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Research Paper

Formulation and Characterization of Lipid-Coated Tobramycin Particles for Dry Powder Inhalation

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Purpose. This study was conducted to develop and evaluate the physicochemical and aerodynamic characteristics of lipid-coated dry powder formulations presenting particularly high lung deposition.
Methods. Lipid-coated particles were prepared by spray-drying suspensions with different concentrations of tobramycin and lipids. The solid-state properties of the formulations, including particle size and morphology, were assessed by scanning electron microscopy and laser diffraction. Aerosol performance was studied by dispersing the powders into a Multistage Liquid Impinger and determining drug deposition by high-performance liquid chromatography.
Results. Particle size distributions of the formulations were unimodal, narrow with more than 90% of the particles having a diameter of less than 2.8 μm. All powder formulations exhibited mass median diameters of less than 1.3 and 3.2 μm, as determined by two different laser diffraction methods, the Malvern's Mastersizer® and Spraytec®, respectively. The fine particle fraction varied within a range of 50.5 and 68.3%.
Conclusions. Lipid coating of tobramycin formulations resulted in a reduced agglomeration tendency and in high fine particle fraction values, thus improving drug deposition. The very low excipients content (about 5% m/m) of these formulations offers the benefit of delivering particularly huge concentrations of antibiotic directly to the site of infection, while minimizing systemic exposure, and may provide a valuable alternative treatment of cystic fibrosis.

KEY WORDS: dry powder inhaler (DPI); lipid-coated particles; pulmonary delivery; spray drying; tobramycin.

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disease affecting more than 60,000 people worldwide (1). Mutations in the gene coding for chloride-channel protein, called the *CF transmembrane conductance regulator*, result in reduced mucociliary clearance, leaving CF patients especially vulnerable to endobronchial infections, particularly to *Pseudomonas aeruginosa* (2,3). Chronic airway infections are closely associated with progressive deterioration in lung function and subsequent mortality in adolescents and adults: patients lose an average of 2% of their lung function per year (4) and about 90% of all CF patients die due to progressive pulmonary disease (5,6).

The standard therapy for *P. aeruginosa* endobronchial infections in CF patients usually involves the administration of two parenteral antipseudomonal antibiotics, including a β-lactam and an aminoglycoside agent. The aim of antibiotic therapy in the chronically infected CF patients is to stabilize lung function and, if possible, to restore some of the lost lung function (7,8).

The bactericidal activity of tobramycin (*O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1-6)-*O*-[2,6 diamino-2,3,6-trideoxy-α-D-ribohexopyranosyl-(1–4)]-2-deoxy-D-streptamine) is accomplished by irreversibly binding to 30S and 50S ribosomal subunits resulting in a defective protein (9).

As aminoglycosides are highly polar, a poor drug penetration into the endobronchial space is generally observed when the parenteral route of administration is used. The mean peak sputum concentration after parenteral administration is only 12–20% of the peak serum concentration (6). Like other aminoglycosides, tobramycin has a comparably narrow safety margin. The therapeutic plasma concentration of tobramycin is in the range of 4–8 mg/L and may cause severe ototoxicity and nephrotoxicity in a long-term therapy (9).

The administration of aminoglycosides by inhalation offers an attractive alternative, delivering high concentrations of antibiotic directly to the site of infection while minimizing systemic bioavailability. Local delivery of medication to the lung is highly desirable, especially in patients with specifically pulmonary diseases, such as cystic fibrosis, asthma, chronic pulmonary infections, or lung cancer. The principal advantages include reduced systemic side effects and higher dose

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levels of the applicable medication at the site of drug action. Unlike the oral route of drug administration, pulmonary inhalation is not subject to first-pass metabolism (10).

The only inhalation device for tobramycin currently available on the market is a nebulization device, the Tobi® (Chiron Corporation, Emeryville, CA, USA), which delivers a solution of the drug. But nebulization has many well-documented disadvantages including extended administration time, high cost, low efficiency, poor reproducibility, risk of bacterial contamination, and the need for bulky compressors or gas cylinders (11).

A different approach in delivering drugs to the lung is the formulation of a dry powder inhalation (DPI) product that is activated and driven by the patient's inspiratory flow. In some cases, they deliver even a higher amount of drug to the lungs leading to the conclusion that lower drug doses delivered with certain DPI devices are as effective as pMDIs (12).

Administration of antibiotic dry powder aerosols to the lung has been attempted, but studies were limited by inefficient delivery devices and/or poorly dispersible lactose formulations (10). One clinical evaluation of dry powder tobramycin, using lipid-based Pulmosphere technology, has already been carried out, giving a mean whole lung deposition of 34 ± 6% (11).

Preparing dry powder formulations for inhalation is an attractive and appreciated approach because many solubility and stability issues can be avoided. Further advantages are low susceptibility to microbial growth and suitability of the technique for delivering both water-soluble and insoluble drugs (13). However, the small particle size necessary to achieve effective lung deposition causes problems in powder processing (poor flowability) and redispersion (strong agglomeration and adhesion) (14).

The percentage of the emitted dose deposited in the lungs is dependent on powder 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, which results in decreased drug deposition in the lung. These forces are proportional to the area of contact and the separation distance between the particles (15).

The micronization process leads to small, irregularly shaped flat particles. The smaller the particles, the stronger the cohesive forces become. Additionally, extensive flat surfaces promote large contact areas, resulting in increased adhesion between the particles. Micronized powders with high energetic surfaces show poor flow properties (16).

Micron-sized particles that are spherical can be obtained by spray drying. Such particles are characterized by a lower area of contact, and a smaller and more homogeneous particle size distribution that results in a higher respirable fraction than mechanically micronized drugs (jet mill) (17,18). This method also allows control over particle shape, morphology, and density, with variations obtained by using different specific spray drying conditions (19).

One of the principal purposes of aerolizing spray-dried powders is to achieve particle diameters of several micrometers with a narrow particle size distribution. This ensures, assuming an appropriate mass median aerodynamic diameter, a maximum deposition of the embedded drugs in the tracheobronchial and deep alveoli regions for normal inhalation rates (20). To reach the lower respiratory tract and optimize systemic drug absorption, dry powder aerosols need to present aerodynamic diameters of between 1 and 5 μm (21). Particles exceeding this range impact in the oropharynx, whereas submicron particles remain suspended in air and are exhaled (22).

In this work, we used lipids for coating tobramycin particles to improve drug targeting to the lung. Lipid deposition results in a modification of the surface properties of micron-sized tobramycin particles, which enables deep deposition in the lung.

A study has already been made in our laboratory on new compositions of solid lipid particles for inhalation and their potential use as carriers or as fillers to overcome problems related with the pulmonary administration of drug (23). Indeed, phospholipids and cholesterol, two physiologically well tolerated components, may present interesting characteristics for the delivery of drugs by the pulmonary route. Like liposomes, they may reduce local irritation, offering a good tolerance in the pulmonary tract as they are mainly constituted of biocompatible and biodegradable material; for example, phosphatidylcholine comprises an estimated 70–80% of the naturally occurring pulmonary surfactant pool (24). A novel approach of delivering liposomes in dry powder form was developed and was advantageous to avoid the detrimental effects of lyophilization and jet milling on leakage of the encapsulated drug (25). But lipids' coating offers better stability and higher encapsulation efficiency than liposomes. Finally, the hydrophobic nature of neutral lipids (cholesterol) reduces the absorption of the ubiquitous vapor, leading to a reduction of the aggregation and the adhesion of particles. Moreover, the presence of a low lipid-coating level allows the preparation of powders with few excipients, thereby delivering more active drug to the lungs.

The objective of this study was to further characterize small lipid-coated particles in terms of aerolization properties, surface composition, and physical state in order to determine which formulations could be the most suitable for pulmonary tobramycin delivery.

MATERIALS AND METHODS

Materials

Tobramycin was supplied as micronized powder (Tobra μ) from Plantex Chemicals (Mijdrecht, The Netherlands).

Cholesterol was purchased from Bufa (Uitgeest, The Netherlands). Phospholipon 90H (Ph90H), hydrogenated soy lecithin, with more than 90% of hydrogenated phosphatidylcholine (consisting of approximately 85% distearol phosphatidylcholine and 15% dipalmitoyl phosphatidylcholine), was donated by Nattermann Phospholipids (Koln, Germany).

All chemicals used were of analytical grade.

Methods

Preparation of the Lipid-Coated Particles

The formulations were prepared, at laboratory scale, by spray drying using a modified Büchi Minispray Dryer B-191a (Büchi Laboratory-Techniques, Flawil, Switzerland). The minispray dryer operates on the principle of nozzle spraying in a parallel flow (the sprayed product and the drying airflow

move in the same direction). Spray drying has been recognized as a successful process to generate powders from solutions in a single step. It converts a liquid feed (solution, coarse suspension, colloidal dispersion) to a dried particulate form. The principal advantages with respect to pulmonary drug delivery are the ability to manipulate and control particle size and size distribution, particle shape, and density in addition to macroscopic powder properties such as bulk density, flowability, and dispersibility. This minispray dryer allows the recovery of a range of particle size from 0.5 to 30 μm. The lower limitation is given by the separation of the cyclone used: smaller particles can no longer be separated and are going into the filter.

The inlet and outlet air temperatures in classical spray dryers are not independently controlled. Typically, the inlet temperature is established at a fixed value and the outlet temperature is determined by such factors as the spraying and drying gas flow rates, chamber dimensions, and feed flow rate.

In this study, we brought some modifications to the commercial minispray dryer to improve its drying efficiency and to avoid softening of the lipidic excipients incorporated in the formulations. The spraying gas was heated, increasing the droplets drying efficacy, and an air cooling system equipped with an air dryer generated cold air in the bottom level of the main drying chamber, permitting a decrease in the outlet temperature. Furthermore, a jacketed cyclone with cold water circulation was used to cool the cyclone separator walls and thus to reduce the adhesion and/or agglomeration of the lipids. The yield of the process, which is the ratio of all the powder collected in the container and the total drug dose in suspension, was about 75%.

Suspensions with different concentrations of tobramycin and lipids were prepared. Although tobramycin is practically insoluble in isopropanol (0.05 mg/mL), lipids are dissolved in it and coat the micron-sized particles during atomization.

First, lipids were dissolved in 50 mL isopropanol. Next, tobramycin was added and the suspension was homogenized with a CAT high-speed homogenizer X620 (CAT M. Zipperer, Staufen, Germany) at 24,000 rpm for 10 min. The suspensions were then spray-dried with constant stirring. The following conditions were used during spray drying: drying airflow, 35 m^3/h heated at 56°C; spraying airflow, 800 L/h; suspension feed rate, 2.7 g/min; nozzle size, 0.5 mm; cold air temperature, −5°C, generated at 10 m^3/h; cold water circulated in the jacketed cyclone at 5°C. The inlet temperature was established at 70°C and, in these conditions the outlet temperature varied between 17 and 20°C. The resultant powder was blown through the cyclone separator and collected in a container. Powders were stored in a dessicator at ambient temperature. The content of tobramycin was quantified by high-pressure liquid chromatography (HPLC) and was between 98.6 and 101.4%.

Particle Size

Particle size was measured by two different techniques based on laser light scattering.

Volume particle size distribution was measured with a Malvern Mastersizer 2000® laser diffractometer using a dry sampling system (Scirocco 2000, Malvern Instruments, Malvern, UK) with a suitable standard operating procedure (SOP) (refractive index: 1.52, vibration feed rate: 25%, measurement time: 7 s, dispersive air pressure: 4 bar).

Particle size distribution is characterized by the mass median diameter ($d_{0.5}$), i.e., the size in microns at which 50% of the sample is smaller and 50% is larger, and the volume mean diameter ($D_{4,3}$). Values presented are the average of at least three determinations.

The second technique used a Malvern Spraytec® (Malvern) 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 Multistage Liquid Impinger (MsLi). The entire assembly is a closed system and allows for 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.

Scanning Electron Microscopy

Evaluation of particle size and morphology was achieved via scanning electron microscopy (SEM), using a JSM-610 microscope (JEOL, Tokyo, Japan). Samples were scattered on a thin film of a two-component epoxy resin and then coated with a platinum layer. Acceleration during the observation was 25 kV.

X-Ray Powder Diffraction

X-ray powder diffraction (XRPD) is a powerful and widely used tool for crystalline state evaluation. Diffraction patterns of tobramycin formulations were determined using a Siemens diffractometer D5000 (Siemens, Munich, 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 2θ range of 2–70°.

Bulk Density

Bulk and tapped density were measured using a tap density tester (Stampfvolumeter, STAV 2003, Jel, Ludwigschafen, Germany). Bulk density was determined by filling the powder in a 10-mL measuring cylinder, and tapped density was measured by tap density measurements following 500 taps, which allowed the density plateau. Bulk and tapped density values allow the determination of the Carr's compressibility index by the formula:

$$\text{Carr's Index (\%)} = \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Tapped Density} \times 100}$$

Determination of Water Content

The water content of the dry powders was assessed by Karl Fisher titration. The measurements were performed with a 756 KF Coulometer (Metrohm Ltd., Antwerp, Belgium).

Table I. Composition of the Spray-Dried Suspensions Used for the Preparation of the Tobramycin DPI Formulations and Lipid Content of the Formulations (Dried Forms)

	Suspensions		Dried forms	
	Tobramycin (% w/v)	Lipids (% w/v)	Lipids (%)[a]	Cholesterol/ phospholipon (%) (w/w)
F1	2	0.10	5	75:25
F2	5	0.25	5	75:25
F3	10	0.50	5	75:25
F4	5	0.10	2	75:25
F5	5	0.50	10	75:25
F6	5	0.25	5	66:34
F7	5	0.25	5	90:10

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

Aerodynamic Particle Size Analysis

The aerodynamic particle size distribution was determined using a Multistage Liquid Impinger (MsLI). A dry powder inhalation device (Cyclohaler®; Novartis, Basel, Switzerland) was filled with a No. 3 Hypromellose (HPMC) capsule (Capsugel, Colmar, France). HPMC capsules were used because gelatin capsules have a tendency to break during the test and to produce agglomerates during particle size measurements. Moreover, with hygroscopic material such as tobramycin, gelatin capsules are not recommended because of their higher water content. The flow rate was adjusted to a pressure drop of 4 kPa, as typical for inspiration by a patient, resulting in a flow rate of 100 L/min during 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 HPLC analysis. For accuracy, each test was repeated three times.

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

The chemical structure indicates that tobramycin has five primary amines, one primary hydroxyl group and four secondary hydroxyl groups. Because of low chromophore in the molecule, direct HPLC methods for tobramycin are not straightforward (9). To increase the UV absorptivity of the molecule, a derivatization method is often applied. The suitable and validated quantification method was described in the USP 25.

All stages of the MsLI were filled with 20 mL water. Deposits in the device, the throat, and the four stages were collected in 100-mL volumetric flask with 1 mL H_2SO_4 1 N and the filter in a 50-mL volumetric flask with 1 mL H_2SO_4 1 N.

For the derivatization procedure, 4.0 mL of each flask was transferred to separate 50-mL volumetric flasks. To each flask were added 10 mL of 2,4-dinitrofluorobenzene reagent [solution of 2,4-dinitrofluorobenzene (Fluka Biochemica, Buchs, Switzerland) in alcohol containing 10 mg/mL] and 10 mL Tris(hydroxymethyl)aminomethane reagent (40 mL of a stock solution of Tris(hydroxymethyl)aminomethane (Pharminnova, Waregem, Belgium) in water containing 15 mg/mL with 160 mL of dimethyl sulfoxide (Merck Darmstadt,

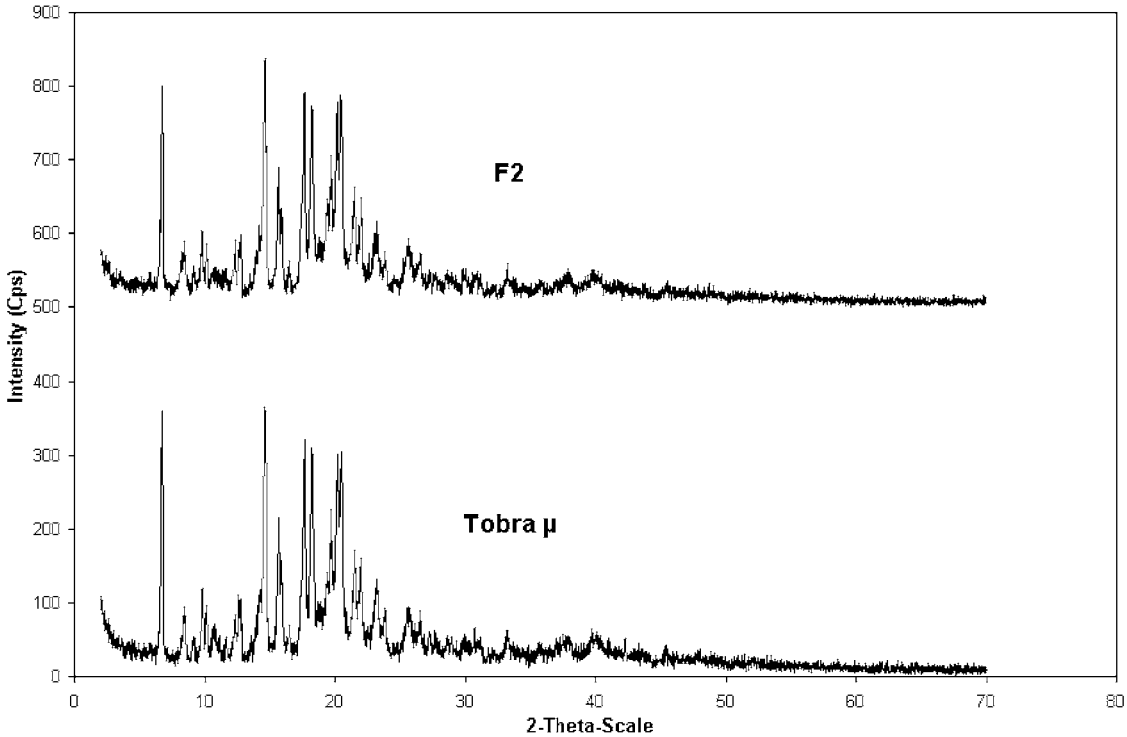

Fig. 1. X-ray powder diffraction patterns of raw tobramycin (Tobra μ) and F2 formulation.

Germany). After shaking, the flasks were placed in a constant temperature bath (GFL 1086, Germany) at 60°C and heated for 50 min with an agitation rate of 0.8 min^{-1}. Next, the flasks were removed from the bath and allowed to stand. After 10 min, acetonitrile was added to the volume and flasks were mixed.

The HPLC system consisted of a High Performance Liquid Chromatography system (HP 1100 series; Agilent Technologies, Brussels, Belgium), equipped with a quartenary pump, an autosampler, and a variable wavelength UV detector set at 365 nm. The separation system was 30 cm × 3.9 mm stainless steel (5 μm particle size) reversed-phase C18 column (Alltima; Alltech, Lokeren, Belgium). Samples of 20 μl volume were injected. The mobile phase was prepared by dissolving 2 g Tris(hydroxymethyl)aminomethane in 800 mL water. After this, 20 mL of H_2SO_4 1 N was added and then the solution was diluted with acetonitrile to obtain 2 L, mixed, and passed through a filter of 0.2 μm. The flow rate was 1.2 mL/min.

RESULTS AND DISCUSSION

Different powder compositions were formulated with the aim of studying the influence of the concentration of tobramycin in drug suspensions used for spray drying, the lipid film composition (cholesterol/Phospholipon ratio), and the coating level (in percentage) on the physicochemical and the aerodynamic characteristics of the powders. Table I gives an overview comparison of all powder formulations studied.

Physicochemical Characteristics

X-ray powder diffraction patterns (Fig. 1) show that the spray drying process did not affect the crystalline form of tobramycin, which is very interesting in terms of guaranteeing the long-term stability of the product. The peaks representing the lipid-coated spray-dried samples correspond to those for the original micronized tobramycin. The absence of peaks characterizing cholesterol and Phospholipon could

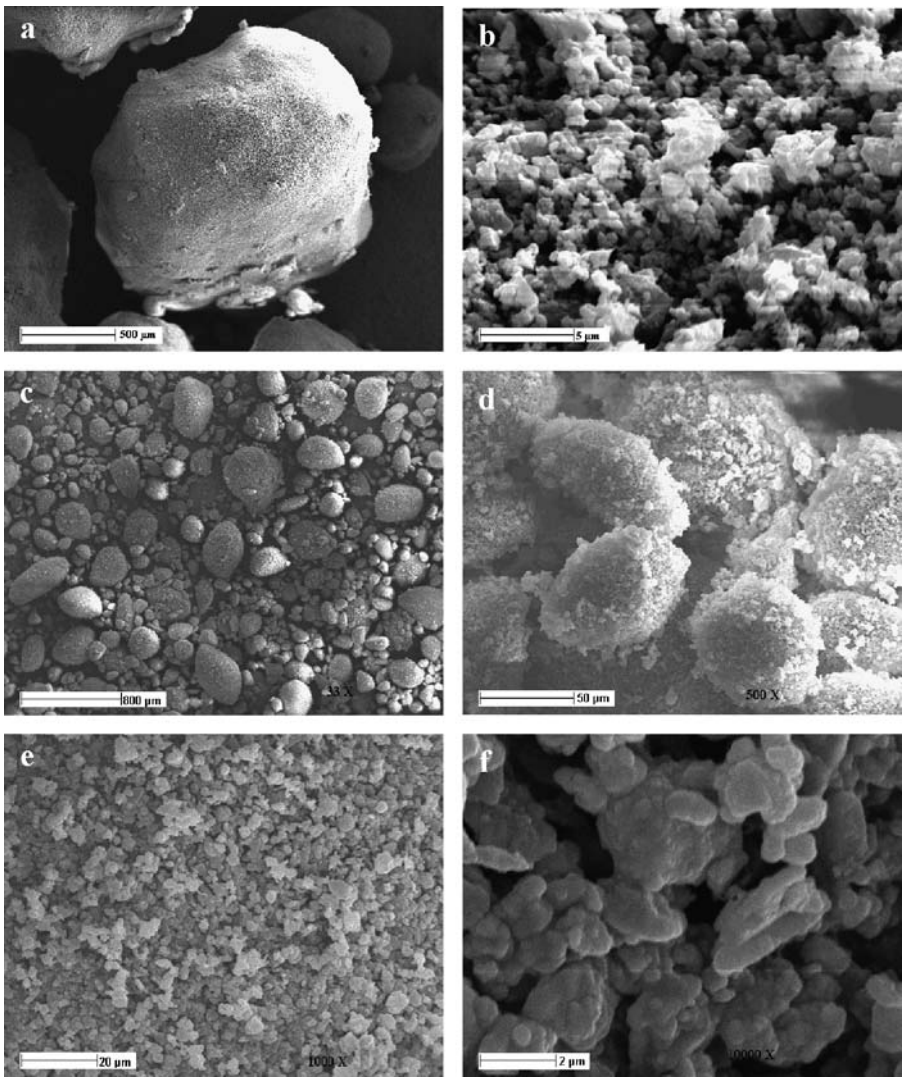

Fig. 2. SEM photographs of micronized tobramycin (raw material) and spray-dried lipid-coated tobramycin powder F2. (a) Micronized tobramycin, magnification 50×; (b) micronized tobramycin, magnification 1,000×; (c) F2 formulation, magnification 33×; (d) F2 formulation, magnification 500×; (e) F2 formulation, magnification 1,000×; (f) F2 formulation, magnification 10,000×.

Table II. Particle Size Characteristics of the Formulations $d_{0.5}$, $D_{4,3}$, and % < 5.0 μm (mean ± SD, $n = 3$) Measured with the Mastersizer 2000® Laser Diffractometer in Dry Powder Form

	$d_{0.5}$	$D_{4,3}$	% < 5.0 μm
Tobra μ[a]	1.29 ± 0.02	1.54 ± 0.01	99.3 ± 0.2
F1	1.24 ± 0.02	1.46 ± 0.03	99.8 ± 0.1
F2	1.28 ± 0.03	1.48 ± 0.05	99.7 ± 0.1
F3	1.23 ± 0.01	1.46 ± 0.01	99.6 ± 0.1
F4	1.27 ± 0.01	1.50 ± 0.01	99.6 ± 0.1
F5	1.38 ± 0.03	1.54 ± 0.04	99.9 ± 0.1
F6	1.38 ± 0.02	1.55 ± 0.01	99.8 ± 0.1
F7	1.29 ± 0.01	1.50 ± 0.01	99.6 ± 0.1

[a] Micronized tobramycin.

Table IV. Bulk Density, Tapped Density, and Carr's Index Values of All Formulations Tested (Mean, $n = 3$)

	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's index (%)
Tobra μ	0.229	0.300	23.6
F1	0.175	0.255	31.3
F2	0.169	0.242	30.2
F3	0.180	0.247	26.9
F4	0.162	0.221	26.9
F5	0.201	0.316	36.2
F6	0.171	0.260	34.2
F7	0.155	0.237	34.4

be explained by the lack of sensitivity of the method and the limited coating level (5% of tobramycin weight). Moreover, lipids are most probably partially present in an amorphous state as they are dissolved in isopropanol and obtained by rapid solvent elimination when using a spray drying process.

The Morphology and surface structure of the formulations were analyzed via SEM. The bulk tobramycin was formed by big, compact agglomerates of micron-size particles. Agglomerate sizes ranged up to 1 mm (Fig. 2a, b). The small tobramycin particles tended to form a very dense and cohesive structure. On the other hand, processing the suspensions by spray drying yielded more regularly shaped and micron-sized particles. The powder formulation F2 consisted of loose agglomerates of about 50 to 200 μm in size (Fig. 2c). At higher magnifications, we observe (Fig. 2e, f) that agglomerates are composed of small, smooth particles with a size of about 1–2 μm. The smoother particle surfaces probably explain the better dispersibility results obtained for the lipid-coated formulations in comparison with the bulk tobramycin (see Aerodynamic behavior). As a result of this morphology, the particles became light, as confirmed by bulk density measurements, and the spray-dried formulations presented good flowability.

The physical properties of the different formulations are summarized in Tables II, III and IV.

The median particle sizes seemed to be similar for all powder formulations, exhibiting a $d_{0.5}$ value of about 1.2–1.4 μm with Mastersizer 2000® and about 2.9–3.3 μm with Spraytec® (Tables II and III). Differences in size determination results obtained by the two laser diffraction methods can be explained by the differences in the particle dispersion capacity of the methods used. The higher compressed air values applied in the dispersion unit of the Mastersizer 2000® (up to 4 bar) permitted all the agglomerates to break down, especially for micron-sized powders, as is the case for DPI formulations. In contrast, the airflow generated in the Spraytec®, which simulates normal breathing conditions, was much lower and did not allow the deagglomeration of all particles. In other words, the Mastersizer 2000® permitted the determination of the size characteristics of totally individualized particles, whereas size results obtained from the Spraytec® included the presence of some agglomerates, probably corresponding to the population's finest particles. There was no correlation between the results obtained from Mastersizer 2000® and Spraytec®, probably because the size characteristics of the different powder formulations were very close: size results obtained with Mastersizer 2000®, at 4 bar, showed that the percentage of particles below 5 μm range from 99.3 to 99.9%.

Table III. Particle Size Characteristics of the Formulations $d_{0.5}$, $D_{4,3}$, and % < 5.0 μm (Mean ± SD, $n = 3$) Measured with Spraytec®

	$d_{0.5}$	$D_{4,3}$	% < 5.0 μm
Tobra μ	2.9 ± 0.7	8 ± 3	72 ± 7
F1	3.11 ± 0.01	3.22 ± 0.02	93.7 ± 0.1
F2	3.3 ± 0.3	3.16 ± 0.02	97 ± 1
F3	3.27 ± 0.09	3.14 ± 0.07	97 ± 1
F4	2.9 ± 0.3	4.2 ± 0.4	88 ± 2
F5	3.3 ± 0.1	3.43 ± 0.09	92 ± 1
F6	3.33 ± 0.08	3.06 ± 0.09	90.8 ± 0.3
F7	3.29 ± 0.01	3.12 ± 0.03	90.3 ± 0.5

Moreover, the particle size distributions of the formulations, obtained from Mastersizer 2000®, are unimodal, narrow, and range from 0.24 to 6 μm (Fig. 3), with more than 90% of the particles having a diameter below 2.8 μm, which is required for an optimal deep lung deposition. The mass median diameters and the volume mean diameters of the formulations were very tiny and ranged from 1.23 to 1.38 μm and from 1.46 to 1.55 μm, respectively. There were no major differences between lipid-coated formulations and the micronized tobramycin. Thus the coating of the micronized tobramycin particles with lipidic excipients did not affect the particle size of the raw material.

However, the volume mean diameter of the micronized tobramycin measured with Spraytec® was about 8 μm, which is more than two times greater than the results from the lipid coated formulations. Figure 4 shows that the F2 formulation has a Gaussian curve (log normal distribution), with 97% of particles below 5 μm, whereas micronized tobramycin shows a very large particle size distribution spread up to a few tens of microns, with only 72% 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 shown by these results, the application of a lipid coating around the active particles allowed an improvement in particle dispersion from the inhalator, thus enhancing the drug deposition deep in the lungs (loose agglomerates were easily scattered into small particles).

Carr's index values of less than 25 are usually taken to indicate good flow characteristics, whereas values above 40 indicate poor powder flowability. The results (Table IV) showed slightly higher Carr's index values for lipid-coated

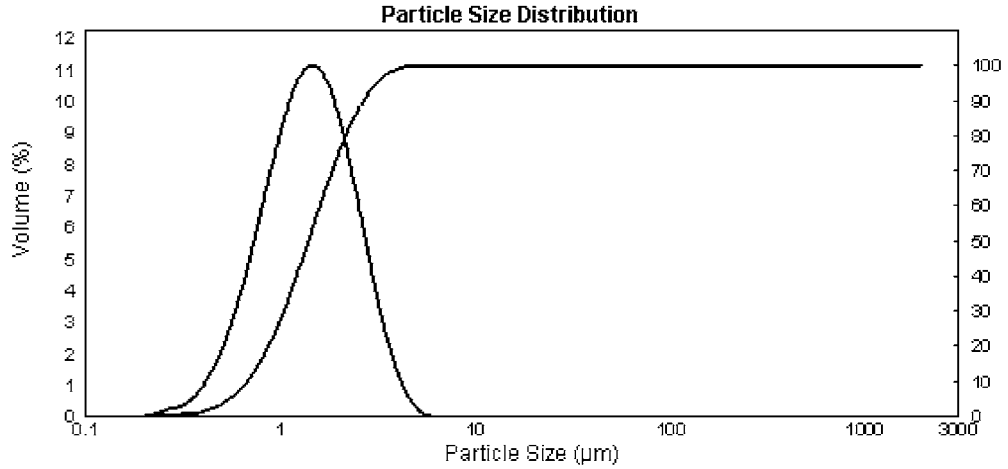

Fig. 3. Laser diffraction particle size distribution and undersize curve of F2 formulation measured with the Mastersizer 2000®.

formulations than for micronized tobramycin. Moreover, these results seem to be influenced by the amount of lipids and the lipids' composition in formulations. More particularly, drug particles covered with higher amounts of lipids (F5, 10% lipids) or with lipid compositions containing cholesterol/Phospholipon ratios of 66:34 (F6, particles with higher tendency to stick together when phospholipid content is increased) and 90:10 (F7, generation of electrostatic charges when phospholipid content is decreased) give the highest Carr's index values and thus poorer flowability. Nevertheless, the flow characteristics of all formulations were acceptable.

Except for the F5 formulation containing the greatest amounts of lipids (higher tendency to stick), the bulk and the tapped density values obtained for the lipid-coated formula-

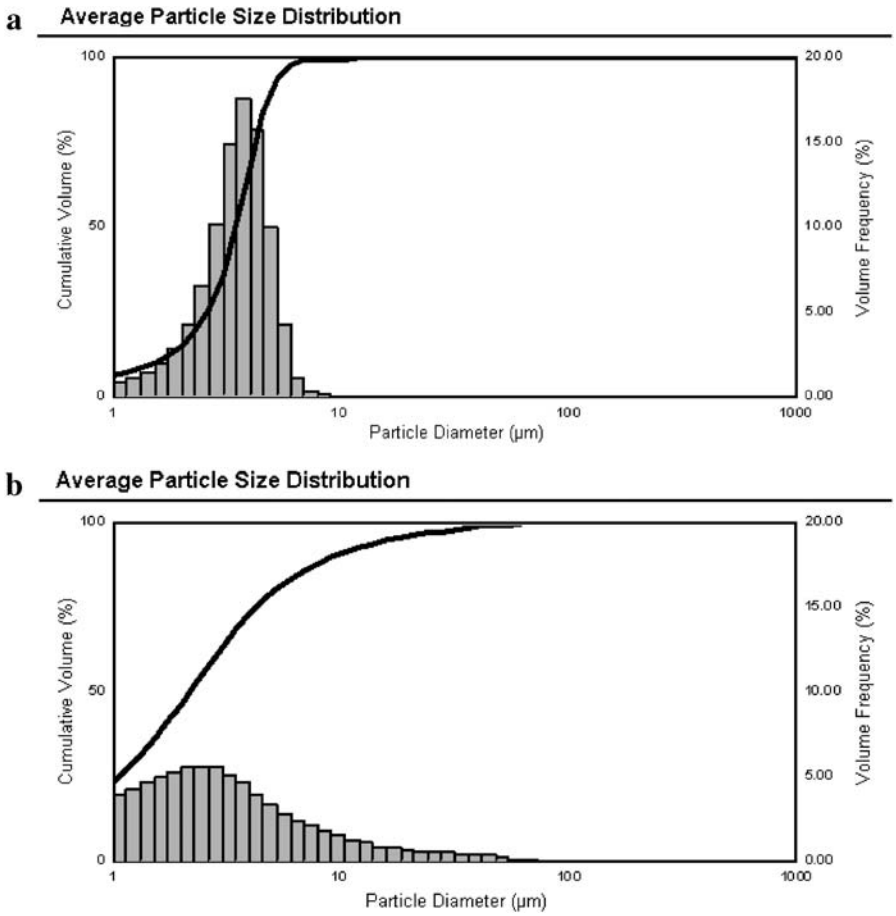

Fig. 4. Average particle size distribution and undersize curve measured with the Spraytec® of (a) F2 formulation and (b) micronized tobramycin (raw material).

Table V. Fine Particle Deposition mg (mean ± S.D., $n = 3$) of the Different Formulations Measured by an MsLI

	Tobra µ	F1	F2	F3	F4	F5	F6	F7
Device	4.7 ± 0.2	2.5 ± 0.1	1.9 ± 0.2	2.4 ± 0.3	4.2 ± 0.3	1.47 ± 0.09	2.12 ± 0.01	2.0 ± 0.4
Throat	0.34 ± 0.07	0.53 ± 0.05	0.74 ± 0.08	0.8 ± 0.2	0.6 ± 0.2	1.9 ± 0.2	1.4 ± 0.2	1.5 ± 0.2
Stage 1	0.76 ± 0.07	0.3 ± 0.3	0.27 ± 0.06	0.29 ± 0.08	0.9 ± 0.2	0.43 ± 0.08	0.4 ± 0.1	0.35 ± 0.06
Stage 2	0.8 ± 0.1	0.4 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.64 ± 0.08	1.2 ± 0.2	0.91 ± 0.01	0.9 ± 0.2
Stage 3	2.8 ± 0.4	2.8 ± 0.3	3.0 ± 0.2	2.9 ± 0.3	2.4 ± 0.2	3.6 ± 0.2	3.1 ± 0.2	3.05 ± 0.01
Stage 4	2.9 ± 0.1	3.6 ± 0.1	3.9 ± 0.1	3.5 ± 0.3	3.1 ± 0.2	3.58 ± 0.05	3.77 ± 0.03	3.63 ± 0.04
Filter	1.64 ± 0.07	3.4 ± 0.2	3.5 ± 0.3	3.9 ± 0.3	2.08 ± 0.08	2.1 ± 0.3	1.9 ± 0.2	2.1 ± 0.1
FPD	7.2 ± 0.6	9.8 ± 0.5	10.2 ± 0.2	10.3 ± 0.8	7.6 ± 0.5	9.1 ± 0.2	8.7 ± 0.4	8.7 ± 0.1
Metered dose	13.8 ± 0.6	13.6 ± 0.4	13.8 ± 0.2	14.4 ± 0.9	14.0 ± 0.1	14.3 ± 0.2	13.7 ± 0.1	13.5 ± 0.1

tions were slightly lower than those obtained for the micronized tobramycin (Table IV). The small decrease in powder density can probably be explained by the preparation process used to obtain the lipid-coated particles, as spray drying usually generates "light" particles with higher porosity.

Because the amount of free water in a powder influences its physical stability and controls the magnitude of capillary forces holding particles in aggregates, residual moisture content was measured. Water content was 3.64% for the micronized tobramycin and 2.26% for F2 formulation. The lipid-coated formulations seemed to be slightly dryer than the raw material. Lipid coating with spray drying decreased the reabsorption of water by tobramycin particles, which are very hygroscopic. Thus, physical stability in long-term storage and deagglomeration of tobramycin powder are improved.

Aerodynamic Behavior

Besides the particle size of a drug powder, the deagglomeration behavior in air stream as well as the flowability are important indicators of how the powder deposits in the lungs and how drug delivery to the lung might occur. Laser diffraction gives geometric particle dimensions, whereas MsLI measures the aerodynamic diameter (26). The aerodynamic behavior of the different tobramycin formulations analyzed in an MsLI is shown in Table V.

The results indicated that the FPD, which roughly corresponds to the drug deposition at stages 3, 4, and the filter (cutoff diameters of 5.27, 2.40, and 1.32 µm, respectively), varied within a range of 7.6 and 10.3 mg. The tobramycin recoveries from the inhalator and the different parts of the MsLI are particularly elevated for all the formulations evaluated as they range between 13.5 and 14.4 mg (between 89.7 and 96.2% of the total loaded drug, respectively).

As shown in Fig. 5, there was a good correlation between the particle size data generated via Spraytec® and the MsLI for the different lipid-coated formulations; the R^2 value was found to be 0.9533. Spraytec® can give a good indication of the aerosolization performance of DPI formulations and can thus be used as a rapid preliminary test for this purpose, in association with the recommended methods described in international pharmacopoeias, which require the use of very heavy experimental procedures.

The results presented in Table V also showed that for the same lipid composition of the formulations in the dried powder (5% lipids, cholesterol/Phospholipon 75:25 ratio), the percentage of tobramycin in the suspensions used for spray drying had no significant effect on the FPD of the powders (F1, F2, and F3, FPD values of 9.8, 10.2, and 10.3 mg).

It is interesting to note that the presence of lipids markedly enhances the FPD, which is about 7.2 mg for micronized tobramycin and between 7.6 and 10.3 mg for the

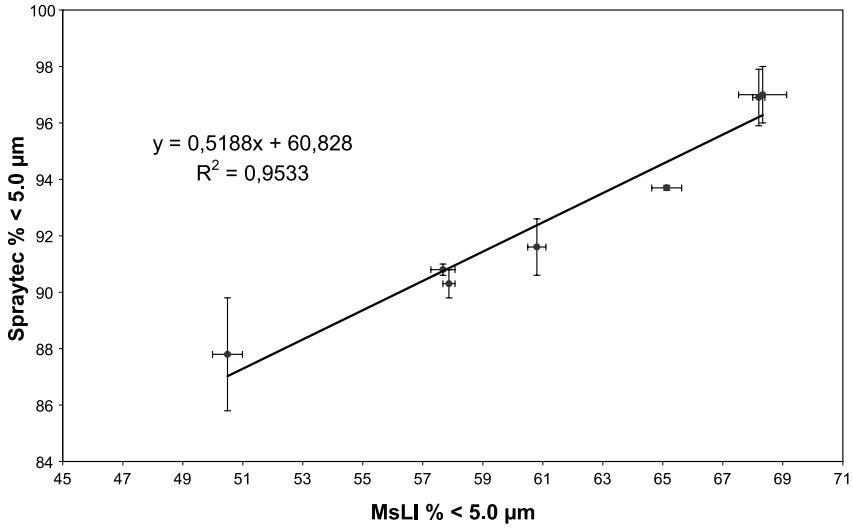

Fig. 5. Correlation between the percentage of particles below 5.0 µm measured with the Spraytec® and with the MsLI and the error bars x and y.

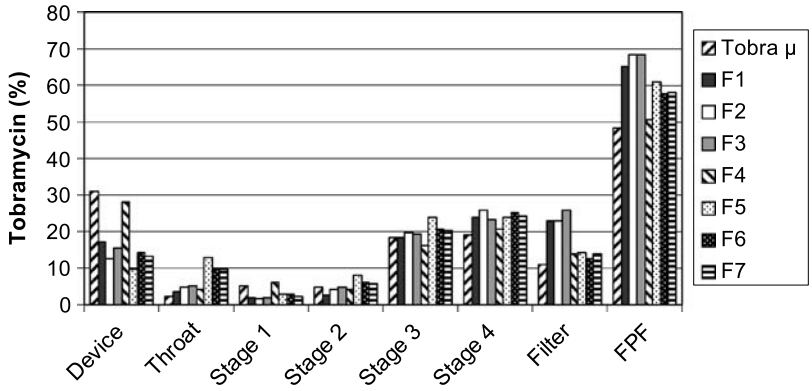

Fig. 6. *In vitro* deposition patterns (MsLI) of all formulations tested.

lipid coated formulations. As shown in Fig. 6, the presence of a lipid coat around tobramycin particles allowed a decrease in deposition in the device of the inhalator, whereas it increased deposition in the filter of MsLI, which is very beneficial for the patient in terms of drug targeting efficiency. The FPF, which is around 48% for the uncoated micronized tobramycin, is increased by up to about 68% for the most effective lipid-coated formulation, in terms of deep lung penetration.

Evaluation of the influence of the coating level (F4, F2, and F5 at 2, 5, and 10% w/w lipids, respectively) showed that the deposition of only 5% w/w lipids (in the dry basis) is sufficient to improve particle dispersion properties during inhalation (Table V, Figs. 6 and 7). As can be expected, the least effective formulation is F4, which has an FPF of 50%. For this formulation, the addition of a very small amount (2% w/w) of lipid coating likely did not permit the homogenous cover of all micronized tobramycin particles, and the efficient reduction of their inherent agglomeration tendency. On the other hand, as discussed above, an increase in the lipid content of the formulations to up to 10% (F5) also seems to induce some particle-sticking and produces an increase in particle density because of the relatively low melting temperature of lipids, and more particularly of the phospholipids present in the formulations. As a consequence, the best results in terms of drug deposition were obtained with compositions containing 5% lipids (F2). These results reveal the need to add sufficient amounts of covering material in order to significantly modify particle surface properties and to reduce their tendency toward agglomeration, while limiting the lipid level in the formulations to prevent any undesirable sticking and to allow the delivery of greater amounts of the active drug to the deep lung.

Similar conclusions can be made if one compares fine-particle deposition results obtained from formulations containing different cholesterol/Phospholipon ratios (F2, F6, F7; Table V, Figs. 6 and 7). An increase in phospholipid content of coatings as in F6 (66:34) tends to increase particle-sticking and agglomeration, which decreases particle deposition in the lungs. Nevertheless, F7, with only 10% of Phospholipon, presents an FPF of 57.9%, which shows the beneficial effect of a relatively low content of phospholipids in the coating because this reduces the generation of electrostatic charges at the particles' surface, as is more particularly observed in particles covered solely by cholesterol (data not shown). Thus it seems that a cholesterol/Phospholipon ratio of 75:25 is the most appropriate one because it reveals the best deposition pattern and gives the highest FPF (Fig. 7).

As shown in Fig. 7, the highest FPF values (about 65 and 68%) were obtained for the formulations prepared by spray drying from suspensions containing 2, 5, or 10% w/v of tobramycin, and coated with 5% of lipids with the most appropriate cholesterol/Phospholipon ratio of 75:25.

These FPF results are especially elevated and very promising comparing to the FPF value of the commercially available tobramycin nebulizer product, Tobi®, which contains 300 mg tobramycin free base in 5 mL sodium chloride at pH 6.0. An *in vivo* study on this product has shown that, after 15 min of nebulization, only 5% of the nominal dose was

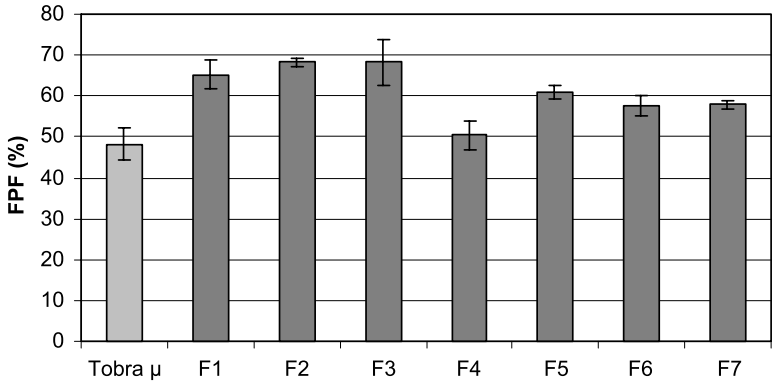

Fig. 7. The FPF assessment (mean ± SD) of the different formulations loaded with 15 mg tobramycin. Each bar represents the average of three repeats.

deposited in the lung (9). Other reports indicate 11% (6) or 20% (27) of delivered dose, depending on the choice of the compressor.

These new lipid-coated tobramycin DPI formulations based on the use of very low excipient levels and presenting very high lung deposition properties offer very important perspectives in improving the delivery of drugs to the pulmonary tract. These formulations are more particularly useful for drugs that are active at relatively high doses, such as antibiotics, because they permit the delivery of a high concentration of antibiotic directly to the site of infection while minimizing systemic exposition. A reduction in administration time and systemic side effects improves the suitability of these formulations to patients.

CONCLUSION

The present study demonstrates that the use of physiological lipid compositions, based on mixtures of cholesterol and phospholipids, to form a coating film around micronized drug particles, offers improved delivery of tobramycin to the pulmonary tract. The resulting powders exhibit a large surface area and a low bulk density. The size and shape of spray-dried powders are suitable for deep lung deposition of drugs. Particles prepared with lipids were small and presented very high FPF results. Due to particle properties, good flowability was observed, making the powders ideally suitable for use in carrier-free dry powder inhalers.

The 15-min nebulization of 300 mg Tobi® would be advantageously replaced with the inhalation of four 15-mg capsules or two 30-mg capsules of lipid-coated tobramycin.

The combination of localized drug delivery and improved lung deposition might be particularly useful, especially for drug substances such as antibiotics that are active at high dose ranges.

However, it still remains to be determined if the formulations are physically and chemically stable in long-term storage, or if they effectively yield better results *in vivo*. A randomized clinical trial on volunteers will be performed to evaluate the efficiency, pharmacokinetics, and bioavailability of these formulations.

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