Spray Freeze-Drying for Inhalable L-leucine, Mannitol-based Microparticles: The Impact of Process Variables, L-leucine, and Crystallinity on Aerosolization Properties

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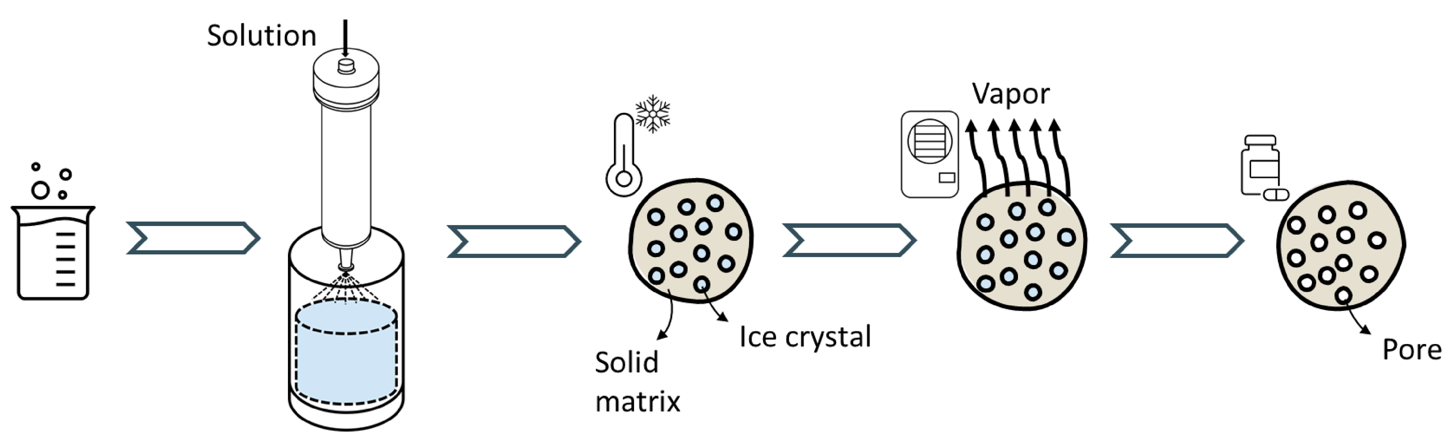
Introduction

Spray freeze-drying (SFD) has emerged as a cutting-edge technology for the manufacturing of temperature-sensitive pharmaceutics. SFD involves the atomisation of a solution into droplets, which are instantaneously frozen into a cryogenic liquid (e.g., N_2) and then dried under vacuum [1]. The formation and subsequent sublimation of ice crystals provide the MPs with a porous structure, constituted by an excipient-based matrix embedding the drug [2]. The high porosity of such MPs reduces their mass density, thus making them extremely aerodynamically performant [3]. Therefore, SFD represents a promising approach to producing drugs to be administered through dry powder inhalers, which require excellent aerodynamic properties to deposit in the target site of the lung and exert their action [4].



Owing to their large surface area, spray freeze-dried MPs are exposed to inter-particle cohesiveness which can affect their flowability [5]. Moreover, these powders are extremely sensitive to humidity-induced deterioration due to their great hygroscopicity. In this study, LL was employed to increase the MPs' flowability, assessing the LL optimal content to reach the highest FPF. In addition, the relationship between the crystallinity of mannitol and LL MPs and their aerodynamic behaviour was uncovered, providing further information about the mechanism of action of this amino acid.

Methods

Production

Formulation	Solid (% w/v)	Mannitol (% w/w_{dw})	SS (% w/w_{dw})	LL (% w/w_{dw})
F2	5, 12.5, 20	99	1	-
F3	5	94	1	5
F4	5	89	1	10
F5	5	79	1	20

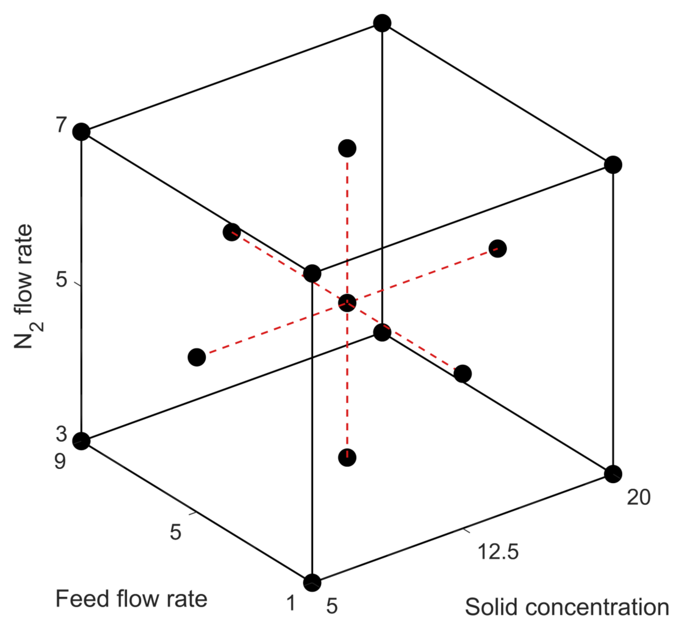
- **Primary drying**: 10 °C, 20 Pa
- **Secondary drying**: 20 °C, 20 Pa, 5 h

Characterisation

- SEM
- XRD
- NGI

Design of Experiment

Face centered design



Response variables:
- y_1 = geometric diameter = d_g
- y_2 = aerodynamic diameter = d_{ae}

Model:

$$y_u = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i,j=1, i\neq j}^{k} b_{ij} x_i x_j + \sum_{i=1}^{k} b_{ii} x_i^2 \qquad u = 1,2$$

Level	Concentration (% w/v)	N_2 flow rate (NL/min)	Feed flow rate (mL/min)
-1	5	3	1
0	12.5	5	5
1	20	7	9
Variable	x_1	x_2	x_3

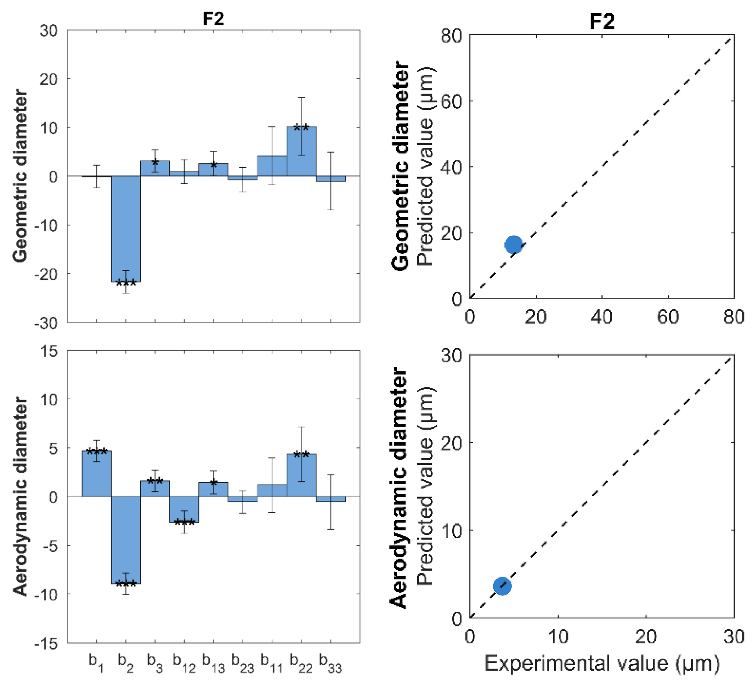
Mannitol + SS MPs

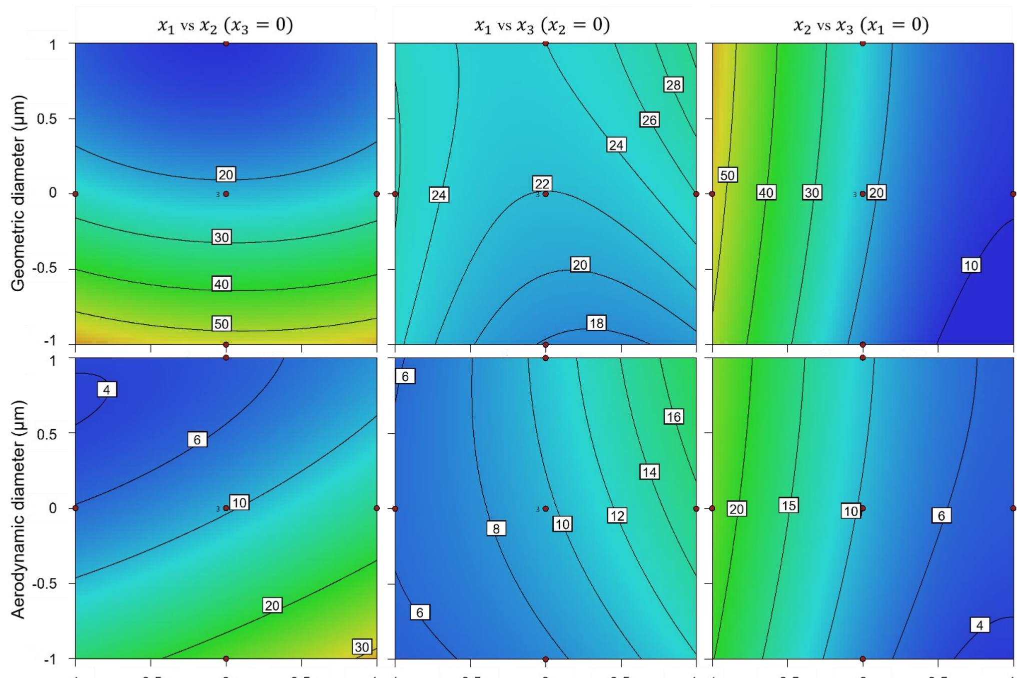
Face centered design

The following model was obtained:

- $y_1 = 21.9 - 0.09x_1 - 21.7x_2 + 3.1x_3 + 0.9x_1x_2 + 2.6x_1x_3 - 0.7x_2x_3 + 4.2x_1^2 + 10.2x_2^2 - 1.04x_3^2$
- $y_2 = 9.6 + 4.7x_1 - 8.9x_2 + 1.6x_3 - 2.6x_2 + 1.4x_1x_3 - 0.55x_2x_3 + 1.2x_1^2 + 4.3x_2^2 - 0.56x_3^2$

The model was validated:
- excellent agreement between experimental and predicted values;
- model predicting the size of MPs in the given domain.





❖ ***Which factors were significant?***

- Geometric diameter:
  - $\uparrow x_2 \rightarrow \downarrow d_g$ ($p < 0.001$)
  - $\uparrow x_3 \rightarrow \uparrow d_g$ ($p < 0.05$)
- Aerodynamic diameter:
  - $\uparrow x_1 \rightarrow \uparrow d_{ae}$ ($p < 0.001$)
  - $\uparrow x_2 \rightarrow \downarrow d_{ae}$ ($p < 0.001$)
  - $\uparrow x_3 \rightarrow \uparrow d_{ae}$ ($p < 0.01$)

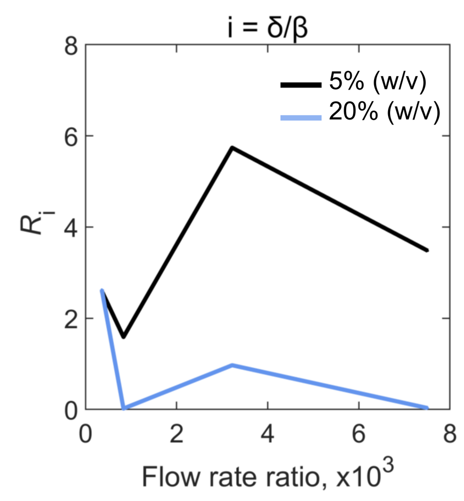
❖ ***Which was the best combination?***

- $x_1 = 5\%$ (w/v)
- $x_2 = 7$ NL/min
- $x_3 = 9$ ml/min

to obtain $\downarrow d_{ae}$ and avoid nozzle clogging.

Cristallinity

- Prevalence of β- and δ-mannitol
- Relationship between size and polymorphism: $\uparrow$ size and $\uparrow R_{\delta/\beta}$ at $x_2 = 3$ NL/min; $\downarrow$ size and $\downarrow R_{\delta/\beta}$ at $x_2 = 7$ NL/min



❖ ***Which is the cause?***

- Only small droplets with nucleation time lower than their falling time could have nucleated in the vapour phase;
- the lower driving force of vapour N_2 could have slowed the freezing process, guaranteeing enough time for the formation of β-mannitol (Ostwald rule [6]);
- bigger droplets nucleated in the liquid phase at a higher freezing rate, promoting the formation of metastable δ-mannitol.

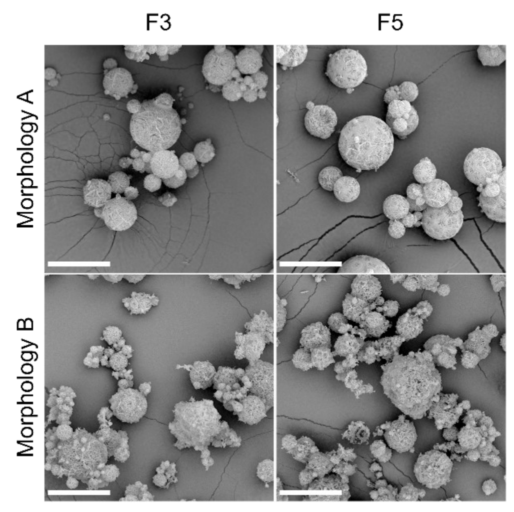
Mannitol + SS + LL MPs

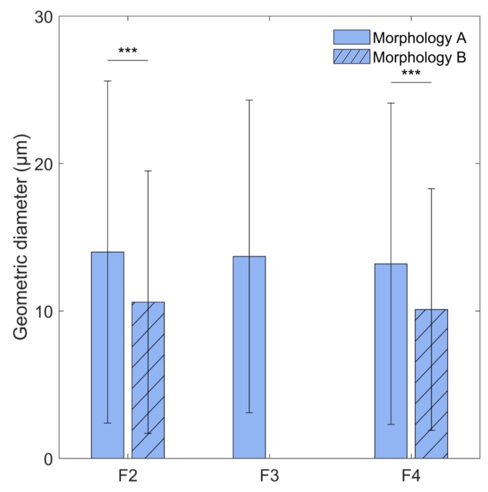
Morphology and size

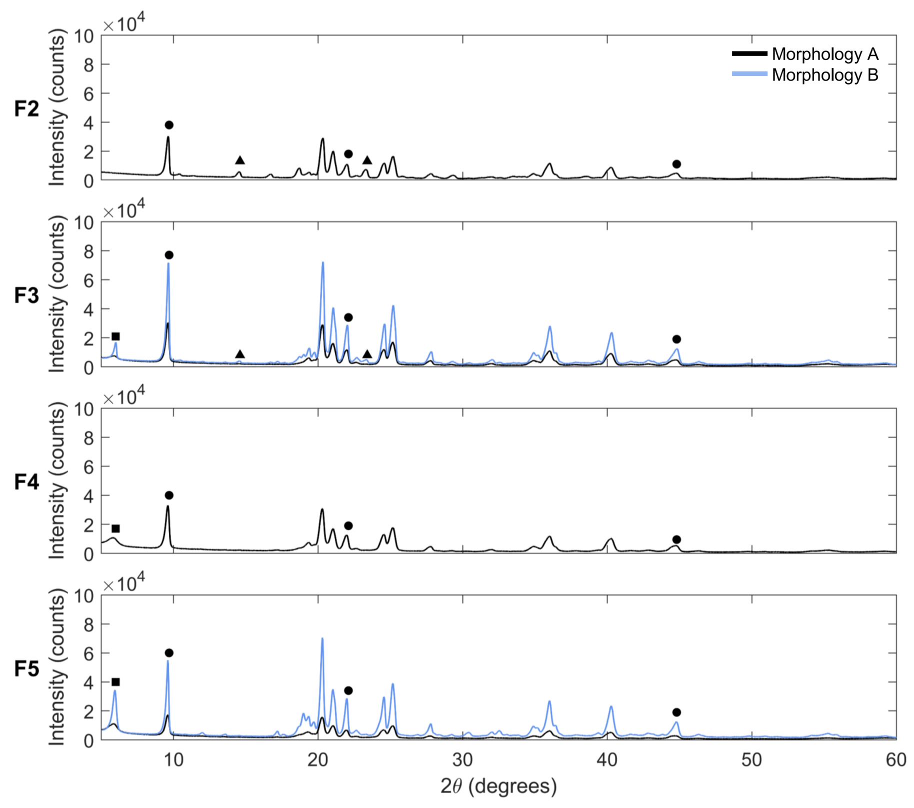
Formation of two morphologies:

Morphology A:
- well-defined shape
- smooth surface
- higher size
- lower cohesion

Morphology B:
- less-defined shape
- rough surface
- lower size
- higher cohesion







Cristallinity

Presence of:
(■) crystalline LL
(▲) β-mannitol
(●) δ-mannitol

- **Morphology A**: $\uparrow R_{\delta/\beta}$ and $\downarrow$ crystallinity
- **Morphology B**: $\downarrow R_{\delta/\beta}$ $\uparrow$ crystallinity

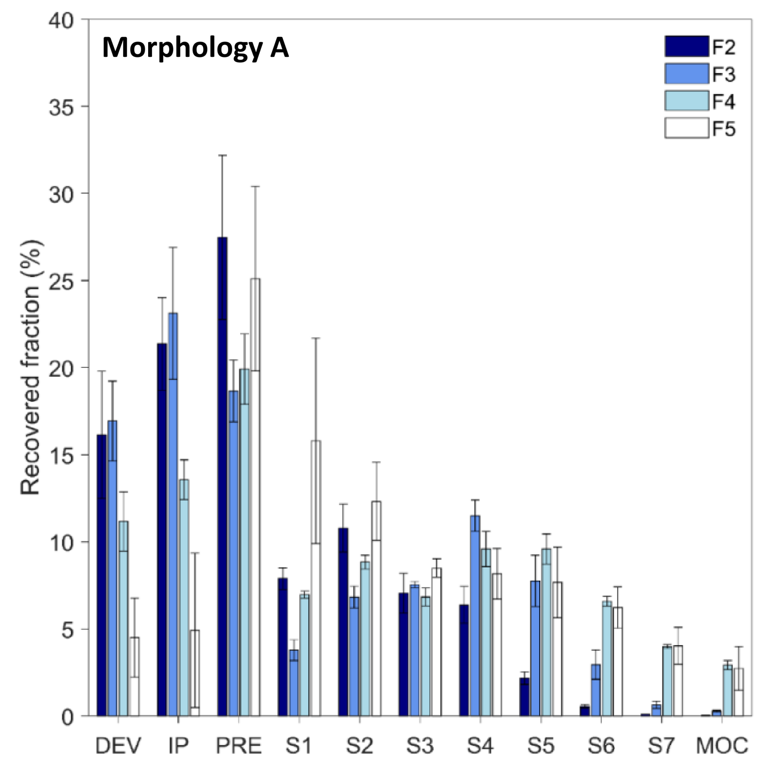
❖ ***Which is the cause of morphology B?***

- Humidity-induced recrystallization of mannitol and LL.

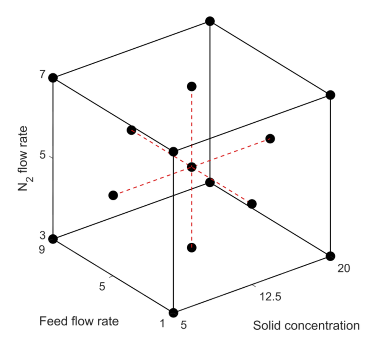
In vitro drug deposition

- **Morphology A**: $\uparrow$ FPF and $\downarrow$ MMAD
- **Morphology B**: $\downarrow$ FPF and $\uparrow$ MMAD
- Best condition: morphology A with 10% (w/w_{dw}) LL

	Morphology	EF (%)	FPF (%)	MMAD (μm)	GSD (-)
F2	-	83.8 ± 3.6	23.6 ± 4.9	4.5 ± 0.2	2.2 ± 0.1
F3	A	83.1 ± 2.3	39.8 ± 3.7	2.4 ± 0.1	2.4 ± 0.1
F3	B	92.1 ± 0.9	21.4 ± 4.1	4.8 ± 0.3	2.1 ± 0.1
F4	A	88.8 ± 1.7	48 ± 3	2.0 ± 0.1	3.2 ± 0.1
F5	A	95.5 ± 2.3	43.2 ± 7.5	3.5 ± 1.1	4.3 ± 0.7
F5	B	93.2 ± 1.1	15.8 ± 4.3	5.1 ± 0.6	2.4 ± 0.1



Conclusions



Face centered design

- Influence of the process variables on MPs size ⇨ $\uparrow$ solid concentration $\rightarrow \uparrow d_{ae}$; $\uparrow N_2$ flow rate $\rightarrow \downarrow d_{ae}$; $\uparrow$ feed flow rate $\rightarrow \uparrow d_{ae}$
- Relationship between MPs size, nucleation time, and polymorphism ⇨ $\uparrow$ size $\rightarrow \uparrow$ δ-mannitol; $\downarrow$ size $\rightarrow \uparrow$ β-mannitol

Optimal condition:
- 5% (w/w) solid
- 7 NL/min
- 9 ml/min

LL

Morphology A: $\downarrow$ crystallinity, Smooth surface, $\downarrow$ cohesiveness ⇨ $\uparrow$ FPF, $\downarrow$ MMAD

Morphology B: $\uparrow$ crystallinity, Rough surface, $\uparrow$ cohesiveness ⇨ $\downarrow$ FPF, $\uparrow$ MMAD

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