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CICLO XXVII

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RESPIRABLE PARTICLES FOR INHALATORY TREATMENT OF PULMONARY BACTERIAL INFECTIONS

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To my family

"Our deepest fear is not that we are inadequate. Our deepest fear is that we are powerful beyond measure. It is our light, not our darkness that most frightens us. We ask ourselves, Who am I to be brilliant, gorgeous, talented, fabulous? Actually, who are you *not* to be? You are a child of God. Your playing small does not serve the world. There is nothing enlightened about shrinking so that other people won't feel insecure around you. We are all meant to shine. It's not just some of us; it's everyone. And as we let our own light shine, we unconsciously give other people permission to do the same. As we are liberated from our own fear, our presence automatically liberates others"

Marianne Williamson (quoted by Nelson Mandela)

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Chapter 1. Introduction and aim

1.1 General consideration of lung infections

The lungs have a large surface area exposed to the external environment (about 100 m^2) and a minimal barrier defence, so they are the most common site of serious infection. The susceptibility of the lungs to infection is due to their physiological function as gas exchangers: 5-10L of air are ventilated each minute and with them also particles, droplets and pathogens. Contrary to the other organs in contact with the environment (skin, GI tract), the lungs have a minimal barrier defence, an epithelial cell monolayer protected by a mobile mucus gel layer, to permit the gaseous diffusion. Most inhaled pathogens are trapped into the mucus and they are expulsed through the mucociliary system. Antimicrobial peptides and antibodies limiting the growth of pathogens and alveolar macrophages removing particles and microorganisms, are present on the lung surface [1]. Respiratory infections include a broad spectrum of diseases with different features and different etiologic agents. The most common are pneumonia, tuberculosis and bacterial infections occurring in cystic fibrosis patients.

Pneumonia is a lung inflammatory condition that affects approximately 450 million people every year. The most common type is the bacterial pneumonia, only 20% has a viral onset. In fact the main etiologic agents are: *Streptococcus pneumoniae* (30-50%), *Chlamydia pneumoniae* (13%), *Mycoplasma pneumoniae* (11%) e *Haemophilus influenzae* (5%). Normally the treatment of pneumonia is the administration of antibiotic (e.g. cephalosporines, macrolids) orally or intravenously according to the seriousness of the disease [2-4].

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. For the World Health Organization (WHO) in 2012 there were 8.6 million patients and 1.3 million died. The treatment is a multi-therapy including three or, more often, four different types of drugs, which are normally orally administered. The most common drugs are: isoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin [5,6].

The chronic bacterial infections in the lungs are facilitated in people with **Cystic Fibrosis (CF)**. CF is the most common lethal genetic disease in Caucasians, with a frequency in the European Union of 1 in 2000-3000 new borns and in the United States of America of 1 in every 3500 births (70.000 worldwide, at now according to WHO). CF is caused by several mutations in a gene named Cystic Fibrosis Transmembrane Regulator (CFTR), which encodes for a cyclic adenosine monophosphate (cAMP)-dependent chloride channel [7]. CF patients have pathological changes in all of the organs that express CFTR (secretory cells, sinuses, lungs, pancreas, liver and reproductive tract). The most striking changes are in airways, where the basic genetic defect causes chronic pulmonary infections. The CFTR mutated leads to a defection of Cl^- in the lumen, this with an abnormal sodium absorption cause an abnormal reabsorption of the water as shown in Figure 1. The bacterial pathogens invading the lung are trapped in the viscous mucus layer: because of its viscosity the mucus can't be removed through the mucociliary clearance [8]. The most common bacterial pathogens are *Haemophilus influenzae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Respiratory infections with *P. aeruginosa* are difficult to treat because of the formation of biofilm-like macrocolonies as shown in Figure 1.1. This pathogen has also an extraordinary capacity to develop resistance through genetic mutations [9].

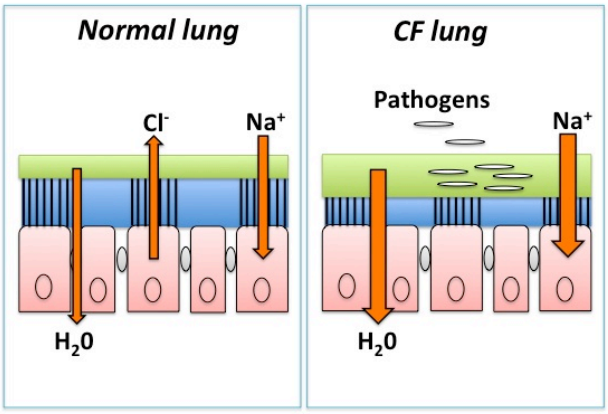

Figure 1.1. Differences between a normal lung and the lung of a Cystic Fibrosis patient

The therapy of CF consists of a combinations of antibiotics (aminoglycosides, macrolides) administered orally or intravenously. In adjunct to the antibiotics, oral anti-inflammatory drugs (corticosteroids, NSAIDs) are used. Because the site of action of these drugs is the lung, inhaled medications has been developed in the past several years to treat this pathology. This solution let to deposit the drug on the site of action limiting the systemic exposure and consequently the side effects.

1.2 Inhalatory treatment of pulmonary infections

The treatment of pulmonary infections requires high payloads of antibiotics into the lungs to achieve sufficient drug concentration in the most distal airways. It is possible to administer these high doses using the pulmonary route through the use of nebulizers or dry powders inhalers (DPIs). Since 2009 tobramycin (aminoglycoside) has been developed for inhalation administration. Tobramycin as solution for inhalation is present on the market as TOBI® (300 mg/5 ml), Novartis, Switzerland, as Tymbrineb® (300 mg/5 ml), TEVA Ltd, UK and as Bramitob® (300 mg/4 ml) Chiesi Farmaceutici S.p.A., Italy. Another antibiotics administrated as solution for nebulization are colistimethate sodium, a polymyxyn (Colomycin® and Promixin®) and aztreonam lysine (AZLI, with the trademark name Cayston®), a monobactam [9,10]. A product under development is Arikace® (Insmed) a liposomial formulation of amikacin for nebulization. Arikace® has been granted orphan drug designation in the U.S. by the FDA and in Europe by EMA for the treatment of Pseudomonas infections in patients with CF. The Phase III study of Arikace® demonstrated that it is an effective inhaled antibiotic and suggest that one once-daily treatment with the liposomial amikacin was comparable to the twice-daily TOBI® treatment [11,12]. However, several drawbacks are related to this administration technique; in fact, nebulizers are used mostly in hospital and ambulatory care settings and are not typically used for chronic-disease management because they are cumbersome, and the aerosol is delivered continuously over an extended period of time with low efficiency and poor reproducibility. Dry powder inhalers (DPIs) let the administration of large amount of drug obtained by patient inhalation through the device, thereby overcoming the problem of co-ordination between aerosol production and the inspiratory act. Device

and formulation joined together constitute the inhalation product since both affect the deposition of particles into lung. These devices are activated by the patient inspiration effort capable to de-aggregate the powder and extract the dose for the aerosol formation. Tobramycin is available on the market also as inhalation powder TOBI™ Podhaler™ (Novartis AG, Switzerland). Two phase III studies assessed its efficacy and safety with a total dose of 112 mg of the tobramycin base divided in 4 capsules, twice daily. Only 71% of the formulation is drug substance (in each capsule there are only 28 mg of tobramycin on 50 mg of formulation), there is a several amount of excipients (distearoylphosphatidylcholine and calcium chloride) [13]. Another DPI approved is Colobreathe® (Forrest, USA). Each capsule contains 1,662,500 IU, which is approximately equal to 125 mg of colistimethate sodium. One capsule is administered twice daily. There are no excipients [14]. So the only DPI on the market comprising an aminoglycoside is TOBI ™ Podhaler ™ and in this formulation there is a large amount of excipients. Colobreathe® on the other hand, have not excipients and contains only the drug (the colistimethate sodium, a polymyxyn). Table 1.1 summarizes the products commercially available for CF patient treatment.

Table 1.1 List of the approved products for inhalation available for CF patients.

Drug	Product	Dosage form	Dose
Tobramycin	Bramitob® (Chiesi Farmaceutici Spa)	Solution for Nebulization	300 mg/4 mL
	Tobi® (Novartis AG)	Solution for Nebulization	300 mg/5 mL
	Tymbrineb® (TEVA Pharm. Ind. LTD)	Solution for Nebulization	300 mg/5 mL
	Tobi PodHaler® (Novartis AG)	Powder for Inhalation	112 mg in 200 mg powder
Colistimethate sodium	Promixin® (Profile Pharma LTD)	Powder for nebulizer solution	80 mg/1 mL
	ColiFin® (PARI Pharma GmbH)	Powder for nebulizer solution	80 mg/3 mL and 160 mg/4 mL
	Colobreathe® (Forest Laboratories Inc.)	Powder for Inhalation	125 mg
Aztreonam lysine	Cayston® (Gilead Sciences Inc.)	Powder for nebulizer solution	75 mg/1 mL
Dornase Alfa (rhDNase)	Pulmozyme® (Genentech Inc.)	Solution for Nebulization	2.5 mg/2.5 mL
Na Cl	Hyaneb® (Chiesi Farmaceutici SpA)	Solution for Nebulization	350 mg/5 mL
Mannitol	Bronchitol (Pharmaxis)	Powder for Inhalation	400 mg

Other inhaled antibiotics in late-phase development for CF lung infections include Aptalis Pharma's Aeroquin (levofloxacin for inhalation solution) and Insmed's Arikace (amikacin for inhalation liposomial suspension). Both medications are administered by a nebulizer; Aeroquin is taken twice daily and Arikace is taken once daily.

Arikace® liposomal product is the only amikacin-containing medicinal product in pipeline for the inhalation treatment of *Pseudomonas aeruginosa* lung infection in

cystic fibrosis. These liposomial nano-suspension of amikacin (250-300 nm), are made with dipalmitoylphosphatidylcholine and cholesterol in the 2:1 weight ratio [15]. Amikacin concentration in the liposomal formulation is 70 mg/mL. The nebulization of 560 mg, corresponding to 8 mL of liposomal suspension, requires long time for the administration (15-20 min). As a further drawback it should be underscored that this treatment would imply the loading of the lung with a significant amount of non-active ingredients.

Amikacin is a semisynthetic aminoglycoside, it has not only an antibacterial activity, it is also able to suppress a premature stop mutation in a CF transgenic mouse model [16]. Amikacin is not a first choice antibiotic because its toxicity. As for the other aminoglycosides the side effects, associated with their use, are kidney damage and hearing loss. There are these side effects when aminoglycosides reach these sites because there are taken inside these cells and here they cause the reduction of the activity of a number of enzymes and the production of free radical species [17]. The local administration of aminoglycosides to the lungs allows to avoid these side effects because the drug doesn't reach the inner ear and the kidney. Dry powders for inhalation are a promising alternative of the solution for nebulization, because of rapid dose administration, high local deposition and concentration and stability improvement compared to liquid formulation.

The advantages of a formulation in form of a powder for inhalation in comparison to a nebulized liquid formulation include the lightweight and portability which enable the dose to be taken quickly and easily in any location.

Furthermore, compared to liquid solutions for nebulization, the use of dry powder formulations leads to the decrease of the nominal dose, since the drug loss in the device, in the exhaled air and the environment is minimized.

There are not dry powders for inhalation of amikacin on the market. A dry powder of amikacin has been patent in 2010 (WO2010003465). Tobramycin and amikacin powders with the adjunct of a salt of fatty acids (sodium stearate in the case of amikacin) were studied. The salt of fatty acid was dissolved in ethanol heating and stirring until to obtain a limpid transparent solution. The amikacin was dissolved in deionized water and the solution obtained was heating before the addiction of the sodium stearate solution. The ratio water-ethanol was 70:30 and the concentration of solids (drug + salt of fatty acid) in the final solution was 1% w/v. The ratio drug-excipient was 99.5:0.5. The hydroalcoholic solution was spray dried (yield 79.4% ± 2.8) and the dry powder obtained showed a $d_{v0.5}$ of 3.28 ± 0.19 μm. The aerodynamic behaviour was investigating using the Andersen Cascade Impactor. The Fine Particle Fraction was 41.7% ± 3.6 and the Median Mass Aerodynamic Diameter was 4.6 µm [18,19].

1.3 Formulation and device features to deliver particles to the lungs

In order to have a lung deposition of drug particles it is necessary to have adequate dimension. Solid or liquid particles suspended in a gaseous medium produce an aerosol. These particles are associated to spherical particles having the same sedimentation velocity of the suspended particles to obtain an equivalent diameter called aerodynamic diameter. The **aerodynamic diameter (d_{ae})** can be calculated with the following formula:

$$d_{ae} = d_v \sqrt{\frac{\rho_{part}}{\chi \rho_0}}$$

where d_v is the equivalent diameter of a sphere having the same volume of the particle suspended, ρ_{part} is the particle density, ρ_0 is a reference density of 1g/cm^3 and χ is a form parameter that for a perfect sphere is 1. The aerodynamic diameter is used to describe the behaviour of the particles in the gaseous medium in function of their volume, their density and their shape.

The particles having a d_{ae} bigger than 5µm can't reach the lungs: they impact in the oropharynx region. Particles with a d_{ae} between 2 µm and 5 µm reach the proximal district of the lungs, that is the primary site of inflammation and where happen the attack by pathogenic microorganisms. The finest particles (smaller than 2µm) reach the alveoli, where they are absorbed by the systemic circulation. This has opened up new chances of drug delivery, assuming the lungs as the door for the systemic administration. Particles smaller than 1 µm are immediately exhaled [20].

There are three types of devices used to obtain a pulmonary administration:

- Nebulizers,
- Pressured Metered Dose Inhalers (pMDI),
- Dry Powders Inhalers (DPI) [21].

The **nebulizers** are able to produce an aerosol from a solution or a suspension.

The are mainly three types of nebulizers:

- Air-jet nebulizers: they are the traditional nebulizers type (pneumatic nebulizers). The gas (normally air) is forced by a compressor through a narrow hole and direct against the solution or the suspension. For the impact of the high-velocity airstream to the liquid the droplets are formed. Only the smaller droplets can pass the baffle and become available for inhalation.
- Ultrasonic nebulizers: they use the vibration of a piezoelectric crystal to aerosolize the solution or the suspension. The ultrasonic vibrations generate droplets with a wide range d_{ae} (between 0.5 μm and 8 μm). There are restrictions about the drugs that can be used because heat is transferred to the solution [22].
- Electronic nebulizers: they are the newest nebulizers (eFlow® produced by Pari and I-neb® produced by Philips). They use a perforated oscillating membrane (vibrating mesh technology) to generate the aerosol. The holes of the membrane, made with a precision laser, have a diameter around 2 μm. The latest I-nebs® are coupled with the Adaptive Aerosol Delivery technology (AAD). The I-neb® AAD systems are called intelligent nebulizers because they have a software able to pulse aerosol only during the patient inspiration [23,24].

The **Pressured Metered Dose Inhalers (pMDIs)** are widely used devices for the inhalation administration. They are pressurized canister containing the drug dissolved o suspended in the propellant. The actuation of the device produces an aerosol previously metered by the valve. The solvent evaporates and the small drug particles are inhaled [25]. The "Montreal protocol on Substances that Deplete the Ozone Layer" published in 2000 bans chlorofluorocarbons (CFCs) so the CFC used in pMDIs are replaced by hydrofluoroalkanes (HFA) such as HFA 134a (1,1,1,2-tetrafluoroethane) or HFA 227 (heptafluoropropane) [26]. These devices are very common because they are portable, inexpensive, and they are able to generate a reproducible aerosol independent of the patient inspiratory flow rate. On the other hand they have some disadvantages. It is necessary in order to have a good deposition of the drug particles in the lung, a proper coordination between device actuation and the inhalation act. This problem can be solved with the use of spacer devices. These devices are connected to the pMDIs so the aerosol is discharged into them and the patient can breath normally and inhale it from the spacer. An additional problem in pMDIs is the potential presence of toxic compounds extracted by the propellant from the valve. Furthermore if the drug is suspended in the propellant they have an unstable physical nature so they can change during the time [27].

The **Dry Powder Inhalers (DPIs)** have some advantages over the pMDIs: they don't contain any propellants, the drug is in the solid phase so more stable and it is not necessary the coordination between the device actuation and the inhalation act. The aerosol is generated by the inspiration flow of the patient. There are two main types of DPI devices: the single-unit dose devices that contain only one of the doses and multiple-doses devices that contain inside all of them. The single doses devices can be capsule devices or disposable devices. The multiple-unit dose devices can be pre-metered or reservoir devices [28]. The DPI devices types are schematically presented in the Figure 1.2.

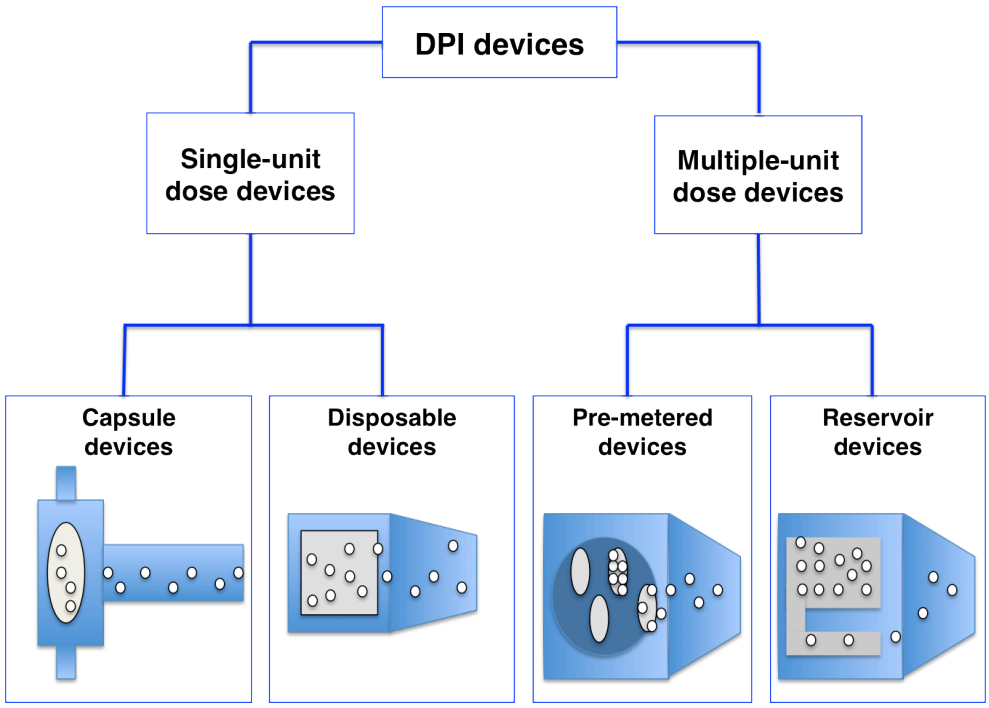

Figure 1.2. Different types of DPI devices.

In the capsule single-unit dose inhalers the drug is formulated and metered in a HMPC capsule. The patient loads the capsule into the device before use. The capsule is pierced by one or two needles and the inspiration flow of the patient make

it turns and the powder is pushed out of the inhaler. It is a very common type of DPI device available on the market (Aerolizer®, Handihaler® and Turbospin®). The second type of single-unit dose inhaler is a new concept, it doesn't require the loading of the single dose, it is ready to be employed and discarded (disposable) after its use. Nowadays there are not available disposable single-unit dose devices on the market in Europe. In Japan, Twincaps® (Hovione) a disposable inhaler, has been approved for the market [29]. An example of a disposable inhaler under development is Twincer® patent by University of Groningen. Twincer® is a multiple classifier inhaler for high powder doses under development [30].

The multi-unit dose devices contain more than one dose of drug. The doses can be pre-metered (generally in blisters) such as in Diskhaler® and in Diskus®. On the other hand, an amount of bulk powder can be loaded inside a chamber device (reservoir). The device has a mechanism to meter a single dose from the reservoir and releases it with each actuation. Examples of reservoir DPIs are Next DPI® and Turbohaler® [28,31].

1.4 Aim

Antibiotics, in particular aminoglycosides, have a relevant role for the treatment of Cystic Fibrosis (CF) lung infections by inhalation. Only tobramycin is available on the market as dry powder for inhalation (TOBI™ Podhaler™, Novartis AG, Switzerland). Tobramycin is formulated with a huge amount of excipients (only the 56% of this formulation is drug substance). Another antibiotic product approved as powder for inhalation is colistimethate sodium, a polymyxyn (Colobreathe®, Forrest, USA). Each capsule contains 125 mg of drug substance without any excipients [13,14].

Amikacin, an aminoglycosides antibiotic, has been used off-label for lung infections in CF patients since many years. Its advantage is to be active against resistant strains of *Pseudomonas aeruginosa* (PA), one of the most common infective agents in CF. Recently, amikacin demonstrated in animals the ability to suppress the CFTR premature stop mutation [16].

Since there are no amikacin dry powders for inhalation on the market, the purpose of this thesis was the study of amikacin dry powders for inhalation to be used in CF PA infected patients. Due to the complexity of the parameters involved in the production process by spray drying, experimental designs were adopted for the identification of the formulation optimized in terms of respirability parameters. Initially, a half-fractional factorial design was performed. The parameters investigated were the drying temperature, the feed rate, the solvent composition, the presence of waxy excipient and the concentration of solids in the final solution.

In the second part of the study, a Central Composite Design (CCD) was used to confirm the optimum region identified in the previous half-fractional factorial design. Three factors, namely drying temperature, feed rate and ethanol proportion, out of

the previous five studied, have been selected. In addition, the levels of these factors were increased from two to three and their effect on amikacin respirability was evaluated. More in particular, focus was given on the role of ethanol presence concerning this specific quality attribute.

In the third part of this thesis, amikacin spray dried powders prepared in different conditions of pH and composition of drug feed solution were studied. The aerodynamic performance of amikacin powders obtained was assessed using two types of inhaler: the Aerolizer® RS01 (a single unit capsule device) and the Twincer® (a single unit disposable device).

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Chapter 2. Spray dried amikacin powder for inhalation in cystic fibrosis patients: A quality by design approach for product construction

An amikacin product for convenient and compliant inhalation in cystic fibrosis patients was constructed by spray-drying in order to produce powders of pure drug having high respirability and flowability. An experimental design was applied as a statistical tool for the characterization of amikacin spray drying process, through the establishment of mathematical relationships between six Critical Quality Attributes (CQAs) of the finished product and five Critical Process Parameters (CPPs). The surface-active excipient, PEG-32 stearate, studied for particle engineering, in general did not benefit the CQAs of the spray dried powders for inhalation. The spray drying feed solution required the inclusion of 10% (v/v) ethanol in order to reach the desired aerodynamic performance of powders. All desirable function solutions indicated that the favourable concentration of amikacin in the feed solution had to be kept at 1% w/v level. It was found that when the feed rate of the sprayed solution was raised, an increase in the drying temperature to the maximum value (160°C) was required to maintain good powder respirability. Finally, the increase in drying temperature always led to an evident increase in emitted dose (ED) without affecting the desirable fine particle dose (FPD) values. The application of the experimental design enabled us to obtain amikacin powders with both ED and FPD, well above the regulatory and scientific references. The finished product contained only the active ingredient, which keeps low the mass to inhale for dose requirement.

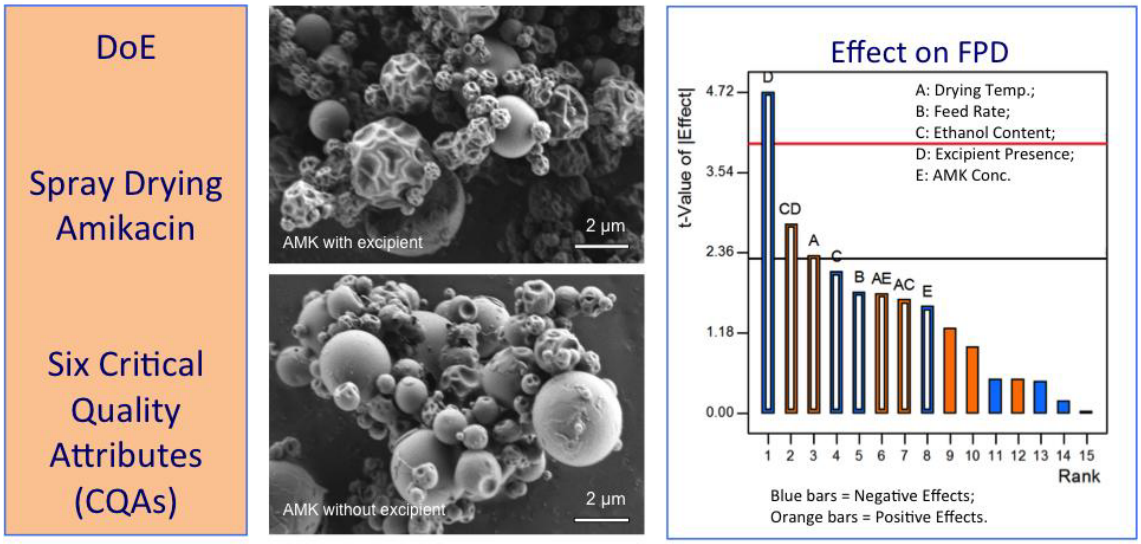

Figure 2.1. Graphical abstract

2.1 Introduction

Cystic fibrosis (CF) is an autosomal recessive genetic disease in Caucasians. Mutations in the gene cystic fibrosis transmembrane regulator (CFTR) led to a defective chloride ion transport in the airway lumen, causing an abnormal accumulation of viscous mucus. CFTR mutations are grouped into different classes:

- Class I: the protein is not synthetized
- Class II: there is a defective processing of the protein
- Class III: there is a defective regulation of the protein
- Class IV: there is a defective Cl- channel activity (decreased conduction)
- Class V: there can be a partly defective production or processing

A 6th mutation class (class VI) has subsequently been proposed and it is associated with a defective regulation of other channels present on the cell surface [1].

The most common are the class II mutations (including the prevalent, F508del), which affect about 90% of CF patients. In this class, the misfolded protein is retained at the endoplasmic reticulum, and subsequently degraded in the proteasome [2]. According to the class of mutation, there are several pharmacological approaches to correct the CFTR defect:

- Class I: Aminoglycoside antibiotics (gentamicin and amikacin) are able to bind the ribosomes and avoid the premature stop of the sequence coding the protein.
- Class II: Chemical chaperones (glycerol,dimethylsulphoxide and others) are able to stabilize the protein structure and help it to assemble correctly.
- Class III and IV: CFTR activators such as the alkylxantine 8-cyclopentyl-1.3-dipropylxanthine (CPX) and the flavonoid genistein act as channel ‘potentiators’ (they directly activate mutant CFTR, which may be either completely inactive or may show

a reduced Cl^- conduction). Potentiators are: phosphate inhibitors (such as deltametrin), or inhibitors of phosphodiesteras (such as milrinone, that promote activation of CFTR by protein kinase A).

- Class V: splicing factors that promote the protein production and potentiators to increase the activity of the channel present at the cell surface. [3,4]

Cystic fibrosis in the lungs often gives rise to chronic pulmonary infections. Bacteria entering the lung and trapped in the mucus layer are difficult to remove through mucociliary clearance. The therapy of CF infections consists of antibiotics administered orally or intravenously (aminoglycosides, macro- lides). As the lung is the site of action, inhaled medications are advantageously prescribed [5-8]. This administration route concentrates the drug at the site of infection, limiting systemic exposure and side effects. However, substantial differences in inhalation administration techniques exist. For instance, nebulizers, mostly used in hospitals and outpatient care, are cumbersome for chronic disease management, showing low efficiency and reproducibility. Dry powder inhalers (DPI), activated by patient inspiration effort, are a substantial alternative because of rapid dose administration, high local drug deposition and concentration, and stability [9]. Among the prescribed antibiotics, aminoglycosides bind bacteria ribosomes and inhibit the protein synthesis [10]. Gentamicin and amikacin also bind the eukaryote ribosomes [11,12]. Gentamicin suppressed the G542X premature stop mutation (class II) in a CF transgenic mouse model [13]. This effect was observed in vivo at peak serum concentrations well above the level required for antibiotic activity. However, the clinical use of gentamicin for this activity is limited because of its systemic side effects. In alternative, amikacin in mouse model was shown to be safer and more

effective than gentamicin in suppressing the CFTR protein premature stop mutation [14]. A liposomial formulation of amikacin for nebulization has recently been granted orphan drug designation for the treatment of Pseudomonas aeruginosa infections in patients with CF [15,16]. Similarly to other recently marketed pulmonary antibiotics, amikacin dry powder inhaler could represent an advancement of pulmonary therapy in CF patients. Spray dried powders of amikacin and other aminoglycosides with the adjunct sodium stearate in order to increase the respirability and stability of the product have been described [17,18]. The purpose of this work was to construct an amikacin spray dried powder for inhalation that would afford convenience and compliance in usage by cystic fibrosis patients. As spray drying is a multi parametric process, the objective was to systematically gain process understanding of the spray drying method for producing amikacin powders characterized by high drug content, respira- bility and flowability. Quality by Design (QbD) is a risk-based, scientific and proactive approach governing the current regulatory framework with regard to pharmaceutical process and product development [19]. Within this context, an experimental design was applied as a statistical tool for the characterization of the amikacin spray drying process, through the establishment of mathematical relationships between the Critical Quality Attributes (CQAs) of the finished product and the Critical Process Parameters (CPPs) [20,21]. In particular, this research was devoted to the understanding of the most influential process parameters affecting the inhalation performance of amikacin spray dried powders. This step opens up the way for optimization exercises capable of revealing the roadmap to further improvement. Finally, in consideration of the typical bulkiness and cohesiveness of the spray dried

powders, an agglomeration process for improving the flow characteristics was performed and evaluated.

2.2 Materials and methods

2.2.1 Materials

Amikacin sulphate was retrieved by ACS DOBFAR S.p.a. (Milan, Italy) and the surface-active excipient, PEG-32 stearate (Gelucire 48/16), was donated by Gattefossé (Nanterre Cedex, France). All solvents were of analytical grade. Water was purified by reverse osmosis (MilliQ, Millipore, Guyancourt, France). Hard hydroxy-propyl methylcellulose (HPMC) capsules (size 3) were received from Capsugel (Colmar, France). RS01 Dry Powder Inhaler device (Aerolizer-like type) was a gift of Plastiape S.p.a. (Osnago, LC, Italy).

2.2.2 Design of experiments (DoE)

A half-fractional factorial design with 5 factors (n) at two levels with resolution V was employed, allowing for the estimation of the main effects and the two factor interactions. The number of experiments to perform for the half-fractional factorial design was 2^{n-1}. The design matrix, reported in Table 2.1, included 16 experiments, plus a centre point (exp. #17) replicated twice, allowing for the estimation of experimental error. The design space was constructed and analysed using the Design-Expert Software, Version 8.0.7.1 (Stat-Ease, Inc., USA).

2.2.3. Preparation of spray dried powders

2.5 g of amikacin sulphate raw material and the reported percentage of excipient (PEG-32 stearate) (Table 2.1) were dissolved in 60 mL of water at room temperature. Water and ethanol were added under stirring to obtain a final solution having the composition and concentration as reported in Table 2.1. The solutions obtained were spray dried using a Büchi Mini Spray Dryer B-290 (Büchi Labortechnik, Flawil, Switzerland) coupled to a B-296 dehumidifier, according to the process parameters reported in Table 2.1. The aspirator rate and atomizing air rate were kept constant at 90% of the total capacity and at 600 L/h, respectively. In addition, nozzle cleaning interval was adjusted at level 5 (one pressure blow every 7 s). The spray dried powder was quantitatively recovered from the product collection vessel, weighed on an analytical balance (sensitivity 0.1 mg) (Mod. E50S, Gibertini, Italy) and expressed as percentage of the amount of solid dissolved in the sprayed solution. The dry product was stored at room temperature in a 25 mL cylindrical glass vial sealed with a rubber stopper and aluminium cap.

2.2.4. Agglomeration procedure

The spray dried powder was placed on the top of a stack of two sieves with nominal apertures of 600 mm and 106 mm, respectively (10 cm diameter sieves, Endecotts Ltd., London, UK); the final collector was added to the stack. The sieve stack was closed with the glass cover and vibrated for 5 min on a laboratory sieve shaker (amplitude 3; Analysette 3 Fritz model, Fritsch GMBH, Germany). Agglomerates retained between 600 mm and 106 mm were collected. The non-agglomerated

powder in the collector was reprocessed, and the large agglomerates on the first sieve were crushed. The entire process was repeated twice.

Table 2.1. Matrix of half-fractional factorial design including the replicated center point 17.

#	Factor 1 Drying Temp. (°C)	Factor 2 Feed rate (ml/min)	Factor 3 Ethanol (% v/v)	Factor 4 Excipient (% w/w)	Factor 5 AMK conc. (% w/v)
1	130	2.0	20	0.0	3
2	160	2.0	20	0.0	1
3	130	5.0	20	0.0	1
4	160	5.0	20	0.0	3
5	130	2.0	10	0.0	1
6	160	2.0	10	0.0	3
7	130	5.0	10	0.0	3
8	160	5.0	10	0.0	1
9	130	2.0	20	1.0	1
10	160	2.0	20	1.0	3
11	130	5.0	20	1.0	3
12	160	5.0	20	1.0	1
13	130	2.0	10	1.0	3
14	160	2.0	10	1.0	1
15	130	5.0	10	1.0	1
16	160	5.0	10	1.0	3
17	145	3.5	15	0.5	2
17bis	145	3.5	15	0.5	2
17ter	145	3.5	15	0.5	2

2.2.5. Powder and agglomerate characterization

2.2.5.1. Scanning electron microscopy

The morphology of the spray dried powders was assessed by scanning electron microscopy (SEM) (Sigma HD, Carl Zeiss, Germany), at EHT 1.00 kV. The samples were placed on a double-sided adhesive tape pre-mounted on an aluminium stub and analysed after a 30 min depressurization.

2.2.5.2. Particle size distribution

The particle size distribution of the spray dried powders was determined by laser light scattering (SprayTec, Malvern, UK). Approximately 10 mg of the sample was dispersed in 20 mL solution of 0.1% (w/v) Span 80 in cyclohexane and sonicated for 5 min. The particle size distribution was measured in triplicate with an obscuration threshold of 10%. Data were expressed in terms of median volume diameter and percentiles, $D_{(v,0.1)}$, $D_{(v,0.5)}$, $D_{(v,0.9)}$.

2.2.5.3. Termogravimetric analysis (TGA)

TGA was performed with a TGA/DSC1 (METTLER Toledo, USA). The samples were placed in 70 mL alumina pans with a pierced cover and heated under a flux of nitrogen (80 mL/min) from 25°C to 170°C at 10°C/min. The weight loss was measured in the range between 25°C and 170°C.

2.2.5.4. Bulk density

The bulk density was calculated as gram/cubic centimetre from the ratio between the mass of powder sample and its unsettled apparent bulk volume. The apparent volume was directly measured inside the cylindrical glass vial used for storage (capacity 25 mL), by calculating the diameter and the height of the powder bed in the container.

2.2.5.5. In vitro aerodynamic assessment

The aerodynamic assessment of the spray dried powders was carried out using the Fast Screening Impactor (FSI) (Copley Scientific, UK). The FSI divides the particles discharged from the inhaler into two parts, namely a coarse fraction and a fine fraction (lower than 5 mm as aerodynamic diameter), respectively. The Coarse Fraction Collector (CFC) is equipped with the insert that enables a cut-off of 5 m at 60 L/min. The particles not captured in the CFC keep following the airstream and deposit in the fine fraction collector (FFC) where a filter captures all of them. An amount of 10 ± 0.2 mg of powder, accurately weighed, was manually introduced into a size 3 hard HPMC capsule. The capsule was then inserted into the holder chamber of the DPI device and pierced. The device was connected to the FSI and passed by the air stream for 4 s at 60 L/min. The type A/E glass filter (76 mm, Pall Corporation, USA) of FFC was weighed before and after the air actuation, in order to determine the amount of powder deposited, termed as fine particle dose (FPD). Each powder was tested in triplicate before and after the agglomeration process.

2.3 Results and discussion

Among the five CPPs identified for investigation, feed rate and amikacin concentration were the variables linked to the productivity, whereas the remaining three, i.e. drying air temperature, solvent composition and presence of PEG-32 stearate, mainly connected the particle formation process to the inhalation performance of the dried powders. Other process variables, such as spray drying equipment or drying and atomizing air flow rate, were kept constant. The quality target profile of the product was oriented by a recently published risk analysis [22]. Six CQAs of the spray dried powders produced were selected and the response values were measured on the dried powders before and after the particle agglomeration step. The yield of the process and the loss on drying (LOD) quantified the productivity, whereas the particle size, bulk density and powder aerodynamic behaviour (i.e. amount of powder emitted from the device and FPD) quantified the particle/powder quality. The FPD is the amount of drug particles smaller than 5 mm deposited in the lower stage of the impactor. It is the most significant attribute to consider, and it indicates the product respirability. A value of FPD > 40% of the dose loaded in the capsule was regarded as adequate for an inhalation antibiotic product [23]. The emitted dose (ED) is a compendial quality attribute. Its relevance derives from the Pharmacopoeia specification establishing that more than 75% of the loaded dose should leave the device upon inhalation. The powder size, expressed as volume diameter of particles, reflects the capability of the spray drying process to provide a particle size distribution at micron level, in order to obtain a favourable aerodynamic diameter. In fact, the aerodynamic diameter is the critical spherical equivalent diameter to refer to in inhalation. The volume diameter is the most relevant

parameter determining the aerodynamic size of particles. The density and shape contribute as well to the particle aerodynamic size [24]. The residual water of the dried powder could affect not only its chemical stability, but also its respirability over time as it could modify the powder properties. Finally, with respect to the yield, the range of the measured values (65–90% of the total amount processed) was regarded as positive in consideration of the size of the batch and in agreement with the literature [25]. Once the process factors and the CQAs were fixed, nineteen powders were prepared and tested. A resolution V half fractional factorial design was applied for correlating formulation and process factors to the responses, which, when combined, reflected the desired product quality target profile. The results of the measurements are presented in Tables 2.2 and 2.3. ANOVA analysis of the responses for the selected factorial model was performed. All the main effects and two-factor interactions were estimated with the replicated centre points included in the analysis. The comparative significance of the factors and their interactions were evaluated graphically using perturbation plots, interaction plots and the relevant Pareto charts. ANOVA revealed significant models for all the responses under study and acceptable correlation was denoted by high R^2 and adjusted R^2 values (Table 2.4). The selection of parameters kept in the models was based on statistical significance, term hierarchy and prediction ability.

Table 2.2 Yield of the process, loss on drying (LOD), volume diameter and bulk density before (BA) and after agglomeration (AA) of the seventeen amikacin spray-dried powders.

#	Yield (%)	LOD (%)	Volume Diameter (μm)			Bulk density (g/cm^3)	
			$D_{(v,10)}$	$D_{(v,50)}$	$D_{(v,90)}$	BA	AA
1	81.82	9.7 ± 0.4	1.27 ± 0.10	2.46 ± 0.09	5.20 ± 0.91	0.37	0.42
2	73.49	8.0 ± 0.0	0.89 ± 0.01	2.22 ± 0.04	6.62 ± 0.11	0.16	0.26
3	81.84	9.0 ± 0.0	1.17 ± 0.01	2.57 ± 0.01	7.47 ± 0.22	0.21	0.21
4	81.20	8.2 ± 0.1	1.42 ± 0.01	3.52 ± 0.01	9.21 ± 0.33	0.27	0.31
5	72.28	8.6 ± 0.4	1.12 ± 0.02	3.02 ± 0.16	9.79 ± 0.37	0.30	0.34
6	80.00	7.6 ± 0.0	1.15 ± 0.01	2.43 ± 0.02	6.30 ± 0.01	0.24	0.28
7	88.03	9.5 ± 0.1	1.26 ± 0.01	2.75 ± 0.03	7.02 ± 0.22	0.25	0.29
8	79.09	9.0 ± 0.1	0.99 ± 0.04	2.23 ± 0.07	6.27 ± 0.90	0.08	0.11
9	70.47	8.8 ± 0.0	0.99 ± 0.01	1.88 ± 0.01	3.89 ± 0.03	0.19	0.24
10	81.40	9.7 ± 0.2	1.32 ± 0.04	2.44 ± 0.02	4.69 ± 0.28	0.22	0.20
11	77.20	9.5 ± 0.2	1.42 ± 0.01	3.23 ± 0.02	7.52 ± 0.17	0.59	0.68
12	67.25	8.8 ± 0.1	1.16 ± 0.01	2.53 ± 0.05	6.05 ± 0.16	0.15	0.15
13	68.52	8.9 ± 0.1	1.29 ± 0.01	2.55 ± 0.04	5.12 ± 0.10	0.32	0.40
14	73.06	9.5 ± 0.1	1.04 ± 0.01	2.04 ± 0.01	4.26 ± 0.02	0.33	0.36
15	72.23	9.3 ± 0.2	1.26 ± 0.01	2.52 ± 0.00	5.94 ± 0.04	0.63	0.80
16	70.35	8.4 ± 0.0	1.35 ± 0.01	2.86 ± 0.00	6.34 ± 0.09	0.30	0.35
17	85.81	9.6 ± 0.0	1.36 ± 0.05	2.89 ± 0.24	6.62 ± 0.89	0.33	0.42
17bis	84.81	9.2 ± 0.1	1.22 ± 0.04	2.45 ± 0.05	5.33 ± 0.21	0.34	0.42
17ter	84.47	8.4 ± 0.1	1.23 ± 0.02	2.59 ± 0.09	6.10 ± 0.36	0.40	0.40

Table 2.3 Emitted Dose (ED) and Fine Particle Dose (FPD) below 5 μm, determined by Fast Screening Impactor, before and after agglomeration of the seventeen amikacin powders (mean values ± standard deviation, n=3).

#	Aerodynamic Performance			
	Before agglomeration		After agglomeration	
	ED (mg)	FPD < 5μm (mg)	ED (mg)	FPD < 5 μm (mg)
1	7.83 ± 0.64	4.26 ± 0.37	8.23 ± 0.64	5.64 ± 0.86
2	8.57± 1.25	5.35 ± 0.67	9.17 ± 0.25	5.48 ± 1.14
3	8.57 ± 0.29	4.73 ± 1.07	7.47 ± 0.15	4.20 ± 0.43
4	8.53 ± 0.25	5.08 ± 1.31	8.17 ± 0.92	5.16 ± 1.26
5	8.53 ± 0.35	6.12 ± 0.20	8.27 ± 0.42	5.60 ± 0.18
6	6.97 ± 0.95	5.77 ± 0.44	8.30 ± 0.10	5.29 ± 0.31
7	7.67 ± 0.51	4.97 ± 0.93	7.40 ± 0.20	4.36 ± 0.20
8	8.73 ± 0.21	5.68 ± 0.13	8.87 ± 0.42	5.82 ± 1.18
9	5.80 ± 0.36	4.67 ± 0.27	5.70 ± 0.10	4.33 ± 0.23
10	6.33 ± 0.75	4.94 ± 0.30	5.93 ± 0.15	4.24 ± 0.30
11	6.93 ± 0.74	3.87 ± 0.24	6.63 ± 0.49	3.49 ± 0.26
12	6.30 ± 0.62	4.71 ± 0.18	6.13 ± 0.32	4.22 ± 0.28
13	6.37 ± 0.59	4.56 ± 0.19	6.63 ± 0.12	3.92 ± 0.08
14	5.67 ± 0.32	4.41 ± 0.13	5.70 ± 0.44	4.08 ± 0.21
15	6.67 ± 0.25	4.28 ± 0.12	6.93 ± 0.15	4.24 ± 0.13
16	7.20 ± 0.52	4.48 ± 0.09	6.67 ± 0.15	3.97 ±0.19
17	6.27 ± 0.85	4.09 ± 0.73	7.13 ± 0.85	3.97 ± 0.83
17bis	6.30 ± 0.72	4.52 ± 0.38	8.20 ± 0.46	4.67 ± 0.44
17ter	6.90 ± 0.44	5.17 ± 0.11	7.50 ± 2.10	4.59 ± 0.72

Table 2.4. Probability values for the model terms relating the Critical Process Parameters (Factors A-E) to selected Critical Quality Attributes (yield, loss on drying, volume diameter, density, emitted dose and fine particle dose)

Term	Yield	LOD	$D_{(v, 0,5)}$	Bulk Density*	Emitted Dose*	FPD*
Model	0.0006	0.0473	0.0112	0.0006	< 0.0001	0.0059
A-Drying Temp	0.5601	0.0331	0.4674	0.0005	-	0.0458
B-Feed Rate	0.1665	0.5812	0.0080	0.1163	0.0045	0.1099
C-Ethanol Content	-	0.6489	0.6421	0.1827	-	0.0678
D-Excipient	0.0003	0.0850	-	0.0027	< 0.0001	0.0011
E-AMK conc	0.0045	0.7856	0.0073	0.0333	0.4632	0.1520
AB	0.0067	-	-	0.0114	-	-
AC	-	-	0.0794	-	-	0.1305
AD	-	0.0370	-	-	-	-
AE	-	0.0730	-	-	-	0.1126
BC	-	0.1669	0.0242	-	-	-
BD	0.0222	0.1129	-	0.0024	-	-
BE	-	-	0.0880	-	-	-
CD	-	-	-	0.0209	-	0.0214
CE	-	0.0208	0.1080	0.0012	-	-
DE	-	-	-	-	0.0007	-
R^2	0.847	0.842	0.824	0.939	0.918	0.850
Adjusted R^2	0.764	0.615	0.668	0.870	0.893	0.717

* Corresponding to the powders before agglomeration.

2.3.1. Yield of the process and LOD

In general, the results indicate that the process was quite efficient in terms of amount of powder produced (Table 2.2). The minimum yield observed was 67%, whereas the vast majority of experiments led to yields greater than 80%, despite the small scale of each individual batch. The generated model was significant (see Table 2.4). The positive or negative effects of variables on the yield and of their interactions show that the spray dried powder yield was very negatively affected by the presence of the PEG-32 stearate excipient (factor D) and enhanced at high amikacin concentrations (factor E). The presence of the PEG-32 stearate caused the powder to adhere to the surface of the spray cylinder of the instrument. The LOD of all powders ranged between 7.6% and 9.7% (w/w). The perturbation plot in Figure 2.2 illustrates the effects of the process factors on residual water content. In order to be able to plot all the process factors on the same axis system, the graph has on the X-axis coded normalized values for each factor, whereas on the Y-axis the actual LOD is reported. The slope of each line in the plot is related to the factor relevance. As expected, there was a significant effect of drying temperature (factor A) on the residual product moisture, which was at the lowest value when the factor was set at 160°C whereas it increased when the product was dried at 130°C. While the differences in this response were small for factors B, C and E, a clear effect of the presence of the surface-active agent (factor D) was identified, leading to higher LOD values when this excipient was in the formulation. This could be due to the hydrophilic PEG portion of the Gelucire 48/16 being able to coordinate water molecules [26].

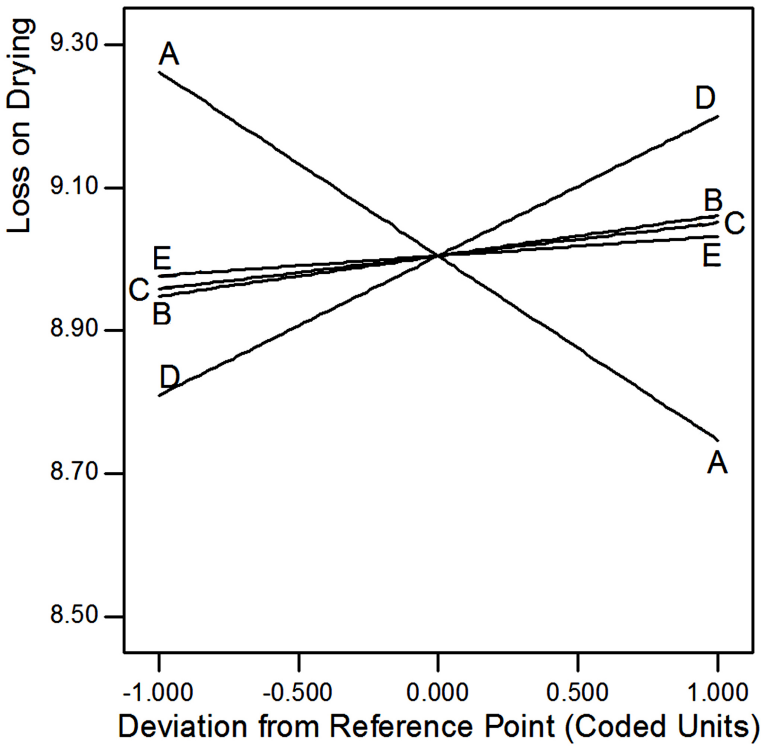

Figure 2.2. Perturbation graph of LOD versus the CPPs plotted as deviation from the reference point. Factors and reference points: A: drying temp. = 145.00; B: feed rate = 3.50; C: ethanol content = 15.00; D: excipient content = 0.50; E: AMK conc. = 2.00. The X-axis values denote the levels of each factor (-1 the minimum, 0 the centre, +1 the maximum).

The produced particles examined by SEM showed amorphous, roundish and empty structures. The clusters of shrunken smaller microparticles were observed when the surface-active excipient was in the feed solution. The presence of this surface-active substance at the air/droplet interface during drying could explain the particle surface configuration as it may affect the evaporation rate of the droplets. Figure 2.3 shows two representative powders selected among all those produced, differentiated by the presence (1% w/w) and absence of the PEG 32-stearate.

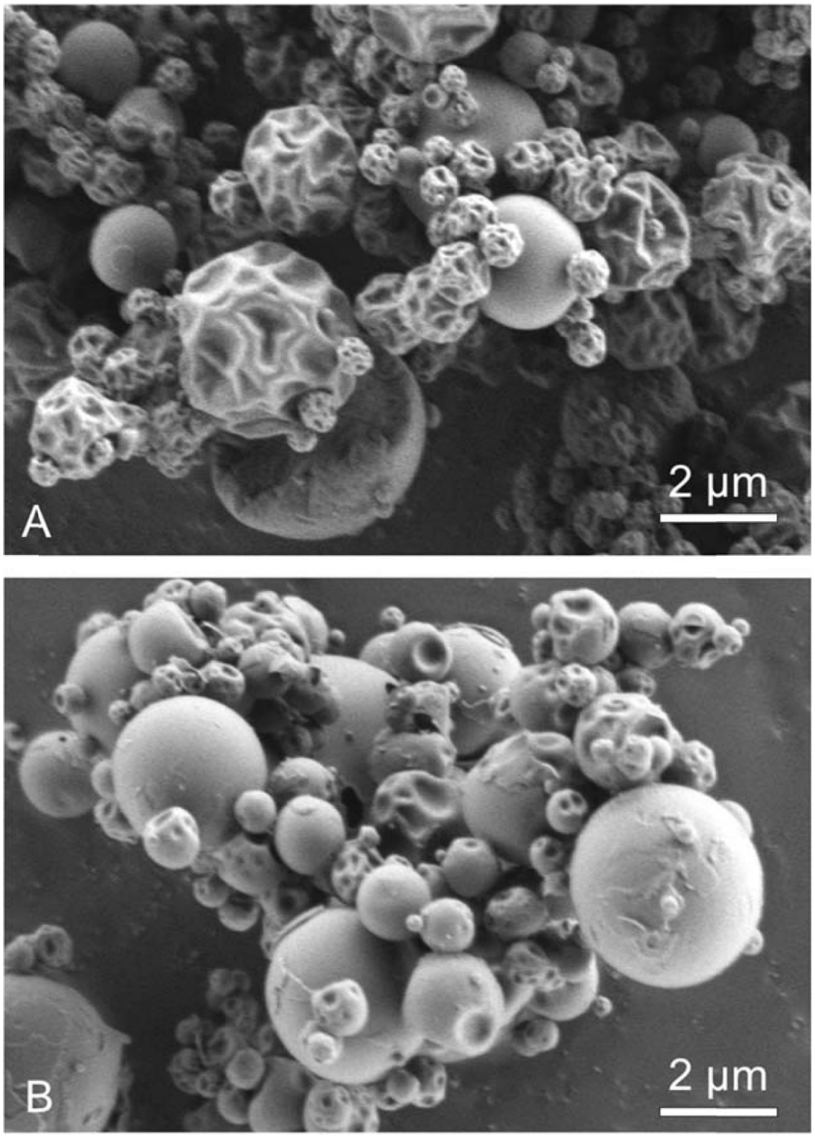

Figure 2.3. SEM pictures of the powders obtained from formulations with (A) and without (B) the excipient

Qualitative differences among the powders were also observed, as some of them appeared to be stickier, more cohesive or more electrostatically charged compared to others. Such bulk characteristics could negatively affect the flowability of powders intended for pulmonary products, which have to be dosed and aerosolized in the device reservoir. For this reason, an agglomeration process was employed to modify these unfavourable bulk properties. Agglomeration was easier with the powder

without the surface-active agent. An example of a powder before and after agglomeration is shown in Figure 2.4.

Figure 2.4. Visual aspect of powder #8 before and after agglomeration process.

2.3.2. Particle size distribution (volume diameter)

The particle size of the powders produced was measured by laser light scattering as volume diameter. The dried powders obtained showed a median diameter, $D_{(v,0.5)}$, between 1.88 mm and 3.52 mm, which is a range considered suitable for respiratory application [6]. The results were analysed in the experimental design, performing ANOVA for particle size focusing only on the $D_{(v,0.5)}$, and the model was found to be significant. The Pareto chart in Figure 2.5 illustrates the positive and negative effects of the selected factors on particle size, remembering that a positive effect must be interpreted as size increase. It has to be underlined that in inhalation an increase in particle size is not desirable. Table 2.2 and Figure 2.5 show that $D_{(v,0.5)}$ shifted towards its highest values when amikacin concentration (factor E) and the feed rate

(factor B) were at their highest levels. In contrast, when these factors were kept at low values (e.g. powders #2, #9, #14, etc.), particles showed very small $D_{(v,0.5)}$. Another significant positive effect (increase in size) was also determined by the interaction between feed rate (factor B) and ethanol presence (factor C) in the spraying solution, showing that the contribution of ethanol to the formation of a larger particle became significant only at high feed rates. In other words, smaller particles useful for inhalation should be produced by drying diluted amikacin solutions at low feed rates. The benefit of these drying operating conditions on the particle size has been demonstrated for other substances [27-29]. Finally, AC, BE and CE were identified as marginally important positive interactions with p-values around 0.10.

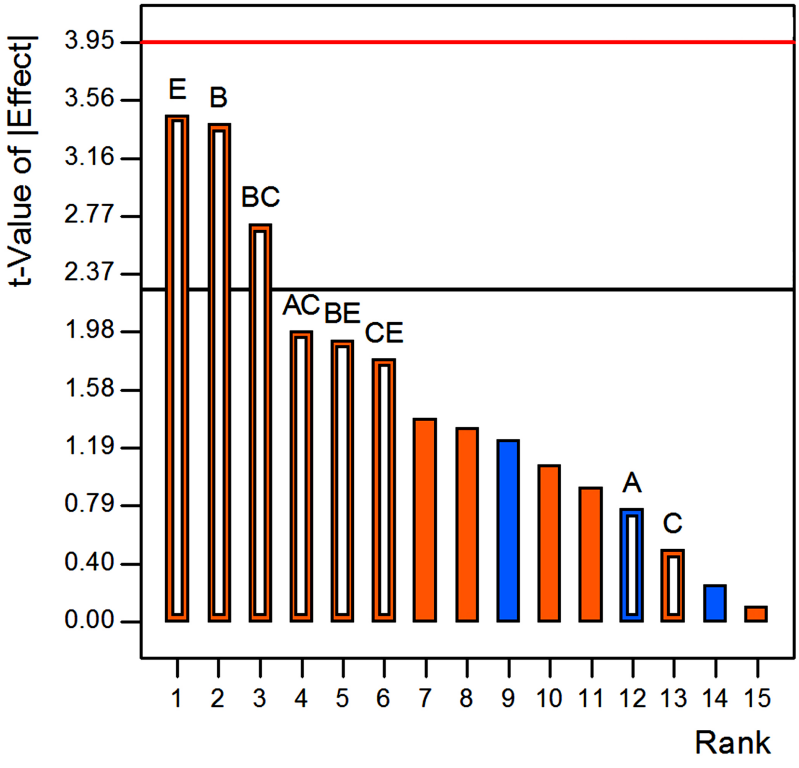

Figure 2.5. Pareto chart illustrating the rank of the t-values corresponding to the effect on $D_{(v,0.5)}$ of each factor and their interactions (empty bars: significant; full bars: non-significant). Blue bars = negative effects; orange bars = positive effects. A: Drying temp.: B: feed rate; C: ethanol content; D: excipient presence; E: AMK conc. The orange line corresponds to the Bonferroni limit and the black one to the t-value limit.

2.3.3. Bulk density

The bulk density was measured in unsettled conditions before and after agglomeration. In powders for inhalation, the bulk density exhibits a paradoxical feature: the decrease in bulk density is beneficial for the aerosolization of the powder bed but, at the same time, a bulk density increase improves the flow properties of the powder bed. Both the size and flow are crucial in inhalation technology as they affect dosing, aerosolization and deposition. The bulk density attribute was decreased mainly by high drying temperatures (factor A) and, to a lesser degree, by ethanol content (factor C) as shown in Fig. 2.6a. The presence of PEG-32 stearate (factor D), high amikacin concentration (factor E) and high feed rate (factor B) gave rise to a lower powder bed volume. Ethanol content and amikacin concentration showed a very strong interaction. The interaction plot (Fig. 2.6b) shows that at low ethanol level (10%) the influence of amikacin solution concentration on bulk density was marginal compared to the evident different effect at high ethanol level. This was attributed to the observed low solubility of amikacin when the ethanol concentration in water was higher than 30% v/v that could affect the droplet solvent evaporation and particle formation. The bulk density of the powders was modified as a consequence of particle agglomeration performed with the aim of obtaining a product with improved flow properties. The agglomeration was carried out by sieve vibration, which allowed for the collection of a definite size range of agglomerates. In general, the spray dried powders from the formulations without the surface-active agent spontaneously agglomerated upon short vibration, producing a non-significant increase in bulk density values. However, a pronounced positive effect on the flow properties was

attained, which is expected to be beneficial for dry powder inhaler manufacturability and performance.

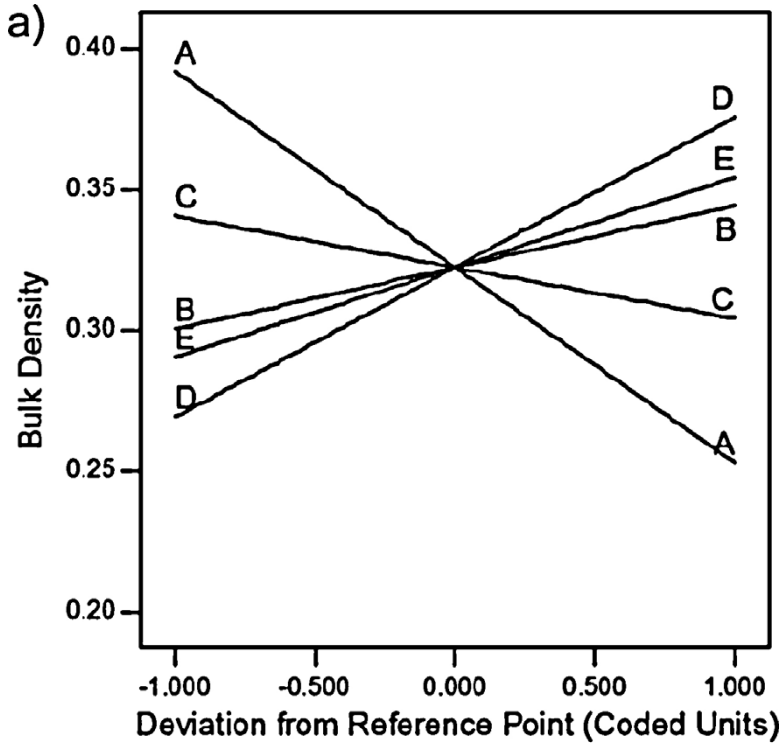

Figure 2.6a. Perturbation graph (a) of bulk density before agglomeration versus the CPPs plotted as deviation from the reference point. Factors and reference points: A: drying temp. = 145.00; B: feed rate = 3.50; C: ethanol content = 15.00; D: excipient content = 0.50; E: AMK conc. = 2.00. Interaction plot

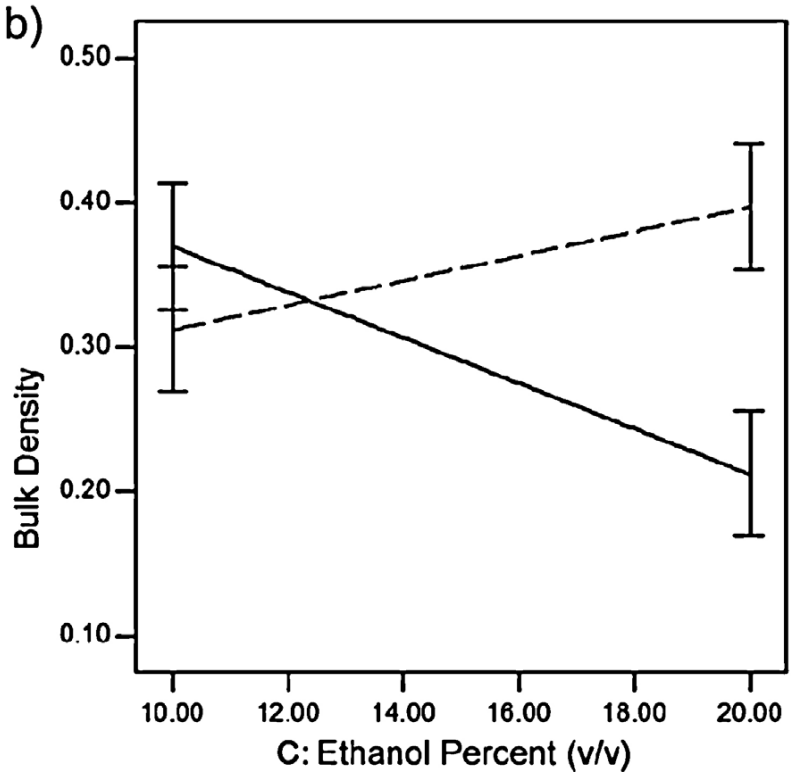

Figure 2.6b. Perturbation graph (b) of bulk density of powders before agglomeration versus ethanol content, interacting with amikacin concentration. Factors: A: drying temp. = 145.00; B: feed rate = 3.50; D: excipient content = 0.50; E: AMK conc.: dotted line = 3.00; full line = 1.00.

2.3.4. Aerodynamic performance

The ED, i.e. the amount of powder leaving the device after actuation and entering the impactor, was measured on the spray dried powders before and after agglomeration (Table 2.3, Fig. 2.7a). According to pharmacopoeia requirements, the ED should exceed 75% of the dose loaded in the device, which in this case was 10 mg. From Table III, it is obvious that the compendial specification was met for almost 50% of the cases, which corresponded to those without the excipient, both before and after agglomeration. In other words, the ED attribute was very negatively affected by the excipient (factor D) at the highest level (1% w/w). The predominant effect of this

factor was confirmed through statistical analysis (Table 2.3) and the relevant Pareto chart (Figure 2.8). It is likely that PEG-32 stearate increased powder cohesiveness, thus hindering de-aggregation by air stream, as already observed for the production yield. As for the remaining factors, the feed rate of the sprayed solution (factor B) showed a weaker but positive influence on this critical quality attribute. A significant interaction was identified between the presence of the excipient and amikacin concentration (DE) with a positive coefficient, despite the fact that factors D and E had individually negative effects. The fact that the individual contribution of these two variables was negative, but their interaction was positive, indicates that the interaction produced a bigger effect to the response compared to the individual contributions. Thus, the interaction in this case is synergistic. Therefore, it is not always true that factors with individual negative effects will also have a negative interaction, as this has to do with the magnitude of the change they cause to the response at their different levels of utilization. The agglomeration of the dried powders did not significantly modify the values of the ED (see Figure 2.7a). This means that the interaction forces holding the particles together are sufficiently weak to be broken when the powder bed is fluidized by the air stream. However, the overall effect of the agglomeration process was favourable as the agglomerated powders were free-flowing compared to the original ones.

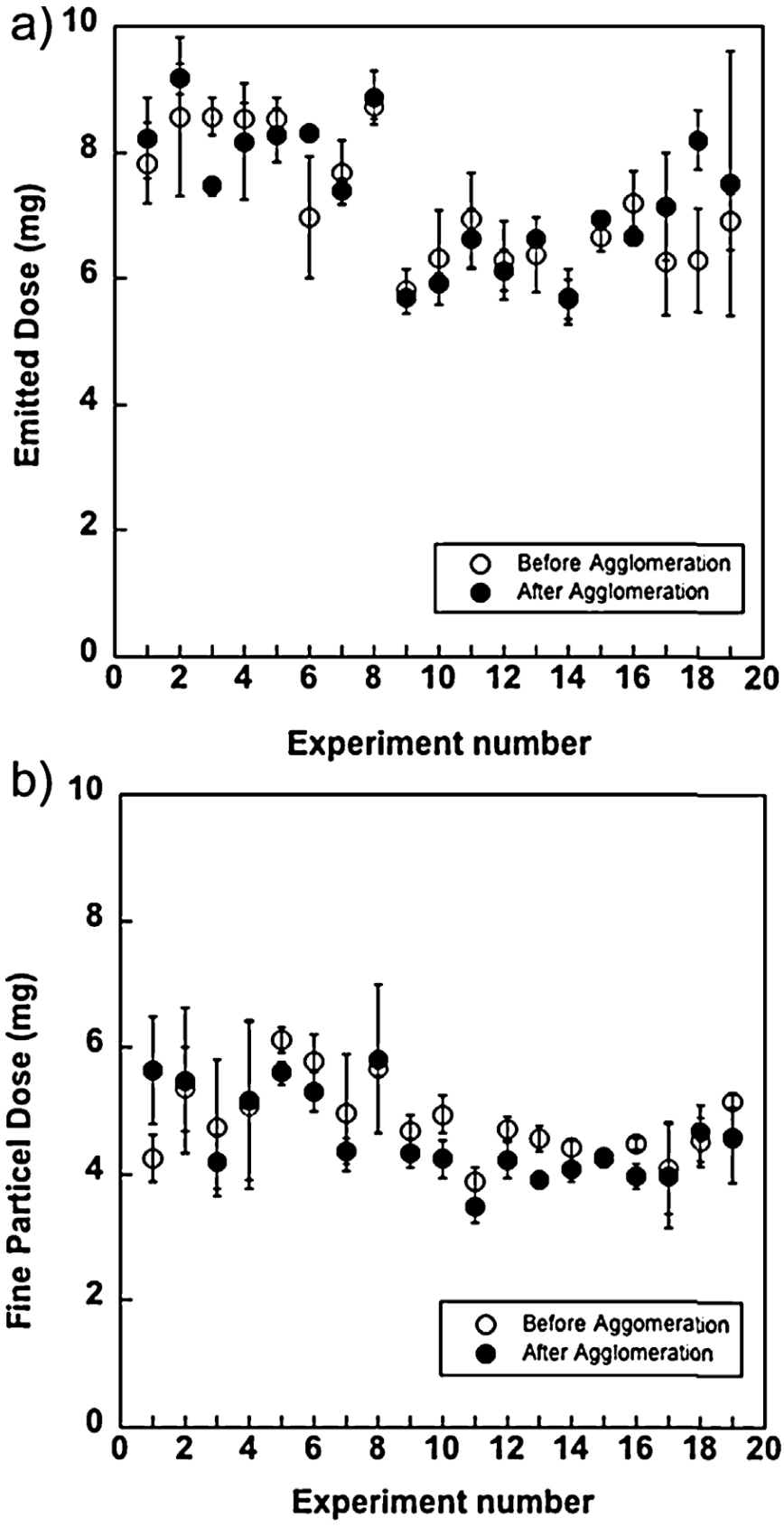

Figure 2.7. Values of ED (a) and FPD (b) of amikacin spray dried powders before and after agglomeration (mean value and standard deviation; n = 3)

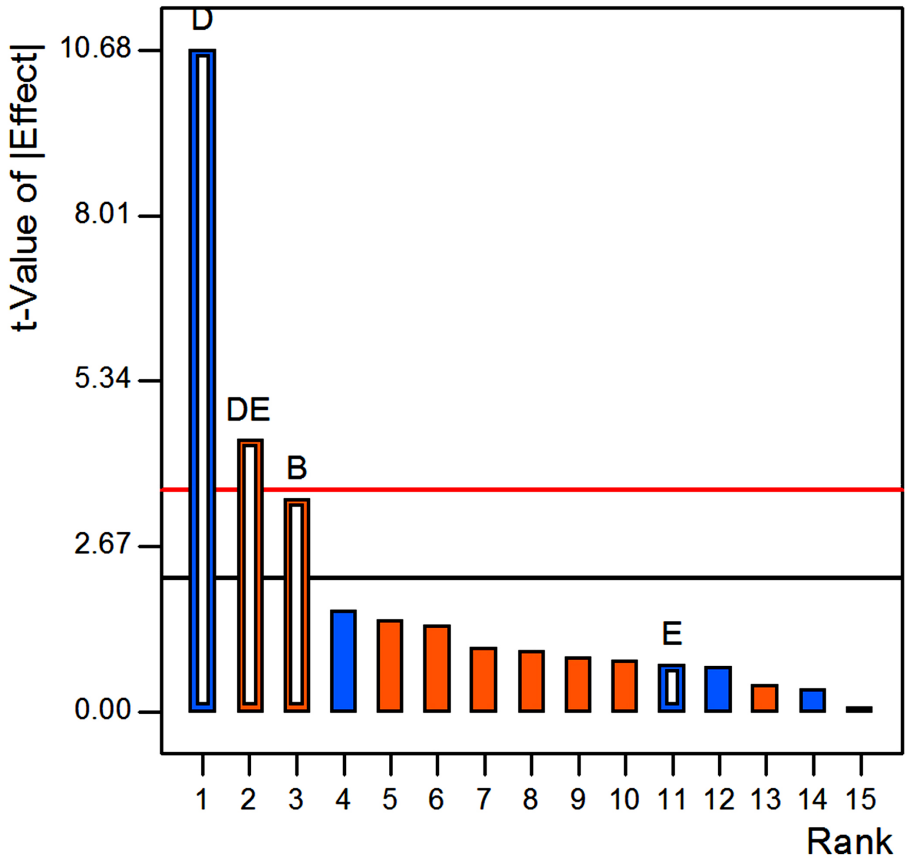

Figure 2.8. Pareto chart illustrating the rank of the t-values corresponding to the effect on the ED of each factor and their interactions. (empty bars: significant; full bars: non-significant). Blue bars = negative effects; orange bars = positive effects. A: Drying temp.; B: feed rate; C: ethanol content; D: excipient presence; E: AMK conc. The orange line corresponds to the Bonferroni limit and the black one to the t-value limit.

The second quality attribute describing the aerodynamic performance of a DPI is the FPD (aerodynamic diameter < 5 μm). The FPD values measured for the experiments of the design are reported in Table 2.3 and plotted in Fig. 2.7b before and after agglomeration. The Pareto chart (Figure 2.9) illustrates the effect on FPD of each factor and their interaction. The presence of the surface-active excipient (factor D) had a large negative effect on respirability. With the excipient, the microparticles tended to spontaneously aggregate, affecting the in vitro deposition level. In contrast,

the drying temperature (factor A) increased the amount of deposited powder smaller than 5 mm. This can be related to the decreased particle size (negative effect) observed as a consequence of the increase in drying temperature (see Figure 2.5). Interaction between excipient presence and ethanol content (CD) was identified as the only statistically significant one, as shown in the Pareto chart (Figure 2.9).

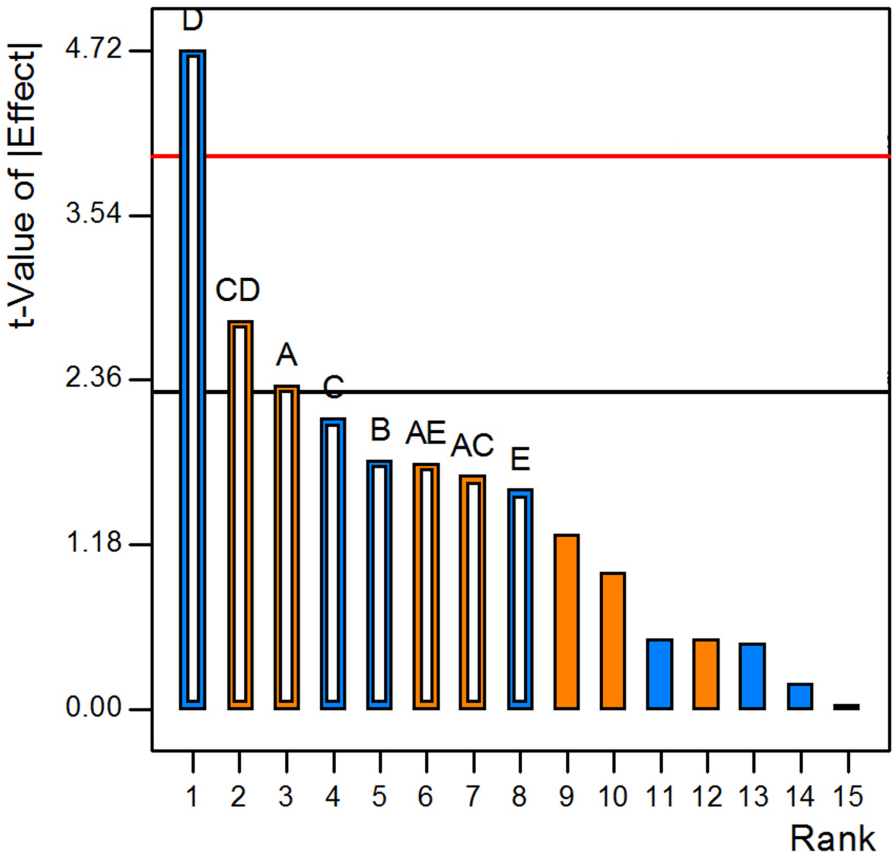

Figure 2.9. Pareto chart illustrating the rank of the t-values corresponding to the effect on the FPD of each factor and interactions (empty bars: significant; full bars: non-significant). Blue bars = negative effects; orange bars = positive effects. A: Drying temp.; B: feed rate; C: ethanol content; D: excipient presence; E: AMK conc. The orange line corresponds to the Bonferroni limit and the black line to the t-value limit.

The relevant interaction plot revealed that when the excipient was present, FPD was always low irrespectively of ethanol content, whereas FPD was enhanced at low

ethanol level when the excipient was absent (Figure 2.10). Similarly to what was observed for the ED, agglomeration of the powders did not significantly affect respirability. Figure 2.7b gives a comprehensive view of the effect of agglomeration on respirability. A general trend towards lower FPD values was recognized as a consequence of the agglomeration, although in most cases this reduction was not significant. Therefore, the agglomeration of amikacin microparticles changed neither the ED nor FPD.

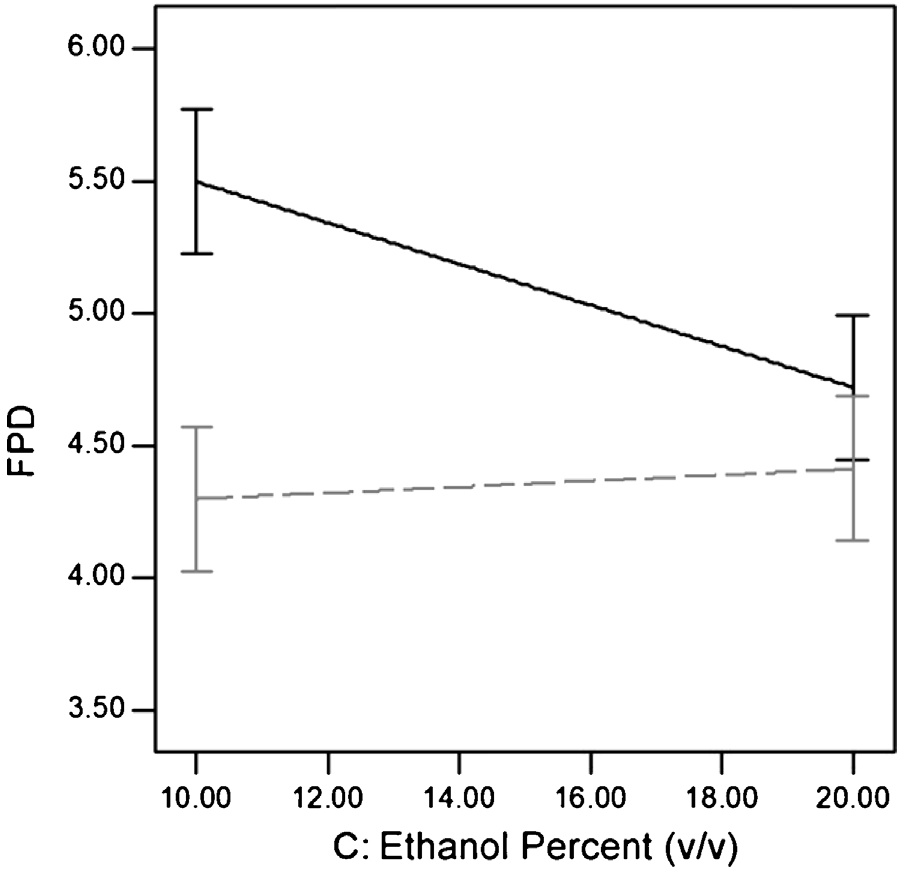

Figure 2.10. Interaction plot of FPD of powders before agglomeration versus ethanol content, interacting with excipient presence. Factors: A: drying temp. = 145.00; B: feed rate = 3.50; E: AMK conc. = 2.00; D: excipient content = solid line = 0.00, dotted line = 1.00.

Finally, the analysis of the DoE results revealed, through the statistically significant correlations, the importance of formulation and process factors and their interactions on the inhalation powder quality attributes. This knowledge provides the scientific basis for optimizing the process under study with response surface methodologies. The first step towards this approach comprised a preliminary statistical exercise using the desirability function. The function reveals specific factor settings concurrently providing acceptable values for several responses and defines the roadmap to a subsequent optimization phase. The purposeful adjustment of the manufacturing spray drying parameters and formulation factors could enable further improvement in the aerodynamic performance of spray dried powders and in the manufacturability of the dry powder inhaler. Desirability function is a mathematical expression related to the coverage extent of the fixed objectives by the proposed combinations of the factors under study. The closest this function is to 1 the better. The criteria set out in this work were the maximization of FPD and the ED values over 75% after powder agglomeration. These were considered as the most important CQAs, reflecting compendial requirements for dry powder inhalers, and their potential in vivo performance. Focusing on the process solutions with the highest possible desirability (in this case >0.800), twenty combinations generated by the software covering the aforementioned criteria are presented in Table 2.5.

Table 2.5. Parameter combinations showing a desirability factor higher than 0.800 (emitted dose ED>7.5 and FPD maximized, after agglomeration with replications).

Number	Factors					Criteria Set		Desirability
	Drying Temp	Feed Rate	Ethanol Content	Excipient	AMK Conc.	FPD	ED	
1	130.00	2.00	10.00	0.00	1.00	5.64	8.48	0.921
2	149.21	2.00	10.00	0.00	1.00	5.58	8.98	0.893
3	130.00	2.03	10.02	0.02	1.12	5.57	8.41	0.893
4	160.00	5.00	10.00	0.00	1.00	5.56	8.75	0.887
5	154.65	2.00	10.09	0.00	1.00	5.55	9.13	0.884
6	159.99	3.77	10.00	0.00	1.00	5.55	8.96	0.883
7	147.48	2.03	10.00	0.03	1.00	5.55	8.85	0.881
8	150.67	2.00	10.48	0.01	1.00	5.54	9.00	0.880
9	160.00	4.65	10.00	0.01	1.01	5.54	8.78	0.879
10	160.00	4.76	10.48	0.00	1.00	5.54	8.79	0.878
11	158.75	3.58	10.00	0.00	1.00	5.54	8.96	0.877
12	158.47	3.72	10.00	0.00	1.00	5.53	8.93	0.875
13	160.00	3.41	10.80	0.00	1.00	5.52	9.02	0.869
14	160.00	2.14	10.00	0.02	1.00	5.51	9.16	0.867
15	130.00	2.12	13.90	0.00	1.00	5.46	8.46	0.843
16	134.20	2.00	13.79	0.00	1.19	5.45	8.55	0.841
17	156.73	2.05	12.95	0.00	1.00	5.44	9.17	0.833
18	130.00	2.00	13.09	0.00	1.48	5.43	8.38	0.833
19	130.00	2.03	16.09	0.00	1.00	5.40	8.47	0.818
20	151.76	4.42	10.00	0.01	1.00	5.40	8.62	0.816

Amikacin powder exhibiting an ED higher than 75% of the amount loaded in the device, together with a maximized FPD around 5.5 mg (55% of the 10 mg dose loaded), could be prepared by appropriately setting the selected process parameters within the design space. The factor combination demonstrated the robustness of the quality level obtained. In detail, the surface-active excipient PEG-32 stearate at the level studied, in general, did not benefit the CQAs of the spray dried powders for inhalation. The spray drying feed solution required the inclusion of ethanol around 10% (v/v) in order to reach the desired aerodynamic performance of spray dried powders. In a similar manner, all desirable function solutions indicated that the favourable concentration of amikacin in the feed solution had to be kept at the lowest level (1% w/v). The remaining two process factors (feed rate and drying air temperature) provided respirability results in a broader range. However, it was noticed that when the feed rate of sprayed solution was raised, an increase in the drying temperature to the maximum value (160°C) was required to properly dry the droplets and maintain good powder respirability. Finally, an increase in drying temperature always led to an evident increase in ED without affecting the desirable FPD values.

2.4 Conclusions

The maximization of amikacin FPD reducing to a minimum the amount of powder to inhale so increasing patient convenience and compliance, was achieved in the current work by a statistical tool based on DoE. The outcome was that both ED and FPD were enhanced well above the regulatory and scientific references. As all desirable solutions practically excluded the use of the surface-active excipient, the finished product contained only the active ingredient meeting the target to minimize the quantity of inhalable powder for a fixed dose. Future work will optimize the manufacturing of the amikacin primary microparticles and the agglomeration process for assuring dose uniformity of the product by improving the flowability of powders and further improving the respirability performance achieved so far.

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Chapter 3. Spray dried amikacin powder for inhalation in cystic fibrosis patients: the role of ethanol in particle formation

A Central Composite Design (CCD) was used to confirm the optimum region identified in a previous half-fractional factorial design applied for the formulation of amikacin spray dried powders for inhalation. Three factors, namely drying temperature, feed rate and ethanol proportion, have been selected out of the initial five. In addition, the levels of these factors were increased from two to three and their effect on amikacin respirability was evaluated. More in particular, focus was given on the role of ethanol presence on the formation of the microparticles for inhalation.

The overall outcome of the CCD was that amikacin respirability was not substantially improved, as the optimum region coincided with areas already explored with the fractional factorial design. However, expanding the design space towards smaller ethanol levels, including its complete absence, revealed the crucial role of this solvent on the morphology of the produced particles. Peclet number and drug solubility in the spraying solution helped to understand the formation mechanism of these amikacin sulphate spray dried particles.

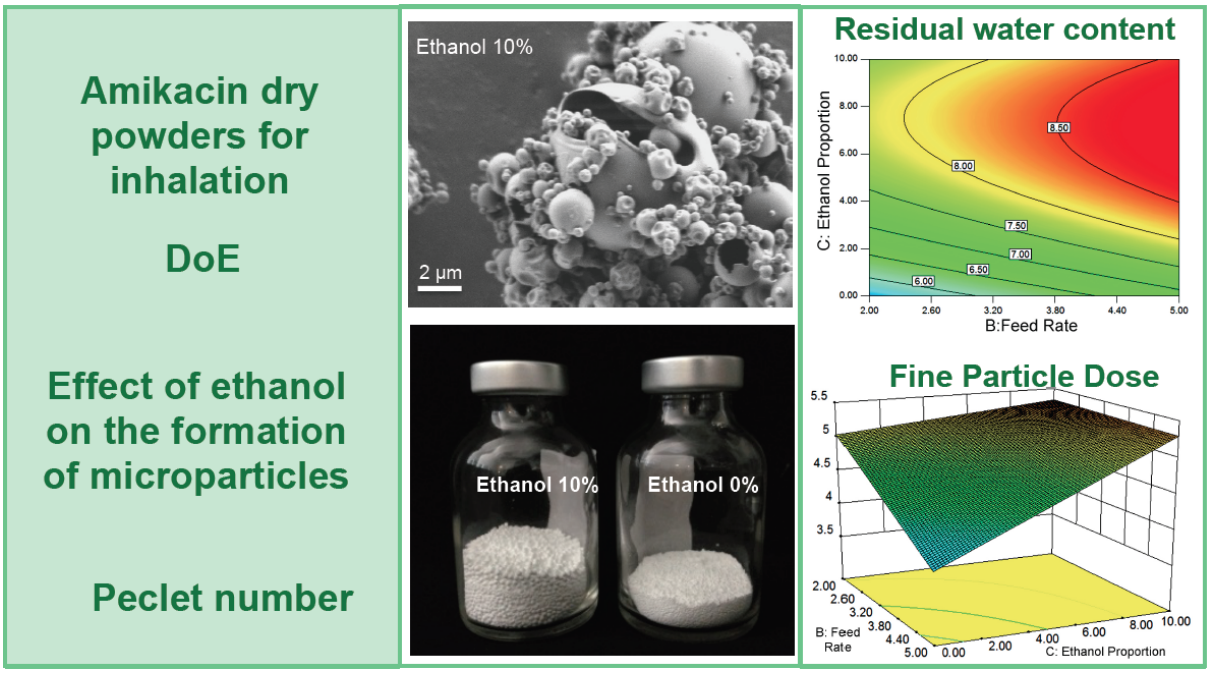

Figure 3.1 Draft of the graphical abstract

3.1 Introduction

Lung infections in Cystic Fibrosis (CF) patients caused by Pseudomonas aeruginosa are efficiently managed with antibacterial drugs. These treatments require high doses of antibiotic. However, using the pulmonary route, the inhaled drug is directly deposited on the site of infection providing higher local concentrations with lower doses compared to systemic administration. Dry powder inhalers are able to deliver high payloads of drug in shorter time, offering a convenient alternative to solutions for nebulization [1]. However, high doses of powders can raise adverse effects during the administration, such as cough and choking. Consequently, there are two approved administration strategies for delivering high doses of powdered drugs to the lung of the patients [2]. The first used a single pre-metered capsule reservoir containing the whole dose to be extracted by successive inhalation acts, such as with the Colobreathe product [3]. The second strategy consisted in splitting the dose in multiple capsule reservoirs. In Tobi Podhaler, the dry powder of tobramycin formulation (112 mg dispersed in approximately 200 mg of powder) is administered by the consecutive inhalation of four capsules content. An evolution of these delivery systems is the use of new disposable devices, capable to gradually release the dose loaded in the device reservoir in alternative to hard capsules [4,5].

The performance of a dry powder inhaler is governed by formulation characteristics. Particle engineering strategies have been adopted to optimize size, morphology and structure of microparticles, in order to maximize the respirable fraction of the drug, without compromising the powder flow properties [6,7]. Since the antibiotics are administered at high doses (up to 100-150 mg), formulation techniques should avoid the use of carrier excipients, to limit the mass of the powder to be inhaled [8].

Spray drying is a suitable technology towards this direction, as it is capable of providing microparticles for lung administration with acceptable flow properties [9]. The method has been used for the preparation of antibiotic [10-12], anti-inflammatory compounds [13,14] and insulin dry powder [15,16]. The shape and density of the spray dried particles can be modified by controlling the parameters affecting the evaporation process of the sprayed droplets [17 - 19].

In a previous study [20], a half-fractional factorial experimental design was applied as a statistical tool for the construction of amikacin sulphate spray dried pulmonary powders. The mathematical relationships between six Critical Quality Attributes (CQAs) of the finished product and five Critical Process Parameters (CPPs) were established. Drying temperature, feed rate, ethanol:water ratio, concentration of amikacin in spraying solution and presence of PEG-32 stearate, as respirability adjuvant, were investigated. The results obtained showed that the proposed adjuvant did not benefit the quality of the spray dried powders and the best factor combination led to an amikacin powder with an Emitted Dose of 85% and a respirable fraction reaching 58%of the loaded dose.

In the present study, a Central Composite Design has been applied, with the intent to expand the experimental space previously defined in the hypothesis to discover further positive combinations of the manufacturing parameters. Therefore, among the previous CPPs, the three most important were amplified at three levels including unexplored regions assumed favorable for increasing amikacin powder respirability. In detail, ethanol proportion, drying temperature and feed rate were evaluated at three levels, including new settings for the first two factors. Special attention has been given to the role of ethanol as solvent in the sprayed solution, with regards to

the effect of its absence/presence on final product structure and inhalation performance.

3.2 Materials and methods

3.2.1 Materials

Amikacin sulfate was obtained by ACS DOBFAR S.p.a. (Milan, I). All solvents used were of analytical grade. Water was purified by reverse osmosis (MilliQ, Millipore, Guyancourt, France). Hydroxy-propyl methylcellulose (HPMC) capsules (size 3) were received from Capsugel (Colmar, France). RS01 Dry Powder Inhaler device flow rate 60 L/min (gift of Plastiape S.p.a. (Osnago, LC, I).

Amikacin sulphate solubility was measured in purified water, in ethanol 95.6° and water ethanol mixtures, using the amikacin assay method of Ph.Eur. 8.

3.2.2 Design of Experiments (DoE)

A face centered-Central Composite design (CCD) at three levels was employed. The experiments to perform are fifteen, plus two replications of the center point. In details,

- 8 experiments from the full factorial design (two levels for each factor: 2^3; red points in the Figure 3.2)
- 6 experiments (star points: the center of each face of the factorial space; yellow stars in the Figure 3.2)
- 1 experiment (center point; green point in the Figure 3.2) in triplicate.

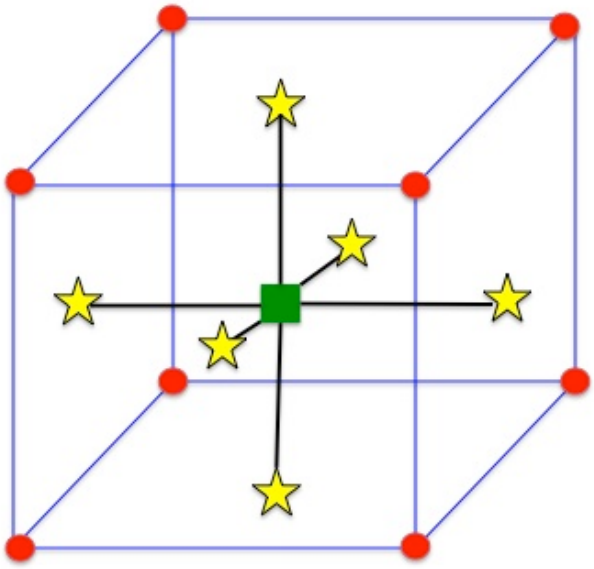

Figure 3.2. Central Composite Design 'face centered'

The design matrix, reported in Table 3.2, included the fifteen experiments plus the replications of the center point (#15 bis, #15 ter). The design was constructed and analyzed using Design-Expert® Software, Version 9.0.1 (Stat-Ease, Inc., Minneapolis, USA).

Table 3.1. Matrix of the face centered-CCD showing the studied parameters, their levels and the experiment number (#) including the replicated center points (#15).

Exp. #	A. Drying Temp (°C)	B. Feed Rate (ml/min)	C. Ethanol (%w/w)
1	150	2.0	10
2	180	2.0	10
3	150	5.0	10
4	180	5.0	10
5	150	2.0	0
6	180	2.0	0
7	150	5.0	0
8	180	5.0	0
9	150	3.5	5
10	180	3.5	5
11	165	2.0	5
12	165	5.0	5
13	165	3.5	10
14	165	3.5	0
15	165	3.5	5
15 bis	165	3.5	5
15 ter	165	3.5	5

3.2.3 Preparation of spray dried powders

2.5 g of amikacin sulphate were dissolved in water at room temperature. Ethanol was added under stirring to obtain the proportions reported in Table 3.1, while drug concentration was kept 2% w/v. The solutions prepared were spray dried using a Büchi Mini Spray Dryer B-290 (Büchi Labortechnik, Flawil, Switzerland) coupled to a B-296 de-humidifier, adopting the process parameters reported in Table 1. Aspirator rate was kept constant at 90%, while atomizing air velocity and nozzle cleaning interval were adjusted at 600 L/h and level 5 respectively.

The spray dried powder was quantitatively recovered from the product collection vessel and weighed on an analytical balance (E50S, Gibertini, Italy). The yield was expressed as percentage of the solid dissolved in the sprayed solution. The dry product was then stored at room temperature in a 25 ml cylindrical glass vial, sealed with a rubber stopper and aluminum cap. Part of the product was agglomerated into microparticle clusters by sieving as described in a previous publication [20].

3.2.4 Powder and agglomerate characterization

The morphology of the spray dried powders was assessed by Scanning Electron Microscopy (SEM) (Sigma HD, Carl Zeiss, Germany), at extra high tension of 1.00 kV. Microparticle samples were placed on a double-sided adhesive tape pre-mounted on an aluminum stub and analyzed after a 30 min depressurization.

Particle size distribution of spray dried powders was measured by laser light scattering (SprayTec, Malvern, UK). Approximately 10 mg of sample were dispersed in 20 ml of cyclohexane containing 0.1% (w/v) of sorbitan monooleate (Span 80) and sonicated for 5 min. The results were expressed in terms of median volume diameter

$D_{(v,90)}$, percentiles $D_{(v,10)}$, $D_{(v,50)}$ and Span.

The residual water content (%) of the spray dried powders was measured by Karl Fischer volumetric titration using TitroMatic Karl Fischer (Crison Instruments, S.A., Barcelona, Spain).

The bulk density was determined as the ratio of the sample mass and its unsettled apparent bulk volume. The latter was directly measured in a 25 ml cylindrical glass vial.

The true density was measured using a helium pycnometer (APS AccuPyc 1330 Gas Pycnometer, Micromeritics, Norcross, GA, USA).

The agglomerates were pictured by optical microscopy (magnification 3x), and the diameter of the projected area assumed as spherical, was measured using Image J software (U. S. National Institutes of Health, Bethesda, Maryland, USA).

The aerodynamic assessment of the spray dried powders was carried out using the Fast Screening Impactor (FSI) (Copley Scientific, UK). The FSI divides the aerosol particles emitted from the inhaler into two parts, i.e. the coarse and the fine fractions, the latter corresponding to sizes lower than 5 µm considered as respirable fraction. The Coarse Fraction Collector (CFC) is equipped with an insert that enables the 5µm cut-off at 60 L/min. The particles not captured in the CFC follow the airstream and deposit in the fine fraction collector (FFC) where they are captured by a filter (A/E glass filter, 76 mm, Pall Corporation, USA).

In detail, an accurately weighed amount of powder equal to 10 ± 0.2 mg, was manually introduced into a size 3 hard HPMC capsule. The capsule was then inserted into the holder chamber of the RS01 device and pierced. The latter was connected to the FSI and flushed by the air stream for 4 s at 60 L/min. The FFC filter

was weighed before and after the air actuation, in order to determine the amount of powder deposited, termed as Fine Particle Dose (FPD). Each powder was tested in triplicate before and after the agglomeration process.

3.2.5 Determination of evaporation rate of spray dried solutions

The evaporation rates of the spray dried solutions were measured by thermogravimetric analysis (TGA, Mettler Toledo, Columbus, OH, USA). An accurate amount of solution was introduced in an aluminum-crucible 40 μl pan (Me-26763 without pin, Mettler Toledo). The sample was heated into the apparatus furnace at constant temperature of 85°C, corresponding to the outlet temperature of spray drying, while purging nitrogen at a flow rate of 20 ml/min. The weight loss was recorded as a function of time [19].

3.3. Results and discussion

In all the experiments the yields of amikacin spray dried powders exceeded 80%. The residual water content was lower than that of amikacin sulphate active substance, which was 10.7% (Table 3.2). The lowest residual water content value (4.92%) was obtained for the combination of the high drying temperature (180°C), low feed rate (2 ml/min) and absence of ethanol in the feed solution (experiment # 6). On the contrary, the maximum water content was measured when the low drying temperature (150°C) was combined with the high level of feed rate (5 ml/min) and ethanol proportion (10%) (experiment # 3).

The ANOVA analysis of residual water content (numerical data not shown) and the corresponding contour plot (Figure 3.3) indicated feed rate (B) and ethanol proportion (C) as the most influential factors. The contour plot illustrates that at the drying temperature of 165°C, the highest residual water values are in the red zone, where high percentages of ethanol and feed solution rates are used. Although the effect of increasing the feed rate on residual water is practically self-explanatory, attributing higher water content of dry particles to the increase of ethanol in feed solution required further consideration. This result could be attributed to the different vapor tension of the two miscible liquids. During the drying process, different composition between the solution to evaporate and the condensed vapor, richer in ethanol, is obtained. Since the evaporation time of the droplets and the drying temperature are constant, the more volatile ethanol, when present, subtracts part of available heat energy to water evaporation, so leaving more residual water in the solid.

Table 3.2. Residual water content and particle size distribution (volume diameter) of amikacin spray dried powders (n=3)

#	Residual water (%)	Volume Diameter (μm)			
		$D_{(v,10)}$	$D_{(v,50)}$	$D_{(v,90)}$	Span
1	6.98 ± 0.35	1.23 ± 0.07	2.80 ± 0.25	7.41 ± 0.58	2.19 ± 0.04
2	7.99 ± 0.41	1.19 ± 0.03	2.72 ± 0.08	7.21 ± 0.25	2.22 ± 0.02
3	9.02 ± 0.27	1.32 ± 0.06	3.23 ± 0.33	8.71 ± 0.83	2.29 ± 0.01
4	7.70 ± 0.42	1.39 ± 0.02	3.25 ± 0.12	8.64 ± 0.68	2.40 ± 0.09
5	6.53 ± 0.51	1.45 ± 0.01	2.53 ± 0.07	4.48 ± 0.39	1.20 ± 0.12
6	4.92 ± 0.28	1.35 ± 0.01	2.37 ± 0.01	4.08 ± 0.03	1.16 ± 0.01
7	7.45 ± 0.41	1.44 ± 0.07	2.32 ± 0.09	4.69 ± 0.32	1.40 ± 0.08
8	6.57 ± 0.51	1.36 ± 0.04	2.49 ± 0.07	4.52 ± 0.05	1.29 ± 0.04
9	8.19 ± 0.23	1.41 ± 0.03	2.60 ± 0.04	4.71 ± 0.00	1.27 ± 0.03
10	8.29 ± 0.40	1.29 ± 0.01	3.21 ± 0.12	8.88 ± 0.42	2.37 ± 0.04
11	7.70 ± 0.19	1.21 ± 0.02	2.64 ± 0.09	6.89 ± 0.37	2.19 ± 0.06
12	8.84 ± 0.39	1.40 ± 0.04	3.68 ± 0.13	9.68 ± 0.22	2.25 ± 0.03
13	8.81 ± 0.35	1.18 ± 0.10	2.73 ± 0.12	8.30 ± 0.08	2.37 ± 0.08
14	5.50 ± 0.10	1.44 ± 0.01	2.60 ± 0.08	4.59 ± 0.18	1.21 ± 0.03
15	8.30 ± 0.11	1.32 ± 0.02	3.33 ± 0.07	8.29 ± 0.30	2.26 ± 0.00
15 bis	8.07 ± 0.22	1.36 ± 0.01	3.22 ± 0.11	8.26 ± 0.79	2.14 ± 0.18
15 ter	7.60 ± 0.06	1.37 ± 0.03	3.54 ± 0.01	9.54 ± 0.20	2.31 ± 0.04

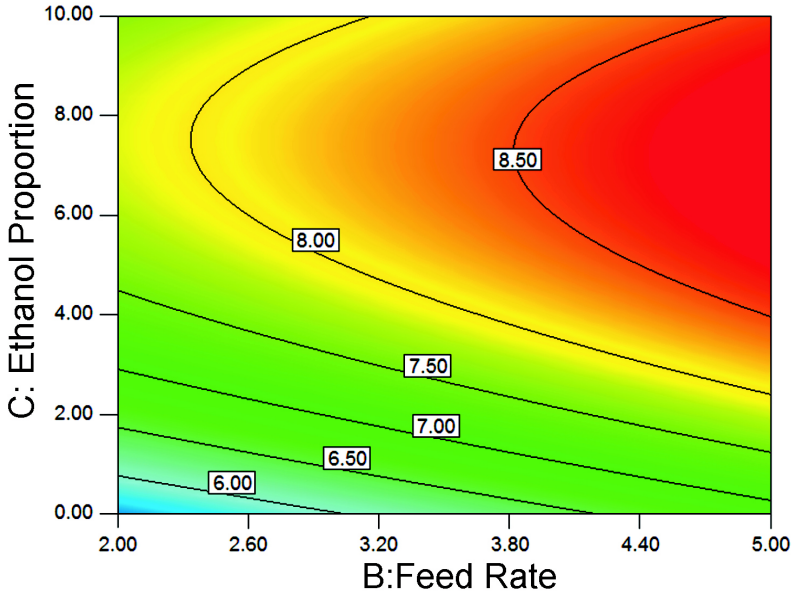

Figure 3.3. Contour plot of water content as function of feed solution rate and ethanol proportion at the drying temperature of 165 °C (red: high water content; blue low water content).

3.3.1 Morphological analysis

The SEM images of the powders produced at different ethanol concentrations (experiments # 13, 14, 15) reveal peculiar morphological differences between the microparticles (Figure 3.4). Almost all the particles produced without ethanol (experiment # 14) are shrunk. On the contrary, in the powders prepared from ethanol solution (experiments # 13 and 15), together with shriveled particles, numerous large spherical particles have been observed, captured as either swollen by pressure from inside or 'exploded'. This condition was more apparent at high level of ethanol in the feed solution The blown or ruptured particles compared to the shriveled (collapsed) ones in the absence of ethanol indicated that water/ethanol evaporation rate during the drying process was the determinant of particles morphology.

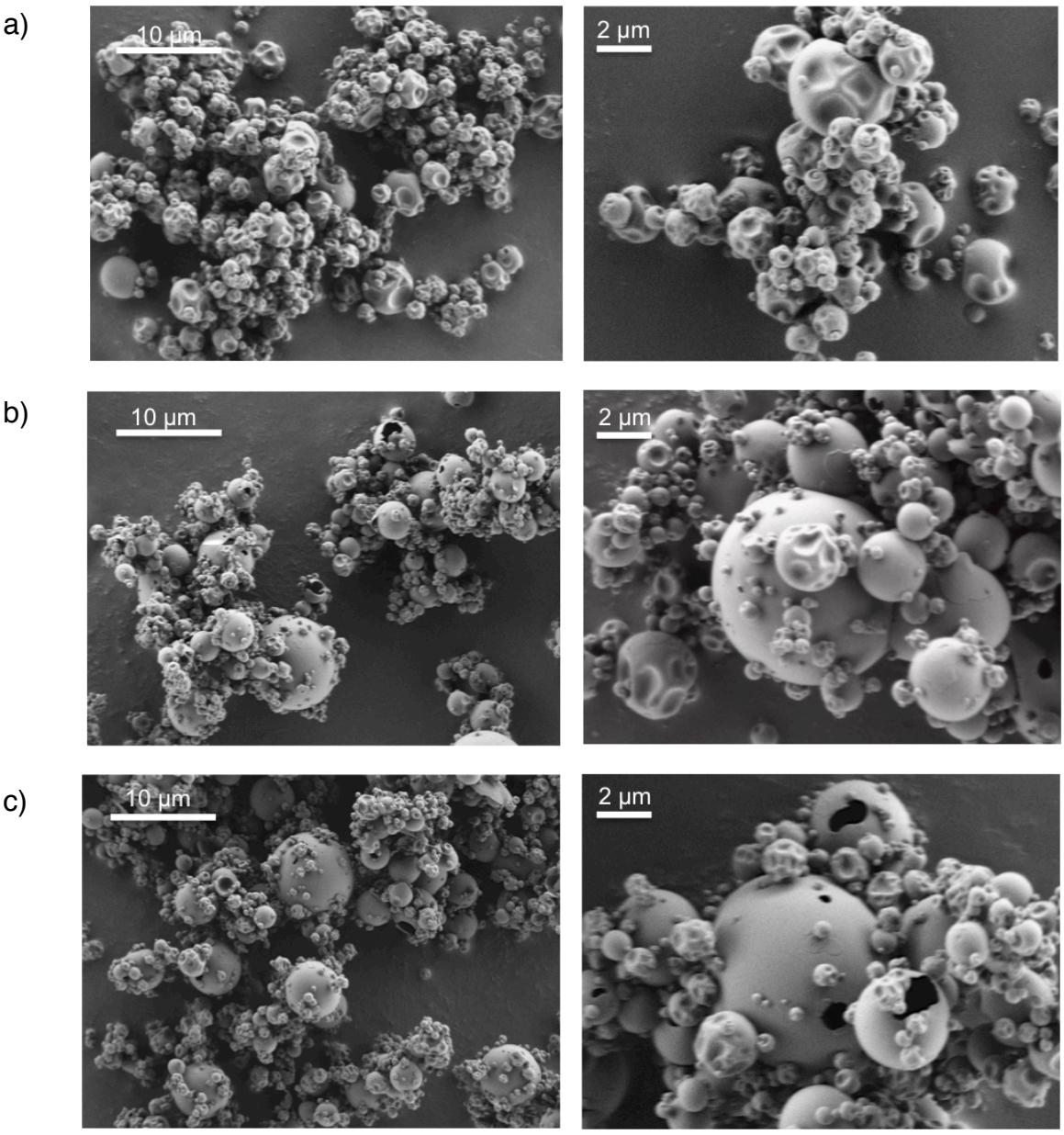

Figure 3.4. SEM pictures of three spray dried powders at two magnifications (into brackets combinations of factor levels are presented): a) powder #14 (0 % EtOH – 3.5 ml/min – 165 °C); b) powder #15 (5 % EtOH – 3.5 ml/min – 165 °C); powder #13 (10 % EtOH – 3.5 ml/min – 165 °C).

3.3.2 Particle size distribution and density of powders and agglomerates

All spray dried powders showed a median diameter, $D_{(v,50)}$, between 2.49 and 4.36 µm, suitable for the pulmonary administration (Table 3.2). Confirming the SEM pictures, the presence of ethanol and the increase of feed rate resulted in particles with larger volume diameter and span.

With regards to true density, no significant differences were measured, as values ranged between 1.5 and 1.6 g/cm^3 (data not shown). On the contrary, bulk density was strongly affected by ethanol presence. As shown in Figure 3.5, the powders with the highest bulk density values (experiments # 5 to 8, and 14) were obtained from feed solutions without ethanol. In agreement with the SEM pictures, the presence of ethanol resulted in large exploded microparticles increasing the powder volume and thus, reducing its bulk density (see Table 3.3).

In general, the spray dried amikacin powders flowed poorly, since they gave rise to lumps of particles having different sizes. This behavior made the powder non homogenous, anticipating negative expectations on the operations of the device reservoir loading for drug product (dry powder inhaler) preparation. In consequence, the powders were agglomerated in soft pellets, in order to homogenize the lumps and improve flowability and packing characteristics. The agglomeration made the powders free-flowing and increased the bulk density with few exceptions (see Figure 3.4).

Table 3.3. Bulk and density of the spray-dried powders and diameters of agglomerates (n = 20)

#	Bulk density		Agglomerate diameter (mm)
	Before agglomeration (g/cm^3)	After agglomeration (g/cm^3)	
1	0.35	0.33	0.834 ± 0.110
2	0.29	0.40	0.602 ± 0.131
3	0.32	0.33	0.698 ± 0.113
4	0.25	0.25	0.750 ± 0.163
5	0.67	0.58	0.455 ± 0.156
6	0.63	0.73	0.445 ± 0.152
7	0.68	0.78	0.399 ± 0.155
8	0.65	0.80	0.465 ± 0.108
9	0.30	0.35	0.699 ± 0.262
10	0.22	0.29	0.854 ± 0.199
11	0.38	0.38	0.642 ± 0.107
12	0.27	0.31	0.679 ± 0.110
13	0.25	0.27	0.602 ± 0.131
14	0.71	0.64	0.162 ± 0.084
15	0.25	0.38	0.674 ± 0.091
15 bis	0.27	0.31	0.791 ± 0.314
15 ter	0.28	0.32	0.585 ± 0.158

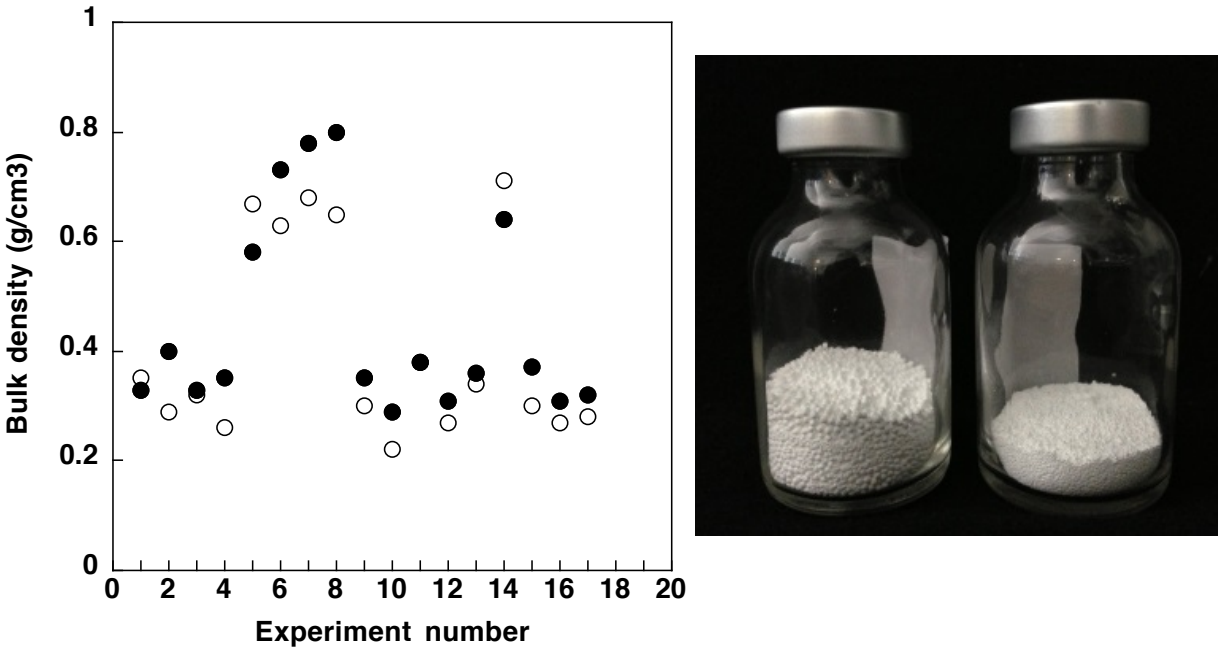

Figure 3.5. Bulk density of the spray-dried powders (Open circle: before agglomeration; Black circle: after agglomeration). In the picture agglomerates of powders #10 and #3

During the agglomeration process it was observed that the spray dried powder gave rise to distinct size groups of soft pellets, one with a diameter smaller than 0.5 mm (0.16 to 0.47 mm), (experiments # 5 to 8 and 14), and a second group with a diameter larger than 0.5 mm (experiments 0.58 to 0.85 mm) as reported in Table 3.3. Agglomerates obtained from powders prepared with feed solutions without ethanol belonged to the first group whereas the powders prepared with 5 or 10% of ethanol entered the second group (Figure 3.6). Having discovered that ethanol in the spray dried amikacin solution resulted in different particle morphology, it can be reasonably assumed that bulk density, water content and size of agglomerates are connected with ethanol presence in the feed solution.

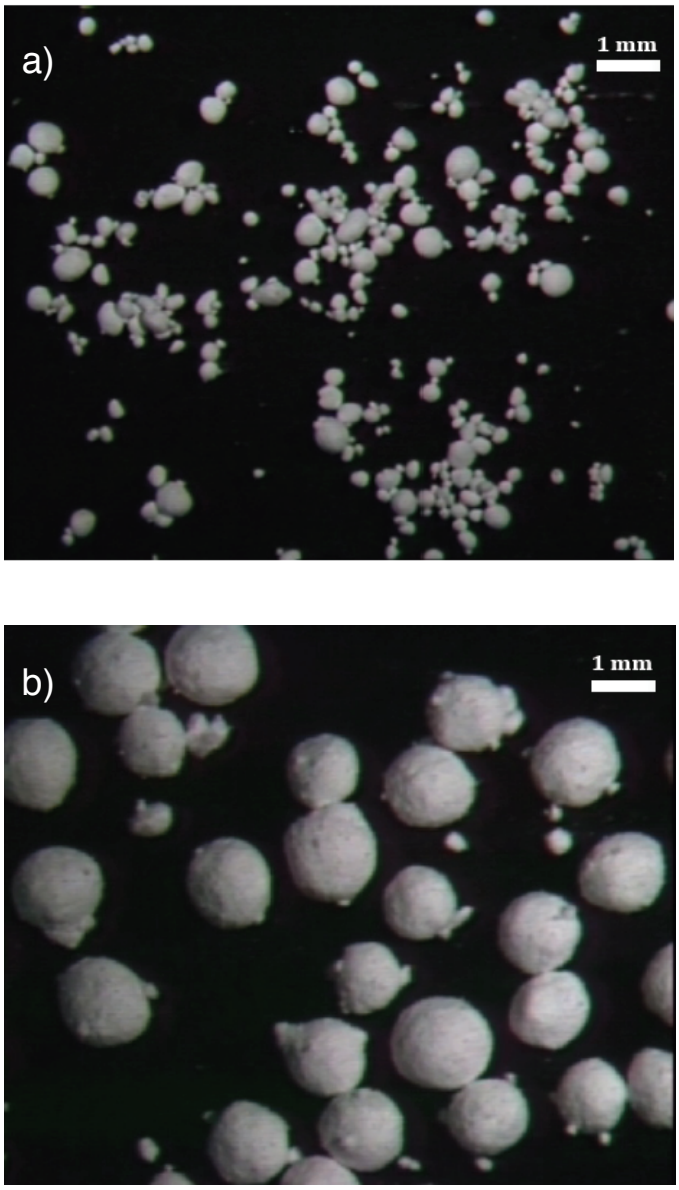

Figure 3.6. Optical microscope pictures of agglomerated powders: a) experiment #14, b) experiment #15 bis.

In summary, the agglomeration process, performed by short sieve vibration of powders, produced free flowing powders which facilitate dosing in the reservoirs of the inhalation devices.

3.3.3 Aerodynamic performance

The aerodynamic performance of the powders before and after agglomeration was tested *in vitro* using the Fast Screening Impactor. The values of Emitted Dose (ED) and Fine Particle Dose (FPD) obtained are shown in Table 3.4.

Table 3.4. Aerodynamic assessment of the spray dried powders. Emitted Dose (ED) and Fine Particle Dose (FPD), (n=3)

#	Before agglomeration		After agglomeration	
	ED (mg)	**FPD <5μm (mg)**	**ED (mg)**	**FPD <5μm (mg)**
1	8.80 ± 0.20	5.54 ± 0.59	8.47 ± 0.93	5.30 ± 1.00
2	8.60 ± 0.30	5.59 ± 0.17	7.53 ± 1.04	4.48 ± 0.91
3	8.93 ± 0.23	5.02 ± 0.88	7.83 ± 0.67	5.09 ± 0.92
4	8.77 ± 0.25	5.41 ± 0.46	9.27 ± 0.81	5.28 ± 0.42
5	7.27 ± 0.76	4.70 ± 0.44	6.77 ± 0.55	3.72 ± 0.14
6	7.77 ± 0.47	5.65 ± 0.21	7.70 ± 1.00	3.70 ± 1.10
7	7.00 ± 0.69	3.45 ± 1.14	8.43 ± 1.40	4.24 ± 0.17
8	7.23 ± 0.51	3.87 ± 0.79	7.53 ± 0.49	3.94 ± 0.80
9	8.63 ± 0.23	4.67 ± 0.83	7.93 ± 0.67	3.39 ± 0.27
10	8.93 ± 0.46	5.47 ± 0.53	8.17 ± 0.23	3.56 ± 0.34
11	8.10 ± 0.85	4.19 ± 0.20	7.87 ± 0.92	3.64 ± 0.43
12	8.83 ± 0.98	4.84 ± 0.79	8.53 ± 0.64	3.71 ± 0.50
13	7.80 ± 0.30	5.30 ± 0.39	7.87 ± 0.06	5.18± 0.51
14	7.87 ± 0.38	4.81 ± 0.48	6.80 ± 0.10	2.86 ± 0.35
15	8.47 ± 0.63	4.72 ± 0.62	8.67 ± 0.45	4.10 ± 0.65
15 bis	8.97 ± 0.60	4.98 ± 0.53	8.50 ± 0.17	3.97 ± 0.14
15 ter	8.43 ± 0.55	4.58 ± 0.53	9.10 ± 0.52	4.63 ± 0.47

The Emitted Doses of the powders and agglomerates studied in this work exceeded 72% in many cases, with few significant differences before and after agglomeration. However, it was noticed that the lowest ED values, both for powders and agglomerates, were found when powders were produced without ethanol in the feed solution.

FPD values before and after agglomeration ranged between 3.45 - 5.59 μm and 2.86 - 5.30 μm respectively. The highest FPD values were obtained for powders produced using a feed solution containing 10% of ethanol, which was also the optimum region identified for this factor in the previous fractional factorial design studying the process.

The graphs of Figure 3.7 illustrate the values of ED and FPD before and after agglomeration for each powder produced. From these graphs groups of powders and agglomerates having similar aerodynamic behavior, differentiated in dependence on the CPPs. were not easy to clearly discern.

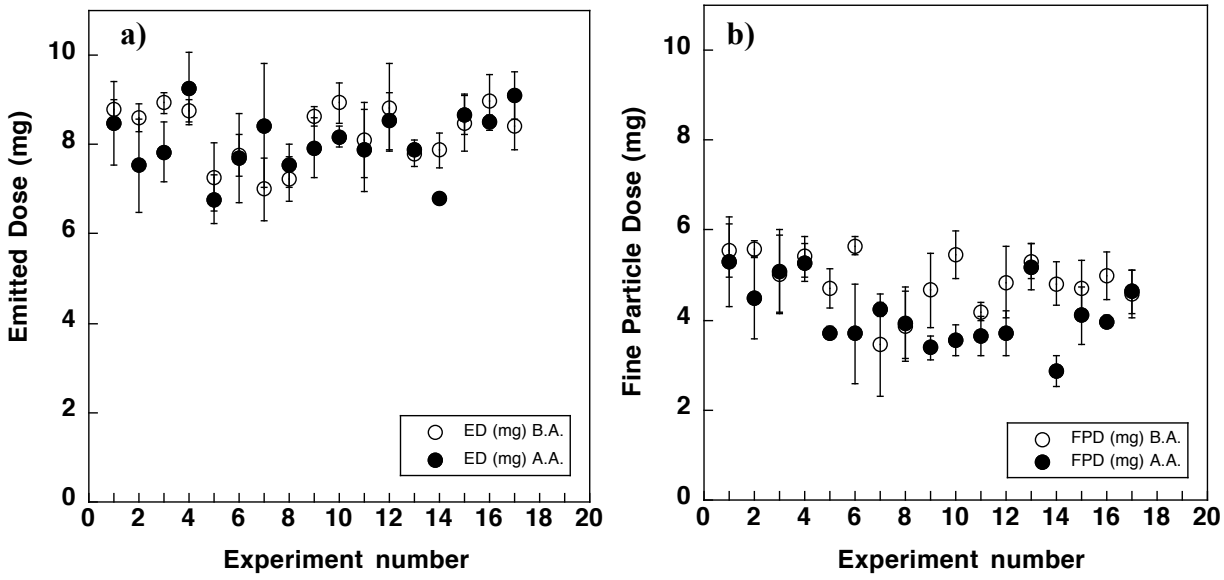

Figure 3.7. Emitted Dose (a) and Fine Particle Dose (b) of spray dried powders and agglomerates.

Instead, statistical analysis revealed the role of ethanol proportion in the feed solution. This is the major parameter influencing the powder aerodynamic behavior, in consequence of the variations in particle structure determined by the presence or absence of this solvent. This is depicted in the perturbation plots and tridimensional graphs on ED and FPD obtained from the design analysis (Figures 3.8 and 3.9).

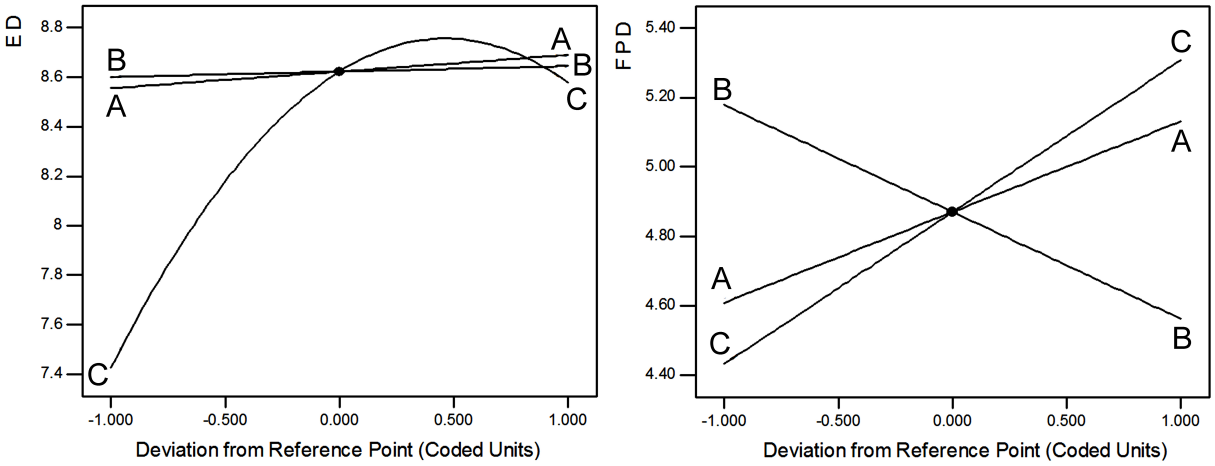

Fig.3.8: Perturbation plot for Emitted Dose (ED) and Fine Particle Dose (FPD). A: drying temperature; B: feed rate; C: Ethanol proportion

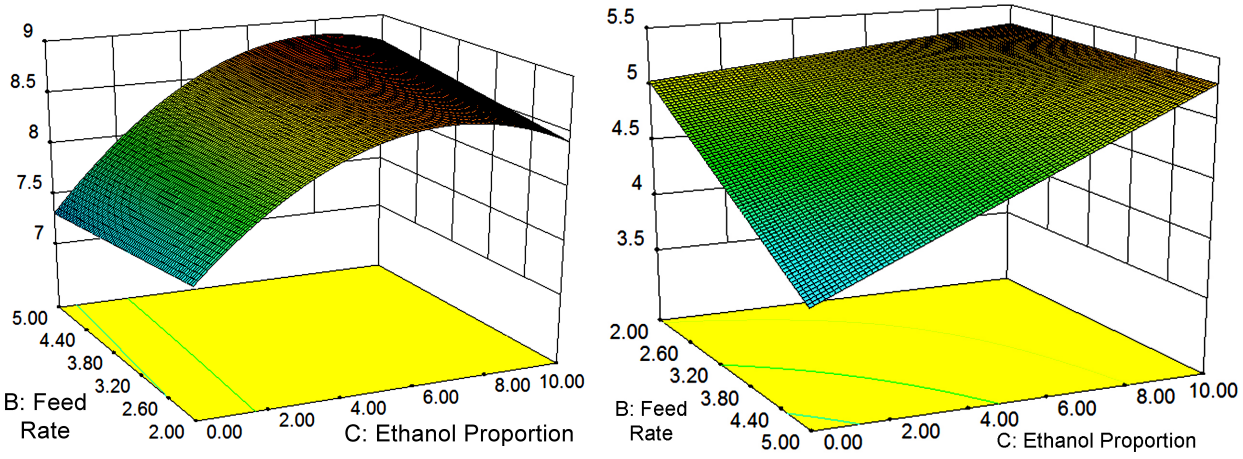

Figure 3.9. 3D plots for Emitted Dose (left) and Fine Particle Dose (right) as a function of feed rate and ethanol proportion at the medium level of drying temperature (165°C).

While ED is clearly affected only by ethanol presence, FPD is influenced by all three studied factors. In other words, it is evident that ethanol proportions govern ED values, irrespectively of the other two CPPs. Furthermore, a curvature occurs at high ethanol proportions towards the maximum of 10%. This was the optimum ethanol concentration also identified previously [20], in which its levels ranged between 10 and 20%, thus validating the former fractional factorial design.

At the same time, ethanol proportion in the same region promotes FPD, while the contribution of Feed Rate and Drying Temperature for this CQA is also significant.

As a result of the above, robust regions for the CPPs can be identified for optimizing both CQAs simultaneously. For instance, settings assuring high ED are located at 10% ethanol levels, where FPD also maximizes with appropriate adjustment of the other two CPPs, which in turn do not deteriorate ED, as the latter is practically unaffected from their changes.

3.3.4 Mechanism of particle formation

The amikacin sulphate particle formation mechanism can be studied by determining the Peclet number (P_e) applied to the evaporation of the sprayed droplets. P_e depends on the drying rate (k) of the droplet and the diffusion coefficient (D) of drug in the droplet solution, according to the following equation:

$$P_e = \frac{k}{8D} \qquad \text{(equation 1)}$$

where k is the evaporation rate constant in cm^2s^{-1} and D is the diffusion coefficient of dissolved substance in the solution.

When $P_e \leq 1$, the diffusion velocity of drug molecules in the droplet is faster or of the same magnitude of the drying rate. In this case, if the solute has a large solubility in

the solvent, during the evaporation process drug precipitation is delayed, leading to dense particles.

When $P_e > 1$, drying rate is faster than diffusion rate of solute molecules which accumulate and precipitate at the droplet surface, leading to empty shell particles [9].

In this work, evaporation rates of amikacin sulphate in the different feed solutions have been determined by TGA. Solutions containing amikacin show a slower evaporation rate compared to the solvent mixtures. The profiles of mass fraction evaporated versus time were linear and the slope was measured as s^{-1}. Since for P_e calculation the evaporation rate constant is measured in surface over time units (cm^2/s), the slope of the evaporation curves (1/s) was multiplied by the evaporating area exposed in the TGA pan (0.26 cm^2) which remained constant during the analysis. In the Figure 3.10 the evaporation curves obtained from TGA analysis on the amikacin feed solutions are show.

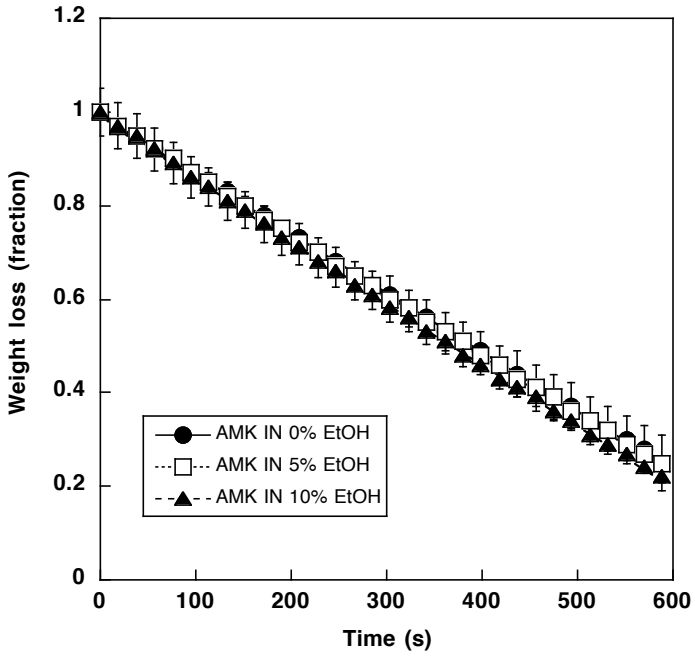

Figure 3.10. Evaporation curve of the solution made with amikacin in water:ethanol mixtures

The values obtained are shown in Table 3.5.

The amikacin coefficient of diffusion was calculated at 298K using the following equation [11]:

$$\text{Log } D = -4.113 - 0.4609 \text{ logMw} \qquad \text{(equation 2)}$$

D value of amikacin sulphate in water was determined as 3.58 10^{-6} cm^2 s^{-1}. Then, assuming that the temperature of evaporating solution equals the outlet temperature (85°C, i.e. 358 K) of the spray drying process and applying the Stokes-Einstein equation [12], D value at 85°C was determined. Disregarding the presence of ethanol, D approximated equal to 1.30 10^{-5} cm^2 s^{-1}.

The P_e values obtained in this study were higher than 1 (Table 3.5) and not significantly modified by the ethanol presence. This indicates that molecules did not diffuse to the inner part of the droplet because the evaporation rate was faster than diffusion. Thus, amikacin sulphate particle formation was described as a fast recession of the droplet surface with the precipitation of solute at the surface, resulting in formation of a shell and a void particle. SEM pictures confirmed this predicted formation of void particles. In the particle pictures, the shell of some broken particles and differences in size depending on the feed solution composition are clearly visible. In fact, the particles obtained from the feed solution without ethanol were smaller, shriveled and evidently empty. On the contrary, numerous swollen and often exploded particles have been obtained from the feed solutions containing ethanol.

The formation of these different particle populations has to be attributed to the different amikacin solubility. Amikacin sulphate is freely soluble in water but

practically insoluble in ethanol. The measured solubility of amikacin sulphate in the solvents and their mixtures used is shown in Table 3.5.

Table 3.5. Ethanol:water ratio, mean slope of the TGA straight lines, evaporation rate constants (k), Peclet numbers and solubility of amikacin sulphate in the feed solution solvents

EtOH: Water	Slope (s^{-1})	k (cm^2/s)	Peclet Number	Amikacin Sulphate solubility (mg/ml)
0 : 100	$1.27\ 10^{-3}$	$3.30\ 10^{-4}$	3.17	309 ± 2
5 : 95	$1.28\ 10^{-3}$	$3.33\ 10^{-4}$	3.20	298 ± 3
10 : 90	$1.32\ 10^{-3}$	$3.43\ 10^{-4}$	3.29	104 ± 3
100 : 0	-	-	-	$3.6\ 10^{-3} \pm 0.4\ 10^{-3}$

The presence of several large particles in the powders made from ethanol:water solutions was justified by considering that amikacin sulphate dissolved in water droplets precipitated at the surface later than in droplets containing ethanol. This is due to the solubility of amikacin sulphate in water higher than in the mixtures with ethanol. Thus, ethanol, decreasing the drug solubility, anticipated its surface precipitation and promoted the formation of swollen, void, often exploded microparticles due to the vapor tension of the ethanol entrapped inside the particle. Particles obtained from the feed solution without ethanol are also void but remain smaller.

3.4 Conclusions

The study concluded that, using a Central Composite Design including new combinations of the three selected spray drying process and formulation parameters, no further aerodynamic improvement of powders and agglomerates was observed, compared to the previous design.

In this study, the role of ethanol in amikacin sulphate solution to be sprayed was identified as crucial in particles' formation. The solubility of amikacin and the evaporation rate of the solution had a critical effect on the structure of the particles obtained. Large particles with low aerodynamic diameter and enhanced respirability were obtained due to the presence of ethanol in solution, in particular close to 10%. This confirms the optimum region identified through application of statistical tools in a previous study. Here, Peclet number and drug solubility in the spraying solution helped to understand the formation mechanism of these amikacin sulphate spray dried particles. The presence of ethanol in the spray dried solution contributed to the appearance in the powder of swollen from inside, empty, often exploded large particles due to the faster precipitation of amikacin in the drying droplet and to the pressure of ethanol entrapped into the shell particle. These particles benefit the aerodynamic performance of the amikacin inhalation powders.

3.5 References

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Chapter 4. Spray dried amikacin powders for inhalation with sodium stearate obtained from a basic feed solution tested with different device types

This study intended to improve the properties of an amikacin spray dried powder, patent in 2010, both in terms of respirability and stability. Three variables of the solution to spray were investigated: the pH of the solution, the ethanol concentration and the presence of sodium stearate. An objective of this work was to find the best conditions in order to keep stable the amikacin solution during the spray drying avoiding the precipitation of sodium stearate as stearic acid. The aerodynamic performance of the amikacin powders obtained was assessed using two types of inhaler: RS01 (a single unit capsule device) and Twincer® (a single unit disposable device).

The feed solutions at 45°C having pH 9.0 and 8.5 did not separate stearate particles during the spray-drying. The sodium stearate (1% w/w) and ethanol (30% v/v) shown an important role in the formation of particles with a high emitted dose and fine particle dose. The powders obtained from the feed solutions at high pH and with sodium stearate (batch #2 A_St_9 and batch #4 A_St_8.5) showed the best dispersion performance with the RS01; however, also the performances with the Twincer® device were interesting considering that device was not developed for aminoglycosides.

4.1 Introduction

In the treatment of respiratory infections associated with cystic fibrosis (CF), pulmonary antibiotic delivery allows to directly reach the bacteria in the airways where its action is needed. With inhaled antibiotics it is possible to limit the disadvantages of the systemic route, while obtaining a higher local drug concentration on the lung epithelium. Despite this advantage, the management of pulmonary infections with antibiotic inhalation is performed with relatively high local payloads of drug for the lungs to achieve an effective drug concentration. Commonly, these high doses for pulmonary administration require the use of nebulizers or dry powders inhalers (DPIs). Since 2009 tobramycin, an aminoglycoside antibiotic, has been developed as solution for nebulization for the management of pulmonary infections caused by *Pseudomonas aeruginosa* in CF patients by inhalation [1-3]. In addition, a liposomal formulation of amikacin for nebulization is under development (Arikace®, Insmed). Arikace® has been granted an orphan drug designation in the USA by the FDA and in Europe by EMA for the treatment of Pseudomonas infected patients with CF [4,5].

DPIs offer many advantages over nebulizers such as their portability, which allow a quick and easy administration of the dose everywhere. Nebulisation takes more than fifteen minutes (twice times daily in CF infections), whereas a single dry powder inhalation takes few minutes. Otherwise, nebulizers need regular cleaning and adequate disinfection to prevent contamination with microorganisms. Compared to liquid nebulization, the use of DPIs, in addition to a reduction of administration time, leads to a decrease of the labelled dose, since the drug loss in the nebulizer DPI, in the exhaled air and or in the environment is limited. Only two DPIs containing

antibiotic are currently available on the market: Tobi PodHaler® (Novartis, Switzerland) containing tobramycin and Colobreathe® (Forest, UK) containing colistimethate sodium [6].

There are basically two different strategies to design and develop an efficient dry powder product for inhalation. The first one is to select a suitable commercially available inhaler, such as the Aerolizer® (single dose capsule inhaler) and engineer the powder into a drug formulation that can effectively be released and dispersed with the inhaler to give a high fine particle dose. Particle engineering strategies are used to control the aerodynamic properties of the powder in order to improve flow properties and to achieve the best DPI performance in terms of maximising the respirable fraction [7-9]. A strategy used to prepare, by spray-drying, a tobramycin powder with a high aerosolization efficiency and protection from the environmental humidity was described by Parlati et al. [10,11]. This strategy used a small amount of a fatty acid salt (1% w/w), so the amount of powder to inhale for the patient was strongly reduced compared to the PulmoSphere™ formulation [12]. The tobramycin powder obtained had a fine particle fraction of 84.3% compared to the 27.1% of the powder without the excipient. This improvement of dispersability of the powder was attributed to the accumulation of a molecular layer of sodium stearate on the particle surface during the drying process. The same technology was applied to other aminoglycosides such as amikacin. Amikacin spray dried powder prepared accordingly to Parlati et al. showed a geometric diameter of 3.28 μm and an aerodynamic diameter of 4.6 µm with a FPF of 41.7% [10].

The alternative strategy to develop an efficient dry powder product for inhalation is to use the pure drug, so eliminating the excipients, by means of air jet micronization or

spray drying. This requires the design of an inhaler that meets the specific requirements for effective dispersion of the pure drug. An example is the Twincer® device developed at the University of Groningen for colistin sulphomethate. The Twincer® is a single dose disposable inhaler using air classifier technology for powder dispersion [13,14]. Briefly, the inhaler is made of three parts and a blister strip containing the powder formulation. The formulation sealed inside the blister is protected from the environment which is an important aspect for hygroscopic powders like tobramycin. The three parts of the inhaler are plate-like and when they are assembled the passages for the powder flow are constituted. During inhalation, after the blister is opened by pulling the cover foil, the air passes through the blister and entrains the powder which is divided into the two circular classifiers. It is possible to add to the powder formulation a small amount of sweeper crystals (lactose with a size between 150 and 200 μm) to avoid the adhesion of the drug particles to the classifier walls. The sweeper crystals are retained inside the classifiers because they are larger than the cut-off diameters of the classifiers. So, the sweeper crystals are not inhaled by the patient because they stay entrapped inside the inhaler. Pilot studies with the colistin sulphomethate dry powder Twincer® inhaler in healthy volunteers and patients (25 mg dose) was assessed to evaluate its feasibility and to make a comparison with a single 160 mg nebulizated dose of colistin sulphomethate. The colistin DPI was well tolerated and appreciated by CF-patients but required of some optimization regarding the dose to reach the equivalent pulmonary deposition to the solution for nebulization [14]. Another DPI device designed by the University of Groningen has been recently patented to administer aminoglycosides. This device, called Cyclops, has been developed for the administration of high payloads of pure

drugs such as tobramycin, amikacin and kanamycin.

The purpose of this part of the thesis work was to improve the properties of the amikacin spray dried powder previously described [10] both in terms of dispersibility and stability. Three variables of the solution for spray drying were investigated: the acidity of the solution, the presence of sodium stearate and that of ethanol, needed to dissolve the excipient. An objective of this work was to find the best conditions for keeping the amikacin solution stable during the spray drying by avoiding the precipitation of sodium stearate as stearic acid. The aerodynamic performance of the amikacin powders obtained was assessed using two types of inhaler: RS01 (a single unit capsule device) and Twincer® (a single unit disposable device).

4.2 Materials and methods

4.2.1 Materials

Amikacin sulphate was obtained from ACS DOBFAR S.p.a. (batch 99016700132, Milan, Italy) and sodium stearate was supplied by Magnesia GMBH (batch 170641, Lüneburg, Germany). Sodium tetraborate decahydrate and picrylsulfonic acid solution 1M (TNBSA) were purchased by Sigma-Aldrich (The Netherlands). All solvents were of analytical grade.

Hard hydroxy-propyl methylcellulose (HPMC) capsules (size 3) were donated from Capsugel (Colmar, France). The RS01 Dry Powder Inhaler (DPI) device (Aerolizer® - like type, but with a high resistance to air flow) was a gift of Plastiape S.p.a. (Osnago, LC, Italy). The Twincer® and Cyclops DPIs were provided by University of Groningen (Groningen, The Netherlands) [13,16].

4.2.2 Preparation of spray dried powders

Amikacin sulphate dried powders were prepared by spray drying using a technology previously described [12,13]. Briefly, amikacin sulphate dissolved in water had a pH around 3.5. The acidity of the solution was decreased according to the values reported in Table 4.1 by adding as much 10 M NaOH as needed to achieve the desired pH. Sodium stearate, when it was included in the formulation, was dissolved in ethanol. The aqueous drug solution and alcoholic stearate solution were heated to 45°C and mixed together before spray drying in the ratio reported in Table 4.1.

The solid content in the solution to spray was maintained at 1% w/v and the each solution was dried using a Büchi Mini Spray Dryer B-290 (Büchi Labortechnik Flawil, Switzerland) coupled to a B-296 dehumidifier. The aspirator rate and atomizing air

rate were kept constant at 100% of the total capacity and at 600L/h, respectively. The drying temperature was set at 150°C and the nozzle feed rate at 3.5 ml/min. Finally, nozzle cleaning interval was adjusted at level 5 (one pressure blow every 7 s). The spray-dried powder was quantitatively recovered from the product collection vessel, weighed on an analytical balance (sensitivity 0.1 mg) (Mod. E50S, Gibertini, Italy) and the yield of the production expressed as percentage of the amount of solid dissolved in the sprayed solution.

Table 4.1. Composition of the amikacin sulphate powders prepared by spray drying: sodium stearate content, water:ethanol ratio and pH of the solution spray dried.

#	Batch	NaSt(w/w %)	Water:Ethanol	pH
1	A_St_3.7	1	70:30	3.7 (not modified)
2	A_St_9	1	70:30	9.0
3	A_St_8	1	70:30	8.0
4	A_St_8.5	1	70:30	8.5
5	A_St_8.5_20	1	80:20	8.5
6	A_8.5	0	70:30	8.5
7	A_8.5_0	0	100:0	8.5

4.2.3 Preparation of amikacin agglomerates

Amikacin spray dried powder agglomerates were prepared according to the procedure described by Belotti et al. [15]. Briefly, the spray dried powder was placed on the top of a stack of two sieves with nominal apertures of 600 µm and 106 µm respectively (Endecotts Ltd, London, UK). Agglomerates retained between 600 and 106 µm after 5 min of vibration were collected and the process repeated twice.

4.2.4 Powder and agglomerate characterization

4.2.4.1 Scanning electron microscopy

The morphology of the spray dried powders was assessed by Scanning Electron Microscopy (SEM) (Sigma HD, Carl Zeiss, Germany), at 1.00 kV. Samples were placed on a double-sided adhesive tape pre-mounted on an aluminium stub and analysed after 30 min depressurization.

4.2.4.2 Particle size distribution

Particle size distributions of the spray dried powders was determined by laser diffraction (SprayTec, Malvern, UK). Approximately 10 mg of sample were dispersed in 20 ml of a solution of 0.1% (w/v) of Span 80 in cyclohexane and sonicated for 5 min. Particle size distribution was measured in triplicate with an obscuration threshold of 10%. Data were expressed in terms of median volume diameter and percentiles, $D_{(v,0.1)}$, $D_{(v,0.5)}$, $D_{(v,0.9)}$.

4.2.4.3 Termogravimetric analysis (TGA)

TGA was performed with a TGA/DSC (METTLER Toledo, USA). The samples were placed in 70µl alumina pans with a pierced cover and heated under a flux of N_2 (180 ml/min) from 25°C to 170°C at 10°C/min.

4.2.4.4 Bulk density

The bulk density was calculated as g/cm^3 from the ratio between the mass of powder sample and its unsettled apparent bulk volume. The apparent volume was directly measured inside the cylindrical glass vial used for storage (capacity 25 ml), by

calculation from the diameter and height of the powder bed in the container.

4.2.5 In vitro aerodynamic assessment

4.2.5.1 Dispersion performance in the RS01 DPI

The aerodynamic assessment of all the powders produced before (B.A.) and after agglomeration (A.A.) was carried out using the Next Generation Impactor (Copley Scientific, UK). The induction port was connected to the system and on each stage (1 to 7) a glass microfiber filter (diameter 50 mm, GE healthcare life sciences, Whatman), soaked with 1 ml of the 0.05M Borax buffer, was placed.

10 mg ± 0.1 of powder, accurately weighed, was manually introduced into a size 3 hard HPMC capsule and the content was next aerosolized using the RS01 device. The device was connected to the NGI and operated for 4 s at 60 L/min (corresponding to a pressure drop of 4 KPa through the device). Each powder was tested in triplicate.

Mass Median Aerodynamic Diameter (MMAD) and Fine Particle Dose (FPD) were calculated according to the USP38.

Emitted Dose (ED) was quantified by subtracting the chemically measured amount of drug retained inside the device and the capsule/blister from the loaded dose. It was next expressed as percentage of the loaded dose of drug.

4.2.5.2 Investigation of loading dose on aerodynamic performance

In order to assess the effect of the loaded dose on the *in vitro* respirability, increasing powder amounts were loaded and aerosolised using RS01 and Twincer® device. In detail 10, 20 or 30 mg of 2 selected batches were loaded after agglomeration inside

the device reservoir (capsule or blister) and aerosolized inside the NGI adopting a air suction flow rate in order to have a 4 KPa pressure drop.

4.2.5.3 Investigation of pressure drop effect on aerodynamic performance

The influence of the pressure drop across the device was studied by aerosolizing the two selected amikacin powders (experiments #2 and #4 both agglomerated, composition reported in Table 4.1) inside the NGI at 2, 4, and 6 KPa. In particular the flow rate adjusted was 40, 60 and 70 L/min for the RS01 and 35, 55 and 65 L/min for the Twincer®, respectively.

4.2.5.4 Chemical analysis of amikacin

0.05M Borax Buffer was used to dissolve the amikacin powder deposited on the stages of the NGI. The samples collected from the stages 1 to 7 were individually filtered (0.2 μm filters, cellulose acetate, Whatman). 1 ml of a 0.01% w/v TNBSA-solution was next added to 2 ml of each sample. After 2 hours rest at room temperature 0.5 ml of 1M HCl was added to each sample, to stop the colouring reaction. The absorbance of the samples placed in polystyrene cuvettes was measured at 340 nm (Unicam UV500 spectrophotometer, thermo electron-visionpro software V4.20)

4.3. Results and discussion

Batch #1 (named A_St_3.7) is the amikacin powder prepared according to the patent of Buttini et al. [10]. To avoid the precipitation of sodium stearate as stearic acid in the amikacin solution during the production process, the temperature of the drug solution fed to the spray drier was increased (from 30°C to 50°C) and the pH of 3.7 of the solution was increased to slightly alkaline values. In detail, Batch #2 A_St_9, batch #3 A_St_8 and # 4 A_St_8.5 had the same composition of the patented powder (1% w/w of sodium stearate and water:ethanol ratio 70:30) but different pH of the drug solution, respectively 9.0, 8.0 and 8.5. The last batches also had a pH of 8.5 but different compositions. Batch #5 A_St_8.5_20 had 20% of ethanol instead of 30% for the other batches, batch #6 A_8.5 is without sodium stearate and batch #7 A_8.5_0 is without sodium stearate and without ethanol. The pH was kept constant at 8.5 for these last batches because no precipitation at this pH occurred during the spray-drying process. From these choices, the influence of pH and that of the presence of excipient and ethanol on dispersion could be investigated.

The yield of the spray drying process for all samples was higher than 70%.

Values for the loss on drying (LOD) for the spray dried powders from TGA are shown in Table 4.2. The highest value is for the powder prepared from the feed solution with non-adjusted pH. A relatively high value was also obtained for the powder prepared from the feed solution without excipient and without ethanol, as previously reported [15,17]. The lowest value was obtained from the powder prepared with the highest pH in the feed solution.

The particle size distributions of the powders produced were expressed in terms of 10, 50 and 90% volume diameters. All spray dried powders obtained showed volume

median diameters, $D_{(v,50)}$, between 2.8 and 1.6 µm, which suggests that they are suitable for pulmonary administration (Table 4.2).

The agglomeration increased the bulk density of the powders (Table 4.2) and allowed to obtain free flowing powders easy to dose in the devices for inhalation.

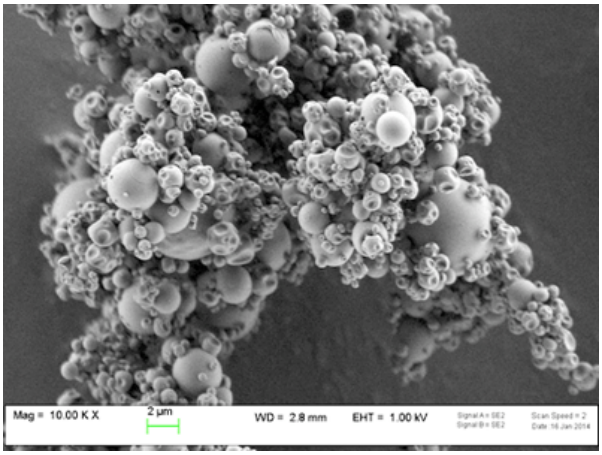
Table 4.2. Loss on drying (LOD) of the spray dried powders (n=3), particle size characteristics (n=3) and bulk densities before and after agglomeration.

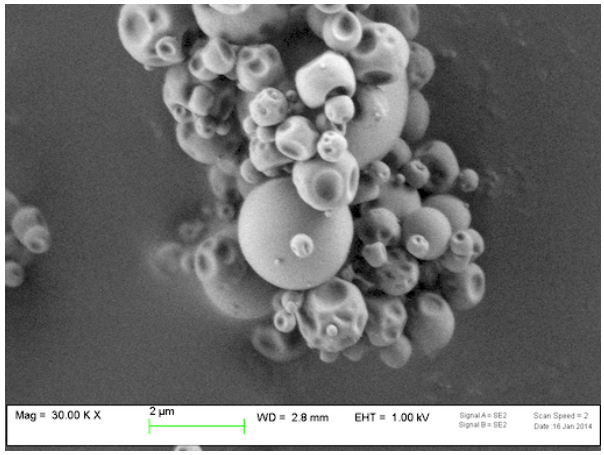
#	Batch	LOD (%)	Particle size analysis (µm)			Bulk density (g/cm^3)	
			D (v0.1)	D (v0.5)	D (v0.9)	Before Agglomeration	After Agglomeration
1	A_St_3.7	7.6 ± 0.2	1.0 ± 0.0	2.8 ± 0.4	6.7 ± 0.7	0.23	0.75
2	A_St_9	4.7 ± 0.1	0.8 ± 0.0	1.6 ± 0.1	3.8 ± 0.6	0.18	0.34
3	A_St_8	5.3 ± 0.2	0.8 ± 0.0	1.8 ± 0.1	4.3 ± 0.2	0.29	0.50
4	A_St_8.5	4.8 ± 0.2	0.9 ± 0.0	2.0 ± 0.1	6.0 ± 1.5	0.18	0.35
5	A_St_8.5_20	5.1 ± 0.0	0.9 ± 0.0	1.7 ± 0.1	4.9 ± 1.0	0.07	0.23
6	A_8.5	5.9 ± 0.0	1.3 ± 0.0	2.6 ± 0.0	6.3 ± 0.6	0.17	0.22
7	A_8.5_0	6.4 ± 0.0	1.5 ± 0.0	2.8 ± 0.1	5.1 ± 0.1	0.34	0.46

In Figure 4.1 the SEM pictures of the spray dried particles are shown. The presence of the excipient and 30% ethanol allowed to obtain unbroken particles as previously reported [10] for the molecular deposition of the sodium stearate on the particle surface (batch #1 A_St_3.7). The alkaline feed solution produced broken particles in presence of 30% v/v of ethanol and sodium stearate (batch #2 A_St_9, #3 A_St_8, #4 A_St_8.5). There are no significant differences between the samples obtained from 30% and 20% of ethanol in the feed solution (batch #4 A_St_8.5 and #5 A_St_8.5_20 respectively). It is possible to see in both samples large broken

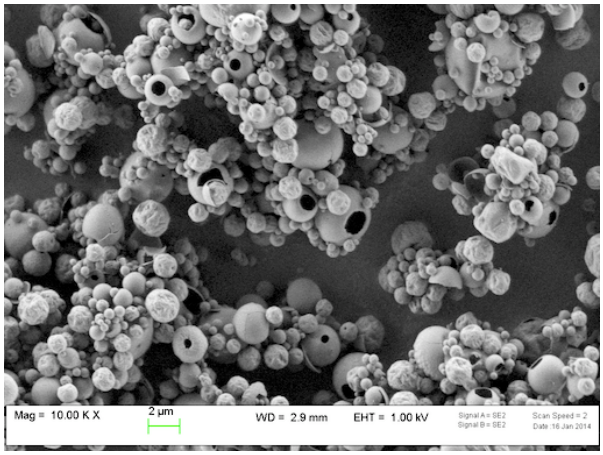
particles agglomerated with smaller particles. When ethanol was absent in the feed solution at pH 8.5 (batch #7 A_8.5_0) particles maintained their integrity. On the other hand, when sodium stearate is absent and ethanol present (at pH 8.5) the particles are partly broken (batch #6 A_8.5) as for all the other batches with ethanol and sodium stearate in the feed solution with adjusted pH. Furthermore, in this batch (#6 A_8.5), the larger particles are broken but the smaller are shriveled. In conclusion, ethanol did not result in fragmentation of the larger particles when there is sodium stearate in the formulation and the pH is not adjusted (#1 A_St_3.7). Ethanol and pH-adjustment contributed to fragmentation of particularly the larger particles in the presence of the fatty excipient (#2 A_St_9, #3 A_St_8, #4 A_St_8.5, #5 A_St_8.5_20). Without sodium stearate but with ethanol and adjusted pH (#6 batch A_8.5), large broken particles together with small shriveled particles were obtained. When sodium stearate and ethanol were both absent (#7 A_8.5_0), all the particles became shriveled.

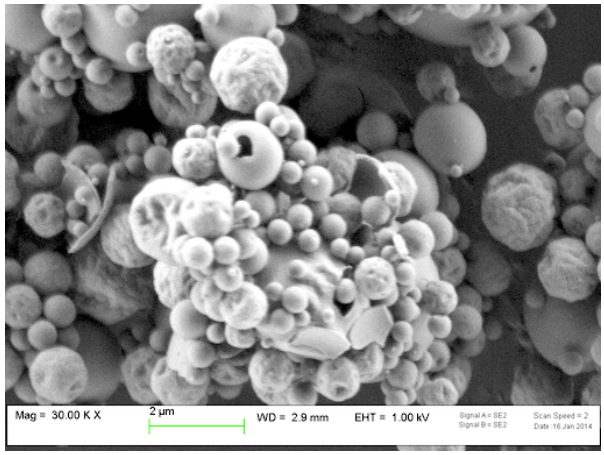
Batch #1 A_St_3.7



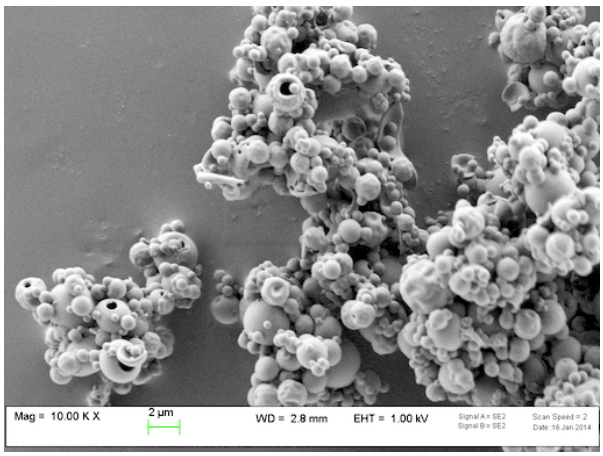


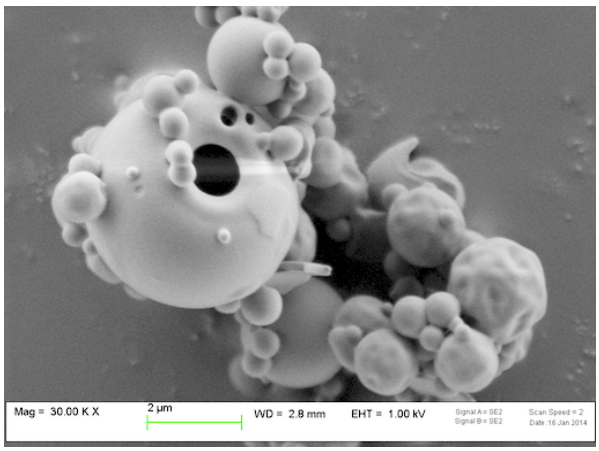
Batch #2 A_St_9



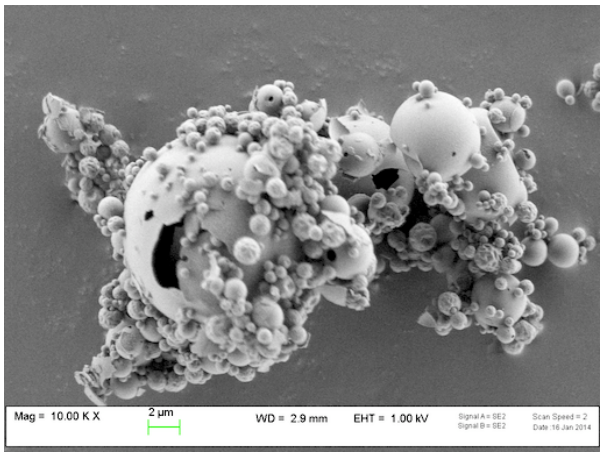


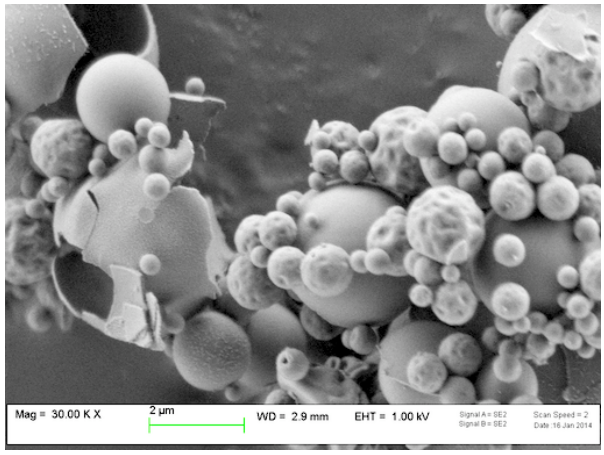
Batch #3 A_St_8



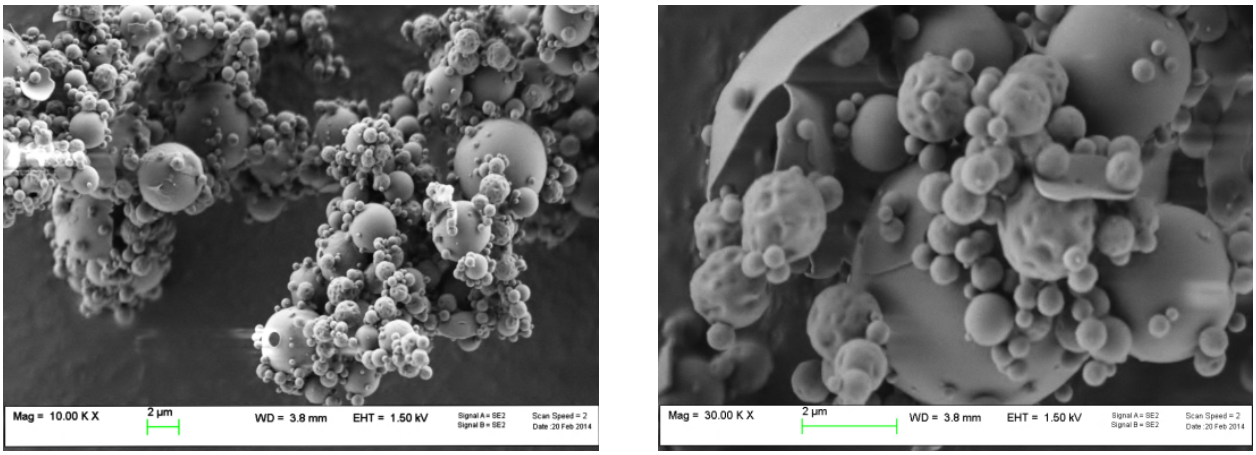


Batch #4 A_St_8.5

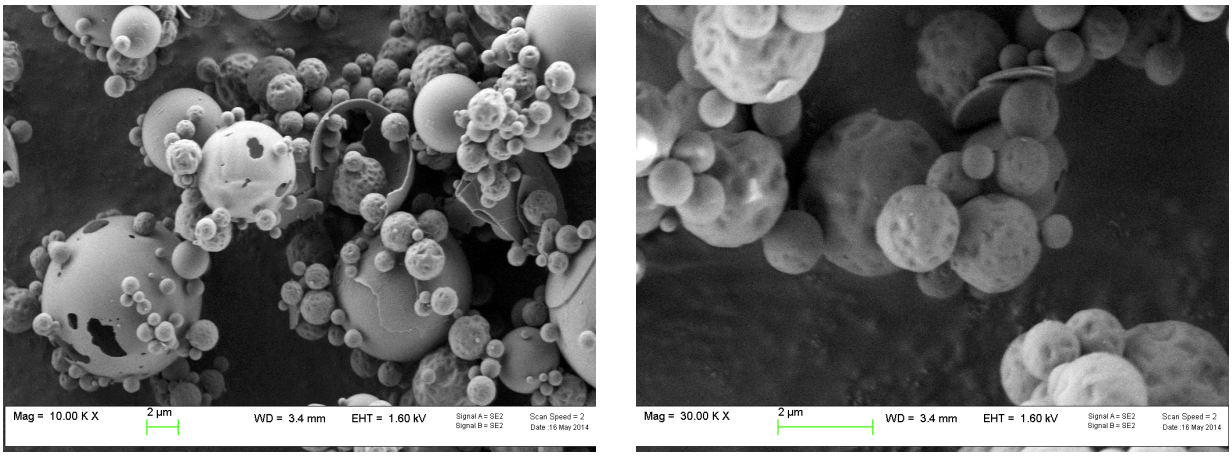




Batch# 5 A_St_8.5_20



Batch #6 A_8.5



Batch #7 A_8.5_0

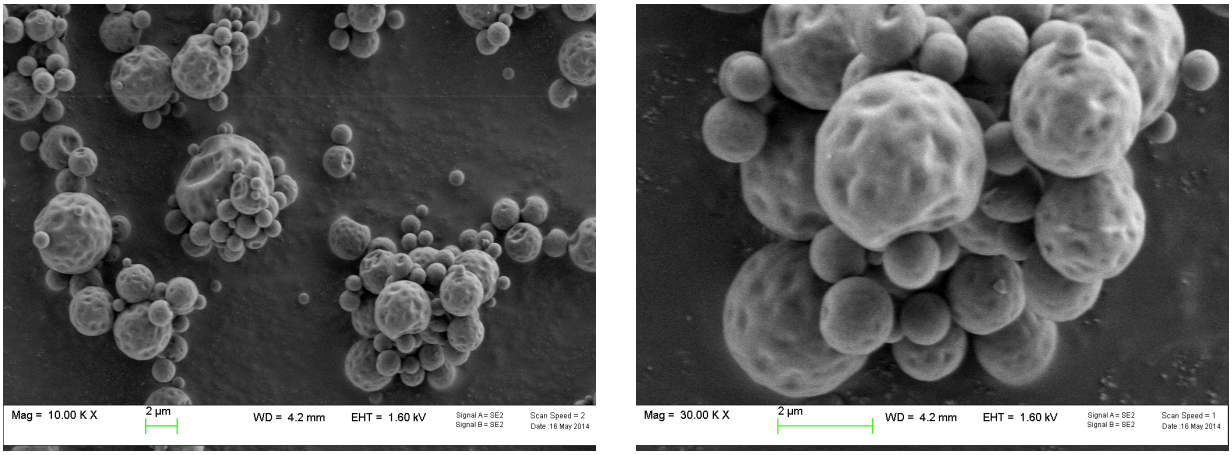


Figure 4.1. SEM pictures of the spray dried powders.

4.3.1 Delivered dose and in vitro aerodynamic assessment

4.3.1.1 Delivered doses from the RS01 device

The assessment of the dispersion performance of all the batches was performed in order to study the influence of sodium stearate, ethanol and pH on the delivered dose. RS01 was the device used for this investigation and it was loaded with a fixed amount of the powder (10 ± 0.1 mg) weighed into an HPMC capsule size 3. In the Table 4.3 the emitted dose (as percent of loaded dose) values of all the batches are shown.

Table 4.3. Emitted Dose (%)

#	Batch	Emitted Dose (%)	
		Before Agglomeration	After Agglomeration
1	A_St_3.7	72.6 ± 4.0	79.8 ± 2.9
2	A_St_9	87.1 ± 1.3	87.1 ± 4.5
3	A_St_8	77.6 ± 1.2	81.0 ± 3.9
4	A_St_8.5	80.9 ± 2.5	82.8 ± 2.3
5	A_St_8.5_20	91.4 ± 3.5	83.8 ± 0.5
6	A_8.5	79.0 ± 0.7	86.4 ± 0.2
7	A_8.5_0	77.0 ± 0.3	78.1 ± 5.2

The agglomeration of microparticles either improved, or did not cause significant changes in the emitted dose percentages. The batch #1 A_St_3.7 and #7 A_8.5_0 had a smaller ED (%) at first, but after agglomeration it became higher, whereas on the other hand batch #5 A_St_8.5_20 has a significantly lower ED after agglomeration. This was due to a different retention of the powders in the RS01 device after they were transformed in agglomerates as shown in the Figure 4.2a.

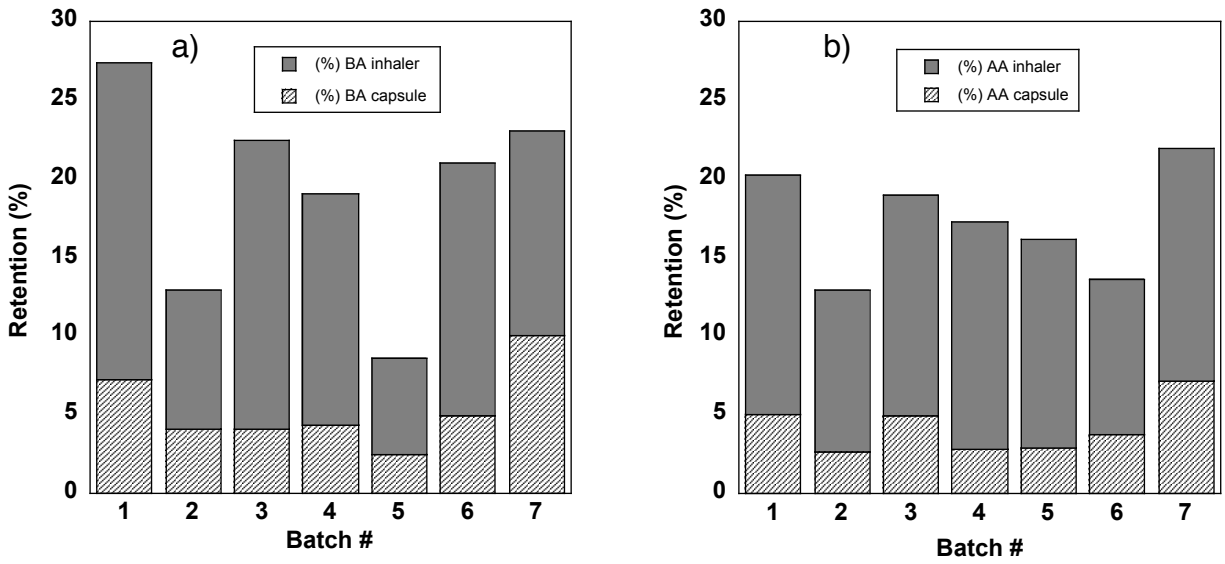


Figure 4.2. Retention in the capsule and in the RS01 device before (a) and after agglomeration (b).

In Figure 4.2 the retention in the capsule and device of powders before and after agglomeration is shown. The largest retention in the capsule for the set of powders before agglomeration was in batch # 7 A_8.5_0 (more than 10% of the loaded dose).

The device retention for batch #1 A_St_3.7 and batch #6 A_8.5 after agglomeration is significantly decreased. On the contrary the device retention of batch #5 A_St_8.5_20 increased from about 10% of the loaded dose to more than 15%.

In Figure 4.3 the retention in the throat (induction port to the NGI) for the all batches, before and after agglomeration, is shown.

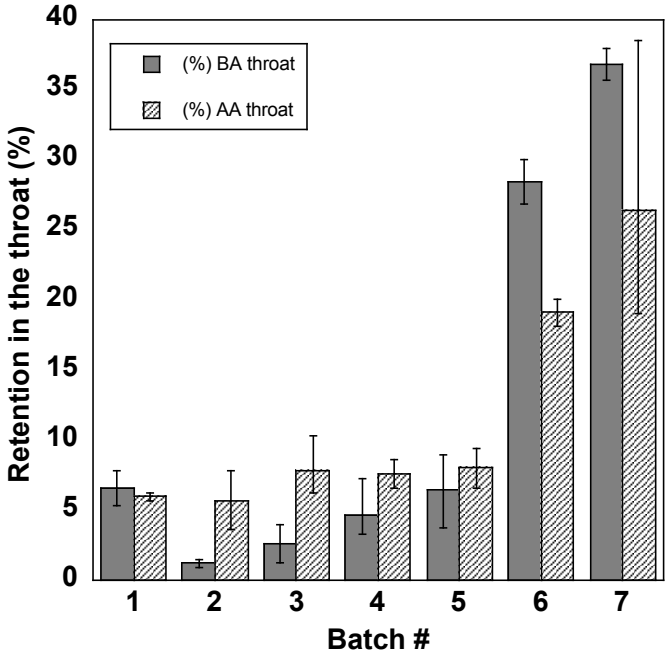


Figure 4.3. Retention of powder in the induction port of the NGI after aerosolization using RS01 device. The powder was loaded in the capsule as obtained from spray drying (BA) and after agglomeration (AA).

For the powder from a feed solution with unmodified pH (batch #1 A_St_3.7) the retention in the throat before and after agglomeration is the same, around 6% of the loaded dose. For the batches from #2 A_St_9 to #5 A_St_8.5_20 (feed solution with modified pH to values of 9.0 and 8.5 respectively) after agglomeration the retention in the throat increased. In contrast, batch #6 A_8.5 (amikacin spray-dried at pH 8.5 without excipient), after agglomeration, showed a reduction of the throat retention and batch #7 A_8.5_0 (without excipient and without ethanol) showed no significantly different behaviour before and after agglomeration. The retention in the throat for these two last batches was significantly higher than the retention for the other batches. The absence of sodium stearate (batch #6 A_8.5) in combination with the absence of ethanol appeared to increase the retention in the throat. The powder from the solution with unmodified pH (batch #1 A_St_3.7) did not behave different from the

powders obtained after the pH of the solution was adjusted to alkaline (batches #2 to #5).

4.3.1.2 Aerodynamic assessment using the RS01 DPI

The MMAD (*μ*m) and the FPD (mg) values from NGI analysis are shown in Table 4.4. The MMAD values obtained were between 2.4 and 4.4 *μ*m. The batches prepared from feed solutions containing sodium stearate and ethanol at 30% v/v had the lowest MMAD values (MMAD smaller than 3 *μ*m) and the best dispersion performance (highest FPD). The highest values for FPD were obtained with the powders from a feed solution with a modified pH containing both ethanol and sodium stearate. Agglomeration did not improve the dispersion behaviour of the powders when FPD was already high (FPD $\geq$ 4.7 mg). For powders with an FPD < 4.3 mg, agglomeration had a positive effect on dispersion.

Table 4.4. *In vitro* aerodynamic assessment of the batches before and after agglomeration (MMAD, Fine Particle Dose, n = 3). (BA= before agglomeration, AA=after agglomeration)

#	Batch	MMAD (*μ*m)		FPD (mg)	
		BA	AA	BA	AA
1	A_St_3.7	2.8 ± 0.3	2.6 ± 0.0	4.3 ± 0.2	5.1 ± 1.0
2	A_St_9	2.5 ± 0.0	2.4 ± 0.1	6.3 ± 0.1	5.9 ± 0.5
3	A_St_8	2.6 ± 0.2	2.4 ± 0.0	5.4 ± 0.3	4.5 ± 0.0
4	A_St_8.5	2.6 ± 0.3	2.6 ± 0.3	5.1 ± 0.4	4.8 ± 0.6
5	A_St_8.5_20	3.0 ± 0.1	3.1 ± 0.2	4.7 ± 0.7	4.3 ± 0.1
6	A_8.5	3.9 ± 0.0	4.2 ± 0.1	2.6 ± 0.4	3.4 ± 0.1
7	A_8.5_0	4.2 ± 0.1	4.4 ± 0.0	2.3 ± 0.0	3.3 ± 0.2

The results show that a favourable aerodynamic size is obtained in the presence of ethanol and the excipient in the feed solution, confirming previously presented data [10,11,17]. Bringing the pH to alkaline values also has a positive influence on the dispersion performance of the powders obtained in the presence of sodium stearate.

4.3.1.3 Effect of loaded dose on the dispersion performance with the RS01 DPI and Twincer® DPIs

In order to assess the effect of the loaded dose on the *in vitro* deposition, increasing powder amounts were loaded and aerosolised using RS01 and Twincer® devices. The agglomerated powders were chosen for these experiments because agglomeration improved the flowability and the handling of the powders, whereas it did not decrease their respirability.

The agglomerated batches with the best fine particle dose and emitted dose (ED) values were the #1 A_St_3.7, #2 A_St_9, #4 A_St_8.5 and #5 A_St_8.5_20. Batch #1 A_St_3.7 had the lowest ED in this selection and ED before agglomeration was lower than 75%. Batch #5 A_St_8.5_20 after agglomeration had the lowest bulk density of these selected powders, and as a result of that it was difficult to increase the loaded dose in HPMC capsule size 3. So, the batches #2 A_St_9 and #4 A_St_8.5 were selected for this part of the study.

The tests were performed for 10, 20 and 30 mg of powder. In Table 4.5 the emitted doses and aerodynamic parameters obtained are shown. There were not substantial differences in the aerodynamic parameters between the loaded doses for both of the powders tested. The emitted doses (%) from the RS01 were higher than 80% for both

batches at the different loading doses. The MMADs were between 2 and 3 μm for both the batches.

Because there were not great differences between these two batches, the influence of the loaded doses on the dispersion performance with the Twincer® device was studied only with batch #4 A_St_8.5 AA. With the Twincer® a sweeper (coarse lactose crystals, approximately 150 micron particles) was used to minimise powder accumulation in the device. The sweeper crystals do not leave the device and they are weighed into the blister on top of the powder.

Different amounts of the sweeper lactose were used: above the 10 ± 0.1 mg of powder, about 5 mg (ratio 2:1) or 10 mg (ratio 1:1) of sweeper crystals were used. The aerodynamic assessment of batch #4 A_St_8.5 AA without and with sweeper was investigated in triplicate. The use of the sweeper improved the dispersion of the powder but there were no significative differences between the two ratios used. Therefore, Table 4.5 shows only data for 10 mg of sweeper. The emitted dose (%) was higher than 70% The MMAD was the same as that from RS01 for this specific batch.

Table 4.5. Emitted Dose (ED (%)), MMAD (μm) and Fine Particle Dose (FPD (mg)) for agglomerated batches #2 A_St_9 and #4 A_St_8.5 at different loaded doses from RS01 and Twincer®, at a pressure drop of 4 KPa, n=3

Device	Batch	Loaded dose (mg)	ED (%)	MMAD (μm)	FPD (mg)
RS01	**A_St_9**	10	87.6 ± 3.9	2.4 ± 0.1	5.7 ± 0.7
		20	87.2 ± 2.4	2.6 ± 0.2	9.7 ± 2.0
		30	88.8 ± 0.9	2.7 ± 0.0	14.8 ± 0.7
RS01	**A_St_8.5**	10	83.2 ± 3.0	2.6 ± 0.3	4.8 ± 0.6
		20	85.5 ± 1.2	2.7 ± 0.1	9.7 ± 0.1
		30	86.9 ± 3.6	2.9 ± 0.3	13.34 ± 0.7
Twincer®	**A_St_8.5**	10	76.7 ± 2.9	2.6 ± 0.1	4.8 ± 0.2
		20	71.1 ± 12.3	2.8 ± 0.1	10.20 ± 2.6
		30	78.1 ± 1.9	2.7 ± 0.2	10.25 ± 0.7

The fine particle dose (FPD) obtained with the two different devices as function of the loaded dose for batch #4 A_St_8.5 AA is shown in Figure 4.4.

For the RS01, the FPD value increased linearly when higher amounts of powder were loaded in the capsule. The Twincer® did not show the same good result when 30 mg of powder was aerosolized with the device. Twincer® was designed for high doses and manages to disperse 50 to 60 mg of colistimethate sodium with excellent efficiency. The reason of these results is likely the low bulk density and the dispersion mode of these powders. When powders are very fluffy and break up quite easily and rapidly into primary particles, they overload the classifiers. This has the consequence that a high density cloud of small particles leaves the classifiers which

creates a drag for particles that are larger than the cut-off diameter of the classifier and this has a negative effect on the fine particle dose (and the MMAD of this dose). This could also explain the positive effect of the sweeper crystals on dispersion: they have a higher inertia and contribute to the tangential drag in the classifier chamber.

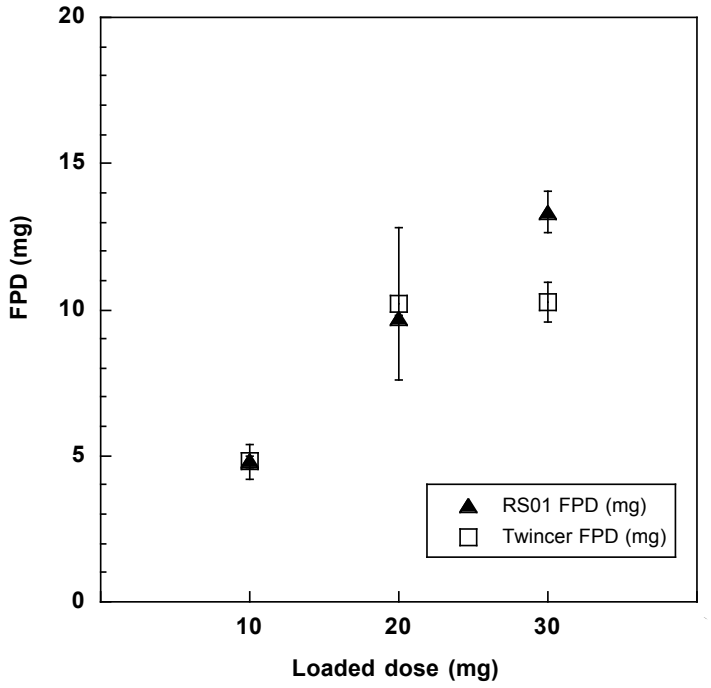


Figure 4.4. Fine Particle Dose (mg) of batch #4 A_St_8.5 AA with Aerolizer RS01 and Twincer at different loading doses.

4.3.1.4 Effect of the pressure drop on the dispersion performance

The influence of the pressure drop on dispersion efficiency was studied using the agglomerated powders from the batches #2 and #4 (#2 A_St_9 AA and #4 A_St_8.5 AA) with the RS01. The results obtained are shown in Table 4.6. The emitted dose (%) was lower than 75% for the powder #4 A_St_8.5 AA at 2 KPa. In comparison the powder #2 A_St_9 AA yielded an ED (%) of 79% at the same pressure drop. The fine particle dose (FPD) slightly decreased for batch #2 A_St_9 AA at 2 KPa. On the other hand the FPD of the powder #4 A_St_8.5 AA slightly increased at 2 KPa

compared to 4 KPa. For both powders, FPD was lower at 6 KPa than at 4 KPa. To study the influence of the pressure drop on the aerodynamic assessment with the Twincer®, only batch # 4 A_St_8.5 AA was used and the results obtained are shown in Table 4.6 too. For the Twincer® a decrease of the pressure drop to 2 KPa increased the variability in the delivered fine particle dose for batch # 4 A_St_8.5 AA. Furthermore with the Twincer® the pressure drop had a greater influence on the dispersion efficiency for powder #4 A_St_8.5 AA than the RS01: at increasing pressure drop, the dispersion performance for powder #4 A_St_8.5 AA was improved.

Table 4.6. Agglomerated batch #2 A_St_9 and #4 A_St_8.5 at different pressure drops, Emitted Dose (%), MMAD (*μ*m) and Fine Particle Dose (mg), Device: RS01 and Twincer®, Loading dose: 10 mg, n=3

Device	Batch	P drop (KPa)	ED (%)	MMAD (*μ*m)	FPD (mg)
RS01	**A_St_9**	2	79.4 ± 3.3	2.2 ± 0.2	5.3 ± 0.1
		4	87.6 ± 3.9	2.3 ± 0.1	6.0 ± 0.7
		6	86.5 ± 1.6	2.6 ± 0.0	5.5 ± 0.1
RS01	**A_St_8.5**	2	72.4 ± 3.8	2.5 ± 0.0	4.5 ± 0.1
		4	83.2 ± 3.0	2.6 ± 0.3	4.8 ± 0.6
		6	83.2 ± 2.6	2.7 ± 0.0	4.8 ± 0.1
Twincer®	**A_St_8.5**	2	39.4 ± 12.8	4.4 ± 2.0	1.1 ± 0.5
		4	75.9 ± 9.7	2.9 ± 0.1	3.5 ± 0.4
		6	81.8 ± 3.0	2.8 ± 0.3	5.0 ± 0.6

In Figure 4.5 the FPD for batch #4 A_St_8.5 AA obtained with the two different devices is shown. There is not a strong influence of the pressure drop on the FPD using the RS01 device which increases from 4.5 mg at 2 KPa to 4.8 mg at 6KPa with the RS01 device. Otherwise increasing the pressure drop results in an increase of FPD from 1.1 mg to 5.0 mg with the Twincer® device.

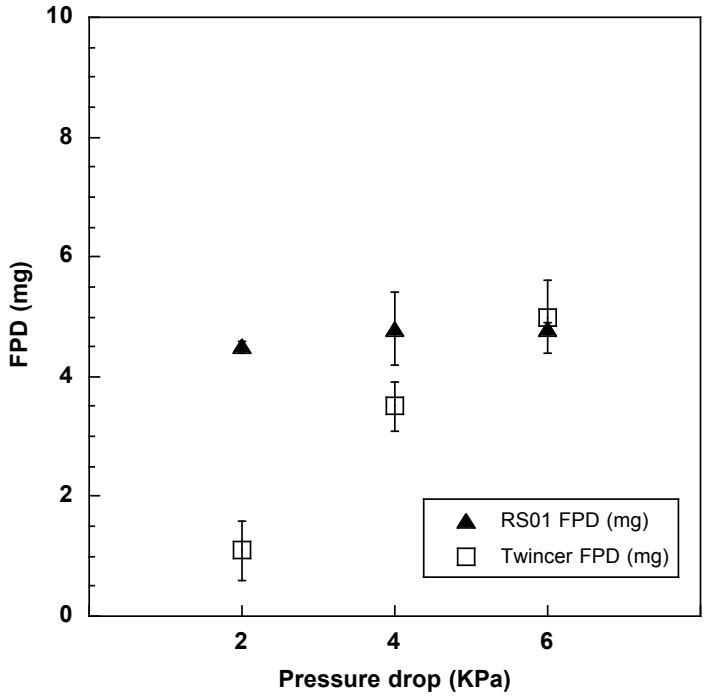


Figure 4.5. Fine Particle Dose (mg) of batch #4 A_St_8.5 AA with Aerolizer RS01 and Twincer at different Pressure drop.

4.3.1.5 Comparison of the RS01 with Twincer®

To make a further investigation on the device, the two devices were tested with three different batches after agglomeration (AA) being #4 A_St_8.5 AA (containing the excipient, ethanol in the feed solution and pH adjusted), #6 A_8.5 AA (without sodium

stearate, with ethanol in the feed solution and pH adjusted), #7 A_8.5_0 AA (without excipient and ethanol in the feed solution, pH adjusted).

In Table 4.7 the emitted doses and aerodynamic parameters obtained are shown. The emitted dose was always higher than 75%, the fine particle dose (FPD) was in all the cases lower with the Twincer® than with the RS01. The MMAD values are not significantly different for the two devices for the batch #4 A_St_8.5 AA and batch #7 A_8.5_0 AA. However, batch #6 A_8.5 AA yielded a larger MMAD value with Twincer® compared to the RS01.

The use of ethanol and sodium stearate improved the dispersion performance of the amikacin powder: batch #4 A_St_8.5 AA with both the devices produced a higher FPD and a smaller MMAD (ED is always higher than 75%).

Table 4.7. Agglomerated batch #4 A_St_8.5, #6 A_8.5 and #7 A_8.5_0. Loaded dose: 10 mg, Pressure drop: 4KPa, ED (%), MMAD (μm) and FPD (mg). Device: RS01 and Twincer (with 5 mg of sweeper), n=3

Batch	Device	ED (%)	MMAD (μm)	FPD (mg)
A_St_8.5	RS01	83.19 ± 1.83	2.4 ± 0.0	5.9 ± 0.9
	Twincer	75.85 ± 9.66	2.7 ± 0.1	3.9 ± 0.4
A_8.5	RS01	86.41 ± 0.16	4.2 ± 0.1	3.4 ± 0.0
	Twincer	83.69 ± 6.88	7.5 ± 2.0	1.0 ± 0.4
A_8.5_0	RS01	78.11 ± 5.19	4.4 ± 0.0	3.2 ± 0.2
	Twincer	83.85 ± 0.57	4.4 ± 0.1	2.1 ± 0.1

4.4. Conclusions

The feed solutions at 45°C having pH 9.0 and 8.5 did not separate stearate particles during the spray-drying process. The sodium stearate (1% w/w) and ethanol (30% v/v) showed to have an important role in the formation of the particles and their use resulted in high emitted doses and fine particle doses. The powders obtained from the feed solutions with a high pH and with sodium stearate (batch #2 A_St_9 and batch #4 A_St_8.5) showed the best dispersion performance with the RS01; however, also the performances with the Twincer® device were interesting considering that device was not developed for aminoglycosides.

The next step will be to study the aerodynamic behaviour of the batch #4 A_St_8.5 after agglomeration with Cyclops, the new device recently patented by the University of Groningen, and developed for the administration of aminoglycosides.

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Chapter 5: General Conclusions

The application of the half-fractional factorial design to the preparation of spray dried amikacin powders from drug feed solution with an unmodified pH (3.7) enabled us to obtain inhalation powders with both Emitted Dose and Fine Particle Dose, meeting the regulatory and scientific references. All desirable powders practically excluded the use of the PEG-32 stearate excipient. The finished product contained only the active ingredient meeting the target to minimize the quantity of inhalable powder for a fixed dose. No more advantages in the additional design space studied using the Central Composite Design have been found. The amikacin respirability did not show a significant improvement compared with the previous design space data.

The role of ethanol presence in amikacin solution to be sprayed was identified as crucial in particle formation. The solubility of amikacin and the evaporation rate of the solution had a relevant effect on the structure of particles obtained. Ethanol in the spray dried solution contributed to the appearance in the powder of large, empty, swollen often exploded particles, due to the earlier precipitation of amikacin in the drying droplet and to the pressure of ethanol entrapped into the shell particle.

The preparation of the spray dried powder was also studied in different conditions of pH and composition of drug feed solution. The amikacin solutions at 45°C with pH 9.0 and 8.5 were stable during the spray-drying and no precipitation of sodium stearate (1% w/w) occurred. The aerodynamic behaviour (Emitted Dose and Fine Particle Dose) of powders containing sodium stearate and ethanol shown the important role of these substances in the formation of particles. These powders tested with two devices RS01 (a single unit capsule device) and the Twincer® (a

single unit disposable device) showed a superior aerodynamic assessment with the RS01. Twincer® device performed interestingly considering that device was not developed for aminoglycosides.

A study on the aerodynamic assessment of amikacin powder with Cyclops, a new device recently patent by the University of Groningen and developed for the administration of aminoglycosides, is under way.

Chapter 6: Publications

During this PhD five abstracts were presented as posters to international congress. Three of them are about Self Emulsifying Lipid Formulation (SELF). They were presented at AAPS Annual Meeting and Exposition in San Antonio, USA (2013). This work was performed during the internship in Pharmaceutical Research and Development in Gattefossè (Saint-Priest, France) under the supervision of dr. Vincent Jannin. The purpose of the internship was to learn how to work in an R&D team and to improve the knowledge about lipid excipients and lipid-based formulation. One of the Gattefossè excipients studied there was used in the half-fractional factorial design. The experience in Gattefossè was the second experience performed in a pharmaceutical company after the time spent in Lisapharma S.p.a. for the Spinner scholarship.

In the same congress (AAPS 2013) also the work on the half-fractional factorial design was presented as a poster.

The work on the central composite design was presented as a poster at DDL 25 in Edinburgh, UK (December 2014).

In 2014 the work on the half-fractional factorial design was published.

In January 2015 the work on the Central Composite Design was submitted to European Journal of Pharmaceutics and Biopharmaceutics.

The third part of the thesis has to be discussed with the others authors and enlarged with the Cyclops experiments.

In the following pages the posters and the first page of the paper are shown.

SELF FORMULATION PROTOCOL: PART I - SOLUBILITY DETERMINATION IN LIQUID AND SOLID EXCIPIENTS

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1 - PURPOSE

The aim of this study was to develop a sound formulation protocol for Self Emulsifying Lipid Formulation (SELF) with the latest characterization tools proposed by the LFCS Consortium [1,2]. These SELF are generally used in order to increase the solubility and bioavailability of poorly water-soluble drugs, mainly BCS class II drugs. The bioavailability of this class of molecules is limited by their low solubility in aqueous fluids. Lipid-based formulations are able to increase their bioavailability by delivering the drug directly as a solution in the gastrointestinal tract. To do so, formulators have to select the best solvents and surfactants to be able to dissolve the dose in a self-emulsifying formulation. In this first part, we assayed the solubility of four BCS class II model compounds in thirteen liquid and solid lipid-based excipients.

2 - METHODS

Ibuprofen, cinnarizine, piroxicam, and fenofibrate are chosen as model poorly water-soluble drugs possessing a log P between 3 and 6. The physicochemical properties of these model drugs are shown in Table 1.

Drugs	Water solubility (mg/L) – 25°C	Melting point (°C)	Log P	pKa	Molecular weight	Therapeutic dose (mg)
Ibuprofen	21	76	3.97	4.91	206	200
Cinnarizine	750	120	5.77	7.88	368	25
Piroxicam	23	198	3.06	1.87-6.3	331	10
Fenofibrate	250	81	5.3	NA	360	67

NA = Not applicable

Table 1. Physico-chemical properties of four model BCS class II drugs: Ibuprofen, Cinnarizine, Piroxicam, Fenofibrate.

Liquid and solid excipients tested in this study are presented in Table 2. These excipients are classified in four types: water-soluble surfactants, water-insoluble surfactants a.k.a. co-surfactants, oils, and hydrophilic cosolvents (Table 2).

Functionality	Chemical name	Commercial name	HLB
Surfactants (water-soluble)	PEG-32 stearate	Gelucire® 48/16	12
	Lauroyl polyoxyl-32 glycerides	Gelucire® 44/14	11
	Caprylo caproyl polyoxyl-8 glycerides	Labrasol® ALF	12
	Polyethylene-20 sorbitan oleate	Tween® 80	15
	Polyethylene-40 hydrogenated castor oil	Cremophor® RH40	14
Co-surfactants (water-insoluble)	Oleyl polyoxyl-6 glycerides	Labrafil® M1944CS	9
	Propylene glycol monocaprylate	Capryol™ 90	5
	Propylene glycol monolaurate	Lauroglycol™ 90	3
	Polyglyceryl-3 dioleate	Plurol® Oleique CC 497	3
Oils	Medium chain triglycerides	Labrafac™ CC	1
	Glyceryl monolinoleate	Maisine™ 35-1	1
	Glyceryl mono-oleate	Peceol™	1
Hydrophilic cosolvent	Diethylene glycol monoethylether	Transcutol® HP	NA

HLB=Hydrophilic Lipophilic Balance, NA=Not applicable

Table 2. Classification of lipid-based excipients.

• Solubility of drugs in liquid excipients

Solubility of drugs in liquid excipients is assayed by HPLC analysis. Solubility is determined at 37°C because it was not possible to assay viscous excipients (e.g. Plurol® Oleique or Tween® 80) at 25°C, and there is also only a slight increase of solubility from 25 to 37°C (about 5%, see Table 3 for the example of Ibuprofen and Cinnarizine).

Excipients	Ibuprofen			Cinnarizine		
	Solubility (mg/mL)		Difference (%)	Solubility (mg/mL)		Difference (%)
	25°C	37°C		25°C	37°C	
Labrasol® ALF	351.9	357.0	1.4	27.8	28.3	1.8
Labrafil® M1944CS	17.4	18.1	3.9	10.5	12.4	15.3
Capryol™ 90	274.8	296.0	7.2	36.1	37.8	4.5
Lauroglycol™ 90	248.7	249.9	0.5	28.0	30.5	8.2
Labrafac™ CC	16.1	17.3	6.9	24.4	26.6	8.3
Transcutol® HP	434.1	437.0	0.7	41.8	42.7	2.1

Table 3. Influence of temperature on the solubility of Ibuprofen and Cinnarizine in liquid lipid-based excipients.

The drug is added in excess to 60 mL amber borosilicate glass bottle containing 10 g lipid-based excipient. Bottle are allowed to equilibrate at 37°C under magnetic stirring with periodic vortex mixing to ensure that undissolved drug particles are homogeneously suspended in the lipid slurry. At intervals, bottles are sampled and these aliquots are centrifuged (Universal centrifuge 320R) at 2800g and 37°C for 30 min [1]. This separated samples into a solid pellet phase and a particle-free supernatant. The supernatant is sampled and diluted with an appropriate solvent (a solution of valerophenone in acetonitrile (0.35 mg/mL) for Ibuprofen, acetonitrile for Cinnarizine and Piroxicam, and methanol for Fenofibrate) for further HPLC analysis. The equilibrium solubility is considered as reached when the difference between two consecutive values is less than 5%. For most of the excipients, the equilibrium is reached in less than 3 days.

• Solubility of drugs in solid excipients

For solid lipid-based excipients (Gelucire® 48/16 and Gelucire® 44/14), the solubility of drugs is assayed by differential scanning calorimetry (DSC). According to the Raoult's law, the variation of melting enthalpy is linked with the concentration of drug dissolved within the excipient. Different concentrations of the drug in the excipient are prepared to determine its solubility in the solid-state excipient. Samples are prepared by melting the excipient and dispersing a specified amount of drug under stirring. These samples are let at 50°C to equilibrate overnight, vortexed, then aliquoted in aluminum pans, and let at 25°C to solidify / crystallize for 24 hours before DSC analysis.

Sample (2.5-5 mg) was sealed in aluminum pan and analyzed using a DSC (Pyris Diamond, Perkin-Elmer, USA) calibrated with Indium (T_m = 156.6°C, ΔH_m =28.6 $J.g^{-1}$). Thermal analysis is carried out between -20 and 120°C at a heating rate of 3°C.min^{-1}.

The drug solubility is observed when there is a change of slope in the curve fitting the evolution of melting enthalpy of the excipient as a function of the drug concentration in the sample [3].

3 - RESULTS AND DISCUSSION

Figure 1 presents the evolution of the melting enthalpy of Gelucire® 48/16 as a function of the concentration of two model drugs as an example. Cinnarizine and Ibuprofen decreases the melting enthalpy of this excipient indicating its solubility within the solid excipient and as a consequence the disorganization of its crystalline matrix. When the solubility of these drugs is reached, the melting enthalpy either stays constant or increases again. Depending on the drugs, the decrease of enthalpy can be more or less pronounced: 16 J/g for Cinnarizine and 130 J/g for Ibuprofen.

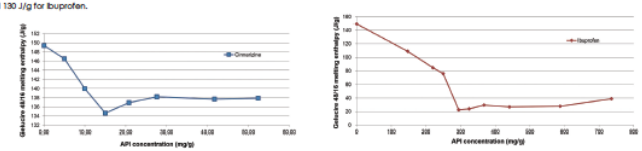

Figure 1. Evolution of the melting enthalpy of Gelucire® 48/16as a function of the concentration of Ibuprofen or Cinnarizine.

Table 4 summarizes the equilibrium solubility determined for all four model drugs. The same group of excipients always shows the highest solvent capacity: the hydrophilic cosolvent Transcutol® HP, all the water-soluble surfactants (Labrasol®, Gelucire® 44/14, and Gelucire® 48/16 for example), and among co-surfactants Capryol™ 90 is always the most efficient. This confirms the need of water-soluble components such as surfactants with high HLB and solvents to dissolve this type of poorly soluble drugs.

Excipients		Solubility (mg/mL)			
		Ibuprofen	Cinnarizine	Piroxicam	Fenofibrate
Surfactants	Gelucire® 48/16	300	15	20	150
	Gelucire® 44/14	300	6	20	150
	Labrasol® ALF	357.0	28.3	15.0	101.9
	Tween® 80	306.6	20.0	18.5	107.2
	Cremophor® RH40	368.9	28.9	28.3	86.9
Co-surfactants	Labrafil® M1944CS	18.1	12.4	3.5	34.2
	Capryol™ 90	296.0	37.8	6.4	127.8
	Lauroglycol™ 90	249.9	30.5	3.7	94.6
	Plurol® Oleique CC 497	14.6	7.7	2.6	32.4
Oils	Labrafac™ CC	17.3	26.6	2.5	54.4
	Maisine™ 35-1	168.9	11.9	2.9	59.8
	Peceol™	162.5	11.7	2.5	62.3
Hydrophilic solvent	Transcutol® HP	437.0	42.7	19.9	123.0

Table 4. Solubility of four model BCS class II drugs in liquid and solid lipid-based excipients.

4 - CONCLUSIONS

This case study shows that BCS class II drugs with a log P between 3 and 6 are more soluble in hydrophilic cosolvents and water-soluble surfactants than in oils (with long or medium fatty acid chain). It also demonstrates the ability of DSC to determine the solubility of drug in solid lipid-based excipients.

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SELF FORMULATION PROTOCOL: PART II - EXCIPIENT SELECTION, DIAGRAM DESIGN AND SELECTION OF PROTOTYPE FORMULATIONS

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1 - PURPOSE

The aim of this study was to develop a sound formulation protocol for Self Emulsifying Lipid Formulation (SELF) with the latest characterization tools proposed by the LFCS Consortium [1,2]. In this second part, we constructed binary and ternary diagrams composed of excipients possessing the higher solvent capacity for BCS class II model drugs. We also tested the ability of these prototype formulations to self-emulsify in contact with aqueous media in order to select prototype formulations.

2 - METHODS

Lipid-based excipients exhibiting the highest solvent capacity for Ibuprofen, Cinnarizine, Piroxicam, and Fenofibrate were Transcutol® HP, water-soluble surfactants, and Capryol™ 90 as summarizes in Table 1.

Table 1. Selection of excipients (in bold) able to dissolve the dose of four model BCS class II drugs in 1g.

Excipients		Solubility (mg/mL)			
		Ibuprofen	Cinnarizine	Piroxicam	Fenofibrate
Surfactants (HLB>10)	Gelucire® 48/16	**300**	15	20	**150**
	Gelucire® 44/14	**300**	6	20	**150**
	Labrasol® ALF	**357.0**	**28.3**	**15.0**	101.9
	Tween® 80	**306.6**	20.0	**18.5**	107.2
	Cremophor® RH40	368.9	28.9	**28.3**	86.9
Co-surfactants (HLB<10)	Capryol™ 90	296.0	**37.8**	6.4	127.8
	Lauroglycol™ 90	249.9	30.5	3.7	94.6
Oils	Labrafac™ CC	17.3	26.6	2.5	54.4
Hydrophilic solvent	Transcutol® HP	**437.0**	**42.7**	19.9	123.0
Therapeutic dose (mg)		200	25	10	67

To develop lipid-based formulations we constructed binary or ternary phase diagrams consisting of surfactants to promote the self-emulsification, and solvents and/or co-surfactants to allow for the dissolution of the dose into a capsule.

+ Miscibility of selected excipients

Miscibility of excipients is checked at 37°C overnight. In the case of mixtures containing a solid lipid-based excipient (Gelucire® 48/16 or Gelucire® 44/14), the miscibility is additionally checked at 25°C overnight to avoid the exudation of liquid from the solid mixture.

+ Dispersion of miscible mixtures

A dispersion test is performed on miscible mixtures in a USP dissolution bath. One gram of miscible mixture is introduced in 200 mL of purified water or simulated gastric fluid (SGF) at 37°C under agitation with a paddle at 60 rpm [3]. Performance criteria are: ease of emulsification, homogeneity and fineness of the dispersion. The fineness of the dispersion is assayed by dynamic light scattering (DLS, Particle Sizing Systems Nicomp).

+ Solubility of the drug within selected mixtures

The theoretical solubility of the model drug is calculated in each selected mixture with Equation 1 to verify that it could theoretically dissolve the selected dose. Then the equilibrium solubility of the drug is measured with the same method as for excipients. In brief, the drug is added in excess to 60 mL amber borosilicate glass bottle containing 10 g lipid formulation. Bottle are allowed to equilibrate at 37°C under magnetic stirring with periodic vortex mixing to ensure that undissolved drug particles are homogeneously suspended in the lipid slurry. At intervals, bottles are sampled and these aliquots are centrifuged (Universal centrifuge 320R) at 2800g and 37°C for 30 min [1]. This separated samples into a solid pellet phase and a particle-free supernatant. The supernatant was sampled and diluted with an appropriate solvent (a solution of valerophenone in acetonitrile (0.35 mg/mL) for Ibuprofen, acetonitrile for Cinnarizine and Piroxicam, and methanol for Fenofibrate) for further HPLC analysis. The equilibrium solubility was considered as reached when the difference between two consecutive values is less than 5%. This equilibrium solubility is then used to calculate the percentage of saturation of the drug within the mixture, with Equation 2.

Equation 1 $C_{ST} = \sum (\%_{ex} * S_D)$

C_{ST}: theoretical equilibrium solubility
$\%_{ex}$: percentage of excipient in the mixture
S_D: equilibrium solubility of the drug in excipient

Equation 2 $\%Sat = D / (V * C_S)$

%Sat: percentage of saturation of the drug within the mixture
D: therapeutic dose of the drug
V: volume of the dosage form (1mL)
Cs: equilibrium solubility

3 - RESULTS AND DISCUSSION

+ Ibuprofen

For Ibuprofen, the best solvent is Transcutol® HP, then all water-soluble surfactants. The quantity of hydrophilic cosolvent that can be introduced in the formulation is generally limited to 15% in order to avoid massive precipitation of the drug after dispersion in aqueous media and incompatibility with capsules. All liquid water-soluble surfactants gave homogeneous mixtures with up to 15% of Transcutol® HP. For Gelucire® 48/16, a solid surfactant, the maximum quantity of liquid cosolvent that can be added is limited to 6% because for higher quantities, non homogeneous mixtures are obtained after storage at 25°C. These formulations are able to quickly self-emulsify in contact with aqueous media and form micellar solutions with mean particle size ranging from 2 to 7 nm (Figure 1B, 2). Mixtures containing Labrasol® ALF as water-soluble surfactant showed the quickest emulsification time when compared to other surfactants that are either viscous liquids or semi-solids.

+ Cinnarizine

For Cinnarizine, the best solvents are Transcutol® HP and Capryol™ 90, then most of the water-soluble surfactants. The same combinations are chosen for Cinnarizine as for Ibuprofen. Capryol™ 90 is also a good solvent for Cinnarizine and was used to draw binary and ternary phase diagrams. This co-surfactant is miscible with Labrasol® ALF alone or in combination with Transcutol® HP in all proportions. However, these mixtures are only able to give turbid and homogeneous dispersions after dispersion in aqueous media when the quantity of Labrasol® ALF is above or equal to 85%. These dispersions are very fine emulsions with mean particle size ranging from 150 to 450 nm (Figure 1A, 3).

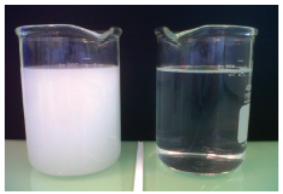

Figure 1. Example of turbid and transparent homogeneous dispersion:
A. Turbid and homogeneous dispersion of 94% Labrasol® ALF + 6% Transcutol® HP;
B. Transparent and homogeneous dispersion of 94% Gelucire® 48/16 + 6% Transcutol® HP.

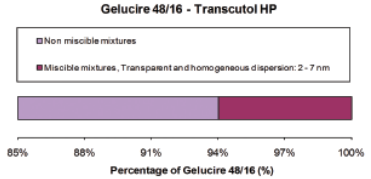

Figure 2: Gelucire® 48/16 - Transcutol® HP binary diagram.

+ Piroxicam

For Piroxicam, only the water-soluble surfactants and Transcutol® HP are able to dissolve the dose within 1g. In order to compare the efficiency of these surfactants in term of emulsification and ability to maintain the drug in solution after dispersion and digestion, formulations containing each surfactant individually are selected.

+ Fenofibrate

For Fenofibrate, the best solubilizers are the solid water-soluble surfactants: Gelucire® 44/14 and Gelucire® 48/16. As for Piroxicam, simple one-excipient formulations are selected to compare both solid surfactants.

All selected prototype formulations are listed in Table 2. The key characteristics of these formulations are presented: LFCS type of formulation, mean particle size after dispersion in water (expressed in volume, mean ± standard deviation), theoretical and measured equilibrium solubility, as well as the percentage of saturation of the drug.

Table 2. Selected prototype formulations for each model BCS class II drugs.

Drug	Prototype formulations	LFCS Type	Size of aqueous dispersion (nm)	CsT (mg/mL)	Cs (mg/mL)	%Sat
Ibuprofen	94% LAS / 6% TRA	IIB	185±41	361.8	330.1	61
	94% 4816 / 6% TRA	IV	260.2	308.1	231.5	86
	94% TBO / 6% TRA	IV	<5	314.4	267.7	75
	100% 4816	IV	7±1	300	300	67
	100%4414	IIB	15±2	300	300	67
Cinnarizine	90% LAS/10% C90	IIB	411±71	29.3	34.9	72
	75% LAS/10% C90/15% TRA	IIB	373±76	31.4	23.9	105
Piroxicam	100% LAS	IIB	412±70	15.0	15.0	67
	100% TBO	IV	9.9±2	18.5	18.5	54
	100% RH40	IV	9±2	28.3	28.3	35
	100% 4816	IV	7±1	20	20	50
	100% 4414	IIB	15±2	20	20	50
Fenofibrate	100% 4816	IV	7±1	150	150	45
	100% 4414	IIB	15±2	150	150	45

with: LAS=Labrasol® ALF, TRA=Transcutol® HP, 4816= Gelucire® 48/16, TBO=Tween® 80, 4414=Gelucire® 44/14, C90=Capryol™ 90, RH40=Cremophor® RH40.

Figure 3: Labrasol® ALF – Capryol™ 90 - Transcutol® HP ternary diagram.

4 - CONCLUSIONS

The development of binary and ternary diagrams permits to define the percentage of each component in order to obtain a compromise between solubilisation in the dosage form, ease of dispersion and homogeneity of the aqueous dispersion. Formulations leading to homogeneous aqueous dispersions and able to dissolve the dose are selected as prototypes for the next step of SELF formulation protocol: the digestion assay.

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SELF FORMULATION PROTOCOL: PART III - IMPACT OF LIPOLYSIS ON PROTOTYPE FORMULATIONS

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1 - PURPOSE

The aim of this study was to develop a sound formulation protocol for Self Emulsifying Lipid Formulation (SELF) with the latest characterization tools proposed by the LFCS Consortium [1,2]. In this third and last part, we evaluated the ability of prototype formulations to maintain model drugs in solution after dispersion and in vitro lipolysis in fasted state.

2 - METHODS

The therapeutic dose of each model drug (200 mg of Ibuprofen, 25 mg of Cinnarizine, and 10 mg of Piroxicam) is loaded in 1 mL of prototype formulations. Table 1 summarizes lipid-based formulation types selected and the percentage of saturation of the drug in each formulation.

- Preparation of media and enzyme suspension

The lipolysis buffer is prepared by adding Tris (0.474 g/L), $CaCl_2$ (0.208 g/L) and NaCl (8.810 g/L) in Milli-Q water. The pH is adjusted at 6.5 with either HCl 1M or NaOH 0.6 M. The lipolysis medium is prepared by adding L-a-phosphatidylcholine (0.576 g/L) and NaTDC (0.576 g/L) in the lipolysis buffer. The medium is stirred overnight to allow the complete solubilisation of lecithin.
The pancreatin solution is prepared by adding 1 g of pancreatin powder in 5 mL of lipolysis buffer. After 10 minutes of magnetic stirring the solution is centrifuged (Universal centrifuge 320R) at 2800g and 5°C for 10 min. The supernatant is sampled to be used in the lipolysis test.

- In vitro lipolysis test

The experimental setup consists in a pH stat apparatus (Metrohm® AG, Switzerland), comprising a Titrando 802 propeller stirrer/804 Ti Stand combination, a glass pH electrode (Unitrode) and one 800 Dosino dosing units coupled to 5 mL autoburette. The apparatus is connected to a PC and operated using Tiamo 2.0 software. The equipment is thermostated at 37°C and filled with lipolysis medium, to mimic fasted state in the small intestine. During the digestion of lipid-based formulations by pancreatic lipases, pH is maintained constant by the addition of either NaOH 0.1N for formulations containing long chain fatty acids (Gelucire® 48/16 for example) or NaOH 0.6N for formulations containing medium chain fatty acids (Labrasol® ALF for example).
Before the lipolysis test, the specific activity of pancreatic lipases is checked on tributyrine (model substrate). The substrate must be in excess to have a reaction rate directly proportional to the enzyme concentration. Five hundred microlitre of tributyrine and 1.5 µL of pancreatin solution are introduced in 28 mL of lipolysis medium (without lecithin). The specific activity of pancreatic lipases should be higher than 900 U/mg.
One gram of lipid-based formulations in hard gelatine capsules (Ucaps, Capsugel), is added to 36 mL of lipolysis medium at 37°C. After 10 minutes (dispersion / emulsification time), 4 mL of pancreatin solution is added. The duration of the test is 1 hour. Aliquots of 1 mL are sampled at various time points to determine the solubility of the drug in the micellar phase: t=-5 min (during the dispersion time), t=0 min (before the addition of the pancreatic solution), t=5 min, t=15 min, t=30 min and t=60 min.
Five microlitres of the inhibitor solution (1M: 100 mg of 4-bromophenylboronic acid in 0.5 mL of methanol) is added immediately to each sample. They are then centrifuged at 21000g and 37°C for 30 minutes. The supernatant is sampled and diluted with an appropriate solvent (a solution of valerophenone in acetonitrile (0.35 mg/mL) for Ibuprofen, and acetonitrile for Cinnarizine and Piroxicam) for further HPLC analysis.

Placebo formulations are also tested in order to determine the solubility of the drug in the lipolysis medium containing the digested lipid formulation. In this case only one sampling is performed at t=60 min. The micellar phase is obtained at the end of lipolysis experiment after centrifugation at 21000g and 37°C for 30 minutes of aliquots of the pH-stat medium. The supernatant is sampled and drug is added in excess. Mixtures are let at 37°C under agitation for 4 hours. Aliquots are then centrifuged as described above and diluted with the appropriate solvent for HPLC analysis.

3 - RESULTS AND DISCUSSION

- Piroxicam formulations

The ease of emulsification of Piroxicam formulations is clearly related to the physical state of excipients and their ability to form mesophases. Labrasol® ALF is liquid and self-emulsifies in a few seconds, allowing the complete dissolution of Piroxicam in the aqueous phase in less than 5 minutes. This is the only excipient able to dissolve and maintain the 10 mg of Piroxicam within the aqueous phases during the dispersion phase. Tween® 80 is slower as it is a viscous liquid that forms viscous mesophases when in contact with water. Gelucire® 44/14 and Cremophor® RH40 are even slower as they are semi-solid excipients needing more time to hydrate and form viscous mesophases that erode slowly. Finally, the new Gelucire® 48/16, a solid surfactant design for solid oral dosage forms, is even slower due to its higher melting point.
These formulations composed of water-soluble surfactants able to dissolve the dose of Piroxicam showed comparable behaviour after digestion even though saturation levels were different. Formulations containing medium chain fatty acids (Labrasol® ALF) are more digested than formulations with long chain, but can in this case keep the drug in solution more efficiently. The turbid dispersion obtained with Labrasol® (67% of saturation) is more efficient in dissolving and maintaining Piroxicam in the aqueous phase than other surfactants leading to micellar solution (for example Cremophor® RH40 (35% of saturation), Figure 1). It should be noted that even if Gelucire® 48/16 is slower to erode because of its higher melting point, it leads to the higher and more constant concentration of Piroxicam in the aqueous phase during the 1-hour digestion.

- Cinnarizine formulations

Solution of Cinnarizine with Labrasol® ALF led to incomplete release of the drug in the micellar phase and a rapid precipitation after the first minutes of digestion (Figure 2). Adding a hydrophilic cosolvent such as Transcutol® HP to increase the solvent power of the formulation gave the same result. This is due to the low solubilising capacity of the digested micellar phase containing Labrasol®. Table 1 presents the solubility of the drug within the digested micellar phases and the corresponding maximum supersaturation ratio (SR^M) calculated with Equation 1. When this ratio is higher than 3, significant precipitation of the drug is expected as explained in [2].

Equation 1

$$SR^M = \frac{Maximum\ drug\ solubility\ in\ AP}{Drug\ solubility\ in\ AP\ digested}$$

SR^M: maximum supersaturation ratio
AP: aqueous phase

Cinnarizine is known to have a good solubility in mixed micelles containing oleic acid [3]. Hence 15% of Maisine™ 35-1 was added to a Labrasol®-based formulation even if this lipid excipient did not show good solvent capacity during the initial solubility screening. After dispersion, the concentration of cinnarizine dissolved in the aqueous phase is equivalent to the previous formulation, but after 5 minutes of digestion, the release of oleic acid by Maisine™ 35-1 led to a dramatic increase of the solubility of Cinnarizine within the aqueous phase (Figure 2). However due to the low stability of the mixed micelles this concentration steadily decreases during the following hour. This case study shows the importance of measuring the solubility of the drug within the digested micellar phase and calculating the SR^M to select the good excipient and anticipate the impact of supersaturation.

- Ibuprofen formulations

All formulations led to low supersaturation $SR^M \leq 3$ and no significant precipitation was observed (Figure 3). This confirms the utility of SR^M as an indicator of the ability of the formulation to keep the drug in solution after in vitro digestion.

Table 1. Selected prototype formulations for Ibuprofen, Cinnarizine, and Piroxicam – percentage of saturation, solubility in digested aqueous phase and supersaturation ratio.

Drug	Prototype formulations	LFCS Type	%Sat	Solubility in digested aqueous phase (mg/mL)	SR^M
Ibuprofen	94% LAS / 6% TRA	IIIB	61	2.20	2.5
	94% 4816 / 6% TRA	IV	86	1.75	3.2
	94% T80 / 6% TRA	IV	75	2.69	2.1
	100% 4816	IV	67	2.01	2.8
	100%4414	IIIB	67	1.90	2.97
Cinnarizine	90% LAS/10% C90	IIIB	72	0.02	43.4
	75% LAS/10% C90/15% TRA	IIIB	105	0.02	43.4
	75% LAS/10% C90/15% MAI	IIIB	63	1.08	0.6
Piroxicam	100% LAS	IIIB	67	ND	ND
	100% T80	IV	54	ND	ND
	100% RH40	IV	35	ND	ND
	100% 4816	IV	50	ND	ND
	100% 4414	IIIB	50	ND	ND

with: LAS=Labrasol® ALF, TRA=Transcutol® HP, 4816= Gelucire® 48/16, T80=Tween® 80, 4414=Gelucire® 44/14, C90=Capryol™ 90, RH40=Cremophor® RH40, %Sat=percentage of saturation, SR^M=maximum supersaturation ratio, ND=not determined

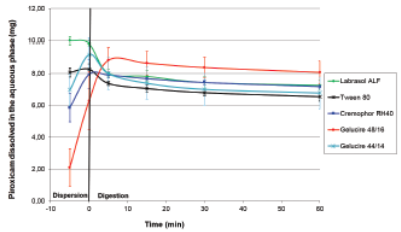

Figure 1. Evolution of the weight of Piroxicam dissolved in the aqueous phase during in vitro dispersion and digestion.

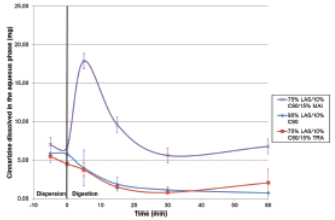

Figure 2. Evolution of the weight of Cinnarizine dissolved in the aqueous phase during in vitro dispersion and digestion.

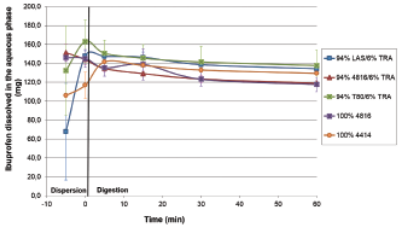

Figure 3. Evolution of the weight of Ibuprofen dissolved in the aqueous phase during in vitro dispersion and digestion.

4 - CONCLUSIONS

During in vitro lipolysis test simulating small intestine fasted state, the percentage of saturation and the extent of digestion (volume of fatty acid titrated) are not always predictive parameters to compare formulations. The dosage of the drug in the micellar phase is mandatory to assay the ability of the formulation to maintain drug in solution. This study also demonstrates that turbid formulations consisting of digested excipients can be more efficient than supposed non-digestible micelle-forming formulations.

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Development and characterization of a spray-drying process for the production of aminoglycoside powders for inhalation using experimental design

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PURPOSE

Inhalation delivery demonstrated to be an effective approach for the administration of various active moieties both for local and systemic actions [1, 2]. Powders for inhalation delivery are produced with multi parametric methods such as spray drying.

The recent regulatory "Quality by Design" (QbD) framework [3] for Pharmaceutical Development requires the extensive use of statistical tools, which will eventually lead to process knowledge, through the identification of mathematical relationships between the Critical Quality Attributes (CQAs) of the finished product and the Critical Process Parameters (CPPs). This is mainly achieved by applying experimental designs (DoE) for process characterization and optimization, allowing for lean, robust and capable manufacturing methods [4].

The purpose of this work was to study the spray-drying process for producing drug powders for inhalation, in compliance with the current (QbD) framework, in order to have an approach capable to control the multitask process.

More in particular, this research work deals with the identification of the most influential process factors affecting the quality characteristics of powders intended for inhalation. The factors studied were: the drying temperature, the feed rate, the solvent composition, the presence of waxy excipients and the drug concentration. The study was carried out by employing a resolution V half fractional factorial design for studying five formulation and process factors to several responses, which when combined reflect the desired product quality.

METHODS

Preparation of powders for inhalation:

The solids were dissolved in the solvent before spray-drying. The finished product was produced via a spray-drying method (Mini Spray Dryer B-290 BUCHI, Switzerland), using various formulation and process settings according to the experimental design plan.

Experimental Design:

The process was assessed using a resolution V half fractional factorial design, with 16 experiments.

In particular, drying temperature (A), feed rate (B), solvent composition (C), the presence of the waxy excipient (D) and the concentration of the API (E) were selected as factors to be screened. The results were evaluated using Design Expert® V. 8.0.0, Stat-ease Inc, Minneapolis.

Characterization of powders for inhalation:

Several Critical Quality Attributes (CQAs) of the produced spray dried powders were evaluated, following their preparation and after a sieve agglomeration step, using official methods.

CQAs considered as responses included powder density, proportion of powder emitted from the device (%Emitted Dose), fine particle dose (FPD), fine particle fraction (%FPF) and the relevant standard deviations.

Finally, the effect of the factors on the yield of the process was also evaluated.

RESULTS AND DISCUSSION

The relevant significance of the factors and their interactions was evaluated using half normal probability plots and the relevant Pareto charts, two of which namely for spray drying Yield and %Emitted Dose are presented in Figure 1. ANOVA revealed significant models for all the responses under study, as denoted by high R^2 and adjusted R^2 values. The relationship between the formulation and process factors with selected CQAs are depicted in the perturbation plots of Figure 2.

Summarizing the findings for each response:

✓Density: this attribute was mainly affected by the presence of the waxy excipient and the drying temperature, while four significant two factor interactions i.e. CE, BD, AB and CD were also identified.

✓%Emitted Dose: the presence of the waxy excipient was by far the most significant factor affecting this CQA, in a negative manner, while the opposite effect was observed with the increase of feeding rate. Moreover, the effect of the excipient was found to be different at the two levels of the API concentration, as revealed by the significance of DE interaction.

✓Fine Particle Dose: the presence of the waxy excipient had a negative effect, followed by feed rate, while the two other significant main factors A and C increased this response. CD, AE and AC were identified as important interactions.

✓Fine Particle Fraction: this particular CQA was significantly affected by A, B, C and D, while almost every possible interaction participated in the model, whose predicted R^2 was very high (>0.9500). High %FPF are achieved at the low level of B and high levels of A, C and D.

✓Yield: the process output was negatively affected by the presence of the waxy excipient and enhanced at high API concentrations, while AB and BD were identified as significant interactions.

✓SD: they were found generally low for all responses studied. As an example the perturbation plot for the variability of the Emitted Dose SD showed that it can be adequately managed by controlling the factors A, B, C and E.

✓Agglomeration of powders did not in general affect the above mentioned patterns.

Preliminary exercises using the desirability function, revealed specific factor settings providing acceptable values for all responses simultaneously and defined the roadmap to the optimization phase. The purposeful adjustment of the manufacturing spray-drying parameters and formulation factors allowed the improvement of the aerodynamic performance of the produced powders and the manufacturability of the inhalation dosage form.

Finally, the analysis of the design revealed the statistically significant correlation of the CQAs with the important factors and their interactions, providing the scientific basis for optimizing the process under study with Response Surface Methodologies, as a next step.

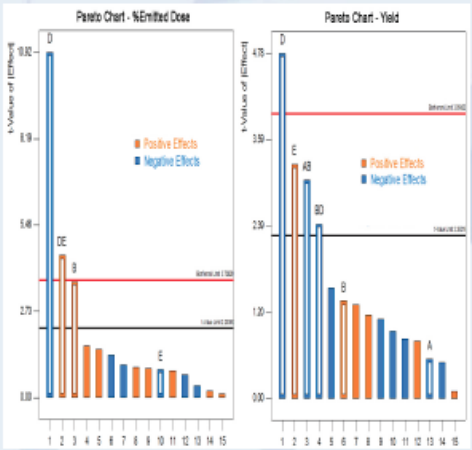

Figure 1. Pareto charts for the responses %Emitted Dose and Yield.

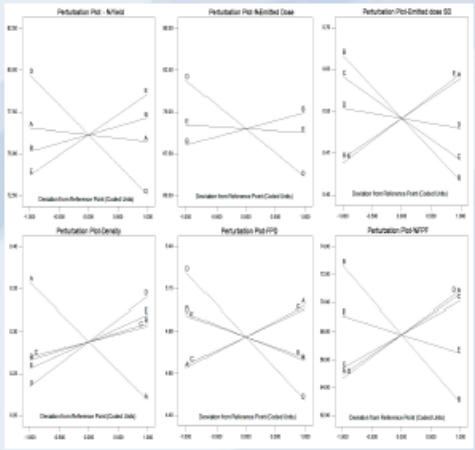

Figure 2. Perturbation Plots for the CQAs.

CONCLUSIONS

Particle engineering by spray drying is a complex process, the variability of which can be successfully managed by controlling the significant factors and their interactions. This is exceptionally important when combined with the fact that powders for inhalation are technologically advanced delivery systems, requiring conformance to a well defined and restricted Quality Target Product Profile. Using DoE techniques in the QbD framework it was feasible to fully characterize a manufacturing process intended for the optimized production of drug powders for inhalation administration.

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Respirable amikacin dry powders for inhalation by a Quality by Design procedure

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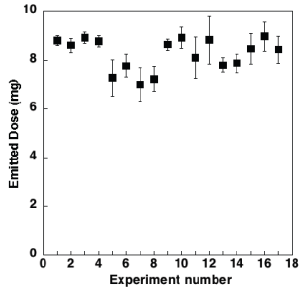
Introduction

Amikacin as a liposomal solution for nebulization is an antibiotic under study for the treatment of *Pseudomonas aeruginosa* infections in cystic fibrosis patients. In a previous study, the respirability of amikacin dry powders obtained by spray drying was maximized using a Design of Experiment (DoE) approach. This study intended to explore the most influential process and formulation DoE parameters in the production by spray drying of amikacin inhalation powders. The aim was to discover using a Central Composite Design (CCD) the factor combination for the best product respirability.

Methods

Three DoE parameters namely drying temperature, feed rate and ethanol proportion have been selected out of the previous five. In addition, the number of this factors levels was increased from two to three, as shown in the Table 1. The design was constructed and analyzed using Design-Expert® Software, Version 9.0.1 (Stat-Ease, Inc., Minneapolis, USA).
The spray dried (SD) powders were characterized by scanning electron microscopy (SEM), water content (%) by Karl Fisher titration and volume diameter measured by laser light scattering. The in vitro aerodynamic assessment was performed using Fast Screening Impactor.

Results and Discussion

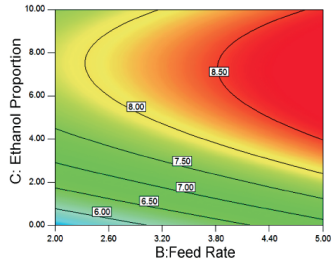


***In vitro* aerodynamic assessment of the spray dried powders (ED and FPD)**

The feed solution containing ethanol gave powders with higher Emitted Dose values without significant differences between the two amounts of ethanol (5% or 10%) in the feed solution. The factors affecting the respirability were ethanol proportion and feed rate. Increasing both increased the Fine Particle Dose (particles < 5 µm).

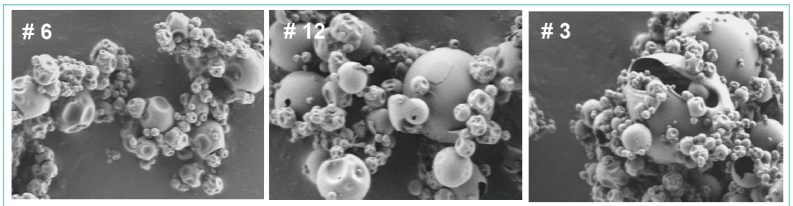
Table 1. Matrix of the Central Composite Design (three factors studied at three levels) and particle size distribution (volume diameter, n = 3).

#	Factor 1 Drying Temperature (°C)	Factor 2 Feed Rate (ml/min)	Factor 3 Ethanol (% w/w)	D_{v50}
1	150	2.0	10	2.80 ± 0.25
2	180	2.0	10	2.72 ± 0.08
3	150	5.0	10	3.23 ± 0.33
4	180	5.0	10	3.25 ± 0.12
5	150	2.0	0	2.53 ± 0.07
6	180	2.0	0	2.37 ± 0.01
7	150	5.0	0	3.11 ± 0.05
8	180	5.0	0	2.49 ± 0.07
9	150	3.5	5	2.60 ± 0.04
10	180	3.5	5	3.21 ± 0.12
11	165	2.0	5	2.64 ± 0.09
12	165	5.0	5	3.68 ± 0.13
13	165	3.5	10	2.73 ± 0.12
14	165	3.5	0	2.60 ± 0.08
15	165	3.5	5	3.33 ± 0.07
15 bis	165	3.5	5	3.22 ± 0.11
15 ter	165	3.5	5	2.80 ± 0.25



Contour plot of water content as function of feed rate and ethanol proportion (drying temperature 165 °C)

SEM pictures showed morphological differences among the samples according to the composition of the feed solution. Powders obtained from ethanol solutions (#3 and #12) exhibited small and big spherical particles merged together. On the contrary, those (for example #6) obtained from a feed solution without ethanol (100% water) contained small, similar sized shrivelled particles.



SEM of amikacin SD powders prepared using different operating parameters and feed solutions.

Conclusion

DoE with CCD indicates the optimal levels of formulation and production process parameters for respirability of amikacin powders for inhalation.
The respirability, the most important Critical Quality Attribute for an inhalation powder, was positively affected by the presence of ethanol in the feed solution.
Further investigations on this parameter are needed to understand its role in the formation of particles.

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Spray dried amikacin powder for inhalation in cystic fibrosis patients: A quality by design approach for product construction

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ABSTRACT

An amikacin product for convenient and compliant inhalation in cystic fibrosis patients was constructed by spray-drying in order to produce powders of pure drug having high respirability and flowability.
An experimental design was applied as a statistical tool for the characterization of amikacin spray drying process, through the establishment of mathematical relationships between six Critical Quality Attributes (CQAs) of the finished product and five Critical Process Parameters (CPPs).
The surface-active excipient, PEG-32 stearate, studied for particle engineering, in general did not benefit the CQAs of the spray dried powders for inhalation. The spray drying feed solution required the inclusion of 10% (v/v) ethanol in order to reach the desired aerodynamic performance of powders. All desirable function solutions indicated that the favourable concentration of amikacin in the feed solution had to be kept at 1% w/v level. It was found that when the feed rate of the sprayed solution was raised, an increase in the drying temperature to the maximum value (160 °C) was required to maintain good powder respirability. Finally, the increase in drying temperature always led to an evident increase in emitted dose (ED) without affecting the desirable fine particle dose (FPD) values.
The application of the experimental design enabled us to obtain amikacin powders with both ED and FPD, well above the regulatory and scientific references. The finished product contained only the active ingredient, which keeps low the mass to inhale for dose requirement.

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