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

S. Belotti[a], A. Rossi[a], P. Colombo[a], R. Bettini[a], D. Rekkas[b], S. Politis[b], G. Colombo[c], A.G. Balducci[d], F. Buttini[a]

[a] Department of Pharmacy, University of Parma, Viale delle Scienze 27/A, Parma 43124, Italy

[b] School of Pharmacy, University of Athens, Panepistimiopolis Zografou, Athens 15771, Greece

[c] Department of Life Sciences and Biotechnology, University of Ferrara, Via Fossato di Mortara 17/19, Ferrara 44121, Italy

[d] Interdepartmental Center, Biopharmanet-TEC, University of Parma, Viale delle Scienze 27/A, Parma 43124, Italy

Summary

Background: 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 at three levels were explored: drying temperature, ethanol percentage in amikacin feed solution and feed rate. The spray dried powders were characterized such as morphology by scanning electron microscopy, water content (%) and volume diameter. The *in vitro* aerodynamic assessment was performed using Fast Screening Impactor.
Results: The yield of the spray drying process was always higher than 80% and the residual water content (%) was always lower than 10%. The $D_{(v,50)}$, was between 2.37 and 3.68 μm. The most influent factors on the mentioned Critical Quality Attributes were the feed rate and the ethanol percentage in the feed solution. Increasing feed rate or the percentage of ethanol in the feed solution allowed obtaining particles with a smaller size.
The powders obtained from feed solutions without ethanol showed a very low emitted dose. Moreover, a solution without ethanol showed the lowest fine particle dose (3.45 mg ± 1.14).
Conclusions: The presence of ethanol in the feed solution promoted the amikacin spray dried powder respirability. The CCD let to identify the process factors combination to optimize the production of respirable amikacin spray dried powders for inhalation.

Introduction

Amikacin, an aminoglycosidic antibiotic, is used off-label for the inhalation treatment of *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients by nebulizing the marketed injectable solutions. Nowadays, no amikacin inhalation products are available on the market and the one in pipeline is an amikacin liposomal formulation for nebulization (Arikace™) [1]. Dry powder inhalers (DPIs) can deliver high payloads of drug directly to the lung. They are the alternative to the solution for nebulization. The advantages of DPIs include their lightweight and portability which allow a quick and ease administration of the dose everywhere. Furthermore, compared to liquid solutions for nebulization, the use of DPIs leads to a reduction of administration time and a decrease of the labelled dose, since the drug loss in the nebulizer, in the exhaled air and or in the environment is reduced.
In a previous study [2], the respirability of spray-dried amikacin powders with and without the presence of a surface-active excipient was studied using a half-fractional factorial design. The aerodymanic performance of amikacin inhalation powder was related to the following process factors: drying temperature, feed rate, surface-active agent presence, drug content and ethanol presence in the feed solution. This study intended to explore the most influential process and formulation Design of Experiment (DoE) parameters in the production by spray drying of amikacin inhalation powders. The aim was to discover the factor combination for the best product respirability using a Central Composite Design (CCD). This was obtained studying the effect on Critical Quality Attributes of three factors (out the previous five) set at three level values. In particular, the role of drying temperature (150-180 °C), ethanol concentration (0-10% v/v) in the amikacin solution to be sprayed and the feed rate (2-5 ml/min) was deepened.

Materials and methods

Materials
Amikacin sulfate was supplied by ACS DOBFAR S.p.a. (Milan, Italy). Water was purified by reverse osmosis (Millipore, France). For the aerodynamic experiments hydroxy-propyl methylcellulose (HPMC) hard capsules (size 3, Capsugel, Colmar, France) and RS01®,dry powder inhaler (Plastiape S.p.a. Osnago, LC, Italy) were employed. All the other chemicals were of analytical grade.

Central Composite Design (CCD)
A CCD 'face centered' with three factors was applied. Fifteen experiments were performed for this design, plus a double replication of the center point. In details:
- 8 experiments of the full factorial design;
- 6 experiments star points (the center of each face of the factorial space);
- 1 experiment (center point) in triplicate.

The design matrix, reported in Table I, included the fifteen experiments plus the replicates of the center point. The design space was constructed and analysed using the Design-Expert® Software, Version 8.0.7.1 (Stat-Ease, Inc., USA).

Preparation and characterization of spray-dried amikacin powders
Amikacin sulphate raw material was dissolved in water or in water-ethanol mixture (2% w/v) at room temperature (see Table I). Amikacin solutions obtained were spray dried using a Büchi Mini Spray Dryer B-290 (Büchi Labortechnik, Flawil, CH), according to the process factors reported in Table I. The spray dried powder was quantitatively recovered, weighed on an analytical balance (sensitivity 0.1 mg) (Mod. E50S, Gibertini, Italy). The yield, as percentage of the amount of solid dissolved in the sprayed solution, was calculated.
The morphology of the spray dried powders was assessed by Scanning Electron Microscopy (SEM) (Sigma HD, Carl Zeiss, Germany) and residual water was measured by Karl Fischer volumetric titration using TitroMatic Karl Fischer (CRISON INSTRUMENTS, S.A., Barcelona, Spain).
Particle size distribution of the spray dried powders was determined by laser light scattering (SprayTec, Malvern, UK). Approximately, 10 mg of sample were dispersed in 20 ml of cyclohexane 0.1% (w/v) of Span 85 solution and sonicated for 5 minutes. Data were expressed in terms of median volume diameter D(v,0.5).

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 Coarse Fraction Collector (CFC) was equipped with the insert that enables a cut-off of 5 microns at 60 L/min. An amount of 10 ± 0.2 mg of powder was loaded into a HPMC size 3 hard capsule and aerosolized using a RS01® device. Particles with aerodynamic size lower than < 5 µm deposited in the fine fraction collector (FFC) captured by a type A/E glass filter (76 mm, Pall Corporation, USA). This filter was weighed before and after the air actuation, in order to determine the amount of powder deposited, termed as fine particle dose. Each powder was tested in triplicate.

Results and discussion

As shown in Table I, the yield of the process was in all the cases higher than 80%. All the spray dried powders had a residual water content lower than the amikacin sulphate raw material (10.7%). ANOVA analysis (data not shown) showed that the most influent factors significatively affecting the water content were the feed rate and the ethanol percentage in the feed solution. It is noticeable that the highest percentage of ethanol in the feed solution was associated with the highest water content in the powder (Table I).
The spray dried powders obtained showed a median diameter, $D_{(v,50)}$, between 2.37 and 3.68 µm, a range size suitable for the pulmonary administration (Table I). The two most significant factors that influenced the variation of the $D_{(v,50)}$, were the feed rate and the ethanol presence in the feed solution. Particles with a larger volume diameter were obtained by increasing the feed rate. This effect has been also reported by Verhing [3].
An increase of the ethanol content in the feed solution increased the mean size of particles. This effect could be addressed to the higher evaporation rate of ethanol and giving rise to the formation of inflated large particles in the powder which shifted the size distribution to larger sizes. This data was confirmed also by SEM analysis.

Table I. Matrix of the Central Composite Design (three factors studied at three levels). Characterization of the amikacin spray dried powders: Yield (%), Water Content (%, n= 3) and Particle size distribution (volume diameter, n = 3).

#	Factor 1 Drying Temp (°C)	Factor 2 Feed Rate (ml/min)	Factor 3 Ethanol (% w/w)	Yield (%)	Water Content (%)	$D_{(v, 50)}$
1	150	2.0	10	83.12	6.98 ± 0.35	2.80 ± 0.25
2	180	2.0	10	82.13	7.99 ± 0.41	2.72 ± 0.08
3	150	5.0	10	86.76	9.02 ± 0.27	3.23 ± 0.33
4	180	5.0	10	88.38	7.70 ± 0.42	3.25 ± 0.12
5	150	2.0	0	85.09	6.53 ± 0.51	2.53 ± 0.07
6	180	2.0	0	82.81	4.92 ± 0.28	2.37 ± 0.01
7	150	5.0	0	88.73	7.45 ± 0.41	3.11 ± 0.05
8	180	5.0	0	86.18	6.57 ± 0.51	2.49 ± 0.07
9	150	3.5	5	85.69	8.19 ± 0.23	2.60 ± 0.04
10	180	3.5	5	84.87	8.29 ± 0.40	3.21 ± 0.12
11	165	2.0	5	84.88	7.70 ± 0.19	2.64 ± 0.09
12	165	5.0	5	88.00	8.84 ± 0.39	3.68 ± 0.13
13	165	3.5	10	84.12	8.81 ± 0.35	2.73 ± 0.12
14	165	3.5	0	86.31	5.50 ± 0.10	2.60 ± 0.08
15	165	3.5	5	83.88	8.30 ± 0.11	3.33 ± 0.07
15 bis	165	3.5	5	85.68	8.07 ± 0.22	3.22 ± 0.11
15 ter	165	3.5	5	85.53	7.60 ± 0.06	3.54 ± 0.01

SEM pictures showed that there were morphological differences among the spray dried powders according to the composition of the feed solution. In particular, the powders obtained from ethanol solutions (sample #3 and #12) (Figure 1, left and centre) exhibited spherical particles with different sizes (small and big spherical particles together). On the contrary, when ethanol was absent in the feed solution and amikacin was spray dried from water feed solution, the powder (sample #6) contained small, similar sized particles having a collapsed profile (Figure 1, right).

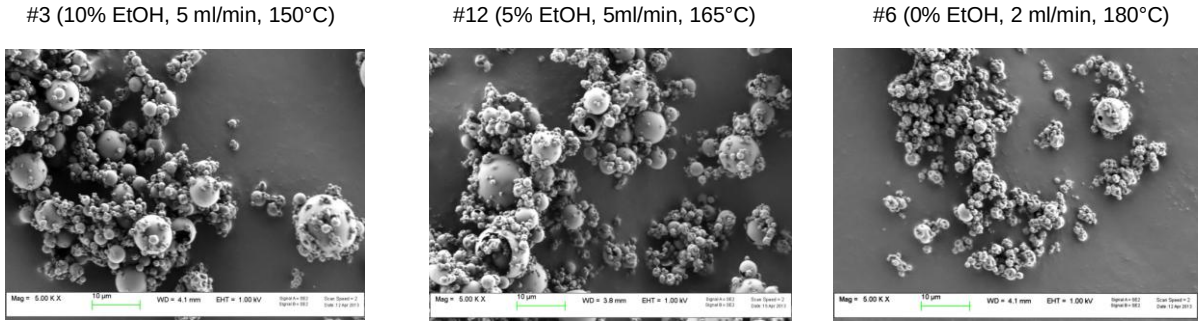

Figure 1. Scanning electron microscopy of amikacin powders prepared by spray drying using different operating parameters and feed solutions.

Emitted dose (ED) and fine particle dose (FPD) values of amikacin powders aerosolized inside the Fast Screening Impactor are shown in Figure 2 a-b. The ED values were between 7 and 9 mg over a labelled dose of 10mg. There are similar ED values for the powders having the same composition of feed solution in ethanol. The smallest ED was obtained for the powders with a feed solution without ethanol (100% water: #5, #6, #7, #14).

The feed solution containing ethanol gave powders with higher ED values without significant differences between the two amounts of ethanol (5% or 10%) in the feed solution.

FPD values (particles smaller than 5 µm) of the powders were between 3 and 6 mg, and powder #7 (obtained from a the feed solution without ethanol) had the lowest value of FPD (3.45 mg ± 1.14). The drying temperature had not a significant influence on the FPD, whereas the predominant factors affecting the respirability were the composition of the feed solution and the feed rate. Increasing the feed rate or the ethanol percentage in the feed solution increased the FPD.

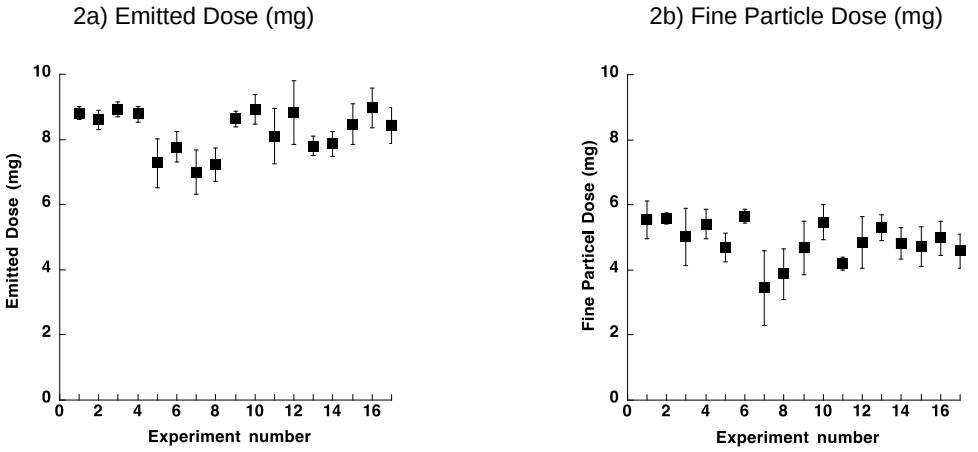

Figure 2. *In vitro* aerodynamic assessment of the spray dried powders (ED and FPD)

Conclusion

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

References

1. Clancy, J.P., et al., “Phase II studies of nebulised Arikace in CF patients with Pseudomonas aeruginosa infection”. Thorax 68 (2013) 818–825.

2. Belotti, S., et al., “Spray dried Amikacin Powder for Inhalation in Cystic Fibrosis Patients: a Quality by Design Approach for Product Construction”. Int. J. Pharm. 471 (2014) 507-5015.

3. Verhing R., “Pharmaceutical Particle Engineering via Spray Drying”. Pharm. Research, Vol. 25, No. 5 (2008) 999-1022.