Substitution of L-Leucine with D-Leucine in spray-dried respirable powders for control of *Pseudomonas aeruginosa* infection

Susan Hoe, Sadaf Matinkhoo, Mohammed Boraey, James Ivey, Abouzar Shamsaddini-Shahrbabak, Warren Finlay, Reinhard Vehring

University of Alberta, Edmonton, AB, Canada

INTRODUCTION

Opportunistic *Pseudomonas aeruginosa* infections are common in cystic fibrosis patients, and usually exist in the form of bacterial biofilms, which are difficult to clear.

L-Leucine (L-Leu) is a known dispersibility agent, and is used as a formulation excipient to improve aerosol performance[1]. However, L-Leu has also been implicated as a nutrition source for *P. aeruginosa* biofilm[2]. Thus, any prospective aerosol formulations for the lung delivery of antipseudomonal compounds will likely need to replace L-Leu as an excipient.

In this study, we propose the substitution of L-Leu with its enantiomer, D-Leu, in a spray-dried powder formulation designed for respiratory drug delivery.

MATERIALS AND METHODS

Spray-drying process

Trehalose (Tre)/D-Leu and Tre/L-Leu (both 80:20 %w/w) were manufactured with the Büchi B90 spray drier under the following conditions:

Feed concentration: 27 mg/mL
Feed rate: 0.2-0.4 mL/min
Drying gas flow rate: 100 L/min
Inlet temperature: 75°C

Three batches were manufactured for each formulation.

Solid-state characterisation

The powders were subjected to modulated differential scanning calorimetry (mDSC) with the following settings: modulation ±0.618°C every 60s, and ramp from 20 to 250°C at 2°C/min.

A custom-built dispersive low-frequency Raman spectroscopy setup was used to observe the solid state characteristics of the spray-dried powders.

Particle characteristics

The compressed bulk density of the powders was measured with an in-house novel technique which applies uniaxial compaction pressure across a range of 0 to 1 MPa, and reports density versus compaction pressure.

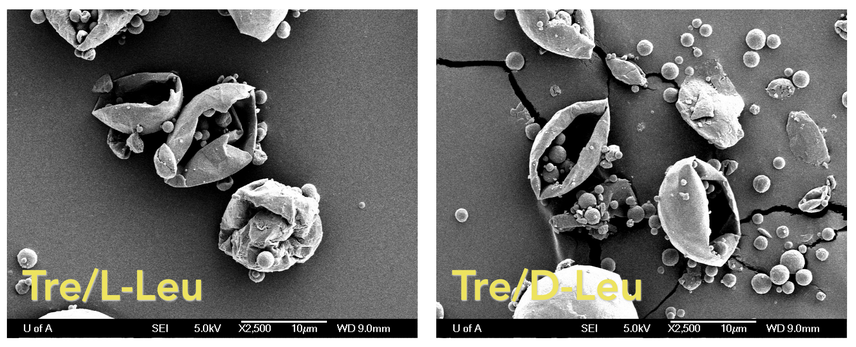
The particle morphology of the powders was observed through scanning electron microscopy (SEM).

Aerodynamic particle size distributions of the spray-dried powders were determined with the TSI Aerodynamic Particle Sizer.

In vitro aerosol deposition

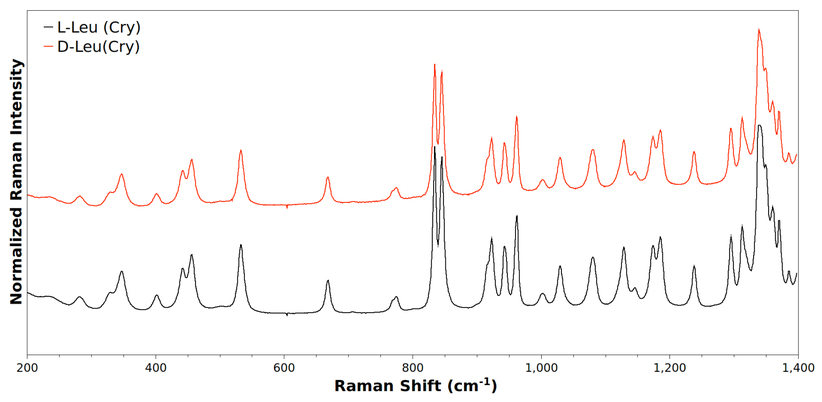
Each formulation was studied for aerosol drug delivery by dispersing 20mg of powder from an Aeroliser dry powder inhaler device, into an Alberta Idealised Throat and filter assembly, at an air flow rate of 60L/min. This was performed in triplicate, for each batch of powder (n = 9). The drug deposited in the filter was defined as “lung dose”.

RESULTS

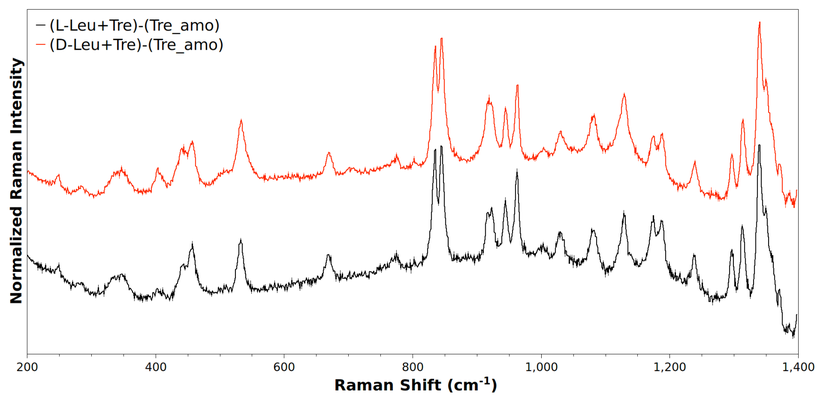


The SEM images above show that spray-dried particles are similar for both Tre/L-Leu and Tre/D-Leu. Large collapsed particles indicate that a shell structure with a large internal void was formed during spray-drying. The smaller spherical particles are similar to spray-dried pure trehalose (not shown).

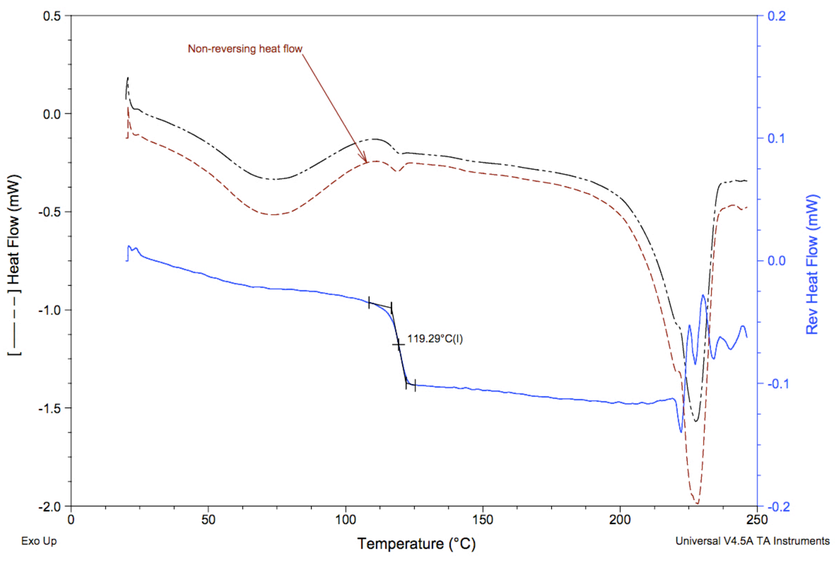
The reference Raman spectra for crystalline L-Leu and D-Leu (below) appear to be identical.



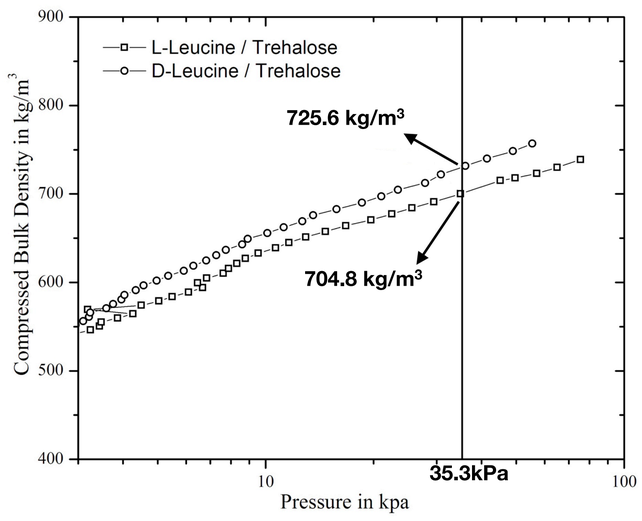
After subtraction of reference amorphous Tre spectra from the Raman spectra for Tre/L-Leu and Tre/D-Leu, the resultant spectra are similar to the reference crystalline spectra above. This indicates that D- and L-Leu are crystalline in the spray-dried material.



The mDSC experiments detected a single glass transition at 119°C for both formulations, corresponding to amorphous trehalose (Tre/D-Leu trace shown below).



The compressed bulk density (CBD) for the two powders are similar across the pressure range.



The *in vitro* aerosol performance of Tre/L-Leu and Tre/D-Leu are shown below:

	TRE/L-LEU	TRE/D-LEU
Mass median aerodynamic diameter (µm; *n* = 3)	3.82 ± 0.04	3.25 ± 0.03
Emitted dose (% of loaded dose; *n* = 9)	74.97 ± 8.25	82.82 ± 17.74
Throat deposition (% of loaded *dose*; *n* = 9)	28.44 ± 11.12	30.92 ± 6.58
Lung dose fraction (% of loaded dose; *n* = 9)	46.43 ± 13.68	52.62 ± 13.22

CONCLUSIONS

L-Leu and D-Leu are identical in crystalline structure in the spray-dried formulations (according to mDSC results and Raman spectra).

L-Leu is expected to form a crystalline shell in a spray-dried Tre/L-Leu particle, which collapses to form the final particle morphology[1]. The SEM images and compressed bulk density measurements show that Tre/D-Leu forms similar particles to Tre/L-Leu.

The spray-dried powders also demonstrated comparable *in vitro* aerosol performance, exceeding 40% lung dose fraction. L-Leu is a known powder dispersibility agent. D-Leu appears to perform the same function.

L-Leucine may be substituted with D-Leucine, without an adverse effect on aerosol performance, while also removing a bacterial biofilm nutrition source from a respirable powder formulation.

REFERENCES

1. Feng AL, Boraey MA, Gwin MA, Finlay PR, Kuehl PJ, Vehring R: Mechanistic models facilitate efficient development of leucine containing microparticles for pulmonary drug delivery. Int J Pharm. 2011;409:156-163.
2. Bernier SP, Ha D-G, Khan W, Merritt JH, O’Toole GA: Modulation of *Pseudomonas aeruginosa* surface-associated group behaviors by individual amino acids through c-di-GMP signaling. Res Microbiol. 2011;162:680-688.