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NEW HIGHLY EFFECTIVE DRY POWDER TOBRAMYCIN FORMULATIONS FOR INHALATION IN THE TREATMENT OF CYSTIC FIBROSIS

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ABBREVIATIONS

^{99m}Tc	Sodium Pertechnetate
ACI	Andersen Cascade Impactor
AUC	Area Under the Curve
C_{max}	Maximal plasma concentration
CF	Cystic Fibrosis
CFC	Chlorofluorocarbon
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
COPD	Chronic Obstructive Pulmonary Disease
CV	Coefficient of Variation
Daer	Aerodynamic Diameter
Dgeo	Geometric Diameter
DPI	Dry Powder Inhaler
DPPC	Dipalmitoylphosphatidylcholine
DSC	Differential Scanning Calorimetry
EDTA	Ethylene Diamine Tetraacetic Acid
FEV_1	Forced Expiratory Volume in one second
FDA	Food and Drug Administration
FPD	Fine Particle Dose
FPF	Fine Particle Fraction
FVC	Forced Vital Capacity
GSD	Geometric Standard Deviation
HFA	Hydrofluoroalkanes
KF	Karl Fisher titration method
HPH	High Pressure Homogenization
HPLC	High Pressure Liquid Chromatography
HPMC	Hydroxypropyl Methyl Cellulose
HSH	High Speed Homogenization
ICH	International Conference on Harmonisation
MDI	Metered Dose Inhaler
MMAD	Mass Median Aerodynamic Diameter
MOC	Micro-Orifice Collector
MsLI	Multi-stage Liquid Impinger
NAL	Nacystelyn
NGI	New Generation Impactor
PLGA	Poly(lactic-co-glycolic) acid polymer
PSD	Particle Size Distribution
RH	Relative Humidity
SCF	Supercritical Fluid
SD	Standard Deviation
SEM	Scanning Electron Microscopy
S.E.M.	Standard Error Mean
T_{max}	Time to reach maximum plasma concentration
TGA	Thermogravimetric Analysis
XRPD	X-Ray Powder Diffraction

I. SUMMARY

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Local delivery of medication to the lung is highly desirable as the principal advantages include reduced systemic side effects and higher dose levels of the applicable medication at the site of drug action. This administration could be particularly useful for patients with specifically chronic pulmonary infections or pulmonary diseases, such as cystic fibrosis, asthma or lung cancer.

In order to deliver a high dose range of medication for highly-dosed drugs such as antibiotics, "carrier-free" DPI formulations of tobramycin were developed with the aim of minimizing the use of excipients. Briefly, dry powders were prepared by spray drying various suspensions of tobramycin in isopropanol.

First, as particle size is a key parameter in defining drug deposition in the lungs, the new Spraytec® laser diffraction method specifically modified for measuring the PSD of aerosolized drug was evaluated. The dispersion properties of various dry powder formulations were investigated using different laser diffraction and impaction apparatuses at different flow rates and using different inhalator devices. Different correlations between geometric and aerodynamic size data were demonstrated in this study. As a potential application, for the flow rate, the different inhalation devices and the drug formulations examined, the tobramycin fine particle fraction could be predicted from measurements obtained from the Spraytec® using linear relationships. Correlations ($R^2 > 0.9$) between the MMAD and the percentage of particles with a diameter below 5 μm could be demonstrated between the results obtained from the laser diffraction technique and the impaction method. Consequently, the Spraytec® laser diffraction technique was proved to be an important tool for initial formulation and process screening during formulation development of DPIs.

In order to modify the surface properties of the raw tobramycin powder, 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 aerodynamic characteristics of the antibiotic.

The results indicated that the application of a lipid coating around the active particles allowed an improvement in particle dispersion from the inhalator, decreasing raw powder agglomeration and thus enhancing drug deposition deep in the lungs. Moreover, these results seemed to be influenced by the amount and composition of the lipids in the formulations. The evaluation of the influence of the coating level showed that the deposition of only 5% w/w lipids (on a dry basis) was sufficient to improve particle dispersion properties during inhalation. The FPF, which is around 36% for the uncoated micronized tobramycin, was increased to up to about 68% for the most effective lipid-coated formulation. Of particular importance, these results revealed the need to add sufficient amounts of covering material in order to significantly modify the particle surface properties and reduce their tendency to agglomeration, while limiting the lipid level in the formulations in order to avoid any undesirable sticking and to allow the delivery of more of the active drug to the deep lung.

Another approach used to modify the surface properties of raw tobramycin was to coat the micronized particles with nanoparticles of the drug, produced by high pressure homogenization. The evaluation of the influence of the level of nanoparticle coating of the micronized particles showed that the presence of nanoparticles in the formulations improved the particle dispersion properties during inhalation. One microparticle was completely covered with a single layer or several layers of nanoparticles, in function of the percentage of nanoparticles in the mixture. Coating the fine drug particles with particles in the nanometer range was believed to reduce Van Der Waals forces and powder agglomeration. These various layers of nanoparticles also allowed a decrease in the cohesion of the powder by improving the slip between the particles.

On the other hand, suspensions containing solely nanoparticles were spray dried with various concentrations of surfactant in order to produce easily dispersible and reproducible micron-size agglomerates of nanoparticles during inhalation. The evaluation of the influence of the concentration of surfactant showed that deposition of only 2% w/w (on a dry basis) of Na glycocholate is sufficient to improve particle dispersion properties during inhalation. Consequently, the use of nanoparticles in dry powder formulations increased the FPF from 36% for the uncoated micronized tobramycin to about 61% for this latter formulation.

To modify the balance between the different forces of interactions without the need for any excipient, the influence of formulation components on the aerosolization characteristics of spray-dried tobramycin through the use of various proportions of water in the solvent used to prepare initial suspensions was investigated. These results showed that it is possible to modify the surface properties of the particles by coating the particles of drug with a homogeneously distributed film of the active compound dissolved in a solvent system containing a mixture of different solvents such as isopropanol and water. During nebulization of the suspension, droplets are composed of one or more particles in solid state surrounded with solvent containing the dissolved drug. It is hypothesized that during the drying step, dissolved tobramycin forms a coating of the amorphous drug around particles in suspension. The coating of drug particles can thus be used as an alternative approach that permits the modification of the surface properties of the particles, increasing the flowability, the desagglomeration tendency and the fine particle fraction deposited in the deep lung. So, the evaluation of the influence of the water content of the suspensions and the effect of the inlet temperature during spray-drying showed that the addition of 2% water v/v is sufficient to improve particle dispersion during inhalation. Of particular interest, as tobramycin is a very hygroscopic drug, the addition of water turned out to be a critical step. It was thus important to add a small amount of water to the solvent system and to process the drying step at a high temperature to produce formulations containing solely the active drug and showing a FPF of up to 50%.

Moreover, stability studies demonstrated that these optimized formulations (lipid-coated formulation, nanoparticle formulation and amorphous drug-coated formulation) were stable over a long time period at various ICH temperature and relative humidity storage conditions (25°C/60% RH, 30°C/65% RH and 40°C/75% RH). The formulations were shown to keep their crystalline state, initial PSD, redispersion characteristics and deposition results for more than twelve months.

In order to confirm these encouraging results, two optimized formulations (one with a lipid coating and another with amorphous drug coating) were selected and compared to the only commercially available tobramycin formulation for inhalation, Tobi® (nebulizer

solution), by performing a combined *in vivo* scintigraphic and pharmacokinetic evaluation of tobramycin DPIs in nine CF patients.

In comparison with Tobi®, it was estimated that lung deposition, expressed as a percentage of the nominal dose, was 7.0 and 4.5 times higher for the lipid-coated and amorphous tobramycin-coated formulations, respectively. Moreover, the pharmacokinetic data, adjusted to the same drug dose as that of the Tobi® deposited in the lungs, showed that the AUC values were found to be 1.6 times higher for Tobi® than for DPI formulations. So this evaluation confirmed the superiority of dry powder formulations in terms of drug deposition and reduced systemic exposure in comparison with the conventional comparator product, Tobi®.

Thus, these new and orginal tobramycin DPI formulations based on the use of very low excipient levels and presenting very high lung deposition properties, were shown to offer very good prospects for improving the delivery of drugs to the pulmonary tract and to the widest possible patient population.

II. INTRODUCTION

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II. 1. DRUG DELIVERY ROUTES TO THE LUNGS

A drug delivery system is defined as a formulation or a device or a combination of a formulation and a device that enables the introduction of a therapeutic substance into the body, and improves its efficacy and safety by controlling the rate, time and place of release of drug in the body. This process includes the administration of the therapeutic product, the release of the active ingredients by the product, and the subsequent transport of the active ingredients across the biological membranes to the site of action (Jain, 2008).

Drugs may be introduced into the human body by various anatomical routes. The choice of the route of administration depends on the disease, the effect desired, and the product available. As the systemic circulation supplies the whole lung, drugs to the pulmonary system may be given systemically by oral or parenteral delivery or administered directly to the organ affected by the disease.

II.1.1. Oral drug delivery

Historically, the oral route of drug administration has been the one most used for both conventional as well as novel drug delivery. The reasons for this preference are obvious because of the ease of administration and its widespread acceptance by patients. Major limitations of the oral route of drug administration are as follows (Jain, 2008):

1. Drugs taken orally for systemic effects have variable absorption rates and variable plasma concentrations, which may be unpredictable.
2. The high acid content and ubiquitous digestive enzymes of the digestive tract can degrade some drugs well before they reach the site of absorption into the bloodstream. This is a particular problem for aminoglycosides, ingested peptides and proteins.
3. The drug may be inactivated in the liver on its way to the systemic circulation.

4. As there is a dilution of the drug into the systemic circulation, high doses of drugs have to be administered in order to reach the therapeutic concentration in the lung, therefore causing enhanced systemic side effects. So the oral route may not be suitable for drugs targeted to specific organs.

II.1.2. Parenteral drug delivery

Parenteral literally means introduction of substances into the body by routes other than the gastrointestinal tract but in practice the term is applied to injection of substances by subcutaneous, intramuscular, intravenous, and intra-arterial routes. Parenteral administration of drugs is now an established part of medical practice and is the most commonly used invasive method of drug delivery. Major drawbacks of parenteral administration are as follows (Jain, 2008):

1. Injection is not an ideal method of drug delivery because it involves pain and patient compliance becomes a major problem.
2. As with the oral route, there is a dilution of the drug into the systemic circulation and it may not be suitable for drugs targeted to specific organs.

II.1.3. Pulmonary drug delivery

Drugs may be delivered directly to the lungs for local treatment of pulmonary conditions. Although simple inhalation devices and aerosols containing various drugs have been used since the early 19th century for the treatment of respiratory disorders, the interest in the use of the pulmonary route for systemic drug delivery is recent. Interest in this approach has been further stimulated by the demonstration of the potential utility of the lung as a portal for the entry of peptides and of the feasibility of gene therapy for cystic fibrosis. The advantages of pulmonary drug delivery are as follows (Jain, 2008):

1. Useful for local treatment and systemic distribution
2. A large surface area available for absorption
3. Close proximity to blood flow, highly vascularised tissue
4. Rapid absorption
5. Avoidance of first pass effect
6. Avoidance of the effects of gastric stasis and pH
7. Smaller doses required than by the oral route to achieve equivalent therapeutic effects.

II.2. CHARACTERISTICS OF THE LUNG

II.2.1. Anatomy of the airways

The airways may be viewed as a series of dividing passageways originating at the trachea and terminating at the alveolar sacs. The respiratory system consists of two tracts, upper and lower. The **upper respiratory tract**, consisting of the naso- and oropharynx and larynx, extends from the nostrils to the junction of the larynx and trachea. The oropharynx communicates with the mouth and serves as a passageway for food and air. The larynx connects the pharynx to the trachea, and conducts air to and from the lungs. The **lower respiratory tract** consists of tracheobronchial, gas-conducting airways and gas exchanging acini (Fig. 1).

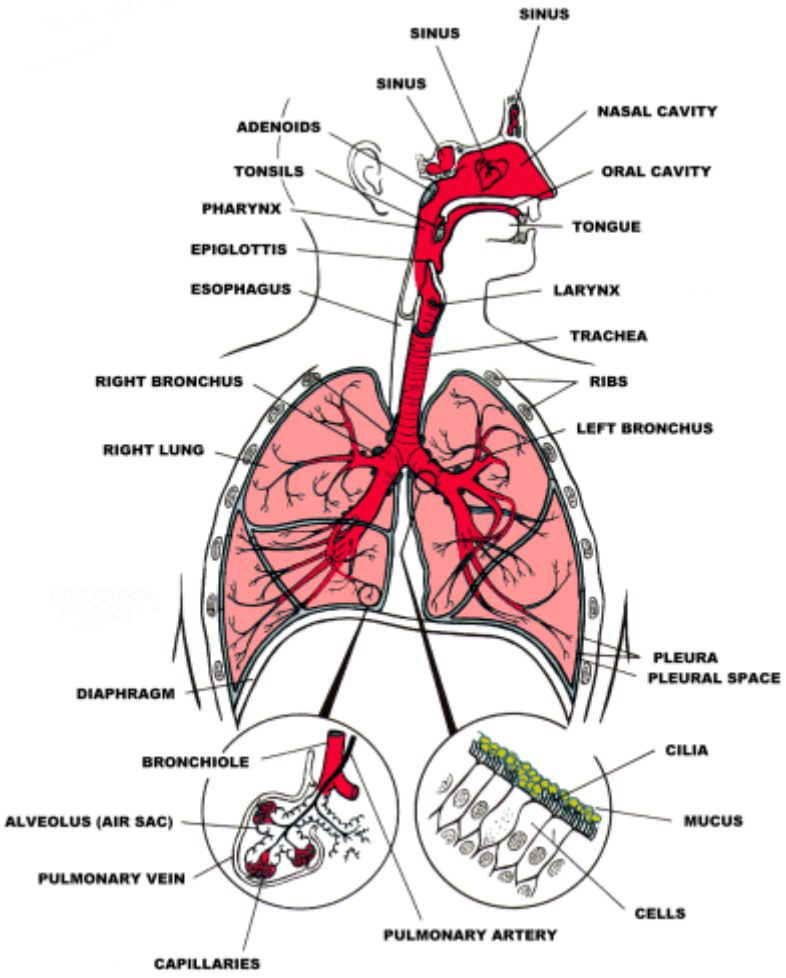


Figure 1: Schema of the respiratory system (The respiratory system, 2008)

The bronchial tree trunk begins with the trachea of the airways, which bifurcates to form main bronchi. As the trachea passes behind the arch of the aorta, it divides into two smaller branches: the left and right primary bronchi. Each primary bronchus divides into still smaller secondary bronchi, or lobar bronchi - one for each lobe of the lung. The secondary bronchi branch into many tertiary (or segmental) bronchi that further branch several times, ultimately giving rise to tiny bronchioles that subdivide many times, finally forming terminal bronchioles and respiratory bronchioles (Fig. 2). Each respiratory bronchiole subdivides into several alveolar ducts that end in clusters of small thin-walled air sacs called alveoli, which open into a chamber called the alveolar sac (Parks, 1994; Ross et al., 1995).

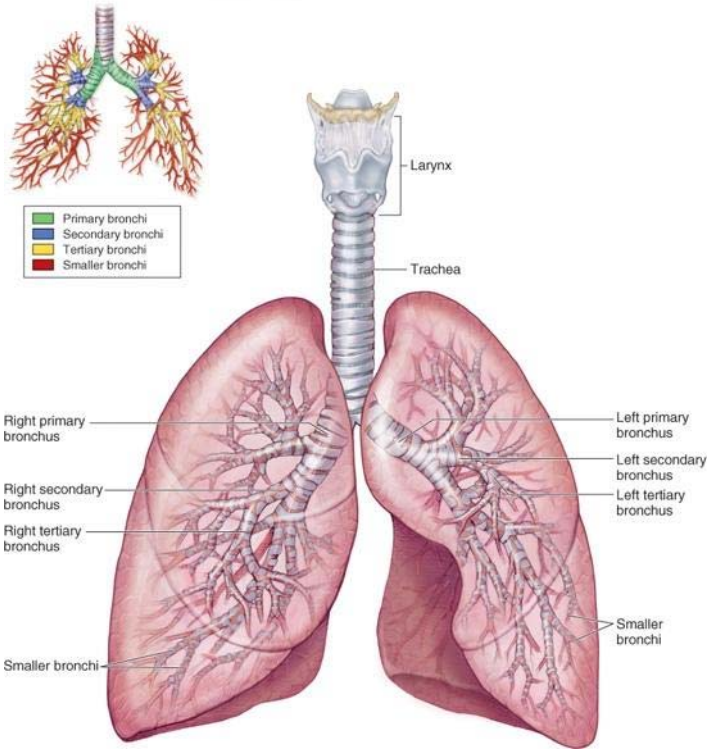


Figure 2: Representation of the bronchial tree (Learning the respiratory system, 2008)

In the classic model of the airways as described by Weibel (Weibel, 1963), each airway divides to form two smaller "daughter" airways. As a result, the number of airways at each generation is double that of the previous generation. The model proposes the existence of 24 airway generations in total, with the trachea being generation 0 and the alveolar sacs being generation 23. In reality, the branching is not perfectly symmetrical (Hickey and Thompson, 2004).

The walls of the primary bronchi, like the trachea, are supported by incomplete cartilage rings. In the lungs, the rings are replaced by small plates of cartilage of irregular shape that completely encircle the bronchus, giving the bronchi a cylindrical shape, in contrast to the ovoid shape with a flattened posterior wall of the trachea. With further branching, the cartilage plates gradually become smaller and fewer in number and the smooth muscles that surround the air passageways become more prevalent. Cartilage ultimately disappears at the point where the airway reaches a diameter of about 1 mm, whereupon it is designated a bronchiole (Parks, 1994; Ross et al., 1995).

The various levels of the airways may also be categorized functionally as being either conducting or respiratory airways. Those airways not participating in gas exchange constitute the **conducting zone** of the airways and extend from the trachea to the terminal bronchioles. The **respiratory zone** includes airways involved with gas exchange and comprises respiratory bronchioles, alveolar ducts, and alveolar sacs (Fig. 3) (Adjei et al., 1996; Altiere and Thompson, 1996).

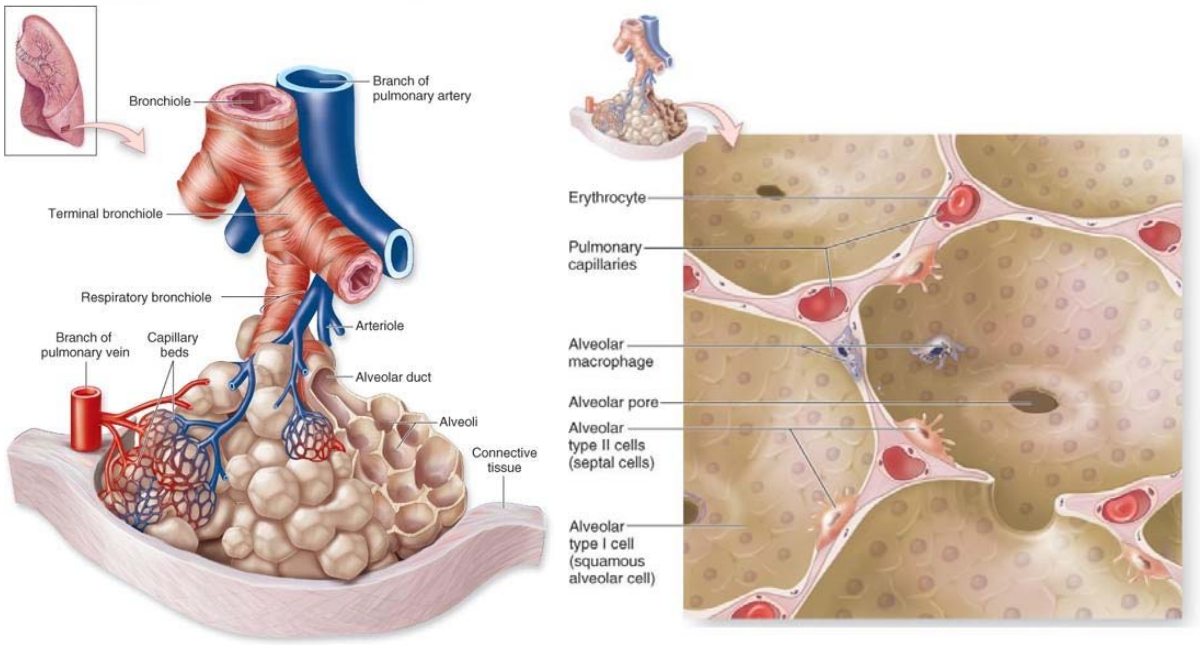
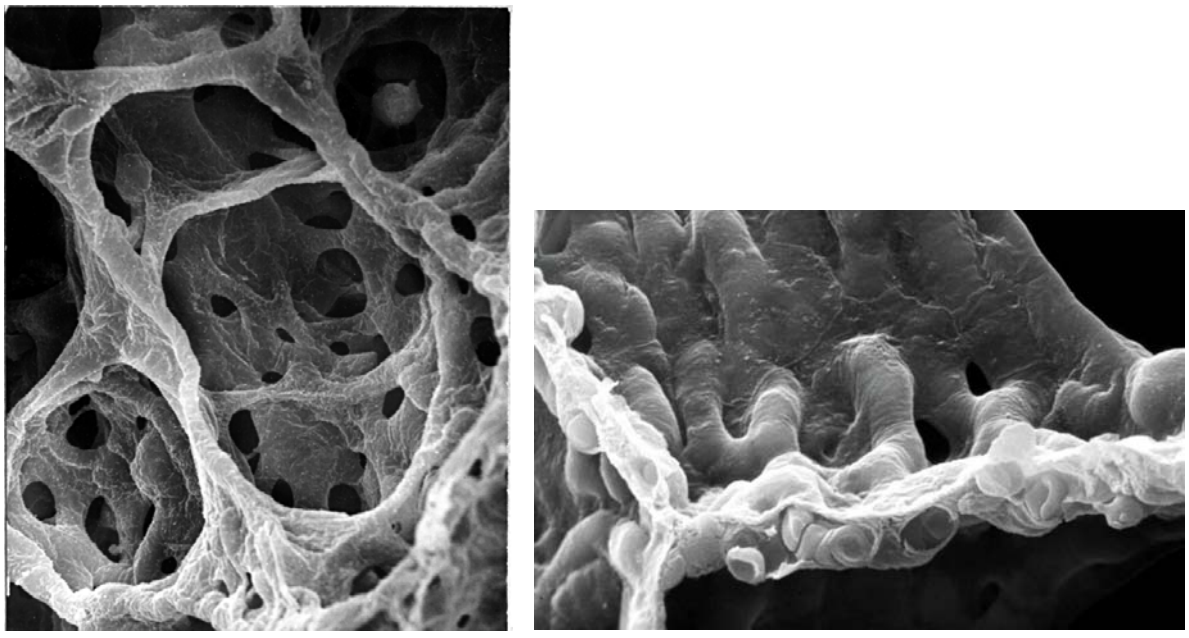


Figure 3: Representation of the alveoli (Learning the respiratory system, 2008)

In passing from the trachea to the alveolar sacs, two physical changes occur in the airways that are important in influencing airway function. Firstly, the airway calibre

decreases with the increasing generations - for example, a tracheal diameter might be of 1.8 cm compared to an alveolar diameter of 0.04 cm. This permits adequate penetration of air to the lower airways for a given expansion of the lungs. Secondly, the surface area of the airways increases with each generation, to the extent that the total lung area at the level of the human alveolus is in the order of 140 m² (Hickey and Thompson, 2004). Alveoli are the terminal air spaces of the respiratory system and are the actual site of gas exchange between air and the blood. About 100 million alveoli are found in each lung (Stone et al., 1992). Each alveolus is a thin-walled polyhedral chamber of approximately 0.2 mm in diameter (Fig. 4.A). Each alveolus is confluent with a respiratory bronchiole at some point, by means of an alveolar duct and an alveolar sac. Alveoli are surrounded and separated from one another by a thin connective tissue layer that contains numerous blood capillaries (Fig.4. B). The tissue between adjacent alveolar air spaces is called the alveolar septum. In traversing the air-blood barrier, gases in the alveolus must cross the alveolar epithelium, the capillary endothelium, and their basement membranes before reaching the blood, a distance in all of approximately 500 nm (Hickey and Thompson, 2004).



A B

Figure 4: A. SEM micrograph (2000x) of three alveoli in a secondary alveolar duct from the upper portion of the left main alveolar duct. The dark round openings are pores between alveoli. A macrophage in its spherical form is in the base of the alveolus in the upper right.
B. Close-up version of the alveolar wall. The red blood cells in a capillary are separated from the air by a very thin tissue barrier (Lung structure tour, 2008).

So, the alveolus is the principal site of gas exchange in the airways, a function compatible with the increased surface area, which promotes extensive and efficient diffusional gas exchange between the alveolar space and the blood in alveolar capillaries (Gehr et al., 1978).

II.2.2. Physiology of the airways

The epithelium of the airways is a continuous sheet of cells lining the luminal surface of the airways. The airway epithelium comprises a variety of cell types, the distribution of which confers different functions according to the airway region. Connecting adjacent epithelial cells are specialized tight junctional processes that limit the penetration of inhaled substances by the intercellular route (Hickey and Thompson, 2004).
Extending from the trachea to the terminal bronchus, the luminal surface of the airways is lined by **ciliated cells**, the most numerous of the cell types.

Mucus cells, which are similar in appearance to the intestinal goblet cells, are interspersed among the ciliated cells and also extend through the full thickness of the epithelium. Mucus, a viscous fluid containing mucin glycoproteins and proteoglycans, floats on a watery layer of pericilliary fluid and covers the luminal surface of the epithelium.
The secretions fulfil four important functions. Firstly, they protect the epithelium from becoming dehydrated. Secondly, the water in the mucus promotes saturation of inhaled air. Thirdly, the mucus contains antibacterial proteins and peptides, such as defensins and lysozyme, that serve to repress microbial colonization of the airways. Fourthly, the mucus is involved in airway protection from inhaled xenobiotics or chemicals (Finkbeiner, 1999; Schutte and Cray, 2002). The cilia, which appear as short hair-like projections from the apical surface, provide a coordinated sweeping motion of the mucus coat, providing a "mucociliary escalator" that serves as an important protective mechanism for removing small inhaled particles from the lungs (Philipps, 1981). Coordinated beating of the epithelial cilia propels the blanket of mucus toward the upper airways and pharynx, where the mucus may be either swallowed or ejected. The rate of mucus propulsion varies according to the airway

region such that movement in the smaller airways is slower than in the larger airways, a situation that arises from the proportionately larger number of ciliated cells and higher ciliary beat frequency in the larger airways (Gail and Lenfant, 1983). Mucus clearance from the airways is also enhanced by coughing, which rapidly propels the mucus toward the pharynx.

Failure to clear mucus from the airways as a result of ciliary disfunction or mucus hypersecretion (as may occur in cystic fibrosis or chronic bronchitis) can result in airway obstruction and infection. Such a situation may adversely affect the therapeutic activity of an inhaled drug by increasing the thickness of the mucus layer through which the drug must diffuse to reach its site of action (Hickey and Thompson, 2004).

The terminal bronchioles are lined with a simple cuboidal epithelium in which **Clara cells** are found among the ciliated cells. Clara cells are nonciliated cells that have a rounded or dome-shaped apical surface. They secrete a surface-active agent, a lipoprotein, to prevent luminal adhesion, particularly during expiration. A small amount of connective tissue underlies the epithelium, and a circumferential layer of smooth muscle underlies it in the conducting portions (Parkes, 1994; Ross et al., 1995).

Alveolar epithelium is composed of Type I and Type II alveolar cells and occasional brush cells. **Type I pneumocytes** are extremely thin squamous cells that line most of the surface of the alveoli - about 95% of the surface area. These cells are joined to one another and to the other cells of the alveolar epithelium - the **Type II pneumocytes** or septal cells, and the occasional **brush cells**, by **zonulae occludentes**. These tight junctions enable the cells to form an effective barrier between the air space and the components of the septal wall. In places, the cell membrane is invaginated to form pinocytic vesicles capable of ingesting macromolecules and particles that may be present in alveolar spaces, affording their transfer to the interstitium. Type II cells are cuboidal secretory cells interspersed among the Type I cells but tending to concentrate at septal junctions. They are the stem cells from which Type I cells differentiate and are replaced after injury. Type II cells are as numerous as Type I cells, but cover only 5% of the alveolar surface (Notter, 2000a; Notter 2000b). Their apical

cytoplasm is filled with lamellar bodies that are rich in phospholipids which are present in the surface-active agent, the surfactant (Parkes, 1994).

In fact, the **surfactant** is composed by weight of approximately 90% lipids and 10% proteins. The lipids are characterized by an unusually high level of saturated fatty acid chains, such as the predominant dipalmitoylphosphatidylcholine (DPPC), which represents 40% in weight and contributes substantially to the unique properties of pulmonary surfactant (Fehrenbach, 2001). In addition to DPPC, lung surfactant contains unsaturated phosphatidylcholines (~35%), phosphatidylglycerol (~10%), phosphatidylinositol (~2%), phosphatidylethanolamine (~3%) and sphingomyelin (~2.5%). There is also a small amount (~3%) of neutral lipid, mainly cholesterol (Possmayer et al., 2001). The protein fraction comprises a highly variable amount of serum proteins and four apoproteins (SP-A, SP-B, SP-C and SP-D), which are associated with surfactant and contribute to its specific functions (Fehrenbach, 2001).
Lung surfactant acts to stabilize the lung alveoli during the respiratory cycle: it decreases the tendency for alveolar collapse during expiration by reducing surface tension in the terminal airways, and makes lung inflation during inspiration easier. Host defense is another function of alveolar surfactant that relies on the nature of SP-A and SP-D (Fehrenbach, 2001).

II.2.3. Blood supply

The lung has both a pulmonary circulation and a systemic circulation.The **systemic circulation** via bronchial arteries that branch from the aorta, supplies oxygenated blood and nutrients to the whole lung tissue other than the alveoli. It participates in air conditioning by assisting in the humidification and warming of inspired air in the trachea and bronchi. It also plays a central role in inflammatory conditions of the lung by contributing to mucosal oedema and delivery of inflammatory cells and mediators to the airways.

In contrast, the **pulmonary circulation** has a principal function of gas exchange of oxygen and carbon dioxide with air in the alveoli. The pulmonary circulation supplies the

dense, sheet-like capillary bed of the alveolar septum and is derived from the pulmonary artery that leaves the right ventricule of the heart. The blood is oxygenated and collected by pulmonary venous capillaries that ultimately coalesce to form the four pulmonary veins that return blood to the left atrium of the heart. Thus, 100% of the cardiac output flows through the pulmonary circulation, at a flow rate of 5l/min. It operates as a low-pressure, low-resistance vascular bed.

Both circulations anastomose at the level of the junction between the conducting and respiratory passages. Thus, drug delivered to the lower airways can enter the systemic circulation through absorption into the systemic circulation or into the alveolar capillaries of the pulmonary vascular bed (Ross et al., 1995; Altiere and Thompson, 1996).

II.2.4. Lung volumes

In healthy young males, the lungs have a total capacity of approximately 5900 ml. About 1200 ml, called the residual volume, always remains in the lungs no matter how forced the expiration. During normal quiet respiration, about 500 ml of air moves into and out of the lungs, which is the tidal volume. The inspiratory reserve is the extra volume of air, approximately 3000 ml, that can be inspired in addition to the normal tidal volume. At the opposite extreme, additional air can be forced out (the expiratory reserve) and this has a volume of about 1200 ml. The vital capacity is the maximal amount of air that can be moved into and out of the lungs, and thus represents the sum of the inspiratory reserve, the tidal volume, and the expiratory reserve. In healthy young men the vital capacity is about 4700 ml. All volumes are somewhat smaller in women because they tend to have smaller thoracic cages and smaller lung capacities (Parkes 1994, Ross et al., 1995).

II.3. PULMONARY DEPOSITION

II.3.1. Deposition mechanisms of inhaled particles

There are five mechanisms by which particles deposit in the respiratory tract. These are impaction, sedimentation, Brownian diffusion, interception and electrostatic precipitation.

Impaction is the inertial deposition of a particle onto an airway surface. It occurs principally at or near airway bifurcations, most commonly in extrathoracic and large conducting airways, where flow velocities are high and where rapid changes in the direction of bulk airflow often take place, generating considerable inertial forces. The probability of impaction increases with increasing air velocity, rate of breathing, particle size (> 5μm) and density (Martonen and Yang, 1996).

Gravitational sedimentation is an important mechanism for deposition of particles over 0.5 μm and below 5 μm in size in the small conducting airways where the air velocity is low. Deposition due to gravity is increased by large particle size and by longer residence times, and decreases with increasing breathing rate (Martonen and Yang, 1996).

Submicrometer-sized particles (especially those < 0.5 μm) acquire a random motion caused by the impact of surrounding air molecules. This Brownian motion may then result in particle deposition by **diffusion**, especially in small airways and alveoli, where bulk airflow is very low.

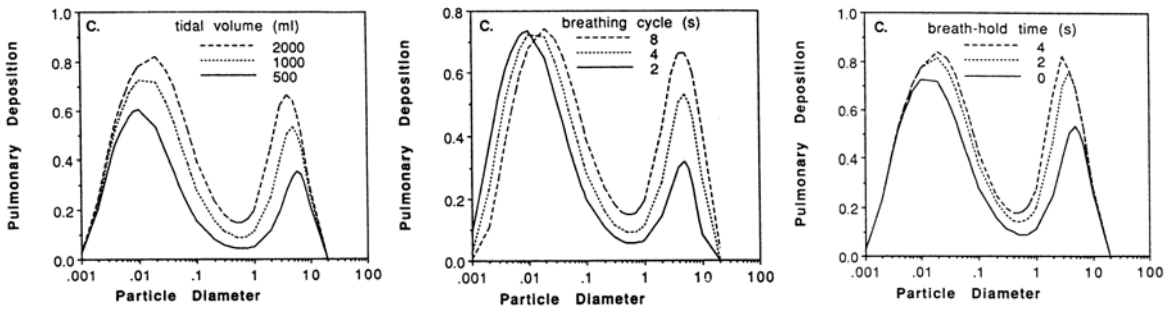
Interception is usually significant only for fibers and aggregates. For such particles, deposition can occur when a particle contacts an airway wall, even though its centre of mass might remain on a fluid streamline (Martonen and Yang, 1996).

Some freshly generated particles can be **electrically charged** during the mechanical generation of aerosols and may exhibit enhanced deposition, due to charges induced on the airway surface by these particles. However, this mechanism is a minor contributor to particle deposition (Lippmann and Schlesinger, 1984; Schlesinger, 1995)

In fact, the deposition patterns of inhaled particles may be expressed as functions of three classes of variables: ventilatory parameters, respiratory tract morphologies and aerosol characteristics (e.g., particle size, shape, and density). The efficiencies of the different deposition mechanisms of inertial impaction, sedimentation and diffusion can, in turn, be formulated in terms of these variables.

II.3.2. Influence of ventilatory parameters

Studies have demonstrated that total lung deposition can be markedly influenced by breathing profiles (Fig. 5) (Martonen and Katz, 1993). For instance, in the upper tracheobronchial tree the deposition of large (> 5 μm) particles can be primarily attributed to inertial impaction, whereas in the more peripheral airways it may be ascribed to sedimentation. By recognizing the effect of ventilatory parameters, deposition due to inertial impaction can be markedly enhanced in the upper lung by increasing the inspiratory flow rate, or, conversely, deposition in the lower lung can be promoted by increasing the duration of a postinspiratory pause (i.e. breath-holding time) (Martonen and Yang, 1996).



A B C

Figure 5: Calculated particle pulmonary deposition in function of A. the tidal volume, B. the breathing cycle and C. breath-hold time. Particles sizes are in micrometers (Martonen and Katz, 1993)

Quiet breathing is appropriate for an inhalation therapy aimed at targeting particles into the alveolar region. An increase in inhalation velocity may result in the development of turbulence, which tends to enhance deposition by impaction in the upper respiratory tract (Schlesinger, 1995). A slow inhalation rate (25 l/min) with breath holding showed maximal deposition of terbutaline sulphate compared to a faster rate (80 l/min) of inhalation (Newman et al., 1989).

Because the velocity distribution of air within the lung is determined by the tidal volume and breathing frequency parameters, the mass delivered to the alveoli can also be enhanced by increasing tidal volume, i.e., the volume of air inhaled during a single breath-hold of 2-6 s at the end of inspiration (Martonen and Yang, 1996). An inspiratory volume of 3000 ml with an inhalation rate of 60 l/min resulted in the highest deposition in the pulmonary region of the lung (Musante et al., 2002).

So, for a given patient (i.e. fixed morphology) and drug (i.e. prescribed aerosol), breathing is the only parameter that can be regulated

II.3.3. Influence of respiratory tract morphology

Among humans, individual variations in airway anatomy affect particle deposition in several ways: the diameter of the airway influences the displacement required by the particle before it contacts the airway surface; the cross section of the airway determines the low velocity for a given flow rate; and the variations in diameter and branching patterns along the bronchial tree affect the mixing characteristics between the tidal and reserve air in the lungs. There are also significant individual differences in respiratory tract anatomy, such as variations in the average alveolar-zone airspace size in humans (Lippmann and Schlesinger, 1984; Martonen and Yang, 1996).

Moreover, some diseases such as asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF) and lung cancer may cause changes in the pulmonary tract by obstruction due to excessive production of mucus or constriction of the airways, influencing the diameter of the various bronches and bronchioles.

II.3.4. Influence of aerosol characteristics: diameter

Particle size is one of the most important design variables in an aerosol formulation, along with shape, density, electrical charge and hygroscopicity. Aerodynamic diameter is the most appropriate measure of aerosol particle size because it relates to particle dynamic behaviour and describes the main mechanisms of aerosol deposition as both gravitational settling and inertial impaction, depending on aerodynamic diameter. In order to reach the lower respiratory tract and optimize pulmonary drug deposition, dry powder aerosols need to present aerodynamic diameters of between 1 and 5 μm (Zanen et al., 1994; Zanen et al., 1995; Zanen et al.1996; Elversson et al., 2003). Particles larger than 5 μm usually deposit in the oropharynx, from which they are easily cleared. In contrast, particles smaller than 0.5 μm may not deposit at all, since they move by Brownian motion and settle very slowly (Heyder et al., 1986; Bosquillon et al., 2004).

The aerodynamic diameter (D_{aer}) is defined by the equation:

$$D_{aer} = D_{geo} \sqrt{(\varrho_P/\varrho_0\chi)} \qquad (1)$$

where D_{geo} is the geometric diameter, ϱ_P and ϱ_0 are particle and unit densities, and χ is the dynamic shape factor.

Pharmaceutical powders are rarely spherical, and shape factors are dimensionless measures of the deviation from sphericity. The dynamic shape factor is the ratio of the actual resistance force experienced by a nonspherical falling particle to the resistance force experienced by a sphere having the same volume (Hinds, 1999). So the aerodynamic diameter can be decreased by decreasing the particle size, decreasing particle density, or increasing the dynamic shape factor.

Aerosol particle design therefore involves two basic strategies. Either particles are made with standard unit density with a geometric size in the 1-5 μm range, or they are created with a non-standard density, and therefore with geometric sizes outside the standard range (Edwards and Dunbar, 2002) (see part II.5.1.5, p 56). As an example, particles exhibiting a high respirable fraction with mean geometric diameters ranged between 3 and 15 μm and tap densities between 0.04 and 0.6 g/cm³ could be produced (Vanbever et al., 1999).

II.4. DELIVERY DEVICES

A good delivery device has to generate an aerosol of suitable size, ideally in the range 0.5-5 μm, and a reproducible drug dosing. It must also protect the physical and chemical stability of the drug formulation. Moreover, the ideal inhalation system must be a simple, convenient, inexpensive and portable device (Dolovich et al., 2005).

Inhaled drug delivery devices can be divided into three principal categories: nebulizers, pressurized metered-dose inhalers (MDIs) and dry powder inhalers (DPIs), each class with its unique strengths and weaknesses.

II.4.1. Nebulizers

Nebulizers have been used in inhalation therapy since the early 19th century. Marketed respiratory solutions are generally composed of drug dissolved in aqueous, isotonic solvent systems that may contain preservatives to reduce microbial growth. There are two traditional devices: air-jet and ultrasonic nebulizers.

For a typical **jet nebulizer** (Fig.6), compressed air passes through a narrow hole and entrains the drug solution from one or more capillaries mainly by momentum transfer. The complex liquid break-up process largely depends on the nozzle design and is usually a combination of turbulent rupture of the instable liquid column and secondary droplet break-up. Large droplets impact on one or more baffles in order to refine the droplet size distribution to the required range for inhalation. Only smaller droplets with less inertia can follow the streamlines of the air and pass the baffle (Le Brun et al., 2000). Approximately 50-60% of the particles produced are in the respirable range.

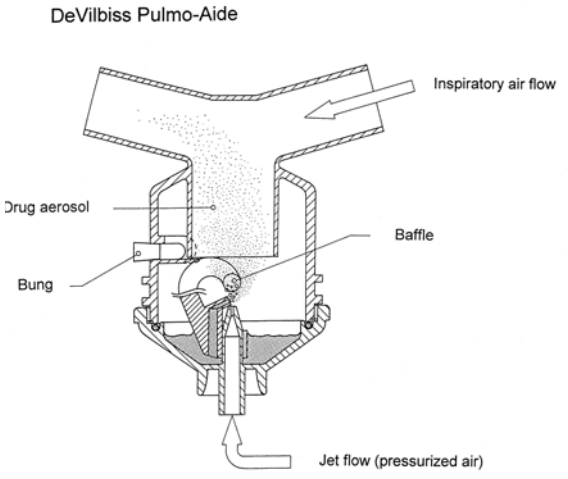


Figure 6 : Schematic presentation of a jet nebulizer (Le Brun et al., 2000)

Alternatively, **ultrasonic nebulizers** use a high frequency vibrating plate to provide the energy needed to aerosolize the liquid. The frequency of the vibrating piezoelectric crystal determines the droplet size for a given solution. Approximately 70% of the particles produced present sizes of between 1 and 5 μm. Nevertheless, heat resulting from frictional forces induced by movement of the transducing crystal may be detrimental to thermolabile formulations.

In fact, early nebulizers were cumbersome and unreliable. Currently, they have the ability to generate small droplets capable of penetrating deeply into the lung, they have high dose delivery capacity (e.g. antibiotics), and some are miniaturized, high-output devices. New developments in liquid spray delivery devices include the use of piezo-electric atomization, high pressure micro-spray nozzle systems and electrostatic generation of aerosol clouds (Smith, 2002). Moreover, coordination between aerosol generation and breathing, which is required for successful metered-dose inhaler use (see below part II.4.2, p 33), is not essential for nebulizers, making them useful for elderly and very young patients (Clay and Clarke, 1987).

Some of the most commonly available nebulizers on the market are: Ventolin® (Salbutamol, ß2-mimetic bronchodilator), Bricanyl® (Terbutaline, ß2-mimetic bronchodilator), Atrovent® (Ipratropium, anticholinergic bronchodilator), Pulmozyme® (Dornase alpha, mucolytic) and Tobi® (Tobramycin, antibiotic).

But nebulization has many well-documented disadvantages, including extended administration time, high cost, low efficiency, poor reproducibility and great variability, risk of bacterial contamination and constant cleaning requirements, and sometimes the need for bulky compressors or gas cylinders (Newhouse et al., 2003). Moreover, in some cases, the presence of preservatives such as sodium metabisulfite, benzalkonium chloride and ethylene diamine tetraacetic acid (EDTA) has caused coughing and bronchoconstruction (Dalby et al., 1996).

One of the principal problems of nebulizers is that the device includes a large "dead volume" of solution. A large fraction of the amount (up to 50%) can thus remain trapped in the

apparatus. Moreover, the aerosolized drug is generated continuously, leading to drug waste (Dalby et al., 1996). With a continuously working compressor (continuous droplet generation), part of the aerosol cloud may be wasted into the environment through the vent when the patient stops or interrupts inhalation or does not inhale fast enough. The amount inspired is equivalent, more or less, to half of the delivered amount. Of this inhaled amount, it is still necessary to remove a fraction of particles that are not in the "respirable range". In conclusion, the pulmonary fractions obtained using a nebulizer may vary from 2-10% of the nominal dose. As an example, 2.5 ml of Pulmozyme at 1 mg/ml is delivered by a jet nebulizer with an estimated delivery efficiency of 10% (Cipolla et al., 1994).

Reduction of the waste by at least 50% of the nebulized dose may be achieved by so-called breath-assisted open vent nebulizers. The vent has a flexible membrane (valve) that opens only during inhalation. Meanwhile, a similar membrane in the outlet tube closes the route for exhalation. When the patient does not inhale, both valves are closed in order to prevent waste of the produced drug aerosol to the environment (Le Brun et al., 2000).

Nevertheless, it is a known fact that patient compliance is poor in inhalation therapy. There are only a few evaluation studies that refer to compliance. In a study on children with respiratory diseases a compliance of 47.6% with the prescribed inhalation therapy was found (Schöni, 1993), whereas another study found a mean compliance of only 56.8% for adult patients on inhalation therapy (Cochrane, 1997). This low compliance is understandable because it usually takes a lot of time and energy to inhale the prescribed medication on a daily basis. Another aspect of the daily routine is the maintenance of the nebulizer. Cleaning and disinfection is necessary in order to prevent contamination of the nebulizer and subsequent possible infections (Reychler et al., 2007).

II.4.2. Pressurized Metered-Dose Inhalers

Traditional asthma therapy has primarily used the pressurized metered-dose inhaler (MDI). Since the 1950s, they have been the mainstay of inhalation therapy (Crompton, 2006). In an MDI, the drug is either suspended or dissolved in a propellant that is pressurized until it liquefies in a canister. Releasing a metered volume of the fluid through a control valve causes the propellant to expand and evaporate, and leaves the drug in the form of a high velocity aerosol. The pressurized formulation is expelled rapidly into the valve stem, which, together with the actuator seating, forms an expansion chamber in which the propellant begins to boil (Fig. 7). The liquefied propellant serves both as a source of energy for expelling the formulation from the valve in the form of rapidly evaporating droplets and as a dispersion medium for the drug and other excipients.

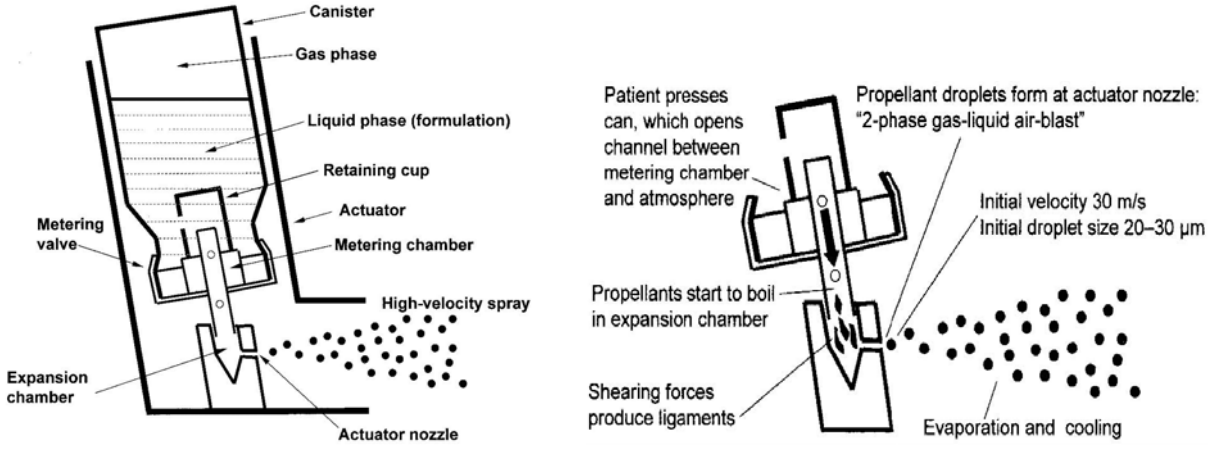


Figure 7: Schematic representation of a typical pressurized metered-dose inhaler (Newman, 2005)

The particle size distribution of an MDI aerosol depends on the physicochemical properties of the formulation. For instance, studies have shown that the aerosol size for suspension formulations may be reduced if the formulation has a high vapor pressure, a small drug particle size, or a low drug concentration (Poli et al., 1969).

A surfactant such as sorbitan trioleate (SPAN 85), oleic acid, and lecithins, at levels between 0.1% and 2.0% w/w, is typically present to aid dispersion of suspended drug particles or dissolution of a partially soluble drug, and to lubricate the metering mechanism (Newman, 2005). Flavors and suspended sweeteners may be present to combat the unpleasant taste. To

enhance chemical stability, antioxidants (ascorbic acid) or chelating agents (EDTA) may be added to formulations (Dalby et al., 1996).

The essential components of an MDI are the container, the metering valve, and the actuator. Usual valve volumes range from 25-100 µl, which deliver a drug dose of about 50 µg to 5 mg.
The most commonly available MDIs on the market are: Flixotide® (Fluticasone, corticosteroid), Atrovent® (Ipratropium bromide, anticholinergic bronchodilator), Ventolin® (Salbutamol, ß2-mimetic bronchodilator) and Combivent® (Ipratropium bromide + Salbutamol, anticholinergic + ß2-mimetic bronchodilator).

Chlorofluorocarbon (CFC)-based MDIs generally contained a combination of a liquefied low boiling-point propellant, CFC 12 (dichlorodifluoromethane), and a liquefied higher boiling-point propellant, CFC 11 (trichlorofluoromethane) or CFC 114 (dichlorotetrafluoromethane). However, the success of CFC propellant-driven MDIs has been overshadowed by their contribution to ozone depletion in the upper atmosphere and the concomitant health effects (Molina and Rowland, 1974). In fact, the international community agreed to phase out CFC propellants in 2000. So nowadays, the main emphasis in MDI developments is on the introduction of non-CFC propellants. But the non-CFC propellant hydrofluoroalkanes, HFA 134a (1,1,1,2-tetrafluoroethane) and HFA 227 (heptafluoropropane), have very different physical properties, and, in particular, extremely poor solvent properties. This feature is beneficial in preventing the dissolution of small drug particles, but it is also disadvantageous in that the commonly used surface-active agents are almost totally insoluble and not able to provide any physical stabilization of drug particles in suspension. There have been numerous approaches taken to overcome the problems of drug-particle instability in the HFA propellants, including addition of a co-solvent, developing new specific surface-active agents, reducing the interfacial tension by modifying the particle surface properties, and particle engineering to produce a more HFA-compatible material (Smith, 2002).

However, MDIs have several other disadvantages. Some patients suffer from the so-called "cold-Freon effect", in which the arrival of the cold propellant spray on the back of the throat causes the patient to stop inhaling (Crompton, 1982). Moreover, because an MDI is pressurized, it emits the dose at high velocity, which makes premature deposition in the oropharynx more likely (Newman and Clarke, 1993; Ganderton, 1997). Thus MDIs require careful coordination of actuation and inhalation. Only a small fraction of the drug (10-20%) escaping the inhaler penetrates the patient's lungs due to a combination of high particle exit velocity and poor coordination between actuation and inhalation. The deposition of aerosolized drugs in the mouth and the oropharyngeal regions varies considerably according to the application technique, but losses using the pressurized devices are routinely greater than 70% and can exceed 90%. Particle losses that occur proximally to the lung are a long-documented problem that continues to compromise the effectiveness of current aerosol therapy protocols.

Spacer devices and reservoirs were developed to allow the deceleration of the aerosol cloud before reaching the throat and to make perfect coordination between actuation and inhalation slightly less important (Fig. 8). Nevertheless, adding a chamber to an MDI makes it less portable, and many chambers can develop static electrical charges on the inner walls and thereby reduce the lung delivery (Geller, 2005). Despite these enhancements, incorrect use of MDIs is still a prevalent problem (Newman and Newhouse, 1996; Giraud and Roche, 2002). About 75% of patients made at least one error when using an MDI (Molimard et al., 2003; Khassawneh et al., 2008).

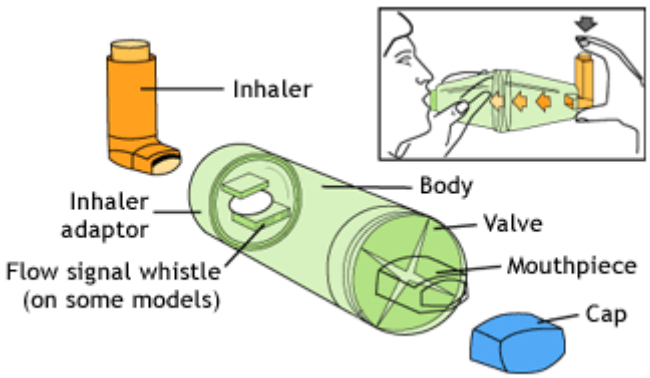


Figure 8: Representation of the use of a spacer device with an MDI (Asthma, 2008)

The introduction of "breath-actuated" MDI devices has provided a convenient and portable means of overcoming the major disadvantage of the need to coordinate the actuation with inhalation. Breath-actuated inhalers sense the patient's inhalation through the actuator and fire the inhaler automatically in synchrony. Examples are the Autohaler® (3M Pharmaceuticals, U.S.A) or Easybreathe® (Norton Healthcare, U.K.) (O'Callaghan and Wright, 2002; Rubin and Fink, 2005).

II.4.3. Dry Powder Inhalers

As a result of the problems encountered with MDIs, the design and use of alternative inhalers that do not use propellants were developed. These devices combine powder technology with device design in order to disperse dry particles as an aerosol in the patient's inspiratory airflow (Delaby et al., 1996).

All DPIs have four basic features: a dose-metering mechanism, an aerosolization mechanism, a deaggregation mechanism, and an adaptor to direct the aerosol into the mouth. DPIs are subject to strict pharmaceutical and manufacturing standards by regulatory bodies, the most challenging of which is the demonstration of device reliability in terms of delivered dose uniformity and delivered dose deposition (Newman and Busse, 2002). Indeed, comparative *in vitro* data for a generic product versus the innovator product must be provided on the complete individual stage particle size distribution profile using a multistage impactor/impinger with various impaction stages, such as the NGI (see part IV. 2.12.1.1.4, p 97) (EMEA, 2006).

For DPIs, the dose received by the patient is dependent on four interrelated factors (Atkins, 2005):

1. The properties of the drug formulation, particularly powder flow, particle size and drug carrier interaction
2. The performance of the inhaler device, including aerosol generation and delivery
3. Correct inhalation technique for deposition in the lungs
4. The inspiratory flow rate

Therefore, a balance between the design of an inhaler device, drug formulation, and the inspiratory flow rate of patient is required (Steckel and Muller, 1997; Srichana et al., 1998).

DPIs are a widely-accepted inhaled delivery dosage form, particularly in Europe where they are currently used by an estimated 40% of patients to treat asthma and chronic obstructive pulmonary disease. Their use will continue to grow (Atkins, 2005).
Presently, over 20 DPI devices are available on the market (Table 1) and more than 25 are in development (Table 2). Today there are essentially two types of DPIs: those in which the drug is packaged in individual doses (capsules) (Single dose or Unit-dose devices) and those that contain multiple doses in a foil-foil blister or a reservoir of drug from which the doses are metered out (Multi-unit and Multi-dose devices).

Table 1: DPI devices currently available on the market (Islam and Gladki, 2008)

Device	DPI type	Company	Delivery method	Drug(s)
Breath-actuated single unit dose				
Spinhaler®	Single dose	Aventis	Capsule	Sodium cromoglycate
Rotahaler®	Single dose	GlaxoSmithKline	Capsule	Salbutamol sulphate
				Beclomethasone dipropionate
Inhalator®	Single dose	Boeringher-Ingelheim	Capsule	Fenoterol
Handihaler®	Single dose	Boeringher-Ingelheim	Capsule	Tiotropium
Aerolizer®	Single dose	Novartis	Capsule	Formoterol
FlowCaps®	Single dose	Hovione	Capsule	/
TwinCaps®	Single dose	Hovione	Capsule	Zanamivir
Breath-actuated multiple dose				
Turbuhaler®	Multi-dose	Astra Zeneca	Reservoir	Salbutamol sulphate
				Terbutaline sulphate
				Budesonide
Diskhaler®	Multi-unit	GlaxoSmithKline	Blister	Salmeterol xinafoate
				Beclomethasone dipropionate
				Fluticasone propionate
				Zanamivir
Diskus®	Multi-unit	GlaxoSmithKline	Strip pack	Salbutamol sulphate
				Salmeterol xinafoate
				Fluticasone propionate
Aerohaler®	Multi-unit	Boeringher-Ingelheim		Ipratopium bromide
Easyhaler®	Multi-dose	Orion Pharma	Reservoir	Salbutamol sulphate
				Beclomethasone dipropionate
Ultrahaler®	Multi-dose	Aventis	Reservoir	
Pulvinal®	Multi-dose	Chiesi	Reservoir	Salbutamol sulphate
				Beclomethasone dipropionate
Novolizer®	Multi-dose	ASTA	Reservoir	Budesonide
MAGhaler®	Multi-dose	Boeringher-Ingelheim	Reservoir	Salbutamol sulphate
Taifun®	Multi-unit	LAB Pharma	Reservoir	Salbutamol sulphate
Eclipse®	Multi-unit	Aventis	Capsule	Sodium cromoglycate
Clickhaler®	Multi-dose	Innoveta Biomed	Reservoir	Salbutamol sulphate
				Beclomethasone dipropionate
Twisthaler®	Multi-dose	Schering-Plough	Reservoir	Mometasone furoate
Active device				
Airmax®	Multi-dose	Norton Healthcare	Reservoir	Formoterol
				Budesonide
Inhance®	Single dose	Pfizer	Blister	Insulin

Table 2: Future/next generation DPIs (approved or in development stage) (Islam and Gladky, 2008)

Device	DPI type	Company	Delivery method	Drug(s)
Aspirair	Multi-dose	Vectura	Powder/Active	Apomorphine hydrochloride
Omnihaler	Single dose	Innoveta Biomeds Ltd	Powder/Active	/
Actispire	Single dose	Britania	Powder/Active	/
NEXT DPI	Multi-unit	Chiesi	Reservoir	/
DirectHaler	Multi-unit	Direct-Haler	Pre-metered	/
JAGO	Multi-dose	SkyPharma	Reservoir	Salbutamol sulphate
Airmax	Multi-dose	Norton Healthcare	Reservoir	Formoterol Budesonide
Turbospin	Single dose	PH&T	Capsule	/
AIR	Single dose	Alkermes	Capsule	/
MicroDose	Multi-unit	MicroDose/ 3M	Powder/ Electronic activated	Insulin
Cyclovent	Multi-dose	Pharmachemie	Reservoir	Morphine
Dispohaler	Multi-dose	AC Pharma	/	/
Conix One	Single dose	Cambridge Consultant	Foil seal	Vaccines
Microhaler	Single dose	Harris Pharmaceutical	Capsule	Sodium cromoglycate
Technohaler	Multi-unit	Innoveta Biomeds Ltd	Blister	/
Spiros	Multi-unit	Dura	Blister/Active	Albuterol sulphate
Bulkhaler	Multi-unit	Asta Madica	Reservoir	/
Miat-Haler	Multi-unit	MiatSpA	Reservoir	Formoterol Fluticasone propionate Budesonide
Prohaler	Multi-unit	Valois	Blister	/
Otsuka DPI		Otsuka Pharmaceutical	Compact cake	/
Acu-Breath	Multi-dose	Respirics	Powder	Fluticasone propionate
MF-DPI	Multi-unit	/	Reservoir	Mometasone furoate
Swinhaler	Multi-dose	Otsuka Pharmaceutical	Powder	Budesonide
Pfeiffer	Single dose	Pfeiffer GmbH	Active	/
Certihaler	Multi-dose	Novartis	Powder	Formoterol

II.4.3.1. Unit-dose devices

The **Spinhaler®** (Aventis) was the first dry powder device, described in 1971 (Bell et al., 1971). It has a mechanism for piercing the capsule. The cap of the capsule fits into an impeller, which rotates as the patient breathes through the device, projecting particles into an airstream (Fig. 9). Shear force and relative motion are the predominant mechanisms of powder deaggregation. This low-resistance device has presented low *in vitro* fine particle fractions (FPF) (% < 5 μm) (see part IV.2.12.1.2, p 99) of 4-12% (Dunbar et al., 1998).

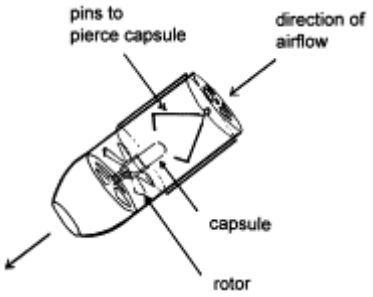


Figure 9: Schematic presentation of the Spinhaler® (Hickey and Concessio, 1997)

A similar DPI, the **Rotahaler®** (GlaxoSmithKline) has a mechanism for breaking the capsule into two pieces. The capsule body containing the dose falls into the device, while the cap is retained in the entry port for subsequent disposal. As the patient inhales, the portion of the capsule containing the dug experiences erratic motion in the airstream, causing dislodged particles to be entrained and subsequently inhaled. Particle deaggregation is mainly caused by turbulence promoted by the grid upstream of the mouthpiece. A FPF of 26% has been reported for this low resistance device (Dunbar et al., 1998).

In the **Aerolizer®** (Novartis), the capsule is pierced on each side by four piercing pins. During inhalation, the capsule whirls and the particles are dispersed by turbulence generated by a spinning motion. Deagglomeration of the powder occurs through its passage through a plastic grid. Interestingly, studies using computational fluid dynamics have been made on the Aerolizer® in order to evaluate the influence of the mouthpiece geometry, the mouthpiece length, the grid structure, the air inlet size and the role of the capsule (Coates et al., 2004, 2005a, 2005b, 2007). These studies have shown that when modifications to the design of the device are made, the flowfield generated within the device can change significantly, and that these changes may have a significant effect on the overall performance of the inhaler.

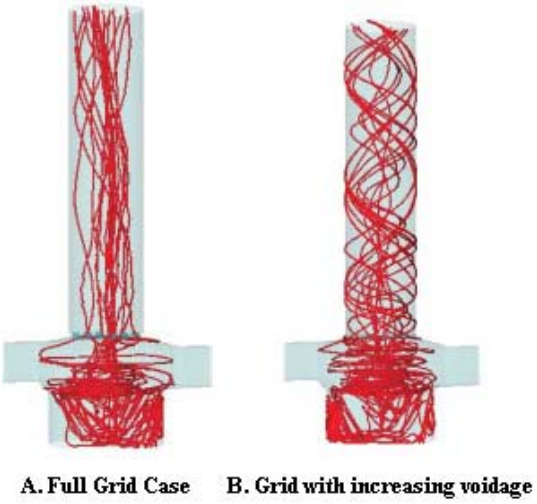


Figure 10: Particle tracks of the dispersed powder indicating that the grid affects the tangential component of the flowfield in the mouthpiece (Coates et al., 2004)

The grid has been demonstrated to play a significant role (Fig. 10): as the voidage of the grid increases, a reduction in the FPF is observed. Therefore, increasing the voidage of the grid reduces the deagglomeration potential of the flow field generated in the device, due to fewer particle-grid impactions. The air inlet size controls the levels of turbulence and particle impaction velocities generated in the device, as well as the flow development rate and device emptying times. Beyond the capsule deagglomeration effects, the break-up mechanism produced by forcing powder agglomerates through the capsule holes plays an important role in the powder dispersion. Nevertheless, the potential role of the capsule acting as a rotor to cause deagglomeration by mechanical impaction and capsule-particle impaction seems to be a weak mechanism for deagglomeration.

The **Handihaler**® (Boehringer Ingelheim) operates by dispensing drug contained in a capsule via a rumbling motion once the capsule has been opened by piercing pins. The particles are dispersed through the turbulence generated by a plastic grid at the time of inhalation. This device seems more complex as it requires at least 7 distinct steps to deliver the dose. For some patients, 2 inhalations are required to completely empty the capsule and achieve the therapeutic dose (Atkins, 2005).

In fact, unit-dose devices are considered as not patient-friendly and not easy to use because there are several manoeuvres to accomplish before inhalation, such as taking the

capsule from the package, loading it and piercing it within the device. Furthermore, there have been recent reports (Tezky and Holquist, 2005) of patients ingesting the capsule instead of placing it in the device and inhaling the contents. In fact, some studies (Melani et al., 2004) show that adherence to correct inhaler use depends on the importance given to appropriate training prior to product use and device education by health-care providers.

II.4.3.2. Multi-dose devices

Multi-dose DPIs have been developed, either as multi-unit dose or as multi-dose reservoir devices.

Inhalator M® (Boehringer Ingelheim) has a rotating drum magazine for the storage of six capsules. The capsule is pierced at both ends and remains stationary while emptying occurs by fluidization due to the high pressure drop across the capsule. Deaggregation is caused by shear stress and collision (Dunbar et al., 1998).

The **Diskhaler®** (GlaxoSmithKline) employs individual doses packaged in blister packs on a disk cassette. Following piercing, inspiratory flow through the packaging depression containing the drug induces dispersion of the powder (Fig.11). The aerosol stream is mixed with a bypass flow entering through two holes in the mouthpiece that, together with a grid, gives rise to turbulence that promotes deagglomeration.

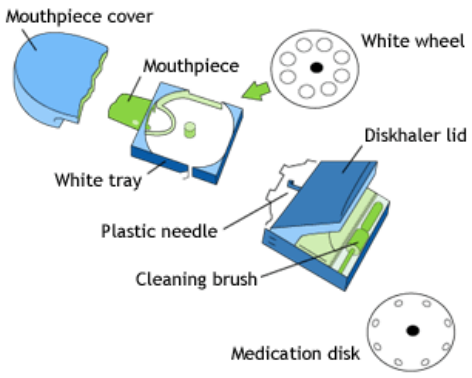


Figure 11: Schematic presentation of the Diskhaler® (Asthma, 2008)

The **Diskus®** (GlaxoSmithKline) is quite similar except that it contains a foil strip with 60 single dose blisters (Fig. 12). FPF have been reported to be approximately 23-30% for these two low resistance devices (Dunbar et al., 1998).

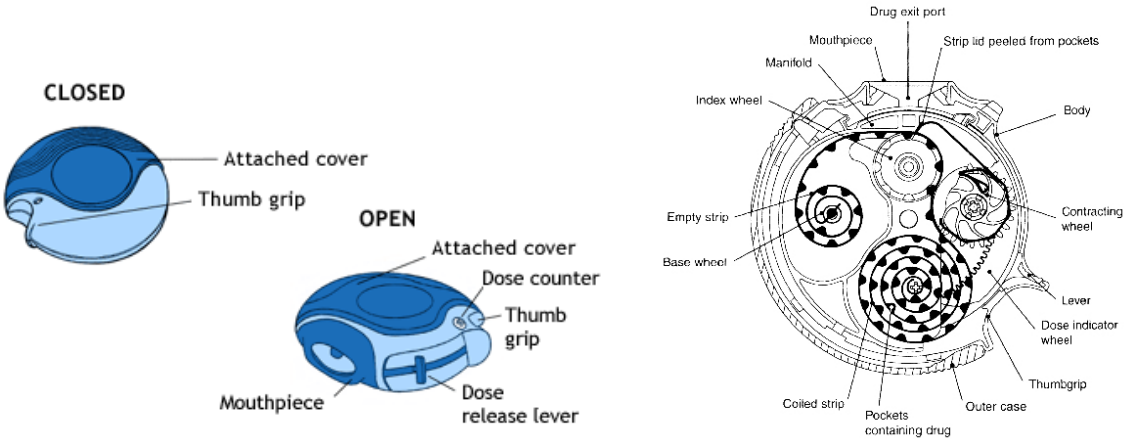


Figure 12: Schematic presentation of the Diskus® (Asthma, 2008)

One of the more sophisticated multi-dose reservoir systems is the **Turbuhaler®** (AstraZeneca). It contains 200 doses of small pellets of micronized drug that disintegrate into their primary particles during metering and inhalation. One dose can be dispensed into the dosing chamber by a simple back-and-forth twisting action on the base of the reservoir (Fig. 13).

Scrapers actively force drug into conical holes which cause the pellets to disintegrate. Fluidization of the powder is done by shear force as air enters the inhaler. Particle deagglomeration occurs by turbulence (from a series of tortuous channels), impaction on the bottom of the mouthpiece and high shear stress in the swirl nozzle of the mouthpiece.

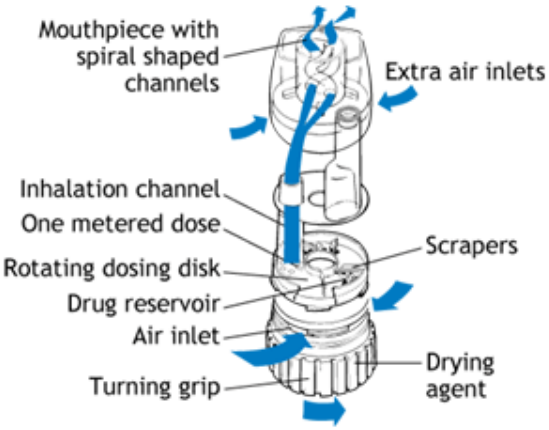


Figure 13: Schematic presentation of Turbuhaler® (Asthma, 2008)

This device of medium resistance has presented an FPF of 39-45% (Dunbar et al., 1998).

The advantages of the reservoir systems are their relative ease and low cost of manufacture and the ease of including a large number of doses within the device (Prime et al., 1997). Nevertheless, reproducible dose metering remains the most difficult challenge in device design (Everard et al., 1997; Sumby et al., 1997). Indeed, the variability of dose emissions from DPIs, and in particular from the reservoir system, at the recommended flow

rate has been found to be relatively high, with a total relative standard deviation (SD) of more than 15% about the average emitted dose for the Pulmicort Turbuhaler® (Handle and Byron, 1995). Moreover, powders contained in reservoirs may be more susceptible to deterioration through ingress of moisture, and the use of a desiccant is recommended. For these reasons, pre-metered doses from multi-unit dose systems are more consistent than doses delivered from the reservoir devices, as they are individually sealed and protected from the environment until the point of use by the patient. It is also vital to ensure that no accidental or additional dose is inhaled. This has led to the incorporation of dose counters on new reservoir devices.

II.4.3.3. Deaggregation and deagglomeration

Deaggregation and deagglomeration are the processes by which particle aggregates (carrier-drug mixture) and agglomerates (drug clusters) are dispersed into primary particles of suitable size for deep lung deposition by interaction with airflow (Houzego, 2002) (Fig. 14).

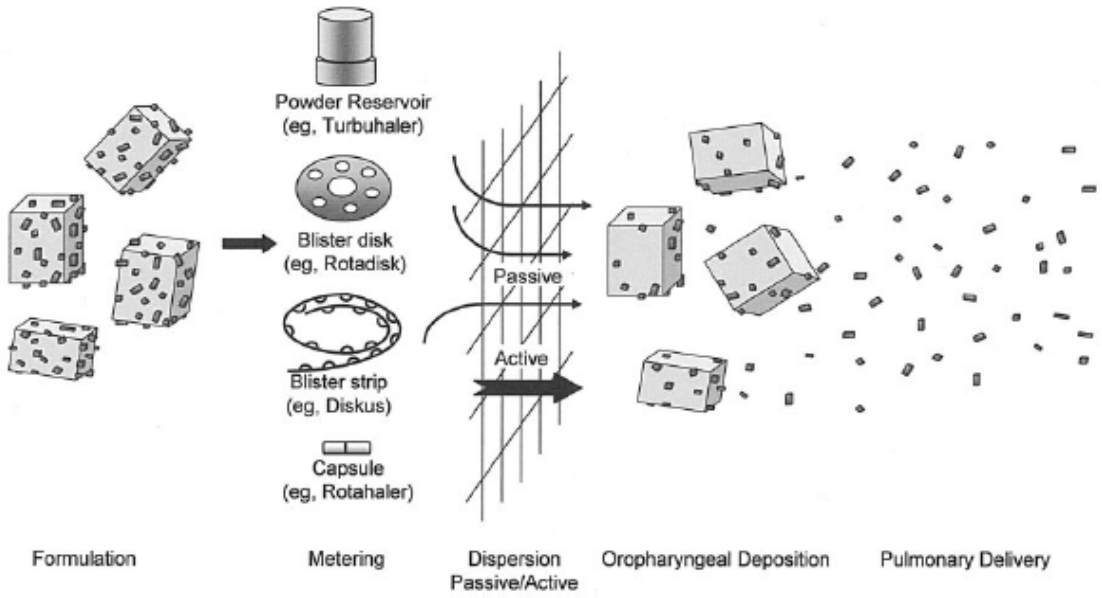


Figure 14: Principle of dry powder inhaler design (Telko and Hickey, 2005)

Deaggragation principally occurs in two regions: the device and the oropharynx. The majority of DPIs are therefore composed of short tubes and complex geometries through

which an airflow passes that consists of a turbulent core surrounded by a laminar envelope (Dunbar et al., 1998). It is known that there is a correlation between the turbulence velocity and the deaggregation that occurs: as the turbulence increases, the deaggregating force increases. However turbulence may not be the only effective deaggregation mechanism in dry powder inhalers (Voss and Finlay, 2002). In fact, the deagglomeration of drug particles to form a fine respirable aerosol cloud is thought to be achieved by three major mechanisms: Particle interaction with shear flow and turbulence, particle-device impaction, and particle-particle impaction (Coates et al., 2004).

II.4.3.4. Importance of the inspiratory airflow

Since DPIs are activated by the patient's inspiratory airflow, they require little or no coordination of actuation and inhalation. This has frequently resulted in better lung delivery than was achieved with comparable MDIs (Borgstrom et al., 1996). Since DPIs are typically formulated as one-phase, solid-particle blends, they are also preferred from the standpoints of stability and processing (Ashurst et al., 2000). Moreover, they are a propellant-free design and thus environmentally friendly.

In contrast, the effectiveness of DPIs is much more susceptible to the vagaries of age, gender, disease, and the breathing cycle of the device user. One of the most important disadvantages includes the fact that DPIs require moderate inspiratory effort to draw the formulation from the device, and some patients are not capable of such effort. Low-resistance passive DPIs are generally less dependent on flow rate than high-resistance devices. In fact, devices with higher resistance need a higher inspiratory force from patients to achieve the desired air flow. This could be difficult for patients with severe asthma and for children and infants. Therefore, a balance between resistance and turbulence is necessary to achieve the desired therapeutic effect from DPI formulations. Moreover, it has been demonstrated that drug deposition deep in the lung from DPI formulations is determined not only by the peak flow rate but also by the flow increase rate. It was found that a high peak flow rate did not

necessarily guarantee a high aerosol deposition if the initial flow increase rate was insufficiently high (De Boer et al., 1996a; Martin et al., 2006a).

Some DPIs require inspiratory flows of more than 60 l/min to effectively de-aggregate the powder (De Boer et al., 1996b; Meakin et al., 1998), which cannot always be achieved by all asthmatic patients, particularly infants. Thus, it is generally thought that DPIs should not be used with patients who have low levels of lung function. However, Borgstrom has shown that in several clinical situations viewed as constrained, patients are still capable of generating enough flow to operate a DPI effectively, including patients with COPD and acute asthma, and children with asthma (Borgstrom, 2001).
Furthermore, some DPIs such as the Clickhaler® and the Easyhaler® (Newhouse et al., 1999; Palander et al., 2000; Tarsin et al., 2004) have shown a uniform delivery of doses independent of flow rate compared to delivery by the Turbuhaler®.

While most DPIs are breath-activated, relying on inhalation for aerosol generation, several power-assisted devices (pneumatic, impact force, and vibratory) (Tobyn et al., 2004; Crowder et al., 2001) have been developed or are currently under development (Tables 1 and 2, pp 37-38). These "active" inhalers are not subject to the same limitations as passive inhalers and have a different advantage/disadvantage profile. It has been suggested that if shear and turbulence could be standardized by using a dispersion mechanism that is independent of the patient's breath, high delivery efficiency and reproducibility might be achieved.
Whilst passive inhalation is commonly used in DPIs designed for topical respiratory drug delivery, active dispersion mechanisms (i.e. where the device inputs the energy) are considered desirable for drugs intended for systemic action, which have to penetrate more deeply into the lungs (Schultz et al., 1992).

The very first approved active device (Exubera®, Pfizer) with compressed air for aerosolizing a drug formulation for DPI insulin delivery was until recently available on the market. This DPI with insulin was anticipated to be cost effective compared to insulin injection. However, this large and clumsy device has failed to achieve recognition by

physicians and patients. It was estimated that the bio-availability and bioefficacy of the current inhalation systems was approximately 10% compared to subcutaneaous insulin. Most of the inneficiencies of the systems were due to losses within the device and in the patient's mouth and oropharynx. Moreover, approximately 30-50% of the insulin that reached the lungs was absorbed systemically (Mastrandrea and Quattrin, 2006).

II.4.3.5. Compliance of patients

Of particular importance, a study by Molimard et al. (2003) has shown that approximately half of the subjects made at least one error when using the Aerolizer®, Diskus® or Turbuhaler®. Concerning the DPIs, critical errors that would result in almost no medication reaching the lungs were made by 11-12% of patients using the Aerolizer® and Diskus® and 32% using the Turbuhaler®.
A study in March 2008 tended to confirm these results as the Aerolizer® was incorrectly handled by 16.9% of patients, the Diskus® by 6.8%, and the Turbuhaler® by 43.2% of patients. Failure to hold the inhaler upright with the grip downwards and failure to turn the grip until it clicked were the most common critical errors in handling the Turbuhaler®. In handling the Aerolizer®, failure to push the buttons to pierce the capsule was the most common critical error, and with the Diskus® it was failure to slide the lever until it clicked (Khassawneh et al., 2008).

So, in choosing a drug/device combination for a patient, the clinician must take into account several factors, including the cognitive and physical ability of the patient, ease of use, convenience, costs, and patient preferences. Moreover, proper use of most current devices is not intuitive and requires education and re-education of the patient. So, health-care providers such as pharmacists, respiratory therapists, physicians and nurses should be able to teach appropriate use of aerosol devices to their patients.
A study in 2007 in Europe demonstrated the importance that pharmacists attach to their role of advising on medication for asthma. Two-thirds of respondents reported giving advice on dry powder inhalers often/ very often/ all the time, and half of their monthly consultations

with patients with asthma involved advice on dry powder inhalers (Williams and Chrystyn, 2007).

II.4.3.6. Future research and new developments

Since the creation of the first DPI Spinhaler®, device technology has continued to grow and a lot of devices are now currently available on the market. However, no devices have shown remarkable efficiency in delivering drugs from the formulation.
Furthermore, the concept of powder interaction with the device on powder dispersion has generally been poorly understood. Recently, computational fluid dynamics has enhanced understanding of the impact of inhaler design on powder dispersion and deposition, and has demonstrated that small variations in device design can produce significant variations in performance (Coates et al., 2004; Chan, 2006).

Nowadays, ways to improve the efficiency of drug delivery from DPIs are developed by changing formulation technology, drug and carrier particle engineering and designing new devices. Indeed, the design of a device needs to be coordinated with drug formulations (i.e., powder in capsules, disks, bulk powders or agglomerates), so that the drugs are aerosolized during inhalation and deliver a dose to the lungs that achieves maximum therapeutic benefits. It is often the case that the drug formulation and inhaler device need to be optimised together to ensure reliable and effective drug delivery. Therefore, the inhaler-drug combination is generally considered as a single medication whose *in vitro* performance and *in vivo* efficacy must be demonstrated.

So, in the design of a new DPI, consideration must be given to optimizing the formulation of the powder containing the drug substance to ensure a chemically stable and consistent dose, and to optimizing the design of the metering system within the inhaler itself to produce a convenient device that is comfortable and easy to use for the patient.

II.5. FORMULATION OF DRY POWDER INHALERS

II.5.1. Types of formulation

In order to obtain an effective delivery of drugs to the lungs, various ways of formulations were developed. Often, the drug is mixed with carrier particles or encapsulated in liposomes and cyclodextrins. Specific biodegradable microspheres or large porous particles were also created for DPI administration.

II.5.1.1. Excipients

The particle size distribution affects the deposition of drug in the respiratory tract. However, the smaller the particles, the stronger the cohesive forces, resulting in increased adhesion between the particles and subsequently poor flow properties (Feeley et al., 1998).
So, one way to improve the flow properties of a drug is through the addition of excipients. In general, excipients are used to enhance the physical or chemical stability of the active pharmaceutical ingredient, its mechanical properties, and/or its pharmaceutical properties, such as dissolution and permeation. In DPI formulations, excipients function first and foremost as carrier particles. Usually, no more than a few milligrams of drug need to be delivered (e.g. corticosteroids for asthma therapy), and excipients provide bulk, which improves handling, dispensing, and metering of the drug.

II.5.1.1.1. Nature of excipients

The primary function of the lungs is respiration. To fulfil this purpose, the lungs have a large surface area and thin membranes. Unlike the gastrointestinal tract, the lungs have limited buffering capacity. Many compounds that could enhance drug delivery outcomes also have the potential to irritate or injure the lungs (Telko and Hickey, 2005). Consequently, the array of potential excipients is limited to compounds that are endogenous to the lung and

can easily be metabolized or cleared. Currently, lactose is the most commonly used excipient in marketed DPIs. The reasons for this are as much historical as they are physicochemical/pharmaceutical in nature: lactose has long been used as an excipient in oral dosage forms before being deployed in DPIs. It has an established safety and stability profile, different manufacturing processes with tight controls over purity and physical properties, and is easily available at different grades and is inexpensive. Moreover, lactose is highly crystalline, less hygroscopic than other sugars and has the smooth surfaces and satisfactory flow properties desirable for a DPI carrier particle (Smyth and Hickey, 2005).

Other sugars, such as glucose, mannitol and trehalose (Steckel and Müller, 1997; Tee et al., 2000; Stahl et al., 2002; Mao and Blair, 2004; Steckel and Bolzen, 2004; Glover et al., 2008) have been shown to be feasible alternatives to lactose, and it is expected that these sugars will eventually find their way into approved products.
An additional benefit that may be gained from the use of a sugar carrier is the taste/sensation on inhaling, which can assure the patient that a dose has been taken (Prime et al., 1997). Phospholipids and cholesterol have also been used in experimental liposomal formulations (Shah and Misra, 2004) or as solid lipidic carriers or fillers (Sebti and Amighi, 2006).

The availability of the active drug depends afterwards on the redispersion of the particles in the inspired air, as a function of the cohesive forces between drug particles and the adhesive forces between drug and carrier particles. The larger carrier particles deposit on the oropharynx, while the fine drug particles partly reach the deep lung. These adhesive forces must be carefully considered, as inadequate separation of drug and carrier is the main reason for deposition problems.

So, as excipients can make up over 99% of the product by weight, their choice is a crucial determinant of overall DPI performance. Despite the apparent lack of choices, the excipient must be carefully selected, as its physicochemical properties, such as size and morphology, profoundly affect the performance of the formulation (Chew and Chan, 2002a; Islam et al., 2004).

In general, the morphology and roughness of carrier particles are not uniform, containing regions that exhibit different roughness parameters. Clearly, these variations in physico-chemical properties in the surface of a carrier material may lead to differences in apparent adhesion properties of drug particles. Furthermore, during the dynamic process of mixing, the adherence of drug particles to the more adhesive areas of the carrier surface is likely to occur (Young et al., 2005). Indeed, Hersey (1975) proposed that the surfaces of larger particles consisted of distinct regions containing so-called "**active sites**". It was further suggested that when the amount of fine carrier particles in the mixture is below the saturation limit of the large carrier particles' adhesive potential, the fine particles will preferentially bind to these active sites. When these active sites have been completely occupied by fine particles, a binary carrier system will then exist, i.e., carrier with strongly bound fine particles, and carrier with weakly bound fine particles or free fine particles (Fig. 15).

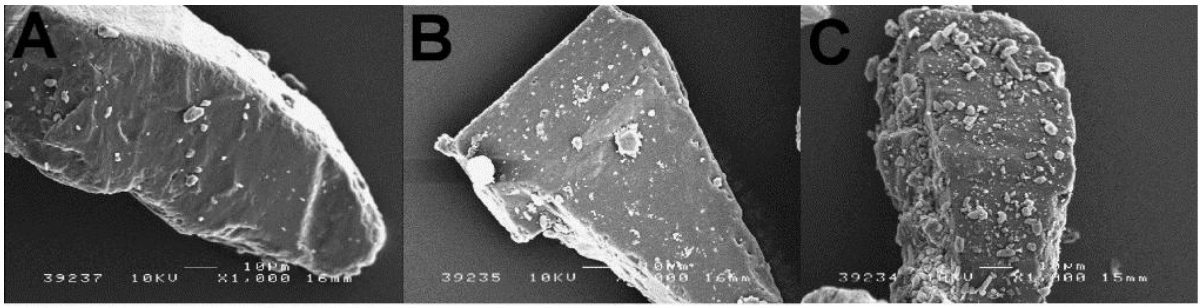


Figure 15: Scanning electron microscope images of A. 12µg, B. 135µg and C. 450µg blends of salbutamol sulphate and lactose (Young et al., 2005)

This presence of active sites has obvious implications for DPI drug delivery since retention of drug particles on these relatively high-energy sites during processing and aerosolization would result in a decrease in apparent respirable drug fraction, as suggested by Staniforth (1996). Furthermore, it is suggested that the active sites present on the surface of the carrier will have a specific energy distribution with a critical, average adhesion point below which particles - drug or lactose - could be removed.

Current methods for overcoming such issues include "filling" the potential active sites by increasing the fine particle content present on the carrier surface (Zeng et al., 1999) or pacifying the effects of active sites by the addition of so-called "force control agents" such as magnesium stearate (Young et al., 2002a; Ferrari et al., 2004). This additional or ternary

component can be added to occupy and presaturate higher-energy binding sites on the carrier before the drug is added, consequently increasing the release and the respirable fraction of the active drug.

II.5.1.1.2. Blending

After drug and excipient(s) have individually been brought to their desired forms, they are combined in the blending process. The flow properties of the components of the powder blend will play an important role in the efficiency of blending and, ultimately, in aerosol dispersion. Blended formulations consist of small drug particles that are mixed with large excipient carrier particles (50-200 μm). It is a critical step in the manufacture of a DPI product and is in fact subject to substantial optimization work during development. When mixing powders with different properties, particle sizes, and ratios, as is the case with DPI formulations, inadequate mixing can cause poor dose uniformity. In many cases, inadequate mixing cannot be overcome simply by increasing the mixing time. Mixer selection, rotation speed, capacity, and fill level are all subject to optimization, as they can all affect the blend homogeneity (Sudah et al., 2002; Alexander et al., 2004). Different powders may have different mixing requirements, depending on the interaction forces present between the various particles (Staniforth et al., 1982). For low concentration (drug-carrier ratio) blends, geometric dilutions are necessary, using multiple preblending steps.

Various blending options are available: low-energy tumbling blending, tumbling blending with sieving to break up agglomerates of micronized drug and aid distribution with the powder mass, and high energy blending, with paddles, impeller blades or planetary augers actively cutting the powder bed and redistributing powder within the blending vessel (Malcomson and Embleton, 1998).

An interactive mixture of the two components is prepared by blending until the mixture can remain intact during the filling process (to produce an accurate metered dose) and then freely separate into its primary components during inhalation. There, the drug particles separate from the carrier particles and are carried deep into the lungs, while the

larger carrier particles impact in the oropharynx and are cleared. Inadequate drug/carrier separation is one of the main explanations for the low deposition efficiency encountered with DPIs (Zeng et al., 2000).

II.5.1.2. Liposomes

Liposomes are small vesicles with an aqueous compartment enclosed by one or more concentric lipid bilayers. They are one of the most extensively investigated systems for controlled delivery of drug to the lung since they can be prepared with phospholipids endogenous to the lung as surfactants. Liposomes can be produced with a wide range of sizes and are able to encapsulate a variety of both hydrophilic and lipophilic drug molecules in their core or within their membrane bilayer. Cytotoxic agents, bronchodilators, anti-asthma drugs, antimicrobial and antiviral agents, and drugs for systemic action, such as insulin and proteins, have been investigated (Liu et al., 1993; Zeng et al., 1995; Desai et al., 2003; Lu and Hickey, 2005; Changsan et al., 2008).

Liposomes are known to promote an increase in the drug retention time and reduce the toxicity of drugs after administration. Intratracheal administration of tobramycin in liposomes was associated with improved plasma concentrations and half-life compared to conventional formulations, and showed a trend for a prolonged efficacy against resistant bacteria (Marier et al., 2002).

They are commonly delivered either in aqueous form via nebulization (Finlay and Wong, 1998) or in dry powder form (Schreier et al., 1994; Desai et al., 2003). Dry powder liposome formulations are generally prepared by lyophilisation of the aqueous liposome dispersions, followed by micronization by jet-milling to achieve particles in the desired range. However, this process could cause deleterious effects on liposome integrity, thereby causing leakage of the entrapped drug (Desai et al., 2002).

II.5.1.3. Cyclodextrins

Native cyclodextrins are polysaccharides made up of six to eight cyclic linked oligosaccharides of D-glucopyranose monomers connected by α-1, 4- indican bonds. These compounds form cone-shaped molecules with primary hydroxyl groups (6-OH) arranged in an inner hydrophobic cavity of 5.7, 7.8 and 9.5 Å respectively for α-, β- and γ-cyclodextrins, and secondary hydroxyl groups (2- and 3-OH), rendering external walls hydrophilic (Szetjtli, 1998). Partial or entire encapsulation occurs by hydrophobic forces, van der Waals interactions, ion pairing and hydrogen bonding (Cao et al., 2003). This complexation improves the aqueous solubility and stability of drugs, ameliorates the dissolution rate and bioavailability of the hydrophobic drugs, decreases toxicity and controls drug release (Loftsson and Brewster, 1996; Stella and Rajewski, 1997; Uekama et al., 1998).

Recently, pulmonary delivery has emerged as a possibility for non-invasive delivery of protein and macromolecular drugs. A major obstacle to the widespread use of pulmonary drug delivery of peptide and macromolecular drugs is the relative impermeability of the lung to many of these substances when they are administered without an absorption enhancer. Cyclodextrins have been reported as potential absorption enhancers in pulmonary protein delivery such as human growth hormone, insulin, cyclosporine A, etc. (Shao and Mitra, 1994; Shao and Mitra, 1996; Hussain et al., 2004; Ungaro et al., 2006; Matilainen et al., 2006; Jalalipour et al., 2008).
Nevertheless, little has yet been published concerning the potential toxicity of cyclodextrin formulations on the lung parenchyma and bronchial tree (Evrard et al., 2004).

II.5.1.4. Biodegradable microspheres

Micron-size hollow-porous microspheres (PulmoSpheres®) are prepared using a two-step process. First, a fluorocarbon-in-water emulsion is prepared by high pressure homogenization, using a saturated phosphatidylcholine as the surfactant. The emulsion is then combined with a second aqueous solution containing the active agent and other wall-

forming material (e.g., co-surfactants, sugars and salts). The second step involves spray drying the aqueous dispersion. The oil phase functions as a "blowing" or "inflating agent" during spray-drying, retarding droplet shrinkage during the drying process, while simultaneously creating pores and voids in the particles. Mean geometric and aerodynamic diameters are about 5 and 7 μm, respectively, and the bulk density is about 0.4 g/cm^3. Morphologically, the particle walls have a sponge-like appearance with pores (Fig. 16) (Dellamary et al., 2000; Duddu et al., 2002). Particle size, morphology and density can be controlled through the selection of the blowing agent type and its concentration.

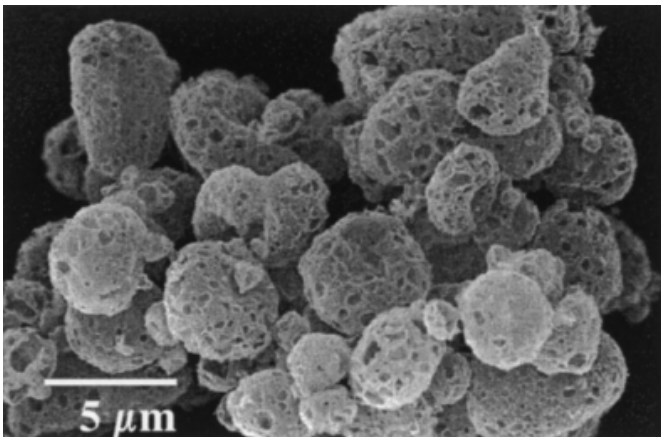


Figure 16: SEM of PulmoSpheres® of budesonide (Duddu et al., 2002)

High respirable fractions are obtained *in vitro* as a result of a significant decrease in interparticle attractive forces and improved aerodynamic properties due to the hollow porous design. One clinical evaluation on healthy volunteers of dry powder tobramycin, using lipid-based Pulmosphere technology, has already been made, giving a mean whole lung deposition of 34 ± 6 % (Newhouse et al., 2003).

Biodegradable microspheres produced from natural and synthetic polymers (e.g. poly(lactic-co-glycolic) acid polymer (PLGA)) have been extensively investigated as drug carriers for administration via a number of different routes in order to ensure targeting and sustained drug release. PLGA microspheres have been used for controlled release of a wide range of drugs including peptides and proteins, and their potential use in pulmonary delivery has been explored, principally for anti-asthmatic drugs (Zeng et al., 1995). A number of microspheres have proved to be non-toxic, biodegradable and non-immunogenic following systemic injection.

II.5.1.5. Large porous particles

In general, therapeutic dry powder aerosols are made with drug particle mass densities of approximately 1 ± 0.5 g/cm³ and mean geometric diameters of less than 5 μm to avoid deposition in the oropharyngeal cavity and in the DPI devices. Now, a new type of inhalation aerosol characterized by particles of mass density significantly lower than 1 (0.1-0.5 g/cm³) has been recently proposed, so that an aerodynamic diameter in the respirable size range can be achieved with a geometric particle size greater than 10 μm (Musante et al., 2002). These large porous particles represent an important step in pulmonary delivery for future applications (Edwards et al., 1997). The increased aerosolization efficiency lowers the probability of deposition losses before particle entry in the intrapulmonary airways, thereby increasing the systemic bioavailability of an inhaled drug. This is due to a lower tendency for powder aggregation as a result of a reduced fractional surface area of particle-particle contact in the dry powder. Moreover, these large porous particles can escape the natural clearance mechanisms of the lungs, such as the mucociliary escalator and phagocytosis by alveolar macrophages, since very large particles may escape that kind of clearance, giving enough time for the drug to be effectively delivered (Edwards et al., 1998).

Thin-walled large porous particles have also been prepared by macroscale aggregation of nanoparticles via a spray-drying process with an additional control over physical characteristics by adding other components to the spray-dried solutions such as sugars, lipids, polymers and proteins. The drug release and delivery potential of nanoparticle systems are then combined with the ease of flow, processing, and aerosolization potential of large porous particle systems (Tsapis et al., 2002).

II.5.1.6. Carrier-free

As previously dicussed, in order to increase the flow and dispersing properties of the micronized drugs, inhalation formulations are aften composed of a mixture of excipient and active drug. Nevertheless, the addition of an inert ingredient in elevated ranges decreases the

dose of active drug in a capsule. Most current DPIs can only deliver microgram quantities of drug rather than the milligram quantities needed for inhaled antibiotic treatment, for example. So there is an increasing interest in formulations that do not need a coarse carrier to aid the flowability of micronized drug, namely "carrier-free" formulations.

For example, sodium cromoglycate has been "pelletized" in weak and redispersible agglomerates without the need for any excipient (Edwards and Chambers, 1989). Some commercially available dry powder inhalers have already employed carrier-free formulations, such as those contained in the Turbuhaler® (Wetterlin, 1988). In this case, spheronization controlled the aggregation of micronized particles. The aggregates are formed in rotating blenders, with the resulting large particle size providing the requisite flow properties or accurate dosing. The deaggregation of the agglomerates occurs by turbulent dispersion generated in the inhaler (Turbuhaler®), which is sufficient to overcome the interparticulate forces holding the micronized particles together.

II.5.2. Forces of interaction

The homogeneity of the blend and the deaggregation and dispersion properties of the respirable particles upon activation (driven by the patient's inspirational energy) are, on a microscopic scale, governed by the resulting cohesive (drug-drug) and adhesive (drug-excipient) interaction forces within the formulation. Excessive adhesive forces may prevent elutriation of the respirable particles from the carrier surfaces, leading to upper airway deposition. Similarly, strong cohesive forces may enhance agglomerate formation, which could directly affect the fluidization and dispersion characteristics of the formulation (Begat et al., 2004a). Ideally the balance between adhesive and cohesive forces should be adjusted to a level that provides enough adhesion between drug and carrier to provide a stable formulation, yet allows easy separation during inhalation.

Particle separation is the most important performance characteristic for effective aerosol generation. To separate particles, the specific force of interaction must be overcome.

There are four major forces of interaction between particles: mechanical interlocking due to surface asperities, capillary forces from the presence of water, electrostatic arising from the insulating nature of the material, and van der Waals forces from the fundamental electromagnetic nature of matter (Telko and Hickey, 2005).

On a large scale, physical interactions are barriers to aerosol generation. Mechanical interlocking due to surface features or roughness is a prominent mechanism preventing particle dispersion. The magnitude of these forces is related to the diameter of the pores between particles and interfacial tension due to hydrogen bonding of water. Controlling moisture content will aid in reducing capillary forces, but care must be taken to avoid increasing the surface charge of the particles. The origins of the electrostatic charge are atmospheric ionization, chemical composition, contact with charged objects, and triboelectric charging from motion. Electrostatic charging is difficult to study and control. Since most pharmaceutical powders are poor conductors, electrostatic charge plays a role in their dispersion. Electrostatic forces are reciprocally but not linearly related to capillary forces in magnitude.

The forces of interaction between pharmaceutical powders are difficult to characterize and control because of heterogeneity in particle composition and physicochemical characteristics. Consequently, it is difficult to consider each of the forces independently or to apply rigid controls. Specifications can be placed on key features that may be responsible for particle interactions, such as crystallinity, presence of impurities, surface asperities, roughness, shape, moisture content, density, particle size and size distribution.

II.5.2.1. Surface morphology

Since the adhesion of drug to carrier is a surface phenomenon, morphological differences may have a significant effect on the ability of the air flow to overcome these adhesive forces and to generate well-dispersed aerosol particles. Indeed, surface area is not solely determined by particle size and shape but also by the surface morphology: corrugated particles have more surface area than smooth particles that occupy the same volume. By

modifying drug particles or selecting carrier particles to obtain a specific surface morphology, the interparticle forces can be modulated to enhance lung deposition.
Moreover, variation in surface roughness will alter the contact area between contiguous surfaces and thus alter the total van der Waal's force of interaction and also the propensity for surface charging or the degree of water condensation (Young et al., 2007). It has been reported in several previous studies that increasing the surface smoothness of the carrier particles increased the FPF of the drug (Zeng et al., 2000; Larhrib et al., 2002; Young et al., 2002a).

II.5.2.2. Moisture content and hygroscopicity

Hygroscopicity is the intrinsic tendency of a material to take moisture from its surroundings. Hygroscopic drugs present a greater risk of physical and chemical instability. Moisture uptake and loss due to changes in relative humidity (RH) can result in local dissolution and recrystallization, leading to irreversible aggregation though solid bridge formation, which can adversely affect aerosol generation and lung deposition (Hickey et al., 1990; Braun et al., 1996; Maggi et al., 1999).

Capillary forces arise from the dynamic condensation of water molecules onto particle surfaces. If the amount of condensed water is sufficient, a meniscus is formed between the contact points of the adjacent surfaces as liquid is drawn by capillary action around the contact points, inducing an attractive force (Begat, 2004b). In fact, at a low RH, a crystalline material only adsorbs water onto its surface and moisture uptake is a function of the specific surface area of the material and the RH of the environment (Van Campen et al., 1983). As the RH is increased, a complete monolayer of water molecules will be formed on the surface of the crystalline solid particles (Hancock and Shamblin, 1998). It has long been recognized that water condensation on the surface of the drug and/or carrier particles markedly increases the magnitude of cohesive forces (between micronized powder particles) and adhesive forces (between drug and carrier particles) (Jashnani et al., 1995; Price et al., 2002; Begat et al., 2004a).

Several studies have suggested that non-surface-specific capillary forces play a dominant role in the adhesion between drug and carrier, which may significantly reduce the deaggregation and dispersion properties of a dry formulation (Price et al., 2002; Young et al., 2002b; Young et al., 2003a; Young et al., 2003b). A study has shown that after exposure to 75% RH for up to 6 days, dry powder formulations containing micronized salbutamol base and a fraction of sorbitol, dextrose or spray-dried maltose as carrier were found to take up a large quantity of water (15-40%), which resulted in a marked change in the surface textures of the particles and a drastic reduction in the FPF. In contrast, the formulation containing lactose monohydrate was found to take up moisture on its surface only and there was only a small reduction in the FPF after storage at 75% RH (Young et al., 2007; Zeng et al., 2007). So excipients that modify the hygroscopic properties or coat the drug particles with hydrophobic films, such as cholesterol for example, may need to be considered (Hickey et al., 1990; Hickey and Martonen, 1993).

II.6. MICRONIZATION

There are several options for reducing the particle size. The first size reduction technique is typically milling.

II.6.1. Milling

There are many different mills, but only a few are able to mill powder to the required particle size range of 1-5 μm. The three main types of mills used are: fluid-energy mills, such as the jet mill; high peripheral speed mills, such as the pin mill; and the ball mill. These basic designs are shown in Fig. 17.

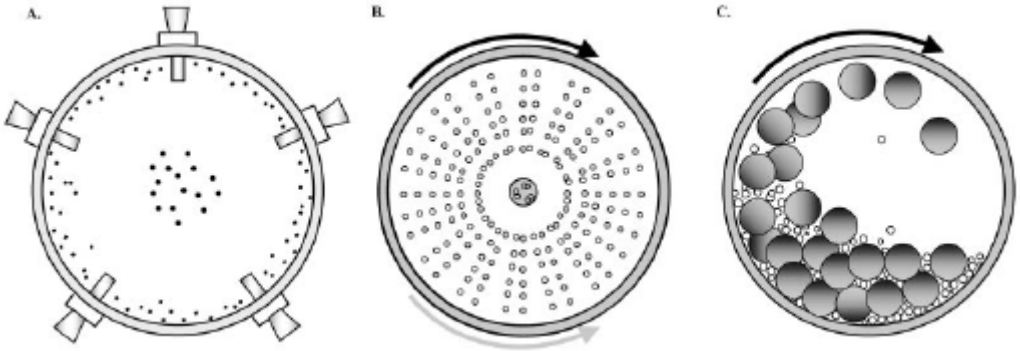


Figure 17: Three mills commonly used to create micron-size particles: A. Jet mill, B. Pin mill, C. Ball mill (Telko and Hickey, 2005)

Jet milling is the most useful technique; it reduces particle size via high velocity particle-particle collisions. Unmilled particles are introduced into the milling chamber. Air or nitrogen is fed through nozzles at high pressure and accelerates the solid particles to sonic velocities. The particles collide and fracture. While flying around the mill, larger particles are subjected to higher centrifugal forces and are forced to the outer perimeter of the chamber. Small particles exit the mill through the central discharge stream. Depending on the pressure and powder feed rate, particles of down to 1 μm in diameter can be produced (Telko and Hickey, 2005).

A **pin mill** uses mechanical impact to grind material, both by particle-particle and particle-solid collisions. A pin mill is equipped with a series of concentrically mounted pins located on a spinning rotor and stationary stator plate. The pin mill cannot produce particles as small as those from the jet mill (Drogemeier and Leschonski, 1996).

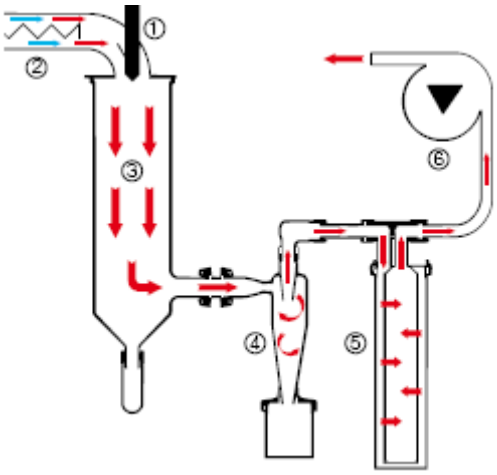
The **ball mill** is essentially a rotating cylinder loaded with drug and "milling media" (ie, balls that grind the drug between each other as they tumble inside the mill). The size and material of the milling media can be varied. Ball milling is very slow and the process is poorly scalable, which is why tumbling-ball mills are used only in the laboratory (Telko and Hickey, 2005).

However, strong mechanical processing, such as milling, has been shown to affect the crystallinity of the material. Amorphous regions might be generated at the surface of the fractures' crystals (Malcolmson and Embleton, 1998). The micronized particles formed carry high amounts of electrostatic charges and are, therefore, very cohesive. The smaller the particles are, the stronger are the cohesive forces. Moreover, the micronization process leads to small, irregularly shaped flat particles and extensive flat surfaces promote large contact areas, resulting in increased adhesion between the particles. Micronized powders with high energetic surfaces show poor flow properties (Feeley et al., 1998). Moreover, this process provides only limited opportunity for control over important particle characteristics such as size, shape and morphology.

Other techniques for making micron-size particles involve direct particle formation from solution. Two noteworthy approaches for controlling particle size are spray drying and supercritical fluid. These techniques are distinctly different from milling in that the particles are built up (i.e., particle size is increased), whereas particle size is decreased during milling.

II.6.2. Spray drying

Spray drying is a one-step process that can convert a liquid feed to a dried particulate form. The feed can be a solution, a coarse or fine suspension, or a colloidal dispersion (e.g., emulsion, liposome, etc.), which is first atomized to a spray form that is put immediately into thermal contact with a hot gas, resulting in the rapid evaporation of the droplets to form dried solid particles. The dried particles are then separated from the gas by means of a cyclone, electrostatic precipitator or bag filter (Fig. 18).



1. Two-fluid nozzle, operated by compressed air to disperse the solution into fine droplets
2. Electric heating of the drying medium
3. Spray cylinder for drying the droplets to solid particles
4. Separation of the particles in the cyclone
5. Outlet filter to remove fine particles
6. Aspirator for generating the flow

Figure 18: Schema of a Mini Spray Dryer (Buchi, 2008)

The three fundamental operations of the spray dying process are atomization, drying, and separation. The design may be in "open cycle" since the drying gas (usually air) is not recirculated and is vented to the atmosphere. When spray drying from organic feeds, the "closed cycle" layout is more suitable than the open one since the risk of flammability and explosion is higher in the latter when organics solvents are heated in the presence of oxygen. In the closed cycle design the heated gas, which is usually nitrogen with less than 5% oxygen, is recirculated, and a condenser is employed to convert organic vapors to the liquid state (Sacchetti and Van Oort, 1996).

Atomization is the process whereby a liquid is broken up into a collection of droplets. The principal atomization methods are centrifugal, pneumatic, high pressure, ultrasonic, electrostatic and effervescent. Pneumatic, or twin-fluid, atomization is suited for generating

particles in the size range of inhalation products and for laboratory-scale research. In this process, the liquid feed is pumped at low flow rates to the orifice of the nozzle, where the liquid comes in contact with a gas under pressure at high velocity, which creates the spray. The nozzle design and the type of atomization can affect droplet size and provide control over particle size distribution. In the "cocurrent" method, the atomizer is positioned at the top of the drying chamber and the spray enters in the same direction as the gas flow. In this approach, the droplets are subjected to the highest temperatures in their most moist state followed by rapid evaporation and cooling. This method is particularly suitable for heat-labile material and compounds with low melting points or glass transition temperatures as the drying time ranges from 100 milliseconds to seconds (Johnson, 1997).

Once the liquid is atomized, droplets dry to solid particles by intimate contact with heated gas in the drying chamber. The important **drying** variables are inlet and outlet temperatures, drying gas medium, gas humidity, gas flow rate and residence time, which all together affect the final size, shape, density, crystallinity and solvent content of the particles. In order to avoid agglomeration in powders, the humidity of the gas medium must be sufficiently low, especially for hygroscopic materials. In the case of organic feed, the vapour must be removed to reduce the solvent content to pharmaceutically acceptable levels (Sacchetti and Van Oort, 1996).

For laboratory-scale work, the most widely-used powder **separation** technique is the cyclone, where a centrifugal force is applied onto spray-dried particles, which then impact on the walls and are removed from the upward-flowing drying gas by collection in a bottom vessel. The collection efficiency can be enhanced by increasing gas inlet velocity and increasing mass loading (Sacchetti and Van Oort, 1996).

Compared to milling, spray-drying can produce more spherical particles. 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 (Chawla et al., 1994; Dellamary et al., 2000; Steckel and Brandes, 2004). Nevertheless, particles from spray drying processes are not always spherical and may have

convoluted surfaces, asperities, holes and voids. In fact, the shape can be influenced by the drying rate, the surface tension and viscosity of the liquid (Johnson, 1997). 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 aerodynamic diameter, a maximum deposition of the embedded drug in the tracheo-bronchial and deep alveoli regions for normal inhalation rates (Elversson et al., 2003).
It is also important to note that spray-dried particles from solutions are mostly amorphous, but in order to keep the crystalline state of the drug, suspensions can be processed.

The principal advantages of spray-drying with respect to pulmonary drug delivery are the ability to manipulate and control a variety of parameters such as solvent composition, solute concentration, solution and gas feed rate, temperature and relative humidty, droplet size, etc., in order to optimize particle characteristics such as size, size distribution, shape, morphology and density, in addition to macroscopic powder properties such as bulk density, flowability and dispersibility (Sacchetti and Van Oort, 1996; Dunbar et al., 2002). This battery of controllable parameters given by spray drying is a great advantage over jet-milling (Hickey et al., 1996). Moreover, spray dryer are available at different laboratory scales and the industrial scaling-up is easy.

The pharmaceutical applications include the preparation of antibiotics, excipients such as lactose and mannitol, proteins and enzymes, vitamins, vaccines and microorganisms. Spray drying is the most commonly-used technique in pharmaceutical industries for preparing dry powders of peptides and proteins for inhalation. However degradation during the atomization process may be a problem for some macromolecules as a result of a number of factors such as thermal stress during droplet drying, high shear stress in the nozzle and peptide/protein adsorption at the greatly expanded liquid/air interface of the spray solution.

II.6.3. Supercritical fluids

The principle of this method relies on the fact that above a fluid's critical point, a solute's solubility can change considerably with a relatively small change in pressure. Rapid depressurization of a solute creates a high degree of supersaturation. This leads to high nucleation rates with large numbers of small crystals. Carbon dioxide, because of its accessible critical point at 31°C and 74 bar, and its low cost and non-toxicity, is the most widely-used solvent in many SCF processes (Johnson, 1997). Its critical temperature makes SCF suitable for processing heat labile solutes at conditions close to room temperature (Dunbar et al., 2002). The three main SCF processes are: rapid expansion of supercritical solutions (RESS), where particles form as the result of rapid expansion of an SCF containing a dissolved solute; gas antisolvent recrystallization, which relies on the capacity of an SCF to act as an antisolvent and cause precipitation within a liquid solution or droplet; and solution-enhanced dispersion by SCF, which involves rapid dispersion and mixing of the drug solution with the SCF, and extraction of the solvent into the SCF, leading to high supersaturation ratios.

Fine drug particles produced via SCF precipitation are less charged than those produced by mechanical means, which makes them better flowing and more dispersible following discharge from a DPI. Moreover, SCF manufactures respirable drug particles that are intrinsically more uniform in terms of crystallinity, morphology, particle size distribution and shape than those produced via jet milling (Malcomson and Embleton, 1998). Particle engineering with supercritical fluids is the subject of intense research in the pharmaceutical industry (Debenedetti et al., 1993; Velaga et al., 2002; Schiavone et al., 2004; Lobo et al., 2005). Despite its potential, SCF is still an emerging technology that is not much exploited in DPI products. Concerns over the potential denaturing effects of the solvents/antisolvents used in this process may arise, however.

So, for each technique it is important to consider the effect it has on the drug. Spray-drying and supercritical fluid methods offer more flexibility and the possibility of morphology control in addition to size control, but they may sometimes yield amorphous material or undesired polymorphs. Milling remains the process of choice for micronizing drugs because it is simpler, more predictable, easier to scale up, and less expensive. However, spray-drying, supercritical fluid processing, and a few other techniques remain alternatives for the formulator to consider when milling does not produce the desired results. Interestingly, these techniques may also be used to produce coated particles or matricial formulations entrapping the active drug.

II.7. CYSTIC FIBROSIS

II.7.1. Disease

Cystic fibrosis (CF) is the most common life-shortening autosomal recessive disorder in the white population, affecting approximately 60000 individuals worldwide (Gibson et al., 2003). It is caused by mutations in a single gene on the long arm of chromosome 7 that encodes the cystic fibrosis transmembrane conductance regulator (CFTR). This mutation results in dysfunction of the apical membrane CFTR protein, which regulates chloride and sodium transport in secretory epithelial cells, with abnormal ion concentrations across the apical membranes of these cells (Ramsey, 1996; Durieu and Nove Josserand, 2008).

Under normal conditions, sodium (followed by chloride counter-ion) is avidly reabsorbed, primarily through apical sodium channels and CFTR. In patients with cystic fibrosis, the absence of functioning CFTR involves abnormal electrolyte transport, resulting in the production of an abnormally viscous secretion (Rowe et al., 2005) (Fig. 19).

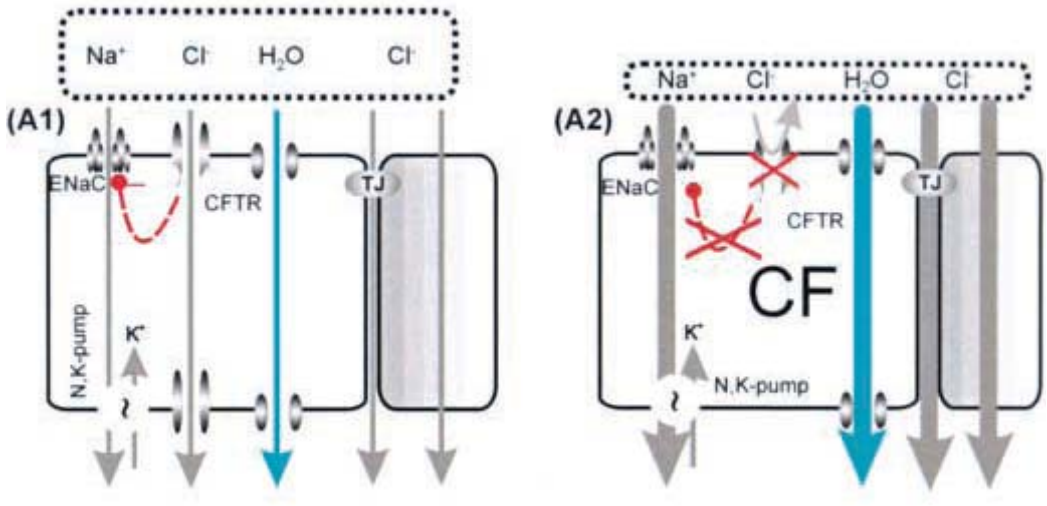


Figure 19: Models explaining the transport of Cl^- across (A1) normal airway epithelium and (A2) airway epithelium in cystic fibrosis (Gibson et al., 2003)

Mutations in the CFTR gene result in defective chloride transport in the epithelial cells in the respiratory, hepatobiliary, gastrointestinal, and reproductive tracts and the pancreas. Proper functioning of the exocrine glands is disturbed, resulting in dehydrated, viscous secretions that are associated with luminal obstruction and destruction and scarring of various exocrine ducts.

The clinical consequences include multi-system disease characterized by progressive pulmonary damage leading to respiratory failure, pancreatic dysfunction, liver disease that may progress to cirrhosis, gut motility problems, infertility and elevated sweat electrolytes. (Kerem et al., 2005; Rowe et al., 2005). Nevertheless, the primary causes of morbidity and mortality in patients with cystic fibrosis are bronchiectasis and obstructive pulmonary disease (Ramsey, 1996). 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 (Hodson et al., 2002) and about 90% of all CF patients die due to progressive pulmonary disease (Koch and Hoiby, 1993; Geller et al., 2002).

Pathogens such as *Pseudomonas aeruginosa, Burkholderia cepacia, Staphylococcus aureus* and *Haemophilus influenzae* become well-established within firmly fixed airway secretions in patients with CF and are not effectively eradicated. By the end of the first decade of life, *Pseudomonas aeruginosa* is the predominant pathogen (Fig. 20).

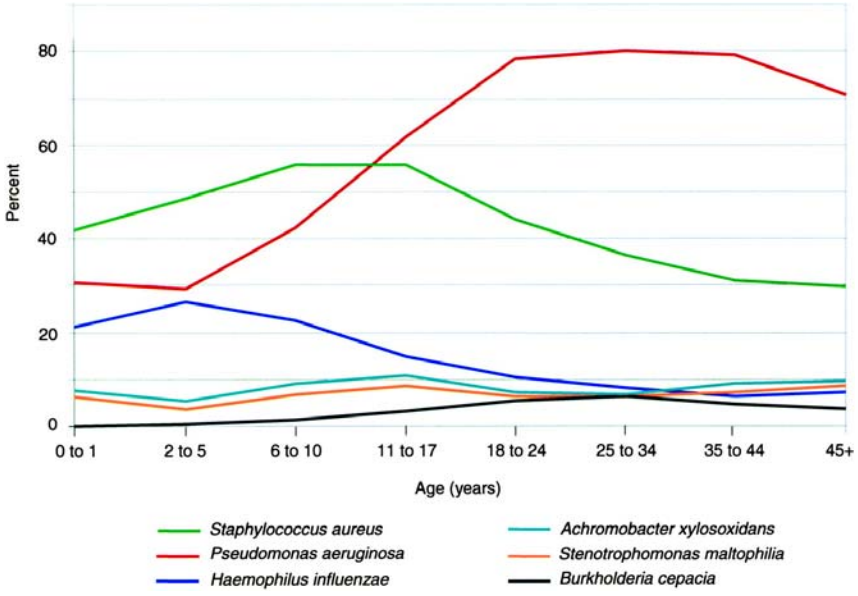


Figure 20: Age-specific prevalence of airway infections in patients with CF (Gibson et al., 2003)

This chronic bacterial endobronchitis is associated with an intense inflammatory response that damages the airways and impairs local host-defense mechanisms. This vicious circle results in progressive bronchiectasis and, ultimately, respiratory failure. *P. aeruginosa,* for example, specifically adapts to the pulmonary microenvironment in patients with cystic fibrosis through the formation of macrocolonies (or biofilms) and the production of a capsular polysaccharide (an alginate product) that inhibits penetration by antimicrobial agents (Ramsey, 1996; Kerem et al., 2005).

II.7.2. Treatments

The treatment of cystic fibrosis is directed toward alleviation of symptoms and correction of organ dysfunction. The cornerstones of treatment have been clearance of lower-airway secretions, treatment of pulmonary infections and pancreatic-enzyme replacement. Improvements in treatment have helped increase median survival from 14 years in 1969 to 32 years in 2004. For patients born in the 1990s, the median survival is predicted to be over 40 years (Fig. 21) (Yankaskas et al., 2004, Durieu and Nove Josserand, 2008).

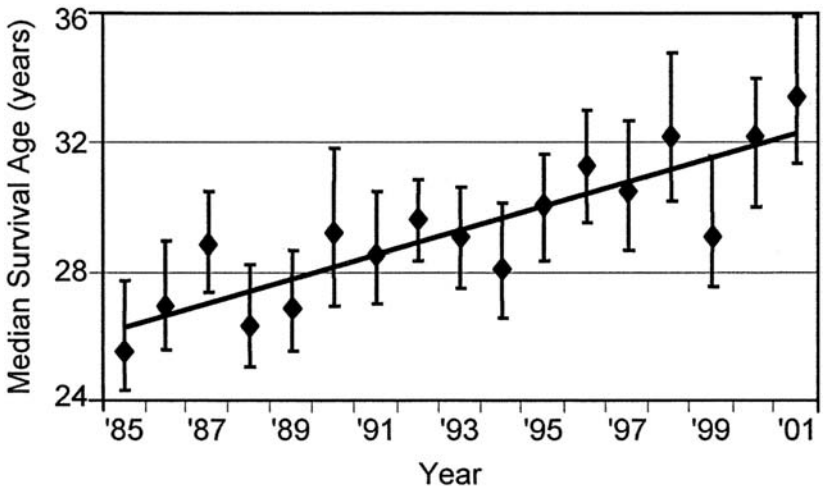


Figure 21: Median survival age in cystic fibrosis from 1985 until 2001. The 95% confidence intervals for the survival estimate are denoted by the vertical bars (Gibson et al., 2003)

II.7.2.1. Clearance of airway secretions

II.7.2.1.1.Chest physiotherapy

The chief non-pharmacologic approach to enhancing clearance of pulmonary secretions has been chest physiotherapy. Standard chest physiotherapy involves postural drainage with chest percussion in several anatomic positions to favour gravitational clearance of secretions from all lobes of the lung (MacIlwaine and Davidson, 1996). The long-term benefits of daily maintenance chest physiotherapy are believed by many clinicians and patients to be beneficial in improving airway clearance (Gibson et al., 2003). Although the procedure may improve pulmonary function, it is time consuming and must be carried out by a trained person.

II.7.2.1.2. Mucolytics

The abnormal viscosity of airway secretions in patients with cystic fibrosis is due primarily to the presence of many polymorphonuclear neutrophils and their degradation products. DNA from dying autolyzing neutrophils aggregates in large fibrils that markedly increase sputum viscosity (Potter et al., 1969). So, a purified recombinant **human deoxyribonuclease I** (rhDNase I) (Pulmozyme) that can digest extracellular DNA reduces the viscoelasticity of sputum specimens from patients with cystic fibrosis.

Classic mucolytics such as **N-acetylcysteine** or **ambroxol** have little effect on lung disease in CF patients (Ratjen et al., 1985). Moreover, the efficacy and safety of N-acetylcysteine at high doses has not been established (Yankaskas et al., 2004).

A well-tolerated new mucoactive drug called **nacystelyn** (NAL), patented by SMB-Galephar (Brussels, Belgium), is an equimolar mixture of L-lysine and N-acetylcysteine and has showen a more potent mucolytic activity than N-acetylcysteine. In fact, its *in vitro* intrinsic mucolytic activity is greater, probably due to the additional effect of L-lysine breaking secondary bridges of mucus polymers. Moreover, NAL is able to increase *in vivo*

the transepithelial potential difference, causing a simulation of chloride and water transports that enhance fluidification of the mucus (Nagy et al., 1997; Vanderbist et al., 1999; App et al., 2002).

II.7.2.1.3. Bronchodilator therapy

The majority of patients with CF demonstrate bronchial hyperreactivity at least at some time. Bronchodilators have therefore become a standard component of the therapeutic regimen. Most of these drugs fall into one of three therapeutic categories: β_2 adrenergic agonists, anticholinergics and corticosteroids.

The lung is the target organ but the site specificities of deposited drugs may be the key factors involved in eliciting therapeutic effects. Some data indicate that certain bronchodilator drugs should be selectively delivered to well-defined, small areas such as tracheobronchial bifurcations.
These clinical observations may be explained at least partially in terms of the spatial distributions of appropriate receptors and nerve endings among lung airways. Most β receptors are located in the alveoli, while anticholinergics target muscarinic receptors, which are moderately distributed throughout the airways and periphery. In fact, the trachea is more densely populated with M3 muscarinic receptors than β receptors, and corticosteroids target inflammatory cells, which are located throughout the airways and alveoli. So, for a maximum therapeutic effect, receptors should be targeted (Martonen and Katz, 1993).

Nebulized β-adrenergic agonsits such as salbutamol, are the most commonly prescribed agents. Therapy with β-adrenergic bronchodilators improved pulmonary function in patients hospitalized for exacerbations of pulmonary infections (Yankaskas et al., 2004).

II.7.2.2. Treatment of pulmonary infections: Antibacterial therapy

Patients with cystic fibrosis have periodic exacerbations of pulmonary infection that are identified primarily on the basis of an increase in pulmonary symptoms and airway secretions.

The standard therapy for *Pseudomonas aeruginosa* endobronchial infections in CF patients usually involves administration of two parenteral antipseudomonal antibiotics, including a **β-lactam** and an **aminoglycoside** agent such as tobramycin. 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) (Fig. 22) is accomplished by binding irreversibly to 30S and 50S ribosomal subunits resulting in a defective protein (Feng et al., 2002).

The aim of antibiotic therapy in chronically-infected CF patients is to stabilise lung function and, if possible, to restore some of the lost lung function (Mendelman et al., 1985; Saiman, 1998).

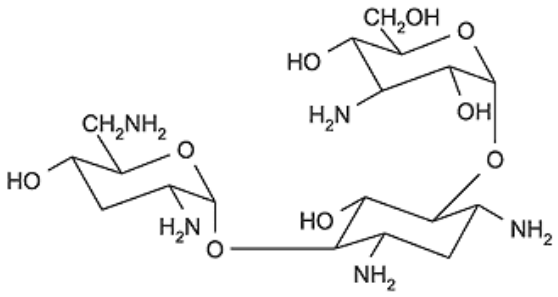


Figure 22: Structure of tobramycin (Rxlist, 2008)

Macrolids such as erythromycin, clarithromycin and azythromycin have also been effective in treatment of chronic infections of the airways with *P. aeruginosa* (Gibson et al., 2003).

Therapy with **fluoroquinolones** is often used for mild to moderate exacerbations. Ciprofloxacin has been effective as intravenous antibiotic therapy in improving pulmonary function (Yankaskas et al., 2004).

Nevertheless, an increasing number of patients with chronic *P. aeruginosa* infection develop multiresistant strains defined as demonstrating resistance to all drugs in at least two of three major classes of antipseudomonal agents, β-lactams, aminoglycosides, and

quinolones (Gibson et al., 2003). Therefore, combined administration of each type of drug is advocated to slow the emergence of antibiotic resistance (Ramsey, 1996).

Moreover, 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 (Geller et al., 2002). Like other aminoglycosides, tobramycin has a 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 (Feng et al., 2002).

Thus, the administration of antibiotics by inhalation offers an attractive alternative, delivering high concentrations of antibiotic directly to the site of infection while minimizing systemic bioavailability. Pulmonary administration of various antibiotics has been found to improve lung function in CF patients with chronic pulmonary infection with *Pseudomonas aeruginosa* and to reduce the frequency of hospital admission. When given by inhalation, the antibiotic is delivered directly to the target organ, which increases the therapeutic index of the drug (Sermet-Gaudelus et al., 2002). Inhalation is recommended in the European Consensus document on antibiotic treatment against *Pseudomonas aeruginosa* (Döring et al., 2000). Several antibiotics, such as colistin sulfate, colistimethate, polymyxin B and carbenicillin, have been investigated for administration by inhalation and, at present, colistin is used in daily practice by CF patients in many countries (Newman et al., 1988; De Boer et al., 2002; Le Brun et al. 2002; Ramsey et al., 2005; Westerman et al., 2007a; Westerman et al., 2007b).

Nevertheless, aerosolized tobramycin has been the most thoroughly studied antibiotic in chronic suppressive therapy (Le Brun et al., 1999a; Le Brun et al., 1999b; Ramsey et al., 1999; Cheer et al., 2003; Lenoir et al., 2007; Poli et al., 2007). In two large, multicenter, double-blind, placebo controlled trials conducted over a 24-week period, treatment with the only nebulized tobramycin product available on the market (TOBI, Chiron Corporation, USA) was found to produce significant improvement in pulmonary function, to decrease the density of *P.*

aeruginosa in sputum, and to decrease the number of days that subjects were hospitalized (Ramsey et al., 1999).

II.7.2.3. Lung transplantation

Double lung or heart-lung transplantation is a treatment option for patients with cystic fibrosis and end-stage lung disease. Overall survival of lung-transplant patients is poorer than for other organ transplantation, with 3-year survival of about 60% in CF patients (Ratjen and Döring, 2003).

II.7.2.4. Pancreatic enzyme remplacements

Poor nutritional status has been linked to worse prognosis of CF patients (Ratjen and Döring, 2003). Introduction of enteric-coated microencapsulated enzymes (Creon®, Solvay Pharma) has greatly improved the weight of CF patients.
As patients with pancreatic insufficiency are prone to malabsorption of the fat-soluble vitamins (i.e., A, D, E and K), a supplementation of these vitamins is often given to CF patients (Yankaskas et al., 2004).

II.7.2.5. Future therapies

II.7.2.5.1. Gene therapy

Cystic fibrosis, as a recessive genetic disorder, is a potential target for gene-replacement therapy. *In vitro* studies have demonstrated that introduction of CFTR complementary DNA into affected cells could correct the chloride channel defect and have provided evidence that gene transfer therapy might be effective in CF (Gibson et al., 2003).

Further studies are needed to develop an optimal system of delivery that results in long-term expression of CFTR and does not evoke local inflammatory or immune responses.

II.7.2.5.2. Pharmacological modulation of ion transport

The physiological defect of CF patients is a combination of defective chloride secretion and excessive sodium absorption that promotes the passive absorption of water from the airway to the lumen. Two pharmacological approaches – inhibition of sodium absorption (amiloride) and activation of alternative chloride channels (UTP, ATP) - have been found to normalize the transport defect (Ramsey, 1996).

So, there is great optimism that the next decade will bring a biological or pharmacological therapy to treat the underlying defect in cystic fibrosis. In the interim, maximal therapy directed toward the secondary effects of the mutation will afford patients the greatest opportunity for prolonged survival.

III. AIM OF THE WORK

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Local delivery of medication to the lung is highly desirable, especially in patients with specifically pulmonary diseases, such as cystic fibrosis or chronic pulmonary infections. The principal advantages include reduced systemic side effects and higher dose levels of the applicable medication at the site of drug action.
Nevertheless, the only inhalation device for tobramycin that is available on the market is a nebulizer, the Tobi® (Chiron Corporation, USA), which delivers a solution of the drug. But, as previously seen, 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.

So, delivering drugs to the lung from a DPI formulation seems to be an attractive and well-appreciated alternative. Most often the active drug is mixed with high amounts of a coarse and inert ingredient (e.g. lactose) in order to increase the flowability and dispersibility of the formulation. Nevertheless, the addition of an excipient to the formulation, generally in the range of 60-99% (w/w), decreases the dose of the active drug that can be filled in a capsule. Most current DPIs can only deliver microgram quantities of drug rather than the milligram quantities needed for an inhaled antibiotic treatment, for example. So there is an increasing interest in formulations that do not need a coarse carrier to aid the flowability of micronized drug, namely **"carrier-free" formulations**.

In this work, different approaches of tobramycin formulation for DPI products were tested with the aim of minimizing the additives used in formulation development, due to the fact that this model drug is a projected highly-dosed drug (20-40 mg of tobramycin per administration).

On the one hand, in order to modify the surface properties of micron-size tobramycin particles, lipids were used in order to improve drug targeting to the lung. The aim was to use low levels of excipient, thus delivering more active drug to the lungs.

On the other hand, using high pressure homogenization and spray drying techniques, novel formulations containing nanoparticles were developed to manufacture DPI formulations. Formulations composed of a mixture of micron-size particles and nanoparticles and formulations containing solely nanoparticles were produced in order to modify the surface properties of the raw product and decrease the agglomeration tendency of the powder.

Another approach that aimed to modify the balance between the different forces of interactions and the surface properties of the particles was the investigation of the influence of formulation components on the aerosolization characteristics of spray-dried tobramycin through the use of various proportions of water in the solvent used to prepare the initial suspensions.

For each formulation strategy, the common objective was clearly to modify significantly particle surface properties and reduce their tendency to agglomeration, while limiting the additive level in order to allow delivery of more of the active drug to the deep lung.

Therefore, all these spray-dried powders were further characterized in terms of aerosolization properties, stability, surface composition and physical state in order to determine which formulations could be the most suitable for pulmonary delivery. Moreover, a clinical trial was performed in order to evaluate the efficiency, pharmacokinetics and bioavailability of these formulations.

IV. MATERIALS AND METHODS

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IV.1. MATERIALS

Tobramycin (purity > 90%, USP 29) was supplied as micronized powder with not less than 90% of the particles having a diameter less than 10 μm (Tobra μ) from Teva (Petah Tiqva, Israel). Cholesterol (purity > 95%, Eur. Ph. 6) was purchased from Bufa (Uitgeest, The Netherlands). Phospholipon 90H, hydrogenated soy lecithin with more than 90% hydrogenated phosphatidylcholine, consisting of approximately 85% distearol phosphatidylcholine and 15% dipalmitoyl phosphatidylcholine, was donated by Nattermann Phospholipids GmbH (Koln, Germany).
Sodium glycocholate (purity > 98%, Eur. Ph. 6) was purchased from Acros Organics (Geel, Belgium) and sodium taurocholate (purity > 97%, Eur. Ph. 6) was purchased from Sigma (Steinheim, Germany).

All chemicals used were of analytical grade.

IV.2. METHODS

IV.2.1. High speed homogenization

High speed homogenization (HSH) with a CAT high speed homogenizer X620 (CAT M.Zipperer, Staufen, Germany) was used as the first size-reduction step. This operation is also useful for obtaining a complete and homogenous dispersion of the drug to be processed using high pressure homogenization or spray-drying.

The procedure for this homogenizing operation was set with a homogenizing time of 10 min (for a 50 ml sample of tobramycin (2-10% w/v) in isopropanol) and a stirring rate of 24000 rpm. This operation was run in an ice bath to prevent sample temperature increase.
HSH was also used during the high pressure homogenization operation as it was placed inside the sample reservoir (stirring rate of 8000 rpm) to ensure suspension homogeneity and avoid sedimentation of the particles.

IV.2.2. High pressure homogenization

High pressure homogenization (HPH) is a method for reducing the size of the particles and producing drug nanosuspensions.
Effective particle size reduction in piston-gap high pressure homogenizers is resultant from enormous impaction forces brought in by intense interparticular collisions, high fluid shear forces and cavitation forces, the latter being the most pronounced (Kipp, 2004).
The cavitation forces created during HPH operation are the consequence of the reduced tubular diameter through which the suspension is processed.
In fact, the suspension reservoir has a terminal diameter of approximately 3 mm and the diameter at the homogenizing valve can be as narrow as 25 μm (size of the homogenizing gaps at a homogenizing pressure of 1500 bar) (Müller et al., 2001), thus leading to a very high streaming velocity (Fig. 23). According to Bernouilli's law, which states that the flow volume of a liquid per cross-section is constant in a closed system, the reduction of the diameter through which the suspension is passed leads to a tremendous increase in the fluid dynamic pressure and simultaneously to a decrease in the fluid static pressure at the homogenization gaps. This drop brings the static pressure below the vapour pressure of the liquid at ambient temperature meaning that water starts to boil, with formation of gas bubbles (cavitation bubbles). When the suspension leaves

Figure 23: Photograph of the Emulsiflex® C5

the gap and the initial pressure is restored, these cavitation bubbles implode and the high energy accompanying this implosion (heat, turbulent flow, acoustic shockwaves) is responsible for the breaking up of the drug particles (Kipp, 2004; Keck and Müller, 2006).

The HPH particle size reduction efficiency is dependant upon the homogenizing pressure applied, the number of homogenizing cycles, the drug hardness characteristics, and eventually the processing temperature (Hecq, 2006).

HPH is, in general, a rapid method for engineering drug nanosuspensions as reported protocols rarely exceed 20-30 homogenizing cycles. These protocols generally involve the processing of previously micronized drugs and a gradual increase in homogenizing pressure in order to prevent the blocking of the homogenizing gaps (since the gap aperture becomes smaller as the homogenizing pressure is increased) (Hecq, 2006).

Tobramycin powder was poured into a surfactant solution of isopropanol (5% Tobra, w/v, suspension) under magnetic stirring (500 rpm). After dispersion and HSH, nanosuspensions were then prepared using an EmulsiFlex-C5 high pressure homogenizer (Avestin Inc., Ottawa, Canada). Low pressure homogenization cycles were first conducted on the tobramycin suspension to further decrease particle size (10 cycles at 12000 PSI). HPH was then finally applied for 10-20 cycles at 24000 PSI. These cycles were conducted by re-circulating the processed suspension directly into the sample reservoir (closed loop).
Since HPH causes sample temperature increase (increase of 30°C following 20 cycles at 24000 PSI), all operations were carried out using a heat exchanger, placed ahead of the homogenizing valve, with sample temperature maintained at 10 ± 1°C. Samples were withdrawn after the different size reduction steps for particle size analysis.

IV.2.3. Spray drying

In order to retrieve the drug particles in dried-powder state from the suspensions in isopropanol, spray-drying was applied for all the DPI formulations produced.

The formulations were prepared, at laboratory scale, by spray-drying using a Büchi Mini Spray Dryer B-191a (Büchi laboratory-Techniques, Flawil, Switzerland). The Mini Spray Dryer operates on the principle of nozzle-spraying in a parallel flow. During the drying process the material temperature remains significantly below that of the drying air due to evaporative cooling, as the drying time ranges from 100 milliseconds to seconds. The temperature of the sample is in general a little below the outlet temperature. This Mini Spray Dryer allows the recovery of a range of particle sizes, from 0.5-30 μm. The lower limit is given by the particle separation capacity of the cyclone used: smaller particles can not be removed any further from the drying gas and go into the filter.

IV.2.3.1. Preparation of formulations containing nanoparticles

Spray drying was applied in order to retrieve nanoparticles in dried-powder state from the nanosuspensions obtained after HPH. The nanosuspensions (tobramycin 5% w/v in isopropanol) 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 and, in these conditions the outlet temperature was about 40°C. The resultant powder was blown through the cyclone separator and collected in a container. Powders were stored in a dessicator at ambient temperature.

IV.2.3.2. Preparation of carrier-free formulations

Tobramycin powder (5% w/v) was poured into solvent systems composed of isopropanol:water (v/v) in ratios between 100:0 and 80:20. After dispersion and HSH, the suspensions 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 varied between 120°C and 200°C, and in these conditions the outlet temperature turned around 60°C and 100°C in function of

the various inlet temperatures applied. The resultant powder was blown through the cyclone separator and collected in a container. Powders were stored in a dessicator at ambient temperature.

IV.2.3.3. Preparation of lipid-coated particles

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 factors such as the spraying and drying gas flow rates, chamber dimensions, and the feed flow rate.

In this part of the study, some modifications were brought to the commercial mini spray dryer in order to improve drying efficiency and to avoid softening of the lipidic excipients incorporated in the formulations. The spraying gas was heated, increasing the droplet drying efficacy, and an air cooling system equipped with an air dryer (Technifluid, Belgium) 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 (HAAKE K15 Water bath cooler, Germany) was used to cool the cyclone separator walls and thus reduce the adhesion and/or agglomeration of the lipids.

Suspensions with different concentrations of tobramycin and lipids were prepared. While tobramycin is practically insoluble in isopropanol (0.05 mg/ml), lipids dissolve in it and coat the micron-size particles during atomization.

Firstly, lipids were dissolved in 50 ml isopropanol. Then, tobramycin was added and the suspensions were processed by HSH. The suspensions were then spray dried with constant stirring. The following conditions were used during spray drying: spraying air flow, 800 l/h heated to 56°C; drying air flow, 35 m^3/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°C 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.

IV.2.4. Scanning electron microscopy

Scanning electron microscopy (SEM) is a technique that uses electrons instead of light for image viewing. The electron beam is produced by heating a metallic filament that functions as the cathode, generally a loop of tungsten. An anode placed right below the cathode forms powerful attractive forces for the electrons, causing them to accelerate down the microscope column towards the sample to be analyzed. Before reaching the sample, the electron beam is condensed and focused as a very fine point on the material to be analyzed. Once the electron beam hits the sample, there is production of secondary electrons, which are collected, converted to voltage and amplified. The image viewed consists of thousands of spots of varying intensity, which are dependent on the topography of the sample. The SEM column must always be in vacuum in order to prevent electron beam instability.
Since the SEM uses electrons to produce an image, it requires samples be electrically conductive. In order to view non-conductive samples, such as most organic drugs, these must be covered with a thin layer of conductive material (gold, platinium, etc.) using a sputter coater.

Evaluation of particle size and morphology was achieved by SEM, using a JSM-610 scanning electron microscope (EDAX CDU "Leap" detector, Jeol, Japan). Samples were scattered onto a thin film of a two-component epoxy resin and then coated with a platinum layer of 300 Å for 3 min under vacuum with a SCD030 sputter coater (Blazers Union, Liechtenstein). Acceleration during observation was 25 kV.

IV.2.5. X-ray powder diffraction

X-ray powder diffraction (XRPD) is a powerful and widely-used tool for crystalline state evaluation. X-rays are the part of the electromagnetic spectrum lying between ultraviolet and gamma rays. When X-rays are incident on a crystalline sample, they are scattered in all directions; in some of these directions the scattered beams are completely in phase and reinforce one another to form the diffracted beams. As defined by Bragg's law, diffraction will occur if a perfectly parallel and monochromatic X-ray beam, of wavelength λ, is incident on a crystalline sample at an angle θ that satisfies the Bragg equation:

$$n\lambda = 2d \sin\theta \quad (2)$$

where n is the order of reflection and d the distance between planes in crystals.

The XRPD pattern consists of a series of peaks collected at different scattering angles. If the sample is amorphous, then X-rays are not coherently scattered and no peaks can be observed.

Diffraction patterns of tobramycin formulations were determined using a Siemens D5000 Diffractometer (Siemens, Germany), with a Cu line as the source of radiation (WL1 = 1.5406 Å, WL2 = 1.54439 Å), and standard runs using a 40 kV voltage, a 40 mA current and a scanning rate of 0.02°/min over a 2 θ range of 2° to 70°.
The evaluation of the amorphous percentage present in the formulations consisted of measuring the diffraction pattern deviation from the measured baseline. The measured area (i.e. diffraction pattern vs. baseline) is thus indicative of the percentage of amorphous material in the formulation (Diffrac Plus Software, EVA, Bruker, Brussels, Belgium).

IV.2.6. Differential scanning calorimetry

Differential scanning calorimetry (DSC) can be used to measure any kind of phase transition, for a given compound, that is associated with heat transfer. The principle behind DSC is that two cups, placed in an oven, one containing the sample and the other nothing (or

an inert material), are heated and kept at the same temperature. Once an endothermic or an exothermic phase transition occurs within the sample, the heat flow provided to keep the two cups at the same temperature needs to be respectively increased or decreased thus giving rise to an endotherm or an exotherm. DSC is a technique that is frequently used in preformulation studies in order to evaluate the crystalline state, the presence of polymorphs or the existence of an amorphous form of a given drug.

Thermal properties of the powder samples were investigated with a differential scanning calorimeter Q 2000 (TA Instruments, Zellik, Belgium) with a refrigerated cooling system (TA Instruments, Zellik, Belgium). The amount of product analyzed was about 10 mg and was placed in aluminium sealed pans. Runs were set from 30-300°C at 20°C/min, using nitrogen as a blanket gas.

IV.2.7. Bulk density

Bulk and tapped density were measured using a tap density tester (Stampfvolumeter, STAV 2003, Jel, Germany). Bulk density (ϱ_B) was determined by filling about 1 g of the various powders produced into a 10 ml measuring cylinder and tapped density (ϱ_T) was measured by tap density measurements following 1000 taps, which allowed the density plateau.

Bulk and tapped density values allow the determination of the Carr's compressibility index by the formula:

$$\textbf{Carr's Index (\%): } 100 \times (\varrho_T - \varrho_B)/\varrho_T \qquad (3)$$

Carr's Index values of less than 25 are usually taken to indicate good flow characteristics, values above 40 indicate poor powder flowability.

IV.2.8. Determination of water content

The water content of the dry powders was assessed by three different methods.

Measurements were performed by the Karl Fisher (KF) volumetric titration method with a 756 KF Coulometer (Metrohm Ltd., Antwerp, Belgium).
This method is designed to determine the water content in substances, utilizing the quantitative reaction of water with iodine and sulfur dioxide in the presence of a lower alcohol, such as methanol, and an organic base (B), such as imidazole or pyridine, as shown in the following formulae:

$$CH_3OH + SO_2 + B \rightarrow HB^+ + CH_3SO_3^-$$
$$H_2O + I_2 + CH_3SO_3^- + 2B \rightarrow CH_3SO_4^- + 2HB^+ + 2I^-$$
$$SO_2 + CH_3OH + H_2O + I_2 + 3B \rightarrow 3HB^+ + CH_3SO_4^- + 2I^- \quad (4)$$

In the coulometric titration method, first, iodine is produced by electrolysis of the reagent containing iodide ions. Then, the water content in a sample is determined by measuring the quantity of electricity which is required for the electrolysis (i.e., for the production of iodine), based on the quantitative reaction of the generated iodine with water.
The water content was measured in triplicate on approximately 12 mg of powder. The determination limits were set between 10 μg and 200 mg of water.

Thermogravimetric analysis (TGA) was done with a Q500 (TA Instruments, Zellik, Belgium). TGA measures the amount and rate of weight change in a material, either as a function of increasing temperature, or isothermally as a function of time, in a controlled atmosphere. It can be used to characterize any material that exhibits a weight change due to decomposition, oxidation or dehydration. Runs in triplicate were set from 30°C to 350°C at 10°C/min with samples of between 5 and 10 mg in a platinium pan.

Finally, the amount of water in the powder was determined using a Sartorius WDS 400 Water Detection System (Sartorius, Goettingen, Germany).

Samples of 10 mg (n=3) were weighed and heated in a stainless steel oven. The water vapour produced is transferred to a phosphorus pentoxide-coated electrolytic cell by the N_2 carrier gas. A chemical reaction takes place between the extremely hygroscopic phosphorus pentoxide and water, which results in the electrolytic dissociation of the water molecules. The electrical charge required for this process is quantitatively measured. Then, applying Faraday's law, the charge measured is converted into the amount of water initially contained in the sample tested. The detection limit of this method is about 1 μg of water and, depending on the sample, covers a measuring range of 15% water content down to just a few parts par million (ppm).

IV.2.9. Laser diffraction

Laser diffraction measurements are based on the phenomenon that particles scatter light in all directions with an intensity pattern dependent on particle size: the smaller the particle size, the higher the scattering angle and the smaller the scattering intensity. The principle behind laser diffraction measurements is that a representative sample, dispersed at an adequate concentration in a suitable liquid or gas, is passed through the beam of a monochromatic light source followed by subsequent recording of the light scattered at various angles by a multi-element detector. These numerical scattering values are then transformed, using an appropriate optical model, to yield the proportion of total volume to a discrete number of size classes forming a volumetric particle size distribution.
Mean particle size and particle size distribution (PSD) are thus obtained through a matrix conversion of the scattered intensity measurements as a function of the scattering angle and the wavelength of the light on applicable scattering theory. In the Mie theory (used by Malvern, U.K.), the scattered light intensity of a particle is a function of different variables: particle size, particle refractive index, medium refractive index, light wavelength, scattering angle.

Laser diffraction analysis yields a size distribution expressed in volume. Calculations are based on the theory of "equivalent spherical diameter" in order to determine particle

sizes; that is to consider the diameter of a theoretical sphere that would produce the same signal as the studied particles. Using this theory, numerous equivalent diameters, including other parameters, such as surface, volume or weight, can be calculated.

The characterization parameters used were:

D(0.1), which is the size (μm) of particle below which 10% of the sample lies,

D(0.9), which is the size (μm) of particle below which 90% of the sample lies,

D(0.5), which is the size (μm) of particle below which 50% of the sample lies (volume median diameter),

and **D[4.3]**, which is the mean volume diameter, calculated using the following formula:

$$\mathbf{D[4.3]: \Sigma d^4 / \Sigma d^3} \quad (5)$$

Values presented are the average of at least 3 determinations.

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

IV.2.9.1. Mastersizer 2000®

The volume particle size distribution was measured with a Malvern Mastersizer 2000® laser diffractometer (Malvern, Worcestershire, U.K.) equipped with dry and wet sampling systems (Scirocco 2000 and Hydro 2000 S, respectively), the latter being used for nanoparticle suspension measurements. A 2 mW 632.8 nm Helium Neon laser, along with a shorter wavelength 466 nm blue light source for improved fine particle resolution, was used. Detection limits for the equipment range from 0.02-2000 μm.

The particle size distribution of the dry powders produced after spray-drying was measured with the dry sampling system with a suitable standard operating procedure (SOP): refractive index, 1.54; vibration feed rate, 25%; measurement time, 7 sec; dispersive air pressure, 4 bar; n=3.

The size and size distribution of the particles in suspension were determined by laser diffraction with the wet sampling system. 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). The refractive indices used for the measurements were 1.54 and 1.39 for the drug and the solvent, respectively.

IV.2.9.2. Spraytec®

The second laser diffraction-based technique consisted of a Malvern Spraytec® (Malvern, Worcestershire, U.K.) equipped with an inhalation cell, specifically modified for measuring the particle size diameter generated from medicinal aerosols, including MDIs, DPIs 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) (see part IV.2.12.1.1.3, p 97) (Fig. 24).

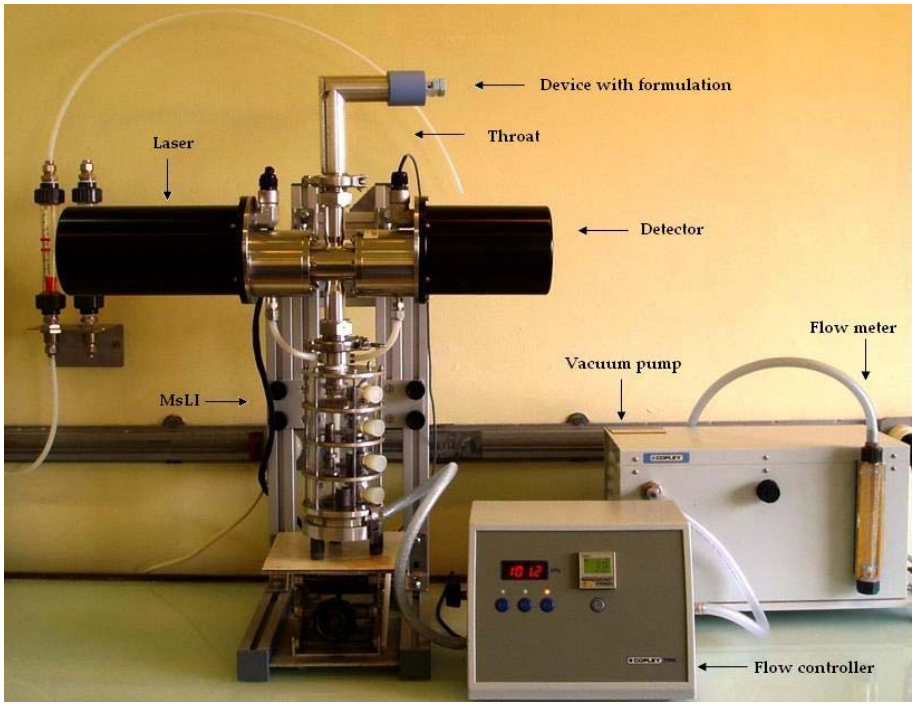


Figure 24: Photograph of the Spraytec connected to a multi-stage liquid impinger

The focal length of the Spraytec® lens used was 100 mm, which has a particle size range of 0.5-200 µm. The D(0.5) and the percentage of particles having a diameter of < 5.0 µm

-considered as the FPF- were obtained using the Spraytec® software. Values presented are the average of at least 3 determinations.

The entire assembly is a closed system and allows for a controlled airflow rate in the measurement zone. This allows the size properties of DPIs to be measured under simulated breathing conditions (Haynes et al., 2004). The difference between the Mastersizer 2000® and the Spraytec® is the particle dispersion capacity of the two methods. The compressed air values applied in the dispersion unit of the Mastersizer 2000® (up to 4 bar) allow all the agglomerates to break down, whereas the air flow generated in the Spraytec® (in the range of 30-100 l/min, similar to a patient inspiratory airflow) is much lower and does not permit the de-agglomeration of all particles.

The assembly was connected to an impactor in order to allow the same aerosol, generated under inspiratory air flow, to pass through both the laser beam crossing the end of the metal throat and the stages of the inertial impactor. Sizing could therefore be carried out using the two techniques in line. Importantly, this modification did not change the determination of the size results from the MsLI. Thus, this assembly allowed the simultaneous determination of the geometric (laser diffraction) and aerodynamic (MsLI) diameters. Each formulation produced was evaluated with the Spraytec® for a rapid screening of the properties of the powder.

IV.2.10. Uniformity of delivered dose

The delivered dose is the dose delivered from the inhaler. The dose collection apparatus must be capable of quantitatively capturing the delivered dose. The apparatus consists of a filter support base with an open-mesh filter-support, a collection tube that is screwed to the filter-support base and a mouthpiece adapter to ensure an airtight seal between the collection tube and the mouthpiece. The apparatus was operated at an airflow rate of 100 l/min, which produces a pressure drop of 4 kPa over the inhaler, and for a duration of 2.4 s consistent with the withdrawal of 4 l of air from the mouthpiece of the inhaler (Eur. Ph. 6; USP 29). The content of the apparatus was quantitatively collected and

the amount of active substance was determined with the suitable and validated HPLC method (see part IV.2.12.1.3.1, p 101). The procedure was repeated 10 times for each formulation tested. The preparation complies with the test (Eur. Ph. 6; USP 29) if nine out of ten results lie between 75% and 125% of the average value and all lie between 65% and 135%.

IV.2.11. Uniformity of drug content

For each batch produced, 5 samples of 15 mg of powder were collected and the amount of tobramycin was determined by the suitable and validated HPLC method after a derivatization step (see part IV.2.12.1.3.1, p 101).

IV.2.12. Assessment of lung deposition

IV.2.12.1 In vitro assessment of lung deposition

IV.2.12.1.1. Impactors and impingers

Inertial impactors have been employed to characterize pharmaceutical aerosols for more than 30 years. Their use allows the prediction of the deposition of powder particles within the lung. Inertial methods of aerosol particle sampling are carried out by passing the airflow and suspended aerosol through an orifice of known dimensions and impinging it onto a collection surface at a fixed distance from the orifice. The aerosol is passed through the orifice and the output stream is directed against a flat plate, called an impaction plate. The plate deflects the flow to form an abrupt 90° bend in streamlines. Particles with an inertia exceeding a certain value are unable to follow the streamlines and collide/impact on the plate. Smaller particles remain airborne, follow the streamlines and avoid hitting the impaction plate. The velocity of the particles increases from one stage to another and

particles are progressively impacted at the lower stages as they present smaller and smaller sizes (Fig. 25).

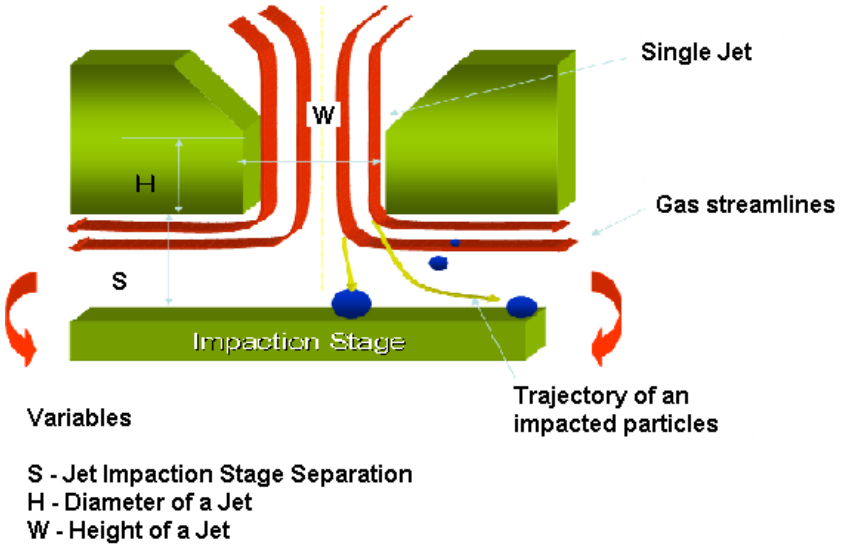


Figure 25: Schema of an impaction stage

All impactors are made according to well-defined design specifications (Eur. Ph. 6; USP 29). The geometrical factors that influence the collection efficiency at each stage, that is, the orifice diameter and orifice-to-surface distance, are constant. Flow rate is the only remaining variable influencing the collection efficiency of each stage of an impactor (Hinds, 1999). The cut-off diameter of a stage is that of the particle size collected with 50% efficiency. Many impactors are designed to operate at fixed flow rates such that the cut-off diameters are constant. However many dry powders are delivered from dry powder inhalers by the inspiratory effort of the patient, which, together with the resistance of the device, therefore dictates the aerosol dispersion. Variation of airflow rate between 30 and 100 l/min (for 8.0-2.4 s respectively so that a volume of 4 litres of air is drawn through the inhaler), which is therefore very useful, affects the collection efficiency of each stage, requiring recalibration of the cut-off diameters (Dunbar et al., 1998).

Four apparatuses are used in the European and US Pharmacopoeias, i.e., the glass impinger, the Andersen cascade impactor (ACI), the multi-stage liquid impinger (MsLI) and the new generation impactor (NGI).

IV.2.12.1.1.1. Glass impinger

This apparatus is a glass system device composed of interconnecting flasks used for the aerodynamic evaluation of the fine particles from nebulizers, MDIs and DPIs (Fig. 26.A). Samples are collected into a suitable liquid. The device is designed to be operated at 60 l/min and consists of one stage with an operational cut-off diameter of 6.4 μm. As this apparatus results in a relatively inefficient size-selective sampling, it was not used during this work, to characterize the formulations. Nevertheless, it can still be used in the first phases of development because of its simplicity and the little time required for carrying out a test.

IV.2.12.1.1.2. Andersen cascade impactor

The Andersen cascade impactor consists of 8 aluminium stages together with a final filter (Fig. 26.B). This allows a more detailed particle size distribution, and can be operated at various flow rates. Nevertheless, it was not used during this work, because the manipulation is time-consuming due to the elevated number of plates, and the *in vitro/in vivo* correlation is sometimes hazardous because the apparatus does not contain water and therefore does not evaluate the influence of humidity on the aerodynamic behaviour of the particles.

IV.2.12.1.1.3. Multi-stage liquid impinger

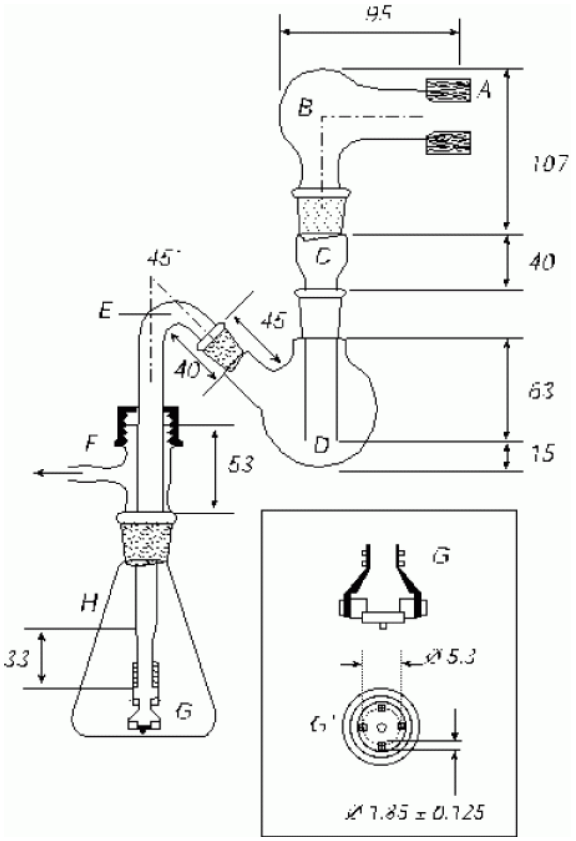
The MsLI is a four stage apparatus with a filter, which is widely used for the aerodynamic evaluation of the fine particles from MDIs and DPIs (Fig. 26.C.). This apparatus was used to characterize the developped formulations as it quickly provides information on the distribution of particle size in the respirable range. The instrument is arranged vertically and samples are collected into an appropriate liquid medium at various operating flow rates between 30 l/min and 100 l/min, making it suitable for the evaluation of dry powder inhalers. At 100 l/min, the cut-off diameters are about 5.27, 2.40 and 1.32 μm at stages 3, 4 and 5 (filter), respectively.

IV.2.12.1.1.4. New generation impactor

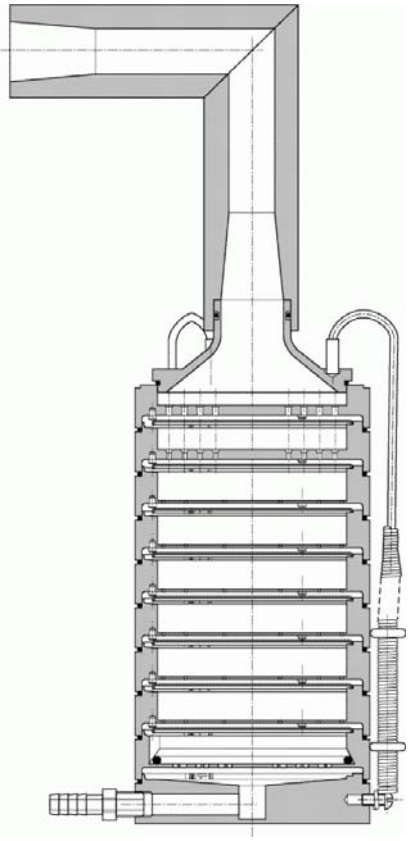
The NGI is an horizontal cascade impactor designed with 7 stages and a micro-orifice collector (MOC) (Fig. 26.D). The terminal MOC, which is an impactor plate with 4032 holes - each approximately 70 μm in diameter - eliminates the need for a final filter.

Over the flow rate range of 30-100 l/min the cut-off diameters range between 0.24 μm and 11.7 μm, evenly spaced on a logarithmic scale. In this flow range, there are always at least 5 stages with cut-off values between 0.5 μm and 6.5 μm. The collection efficiency curves for each stage are sharp and minimise overlap between stages, unlike the ACI.

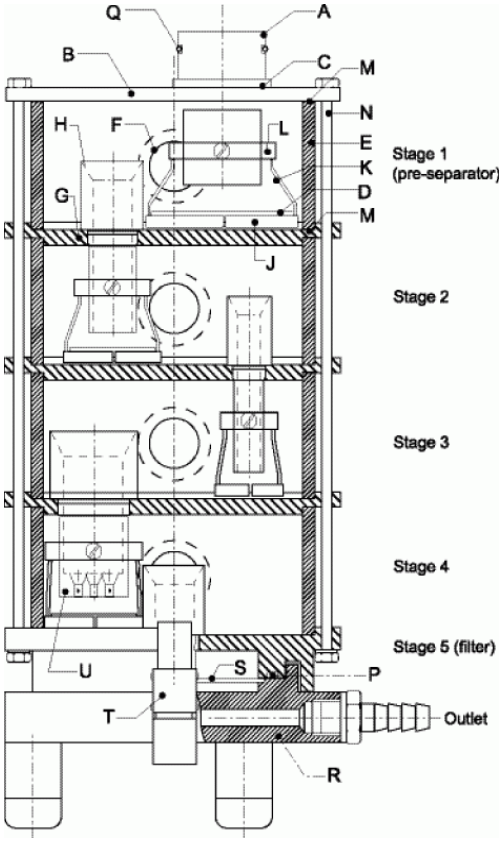
There are three main sections to the impactor: the bottom frame, which holds the impaction cups; the seal body, which holds the jets; and the lid, which contains the interstage passageways. As the impactor configuration has removable impaction cups with all the cups in one plane, the NGI is easier to use than the ACI and will advantageously replace it.



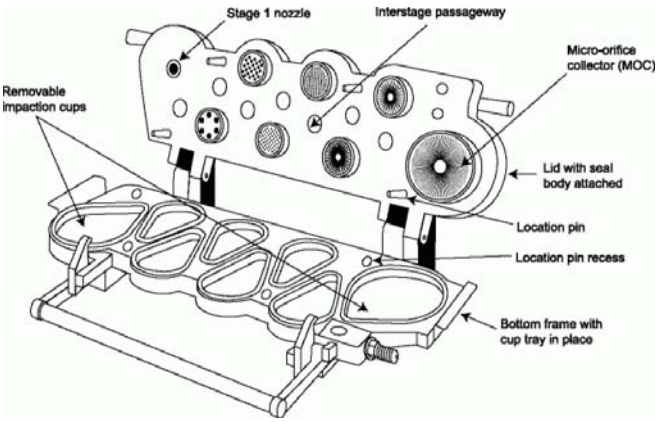
A.



B.



C.



D.

Figure 26: Schema of A. Glass impinger, B. ACI, C. MsLI, D. NGI (Eur. Ph. 6.)

IV.2.12.1.2. Interpretation of results

Data interpretation is done by plotting the amount of drug sampled at each stage of the impactor against the cut-off diameter for the stage, resulting in a collection efficiency curve.

The experimental mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) can be derived from the log-probability scale plot. The MMAD of the particles is defined from this graph as the particle size at which the line crosses the 50% mark and the GSD as

$$\mathbf{GSD = \sqrt{(SizeX/SizeY)}} \quad (6)$$

where size X is the particle size for which the line crosses the 84% mark and Size Y the size for which it crosses the 16% mark.

Since particles are generally inhaled not singly but as constituents of aerosols, an experimental aerodynamic diameter, or MMAD, is an appropriate parameter to use rather than the theoretical D_{aer} (see part II.3.4, p 29) or the geometric size (Schlesinger, 1995). The MMAD corresponds to the diameter of the particles deposited in the impactor for which 50% w/w of particles have a lower diameter and 50% w/w have a higher diameter (Courrier et al., 2002). This parameter better reflects the possible aerosolization of particles as aggregates and not only as individual particles.

Often delivery devices produce a polydisperse aerosol, that is, particles of widely different sizes, rather than a monodisperse one. This parameter, which influences the deposition of particles, is described by the GSD. If the GSD is smaller than 1.2, an aerosol is termed “monodisperse” (Schulz, 1998). However, many pharmaceutical aerosols will exhibit more than one mode. A therapeutic aerosol with excipients exhibits a GSD of approximately 2. It is conceivable that two completely different aerosol distributions (e.g., small median size with narrow distribution or large median size with broad distribution) could give exactly the same fine particle fraction.

However, within the FPF, the aerosol would exhibit different sizes, leading to differences in regional lung deposition, resulting in variations in therapeutic effect. Thus, the degree of

dispersity is an important consideration for both the quality and the efficacy of pharmaceutical aerosols (Chew and Chan, 2002b). These considerations are particularly important for a standard DPI formulation, which is frequently bimodal, because it contains micronized drug and substantially larger carrier particles.

The total dose of particles with aerodynamic diameters smaller than 5 μm was also calculated by interpolation from the collection efficiency curve 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.
The FPF or FPD is defined as the fraction of the powder that can be deeply inhaled and is theoretically available for pharmacological activity (Dunbar et al., 1998).
The emitted dose is the dose delivered by the inhaler to the patient or to the impactor device. Hence, the emitted dose equals the dose previously contained in the capsule minus the fractions of the dose remaining in the DPI device.

IV.2.12.1.3. Aerodynamic particle size analysis

The aerodynamic particle size distribution was determined using an MsLI or an NGI (Copley Instruments, U.K.) operated under pharmacopoeial conditions. Dry powder inhalation devices (Aerolizer®, Spinhaler® and Handihaler®) were filled with HPMC size 3 capsules (Capsugel, Colmar, France). HPMC capsules were used because gelatine capsules have a tendency to break during the test and to produce agglomerates during particle size measurement. 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. Apparatuses were operated at various airflow rates ranging between 30 and 100 l/min for 8.0-2.4 s respectively so that a volume of 4 litres of air was drawn through the inhaler as recommended by the pharmacopoeias (Eur. Ph. 6 and USP 29). Three capsules loaded with 15 mg powder were taken for each test. Drug deposition in the device, the throat, and all the stages was determined by HPLC analysis. For accuracy, each test was repeated three times.

IV.2.12.1.3.1. Derivatization procedure

The chemical structure indicates that tobramycin has five primary amines, one primary hydroxyl group and four secondary hydroxyl groups (Fig. 22, p 73). Due to the low chromophore in the molecule, direct HPLC methods for tobramycin are not straightforward (Feng et al., 2002). In order 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 29.

All stages of the MsLI were filled with 20 ml water. For the two apparatuses, deposits in the device, throat and the stages were collected in 100 ml volumetric flask with 1 ml H_2SO_4 1N, and deposits in the filter or terminal MOC were collected in a 50 ml volumetric flask with 1 ml H_2SO_4 1N.

For the derivatization procedure, 4.0 ml of each flask were 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 of 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, Germany)). After shaking, the flasks were placed in a constant temperature bath (GFL 1086, Germany) at 60°C and heated for 50 minutes with an agitation of 0.8 min^{-1}. Then, the flasks were removed from the bath and allowed to stand. After 10 minutes, acetonitrile was added to volume and the contents of the flasks were mixed.

As can be seen in Fig. 27, there is a nucleophile substitution between an amine of the tobramycin and the fluor of the 2,4 dinitrofluorobenzene with the apparition of a yellow-colored product soluble in dimethylsulfoxide that absorbed at 365 nm. As there is formation of HF, a basic solution of tris(hydroxymethyl)aminomethane was added to help the reaction.

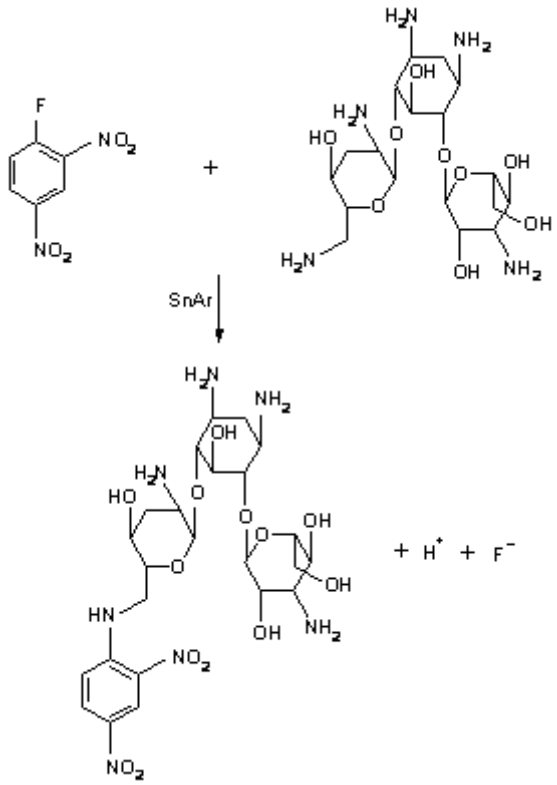


Figure 27: Reaction of the derivatization procedure

IV.2.12.1.3.2. HPLC system

The high pressure liquid chromatography method was used to determine the uniformity of the delivered dose, the aerodynamic particle size distribution and the uniformity content of the formulations.

The equipment consisted of a HPLC (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 a 30 cm x 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 of Tris(hydroxymethyl)aminomethane in 800 ml of 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 0.2 μm filter. The flow rate was 1.2 ml/min.

IV.2.12.2. *In vivo* assessment

IV.2.12.2.1. Pharmacokinetic analysis

Venous blood samples (7 ml) were collected at pre-dose and at 30 min, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 4 h, 6 h, 8 h and 10 h post-dose in order to quantify plasma levels of tobramycin. After centrifugation, the plasma samples were decanted and divided into two approximately equal portions of not less than 2 ml per tube (aliquot) and rapidly stored at -80° C, in an upright position.

The extraction of the tobramycin was performed by passing the plasma samples through a solid phase extraction cation exchange column (MCX, Waters, Belgium) and the tobramycin was eluated with a mixture of NH_4OH:CH_3OH 50:50. The sample was then evaporated to dryness and the residue was reconstructed and vortex-mixed with solvents A and B 40:60 (v/v). Solvents A and B consisted of methanol:water 50:50 and 0:100, respectively, both containing 5mM HFBA (heptofluorobutyric acid). An aliquot was analyzed with the validated LC/MS-MS method described below (see VIII.Annexe p 248).

An HPLC system (HP 1100 series, Agilent Technologies, Belgium), coupled to an API365 quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Concord, Canada) was used to measure tobramycin in plasma samples (n=2). The separation system was a 150 x 2 mm Polar-RP Synergi column (Phenomenex, U.S.A). Analyses were performed in a binary gradient mode. Solvents A and B consisted of acetonitrile:water 90:10 and 0:100 (v/v), respectively, both containing 2 mM NH_4Ac and 1% HCOOH. Solvent B also contained 5 mM HFBA. The HLPC gradient started at 20% A and 80% B (1 min hold) and was linearly increased to 100% A over 0.2 min (6.8 min hold) before returning to 20% A over 1 min (7 min hold). The flow was adjusted to 0.150 ml/min. The limit of quantification of the method was 10 ng/ml.

The calculation of pharmacokinetic parameters was as follows:
Maximal plasma concentration (C_{max}) and time to maximal plasma concentration (T_{max}) were taken directly from the plasma concentration *vs.* time curve. The area under the curve (AUC) was calculated by the linear trapezoidal rule from measured data points from the time of administration until the time of the last quantifiable concentration.
The AUC was also adjusted to the same drug dose as that of the comparator product (Tobi®) deposited in the lung (based on scintigraphic deposition values) in order to facilitate the comparison of the test and reference products.

IV.2.12.2.2. Gamma scintigraphy analysis

In vivo evaluation of pharmaceutical inhalation products is achieved by γ-scintigraphic imaging of the aerosol deposited in the lung. Imaging provides direct information on the amount and location of the drug deposited in the lung after inhalation. This local bioavailability, rather than the systemic bioavailability after absorption, is pertinent as it reflects the efficacy of drugs that act directly on the lung (Chan, 2002).

To measure lung deposition by imaging, the aerosol must be first labelled or tagged with a suitable radionuclide. Typical radiolabelling methods involve adsorbing Technetium 99m (^{99m}Tc) as sodium pertechnetate onto the surface of drug particles in a dry powder inhaler (Newman et al., 2003). ^{99m}Tc is the most commonly used pure γ-emitter for indirect radiolabelling of pharmaceutical aerosols. The γ-ray of ^{99m}Tc has sufficient energy (140 keV) to penetrate body tissues, and when it reaches the detector of the γ-camera, it is absorbed and converted into light photons, thus optimizing γ-camera imaging. The half-life of ^{99m}Tc is 6 h, which is long enough for handling and imaging but not so long that it increases the radiation dose to the subject unnecessarily (Newman et al., 1998).

Lung imaging is achieved using a γ–camera, which creates an image of the γ-rays emitted by the radionuclide in the lung. One of the most commonly used techniques, known as gamma scintigraphy, involves taking two-dimensional "planar" views of radionuclide

distributions with a single-headed or dual-headed gamma camera (Mobley and Hochlaus, 2001).

Each labelled capsule was first measured at distance by a non-specific gamma counter Ortec Elscint (AC Joure, The Netherlands) and the activity compared with that of a syringe containing approximately 30% of the capsule activity using ^{99m}Tc. This syringe was injected into a perfusion bag, which was placed near the patient's chest. This was considered to be the standard and was used to assess radioactive decay over time.

Immediately following the administration of the radiolabelled aerosol, scintigraphic images of the chest (posterior and anterior) and lateral oropharynx were recorded (DHD-SMV, Sopha Medical, France). A flat flood source was interposed between the lower detector and the posterior aspect of the trunk of the subject, and the activity was recorded with the upper detector. This was done to take into account the attenuation of the radioactivity resulting from absorption by tissues and to allow the lung fields to be outlined. A background count was recorded for each camera head.
The empty device and capsule (Aerolizer®), and the exhalation filter were also counted (Vanderbist et al., 2001; Ball et al., 2002).

Regions of interest were drawn around the oropharynx, oesophagus, stomach, and whole lung. The edges of the lungs were delineated using a ^{99m}Tc transmission scan and the lungs were subdivided into central, intermediate and peripheral regions of interest, corresponding approximately to large, medium and small airways. The ratio of peripheral to central lung deposition (P/C ratio) was calculated as an index of regional lung deposition (Newman et al., 1989; Newman and Wilding, 1998; Newman et al., 2001; Dolovich, 2004).
The counts in each named area were expressed as a percentage of the nominal dose, which was determined from the sum of the total body counts and those from the Aerolizer® inhaler device and capsules, and the exhalation filter.
The counts obtained within these regions were corrected for background radioactivity, radioactive decay, and tissue attenuation of gamma rays (Pitcairn and Newman, 1997). In

regions where both anterior and posterior images were recorded, the geometric mean of counts in both images was calculated.

IV.2.12.2.3. Radiolabelling method

A method of wide application to radiolabelling dry powders consists to adsorb the radiolabel onto the surface of the active particles. This is achieved by wetting the drug particles with a non-solvent containing the radiolabel, followed by the evaporation of the solvent, leaving the radiolabel on the surface of the drug particles (Chan, 2002).

Firstly, ^{99m}Tc as sodium pertechnetate was eluted from a ^{99}Mo-^{99m}Tc generator. Then, the ^{99m}Tc was extracted into methylethylketone by shaking the pertechnetate solution with an approximately equal volume of methylethylketone. The aqueous and methylethylketone phases were separated in a separating funnel and the methylethylketone phases containing the pertechnetate were collected and evaporated to dryness in a bath at 60°C for 10 min and 80°C for 5 min. After this, the pertechnetate was re-dissolved in isopropanol and then tobramycin (and, if applicable, lipids) was added to the solution. The suspension was processed through HSH and then spray dried with constant stirring.

Nebulized tobramycin was radiolabelled by simply dissolving an appropriate amount of ^{99m}Tc-DTPA (diethylenetriamine penta-acetic acid) complex in the aqueous nebulized tobramycin solution (Newman et al., 1988; Kuni et al., 1992).

IV.2.12.2.4. Validation of radiolabelled powder

Before initiation of the clinical phase of the study, *in vitro* validation experiments were conducted to demonstrate that significant alteration of the PSD did not occur during the labelling process, and that the PSD of the radiolabel matched the PSD of the drug.

For each formulation, the FPD and the PSD of the unlabelled drug were determined then compared against those of the labelled drug and of the radiolabel (n=3). The measurements were made with an MsLI operating at an air flow rate corresponding to a pressure drop of 4 kPa. The test was carried out at 100 l/min for 2.4 seconds.
The drug and radiolabel content at each stage of the MsLI were determined by the validated analytical HPLC method and by gamma counting (using a Cobra gamma 5003 counter, Packard Bioscience; GMI, Minnesota, U.S.A.), respectively. Quantification of tobramycin was done by the US Pharmacopeia method, which involves derivatization of the tobramycin with 2,4-dinitrofluorobenzene, and quantification by HPLC (part IV.2.12.1.3.1, p 101).
It should be noted that since the radiolabel is adsorbed onto the surface of the drug particles, its particle size distribution (as determined by a gamma counting technique) corresponds to that of the drug particles.

IV.2.13. Statistical analysis

The Student's *t* test was used to compare the lung deposition pattern between the two DPI products. For all tests, the significance level was set at $p = 0.05$.

The repeated-measures ANOVA test was used to compare the *in vitro* deposition data obtained with the MsLI, to validate the radiolabelling method and to compare the pharmacokinetic data obtained with the three formulations. The significance level was set at $p = 0.05$.

For the stability studies, the multiple comparison procedure of Tukey-Kramer was used to compare simultaneously the sets of data for the values at the initial times and at the different times of analyses. The significance level was set at $p = 0.05$.

For the validation of the acquisition, computation and comparison of the different sets of data from different apparatuses and methods, the Deming regression was used.

In practice, the quality of the analytical input data is crucial for the interpretation of method comparison studies. A first criterion generally used is the correlation coefficient R^2, which measures the strength of a linear relation between two variables, not the agreement between them (Bland and Altman, 1986). Nevertheless, as R^2 is still commonly used, it was computed in this study. A second method is to draw a comparison plot (one method per axis) coupled to a linear regression study (Westgard, 1998). Ordinary least-square regression (OLR) has the disadvantage of assuming an error-free x variable and a constant analytical imprecision of the y variable (homoscedatic variance), assumptions that are seldom met in practice (Stockl et al., 1998). If both measurement sets are subject to random errors, an alternative to OLR is the Deming regression computed in this study, also named the "principal component analysis", which requires specifications of the ratio between variances of both analytical methods (Linnet, 1990; Linnet, 1998; Stockl et al., 1998).

V. EXPERIMENTAL PART

PART V.1: CORRELATIONS BETWEEN CASCADE IMPACTOR ANALYSIS AND LASER DIFFRACTION TECHNIQUES FOR THE DETERMINATION OF THE PARTICLE SIZE OF AEROSOLIZED POWDER FORMULATIONS

Int.J.Pharm., 358(1-2): 75-81 (2007)

V.1.1. Introduction

As particle size is a key parameter in defining the deposition pattern and bioavalability of drug material delivered to the respiratory system using inhalers, it is clearly important to have an efficient measurement technique during the research development process for inhalation formulations.

The aerodynamic diameter is routinely measured using sizing techniques that are based on inertial impaction. Inertial impaction of particles in movement in an air stream is well-understood and, as previously seen, many different types of impactors and impingers have been proposed for inhalation aerosols in the last few decades. They vary from simple devices, such as the glass impinger, to more complex apparatuses that have more collection plates, such as the Andersen cascade impactor (ACI), the multi-stage liquid impinger (MsLI), or the recently developed new generation impactor (NGI). Impingers and impactors have been widely employed for particle size characterization for lung and nasal drug delivery devices.

Nevertheless, laser diffraction is the most widely-used technique for particle size analysis. Instruments employing this technique are considered easy to use and particularly attractive for their capability to analyse particles over a broad size range in a variety of dispersion media (Clark, 1995). However, laser diffraction provides measurement of geometrical instead of aerodynamic particle size, and the apparent particle density and dynamic shape factors of drug agglomerates are not considered. Nevertheless, laser diffraction has been successfully employed to examine aerosols for inhalation from nebulizers (Clarck, 1995; Mc Callion et al., 1996; Bridges and Taylor, 1998) and MDIs (Moren, 1981), although its application to DPIs has been little studied.

The increasing popularity of DPI in the development of drugs for pulmonary administration requires new advances in the evaluation of the particle size distribution of aerolised formulations (Olsson et al., 1988; Martin et al., 2006b; Zeng et al, 2006).

Therefore the purpose of this part of the study was to establish whether the new Spraytec® laser diffraction method has the potential to characterize the properties of various dry powder aerosol formulations, and to compare the results with those obtained from inertial impaction methods. Dispersion properties of various dry powder formulations were investigated using different laser diffraction and impaction apparatuses at different flow rates and using different inhalator devices.

V.1.2. Results

Dry powders considered as "carrier-free" and presenting different degrees of particle aggregation were prepared by spray-drying as described in parts IV. 2.3.2 and 2.3.3, pp 84-85.

Briefly, various concentrations of lipids were dissolved in micronized tobramycin suspensions, which were spray dried at 70°C, resulting in coating of the drug particles and in modification of their surface properties and agglomeration tendency (F1 formulations). On the other hand, micronized tobramycin was added to a solvent system composed of isopropanol:water 80:20 and spray dried at various temperatures (from 120°C to 200°C), leading to differences in residual humidity content and various aggregation and dispersion properties (F2 formulations).

HPMC size 3 capsules were hand-filled with 15 mg of these various powders.

Table 3 gives an overview comparison of all the powders studied. All powders presented a low bulk powder tap density (< 0.3 g/cm^3) and sizes theoretically suited for administration to the deep lungs. In both cases, all powder formulations were considered as homogeneous and presented a Gaussian size distribution curve (log-normal).

Table 3: Composition of the spray-dried suspensions F1 and F2 formulations

	Suspensions			
	Tobramycin (% w/v)	**Lipids (% w/v)**	**Ratio Cholesterol: Phospholipon (%)**	**Inlet temperature (°C)**
F1.1	5	0.1	75:25	70
F1.2	5	0.25	66:44	70
F1.3	5	0.25	90:10	70
F1.4	5	0.5	75:25	70
F1.5	2	0.1	75:25	70
F1.6	10	0.5	75:25	70
F1.7	5	0.25	75:25	70
F2.1	5	/	/	120
F2.2	5	/	/	140
F2.3	5	/	/	160
F2.4	5	/	/	180
F2.5	5	/	/	200

V.1.2.1. Particle size distribution by laser diffraction

The particle size distribution results for the F1 and F2 formulations are presented in Table 4.

All particle size distributions for the F1 and F2 formulations obtained from the Mastersizer 2000® were unimodal and narrow. The measured particle size distributions of these formulations were found to be accurate, with errors in geometric mean diameter measurements of less than 10%, as reported previously in the sizing of narrow unimodal systems of similar particle size (Annapragada and Adjei, 1996).

The median particle sizes for F1 appeared to be similar for all powder formulations, exhibiting D(0.5) values between about 1.2–1.4 μm with the Mastersizer 2000® and about 2.6-3.4 μm with the Spraytec®. For the F2 formulations, the median particle sizes appeared to be different for all powder formulations, exhibiting D(0.5) values between about 1.4–2.9 μm with the Mastersizer 2000® and about 1.8–35.0 μm with the Spraytec®. Concerning F2.1, the D(0.5) measured with the Spraytec® appeared to be twelve times greater than that measured with the Mastersizer 2000®.

Table 4: Particle size characteristics of the F1 and F2 formulations D(0.5) and % < 5 μm (mean ± SD, n=3), measured with the Mastersizer® 2000 laser diffractometer dry sampling system (4 bar) and with the Spraytec® (100 l/min, 2.4 s)

	D (0.5)		% < 5 μm	
	Mastersizer	Spraytec	Mastersizer	Spraytec
F1.1	1.27 ± 0.01	2.6 ± 0.9	99.6 ± 0.1	88 ± 1
F1.2	1.38 ± 0.02	3.0 ± 0.5	99.8 ± 0.1	90.8 ± 0.3
F1.3	1.29 ± 0.01	2.9 ± 0.4	99.6 ± 0.1	90.3 ± 0.5
F1.4	1.38 ± 0.03	3.3 ± 0.6	99.9 ± 0.1	92 ± 1
F1.5	1.24 ± 0.02	3.3 ± 0.8	99.8 ± 0.1	93.7 ± 0.1
F1.6	1.28 ± 0.03	3.4 ± 0.7	99.7 ± 0.1	96.0 ± 0.9
F1.7	1.23 ± 0.01	3.3 ± 0.9	99.6 ± 0.1	98 ± 1
F2.1	2.96 ± 0.02	35 ± 6	88 ± 1	36 ± 6
F2.2	2.47 ± 0.05	18 ± 2	93 ± 1	59 ± 9
F2.3	1.6 ± 0.1	7 ± 1	96.4 ± 0.7	79 ± 6
F2.4	1.41 ± 0.08	2.0 ± 0.8	97.3 ± 0.3	84 ± 9
F2.5	1.7 ± 0.1	1.8 ± 0.1	98.33 ± 0.06	90.6 ± 0.3

As can be seen in Table 4, particle agglomeration problems are more noticeable with the Spraytec® than with the Mastersizer 2000®. The differences in the size determination results obtained by the two laser diffraction methods can be explained by the differences in the particle dispersion capacity of the two 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-size powders, as is the case for DPI formulations. For all the powders investigated, the mass median diameter measured by the Mastersizer 2000® in the dry state rapidly decreased with increased pressure of compressed air used to deaggregate the powder. Moderately to strongly aggregated powders can be dispersed by pressures between 3 and 5 bars (Bosquillon et al., 2001). 4 bars presented a good compromise between separation of the agglomerates and breaking of the particles. In contrast, the air flow generated in the Spraytec® was much lower and did not allow the de-agglomeration of all particles. In fact, the entire assembly is a closed system and allowed for a controlled airflow rate (100 l/min for 2.4 s) in the measurement zone. This permitted the size properties of DPIs to be measured under simulated breathing conditions. In other words, the Mastersizer 2000® permitted the determination of the size characteristics of totally individualized particles, whereas the size results obtained with the Spraytec® included the

presence of some agglomerates, as appear under the normal conditions of use of an inhaler by a patient.

As can be shown from these results, the behaviour of the two types of formulations, F1 and F2, are very different. For the F2 formulations, increasing the spray-drying temperature allowed a decrease in the moisture content of the formulations from 9.3% to 5.9% for F2.1 and F2.5 respectively, which had implications in aggregation and dispersion properties. Moreover, the application of a lipid coating around the active particles (F1 formulations) allowed an improvement in the particle dispersion from the inhalator, thus enhancing the drug deposition deep in the lungs (loose agglomerates were easily scattered into small particles).

For the F1 formulations, there was no correlation between the results obtained from the Mastersizer 2000® and the Spraytec®, probably due to the fact that the size characteristics of the different powder formulations were very close: for example, the size results obtained with the Mastersizer 2000® at 4 bar showed that the percentage of particles below 5 µm ranged from 99.3% to 99.9%. Nevertheless, after computation of the Deming regression, linear relationships between the most important size characterization parameters, i.e. D(0.5) and the percentage of particles below 5 µm, could be demonstrated between the results obtained with the Mastersizer 2000® and the Spraytec® for the F2 formulations. The best index of correlation (R^2: 0.99) was obtained for the percentage of particles below 5 µm (Fig. 28), which is the size range that is considered to be "respirable".

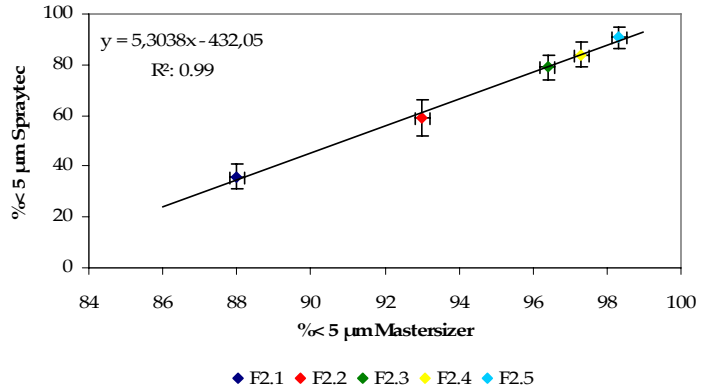


Figure 28: Deming regression of the percentage of particles under 5 µm measured with the Spraytec® (mean ± SD, n=3) against the percentage of particles under 5 µm measured with the Mastersizer® 2000 (mean ± SD, n=3) for the F2 formulations

V.1.2.2. Particle size distribution by inertial impaction

The most effective formulation of each of the two types of formulations produced (F1.7 and F2.5) were tested with an MsLI and an NGI apparatus at 100 l/min for 2.4 s, consistant with the withdrawal of 4 l of air.

It is important to note that for this part (Part V.1), the FPF was expressed as a percentage of the emitted dose that was determined as the percentage of total powder mass exiting the capsule and device. This exception was made in order to compare the results with those from the Spraytec®, for which only the size of particles reaching the throat are counted.

The fine particle fraction for F1.7 was about 77.3 ± 0.9% with the MsLI and about 76.6 ± 0.6% for the NGI. The fine particle fraction for F2.5 was about 60.3 ± 2.0% with the MsLI and about 62.5 ± 2.3% with the NGI. These results are significantly similar ($p > 0.05$).

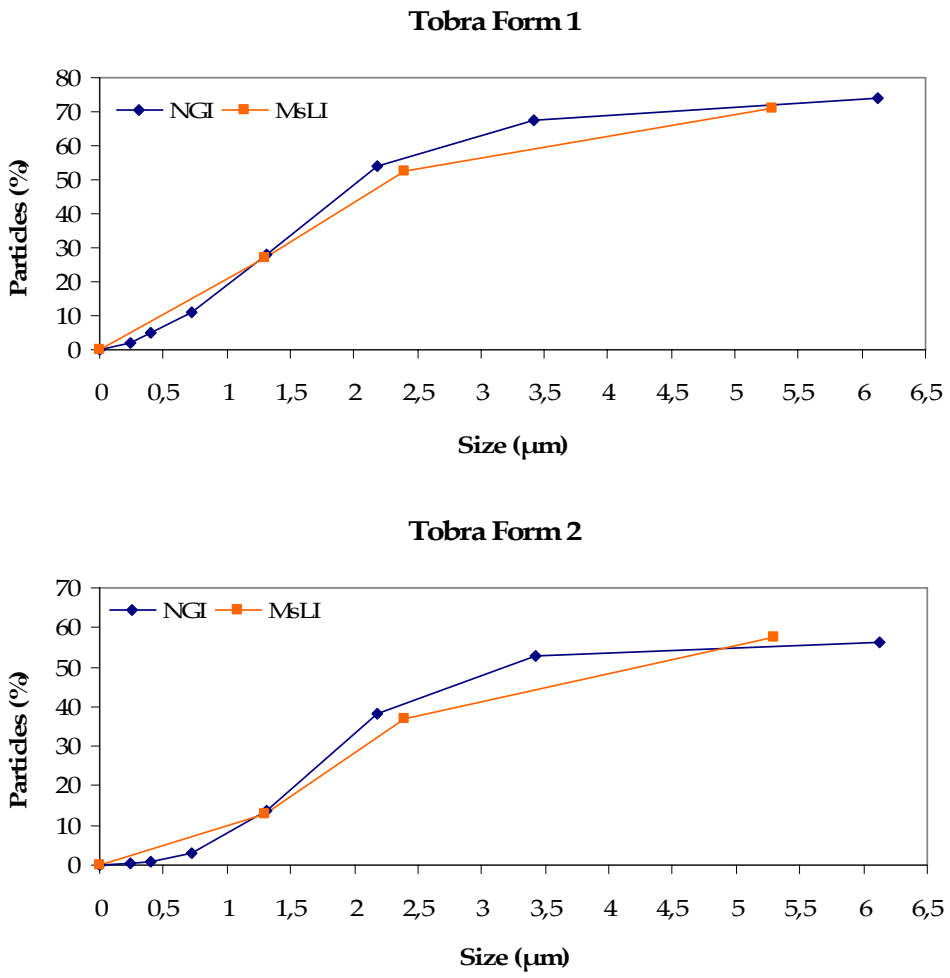


Figure 29: Aerodynamic particle size distributions for F1.7 and F2.5, determined by the MsLI (100 l/min, 2.4 s) (mean ± SD, n=3) and the NGI (100 l/min, 2.4 s) (mean ± SD, n=3) for particles below 6 µm

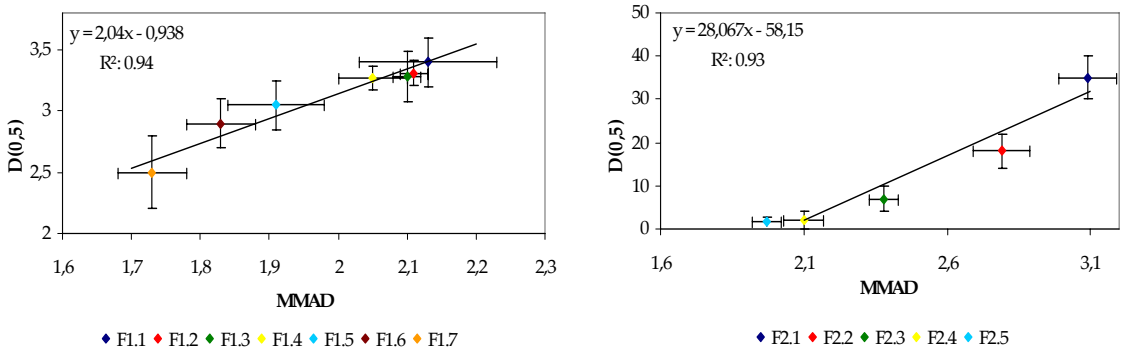
As can be seen in Fig. 29, the cumulative percent undersize curves of the two impactors were similar. However, the reduced number of stages of the MsLI may cause an approximation in the evaluation of the particle size distribution of powders, especially in the smaller sizes ranging from 0.2-3.5 μm. In fact, the principal difference between the two apparatus is the number of stages: the MsLI is divided into 5 stages with cut-off diameters at 100 l/min about 5.27, 2.40 and 1.32 μm at stages 3, 4 and 5, respectively. In contrast, the NGI has 7 stages, of which 6 have a cut-off diameter below 6.2 μm at 100 l/min: 6.12, 3.42, 2.18, 1.31, 0.72, 0.40 and 0.24 μm. This allows a more detailed particle size distribution, in particular for small particles. Nevertheless, the manipulation of the NGI is more time consuming than the manipulation of the MsLI.

V.1.2.3. Correlation between inertial impaction and Spraytec® laser diffraction

In general, when the aerodynamic diameter of a particle is related to its geometric diameter (part II.3.4, p 29), the dynamic shape factor χ is considered as insignificant. Nevertheless, it has been demonstrated that the physical implications of slip and shape on aerosol behaviour are of extreme importance. Without correction for these factors, calculation of aerodynamic particle properties and deposition probabilities would be inaccurate (Crowder et al., 2002). Of primary interest is also the use of the particle density in the calculation of a particle's aerodynamic diameter. The particle density is often evaluated by tap density measurements. Nevertheless, assuming efficient packing, the tap density of a monodisperse assay of spheres underestimates the true particle density by 21% because of the void spaces between particles (Vanbever et al., 1999). Considering the difficulty of a true measurement of the particle's density and the approximation of the shape and slip factors, no conversion of the geometric diameter into aerodynamic diameter has been performed.

In order to evaluate the Spraytec® as a reliable measuring instrument for characterizing powders for inhalation, the MMAD for tobramycin particles measured by inertial impaction were compared with and plotted against the D(0.5) measured by laser diffraction. As can be seen from Fig. 30, a linear relationship calculated with the Deming

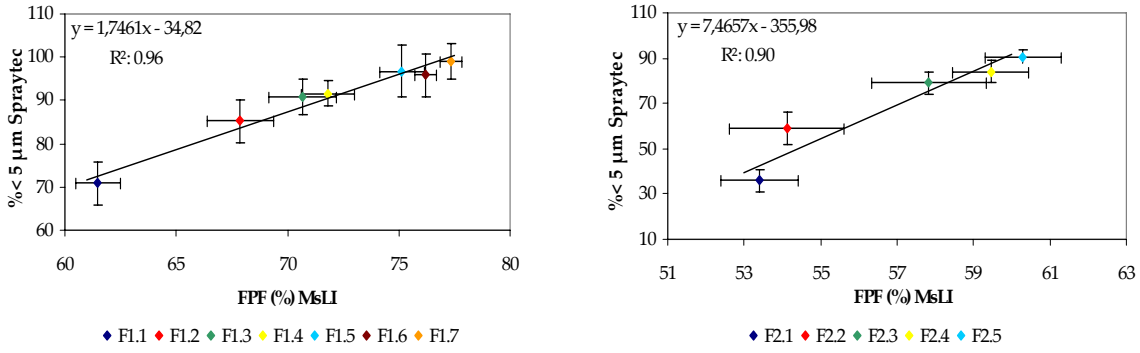
regression existed between the MMAD and the D(0.5), on the one hand, among the F1 formulations (R^2: 0.94) and, on the other hand, among the F2 formulations (R^2: 0.93).



A. B.

Figure 30: Deming regressions between the MMAD measured with the MsLI (100 l/min for 2.4 s, mean ± SD, n=3) and the D(0.5) measured with the Spraytec (mean ± SD, n=9) for A. F1 formulations, and B. F2 formulations

Similar correlations for the F1 and F2 formulations for the percentage of particles below 1.3 µm, between 1.3 and 2.4 µm, and between 2.4 and 5.3 µm corresponding to the three last stages of the MsLI (stage 3, 4 and 5) were also demonstrated. The R^2 ranged between 0.90 and 0.98. The existence of these linear relationships demonstrated that the results of the Spraytec® are useful for size evaluation of particles below 5 µm, which were considered to be the respirable fraction. Of particular interest for pulmonary delivery, regression analysis of the data between the FPF and the % of particles < 5 µm for the F1 and F2 formulations demonstrated a good correlation (R^2 of 0.96 and 0.90, respectively) between the two techniques (Fig. 31).



A. B.

Figure 31: Deming regressions between the percentages of particles below 5 µm measured with the MsLI (100 l/min for 2.4 s, mean ± SD, n=3) and with the Spraytec (mean ± SD, n=3) for A. F1 formulations, and B. F2 formulations

In order to evaluate if it is possible to determine the FPF of dry powders from this correlation, 5 different batches of the F1.7 formulation were tested with the Spraytec® and the MsLI. Table 5 gives a comparison of the experimental FPF, determined by the MsLI, and the theoretical FPF, calculated from the equation of the Deming regression y = 1.7461x - 34.82 (Fig. 31), where y is the percentage of particles below 5.0 µm measured by the Spraytec®, and x, the FPF measured by the MsLI.

Table 5: Comparison of the experimental and theoretical FPF of 5 different F1.7 batches (mean ± SD, n=5)

Powder	% < 5 µm (Spraytec)	Experimental FPF (MsLI)	Theoretical FPF	
1	97.6 ± 1.0	76.8 ± 1.0	75.8 ± 0.6	$p > 0.05$
2	96.7 ± 0.7	75.7 ± 0.9	75.3 ± 0.4	$p > 0.05$
3	98.7 ± 0.8	76.9 ± 0.3	76.6 ± 0.4	$p > 0.05$
4	98 ± 1.1	75.7 ± 0.5	76.5 ± 0.6	$p > 0.05$
5	95.5 ± 1.0	74.3 ± 0.8	74.6 ± 0.6	$p > 0.05$

These results show that the experimental and the theoretical FPF appeared to be statistically similar ($p > 0.05$) and that the Spraytec® could quickly provide a very good evaluation of the aerodynamic behaviour of the powders produced.

A similar correlation was also demonstrated between the results of the Spraytec® and the NGI, showing that the simultaneous measurements of laser diffraction and impaction could be provided with different impaction apparatus.

The shift of the values obtained by the two methods (laser diffraction vs impaction) can be explained, as mentioned above, by the influence of slip, shape and, especially, density of the particles. As expected, the d_{geo} presented higher values than the d_{aer}, since the density of the particles was lower than 1 g/cm³. This implication of the combination of physicochemical factors was also found in the fact that it proved to be impossible to have a correlation between the F1 and F2 formulations because the surface morphology and the density of the two types of formulation appeared to be too different.

V.1.2.4. Evaluation of influence of the device

The quality of a DPI product is determined by the formulation, device design and interaction between these two factors. Since different devices can be used to deliver the same active ingredient, it is still common practice to screen a large number of candidate formulations and devices before identifying the most suitable formulation for a specific and/or selected device (Kamiya et al., 2004). Tests with the Aerolizer® (100 l/min, 2.4 s), the Spinhaler® (100 l/min, 2.4 s) and the Handihaler® (60 l/min, 4 s) were carried out with capsules filled with 15 mg of powder (Fig. 32). The devices were operated at an adjusted airflow rate that produces a pressure drop of 4 kPa over the inhaler and for a duration consistent with the withdrawal of 4 l of air from the mouthpiece of the inhaler (Eur. Ph. 6; USP 29).

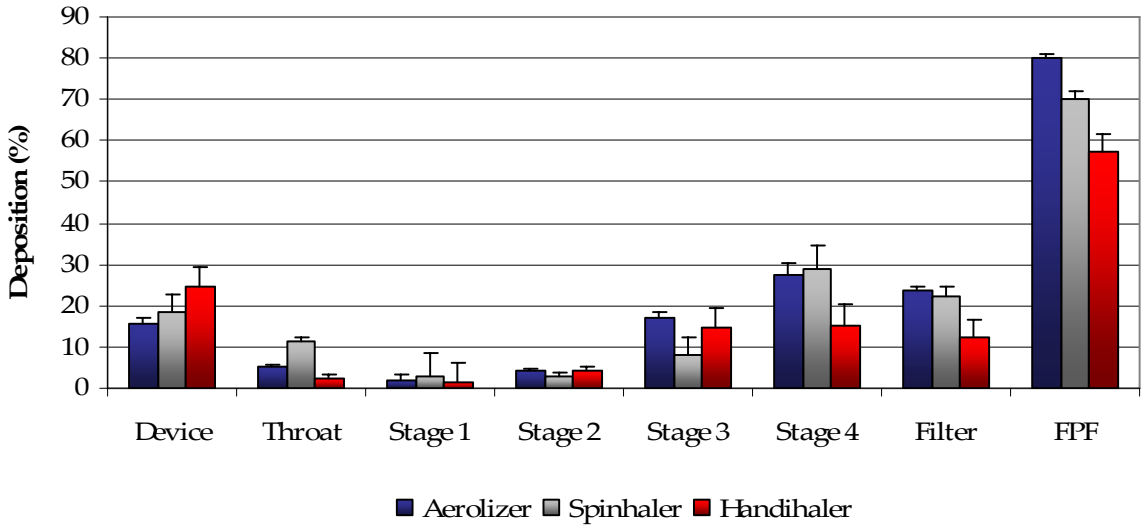


Figure 32: Deposition patterns of the F1.7 formulation with the Aerolizer®, the Spinhaler® and the Handihaler® (MsLI, mean ± SD, n=5)

The results of the particle deposition of the F1.7 formulation measured with the MsLI, revealed that the Aerolizer® seemed to be the most effective device, with an FPD about 9.7 ± 0.1 mg, in comparison with the Spinhaler® (8.1 ± 0.3 mg) and the Handihaler® (6.2 ± 0.6 mg). These deposition results are significantly different ($p < 0.05$) for the three devices. The Spinhaler® presented a deposition profile slightly different from the two others, with a deposition level more elevated in the throat and in the stage 4 (Fig. 32). The Handihaler® had the highest level of retention of powder in the capsule and device (3.50 mg). In fact, in

contrast to the Aerolizer® and the Spinhaler®, the capsule did not whirl during inhalation and the particles were probably less dispersed in the airstream.

Regression analysis of the data from the percentage of particles below 5 µm and the FPF of F1.7 with the different inhalers demonstrated a linear relationship with good correlation (R^2: 0.99) (Fig. 33). The Spraytec® can thus be a useful tool for rapid screening for the most suitable device for a specific formulation.

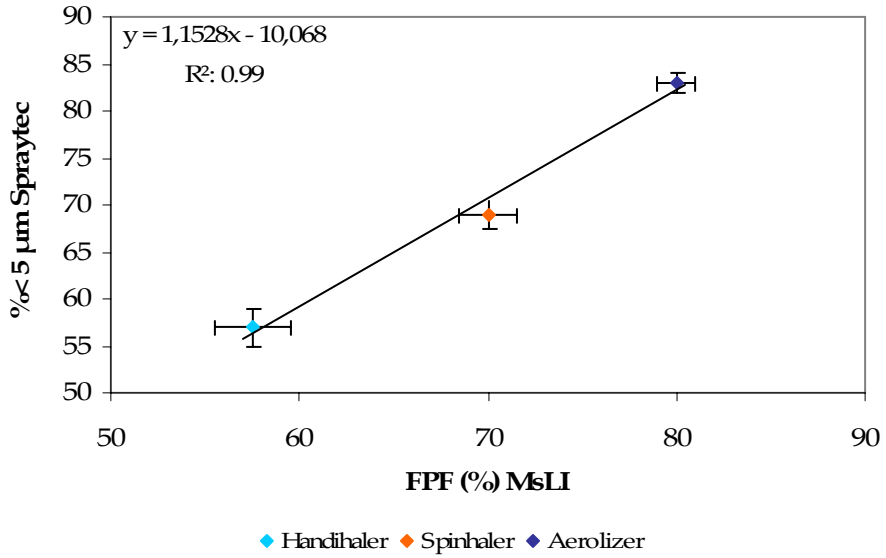


Figure 33: Deming regression of the tobramycin FPF obtained with the MsLI (mean ± SD, n=5) plotted as a function of corresponding FPF obtained with the Spraytec® (mean ± SD, n=15) for the F1.7 formulation, generated with various devices

V.1.2.5. Evaluation of influence of the flow rate

Experiments at different flow rates (30 l/min for 8 s; 40 l/min for 6 s; 60 l/min for 4 s and 100 l/min for 2.4 s) were carried out on the F1.7 formulation with the Aerolizer® (Fig. 34) in order to evaluate the influence of the airflow on the dispersion of particles. The duration of the test was consistent with the withdrawal of 4 l of air through the apparatus (Eur. Ph. 6; USP 29).

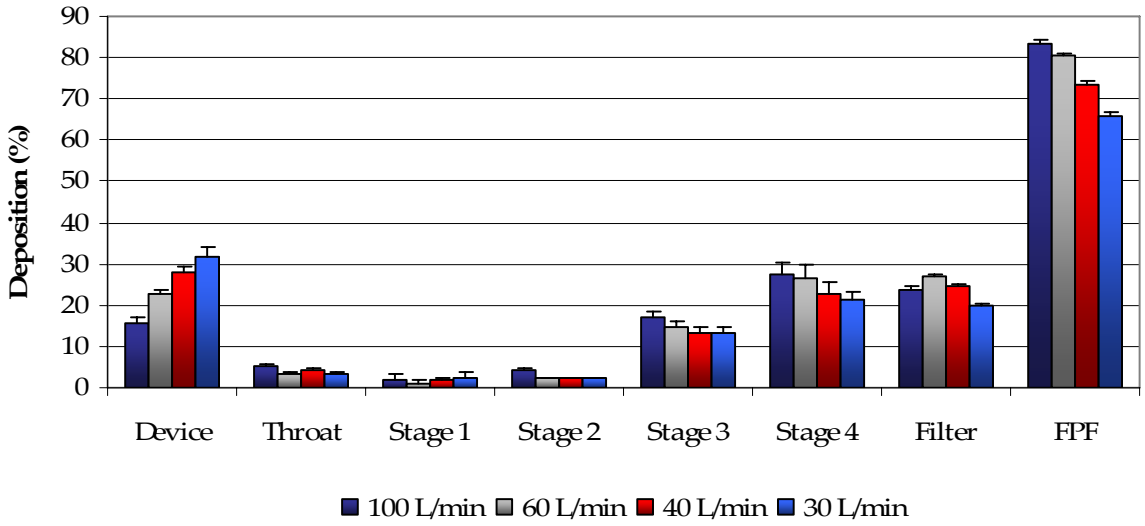


Figure 34: Deposition patterns of the F1.7 formulation with the Aerolizer® at different flow rates (MsLI, mean ± SD, n=5)

The FPD appeared to decrease with reduction of the flow rate. The FPD decreased from 9.6 mg at 100 l/min to 6.4 mg at 30 l/min. The more the flow decreased, the more the powder was retained inside the capsule and device (2.2 mg vs. 4.5 mg) and a lower deposition of tobramycin was observed in the stages 3, 4 and 5 (Fig. 34). The decrease in deposition is significantly different below 60 l/min ($p < 0.05$). These results may suggest that a flow rate superior to 60 l/min is necessary for the breakdown of most of the drug particle agglomerates.

Deming regression analysis of the data from the percentage of particles below 5 µm and the FPF of the F1.7 formulation with the Aerolizer® at different flow rates demonstrated a linear relationship with a good correlation (R^2: 0.99) (Fig. 35).

So, as opposed to other laser diffraction techniques, the Spraytec® presents the advantage that the analysis of particle size distribution depends on controlled flow rates that are near those produced by a patient. This provides more realistic results for the tendency of the powder to deagglomerate during inhalation.

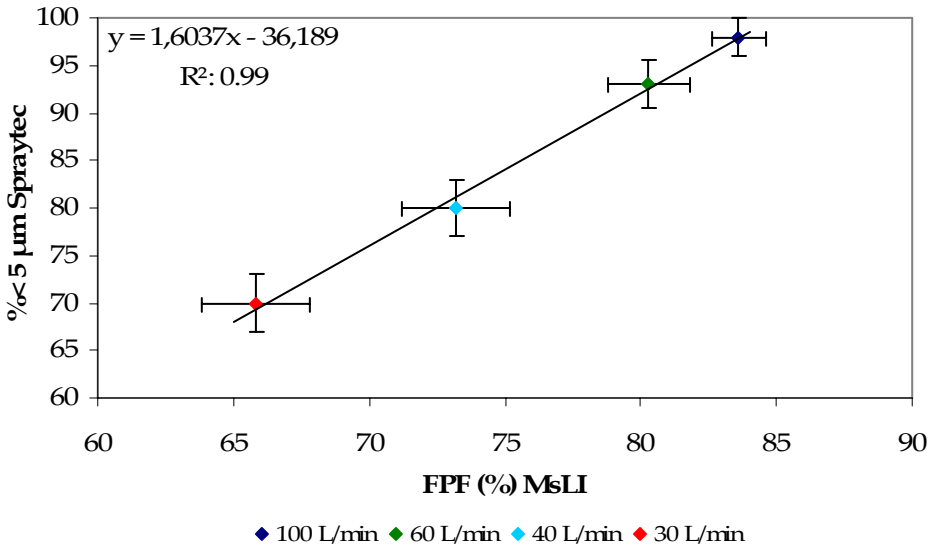


Figure 35: Deming regression of the tobramycin FPF obtained with the MsLI (mean ± SD, n=5) plotted as a function of the corresponding fine fraction obtained with the Spraytec® (mean ± SD, n=15) for the F1.7 formulation with the Aerolizer®, generated at various flow rates

V.1.2.6. Evaluation of maximal capacity of the capsule

A study on the maximal capacity of the capsule was done in order to evaluate what maximal dose could be delivered without a decrease in the aerodynamic properties of the powder. Size 3 HPMC capsules were hand-filled with 8, 15, 25, 30, 40, and 45 mg of F1.7 and of F2.5 powder and were tested with an MsLI (Aerolizer®, 100 l/min for 2.4 s, 3 capsules/test, n=3).

Table 6: Maximal capacity of a capsule with the F1.7 formulation

Dose F1.7 (mg)	FPF (MsLI)	% < 5 µm (Spraytec)
8	64.5 ± 0.5	98 ± 1
15	65.6 ± 0.6	98 ± 1
25	66.3 ± 0.3	98 ± 1
30	66.8 ± 0.3	98 ± 1
40	69.3 ± 0.5	98 ± 1
45	72.1 ± 0.5	98 ± 1

Table 7: Maximal capacity of a capsule with the F2.5 formulation

Dose F2.5 (mg)	FPF (MsLI)	% < 5 μm (Spraytec)
8	55.9 ± 0.8	91 ± 1
15	56.6 ± 0.9	91 ± 1
25	57.5 ± 0.8	91 ± 1

For the F1.7 formulation, a size 3 capsule was totally filled with 45 mg of powder, whereas for F2.5 the maximal capacity of the capsule was found to be about 25 mg. This difference can be explained by the difference in density of the two formulations as the density of the F2.5 formulation is lighter than that of the F1.7, which has a lipid coating.

As can be seen in Tables 6 and 7, the FPF and the percentage of particles with a diameter below 5 μm did not decrease with the increase of powder volume in the capsule. In fact, the FPF tended to be slightly enhanced with the increase of the dose in the capsule. This phenomenon can be explained by the fact that there is always a fine layer of powder that is retained on the walls of the capsule because of the generation of electrostatic charges. As the FPF is expressed as a percentage of the total drug dose, when the dose loaded increases, the calculation of the FPF tends to minimize this loss in powder, which is constant regardless of initial dose. This explanation is corroborated by the fact that the size of the particles measured by the Spraytec® was similar at the various loading doses.
In conclusion, higher doses than 15 mg can be delivered in a size 3 HPMC capsule with the Aerolizer® on only one inhalation.

V.1.3. Discussion

As previously explained, the aerodynamic particle size diameter is routinely measured using sizing techniques that are based on inertial impaction. The principle of classification by inertial separation is well-established and different types of apparatus - such as the MsLI, the ACI and the NGI - and test procedures have been adopted by the European and US Pharmacopoeias. Impingers and impactors have been widely employed for product development. However, such impaction techniques are invariably laborious and time-

consuming to operate and thus are not the best choice for screening many candidate formulations during the early stages of product and process development. Moreover, in the last decade, cascade impactor analysis has been subjected to critical evaluations and to some developments in their use (Hickey, 1990): high inter- and intra-laboratory variations have been described with impactors of the same design. Variation for the fine particle dose may be quite large, ranging from 5.5-20% for DPIs (Olsson et al., 1996). Comparison of results from different types of impactors with different upper class limits for fine particle fractions at the same flow rate are even more problematic. This is especially so when the number of size classes is low and the composition of a cumulative mass distribution curve as a function of particle diameter is impossible.

Therefore, alternative techniques need to be identified in order to cope with the limitations of inertial impaction techniques. Laser diffraction may prove to be such a technique since it is fast, reproducible and, above all, offers a much higher number of size classes for the relevant fine particle fractions that can be obtained from inertial impaction. However the use of laser diffraction to characterize a dry powder aerosol is limited for many reasons. First, most dry powder aerosol formulations are composed of micronized drug blended with a coarse carrier. Since the carrier almost always contains small particles that are similar in size to the drug, it is impossible to differentiate between drug and fine carrier particles. Second, particle size measurement by laser diffraction is based on the assumption that the particles are spherical. In fact, here, for micronized particles, deviation from spherocity could be considered as negligible (Marriott et al., 2006). Finally, the method provides data on geometric instead of aerodynamic diameter and the transformation of the results requires a good knowledge of the density and the shape factor of the particles. So, it requires good understanding of the working principle of a DPI and the properties of powder formulations for inhalation to draw correct conclusions. This could limit the application of laser diffraction for DPI development.

However, different correlations between geometric and aerodynamic size data were demonstrated in this part of the study. Within the flow rates, the different inhalation devices and the drug formulations examined in this work, the tobramycin fine fraction could be

predicted from measurements obtained from the laser diffraction technique using one linear relationship for each type of formulation. Indeed, a combination of physicochemical properties, particle size, density, shape, surface area, and morphology affects the forces of interaction between the drug particles, and these can subsequently change the aerodynamic behaviour of the powder (Telko and Hickey, 2005). Therefore, it appears that it is not possible to predict with exactitude the fine particle fraction of all inhalation powders with only one linear relationship since the inhaled drug properties vary not only in size distribution but also in particle density, shape and velocity (De Boer et al., 2002). Deposition of the drug depends upon a complex interaction between the device, the formulation, and the patient, who controls the flow rate of inhaled air through the system.

Therefore, the Spraytec® may be a very useful tool in pharmaceutical development for screening many formulations, devices and flow rates because the particle size distributions of powders from the Spraytec®, as opposed to the conventional laser diffraction method, are dependent on those factors that also influence the fine particle fraction.

Consequently, the laser diffraction technique has been proved to be an important tool for initial formulation and process screening for a specific type of formulation. Moreover, the use of the Spraytec® could be beneficial, especially in the process control and quality control of finished products as it allows rapid screening of many products. It is a robust technique that is capable of conducting in-line measurement of particle size distribution to ensure that a predefined quality can be achieved at the end of the manufacturing process.

V.1.4. Conclusion

The aerodynamic particle size distribution of aerolized drugs is an essential parameter to evaluate in formulation screening and the subsequent quality control of the final product. In this part of the work, the applicability of the laser diffraction technique was evaluated as an alternative to, not a substitute for, cascade impactor analysis for *in vitro*

characterization of inhalation particles. The method has the potential to solve some of the major problems related to cascade impactor analysis as it quickly permits the generation of a sizing parameter that corresponds to the aerosol fine particle fraction. The most useful features of laser diffraction are time savings, reproducibility, high size resolution, and automatic data recording and processing, which could be very interesting and useful in product development, production and quality control of inhalation products.

In this part of the study, only "carrier-free" dry powders were tested. As most dry powder inhaler formulations are binary interactive mixtures composed of micronized drug blended with a coarse carrier, it would be interesting in the near future to examine the potential of the technique on such complex blends.

As the Spraytec® is now considered as an important tool in the early stages of formulation and development, its use to evaluate the new "carrier-free" dry powders produced by spray-drying will be discussed in the next sections in addition to other techniques such as tap density measurements, XRPD, SEM, etc. Moreover, impaction analyses performed with an MsLI in order to characterize the aerodynamic behaviour of the new powders in an airstream will also be discussed.

PART V.2: FORMULATION AND CHARACTERIZATION OF LIPID-COATED TOBRAMYCIN PARTICLES FOR DRY POWDER INHALATION

Pharm. Res., 23(5): 931-940 (2006)

V.2.1. Introduction

As the aim of the work was to abolish the use of carriers and minimize the use of excipients to allow the admnistration of higher doses of active drug, the idea of the formulation of a coating modifying the surface properties of the drug was explored.

In this part of the study, lipids were used for coating tobramycin particles in order 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 done 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 (Sebti and Amighi, 2006). Indeed, phospholipids and cholesterol, two physiologically well-tolerated components, could present interesting characteristics for the delivery of drugs by the pulmonary route. Like liposomes, they could 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. Moreover coating with lipids offers better stability and higher encapsulation efficiency than the use of liposomes. Finally, the hydrophobic nature of neutral lipids (cholesterol) reduces the absorption of the ubiquitous vapour, leading to a reduction in the aggregation and the adhesion of particles. Moreover, the presence of a low lipid-coating level allows the preparation of powders with low levels of excipients, thus delivering more active drug to the lungs.

The solid-state properties of the formulations, including particle size and morphology, were assessed by SEM (see part IV.2.4, p 86), XRPD (see part IV.2.5, p 87), density measurements (see part IV.2.7, p 88) and laser diffraction (see part IV.2.9, p 90). Aerosol performance was studied by dispersing the powders into an MsLI and determining drug deposition by HPLC (part IV.2.12.1.3, p 100).

V.2.2. Results and discussion

Suspensions with different concentrations of tobramycin and lipids were prepared. Firstly, lipids were dissolved in 50 ml isopropanol. Then, tobramycin was added and the suspensions were processed by HSH. The suspensions were then spray dried at an inlet temperature of 70°C with an outlet temperature ranging between 17°C and 20°C (see part IV.2.3.3, p 85).

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 8 gives an overview comparison of all the powder formulations studied.

Table 8: Composition of the spray-dried suspensions used for the preparation of the tobramycin DPI formulations and lipid content of the formulations (dried forms)

	Tobramycin (% w/v)	Lipids (% w/v)	Lipids (%)*	Cholesterol:Phospholipon (%) (w/w)
T1	2	0.1	5	75:25
T2	5	0.25	5	75:25
T3	10	0.5	5	75:25
T4	5	0.1	2	75:25
T5	5	0.5	10	75:25
T6	5	0.25	5	66:34
T7	5	0.25	5	90:10

* Data expressed as a percentage of tobramycin weight

V.2.2.1. Physicochemical characteristics

The X-ray powder diffraction patterns (Fig. 36) 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.

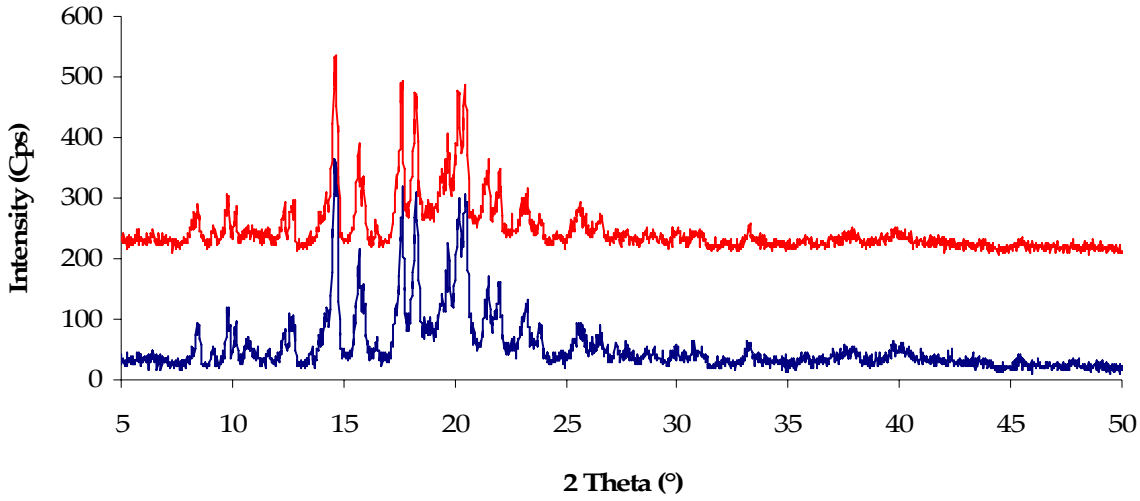
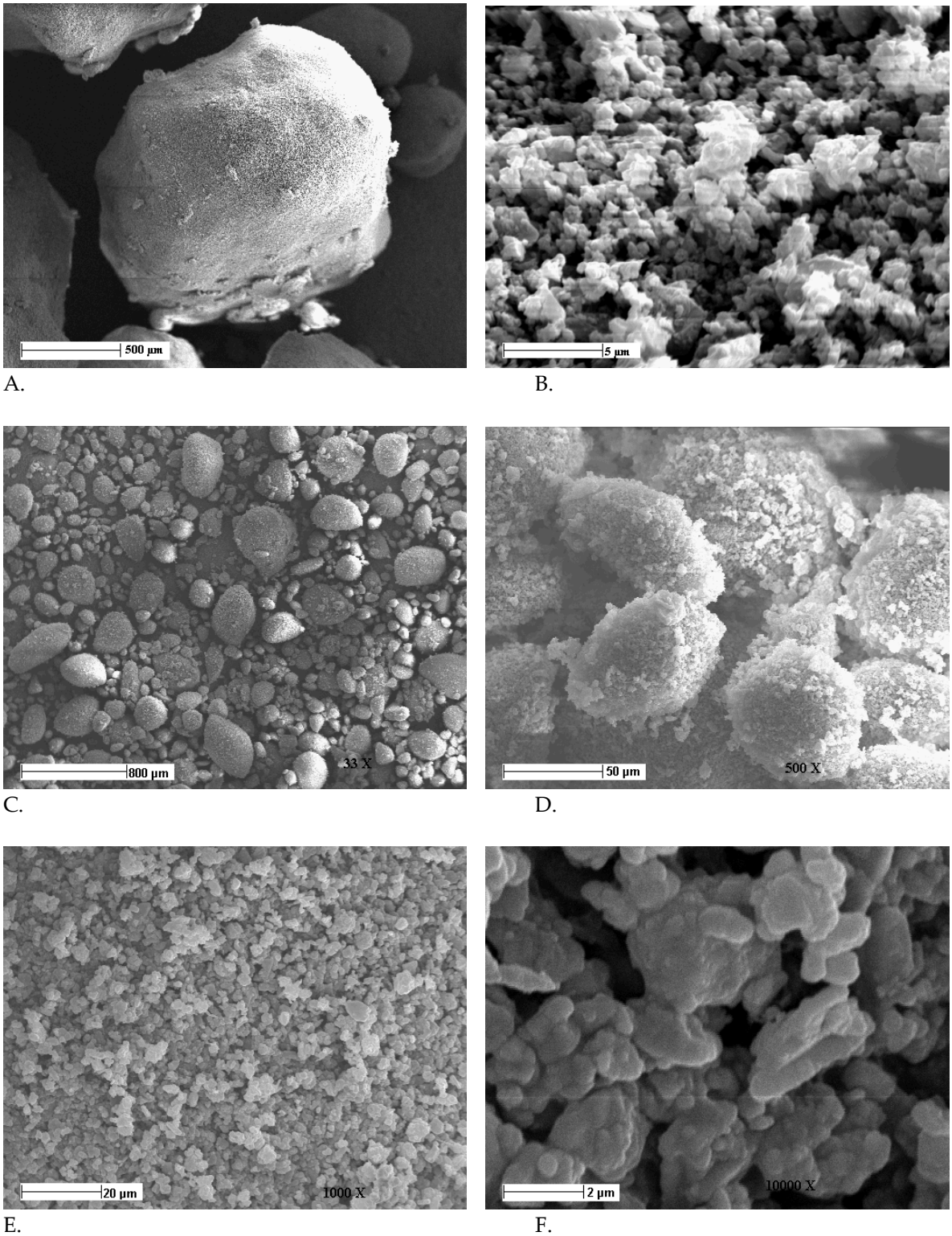


Figure 36: XRPD patterns of raw tobramycin (-) and the T2 formulation (-)

The absence of peaks characterizing cholesterol and Phospholipon could be explained by the lack of sensitivity of the method and the fact that lipids are most probably present partially 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 analysed by SEM. The bulk tobramycin was formed by big, compact agglomerates of micron-size particles. The size of the agglomerates ranged up to 1 mm (Fig. 37, A, B). The small tobramycin particles tended to form a very dense and cohesive structure. In contrast, processing the suspensions by spray-drying yielded more regular-shaped and micron-sized particles. The T2 powder formulation consisted of loose agglomerates of about 50-200 μm in size (Fig. 37, C, D). At bigger magnifications, we can observe (Fig. 37, E, F) that the agglomerates are composed of small particles with a size of about 1-2 μm.

Due to this morphology, the particles became light, as was confirmed by the bulk density measurements, and the spray-dried formulations presented good flowability.



A. B.

C. D.

E. F.

Figure 37: SEM photographs of micronized tobramycin (raw material) and spray-dried lipid-coated tobramycin powder T2

A. micronized tobramycin: magnification 50x ; B. micronized tobramycin: magnification 1000x : C. T2 formulation: magnification 33x ; D. T2 formulation: magnification 500x ; E. T2 formulation: magnification 1000x ; F. T2 formulation: magnification 10000x

The physical properties of the different formulations are summarized in Table 9.

Table 9: Particle size characteristics of the formulations D(0.5), D[4,3] and % < 5 μm (mean ± SD, n=3) measured with the Mastersizer 2000® laser diffractometer dry sampling system (4 bar) and the Spraytec® (100 l/min, 2.4 s)

	Mastersizer			Spraytec		
	D (0.5)	**D [4, 3]**	**% < 5 μm**	**D (0,5)**	**D [4,3]**	**% < 5 μm**
Tobra μ	2.6 ± 0.1	2.9 ± 0.1	78 ± 1	3.1 ± 0.9	11 ± 4	68 ± 8
T1	1.24 ± 0.02	1.46 ± 0.03	99.8 ± 0.1	3.3 ± 0.8	3.22 ± 0.02	93.7 ± 0.1
T2	1.28 ± 0.03	1.48 ± 0.05	99.7 ± 0.1	3.3 ± 0.3	3.16 ± 0.02	97 ± 1
T3	1.23 ± 0.01	1.46 ± 0.01	99.6 ± 0.1	3.27 ± 0.09	3.14 ± 0.07	97 ± 1
T4	1.27 ± 0.01	1.50 ± 0.01	99.6 ± 0.1	2.6 ± 0.3	4.2 ± 0.4	88 ± 2
T5	1.38 ± 0.03	1.54 ± 0.04	99.9 ± 0.1	3.3 ± 0.1	3.43 ± 0.09	92 ± 1
T6	1.38 ± 0.02	1.55 ± 0.01	99.8 ± 0.1	3.0 ± 0.5	3.06 ± 0.09	90.8 ± 0.3
T7	1.29 ± 0.01	1.50 ± 0.01	99.6 ± 0.1	2.9 ± 0.4	3.12 ± 0.03	90.3 ± 0.5

The median particle sizes appeared to be similar for all powder formulations, exhibiting a D(0.5) value of about 1.2–1.4 μm with the Mastersizer 2000® and about 2.6-3.3 μm with the Spraytec® (Table 9). As previously seen, the differences in the size determination results obtained by the two laser diffraction methods can be explained by the differences in the particle dispersion capacity of the two 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-size powders, as is the case for DPI formulations. In contrast, the air flow generated in the Spraytec®, which simulates normal breathing conditions, was much lower and did not allow the de-agglomeration of all the 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 made up of the population's finest particles.

Moreover, the particle size distributions of the formulations, obtained from the Mastersizer 2000®, are unimodal, narrow and range from 0.2-6.0 μm (Fig. 38), with more than 90% of particles having a diameter below 3 μm, which is required for an optimal deep lung deposition.

As can be seen in Fig. 38, there were differences between the curves of the lipid-coated formulation and the micronized tobramycin, resulting in differences in median diameter of about 2.6 and 1.3 μm for the raw material and the T2 formulation, respectively.

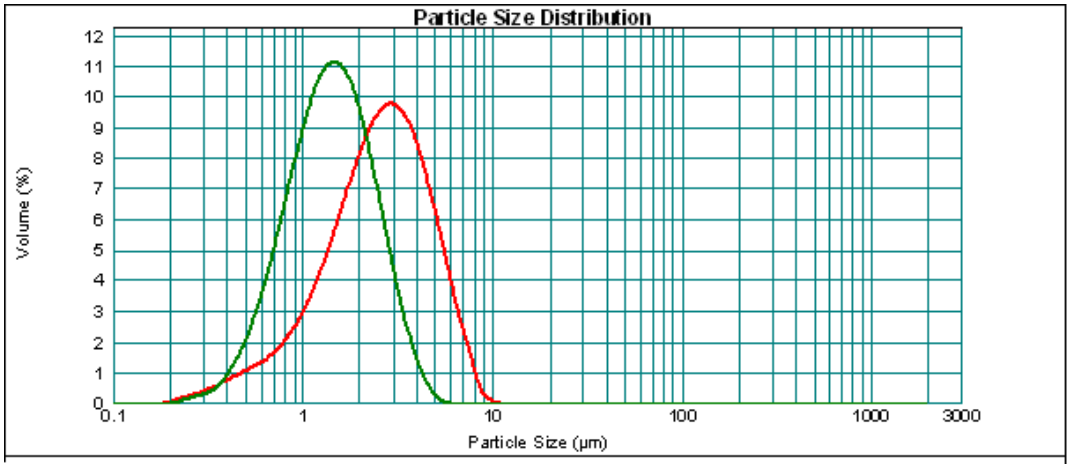
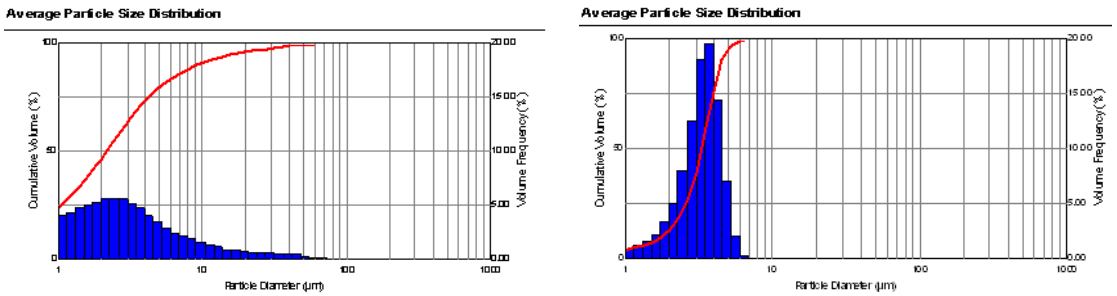


Figure 38: Laser diffraction particle size distribution curve for micronized tobramycin (raw material) (-) and the T2 formulation (-), measured with the Mastersizer 2000®

The volume mean diameter of the micronized tobramycin measured with the Spraytec® was about 11 μm, which is about three times greater than the results from the lipid-coated formulations. Figure 39 shows that the T2 formulation presented a Gaussian curve (log-normal distribution), with 97% of particles below 5 μm, whereas micronized tobramycin showed a very large particle size distribution spread up to a few tens of microns, with only 68% of particles below 5 μm.



A. B.

Figure 39: Average particle size distribution and undersize curve measured with the Spraytec® of A. Tobra μ (raw material) and B. the T2 formulation

There was probably a problem of the raw powder agglomeration, which is very prejudicial for pulmonary administration, decreasing the FPD. As can be shown from these results, the application of a lipid coating around the active particles allowed an improvement in the particle dispersion from the inhalator, thus enhancing the drug deposition deep in the lungs (loose agglomerates were easily scattered into small particles).

Table 10: Bulk density, tapped density and Carr's Index values of all formulations tested

	Bulk density (g/cm³)	Tapped density (g/cm³)	Carr's Index (%)
Tobra μ	0.229	0.300	23.6
T1	0.175	0.255	31.3
T2	0.169	0.242	30.2
T3	0.180	0.247	26.9
T4	0.162	0.221	26.9
T5	0.201	0.316	36.2
T6	0.171	0.260	34.2
T7	0.155	0.237	34.4

The results (Table 10) showed slightly higher Carr's Index values for lipid-coated formulations than for micronized tobramycin. Moreover, these results seem to be influenced by the amount and composition of the lipids in the formulations. More particularly, drug particles covered with higher amounts of lipids (T5, 10% lipids) or with a lipid composition containing a cholesterol:Phospholipon ratio of 66:34 (T6, particles have a higher tendency to stick together when the phospholipid content is increased) or 90:10 (T7, generation of electrostatic charges when the phospholipid content is decreased) gave the highest Carr's Index values and thus poorer flowability. Nevertheless, the flow characteristics of all the formulations were acceptable.

Except for the T5 formulation, which contained the greatest amounts of lipids (higher tendency to stick), the bulk and the tapped density values obtained for the lipid-coated formulations were slightly lower than those obtained for the micronized tobramycin (Table 10). The small decrease in the 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 as it can be seen on SEM photographs in Fig. 37, p 132.

Because the amount of free water in a powder influences its physical stability and controls the magnitude of the capillary forces that hold particles in aggregates, residual moisture content was measured by the KF titration method (part IV.2.8, p 89). Water content was 3.6% for the micronized tobramycin and 2.3% for the T2 formulation. The lipid-coated formulations seemed to be slightly dryer than the raw material. The lipid coating with spray-drying decreased the re-absorption of water by the tobramycin particles, which are very hygroscopic. Thus, physical stability in long-term storage and the de-agglomeration behaviour of tobramycin powder were improved.

V.2.2.2. Aerodynamic behaviour

Besides the particle size of a drug powder, the de-agglomeration behaviour 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. The aerodynamic behaviour of the different tobramycin formulations analyzed with an MsLI is shown in Table 11.

Table 11: Particle deposition in mg for one capsule (mean ± SD) of the different formulations measured with an MsLI (Aerolizer®, 100 l/min 2.4 s, 3 capsules of 15 mg/test, n=3)

	Tobra µ	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Device	6 ± 1	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	0.4 ± 0.1	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	0.9 ± 0.5	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	0.6 ± 0.2	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	2.1 ± 0.9	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	1.7 ± 0.6	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	1.4 ± 0.5	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	5.5 ± 0.9	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
Met.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

The results indicated that the FPD, which roughly corresponds to the drug deposition at stages 3, 4 and the filter (cut-off diameters: 5.27 µm, 2.40 µm and 1.32 µm, respectively), varied within a range of 5.5-10.3 mg. The tobramycin recoveries from the inhalator and the different parts of the MsLI were particularly elevated for all the formulations evaluated as

they ranged between 13.5 mg and 14.4 mg (between 89.7% and 96.2% of the total loaded drug, respectively).

The results presented in Table 11 also show 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 (T1, T2 and T3, FPD values of 9.8 mg, 10.2 mg and 10.3 mg).

It is interesting to note that the presence of lipids markedly enhances the FPD, which is about 5.5 mg for micronized tobramycin and between 7.6 mg and 10.3 mg for the lipid-coated formulations.

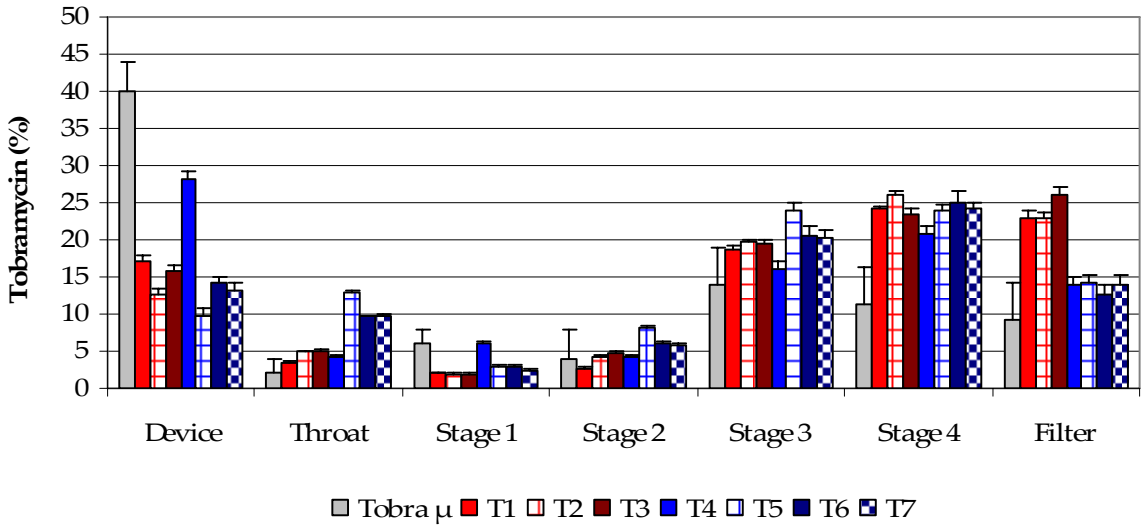


Figure 40: In vitro deposition pattern of the lipid-coated formulation measured with an MsLI (Aerolizer®, 100 l/min 2.4 s, 3 capsules of 15 mg/test) (mean ± SD, n=3)

As it can be seen in Fig. 40, the presence of a lipid coat around the tobramycin particles permitted a decrease in deposition in the device of the inhalator, while it increased deposition in the filter of the MsLI, which is very beneficial for the patient in terms of drug-targeting efficiency. The FPF, which is around 36% for the uncoated micronized tobramycin, increased to up to about 68% for the most effective lipid-coated formulation, in terms of deep lung penetration.

The evaluation of the influence of the coating level (T4, T2 and T5, 2%, 5% and 10% w/w lipids, respectively) showed that the deposition of only 5% w/w lipids (on a dry basis) is

sufficient to improve particle dispersion properties during inhalation (Table 11, Figs. 40 and 41). As can be expected, the least effective formulation was the T4, which had an FPF of 50%. For this formulation, the addition of a very small amount (2% w/w) of lipid coating probably did not permit homogenous cover of all the micronized tobramycin particles and so 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% (T5) also seemed to induce some particle-sticking and produce 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 (T2). 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 lipid level in the formulations in order to avoid any undesirable sticking and to allow the delivery of more of the active drug to the deep lung.

Similar conclusions can be made if one compares the fine particle deposition results obtained from formulations containing different cholesterol:Phospholipon ratios (T2, T6, T7: Table 11, Figs. 40 and 41). An increase of the phospholipid content of coatings, as in T6 (66:34) tends to increase particle-sticking and agglomeration, which decreases particle deposition in the lungs. Nevertheless, T7, with only 10% of Phospholipon, presented 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. So, it seems that a cholesterol:Phospholipon ratio of 75:25 is the most appropriate one as it revealed the best deposition pattern and gave the highest FPF (Fig. 41).

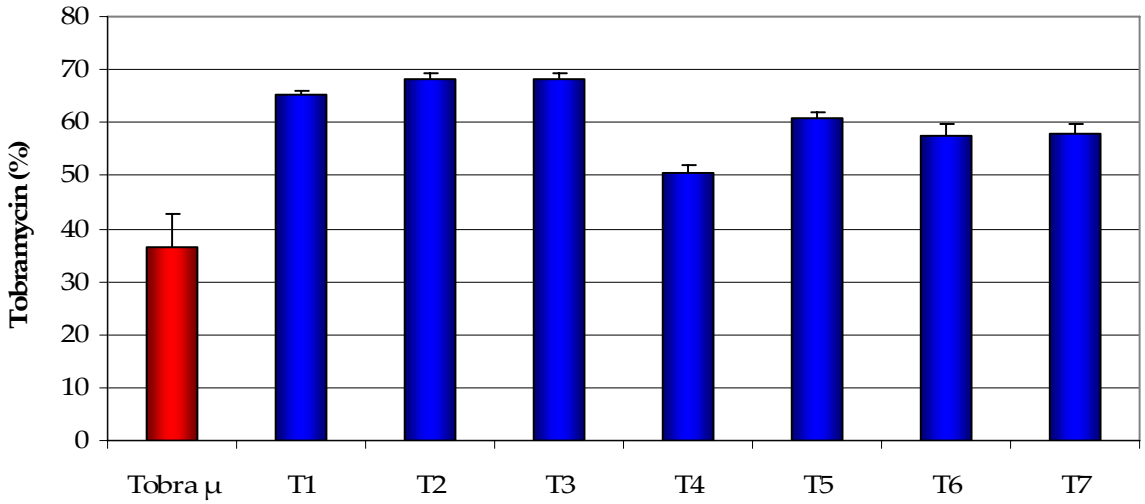


Figure 41: The FPF assessment (MsLI, Aerolizer®, 100 l/min 2.4 s, 3 capsules/test) (mean ± SD) of the different formulations loaded with 15 mg of tobramycin. Each bar represents the average of three repeats

As can be seen from Fig. 41, the highest FPF values, of 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 were especially elevated and very promising compared to the FPF value of the commercially available tobramycin nebulizer product Tobi®, which contains 300 mg of tobramycin free base in 5 ml of sodium chloride at pH 6.0. *In vivo* studies on this product have shown that, after 15 minutes of nebulization, between 5% and 11% of the nominal dose was deposited in the lung depending on the choice of the compressor used (Feng et al., 2002; Geller et al., 2002).

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 promising perspectives in improving the delivery of drugs to the pulmonary tract. These formulations are especially useful for drugs that are active at relatively high doses, such as antibiotics, as 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 in systemic side effects allows improved suitability of these formulations for patients.

V.2.3. Conclusion

This part of the 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 size, shape and density of the 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 the particle properties, good flowability was observed, making the powders ideally suitable for use in carrier-free dry powder inhalers.

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

Nevertheless, these physiological lipids are considered as new excipients and are therefore not yet recognized by the Food and Drug Administration (FDA). Thus, toxicological studies have to be done to prove their safety for human pulmonary administration. Therefore, new formulations with a coating composed of solely the active drug and containing no lipids were developed in the next sections.

PART V.3: PREPARATION AND CHARACTERIZATION OF SPRAY-DRIED TOBRAMYCIN POWDERS CONTAINING NANOPARTICLES FOR PULMONARY DELIVERY

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V.3.1. Introduction

In order to modify the agglomeration tendency of the micronsized tobramycin raw material, novel formulations were developed for manufacturing dry powder for inhalation, composed of a mixture of micro- and nanoparticles of the active drug in order to enhance lung deposition.

Due to rapid advances in nanotechnology, the use of nanoparticles has become a subject of very active research. 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 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 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, which results in decreased drug deposition in the lung.
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.

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; Hadinoto et al., 2006; Grenha et al., 2007; Hadinoto et al., 2007a; Hadinoto et al., 2007b).

Therefore, the objective of this part of the study was to produce tobramycin nanosuspensions containing an appropriate surfactant system for drug stabilization that is potentially well-tolerated for inhalation and to establish a protocol for further industrial production. In order to retrieve tobramycin as a dry powder, spray drying at 80°C (see part IV.2.3.1, p 84) was applied and formulations composed of a mixture of micro- and nanoparticles of tobramycin in order to improve the desagglomeration and dispersion properties of the raw material were developed.

These powders were further characterized in terms of particle size distribution (Mastersizer Hydro 2000® wet sampling system (see part IV.2.9.1, p 91) and Spraytec® (see part IV.2.9.2, p 92) aerosolization properties (MsLI and quantification by HPLC, part IV.2.12.1.3, p 100), tap density measurements (part IV.2.7, p 88), surface composition (SEM, part IV.2.4, p 86) and physical state (XRPD, part IV.2.5, p 87) in order to determine which formulations could be the most suitable for pulmonary delivery.

V.3.2. Results and discussion

V.3.2.1. Preparation of nanosuspensions

Tobramycin powder was poured into a surfactant solution of isopropanol (5% Tobra, w/v, suspension). After dispersion and HSH (see part IV.2.1, p 81), nanosuspensions were then prepared using a high pressure homogenizer. Low pressure homogenization cycles were first conducted on the tobramycin suspension to further decrease particle size (10 cycles at 12000 PSI). HPH was then finally applied for 10-20 cycles at 24000 PSI (see part IV.2.2, p 82). Samples were withdrawn after the different size reduction steps for particle size analysis with the Mastersizer Hydro 2000® wet sampling system (see part IV.2.9.1, p 91).

V.3.2.1.1. 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. The surfactants used need to be well-tolerated and to have appropriate physicochemical characteristics with regards to further processing of the nanosuspensions. They are listed in Table 12.

Table 12: Composition of Tobramycin (5% w/v) suspensions with various surfactants (% w/w relative to tobramycin content)

	Phospholipon (%)	**Na taurocholate (%)**	**Na glycocholate (%)**
S1	2		
S2		2	
S3			2
S4	1	1	
S5	1		1
S6		1	1

The results in Figure 42 show the size of suspensions of tobramycin in isopropanol (5% w/v) before spray-drying, processed using low pressure homogenization cycles (10 cycles at 12000 PSI) and 10 cycles at 24000 PSI.

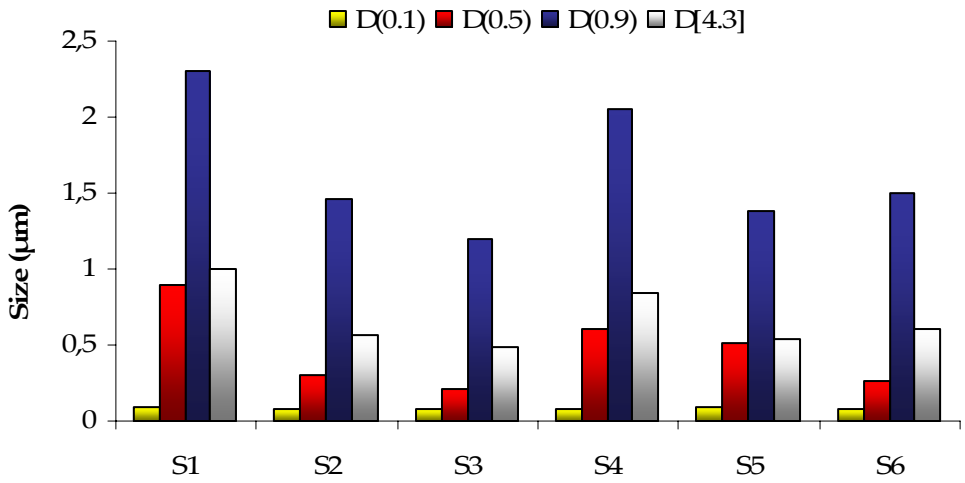


Figure 42: Laser diffraction diameters D(0.1), D(0.5), D(0.9) and D[4.3] after 10 homogenization cycles at 24000 PSI for formulations T1 to T6 measured with Mastersizer Hydro 2000® wet sampling system

Judging from the results shown in Fig. 42, it is obvious that all formulations containing Phospholipon (S1, S4 and S5) 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 S1 was about 2 µm 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 S3. 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 S2, 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.

So, stabilizing is successful or not depending on the physico-chemical properties of the surfactants. 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.

V.3.2.1.2. Influence of pre-homogenization and HPH operations

To investigate the homogenization process in more detail, formulation S3 was chosen and particle size reduction as a function of the homogenization cycles applied was determined. Depending on the hardness of the drug powder and the required fineness of the particle material, the homogenization process can take from 3 up to 20 cycles (Hecq, 2006). For each drug and application, depending on the size requirements of the application route, the number of cycles has to be optimized.
Prior to the HPH operation, tobramycin has to follow a preliminary homogenization step by HSH as the different batches showed a high variability in particle size distribution.

Table 13: Laser diffraction results from Mastersizer Hydro 2000® wet sampling system following successive size reduction steps for the S3 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
10C 12 000 PSI	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

The results given in Table 13 show that the HSH operation (10 min, 24000 rpm – suspension placed in an ice bath) allowed a reduction 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 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, limitations in particle size reduction can be observed at a homogenizing pressure of 24000 PSI. Little change in D(0.5) was observed between 10-20 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 reduction operations, primarily for time saving purposes.

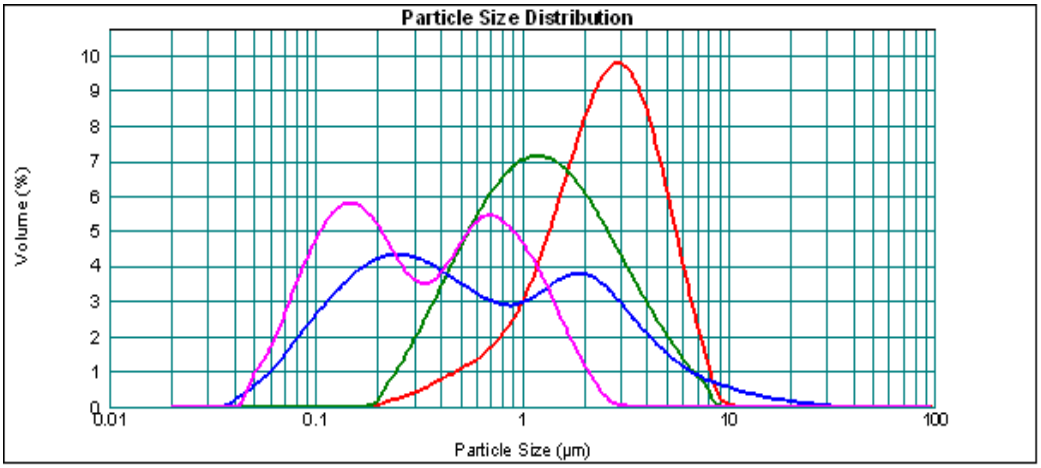


Figure 43: Particle size distribution curves by Mastersizer Hydro 2000® wet sampling system of tobramycin in isopropanol: raw material (-), suspension S3 following HSH (-), 10C 12000 PSI (-) and HPH 10C 24000 PSI (-)

The particle size distribution curves (Fig. 43) following the different size reduction steps also indicate that the low pressure homogenization cycles were 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.

V.3.2.2. Evaluation of 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 14).

Table 14: Composition of the spray-died suspensions used for the preparation of the tobramycin DPI formulations and Na glycocholate content of the formulations (dried forms)

	Tobramycin suspensions 5 % w/v		Dried Form
	Tobra (% w/w)	**Tobra nanoparticles (% w/w)**	**Na glycocholate (%) ***
N1	95	5	0.1
N2	90	10	0.2
N3	50	50	1
N4		100	1
N5		100	2
N6		100	5

* Data expressed as a percentage of tobramycin weight

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. 44.A). Different proportions of these nanosuspensions were added to a suspension of micronized tobramycin in isopropanol and homogenized with the HSH and then spray-dried at 80°C (N1, N2 and N3) (see part IV.2.3.1, p 84).

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. 44.B). In order to retrieve nanoparticles in dried-powder state, nanosuspensions with different concentrations of Na glycocholate were spray dried at 80°C (N4, N5 and N6)(see part IV.2.3.1, p 84).

A **B**

Figure 44: Type of formulations evaluated: A. Coated nanoparticles; B. Agglomerates of nanoparticles

V.3.2.2.1. Physicochemical characteristics

The X-ray powder diffraction patterns (Fig. 45) 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 preserved for nanoparticles. This is very useful in terms of guaranteeing the long term stability of the product in comparison with amorphous drugs.

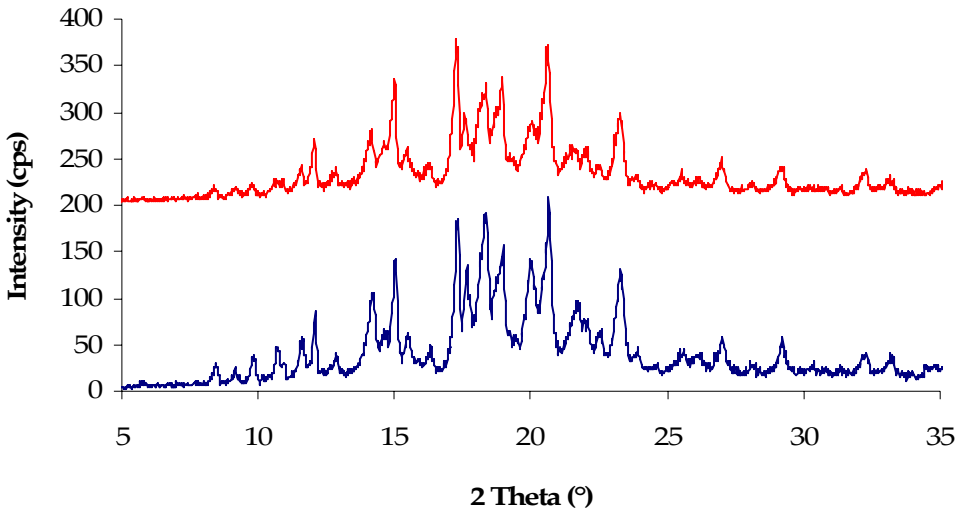
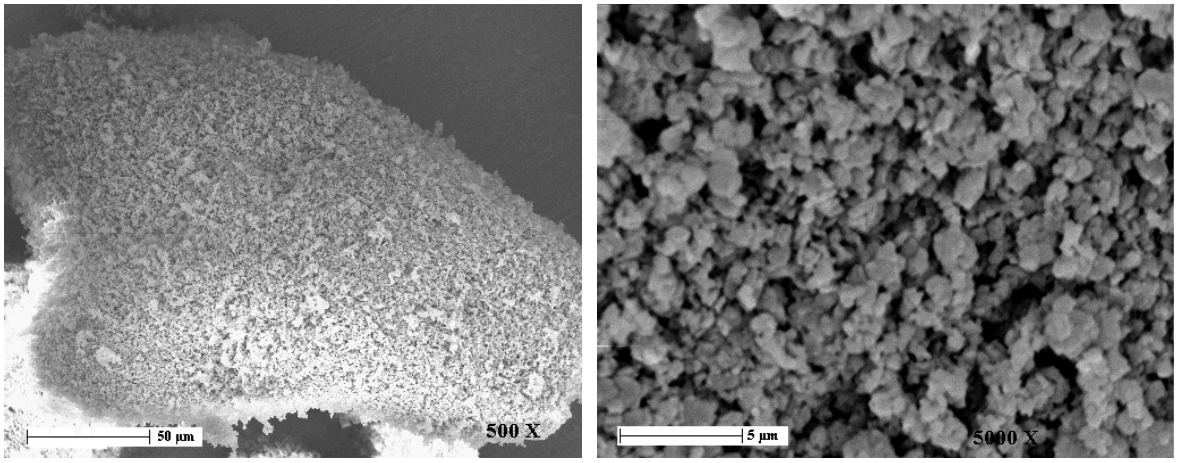


Figure 45: XRPD patterns of raw micronized tobramycin (-) and N5 formulation (-)

The only difference observed between the micronized tobramycin and the nanoparticles lay in the 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 analysed by SEM. Processing the nanosuspensions by spray-drying yielded looser agglomerates that were less smooth, less regular and less cohesive than the micronized tobramycin (see Fig.37, p 132). The yield consisted of smaller agglomerates of about 50-200 μm in size (Fig. 46.A). At larger magnifications, we can observe (Fig. 46.B) that these agglomerates were composed of small particles of about the nanometer range that had a structure that tended 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 during inhalation compared to raw micronized tobramycin.



A. B.

Figure 46: SEM photographs of: A. N5 formulation, magnification 500x; B. N5 formulation, magnification 5000x

The physical properties of the different formulations are summarized in Table 15.

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 N1 vs. 0.124 for N3). Consequently, as expected, the formulations made up exclusively of nanoparticles presented an even lower density (0.083 for N5). However, the increase in density for the N6 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.

Table 15: Physical properties of all formulations tested: particle size characteristics (mean ± SD, n=3) measured with the Mastersizer Hydro 2000® wet sampling system and the Spraytec® (100 l/min, 2.4 s), 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/cm³)	Tapped density (g/cm³)	Carr's Index (%)
Tobra	3.57 ± 0.05	10.4 ± 0.1	71.0 ± 0.1	3.1 ± 0.9	11 ± 4	68 ± 8	0.229	0.300	23
N1	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
N2	0.86 ± 0.03	1.48 ± 0.04	90.4 ± 0.1	2.3 ± 0.7	5 ± 1	80 ± 5	0.140	0.198	29
N3	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
N4	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
N5	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
N6	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

As expected, the Carr's Index is higher for the formulations with nanoparticles. The nanometer-size dimensions led to a severe aggregation problem arising from the small size, which would make the physical handling of the particles extremely difficult for DPI delivery. For the N1, N2 and N3 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 in the flow properties (47 for N4 vs. 34 for N6). Nevertheless, the flow characteristics of all the formulations, except N4, were acceptable.

As can be seen from Fig. 47, the particle size distributions of both the micronized tobramycin and the N5 formulation obtained with the Mastersizer 2000® are unimodal and range from 0.2-10 µm. Nevertheless, most of the population for the N5 formulation lies in the smaller sizes, below 1 µm, as opposed to the micronized tobramycin.

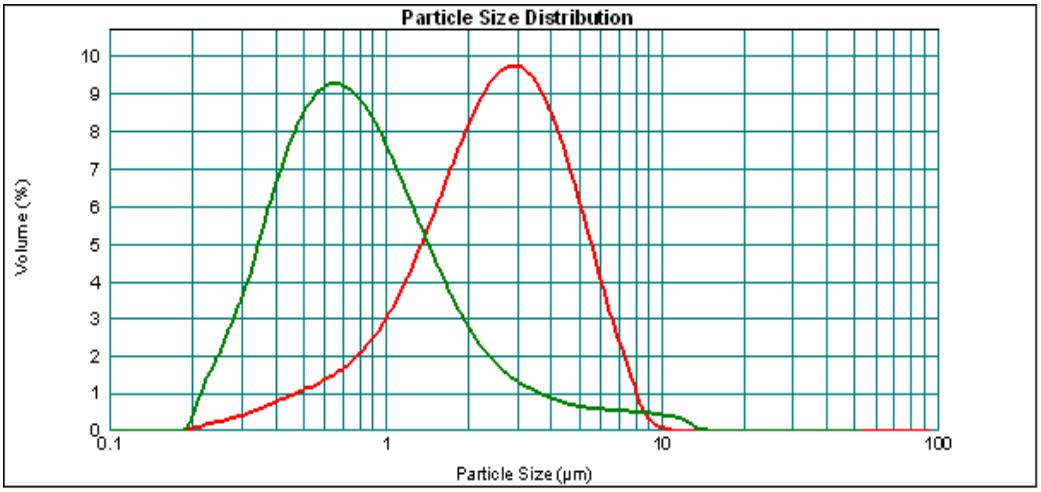


Figure 47: Laser diffraction particle size distribution of micronized tobramycin in isopropanol (-)and the N5 formulation after spray-drying (-) measured with the Mastersizer Hydro 2000® wet sampling system

It is important to note that the particle size distribution of the N5 formulation obtained before and after spray-drying is different. Indeed, instead of the bimodal curve (see Fig. 43, p 147), 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 spray drying in order to form a more homogeneous population of particles.

The D(0.5) measured with the Mastersizer Hydro 2000® for all the formulations containing nanoparticles varied within a range of between 0.75 µm for N1 and 0.87 µm for N6. As expected, the particle size values of the nanoparticulate 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 nanoparticulate formulations, exhibiting a D(0.5) value of about 3.6 µm and 0.8 µm, respectively. Consequently, the percentage of particles below 5.0 µm increased from 71% to 97% for the most effective nanoparticle formulation (N6).

However, the D(0.5) obtained for the nanoparticle formulations with the Spraytec® was higher than that obtained with the Mastersizer Hydro 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 three times greater than the results for the nanoparticulate formulations. Indeed, formulations containing nanoparticles present looser agglomerates with higher porosity, which are more easily dispersible into smaller agglomerates during inhalation. Fig. 48 shows that the N5 formulation had a Gaussian curve (log-normal distribution), with 84% of particles below 5 µm, whereas micronized tobramycin showed a very large particle size distribution spread of up to more than 70 microns, 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.

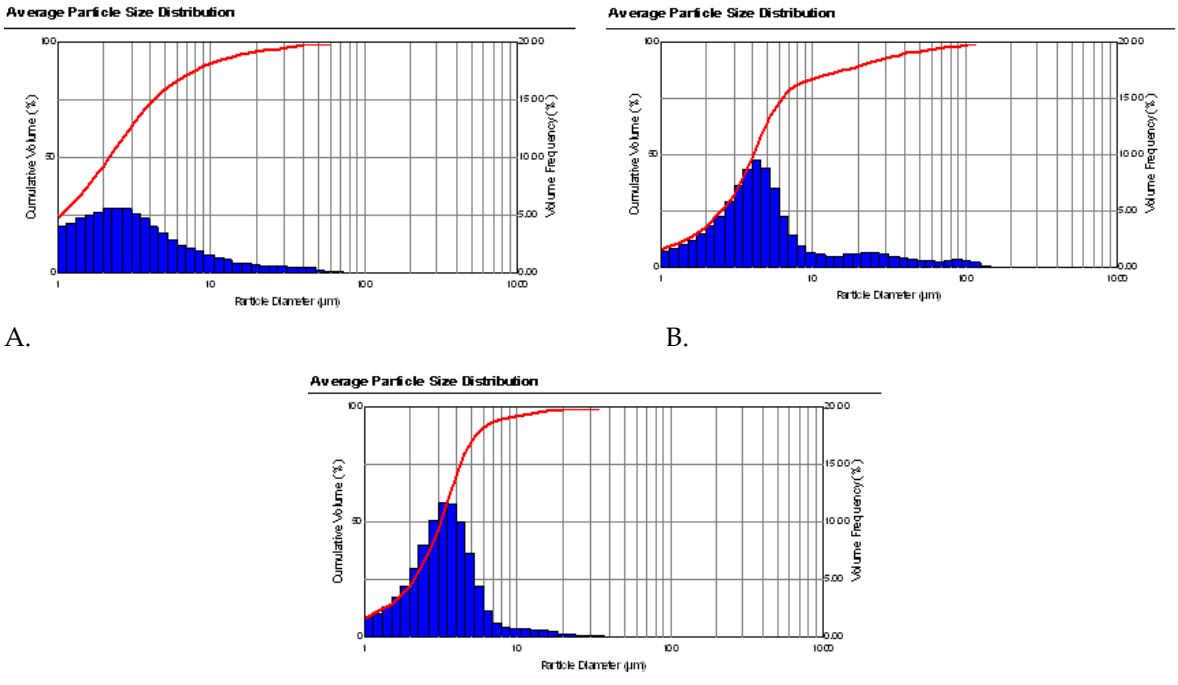


Figure 48: Average particle size distribution and undersize curve measured with the Spraytec® (100 l/min 2.4 s) of A. Micronized tobramycin, B. N3 formulation and C. N5 formulation

As can be seen from the results, the presence of a coating of nanoparticles around micronized tobramycin particles allowed an increase in powder desagglomeration, with an increase in the percentage of particles below 5 µm of around 80% (N1, N2 and N3).

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):

$$F_{VW} = AD/12z^2 \quad (7)$$

where A is the Hamaker constant dependant on the particles' densities, z the shortest distance between the particles, and $D = d_1d_2/(d_1+d_2)$, 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 by 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 (N4, N5 and N6): the percentage of particles below 5 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 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.

V.3.2.2.2. 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 (Fig. 49 and Table 16). The aerodynamic behaviour of the different

spray-dried tobramycin formulations was analyzed with an MsLI at a flow rate of 100 l/min for 2.4 s (see part IV.2.12.1.3, p 100). A dry powder inhalation device, the Aerolizer®, was filled with a size 3 HPMC capsule loaded with 15 mg of powder. Three capsules 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 (see part IV.2.12.1.3.2, p 102). For accuracy, each test was repeated three times.

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

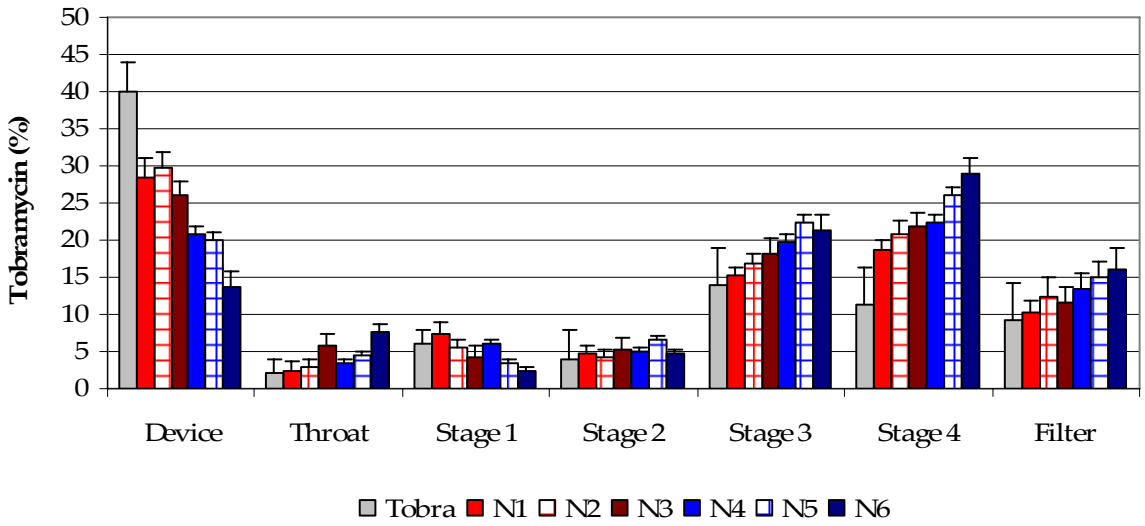


Figure 49: In vitro deposition pattern of formulations containing nanoparticles measured with an MsLI (Aerolizer®, 100 l/min 2.4 s, 3 capsules of 15 mg/test) (mean ± SD, n=3)

The evaluation of the influence of the level of coating with nanoparticles (N1, N2 and N3) showed that the presence of nanoparticles in the formulations improved the particle dispersion properties during inhalation (Fig. 49).

The FPD increased with the increase in the nanoparticle content. The FPD increased from 5.5 mg to 7.6 mg for the micronized tobramycin and the N3 formulation, respectively (Table 16). This could be explained by the fact that a low content of nanoparticles probably did not permit homogenous cover of all the 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 between the particles.

Table 16: Fine particle deposition in mg for one capsule (mean ± SD, n=3) of the different formulations measured with an MsLI (Aerolizer®, 100 l/min 2.4 s, 3 capsules of 15 mg/test).

	FPD
Tobramycin	5.5 ± 0.9
N1	6.7 ± 0.6
N2	7.6 ± 0.5
N3	7.5 ± 0.5
N4	8.0 ± 0.5
N5	9.0 ± 0.5
N6	9.1 ± 0.3

On the other hand, suspensions containing solely nanoparticles were spray dried with various concentrations of surfactant (N4, N5, N6) in order to produce easily dispersible and reproducible micron-size agglomerates of nano-particles 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 (N4) to 9.0 mg with 2% sodium glycocholate (N5). 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 can be seen in Fig.49, 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 (on a 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 modify significantly 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.

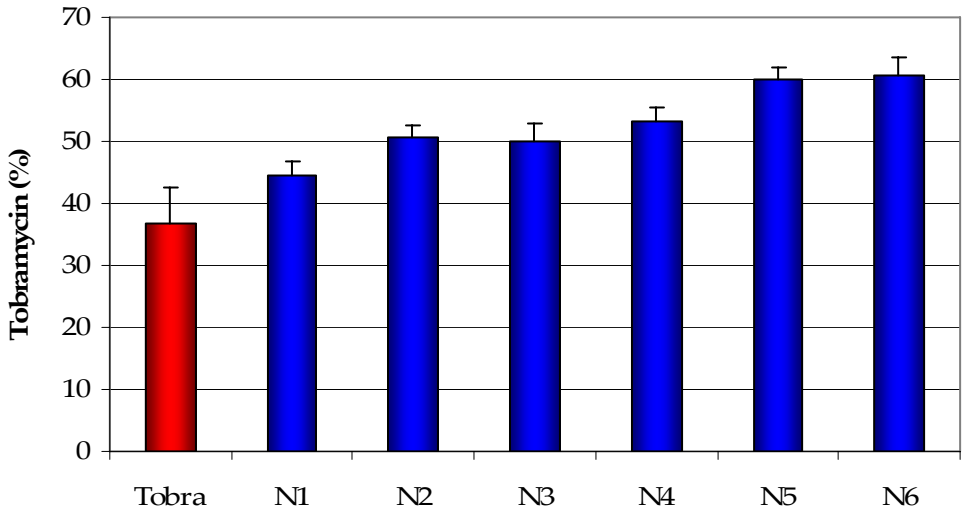


Figure 50: The FPF assessment (MsLI, Aerolizer®, 100 l/min 2.4 s, 3 capsules 15 mg/test) (mean ± SD) of the different formulations of tobramycin. Each bar represents the average of three repeats

Consequently, the use of nanoparticles in dry powder formulations increased the fine particle fraction (FPF) from 36% for the uncoated micronized tobramycin to about 61% for the most effective formulation in terms of deep lung penetration (Fig. 50).

V.3.3. Conclusion

This part of the study demonstrates the possibility of delivering to the lungs formulations 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 solely of 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, presented high lung deposition properties

Nevertheless, even though these powders are considered carrier-free, they still contained 0.1-5% of Na glycocholate. Therefore, an approach to producing a totally carrier-free formulation containing 100% tobramycin was evaluated in the next part. The presence of a nanoparticle coating was replaced by an amorphous coating of the active drug particles, allowing modification of the surface properties of the particles.

PART V.4: SPRAY-DRIED CARRIER-FREE DRY POWDER TOBRAMYCIN FORMULATIONS WITH IMPROVED DISPERSION PROPERTIES

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V.4.1. Introduction

Particle separation is the most important performance characteristic for effective aerosol generation. To separate particles, specific forces of interaction must be overcome. Controlling the surface charges of the particles helps in reducing electrostatic forces. In order to modify the balance between the different forces of interactions, modify the surface properties of the particles and permit an efficient reduction in the inherent agglomeration tendency of the powder, the influence of formulation components on the aerosolization characteristics of spray-dried tobramycin with regard to the use of a various proportions of water (1-20% v/v) in the solvent used to prepare initial suspensions was investigated.

The aim of this part of the work was to produce carrier-free tobramycin formulations with 100% active compound and high lung deposition properties. Powders were produced by spray-drying in a one step process which is very useful for time saving purpose, industrial scaling-up and production. The idea was to modify the surface properties of the particles by coating the particles of drug with a homogeneously distributed continuous film of the active compound dissolved in a solvent system containing a mixture of different solvents such as isopropanol and water. During nebulization of the suspension, droplets are composed of one or more particles in solid state that are surrounded with solvent containing the dissolved drug. It is hypothesized that during the drying step, the dissolved particles form the coating film and allow an improvement of the aerodynamic behaviour of the drug during inhalation.

Tobramycin powder (5% w/v) was poured into solvent systems composed of isopropanol:water (v/v) in ratios between 100:0 and 80:20. After dispersion and HSH, the suspensions were spray dried at various inlet temperature between 120°C and 200°C (see part IV.2.3.2, p 84).

The physical properties of the formulations were assessed by SEM (see part IV.2.4, p 86), XRPD (see part IV.2.5, p 87), DSC (see part IV.2.6, p 87) and tap density measurements (see part IV.2.7, p 88). Water content considered as a key parameter in agglomeration

tendency was evaluated with a Sartorius water detection system (see part IV.2.8, p 89). Particle size distribution was evaluated by laser diffraction with the Mastersizer Scirocco 2000® dry sampling system (see part IV.2.9.1, p 91) and the Spraytec® (see part IV.2.9.2, p 92). Aerosol performance was studied by dispersing the powders into an MsLI at a flow rate of 100 l/min for 2.4 s (see part IV.2.12.1.3, p 100). A dry powder inhalation device, the Aerolizer®, was filled with a size 3 HPMC capsule loaded with 15 mg of powder. Three capsules 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 (part IV. 2.12.1.3.2 p 101). For accuracy, each test was repeated three times.

V.4.2. Results and Discussion

V.4.2.1. Influence of spray-drying temperature

In order to retrieve the tobramycin particles in dried powder state from the suspensions containing an 80:20 mixture of isopropanol:water, spray-drying at temperatures between 120°C and 200°C was applied (see part IV.2.3.2, p 84). Approximately 4.1 mg/ml of tobramycin was dissolved in the solvent system.

The yield of the process varied with the conditions of production, with powders such as T180 80:20 and T200 80:20 performing well in the spray-dryer and generating yields of almost 75%, whilst other powders (T120 80:20 and T140 80:20) generated yields of about 60%. In general, increasing the atomization temperature increased the yield of the resultant powder. Indeed, at low temperatures, the higher moisture content of the powder caused an agglomeration of the particles on the walls of the spray-dryer, decreasing the yield of the process.

The particle size distributions of the formulations obtained with the Mastersizer 2000® dry sampling system are unimodal, narrow, and range from 0.1-10 µm (Fig. 51). Nevertheless, a shift of the curves towards the left, showing a decrease in the particle size population, is noted as the temperature increases.

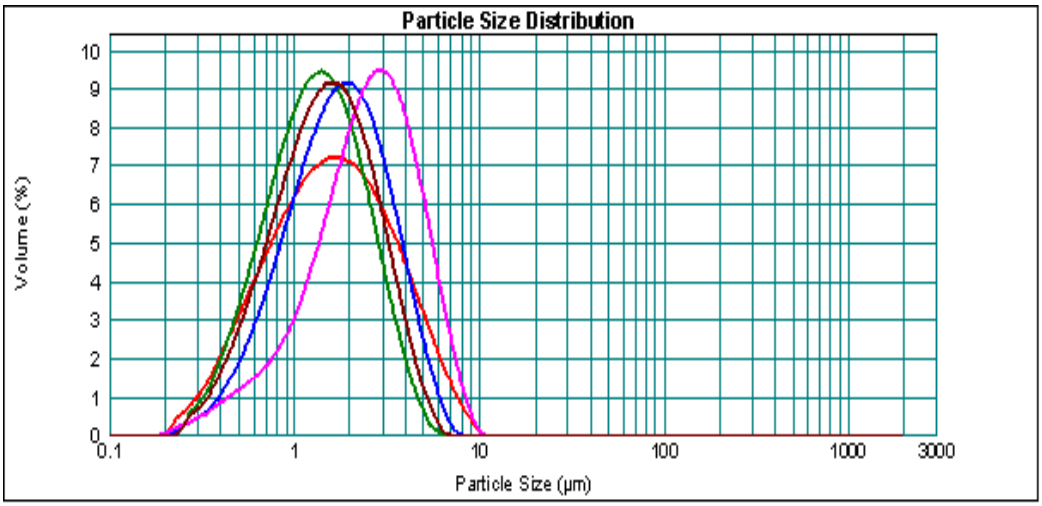


Figure 51: Laser diffraction particle size distribution of powders spray dried at different temperatures from an isopropanol:water 80:20 ratio, measured with the Mastersizer Scirocco 2000® dry sampling system (4 bar): T120 80:20 (-), T140 80:20 (-), T160 80:20 (-), T180 80:20 (-) and T200 80:20 (-)

As can be seen in Table 17, the D(0.5) decreased progressively with the increase in temperature of spray-drying. The mean diameter varied from 3.0 µm to 1.8 µm for T120 80:20 and T200 80:20, respectively. Of course, this reduction in particle size distribution allowed an increase in the percentage of particles below 5 µm from 88% to 98%.

Table 17: Physical properties of formulations obtained from suspensions with 20% v/v water and spray-dried at different drying temperatures (120°C to 200°C): particle size characteristics (mean ± SD, n=3) measured with the Mastersizer Scirocco 2000® (4 bar) and the Spraytec® (100 l/min, 2.4 s), and bulk density, tapped density and Carr's index values

			Mastersizer 2000®			Spraytec®			Density		
	Inlet T°	Outlet T°	D(0.5)	D[4.3]	%< 5µm	D(0.5)	D[4.3]	%< 5µm	Bulk density (g/cm³)	Tapped density (g/cm³)	Carr's Index (%)
T120 80:20	120°	60°	3.0 ± 0.1	3.8 ± 0.1	88 ± 1	35 ± 6	43 ± 7	36 ± 6	0.205	0.321	36
T140 80:20	140°	70°	2.5 ± 0.1	2.8 ± 0.1	93 ± 1	18 ± 2	24 ± 4	59 ± 9	0.200	0.313	36
T160 80:20	160°	80°	1.6 ± 0.1	3 ± 2	96 ± 1	7 ± 1	10 ± 5	79 ± 6	0.173	0.259	33
T180 80:20	180°	90°	1.4 ± 0.1	1.7 ± 0.2	97 ± 1	2.0 ± 0.8	4 ± 2	84 ± 9	0.154	0.225	32
T200 80:20	200°	100°	1.8 ± 0.1	2.2 ± 0.4	98 ± 1	1.8 ± 0.1	4 ± 1	90.6 ± 0.3	0.141	0.205	31

Measurements with the Spraytec® confirmed this reduction in particle size distribution in line with the temperature increase. Here, the influence of the temperature on the properties of the powder appeared more obvious: an increase of 20°C - from 120°C to 140°C - caused a reduction in the D(0.5) of almost 20 μm (35 μm for T120 80:20 vs. 18 μm for T140 80:20). Thus for the same initial suspension containing 20% water (v/v) and used for spray drying, the median diameter of the resultant powder varied from 35 μm to 1.8 μm in function of the drying temperature used for the process. This suggests that the larger size obtained at low temperatures reflected the cohesion of individual particles, forming larger aggregates that failed to disperse during the sizing procedure. Furthermore, this also suggests that these formulations exhibited poor powder flow properties, and were therefore unlikely to display optimal aerosolization characteristics.

As previously demonstrated, the problems of agglomeration of particles were more noticeable with the Spraytec® than with the Mastersizer 2000®. The differences in the size determination results obtained by the two laser diffraction methods can be explained by the differences in the particle dispersion capacity of the two 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 those of micron-size powders, as is the case for DPI formulations. In contrast, the air flow generated in the Spraytec®, which simulates normal breathing conditions, was much lower and did not allow de-agglomeration of all particles.

Figure 52 shows that the T200 80:20 formulation presented a size distribution curve with 90% of particles below 5 μm, whereas T120 80:20 showed a very broad particle size distribution spread up to a few hundreds of microns, with only 36% of particles below 5 μm. There was probably a problem of strong powder agglomeration, which is very prejudicial for pulmonary administration as it decreases the fine particle dose.

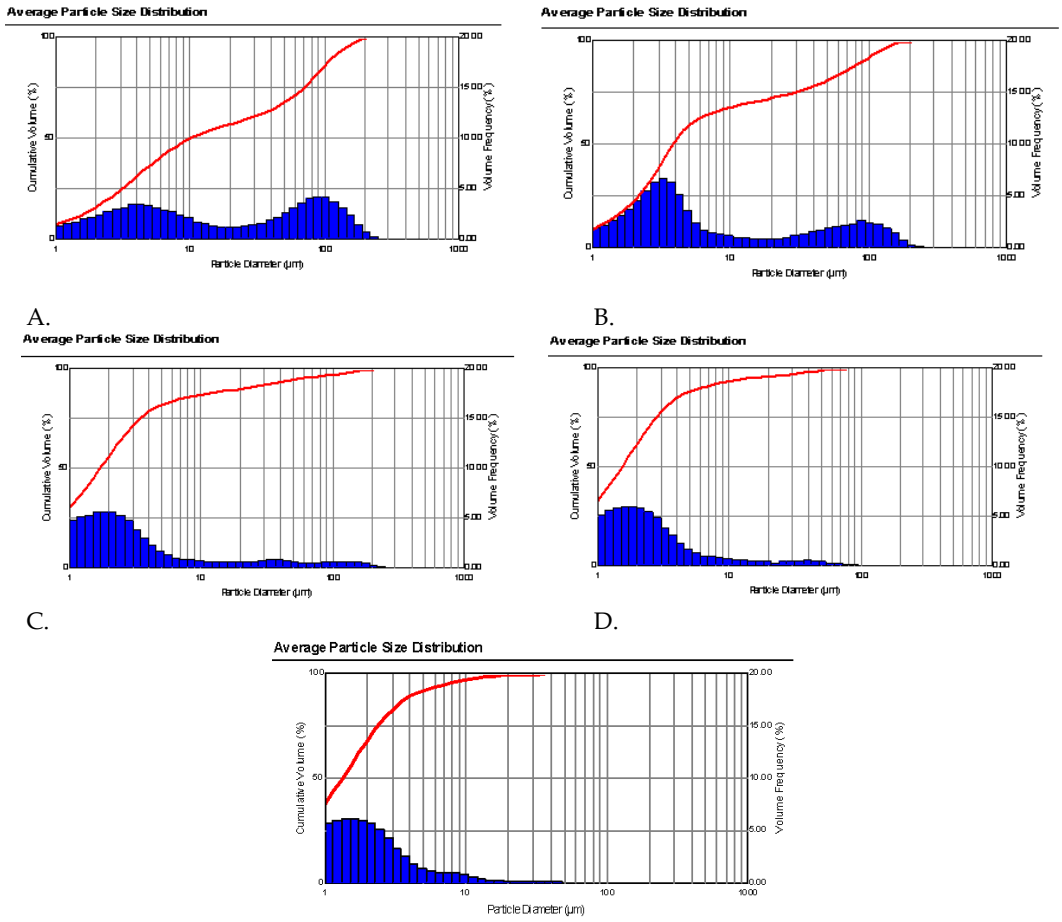


Figure 52: Average particle-size distribution and undersize curve measured with the Spraytec® (100 l/min, 2.4 s) of powders spray dried at different temperatures from an isopropanol:water ratio of 80:20: A. T120 80:20, B. T140 80:20, C. T160 80:20, D. T180 80:20 and E. T200 80:20

As can be shown from these results, the increase in the spray-drying temperature allowed an improvement in the particle dispersion from the inhalator, thus enhancing drug deposition deep in the lungs (loose agglomerates were easily scattered into small particles). This phenomenon could be linked to the difference in the amount of free water in the powders. Indeed, while the water content of raw tobramycin is about 3.4%, the water content increased to 5.9% for the T200 80:20 formulation and to 9.3% for the T120 80:20 formulation. This reduction in particle size distribution, related to the temperature of atomization, could be explained by the fact that tobramycin particles are very hygroscopic so that it was necessary to reach a high temperature in order to sufficiently dry the resultant powder. Otherwise, in addition to the van der Waals forces that tend to bring the particles between them closer, it is then possible that liquid bridges formed, due to the capillary forces between the particles, and thus, stronger aggregates formed in the powder.

The aerodynamic behaviour of the different tobramycin formulations analyzed in the MsLI is shown in Table 18.

Table 18: *In vitro* deposition profile and fine particle deposition in mg for 1 capsule (mean ± SD) of the formulations obtained from spray-dried suspensions with 20% v/v water with an MsLI (Aerolizer®, 100 l/min 2.4 s, 3 capsules of 15 mg/test, n=3)

	T120 80:20	**T140 80:20**	**T160 80:20**	**T180 80:20**	**T200 80:20**
Device	2.07 ± 0.05	1.5 ± 0.1	1.9 ± 0.3	2.7 ± 0.4	3.8 ± 0.6
Throat	0.25 ± 0.05	0.23 ± 0.04	0.5 ± 0.3	0.6 ± 0.3	0.8 ± 0.7
Stage 1	3.5 ± 0.3	3.3 ± 0.2	2.6 ± 0.6	2.2 ± 0.7	1.4 ± 0.4
Stage 2	1.63 ± 0.07	1.91 ± 0.05	1.94 ± 0.09	1.7 ± 0.2	0.76 ± 0.02
Stage 3	2.4 ± 0.3	2.0 ± 0.4	2.3 ± 0.4	2.3 ± 0.3	2.3 ± 0.5
Stage 4	2.00 ± 0.06	2.1 ± 0.4	2.04 ± 0.07	2.3 ± 0.3	2.39 ± 0.03
Filter	0.8 ± 0.3	1.4 ± 0.2	1.4 ± 0.3	1.29 ± 0.09	1.5 ± 0.4
FPD	5.02 ± 0.01	5.4 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	6.1 ± 0.1
Metered dose	12.7 ± 0.3	12.5 ± 0.3	12.8 ± 0.1	13.1 ± 0.1	13.0 ± 0.8

The results presented showed that for the same compositions of the suspensions used for spray drying, the inlet temperature had an effect on the FPD of the powder. Indeed, the results of deposition were related to the particle size distribution: the FPD significantly increased from 5.0 mg to 6.1 mg ($p < 0.05$) for T120 80:20 and T200 80:20, respectively. At the highest temperature, the deposition in stage 4 and especially in the filter, which roughly corresponded to the finest particle population, increased from 0.8 mg to 1.5 mg ($p < 0.05$), for T120 80:20 and T200 80:20, respectively. It is hypothesized that interparticle cohesion, as observed for powders spray-dried at a low temperature during the particle sizing, could not be overcome during the aerosolization process, resulting in the emission of large aggregates that could not deposit in the lower stages of the MsLI and consequently deep in the lung. The increase in the adhesive interactions operating between the tobramycin spray-dried particles may be a result of the increase in water content in the powder. It thus seemed that the inertial force produced by the capsule deagglomeration effects within a device such as the Aerolizer® during the aerosolization process was not sufficient to help the adhered and/or agglomerated particles to escape completely from the capsules.

V.4.2.2. Influence of amount of water in the suspensions

Attempts have been made to modify the electrostatic charges of the dry powder particles to affect drug deposition, but, as can be seen, care had to be taken to avoid increasing the moisture content. The presence of moisture, even in small quantities, will bring about capillary forces. The magnitude of these forces is related to the diameter of the pores between particles and the interfacial tension due to hydrogen bonding of water. Temperature and humidity cycling or poor drying may result in solid bridging through crystallization phenomena at the particle surface. In fact, the forces of interaction between pharmaceutical powders are difficult to characterize and control because of heterogeneity in particle composition and physicochemical characteristics.

In order to decrease the moisture content of the above powders and control the magnitude of the capillary forces holding particles in aggregates, suspensions of tobramycin with isopropanol:water ratios from 80:20 to 100:0 were spray dried at 200°C. Whilst 4.1 mg/ml of tobramycin dissolved in an 80:20 solvent system; approximately 1.0 mg/ml, 0.2 mg/ml and 0.1 mg/ml of tobramycin dissolved in ratios of 90:10, 95:5 and 98:2 isopropanol:water, respectively. Without being linked to any particular theory, it was hypothesized that the amount of particles dissolved in the solvent system would influence the thickness of the coating around the tobramycin particles and would modify differently the surface properties of the drug.

Table 19: Physical properties of raw tobramycin and all formulations obtained from suspensions with 10%, 5%, 2%, 1% and 0% v/v water and spray-dried at 200°C: particle size characteristics (mean ± SD, n=3) measured with the Mastersizer Scirocco 2000® (4 bar) and the Spraytec® (100 l/min, 2.4 s), and 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]	%< 5µm	Bulk density (g/cm³)	Tapped density (g/cm³)	Carr's Index (%)
T200 90:10	1.5 ± 0.1	2.2 ± 0.1	97.8 ± 0.3	1.54 ± 0.02	2.8 ± 0.1	91.0 ± 0.7	0.129	0.173	25
T200 95:5	1.55 ± 0.09	3.6 ± 0.7	97.5 ± 0.9	1.6 ± 0.7	3.1 ± 0.2	89 ± 1	0.130	0.177	26
T200 98:2	1.30 ± 0.01	2.00 ± 0.03	98.9 ± 0.3	1.3 ± 0.1	2.06 ± 0.08	91.4 ± 0.9	0.142	0.191	26
T200 99:1	1.4 ± 0.9	3.1 ± 0.9	98.6 ± 0.8	1.4 ± 0.9	2.8 ± 0.2	91.6 ± 0.8	0.165	0.220	25
T200 100:0	1.32 ± 0.09	1.58 ± 0.02	98.8 ± 0.3	3.21 ± 0.08	4.7 ± 0.7	88 ± 1	0.181	0.225	20
Tobra	2.6 ± 0.1	2.9 ± 0.1	78 ± 1	3.1 ± 0.9	11 ± 4	69 ± 8	0.229	0.300	24

The median particle sizes results obtained with the Mastersizer 2000® dry sampling system (Table 19) appeared to be similar for all powder formulations produced from suspensions containing water, exhibiting a D(0.5) value of about 1.3–1.6 µm. The formulations obtained from suspensions containing less than 2% (v/v) water showed the best size characteristics, thus revealing a reduction in agglomerates due to the decrease of water in the powder.

The results with the Spraytec®, shown in Table 19, confirmed this tendency. The median particle size of the powder decreased with the amount of water in the suspension. The volume mean diameter of the micronized tobramycin measured with the Spraytec® was about 11 µm, which was four times greater than the results of T200 98:2 formulation.
The T200 98:2 formulation exhibited a Gaussian curve (log-normal distribution) with 91% of particles below 5 µm, whereas micronized tobramycin showed a very wide particle size distribution spread up to a few tens of microns, with only 68% of particles below 5.0 µm (Fig. 53).

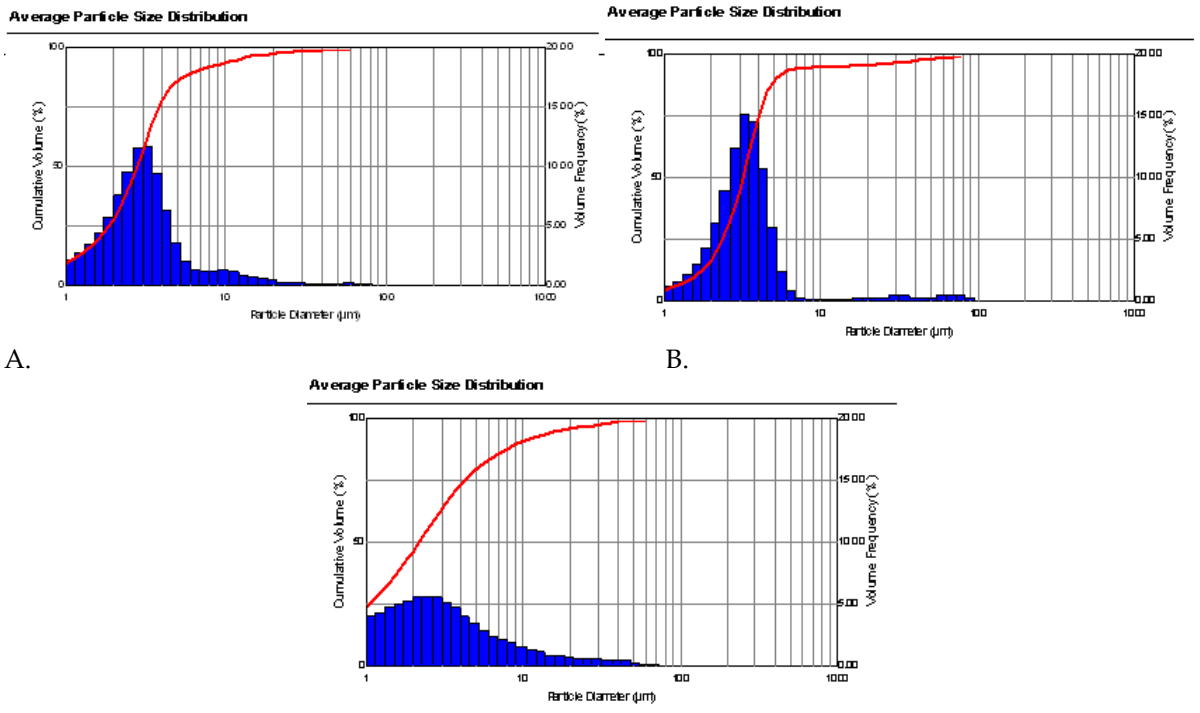


Figure 53: Average particle size distribution and undersize curve measured with the Spraytec® (100 l/min, 2.4 s) of A. T200 98:2 formulation, B. T200 100:0 formulation and C. Tobra µ (raw material)

Thus, it seems that the presence of water in the suspension allowed improvement in the flow characteristics of the dry powder. These results showed that it is possible to modify the surface properties of the particles by coating the particles of drug with a homogeneously distributed continuous film of the active compound dissolved in a solvent system containing a mixture of different solvents such as isopropanol and water. During nebulization of the suspension, droplets are composed of one or more particles in solid state that are surrounded with solvent containing the dissolved drug. It is hypothesized that during the drying step, the dissolved particles form the coating film. The coating of drug particles can thus be used as an alternative that permits the modification of the surface properties of the particles, increasing the flowability, the desagglomeration tendency and the fine particle fraction deposited in the deep lung.

Interestingly, it is important to note that the T200 100:0 formulation, which did not contain water in the initial suspension used for spray drying, allowed an increase in the percentage of particles below 5 μm compared with the raw material (88% vs. 69%, respectively). This result showed the effect of the initial HSH step and of the spray-drying process. Indeed, the principal advantage of spray-drying is the better desagglomeration tendency of the powder due to the production of loose agglomerates that are easily scattered into small particles during inhalation. The enhanced dispersion of spay-dried vs. micronized powders could be explain by the partial dissolution of the surface layers of the active particles in isopropanol, resulting, upon spray-drying of the powder, in the formation of a very thin coat of active material onto the particles surface. Moreover, processing the suspensions by spray-drying yielded more regular-shaped and micron-sized particles.

As expected, the amount of free water in the powders decreased with the ratio of water to isopropanol in the suspensions. Water content was about 5.9% for the T200 80:20 formulation, which contained 20% water (v/v) in the initial suspension, and about 2.9% for the T200 98:2 formulation, which contained 2% water (v/v) in the initial suspension. This latter formulation thus seemed to present more suitable characteristics for the pulmonary delivery of the powders and the long-term stability of the product.

Besides the particle size of a drug powder, the deagglomeration behaviour in an 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. The aerodynamic behaviour of the different tobramycin formulations analyzed in the MsLI is shown in Table 20.

Table 20: *In vitro* deposition profile and fine particle deposition in mg for 1 capsule (mean ± SD) of the formulations obtained from spray-dried suspensions with 10, 5, 2, 1 and 0% v/v water formulations measured by an MsLI (Aerolizer®, 100 l/min 2.4 s, 3 capsules of 15 mg/test,n=3)

	T200 90:10	T200 95:5	T200 98:2	T200 99:1	T200 100:0	Tobra µ
Device	3.1 ± 0.3	1.5 ± 0.1	1.9 ± 0.3	2.0 ± 0.5	4.8 ± 0.4	6.0 ± 0.7
Throat	0.3 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.6 ± 0.3	0.36 ± 0.06	0.3 ± 0.1
Stage 1	0.9 ± 0.1	1.8 ± 0.2	1.1 ± 0.4	1.2 ± 0.7	0.7 ± 0.1	0.9 ± 0.3
Stage 2	1.0 ± 0.1	1.91 ± 0.05	0.9 ± 0.1	0.7 ± 0.2	0.8 ± 0.1	0.6 ± 0.4
Stage 3	3.5 ± 0.3	3.0 ± 0.6	3.6 ± 0.2	3.9 ± 0.9	2.9 ± 0.3	2.1 ± 0.9
Stage 4	3.2 ± 0.3	3.1 ± 0.4	3.4 ± 0.1	3.0 ± 0.3	2.9 ± 0.1	1.7 ± 0.6
Filter	1.0 ± 0.1	1.4 ± 0.5	2.3 ± 0.3	1.9 ± 0.3	1.68 ± 0.09	1.4 ± 0.3
FPD	7.3 ± 0.9	7.0 ± 0.9	8.5 ± 0.1	8.1 ± 0.6	7.4 ± 0.5	5.5 ± 0.9
Metered dose	13.0 ± 0.4	12.9 ± 0.3	13.7 ± 0.1	13.3 ± 0.5	14.2 ± 0.7	12.9 ± 0.3

It is interesting to note that the presence of small amounts of water in the suspensions used for spray drying markedly enhanced the FPD, which was about 5.5 mg for raw tobramycin, about 7.4 mg for spray-dried tobramycin from suspensions with an isopropanol:water ratio of 100:0 and about 8.5 mg for powder from suspensions with an isopropanol:water ratio of 98:2 ($p < 0.05$) (Table 20). The results presented in Figure 54 show that the percentage of water in the suspensions used for spray drying had a significant effect on the FPF of the powders. The FPF, which was about 40% for T200 80:20, increased to up to 48% for the T200 90:10 formulation, which contained 10% v/v water in the initial suspension, and to 57% for T200 98:2 formulation, which contained 2% v/v of water in the initial suspension ($p < 0.05$). The results of the present example show that the spray dried coated powder compositions yielded better results than the micronized ones. It is hypothesized that the dissolved particles coat the active particles of tobramycin and modify the properties of the surface, the flowability and the aerosolization of the active drug particles. So, the evaluation of the influence of the water content in the suspensions and the effect of the spray-drying process showed that the addition of 2% water v/v was sufficient to improve particle dispersion during inhalation. This is of particular interest: as tobramycin is a very

hygroscopic drug, the addition of water is a critical step. It is therefore important to add a small amount of water to the solvent system and to process the drying step at a high temperature.

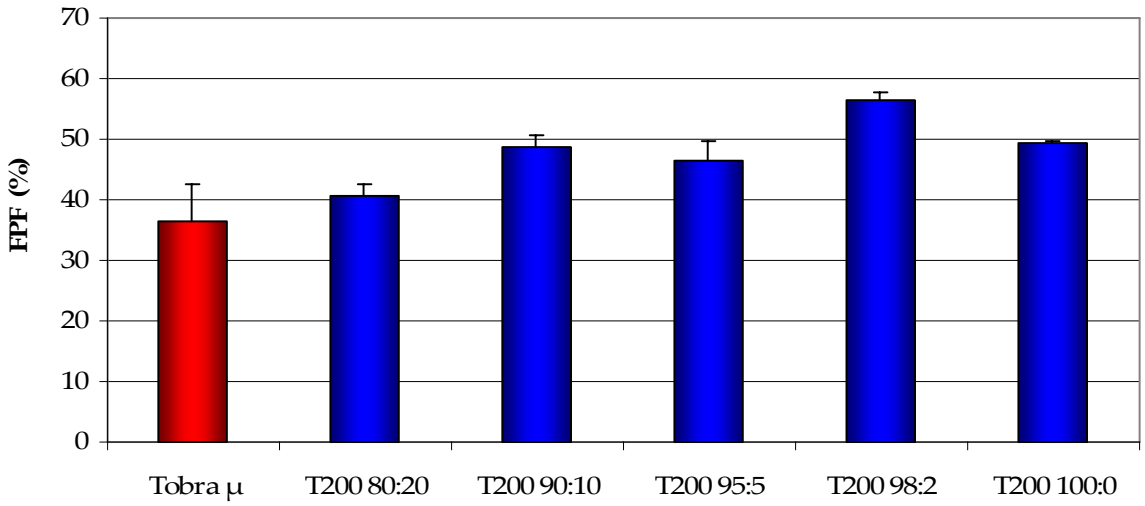
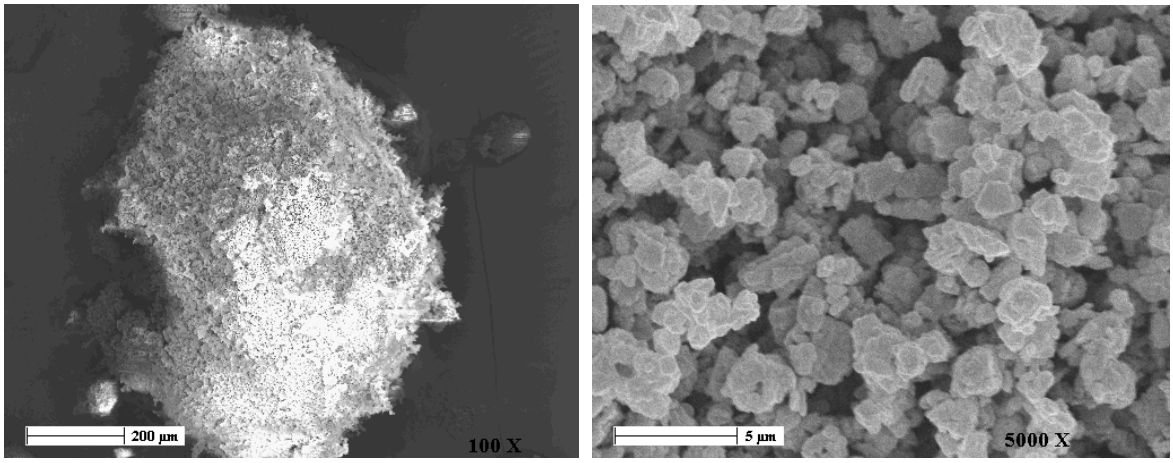


Figure 54: The FPF (MsLI, Aerolizer®, 100 l/min 2.4 s, 3 capsules of 15 mg/test) assessment (mean ± SD) of the different formulations. Each bar represents the average of three repeats

The morphology and surface structure of the T200 98:2 formulation were analyzed by SEM. Processing the suspensions by spray-drying yielded looser agglomerates that were less smooth, less regular and less cohesive than the raw material (Fig.37, p 132). Increasing the proportion of water in the feed was shown to change the size and shape of the spray-dried tobramycin particles. The powder consisted of smaller agglomerates of about 50-200 µm in size (Fig. 55.A). A corrugated surface that was consistent with rapid particle drying, cavity formation and particle collapse was observed. At larger magnifications, we could observe (Fig. 55.B) that these agglomerates were composed of small particles and tended to being porous. These loose agglomerates made up of small particles probably explain the better dispersion of the particles, compared to raw micronized tobramycin, during inhalation. Due to this morphology, the particles became light, as was confirmed by the bulk density measurements, and the spray-dried formulations presented good flowability (Table 19, p 166).

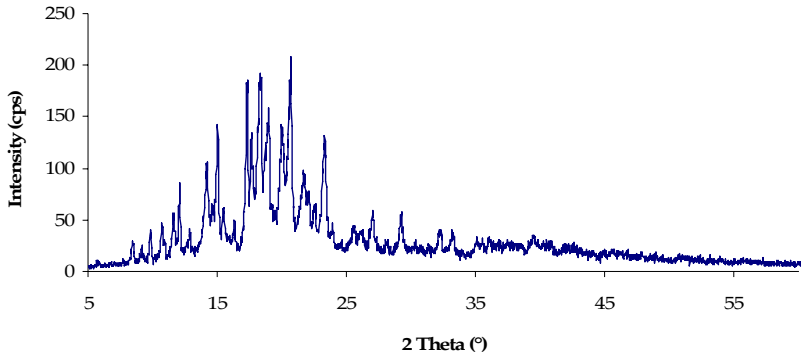


A. B.

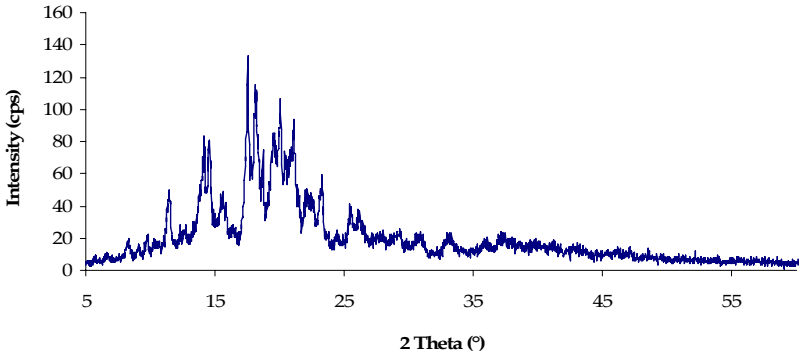
Figure 55: SEM photographs of: A. T200 98:2 formulation, magnification 500x; B. T200 98:2 formulation, magnification 5000x

The sharp peaks presented in the XRPD diffraction patterns (Fig. 56) for raw tobramycin suggest a crystalline state with a high degree of order. Increasing the amount of water from 0% to 20% v/v, with respect to isopropanol, resulted in a decrease in the crystallinity of the corresponding spray-dried material. When water was used in the feed, the spray-dried materials presented diffractograms with a reduction in peaks intensity in comparison to those of the reference material. The presence of water in the feed favoured the solubilisation of tobramycin and thus the formation of amorphous particles during the spray-drying process. The evaluated amorphous percentage in the formulations was about 15.8%, 16.4%, 30.1% and 40.9% for the raw tobramycin, T200 98:2, T200 90:10 and T200 80:20 formulations, respectively. Overall, the crystalline content was predominant in all samples spray-dried in the presence of water.

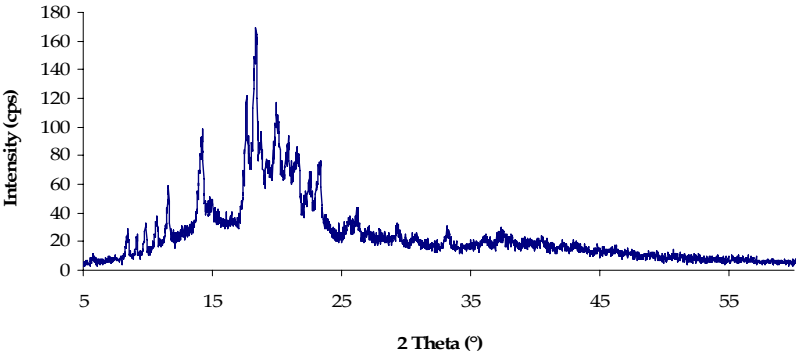
In order to characterize this amorphous phase, glass transition (Tg) measurements were done on a totally amorphous sample obtained by spray-drying a solution of tobramycin in isopropanol:water 70:30 at 200°C. As an example, amorphous tobramycin powder containing 10.0% (w/w) of water presented an onset point about 58.5°C. Nevertheless, this value corresponds to a totally amorphous powder with high water content whilst for the formulations herein above, the crystalline content is predominant. It should be noted that because of the low amorphous content in the formulations, it was not possible to characterize the amorphous layer of the developed formulations. So, this value gives only an estimate but is not believed to predict with exactitude the stability of the optimized formulations.



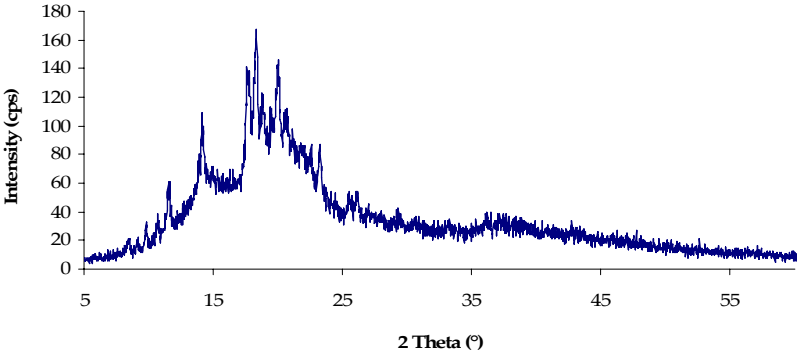
A.



B.



C.



D.

Figure 56: X-ray diffractograms of tobramycin samples spray dried from different water:isopropanol ratios: A. micronized tobramycin, B. T200 98:2, C. T200 90:10 and D. T200 80:20.

The bulk density values obtained for all formulations produced from suspensions containing water were lower than those obtained for the micronized tobramycin (Tables 17, p 162 and 19, p 166). The results seemed to be influenced by the amount of water in the suspensions used for spray-drying. The values showed that the powders with a high moisture content, such as the T120 80:20 or T200 80:20 formulations, presented a higher Carr's index and thus poorer flowability. The elevated humidity in the powder led to a severe aggregation problem arising from capillary forces, which made the physical handling of the particles difficult for DPI delivery. Carr's Index values of less than 25 are usually taken to indicate good flow characteristics, whereas values above 40 indicate poor powder flowability. Considering that the values are about 25 and 26, the flowability is not considered as ideal but the results suggest that the powder flow properties are acceptable. The aim of the work was to develop powders with improved deposition results while limiting the addition of excipient. Adding lactose carrier will decrease the content of tobramycin in the capsule, which is not desired for projected high dose drugs such as antibiotics. With the Carr's index about these values, it is expected that the flowability of the powder would not necessarily be the limiting factor during the machine filling process. Of course, this question will be evaluated during the industrial scaling-up.

V.3. Conclusion

The aim of this part of the study was to produce carrier-free formulations with high deposition properties for projected highly-dosed drugs for use as dry powders for inhalation. The resulting powders exhibited different characteristics according to the proportion of water in the feed and the temperature of atomization. The effect of the water:isopropanol ratio on particle size, bulk density, moisture content and aerodynamic behaviour was evident. The spray-dried samples produced different deposition profiles, resulting from their different physical properties. The presence of water in small amounts in the suspensions (2% v/v) resulted in the production of particles with modified surface properties, due to an amorphous coating, and with improved dispersion results. The study

has shown that delivery of antibiotics from carrier-free formulations via DPI is feasible and could, with further improvement, ultimately be used to improve therapeutic outcomes for patients suffering from critical pulmonary illnesses.

However, it still remains to be determined if the formulations effectively yield better results *in vivo*. A randomized clinical trial on CF patients will be performed to evaluate the efficiency, pharmacokinetics, and bioavailability of these formulations.

PART V.5: PHARMACOSCINTIGRAPHIC AND PHARMACOKINETIC EVALUATION OF TOBRAMYCIN DPI FORMULATIONS IN CYSTIC FIBROSIS PATIENTS

Eur.J.Pharm.Biopharm., 68(2): 413-421 (2008)

V.5.1. Introduction

As previously explained, lipid-coated and amorphous-coated tobramycin dry powder formulations for inhalation were developed during this work. These formulations were characterized in terms of aerosolization properties, surface composition and physical state. These powders were shown to deliver drugs efficiently to the lower respiratory tract using relatively simple and inexpensive inhalation devices. When tested *in vitro* with an MsLI, these powder formulations produced an FPF of at least 68% and 57% of the nominal dose, for the lipid-coated (T2) and amorphous-coated tobramycin (T200 98:2) formulations, respectively.

In order to confirm these encouraging results, two formulations were selected and compared to Tobi® (nebulizer solution) (Chiron Corporation; Seattle, WA) by performing a combined *in vivo* scintigraphic and pharmacokinetic evaluation of tobramycin after inhalation of a single oral dose in nine CF patients. Gamma scintigraphic imaging provides direct information on the amount and site of drug deposition in the lung after inhalation and the pharmacokinetic evaluation gives valuable information on the rapid elimination of the drug from the site of deposition by absorption and on the systemic bioavailability of the drugs.

V.5.2. Study protocol

V.5.2.1. Dry powder formulations

Two tobramycin DPI formulations were selected.

The first formulation (Tobra Form 1) consisted of lipid-coated micronized tobramycin particles. The powder was obtained by spray-drying a suspension of isopropanol containing micronized tobramycin 5% w/v and lipids 0.25% w/v in an appropriate ratio of 75% of cholesterol and 25% of Phospholipon® 90H (T2 formulation).

The second formulation (Tobra Form 2) consisted of micronized tobramycin without excipient (T200 98:2 formulation).
Size 3 HPMC capsules were filled with 25 mg of these powders, which contained nominally 95% w/w tobramycin for the first formulation and 100% w/w tobramycin for the second formulation. The fill-mass specification was set at 25 ± 0.5 mg, and this target was met for all hand-filled capsules.
The DPI device used was the Aerolizer®, a passive breath-actuated, single-dose dry powder inhaler.

The third formulation was the comparator product Tobi®, which contains 300 mg of tobramycin free base in 5 ml of sodium chloride (2.25 mg/ml) adjusted to pH 6.0. Nebulized tobramycin was administered as directed using a hand-held Pari LC Star nebulizer (PARI GmbII; Starnberg, Germany) in combination with the Pari Turbo Boy N compressor (PARI GmbII; Starnberg, Germany), as recommended by the manufacturer, until dry running.

V.5.2.2. Study design

The study design was an open single-dose, three-treatment, three-period, cross-over study with a wash-out period of at least 6 days between the three phases of the study.

All patients received the drug treatment as one capsule of 25 mg tobramycin (Tobra Form 1 or 2) or one dose of Tobi® (nebulizer solution) on each occasion (Table 21).

Table 21: Study design

	Period I			**Period II**			**Period III**		
Subjects	***Day 1***	***Day 2***	***Day 3***	***Day 8***	***Day 9***	***Day 10***	***Day 15***	***Day 16***	***Day 17***
1,4,7	Tobra Form 1	X	X	Tobi®	X	X	Tobra Form 2	X	x
2,5,8	X	Tobra Form 2	X	X	Tobra Form 1	X	X	Tobi®	x
3,6,9	X	X	Tobi®	X	X	Tobra Form 2	X	X	Tobra Form 1

Immediately following the administration of drug, scintigraphic images of the chest and lateral oropharynx were recorded as described in part IV.2.12.2.2, p 104.
To measure lung deposition by gamma scintigraphy, ^{99m}Tc was adsorbed onto the surface of tobramycin particles as described in part IV.2.12.2.3, p 106. The amount of ^{99m}Tc was adjusted so that the maximum amount of radioactivity inhaled by the subjects on any occasion did not exceed 28 MBq of ^{99m}Tc.

Before inhalation of the drug, patients took Atrovent® (nebulizer solution) and Pulmozyme® (nebulizer solution) and underwent 30 min of chest physiotherpy in order to clear a maximum their respiratory tract.

Furthermore, a total of 50 g of activated charcoal (Carbomix, Norit, The Netherlands) was given orally before and over the 30 minutes (divided in two doses of 25 mg) after each administration of drug treatment (Thorsson et al., 1994). Even though anti-pseudomonas agents have low oral bioavailability, activated charcoal was given to prevent any risk of gastrointestinal absorption of tobramycin (Table 22).

Table 22: Study day protocol

Sample N°	Theoretical time (h :min)	Time post-dose (h:min)	Remarks	Blood Sample volume
P0	07:00	-01:00	Predose sample Administration of Atrovent® + Pulmozyme® 30 min chest physiotherapy	7 ml
	07:30	-00:30	Spirometer	
	07:55	-00:05	Ingestion of 25 g of Norit-carbomix®mixed with 1 glass of water	
	08 :00	00 :00	Administration of 1 capsule of 25 mg tobramycin by inhalation or one nebulization of Tobi®	
	08:25	00:25	Ingestion of 25 g of Norit-carbomix®mixed with 1 glass of water	
P1	08:30	00.30		7 ml
	08:35	00:35	Spirometer	
P2	09:00	01:00		7 ml
	09:05	01:05	Breakfast	
P3	09:30	01:30		7 ml
P4	10:00	02:00		7 ml
P5	10:30	02:30		7 ml
P6	11:00	03:00		7 ml
P7	12:00	04:00		7 ml
	12:05	04:05	Lunch	
P8	14:00	06:00		7 ml
P9	16:00	08:00		7 ml
	16:05	08:05	Snack	
P10	18:00	10:00	Dinner	7 ml

V.5.2.3. Subjects

Nine patients (5 men and 4 women; 34 ± 5 years) were recruited. Each patient was clinically well (without infective exacerbation) and had mean values for FEV_1 (forced expiratory volume in 1 second) and for FVC (forced expiratory vital capacity) > 50% of the

predicted value (Table 23). Excluded from this study were patients that were receiving continuous home intravenous antibiotic therapy, pregnant or participating in another study within 4 weeks of the proposed study.

Patients were seen before entering the study and were carefully instructed in the use of the Aerolizer® and the Tobi® nebulizer set.

Table 23: Demographic data of the 9 CF patients

Patient n°	Gender	Age (years)	Size (cm)	Weight (kg)	FEV_1 (%)
1	F	35	174	62	73
2	M	37	170	63	55
3	M	33	176	66	63
4	F	37	160	60	60
5	F	38	165	58	61
6	M	42	170	62	67
7	M	30	178	60	85
8	M	32	180	58	64
9	F	25	166	59	59

Before starting the study, the nature of the clinical trial was explained and written consent was obtained from all patients. The study was conducted at the Erasme Hospital (Brussels, Belgium), in accordance with the principles stated in the Declaration of Helsinki, and approval was obtained from the Ethics Committee of Erasme Hospital (Ref.: P2006/072) and the Belgian Minister of Social Affairs and Public Health (Ref.: EudraCT n° 2006-000456-40) (see part VIII. Annexes, p 247).

V.5.2.4. Safety assessment

Pulmonary function (FVC, FEV_1) and vital signs were recorded before and 30 min after dose. Adverse events were monitored throughout the study. In addition, each subject underwent a physical examination, pulmonary function testing, routine clinical chemistry, haematology and urinalysis at the beginning and the end of the study.

V.5.3. Results

V.5.3.1. Validation of the radiolabelling method

In vitro assessment (as described in part IV.2.12.2.4, p 106) of the output of radiolabelled tobramycin with the Aerolizer® was used to ensure that the radiolabelling method did not significantly modify the PSD of the aerosol generated by the device and also to confirm that the distribution of ^{99m}Tc reflected that of the drug, thus acting as a suitable marker for tobramycin.

In vitro deposition was compared for unlabelled tobramycin, labelled tobramycin and the ^{99m}Tc with the MsLI at 100 l/min for 2.4 s. The PSD for Tobra Form 1 are given in Fig. 57. The FPF values (mean ± SD) obtained for the drug before labelling (69.8 ± 1.0%), after labelling (68.4 ± 1.9%) and for the radiolabel (67.7 ± 2.3%) were not significantly different (p > 0.05).

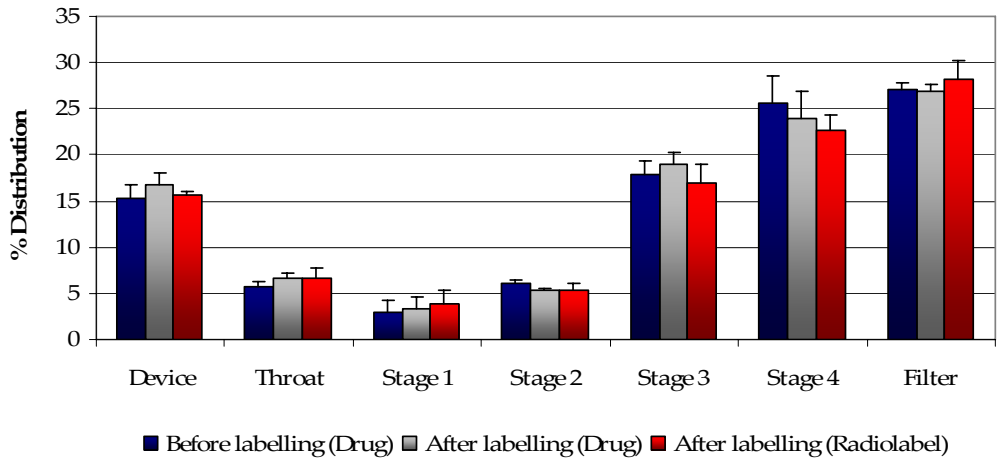


Figure 57: *In vitro* comparative deposition of Tobra Form 1 (MsLI, Aerolizer®, 100 l/min 2.4 s, 3 capsules of 25 mg/test, n=9): before labelling, after labelling (drug) and after labelling (radioactive)

The data for Tobra Form 2 are shown in Fig. 58. There was also a good match between the FPF values (mean ± SD) of the drug before labelling (57.6 ± 2.3%), after labelling (56.5 ± 2.8%) and for the radiolabel (56.1 ± 2.9%) (p > 0.05). The results did not show any significant differences among the three measurements on any stages of the MsLI. The amount remaining in the device was also similar for the three measurements.

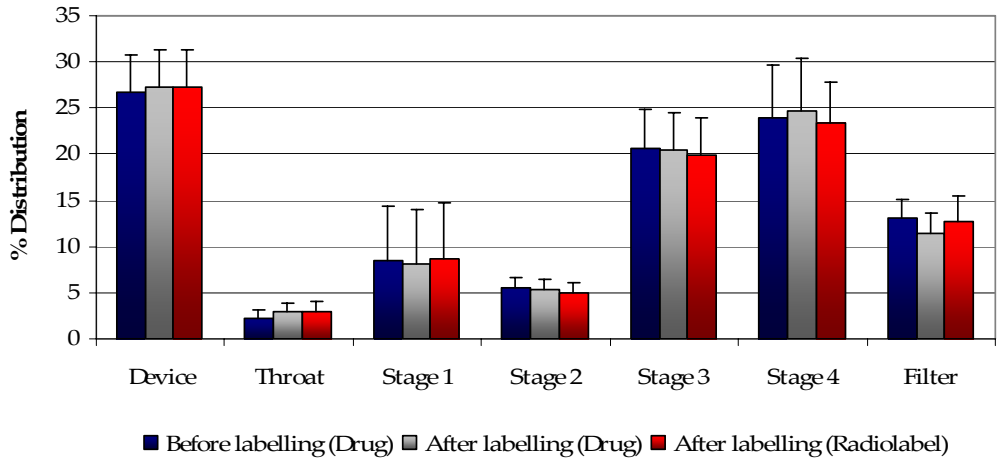


Figure 58: *In vitro* comparative deposition of Tobra Form 2 (MsLI, Aerolizer®, 100 l/min 2.4 s, 3 capsules of 25 mg/test, n=9): before labelling, after labelling (drug) and after labelling (radioactive)

This validation demonstrated that the PSD of the radiolabel and Tobra Form 1 and 2 were well-matched, with no alteration of aerosol properties of the formulations after radiolabelling. So, for both formulations, a homogenous cover of ^{99m}Tc that did not affect the size of the particles was obtained around the active drug. Moreover, this method was proven to be reproducible as the deposition results for the two forms for 3 different days were significantly similar ($p > 0.05$).

For nebulized tobramycin, the radiolabel and drug were both dissolved in the aqueous phase, and hence the concentration of drug and radiolabel were uniformly distributed in a given nebulized droplet (Newhouse et al., 2003).

V.5.3.2. Lung function tests

The mean FEV_1 (as predicted value) measured before the beginning of the study for all subjects was 68 ± 11% (ranging from 55-89%). Moreover, the FEV_1 and FVC values, measured prior to and 30 min after dosing, were similar on each study day before and after inhalation of the drug (Table 24).

Table 24: Lung function values for the nine CF patients

Patient 1		Formulations		
		Tobra Form 1	Tobra Form 2	Tobi
FEV₁	Pre-inhalation	89	85	73
	Post-inhalation	92	90	83
FVC	Pre-inhalation	87	88	74
	Post-inhalation	95	93	85

Patient 2		Formulations		
		Tobra Form 1	Tobra Form 2	Tobi
FEV₁	Pre-inhalation	56	71	68
	Post-inhalation	68	68	60
FVC	Pre-inhalation	87	79	86
	Post-inhalation	87	74	81

Patient 3		Formulations		
		Tobra Form 1	Tobra Form 2	Tobi
FEV₁	Pre-inhalation	88	85	88
	Post-inhalation	92	104	88
FVC	Pre-inhalation	85	83	74
	Post-inhalation	82	80	80

Patient 4		Formulations		
		Tobra Form 1	Tobra Form 2	Tobi
FEV₁	Pre-inhalation	61	60	59
	Post-inhalation	58	55	57
FVC	Pre-inhalation	76	99	90
	Post-inhalation	92	93	77

Patient 5		Formulations		
		Tobra Form 1	Tobra Form 2	Tobi
FEV₁	Pre-inhalation	60	65	63
	Post-inhalation	61	69	61
FVC	Pre-inhalation	64	74	73
	Post-inhalation	69	76	66

Patient 6		Formulations		
		Tobra Form 1	Tobra Form 2	Tobi
FEV₁	Pre-inhalation	70	64	77
	Post-inhalation	69	82	67
FVC	Pre-inhalation	86	81	89
	Post-inhalation	88	90	82

Patient 7		Formulations		
		Tobra Form 1	Tobra Form 2	Tobi
FEV₁	Pre-inhalation	68	66	63
	Post-inhalation	65	64	63
FVC	Pre-inhalation	94	108	91
	Post-inhalation	95	114	92

Patient 8		Formulations		
		Tobra Form 1	Tobra Form 2	Tobi
FEV₁	Pre-inhalation	67	60	68
	Post-inhalation	69	71	65
FVC	Pre-inhalation	100	89	109
	Post-inhalation	94	113	93

Patient 9		Formulations		
		Tobra Form 1	Tobra Form 2	Tobi
FEV₁	Pre-inhalation	65	60	59
	Post-inhalation	62	81	61
FVC	Pre-inhalation	82	76	85
	Post-inhalation	85	79	82

The results indicated that the inhalation of 25 mg of tobramycin in the presence of lipids or alone did not have any significant effect on lung function. Furthermore, no major or minor complications were observed during the clinical trial and there was no evidence of bronchoconstriction, suggesting good tolerance of these products.

V.5.3.3. Scintigraphic results

The percentage of the radiolabelled tobramycin deposited in the whole lung, the oropharynx, the stomach and the device, as determined by gamma scintigraphy, is shown in Table 25.

Table 25: A. Mean ± SD (CV) fractionation of the dose between lungs, oropharynx, stomach and device measured by gamma scintigraphy for Tobra Form 1, Tobra Form 2 and nebulized tobramycin (Tobi®) in 9 CF patients
B. Deposition in mg (mean ± SD) in the lungs measured by gamma scintigraphy for the three formulations in 9 CF patients

	Tobra Form 1	**Tobra Form 2**	**Tobi®**
Lungs	53.0 ± 10.0 (19)	34.1 ± 12.4 (36)	7.6 ± 2.7 (39)
Oropharynx	16.0 ± 6.0 (38)	15.2 ± 7.1 (47)	2.7 ± 1.3 (50)
Stomach	3.1 ± 3.2 (107)	2.1 ± 2.0 (93)	1.8 ± 1.2 (68)
Device	20.4 ± 8.5 (41)	43.4 ± 14.6 (33)	/

A.

	Lungs (mg)
Tobra Form 1 (25 mg)	12.6 ± 2.5
Tobra Form 2 (25 mg)	8.5 ± 3.1
Tobi® (300 mg)	22.8 ± 8.1

B.

Mean ± SD lung deposition for Tobra Form 1 for all subjects was 53.0 ± 10.0% (range from 35.7% to 62.7%), for an overall coefficient of variation (CV) of 19%. For Tobra Form 2, the mean ± SD lung deposition was 34.1 ± 12.4% (range from 15.3% to 50.0%), for an overall CV of 36%.

The mean lung deposition for Tobra Form 1 of 53.0 ± 10.0% was significantly greater ($p < 0.01$) than that for Tobra Form 2 (34.1 ± 12.4%). These results corresponded to 13.3 mg (12.6 mg of tobramycin) and 8.5 mg tobramycin deposited in the lung, assuming a total delivered dose of 25 mg via the Aerolizer® loaded with Tobra Form 1 and Form 2, respectively.

Approximately the same percentage of the tobramycin dose was deposited in the oropharynx (16.0% vs. 15.2% ($p > 0.05$)) and the stomach (3.1% vs. 2.1% ($p > 0.05$)) for Tobra Form 1 and Tobra Form 2, respectively.
Nevertheless, the dose retained inside the capsule and inhaler was significantly higher ($p < 0.01$) for Tobra Form 2 than for Tobra Form 1 (43.4% vs. 20.4%). The exhaled fraction was found to be less than 0.2% for both products and was assumed to be negligible.

The high variability observed in lung deposition in patients is attributable in part to the very low deposition results observed in two subjects (Patients 5 and 9) because of poor lung function (see Fig. 59, pp 187-189). If we perform the calculation without including these subjects (n=7), the lung deposition results are higher and the SD and CV is lower: the mean ± SD (CV) lung deposition for Tobra Form 1 is about 57.7 ± 4.1 (8%) and for Tobra Form 2 is about 39.4 ± 7.4 (18%).

Concerning the amount of reference product (Tobi®), after the time of 20 min necessary to nebulize to dryness the product, only 7.6 ± 2.7% (CV of 39%) of the radiolabelled tobramycin (range from 3.6% to 13.2%) was deposited in the lung and 2.7 ± 1.3% in the oropharynx. This result corresponded to 22.8 mg tobramycin deposited in the lung, assuming a total delivered dose of 300 mg after nebulization.

Regional deposition results after inhalation of radiolabelled tobramycin are shown in Table 26.

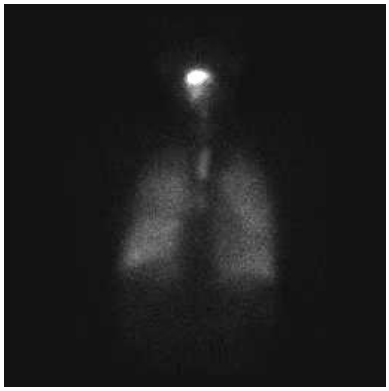
Table 26: Regional lung deposition: mean ± SD (CV) percentage deposition in peripheral, intermediate and central lung zones, and mean ± SD (CV) peripheral zone/central zone deposition ratio (P/C ratio) measured by gamma scintigraphy

	Tobra Form 1	**Tobra Form 2**	**Tobi®**
Central zone	19.7 ± 4.4 (22)	13.0 ± 4.8 (37)	2.9 ± 1.1(36)
Intermediate zone	16.0 ± 3.7 (23)	10.1 ± 4.0 (39)	2.2 ± 0.8 (38)
Peripheral zone	17.3 ± 3.3 (19)	11.0 ± 4.2 (39)	2.1 ± 0.9 (44)
P/C ratio	0.9 ± 0.2 (20)	0.8 ± 0.2 (21)	0.7 ± 0.5 (50)

The relative distribution (P/C ratio) of radiolabel within the central, intermediate, and peripheral airways was relatively similar for Tobra Form 1, Tobra Form 2 and the nebulized solution (0.9, 0.8 and 0.7). Even if the P/C ratio did not vary significantly ($p > 0.05$), it indicated that more of the dose was deposited in the medium- and small-diameter airways and alveoli, rather than in primarily large-diameter airways for the DPI than for the nebulizer. The P/C ratio results ranged from 0.7 to 1.2 for both Tobra Form 1 and 2, while they ranged between 0.3 and 1.0 for Tobi®.

The scintigraphic images showing deposition patterns for each formulation for the nine patients are illustrated in Fig.59. As can be seen, patients 5 and 9 presented a low respiratory function with a deficient lung, resulting in poor deposition results.

PATIENT 1

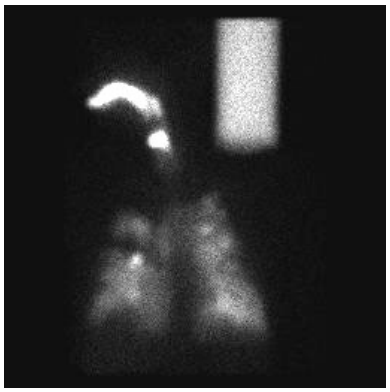


Tobra Form 1

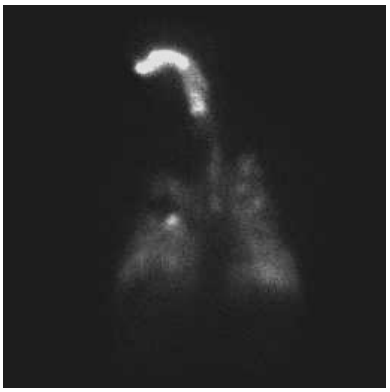
Tobra Form 2

Tobi

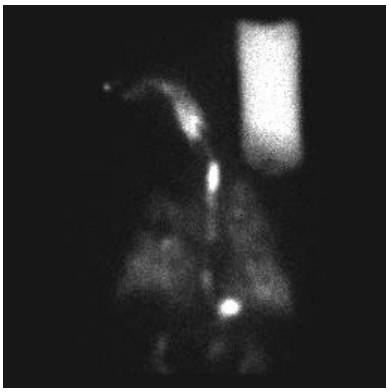
PATIENT 2



Tobra Form 1



Tobra Form 2

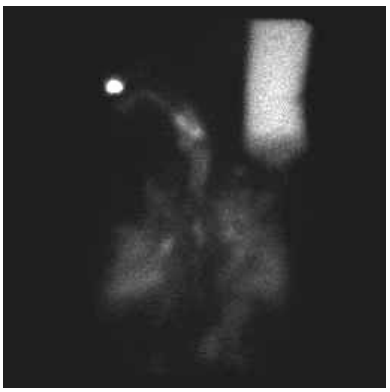


Tobi

PATIENT 3

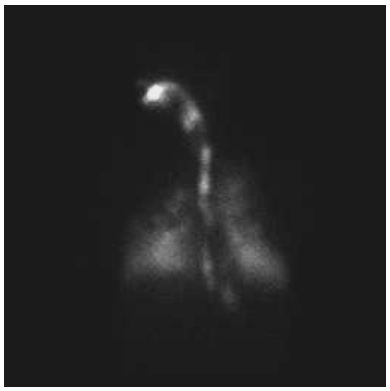
Tobra Form 1

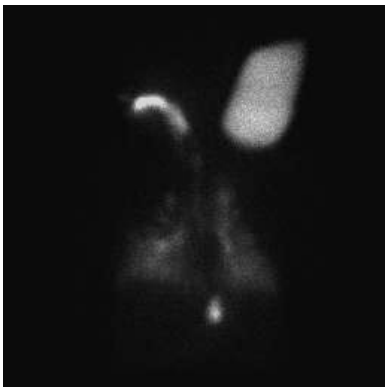
Tobra Form 2



Tobi

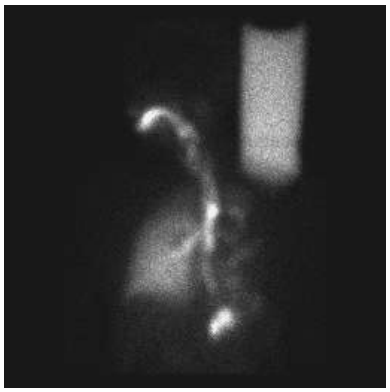
PATIENT 4


Tobra Form 1


Tobra Form 2


Tobi

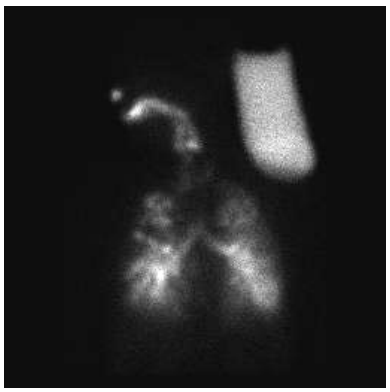
PATIENT 5

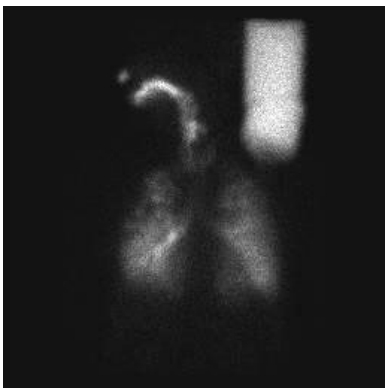

Tobra Form 1

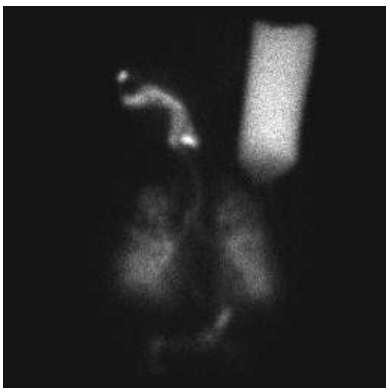

Tobra Form 2


Tobi

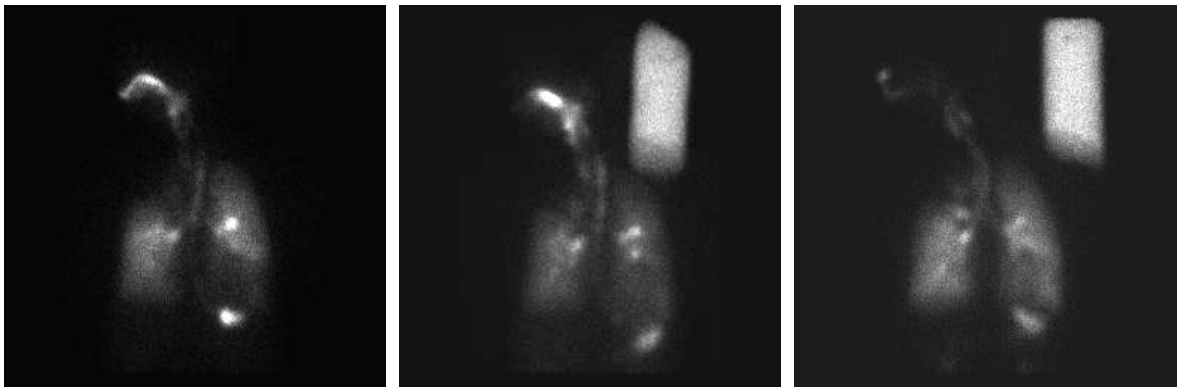
PATIENT 6


Tobra Form 1


Tobra Form 2

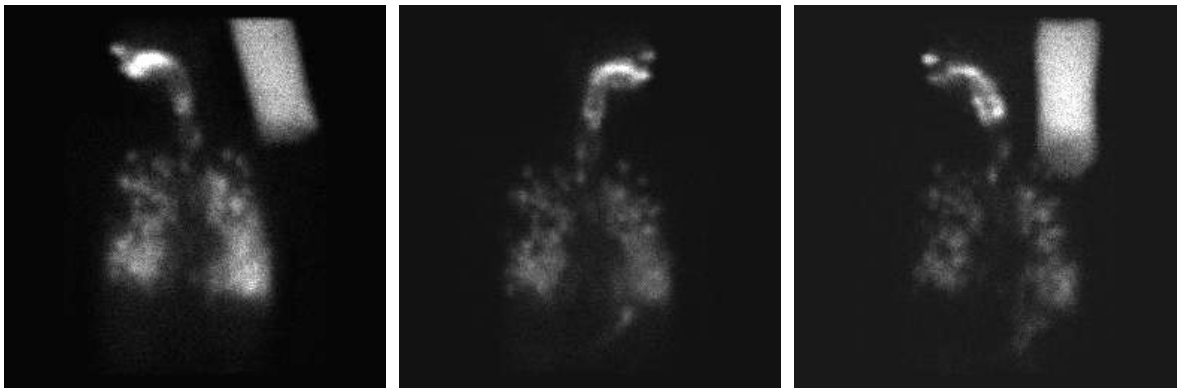

Tobi

PATIENT 7



Tobra Form 1 | Tobra Form 2 | Tobi

PATIENT 8



Tobra Form 1 | Tobra Form 2 | Tobi

PATIENT 9

Tobra Form 1 | Tobra Form 2 | Tobi

Figure 59: Scintigraphic images obtained for Tobra Form 1, Tobra Form 2 and Tobi® for the 9 patients

V.5.3.4. Pharmacokinetic data

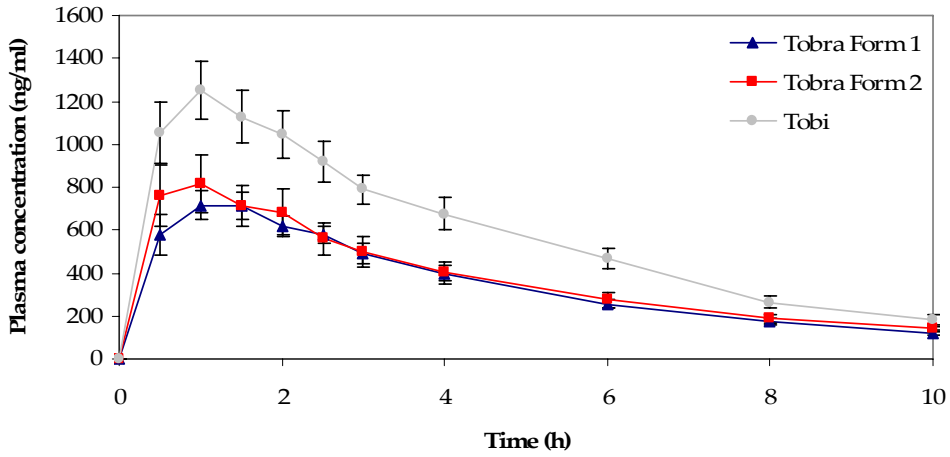


Figure 60: Mean plasma concentrations of tobramycin adjusted to correspond to the same drug dose as that of the comparator product (Tobi®) deposited in the lung (based on the scintigraphic deposition values (22.8 mg)) following administration of Tobra Form 1, Tobra Form 2 and nebulized tobramycin (Tobi®) (mean ± S.E.M, n=9)

The examination of Figure 60, representing the plasma concentration-time profile adjusted to give the same drug dose deposited in the lung as that of the comparator product (Tobi®) (based on scintigraphic deposition values (22.8mg)), showed a rapid plasma concentration peak for the DPI formulation as well as for the comparator product, followed by a progressive decrease in tobramycin plasma concentrations over 10 hours. Nevertheless, Tobi® (nebulizer solution) was absorbed a little faster than the Tobra Form 1, with maximum plasma concentrations (C_{max}) occurring at 0.9 h and 1.4 h, respectively. So, the droplets, with their hydrophilic properties and a median diameter about 2.2 μm, were dissolved more easily than the particles of tobramycin in the bronchial fluid and then passed in the systemic circulation.

The mean mass of tobramycin deposited in the lung as determined by gamma scintigraphy for the DPI formulations correlates well with the pharmacokinetic data herein below. Indeed, the mean deposition in the lung was found to be 1.5 times higher for Tobra Form 1 than for Tobra Form 2 and the mean AUC was also found to be 1.4 times higher for Tobra Form 1 than for Tobra Form 2.

As can be seen in Table 27, the mean AUC and C_{max} of the Tobi® were approximately 3 and 4 times higher than those of Tobra Form 1 and Tobra Form 2, respectively.

Nevertheless, the administrated dose of Tobi® was 12 times greater than for the DPI forms (300 mg vs. 25 mg). Only one inhalation was performed and the DPIs were loaded with about twelve fold less drug, but there is no reason why multiple inhalations could not be performed.

Table 27: Mean ± SD (CV) pharmacokinetic parameters for tobramycin (n=9)

	Tobra Form 1 (25 mg)	**Tobra Form 2 (25 mg)**	**Tobi (300 mg)**
Tmax (h)	1.4 ± 0.5 (35)	1.2 ± 0.5 (43)	0.9 ± 0.3 (32)
Parameters			
AUC (ng x h/ml)	1881 ± 475 (25)	1348 ± 622 (46)	5687 ± 1590 (28)
C max (ng/ml)	422 ± 105 (25)	331 ± 184 (56)	1274 ± 389 (30)
Adjusted to the same drug dose of the Tobi deposited in the lung			
AUC (ngx h/ml)	3404 ± 860 (25)	3615 ± 1669 (46)	5687 ± 1590 (28)
C max (ng/ml)	764 ± 190 (25)	887 ± 494 (56)	1274 ± 389 (30)

As can be observed from the examination of the pharmacokinetic data adjusted to the same drug dose of the comparator product (Tobi®) deposited in the lung, shown in Table 27, the C_{max} and AUC values were found to be significantly higher for Tobi® than for the DPI formulations ($p < 0.05$). For example, the C_{max} values were found to be 1274 ng/ml, 764 ng/ml and 887 ng/ml for the Tobi®, Tobra Form 1 and Tobra Form 2, respectively.
Consistent with the difference in C_{max} between the two methods of administration, the AUC after inhalation of the nebulized tobramycin was 1.6 times greater than after inhalation of Tobra Form 1 and Form 2 (5687 ng x h/ml vs. 3404 ng x h/ml h and 3615 ng x h/ml, $p < 0.01$).

The high variability observed is again attributable in part to the very low lung deposition observed in two subjects (Patients 5 and 9) because of poor lung function. As the inter-subject variability was relatively high, the clinical trial should be carried out on a higher number of volunteers and with the drug dose adjusted to that of the Tobi® in order to confirm these results.

V.5.4. Discussion

In this study, two kinds of tobramycin dry powder formulations for inhalation were compared to Tobi® (nebulizer solution) using a pharmacoscintigraphic method.

In comparison with Tobi® (nebulizer solution), it was estimated that lung deposition expressed as a percentage of the nominal dose, was 7.0 and 4.5 times higher for Tobra Form 1 and Form 2, respectively. The difference in deposited dose values between Tobra Form 1 and Form 2 could be explained by the fact that the presence of a lipid coating around tobramycin particles allowed a decrease in deposition in the device of the inhalator, and allowed a more complete redispersion of tobramycin as individual particles, thus increasing deposition in the lung, which is beneficial in terms of drug targeting efficiency. As previously demonstrated, lipids improve the dispersion properties of particles during inhalation with efficient reduction of their inherent agglomeration tendency. Moreover, they may reduce local irritation, offering a good tolerance in the pulmonary tract as they are mainly constituted of biocompatible and biodegradable material. Finally, the hydrophobic nature of neutral lipids (cholesterol) reduces the absorption of the ubiquitous vapour leading to a reduction in aggregation and adhesion of the particles.

Furthermore, the absence of carrier particles enables a much greater drug dose to be loaded into capsules. These formulations are more particularly useful for drugs that are active at relatively high doses, such as antibiotics, as they permit the delivery of a high concentration of antibiotic directly to the site of infection while minimizing systemic exposition.

This technique indicated that 53.0% of the nominal dose was deposited in the lungs for Tobra Form 1, which was found to be 1.5 times higher than the 34.1% total lung deposition for Tobra Form 2. These results therefore corresponded with the *in vitro* fine particle assessment in which the FPF of tobramycin from Tobra Form 1 was 1.2 times higher than that from Tobra Form 2. The lung deposition in patients was lower than the *in vitro* FPF, indicating, as expected, that the nature of the disease and, probably, its severity play key roles in the deposition of drugs in the lungs.

These deposition results are especially elevated and very promising compared to the values of the clinical evaluation of dry powder tobramycin in healthy volunteers of Newhouse et al. (2003), which used lipid-based Pulmosphere technology and gave a mean whole lung deposition of 34 ± 6%.

The deposition results of the Tobi® are in accordance with other studies of nebulized antibiotic in CF patients, where the mean pulmonary deposition varied between 5% and 11% (Ilowite et al., 1987; Mukhopadhyay et al., 1994; Geller et al., 2002; Newhouse et al., 2003). The other 90% of active drug either remained attached to the wall of the nebulizing system or impacted in the oropharynx and was swallowed or exhaled into the surrounding atmosphere. It should be noted that because of the shape of the nebulizer system, the activity of the drug remaining inside the device could not be counted with the gamma counter.

There is no correlation between the P/C ratio and the FEV_1 and FVC results. Patients with low or high respiratory capacity showed relatively similar regional deposition results. In fact, deposition of drug depends upon a complex interaction between the device, the formulation, and the patient, who controls the rate of flow of inhaled air through the system. These P/C ratio results below 1 demonstrate the thickening of the peripheral airways in CF, causing a reduction in maximal expiratory flow. Indeed, it has been described that airway disease in CF begins in the small peripheral airways and progresses to the development of widespread bronchiectasis. It has been reported that the inflammatory process, and geometrical airway changes resulting from that, are significantly more severe in the peripheral than in the central airways (Tiddens, 2002).

On the other hand, when adjusted to give the same drug dose deposited in the lung as that of the comparator product (Tobi®), the proportion of the drug delivered to the lung that reached the systemic circulation was not the same for the nebulization form as for the DPI forms. This can be explained by differences in the physical state (solution vs. solid form) and the lipophilicity of the two forms. In the lungs of CF patients especially, where secretions are of extremely high viscosity, the solid particles of tobramycin from the DPI forms seem to present greater retention in the pulmonary tissue, leading to a much slower rate of

dissolution in the bronchial fluid (delay in the T_{max}), than the tobramycin droplets. This is beneficial for the treatment of the CF patient as it decreases systemic exposure.

Such an evaluation, in accordance with the *in vivo* scintigraphic deposition, confirms the superiority of the dry powder formulations Tobra Form 1 and 2, in terms of drug deposition and reduced systemic exposure in comparison with the conventional comparator product, Tobi® (nebulizer solution).

Moreover, aerosol administration via nebulization takes much longer, approximately 30 min if set-up, drug administration and cleaning are taken into account (Newhouse et al., 2003). This imposes a significant time burden on patients with CF, who are commonly treated with multiple inhaled medications. Thus, further improvement in aerosol delivery systems with greater efficiency and portability and shorter administration time could improve patient quality of life and compliance.

V.5.4. Conclusion

The results of this clinical trial demonstrated that Tobramycin DPI formulations containing high drug concentrations allow high lung deposition in patients. The inhalation of one capsule of 40 mg or two capsules of 20 mg of Tobra Form 1, or two capsules of 30 mg of Tobra Form 2 could be an advantageous substitute for the administration of Tobi®.
Thus, these new tobramycin DPI formulations based on the use of very low excipient levels and presenting very high lung deposition properties offer very good prospects for improving the delivery of drugs to the pulmonary tract to the widest possible CF patient population.

However, it still remains to be determined if the formulations are physically and chemically stable in long-term storage.

PART V.6: STABILITY STUDIES

V.6.1. Introduction

Stability can be defined as the ability of a pharmaceutical product and/or drug substance not to undergo specific chemical, physical, microbiological or biopharmaceutical alterations during the product shelf-life. Therefore, during initial formulation development or feasability studies, the long-term stability profiles of a new formulation have to be reliably predicted. One approach is to expose the formulation to elevated humidities and temperatures (high stress conditions) and analyse the physicochemical and pharmaceutical properties of the formulation at predetermined time intervals. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as, mainly, temperature and humidity. The recommended storage conditions (ICH- Q1A, 2003) are:

- 25 ± 2°C and 60 ± 5% RH (long-term conditions)
- 30 ± 2°C and 65 ± 5% RH (intermediate conditions)
- 40 ± 2°C and 75 ± 5% RH (accelerated conditions)

Concerning DPI, in addition to the physicochemical stability and the amorphous state of the samples, it is essentially the degree of agglomeration of the powder that must be evaluated by laser diffraction measures and *in vitro* deposition tests during the storage time. Indeed, as previously seen, it is widely known that the RH is one of the main factors influencing the stability of the dry powders.
The evolution of the moisture content at the different storage conditions, considered as a key parameter in agglomeration tendency, was evaluated by TGA (see part IV.2.8, p 89).
During the stability study, the preservation of the crystalline state was evaluated by XRPD (see part IV.2.5, p 87).

The particle size distribution and the agglomeration tendency (e.g. D(0.5) and % < 5 µm) was measured by the Spraytec® (see part IV.2.9.2, p 92). The entire assembly is a closed system and allowed for a controlled airflow rate (100 l/min for 2.4 s) in the measurement zone. This permitted the size properties of DPIs to be measured under simulated breathing

conditions. As previously seen, different correlations between geometric and aerodynamic size data were demonstrated in the part V.1 of this work. The tobramycin fine fraction could be predicted from measurements obtained from the laser diffraction technique. Moreover, the size results obtained with the Spraytec® included the presence of some agglomerates, as appear under the normal conditions of use of an inhaler by a patient. Values presented are the average of at least 9 determinations.

The uniformity of drug content (n=3) (see part IV.2.11, p 94) was determined by the suitable and validated HPLC method after a derivatization step for each formulations and for the various conditions after 1, 3, 6, 9 and 12 months of storage.

Aerosol performance and FPF were studied by dispersing the powders into an MsLI at a flow rate of 100 l/min for 2.4 s with the Aerolizer®. Three capsules 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 (see part IV. 2.12.1.3, p 100). For accuracy, each test was repeated three times.

Moreover, the uniformity of the delivered dose from the inhaler was evaluated (see part IV.2.10, p 93). The apparatus was operated at an airflow rate of 100 l/min for 2.4 s consistent with the withdrawal of 4 l of air. The content of the apparatus was quantitatively collected and the amount of tobramycin was determined with the suitable and validated HPLC method. This quantified amount of tobramycin was expressed in percentage of the nominal dose and known as the percentage delivered. The procedure was repeated 10 times for each formulation tested. The preparation complies with the test if nine out of ten results lie between 75% and 125% of the average value (% / mean) and all lie between 65% and 135%.

Four of the most promising formulations developed during this work were selected on the basis of their aerodynamic behaviour and FPF values:

- Formulation T2 (95% w/w micronized tobramycin with 5% w/w lipids (ratio cholesterol:Phospholipon 75:25)), 40 ± 0.5 mg/capsule
- Formulation T200 98:2 (100% w/w micronized tobramycin with amorphous coating), 25 ± 0.5 mg/capsule
- Formulation N2 (90% w/w micronized tobramycin, 9.8% w/w nanoparticles tobramycin, 0.2% w/w Na glycocholate), 25 ± 0.5 mg/capsule
- Formulation N5 (98% w/w nanoparticles tobramycin, 2% w/w Na glycocholate), 25 ± 0.5 mg/capsule

The capsules loaded with the formulations were placed in hermetically closed container of 30 ml of high-density polyethylene with a dessicant of 2 ± 15% g of silica gel and also in aluminium/aluminium blisters. Blisters were formed and sealed using a Fantasy Plus model Blister Mac VT170W blistering unit (OMAR di Cericola Giorgo, Milan, Italy). The sealing temperature was set at 150°C and the sealing time at 2.5 s. These pre-conditioning operations took place in a chamber with controlled humidity and temperature at 22°C and 40% RH. The boxes and blisters were then stored into ovens at ICH conditions of 25°C/60% RH, 30°C/65% RH and 40°C/75% RH.

V.6.2. Results

V.6.2.1. XRPD analyses

Drug stability for T2 and T200 98:2 was assessed for the three storage conditions for 12 months.

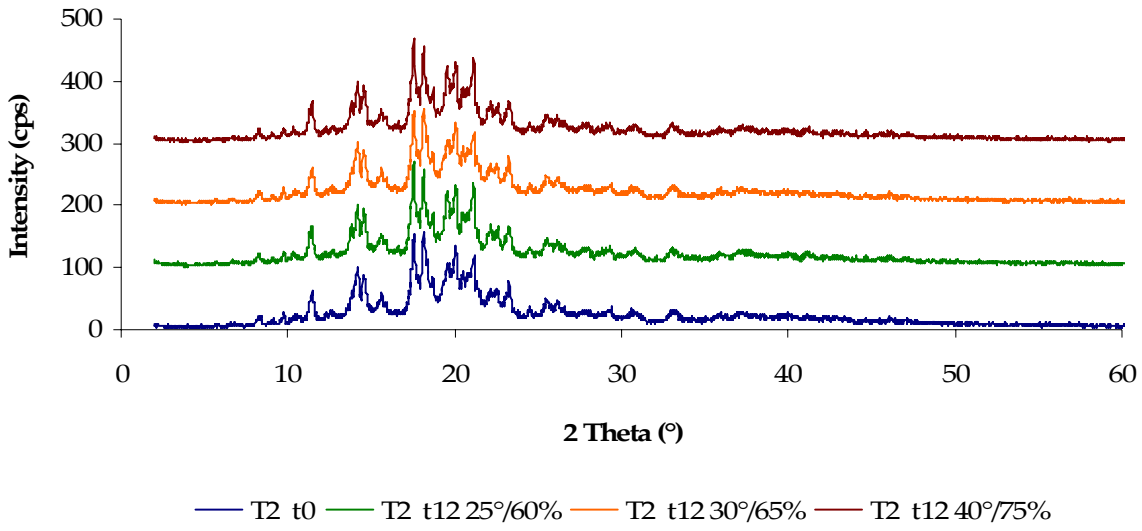


Figure 61: XRPD diffractograms for T2 at t0 and t12 for the 3 storage conditions

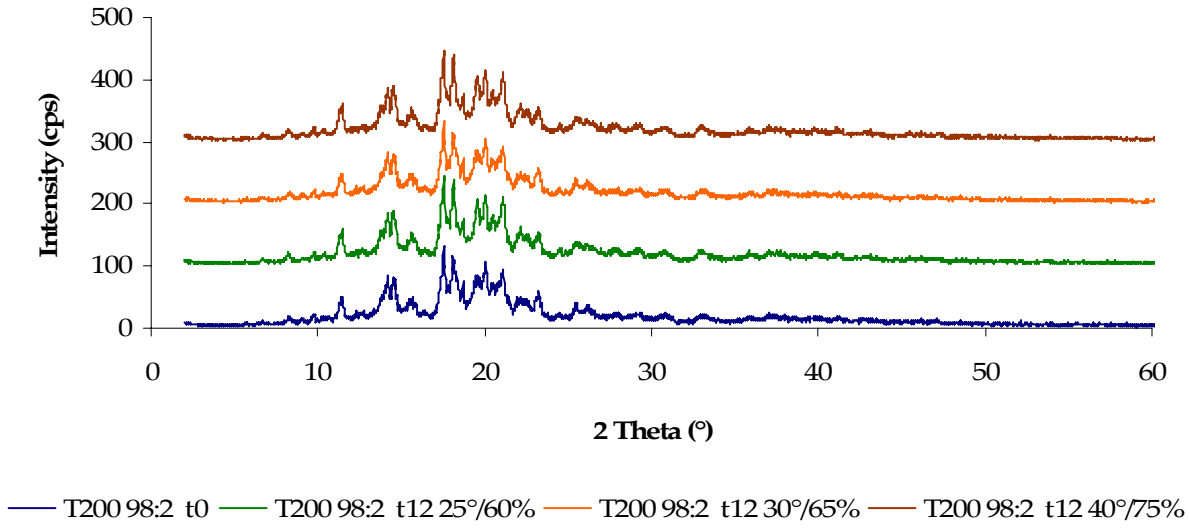


Figure 62: XRPD diffractograms for T200 98:2 at t0 and t12 for the 3 storage conditions

Drug stability for N2 and N5 was assessed for the three storage conditions for 6 months.

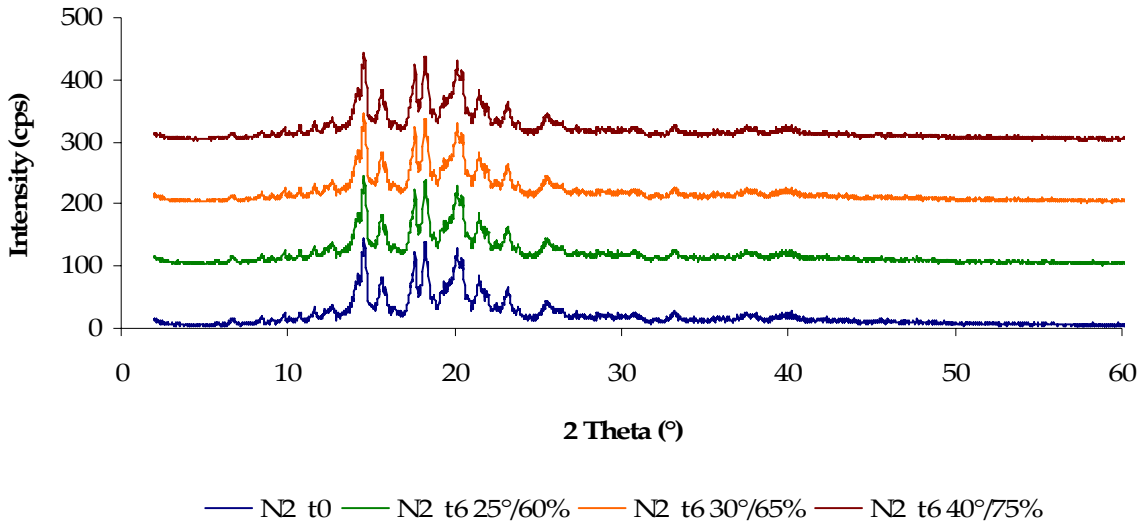


Figure 63: XRPD diffractograms for N2 at t0 and t6 for the 3 storage conditions

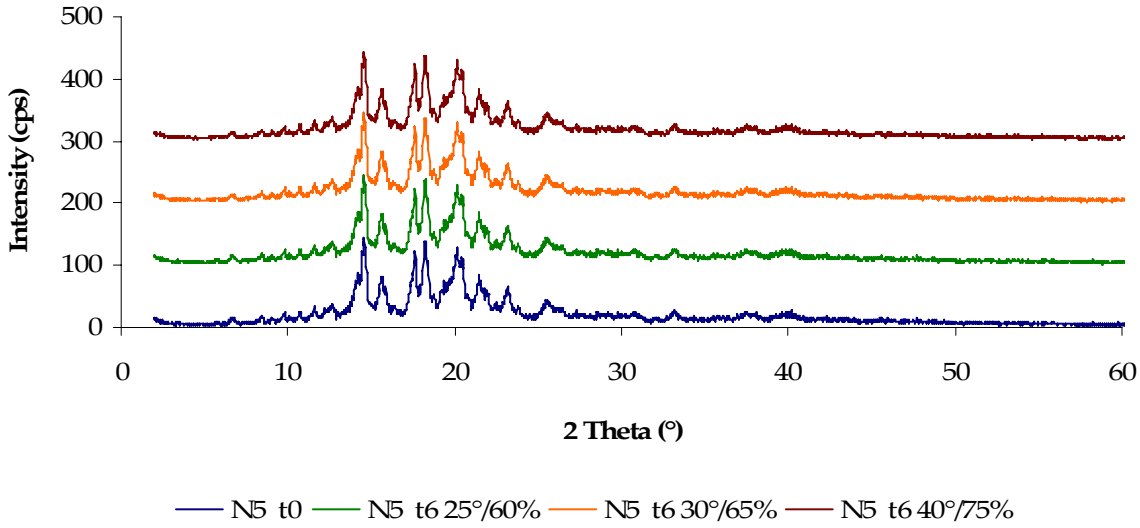


Figure 64: XRPD diffractograms for N5 at t0 and t6 for the 3 storage conditions

From the XRPD pattern observed (Figs. 61-64), it can be seen that tobramycin crystalline state for the four formulations seemed unaltered following 12 months (T2 and T200 98:2) and 6 months (N2 and N5) of storage at 25°C/60% RH, 30°C/65% RH and 40°C/75% RH.

An evaluation of the amorphous percentage present in the formulations studied was also made with comparison of the data obtained at t0 and that obtained following 6 and 12 months of storage (Table 28).

Table 28: Evaluation of the amorphous content of the formulations by XRPD at the various storage conditions at t0, t6 and t12

	t0	t6			t12		
		25°C/60%RH	30°C/65%RH	40°C/75%RH	25°C/60%RH	30°C/65%RH	40°C/75%RH
T2	15.9	15.9	15.9	15.8	15.7	15.8	15.9
T200 98:2	16.3	16.3	16.3	16.3	16.3	16.2	16.2
N2	14.9	14.9	15.0	14.9	/	/	/
N5	15.4	15.4	15.4	15.4	/	/	/

The evaluated amorphous percentage of the T2 and T200 98:2 formulations remained similar for one year with values about 15.9% and 16.3%, respectively.

Similar conclusions can be drawn for the formulations composed of nanoparticles, N2 and N5. The amorphous percentage of 14.9% and 15.4% remained stable for 6 months, respectively. It can be seen from these results that these optimized tobramycin formulations are stable, crystalline-state-wise, over a long storage period.

V.6.2.2. Uniformity of delivered dose

Table 29: Uniformity of the delivered dose at t0 and after 6 months at 40°C/75% RH for T2, T200 98:2, N2 and N5 formulations (Aerolizer®, 100 l/min 2.4 s)

	T2		**T200 98:2**		**N2**		**N5**	
Discharge T0	*% delivered*	*% / mean*	*% delivered*	*% / mean*	*% delivered*	*% / mean*	*% delivered*	*% / mean*
1	85.3	98.1	83.8	101.9	77.2	100.9	83.6	105.1
2	80.6	92.7	83.2	101.2	75.6	98.8	76.6	96.2
3	90.4	103.9	85.9	104.5	79.1	103.3	78.9	99.2
4	91.7	105.4	84.3	102.5	74.9	97.9	79.2	99.6
5	88.8	102.1	77.8	94.6	80.5	105.2	81.8	102.9
6	90.0	103.5	81.2	98.8	79.6	104.0	78.7	98.9
7	87.3	100.4	83.3	101.3	78.2	102.2	80.3	100.9
8	89.5	102.9	80.7	98.2	73.0	95.3	77.6	97.6
9	83.5	96.0	79.4	96.6	74.4	97.2	79.5	99.9
10	82.6	95.0	82.5	100.4	72.8	95.1	79.5	99.9
Mean ± SD	87.0 ± 3.8		82.2 ± 2.4		76.5 ± 2.8		79.6 ± 2.0	
Discharge T6								
1	93.5	106.2	78.5	96.3	73.5	98.0	86.4	107.5
2	90.6	102.9	79.6	97.7	74.8	99.8	83.6	104.0
3	91.3	103.7	84.5	103.7	76.5	102.0	81.8	101.8
4	88.6	100.6	85.7	105.2	72.5	96.7	77.2	96.1
5	80.1	91.0	76.8	94.3	77.4	103.2	78.5	97.7
6	82.4	93.6	77.8	95.5	72.9	97.2	79.5	98.9
7	88.7	100.8	83.2	102.1	73.7	98.3	80.3	99.9
8	87.5	99.4	82.9	101.7	74.5	99.4	78.2	97.3
9	89.4	101.6	82.7	101.5	76.8	102.4	78.7	97.9
10	88.2	100.2	83.1	102.0	77.1	102.8	79.4	98.8
Mean ± SD	88.0 ± 4.0		81.5 ± 3.0		75.0 ± 1.8		80.4 ± 2.8	

As can be seen in Table 29, at the initial time, the mean percentage of the delivered dose was about 87.0 ± 3.8% (discharge between 95-105% of the mean value), 82.2 ± 2.4% (discharge between 97-105% of the mean value), 76.5 ± 2.8% (discharge between 95-105% of the mean value) and 79.6 ± 2.0% (discharge between 96-105% of the mean value) for the T2, T200 98:2, N2 and N5 formulations, respectively. These preparations comply with the pharmacopeial requirements (Eur. Ph. 6 and USP 29) as all the results lie between 65% and 135% of the average value.

Moreover, the results of the delivered dose obtained after 6 months at 40°C/75% RH, are significantly similar to those at t0 ($p > 0.05$). Therefore, no degradation of tobramycin and no

agglomerates of the powder could be observed for the four optimized formulations after storage at accelerated conditions.

V.6.2.3. Particle size distribution and in vitro deposition

The particle size distribution and the agglomeration tendency (e.g. D(0.5) and % < 5 μm) was measured by the Spraytec® and the aerosol performance and FPF were studied by dispersing the powders into an MsLI at a flow rate of 100 l/min for 2.4 sec with the Aerolizer®. Three capsules were taken for each test. For accuracy, each test was repeated three times.

Table 30: Stability results of the particle size evaluation (Spraytec®, n=9), FPF (MsLI, Aerolizer®, 100 l/min 2.4 s, 3 capsules/test, n=3) and tobramycin content (HPLC, n=3) of the T2 formulation

Parameters	Time (month)	Storage conditions						
		Container with dessicant			Blister			
		25°C/60%RH	30°C/65%RH	40°C/75%RH	25°C/60%RH	30°C/65%RH	40°C/75%RH	
FPF (%)	0	68.8 ± 0.5	68.8 ± 0.5	68.8 ± 0.5	68.8 ± 0.5	68.8 ± 0.5	68.8 ± 0.5	
	1	69 ± 1	69 ± 1	69 ± 0.4	71 ± 1	69 ± 1	69 ± 1	
	3	69 ± 1	68,1 ± 0,7	70 ± 1	68,8 ± 0,2	67,5 ± 0,4	69 ± 1	$p > 0.05$
	6	69 ± 1	70 ± 1	72 ± 2	70,2 ± 0,9	69,1 ± 0,7	67 ± 1	
	9	68 ± 1	68,2 ± 0,2	68 ± 1	69 ± 1	70 ± 1	70 ± 1	
	12	70 ± 1	67 ± 2	70 ± 1	68 ± 1	69 ± 1	69 ± 1	
D(0.5) μm	0	3.3 ± 0.5	3.3 ± 0.5	3.3 ± 0.5	3.3 ± 0.5	3.3 ± 0.5	3.3 ± 0.5	
	1	3.3 ± 0.3	3.3 ± 0.6	3.2 ± 0.4	3.3 ± 0.5	3.4 ± 0.2	3.1 ± 0.4	
	3	3.5 ± 0.7	3.2 ± 0.3	3.3 ± 0.3	3.4 ± 0.2	3.5 ± 0.4	3.3 ± 0.2	$p > 0.05$
	6	3.1 ± 0.5	3.2 ± 0.2	3.2 ± 0.7	3.2 ± 0.5	3.3 ± 0.1	3.4 ± 0.3	
	9	3.2 ± 0.3	3.5 ± 0.1	3.4 ± 0.9	3.2 ± 0.3	3.3 ± 0.4	3.4 ± 0.7	
	12	3.1 ± 0.4	3.6 ± 0.3	3.3 ± 0.4	3.3 ± 0.2	3.4 ± 0.2	3.2 ± 0.1	
%< 5 μm (%)	0	97 ± 1	97 ± 1	97 ± 1	97 ± 1	97 ± 1	97 ± 1	
	1	96 ± 1	96 ± 1	97 ± 1	99 ± 1	96 ± 2	99 ± 1	
	3	98 ± 1	96 ± 1	99 ± 1	95 ± 2	94 ± 4	98 ± 1	$p > 0.05$
	6	99 ± 1	98 ± 1	99 ± 1	97 ± 2	96 ± 1	99 ± 1	
	9	98 ± 1	94 ± 2	98 ± 1	99 ± 1	99 ± 1	99 ± 1	
	12	98 ± 1	94 ± 4	98 ± 1	97 ± 1	97 ± 1	98 ± 1	
Content (%)	0	100 ± 1	100 ± 1	100 ± 1	100 ± 1	100 ± 1	100 ± 1	
	1	103 ± 1	100 ± 1	99 ± 1	99 ± 1	100 ± 1	100 ± 1	
	3	103 ± 2	100 ± 1	101 ± 1	101 ± 1	101 ± 1	98 ± 2	$p > 0.05$
	6	101 ± 1	104 ± 2	98 ± 3	101 ± 1	103 ± 1	100 ± 2	
	9	101 ± 1	100 ± 1	99 ± 2	99 ± 2	99 ± 1	102 ± 1	
	12	100 ± 1	98 ± 2	101 ± 1	100 ± 1	99 ± 1	101 ± 1	

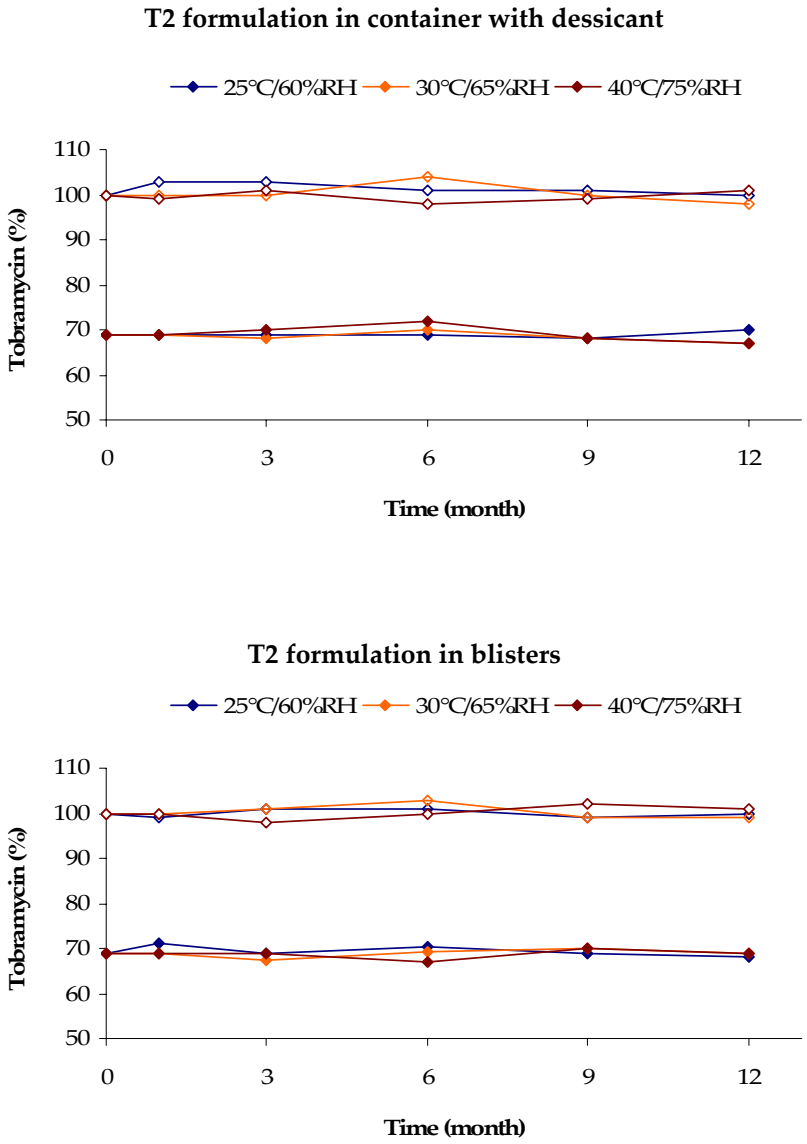


Figure 65: Evolution of the FPF (◆) and tobramycin content (◇) for the T2 formulation over one year for the 3 storage conditions

As can be seen from these results (Table 30, Fig. 65), the lipid-coated formulation T2 seems stable over time and for the various storage conditions, even the accelerated ones. The median particle size (D(0.5)) of 3.3 ± 0.5 µm did not change over one year ($p > 0.05$). Moreover, the FPF and the % of particles below 5 µm remained statistically similar for the three conditions and over 12 months ($p > 0.05$). So, the small amount of lipids present in the coating of the formulation did not undergo any transformations, even at 40°C, which is very interesting for the long term stability. Moreover, the uniformity content of tobramycin remained at 100 ± 2 %, showing that no degradation of the drug could be observed.

Table 31: Stability results of the particle size evaluation (Spraytec®, n=9), FPF (MsLI, Aerolizer®, 100 l/min 2.4 s, 3 capsules/test, n=3) and tobramycin content (HPLC, n=3) of the T200 98:2 formulation

Parameters	Time (month)	Storage conditions						
		Container with dessicant			Blister			
		25°C/60%RH	30°C/65%RH	40°C/75%RH	25°C/60%RH	30°C/65%RH	40°C/75%RH	
FPF	0	55.9 ± 0.8	55.9 ± 0.8	55.9 ± 0.8	55.9 ± 0.8	55.9 ± 0.8	55.9 ± 0.8	
(%)	1	55 ± 1	56 ± 1	56 ± 2	54 ± 3	57 ± 1	54 ± 2	
	3	55 ± 1	55 ± 1	54 ± 2	56 ± 1	57 ± 2	54 ± 2	$p > 0.05$
	6	56 ± 1	55 ± 2	57 ± 2	57 ± 1	56 ± 1	54 ± 2	
	9	55 ± 1	56 ± 1	57 ± 1	57 ± 2	55 ± 1	56 ± 1	
	12	57 ± 1	57 ± 1	56 ± 1	55 ± 2	55 ± 1	55 ± 1	
D(0.5)	0	2.2 ± 0.6	2.2 ± 0.6	2.2 ± 0.6	2.2 ± 0.6	2.2 ± 0.6	2.2 ± 0.6	
µm	1	2.3 ± 0.4	2.3 ± 0.6	2.2 ± 0.4	2.1 ± 0.1	2.4 ± 0.3	2.3 ± 0.8	
	3	2.3 ± 0.5	2.4 ± 0.4	2.3 ± 0.4	2.5 ± 0.3	2.6 ± 0.5	2.3 ± 0.4	$p > 0.05$
	6	2.2 ± 0.2	2.2 ± 0.7	2.2 ± 0.5	2.2 ± 0.4	2.3 ± 0.2	2.5 ± 0.2	
	9	2.4 ± 0.3	2.3 ± 0.3	2.7 ± 0.5	2.2 ± 0.3	2.9 ± 0.5	2.2 ± 0.3	
	12	2.3 ± 0.2	2.3 ± 0.2	2.5 ± 0.4	2.3 ± 0.3	2.4 ± 0.4	2.3 ± 0.5	
%< 5 µm	0	90 ± 2	90 ± 2	90 ± 2	90 ± 2	90 ± 2	90 ± 2	
(%)	1	90 ± 2	90 ± 2	89 ± 2	90 ± 2	91 ± 2	89 ± 2	
	3	90 ± 2	90 ± 1	91 ± 2	89 ± 2	88 ± 2	89 ± 2	$p > 0.05$
	6	89 ± 1	88 ± 2	89 ± 2	91 ± 2	91 ± 2	91 ± 2	
	9	88 ± 3	90 ± 2	91 ± 2	90 ± 2	88 ± 2	90 ± 2	
	12	90 ± 2	89 ± 1	89 ± 2	88 ± 3	89 ± 2	89 ± 2	
Content	0	100 ± 1	100 ± 1	100 ± 1	100 ± 1	100 ± 1	100 ± 1	
(%)	1	101 ± 1	101 ± 1	99 ± 1	97 ± 2	99 ± 1	98 ± 1	
	3	100.9 ± 0.2	96 ± 2	98 ± 1	101 ± 2	101 ± 1	100 ± 2	$p > 0.05$
	6	103 ± 2	102 ± 2	101 ± 2	103 ± 4	102 ± 2	103 ± 3	
	9	99 ± 1	100 ± 1	99 ± 2	98 ± 2	100 ± 1	100 ± 1	
	12	100 ± 1	101 ± 2	101 ± 1	99 ± 2	100 ± 2	100 ± 1	

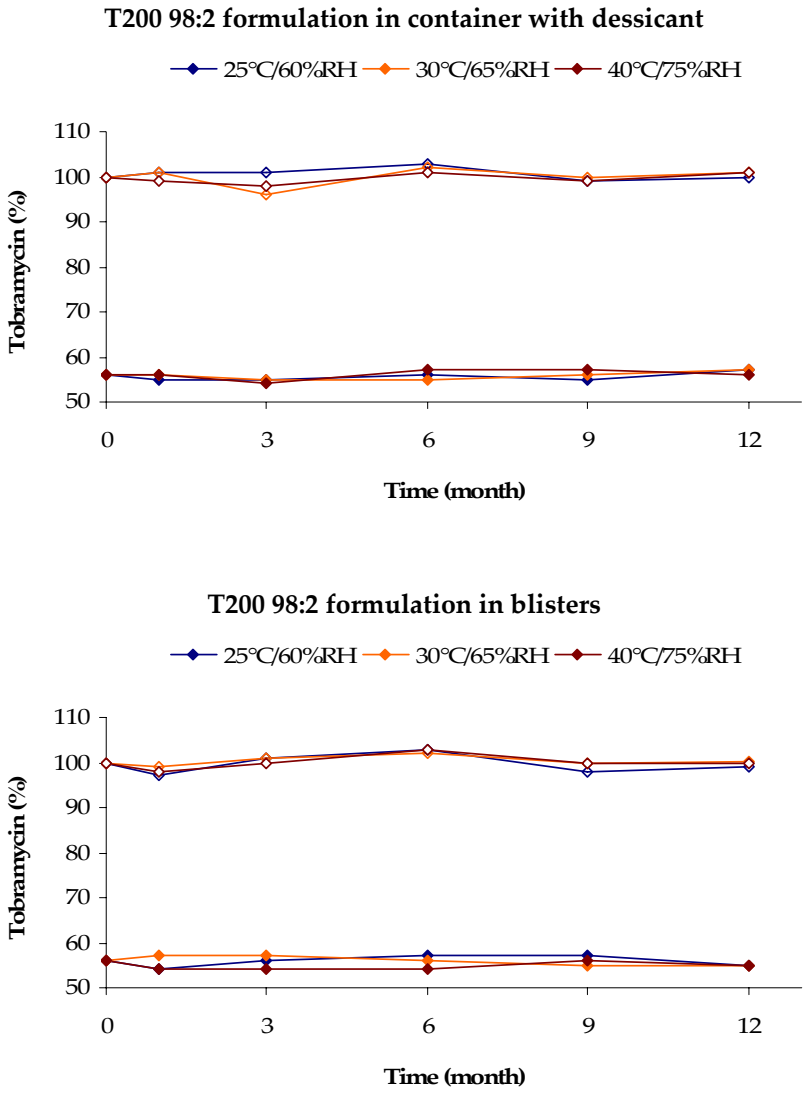


Figure 66: Evolution of the FPF (◆) and tobramycin content (◇) for the T200 98:2 formulation over one year for the 3 storage conditions

After 12 months, the values of the percentage of particle below 5 µm show that no agglomeration of powder was formed during the storage of the formulation. Indeed, the value of 90 ± 2% measured at the initial time seems constant for all the conditions over one year. Therefore, the FPF values remained similar ($p > 0.05$), with values between 55 and 57% for all the storage conditions and at the various times of analysis. So the high temperature of 40°C did not influence the amorphous layer of the active drug, as all the initial parameters seemed unchanged. The T200 98:2 seems to be a stable formulation over a long time period (Table 31, Fig. 66).

Table 32: Stability results of the particle size evaluation (Spraytec®, n=9), FPF (MsLI, Aerolizer®, 100 l/min 2.4 s, 3 capsules/test, n=3) and tobramycin content (HPLC, n=3) of the N2 formulation

Parameters	Time (month)	Storage conditions						
		Container with dessicant			Blister			
		25°C/60%RH	30°C/65%RH	40°C/75%RH	25°C/60%RH	30°C/65%RH	40°C/75%RH	
FPF	0	50 ± 1	50 ± 1	50 ± 1	50 ± 1	50 ± 1	50 ± 1	
(%)	1	51 ± 1	49 ± 2	52 ± 2	51 ± 1	50 ± 1	51 ± 1	$p > 0.05$
	3	50 ± 1	51 ± 1	50 ± 1	51 ± 2	50 ± 2	50 ± 1	
	6	52 ± 1	50 ± 1	49 ± 2	51 ± 1	48 ± 2	51 ± 1	
D(0.5)	0	2.5 ± 0.5	2.5 ± 0.5	2.5 ± 0.5	2.5 ± 0.5	2.5 ± 0.5	2.5 ± 0.5	
μm	1	2.3 ± 0.4	2.7 ± 0.7	2.5 ± 0.4	2.3 ± 0.5	2.4 ± 0.5	2.2 ± 0.3	$p > 0.05$
	3	2.5 ± 0.5	2.2 ± 0.5	2.3 ± 0.4	2.6 ± 0.3	2.5 ± 0.4	2.5 ± 0.3	
	6	2.3 ± 0.6	2.2 ± 0.4	2.6 ± 0.6	2.3 ± 0.4	2.7 ± 0.7	2.5 ± 0.4	
%< 5 μm	0	77 ± 4	77 ± 4	77 ± 4	77 ± 4	77 ± 4	77 ± 4	
(%)	1	78 ± 2	76 ± 2	80 ± 2	77 ± 1	76 ± 2	77 ± 4	$p > 0.05$
	3	78 ± 1	76 ± 4	78 ± 2	76 ± 2	75 ± 4	78 ± 3	
	6	80 ± 3	76 ± 2	76 ± 5	77 ± 2	74 ± 5	79 ± 5	
Content	0	100 ± 1	100 ± 1	100 ± 1	100 ± 1	100 ± 1	100 ± 1	
(%)	1	101 ± 1	103 ± 2	104 ± 5	100 ± 1	98 ± 2	100 ± 1	$p > 0.05$
	3	101 ± 2	100 ± 1	101 ± 2	99 ± 2	103 ± 2	102 ± 2	
	6	101 ± 1	100 ± 2	100 ± 3	101 ± 2	98 ± 3	101 ± 2	

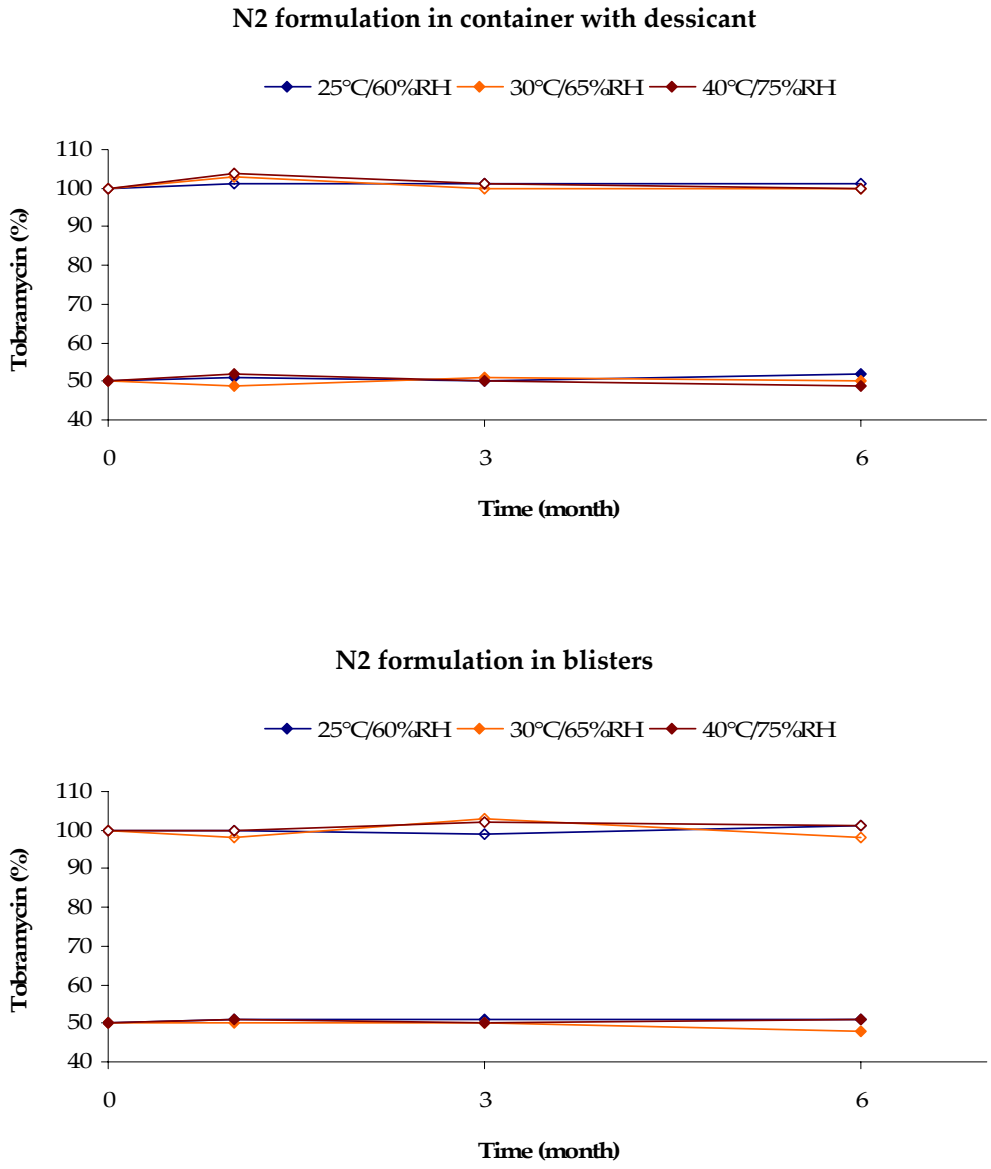


Figure 67: Evolution of the FPF (◆) and tobramycin content (◇) for the N2 formulation over time for the 3 storage conditions

As can be seen from the results herein above (Table 32, Fig. 67), the FPF of the N2 formulation remained stable with values about ranged between 48 ± 2% and 52% ± 2% for all the storage conditions. All the FPF results obtained were statistically similar for the three conditions over 6 months ($p > 0.05$). Similar conclusions can be made for the PSD with a % of particles below 5 µm about 77 ± 4% at time 0 and about 79 ± 5% at time 6 at 40°C/75% RH ($p > 0.05$).

Table 33: Stability results of the particle size evaluation (Spraytec®, n=9), FPF (MsLI, Aerolizer®, 100 l/min 2.4 s, 3 capsules/test, n=3) and tobramycin content (HPLC, n=3) of the N5 formulation

Parameters	Time (month)	Storage conditions						
		Container with dessicant			Blister			
		25°C/60%RH	30°C/65%RH	40°C/75%RH	25°C/60%RH	30°C/65%RH	40°C/75%RH	
FPF	0	61 ± 1	61 ± 1	61 ± 1	61 ± 1	61 ± 1	61 ± 1	
(%)	1	62 ± 1	63 ± 2	61 ± 1	60 ± 1	60 ± 2	59 ± 1	$p > 0.05$
	3	62 ± 2	62 ± 1	61 ± 1	62 ± 1	61 ± 1	61 ± 1	
	6	62 ± 1	61 ± 1	64 ± 2	61 ± 1	61 ± 1	61 ± 1	
D(0.5)	0	2.3 ± 0.4	2.3 ± 0.4	2.3 ± 0.4	2.3 ± 0.4	2.3 ± 0.4	2.3 ± 0.4	
µm	1	2.3 ± 0.3	2.1 ± 0.4	2.3 ± 0.3	2.3 ± 0.5	2.4 ± 0.3	2.5 ± 0.5	$p > 0.05$
	3	2.5 ± 0.4	2.2 ± 0.3	2.3 ± 0.3	2.2 ± 0.3	2.3 ± 0.2	2.4 ± 0.3	
	6	2.1 ± 0.4	2.3 ± 0.3	2.0 ± 0.3	2.3 ± 0.5	2.3 ± 0.3	2.4 ± 0.4	
%< 5 µm	0	84 ± 2	84 ± 2	84 ± 2	84 ± 2	84 ± 2	84 ± 2	
(%)	1	85 ± 2	87 ± 3	86 ± 2	84 ± 1	83 ± 2	81 ± 3	$p > 0.05$
	3	85 ± 1	85 ± 2	85 ± 3	82 ± 2	83 ± 2	83 ± 2	
	6	85 ± 2	85 ± 3	87 ± 3	84 ± 2	83 ± 2	84 ± 1	
Content	0	100 ± 1	100 ± 1	100 ± 1	100 ± 1	100 ± 1	100 ± 1	
(%)	1	100 ± 1	102 ± 2	101 ± 1	101 ± 1	102 ± 1	101 ± 1	$p > 0.05$
	3	101 ± 1	101 ± 1	101 ± 1	101 ± 1	99 ± 1	101 ± 2	
	6	100 ± 1	100 ± 1	102 ± 2	101 ± 1	101 ± 1	101 ± 1	

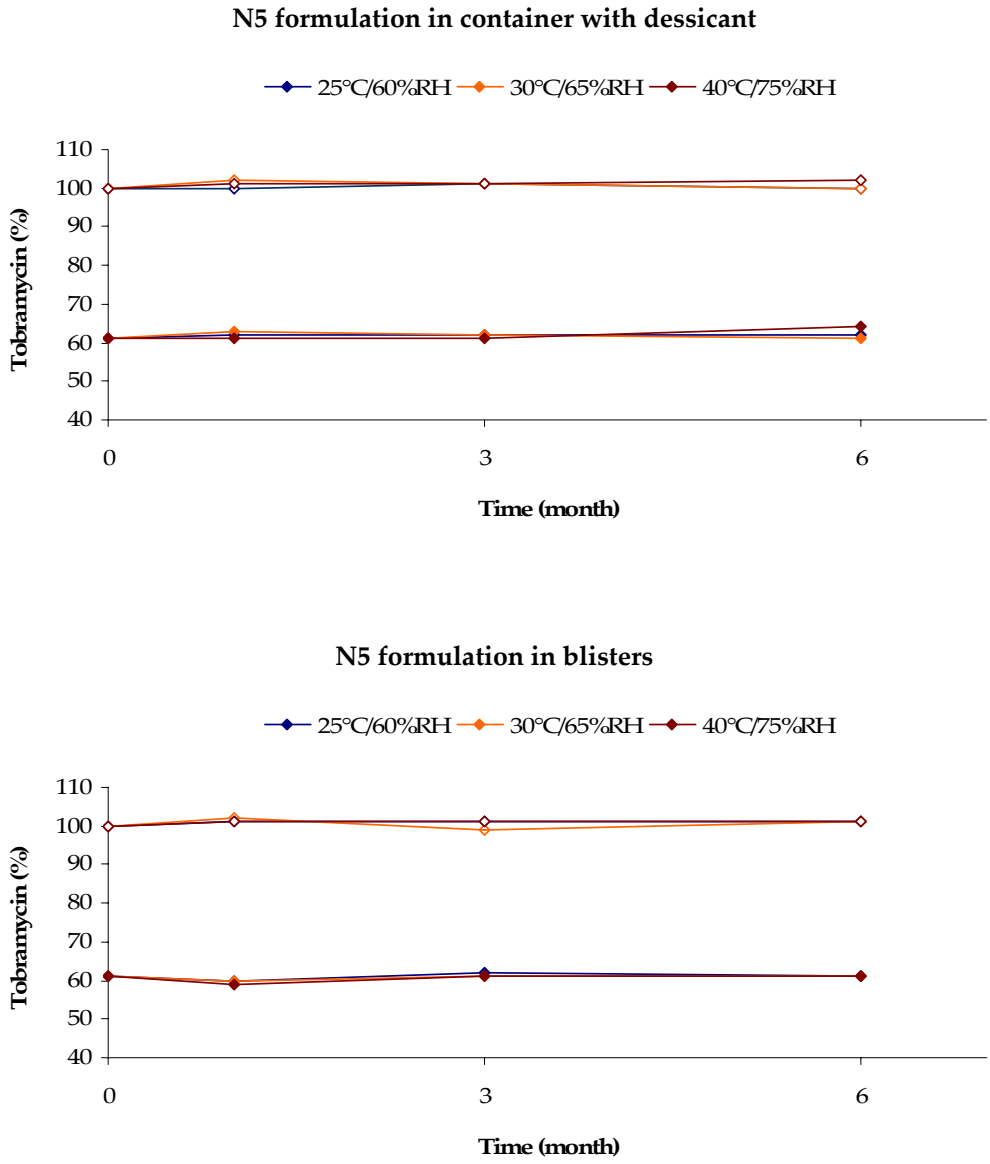


Figure 68: Evolution of the FPF (◆) and tobramycin content (◇) for the N5 formulation over time for the 3 storage conditions

Like the three other formulations, the N5 formulation, composed of agglomerates of nanoparticles, seems to be stable over 6 months at the various storage conditions. The FPF of 61 ± 1% and the % < 5 µm of 84 ± 2% remained statistically similar ($p > 0.05$) over 6 months at 40°C/75% RH. So it appears that the high value of RH did not influence the agglomeration tendency of the formulation, which decreased the FPF. The aerodynamic behaviour and particle size distribution remained stable over 6 months, even at the accelerated storage conditions (Table 33, Fig. 68). As expected, the amount of tobramycin content in the capsule

also remained stable, with values about 100 ± 1%, showing that no degradation of the drug could be observed.

V.6.3. Conclusion

From the results presented in this chapter, it seems that the optimized developed formulations are stable over a long time period at ICH storage conditions of 25°C/60% RH, 30°C/65% RH and 40°C/75% RH. No degradation of tobramycin could be observed over a long storage period. The tobramycin content and crystalline state were shown to be unaltered after 6 (N2 and N5) or 12 months (T2 and T200 98:2) at the three tested conditions. The amorphous/crystalline percentage proved to be unchanged over time. Moreover, for all tested storage conditions, the four selected formulations were shown to keep their initial particle size distribution and aerodynamic behaviour, which is very important for DPI formulations.

VI. GENERAL CONCLUSION

VI. GENERAL CONCLUSION

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 levels of the applicable medication at the site of drug action.

Delivering drugs to the lung from DPI formulations seems to be an attractive and well-appreciated alternative as they are breath-actuated, environmentally friendly, small and portable and they require short treatment time and less patient coordination than nebulizers and MDIs. Unfortunately, most of the DPIs contain excipient to increase flowability and therefore can only deliver microgram quantities of drugs that are useful for asthma therapy. Administration of antibiotic dry powder aerosols to the lung has been attempted, but studies were limited by inefficient delivery designs and/or poorly dispersible lactose formulations. So in this work, various approaches to "carrier-free" formulations were investigated in order to allow the delivery of projected highly-dosed drugs such as tobramycin.

In order to produce particles in an adequate size range for pulmonary delivery, the spray-drying process was chosen. This method has proved to allow control over particle shape, morphology and density, with variations obtained by using different solvent systems or specific spray-drying conditions. 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 MMAD, a maximum deposition of the drugs in the tracheo-bronchial and deep alveoli regions for normal inhalation rates.

The first approach consisted of coating the tobramycin particles with cholesterol and phospholipids, two physiologically well-tolerated components, in order to significantly modify the surface properties of the drug particles. Interestingly, these new lipid-coated particles offered improved delivery of tobramycin to the pulmonary tract. The evaluation of the influence of the coating level showed that the deposition of only 5% w/w lipids relative

to tobramycin weight was sufficient in order to improve particle dispersion properties during inhalation and thus allow a drastic increase in the fine particle fraction of the powder.

Nevertheless, to avoid the use of lipidic excipients, coating the tobramycin particles with nanoparticles of the drug itself was tested. Nanoparticles were prepared by high pressure homogenization, which has the advantage of being a rapid and simple method. In fact, after a rapid screening of the different parameters such as the homogenizing pressure applied, the number of homogenizing cycles and the use and choice of a surfactant, tobramycin particles within a desired size range were easily produced.

On the one hand, these nanoparticles were used to coat micron-size particles of the drug. Interestingly, the presence of a coating of nanoparticles around tobramycin micronized particles was proven to allow an increase in powder desagglomeration, with an increase in the percentage of particles below 5 μm. Coating of the fine drug particles with particles in the nanometer range was believed to reduce van der Waals forces and powder agglomeration and decreased the cohesion of the powder by improving the slip between the particles.

On the other hand, formulations composed solely of nanoparticles were produced in order to form easily dispersible and reproducible micron-size agglomerates of particles. These formulations were light, presented loose agglomerates with high porosity and were more easily dispersible in smaller particles during inhalation than the raw tobramycin.

Due to the fact that these formulations were evaluated especially for drug substances that are active at high dose ranges, minimizing the additives (surfactants, carriers, etc) used in formulation development was further investigated. Formulations with the aim of modifying the balance between the different forces of interactions were developed. To avoid the use of any excipient, these modifications of the surface properties of the particles were obtained through the use of various proportions of water in the solvent used to prepare the initial suspensions. It was hypothesized that the dissolved particles coated the active particles of tobramycin and modified the properties of the surface, the flowability and the aerosolization of the active drug particles. The amount of particles dissolved in the solvent system influenced the thickness of the coating around the tobramycin particles and

differently modified the surface properties of the drug. Of particular interest, as tobramycin is a very hygroscopic drug, it was demonstrated that the addition of water was a critical step. It was revealed that it was important to add a small amount of water to the solvent system and to process the drying step at a high temperature to produce loose agglomerates of drug that are easily scattered into small particles during inhalation.

Moreover, all these optimized developed formulations were proven to be stable over a long time period at storage conditions of 25°C/60% RH, 30°C/65% RH and 40°C/75% RH. The formulations were shown to keep their crystalline state, their original particle size distributions, initial redispersion characteristics and deposition results.

Furthermore, the pharmacokinetic and scintigraphic evaluation of two of the developed formulations (one lipid-coated and one amorphous-coated), confirmed the superiority of dry powder formulation in terms of drug deposition and reduced systemic exposure in comparison with the conventional comparator product Tobi® (nebulizer solution).
So, this work has demonstrated that tobramycin DPI formulations containing high drug concentrations allow high lung deposition in patients. The inhalation of one capsule of 40 mg or two capsules of 20 mg of lipid-coated tobramycin, or two capsules of 30 mg of amorphous-coated tobramycin could be an advantageous substitute for the administration of Tobi®.

As clarithromycin has been proven to enhance *in vitro* the efficiency of tobramycin to eradicate *P. aeruginosa* infections through enhanced biofilm destruction, further improvements would include co-administration of tobramycin with clarithromycin for offering an efficient alternative in the treatment of infections involving biofilm such as in CF. Moreover, tests for industrial scaling-up may be performed in order to produce batches of adequate size for production.

These new and orginal carrier-free DPI formulations based on the use of very low excipient levels and presenting very high lung deposition properties offer very important prospects for 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, as they permit the delivery of a high concentration of antibiotic directly to the site of infection while minimizing systemic exposition. Moreover, a reduction in administration time and in systemic side effects allows improved suitability of these formulations for patients.

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VII. REFERENCES

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VIII. ANNEXES

VIII. ANNEXES

VIII.1. Validation criteria of the LC/MS-MS method

Tobramycin: MH^+: 468.1, fragment ion: 163.35

Sisomycin (S.I.): MH^+: 448.1, fragment ion: 271.3

Stability	
Stock solution at -80°C (T 24H; T 48H; T 7 days; T 1 month)	**Demonstrated**
Plasma	**Demonstrated**
Stability in vials at 4°C (T 24H; T 48 H)	**Demonstrated**
Stability eluate at T° ambient (T 24H; T 48 H)	**Demonstrated**
Stability evaporate at 30°C (T 24H; T 48 H)	**Demonstrated**
Stability plasma on table at T° ambient (T 24H; T 48 H)	**Demonstrated**
Stability plasma at -80°C (T 1 month; T 3 month)	**Demonstrated**
Stability frost / defrost (3 cycles)	**Demonstrated**
Selectivity (blank plasma)	**Demonstrated**
Absolute recovery (%, mean ± SD)	**32,2 ± 6,7**
Linearity (ng/ml; k = 7; n = 3)	**10 - 1000**
Slope	**0,00596**
Intercept	**-0,0122**
R^2	**0,999**
Limit of quantitation (ng/ml)	**10**
Repeatability (RSD %; n = 6)	
10 ng/ml	**4,55**
25 ng/ml	**6,86**
100 ng/ ml	**6,74**
1000 ng/ ml	**1,34**
Reproducibility (RSD %; 3 days; n = 18)	
10 ng/ml	**8,52**
25 ng/ml	**6,21**
100 ng/ ml	**4,62**
1000 ng/ ml	**4,16**
Accuracy (recovery ± SD, %; n = 6)	
10 ng/ml	**107,40 ± 4,93**
25 ng/ml	**94,22 ± 6,52**
100 ng/ ml	**100,53 ± 6,78**
1000 ng/ ml	**112,67 ± 1,51**

VIII.2. Ethics committee approval

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Brussels, the 13/04/2006

COMITE D'ETHIQUE
Agréation : N°OM021

Pr. Karim AMIGHI
Department of Pharmacy
- CP 207 -
CAMPUS DE LA PLAINE
1050 BRUXELLES

Secrétariat : Tél.: 32.2.555.37.07
Fax: 32.2.555.46.20
E-mail : ethique.hopitalerasme@ulb.ac.be

Dear Pr. AMIGHI,

Please find further on the answer of the Ethics Committee concerning the below mentioned study.

ETHICS COMMITTEE APPROVAL

We, undersigned, Pr. A. HERCHUELZ & Mr. G. NISET
Chairman & Secretary of Ethics Committee Erasme Hospital
808, route de Lennik
B-1070 Brussels, Belgium
N° of agreation by "Ordre des Médecins": OM021

confirm that the documents submitted for approval were initially examined during the meeting held on 21/03/2006 (dd/mm/yyyy).

After examination of the answers to the comments and of the new version of the information sheet and consent form received the 07/04/2006, the Ethics Committee gave its agreement on 13/04/2006 to the following documents:

- Protocol (**Our Ref.: P2006/072**) entitled: *"Comparative pharmacoscintigraphic and pharmacokinetic evaluation of the bioavailability of inhaled Tobramycin after oral dose of three formulations of Tobramycin in cystic fibrosis patients : Tobi® (nebulizer solution), Tobramycin form 1, Tobramycin form 2. Single dose, 3 way, cross-over study."* (Your Ref.: TO-02 Version 2, February 2006.)
- French Patient Information Sheet: March 2006. and French Informed Consent Form: March 2006.
- Dutch Patient Information Sheet: March 2006. and Dutch Informed Consent Form: March 2006.
- Investigator's Curriculum Vitae
- Certificate of Insurance: Request in progress, attestation to be furnished before study initiation
- National Application Form Clinical Trial
- A questionnary.

The list of the names and qualifications of the members of the Ethics Committee present at the meeting is added in appendix.

The Committee reminds the investigator of his personal responsibility for this project. We consider **your responsibility**, in accordance with the recommendations of ICH GCP, that **each suspected and unexpected severe adverse reaction** (SUSAR) will be transmitted to this Ethics Committee with **your appreciation** of the relationship between drug and adverse reaction, and the impact on the security of all participants to the assay, that annual trial status and end trial results will be transmitted to the Committee.

If Dutch speaking volunteers have to be recruited for this study, it is your responsibility to submit to the Ethics Committee a Dutch version of the information sheet and consent form.

The Committee reminds also **the investigator** that this positive opinion is valid provided we receive the clinical trial agreements as required by the Belgian law (art. 11, §4, 11°) of may the 7th, 2004.

We hereby confirm that this Ethics Committee is organized and operates according to ICH GCP and the applicable law and regulations.

Mr. G. NISET
Secretary

Pr. A. HERCHUELZ
Chairman

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COMITE D'ETHIQUE
Agréation : N°OM021

List of Members present at the meeting of 3/21/2006

Secrétariat : Tél.: 32.2.555.37.07
Fax: 32.2.555.46.20
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Président : Pr. A. Herchuelz	Pr. André HERCHUELZ, Chairman, Professor, Head of Department, Department of Pharmacology, Faculty of Medicine - U.L.B., Medical Doctor, Male
Vice-Président : Pr. F. Lotstra	Dr. Georges NISET, Secretary, Chief in the Department of Physiotherapy, Erasme Hospital, Physiotherapist, Male
Secrétaire : Dr. G. Niset	Pr. Michel TOUNGOUZ, Member, Assistant Professor, Clinic of Haemobiology, Erasme Hospital, Medical Doctor, Male
Membres : Mr. Y. Dusart	Mr Yves DUSART, Member, Assistant Nursing Director, Department of Radiology, Erasme Hospital, Male nurse, Male
Mr. P. Fischbach	Mr Piet FISCHBACH, Member, Harvey Cushing Center, Psychologist, Male
Me. P.A. Foriers	Pr. Paul-Alain FORIERS, Member, Vice-Dean, Faculty of Common & Criminal Law - U.L.B., Lawyer, Male
Pr. S. Goldman	Pr. Serge GOLDMAN, Member, Head of Department, Department of Nuclear Medicine & PET/Biomedical Cyclotron Unit, Erasme Hospital, Medical Doctor, Male
Dr. A. Kentos	Dr. Alain KENTOS, Member, Assistant Professor, Clinic of Haematology, Erasme Hospital, Medical Doctor, Male
Dr. M. Lipszyc	Dr. Hubert LOUIS, Member, Assistant Professor, Department of Medical Gastroenterology, Erasme Hospital, Medical Doctor, Male
Dr. H. Louis	Mr Marc MAYER, Lay member, Moral Counsel, Erasme Hospital, Lay adviser, Male
Mr. M. Mayer	Dr. Myriam REMMELINK, Member, Assistant Professor, Department of Pathology, Erasme Hospital, Medical Doctor, Female
Pr. R. Naeije	Dr. Jacques ROUBY, Member, General Practitioner, Avenue d'Itterbeek 480, B -1070 Brussel, Medical Doctor, Maleale
Dr. M. Remmelink	
Dr. J. Rouby	
Pr. M. Toungouz	

Edited the 21 March 2006

VIII.3. Directorate-general for medicinal products approval

FPS of Public Health, Food Chain Security and Environment
Directorate-General for Medicinal Products
Amazone
Av. Bischoffsheim 33
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Research and Development

Your letter:
Your reference:

Our reference: DG3-LSA 140/4/06
Date: 6/4/06

Professor Karim Amighi
ULB Service de Pharmacie Galénique et Biopharmacie
Campus de la Plaine, CP-207
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Enclosure(s):

General phone: 32 (0)2/227.55.00
General fax : 32 (0)2/227.55.55

Subject: **COMPARATIVE PHARMACOSCINTIGRAPHIC AND PHARMACOKINETIC EVALUATION OF THE BIOAVAILABILITY OF INHALED TOBRAMYCIN AFTER ORAL DOSE OF THREE FORMULATIONS OF TOBRAMYCIN IN CYSTIC FIBROSIS PATIENTS: Tobi® (nebulizer solution), Tobramycin form 1, Tobramycin form 2 SINGLE DOSE, 3WAY, CROSS-OVER STUDY**

Concerning **Authorisation of clinical trial on the 6th of April 2006**
EudraCT no: **2006-000456-40**

Dear Madam, Dear Sir,

Conform article 14 of the Law of 7 May 2004 concerning experiments on the human person, I have decided to authorize the clinical trial mentioned below. However, the points as mentioned in annex are to be followed up.

COMPARATIVE PHARMACOSCINTIGRAPHIC AND PHARMACOKINETIC EVALUATION OF THE BIOAVAILABILITY OF INHALED TOBRAMYCIN AFTER ORAL DOSE OF THREE FORMULATIONS OF TOBRAMYCIN IN CYSTIC FIBROSIS PATIENTS: Tobi® (nebulizer solution), Tobramycin form 1, Tobramycin form 2 SINGLE DOSE, 3WAY, CROSS-OVER STUDY

2006-000456-40

Yours sincerely,

The Minister of Social Affairs
and Public Health

p.o.

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VIII.4. Declaration of Helsinki

Initiated: 1964

17.C
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WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must

continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.

17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

20. The subjects must be volunteers and informed participants in the research project.

21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

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7.10.2000 09h14

VIII. 5. Patient information

Etude randomisée, en simple aveugle, pour comparer l'efficacité et la tolérance de deux nouveaux produits (ULB-GALTO 1 et ULB-GALTO 2) au Tobi® chez le patient atteint de mucoviscidose.

Protocole

Vous êtes invité à participer à une étude clinique utilisant un médicament couramment utilisé chez les patients atteints de mucoviscidose (le Tobi®) ainsi que deux nouveaux médicaments expérimentaux (ULB-GALTO 1 et ULB-GALTO 2) contenant le même principe actif que le Tobi® (tobramycine).
Il vous appartient de décider si vous souhaitez participer ou non à l'étude. Si vous décidez d'y participer, vous pouvez quitter l'étude à tout moment. Quelle que soit votre décision, vous ne serez pas pénalisé, et elle n'affectera en rien les soins médicaux ou tout autre avantage auquel vous pouvez prétendre. Si vous ne comprenez pas quelque chose après avoir lu la présente information, interrogez le médecin de l'étude ou un membre de son équipe.

Le but de cette étude est de comparer les deux médicaments expérimentaux au Tobi® afin de voir dans quelles mesures ils peuvent améliorer le traitement des infections pulmonaires récurrentes et réduire les effets secondaires du médicament. Vous serez neuf personnes à participer à cette étude durant un peu plus de deux semaines (réparties en neuf périodes de un jour de traitement entrecoupées de jours de repos).
Il vous sera demandé de prendre les trois traitements (ULB-GALTO 1, ULB-GALTO 2 ou Tobi®) le premier jour de chaque période vous concernant. L'ordre de la prise de ces traitements sera déterminé par le hasard. Par exemple, vous prendrez le premier jour, une dose de ULB-GALTO 1, puis vous prendrez une dose de Tobi® le huitième jour et une dose de ULB-GALTO 2 le quinzième jour. Cela se fera en simple aveugle, ce qui signifie que votre médecin saura quel produit vous recevrez tel jour mais vous ne le saurez pas.
Quel que soit le traitement que vous recevrez, votre médecin surveillera vos fonctions respiratoires et des procédures de sécurité très strictes seront appliquées en cas de besoin.

Que se passe-t-il si vous décidez de participer à l'étude?

Comme indiqué dans le tableau ci-dessous, vous devrez vous présenter 5 fois à l'hôpital. Avant le début de l'étude, votre médecin vous posera des questions sur votre état général. Vous subirez un examen physique et on procédera à une prise de sang pour des tests de contrôle (27 ml de sang). On vous demandera également un échantillon d'urine pour des tests de contrôle et la détection de drogues. On mesurera aussi votre pression sanguine et votre pouls. Vos fonctions pulmonaires seront testées au moyen d'un appareil appelé spiromètre. Vous devrez souffler dans l'appareil qui mesure combien d'air vous pouvez exhaler et avec quelle force et quelle vitesse vous pouvez le faire. Enfin, votre médecin vous expliquera en détail comment utiliser les inhalateurs qui serviront à la prise des médicaments.

Le tableau ci-dessous vous indique les tests et activités qui auront lieu à chacune de vos visites pour l'étude. Quelques informations supplémentaires sont expliquées plus en détail après le tableau.

Nombre de visites	**1**	**2**	**3**	**4**	**5**
	Avant le début de l'étude	**Jour 1**	**Jour 8**	**Jour 15**	**Jour 17**
Discussion avec le docteur ou un collaborateur et consentement pour entrer dans l'étude	X				
Antécédents médicaux	X				
Examen Physique	X				
Pression artérielle et pouls	X				
Echantillons d'urine	X				X
Échantillons sanguins *	X	X	X	X	X
Tests des fonctions pulmonaires	X	X	X	X	
Prise des médicaments par inhalation		X	X	X	
Images des poumons		X	X	X	

*Au total, un maximum de 77 ml de sang par jour (jour 1,8 et 15) seront prélevés, via à un cathéter (pour éviter de piquer les veines plusieurs fois dans la journée).

Le premier jour, à 7h00, vous serez admis (à jeûn) à l'hôpital, un numéro vous sera attribué au hasard et un cathéter vous sera placé. A partir de 7h30 et jusqu'à 19h00, on vous prélèvera 11 fois 7ml de sang et puis vous serez autorisé à quitter l'établissement.
À 8h00, un des trois médicaments vous sera administré par inhalation. Ces médicaments contiennent une très faible dose de technétium pertechnéate, un élément radioactif qui s'élimine très vite et qui servira à visualiser le trajet des médicaments dans les poumons.
Immédiatement après l'inhalation des images (une sorte de radiographie) de votre corps seront prises pour localiser, grâce à l'élément radioactif, la position des médicaments.
À 9h00, vos fonctions pulmonaires seront testées à l'aide du spiromètre pour s'assurer que tout va bien. Des repas vous seront fournis à 12h00, 16h00 et 18h00.
Le huitième et le quinzième jour, vous serez de nouveau admis à l'hôpital pour suivre le même protocole avec les deux autres médicaments.
Enfin, une dernière visite à l'hôpital aura lieu le dix-septième jour pour faire une analyse de sang (27ml) et d'urine pour des tests de contrôle et clôturer le dossier.

Quels sont les avantages à participer à l'étude?

Si vous décidez de participer à l'étude, votre état général sera suivi de près et vous n'aurez pas à payer pour la médication à l'étude. Vous bénéficierez d'examens médicaux et de tests de laboratoire gratuits.
Vous n'allez pas tirer un avantage thérapeutique direct en prenant ces médicaments, mais en participant, vous aidez les chercheurs à déterminer si les nouveaux produits testés peuvent améliorer

l'efficacité et la tolérance du traitement des infections bronchiques chez le patient atteint de mucoviscidose.

Y a-t-il des effets secondaires ou des risques?

La plupart des personnes qui prennent la tobramycine (qui est la substance active des médicaments ULB-GALTO 1, ULB-GALTO 2 et Tobi®) ne ressentent aucun effet secondaire. De plus, il y a de grandes chances que vous ayez déjà suivi un traitement de plusieurs jours à base de tobramycine et que vous n'en ayez ressenti aucun effet indésirable.

Quelquefois, une altération de la voix (enrouement), une augmentation de la toux, une respiration courte et des maux de gorge peuvent survenir après inhalation du traitement.

Rarement, un bronchospasme, une gêne respiratoire, une douleur à la poitrine, des tintements d'oreille, des ulcérations de la bouche, une éruption, une sensation de faiblesse, une fièvre, des maux de tête, se sentir malade, une perte d'appétit, des étourdissements, une augmentation des expectorations, des expectorations contenant du sang, des saignements de nez, le nez qui coule et des perturbations du goût peuvent survenir.

Très rarement, de douleurs abdominales, une diarrhée, des douleurs dorsales, des infections à champignons (ex: muguet), un œdème ganglionnaire, une somnolence, une hyperventilation, une agitation et une confusion, des troubles auriculaires ou une sinusite peuvent survenir.

Si à n'importe quel moment de l'étude vous commencez à éprouver des effets secondaires ou un autre problème, contactez le médecin ou ses collaborateurs pour l'en informer.
Le fait de prélever du sang de vos veines peut provoquer des étourdissements, défaillances, douleurs ou inconfort, contusions ou infection à l'endroit du prélèvement.

Que se passe-t-il si je désire stopper l'étude?

Vous pouvez décider de quitter l'étude à tout moment. Votre médecin peut aussi décider de vous retirer de l'étude si vous ne suivez pas les instructions, pour des raisons médicales ou pour d'autres raisons. Il y a aussi une possibilité que l'étude soit arrêtée par le sponsor, ULB Galénique, avant que votre participation ne soit complète. Si cela arrive, vous ne continuerez pas à recevoir la médication à l'étude ni les évaluations planifiées. Si vous quittez l'étude pour quelque raison que ce soit, vous devez retourner chez le médecin de l'étude pour effectuer les examens de clôture de dossier. Toutes les données collectées jusqu'à ce que vous quittiez l'étude et refusiez de continuer les évaluations seront gardées et utilisées par ULB Galénique.

Dédommagement

L'ULB galénique a souscrit une assurance RC (Responsabilité Civile) destinée à vous fournir une compensation financière si vous subissez un dommage lié à l'étude. Par «dommage lié à l'étude», on entend toute lésion physique causée par les médicaments testés ou par des actes médicaux requis pour l'étude pour autant qu'ils soient différents de ceux que vous auriez subis si vous n'aviez pas participé à cette étude clinique.

Remboursement des frais

Il ne vous coûte rien de participer à cette étude. Vous serez remboursé de tout frais raisonnable que vous auriez dépensé pour vous rendre à l'hôpital pour les visites de l'étude.
Vous serez remboursé comme suit pour votre participation:

200,00 € au Jour 1
200,00 € au Jour 8
200,00 € au Jour 17

Si vous interrompez votre participation à l'étude, la rémunération sera au minimum proportionnelle à votre temps de participation.

Confidentialité et traitement des données

Votre médecin notera les informations recueillies dans le cadre de cette étude sur des formulaires qui seront ensuite transmis à ULB Galénique ou ses représentants. Sur ces rapports, vous serez identifié par un numéro de patient. Toutes les données dans lesquelles votre nom apparaît seront gardées par votre médecin et seront toutes confidentielles. Votre nom n'apparaîtra jamais sur aucun formulaire ou dans aucun rapport à publication.

Les personnes autorisées de ULB Galénique, des autorités gouvernementales de la santé compétentes et/ou du Comité d'éthique pourront avoir accès à votre dossier pour vérifier les données.

En signant ce formulaire de consentement éclairé, vous acceptez que les données vous concernant soient utilisées dans le but décrit dans ce document et soient transmises en dehors de la Communauté Européenne incluant les Etats-Unis d'Amérique, en accord avec les lois nationales qui transposent les directives européennes relatives à la protection de la vie privée.

Vos responsabilités

Si vous décidez de participer à cette étude, vous devrez :

* Respecter le calendrier des rendez-vous.

* S'abstenir de l'usage de drogues, et ne participer à d'autres études.

* Informer votre médecin de tout médicament que vous prenez, même s'il s'agit d'un médicament acheté sans ordonnance.

* Informer votre médecin de tout problème médical survenu.

Si vous ne suivez pas les indications qui précèdent, vous pouvez être retiré de l'étude.

Si vous avez des questions concernant vos responsabilités ou les activités de l'étude, contactez:
Dr. Knoop Service de Pneumologie Tel: 02/5553985

VIII. 6. Patient declaration

Etude randomisée, en simple aveugle, pour comparer l'efficacité et la tolérance de deux nouveaux produits (ULB-GALTO 1 et ULB-GALTO 2) au Tobi® chez le patient atteint de mucoviscidose.

- ✓ J'accepte de mon plein gré de participer à cette étude.
- ✓ Je sais que le sponsor de l'étude, ULB Galénique, peut interrompre l'étude à tout moment. Si cela se produit, je ne recevrai plus les médicaments à l'étude ni les évaluations prévues.
- ✓ J'ai lu et je comprends cette déclaration de consentement éclairé ainsi que les risques qui y sont décrits.
- ✓ Je sais que je recevrai une copie signée et datée de ce formulaire de consentement.
- ✓ Je sais que je peux retirer mon consentement à tout moment.
- ✓ J'ai eu la possibilité de poser des questions et j'ai compris les réponses qui ont été données à toutes mes questions.

Nom du patient : _____
(en caractères d'imprimerie)

Signature du patient : _____ Date : _____

_____

Nom de la personne ayant conduit la discussion de consentement éclairé :
(en caractères d'imprimerie)

_____

Signature de la personne ayant conduit la discussion de consentement éclairé :

_____

Date : _____