On the stability of spray freeze-dried microparticles for pulmonary drug delivery

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Summary

This study deals with the stability of mannitol-based spray freeze-dried microparticles (MPs) carrying salbutamol sulphate (SS). Although spray freeze-drying (SFD) produces porous MPs suitable for inhalation, such powders are prone to inter-particle cohesiveness and recrystallisation during storage. These features may affect their flowability and aerodynamics, especially in the presence of mannitol, which is a crystalline excipient. The research aimed to stabilize these MPs by adjusting storage temperature, freezing conditions, and formulation.

MPs were produced by a 5% (w/v) mannitol solution, 1% (w/w_{dw}) SS, and different excipients, i.e., L-leucine (LL), dextran (Dex), and polysorbate 80 (PS80). The freezing process included an "intermediate freezing" (IF) step, where freezing was held for 1 or 5 h to stabilise crystallinity. Scanning electron microscopy (SEM) and X-ray diffraction (XRD) were used to assess particle size, morphology, and crystallinity over time.

MPs stored at room temperature (T_{amb}) exhibited rougher morphology and higher cohesiveness, especially after a 5 h IF, due to the transition of δ-mannitol to β-mannitol. Storing MPs at -20 °C with a 1 h IF step stabilized their morphology and reduced recrystallisation. However, achieving stability at T_{amb} required an effective excipient. LL did not prevent recrystallisation and led to two distinct morphologies with varying aerodynamic properties. Similarly, PS80 did not improve the stability of MPs. By contrast, Dex successfully maintained the δ-mannitol form and prevented morphological changes, ensuring the stability at T_{amb}. Thus, Dex emerged as a superior stabilizing excipient for mannitol-based SFD formulations, thus encouraging their use in pulmonary drug delivery.

Key Message

The stability of mannitol-based spray freeze-dried MPs stored at T_{amb} was achieved by employing the amorphous excipient dextran. This excipient avoids the recrystallisation of mannitol during storage, thus preventing morphological changes and cohesiveness among MPs.

Introduction

Spray freeze drying (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 MPs present a porous structure, consisting of an excipient-based matrix embedding the drug [2]. The high porosity of such MPs reduces their mass density, thus providing them with excellent inhalation properties [3]. Moreover, SFD is preferred to spray drying for the manufacturing of thermosensitive drugs, such as biologics. Although SFD has emerged as a cutting-edge technology in the field of pulmonary delivery of drugs, spray freeze-dried MPs are exposed to inter-particle cohesiveness which can affect their flowability [4]. Moreover, these powders are extremely sensitive to recrystallisation during storage, which leads to morphological modifications and poor aerodynamics. Therefore, the stabilisation of spray freeze-dried powders during long-term storage is of paramount importance. This study involved the stabilisation of spray freeze-dried MPs whose size had been previously optimised. Indeed, the optimal size for inhalation purposes had been assessed by a design of experiments (DoE) investigating the mutual interaction of the solid content, the atomisation flow rate, and the feed flow rate. 5% (w/v) of solute, 7 NL/min of N_2 flow rate, and 9 mL/min of feed flow rate were selected as the best combination to obtain MPs with a d_{ae} lower than 4 µm. Despite the appropriate size, the MPs were affected by high inter-particle cohesiveness and poor aerodynamics when stored at T_{amb}. Moreover, recrystallisation of mannitol during storage was detected. To overcome these issues, the stability of spray freeze-dried MPs was first assessed as a function of the freezing conditions and the storage temperature. Then, different excipients were added to the formulation to induce a stabilising effect.

Materials and methods

Spray freeze-dried MPs were produced at 5% (w/v) of solute, i.e., D-mannitol added with 1% (w/w_{dw}) SS. The formulation was then added with L-leucine (LL) 5% (w/w_{dw}), dextran (Dex) 5% (w/w_{dw}), or polysorbate 80 (PS80) 0.1% (w/w_{dw}) as explained in Table 1.

Table 1. Formulations involved in the study.

Formulation #	Mannitol % (w/w_{dw})	SS % (w/w_{dw})	LL % (w/w_{dw})	Dex % (w/w_{dw})	PS80 % (w/w_{dw})
1	99	1	-	-	-
2	94	1	5	-	-
3	94	1	-	5	-
4	98.9	1	-	-	0.1

During SFD, the atomisation N_2 and feed flow rates were maintained at 7 NL/min and 9 mL/min, respectively. The frozen droplets were stored at -50°C for 1 h or 5 h before drying. Primary drying was conducted at 10 °C and 20 Pa, while secondary drying was performed at 20 °C and 20 Pa for 5 h. Following drying, the powders were transferred into vials, sealed, and stored at room temperature (T_{amb}), at 5°C, or at -20°C. According to the protocols reported by [5], scanning electron microscopy (SEM), and X-ray diffraction (XRD) were employed to evaluate the MPs' size, morphology, crystallinity, and stability at different times after the production, i.e., one week (t_0), and three months (t_2). The open-source ImageJ software (NIH, Bethesda, MD, USA) was then used to determine the geometric diameter (d_g) and the d_{ae} of 400 MPs, as reported in [5]. To assess the changes in the crystallinity of mannitol, the peak ratio R between δ- and β-mannitol at 9.7° and 14.6° was calculated. The fine particle fraction (FPF) and the mass median aerodynamic diameter (MMAD) were obtained through a Next Generation Impactor following the protocol reported by [5].

Results

This study aimed at stabilising mannitol-based spray freeze-dried MPs since, being a crystalline excipient, mannitol is particularly prone to recrystallisation over time. The freezing step of SFD and the storage temperature were tuned to mitigate this phenomenon. An intermediate stage was introduced between spray freezing and drying, aiming at stabilising the frozen MPs. For the sake of simplicity, this step can be referred to as "intermediate freezing" (IF). Figure 1 reports the morphology of spray freeze-dried MPs, containing mannitol and SS, which undergone IF and were stored at different temperatures. At t_0 MPs stored at T_{amb} exhibited a rougher morphology and higher cohesiveness than MPs stored at lower temperatures. Moreover, these features appeared magnified by an IF of about 5 h. After three months, the morphology of MPs either produced at IF equal to 1 h or stored at -20 °C remained unvaried. Instead, the MPs which underwent IF equal to 5 h faced a dramatic transition to a rough morphology and needle-like mannitol crystals.

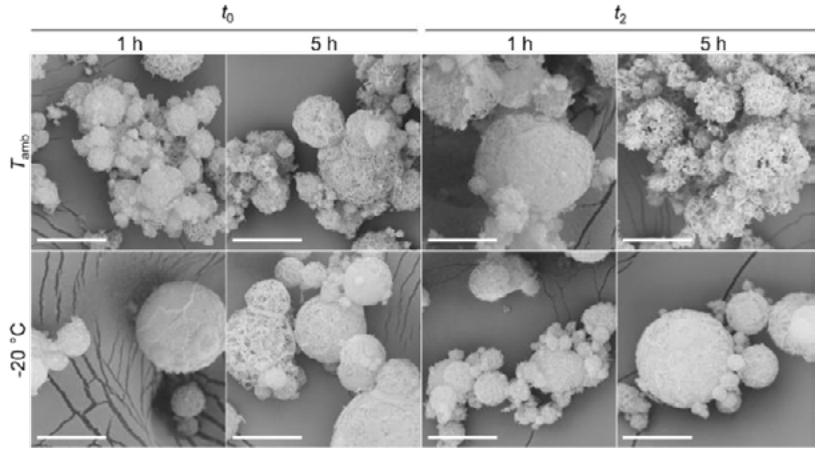


Figure 1. Morphology of mannitol and SS MPs stored at different temperatures and produced at IF equal to 1 and 5 h. Samples were characterised one week (t_0) and three months (t_2) after production. Scale bars refer to 50 µm and 1450x magnification.

The morphological change of MPs from rod-shaped crystals to needle-like ones was ascribable to the transition of δ-mannitol to β-mannitol [6]. Overall, an IF equal to 1 h led to a higher R, which progressively increased as the storage temperature decreased (Figure 2). The MPs' R was quite constant during the three months storage, except for those produced at IF equal to 5 h and stored at T_{amb} where a remarkable decrease of R was registered. This reduction was coherent with the morphological modification shown in Figure 1 and pointed out that an IF of about 5 h could easily induce the formation of β-mannitol.

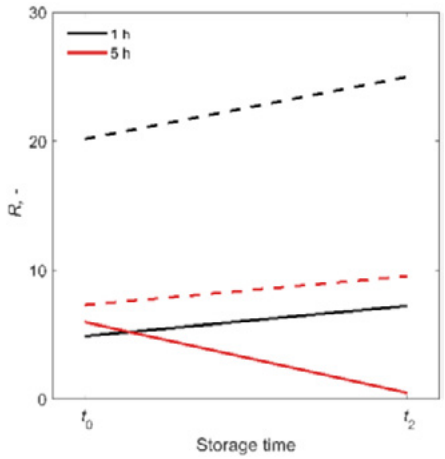
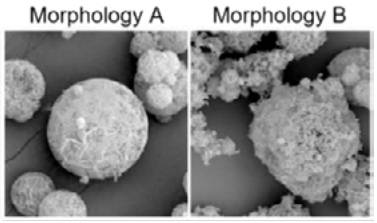


Figure 2. R of mannitol and SS MPs, produced either at (black) 1 h or (red) 5 h IF. MPs were stored at (solid line) T_{amb} and (dashed line) -20 °C. R is the XRD peak ratio between δ- and β-mannitol at 9.7° and 14.6°.

Given that IF equal to 1 h and storage at -20 °C effectively stabilised the MPs, we aimed to obtain a similar outcome at T_{amb}. Indeed, DPIs are usually employed at T_{amb} and we observed that thawing frozen MPs rapidly caused a reduction of R around 50%, inducing cohesiveness. Since amino acids are commonly employed in the inhalation field, 5% (w/w_{dw}) LL was added to protect the MPs from cohesiveness, proving to be efficient only when recrystallisation was avoided. The addition of LL, indeed, led to the formation of two morphologies (A and B) characterised by different aerodynamic behaviours (Figure 3). When recrystallisation occurred (morphology B), MPs were affected by higher β-mannitol content, higher cohesiveness, and poorer flowability.



MMAD (μm)	2.4 ± 0.1	4.8 ± 0.3
FPF (%)	39.8 ± 3.7	21.4 ± 4.1

Figure 3. (*top*) morphology and (*bottom*) MMAD and FPF of mannitol and SS MPs added with LL. Morphology A displayed lower cohesiveness and better aerodynamic properties than morphology B.

Since LL was not able to prevent recrystallisation, the action of different excipients was tested and R is reported in Figure 4. Dex (Figure 4) excellently stabilised the spray freeze-dried MPs, since R was constant at different storage temperatures for three months. No morphological changes occurred employing Dex, indicating good stability. Moreover, R was remarkably higher compared to the additive-free powders, pointing out the prevalence of δ-mannitol.

PS80, instead, performed worse at stabilising the MPs. Indeed, while an increase of R was registered at t_0, R was comparable with the additive-free case at t_2. Furthermore, MPs surfaces appeared rough and cohesive after three months of storage at T_{amb}. Frozen PS80-based MPs, instead, preserved their morphology and constant R.

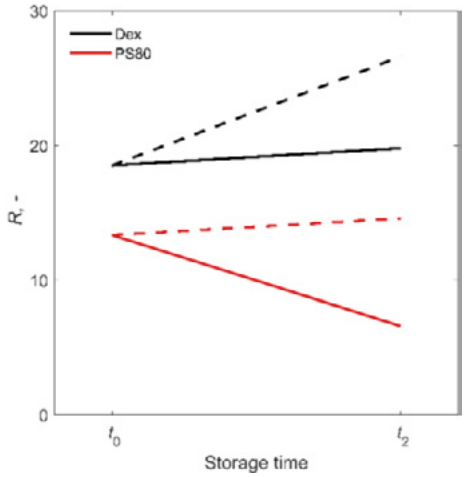


Figure 4. R of MPs composed of mannitol and SS, added with either (black) Dex 5% or (red) PS80 0.1%. MPs were stored at (solid line) T_{amb} and (dashed line) -20 °C and produced at IF equal to 1 h. R is the XRD peak ratio between δ- and β-mannitol at 9.7° and 14.6°.

Discussion and conclusion

In this work, the intermediate step IF was added to the SFD process to tune MPs' crystallinity. An IF equal to 5 h induced recrystallisation of the powders during storage, causing the conversion of δ-mannitol to β-mannitol [6]. Storing the frozen MPs, after freezing, at -50 °C for a longer time could have provided mannitol crystals with more time to rearrange into the most stable polymorph β due to the Ostwald's rule [7]. Although being the most thermodynamically stable polymorph, β-mannitol increased the cohesiveness among MPs. Instead, stability was achieved for MPs undergoing IF equal to 1 h and stored at -20 °C where the highest δ-mannitol content was registered. Since frozen MPs rapidly recrystallized during thawing, the stability at T_{amb} was pursued. The aim was to find an excipient able to replace LL, which is suitable for enhancing the flowability of spray freeze-dried MPs but is also prone to recrystallisation. Dex proved to increase the δ-mannitol content both at T_{amb} and -20 °C. As a result, the MPs' morphology remained unvaried during the three-month storage period, and cohesiveness was not induced. Therefore, stability of spray freeze-dried MPs stored at T_{amb} was achieved, paving the way for the use of Dex as standard excipients for mannitol-based formulations for SFD.

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