

Formulation of Inhalable Voriconazole Dry Powders Using Spray Freeze-Drying Technique

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Summary

Systemic administration of antifungal agents intended for the treatment of pulmonary aspergillosis is limited by the poor lung distribution and severe adverse effects. Pulmonary delivery is desirable as it allows deposition of drug at high concentrations directly in the site of infection. Voriconazole is the primary treatment of pulmonary aspergillosis with potent and wide-spectrum activity. This study aimed to develop inhalable voriconazole dry powder formulation with excellent aerodynamic performance by constructing porous particles using spray freeze-drying technique. Mannitol was included in the formulation as a bulking agent. Since voriconazole has a poor aqueous solubility, tert-butyl alcohol (TBA) was used as a co-solvent. A two-level full factorial design was employed to systematically investigate the effect of three factors and their interactions on the aerosol performance of the formulations. These factors were (i) solute concentration of the feed solution; (ii) the voriconazole concentration; and (iii) the co-solvent composition. The cascade impactor study revealed that spray freeze-dried powder containing high level of voriconazole concentration could reach the highest fine particle fraction (FPF, <5 µm) of 47.4%. After analysing the factorial design using Minitab® 18 statistical software, the voriconazole concentration was found to be the most significant factor that can positively affect the fine particle fraction and negatively affect the emitted fraction. This result suggests that, with current production method, increasing the voriconazole concentration in the feed solution could not only improve the delivery efficiency as higher percentage of voriconazole was included in same amount of powder, but also enhance the aerosol performance of powder formulation as higher FPF was achieved.

Key Message

Porous inhalable voriconazole powder formulation that showed good aerosolization performance (fine particle fraction > 40%) was successfully produced by spray freeze-drying. The results of a full factorial design for the formulation optimization suggests that the voriconazole concentration in the feed solution could significantly enhance the aerosol performance.

Introduction

Pulmonary aspergillosis caused by *Aspergillus* species has been showing a significant rise on morbidity and mortality especially in immunocompromised patients during the last decade^[1]. While a second-generation triazole – voriconazole is the primary treatment of pulmonary aspergillosis, the currently available intravenous and oral formulations are suffering from poor lung distribution and severe side-effects^[2]. Pulmonary delivery is an attractive approach as it allows deposition of high drug concentration at the infected site with reduced risk of systemic exposure. This study aimed to develop inhaled voriconazole powder formulation with excellent aerosol performance by spray freeze-drying technique. Mannitol and TBA served as a bulking agent and a co-solvent for voriconazole, respectively. A full factorial design was adopted to examine how the operating conditions affect the aerosol performance and to optimize the formulation for further pharmaceutical and clinical development.

Experimental Methods

Materials

Voriconazole was purchased from Tecoland Corporation (Irwin, CA, USA). Mannitol (Pearlitol 160C) was obtained from Roquette (Lestrem, France). T-butyl alcohol (TBA) and formic acid were obtained from Sigma (Poole, UK). Acetonitrile was obtained from Anaqua Chemicals Supply (Cleveland, OH, USA). All solvents and reagents were of analytical grade or better unless otherwise stated.

Experimental design – factorial design

A 2³ full factorial design generated by Minitab® 18 was used for designing the experiments. The studied factors were: solute concentration (A); voriconazole concentration (B) and TBA concentration (C). The response studied were: fine particle fraction (FPF) and emitted fraction (EF), both were obtained from aerosol performance. The levels of each variable were designated as -1, 0 and +1, respectively, and the corresponding actual values for each factor are shown in Table 1.

Table 1 – A 2³ full factorial experimental design. The levels of each factor are designated as -1 (low level), 0 (middle level), and +1 (high level).

Factors	Levels		
	-1	0	1
A – Solute concentration (w/v%)	1	2.5	4
B – Voriconazole concentration (w/w%)	20	30	40
C – TBA concentration (v/v%)	40	55	70

Preparation of spray freeze dried powders

Solutions with different levels of solute concentration, voriconazole concentration and TBA concentration were prepared (table 2) and fed to an ultrasonic nozzle (130K50ST, Sonaer®, Farmingdale, NY, USA) operating at 130 kHz powered by digital ultrasonic generator for atomizers (Sonaer®, NY, USA). The atomized droplets were collected in liquid nitrogen instantly and transferred into freeze dryer for lyophilization. The samples were kept under vacuum with a chamber pressure below 0.133mBar at -25 °C for 20 h, followed by secondary drying at 20 °C for 20 h, and kept at room temperature for 25 h. The dried powders were collected and stored in desiccator with silica gel at ambient temperature until further analysis.

Table 2 – Compositions of spray freeze dried formulations. The formulation S2.5-V30-T55 was prepared in triplicate as the centre point of factorial design.

Formulation	Solute Concentration (% w/v)	VRC percentage (% w/w)	TBA Concentration (% v/v)
S1-V20-T40	1	20	40
S1-V20-T70	1	20	70
S1-V40-T40	1	40	40
S1-V40-T70	1	40	70
S4-V20-T40	4	20	40
S4-V20-T70	4	20	70
S4-V40-T40	4	40	40
S4-V40-T70	4	40	70
S2.5-V30-T55	2.5	30	55

Morphology study

Spray freeze dried powder morphology was observed using scanning electron microscopy (SEM, S4800 FEG SEM, Hitachi, Tokyo, Japan) at 5 kV.

Quantification of voriconazole

Voriconazole was quantified chromatographically using high performance liquid chromatography (HPLC; Agilent 1260 Infinity; Santa Clara, USA) equipped with a C18 column (Agilent Prep – C18, 4.6 x 250 mm, 5 µm). The mobile phase was acetonitrile and 0.5% formic acid (50:50, v/v) running at an isocratic flow rate of 1 ml/min. Voriconazole was detected at the wavelength of 254 nm and quantified against a standard curve in the range of 3.125 to 100 µg/ml at retention time of 6.8 min.

In vitro aerosol performance evaluation

The aerosol performance of the spray freeze dried powders were evaluated using Next Generation Impactor (Copley Scientific, United Kingdom) coupled with a Breezhaler® (Novartis Pharmaceuticals, Hong Kong) operated at 100 L/min for 2.4 s. For each dispersion, approximately 3.0 ± 0.5 mg of powder were loaded in a size 3 hydroxypropyl methylcellulose (HPMC) capsule (Capsugel, West Ryde, NSW, Australia). Voriconazole deposited on each stage was assayed using the HPLC as described above. Recovered dose was defined as the sum of powder mass assayed on inhaler and all NGI stages in a single run. The EF was the fraction of powder exited the inhaler with respect to the recovered dose. FPF (<5 µm) was defined as the fraction of powder with aerodynamic diameter less than 5 µm of the recovered dose.

Dissolution study

Fine particle doses (FPD, $d_{ae} < 5\mu\text{m}$) of S1-V40-T40, S1-V40-T70, S4-V40-T40, and S4-V40-T70 were captured by a glass fibre filter paper using Fast Screening Impactor (Copley Scientific, United Kingdom) operated at 100 L/min for 2.4s. The paddle over disk method was adopted to examine the dissolution profile of the FPD of voriconazole after transferred into a dissolution apparatus containing 400 ml of PBS buffer (pH 7.4) as dissolution medium. Same method was also applied to study the dissolution profile of an equivalent amount of raw voriconazole power placed on a glass fibre filter paper.

Results and discussion

Spherical particles with porous structure and minor agglomeration were observed from all the spray freeze dried powder formulation by SEM. Figure 1 showed representative SEM images of three formulations that contained different voriconazole concentration (two corner points and one centre point of the factorial design), where the powders contain higher voriconazole concentration exhibited more porous structure and finer network.

The powder formulations containing high voriconazole concentration (40%) had highest FPF and lowest EF (figure 2). Among all the formulations, S1-V40-T70 and S4-V40-T70 showed best aerosol performance with FPF of 47.4% and 41.1%, respectively.

The results of *in vitro* aerosol performance were analysed systematically using statistical software Minitab® 18. The pareto charts identified the significant factors and interaction among factors, ranked the factors and their interactions from largest effects to smallest effect (figure 3). Any factor or their interaction that cross the vertical reference line is considered as significant. Voriconazole concentration was found to be the most significant factor affecting the FPF. At the same time, the EF was only significantly affected by voriconazole concentration. Interestingly, according to the results of aerosol performance (figure 2), it can be observed that the formulations with higher voriconazole concentration resulted in higher FPF (>40%) and lower EF (approx. 80%). A high FPF is desirable for a good aerosol powder formulation as it indicates effective deposition in the deep lung. On the other hand, since EF represents the portion of powder that exposed to the patient regardless of site of deposition, EF and FPF should be closed with each other. An inhalable powder formulation that has high FPF but relatively low EF will cause minimal exposure of antifungal drug at unintended site, such as the throat and the upper airways, but effective drug deposition in the lower respiratory tract. Additionally, including higher voriconazole concentration in the powder formulation can increase the delivery efficiency as a higher dose of voriconazole can be delivered with a lower powder dose. Overall, increasing the voriconazole concentration in the formulation can not only enhance its aerosol performance but also allow more drug to be deposited at the target site.

The dissolution profile of FPD of S1-V40-T40, S1-V40-T70, S4-V40-T40, and S4-V40-T70 formulation, as well as the raw voriconazole were evaluated (figure 4). The dissolution rate of raw voriconazole was slow, as the accumulative percentage of dissolved drug reached 95% after 90 min. On the contrary, the FPD of the formulated powders containing high voriconazole concentration showed fast dissolution behaviour as 95% accumulative percentage of drug was dissolved within 5 min. Given the low aqueous solubility of voriconazole, it is important for voriconazole aerosol powder formulation to have a fast dissolution behaviour. The results showed that the dissolution rate of voriconazole can be accelerated by formulating inhalable powder using spray freeze drying technique, as particles with porous structure and increased surface area were successfully produced.

Conclusions

Inhaled voriconazole powders with mannitol as a bulking agent were prepared by spray freeze drying in this study. Spherical and porous particles were constructed, and the more porous structure and finer network were observed in the powders containing higher voriconazole concentration. According to the factorial design analysis, voriconazole concentration was found to be the most significant factor that can positively affect FPF and negatively affect EF in the *in vitro* aerosol performance evaluation. The FPD of powders containing high voriconazole concentration showed improved dissolution behaviour compared to unformulated voriconazole powders. Further studies will be conducted to evaluate the pharmacokinetic profiles and biodistribution of spray freeze dried powders following pulmonary delivery in animal models.

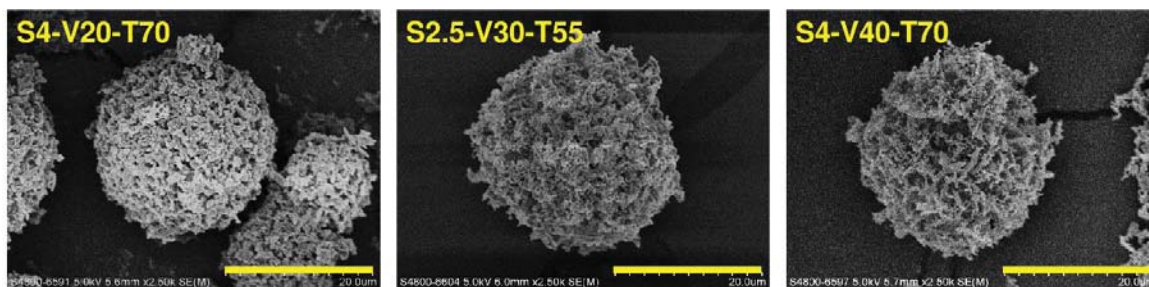


Figure 1 – Representative SEM images of spray freeze dried powders at 2,500x magnification. Scale bar represents 20 μm . Nomenclature: S – solute concentration (% w/w); V – voriconazole concentration (% w/w); T – TBA concentration (% v/v).

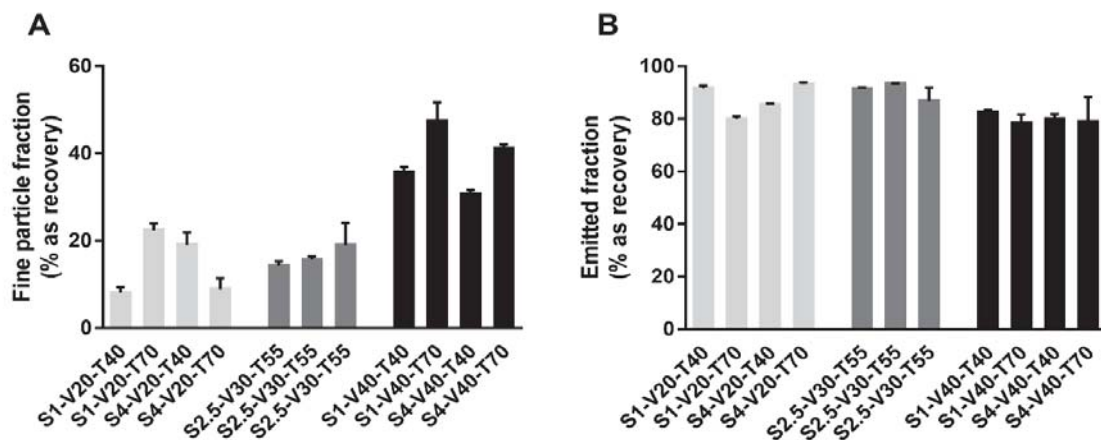


Figure 3 – *in vitro* aerosol performance of spray freeze dried powders evaluated by NGL. (A) Fine particle fraction and (B) emitted fraction were expressed as percentage relative to the recovered dose. Nomenclature: S – solute concentration (% w/v); V – voriconazole concentration (% w/w); T – TBA concentration (% v/v). Data was presented as mean ± standard deviation (n=3).

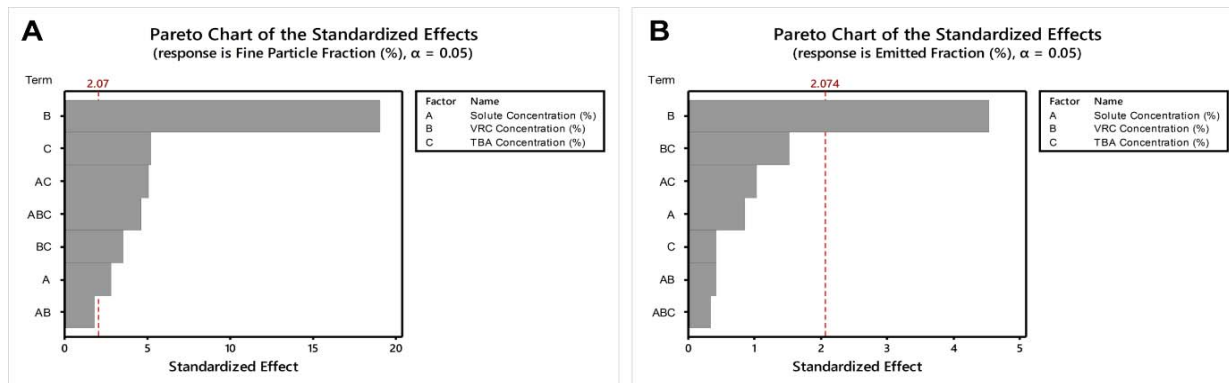


Figure 4 – pareto charts of the standardized effects where the responses were (A) fine particle fraction and (B) emitted fraction. The vertical line represents minimum statistically significant effect magnitude for a 95% confidence level, any terms that cross the vertical line are considered statistically significant.

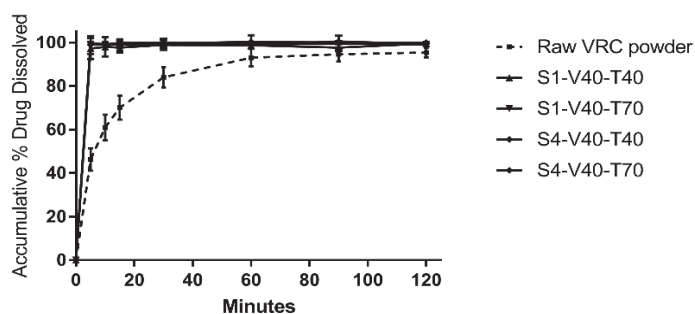


Figure 2 – dissolution profiles of raw voriconazole powder and fine particle dose (FPD) of the formulated powders containing 40% voriconazole concentration. Nomenclature: S – solute concentration (% w/v); V – voriconazole concentration (% w/w); T – TBA concentration (% v/v). Data was presented as mean ± standard deviation (n=3).

References

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- 2 Beinborn NA, Du J, Wiederhold NP, Smyth HDC, Williams RO. *Dry powder insufflation of crystalline and amorphous voriconazole formulations produced by thin film freezing to mice*. European Journal of Pharmaceutics and Biopharmaceutics. 2012;81(3):600-8.