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ECPS2011

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Communication

The Role and Interaction Effects of Amino Acids on the Particle Engineering of a Mannitol-based Powder Formulation

David A.V. Morton[1,*] Tomás Sou[1], Lisa M. Kaminskas[1], Michelle P. McIntosh[1] and Laurence Orlando[1]

[1] Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Australia

E-Mail: david.morton@monash.edu

* Author to whom correspondence should be addressed; Tel.: +61-31-9903-9523; Fax: +61-3-9903-9583

Received: / Accepted: / Published:

Abstract:
The purpose of the present study was to examine the potential to engineer a new formulation platform for delivering biomolecules to the lung: a design of experiments approach to determine the impact of adding amino acids (leucine, glycine and alanine) on the dispersibility and morphology of a mannitol-based spray-dried powder formulation. A range of compositions comprising mannitol and varying amino acids were spray-dried. A 2^3 factorial design in which 3 amino acids selected for varying hydrocarbon chain length were added to control specific properties of the formulations. The aerosolisation efficiency was assessed by dispersing the powders from a Monodose inhaler into a Spraytec laser diffraction system. The morphology of the particles was examined under scanning electron microscope (SEM). The mannitol had a median volume equivalent diameter of 2.8 (±0.1) µm while the formulations containing amino acid additives were mostly larger in laser diffraction measured diameter, although the DoE central point formulations containing leucine, glycine and alanine as additives demonstrated the smallest median diameter of 2.4 (±0.3) µm. The particles containing leucine, with or without others provided relatively efficient aerosolisation, but particle shape revealed distinctive morphological features. However, particles with alanine and/or glycine were more substantially agglomerated, and less easily dispersed. This DoE approach clearly identified the beneficial impact of inclusion of leucine on the aerosol performance and morphology of the formulations. The benefit provided by the leucine appears associated with its enrichment at the particle surface, as well as impact on particle morphology.

Keywords: powder formulation, spray drying, self-assembly, surface modification

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1. Introduction

A new generation of 'smart' platform powder formulations for inhalation of biomolecules have been proposed in recent years, often formed via spray drying (1). In many cases, these formulations comprise a cocktail of excipients each of which is proposed to provide one or more functional roles in the solid phase. Formulation design however has been largely based on an empirical approach. The purpose of the present study was to examine the potential to engineer a new formulation platform for delivering biomolecules to the lung. A design of experiments approach was employed to determine the impact of adding amino acids (leucine, glycine and alanine) on the dispersibility and morphology of a mannitol-based spray-dried powder formulation.

The potential advantageous properties provided by adding L-leucine, either by co-milling or by condensation/precipitation was first demonstrated by Staniforth and Ganderton et al. (2, 3, 4). Several groups have since studied the benefit that this additive provides to powder aerosolisation, especially when co-sprayed with actives and excipients (5)

2. Methodology

Design of Experiment (DoE)

This study utilised a 2^3 factorial design in which 3 amino acids selected for varying hydrocarbon chain length were added to control specific properties of the formulations. Two levels of 0% and 30% molar concentration of the amino acids were selected. The mid-point concentration, 15% of each amino acids, was used to formulate a central point (CP) composition (Table 1).

Mannitol Factorial Design (Additives in molar percentage%)			
Trial	Leu (1)	Gly (2)	Ala (3)
CP	15	15	15
T1	30	30	30
T2	0	30	30
T3	30	0	30
T4	0	0	30
T5	30	30	0
T6	0	30	0
T7	30	0	0
T8	0	0	0

Table 1. Mannitol factorial design compositions.

In situ real-time particle sizing

A Spraytec (Malvern Instruments Ltd, UK) laser diffraction system was used to measure the aerosolisation efficiency of the dry powder formulations. The median particle size (Dv50) was measured at a flow rate of 60 L/min using the Monodose Inhaler (Miat, Italy) as the dispersion device. Size 3 HPMC capsules were filled with 20 (±0.5) mg of powder for the tests which were performed under controlled temperature (18-25ºC) and relative humidity (40-60% RH).

In vitro powder aerosolisation

The fine particle fraction (FPF) and *in vitro* aerosol deposition was determined using a twin stage impinger (TSI, Apparatus, A; British Pharmacopoeia, 2000) (Copley Scientific Ltd., Nottingham, UK) and a Monohaler. A vacuum pump (Model OD 5/2, Dynavac Engineering, Melbourne, Australia) fitted to the mouthpiece of TSI is used to create the airflow and the flow rate is adjusted to run for 4 seconds at 60 (±5) L/min for each measurement. The amount of powder deposited at different stages of the TSI was determined using a UV spec.

Cohesion value

The Freeman FT4 powder rheometer (Freeman technology, UK) was used to perform the shear test in order to estimate the cohesion force between the particles. The measurements were performed under controlled temperature (18-25°C) and relative humidity (40-60% RH). The results were presented in the form of a chart where the shear force measured is plotted versus the normal force exerted.

Surface morphology

The surface morphology of the particles was examined a scanning electron microscope (SEM) (Phenom, FEI Company, USA.

3. Results and Discussion

In situ real-time particle sizing

With the exception of mannitol alone (T8) as the foundation material, all other formulations without leucine had a mean Dv50 greater than 5 µm (Fig. 1). Mannitol alone (T8) had a mean Dv50 of 2.83 (±0.05) µm while the formulations containing 30% glycine and 30% alanine (T2), 30% alanine alone (T4), and 30% glycine alone (T6) had mean Dv50s of 28.55 (±2.73) µm, 12.02 (±1.58) µm and 5.52 (±0.37) µm, respectively. Statistical analysis demonstrates the significant dispersibility enhancing effect of leucine, even in the presence of glycine and alanine, which significantly increase Dv50 when used alone (Fig. 2).

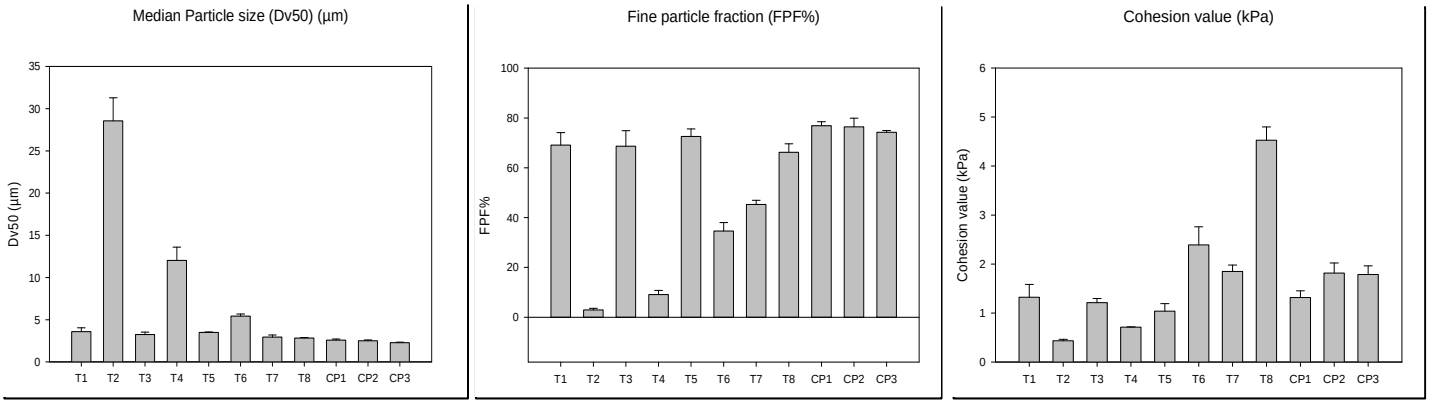

Figure 1. Results of median particle size (Dv50) (µm), fine particle fraction (FPF%) and cohesion value (kPa) (n = 3, data are represented as mean ± S.D.).

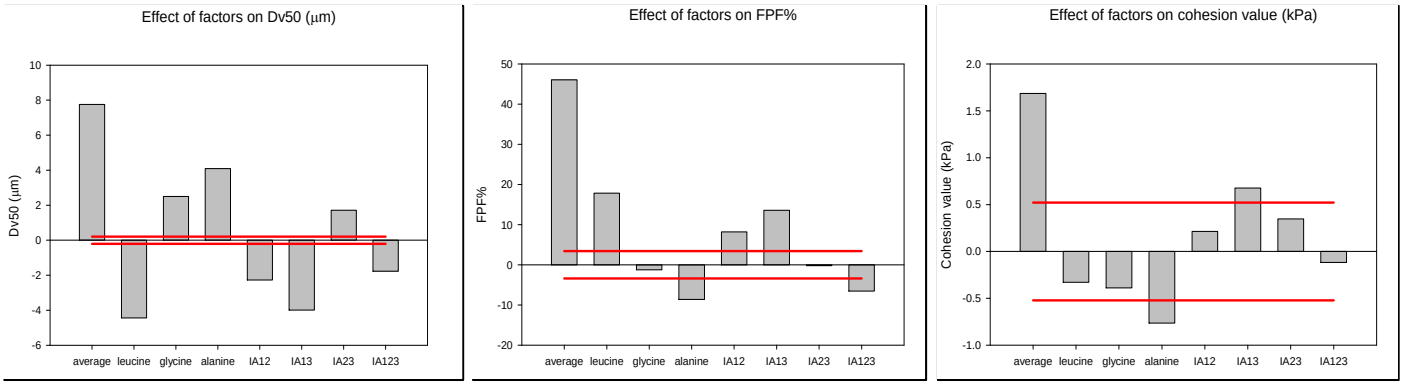

Figure 3. Effect of each and interaction (IA) of amino acids (1, leucine; 2, glycine; 3, alanine.) on median particle size (Dv50) (µm), fine particle fraction (FPF%) and cohesion value (kPa). Red lines show the level of significance derived from the statistical analysis of the factorial design.

In vitro powder aerosolisation

Fine particle fraction (FPF) measured from TSI correlates well with Dv50 results, with particles in the range of 1-5 µm demonstrating the highest FPFs. Particles with Dv50 above this range show significantly lower FPFs as demonstrated from T2, 4 and 6, which are the formulations without leucine (Fig. 1). Statistical analysis shows that FPF is significantly higher in the presence of leucine (Fig. 2). This result demonstrates the improved dispersibility and subsequently lung deposition achieved by inclusion of leucine.

Cohesion value

Mannitol alone (T8) is a very cohesive powder (Fig. 1). The presence of amino acids clearly reduces the cohesion value of the dry powders. However, leucine is the only amino acid that reduces cohesion without increasing particle size. Inter-particle forces are known to be prominent as particle size reduces. In the absence of leucine, the inclusion of glycine and alanine reduces cohesion at the expense of increasing particle size.

Surface morphology

Spherical particles were found in all formulations containing leucine (CP, T1, 3, 5, 7) and mannitol (T8) as a foundation material alone (Fig. 3). Other formulations without leucine formed wrinkled particles with irregular and rough surfaces. This result suggests that the presence of leucine help to form spherical particles which is dispersed more easily as demonstrated with the lower Dv50 and higher FPFs.

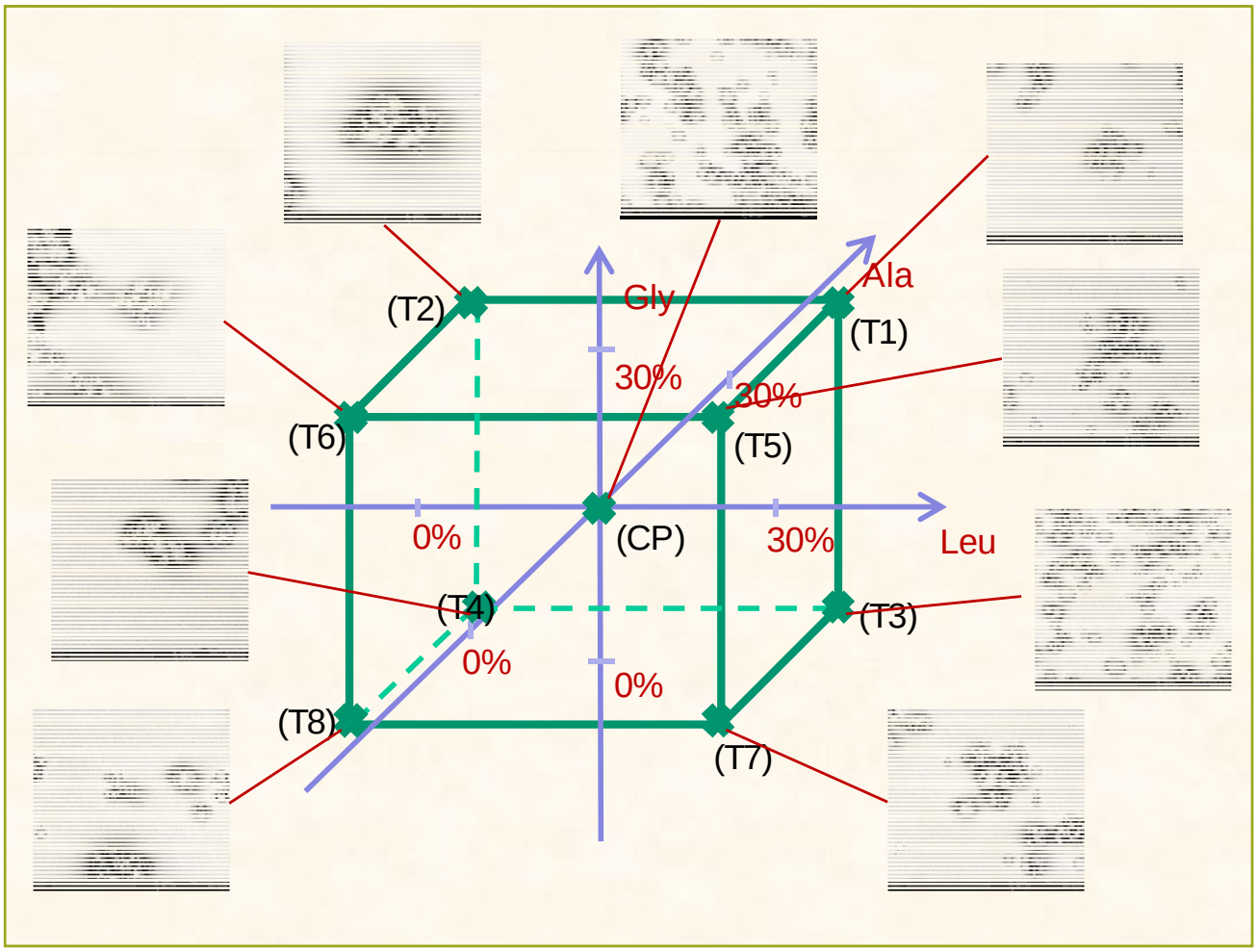

Figure 3. Cube plot of a 2^3 factorial design showing scanning electronic microscope (SEM) images.

4. Conclusions

- This study clearly demonstrates the impact achieved by inclusion of leucine on the aerosol performance and morphology of mannitol dry powder formulations.
- In addition, this study also shows that, under the experimental condition, this effect is unique to leucine and is not achieved using similar amino acids with varying hydrocarbon chain lengths.

- The effect of leucine is very influential and is able to offset the negative effect on the aerosolisation performance produced from glycine and alanine under the experimental condition.
- Further investigation is proposed to understand the interaction effect produced from the amino acids, and will be applied in the design of a novel baseline carrier for inhalable dry powder pharmaceuticals.

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