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Surface composition and aerosolization stability of an inhalable combinational powder formulation spray dried using a three-fluid nozzle

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Abstract

Purpose—This study aims to understand the impact of spray drying nozzles on particle surface composition and aerosol stability.

Methods—The combination formulations of colistin and azithromycin were formulated by 2-fluid nozzle (2N) or 3-fluid (3N) spray drying in a molar ratio of 1:1. A 3-factor, 2-level (2³) factorial design was selected to investigate effects of flow rate, inlet temperature and feed concentration on yield of spray drying and the performance of the spray dried formulations for the 3N.

Results—FPF values for the 2N formulation (72.9 ± 1.9% for azithromycin & 73.4 ± 0.8% for colistin) were higher than those for the 3N formulation (56.5 ± 3.8% for azithromycin & 55.1 ± 1.6% for colistin) when stored at 20% RH for 1 day, which could be attributed to smaller physical size for the 2N. There was no change in FPF for both drugs in the 2N formulation after storage at 75% RH for 90 days; however, there was a slight increase in FPF for colistin in the 3N formulation at the same storage conditions. Surface enrichment of hydrophobic azithromycin was measured by X-ray photoelectron spectroscopy for both 2N and 3N formulations and interactions were studied using FTIR.

Conclusions—The 3-fluid nozzle provides flexibility in choosing different solvents and has the capability to spray dry at higher feed solid concentrations. This study highlights the impact of hydrophobic azithromycin enrichment on particle surface irrespective of the nozzle type, on the prevention of moisture-induced deterioration of FPF for hygroscopic colistin.

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Keywords

Dry powder inhaler; spray drying; three-fluid nozzle; surface composition; aerosol performance

INTRODUCTION

Respiratory infections are among the deadliest diseases. Infections in the lower respiratory tracts are not easy to treat because many antibiotics cannot be delivered to the infectious site in the deep lungs when administered systemically due to pharmacokinetics (1–4). For instance, colistin is an effective antibiotics for treating multi-drug resistant Gram-negative bacteria (5). However, only a small fraction of the drug can reach the infectious site in the deep lung if colistin is administered intravenously (6, 7). On the other hand, increasing the dose of colistin can cause nephrotoxicity in 45%–55% of patients (8, 9). Recent studies have demonstrated that inhaled colistin showed much higher drug concentrations in the airway in a rat model (10) and improved *in vivo* bacterial killing in a mouse lung infection model (11–13) when compared to the parenteral administration.

Since their discovery, antibiotics have saved billions of lives. However, the past decades have seen a remarkable increase of multi-drug resistance (MDR) bacteria; while the new antibiotics pipeline experiences draught (14). Among all infections caused by MDR Gram-negative pathogens, those in the lungs are the most deadly (15). These bacteria typically include *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* which are responsible for the majority of acute and chronic pulmonary infections (15). The resistance build-up of these pathogens to the first-line antibiotics is rapid (14). Although colistin is often the only effective antibiotic against many MDR Gram-negative pathogens, an emergence of colistin-resistance has occurred in the past a few years (14). Mono-therapy of colistin may lead to resistance (16–18), making combination therapy is a promising strategy to combat colistin resistance (14, 19).

Formulating colistin into pulmonary drug delivery systems as a combination therapy has a great potential in re-inventing this ‘old’ drug to be a better utilized antibiotic. Literature demonstrates that the combination of colistin with azithromycin has excellent synergistic activities against Gram-negative *P. aeruginosa* (20). Azithromycin has been broadly used to treat various bacterial infections (21) but azithromycin monotherapy could also lead to resistance (22). Hence, the inhalation route may be a viable option for delivery of the antibiotic combination of colistin-azithromycin.

Dry powder inhaler (DPI) is a viable choice for inhaled colistin therapy because colistin is chemically more stable in the powder form. In the aqueous solution, colistin is stable for only 60 days and 120 hours at 4°C and 37°C, respectively (23). In contrast, the powder form of colistin is stable for at least two years. There are several approaches to manufacturing fine drug powders for DPIs; spray drying is among the most popular particle engineering techniques because it enables effective particle size control (24).

One of the challenges for spray dried colistin DPIs is their hygroscopicity (25). After exposure to humidity, the fine colistin particles tend to agglomerate and may not reach deep

lungs. An earlier study by *Zhou et al.*, showed that when the spray dried colistin was stored at the relative humidity of 75% or higher, it absorbed up to 30% (w/w) water (5). Such moisture absorption led to substantial reduction in aerosolization, from $80.1 \pm 1.3\%$ at 60% RH storage to $54.7 \pm 7.1\%$ at 75% RH storage. But when colistin was co-spray dried with 50% (w/w) hydrophobic azithromycin, there was an enrichment of azithromycin (96.5%) on the particle surface, confirmed by XPS (25). Such surface coating of hydrophobic azithromycin has been shown to prevent moisture-induced reduction in colistin aerosol performance (25).

The current co-solvent spray drying method has limitations in developing combination formulations containing components with very different solvent solubilities, because traditional 2-fluid nozzle (2N) spray drying only uses one liquid channel. It may not be possible to dissolve two or more components with very different solubilities in a single solvent with the desired drug amount for 2N spray drying, which may lead to very low output for manufacturing. The 3-fluid nozzle (3N) spray drying can circumvent this problem by providing two liquid channels for each the colistin-water solution and azithromycin-ethanol solution. The 3-fluid nozzle has an inner channel and an outer channel that allow two separate drug solutions to be pumped and mixed only at the tip of nozzle (26–28). This study aimed to examine the surface composition and aerosolization behavior of co-spray dried combination DPIs containing colistin and azithromycin. We also determined the short-term aerosol stability of such formulation stored at an elevated humidity. Characteristics of the formulations prepared by the 3N spray drying were compared with those of 2N spray drying.

MATERIAL AND METHODS

Materials

Azithromycin dihydrate and colistin sulphate were purchased from Beta Pharma (Beta Pharma (Shanghai) Co. Ltd, Wujiang City, China). Acetonitrile (HPLC grade) was purchased from Merck (Fair Lawn, New Jersey, USA). Sodium sulphate anhydrous was purchased from Fisher Chemicals (Fair Lawn, New Jersey, USA). Potassium phosphate dibasic trihydrate was purchased from VWR Chemicals (Solon, Ohio, USA).

Solubility

To determine the solubility of azithromycin and colistin in water-ethanol co-solvent, excess amounts of azithromycin and colistin were added to water-ethanol (1:1 v/v) co-solvent (80 mg of azithromycin in 10 mL water-ethanol co-solvent, and 240 mg of colistin in 10 mL water-ethanol co-solvent.) and then stirred for 24 hours at the room temperature. The solutions were centrifuged at 50 rpm under 25°C for 15 minutes and the supernatant was drawn and diluted. Then the drug concentration was analyzed using HPLC.

Drug Quantification by HPLC

Concentrations of azithromycin and colistin were determined using the established HPLC methods (29). An Agilent Eclipse Plus (5 μ m C18 150 $\times$ 4.60 mm) column was used (Agilent, Waldbronn, Germany). For azithromycin, the mobile phase consisted of 50 mM

dipotassium phosphate trihydrate (adjusted to pH 8.2) (A) and acetonitrile (B). The isocratic elution program used for azithromycin detection was 35% A and 65% B (v/v) for 15 min at the flow rate of 1.5 mL/min at the wavelength of 210 nm. The standard curve was prepared in the concentration range of 0.008 – 1.0 mg/mL ($R^2 > 0.999$). For colistin, the mobile phase consisted of 30 mM sodium sulfate (adjusted to pH 2.5 with H_3PO_4) (A) and acetonitrile (B). The isocratic elution program used for colistin detection was 76% A and 24% B (v/v) for 11 min at the flow rate of 1.0 mL/min and the wavelength of 210 nm. A standard curve of colistin was prepared in the range of 0.008 – 1.0 mg/mL ($R^2 > 0.999$).

Spray drying

A Büchi mini spray dryer B-290 (Büchi Labortechnik AG, Fällwil, Switzerland) was used for spray drying colistin and azithromycin powders in a molar ratio of 1:1. The combinational formulations were prepared using 2N and 3N nozzles. The spray dried formulations of pure azithromycin or pure colistin were prepared using the 2N nozzle.

Two-fluid nozzle—Colistin was dissolved in water and azithromycin was dissolved in ethanol, then the two solutions were carefully mixed without precipitation in the molar ratio of 1:1. The mixed solution was spray dried using a 2N (Figure 1A) to produce fine dry powder. The spray drying conditions for the 2N were: inlet temperature 150 °C; outlet temperature 70 ± 2 °C; aspirator 35 m³/h; atomizer setting 700L/h; feed concentration 10 mg/ml; flow rate 8 mL/min.

Three-fluid nozzle—A 3N (Figure 1B) was used to spray dry colistin and azithromycin, which were dissolved separately in water and ethanol, respectively. The two solutions were pumped into separated channels in the 3N and spray dried with colistin in the inner channel and azithromycin in the outer channel.

Design of experiments—To understand the effect of various formulation and processing parameters on the performance of the spray dried formulation, and to optimize their levels, a structured approach towards experimental design was used. Design of experiments (DOE) offers several advantages over the traditional one-variable-at-a-time approach including the ability to determine the interactions between variables as well as better predictability using the statistical models (30). Based on the risk assessment, literature review and preliminary data, the flow rate, inlet temperature, and feed concentration were selected as critical parameters for 3N spray drying in this study. A 3-factor, 2-level (2^3) factorial design was selected using Design-Expert version 11 (Stat-Ease, Minneapolis, USA) to investigate the effect and interaction of these three variables on yield of spray drying and the performance of the spray dried formulations (Table S1). Table 1 shows the variables used for designing the experiments along with their levels. Figure S1 shows the schematic of a 3-factor and 2-level factorial design where each corner of the cube represents a factorial point experiment and the central dot represents a center point experiment. In addition to the eight factorial points, three center points were selected to estimate the curvature and the variability in the spray drying process resulting in eleven DOE experiments. These parameters were used when performing 3N spray drying and the formulations were characterized for their aerosol performance. Statistical analysis was performed on the results and models were built using

ANOVA and regression to estimate the relationship between these three variables and the performance attributes of the formulation. These models were then used for optimizing the formulation and predicting the range of variables that would give the desired product characteristics.

Particle size distribution

The particle size distribution of the co-spray dried samples was determined by laser diffraction using a Mastersizer 3000 with an Aero-S dry powder dispersion unit (Malvern Instruments, Worcestershire, UK). Each powder sample (approximately 10 – 20 mg) was fed into the dispersion system. Compressed air at 4 bar was used to disperse the powder through the measuring zone. D10, D50 and D90 were calculated using a built-in software.

Powder X-ray diffraction (PXRD)

A Rigaku Smartlab™ diffractometer (Rigaku Americas, Texas, USA) was used to determine powder crystallinity. Equipped with a Cu-K α radiation source and a highly sensitive D/tex ultra-detector, the PXRD patterns were acquired from 5 to 40° 2 θ at a scan speed of 4°/min and a step size of 0.02°. The voltage and current used were 40 kV and 44 mA, respectively.

Scanning electron microscopy (SEM)

A field emission scanning electron microscope (NOVA nanoSEM, FEI Company, Hillsboro, Oregon, USA) was used to examine the morphology of spray dried particles. A small amount of sample powder (5 – 10 mg) was placed on a stainless-steel stub covered with adhesive carbon tape. The stub was sprayed with pressurized air to remove excessive powder. Then the stub was coated with platinum using a sputter coater (208 HR, Cressington Sputter Coater, Watford, UK) at 40 mA for 1 min. Images were captured at an acceleration voltage of 5 kV.

Dynamic water vapor sorption (DVS)

Moisture sorption was measured using a dynamic vapor sorption (31). For each test, approximately 15 mg of powder was used. The machine was set to 0% relative humidity (RH) as the baseline, then the sample was placed on the pan. A sorption cycle ran from 0% RH to 90% RH with 10% increases for each step. A desorption cycle ran from 90% RH to 0% RH. We deemed the sample to be equilibrated at the mass change rate smaller than 0.002% per minute.

Thermal gravimetric analysis (TGA)

The weight loss on heating was measured using a thermogravimetric analyzer (Shimadzu DSC-60, Kyoto, Japan). Approximately 5 mg sample was heated from room temperature to 150°C at a rate of 10°C/minute in a nitrogen environment. The weight loss was analyzed from room temperature to 120°C (n=3).

X-ray photoelectron spectroscopy (XPS)

Surface composition (approximately top 10 nm) of powder formulations was determined using X-ray photoelectron spectroscopy (XPS) (AXIS Ultra DLD spectrometer, Kratos

Analytical Inc., Manchester, UK) using the established method (29). Curve-fitting was performed following a Shirley background subtraction using standard procedure in the CasaXPS software with model peaks obtained from pure compounds. O1s data were used to calculate surface composition.

Attenuated Total Reflectance–FTIR spectroscopy

FTIR analysis was conducted on an attenuated total reflectance (ATR) FT-IR spectrometer (Cary 600 series FTIR spectrometer, Agilent Technologies, Santa Clara, CA, USA). Spectra were collected from 800 to 4000 cm^{-1} with a resolution of 4 cm^{-1} . The scan number of 256 was used for both background and samples. The obtained spectra were analyzed to using ResolutionPro software (Version 5.2.0, Agilent Technologies, Santa Clara, CA, USA).

In-vitro aerosolization

A next-generation impactor (NGI, Apparatus X, USP X, Copley, Nottingham, UK) was used to determine in-vitro aerosolization performance of the formulations. To minimize particles bouncing in stages, each stage was coated with silicon spray. The capsule (each was filled with 10 ± 0.5 mg sample) was fired through a low-resistance RS01 DPI device (Plastiaple S.p.A., Osnago, Italy), with 4 L of air passing through the inhaler at an airflow of 100 L/min for 2.4 s, which corresponded to a pressure drop of approximately 4 kPa. Capsule, inhaler device, USP throat and Stage 1–8 of the NGI were washed with 20 mL co-solvent consisting of 1:1 (v/v) of 20 mM potassium dihydrogen phosphate (adjusted to pH 5 with 10 % (w/v) sodium hydroxide) and methanol. The solutions with dissolved drugs were collected, centrifuged, and transferred to HPLC vials. Fine particle fraction (FPF) was calculated as the fraction of drugs with an aerodynamic diameter $< 5 \mu\text{m}$ over the recovered dose. Emitted dose was determined as the percentage of powder not retained in the capsule and device over the recovered dose. Median mass aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were also calculated based on NGI data (32).

Statistical analysis

GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA) was used to perform statistical analysis. Results were analyzed using independent t-test or one-way analysis of variance (ANOVA) with Tukey–Kramer post-hoc tests and displayed as mean $\pm$ standard deviation (SD). A p value of < 0.05 is deemed as statistically different.

RESULTS

Solubility

The solubility of azithromycin dihydrate in ethanol is approximately 16 mg/ml and the solubility of colistin sulfate in water is 50 mg/ml, based on the literature (33). The measured solubility of azithromycin dihydrate was found to be 7.9 mg/ml in 1:1 (v/v) water-ethanol co-solvent system and measured solubility of colistin sulfate was 24 mg/ml in the 1:1 (v/v) water-ethanol co-solvent system. One of the disadvantages of using a 2-fluid nozzle is that the water-ethanol co-solvents need to be premixed; when using the three-fluid nozzle, two solutions can be sprayed through two different feed channels (Figure 1). It is worth noting that it would not be feasible to spray dry a solid concentration of 30 mg/ml using a two-fluid

nozzle due to poor solubility of azithromycin in the water-ethanol system. With the recent molecules in discovery and development being poorly water soluble it is very challenging to select a solvent system in which both API and excipient are soluble. Thus, the three-fluid nozzle offers flexibility in choosing different solvent systems and the capability to spray dry at higher solid concentrations to improve output. Based on the measured solubility information, drug concentrations ranging from 10 to 30 mg/ml were selected for the DOE study using three-fluid nozzle.

Formulation and process optimization for 3-fluid nozzle

Impacts of three independent variables (i.e. feed concentration, inlet temperature and flow rate) on the three dependent variables (i.e. spray drying yield, emitted dose, and fine particle fraction) of spray dried formulation were studied using the design of experiments (DOE). The experimental design is shown in Table S1 along with the results for spray drying yield, emitted dose, and fine particle fraction. These results were then used to model the relationship between the dependent and independent variables which are described in this section. SEM images, PXRD graphs and deposition profiles of DoE formulations are presented in Figure S2–S4, representatively.

Spray drying yield—The spray drying yield was found to be affected by the studied variables as shown by the contour plots in Figure 2 where the change in the color from green towards red represents an increase in the spray drying yield, and the numbers on the contour lines indicate the value of spray drying yield on that line. An increase in the yield was observed with an increase in the inlet temperature as shown in Figure 2a. At the low inlet temperature, an increase in flow rate decreased the yield. Increasing drying temperature provides efficient drying and high yield is obtained even at high flow rates as shown in Figure 2a. An increase in the yield was observed with increased feed concentration as shown in Figure 2b. Such effect was more remarkable at the lower temperature than at the higher temperature.

Fine particle fraction (FPF)—FPF was found to be influenced by the feed concentration. A decrease in FPF of colistin and azithromycin was observed with an increase in the feed concentration as depicted in Figure 3a and Figure 3b, respectively.

Emitted dose (ED)—ED was also found to be influenced by the feed concentration. An increase in ED of colistin and azithromycin was observed with an increase in the feed concentration as depicted in Figure 3c and Figure 3d, respectively.

Optimized formulation—Using the models generated by DOE, a 10 mg/mL feed concentration, 4 mL/min flow rate, and 150 °C inlet temperature was suggested to obtain high FPF using the three-fluid nozzle. The spray dried formulation manufactured using these optimized settings was used for further studies.

SEM

SD azithromycin are the sphere-shaped particles in Figure 4a, and SD colistin particles are the dimpled polyhedrons shown in Figure 4b. The 3N formulations consisted of small

spherical shaped particles attached to larger polyhedron particles (Figure 4c). However, the 2N particles formed a mixture of both hollow and solid spherical particles (Figure 4d).

Figures 4e–f shows SEM images of 2N and 3N spray dried formulations stored at the relative high humidity of 75% for 90 days. No change in morphology was observed for both formulations at 75% RH for 90 days as compared to those stored at 20% RH for 1 day (Figure 4c–d).

PXRD

Figure S5 show that there is no sharp peak in the PXRD diffractogram, which suggests that the formulations were amorphous when stored at 20%RH for 1 day and remained amorphous after storage at 75% RH for 90 days.

In-vitro aerosol performance

Figure 5 shows the FPF and ED of the 2N and 3N spray dried formulations. The 2N formulation has an average FPF of $72.9 \pm 1.9\%$ for azithromycin and $73.4 \pm 0.8\%$ for colistin which is significantly higher than the 3N ($56.5 \pm 3.8\%$ for azithromycin and $55.1 \pm 1.6\%$ for colistin) when stored at 20% RH for 1 day ($p < 0.05$). As for the emitted dose, no significant difference was found between the 2N and 3N formulations for azithromycin. Significant difference was observed for colistin ($p < 0.05$).

Figure 5C reports the FPF for 3N stored at 20% RH day 1 and at 75% RH for up to 90 days. It is evident that there are significant changes in the FPF of colistin in the 3N formulation stored at 75% RH after 90 days. FPF values for the 3N formulation were $56.5 \pm 3.8\%$ for azithromycin and $55.1 \pm 1.6\%$ for colistin upon storage at 20% RH for 1 day. Upon further storage at 75% RH for up to 90 days there was significant increase in FPF for azithromycin ($68.3 \pm 4.3\%$) and colistin ($64.2 \pm 2.2\%$).

However, there was no significant difference in the FPF on storage of spray dried powder obtained by 2N ($72.9 \pm 1.9\%$ for azithromycin and $73.4 \pm 0.8\%$ for colistin) at 20% RH for 1 day as compared to those stored at 75% RH for 90 days ($74.3 \pm 2.9\%$ for azithromycin and $74.6 \pm 4.4\%$ for colistin) (Appendix A5, $p > 0.05$). This observation for the 2N formulation is in agreement with the previous report (25). Particle deposition profiles are presented in Figures S6 – S9.

MMAD and GSD

Table 2 shows the MMAD and GSD of all co-spray dried colistin-azithromycin formulations prepared using 3N or 2N nozzles. The 2N co-spray dried colistin-azithromycin has a MMAD (azithromycin: 2.2 ± 0.1 , colistin: $2.2 \pm 0.2 \mu\text{m}$) which is comparatively smaller than 3N (azithromycin: 2.9 ± 0.1 , colistin: $3.2 \pm 0.6 \mu\text{m}$) when stored at 20% RH for 1 day. There is no significant difference in the MMAD when the respective formulations are stored at 75% RH for 90 days ($p > 0.05$).

X-ray photoelectron spectroscopy (XPS)

XPS results show that azithromycin was the dominant molecule at the surface of both the co-spray dried formulations prepared using two different nozzle types (Table 3). The 2N formulation has slightly higher azithromycin on the particle surface than that of the 3N formulation ($p < 0.05$). The two-fluid nozzle data are in agreement with our previous studies. The solubility of the different components in the feed solution plays a critical role in determining the surface composition. For the two-fluid nozzle formulation, as azithromycin has lower solubility in the water-ethanol co-solvent system as compared to colistin, azithromycin reaches supersaturation earlier and precipitates at the particle surface, leading to enrichment at the particle surface after spray drying. Interestingly, the 3N formulation also exhibited azithromycin-enrichment at the particle surface, although with a slightly lower surface coverage of azithromycin than the 2N formulation.

Particle size distribution

Table 4 shows particle size distributions of the co-spray dried colistin-azithromycin (1:1) stored at 20% RH. The 2N drug particles have the smallest D50 of 1.9 μm , which could be the reason for the relative higher aerosol performance of 2N formulation. When spray drying using 3N the formulation has a D50 of 2.8 μm .

Attenuated Total Reflectance–FTIR spectroscopy

FTIR spectra are shown in Figure 6. In our previous report, the amide structure was shown to be the most sensitive to interact with azithromycin, so we focused on analyzing the differences in the associated spectral regions (29). The information of IR peak wavenumber and their degree of changes are presented in Table 5 to demonstrate more detailed differences between formulations. In the IR spectrum of COL, two peaks corresponding to the characteristic of amide A and B were found at 3251.1 cm^{-1} and 3044.9 cm^{-1} , respectively. The representative peaks from the stretching vibrations of amide I C=O stretching and amide II N-H bending were observed at 1642.1 and 1516.6 cm^{-1} , respectively (34). Similar to our previous report, the IR peaks of 2N that correspond to amide A, B and amide II N-H bending were shifted to a higher wavenumber with the extents of shifts larger than the resolution of 4 cm^{-1} used in the measurement, compared to those of pure colistin. However, for the 3N sample, the shift of these peaks was much less than those of 2N (Table 5).

DVS

Figure S10 shows the water sorption and desorption behavior of the five spray dried formulations. The SD azithromycin absorbs the least amount of water (6.98% w/w) at 90% RH.

TGA

Table S4 displays the percent weight loss measured by TGA. SD Colistin has the highest weight change of $8.8 \pm 1.7\%$; while SD Azithromycin has the lowest weight change of $0.7 \pm 0.1\%$. Two combinational formulations have weight loss values between SD Colistin and SD Azithromycin. The TGA data agree with the DVS data that colistin is hygroscopic and more

difficult to remove solvent residues during spray drying and azithromycin is hydrophobic and easier to remove solvent residues.

DISCUSSION

Spray drying has been an established technique for producing inhalable particles. Newer modifications are made, such as incorporating additional nozzles in the spray drying process for further improving the efficacy of the process. The objective of our study was to understand the impact of three-fluid nozzle spray drying technique on the final powder characteristics particularly related to aerosol performance at higher humidity conditions. The three-fluid nozzle has the advantage of incorporating multiple drug combinations in a single formulation with broader solvent choices (26).

Interesting observations were made by *Leng et al.*, who compared the two-fluid nozzle and three-fluid nozzle spray drying processes in formulating inhalable dry powders (35). Budesonide nanocomposite microparticles (BNMs) were prepared using a two-fluid nozzle spray drying process wherein Budesonide nanocrystals suspended in an aqueous mannitol solution were co-spray dried. Budesonide-mannitol microparticles (BMMs) were prepared using a spray drier with a three-fluid nozzle by concomitant spray drying of a budesonide solution and an aqueous mannitol solution. They noted that budesonide remained crystalline in the BNMs, whereas budesonide was amorphous in the BMMs and the yield of BNMs was higher than that of BMMs. Hence, it was concluded that the two-fluid nozzle spray drying was superior to three-fluid nozzle spray drying in terms of yield (35). In our study azithromycin co-spray dried with colistin was found to be amorphous when spray dried with either two- or three-fluid nozzle and in fact spray drying yield was higher with the three-fluid nozzle as compared to the two-fluid nozzle.

For the three-fluid nozzle formulations, we observed that the geometric size of the particles could have an impact on the yield at the high drug concentration. Higher feed concentration results in an increase in the geometric particle size and lower FPF, according to Equation 1 (24).

$$Dg = \sqrt[3]{\frac{CF}{\rho}} Dd \quad \text{Eq. 1}$$

Where Dg is geometric diameter, CF is feed solution concentration, ρ is particle density and Dd is droplet diameter. Negative correlation was observed between D50 of spray dried particles and the FPF whereas positive correlation was observed between the D50 of spray dried particles and the emitted dose. Emitted dose is known to be influenced by the particle size which governs the surface energy, flowability and fluidization (36). Larger particles with less tendency to agglomerate would lead to better flowability and fluidization properties as compared to the smaller particles, and thereby are more easily emitted from the device (36).

An earlier study showed that when the spray dried colistin was stored at the relative humidity of 75% or higher, it absorbed up to 30% (w/w) water (5). Such moisture absorption led to substantial reduction in aerosolization from $80.1 \pm 1.3\%$ at 60% RH storage to $54.7 \pm$

7.1% at 75% RH storage. But when colistin was co-spray dried with 50% (w/w) hydrophobic azithromycin (Figure S10), there was an enrichment of azithromycin (96.5%) on the particle surface confirmed by XPS (25). Such surface coating of hydrophobic azithromycin has been shown to prevent moisture-induced reduction in colistin aerosol performance (25). The current co-solvent spray drying method has limitations in developing combination formulations containing components with very different solvent solubilities, because traditional two-fluid nozzle spray drying only uses one liquid channel. One of the disadvantages of using a two-fluid nozzle is that the water-ethanol co-solvents need to be premixed; when using the three-fluid nozzle, two solutions can be sprayed through two different feed channels (Figure 1). It is worth noting that it is not feasible to spray dry a solid concentration of 30 mg/mL using a two-fluid nozzle due to poor solubility of azithromycin in the water-ethanol system. Although lower feed concentrations may lead to smaller aerodynamic particle sizes, it will result in lower spray drying yield (Figure 2) and lower output, which may increase manufacturing costs and reduce production efficiency at the industrial scale. With the recent molecules in discovery and development being poorly water soluble it is very challenging to select a solvent system in which both API and excipient are soluble. Two or more components with very different solubilities may not be able to be dissolved in a single solvent with desirable drug amount for 2N spray drying, which may lead to very low output for industrial manufacturing. The three-fluid nozzle spray drying can circumvent this problem by providing two liquid channels for each of the colistin-water solution and the azithromycin-ethanol solution. The three-fluid nozzle has an inner channel and an outer channel that allow two separate drug solutions to be pumped and mixed only at the tip of nozzle.

Upon storage of the 3N formulation at 75% RH for 90 days, there is no reduction but a slight increase in FPF for both colistin and azithromycin in the co-spray dried 3N formulation (Figure 5). There is no significant change in particle morphology and solid-state upon storage of both 3N and 2N at 75% RH for up to 90 days compared to formulations stored at 20% RH for 1 day. Prevention of moisture-induced reduction in FPF after storage at 75% RH is attributed to enriched azithromycin on the surface of the co-spray dried particles for the 3N formulation as shown by the XPS data (Table 3). The slight increase in FPF is likely due to dissipation of charge over storage at high humidity, which deserves further investigation (37).

FPF values of the 2N formulation ($72.9 \pm 1.9\%$ for azithromycin and $73.4 \pm 0.8\%$ for colistin) were higher than those of the 3N ($56.5 \pm 3.8\%$ for azithromycin & $55.1 \pm 1.6\%$ for colistin) when stored at 20% RH for 1 day (Figure 5). This could be attributed to smaller physical size (D50) for 2N ($1.9 \mu\text{m}$) as compared to the 3N ($2.8 \mu\text{m}$) (Table 4). As shown in Table 5, the remarkable peak shifts towards higher wavenumbers in FTIR spectra corresponding to amide A, B and amide II N-H bending could suggest the carbonyl oxygen or hydrogen of amine group in amide structure of colistin molecule can work as a hydrogen bond acceptor or donor, respectively, to interact with the hydrogen bond donating or accepting group of azithromycin (29). In contrast, those spectral changes are less in the 3N sample, suggesting less molecular interaction between azithromycin and colistin compared to the 2N sample. The 2N formulation is formed by rapid solidifying from a homogeneous solution state in which the two substances are completely dissolved while maintaining the

interaction at the molecular level. Thus, the colistin-azithromycin particles prepared using 2N can be observed with greater interaction between the two substances in the solid state.

CONCLUSION

This study has examined the impact of nozzle design (2N vs. 3N) during the spray drying process on solid-state, morphology and aerosol performance of the resulting co-spray dried colistin-azithromycin particles. The 2N formulation has higher FPF values for both colistin and azithromycin than those of the 3N formulation likely due to much smaller physical size. As for the 2N formulation, there was no change in FPF after storage at 75% RH for 90 days, but there were significant increases in the FPF of azithromycin and colistin in the 3N formulation as stored at 75% RH for 90 days, likely due to dissipation of charge. Surface enrichment of hydrophobic azithromycin was observed for both 2N and 3N formulations, which contributed to the prevention of moisture-induced deterioration of FPF for hygroscopic colistin upon storage at the high RH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

2N	Two-fluid nozzle
3N	Three-fluid nozzle
ColAz (1–1)	Co-spray dried colistin and azithromycin at a molar ratio of 1:1
DOE	Design of experiments
DPI	Dry powder inhaler
DVS	Dynamic vapor sorption
ED	Emitted dose
FPF	Fine particle fraction
FTIR	Fourier-transform infrared spectroscopy
GSD	Geometric standard deviation
MMAD	Median mass aerodynamic diameter
NGI	Next generation Impactor

PXRD	Powder X-ray diffractometer
RH	Relative humidity
SD	Spray drying
SEM	Scanning electron microscopy
XPS	X-ray photoelectron spectroscopy

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Figure 1.
Structural sketches of (A) 2N nozzle and (B) 3N nozzle.

Figure 2.

(a) Contour plot shows the effects of flow rate and inlet temperature on spray drying yield at the feed concentration of 20 mg/mL. (b) Contour plot shows the effects of feed concentration and inlet temperature on the spray drying yield at the flow rate of 6 mL/min. For both the plots, the change in the color from green towards red represents an increase in the spray drying yield, and the numbers on the contour lines indicate the value of spray drying yield on that line.

Figure 3. Effect of feed concentration on **(a)** FPF of colistin, **(b)** FPF of azithromycin, **(c)** ED of colistin, and **(d)** ED of azithromycin.

Figure 4. Scanning electron microscopy images of (a) SD azithromycin, (b) SD colistin, (c) 3N formulation stored at 20% RH for 1 day (d) 2N formulation stored at 20% RH for 1 day; (e) 3N formulations stored at 75% RH for 90 days and (f) 2N formulations stored at 75% RH for 90 days.

Figure 5.

(A) Fine particle fraction and (B) emitted dose data of co-spray dried colistin-azithromycin formulations prepared using 2N or 3N at 20% RH for 1 day. (C) FPF and (D) ED of the 3-fluid nozzle formulation stored at 75%RH for up to 90 days (mean $\pm$ SD, $n = 3$). * $p < 0.05$; ** $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$; NS, no significant difference

Figure 6.

FTIR spectra of the spray dried colistin, azithromycin, and their co-spray dried samples using 2N and 3N at the specific spectral ranges of (a) 3400 – 3000 cm^{-1} and (b) 1800 – 1400 cm^{-1} (ν : stretching, δ : bending).

Table 1.

Variables and their levels used for the design of experiments.

Name	Units	Low	Medium	High
Flow rate	mL/min	4	6	8
Inlet temperature	°C	120	135	150
Feed solid concentration	mg/mL	10	20	30

Table 2.

MMAD and GSD of co-spray dried colistin-azithromycin formulations

Formulation	MMAD (μm)		GSD (μm)	
	Azithromycin	Colistin	Azithromycin	Colistin
2N 20% RH-Day 1	2.2 $\pm$ 0.1	2.2 $\pm$ 0.2	1.8 $\pm$ 0.1	1.7 $\pm$ 0.0
2N 75% RH Day 90	2.2 $\pm$ 0.6	2.4 $\pm$ 0.9	2.0 $\pm$ 0.2	1.9 $\pm$ 0.2
3N 20% RH Day 1	2.9 $\pm$ 0.1	3.2 $\pm$ 0.6	2.8 $\pm$ 0.2	1.8 $\pm$ 0.4
3N 75% RH Day 90	2.5 $\pm$ 0.2	2.5 $\pm$ 0.1	2.1 $\pm$ 0.1	2.5 $\pm$ 0.1

Table 3.

Theoretical and XPS-measured surface compositions based on O1s for the co-spray dried colistin-azithromycin formulations using different nozzle types (mean $\pm$ SD, n = 3).

Formulation	Theoretical Surface Composition based on O1s (%)		Measured Surface Composition (%)	
	colistin	azithromycin	colistin	azithromycin
2N	55	45	16 $\pm$ 1	84 $\pm$ 1
3N	55	45	22 $\pm$ 4	77 $\pm$ 4 [*]

*
 $p < 0.05$ as compared to 2-fluid nozzle.

Table 4.

Particle size distribution of co-spray dried colistin-azithromycin formulation stored at 20% RH for 1 day (mean $\pm$ SD, n = 3).

	D10 (μm)	D50 (μm)	D90 (μm)
SD_COLISTIN	1.6 $\pm$ 0.0	4.0 $\pm$ 0.2	9.6 $\pm$ 0.4
SD_AZITHROMYCIN	1.0 $\pm$ 0.0	2.4 $\pm$ 0.1	5.9 $\pm$ 0.2
2N	0.8 $\pm$ 0.0	1.9 $\pm$ 0.0	9.2 $\pm$ 0.2
3N	1.2 $\pm$ 0.0	2.8 $\pm$ 0.0	6.4 $\pm$ 0.0

Table 5.

FTIR band assignments for the spray dried formulations.

Band assignment	Wavenumber (cm ⁻¹)		Colistin
	Combination formulations 2N	3N	
Amide A	3259.2 (8.1)	3254.3 (3.2)	3251.1
Amide B	3052.0 (7.1)	3050.9 (6.0)	3044.9
Amide I C=O stretching	1643.6 (1.5)	1643.2 (1.1)	1642.1
Amide II N-H bending	1523.3 (6.7)	1519.9 (3.3)	1516.6

The differences in wavenumber of peaks as compared to those of the spray dried pure colistin are provided in the bracket.