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Spray Drying D-Amino Acids to Develop Respirable Dry Powder for the Treatment of Pseudomonas Aeruginosa Biofilms

S. Matinkhoo, S. Hoe, M. Boraey, A. Shamsaddini-Shahrbabak, W. H. Finlay, R. Vehring

Department of Mechanical Engineering, University of Alberta, Edmonton, AB, Canada

Particle Engineering

Abstract

Purpose

Pseudomonas aeruginosa bacteria form biofilms in the lungs of cystic fibrosis patients. A 1:1:1:1 mixture of D-leucine, D-tyrosine, D-methionine, and D-tryptophan of 5 nM each has been proven to break down these biofilms *in vitro* (1). Biofilm breakdown facilitates the delivery of antimicrobial drugs to the bacteria, and can be used as the first step to treat the *P. aeruginosa* infection. One way of administering the D-amino acids to the lung is via respirable dry powder. Research has shown that L-leucine can be spray dried into respirable dry powder with good aerosol performance (2). This study investigates the effect of substituting L-leucine with D-leucine on powder properties and aerosol performance.

Methods

Dry powder was developed from aqueous solutions containing Trehalose and one of D- or L-leucine by low temperature spray drying using a Büchi B-90 spray dryer. Trehalose was added to the formulation as a bulking agent for dose adjustment, and due to previous success in spray drying with L-leucine, a known powder dispersibility enhancer. The aerosol performance of the spray-dried powder was tested in an Alberta Idealized Throat and filter assembly with an air flow rate of 60 L/min using the Aerolizer®, a commercial dry powder inhaler. Physical characterisation of the powders included scanning electron microscopy, particle sizing (TSI Aerodynamic Particle Sizer), low frequency shift Raman spectroscopy, and compressed bulk density measurements (both developed by the research group).

Results

The SEM pictures illustrate that the particles containing L- and D-leucine are similar in shape and size, and their morphology is more ideal for lung delivery compared to the solid Trehalose particles (Figure 1). Both powders had acceptable leucine lung dose fraction, exceeding 25% of capsule load. Particles from both powders had similar count aerodynamic diameter distribution. Raman spectroscopy showed that both L- and D-leucine contents of the powders were mostly crystalline, and had similar crystalline structure.

Conclusion

D-leucine can be used as dispersibility enhancer to produce respirable dry powders that have similar aerosol behaviour and properties to spray dried Trehalose/L-leucine.

Purpose

Research shows that L-leucine enhances the growth of bacteria biofilms (3); hence, cannot be used to treat Pseudomonas Aeruginosa infection in cystic fibrosis patients. This study attempts to replace L-leucine with D-leucine in aqueous formulations for spray drying to develop respirable dry powder for pulmonary delivery.

Methods

➢ Spray drying was performed on a Büchi B-90 spray dryer. Process conditions were chosen to provide a low outlet temperature of 40 – 45°C and an outlet RH of less than 7% RH to prevent chemical degradation and depression of glass transition temperature (2). Calculated formulation parameters are listed in table 1.

Table 1: Formulation parameters of the spray-dried microparticles

Formulation	TL	TD	TDM
Mass fraction, trehalose	0.8	0.8	0.8
Mass fraction, L-leucine	0.2	NA	NA
Mass fraction, D-leucine	NA	0.2	0.1
Mass fraction, D-methionine	NA	NA	0.1
Feed concentration in mg/mL	27	27	27

T, L, D, and M denote trehalose, L-leucine, D-leucine, and D-methionine respectively.

➢ Aerosol performance of the dry powder was tested in a commercially available dry powder inhaler, the Aerolizer® and the Alberta Idealized Throat.

➢ Modulated differential scanning calorimetry was performed on each sample using a TA Instrument Q1000 DSC.

➢ The crystallinity of each powder sample was investigated using a customized Raman spectroscopy system.

➢ The UAlberta Density Tester, a new instrument developed in the University of Alberta Particle Engineering group, was used to measure the bulk density of each powder under a range of pressures.

➢ The mass median aerodynamic diameter of the particles was measured using a TSI Aerodynamic Particle Sizer.

Results

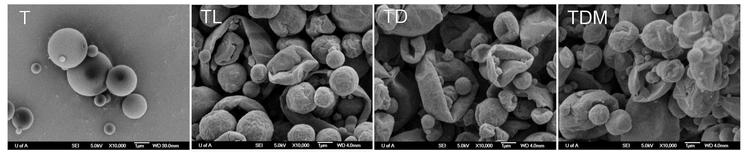

Figure 1: Morphology of the spray -dried micro particles containing only trehalose (left), trehalose/L-leucine (middle left), trehalose/D-leucine (middle right), and trehalose/D-leucine/D-methionine (right)

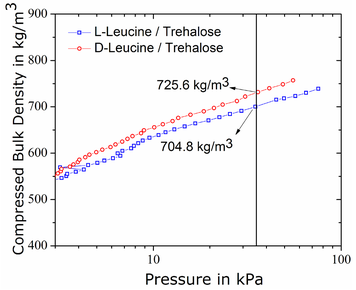

Figure 2: Compressed Bulk Density of the spray dried leucine powders as a function of pressure

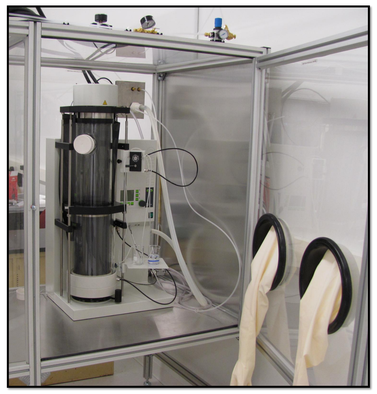

Figure 3: Büchi B-90 spray dryer in moisture controlled enclosure

Table 2: Aerosol performance of the spray-dried micro particles

Formulation	TL	TD	TDM
Particle MMAD in µm	3.82±0.04 (n=3)	3.25±0.03 (n=3)	---
Particle density in kg/m^3 at 35 kPa	704.8	725.6	---
Emitted mass in % of capsule mass	70.1, 73.9	72.8±5.2 (n=3)	78.5, 74.7
Lung dose fraction in % of emitted mass	42.7, 79.0	59.1±0.6 (n=3)	47.5, 26.4

T, L, D, and M denote trehalose, L-leucine, D-leucine, and D-methionine respectively.

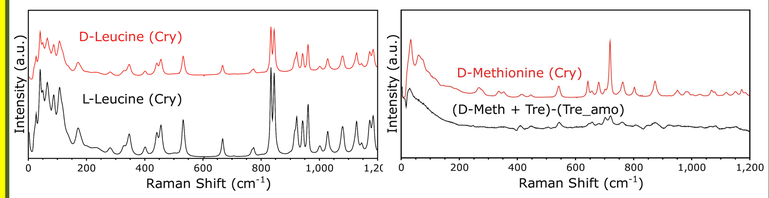

Figure 4: Raman spectroscopy results for crystalline L-leucine compared with D-leucine (left), and D-methionine compared with trehalose/D-methionine minus amorphous trehalose (right)

Conclusions

➢ The proposed approach for spray drying was fast and efficient, and had acceptable manufacturing yields (average of 50%) using only a small amount of material.

➢ Aerodynamic particle diameter (2-5µm) and morphology (hollow and wrinkled) for particles containing L- or D-leucine were suitable for pulmonary delivery (Figure 1).

➢ The spray-dried powders containing D- or L-leucine both had good aerosol performance (Table 2).

➢ Raman and mDSC showed crystalline L- and D-leucine content in the spray-dried powders (Figure 4).

➢ D-methionine can be added to the spray drying formulation while maintaining desirable morphology and aerosol performance for pulmonary delivery.

➢ **L-leucine can be replaced by D-leucine in formulations for spray drying.**

References

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UNIVERSITY OF ALBERTA

4-9 Mechanical Engineering Building, University of Alberta
Edmonton AB T6G 2G8, Canada