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Review article

Formulation strategies, preparation methods, and devices for pulmonary delivery of biologics

Kai Berkenfeld [a,j,1], Simone Carneiro [b,c,j,1], Carolina Corzo [d,j,1], Flavia Laffleur [e,j,1], Sharareh Salar-Behzadi [d,f,j,1], Benjamin Winkeljann [b,c,g,h,j,1], Golbarg Esfahani [i,j,*]

[a] Laboratory of Pharmaceutical Technology and Biopharmaceutics, Institute of Pharmacy, University of Bonn, Gerhard-Domagk-Street 3, 53121 Bonn, Germany
[b] Department of Pharmacy, Ludwig-Maximilians-Universität Munich, Butenandtstrasse 5-13, Haus B, 81377 München, Germany
[c] Center for NanoScience (CeNS), Ludwig-Maximilians-Universität München, München 80799, Germany
[d] Research Center Pharmaceutical Engineering GmbH, Graz, Austria
[e] Department of Pharmaceutical Technology, Institute of Pharmacy, Center for Chemistry and Biomedicine, University of Innsbruck, Innrain 80-82, 6020 Innsbruck, Austria
[f] Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology, University of Graz, Graz, Austria
[g] RNhale GmbH, München 81371, Germany
[h] Comprehensive Pneumology Center Munich (CPC-M), Helmholtz Munich, German Center for Lung Research (DZL), 81377 Munich, Germany
[i] Department of Pharmaceutical Technology, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Wolfgang-Langenbeck-Straße 4, Halle 06120, Saale, Germany
[j] Pharmaceutical Engineering and Technology Research Scientists (PETRS)

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ABSTRACT

Biological products, including vaccines, blood components, and recombinant therapeutic proteins, are derived from natural sources such as humans, animals, or microorganisms and are typically produced using advanced biotechnological methods. The success of biologics, particularly monoclonal antibodies, can be attributed to their favorable safety profiles and target specificity. However, their large molecular size presents significant challenges in drug delivery, particularly in overcoming biological barriers. Pulmonary delivery has emerged as a promising route for administering biologics, offering non-invasive delivery with rapid absorption, high systemic bioavailability, and avoidance of first-pass metabolism. This review first details the anatomy and physiological barriers of the respiratory tract and the associated challenges of pulmonary drug delivery (PDD). It further discusses innovations in PDD, the impact of particle size on drug deposition, and the use of secondary particles, such as nanoparticles, to enhance bioavailability and targeting. The review also explains various devices used for PDD, including dry powder inhalers (DPIs) and nebulizers, highlighting their advantages and limitations in delivering biologics. The role of excipients in improving the stability and performance of inhalation products is also addressed. Since dry powders are considered the suitable format for delivering biomolecules, particular emphasis is placed on the excipients used in DPI development. The final section of the article reviews and compares various dry powder manufacturing methods, clarifying their clinical relevance and potential for future applications in the field of inhalable drug formulation.

1. Introduction

Biological products include a wide variety of substances, for example, vaccines, blood or blood components, and recombinant therapeutic proteins. These products can be composed of sugars and other excipients, proteins, nucleic acids or complex combinations of those. This class also comprises living entities such as cells and tissues. Biologics are derived from diverse natural sources such as humans, animals, or microorganisms. Also, they can be produced through biotechnological methods and other advanced technologies. In contrast to most drug substances that are chemically defined and their structure is known, most biologics are complex mixtures that are not easily

* Corresponding author.
E-mail address: golbarg.esfahani@pharmazie.uni-halle.de (G. Esfahani).
[1] equal shared first-authorship.

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characterized. Biological products, including those manufactured by biotechnology, tend to be heat sensitive and susceptible to microbial contamination. Therefore, it is necessary to use aseptic principles from the initial manufacturing steps, which is also in contrast to most conventional drugs. Biological products are frequently found at the forefront of advanced biomedical research and have the potential to provide the most effective treatments for a wide range of medical illnesses and conditions that currently lack alternative remedies [1]. Nearly 30 % of all drugs approved by the US Food and Drug Administration (FDA) in 2015–2018 were biologics. Currently, more than 60 peptides are approved by the FDA for various indications, and there are even more in clinical trials [2]. Proteins and peptides now constitute a major proportion of therapeutic modalities being pursued for the treatment of various diseases [3]. Monoclonal antibodies (mAbs) have become the leading treatment approach, with more than 50 approved products [4] and over 500 mAb-based therapies currently being evaluated in clinical trials. More than 90 % of the biologics recently approved are mAbs. The growing success of biologics can be ascribed to their favorable safety profile, target specificity, and pharmacokinetics, in comparison to traditional small-molecule drugs [4]. Yet, the high molecular mass of biotechnological drugs presents major challenges as they are unable to passively diffuse across biological barriers [5].

The delivery of biologics via inhalation has particularly gained attention as it enables the non-invasive administration of drugs, either locally or systemically, even at high doses. To give a brief overview on the history of modern aerosol therapy, Fig. 1 shows major events and milestones in its development. The inhalation route has the advantage of rapid absorption and fast onset of drug action due to extensive vascularization, high tissue permeability and large surface area [6]. The amount of proteolytic enzyme activity in the lungs is considerably lower than in the gastrointestinal tract. However, enzymes such as CYP450, CYP2S, CYP2F, esterases and peptidases are expressed in the lung epithelia [78]. The extent of the metabolism of biologics in the lung is still unclear. It has been reported that the level of protease is higher in inflamed lungs [7]. The efficacy of inhaled biologics may be impaired in this case due to the increased metabolic activity. Delivery of biologics is not only affected by enzymatic degradation but also by the physical structures of the airways in the lungs, mucociliary clearance, alveolar macrophages, lung surfactant and epithelial membrane permeability [8].

The main advantage, among many others, of using pulmonary drug delivery (PDD) with the aim of systemic exposition, is that by delivering a drug via the respiratory tract, it can directly enter the circulation system without passing through the liver, thus bypassing the first-pass metabolism, resulting in a higher systemic bioavailability. Although the first-pass effect can be avoided using PDD, it is important to keep in mind that the lungs still represent a metabolizing organ, as comprising Cytochrome P450 enzymes. However, the metabolic rate of the lungs is much lower than that in the liver. In addition to that, the respiratory tract has an estimated internal surface area of 24–69 m^2 per lung lobe [11] in adults, allowing for an efficient and complete absorption of drugs and a rapid onset of action. If the target organ of an administered drug is the lung itself, which to date is the case most of the time when PDD is chosen, the dose of the drug can be lowered (compared to an oral dose) while still achieving an effective drug concentration at the site of action. This can result in fewer adverse drug events and therefore in a higher compliance, or potentially reduce therapy costs. Upon entering the airway system, a drug particle encounters a number of barriers of different nature. Following, we will provide a brief introduction to the anatomy of the airways, the clearance mechanisms present in the lung, and the deposition mechanisms, which all represent difficulties a particle must overcome in order to unfold its desired pharmacological effect.

1.1. *Airway anatomy and mechanical barriers*

One of the first obstacles that a drug is confronted with is the anatomy of the airways, which is highly complex and imposes certain criteria on the particle. The respiratory tract can be divided into the upper airways, lower airways and the so-called gas exchange or alveolar region. The upper airways consist of the nasal and oral cavities, pharynx, and larynx whereas the lower airways include trachea, bronchi, bronchioles as well as the terminal bronchioles [12]. It is important to mention that in total, the airways present about 23 bifurcations, each bifurcation representing an obstacle the drug particle must circumvent [13]. While the structure of the conducting airways is already narrow by nature, a diseased lung shows an even tighter pathway, which is a further hurdle in the way of effectively reaching the deep lung [14].

Pulmonary clearance in the lungs involves three key mechanisms: mucociliary clearance, macrophage uptake, and absorption into the epithelial layer, all of which are crucial for the development of drugs administered via the respiratory tract [15]. Mucociliary clearance relies on a two-layer system where a mucus layer traps inhaled particles and microorganisms, while a periciliary layer lubricates ciliated epithelial

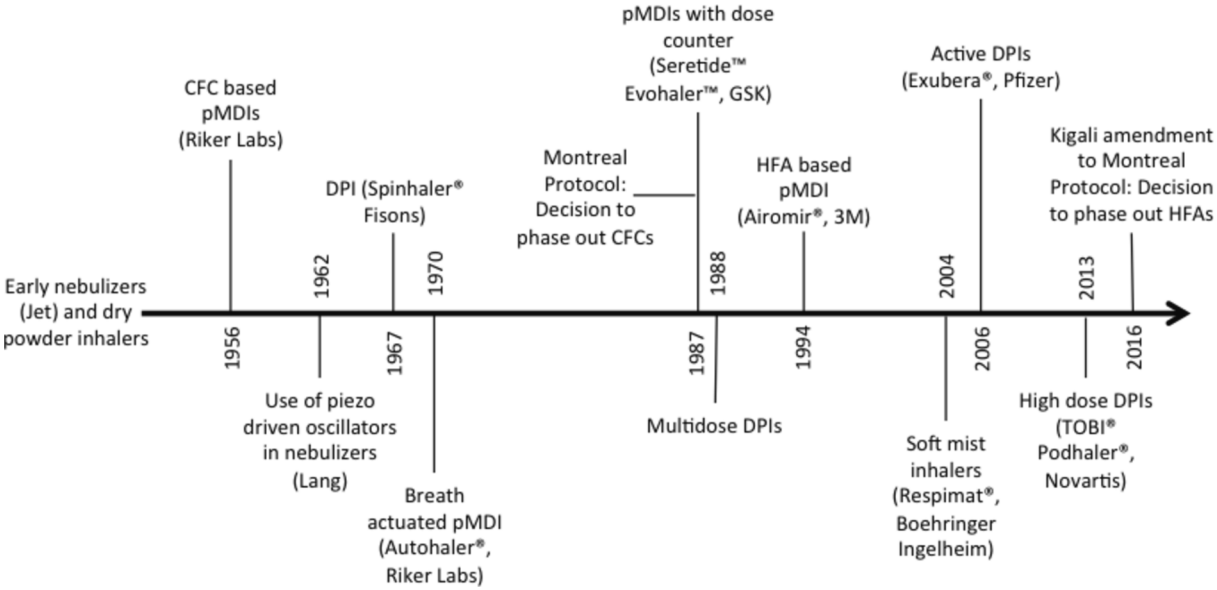

Fig. 1. Timeline of milestones in modern aerosol therapy [9,10].

cells to move mucus at around 10 mm/min in healthy tracheas, ultimately expelling particles via swallowing, coughing, or sneezing [12,16–18] Macrophage uptake, primarily in the alveoli where 97 % of airspace macrophages reside, removes foreign particles through electrostatic or receptor interactions, leading to elimination by lysosomes or transport to the interstitial space for lymphatic clearance[12,17,18]. The third mechanism, absorption into the epithelial layer, involves the dissolution of drug particles in epithelial lining fluid, with small hydrophilic molecules permeating slowly via paracellular diffusion, while lipophilic molecules are absorbed quickly through passive transcellular diffusion, and larger molecules are taken up through receptor-mediated transcytosis. The airway epithelium, consisting of various interconnected cells, regulates permeability through tight junctions, cellular enzymes, and efflux proteins, while the alveoli, lined by type I and type II pneumocytes, maintain alveolar structure and function, with type II pneumocytes secreting surfactant to prevent collapse [12,19–23].

1.2. Deposition mechanisms

When a drug is being inhaled, there are multiple factors which co-decide its fate once it has entered the airways. The most important influencing factor is the structure of the lungs, which is highly complex and rich in bifurcations. Further features need to be taken in consideration such as size and general physical characteristics of the particles [24]. Here, not only the geometric diameter but rather their aerodynamic diameter is relevant. The aerodynamic diameter is a surrogate diameter describing the aerodynamic behavior of the particle of interest combining information about the particle's geometric diameter, density, and shape. It is defined as the diameter of a hypothetical standardized particle (:= spherical shape and density of 1 g/cmˆ3) that shows the same settling velocity as the particle of interest [25].

There are five different mechanisms by which particle deposition can occur in the lungs: inertial impaction, sedimentation, diffusion, interception and electrostatic precipitation. Impaction is the primary mechanism responsible for the deposition of particles in the upper airways, concerning mainly larger particles with an aerodynamic diameter of ≫5 μm. Nevertheless, depending on the flow velocity, impaction under physiological conditions can also occur for particles as small as 2 μm [24]. Sedimentation is a time-dependent process in which particles settle due to the influence of gravity. Hence, breathing maneuvers in which more time is allowed for the particles to sediment (e.g. breath-holding) may increase lung deposition[26]. Diffusion comes about through so-called Brownian motions, namely "Brownian diffusion". As opposed to the mechanisms of impaction and sedimentation, deposition by diffusion increases with decreasing particle mass and is the main settling process for particles that measure < 0.5 μm in aerodynamic diameter [24]. Interception describes the separation of fiber like particles (e.g. asbestos) from the airstream: due to their unfavorable geometric shape, these particles are likely to interact with flow path defining structures falling below a particle specific critical dimension, e.g. when reaching the bifurcations of e.g. bronchioles. Deposition by interception usually does not occur in pharmaceutical preparations though and is more related to toxicology. These mechanisms have been reviewed in detail elsewhere [27,28].

2. Requirements for PDD

Modern inhaled medicines may contain two levels of particle/droplet structures (Fig. 2). The primary structures with particle size in μm range, as a liquid droplet or a dried matrix, determines the formulation's deposition in the lung. On the other hand, the secondary structures with particle size in nm range refers to a formulation containing an encapsulated active pharmaceutical ingredient (API) designed to enhance the physicochemical characteristics of the drug within the respiratory tract. The presence of a secondary particles in primary particles creates what is often referred to as a nano-in- or nano-embedded microparticle (NIM/NEM). Whether or not a secondary particle structure is necessary depends on the therapeutic approach (e.g. proactively supporting macrophage targeting) and the properties of the API, in particular, whether its bioavailability is sufficient without further formulation [28].

Considering the fact that dry powders for inhalation (DPI) are suitable products for the delivery of biomolecules, it is important to note that they are generally existing in carrier-based and carrier-free formulations. The modern products based on mentioned primary and secondary particle structures are carrier-free particles. More information on DPIs in general and each category in particular, together with applied excipients, can be found in next sections.

2.1. Particle size

The aerodynamic diameter of the particle is crucial in determining the path it take once in the airways and even the mechanisms that ensure their removal from the organism. Table 1 lists a correlation between the size of a particle and its elimination and deposition mechanisms.

While the table can be a helpful guideline for defining appropriate quality attributes such as particle size, in drug development, it is important to note that the size of an inhaled particle is not always static. Usually, the lung presents a higher relative humidity than ambient conditions, which can be absorbed accordingly by hygroscopic particles.

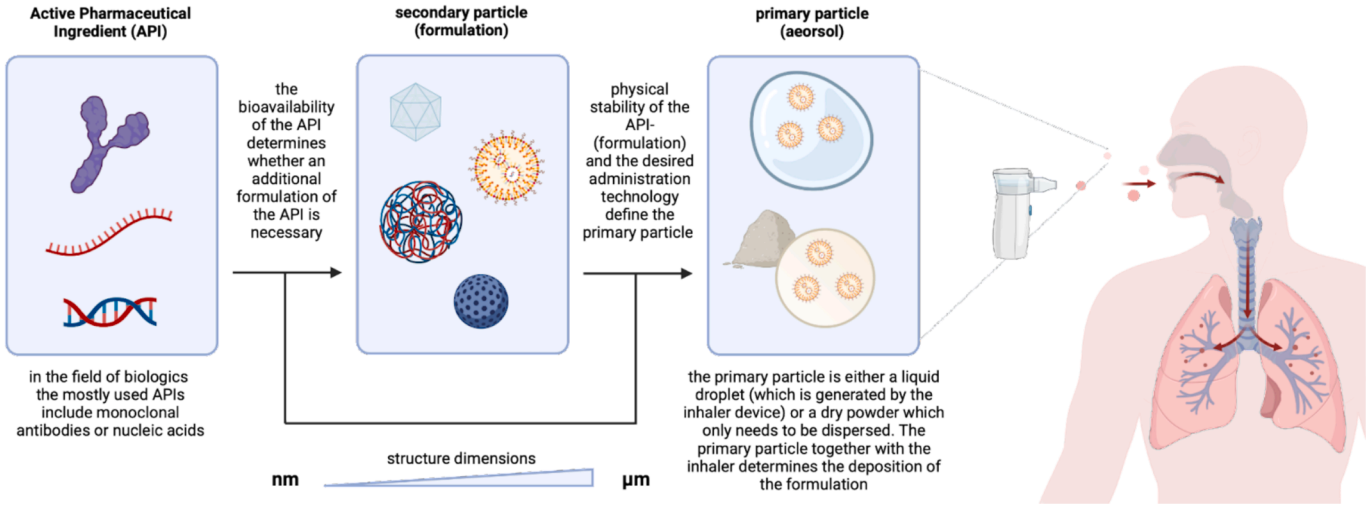

Fig. 2. Primary and secondary structures in modern inhaled medicines.

Table 1
Correlation between particle size, removal mechanism and deposition mechanism.

Particle size	Reached lung region	Clearance mechanism	Deposition mechanism
< 10 nm	Nose, mouth, pharyngeal airways	Exhalation	Brownian diffusion
< 200 nm	Deep lung	Exhalation/macrophage uptake	Brownian diffusion
< 500 nm	Acinus	Exhalation/macrophage uptake	Brownian diffusion
< 2 μm	Alveolar ducts and alveoli	Macrophage uptake	Brownian diffusion
2–5 μm	Central and small airways	Macrophage uptake/mucociliary clearance	Impaction/Sedimentation
5–10 μm	Upper and large airways	Mucociliary clearance	Impaction/Sedimentation
> 10 μm	Oropharynx/nasal cavity	Captured by nose hairs or mucus and then removed through mucociliary clearance	Impaction

This change in size can consequently affect both the mechanism of deposition and the location of deposition[18]. Many studies have been conducted with the goal to find the ideal particle size. Indeed, there is a conflict between overcoming the multiple barriers in the airways and the efficient deposition of a particle. For instance, whereas small particles are able to penetrate mucus more easily, they are also more likely to be exhaled. On the other hand, larger particles can be deposited effectively, but might not be capable of penetrating the mucus layer. One solution to this issue is formulating nano-based microparticles, or "Trojan" particles. After reaching the desired target, these nano-based microparticles disaggregate into nanoparticle subunits, which are able to permeate the mucus layer. Another approach involves designing large porous particles with low mass density ((ρ) < 0.4 g/cm3) and a diameter of > 5 μm. These characteristics enable deep lung deposition while evading macrophage uptake[29]. Another approach proactively exploits the hygroscopicity of certain excipients (e.g. mannitol). This strategy is based on the hypothesis that particles with smaller aerodynamic diameters are more effective at reaching the deep lungs. Once in the lungs, these particles increase their aerodynamic diameter by absorbing humidity, increasing the likelihood of depositing within the lower respiratory tract or the alveolar region (known as Excipient Enhanced Growth, EEG)[30].

2.2. Requirements for secondary particles

Particulate-based drug delivery systems are commonly employed as secondary particles and include matrices such as polymeric nanoparticles, liposomes, dendrimers, and lipid nanoparticles. These formulations significantly improve the overall capacity of the drug to reach the target site within the intricate structure of the lungs. An inherent advantage of secondary particles lies in their ability to protect the API from direct contact with lung fluids and cells, mitigating premature degradation [31]. These formulations address challenges associated with the poor water solubility of some APIs, thereby enhancing bioavailability. Additionally, they offer benefits such as sustained release of the drug [32].

The nanometric size and large surface area of these secondary particles enable them to penetrate through the lungs, evading pulmonary clearance mechanisms and passing through biological barriers such as mucus, cell membranes, and the endolysosomal compartment [32]. Moreover, the versatile composition of nanoparticles, including various polymers and lipids, allows the design of delivery systems with targeting molecules aiming at enhanced specificity [33]. An important criterion for the polymers and lipids incorporated into the formulation of secondary particles intended for inhalation is the possession of biocompatible and biodegradable properties, ensuring minimal toxicity [31].

Considering that the physicochemical characteristics of nanoparticles drive their fate within the lungs, this chapter will explore the requirements of secondary particles for successful pulmonary drug delivery. We aim to deeply understand the intricate interplay between the characteristics of nanoparticles and their role in optimizing drug delivery to the pulmonary system.

2.3. Influence of physicochemical properties of nanoparticles on their fate after pulmonary drug delivery

Physicochemical properties of nanoparticles, such as size [34], hydrophobicity/hydrophilicity [35,36], surface charge [37] and shape [38,39] have a crucial effect on their fate after pulmonary administration. It is widely reported that penetration of a particle through mucus is size-dependent. The mucus consists of mucin fibers that create a tangled and interconnected network, giving mucus its size-filtering capability. The mesh spacing of this network prevents nanoparticles larger than the mesh size from passing through the mucus, causing them to be sterically impeded and potentially eliminated from the lung through mucociliary clearance. By contrast, particles and pathogens smaller than the mesh pore size can rapidly penetrate the respiratory mucus [40–42].

Mucus contains abundant negatively charged groups, causing positively charged nanoparticles to become immobilized within the mucus network through electrostatic association. This enhanced mucoadhesion serves as an effective approach to enhance the pulmonary delivery of drugs by prolonging their retention time in the lungs. Chitosan (CS) is frequently employed as a positively charged mucoadhesive material in the airway mucus [43,44]. Ciprofloxacin loaded chitosan (Cipro-CS) particles, prepared by ionic gelation, with a size in the range of ≤ 200 nm were reported to be internalized in the human lung epithelial cells [45]. Nevertheless, the mechanism by which CS-based nanoparticles are internalized is not yet clear. However, their efficiency in intracellular delivery of nucleic acids is proven by several studies [46–48].

Coating the nanoparticles is one of the most used approaches to modulate surface properties. Hyaluronan is a polysaccharide often used as a coating agent. CS-coated poly(lactic-co-glycolic acid) (PLGA) nanoparticles loaded with voriconazole were proposed to increase retention time at the pulmonary site, consequently promoting cellular internalization [49]. Almeida et al, observed improved cellular internalization of hyaluronan-coated particles [50]. In a study conducted by Ghosh et al [51], hydrophilic, net-neutral charged peptides were identified and used to coat the nanoparticles. The ex-vivo outcomes showed 600-fold better penetration of the particles through human cystic fibrosis mucus compared to uncoated or PEGylated nanoparticles. In a recent study, novel small lipidated sulfoxide polymers based on poly(2-(methylsulfinyl)ethyl acrylate) (PMSEA) as inhalable drug delivery platforms was investigated. Linear PMSEA (5 kDa) was used as a hydrophilic polymer backbone with excellent anti-fouling and stealth properties compared to poly(ethylene glycol). Their data suggest that there is scope to tune the lipid component of the system to control membrane permeability and cellular interactions in the lungs to tailor drug disposition in the lungs [52].

Once the particle reaches the surface of the target cells of the respiratory tract, it has to cross the cellular membrane. Endocytosis is the major cellular uptake pathway known to be involved in non-viral nucleic acid delivery [53]. Particles below 150 nm could avoid macrophage uptake and will enter the cells either by diffusion or by endocytosis depending on the particle size [54]. In a study conducted by Thorley et al [55], it was demonstrated that 50 nm nanoparticles enter largely by passive diffusion and are found in the cytoplasm, whereas

100 nm nanoparticles enter primarily via clathrin- and also caveolin-mediated endocytosis and are found in endosomes. Particles that are taken up by the cells through this pathway are enclosed in clathrin-coated vesicles. They are then transported into early endosomes, which fuse to form late endosomes and subsequently into the lysosomes [56]. Endocytosis can also occur via receptor-mediated mechanisms. Kim et al, reported hyaluronan-receptor mediated endocytosis of nanoparticles formed with hyaluronan (100 kDa) conjugated with vascular endothelial growth factor receptor 1 peptide [57]. After the internalization, polysaccharide-based nanoparticles may remain within the cells, exerting their effects, or be transported transcellularly and expelled in a process named transcytosis. By transcytic pathway, the nanoparticles can permeate the epithelium, and reach blood or lymphatic circulation [58]. Another mechanism that enables nanoparticles to cross the epithelial barrier is through the opening of tight junctions, which allows the movement of solutes via the paracellular pathway. CS is recognized for its capability to open tight junctions, thereby enabling the translocation of nanoparticles of sizes up to 50 nm [59].

Please note, that the described formulation strategies should be defined and tailored carefully for each case of application. Beyond this, the structure of biological compounds can be modified to improve the absorption of biological drug and to maintain its in vivo stability.

Genetic engineering, protein fusion and chemical conjugation are examples for strategies to prevent physical and chemical degradation [60,61]. Genetic engineering of a recombinant protein, by introducing single or multiple amino acid substitutions, can affect properties such as specificity of ligand binding and sensitivity to degradation. Genetic modifications to improve the conformational stability can be further applied for optimization of electrostatic contacts and hydrogen bond network, of hydrophobic and van der Waals interactions [62].Glyco-engineering is another approach, in which new glycosylation sites are introduced to the protein, or the previously available sites are engineered with the aim of improving the in vitro stability [63].

Another strategy to improve the properties of therapeutic proteins is fusion proteins. Examples are the fusion of different biologics such as glucagon-like peptide-1 (GLP-1), fibroblast growth factor 21 (FGF21), canine interferon gamma (IFN)-ɣ, and human serum albumin (HSA), to improve the short life-time and instability of these biologics [64–66].

Covalent linkage of polymers to protein drugs is an approach to improve the pharmacokinetic and in vivo stability of loaded biologics upon pulmonary administration. This approach can provide a controlled and sustained exposure of the lungs to the biologic. The steric hindrance or surface modifications can prevent degradation of the molecules. Conjugation can be performed with a series of carbohydrate moieties or synthetic polymers, e.g. glycans, polyethylene glycol (PEG), dendrimers, polyethylenimine (PEI), hyaluronic acid, etc. Comprehensive research is available on this topic and an extensive description can be found in Marasini et al. [60] among others.

3. Devices for PDD

Aerosols are a suitable option to deliver pharmaceutics to the lungs. As discussed in the previous section, therapeutic aerosols must fulfill certain requirements regarding their aerodynamic particle size. Over the past decades, multiple approaches to generate appropriate aerosols have been developed and transferred into clinical practice, most notably formulation as pressurized metered dose inhalers (pMDIs), dry powder for inhalation (DPI) and different types of nebulizers including soft mist inhalers (SMIs) [67]. For delivery of biomolecules though, pMDIs do not play a major role [68] which might be related to several limitations of the technology e.g. the necessity to formulate as either suspension or solution, which inherently creates a need to provide a formulation of suitable physical and chemical stability over the entire anticipated shelf live. Other concerns might be related to fairly high shear forces occurring during actuation that might have negative effects on the activity of the molecules post delivery, or the limitation to doses in the lower milligram range. DPIs and nebulizers currently are the most prominent technologies to produce respirable aerosols containing macromolecules and will be discussed briefly in the following sections. For an in depth discussion of associated device technology, the reader is referred to the excellent review articles available, e.g. [67,69–73].

3.1. Dry powders inhalers (DPIs)

Formulating as dry powders for inhalation is a viable approach to generate therapeutic aerosols. Here, the API and excipients are formulated and processed so that they form dry powder particles in the suitable size range. Excipients and technologies used within this context are discussed in the corresponding sections of this review. Even though the aerodynamic diameter of the particles may efficiently be manipulated through adjusting the particle density and geometry[25], particles still exhibit a geometric diameter in the lower micrometer range. Due to unfavorable surface to mass ratios, particles in this size range typically are very co– and adhesive so that dry powders for inhalation need to be de-agglomerated prior to or upon inhalation, using dry powder inhalation devices. In most cases, the energy required to overcome the cohesive forces (namely: van-der-Waals, capillary, and electrostatic forces and mechanical interlocking) is provided by the inspirational airflow of the patient and devices using this source of energy are classified as ‚passive‘ devices. Often, the API particles are co-formulated with larger so-called carrier particles (Lactose monohydrate or mannitol) that can aid in increase processability, dose metering, and aerosolizability [67]. This formulation approach comes with the trade-off of having to accept that a certain fraction of the bulk formulation/metered dose (often times in the range of 90+ %) is pharmaceutically inactive [74]. Therefore, also here, dose limitations might play a role, making this formulation strategy less attractive for biomolecules. Also processing technology suitable for biomolecules is often suitable for particle engineering approaches as well, lowering the barrier to decide against the more traditional approach involving interactive blends of jet-milled API and larger carrier particles.

Another advantage of passive devices is that the actuation of the device inherently is synchronized with the inspiration maneuver of the patient, which can be challenging in clinical practice when the inspiration must occur simultaneously with the manual actuation, as e.g. seen in pMDIs [75]. Associated with all types of DPI devices is a device specific intrinsic airflow resistance, which correlates the volume flow occurring over the device with a given pressure drop that is to be provided by the patient. Especially in the case of obstructing conditions e.g. chronic obstructive pulmonary disease (COPD) or asthma, the overall air flow is dominated/regulated by the air flow over the constricted bronchi and bronchioles, so that high resistance devices might be better suited for this patient population. Selection of an unsuitable device might result in a situation where the patient is not capable of providing sufficient pressure drop for the device to function properly, which might lead to incomplete de-agglomeration and consequently in an increase in aerodynamic particle size, which then leads to premature separation of the particles from the air stream and impaction in the upper airways thus delivering an incorrect dose. In contrast, low intrinsic resistance devices are often more effective in de-agglomerating the particles so the devices have to be designed or adapted carefully for the intended purpose [70].

Different DPI devices frequently use unique design features to convert the energy provided through the airflow into particle motion aiding the de-agglomeration of the bulk particles. In one common approach, powder doses are pre-metered in gelatin or hydroxypropylmethylcellulose (HPMC) capsules that are pierced during a priming step and set in motion during the inhalation process. The type of primary motion (e.g. axial gyration (e.g. Handihaler), revolution (e.g. RS01 type)) [76], geometry of the needle tip and the resulting holes [77], and numerous other device characteristics e.g. extent of capsule-wall, particle–particle, or particle–wall interactions, but also other

characteristics [78] as e.g. the exit velocity of the aerosol plume demand that dry powder formulation and DPI device need to be viewed at as one operational unit with distinct product characteristics i.e. very specific aerodynamic particle size distributions. As the overall dose delivered is related to the aerosol characteristics and exit velocity from the device [70], these products are not interchangeable. From the scientist's point of view, this also leads to the conclusion that evaluation of the aerosol properties of dry powders for inhalation without precise knowledge of the device and test conditions used is not very meaningful. Of course, not all DPI devices use a capsule based approach and there is a broad variability not only when it comes to device effectiveness, but also other features as e.g. feed-back mechanisms indicating correct use are of relevance and different use cases demand different solutions. It is to be mentioned though that in the context of research and early stage feasibility studies, capsule-based DPI devices are often preferred, which is related to their ease of use. For an in depth discussion of DPI device technology, the reader is kindly referred to the excellent reviews on this topic [67,69,70].

3.2. Nebulizers

Nebulizers are the most straightforward approach to generate pharmaceutical aerosols. Solutions, suspensions, or emulsions of the API in a suitable solvent or solvent mixture (typically water) are nebulized using either compressed air (jet-nebulizers) or by other means as e.g. oscillation provided by a piezo driver (mesh nebulizers) [79]. Most nebulizers have in common that an external source of energy is required which often makes nebulizers quite bulky and not very easy to carry around [80]. This becomes especially cumbersome when doses are to be applied multiple times a day. Modern hand held devices are better suited for convenient use but also here, user friendliness might be an issue as all nebulizers require substantial effort for priming the device, cleaning, or the need for energy supply. Additionally, the delivery rate is rather low [81,82] when compared to pMDIs and DPI and delivering one dose might take up to more than ten minutes – compared to a couple of seconds using aforementioned devices. Nevertheless, nebulizers play a very important role in modern healthcare, especially when it comes to the treatment of specific patient populations for instance children that might not be able to operate pMDIs or DPIs properly, which is also related to their differences in anatomy and physiology. Firstly, the dimensions of the respiratory tract of children are smaller compared to adults so that excessive oropharyngeal deposition is observed when using e.g. pMDIs. Spacers or valved holding chambers may be used to mitigate this issue (and also to help with synchronizing actuation and inhalation), but often nebulizers are the better choice, as also these systems are linked to certain limitations such as problems with not reproducible doses related to electrostatic charging [83]. Also DPIs are not suitable for children up to a certain age, as the physiology of respiration (volume inhaled, pressure drops, breathing pattern) of the very young differ significantly from the adults and does not allow their proper use [84]. Nebulizers are also widely used in hospitals if patients are supplied with active ventilation to support breathing. In this scenario, the nebulizer head can be incorporated into the system already in use and often this is the only option for aerosol therapy, especially when the patients are unconscious.

Another important field of application is their use in early clinical testing of investigational drug candidates, which is related to the rather low effort to develop suitable test formulations and the potentially increased traceability of quality of the inhalation maneuver. Also, aforementioned limitations are not very critical in a clinical trial setting. Latest developments in nebulizer technology include the development of smart devices, where for example, the device is trained to optimize actuation patterns based on the inspiratory flow patterns of the user so that a smaller fraction of the dose is lost during the application process. For more information on nebulizers, the reader is kindly referred to [71,72,85].

3.3. Choice of inhalation devices

As discussed in the previous sections, it is of utmost importance to select the ‚right' device for each application. Here, not only the suitability of the target patient population to successfully and safely operate the device is relevant, but also other considerations play a role. Besides the limitations that apply to the delivery of biomolecules, in therapeutic approaches where high doses are needed, pMDIs are not the first choice, and other approaches such as DPIs are in most cases better suited. One prominent example is anti-infective aerosol therapy using antibiotic or antifungal agents. Nebulizers are often a suitable alternative; however, they still have some notable drawbacks. In this specific use case, using single-use, disposable devices might be beneficial as the risk of recontamination of the patient through an already used and not properly cleaned device is minimized. Of course, single-use products may not be the first choice when considering environmental aspects, but it is conceivable that future devices could be made from biodegradable polymers. However, the disposal of used and potentially contaminated devices (with APIs and pathogens) could pose a challenge. These exemplary considerations highlight that modern device development must not only consider and meet the requirements to assure a safe, easy to use, and reliable therapy, rather it is to be expected that additional factors, such as environmental safety or the device's carbon footprint will become more important.

3.4. Regulatory pathways for biologics for pulmonary delivery

Besides selection of a suitable formulation and device strategy, selection of the regulatory pathway is another very important decision to be made during the development of biologics intended for administration via the pulmonary route. Depending on the area of the world where the product is intended to be made available, different regulatory pathways are available. It is beyond the scope of this article to discuss all pathways as defined by the different regulatory agencies, but as an example, we would like to very briefly discuss different options as provided by the United States Food and Drug Administration. Choice of the appropriate pathway is to be made on a case dependent basis as different therapeutic approaches, APIs, devices and associated development programs will have their individual needs and constraints for example, shelf life of the device after first use, storage conditions, frequency of use etc. Due to aforementioned requirements regarding aerodynamic PSD, fit for the intended patient population etc., it should be considered state-of-the-art to link drug product and device, though e.g. development as solution for nebulization might be appropriate in some cases. Nevertheless, registration as a combination product will be the preferred option in the majority of cases. Combination products are products where the drug formulation (here called drug constituent part) is either an integral part of the device (device constituent part), or is co-packaged with the device, or is linked to the device that is packaged separately through cross-labeling [86]. Example scenarios might be multidose DPIs or pre-loaded soft mist inhalers, multi-unit dose DPIs or a nebulizer with co-packaged solution for nebulization, or cross-labelling the drug product with e.g. a certain nebulizer, respectively. It is to be mentioned that not only the drug product but also the referenced device must be approved in the respective region, when cross-labelled. Regardless of the regulatory strategy, it is advisable to take this decision early on in the development programs, as the regulatory pathway should be reflected in the CMC aspects of the development. for further information on this topic, the reader is kindly referred to [87]. Additional information on aerosol product development can be found in the respective guidelines including ICH, FDA and EMA.

4. Excipients

Excipients are used in the development of inhalation products to improve the stability, the mechanical properties and the dosing

reproducibility of administered API. The result is improved performance of the inhalation product. Beyond those classical applications, they are used in advanced pharmaceutical manufacturing for encapsulation of both biologics and small molecules, to improve the stability, to modify the active compounds' pharmacokinetics and pharmacodynamics and to enhance the drug targeting [88].

The focus of this section is mostly on the excipients used for the development of dry powder inhaler (DPI) products, as DPI is the most promising delivery system for providing improved stability of the advanced pulmonary formulations. Carrier-based and carrier-free formulations are considered. Whereas a short overview is provided on the carrier excipients, the main focus is on the carrier-free formulations of biologics, divided into primary and secondary particle structures. This is due to the fact that the stress factors involved in the preparation of carrier-based formulations can cause degradation and denaturation of proteins and peptides. Hence, carrier-free development is mainly used for the formulation of biologics for inhalation purposes[89,90]. The manufacturing processes are discussed in the next section.

It is important to note that despite the existence of a huge number of research papers dealing with the functionality of different groups of excipients, the required safety data is the limiting factor for their application in industrial production[91,92].

4.1. Excipients for carrier-based formulations

Coarse excipient particles are required as carriers to improve the dispersing and actuation of cohesive micronized particles of API, hence improving the delivery to the lungs by ensuring dosing accuracy. This is of special importance in case of potent, and low dose micronized APIs such as antibiotics and steroids. Generally, carrier attributes such as particle shape and size distribution, surface morphology, powder porosity, and rheological properties are the key parameters for the functionality. Those properties significantly affect the blend uniformity and powder flowability, among others[90,93]. The ratio of micronized drug to carrier, their physical and chemical compatibility, as well as the interparticular interactions between drug, carrier and container have to be considered for selecting the carrier.

Available as approved excipient in different forms, lactose is the most applied carrier for industrial production. Lactose monohydrate is the most common form in different inhalation-grades with detailed specifications including impurities, protein content and amorphous content [94]. Detailed information on different types of lactose and the effect of critical quality attributes such as particle size distribution (PSD) and surface modifications on the performance of lactose as carrier can be found elsewhere[9,95–97].

The reducing function of lactose, due to the available carbonyl group in its chemical structure, makes it inappropriate as a carrier for proteins and small molecules containing primary amine groups such as formoterol and budesonide. Therefore, alternative carriers have been extensively screened. Examples include mannitol, xylitol, sorbitol, maltitol, trehalose, fructose, galactose, sucrose, dextran and cyclodextrins, among others[85,98–100]. The most researched non-reducing sugar is mannitol, which possesses the additional advantage of a highly sweet aftertaste that can improve patient adherence to therapy and monitor the dose taken by the patient[98,101]. Mannitol is also available as a mucolytic active compound in Bronchitol® to support the treatment of cystic fibrosis.

There are several approaches to improve the performance of carrier-based formulations, in terms of improving blend's rheological behaviour, dispensing properties, increased FPF, and thus dosing accuracy. Such approaches are basically established on adding a second additive to the API-carrier blend and building a ternary mixture as well as carrier engineering.

In the case of providing ternary mixtures, one strategy is the addition of carrier fine particles to the coarse material. Two different theories explain the effect of fine carrier particles. The first one is based on the partial covering of API bonding sites with fine particles, resulting in the fewer remaining number of active sites on API for attaching to the active sites of coarse carrier. The result would be the easier detachment of drug particles from the carrier by actuation. A fundamental understanding of the attributes of coarse carrier that determines the active sites of those particles is required for an effective formulation development. Examples of such critical attributes are PSD, surface energy and morphology, porosity, electrostatic charges and chemical properties of coarse particles [97,102–107]. The second theory is based on the forming of agglomerates of API and fine particles. The de-agglomeration may easily occur due to the smooth surface of fine particles compared to the engineered surface of coarse material. The small API-fine agglomerates with improved flowability can also be directly deposited in the lungs [107–111].

The other strategy is to coat the active sites of the coarse carrier by using the so-called force control agents (FCAs). The aim is to facilitate the detachment of API, hence increasing the FPF and improving the performance of formulation. FCAs possess low surface free energy, and they are supposed to reduce the intrinsic cohesion of the powder, resulting in improved flowability. Magnesium stearate is the famous FCA with approval for inhalation purposes and its mixture with lactose monohydrate has been used in industrial manufacturing of carrier-based inhalation products for decades [112–114]. Examples of marketed products containing lactose and magnesium stearate include Anoro® Ellipta®, Bero® Ellipta®, Incruse® and Trelegy®[115]. Ternary mixtures of mannitol were prepared by adding magnesium stearate or mannitol fine particles to successfully improve the API detachment from coarse mannitol carrier[98,116].

Besides magnesium stearate, several authors reported on the application of excipients such as leucine, lecithine, sucrose stearate, and even polymers, among others [117–120]. Due to the fact that small changes in critical carrier attributes can affect the reproducible and stable performance of the final product, carrier engineering is of significant interest for tailoring those attributes. Different crystallization methods have been used to tailor the polymorphic form of lactose and mannitol. Spray-drying, spray freeze-drying, freeze-drying, supercritical fluid technology and electrospinning are the common processes to tailor the surface properties [121–123]. As mentioned above, application of carrier-based formulations for manufacturing of biologics is uncommon. One example is the use of commercial mannitol to provide a spray freeze-dried mixture with 10 % w/w glycine as carriers for loading of glucagon-like peptide-1 (GLP-1). The peptide was also prepared without excipients via spray drying. GLP-1 delivered dose was significantly improved by using spray freeze-dried glycine-mannitol as carrier[124]. Other examples below focus on using engineered carriers for loading of small molecules.

Leucine is one of the promising excipients used in spray-dried inhalation products, both in ternary mixtures for manufacturing of engineered carriers, as well as for the development of carrier-free products. Enhanced aerosolization, increased FPF, improved stability, preventing moisture, especially in combination with hygroscopic carriers such as lactose, and enhanced API solubility have been reported for formulations developed with leucine [125–132]. Kaialy et al. have investigated the impact of PSD on carrier properties of mannitol by spray-drying of the material to provide consistent properties i.e. homogeneous spherical shape, solid-state (mixture of α and β polymorphic form) and true density with a water content of 1 ± 0.1 %, w/w, but different particle sizes. The spray-dried mannitol was used as carrier to load albuterol sulphate. The formulation with larger particle size had a superior flowability and improved FPF, resulting in a minimized dose variability [101]. In a further study Ferdynand and Nokhodchi have spray-dried the mixtures of lactose and mannitol in different ratios with 5 %w/w leucine, to successfully improve the aerosolization performance of spray-dried ternary mixture for loading salbutamol as small molecule. Presence of mannitol in the samples resulted in the stabilizing of amorphous state of lactose, while mannitol remained in its crystal form

having a mixture of α and β polymorphic forms when the ratio of mannitol was low. The best aerosolization performance belonged to spray-dried ternary mixture with the 1:3 ratio of mannitol to lactose. Consequently, the same ternary carrier delivered the highest FPF (62.42 ± 4.21 %) after loading with API [128].

Detailed information on carrier engineering as well as on the mechanisms of drug detachment from carrier particles can be found in [102].

4.2. Excipients for carrier-free formulations

Carrier-free formulations are divided into primary and secondary particle structures. The primary structure, as a liquid droplet or a dried matrix, determines the formulation's deposition in the lung, while the secondary particle refers to nanoparticles containing an API designed to enhance the physicochemical characteristics of the drug within the respiratory tract. The most applied excipients for the development of such structures are described below and relevant examples are listed in Table 2.

4.2.1. Excipients for primary particles

Primary particles with a size range of a few micrometers are mostly prepared by natural polymers. Natural polysaccharides such as CS, alginate (AG), hyaluronic acid (HA), dextran, and cellulose are extensively used to deliver drugs into the pulmonary tract due to their biodegradability, low toxicity, and ability to blend with a varied range of drug formulations [133]. This review focuses on the most commonly used polysaccharides in pulmonary formulation preparation.

Hyaluronic acid (HA) is a polysaccharide that is naturally present in the lung [134]. Hyaluronic acid protects the lung elastin from inflammatory lung disease injuries and helps to repair lung damage [135]. HA is able to entrap bioactive molecules by forming a stable matrix. For instance, HA amorphous microparticles can be produced by co-spray drying of the aqueous solution of the polymer and a bioactive molecule. Solubility and molecular weight of the bioactive molecule determine the dissolution and release rate. These factors regulate the diffusion of the molecule through the hydrogel matrix that is formed after the exposure of the HA to water [136]. In a study conducted by Blair et al, HA was spray-dried with insulin from aqueous solutions to form inhalable dry powders containing 10 % (w/w) drug. In this study, the authors faced several limitations of spray drying HA at the laboratory scale including: 1. Long spray drying batch times due to low solids concentrations because of the viscous nature of the HA. 2. Extrusion of the viscous solution through the nozzle, rather than atomization in case of lack of steady rate of pump control of the solution to the nozzle [137].

Carboxymethyl cellulose (CMC) is a water soluble cellulose derivative [138]. In a study conducted by Gallo et al., sodium carboxymethylcellulose (CMCNa), sodium hyaluronate (HLNa), and sodium alginate (AlgNa) were selected to co-process sodium cromoglycate (SC), an antiasthmatic and antiallergenic drug, by spray drying. The powder based on SC and CMCNa exhibited the best mucoadhesion and aerosolization performance, the highest process yield and adequate moisture content, hygroscopicity, and stability [139].

CS has widely been studied for the delivery of various drugs, vaccines, genes and chemotherapeutic agents [140–142]. Various types of CS-based carriers have been investigated for pulmonary delivery of various drugs such as antibiotics [143,144] or heparin[145]. In a study, spray-dried powders were produced from 30 % (v/v) aqueous ethanol formulations that contained hydrophilic (terbutaline sulphate) and hydrophobic (beclometasone dipropionate) model drugs, CS (as a drug release modifier) and leucine (aerosolizsation enhancer). Their investigations demonstrated that it was possible to generate highly respirable powders that exhibit sustained drug release profiles of both hydrophilic and lipophilic agents by spray drying[146].

CS and its derivative, trimethyl chitosan (TMC), have also been used as dispersibility enhancers in dry powder formulations for pulmonary deposition. Birchall and Li demonstrated that both CS and its soluble derivative TMC can significantly increase the emitted dose (> 90 %) and the in vitro pulmonary deposition (Fine particle fraction (FPF) > 35 % = fraction of dose with aerodynamic diameter < 5 μm) of dry powder dispersions[147].

Besides natural polymers, synthetic polymers have also been investigated to design novel pulmonary delivery systems. For example, an innovative material approach was explored in a study on disordered mesoporous silica particles (MSP). In this case, MSP was employed as a novel carrier for delivering proteins to the lungs. The disordered structure of the silica particles provided a high surface area and porosity, facilitating efficient encapsulation and controlled release of therapeutic proteins. This method showed promise in enhancing the stability and bioavailability of proteins during pulmonary delivery[148].

Table 2
The most applied excipients for the development of carrier-free formulations.

Material	Active substance	Used device/method	Method of preparation	reference
Sodium hyaluronate	Insulin	surgically prepared permanent tracheostome in dogs	Spray drying	[137]
Guanidinylated chitosan	siRNA	Aeroneb Pro nebulizer	Lyophilization	[207]
Chitosan, Lactose	Rifampicin	DPI	Spray drying	[208]
Sodium carboxymethylcellulose	Sodium cromoglycate	DPI (Breezhaler®)	Spray drying	[139]
Leucine-modified chitosan	Terbutaline sulphate, beclometasone dipropionate	DPI (Spinhaler®)	Spray drying	[146]
Chitosan	Plasmid DNA	DPI (Spinhaler®)	Spray drying	[147]
Diglycerol full ester of behenic acid (PG2-C22)	Rifampicin	DPI	Spray drying	[168]
Diglycerol ester fully esterified with stearic acid	Dexamethasone	Nebulization	Melt-emulsification followed by high pressure homogenization	[216]
Dipalmitoylphosphatidylcholine	Temocillin	DPI	Spray-drying	[217]
Glycerol	Beclomethasone dipropionate	pMDI	Mixing of the formulation components followed by the filling of hydrofluoroalkane as a propellant using a Pamasol 2016 Laboratory plant	[218]
Fumaryl diketopiperazine	Insulin	Dreamboat® inhaler	Mixture of ultrafine aerosol ^{99m}pertechnetate nanoparticles and the insulin-fumaryl diketopiperazine dry powder in a nebulizer chamber. The powder was filled into cartridges for inhalation.	[219]
Cholesterol and distearoyl phosphatidylcholine (DSPC)	Tobramycin	DPI	Spray drying	[220]
DSPC and DPPC	GDC-A	DPI	Spray drying	[221]

4.2.2. Excipients for secondary particles

As introduced above, certain APIs require additional formulation effort besides what is needed for aerosol generation. One of the most prominent examples is nucleic acids (NAs). Naked NAs have almost no bioavailability; they are rapidly cleared by the immune system, and target tissue cells hardly internalize bare NAs. Similarly, proteins or small molecules with low water solubility might require additional formulation to become bioavailable in reasonable amounts. Nano-formulations are one strategy to increase the bioavailability of such payloads. Generally, one can distinguish between viral and non-viral vectors. The latter can be further categorized into polymeric, lipid-based, and inorganic carriers such as silica or gold nanoparticles. Additionally, hybrid systems combining multiple types of materials can also be utilized, offering the advantages of various carrier types and enhancing the overall efficacy of the formulation.

4.2.2.1. Lipids. Lipid-based nano-formulations have emerged as drug carriers made from biocompatible materials designed to efficiently deliver cargo through various routes of administration. These non-viral delivery systems, which include liposomes, lipoplexes, and stable lipid nanoparticles for RNA delivery, offer several advantages to overcome the poor solubility and stability of conventional drugs. While efficiently encapsulating the drugs, these carriers can protect the cargo from degradation and premature release, increasing drug stability [149]. For pulmonary administration, lipid-based systems have shown promise in overcoming lung barriers and interacting efficiently with cell membranes, enhancing overall pharmacokinetics [150].

Among lipid-based nanoformulations, lipid nanoparticles (LNPs) stand out as the clinically most advanced systems. The approval of the Onpattro® (name of the company and location) a few years ago established LNPs as efficient and safe carriers for siRNA delivery [151]. LNPs were later successfully used as platforms for two vaccines loading mRNA against SARS-CoV-2, providing protection against severe COVID-19 disease and playing a crucial role during the pandemic [152]. Due to the highlighted translational potential of LNPs, this section focuses on discussing the main features of LNPs and their application as inhalable therapeutics.

LNPs are essentially composed of four lipids: ionizable cationic lipid, helper lipid, cholesterol, and polyethylene glycol (PEG)-lipid, combined at specific molar ratios. This standard composition ensures a monodisperse system, with increased stability, as well as efficient cell uptake and endosomal escape [153].

The ionizable cationic lipid plays a pivotal role in enabling both payload encapsulation and endosomal escape. The RNA is encapsulated by electrostatic interactions between negatively charged phosphate groups and the tertiary amines that are typically part of the chemical structure of such ionizable lipids. The mechanism is based on the pKa of these lipids, between 6.2 and 6.4, which ensures a positive charge under acidic conditions and a neutral charge at physiological pH. Therefore, preparing LNPs under an acidic pH allows for nucleic acid encapsulation, while the overall particle charge becomes neutral after increasing the pH to a physiological value [153]. This mechanism also explains the endosomal escape. Due to the acidic endosomal environment, the tertiary amine moieties of the ionizable cationic lipids become protonated and thus, interact with the negatively charged endosomal membrane. Consequently, the RNA is released in the cytoplasm to exert its function [154].

Helper lipids are essentially represented by phospholipids, such as 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE). This class of lipids influences the lipid packing of final LNPs, affecting particle stability, cell interaction, and transfection [154–156]. By analyzing the chemical structure of DSPC, for instance, features such as the saturated lipid tails, the melting temperature of approximately 54 °C, and its cylindrical geometry lead to its packing as a lamellar phase. This directly impacts the overall stability of the final LNPs. Comparatively, the conical shape, melting temperature of around 30 °C, and unsaturated tails of DOPE allow for the formation of inverted hexagonal (HII) phases that are likely to interact and disrupt endosomal membranes [156].

Cholesterol mainly modulates the rigidity and fluidity of LNPs. The mechanism explaining these properties lies in the interaction with helper lipids, which drives the lipids to organize themselves into a liquid-ordered phase, balancing both parameters [154]. Additionally, cholesterol impacts the protein corona formation after intravenous administration, therefore interfering with LNPs clearance from the blood circulation and cell uptake [157].

Although PEG-lipids are usually present at a minimum percentage of the total molar ratio in LNPs, they are essential constituents of the carriers. These molecules modify the surface of LNPs by forming a hydrophilic steric layer that is sufficient to prevent particle aggregation. The final formulation presents small particle sizes and monodispersity [154]. Besides the impact on the overall LNP stability over preparation and storage, PEG-lipids also modulate the particles' circulation time in vivo. This feature is mainly due to the stealth properties of PEG-lipids that prevent the binding of complement proteins that would trigger phagocytosis [158]. This class of lipids has also evidenced benefits in successfully overcoming the mucus barrier when administered to the lungs [159]. On the other hand, many reports in the literature have associated multiple administrations of PEG-based formulations with immune responses through the activation of the ABC phenomenon. However, these findings have been related to high payload and repetitive doses and, thus, have not yet demonstrated major impairments in the clinic [158,160].

In the context of pulmonary administration, LNPs have demonstrated their potential in overcoming multiple lung barriers and achieving their target. Zimmermann et al. [161] highlighted the potential of LNPs in ensuring an effective biological response when manufactured for pulmonary administration. They successfully spray-dried siRNA-LNPs that maintained similar physicochemical properties compared with the fresh particles and siRNA integrity. Moreover, the dry powder demonstrated an equivalent performance regarding the gene silencing effect both in vitro and in human lung tissue. A different strategy to ensure the pulmonary delivery of LNPs was reported by Lokugamage et al. [162]. Optimized mRNA-LNPs were nebulized in mice and efficiently transfected pulmonary cells, following a PEG-lipid dose-dependent pattern.

Lipid-based nanoparticles show promise for delivering biologics to the lungs via inhalation. However, there is a lack of studies focusing on encapsulating biologics into LNPs for pulmonary delivery compared to those investigating small molecules or nucleic acids [163]. This discrepancy may be due to variations in physicochemical properties among biologics, such as hydrodynamic size, geometry, surface charge, and molecular weight [164]. Additionally, preparing inhaled biologic therapeutics using LNPs poses risks of vehicle destabilization and cargo degradation. Therefore, a thorough optimization of LNPs loading biologics is necessary for each application.

Some authors successfully designed biologics-LNPs for pulmonary delivery. In a study by Hajos et al.[165], vasoactive intestinal peptide (VIP) was encapsulated into liposomes for nebulization. VIP, known for its bronchodilator and vasodilator effects and the presence of receptors in the airways, has shown efficacy in idiopathic pulmonary artery hypertension. The authors successfully nebulized VIP-liposomes using the Micro Drop Master Jet inhaler, demonstrating sustained VIP release on excised pulmonary arteries from rats. Moreover, VIP-loaded liposomes exhibited prolonged vasorelaxation effects compared to free VIP[165].

In a different approach, the biologics lumacaftor and ivacaftor were encapsulated into nanostructured lipid carriers to evaluate their potential in correcting specific mutations on the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel. The formulation was nebulized to mice, resulting in the successful restoration of CFTR activity and expression. Additionally, the presence of fibrotic tissue in the lungs of treated animals was reduced [166].

In conclusion, LNPs hold promise in revolutionizing the therapeutics of respiratory diseases through pulmonary administration. Their unique composition and capabilities in cargo delivery and endosomal escape make them attractive candidates for further exploration and development in the field.

4.2.2.2. Approved lipids for inhalation. Pharmaceutical research is continuously growing towards the development of novel drug delivery systems that can minimize the patient burden to the greatest extent. Lipid-based excipients (LBEs) play a critical role in this regard and there is an increasing interest in using them in advanced pharmaceutical development. The most famous example is the application of LBEs in the development of mRNA-based vaccines [156]. This group of excipients is functional materials that can provide targeted attributes to the pharmaceutical product. They are biocompatible, low toxic and generally recognized as safe. However, their application in the pharmaceutical industry still encounters difficulties that are associated with the characteristic phase transitions of lipids. Phase transitions such as polymorphism, longitudinal re-arrangements, phase separation, and crystal growth impact the development of manufacturing processes, product performance and stability thereof [167,168]. Although the general safety profile of LBEs is remarkably advantageous, the required data on the path of pulmonary clearance and the in vivo lung safety of each specific lipid-based formulation are the main challenges regarding approved LBEs for pulmonary delivery. Moreover, the complexity of the phase transitions of LBEs is discouraging for their use [167–169]. As yet, only four LBEs are approved by the U.S. Food and Drug Administration (FDA) for application in pulmonary delivery: cholesterol, oleic acid, and the phospholipids, dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC) [170]. DPPC and DSPC constitute the principal components of endogenous lung surfactants. Unlike nonpolar LBEs, such as acylglycerols, which constitute the majority of approved excipients for oral delivery, phospholipids are polar LBEs. They are composed of a glycerol molecule esterified with two fatty acids and one phosphate group, the latter leading to their polar nature. The use of DPPC and DSPC for inhalation has been strongly established through the marketed products Arikayce® [171,172], an amikacin liposomal suspension, and two dry powder inhalation (DPI) products; TOBI®Podhaler® [173], and Inbrija® [169,174]. TOBI®Podhaler® is a tobramycin DPI for suppressive therapy of chronic pulmonary infections due to *Pseudomonas aeruginosa* in cystic fibrosis patients. Inbrija® is a levodopa DPI product for the intermittent treatment of OFF episodes in patients with Parkinson's disease (PD). The other application of phospholipids is in the preparation of co-suspensions formulated as pMDI. Examples are Breztri Aerosphere® and Bvespi Aerosphere®, a dual and triple composition, respectively, as fixed-dose combination (FDC) products for the treatment of COPD [175].

Arikayce®, the only approved liposomal product for inhalation, is composed of the antibiotic amikacin entrapped into liposomes for the treatment of *Mycobacterium avium* complex lung disease. It is administered via nebulization. The development of liposomal products for nebulization is complex, especially in terms of the physical stability of liposomes and entrapped API during aerosolization. This poses a significant challenge in reaching the market. The industry has responded to this by subjecting liposomal suspensions to a drying process (e.g. spray-drying or freeze-drying) to prepare dry powders. Liposomes formulated as dry powders tend to form a multilamellar state that is thermodynamically more stable which in turn increases their aerosolization and long-term stability [176]. However, no product of such kind has reached the market yet. Formulation of dry liposomes requires extensive process control and the use of additives (e.g. carbohydrates) to ensure the retention of API in the liposomes during drying and during in vivo rehydration. As a result, dry powder liposomal formulations typically have API loadings up to 20 %w/w [177].

TOBI®Podhaler® and Inbrija® DPI are composed of solid lipid-microparticles instead of dry liposomes. This helps to improve the API loadings and potential loss of API encapsulation during manufacturing. In both products, phospholipids are used as surface enrichment agents during particle formation via spray-drying. Administration of TOBI®-Podhaler® and Inbrija® is provided through proprietary capsule-based inhalers. TOBI®Podhaler® consists of small porous lipid-microparticles with sponge-like morphology containing Tobramycin for the treatment of bacterial infections in cystic fibrosis patients and is administered as carrier-free DPI. This product was developed under the sound platform Pulmosphere™. This technology involves preparation of a feedstock for spray-drying composed of a sub-micron oil in water emulsion. The oil phase consists of Perfluorooctyl bromide (PFOB), whereas the aqueous phase contains principally DSPC and $CaCl_2$. The API can be incorporated either dissolved or dispersed. During spray-drying, DSPC enriches in the interfaces, followed by evaporation of PFOB leaving pores within the particle which creates the characteristic morphology of Pulmospheres. Inbrija®, also administered as carrier-free DPI, contains Levodopa. Inbrija® consists of large porous lipid-microparticles composed mainly of DPPC. These large particles have shown to circumvent phagocytosis while still being sufficiently deposited in the airways. Manufacturing is carried out through spray-drying of either solutions or suspensions under the ARCUS® technology [178–180]. Breztri® and Bevespi® are based on Aerosphere™ as an aerosol-based co-suspension delivery technology, in which drug crystals are co-suspended with porous phospholipid particles and administered as aerosol [175,181]. Pulmosphere™ and ARCUS® platforms, alongside with other cutting-edge carrier-free DPI technologies, have revolutionized the market of inhalation products. They have shown to significantly improve the aerodynamic performance of conventional DPIs while providing opportunities for local and systemic pulmonary delivery via particle engineering.

4.2.2.3. Polymers. Polymeric nanoparticles have mucoadhesive properties that enhance transepithelial transport [182,183] and impede mucociliary clearance [184] to promote bioavailability. Polymers can be prepared chemically in laboratories or extracted from natural sources such as plants, seaweeds, terrestrial/ marine animals, and fishes [185]. Oceanic animals are widely exploited for the extraction of bioactive peptides and polymers which are used in several therapeutic applications[186]. As mentioned earlier, natural polysaccharides are extensively studied due to their biodegradability, lower toxicity, and ability to blend with a varied range of drug formulations. Furthermore, nano/microparticles prepared from natural polysaccharides such as CS, alginate, hyaluronic acid, carrageenan, cellulose, and agarose have been notably used in pulmonary ailment reduction [187–190]. Polysaccharides can be responsive to particular physiological stimuli, releasing the entrapped therapeutic molecule at the target site and at a suitable time [191,192]. Also, due to their ability to form crosslinked networks and their water swelling capacity, the entrapped drug can be released from the system in a controlled manner when it comes into contact with body fluids [193].

For nanoparticle fabrication from polysaccharides, both bottom-up and top-down methods are applied. Polyelectrolyte polysaccharides that carry surface charges can be made into nanoparticles by ionic gelation (bottom-up method), while for non-polyelectrolyte polysaccharides that do not have any charged groups, top-down methods (including chemical degradation and mechanical approach) are usually applied to break down the bulk structure into nanoscale [194]. As the only positively charged polysaccharide, CS is often applied to prepare CS nanoparticles via electrostatic interactions with polyanions (sodium tripolyphosphate [195], for example), while negatively charged polysaccharides, like alginate, pectin, gum arabic, xanthan gum, etc., can electrostatically complex with polycations (calcium and ferrous, for instance). On the other hand, non-polyelectrolyte polysaccharides, such as cellulose and starch, have highly crystalline structure and thus their

Table 3
Overview of inhaled biologics for respiratory and systemic diseases: clinical stages, indications, and delivery devices.

Biological	Indication	Mode of action	Clinical stage	Device	Identifier
Dornase Alfa (Pulmozyme)	Cystic fibrosis	Recombinant human DNase I enzyme that cleaves extracellular DNA to reduce mucus viscosity in cystic fibrosis.	Approved	Nebulizer	–
Insulin (Afrezza)	Diabetes mellitus (Type 1 and Type 2)	A rapid-acting human insulin powder administered through the lungs for blood sugar control.	Approved	Dry powder inhaler	–
SARS-CoV-2-neutralizing monoclonal antibody (DZIF-10c)	Neutralizing Different SARS-CoV-2 variants	Neutralizes SARS-CoV-2 antibody	Phase 1 and Phase 2	Device not mentioned	NCT04631705
IBIO123	Post-exposure prophylaxis of COVID-19	Neutralising SARS-CoV-2 by targeting several epitopes and triggering antibody-mediated effector mechanisms	Phase 2-completed	Inhalation via an Aerogen Ultra mesh nebulizer	NCT05639166
COVID-19 Specific T Cell derived exosomes (CSTC-Exo)	Treatment of early stage novel coronavirus (NCV) pneumonia.	Possibly a cytokine-mediated inflammatory cell death signaling	Phase 1	Metered dose inhaler	NCT04389385
Alpha 1-Antitrypsin (Kamada-AAT)	Alpha-1-Antitrypsin deficiency	Prevents excess neutrophil elastase accumulation and proteolysis of elastin in the alveoli, reducing the risk of emphysema	Phase 3 – ongoing	Nebulizer	NCT04204252
Ecleralimab (inhaled monoclonal antibody fragment)	Asthma	Binds soluble thymic stromal lymphopoietin, blocking receptor activation and inhibiting inflammatory signaling	Phase 2 – ongoing	Device not mentioned	NCT04410523
Aerosolized Adenovirus Type-5 Vector-based COVID-19 Vaccine (Ad5-nCoV)	Prime-boost Immunization	Encodes the SARS-CoV-2 spike protein	Phase 1 and Phase 2	Device not mentioned	NCT05043259
Aerosol Sargramostim	Treatment of melanoma that has metastasized to the lung	Stimulate the immune system by prompting the bone marrow to produce more white blood cells	Phase 1-ongoing	Nebulizer	NCT05717140
AZD 1402	Asthma	Anti IL-4 Anticalin	Phase 1 and 2 (terminated)	Dry powder nebulizer	NCT03921268 NCT04643158
SNG01	COVID-19 Asthma COPD	Interferon beta1A	Phases 2 and 3	Nebulizer	NCT04732949 NCT04385095 NCT1126177 NCT03570359
ALX0171	Respiratory Syncytival Virus (RSV) infection	Anti RSV Nanobody	Phases 1 and 2	Nebulizer	NCT02979431 and others
VR942	Uncontrolled asthma	Anti IL-13 antibody fragment	Phase 1	Dry powder	NCT02473939
Alteplase	Plastic fibrosis	Tissue-type plasminogen activator	Phase 2	Not reported	NCT02315898
BI767551	COVID-19	SARS-CoV2 neutralizing antibody	Phase 2 and 3 (discontinued)	Nebulizer	NCT04822701 NCT04894474
CSL787	Noncystic-fibrosis bronchiectasisis	Immunoglobulin	Phase 1	Nebulizer	NCT04643587
AP-PA02	Noncystic-fibrosis bronchiectasisis and cystic fibrosis with P. aeruginosa infection	Bacteriophage	Phase 1 and 2	Nebulizer	NCT05616221 NCT04596319
APN01	COVID-19	Recombinant angiotensin converting enzyme 2	Phase 1	Nebulizer	NCT05065645

nanoparticles are commonly prepared by top-down methods, such as chemical hydrolysis (under acidic or enzymatic conditions) to disrupt amorphous domains from semicrystalline granules and generate nanocrystals [196]. Another traditional approach is the mechanical treatment (more commonly applied to cellulose), such as high-pressure homogenization or grinding, to induce the disintegration of bulk structure[197]. To facilitate the break-down of their crystals, the production of starch and cellular nanoparticles is normally associated with various pre-treatments (pre-cutting for cellulose, gelatinization for starch, heating and/or freeze-drying, etc.), which are especially needed for the high-energy consuming mechanical process. For other polysaccharides with a relatively weak crystalline structure, such as dextran, nanoparticles may be prepared by emulsion/solvent evaporation method, while a high-energy process (sonication or high-pressure) is needed to further reduce the particle size down to the nanoscale, otherwise, microspheres are obtained. Given the high water solubility of these polysaccharides, chemical crosslinking is necessary to maintain their nanostructure to avoid complete dissolution in water after evaporation of organic solvent[194].

Since polysaccharides are highly hydrophilic macromolecules with very large molecular weight, high viscosity, and a mix of crystalline and amorphous regions in their structure, it is difficult to fabricate fine nanoparticles with monodispersity. If one aims to design ultra-small and uniform nanoparticles, polysaccharides may not work very well. However, there are a variety of chemical reactions that can modify the structure of polysaccharides, by either reducing their molecular weight or their increasing hydrophobicity. After appropriate modifications, polysaccharides could become functionalized for easy preparation of nanoparticles with high quality. Chemical modifications usually require toxic chemicals or catalysts, which may affect the their final applications [194]. To sum up, charged polysaccharides can be formed into nanoparticles by the ionic gelation method (bottom-up approach) and for neutral polysaccharides, the mechanical and chemical degradation (top-down approach) method is suitable to form nanoscale formulations [194].

CS is generally obtained from biopolymer chitin and is widely used in medicinal applications due to its high bioavailability, mucoadhesiveness, and low toxicity [186,198]. Apart from this, CS oligosaccharides

have also found great applications due to their water-soluble nature. For the delivery of drugs to the lungs, CS is one of the extensively used polymers due to its high mucoadhesive property and cationic nature which enables the nanoparticle to bind to the mucosa. Moreover, CS itself shows certain bactericidal and anti-tubular properties due to the electrostatic interactions and destruction of the bacterial lipopolysaccharide-based membrane which provides an additional advantage over conventional pulmonary drugs. Similarly, other bioactive properties depicted by CS lead to extensive use in pulmonary disease control[133]. CS nanoparticles are produced using different methods including cross-linking ionic gelation, emulsion, and precipitation which have already been reviewed by Rampino et al, [199]. Ionic gelation is one of the most commonly used methods in the preparation of CS nanoparticles due to the low toxicity of the prepared nanoparticles and the high drug encapsulation efficiency [200]. In this method, CS nanoparticles are produced using electrostatic interactions with polyanions such as sodium tripolyphosphate (TPP). However, a high batch-to-batch variability due to the lack of control over electrostatic complexation and the slow production rate are the drawbacks of this method. In a recent study conducted by Greco et al, microfluidics as an efficient mixing technique has proven to be a promising technology to produce CS nanoparticles with narrow size distribution for the delivery of biological macromolecules [5,201]. CS-TPP nanoparticles have been widely studied in the delivery of siRNA. Their ability in entrapment of the siRNA and protection of siRNA to the RNases is reported by several studies [202–204]. siRNA-encapsulated CS nanoparticles were patented by Kjems et al. in 2011 [205] exhibiting enhanced biological activity and therapeutic efficacy against infectious diseases, suitable for nebulization. Likewise, Santra et al [206] patented a method for generating CS nanoparticles as aerosols, enabling targeted delivery of proteins, peptides, and therapeutic drugs.

In a study conducted by Luo et al., the application of the guanidinylated CS carrying siRNA for gene-silencing therapy was explored. They showed the potential of the aerosol approach for future application in delivery of the therapeutic nanocomplexes to the lung [207]. In another study conducted by Shah et al., solid microparticles were achieved through using lactose as the primary carrier of the liquid nanoemulsion based on CS and subjecting the blend to a spray drying process [208].

Trimethyl chitosan (TMC) is one of the most investigated derivatives of CS. It is a biocompatible and biodegradable derivative of CS with three methyl groups on nitrogen which provides positive charges and water-solubility in a wide pH range when the degree of quaternization is higher than 40 % [209,210]. The permanent positive charges ensure unique features such as mucoadhesion [149], reversible opening of tight junctions between epithelial cells[211], and antimicrobial effect via interaction with bacterial cell walls[212]. However, the results of a recent study, investigating the phase behavior of a pulmonary surfactant mimetic in the presence of TMC, anticipated that the neutralization of TMC cationic charges by the pulmonary surfactant will lead to the deactivation of its permeationenhancer capacity[213]. Further studies are needed to evaluate the permeation-enhancing effect of TMC.

Besides natural polymers, synthetic polymers have also been investigated to prepare secondary structures. In a study conducted by Gonsalves et al, the researchers utilized poly(lactic-co-glycolic acid) (PLGA) nanoparticles coated with a biomimetic lung surfactant, Infasurf®. The PLGA served as the core material for encapsulating drugs, while the surfactant coating helped improve compatibility with lung tissue and reduced uptake by alveolar macrophages, thus enhancing retention in the lungs. The formulation allowed for localized and sustained drug delivery, demonstrating potential for treating lung conditions such as cancer [214]. In a recent study conducted by Adams et al., amphiphilic poly(Spermine Acrylamides) was utilized to form micellar structures that efficiently encapsulated and protected siRNA. This material provided enhanced siRNA stability, facilitated cellular uptake, and improved gene silencing efficiency in the lungs [215].

Table 2 provides a list of commonly used excipients for the development of carrier free formulations, while Table 3 highlights recent examples of inhalable biologics, providing detailed information on their therapeutic indications and clinical stages of development.

5. Dry powder manufacturing

The development of inhalable drug formulations involves a variety of preparation technologies, each with its unique advantages, challenges, and clinical relevance (Table 4). Spray drying, a process that transforms a liquid feed into a dry powder by atomization into a hot drying medium, is a widely used and clinically approved method. Freeze drying or lyophilization, another clinically approved technique, involves the dehydration of a frozen product by sublimation and secondary drying. It is particularly suitable for temperature-sensitive drugs and offers long-term stability. On the other hand, some techniques are still under investigation for their potential in preparing inhalable drugs. Spray-freeze drying, a hybrid technique combining the principles of spray drying and freeze drying, can produce highly porous particles with a low density, enhancing aerosol performance. Critical point drying, primarily used in microscopy, involves the use of a fluid above its critical temperature and pressure to avoid surface tension effects. In this chapter, we will delve into each of these techniques, exploring their processes, advantages, challenges, and considerations. We will also discuss their clinical relevance and potential for future applications in the field of inhalable drug formulation.

5.1. Spray drying

5.1.1. Introduction to spray drying

Spray drying is a versatile technique used in various industries, including food processing and pharmaceuticals. In the realm of pharmaceuticals, spray drying is particularly valuable for the production of inhalable drug formulations. This method excels at rapidly producing powders with customized physical properties, ideal for pulmonary delivery. Its primary benefit lies in its ability to precisely control particle size, shape, and other key features. For inhalable drugs, such precision is critical for effective drug deposition in the respiratory tract [222]. Moreover, the ability to readily generate particles in the desired shape and size eliminates the need for post-processing, such as milling. The clinical relevance of spray drying is underscored by several inhalable products formulated through this method. Products like Tobi Podhaler® (tobramycin inhalation powder) and Afrezza® (inhaled insulin) have not only successfully completed clinical trials but have also gained

Table 4
High-level comparison of drying techniques for the production of inhalable drugs. Typically used drying techniques including freeze drying, spray drying, spray freeze drying and critical point drying are compared in terms of throughput, thermal stress on the product, obtained particle size and process costs.

	Freeze drying	Spray drying	Spray-freeze drying	Critical point drying
Throughput	Batch (low)	Continuous & scalable	Batch (low)	Batch (low)
Thermal stress	Low	Short & high	Low	Low
Particle size	Post processing	Inhalable	Inhalable	Inhalable
Process costs	8X	1X	–	12X

regulatory approval for market release [173,223]. This chapter delves into principles and technical aspects of spray drying. It also highlights the role of excipients, which are crucial for handling biologics. We conclude with a focus on biologics, discussing unique considerations and offering insights into the future of spray-dried biologics.

5.1.2. Functioning principle of spray drying

The fundamental principle of spray drying is based on the atomization of a liquid feed into a hot drying medium, typically air. This atomization process breaks the liquid feed into fine droplets. As these droplets come into contact with the hot air, the solvent rapidly evaporates, resulting in the formation of dry particles. Each stage of this process plays a pivotal role in determining the final product's quality and characteristics [224,225].

Atomization: The liquid feed, which contains the drug and any excipients, is atomized into fine droplets using a nozzle or rotary atomizer. The choice of atomization method can significantly influence the size and distribution of the droplets. This stage is crucial as the droplet size directly dictates the size of the final particles, which later affects the drug's deposition in the respiratory tract and its dissolution rate [226,227].

Drying: As the droplets move through the drying chamber, they are exposed to hot air, which leads to rapid solvent evaporation. The temperature and flow rate of the drying medium, along with the droplet size, determine the drying rate. Proper optimization of these parameters is essential to ensure complete drying while preserving the stability of the drug. This stage is especially critical for temperature-sensitive drugs, as even slight deviations can compromise their efficacy and stability [228,229]. Additionally, the selection of process parameters plays a crucial role in particle engineering, i.e., manipulation of the particle's size, shape, density, morphology, surface properties, flowability, and stability.

Particle Recovery: The dried particles are then separated from the drying medium using cyclones or filters. Efficient separation is crucial as it determines the yield and purity of the final product. Problems at this stage may further lead to product contamination, affecting the overall quality and safety of the inhalable formulation [226,230].

5.1.3. Technical aspects of spray drying for pharmaceutical applications

Spray drying, while conceptually straightforward, demands meticulous attention to its technical intricacies. Different types of spray dryers cater to varied needs. Single-stage spray dryers, the most basic form, consist of a singular drying chamber where atomization, drying, and particle recovery transpire in a sequential manner. They are apt for compounds that are stable in the presence of heat. However, for temperature-sensitive biologics, multi-stage spray dryers might be more fitting. These incorporate multiple drying chambers or stages, which allow the feed to undergo partial drying in the initial stages and further drying in subsequent ones. This tiered approach can offer better control over the drying process; however, it comes at the cost of increased process complexity [231]. Another variant, the fluidized bed spray dryer, combines the principles of spray drying with fluidized bed drying. Here, particles that are partially dried in the spray drying phase undergo further drying and conditioning in a fluidized bed[232].

The choice of nozzle is pivotal in determining the characteristics of the atomized droplets across all variants of the spray drying process. Pressure nozzles operate by propelling the liquid feed through an orifice under high pressure. Their ability to handle high-viscosity feeds and their resistance to clogging make them suitable for certain biologic formulations. In contrast, rotary atomizers use a spinning disk to disperse the liquid feed into droplets, with the droplet size being adjustable by tweaking the disk's rotational speed [233].

The operating conditions, encompassing aspects like inlet and outlet temperatures, feed flow rate, and drying medium flow rate, play a decisive role in the spray drying process. These parameters need to be carefully optimized to strike a balance between efficient drying and drug stability [231].

5.1.4. Spray drying of Biologics: Challenges and innovations

Biologics, with their intricate structures and sensitivity, present unique formulation challenges for spray drying [224]. Unlike traditional small molecules, biologics require a more nuanced approach to ensure their stability and efficacy during the spray drying process [222,234]. These large, complex molecules—ranging from proteins and peptides to monoclonal antibodies and RNA-based therapies—are highly susceptible to degradation due to physical stress and environmental factors such as heat, moisture, and shear forces inherent to the spray drying process.

Proteins and peptides are particularly sensitive to the high temperatures and rapid drying rates associated with spray drying. These conditions can lead to protein aggregation, which compromises the biological activity and stability of the final product. The physical stresses during the drying process can also induce conformational changes in the protein structure, leading to denaturation and a loss of therapeutic function. Furthermore, the shear forces during atomization can break fragile protein molecules or cause them to unfold, further reducing efficacy and potentially increasing immunogenicity. Monoclonal antibodies share many of the same vulnerabilities as proteins, but their larger size and more complex structure make them even more prone to aggregation and denaturation. For inhalable formulations of monoclonal antibodies, achieving the right particle size is crucial: particles must be within the 1–5 μm range for effective lung deposition, but maintaining this size without compromising the antibody's stability poses a significant technical challenge. Moreover, monoclonal antibodies require delicate handling to prevent damage during both the atomization and drying phases of the process. Nucleic acid-based drugs, such as siRNA (short double-stranded RNA) and mRNA (long single-stranded RNA), face distinct challenges in spray drying. siRNA, due to its relatively small size and double-stranded structure, is susceptible to shear stress and heat during atomization, which can result in fragmentation or unwinding of the strands. Maintaining the integrity of siRNA during both the spray drying and storage phases is a significant challenge, often requiring the use of encapsulating agents or nanoparticles. Long single-stranded mRNA molecules are even more prone to degradation from both thermal and mechanical stress. Their larger size and single-stranded nature make them highly sensitive to environmental conditions, which can lead to the breakdown of the RNA strands or the formation of secondary structures that reduce their functionality. Formulating stable spray-dried mRNA often requires encapsulation in lipid nanoparticles to protect the molecule from degradation and enhance delivery to target cells.

In addition to the specific challenges associated with different biologic types, several general considerations must be addressed throughout the spray drying process to preserve biologic stability. Solvent selection is one of the most critical factors. Water is the most commonly used solvent, but it may not always provide an ideal environment for biologics, particularly for those requiring specific solubility or stability conditions. Some biologics may require organic solvents or co-solvents for improved solubility or stability. However, the introduction of organic solvents presents challenges, including the potential for denaturation, aggregation, or loss of bioactivity. Choosing the wrong solvent can also lead to phase separation during atomization or drying, which can compromise the product's quality. Technical parameters such as inlet/outlet temperature, feed rate, and atomization conditions are equally crucial to avoiding damage to sensitive biologics. High inlet temperatures can cause thermal degradation, while excessively low outlet temperatures can lead to incomplete drying, resulting in particles that are too moist and unstable during storage. Maintaining the optimal temperature balance is essential to preserving biologic activity. The atomization step, where the liquid formulation is broken into droplets, introduces significant shear stress. This can cause biologics, especially proteins and nucleic acids, to unfold or fragment. Achieving the optimal droplet size is critical, as large droplets may result in incomplete drying,

while overly fine droplets can lead to excessive thermal exposure and molecular degradation. Controlling particle size is crucial for both process efficiency and therapeutic efficacy. For inhalable formulations, the particles must be small enough (typically 1–5 μm) to reach the deep lung, but their size must also be controlled to prevent aggregation or overexposure to heat during drying. Improper size distribution can lead to inconsistent dosing or reduced efficacy of the biologic. The evaporation process must also be carefully managed to avoid irreversible damage. Rapid evaporation can induce local concentration effects, which may cause protein unfolding or RNA degradation. Slow evaporation, on the other hand, can lead to particle agglomeration or excessive moisture content, impacting the long-term stability of the biologic product. The choice of excipients is essential to protect biologics from the stresses of spray drying. Sugars like trehalose and mannitol are commonly used to form protective glassy matrices around proteins or nucleic acids, helping to stabilize them against thermal and mechanical damage. However, the excipients must be carefully selected to avoid interactions that could destabilize the biologic.

A notable example for spray drying of biologics is the ongoing investigation into monoclonal antibodies that target respiratory infections. These antibodies, when formulated into inhalable particles via spray drying, offer a novel treatment modality that could improve the bioavailability and targeting of therapies for respiratory diseases such as influenza, RSV, or even COVID-19 [235–237]. The combination of nanotechnology with spray drying has recently shown promising results for nucleic acid delivery to the lungs: by encapsulating siRNA or mRNA first into lipid or polymeric nanoparticles and subsequently spray drying them, nano-embedded microparticles were obtained that improve the delivery efficacy [161,238,239]. Notably, this strategy has shown the potential to stabilize siRNAs for extended periods, up to 18 months at room temperature, which could be a game-changer for the storage and distribution of RNA-based drugs [240].

While this combination of technologies is promising, further advancements are needed to optimize particle morphology, ensure consistent dosing, and maintain long-term stability during storage. Personal insights from experts with hands-on experience in both nanotechnology and biologics formulation could provide valuable guidance in tackling these challenges. For example, deeper expertise in optimizing lipid composition for RNA encapsulation or fine-tuning spray drying parameters for nanoparticle formulations could significantly accelerate progress in this field.

5.2. Freeze drying

Freeze drying, or lyophilization, is another technique frequently employed in the production of inhalable drug formulations. This process involves the dehydration of a frozen product by sublimation and secondary drying. The freeze-drying process starts with the preparation of a liquid solution or suspension containing the drug and any other formulation components. Once frozen, the product is placed under vacuum, allowing the frozen solvent to sublimate directly from solid to gas. The process concludes with secondary drying, where any remaining bound water molecules are removed [241,242]. Freeze drying is particularly advantageous for the preparation of inhalable drugs as it allows for the production of dry powders from solutions or suspensions without exposure to high temperatures, making it suitable for temperature-sensitive drugs such as proteins or peptides. The resulting dry powder is highly porous, which can enhance the dispersibility of the particles [243–245]. Despite its benefits, freeze drying comes with its own set of challenges. The process is time-consuming and energy-intensive, which can lead to higher production costs. Moreover, the formulation and process parameters need to be carefully optimized to ensure the production of particles with the desired properties. Often, post processing of the lyophilized product is necessary to obtain particles with suitable aerodynamic properties. The choice of excipients, the freezing rate, the primary and secondary drying conditions, and the storage conditions can all significantly impact the characteristics of the final product [246].

5.3. Spray-Freeze drying

Spray-freeze drying is a hybrid technique that combines the principles of spray drying and freeze drying. The process begins with the atomization of a liquid feed into a cryogenic medium, creating frozen particles. These particles are then lyophilized to remove the solvent, resulting in dry powder. This method can produce highly porous particles with a low density, which can enhance the aerosol performance of the inhalable drugs [247]. Moreover, like freeze drying, spray-freeze drying is suitable for temperature-sensitive drugs as it avoids the use of heat. However, spray-freeze drying is a complex, multi-step process that requires careful optimization. The choice of cryogenic medium, the atomization method, and the lyophilization conditions can significantly impact the characteristics of the final product [243,248]. Furthermore, the process is time-consuming and may have substantially higher production costs compared to other drying techniques. Despite its promise, the complexity and higher production costs associated with this method have limited its clinical application thus far, and it remains in the research and development stage.

5.4. Critical point drying

Critical point drying (CPD) is a technique extensively used in microscopy and material sciences for drying delicate samples without causing the structural distortions typically associated with surface tension during air-drying [249]. In CPD, a fluid is heated above its critical temperature and pressure, eliminating the distinction between liquid and gas phases. This approach allows for drying without the surface tension effects of liquid–gas phase transition. The sample is initially saturated with a solvent, usually CO_2, which is then brought to its critical point. Upon gradual depressurization, the CO_2 transitions from supercritical fluid to gas, leaving behind a dry, undistorted sample. CPD is renowned for its ability to preserve the intricate structures of biological specimens and materials at a nanoscale level. Although CPD is predominantly used in microscopy, its potential for creating highly porous particles suggests possible applications in the preparation of inhalable drugs. The ability of CPD to produce porous structures might enhance the dispersibility of drug particles in the lungs. However, applying CPD in pharmaceutical manufacturing, particularly for temperature-sensitive drugs, requires careful exploration. The method's complexity, need for specialized equipment, and considerations for scalability and industrial production are significant factors limiting its current use in drug formulation. Nevertheless, the potential for CPD to contribute to novel drug delivery methods warrants further investigation [250].

6. Perspective

Delivery of biomolecules via the pulmonary route has been gaining interest over the past years. However, despite the increasing number of publications, only a very small number of products have managed to reach the clinical phase or even commercial availability. The reasons for this are manifold and often unclear, as such data are usually not readily available. Nevertheless, in this section, we will discuss several aspects that might currently prevent this therapeutic approach from reaching its full potential.

First of all, it is to be highlighted that the development of aerosol preparations, in general, is highly challenging. This challenge arises from the highly complex interplay of different processes, not only regarding the aerosol and its generation itself, but also its interaction with the complex anatomy and physiology of the human respiratory tract. As mentioned in the section on requirements for therapeutic aerosols, they need to meet certain criteria regarding their physical

properties. Most notably, they need to display a defined aerodynamic particle size that is related to the desired target area. Consequently, aerosol product development usually demands addressing the complexity of combination product development. Moreover, it is mandatory to meet other CMC requirements such as drug product stability, as well as human factor aspects involving applicability, interpatient variability and more. While developing chemically defined active pharmaceutical ingredient (API) products is already very challenging, it becomes even more so when developing biomolecules. These drug substances inherently possess high complexity due to factors such as their molecule size, tendency to self-arrange/rearrange, and other effects originating from associated manufacturing processes. Also, these molecules might show unfavorable toxicology e.g. a certain immunogenic potential that might restrict the use of this application route. Additionally, biomolecules are often sensitive to physical and chemical stresses, such as shear forces during aerosolization, temperature fluctuations, and exposure to moisture, all of which can lead to degradation and loss of therapeutic efficacy. This adds another layer of complexity to both the formulation and delivery of such therapies. To address these challenges, scientists often rely on specialized excipients designed to stabilize biomolecules during aerosolization. However, the choice and use of excipients for pulmonary delivery pose additional difficulties that can hinder the development of these therapies.

Technologies involving biologics, and therefore often secondary particles, frequently require excipients/constituents with very specific properties. This could either involve adding new functionality, such as specific surface charge (e.g., in the case of polymeric particles), or preventing physico-chemical instability, such as undesired phase transitions in the case of lipid excipients. Yet, one major challenge is the relatively limited number of excipients approved for pulmonary delivery.

Introducing new excipients is not only scientifically complex but also financially risky. Large pharmaceutical companies will typically prioritize quick market access for their pipeline molecules and strive to design lean and robust development programs, as the cost and time associated with getting a new excipient approved may not be justified unless there is a clear pathway to market. This is largely due to the extensive toxicological testing required, which can be a significant investment, especially for a compound that might not even be used in a final drug product. This approach might lead them to opt for the use of already approved excipients, different formulation technologies or routes of application. Smaller companies that specialize in excipient development face an even more daunting business case: investing heavily in regulatory approval for an excipient without the guarantee of its inclusion in a drug product is often not financially viable. Since excipients are rarely approved independently of a drug product, many small companies may hesitate to pursue this route unless they have secured partnerships or contracts with larger pharmaceutical firms that can absorb these costs. As a result, many opt for safer, more established excipients that might lack the desired functionalities but reduce regulatory and financial risks. Thus, one of the future challenges for the field is to create an innovation ecosystem that encourages the development of novel excipients, with clearer regulatory frameworks and potentially shared risks between smaller innovators, larger pharmaceutical firms, venture capital and governmental institutions. While university-based research labs may provide an ideal environment for their synthesis and technical testing, they often lack the capacity to conduct extensive toxicological studies. Consequently, the environment for developing novel excipients is more likely to be found with excipient manufacturers themselves or smaller companies originating from university environments that specialize in excipient development, such as start-ups or consultancies. In the coming years, fostering collaborations between academic institutions, smaller excipient-focused companies, and larger pharmaceutical firms will be key to overcoming these financial and regulatory hurdles.

Similar considerations arise when discussing the observation that, to a certain extent, academic research associated with biomolecules is focused solely on the development of secondary particles. This leaves a gap between functional micro- or nano-particles and an inhalable formulation or combination product that demonstrates suitability for effective delivery to the lower respiratory tract. This may be attributed to the unique skill set required for research or development in such systems. Proficiency in biomedical research and adjacent pharmaceutical technological methodology (i.e., preparation of MPs, NPs), cell culture, animal experiments, manufacturing methods for primary particles, aerosol physics, human physiology, biorelevant in vitro/in silico models, and engineering are all essential. Academic research groups often specialize in either end of this spectrum, highlighting the paramount importance of collaborative research. In vivo testing in a preclinical setting is practically limited to rodents which naturally have very different airway physiology from humans. Therefore, testing with the intended (or preliminary) drug-device combination is not possible. Substitute methods, such as instillation, insufflation, or aerosol chambers, are available and allow for testing of secondary particles, but they have certain limitations. To trace primary particle deposition in the patient, suitable direct methods like gamma-scintigraphy are available. However, these studies are typically associated with clinical trial settings and are usually accessible only to companies with respective development programs. Due to these limitations, in silico modelling of airway deposition and biorelevant in vitro experiments (e.g. using oropharyngeal models) have a high relevance in answering different questions. Further advancements in these fields might help improve the quality of dose estimations and provide more confidence when entering clinical testing, thereby fostering further development.

CRediT authorship contribution statement

Kai Berkenfeld: Writing – review & editing, Writing – original draft, Conceptualization. **Simone Carneiro:** Writing – review & editing, Writing – original draft, Conceptualization. **Carolina Corzo:** Writing – review & editing, Writing – original draft, Conceptualization. **Flavia Laffleur:** Writing – review & editing, Writing – original draft. **Sharareh Salar-Behzadi:** Writing – review & editing, Writing – original draft, Conceptualization. **Benjamin Winkeljann:** Writing – review & editing, Writing – original draft, Conceptualization. **Golbarg Esfahani:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [At the time of submission, Kai Berkenfeld is an employee of Boehringer Ingelheim Pharma GmbH & Co. KG].

Data availability

No data was used for the research described in the article.

References

[1] FDA, (n.d.). https://www.fda.gov/about-fda/center-biologics-evaluation-and-research-cber/what-are-biologics-questions-and-answers#:~:text=Biologics can be composed of, such as cells and tissues.
[2] K. Fosgerau, T. Hoffmann, Peptide therapeutics: current status and future directions, Drug Discov. Today. 20 (2015) 122–128.
[3] B. Leader, Q.J. Baca, D.E. Golan, Protein therapeutics: a summary and pharmacological classification, Nat. Rev. Drug Discov. 7 (2008) 21–39.
[4] A.C. Anselmo, Y. Gokarn, S. Mitragotri, Non-invasive delivery strategies for biologics, Nat. Rev. Drug Discov. 18 (2019) 19–40.
[5] A. Greco, B. Gabold, S. Chen, X. Wang, Z. Xu, A. Hartschuh, E. Chiesa, I. Genta, C. L. Ried, T. Merdan, Microfluidic mixing as platform technology for production of chitosan nanoparticles loaded with different macromolecules, Eur. J. Pharm. Biopharm. 188 (2023) 170–181.
[6] R. Uchenna Agu, M. Ikechukwu Ugwoke, M. Armand, R. Kinget, N. Verbeke, The lung as a route for systemic delivery of therapeutic proteins and peptides, Respir. Res. 2 (2001) 1–12.

[7] G.M. Pacifici, M. Franchi, C. Bencini, F. Repetti, N. Di Lascio, G.B. Muraro, Tissue distribution of drug-metabolizing enzymes in humans, Xenobiotica 18 (1988) 849–856.
[8] G.I. Somers, N. Lindsay, B.M. Lowdon, A.E. Jones, C. Freathy, S. Ho, A.J. M. Woodrooffe, M.K. Bayliss, G.R. Manchee, A comparison of the expression and metabolizing activities of phase I and II enzymes in freshly isolated human lung parenchymal cells and cryopreserved human hepatocytes, Drug Metab. Dispos. 35 (2007) 1797–1805.
[9] S.W. Stein, C.G. Thiel, The history of therapeutic aerosols: a chronological review, J. Aerosol Med. Pulm. Drug Deliv. 30 (2017) 20–41.
[10] United Nations Treaty Collection, (n.d.). https://treaties.un.org/Pages/ViewDetails.aspx?src=IND&mtdsg_no=XXVII-2-f&chapter=27&clang=_en.
[11] P.S. Hasleton, The internal surface area of the adult human lung, J. Anat. 112 (1972) 391.
[12] Q. Fei, I. Bentley, S.N. Ghadiali, J.A. Englert, Pulmonary drug delivery for acute respiratory distress syndrome, Pulm. Pharmacol. Ther. (2023) 102196.
[13] A.J. Plaunt, T.L. Nguyen, M.R. Corboz, V.S. Malinin, D.C. Cipolla, Strategies to overcome biological barriers associated with pulmonary drug delivery, Pharmaceutics. 14 (2022) 302.
[14] G. Moeller, M. Bieber, S. Gürzing, A.L. Thiebes, S. Klein, C.G. Cornelissen, M. A. Reddemann, Transparent 23-generation airway model for experimental investigation of aerosol flow and deposition within the human respiratory tract, J. Aerosol Sci. 156 (2021) 105782.
[15] A.K. Thakur, D.K. Chellappan, K. Dua, M. Mehta, S. Satija, I. Singh, Patented therapeutic drug delivery strategies for targeting pulmonary diseases, Expert Opin. Ther. Pat. 30 (2020) 375–387.
[16] D.A. Groneberg, C. Witt, U. Wagner, K.F. Chung, A. Fischer, Fundamentals of pulmonary drug delivery, Respir. Med. 97 (2003) 382–387.
[17] R. Pangeni, T. Meng, S. Poudel, D. Sharma, H. Hutsell, J. Ma, B.K. Rubin, W. Longest, M. Hindle, Q. Xu, Airway mucus in pulmonary diseases: Muco-adhesive and muco-penetrating particles to overcome the airway mucus barriers, Int. J. Pharm. (2023) 122661.
[18] R. Pereira, T. Barbosa, A.L. Cardoso, R. Sá, M. Sousa, Cystic fibrosis and primary ciliary dyskinesia: Similarities and differences, Respir. Med. (2023).
[19] S. He, J. Gui, K. Xiong, M. Chen, H. Gao, Y. Fu, A roadmap to pulmonary delivery strategies for the treatment of infectious lung diseases, J. Nanobiotechnology. 20 (2022) 101.
[20] S. Ehrmann, O. Schmid, C. Darquenne, B. Rothen-Rutishauser, J. Sznitman, L. Yang, H. Barosova, L. Vecellio, J. Mitchell, N. Heuze-Vourc'h, Innovative preclinical models for pulmonary drug delivery research, Expert Opin. Drug Deliv. 17 (2020) 463–478.
[21] Y. He, Y. Liang, R. Han, W.-L. Lu, J.C.W. Mak, Y. Zheng, Rational particle design to overcome pulmonary barriers for obstructive lung diseases therapy, J. Control. Release. 314 (2019) 48–61.
[22] J.M. Borghardt, C. Kloft, A. Sharma, Inhaled therapy in respiratory disease: the complex interplay of pulmonary kinetic processes, Can. Respir. J. 2018 (2018).
[23] M. Ghadiri, P.M. Young, D. Traini, Strategies to enhance drug absorption via nasal and pulmonary routes, Pharmaceutics. 11 (2019) 113.
[24] C. Darquenne, Deposition mechanisms, J. Aerosol Med. Pulm. Drug Deliv. 33 (2020) 181–185.
[25] V.A. Marple, D.L. Roberts, F.J. Romay, N.C. Miller, K.G. Truman, M. Van Oort, B. Olsson, M.J. Holroyd, J.P. Mitchell, D. Hochrainer, Next generation pharmaceutical impactor (a new impactor for pharmaceutical inhaler testing), Part i: Design, J. Aerosol Med. 16 (2003) 283–299.
[26] X.M. Zeng, G.P. Martin, C. Marriott, Medicinal aerosols, Part. Interact. Dry Powder Formul. Inhal. New York, NY Taylor Fr. (2001) 66–91.
[27] I. Gonda, Targeting by deposition, Pharm. Inhal. Aerosol Technol. New York, NY Marcel Dekker. (2003) 65–88.
[28] T.C. Carvalho, J.I. Peters, R.O. Williams III, Influence of particle size on regional lung deposition–what evidence is there? Int. J. Pharm. 406 (2011) 1–10.
[29] S. Gharse, J. Fiegel, Large porous hollow particles: lightweight champions of pulmonary drug delivery, Curr. Pharm. Des. 22 (2016) 2463–2469.
[30] Y.-J. Son, P.W. Longest, G. Tian, M. Hindle, Evaluation and modification of commercial dry powder inhalers for the aerosolization of a submicrometer excipient enhanced growth (EEG) formulation, Eur. J. Pharm. Sci. 49 (2013) 390–399.
[31] M. Motiei, O. Mišík, T.H. Truong, F. Lizal, P. Humpolíček, V. Sedlařík, P. Sáha, Engineering of inhalable nano-in-microparticles for co-delivery of small molecules and miRNAs, Discov. Nano. 18 (2023) 38.
[32] S.G. Carvalho, A.M. Dos Santos, A.L. Polli Silvestre, A.G. Tavares, M. Chorilli, M. p. Daflon Gremião, Multifunctional systems based on nano-in-microparticles as strategies for drug delivery: advances, challenges, and future perspectives, Expert Opin. Drug Deliv. 20 (2023) 1231–1249.
[33] P. Muralidharan, M. Malapit, E. Mallory, D. Hayes Jr, H.M. Mansour, Inhalable nanoparticulate powders for respiratory delivery, Nanomedicine Nanotechnology, Biol. Med. 11 (2015) 1189–1199.
[34] T. Ku, S. Gill, R. Löbenberg, S. Azarmi, W. Roa, E.J. Prenner, Size dependent interactions of nanoparticles with lung surfactant model systems and the significant impact on surface potential, J. Nanosci. Nanotechnol. 8 (2008) 2971–2978.
[35] R.P. Valle, C.L. Huang, J.S.C. Loo, Y.Y. Zuo, Increasing hydrophobicity of nanoparticles intensifies lung surfactant film inhibition and particle retention, ACS Sustain. Chem. Eng. 2 (2014) 1574–1580.
[36] M.-C. Jones, S.A. Jones, Y. Riffo-Vasquez, D. Spina, E. Hoffman, A. Morgan, A. Patel, C. Page, B. Forbes, L.A. Dailey, Quantitative assessment of nanoparticle surface hydrophobicity and its influence on pulmonary biocompatibility, J. Control. Release. 183 (2014) 94–104.
[37] Q. Liu, J. Guan, R. Song, X. Zhang, S. Mao, Physicochemical properties of nanoparticles affecting their fate and the physiological function of pulmonary surfactants, Acta Biomater. 140 (2022) 76–87.
[38] S.I. Hossain, Z. Luo, E. Deplazes, S.C. Saha, Shape matters—the interaction of gold nanoparticles with model lung surfactant monolayers, J. r. Soc. Interface. 18 (2021) 20210402.
[39] Z. Luo, S. Li, Y. Xu, Z. Yan, F. Huang, T. Yue, The role of nanoparticle shape in translocation across the pulmonary surfactant layer revealed by molecular dynamics simulations, Environ. Sci Nano. 5 (2018) 1921–1932.
[40] B.S. Schuster, J.S. Suk, G.F. Woodworth, J. Hanes, Nanoparticle diffusion in respiratory mucus from humans without lung disease, Biomaterials 34 (2013) 3439–3446.
[41] C.S. Schneider, Q. Xu, N.J. Boylan, J. Chisholm, B.C. Tang, B.S. Schuster, A. Henning, L.M. Ensign, E. Lee, P. Adstamongkonkul, Nanoparticles that do not adhere to mucus provide uniform and long-lasting drug delivery to airways following inhalation, Sci. Adv. 3 (2017) e1601556.
[42] S.K. Lai, Y.-Y. Wang, J. Hanes, Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues, Adv. Drug Deliv. Rev. 61 (2009) 158–171.
[43] H. Yamamoto, Y. Kuno, S. Sugimoto, H. Takeuchi, Y. Kawashima, Surface-modified PLGA nanosphere with chitosan improved pulmonary delivery of calcitonin by mucoadhesion and opening of the intercellular tight junctions, J. Control. Release. 102 (2005) 373–381.
[44] M. Zaru, M.-L. Manca, A.M. Fadda, S.G. Antimisiaris, Chitosan-coated liposomes for delivery to lungs by nebulisation, Colloids Surfaces B Biointerfaces. 71 (2009) 88–95.
[45] V. Kucukoglu, H. Uzuner, H. Kenar, A. Karadenizli, In vitro antibacterial activity of ciprofloxacin loaded chitosan microparticles and their effects on human lung epithelial cells, Int. J. Pharm. 569 (2019) 118578.
[46] A. Dey, J. Stenberg, P. Dandekar, R. Jain, A combinatorial study of experimental analysis and mathematical modeling: How do chitosan nanoparticles deliver therapeutics into cells? Carbohydr. Polym. 229 (2020) 115437.
[47] A.E. Caprifico, E. Polycarpou, P.J.S. Foot, G. Calabrese, Biomedical and pharmacological uses of fluorescein isothiocyanate chitosan-based nanocarriers, Macromol. Biosci. 21 (2021) 2000312.
[48] W. Zhang, W. Xu, Y. Lan, X. He, K. Liu, Y. Liang, Antitumor effect of hyaluronic-acid-modified chitosan nanoparticles loaded with siRNA for targeted therapy for non-small cell lung cancer, Int. J. Nanomedicine. (2019).
[49] P. Paul, S. Sengupta, B. Mukherjee, T.K. Shaw, R.H. Gaonkar, M.C. Debnath, Chitosan-coated nanoparticles enhanced lung pharmacokinetic profile of voriconazole upon pulmonary delivery in mice, Nanomedicine 13 (2018) 501–520.
[50] A.P.B. Almeida, G.B.R. Damaceno, A.F. Carneiro, A. Bohr, H.R. Goncalves, M. C. Valadares, T.L. Nascimento, E.M. Lima, Mucopenetrating lipoplexes modified with PEG and hyaluronic acid for CD44-targeted local siRNA delivery to the lungs, J. Biomater. Appl. 34 (2019) 617–630.
[51] J. Leal, X. Peng, X. Liu, D. Arasappan, D.C. Wylie, S.H. Schwartz, J.J. Fullmer, B. C. McWilliams, H.D.C. Smyth, D. Ghosh, Peptides as surface coatings of nanoparticles that penetrate human cystic fibrosis sputum and uniformly distribute in vivo following pulmonary delivery, J. Control. Release. 322 (2020) 457–469.
[52] G.R. Ediriweera, N.J. Butcher, A. Kothapalli, J. Zhao, J.T. Blanchfield, C. N. Subasic, J.L. Grace, C. Fu, X. Tan, J.F. Quinn, Lipid sulfoxide polymers as potential inhalable drug delivery platforms with differential albumin binding affinity, Biomater. Sci. 12 (2024) 2978–2992.
[53] I.A. Khalil, K. Kogure, H. Akita, H. Harashima, Uptake pathways and subsequent intracellular trafficking in nonviral gene delivery, Pharmacol. Rev. 58 (2006) 32–45.
[54] T. Lebhardt, S. Roesler, M. Beck-Broichsitter, T. Kissel, Polymeric nanocarriers for drug delivery to the lung, J. Drug Deliv. Sci. Technol. 20 (2010) 171–180.
[55] A.J. Thorley, P. Ruenraroengsak, T.E. Potter, T.D. Tetley, Critical determinants of uptake and translocation of nanoparticles by the human pulmonary alveolar epithelium, ACS Nano 8 (2014) 11778–11789.
[56] J.-K.-W. Lam, W. Liang, H.-K. Chan, Pulmonary delivery of therapeutic siRNA, Adv. Drug Deliv. Rev. 64 (2012) 1–15.
[57] H. Kim, H.T. Park, Y.M. Tae, W.H. Kong, D.K. Sung, B.W. Hwang, K.S. Kim, Y. K. Kim, S.K. Hahn, Bioimaging and pulmonary applications of self-assembled Flt1 peptide–hyaluronic acid conjugate nanoparticles, Biomaterials 34 (2013) 8478–8490.
[58] P. Detampel, A. Ganguly, S. Tehranian, F. Green, S. Singha, P. Santamaria, A. A. Jeje, C.S. Cho, B. Petri, M.W. Amrein, In vivo clearance of nanoparticles by transcytosis across alveolar epithelial cells, PLoS One 14 (2019) e0223339.
[59] T. Jung, W. Kamm, A. Breitenbach, E. Kaiserling, J.X. Xiao, T. Kissel, Biodegradable nanoparticles for oral delivery of peptides: is there a role for polymers to affect mucosal uptake? Eur. J. Pharm. Biopharm. 50 (2000) 147–160.
[60] N. Marasini, S. Haque, L.M. Kaminskas, Polymer-drug conjugates as inhalable drug delivery systems: A review, Curr. Opin. Colloid Interface Sci. 31 (2017) 18–29.
[61] W. Wang, Advanced protein formulations, Protein Sci. 24 (2015) 1031–1039.
[62] A. Szlachcic, M. Zakrzewska, J. Otlewski, Longer action means better drug: tuning up protein therapeutics, Biotechnol. Adv. 29 (2011) 436–441.
[63] R.J. Solá, K.A.I. Griebenow, Effects of glycosylation on the stability of protein pharmaceuticals, J. Pharm. Sci. 98 (2009) 1223–1245.

[64] B. Li, A. Chen, S. Zou, J. Wu, H. Wang, R. Chen, M. Luo, Albumin fusion improves the pharmacokinetics and in vivo antitumor efficacy of canine interferon gamma, Int. J. Pharm. 558 (2019) 404–412.
[65] H. Watanabe, M. Miyahisa, M. Chikamatsu, K. Nishida, Y. Minayoshi, M. Takano, S. Ichimizu, Y. Kobashigawa, H. Morioka, H. Maeda, Development of a long acting FGF21 analogue-albumin fusion protein and its anti-diabetic effects, J. Control. Release. 324 (2020) 522–531.
[66] W.-F. Dou, J.-Y. Lei, L.-F. Zhang, Z.-H. Xu, Y. Chen, J. Jin, Expression, purification, and characterization of recombinant human serum albumin fusion protein with two human glucagon-like peptide-1 mutants in Pichia pastoris, Protein Expr. Purif. 61 (2008) 45–49.
[67] K. Berkenfeld, A. Lamprecht, J.T. McConville, Devices for dry powder drug delivery to the lung, AAPS PharmSciTech 16 (2015) 479–490.
[68] S. Mossadeq, R. Shah, V. Shah, M. Bagul, Formulation, device, and clinical factors influencing the targeted delivery of COVID-19 vaccines to the lungs, AAPS PharmSciTech 24 (2022) 2.
[69] M. Hoppentocht, P. Hagedoorn, H.W. Frijlink, A.H. De Boer, Technological and practical challenges of dry powder inhalers and formulations, Adv. Drug Deliv. Rev. 75 (2014) 18–31.
[70] A.R. Clark, J.G. Weers, R. Dhand, The confusing world of dry powder inhalers: it is all about inspiratory pressures, not inspiratory flow rates, J. Aerosol Med. Pulm. Drug Deliv. 33 (2020) 1–11.
[71] A.R. Martin, W.H. Finlay, Nebulizers for drug delivery to the lungs, Expert Opin. Drug Deliv. 12 (2015) 889–900.
[72] R. Respaud, L. Vecellio, P. Diot, N. Heuzé-Vourc'h, Nebulization as a delivery method for mAbs in respiratory diseases, Expert Opin. Drug Deliv. 12 (2015) 1027–1039.
[73] W. Longest, B. Spence, M. Hindle, Devices for improved delivery of nebulized pharmaceutical aerosols to the lungs, J. Aerosol Med. Pulm. Drug Deliv. 32 (2019) 317–339.
[74] A. Benassi, I. Perazzi, R. Bosi, C. Cottini, R. Bettini, Quantifying the loading capacity of a carrier-based DPI formulation and its dependence on the blending process, Powder Technol. 356 (2019) 607–617.
[75] Á. Farkas, A. Horváth, A. Kerekes, A. Nagy, S. Kugler, L. Tamási, G. Tomisa, Effect of delayed pMDI actuation on the lung deposition of a fixed-dose combination aerosol drug, Int. J. Pharm. 547 (2018) 480–488.
[76] R. Groß, K. Berkenfeld, C. Schulte, A. Ebert, S. Sule, A. Sule, A. Lamprecht, State of the art in capsule-based dry powder inhalers: deagglomeration techniques and the consequences for formulation aerosolization, Pharmaceutics. 14 (2022) 1185.
[77] N. Wauthoz, I. Hennia, S. Ecenarro, K. Amighi, Impact of capsule type on aerodynamic performance of inhalation products: A case study using a formoterol-lactose binary or ternary blend, Int. J. Pharm. 553 (2018) 47–56.
[78] J. Shur, S. Lee, W. Adams, R. Lionberger, J. Tibbatts, R. Price, Effect of device design on the in vitro performance and comparability for capsule-based dry powder inhalers, AAPS J. 14 (2012) 667–676.
[79] J.G.Y. Chan, J. Wong, Q.T. Zhou, S.S.Y. Leung, H.-K. Chan, Advances in device and formulation technologies for pulmonary drug delivery, AAPS PharmSciTech 15 (2014) 882–897.
[80] J.N. Pritchard, R.H.M. Hatley, J. Denyer, D. von Hollen, Mesh nebulizers have become the first choice for new nebulized pharmaceutical drug developments, Ther. Deliv. 9 (2018) 121–136.
[81] G. Adorni, G. Seifert, F. Buttini, G. Colombo, L.A. Stecanella, I. Krämer, A. Rossi, Aerosolization performance of jet nebulizers and biopharmaceutical aspects, Pharmaceutics. 11 (2019) 406.
[82] K.H. Chang, S.-H. Moon, S.K. Yoo, B.J. Park, K.C. Nam, Aerosol delivery of dornase alfa generated by jet and mesh nebulizers, Pharmaceutics. 12 (2020) 721.
[83] R.M. DiBlasi, Clinical controversies in aerosol therapy for infants and children, Respir. Care. 60 (2015) 894–916.
[84] K. Bass, D. Farkas, A. Hassan, S. Bonasera, M. Hindle, P.W. Longest, High-efficiency dry powder aerosol delivery to children: Review and application of new technologies, J. Aerosol Sci. 153 (2021) 105692.
[85] S.P. Hertel, G. Winter, W. Friess, Protein stability in pulmonary drug delivery via nebulization, Adv. Drug Deliv. Rev. 93 (2015), https://doi.org/10.1016/j.addr.2014.10.003.
[86] FDA, (n.d.). https://www.fda.gov/combination-products/about-combination-products/combination-product-definition-combination-product-types (accessed October 6, 2024).
[87] USA Food and Drug Administartion, (n.d.). https://www.fda.gov/regulatory-information/search-fda-guidance-documents/principles-premarket-pathways-combination-products (accessed October 6, 2024).
[88] R.Y.K. Chang, H.-K. Chan, Advancements in particle engineering for inhalation delivery of small molecules and biotherapeutics, Pharm. Res. 39 (2022) 3047–3061.
[89] T. Ito, E. Yamazoe, K. Tahara, Dry powder inhalers for proteins using cryo-milled electrospun polyvinyl alcohol nanofiber mats, Molecules 27 (2022) 5158.
[90] P. Mehta, Dry powder inhalers: a focus on advancements in novel drug delivery systems, J. Drug Deliv. 2016 (2016).
[91] EMA, GUIDELINE ON THE PHARMACEUTICAL QUALITY OF INHALATION AND NASAL PRODUCTS, (n.d.). https://www.ema.europa.eu/en/documents/assessment-report/ozurdex-epar-public-assessment-report_en.pdf (accessed April 10, 2023).
[92] FDA, (n.d.). https://www.accessdata.fda.gov/drugsatfda_docs/label/2006/018869s014lbl.pdf.
[93] M.M.A. Elsayed, Microstructural characterization of carrier-based dry powder inhalation excipients: Insights and guidance, Int. J. Pharm. 568 (2019) 118482.
[94] E. Benke, Á. Farkas, P. Szabó-Révész, R. Ambrus, Development of an innovative, carrier-based dry powder inhalation formulation containing spray-dried meloxicam potassium to improve the in vitro and in silico aerodynamic properties, Pharmaceutics. 12 (2020) 535.
[95] G.A. Hebbink, M. Jaspers, H.J.W. Peters, B.H.J. Dickhoff, Recent developments in lactose blend formulations for carrier-based dry powder inhalation, Adv. Drug Deliv. Rev. 114527 (2022).
[96] A.H. de Boer, P. Hagedoorn, M. Hoppentocht, F. Buttini, F. Grasmeijer, H. W. Frijlink, Dry powder inhalation: past, present and future, Expert Opin. Drug Deliv. 14 (2017) 499–512.
[97] H. Kinnunen, G. Hebbink, H. Peters, J. Shur, R. Price, An investigation into the effect of fine lactose particles on the fluidization behaviour and aerosolization performance of carrier-based dry powder inhaler formulations, AAPS PharmSciTech 15 (2014) 898–909.
[98] H. Steckel, N. Bolzen, Alternative sugars as potential carriers for dry powder inhalations, Int. J. Pharm. 270 (2004) 297–306.
[99] R. Mahar, A. Chakraborty, N. Nainwal, The influence of carrier type, physical characteristics, and blending techniques on the performance of dry powder inhalers, J. Drug Deliv Sci. Technol. (2022) 103759.
[100] N. Hertel, G. Birk, R. Scherließ, Particle engineered mannitol for carrier-based inhalation–A serious alternative? Int. J. Pharm. 577 (2020) 118901.
[101] W. Kaialy, T. Hussain, A. Alhalaweh, A. Nokhodchi, Towards a more desirable dry powder inhaler formulation: large spray-dried mannitol microspheres outperform small microspheres, Pharm. Res. 31 (2014) 60–76.
[102] O. Abiona, D. Wyatt, J. Koner, A. Mohammed, The optimisation of carrier selection in dry powder inhaler formulation and the role of surface energetics, Biomedicines. 10 (2022) 2707.
[103] X.M. Zeng, G.P. Martin, S.-K. Tee, C. Marriott, The role of fine particle lactose on the dispersion and deaggregation of salbutamol sulphate in an air stream in vitro, Int. J. Pharm. 176 (1998) 99–110.
[104] V.N.P. Le, T.H.H. Thi, E. Robins, M.P. Flament, Dry powder inhalers: study of the parameters influencing adhesion and dispersion of fluticasone propionate, AAPS PharmSciTech 13 (2012) 477–484.
[105] X.M. Zeng, G.P. Martin, S.-K. Tee, A.A. Ghoush, C. Marriott, Effects of particle size and adding sequence of fine lactose on the deposition of salbutamol sulphate from a dry powder formulation, Int. J. Pharm. 182 (1999) 133–144.
[106] S.C. Das, I.G. Tucker, P.J. Stewart, Surface energy determined by inverse gas chromatography as a tool to investigate particulate interactions in dry powder inhalers, Curr. Pharm. Des. 21 (2015) 3932–3944.
[107] H. Kinnunen, G. Hebbink, H. Peters, D. Huck, L. Makein, R. Price, Extrinsic lactose fines improve dry powder inhaler formulation performance of a cohesive batch of budesonide via agglomerate formation and consequential co-deposition, Int. J. Pharm. 478 (2015) 53–59.
[108] M.D. Louey, P.J. Stewart, Particle interactions involved in aerosol dispersion of ternary interactive mixtures, Pharm. Res. 19 (2002) 1524–1531.
[109] P. Lucas, K. Anderson, J.N. Staniforth, Protein deposition from dry powder inhalers: fine particle multiplets as performance modifiers, Pharm. Res. 15 (1998) 562–569.
[110] M.D. Jones, H. Harris, J.C. Hooton, J. Shur, G.S. King, C.A. Mathoulin, K. Nichol, T.L. Smith, M.L. Dawson, A.R. Ferrie, An investigation into the relationship between carrier-based dry powder inhalation performance and formulation cohesive–adhesive force balances, Eur. J. Pharm. Biopharm. 69 (2008) 496–507.
[111] M.D. Jones, R. Price, The influence of fine excipient particles on the performance of carrier-based dry powder inhalation formulations, Pharm. Res. 23 (2006) 1665–1674.
[112] J. Shur, R. Price, D. Lewis, P.M. Young, G. Woollam, D. Singh, S. Edge, From single excipients to dual excipient platforms in dry powder inhaler products, Int. J. Pharm. 514 (2016) 374–383.
[113] K. Thalberg, L. Ivarsson, M. Svensson, P. Elfman, A. Ohlsson, J. Stuckel, A.-M. Lyberg, The effect of mixing on the dispersibility of adhesive mixtures for inhalation. Comparison of high shear and Turbula mixers, Eur. J. Pharm. Sci. 193 (2024) 106679.
[114] Q.T. Zhou, L. Qu, I. Larson, P.J. Stewart, D.A.V. Morton, Improving aerosolization of drug powders by reducing powder intrinsic cohesion via a mechanical dry coating approach, Int. J. Pharm. 394 (2010) 50–59.
[115] Y. Ye, Y. Ma, J. Zhu, The future of dry powder inhaled therapy: Promising or discouraging for systemic disorders? Int. J. Pharm. 614 (2022) 121457.
[116] N. Hertel, G. Birk, R. Scherließ, Performance tuning of particle engineered mannitol in dry powder inhalation formulations, Int. J. Pharm. 586 (2020) 119592.
[117] J.G. Weers, J. Bell, H.-K. Chan, D. Cipolla, C. Dunbar, A.J. Hickey, I.J. Smith, Pulmonary formulations: what remains to be done?, J. Aerosol Med. Pulm, Drug Deliv. 23 (2010) S-5.
[118] Q.T. Zhou, D.A.V. Morton, Drug–lactose binding aspects in adhesive mixtures: Controlling performance in dry powder inhaler formulations by altering lactose carrier surfaces, Adv. Drug Deliv. Rev. 64 (2012) 275–284.
[119] P. Begat, D.A.V. Morton, J. Shur, P. Kippax, J.N. Staniforth, R. Price, The role of force control agents in high-dose dry powder inhaler formulations, J. Pharm. Sci. 98 (2009) 2770–2783.
[120] D. Traini, S. Scalia, H. Adi, E. Marangoni, P.M. Young, Polymer coating of carrier excipients modify aerosol performance of adhered drugs used in dry powder inhalation therapy, Int. J. Pharm. 438 (2012) 150–159.
[121] P. Mehta, Imagine the superiority of dry powder inhalers from carrier engineering, J. Drug Deliv. 2018 (2018).
[122] A.H.L. Chow, H.H.Y. Tong, P. Chattopadhyay, B.Y. Shekunov, Particle engineering for pulmonary drug delivery, Pharm. Res. 24 (2007) 411–437.

[123] M. Thieme, S. Agarwal, J.H. Wendorff, A. Greiner, Electrospinning and cutting of ultrafine bioerodible poly (lactide-co-ethylene oxide) tri-and multiblock copolymer fibers for inhalation applications, Polym. Adv. Technol. 22 (2011) 1335–1344.
[124] M. Babenko, R.G. Alany, G. Calabrese, W. Kaialy, A. ElShaer, Development of drug alone and carrier-based GLP-1 dry powder inhaler formulations, Int. J. Pharm. 617 (2022) 121601.
[125] N. Alhajj, N.J. O'Reilly, H. Cathcart, Leucine as an excipient in spray dried powder for inhalation, Drug Discov. Today. 26 (2021) 2384–2396.
[126] M. Gomez, J. McCollum, H. Wang, M. Ordoubadi, C. Jar, N.B. Carrigy, D. Barona, I. Tetreau, M. Archer, A. Gerhardt, Development of a formulation platform for a spray-dried, inhalable tuberculosis vaccine candidate, Int. J. Pharm. 593 (2021) 120121.
[127] A. Hassan, D. Farkas, W. Longest, M. Hindle, Characterization of excipient enhanced growth (EEG) tobramycin dry powder aerosol formulations, Int. J. Pharm. 591 (2020) 120027.
[128] M.S. Ferdynand, A. Nokhodchi, Co-spraying of carriers (mannitol-lactose) as a method to improve aerosolization performance of salbutamol sulfate dry powder inhaler, Drug Deliv Transl. Res. 10 (2020) 1418–1427.
[129] J. Shur, T.G. Nevell, R.J. Ewen, R. Price, A. Smith, E. Barbu, J.H. Conway, M. P. Carroll, J.K. Shute, J.R. Smith, Cospray-dried unfractionated heparin with L-leucine as a dry powder inhaler mucolytic for cystic fibrosis therapy, J. Pharm. Sci. 97 (2008) 4857–4868.
[130] Y.-X. Chang, J.-J. Yang, R.-L. Pan, Q. Chang, Y.-H. Liao, Anti-hygroscopic effect of leucine on spray-dried herbal extract powders, Powder Technol. 266 (2014) 388–395.
[131] H. Otake, T. Okuda, D. Hira, H. Kojima, Y. Shimada, H. Okamoto, Inhalable spray-freeze-dried powder with L-leucine that delivers particles independent of inspiratory flow pattern and inhalation device, Pharm. Res. 33 (2016) 922–931.
[132] L. Li, S. Sun, T. Parumasivam, J.A. Denman, T. Gengenbach, P. Tang, S. Mao, H.-K. Chan, L-Leucine as an excipient against moisture on in vitro aerosolization performances of highly hygroscopic spray-dried powders, Eur. J. Pharm. Biopharm. 102 (2016) 132–141.
[133] A. Balde, S.-K. Kim, S. Benjakul, R.A. Nazeer, Pulmonary drug delivery applications of natural polysaccharide polymer derived nano/micro-carrier systems: A review, Int. J. Biol Macromol (2022).
[134] Y. Suzuki, T. Yamaguchi, Effects of hyaluronic acid on macrophage phagocytosis and active oxygen release, Agents Actions. 38 (1993) 32–37.
[135] K.G. Rice, The Chemistry, Biology, and Medical Applications of Hyaluronan and Its Derivatives Edited by TC Laurent. Portland Press, London, UK 1998. xvi+ 341 pp. 17× 25 cm. ISBN 1-85578-119-0. $127.50., (1998).
[136] G. Jederström, Low molecular weight hyaluronic acid with peptide or protein, (2001).
[137] K. Surendrakumar, G.P. Martyn, E.C.M. Hodgers, M. Jansen, J.A. Blair, Sustained release of insulin from sodium hyaluronate based dry powder formulations after pulmonary delivery to beagle dogs, J. Control. Release. 91 (2003) 385–394.
[138] R. Kumar, T. Islam, M. Nurunnabi, Mucoadhesive carriers for oral drug delivery, J. Control. Release. 351 (2022) 504–559.
[139] L. Gallo, V. Bucalá, M.V. Ramírez-Rigo, Formulation and characterization of polysaccharide microparticles for pulmonary delivery of sodium cromoglycate, AAPS PharmSciTech 18 (2017) 1634–1645.
[140] E.C. Carroll, L. Jin, A. Mori, N. Munoz-Wolf, E. Oleszycka, H.B.T. Moran, S. Mansouri, C.P. McEntee, E. Lambe, E.M. Agger, The vaccine adjuvant chitosan promotes cellular immunity via DNA sensor cGAS-STING-dependent induction of type I interferons, Immunity 44 (2016) 597–608.
[141] H. Homayoni, J.U. Menon, K.T. Nguyen, Chitosan-based nanoparticles for drug delivery, Rev. Nanosci. Nanotechnol. 3 (2014) 133–148.
[142] N. Islam, V. Ferro, Recent advances in chitosan-based nanoparticulate pulmonary drug delivery, Nanoscale 8 (2016) 14341–14358.
[143] R. Osman, P.L. Kan, G. Awad, N. Mortada, E.-S. Abd-Elhameed, O. Alpar, Spray dried inhalable ciprofloxacin powder with improved aerosolisation and antimicrobial activity, Int. J. Pharm. 449 (2013) 44–58.
[144] Y.-C. Huang, R.-Y. Li, J.-Y. Chen, J.-K. Chen, Biphasic release of gentamicin from chitosan/fucoidan nanoparticles for pulmonary delivery, Carbohydr. Polym. 138 (2016) 114–122.
[145] A. Trapani, S. Di Gioia, N. Ditaranto, N. Cioffi, F.M. Goycoolea, A. Carbone, M. Garcia-Fuentes, M. Conese, M.J. Alonso, Systemic heparin delivery by the pulmonary route using chitosan and glycol chitosan nanoparticles, Int. J. Pharm. 447 (2013) 115–123.
[146] T.P. Learoyd, J.L. Burrows, E. French, P.C. Seville, Sustained delivery by leucine-modified chitosan spray-dried respirable powders, Int. J. Pharm. 372 (2009) 97–104.
[147] H.-Y. Li, J. Birchall, Chitosan-modified dry powder formulations for pulmonary gene delivery, Pharm. Res. 23 (2006) 941–950.
[148] A. Rocío Hernández, E. Bogdanova, J.E. Campos Pacheco, V. Kocherbitov, M. Ekström, G. Pilkington, S. Valetti, Disordered mesoporous silica particles: an emerging platform to deliver proteins to the lungs, Drug Deliv. 31 (2024) 2381340.
[149] A.D. Kulkarni, H.M. Patel, S.J. Surana, Y.H. Vanjari, V.S. Belgamwar, C. V. Pardeshi, N, N, N-Trimethyl chitosan: An advanced polymer with myriad of opportunities in nanomedicine, Carbohydr. Polym. 157 (2017) 875–902.
[150] J.A. Kulkarni, D. Witzigmann, S. Chen, P.R. Cullis, R. Van Der Meel, Lipid nanoparticle technology for clinical translation of siRNA therapeutics, Acc. Chem. Res. 52 (2019), https://doi.org/10.1021/acs.accounts.9b00368.
[151] A. Akinc, M.A. Maier, M. Manoharan, K. Fitzgerald, M. Jayaraman, S. Barros, S. Ansell, X. Du, M.J. Hope, T.D. Madden, The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs, Nat. Nanotechnol. 14 (2019) 1084–1087.
[152] L. Schoenmaker, D. Witzigmann, J.A. Kulkarni, R. Verbeke, G. Kersten, W. Jiskoot, D.J.A. Crommelin, mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability, Int. J. Pharm. 601 (2021) 120586.
[153] J.A. Kulkarni, M.M. Darjuan, J.E. Mercer, S. Chen, R. Van Der Meel, J.L. Thewalt, Y.Y.C. Tam, P.R. Cullis, On the formation and morphology of lipid nanoparticles containing ionizable cationic lipids and siRNA, ACS Nano 12 (2018) 4787–4795.
[154] C.H. Albertsen, J.A. Kulkarni, D. Witzigmann, M. Lind, K. Petersson, J. B. Simonsen, The role of lipid components in lipid nanoparticles for vaccines and gene therapy, Adv. Drug Deliv. Rev. 188 (2022) 114416.
[155] I. Koltover, T. Salditt, J.O. Rädler, C.R. Safinya, An inverted hexagonal phase of cationic liposome-DNA complexes related to DNA release and delivery, Science (80-) 281 (1998) 78–81.
[156] X. Hou, T. Zaks, R. Langer, Y. Dong, Lipid nanoparticles for mRNA delivery, Nat. Rev. Mater. 6 (2021) 1078–1094.
[157] M. Kawaguchi, M. Noda, A. Ono, M. Kamiya, M. Matsumoto, M. Tsurumaru, S. Mizukami, H. Mukai, S. Kawakami, Effect of cholesterol content of lipid composition in mRNA-LNPs on the protein expression in the injected site and liver after local administration in mice, J. Pharm. Sci. 112 (2023) 1401–1410.
[158] T. Suzuki, Y. Suzuki, T. Hihara, K. Kubara, K. Kondo, K. Hyodo, K. Yamazaki, T. Ishida, H. Ishihara, PEG shedding-rate-dependent blood clearance of PEGylated lipid nanoparticles in mice: Faster PEG shedding attenuates anti-PEG IgM production, Int. J. Pharm. 588 (2020) 119792.
[159] E.F. Craparo, B. Porsio, C. Sardo, G. Giammona, G. Cavallaro, Pegylated polyaspartamide–polylactide-based nanoparticles penetrating cystic fibrosis artificial mucus, Biomacromolecules 17 (2016) 767–777.
[160] R. Münter, E. Sørensen, R.B. Hasselbalch, E. Christensen, S.D. Nielsen, P. Garred, S.R. Ostrowski, H. Bundgaard, K.K. Iversen, T.L. Andresen, Investigating generation of antibodies against the lipid nanoparticle vector following COVID-19 vaccination with an mRNA vaccine, Mol Pharm (2023).
[161] C.M. Zimmermann, D. Baldassi, K. Chan, N.B.P. Adams, A. Neumann, D.L. Porras-Gonzalez, X. Wei, N. Kneidinger, M.G. Stoleriu, G. Burgstaller, Spray drying siRNA-lipid nanoparticles for dry powder pulmonary delivery, J. Control. Release. 351 (2022) 137–150.
[162] M.P. Lokugamage, D. Vanover, J. Beyersdorf, M.Z.C. Hatit, L. Rotolo, E. S. Echeverri, H.E. Peck, H. Ni, J.-K. Yoon, Y. Kim, Optimization of lipid nanoparticles for the delivery of nebulized therapeutic mRNA to the lungs, Nat. Biomed. Eng. 5 (2021) 1059–1068.
[163] E.W.X. Leong, R. Ge, Lipid nanoparticles as delivery vehicles for inhaled therapeutics, Biomedicines. 10 (2022), https://doi.org/10.3390/biomedicines10092179.
[164] Y. Li, Z. Ye, H. Yang, Q. Xu, Tailoring combinatorial lipid nanoparticles for intracellular delivery of nucleic acids, proteins, and drugs, Acta Pharm. Sin. b. 12 (2022) 2624–2639.
[165] F. Hajos, B. Stark, S. Hensler, R. Prassl, W. Mosgoeller, Inhalable liposomal formulation for vasoactive intestinal peptide, Int. J. Pharm. 357 (2008) 286–294.
[166] O.B. Garbuzenko, N. Kbah, A. Kuzmov, N. Pogrebnyak, V. Pozharov, T. Minko, Inhalation treatment of cystic fibrosis with lumacaftor and ivacaftor co-delivered by nanostructured lipid carriers, J. Control. Release. 296 (2019) 225–231.
[167] C. Corzo, A. Fuchsbichler, I. Savencu, J.A. Urich, A. Zimmer, D. Lochmann, S. Reyer, S. Salar-Behzadi, Lipid-microparticles for pulmonary delivery of active pharmaceutical ingredients: Impact of lipid crystallization on spray-drying processability, Int. J. Pharm. 610 (2021) 121259.
[168] C. Corzo, D. Crvenjak, K. Sotirov, J.A. Urich, K. Öhlinger, C. Meindl, D. Lochmann, S. Reyer, E. Fröhlich, A. Zimmer, Lipid-based particle engineering via spray-drying for targeted delivery of antibiotics to the lung, Int. J. Pharm. 642 (2023) 123201.
[169] D. Zillen, M. Beugeling, W.L.J. Hinrichs, H.W. Frijlink, F. Grasmeijer, Natural and bioinspired excipients for dry powder inhalation formulations, Curr. Opin. Colloid Interface Sci. 56 (2021) 101497.
[170] U.S.F. and D. Administration, Inactive ingredients database, FDA. Gov, https// Www. Accessdata. Fda. Gov/Scripts/Cder/Iig/Index. Cfm (Accessed 12 Feb. 2019). (2017).
[171] K.M. Rubey, J.S. Brenner, Nanomedicine to fight infectious disease, Adv. Drug Deliv. Rev. 179 (2021) 113996.
[172] Arikayce, amikacin liposome inhalation suspension., (n.d.). https://www.arikayce.com. (accessed November 12, 2023).
[173] D.R. VanDevanter, D.E. Geller, Tobramycin administered by the TOBI® Podhaler® for persons with cystic fibrosis: a review, Med. Devices Evid. Res. (2011) 179–188.
[174] Inbrija, levodopa inhalation powder, (n.d.). https://www.inbrija.com (accessed November 12, 2023).
[175] O.S. Usmani, N. Roche, M. Jenkins, N. Stjepanovic, P. Mack, W. De Backer, Consistent pulmonary drug delivery with whole lung deposition using the aerosphere inhaler: a review of the evidence, Int. J. Chron. Obstruct. Pulmon. Dis. (2021) 113–124.
[176] G. Pilcer, K. Amighi, Formulation strategy and use of excipients in pulmonary drug delivery, Int. J. Pharm. 392 (2010) 1–19.
[177] J. Weers, T. Tarara, The PulmoSphere™ platform for pulmonary drug delivery, Ther. Deliv. 5 (2014) 277–295.
[178] D.A. Edwards, G. Caponetti, J.S. Hrkach, N. Lotan, J. Hanes, A.A. Ben-Jebria, R.S. Langer, Aerodynamically light particles for pulmonary drug delivery, (1999).
[179] Acorda Therapeutics. (2021) ARCUS®: Innovative Technology Platform for Inhaled Medicines., (n.d.).

[180] Y.-J. Son, D.P. Miller, J.G. Weers, Optimizing spray-dried porous particles for high dose delivery with a portable dry powder inhaler, Pharmaceutics. 13 (2021) 1528.
[181] R. Vehring, D. Lechuga-Ballesteros, V. Joshi, B. Noga, S.K. Dwivedi, Cosuspensions of microcrystals and engineered microparticles for uniform and efficient delivery of respiratory therapeutics from pressurized metered dose inhalers, Langmuir 28 (2012) 15015–15023.
[182] S. Al-Qadi, P. Taboada, C. Remuñán-López, Micro/nanostructured inhalable formulation based on polysaccharides: Effect of a thermoprotectant on powder properties and protein integrity, Int. J. Pharm. 551 (2018) 23–33.
[183] Y.-B. Shen, Z. Du, C. Tang, Y.-X. Guan, S.-J. Yao, Formulation of insulin-loaded N-trimethyl chitosan microparticles with improved efficacy for inhalation by supercritical fluid assisted atomization, Int. J. Pharm. 505 (2016) 223–233.
[184] M. Sakagami, K. Sakon, W. Kinoshita, Y. Makino, Enhanced pulmonary absorption following aerosol administration of mucoadhesive powder microspheres, J. Control. Release. 77 (2001) 117–129.
[185] A. Mittal, A. Singh, R.E. Aluko, S. Benjakul, Pacific white shrimp (Litopenaeus vannamei) shell chitosan and the conjugate with epigallocatechin gallate: Antioxidative and antimicrobial activities, J. Food Biochem. 45 (2021) e13569.
[186] A. Balde, P. Raghavender, S. Dasireddy, J. Abraham, S. Prasad, I. Joshi, N. R. Abdul, Crab pentapeptide and its anti-inflammatory activity on macrophage cells, Int. J. Pept. Res. Ther. 27 (2021) 2595–2605.
[187] C. Yee Kuen, M.J. Masarudin, Chitosan nanoparticle-based system: a new insight into the promising controlled release system for lung cancer treatment, Molecules 27 (2022) 473.
[188] E. Fernández-Paz, C. Fernández-Paz, S. Barrios-Esteban, I. Santalices, N. Csaba, C. Remuñán-López, Dry powders containing chitosan-based nanocapsules for pulmonary administration: Adjustment of spray-drying process and in vitro evaluation in A549 cells, Powder Technol. 399 (2022) 117149.
[189] Y.-B. Chen, Y.-B. Zhang, Y.-L. Wang, P. Kaur, B.-G. Yang, Y. Zhu, L. Ye, Y.-L. Cui, A novel inhalable quercetin-alginate nanogel as a promising therapy for acute lung injury, J. Nanobiotechnology. 20 (2022) 272.
[190] T. Sato, T. Takami, H. Saito, Y. Murakami, Thermosensitive polysaccharide particles for pulmonary drug delivery, Colloids Surfaces A Physicochem. Eng. Asp. 580 (2019) 123720.
[191] M. Curcio, L. Diaz-Gomez, G. Cirillo, A. Concheiro, F. Iemma, C. Alvarez-Lorenzo, pH/redox dual-sensitive dextran nanogels for enhanced intracellular drug delivery, Eur. J. Pharm. Biopharm. 117 (2017) 324–332.
[192] H.-M. Ren, L. Han, L. Zhang, Y.-Q. Zhao, C. Lei, Z. Xiu, N. Zhao, B. Yu, F. Zhou, S. Duan, Inhalable responsive polysaccharide-based antibiotic delivery nanoparticles to overcome mucus barrier for lung infection treatment, Nano Today 44 (2022) 101489.
[193] C. Alvarez-Lorenzo, B. Blanco-Fernandez, A.M. Puga, A. Concheiro, Crosslinked ionic polysaccharides for stimuli-sensitive drug delivery, Adv. Drug Deliv. Rev. 65 (2013) 1148–1171.
[194] Y. Luo, Perspectives on important considerations in designing nanoparticles for oral delivery applications in food, J. Agric. Food Res. 2 (2020) 100031.
[195] Y. Luo, B. Zhang, W.-H. Cheng, Q. Wang, Preparation, characterization and evaluation of selenite-loaded chitosan/TPP nanoparticles with or without zein coating, Carbohydr. Polym. 82 (2010) 942–951.
[196] D. Le Corre, J. Bras, A. Dufresne, Starch nanoparticles: a review, Biomacromolecules 11 (2010) 1139–1153.
[197] A. Dufresne, Nanocellulose: a new ageless bionanomaterial, Mater. Today. 16 (2013) 220–227.
[198] A. Singh, A. Mittal, S. Benjakul, Chitosan, chitooligosaccharides and their polyphenol conjugates: preparation, bioactivities, functionalities and applications in food systems, Food Rev. Int. 39 (2023) 2297–2319.
[199] A. Rampino, M. Borgogna, P. Blasi, B. Bellich, A. Cesàro, Chitosan nanoparticles: Preparation, size evolution and stability, Int. J. Pharm. 455 (2013) 219–228.
[200] S.M. Bashir, G. Ahmed Rather, A. Patrício, Z. Haq, A.A. Sheikh, M.Z. ul, H. Shah, H. Singh, A.A. Khan, S. Imtiyaz, S.B. Ahmad, Chitosan nanoparticles: a versatile platform for biomedical applications, Materials (basel) 15 (2022) 6521.
[201] E. Chiesa, R. Dorati, S. Pisani, B. Conti, G. Bergamini, T. Modena, I. Genta, The microfluidic technique and the manufacturing of polysaccharide nanoparticles, Pharmaceutics. 10 (2018) 267.
[202] H. Kolge, K. Kadam, S. Galande, V. Lanjekar, V. Ghormade, New frontiers in pest control: chitosan nanoparticles-shielded dsRNA as an effective topical RNAi spray for gram podborer biocontrol, ACS Appl. Bio Mater. 4 (2021), https://doi.org/10.1021/acsabm.1c00349.
[203] H. Katas, H.O. Alpar, Development and characterisation of chitosan nanoparticles for siRNA delivery, J. Control. Release. 115 (2006), https://doi.org/10.1016/j.jconrel.2006.07.021.
[204] A.M. Abdelgawad, S.M. Hudson, Chitosan nanoparticles: Polyphosphates cross-linking and protein delivery properties, Int. J. Biol. Macromol. 136 (2019) 133–142.
[205] J. Kjems, K.A. Howard, F. Besenbacher, M.Ø. Andersen, Dehydrated Chitosan Nanoparticles (2011).
[206] S. Santra, Chitosan-based nanoparticles and methods for making and using the same, (2011).
[207] Y. Luo, X. Zhai, C. Ma, P. Sun, Z. Fu, W. Liu, J. Xu, An inhalable β2-adrenoceptor ligand-directed guanidinylated chitosan carrier for targeted delivery of siRNA to lung, J. Control. Release. 162 (2012) 28–36.
[208] K. Shah, L.W. Chan, T.W. Wong, Conversion of liquid chitosan-based nanoemulsions into inhalable solid microparticles: Process challenges with polysaccharide, Int. J. Biol. Macromol. 126991 (2023).
[209] E.D. Freitas, C.F. Moura Jr, J. Kerwald, M.M. Beppu, An overview of current knowledge on the properties, synthesis and applications of quaternary chitosan derivatives, Polymers (basel). 12 (2020) 2878.
[210] A. Jintapattanakit, S. Mao, T. Kissel, V.B. Junyaprasert, Physicochemical properties and biocompatibility of N-trimethyl chitosan: effect of quaternization and dimethylation, Eur. J. Pharm. Biopharm. 70 (2008) 563–571.
[211] J.K. Sahni, S. Chopra, F.J. Ahmad, R.K. Khar, Potential prospects of chitosan derivative trimethyl chitosan chloride (TMC) as a polymeric absorption enhancer: synthesis, characterization and applications, J. Pharm. Pharmacol. 60 (2008) 1111–1119.
[212] D.P. Facchi, S.P. Facchi, N, N, N-trimethyl chitosan and its potential bactericidal activity: current aspects and technological applications, J. Infect. Dis Ther (2016).
[213] J. Szabová, F. Mravec, M. Mokhtari, R. Le Borgne, M. Kalina, J.-F. Berret, N, N, N-Trimethyl chitosan as a permeation enhancer for inhalation drug delivery: interaction with a model pulmonary surfactant, Int. J. Biol. Macromol. 239 (2023) 124235.
[214] A. Gonsalves, P. Sorkhdini, J. Bazinet, M. Ghumman, D. Dhamecha, Y. Zhou, J. U. Menon, Development and characterization of lung surfactant-coated polymer nanoparticles for pulmonary drug delivery, Biomater. Adv. 150 (2023) 213430.
[215] F. Adams, C.M. Zimmermann, D. Baldassi, T.M. Pehl, P. Weingarten, I. Kachel, M. Kränzlein, D.C. Jürgens, P. Braubach, I. Alexopoulos, Pulmonary siRNA delivery with sophisticated amphiphilic poly (Spermine Acrylamides) for the treatment of lung fibrosis, Small 20 (2024) 2308775.
[216] C. Corzo, C. Meindl, D. Lochmann, S. Reyer, S. Salar-Behzadi, Novel approach for overcoming the stability challenges of lipid-based excipients. Part 3: Application of polyglycerol esters of fatty acids for the next generation of solid lipid nanoparticles, Eur. J. Pharm. Biopharm. 152 (2020) 44–55.
[217] B. Cuvelier, P. Eloy, C. Loira-Pastoriza, B. Ucakar, A.A. Sanogo, C. Dupont-Gillain, R. Vanbever, Minimal amounts of dipalmitoylphosphatidylcholine improve aerosol performance of spray-dried temocillin powders for inhalation, Int. J. Pharm. 495 (2015) 981–990.
[218] M. Haghi, M. Bebawy, P. Colombo, B. Forbes, D.A. Lewis, R. Salama, D. Traini, P. M. Young, Towards the bioequivalence of pressurised metered dose inhalers 2. Aerodynamically equivalent particles (with and without glycerol) exhibit different biopharmaceutical profiles in vitro, Eur. J. Pharm. Biopharm. 86 (2014) 38–45.
[219] J.P. Cassidy, N. Amin, M. Marino, M. Gotfried, T. Meyer, K. Sommerer, R. A. Baughman, Insulin lung deposition and clearance following Technosphere® insulin inhalation powder administration, Pharm. Res. 28 (2011) 2157–2164.
[220] G. Pilcer, T. Sebti, K. Amighi, Formulation and characterization of lipid-coated tobramycin particles for dry powder inhalation, Pharm. Res. 23 (2006) 931–940.
[221] N. Shetty, J. Hau, E. Yanez, J. Shur, J. Cheng, C.C. Sun, K. Nagapudi, A. Narang, Effect of lipidic excipients on the particle properties and aerosol performance of high drug load spray dried particles for inhalation, J. Pharm. Sci. 111 (2022) 1152–1163.
[222] N.R. Labiris, M.B. Dolovich, Pulmonary drug delivery. Part I: physiological factors affecting therapeutic effectiveness of aerosolized medications, Br. J. Clin. Pharmacol. 56 (2003) 588–599.
[223] D.C. Klonoff, Afrezza inhaled insulin: the fastest-acting FDA-approved insulin on the market has favorable properties, J. Diabetes Sci. Technol. 8 (2014) 1071–1073.
[224] J.M. Baumann, M.S. Adam, J.D. Wood, Engineering advances in spray drying for pharmaceuticals, Annu. Rev. Chem. Biomol. Eng. 12 (2021) 217–240.
[225] E. Lintingre, F. Lequeux, L. Talini, N. Tsapis, Control of particle morphology in the spray drying of colloidal suspensions, Soft Matter 12 (2016) 7435–7444.
[226] J.P. Szczap, I.C. Jacobs, Atomization and spray drying processes, in: Microencapsul. Food Ind., Elsevier, 2023, pp. 59–71.
[227] T.W. Wong, P. John, Advances in spray drying technology for nanoparticle formation, Handb Nanoparticles [Internet], Cham Springer Int. Publ. (2016) 329–346.
[228] K. Cal, K. Sollohub, Spray drying technique. I: Hardware and process parameters, J. Pharm. Sci. 99 (2010) 575–586.
[229] E. Boel, R. Koekoekx, S. Dedroog, I. Babkin, M.R. Vetrano, C. Clasen, G. Van den Mooter, Unraveling particle formation: From single droplet drying to spray drying and electrospraying, Pharmaceutics. 12 (2020) 625.
[230] A. Sosnik, K.P. Seremeta, Advantages and challenges of the spray-drying technology for the production of pure drug particles and drug-loaded polymeric carriers, Adv. Colloid Interface Sci. 223 (2015) 40–54.
[231] A. Ziaee, A.B. Albadarin, L. Padrela, T. Femmer, E. O'Reilly, G. Walker, Spray drying of pharmaceuticals and biopharmaceuticals: Critical parameters and experimental process optimization approaches, Eur. J. Pharm. Sci. 127 (2019) 300–318.
[232] A. Di Renzo, F. Scala, S. Heinrich, Recent advances in fluidized bed hydrodynamics and transport phenomena—progress and understanding, Processes. 9 (2021) 639.
[233] V. Godavarthi, K. Dhivyaraja, R.I. Sujith, M.V. Panchagnula, Analysis and classification of droplet characteristics from atomizers using multifractal analysis, Sci. Rep. 9 (2019) 16218.
[234] B.B. Eedara, W. Alabsi, D. Encinas-Basurto, R. Polt, H.M. Mansour, Spray-dried inhalable powder formulations of therapeutic proteins and peptides, AAPS PharmSciTech 22 (2021) 185.
[235] H.C. Seow, J.-P. Cai, H.W. Pan, C. Luo, K. Wen, J. Situ, K. Wang, H. Cao, S.W. S. Leung, S. Yuan, Neutralisation of SARS-CoV-2 by monoclonal antibody through dual targeting powder formulation, J. Control. Release. 358 (2023) 128–141.

[236] A.D. Brunaugh, L. Ding, T. Wu, M. Schneider, R. Khalaf, H.D.C. Smyth, Identification of stability constraints in the particle engineering of an inhaled monoclonal antibody dried powder, J. Pharm. Sci. 111 (2022) 403–416.
[237] K.B. Shepard, D.T. Vodak, P.J. Kuehl, D. Revelli, Y. Zhou, A.M. Pluntze, M. S. Adam, J.C. Oddo, L. Switala, J.L. Cape, Local treatment of non-small cell lung cancer with a spray-dried bevacizumab formulation, AAPS PharmSciTech 22 (2021) 230.
[238] T.W.M. Keil, C. Zimmermann, D. Baldassi, F. Adams, W. Friess, A. Mehta, O. M. Merkel, Impact of crystalline and amorphous matrices on successful spray drying of siRNA polyplexes for inhalation of nano-in-microparticles, Adv. Ther. 4 (2021) 2100073.
[239] K.P. Friis, S. Gracin, S. Oag, A. Leijon, E. Sand, B. Lindberg, E. Lázaro-Ibáñez, J. Lindqvist, K.A. Whitehead, A. Bak, Spray dried lipid nanoparticle formulations enable intratracheal delivery of mRNA, J. Control. Release. 363 (2023) 389–401.
[240] C.M. Zimmermann, L. Deßloch, D.C. Jürgens, P. Luciani, O.M. Merkel, Evaluation of the effects of storage conditions on spray-dried siRNA-LNPs before and after subsequent drying, Eur. J. Pharm. Biopharm. 193 (2023) 218–226.
[241] S. Wanning, R. Süverkrüp, A. Lamprecht, Pharmaceutical spray freeze drying, Int. J. Pharm. 488 (2015) 136–153.
[242] S.R. Pardeshi, N.S. Deshmukh, D.R. Telange, S.N. Nangare, Y.Y. Sonar, S. H. Lakade, M.T. Harde, C.V. Pardeshi, A. Gholap, P.K. Deshmukh, Process development and quality attributes for the freeze-drying process in pharmaceuticals, biopharmaceuticals and nanomedicine delivery: a state-of-the-art review, Futur. J. Pharm. Sci. 9 (2023) 99.
[243] M. Rostamnezhad, H. Jafari, F. Moradikhah, S. Bahrainian, H. Faghihi, R. Khalvati, R. Bafkary, A. Vatanara, Spray freeze-drying for inhalation application: process and formulation variables, Pharm. Dev. Technol. 27 (2022) 251–267.
[244] S. Hufnagel, H. Xu, S. Sahakijpijarn, C. Moon, L.Q.M. Chow, R.O. Williams, Z. Cui, Dry powders for inhalation containing monoclonal antibodies made by thin-film freeze-drying, Int. J. Pharm. 618 (2022), https://doi.org/10.1016/j.ijpharm.2022.121637.
[245] K. AboulFotouh, H. Xu, C. Moon, R.O. Williams, Z. Cui, Development of (Inhalable) dry powder formulations of AS01B-containing vaccines using thin-film freeze-drying, Int. J. Pharm. 622 (2022), https://doi.org/10.1016/j.ijpharm.2022.121825.
[246] S. Wanning, R. Süverkrüp, A. Lamprecht, Impact of excipient choice on the aerodynamic performance of inhalable spray-freeze-dried powders, Int. J. Pharm. 586 (2020), https://doi.org/10.1016/j.ijpharm.2020.119564.
[247] S. Wanning, R. Süverkrüp, A. Lamprecht, Aerodynamic droplet stream expansion for the production of spray freeze-dried powders, AAPS PharmSciTech 18 (2017), https://doi.org/10.1208/s12249-016-0648-2.
[248] S. Farinha, J.V. Sá, P.R. Lino, M. Galésio, J. Pires, M.Â. Rodrigues, J. Henriques, Spray freeze drying of biologics: a review and applications for inhalation delivery, Pharm. Res. 40 (2023), https://doi.org/10.1007/s11095-022-03442-4.
[249] J.L. Nation, A new method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy, Stain Technol. 58 (1983) 347–351.
[250] D. Vanhecke, W. Graber, D. Studer, Chapter 9 close-to-native ultrastructural preservation by high pressure freezing, Methods Cell Biol. 88 (2008), https://doi.org/10.1016/S0091-679X(08)00409-3.