Co-amorphization: A formulation strategy for amorphous high dose dry powder to treat lung infections

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Summary

High dose antibiotic dry powder has become a treatment approach for effectively treating lung infection in various pathophysiological lung conditions such as cystic fibrosis and non-cystic fibrosis bronchiectasis. This has kindled further interest in reformulating other antibiotics as dry powder to potentially optimize therapeutic outcomes. Among different techniques used for the preparation of such high dose dry powder, spray drying is a promising technique, particularly due to its particle engineering capability. However, in many cases, the technique produces particles in an amorphous state which tend to have poor aerosolization and chemical stability. Here, co-amorphization with amino acid was explored as a potential strategy to overcome limitations associated with spray drying. Ceftazidime, a potent antibiotic against *Pseudomonas aeruginosa*, was used as a model drug and valine was used as a model amino acid to prepare a co-amorphous system. The formulations were characterized using various known techniques of spectroscopy, microscopy, and thermal analysis. X-ray diffraction and infrared spectroscopy suggested that the ceftazidime-valine (CeV) formulation was amorphous and co-amorphization was evident through calorimetry. SEM images suggested increased asperities in CeV formulation. Valine was found to effectively improve the fine particle fraction of spray-dried ceftazidime by 24%. In addition, it improved the chemical stability of the drug by 24%. This study concludes that amino acids can be used to achieve dual edge in terms of aerosolization and chemical stability.

Key Message

To treat lung infections, highly aerosolizable high dose amorphous antibiotic dry powder with improved chemical stability can be prepared by co-amorphization with valine using co-spray drying technique.

Introduction

Formulation of high dose dry powder targeting lung infection is gaining momentum [1, 2]. While various techniques such as direct crystallization, milling, etc. have been employed to generate aerosolizable particle, spray drying has emerged as a promising formulation technique pertaining to its reproducibility and particle engineering capability [3-5]. However, many drugs upon spray drying produce amorphous particles which commonly show poor aerosolization and aerosolization stability. In addition, the chemical stability of the drug molecules can manifest as another problem in amorphous state.

Co-amorphous system refers to a molecular mix of the constituting ingredients [6]. Ceftazidime was chosen as the model drug. The molecule forms poorly aerosolizable amorphous particles upon spray drying. In addition, it exhibits chemical stability issues in amorphous state. The aim of this work was to explore the use of potentially safe endogenous amino acids to engineer the particles via co-amorphization. Amino acids being endogenous molecules have the advantage of being non-toxic compared to other excipients and could add to the limited number of approved-excipients which can be used to formulate highly aerosolizable particles for inhalation therapy. Leucine has been widely used to improve aerosolization; however, the use of other amino acids is limited. Valine is another amino acid which is non-polar, hydrophobic, and has a molar volume similar to Leucine. Hence, valine was used as a model amino acid as a part of extensive study exploring use of amino acids in improving aerosolization and chemical stabilization of small molecules in amorphous state.

Materials and method

Materials

Ceftazidime and L-valine were purchased from Xian Leader Biochemical Engineering Co. ltd (Shanxi, China) and Sigma Aldrich (MI, USA).

Preparation of the spray-dried particles

The dry powders for the drug were prepared using Buchi B-290 mini spray dryer (Buchi Labortechnik AG, Flawil, Switzerland). A feed concentration 0.2% $^{w}/_{w}$ (relative to the drug) in water was used and

spray dried with valine in 1:1 molar ratio to prepare ceftazidime-valine formulation (Cev). In addition, a formulation containing only ceftazidime (Ce) was prepared for comparison. A feed flow rate of 2 mL/min was used, and air was used as spraying gas (670L/h). The inlet temperature was set at 60 °C. Secondary drying was performed using a freeze dryer for 48 hours.

Particle characterization

X-ray diffraction (XRD), Infrared (IR) spectroscopy, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), particle sizer, and scanning electron microscopy (SEM) were performed for complete characterization in terms of amorphicity, molecular interactions, water content, glass transition temperature (Tg), particle size distribution, and morphology.

Aerosol performance

Aerosol performance was assessed using a next generation impactor (NGI) (Copley scientific Ltd., Nottingham, UK) and aerolizer (Novartis Pharmaceutical Limited, UK). The samples collected in various parts of the NGI were quantified using high performance liquid chromatography (HPLC). The fine particle dose (FPD) representing particles with aerodynamic diameter ≤ 5 microns was calculated by interpolation from cumulative mass vs D50 graph. The fine particle fraction (FPF) was calculated relative to recovered dose. Recovered dose (RD) was the amount of drug which could be quantified from the capsule, aerolizer, mouth piece, induction port, and different stages (S1-7) of the NGI. Emitted dose (ED) was the amount of drug which left the aerolizer and was deposited in various parts of NGI (mouth piece, induction port, and the different stages). RD was expressed in percentage relative to the amount of drug loaded in the capsule and ED was calculative relative to RD.

Chemical Stability

Chemical stability of ceftazidime formulations was assessed by measuring drug content on day 0 and at the end of 10 weeks at 25 °C/ <15% relative humidity (RH).

Statistical analysis

One-way analysis of variance (post hoc test - Tuckey's honest significant difference) was applied to assess significance using Rstudio [7].

Results and discussion

Spray-dried formulations containing only ceftazidime (Ce) and ceftazidime-valine (CeV) were prepared. Ceftazidime was amorphous on spray drying alone, whereas valine was crystalline. Upon co-spray drying ceftazidime with valine, both ingredients were found in amorphous state. The water content of Ce formulation was ~7%. CeV showed slightly less water content (~5%), likely due to the hydrophobic nature of the amino acid. Both Ce and CeV showed a Tg of ~50 °C, suggesting non-ideal mixing. Nevertheless, the single Tg of the CeV suggested co-amorphization. In addition, valine did not crystallize upon heating suggesting the system was stable under temperature variation. Both formulations showed a similar D50 value of ~2.5 microns. On SEM, both particles were spherical; however, valine showed higher surface asperities as shown in **Figure 1**. The IR spectra for both the formulations were populated with characteristic peaks of ceftazidime as expected; ceftazidime being the dominant ingredient.

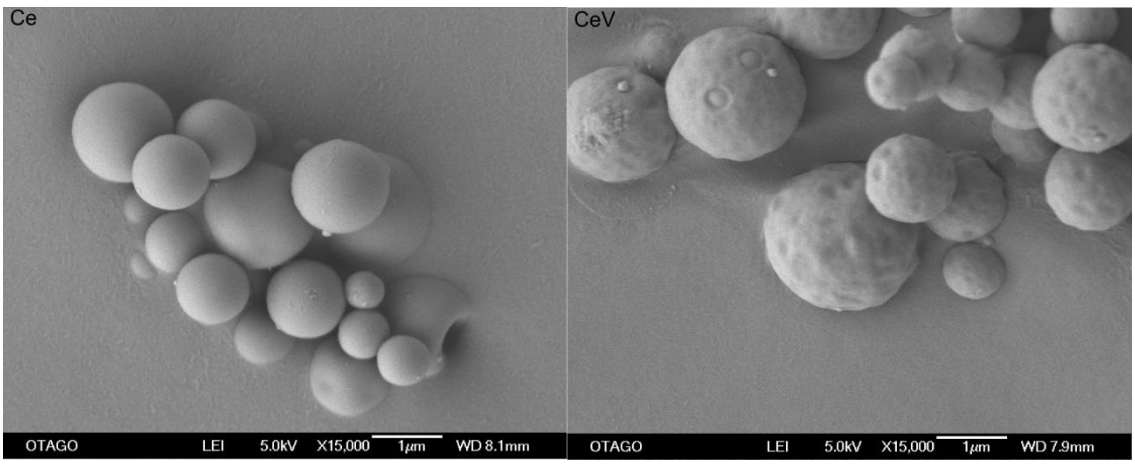


Figure 1. Representative SEM images of the spray-dried particles (Ce= Ceftazidime only, CeV= Ceftazidime-valine formulation).

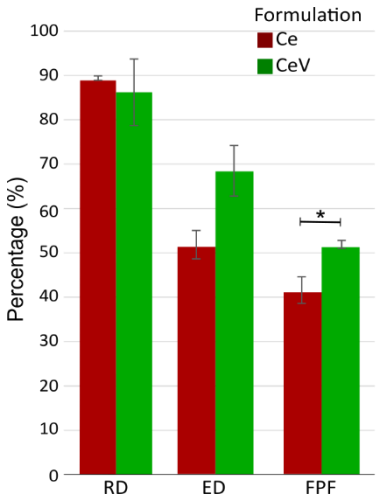


Figure 2. Recovered dose (RD), emitted dose (ED), and fine particle fraction (FPF) of the spray-dried particles (Ce= Ceftazidime only, CeV= Ceftazidime-valine formulation)**.** *The value difference is statistically significant ($p < 0.05$).

Influence of inclusion of valine on aerosolization was determined with NGI (**Figure 2**). The recovered dose for both the formulations was ~90%. Emitted dose increased from ~50 to 68% with inclusion of valine and FPF increased from 41 to 51% ($p < 0.05$However, the improvement in FPF was not observed for another drug, kanamycin [8]. The difference in the influence of valine on improvement of aerosolization between kanamycin and ceftazidime could be ascribed to the surface asperity present only in the ceftazidime-valine particles.

The drug assay on Day 0 was ~92% for both the formulations, and no other peaks apart from the ingredient peaks were present in the chromatogram of the formulations. On the 10th week, the drug content of Ce formulation dropped to 43%; however, the drug content of the CeV was 51%. Although chemical degradation occurred both in the presence and absence of valine in the formulations, the decrease was less pronounced with presence of valine. In fact, the chemical degradation was 24% lower ($p < 0.05$) in the presence of valine in the formulation. The mechanism behind such protective behaviour is currently under investigation. In addition, protective role of other amino acids is unknown; hence, the use of other amino acids is also under investigation to better understand the mechanism and factors affecting protective efficacy of different amino acids.

Conclusion

Ceftazidime-valine co-amorphous co-spray dried particles were prepared. The amino acid was effective in improving emitted dose, fine particle fraction as well as chemical stability of ceftazidime in amorphous state suggesting the potential use of amino acid in achieving dual advantages for pulmonary delivery of amorphous high dose dry powder.

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