Particle Engineering via Spray Freeze-Drying for Pulmonary Drug Delivery Applications

Pulmonary drug delivery has increasingly been recognised as a powerful therapeutic strategy, not only for local treatment of respiratory disorders but also for systemic diseases. Compared with oral or parenteral administration, inhalation offers a range of advantages: a rapid onset of action, direct targeting of the lungs, reduced systemic side effects, and access to an exceptionally large absorptive surface. These features have established the pulmonary route as a highly attractive alternative for the administration of both small molecules and complex biologics. Yet, the translation of this potential into effective therapies is constrained by physiological defence mechanisms such as mucociliary clearance and macrophage activity, and by the technical challenges of designing powders that reach the lower airways in sufficient quantity while remaining physically and chemically stable. Among available inhalation platforms, dry powder inhalers have emerged as a preferred choice. They combine stability, ease of use, and environmental sustainability, offering propellant-free administration. Their success, however, depends critically on the ability to design powders with precise aerodynamic characteristics and robust stability profiles. Historically, micronisation techniques such as milling were used to produce inhalable powders, but these often generated irregular particles with poor flowability and limited control over size distribution. In recent years, particle engineering strategies have transformed this field, enabling the design of powders tailored at the micro- and nano-scale. Techniques such as spray drying, thin film freezing, and supercritical fluid technology have all contributed to advances, but spray freeze-drying has distinguished itself as an especially versatile approach. Spray freeze-drying combines atomisation, rapid freezing, and sublimation, producing porous, low-density microparticles with tunable properties. The low processing temperatures involved make it particularly suitable for heat-sensitive compounds, including proteins and monoclonal antibodies. Moreover, the porosity of the powders generated confers excellent aerodynamic potential, essential for lung deposition. Despite these advantages, however, several challenges remain. Powders produced by spray freeze-drying often exhibit high cohesiveness, which can compromise their flowability and dispersion from inhaler devices. The polymorphic behaviour of crystalline excipients such as mannitol can further complicate long-term stability, as recrystallisation during storage can alter morphology and impair deposition. Furthermore, the role of excipients in protecting active molecules, particularly biologics, during the stresses of atomisation, freezing, and drying, remains incompletely understood. The present doctoral work was conceived to systematically explore spray freeze-drying as a platform for inhalable powders and to address these critical challenges. The research followed a progressive trajectory, beginning with fundamental investigations into the influence of process parameters on excipient-based microparticles, then examining the interplay between crystallinity and stability, and ultimately extending to the development of formulations for biologics. The first part of the research focused on the feasibility of producing mannitol-based microparticles, either alone or in combination with a model drug, using ultrasonic spray freeze-drying. Mannitol was chosen as a model excipient due to its favourable lyophilisation properties, its high eutectic temperature, and its established role in dry powder inhaler formulations. The study aimed to understand how parameters such as solid concentration and feed flow rate influence particle morphology, size, porosity, and crystallinity. At the same time, the effect of incorporating a bronchodilator into the matrix was examined. This stage provided fundamental knowledge on the relationships between process conditions and microparticle properties, as well as highlighting the strengths and limitations of ultrasonic atomisation for pulmonary applications. It became clear that, while this approach could generate highly porous and spherical microparticles, achieving fine particle fractions suitable for efficient lung deposition required further innovation. To address this limitation, the research then turned to pneumatic spray freeze-drying, which offers a different atomisation mechanism and thus new opportunities to optimise powder characteristics. A Design of Experiments was employed to capture the complex interactions between solid concentration, atomisation gas flow, and feed rate, allowing the development of models for particle size and morphology. This methodological shift not only improved understanding of process-property relationships but also laid the groundwork for systematic process optimisation. In parallel, attention was devoted to the role of L-leucine, an amino acid

widely recognised for enhancing the dispersibility of inhalable powders. Incorporating this excipient into formulations offered a way to mitigate cohesiveness, but its mechanism of action remained debated. The work revealed how L-leucine influenced microparticle crystallinity, leading to morphologies with varying degrees of order, and demonstrated the strong dependency of powder aerodynamics on the polymorphic state of both mannitol and leucine. These findings clarified the contribution of leucine to particle engineering, showing that lower crystallinity states favoured improved aerosolisation and dispersibility. Having identified the influence of process parameters and excipients on particle performance, the next step was tackling the issue of long-term stability. One of the recurring challenges observed was the recrystallisation of mannitol during storage, which increased cohesiveness and compromised flowability. To overcome this bottleneck, the research investigated strategies for controlling polymorphism over time. A novel modification to the spray freeze-drying process was introduced in the form of an intermediate freezing step, where powders were conditioned before drying. This additional step allowed for a deeper knowledge of the mechanisms behind polymorphic transition of mannitol in spray freeze-dried microparticles. Alongside process modifications, the study examined the impact of storage temperature and explored the use of excipients such as dextran, hydroxypropyl-β -cyclodextrin, polyvinylpyrrolidone, and polysorbate 80 as stabilising agents. By selectively favouring the δ polymorph of mannitol and suppressing transitions to more cohesive forms, these approaches succeeded in preserving morphology and flowability over time. Importantly, they also linked solid-state control directly to aerodynamic performance, demonstrating that stability strategies were not only about preserving structure but also about ensuring therapeutic efficacy through consistent deposition and dissolution. The final part of the research represented a major advance by extending spray freeze-drying to biologics, specifically monoclonal antibodies. Although these molecules have revolutionised modern medicine, their delivery remains a challenge. Systemic administration is often inefficient for targeting the lung, requiring high doses to achieve therapeutic levels at the site of action, and is associated with significant costs. Pulmonary delivery offers a compelling alternative, but the sensitivity of antibodies to mechanical, thermal, and dehydration stresses makes formulation particularly demanding. Spray freeze-drying, with its mild processing conditions, provided an ideal platform to attempt this translation. The study investigated the use of protective excipients - sugars such as trehalose and mannitol, amino acids such as leucine, cyclodextrins such as hydroxypropyl-β -cyclodextrin, and surfactants such as polysorbate 80 - to shield antibodies during processing and storage. By combining these excipients in carefully designed formulations, it was possible to identify strategies that maintained antibody structural integrity, minimised aggregation, and preserved functional activity. Trehalose, especially in combination with leucine or hydroxypropyl-β -cyclodextrin, proved particularly effective, offering high levels of protection across all stages of the spray freeze-drying process. The resulting powders exhibited outstanding aerodynamic performance, rapid dissolution within the lung, and robust stability across a range of storage conditions. These results confirmed the feasibility of delivering monoclonal antibodies via inhalable powders and underscored the advantages of spray freeze-drying over traditional spray drying for biologics. The research presented in this thesis establishes a comprehensive framework for the optimisation of spray freeze-drying as a platform technology for inhalable formulations. The work addressed successive challenges: elucidating the impact of process parameters, defining the contribution of excipients, developing strategies to control polymorphism and stability, and extending these insights to the formulation of biologics. This progression illustrates how systematic advances can collectively overcome barriers that have hindered the broader industrial implementation of the technique. Beyond its conceptual contribution, the research delivers practical outcomes. By correlating processing conditions and excipient composition with particle morphology, crystallinity, and stability, it outlines concrete strategies for the rational design of dry powders. The successful formulation of monoclonal antibodies demonstrates the potential of spray freeze-drying to enable pulmonary delivery of complex therapeutics that remain underserved by conventional approaches. The translational significance of these findings is reinforced by their integration into industrial research pipelines, laying the foundation for continued development within Chiesi Farmaceutici S.p.A. Overall, the thesis positions spray freeze-drying as a versatile and scalable technology capable of addressing key challenges in pulmonary drug delivery. Through a balanced integration of mechanistic insight and applied formulation design, it advances the scientific understanding of particle engineering while providing solutions with direct industrial relevance. In doing so, it establishes a solid basis for future progress in inhalation therapy and contributes to innovation in advanced pharmaceutical manufacturing.