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Research Paper

Spray-dried amikacin sulphate powder for inhalation in cystic fibrosis patients: The role of ethanol in particle formation

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ABSTRACT

A Central Composite Design (CCD) was applied in order to identify positive combinations of the production parameters of amikacin sulphate spray-dried powders for inhalation, with the intent to expand the experimental space defined in a previous half-fractional factorial design. 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. 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.

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 antibiotics. 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 a 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

Abbreviations: CCD, Central Composite Design; CF, cystic fibrosis; CQAs, Critical Quality Attributes; CPPs, Critical Process Parameters; DoE, Design of Experiments; ED, Emitted Dose; FPD, Fine Particle Dose.

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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 respirable 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 sulphate 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 sulphate 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 (CCD) has been applied, aiming 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 favourable 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 respect to the effect of its absence/presence on final product structure and inhalation performance.

2. Materials and methods

2.1. Materials

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

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.

2.2. Design of Experiments (DoE)

A face centered-CCD with three factors at three levels was employed, and the experimental matrix is presented in Table 1. The design was constructed and analysed using Design-Expert® Software, Version 9.0.1 (Stat-Ease, Inc., Minneapolis, Minnesota, USA).

2.3. Preparation of spray-dried powders

2.5 g of amikacin sulphate was dissolved in water at room temperature. Ethanol was added under stirring to obtain the proportions reported in Table 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.

Table 1
Matrix of the face centred-CCD showing the studied parameters, their levels and the experiment number (#) including the replicated centred point (#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

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 aluminium cap. Part of the product was agglomerated into microparticle clusters by sieving as described in a previous publication [20].

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 aluminium stub and analysed 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 was 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 percentiles, $D_{(v,90)}$, $D_{(v,50)}$, $D_{(v,10)}$ 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, Georgia, USA).

The agglomerates were observed by optical microscopy (magnification 3×), 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, Nottingham, UK). The FSI divides the aerosol particles