Formulation and Evaluation of Inhalable Porous Tobramycin Powder Prepared by Spray Drying Technology

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Abstract: Inhaled tobramycin is one of the ideal ways to treat cystic fibrosis. The main purpose of this work is to prepare inhaled tobramycin dry powder by spray drying through reasonable formula design. The properties of the engineering particles are then characterized to reveal the particle-forming mechanism and structure-activity relationship. Tobramycin, which exists in the form of sulphate, has higher stability compared with its preparation alone. Leucine is enriched and crystallized on the surface of particles, which inhibits the absorption of environmental water by forming a hydrophobic shell and reduces the cohesion between particles. Compared with the organic solvent, ammonium bicarbonate (AB) as a porogen is less toxic and more suitable for scale-up. An optimized formulation was obtained when active pharmaceutical ingredient (API): Leucine: AB = 20: 7: 19. The sample under this formula has a porosity of 78.93 %. The loose and porous structure brings good flowability (CI index is 24.63 ± 1.17 %) and 40.78 % fine particles fraction (FPF). The particle deposition performance has been significantly improved. The drug load reaches 74.1 %, which adapts to the requirement of high-dose delivery of antibiotics.

1 INTRODUCTION

In the research history of pulmonary delivery of antibiotics, the treatment of cystic fibrosis (CF) with inhaled tobramycin powder was a classic case. CF patients were particularly vulnerable to the lung infections caused by gram-negative bacteria such as pseudomonas aeruginosa (PA) (Alhajj, 2022). Patients infected with PA experienced acute lung deterioration, reflected by worsening respiratory symptoms and a sharp decline in lung function. Tobramycin was a well-known fungicide, which was mainly active against aerobic gram-negative bacilli and was considered to be more active against PA than most other aminoglycoside antibiotics (Elborn, 2022).

The first tobramycin inhaled dry powder (TIP, TOBI) inhalation product was developed by Novartis and available in the U.S. market since 2013 (Miller, 2015). TIP should be taken twice a day. Each dose contained 4 capsules, of which contained 200 mg dry powder (including 112 mg tobramycin) (Buttini, 2018). The number of capsules limited the improvement of patient compliance. The excipient used in TIP was DSPC, a human endogenous substance, which would bring high pharmaceutical costs. At the same time, the perfluorooctyl bromide (PFOB) in the formulation could be replaced with a suitable porogen, which helped avoid some safety hazards in the production process (explosion-proof in workshop and equipment) (Miller, 2017).

The purpose of this paper was to explore the effects of different formulations and process parameters on the particle microstructure and final product properties, and to study the correlations among parameters, microstructure, properties and performance. In this work, tobramycin inhaled dry powder was prepared by spray drying, and the function and particle microstructure of the above components were verified by several characterizations, and the particle forming mechanism and structure-activity relationship of tobramycin inhaled powder were explored.

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2 MATERIALS AND METHODS

2.1 Materials

United States Pharmacopeia (USP) tobramycin was purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). 98 % concentrated sulfuric acid and L-leucine (USP) were purchased from Titan Technology Co., Ltd. (Shanghai, China). AB (USP) was purchased from Jizhi Biotechnology Co., Ltd. (Shanghai, China). Distilled water used in all steps was produced from a Milli-Q device (~18.2 MΩ cm).

2.2 Preparation of The Feed Solution and SD Particles

The feed solution composition was shown in Table 1. So as to avoid the crystallization of naked tobramycin, a certain amount of tobramycin was dissolved in the aqueous solution before adding 98 % concentrated sulfuric acid with a mass ratio of 3 to 1 under ultrasonication to acquire a clear solution. The ratio was obtained from the commercial formulations (TIP, TOBI), which had been thoroughly discussed and reviewed regarding their safety (US Patent, 10744098B2). Leucine was then added to the precursor formula. The enrichment of leucine on the particle surface also helped reduce the cohesion between particles and improved the powder delivery efficiency. To obtain a particle of low density, e. g. a porous particle structure similar to Pulmosphere®, AB was eventually added to replace PFOB. In the formula abbreviation, SD represented for spray drying; the subscript numbers of TS and L (leucine) represented the mass ratio between them; the subscript numbers of AB represented their ratio to API; the last digit represented the total solid content of the feed solution.

2.3 Methods

2.3.1 Particle Size Distribution

The particle size distribution was determined by laser diffraction using the Sympatec HELOS system equipped with the INHALER module (Sympatec GmbH, Clausthal-Zellerfeld, Germany). The dispersion pressure of the powders was 4 bars, and the dispersion agent was compressed air. Before measurement, each capsule was filled with approximately 10 mg of sample and subjected to static elimination. The particle size distribution of the powder was calculated by applying the Fraunhofer model preset in the instrument software.

2.3.2 Scanning Electron Microscopy

Scanning electron microscope (SEM, S-4700, Hitachi High Technologies Corporation, Japan) was used to characterize the powder morphology. The accelerating voltage of the SEM was 15 kV and the magnification of the images was 800–20000 times. Before observation, the conductive carbon tape was fixed on the sample preparation table, and an appropriate amount of sample powder was placed on the conductive carbon tape, and then loaded into the ion sputtering coater (MC1000, Hitachi Ltd., Japan). Finally, the powder was platinized by sputtering to make the surface sample conductive and avoid charge build-up during observation in the SEM.

2.3.3 Infrared Spectroscopy

The residual degree of AB was determined by Fourier transform infrared spectroscopy (FTIR) to evaluate the decomposition of AB. These spectra were obtained by a single reflection diamond ATR (Universal ATR) in a Nicolet IS50 FTIR spectrometer (Thermo Fisher Scientific Inc.). The FTIR spectra were obtained at a resolution of 0.09 cm^{-1} over a wavelength range from 400 cm^{-1} to 4000 cm^{-1}.

2.3.4 Pore Size, Porosity and Density

The porosity and pore size distribution of the sample were determined with a mercury porosimeter (AutoPore IV, Micromeritics, US). Under standard atmospheric pressure conditions, 3ml sample was placed in a permeameter, which was bonded to a glass capillary rod. When pressure was applied, the mercury moved down the capillary rod and filled the voids in and around the sample. The change in capacitance due to the loss of mercury in the capillary rod could be analyzed to determine the porosity and pore size distribution.

The bulk density (ρ_b) was measured by measuring the mass of about 4 mL of particles in a glass cylinder before being tapped and the tapped density (ρ_t) was determined by measuring the volume after tapping the above-mentioned particles of known mass. The sample's flowability was characterized by Carr's index (CI) calculated by Eq. (1):

$$CI = \frac{\rho_t - \rho_b}{\rho_t} * 100\% \quad (1)$$

2.3.5 Aerodynamic Properties

The aerodynamic characteristics of inhaled tobramycin particles were studied using the next generation impactor (NGI) (Copley Science,

Nottingham, UK). The samples were dispersed by the Twincaps® (BrightGene Ltd., Soochow, China) at 60 L/min evaluated by a Critical Flow Controller (Erweka, Heusenstamm, Germany). A vacuum pump (Erweka, Heusenstamm, Germany) was used to set the pressure drop across the whole device at 4 KPa. Each formulation would be dispersed for 4 s to attain 4 L volume. The specific operation was carried out according to the weighing method stipulated in the British Pharmacopoeia. The FPF and mass median aerodynamic diameter (MMAD) of the sample could be calculated by the data processing software CITDAS (Copley Science, Nottingham, UK).

3 RESULTS AND DISCUSSION

3.1 Particle Size, Morphology and Density

The sample particles were successfully prepared by spray-drying feed liquids with different formulations (Table 1). Table 2 summarized the physical properties of various spray-dried (SD) microparticles.

After adding AB to the precursor solution (Fig. 1), the morphology of the particles showed that the sphericity of the particles gradually recovered with the increase of the amount of AB added. Since the amount of AB was included in the total solid content, its decomposition and gas escape did not lead to a significant change in particle size. Under the same ratio of TS and leucine, the CI decreased from 29.12 ± 0.82 % (SD-$TS_1L_{0.35}$-AB_1-0.68) to the lowest 24.63 ± 1.17 % (SD-$TS_1L_{0.35}$-$AB_{3.5}$-0.5), indicating that the appearance of pores brought about a significant improvement in fluidity. It should be noted that the addition of excess AB would lead to obvious agglomeration behavior of the particles, the particle size rose to 34.10 ± 1.88 μm, and the CI rose to 28.44 ± 2.79 %, indicating that the flowability also had a certain degree of loss. The inference of this phenomenon would be analyzed in conjunction with the pore structure below.

Table 1: Precursor formulation and process parameters for spray drying.

Sample	Tobramycin (10^{-2} g/ml)	Sulfate (10^{-2} g/ml)	Leu (10^{-2} g/ml)	AB (10^{-2} g/ml)	Total solid content (%w/w)
SD-$TS_1L_{0.35}$-AB_1-0.5	0.228	0.080	0.108	0.083	0.50
SD-$TS_1L_{0.35}$-AB_2-0.5	0.196	0.068	0.093	0.143	0.50
SD-$TS_1L_{0.35}$-$AB_{3.5}$-0.5	0.161	0.056	0.076	0.206	0.50
SD-$TS_1L_{0.35}$-AB_5-0.5	0.137	0.048	0.065	0.250	0.50

Table 2: Physical properties of different formulations.

Sample	Bulk density ρ_b(g/cm^3)	Tapped density ρ_t(g/cm^3)	CI (%)	D_{50} (μm)	Porosity (%)
SD-$TS_1L_{0.35}$-AB_1-0.5	0.353±0.002	0.498±0.003	29.12±0.82	8.19±0.28	-
SD-$TS_1L_{0.35}$-AB_2-0.5	0.164±0.001	0.224±0.002	26.79±1.08	9.95±0.39	56.75
SD-$TS_1L_{0.35}$-$AB_{3.5}$-0.5	0.061±0.001	0.081±0.001	24.69±1.53	10.92±0.36	78.93
SD-$TS_1L_{0.35}$-AB_5-0.5	0.075±0.002	0.105±0.003	28.57±3.83	34.10±1.88	54.07

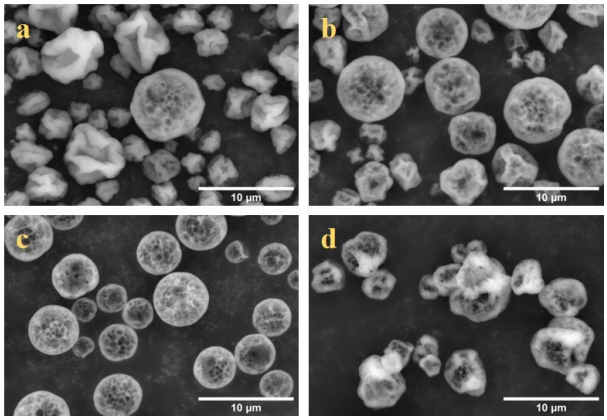

Figure 1: SEM images of (a) SD-$TS_1L_{0.35}$-AB_1-0.5; (b) SD-$TS_1L_{0.35}$-AB_2-0.5; (c) SD-$TS_1L_{0.35}$-$AB_{3.5}$-0.5; (d) SD-$TS_1L_{0.35}$-AB_5-0.5.

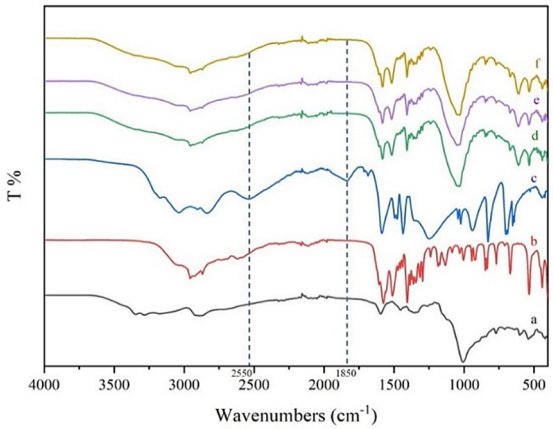

Figure 2: Infrared spectral images of (a) tobramycin sulfate; (b) leucine; (c) ammonium bicarbonate; (d) SD-$TS_1L_{0.35}$-AB_2-0.5; (e) SD-$TS_1L_{0.35}$-$AB_{3.5}$-0.5; (f) SD-$TS_1L_{0.35}$-AB_5-0.5.

3.2 Porous Structure and Residual Ammonium Bicarbonate

Infrared results for TS, leucine, AB, and samples were analyzed (Figure 2). Specifically, AB had unique characteristic peaks near 1850 cm^{-1} and 2550 cm^{-1}, but these characteristic peaks did not appear in the infrared image of the sample. This confirmed that AB has been completely decomposed with negligible residues.

The mercury intrusion analysis results (Fig. 3) showed that from sample SD-$TS_1L_{0.35}$-AB_2-0.5 to SD-$TS_1L_{0.35}$-$AB_{3.5}$-0.5, the porosity increased from 56.75 % to 78.93 %, and the pore size increased from 70 nm to 200 nm. This showed that the decomposition of AB, gas accumulation and final pore formation were stable and controllable in the particle formation process under the current conditions, which was of great significance for the formation of a feasible process flow. However, when the addition of AB continued to increase (SD-$TS_1L_{0.35}$-AB_5-0.5), the pore size expanded to 450 nm, while the porosity was significantly reduced to 54.07 %. Combining the results of the SEM images, particle size, and flowability data for this formulation above, the reason for this phenomenon was that the relative proportions of AB and the rest of the solids (TS and leucine) were too high, while the total solids content of the precursors was constant. In order to maintain the particle structure at a substantially constant particle size, the required solid content in the sample particles was already insufficient, which led to the collapse of the basic structure of the particles, and the porosity that should continue to increase also decreased. The finally obtained sample particles had serious agglomeration, increased cohesion, and decreased dispersibility and fluidity.

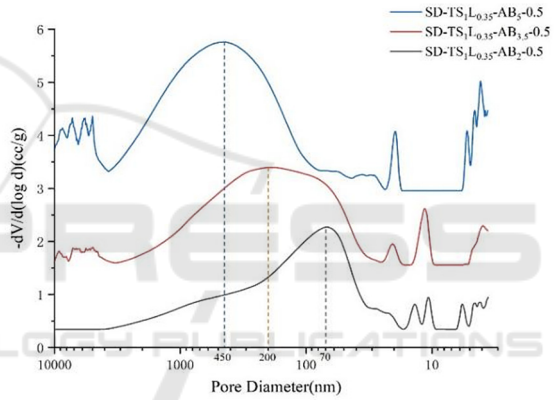

Figure 3: Images of the pore size distribution of (a) SD-$TS_1L_{0.35}$-AB_2-0.5; (b) SD-$TS_1L_{0.35}$-$AB_{3.5}$-0.5; (c) SD-$TS_1L_{0.35}$-AB_5-0.5.

3.3 Aerodynamic Performance

In order to obtain particles suitable for inhalation therapy, proper aerodynamic behavior was required. In general, the aerodynamic particle size of particles for inhalation should be between 0.5 and 5 μm. On this basis, the aerodynamic particle size distribution of the samples was characterized by NGI.

Preliminary experiments found that under the existing conditions, when the ratio of API and leucine was 20:7, the aerodynamic performance of the formulation was relatively optimal. Based on this ratio of leucine and TS, AB was added to the precursor solution and the aerodynamic performance of the samples were characterized (Fig. 4). From the formulation of SD-$TS_1L_{0.35}$-AB_1-0.5 to SD-$TS_1L_{0.35}$-$AB_{3.5}$-0.5, it could be seen that the particle

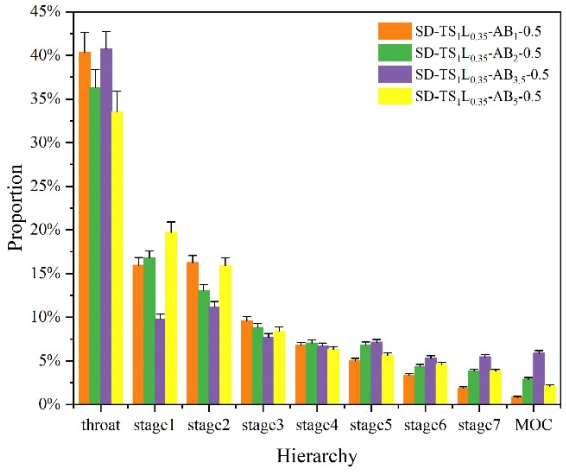

Figure 4: NGI results for different formulations.

distribution of each level had a tendency to gradually aggregate to higher levels. The deposition amount of the formula SD-$TS_1L_{0.35}$-$AB_{3.5}$-0.5 at stage 6, stage 7, and MOC was significantly higher than that of other formulas, indicating that the pores left by AB in the sample particles could significantly improve the aerodynamic performance of samples. The MMAD of the SD-$TS_1L_{0.35}$-$AB_{3.5}$-0.5 formula had dropped to about 2.4 μm, and the FPF had increased to 40.78 %. However, for the SD-$TS_1L_{0.35}$-AB_5-0.5, the FPF did not increase further but decreased to 34.07 %, and the MMAD also increased to 2.8 μm, which was consistent with the characterization results of SEM images, particle size and particle density. At this time, the agglomeration between particles was intensified, the fluidity and dispersion of the powder were also deteriorated, and the unsatisfactory particle structure eventually led to the decline of aerodynamic performance. Eventually, the formulation of SD-$TS_1L_{0.35}$-$AB_{3.5}$-0.5 was optimized.

4 CONCLUSION

This work explored the heat-mass coupling process in the spray drying process by adjusting the formulation and parameters, and finally achieved a FPF of 40.78 % in the optimal formulation. The low-density, loose and porous particle structure in the expected target was verified by multiple characterization results such as SEM images and porosity. This structure provided particles with better dispersibility and flowability and smaller aerodynamic size, making them suitable for efficient pulmonary delivery. AB residues in the final product were also substantially absent as evidenced by infrared spectroscopy. When the preferred ratio of API to leucine is 20: 7, leucine was enriched on the outer surface of the particles to a certain extent. When the ratio of non-porous components to AB was 10: 7, the porous particles had higher porosity and lower density, resulting in lower aerodynamic particle size. The final optimized spray drying formulation was SD-$TS_1L_{0.35}$-$AB_{3.5}$-0.5. The particles prepared under this formula had large geometric size, loose porosity, good deposition performance and high stability. Inhalation of particles was both the core idea of this article and the starting point for continuous improvement in formulation design.

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