

Solid-state characterization of tobramycin powders for inhalation

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Summary

Background: Tobramycin is an aminoglycoside broad-spectrum antibiotic, effective against Gram-negative bacteria, especially *Pseudomonas* infections in CF's patients.

The pulmonary administration of powder formulations offers well known advantages with respect to liquid formulation. When solid dosage forms are considered, the solid state characteristic of the active ingredient, i.e. polymorphism, represents an issue that should be addressed.

Methods: The spray-dried powder was produced starting from an ethanol-water mixture while three tobramycin raw material, namely Powder A, Powder B and jet-milled tobramycin Powder C were obtained from the market. They were characterized in terms of morphology by SEM, thermal behaviour by DSC and TGA, crystallinity by XRPD and water uptake by DVS.

Results: XRPD patterns showed that Powder A and C were crystalline, while SD powder and Powder B were amorphous. In the DSC profiles, Powder A showed two main endothermic peaks and one exothermic event between them. Interestingly, Powder C presented only the endotherm corresponding to the fusion of the high melting phase.

TGA pointed out a water content of 6.0%, 5.0%, 5.6% for Powder A, C and B, respectively. Spray-dried tobramycin showed the highest water content (9.8%). Isothermal DVS on spray-dried powder afforded a crystalline monohydrate phase presenting DSC and XRPD traces similar to those reported in literature.

Conclusions: The analysis on raw materials pointed out the existence of two solid phases of tobramycin. A monohydrate, already described in literature, can be obtained upon isothermal crystallization between 50% and 70% RH.

Introduction

Parenteral delivery is commonly used to treat acute lung infection as in the case of cystic fibrosis, pneumonia and chronic obstructive pulmonary disease (COPD). To overcome poor antibiotic distribution to the lung, high doses (10 mg/kg) are required, especially for aminoglycosides¹.

The administration of antibiotics to the respiratory tract of patients with lung infections is a well-established therapeutic approach for the stabilisation and restoration of lung function².

Thus, the administration of aminoglycosides by inhalation is an attractive route since allows reducing side effects like ototoxicity and nephrotoxicity. Dry powder inhaler formulations incorporate a powder containing the drug as micron-sized particles (aerodynamic diameter less than $<5 \mu\text{m}$) that, upon inhalation are aerosolised from the device to deposit in the respiratory tract. Dry powder inhalers have many advantages over liquid formulation, including relatively high dose delivery, better physico-chemical stability, no need of patient coordination and absence of propellant.

When dry powders are considered, the solid state characteristic of the active ingredient, i.e. polymorphism, represents an issue that should be addressed because it may affect the quality and the performance of a drug product³. Indeed, drug stability and respirability are critical quality attributes which need to be evaluated during pharmaceutical development and kept constant during clinical studies and marketing. Powders may have different polymorphic phases and this topic is crucial during pharmaceutical development^{3,4}.

The aim of this work was to investigate the solid state properties of a spray-dried powder intended for pulmonary delivery in comparison with different tobramycin raw materials.

Materials and Methods

Materials

Tobramycin was supplied by TEVA Pharmaceutical (Debrecen, Hungary) and by Biovet (Razgrad, Bulgaria); a jet-milled tobramycin was obtained by Chiesi Farmaceutici (Parma, Italy). Water was purified by reverse osmosis (MilliQ, Millipore, France). All solvents and chemicals were obtained from VWR International (Milano, Italy) and were of analytical grade.

Methods

Spray-drying process

An ethanol-water mixture (30:70) containing 1% w/v of tobramycin was spray-dried. A B-290 Mini Spray-Dryer with a Dehumidifier B-296 (BÜCHI, Laboratoriums-Technik, Swiss) was employed at the following operating conditions: inlet temperature, 125 °C; feed rate, 3 ml/min; spray flow rate, 600 L/h; and aspirator setting, 100% (35 m³/h). The outlet temperature was approximately 73 °C. The spray-dried powder was collected from the lower part of the cyclone and the collection vessel, and stored in a desiccator.

X-Ray Powder Diffraction (XRPD)

XRPD measurement was carried out at room temperature using a Rigaku Miniflex X-Ray diffractometer (Tokyo, Japan) to investigate tobramycin crystallinity. The powder samples were loaded onto a horizontal aluminium sample holder and measured with a slit-detector Cu K α radiation source ($\lambda = 1.5406$ Å, 40 kV voltage, and 44 mA current). The scanning rate of 1.50°/min over a 2 θ range of 2.0–35.0° was employed.

Scanning Electron Microscopy (SEM)

Raw materials and spray-dried powder morphology were investigated using Scanning Electron Microscopy (SEM, Zeiss SUPRA 40, Oberkochen, Germany). The microscope operated under high vacuum conditions with an accelerating 1.5 kV voltage, at different magnifications. Powders were deposited on adhesive black carbon tabs pre-mounted on aluminium stubs.

Differential Scanning Calorimetry (DSC)

The DSC profiles were determined using DSC mod. 821 (Mettler Toledo, USA). For the measurement, 4-5 mg of powder were weighted into a 40 μ l aluminium pans sealed and double pierced. The samples were scanned at a heating rate of 10 °C/min under nitrogen flow of 100 ml/min in the temperatures range 25-250 °C.

Thermogravimetric Analysis (TGA)

A sample of about 10 mg was placed in a 100 μ l alumina pan in the heating zone of the TGA apparatus (TGA/DSC1, Mettler Toledo, USA). The samples were heated at 10 °C per minute from 25 °C to 250 °C. The mass loss was measured directly by the TGA. Instrument was purged with nitrogen at a constant flow rate (80 ml/min) and data analysis was accomplished using STARe evaluation Software.

Dynamic Vapour Sorption (DVS)

The water absorption tendency of the various samples (10.0–15.5 mg) was evaluated using a dynamic vapour sorption analyser (Aquadyne, Quantachrome Instruments, England). DVS is a gravimetric method, thus the instrument is equipped with a microbalance capable of measuring small changes in the sample mass. The water uptake was monitored as function of the relative humidity (from 0 to 95%) at constant temperature of 22°C.

Results

In Figure 1, X-Ray diffractograms of the powders are reported. Powder A and C (panel a) presented a series of peaks with a drift of the baseline which indicated the crystalline nature of the samples in the presence of an amorphous portion. Powder B and SD (panel b) were completely amorphous as testified by the halo without peaks. Interestingly, the diffraction patterns of Powder A and C were not completely superimposed. In particular, the main differences were noticed in the 15-18°, 19-21° and 31-35° 2 θ regions. This indicates that the crystalline phases of the two materials were different.

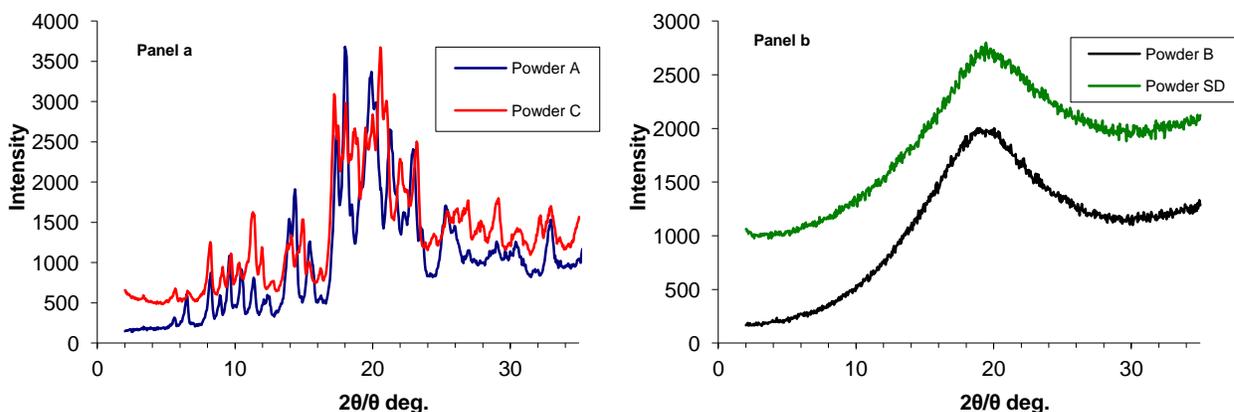


Figure 1. XRPD of Powder A and C (left, panel a) and of Powder B and SD (right, panel b).

The particle and surface morphology were investigated by SEM, as shown in Figure 2. Powder A showed coarse crystal particles with irregular and rough surface; Powder B had flakes-like particles with bigger size than particles of Powder A. Powder C was composed of micronized particles that maintained crystal structure with wrinkled morphology. Finally, spray-dried powder had different morphology and dimensional characteristics respect to the raw materials. In fact, microparticles were spherical with smooth surface and size below 5 μm .

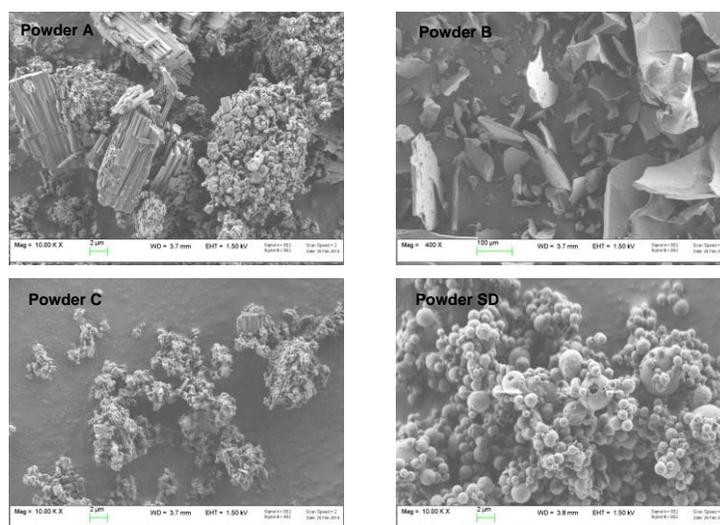


Figure 2. SEM images of Powder A, B, C and SD.

The DSC traces of the raw materials and the spray-dried tobramycin are shown in Figure 3. The traces of Powder SD and Powder B presented only a broad endotherm of moisture evaporation between 40 and 120°C. Also the Powder A showed this kind of thermal event; however it highlighted other interesting phenomena. In particular, two small endothermic peaks at 124 (-10.4 J/g) and 167°C (-5.3 J/g), respectively. Thereafter, a peak at 228°C (-43.3 J/g) immediately followed by an exothermic event at 231°C (9.5 J/g), in turn followed by a much bigger endotherm at 238°C (-120.6 J/g).

Powder C, after the endotherm correspond to the water evaporation, presented a small endothermic event at 123°C (-6.0 J/g) followed by an exothermic peak at 161°C (24.4 J/g), and by the final endotherm at 238°C (-138.7 J/g). Thus, DSC data confirmed that Powder A and C were different crystal phases whereas Powder B and SD were amorphous.

TGA pointed out a water content of 6.0%, 5.0%, 5.6% for Powder A, C and B, respectively. Spray-dried tobramycin showed the highest water content (9.8%).

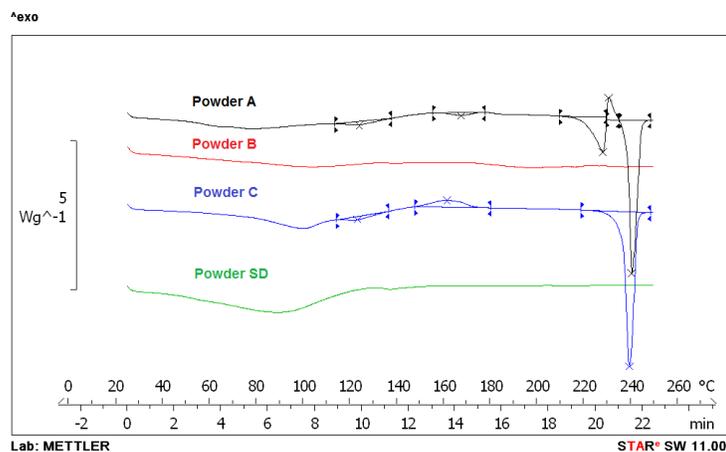


Figure 3: DSC traces of Powder A, B, C and SD.

DVS profiles of Powder A and SD are reported in Figure 4 as mass change percentage and relative humidity as a function of time. It can be appreciated that Powder A showed a progressive increase in mass giving rise to a peak at the highest RH value. The SD powder presented a sudden mass increase between 40 and 60 % RH follow by a decrease. This was interpreted as the consequence of the tobramycin crystallization with “squeezing-out” of entrapped water. In fact, the SD powder obtained upon exposure at RH higher than 50 %, recovered and submitted to DSC and XRPD analysis showed solid state characteristic similar to that of the monohydrate crystal phase already described by Dash and Suryanarayanan⁵.

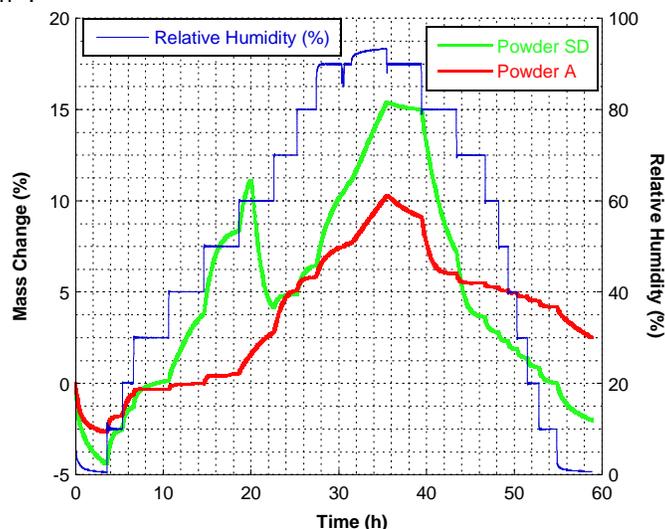


Figure 4. Water uptake for Powder A and SD.

Conclusions

Tobramycin can exist in different crystal phases as pointed out by XRPD and DSC carried out on different marketed samples.

Moreover, a monohydrate can be obtained upon isothermal crystallization of the Powder SD between 50% and 70% RH. These finding must be taken in careful consideration when developing a dry powder inhaler formulation.

References:

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