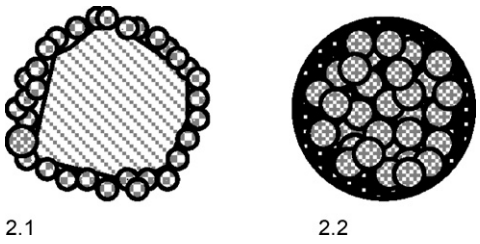


**Fig. 2.** Type formulations: (2.1) coated nanoparticles; (2.2) agglomerates of nanoparticles.

On the other hand, formulations composed solely of nanoparticles were produced in order to form easily dispersible and reproducible micron-size agglomerates of particles with low density and high porosity during inhalation (Fig. 2.2). In order to retrieve nanoparticles in dried-powder state, the nanosuspensions with different concentrations of Na glycocholate were spray dried (F4, F5 and F6).

#### 3.2.1. Physicochemical characteristics

The X-ray powder diffraction patterns (Fig. 3) confirm that the HPH operation and the spray-drying technique do not interfere with the crystalline state of tobramycin particles as the diffraction pattern is conserved for nanoparticles. This is very useful in terms of guaranteeing the long-term stability of the product in comparison to amorphous drugs. The only difference observed between the micronized tobramycin and the nanoparticles lay in peak intensities, which were found to be smaller for the nanoparticles. This difference was attributed to the presence of Na glycocholate in the formulations, where the surfactant is homogenously dispersed around the nanoparticles. So, as demonstrated by Hecq et al. (2005), the results suggest that the reduction in peak intensity is essentially due to the particle size reduction and to the dilution of the particles in the surfactant rather than any change of the polymorphic form of the active drug.

The morphology and surface structure of the formulations were analyzed by SEM. The bulk tobramycin was composed of big, compact agglomerates of micron-sized particles. The size of agglomerates ranged up to 1 mm (Fig. 4.1). The small tobramycin particles tended to form a very dense and cohesive structure (Fig. 4.2). On the other hand, processing the nanosuspensions by spray-drying yielded looser agglomerates that were less smooth, less regular and less cohesive. The yield consisted of smaller agglomerates of about 50–200 μm in size (Fig. 4.3). At larger mag-

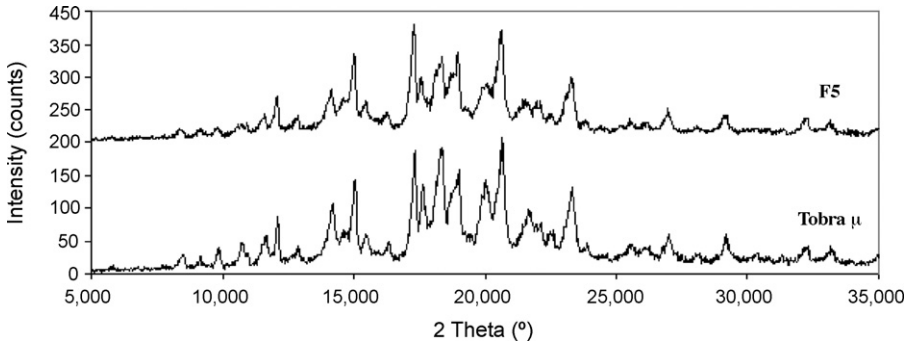


**Fig. 3.** X-ray powder diffraction patterns of raw micronized tobramycin and F5 formulation.

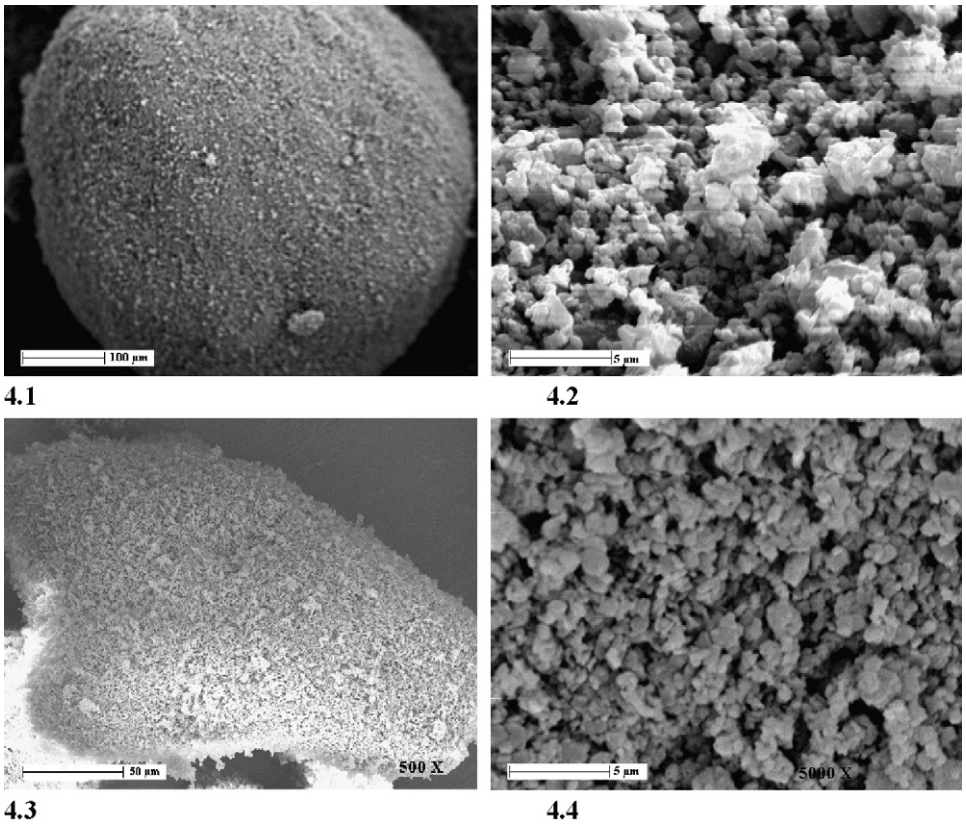


**Fig. 4.** SEM photographs of: (4.1) micronized tobramycin, magnification 500×; (4.2) micronized tobramycin, magnification 5000×; (4.3) F5 formulation, magnification 500×; (4.4) F5 formulation, magnification 5000×.

nifications, we can observe (Fig. 4.4) that these agglomerates are composed of small particles of about the nanometer range that have a structure that tends to being porous. This modification is probably explained by the production processes used. Thus, HPH allowed nanoparticles to be produced and atomization led to a porous powder. The presence of loose agglomerates made up of small particles probably explains the better dispersion of the particles compared to raw micronized tobramycin during inhalation.

The physical properties of the different formulations are summarized in Table 4. The bulk and tapped density values obtained for the spray-dried nanoparticle formulations were lower than those obtained for the micronized tobramycin. For the formulations made up of nano- and microparticles, the more the formulation contained nanoparticles, the lower its density was (0.156 for F1 vs. 0.124 for F3). Consequently, as expected, the formulations made up exclusively of nanoparticles presented an even lower density (0.083 for F5). However, the increase in density for the F6 formulation, which contained only nanoparticles, can be explained by the higher content of Na glycocholate (5%, w/w relative to tobramycin content) compared to all the other formulations, which contained a maximum of 2% (w/w) of surfactant.

Carr's Index values of less than 25 are usually taken to indicate good flow characteristics, values above 40 indicate poor powder flowability. As expected, the Carr's Index is higher for the formulations with nanoparticles. The nanometer-size dimensions lead to a severe aggregation problem arising from the small size, which makes the physical handling of the particles extremely difficult for DPI delivery. For the F1, F2 and F3 formulations, the content of nanoparticles in the coating of the microparticles did not affect the Carr's Index. Indeed, the Carr's Index is around 29 for all the formulations containing between 5% and 50% of nanoparticles. However, the Carr's Index varies for the formulations containing solely nanoparticles. Moreover, it appeared that the amount of Na glycocholate present in the formulations modified the flow properties of the powders. Indeed, an increase in surfactant content allowed an improvement of the flow properties (47 for F4 vs. 34 for F6). Nevertheless, the flow characteristics of all the formulations, except F4, were acceptable.

**Table 4**
Physical properties of all formulations tested: particle size characteristics (mean ± S.D., $n = 3$) measured with the Mastersizer 2000® and the Spraytec®, bulk density, tapped density and Carr's Index values

| | Mastersizer 2000® | | | Spraytec® | | | Density | | |
|---|---|---|---|---|---|---|---|---|---|
| | *D*(0.5) | *D*[4.3] | % <5 µm | *D*(0.5) | *D*[4.3] | % <5 µm | Bulk density (g/cm$^3$) | Tapped density (g/cm$^3$) | Carr's Index (%) |
| Tobramycin | 3.57 ± 0.05 | 2.9 ± 0.1 | 71.0 ± 0.1 | 3.1 ± 0.9 | 11 ± 4 | 68 ± 8 | 0.229 | 0.300 | 23 |
| F1 | 0.75 ± 0.02 | 1.54 ± 0.04 | 90.0 ± 0.1 | 2.9 ± 0.8 | 4.8 ± 0.9 | 80 ± 6 | 0.156 | 0.220 | 29 |
| F2 | 0.86 ± 0.03 | 1.48 ± 0.04 | 90.4 ± 0.1 | 2.3 ± 0.7 | 5 ± 1 | 80 ± 8 | 0.140 | 0.198 | 29 |
| F3 | 0.70 ± 0.04 | 1.48 ± 0.03 | 94.6 ± 0.1 | 2.4 ± 0.8 | 4.7 ± 0.6 | 84 ± 5 | 0.124 | 0.177 | 29 |
| F4 | 0.77 ± 0.01 | 2.0 ± 0.02 | 93.4 ± 0.1 | 3.3 ± 0.3 | 4.8 ± 0.7 | 74 ± 6 | 0.101 | 0.192 | 47 |
| F5 | 0.76 ± 0.01 | 1.40 ± 0.01 | 96.1 ± 0.1 | 2.2 ± 0.2 | 3.8 ± 0.7 | 84 ± 3 | 0.083 | 0.132 | 37 |
| F6 | 0.87 ± 0.01 | 1.96 ± 0.01 | 96.9 ± 0.1 | 2.1 ± 0.2 | 3.2 ± 0.8 | 96 ± 3 | 0.153 | 0.233 | 34 |

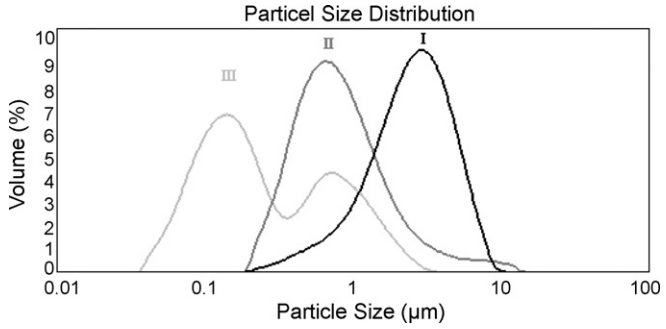


**Fig. 5.** Laser diffraction particle size distribution of (I) micronized tobramycin and (II) F5 formulation after spray-drying (III) F5 formulation before spray-drying measured with the Mastersizer Hydro 2000.

As can be seen from Fig. 5, the particle size distributions of both the micronized tobramycin and the F5 formulation obtained with the Mastersizer 2000® are unimodal and range from 0.2 to 10 μm. Nevertheless, most of the population for the F5 formulation lies in the smaller sizes, below 1 μm, as opposed to the micronized tobramycin. It is important to note that the particle size distribution of the F5 formulation obtained before and after spray-drying is different. Indeed, instead of the bimodal curve, it became a Gaussian curve (log normal distribution), spread up to a few tens of microns. This phenomenon could be explained by the fact that the population's finest particles aggregate during spay-drying in order to form a more homogeneous population of particles.

The $D(0.5)$ measured by the Mastersizer for all the formulations containing nanoparticles varied within a range of between 0.75 μm for F1 and 0.87 μm for F6. As expected, the particle size values of the nanoparticle formulations were smaller than those of the micronized tobramycin raw material. This median particle size appeared to be very different for the micronized tobramycin and the formulations containing nanoparticles, exhibiting a $D(0.5)$ value of about 3.6 and 0.8 μm, respectively. Consequently, the percentage of particles below 5.0 μm increased from 71% to 97% for the most effective formulation composed of nanoparticles (F6).

However, the $D(0.5)$ obtained for the formulations containing nanoparticles with the Spraytec® was higher than that obtained with the Mastersizer 2000, with a value about 3 μm. With these measurements, it was not possible to highlight the nanoparticle population of the formulations. This can be explained by the fact that the size results obtained from the Spraytec® included the presence of some agglomerates, probably corresponding to the population's finest particles. Indeed, spray-drying, used to retrieve particles in a desired powder state suitable for DPI, caused agglomeration of the nanoparticles. However, the volume mean diameter of the micronized tobramycin measured with the Spraytec® was about 11 μm, which is more than two times greater than the results for the nanoparticle formulations. Indeed, formulations containing nanoparticles present looser agglomerates with higher porosity, which are more easily dispersible in smaller agglomerates during inhalation. Fig. 6 shows that the F5 formulation has a Gaussian curve (log normal distribution), with 84% of particles below 5 μm, whereas micronized tobramycin shows a very large particle size distribution spread of up to more than 70 μm, with only 68% of particles below 5 μm. There is probably a problem of the raw powder agglomeration that is very prejudicial for pulmonary administration, consequently decreasing the FPD.

As can be seen from the results, the presence of a coating of nanoparticles around tobramycin micronized particles allowed an increase in the powder disagglomeration, with an increase in the percentage of particles below 5 μm of around 80% (F1, F2 and F3).

The forces of interaction between particles present barriers to their flow and dispersion. The major forces of interaction are Van der Waals, electrostatic, and capillary forces. Nevertheless, electrostatic and capillary forces are smaller than Van der Waals forces, which are derived from the energy of interaction between two molecules (Hickey, 2002):

$$F_{VW} = \frac{AD}{12z^2}$$

where $A$ is the Hamaker constant dependant on the particles' densities, $z$ the shortest distance between the particles, and $D = d_1 d_2/(d_1 + d_2)$, where $d$ is the diameter of the particles.

So, $F_{VW}$ may be decreased by decreasing $A$ or increasing $z$. Theoretically, the Hamaker constant can be decreased by decreasing the densities of the two interacting particles. Since the separation distance plays a significant role in Van der Waals attraction, any means to increase the distance will reduce the attractive force and increase the ease of dispersion. So, the presence of nanoparticles around the micronized particles of tobramycin allows a decrease in the density of the powder and an increase in the distance between particles, which will improve the particle dispersion, reducing the Van der Waals forces.

On the other hand, the powder agglomeration tendency can be decreased by adding Na glycocholate to the formulations composed solely of nanoparticles (F4, F5 and F6): the percentage of particles below 5.0 μm increased from 74% to 96% with an increase in Na glycocholate content from 1% to 5% (w/w relative to tobramycin weight, in dried form). Probably, loose agglomerates of nanoparticles were more easily scattered into small particles with the presence of the surfactant around the nanoparticles. These size properties, measured under simulated breathing conditions, allow a good approximation of the particle size distribution of the powders during inhalation by a patient.

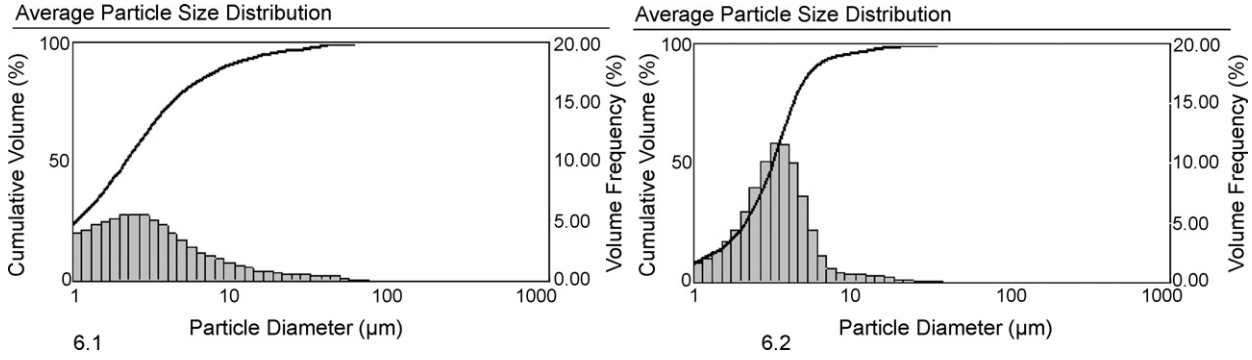


**Fig. 6.** Average particle size distribution and undersize curve measured with the Spraytec® of: (6.1) Micronized tobramycin and (6.2) F5 formulation.

**Table 5**
Fine particle deposition mg (capsule 15 mg, mean ± S.D., n = 3) of the different formulations measured with an MsLI

| | FPD |
|---|---|
| Tobramycin | 5.5 ± 0.9 |
| F1 | 6.7 ± 0.6 |
| F2 | 7.6 ± 0.5 |
| F3 | 7.5 ± 0.5 |
| F4 | 8.0 ± 0.5 |
| F5 | 9.0 ± 0.5 |
| F6 | 9.1 ± 0.3 |

*3.2.2. Aerodynamic behaviour*

Spray-dried formulations containing different proportions of nanoparticles and various concentrations of Na glycocholate were evaluated for their de-agglomeration behaviour in an air stream. The aerodynamic behaviour of the different spray-dried tobramycin formulations analyzed in an MsLI is shown in Table 5.

The tobramycin recoveries from the inhalator and the different parts of the MsLI were elevated for all the formulations evaluated as they range between 13.4 and 14.4 mg (between 89.7% and 96.2% of the total loaded drug, respectively).

The evaluation of the influence of the coating level with nanoparticles (F1, F2 and F3) showed that the presence of nanoparticles in the formulations improved the particle dispersion properties during inhalation. The FPD increased with the increase in the nanoparticle content. The FPD increased from 5.5 to 7.6 mg for the micronized tobramycin and the F3 formulation, respectively. This could be explained by the fact that a low content of nanoparticles probably did not permit homogenous cover of all micronized tobramycin particles and thus efficient reduction of their inherent agglomeration tendency. One microparticle can be completely covered with a single layer – or alternatively with several layers – of nanoparticles in function of the percentage of nanoparticles in the mixture. Coating of the fine drug particles with particles in the nanometer range might reduce Van Der Waals forces and powder agglomeration. These various layers of nanoparticles also decreased the cohesion of the powder by improving the slip of the particles between them.

On the other hand, suspensions containing solely nanoparticles were spray-dried with various concentrations of surfactant (F4, F5, F6) in order to produce easily dispersible and reproducible micron-size agglomerates of nanoparticles during inhalation. The results presented showed that the percentage of sodium glycocholate in the suspensions used for spray-drying had a significant effect on the FPF of the powders, with an increase from 8.0 mg with 1% sodium glycocholate (F4) to 9.1 mg with 2% sodium glycocholate (F5). Easily dispersible agglomerates of micron-size particles composed of nanoparticles of the drug were probably broken down into individual particles in the air stream when the particles were inhaled and were, therefore, more likely to reach the lower lung on inhalation. As it can be seen in Fig. 7, the production of nanoparticles of tobramycin with sodium glycocholate permitted a decrease in deposition in the device of the inhalator, while it increased deposition in the stage 4 and the filter of the MsLI, which is very beneficial for the patient in terms of drug-targeting efficiency. The evaluation of the influence of the concentration of surfactant showed that deposition of only 2% (w/w) (in the dry basis) is sufficient in order to improve particle dispersion properties during inhalation. Due to the fact that this model drug is a projected highly dosed drug, minimizing the additives (stabilizers, carriers, etc.) used in formulation development was necessary. These results reveal the need to add sufficient amounts of covering material in order to significantly modify particle surface properties and reduce their tendency to agglomeration, while limiting the additive level in the formulations in order to allow delivery of more of the active drug to the deep lung.

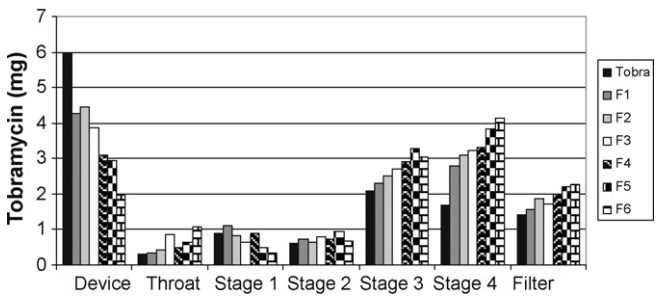


**Fig. 7.** In vitro deposition patterns (MsLI) of tested formulations.

Consequently, the use of nanoparticles in dry powder formulations increased the FPF from 36% for the uncoated micronized tobramycin to about 61% for the most effective formulation, in terms of deep lung penetration.

Moreover, a storage test under accelerated conditions of 40 °C and 75% relative humidity (RH) was carried out to estimate the stability of the samples. After 6 months, no degradation of tobramycin could be observed. All data retrieved show that the original particle size distribution can be restored (data not shown). The formulations were shown to keep their initial particle size distribution and aerodynamic behaviour.

## 4. Conclusion

The present study demonstrates the possibility of delivering formulations to the lungs that are made up of a mixture of nano- and microparticles of the active drug. On the one hand, nanoparticles were used to coat micron-size particles and on the other hand, formulations composed of solely nanoparticles were produced in order to form easily dispersible and reproducible micron-size agglomerates of particles. These new carrier-free dry powders, with only a small amount of surfactant, present high lung deposition properties.

Therefore, these formulations hold great potential for treating diseases that require direct lung delivery with reduced drug dosage and dosing frequency, leading to fewer systemic side effects and improved patient compliance. These carrier-free powders may prove instrumental for the treatment of pulmonary conditions such as tuberculosis and cystic fibrosis as they permit the delivery of high doses directly to the site of infection.

However, the efficiency and pharmokinetics of these formulations still remain to be evaluate in vivo.

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