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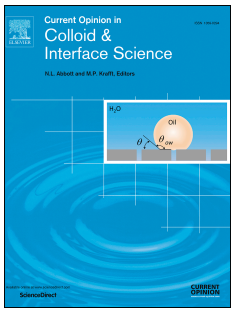

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Natural and bioinspired excipients for dry powder inhalation formulations

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Abstract

Pulmonary drug delivery can have several advantages over other administration routes, in particular when using dry powder formulations. Such dry powder inhalation formulations generally include natural and bio-inspired excipients, which, among other purposes, are used to improve dosing reproducibility and aerosolization performance. Amino acids can enhance powder dispersibility and provide protection against moisture uptake. Sugars are used as drug-carrying diluents, stabilizers for biopharmaceuticals, and surface-enrichers. Lipids and lipid-like excipients can reduce interparticle adhesive forces and are also used as constituents of liposomal drug delivery systems. Lastly, biodegradable polymers are used to facilitate sustained release and targeted drug delivery. Despite their promise, pulmonary toxicity of many of the discussed excipients remains largely unknown and requires attention in future research.

Keywords: bioinspired excipients, drug formulation, dry powder inhalation, natural excipients, pulmonary drug delivery

1. Introduction

Pulmonary drug delivery offers several advantages over more conventional routes of administration, for both systemic and local treatment [1,2]. Some examples are the large surface area of the lungs, its high perfusion, low metabolic activity and absence of a first-pass effect [2]. Furthermore, pulmonary administration can be considered patient-friendly compared to more invasive drug administration routes, especially promising for biopharmaceutical drugs (e.g., vaccines, therapeutic proteins) that are typically administered parenterally [3]. To deliver drugs to the lungs, dry powder inhalers (DPIs) are generally favored over alternatives like nebulizers and pressurized metered-dose inhalers. Compared to the last two, DPIs are small and portable, user-friendly, more effective in deep-lung delivery and propellant free [1,2,4,5]. Furthermore, DPI formulations are generally more stable, since drugs are formulated in a dry solid state [1,2,5]. We kindly refer the reader to extensive reviews on advantages and challenges associated with pulmonary drug delivery [1,3], as well as inhalation systems [2], as the focus of this review is exclusively on DPI formulation excipients.

<Figure 1 here>

A DPI formulation should meet several requirements to serve its purpose. First and foremost, the DPI formulation should consist of drug-containing particles with aerodynamic diameters roughly in the range of 1-5 μm in order to achieve deep lung deposition, often referred to as the fine particle fraction (FPF) [1,5]. Particles larger than 5 μm generally impact on the oropharynx and are subsequently swallowed, while the bulk of particles smaller than 1 μm does not deposit at all and is exhaled (Fig. 1). In addition to a suitable particle size distribution, DPI formulations should have good physical and chemical stability and a relatively low retention in the DPI device (i.e., a high emitted dose (ED)). Furthermore, a DPI formulation should have satisfactory dose reproducibility, by ensuring powder flowability and dispersibility [1]. Meeting these requirements is far from trivial, because micron-sized particles are generally very cohesive and adhesive, which

results in poor flow properties and poor aerosolization performance. Consequently, development of a DPI formulation is typically a delicate process, in respect to particle generation as well as balanced use of excipients.

To generate particles in the desired size range, several preparation techniques can be applied, of which milling and spray-drying are most commonly used. Milling is usually the first technique that is attempted due to its low costs, reproducibility, and ease of use. With milling, larger particles are mechanically broken up into smaller particles in the desirable size range by, for instance, particle-particle collisions. However, milling does not enable much control over the shape, density, and surface properties of the resulting particles. By contrast, more control over these particle characteristics can be achieved by spray-drying. With spray-drying, a solution, suspension, or colloidal dispersion is atomized after which the formed droplets are dried by a hot gas. Typically, spray-drying produces spherical or raisin-like particles [6]. Spray-drying is highly suitable for so-called 'particle engineering', because its various process parameters such as solute concentration, droplet size, and feed rate, strongly affect the particle characteristics and can be easily controlled.

The performance of dry powder formulations can be further improved by the incorporation of excipients. Typically, excipients are added to DPI formulations for four main purposes: (1) to enhance physical and chemical stability of the active pharmaceutical ingredient (API); (2) to enhance mechanical properties of the API; (3) to modify API pharmacokinetics and/or -dynamics; (4) and to improve API dosing reproducibility by functioning as a bulking agent and powder flow enhancer. However, an excipient should be inactive and exert no therapeutic effect at the used dosage.[2,7] Notwithstanding, pulmonary toxicity of excipients that could successfully fulfil one or more of these functions is a common challenge in DPI formulation development, partly due to the limited buffering capacity of the lungs[5]. Furthermore, as toxicity studies are typically very costly and pulmonary drug delivery is a nonconventional delivery method, knowledge on excipient toxicity is generally lacking. This is reflected by the fact that only a limited number of compounds are included in the inactive ingredient list of the Food and Drug Administration (FDA) for inhalation purposes. Consequently, potential excipients for DPI

formulations are preferably natural and bioinspired compounds that are biocompatible and can easily be metabolized and cleared.

The main aim of this manuscript is to review the use of natural and bioinspired excipients (NBEs) for the preparation of inhalation dry powders by using mainly literature published in the previous two years (January 2019 to January 2021). In the context of this review, NBEs are compounds from natural sources or excipients inspired by or based on such compounds. It should be noted that our goal is not to give an extensive list of all NBEs that have been used in this period, but to discuss fundamental and applied research on NBEs frequently used for the preparation of inhalation dry powders. For an extensive list of excipients that have been used in approved pulmonary drug products, we kindly refer the reader to the Food and Drug Administration's (FDA) list of inactive ingredients [8]. The NBEs we reviewed are divided into four main categories, namely: amino acids, sugars, lipids, and biodegradable polymers. Salts and buffers are also important excipients in dry powder inhalation formulations, but they have been scarcely studied during the period covered by this review and are, therefore, not further discussed.

2. Amino acids

Amino acids have been extensively investigated as NBEs in inhalation dry powders. Due to specific characteristics, the amino acid L-leucine, and its tripeptide trileucine, are of special interest. Most often, (tri)leucine is added as excipient to spray-dried inhalation dry powders to enhance their dispersibility and to provide moisture protection (Fig. 2).

<Figure 2 here>

2.1. L-leucine

L-leucine, a nonpolar aliphatic amino acid, is the most widely investigated amino acid in inhalation dry powders. The addition of L-leucine to spray-dried formulations of active

pharmaceutical ingredients (APIs) often results in a better *in vitro* aerosolization performance[9–12]. For example, addition of 20% (w/w) L-Leucine to a range of formulations resulted in absolute increases of 17.3 – 41.5% for EDs [9,10], 13.6 – 43.1% for FPFs[9–12], and 0.63 – 3.5 µm smaller mass median aerodynamic diameters (MMADs) [9–11]. Similarly, Šimková et al. [13] showed an absolute FPF increase of approximately 28% when 37.5% (w/w) leucine was added to a spray-dried nanosuspension containing budesonide. The observed improvements can be attributed to the fact that leucine tends to enrich at the droplet surface during spray-drying due to its surface-active properties. As a consequence, the presence of leucine during spray-drying changes the surface composition and potentially the morphology (e.g., corrugated) of the resulting particles (Fig. 2). This benefits the aerosolization performance of the formulation if it results in lower co- and adhesion forces within the powder and between the particles and the inhaler material, respectively. For an in-depth description of the mechanisms of effect of leucine in spray-dried particles, the reader is referred to a review by Vehring [6]. Sibum et al. [14] showed that the fraction of primary particles $\leq$ 5 µm of a spray-dried isoniazid inhalation formulation increased four-fold when 5% (w/w) leucine was added. It was hypothesized that the addition of 5% (w/w) leucine resulted in a leucine coating that prevented the isoniazid cores from interacting during the crystallization process after spray-drying. In addition to improvement of the aerosolization performance of spray-dried powders, leucine can also increase the FPFs of jet-milled ciprofloxacin and levodopa at concentrations as low as 0.5% and 2% (w/w), respectively [15,16]. This improvement was ascribed to a reduction of the surface energy as well as changes in surface rugosity.

Generally, spray-dried powders are amorphous, and therefore hygroscopic. As a consequence, they are often susceptible to moisture-induced crystallization and agglomeration. To protect spray-dried powders from moisture, and thereby to improve the physical stability of the formulation, leucine has often been added as an excipient. Due to its surface-active properties and its low solubility, leucine can form a hydrophobic shell that protects spray-dried particles from moisture (Fig. 2). For instance, Wang et al. [17] demonstrated an approximate 10% lower weight gain at 90% relative humidity (RH) for a

spray-dried formulation of aztreonam and tobramycin when 34% (w/w) leucine was added. In addition, according to the authors, the particle morphology of the leucine-containing formulation remained better intact after five months of storage at room temperature and 58% RH, compared to formulations not containing leucine. Another study showed that trehalose rapidly recrystallized at ~50% and ~60% RH in spray-dried formulations with 10% and 20% (w/w) leucine, respectively, but not with 30% (w/w) leucine [18]. However, 30% (w/w) leucine negatively impacted the aerosolization performance of the formulation. Hence, a balance between physical stability and aerosolization performance may need to be found when leucine is used as an excipient in spray-dried inhalation formulations. Lu et al. [19] compared the stabilizing effect of leucine to that of tryptophan and lysine in spray-dried formulations. To this end, simvastatin, a model API, was co-spray-dried with either leucine, tryptophan, or lysine at a molar ratio of 1:1. After one month of storage at 25 °C and 60% RH, no recrystallization of simvastatin was observed for the leucine-containing formulation, whereas simvastatin recrystallized in the tryptophan-containing formulation and the lysine-containing formulation became an aqueous slurry. Moreover, the aerosolization performance of only the leucine-containing formulation remained unchanged after the storage period.

Because leucine is an essential amino acid that is approved for intravenous and oral administration [8], its toxicity after pulmonary administration is also likely to be limited. Although leucine is yet to be used in a FDA-approved drug product for inhalation, current clinical studies indicate a low risk for leucine causing local toxicity after inhalation [20,21]. Patients that were given a single dose of a formulation containing 0.6 or 1.2 mg leucine showed no changes in lung function parameters [20]. Similarly, the pulmonary function of patients that inhaled a single dose of a formulation containing 5; 15; 30; or 60 mg leucine was unchanged after inhalation [21]. In addition, a DPI formulation of vancomycin containing leucine, AeroVanc^{TM}, was found to be well-tolerated in both phase 1 [23], with single doses up to 8.8 mg leucine, and phase 2 [24], with daily doses of up to 14 mg, for at least 28 days. The formulation was also well-tolerated when administered for 24 weeks in

phase 3, resulting in daily leucine exposure up to 6.7 mg [22]. Nevertheless, AeroVanc™ was discontinued because it did not meet primary endpoints.

2.2. Trileucine

Trileucine, which consists of three leucine amino acids bound by peptide bonds, has been used for similar purposes in inhalation dry powders as leucine (Fig. 2). For instance, Gomez et al. [25] showed that the lung dose of a spray-dried powder, containing ID93 (a recombinant tuberculosis subunit vaccine candidate) adjuvanted with glucopyranosyl lipid A in a squalene emulsion and trehalose (stabilizer), increased significantly from 18.0 ± 0.5% to 34 ± 6% and 33 ± 6% when it was co-spray-dried with 3% and 6% (w/w) trileucine, respectively. When the formulation was co-spray-dried with 20% (w/w) leucine instead of trileucine, the lung dose increased, however not significantly, to 32 ± 12%. The calculated $d_{a,50}$ (aerodynamic diameter 50% of the powder that deposited in the impactor) was 8.8 ± 2.3 μm for the leucine-containing formulation, whereas the calculated $d_{a,50}$'s were 5.7 ± 0.2 μm and 5.4 ± 0.2 μm for the formulations containing 3% and 6% (w/w) trileucine, respectively. These results could be ascribed to the fact that the addition of trileucine resulted in substantial changes of the particle morphology compared to the leucine-containing particles due to its higher surface activity, lower diffusivity, and lower solubility (6.2 and 23 mg/ml) compared to leucine. More specifically, the addition of trileucine led to geometrically large, thin-shelled and hollow particles, which are easier to disperse. Moreover, the surface enrichment of trileucine may reduce the particle surface energy in addition to reducing the contact area between particles due to the formation of wrinkled particle surfaces.

Sibum et al. [26] showed that spray-dried isoniazid formulations with leucine (1; 2; 3; or 5% (w/w)) were not stable in terms of the fraction of primary particles ≤ 5 μm and the FPF dispersed from the Twincer® inhaler after storage at 43.5% and 75% RH for one week. By contrast, the use of trileucine (1; 2; or 3% (w/w)) resulted in powders that showed almost no decrease in the fraction of primary particles ≤ 5μm as well as FPFs suitable for

pulmonary administration after storage at 43.5% and 75% RH for three months. Dynamic vapor sorption experiments showed that dissolution-recrystallization occurred around 40% RH for the 3% (w/w) leucine-containing formulation. In contrast, dissolution-recrystallization occurred around 70% and 80% RH for the 1% and 3% (w/w) trileucine-containing formulations, respectively. Time-of-flight secondary ion mass spectrometry revealed that the 3% (w/w) trileucine-containing formulation had an approximate 1.3-2.6-fold higher leucine:isoniazid surface ratio than the 3% (w/w) leucine-containing formulation. This finding may be ascribed to a higher encapsulation efficiency of trileucine compared to leucine due to its higher surface activity, lower diffusivity, and lower solubility, resulting in an improved physical stability. However, remarkably, despite having an improved physical stability compared to the 3% (w/w) leucine-containing formulation, time-of-flight secondary ion mass spectrometry showed that the 1% (w/w) trileucine-containing formulation had roughly 0.1-0.2 times the leucine:isoniazid surface ratio. Hence, other mechanisms were also at play that resulted in an improved physical stability when trileucine was used. It was hypothesized that the amorphous trileucine coating was better at delaying diffusion-crystallization than the crystalline leucine coating.

The described results may imply that trileucine is better than leucine at improving the aerosolization performance as well as the physical stability of spray-dried inhalation powders. Moreover, its effect may already be achieved with only a few mass percent. Therefore, trileucine could be of special interest as an excipient in high-dose API inhalation powders. Despite its great potential, little is known about the (local) toxicity of trileucine. However, as trileucine can be converted into leucine by peptidases, it may be expected that the earlier described lack of (local) toxicity of leucine is similar to that of trileucine. Moreover, single doses of a dry powder of ribavirin with trehalose and trileucine as excipients (35:55:10, w/w/w, respectively) were well tolerated in 60 healthy adults. The observed adverse events were mild to moderate in intensity and similar across all doses (7.5; 15; 30; 60 mg) and placebo. [27]

3. Sugars

Sugars, particularly lactose, are used as NBEs in many marketed inhalation dry powders. Their main function is to act as a diluent and powder flow enhancer, which together enable the accurate dosing of small amounts of micronized API particles (Fig. 3A). In addition, sugars are used as stabilizers for biopharmaceuticals in spray-dried inhalation powders (Fig. 3B), and as microparticulate matrices for nanoparticles (Fig. 3C).

<Figure 3 here>

3.1. Lactose

Lactose, a disaccharide composed of galactose and glucose, is primarily used as a diluent and flowability enhancer to improve the dosing reproducibility of micronized API particles in many marketed inhalation powders. Generally, these formulations consist of a mixture of larger (in the range of 50-200 μm) carrier particles to which micronized (1-5 μm) API particles are adhered, and are also known as 'adhesive mixtures' (Fig. 3A). In some instances, the API particles are blended with lactose particles of roughly equal size and then agglomerated in a controlled manner to spherical pellets to improve the flowability of the powder. Upon inhalation of adhesive mixtures, the micronized API particles have to detach from carrier particles in order to reach the lower airways (Fig. 3A). Due to their larger size, the carrier particles themselves (and undetached micronized API particles) are deposited in the throat, and are subsequently rinsed out or swallowed. It has been shown that current marketed inhalation powders generate FPFs in the range of 10-50% of the label claim [28]. Current research on adhesive mixtures using lactose as carrier material mainly focuses on the effects of variables on the formulation, such as the morphology of the carrier [29,30], the addition of fines (micronized carrier particles that can occupy high energy sites of the larger carrier particles) [29–31], and the API content [32]. For instance, Rudén et al. [29] investigated how the morphology of the carrier affects the expression of blend states in adhesive mixtures as a function of surface coverage ratio (i.e., fines to carrier ratio). To this

end, the authors prepared adhesive mixtures of five lactose carriers of different sizes with various amounts of lactose fines (median particle diameter of 2.7 μm), resulting in blends with surface coverage ratios between 0 and 6. The results showed that smaller carriers (approximately 100 μm) with no or little irregularities on their surfaces were much more sensitive to the addition of fines in the sense that self-agglomeration of the fines was observed already at a surface coverage ratio of 0.75. By contrast, self-agglomeration of the fines occurred at surface coverage ratios between 3 and 6 with larger carriers (approximately 200 μm) that had irregular surfaces. These results imply that the size and the morphology of the carrier as well as the addition of fines in adhesive mixtures are important variables that can interact with each other and have a marked impact on the formulation. For a more in-depth review of the variables at play in adhesive mixtures, the reader is referred to the work of Grasmeijer et al. [33]. Although adhesive mixtures with lactose as carrier material are established on the market as safe inhalation formulations, improvements in terms of the aerosolization performance can be expected in the near future due to a better understanding of the effects and interactions of the variables in adhesive mixtures.

In addition to being used as a diluent and flowability enhancer in adhesive mixtures, sugars are used as stabilizers in spray-dried inhalation formulations, since they are known to protect biopharmaceuticals during drying and subsequent storage. The two theories on the mechanism behind the stabilization of biopharmaceuticals by sugars in the solid state are the water replacement theory and the vitrification theory. The latter theory is based on vitrifying the biopharmaceutical in a glassy sugar matrix (Fig. 3B), and thereby slowing down degradation. The concept of stabilizing biologics in a glassy sugar matrix was first introduced in 2000 as the PulmoSol™ technology, by Nektar Therapeutics (formerly known as Inhale Therapeutic Systems) [6,34]. For elaboration on the PulmoSol™ technology and refinement of glass stabilization theories, the reader is kindly referred to the work of Healy et al. [34] and Mensink et al. [35], respectively. Lactose is among the sugars that have been used as stabilizers in spray-dried inhalation formulations. For instance, lactose has been used, together with ciprofloxacin and leucine (at a 1:1:1 weight ratio), in

spray-dried phage PEV20 combination formulations [36,37]. However, the use of lactose as a stabilizer in spray-dried biopharmaceutical formulations is not recommended due to the fact that lactose is a reducing sugar and may, therefore, cause a Maillard reaction with amines, which are abundantly present in biopharmaceuticals. For this reason, non-reducing sugars (e.g., trehalose) are most commonly used as stabilizers in spray-dried inhalation formulations.

3.2. Mannitol

Mannitol, a sugar alcohol, has been investigated as an alternative to lactose in inhalation powders. Unlike lactose, mannitol is a non-reducing compound and is, therefore, compatible with APIs that contain amines. However, mannitol typically rapidly crystallizes after spray-drying and is therefore not ideal as stabilizer. Notwithstanding, in spite of its low glass transitions temperature, mannitol can be used as stabilizer when formulated with other excipients that increase the system's glass transition temperature sufficiently or inhibit crystallization through other mechanism (e.g. inorganic salts, glycine, salmon calcitonin, other sugar derivatives) [38]. This was exemplified by Exubera®, the first approved inhalable human insulin formulation comprising mannitol, sodium citrate, glycine, and sodium phosphate as excipients [39]. Notwithstanding, addition of multiple excipients to a formulation will typically introduce more complexity to the formulation, which is undesirable.

Recently, Hertel et al. [40] compared a commercially available spray granulated mannitol carrier (Parteck® M DPI) with a commonly used lactose carrier (InhaLac® 120) in adhesive mixtures. To this end, the authors produced blends with API concentrations of 0.1-4 wt-% with two different APIs, i.e., salbutamol and budesonide. Aerosolization studies showed that the FPFs with mannitol as carrier and salbutamol as API were similar to those achieved with lactose. By contrast, compared to lactose, higher FPFs were obtained with mannitol as carrier and budesonide as API. These results imply that spray granulated mannitol could be a potential alternative to lactose as carrier material in adhesive mixtures.

The use of mannitol has also been described for spray-dried inhalation formulations [41–44], in which mannitol mainly functions as a readily water-soluble microparticulate matrix. For instance, Lee et al. [43] co-spray-dried bosentan with mannitol at different weight ratios (3:1; 1:1; and 1:3). Of these formulations, the 1:1 weight ratio appeared to be the most optimal formulation in terms of aerosolization performance (FPF of 51.68 ± 6.20% and MMAD of 1.91 ± 0.07 μm). Moreover, the authors showed that the addition of mannitol at a 1:1 weight ratio increased the cumulative *in vitro* dissolution during 60 minutes with approximately 40% compared to raw bosentan. Similarly, others have used mannitol as a microparticulate matrix for so-called 'nanocomposite microparticles' [41,42,44]. These nanocomposite microparticles generally consist of drug-loaded nanoparticles, which on their own are too small to deposit in the lungs, embedded in a microparticulate matrix (Fig. 3C). The micro-size of the matrix and its high water solubility make pulmonary delivery of the embedded nanoparticles possible. A major advantage of using mannitol for this purpose is that mannitol is less hygroscopic than other sugars (e.g., lactose).

Although mannitol is a substance that is generally recognized as safe [45], it is worth noting that mannitol is currently used in cumulative doses of up to 635 mg for osmotic bronchial provocation tests in order to identify bronchial hyper-responsiveness. Bronchial hyper-responsiveness is a key feature of asthma [46]. Therefore, it is not recommended to use high amounts of mannitol, or other excipients creating a hyperosmolar environment, as NBE in inhalation powders for the treatment of asthma. Nevertheless, mannitol is used in a clinical study as excipient for an antibody fragment dry powder formulation (CSJ117) to treat asthma, currently 'recruiting' in phase 2b [47], after passing phase 1 [47]. In addition, mannitol has been approved as add-on maintenance therapy (after passing a tolerance test) to improve the pulmonary function of patients ≥ 18 years of age with cystic fibrosis [48]. For this indication, patients inhale 400 mg mannitol twice a day [48], implying the safety of mannitol in case of non-hyperresponsiveness.

3.3. Trehalose

Trehalose is a non-reducing disaccharide composed of two glucose molecules, which has a relatively high glass transition temperature of ~106 °C (in comparison, sucrose has a glass transition temperature of ~60 °C) [49]. Due to these characteristics, trehalose often functions as a stabilizer for biopharmaceuticals in spray-dried inhalation powders by forming a glassy sugar matrix (Fig. 3B). While the addition of trehalose may improve the stability of biopharmaceuticals during drying and subsequent storage, its use also introduces challenges. As Nieto-Orellana et al. [50] discuss, an amorphous trehalose matrix is hygroscopic and cohesive, which may severely limit the dispersion of the powder formulation into respirable particles. Therefore, trehalose is often combined with other excipients (e.g., leucine) in spray-dried inhalation powders. Over the past two years, trehalose has been mentioned in several publications as NBE for the preparation of inhalation powders [25,50–53]. For example, Faghihi et al. [51] used trehalose to stabilize infliximab during spray-drying in order to prepare an inhalation powder. In this study, trehalose was combined with Tween 20 (a surfactant) and cysteine (as hydrophobic component). The optimal formulation, consisting of 30 mg infliximab, 36 mg trehalose, 12 mg cysteine, and 0.05% Tween 20, resulted in an FPF of 67.75 ± 1.29%. However, the yield was relatively low at approximately 40%. Nevertheless, the authors showed that the optimal formulation was more potent than a formulation containing only infliximab and Tween 20, demonstrating that trehalose (combined with cysteine) acted as a stabilizer. Similarly, Nieto-Orellana et al. [50] used trehalose as stabilizer for the preparation of a spray-dried protein complex for pulmonary delivery. However, the authors showed that the addition of leucine was required to prevent agglomeration.

Keil et al. [52]compared trehalose with mannitol (at 5% or 10% (w/v)) as matrix for spray-dried polyethyleneimine (PEI)/DNA polyplex nanoparticles in order to prepare nanocomposite microparticles (Fig. 3C). The authors showed that the use of 10% trehalose or mannitol resulted in a matrix that allowed for nanoparticle reconstitution without a significant change in primary particle diameter compared to freshly prepared nanoparticle solutions. Powder characterization revealed that the nanocomposite microparticles

containing 10% trehalose had a significantly lower MMAD (3.17 ± 0.21 μm) than the mannitol-containing nanocomposite microparticles (4.67 ± 0.13 μm). Additionally, the trehalose formulation yielded a slightly higher FPF (72.6 ± 3.4%) than the mannitol formulation (67.5 ± 1.3%). Cellular uptake and transfection studies showed that reconstituted trehalose-containing nanocomposite microparticles performed equally or better than freshly prepared nanoparticles that were not spray-dried, depending on the ratio PEI/DNA. With this, the authors showed that trehalose was an excellent stabilizer for a spray-dried nanocomposite microparticle formulation with satisfactory aerodynamic properties.

Despite their potential as a stabilizer for biopharmaceuticals in spray-dried inhalation formulations, a major drawback of amorphous sugars is hygroscopicity after spray-drying. This makes trehalose-containing formulations prone to recrystallisation and cohesiveness during handling and storage. Therefore, trehalose will likely have to be combined with other excipients, in particular moisture protectors (e.g., (tri)leucine). While trehalose is generally recognized as safe as a food ingredient [54], trehalose is not yet included in the inactive ingredient list of the FDA as excipient for inhalation [8]. Trehalose is used as excipient in the aforementioned CSJ117 [55], with ongoing recruitment for a phase 2b trial [47]. Furthermore, it is worth noting that the FDA is currently reviewing a New Drug Application of a dry powder formulation of treprostinil. The formulation contains ~93% trehalose dihydrate by weight, among other excipients [56]. It was well-tolerated in phase 1 [57] to phase 3 [58] trials, with a pulmonary exposure of up to ~105 mg trehalose dihydrate per day, for at least two months [59]. This suggests trehalose is a safe pulmonary excipient.

3.4. Pullulan

Pullulan, a rigid polysaccharide, has been explored as stabilizer for biopharmaceuticals in freeze-dried formulations. Pullulan has a very high glass transition temperature (~261 °C), which, even when exposed to 90% RH for 72 hours, remained well above room

temperature [60]. Additionally, pullulan's rigidity promotes vitrification of biopharmaceuticals [35]. In spray-dried inhalation formulations, pullulan is not readily explored. However, recently Carrigy et al. [53] produced several amorphous pullulan trehalose blends (mass ratios of 0:100; 5:95; 10:90; 17:83; 20:80; 30:70; 40:60; and 100:0) by spray-drying. Scanning electron microscopy images of the powders showed non-uniform microparticles that increased in surface folding with increasing amounts of pullulan, which may be explained by the fact that pullulan enriched at the surface of the microparticles. This surface enrichment of pullulan was corroborated by differential scanning calorimetry, which revealed that the surface of the microparticles was characterized by a higher glass transition temperature than the interior of the microparticles. The formulations showed aerosolization characteristics suitable for pulmonary delivery, with an average ED of 93-94%, a total lung dose of 37-46%, an FPF of 33.6-40.1%, and an MMAD of 2.16-2.38 μm, depending on the ratio pullulan:trehalose. By increasing the pullulan:trehalose ratio, the pullulan enrichment and thus the glass transition temperature of the surface could be increased, potentially protecting against higher temperature and relative humidity. In another study, Carrigy et al. [61] compared bacteriophage stability after spray-drying with different combinations of leucine, trileucine, trehalose, and pullulan. While the authors did not assess aerosolization behavior, they found that surface-enriching excipients with high glass transition temperatures (i.e., pullulan) outperformed the conventional shell-former leucine in bacteriophage stabilization. Since biopharmaceuticals also often reside at microparticle surfaces, due to their large size and low diffusion coefficient, the addition of pullulan may be beneficial for the physical stability of the biopharmaceutical and the microparticles in general.

Overall, pullulan shows great potential as NBE in inhalation formulations. Due to its high glass transition temperature, pullulan may hold advantages over other surface-enriching excipients, such as the previously discussed (tri)leucine. Like trehalose, pullulan is generally recognized as safe in the United States [62] and has a history of safe use for over 30 years as a food additive in Japan [63]. Notwithstanding these facts, caution is advised when extrapolating oral toxicity to pulmonary toxicity. If pullulan is to be used as

an excipient in a commercialized pulmonary formulation, toxicology studies are warranted, as pullulan is only listed in the inactive ingredient list of the FDA for oral administration [8].

4. Lipids

Lipids are a type of NBE that can be found in marketed inhalation dry powders. For example, magnesium stearate is used to partially dry-coat the surface of lactose carriers in several marketed adhesive mixtures via mixing (Fig. 4A) and API particles via co-jet-milling (Fig. 4B). In addition, phospholipids are currently mainly used to prepare liposomes (Fig. 4C). These liposomes are generally embedded in a microparticulate matrix composed of other excipients (e.g., sugars) (Fig. 3C) to generate liposomal inhalation dry powders.

<Figure 4 here>

4.1. Magnesium stearate

Magnesium stearate is a synthetically produced salt composed of two molecules stearic acid, which is a saturated fat found in various animal and vegetable fats, and the essential mineral magnesium. Currently, magnesium stearate is used in several dry powder inhalation products (e.g., Breo® Ellipta®, Foster® NEXThaler®, and Seebri™ Neohaler® [64–66]) as a so-called 'force control agent'. The addition of magnesium stearate to lactose carrier particles prior to blending with micronized API particles reduces the adhesive forces between the lactose carrier particles and micronized API particles by partially coating the surface. This improves the detachment of micronized API particles from the lactose carrier particles and, consequently, improves aerosolization performance (Fig. 4A).

In addition, magnesium stearate has been investigated to mechanically dry-coat API particles via co-jet-milling to improve the aerosolization performance of high-dose inhalation powders [14,15] (Fig. 4B). For instance, Mangal et. al [15] co-jet-milled

ciprofloxacin with different contents of magnesium stearate (0; 0.5; 1; 5; and 10% (w/w)). The authors showed that co-jet-milling ciprofloxacin with ≥ 5% (w/w) magnesium stearate significantly increased the FPF compared to jet-milled ciprofloxacin from 62.1% to approximately 66.5-67.5% of the recovered drug. Above 5% (w/w) magnesium stearate, the FPF plateaued, and below 5% (w/w) magnesium stearate the FPF slightly increased compared to jet-milled ciprofloxacin, however, not significantly. Interestingly, the authors revealed with X-ray photoelectron spectroscopy and time-of-flight secondary ion mass spectrometry that magnesium stearate primarily acted by reducing interparticulate interaction forces by the formation of low surface energy coating films. Similarly, Sibum et al. [14] co-jet-milled isoniazid with different contents of magnesium stearate (0; 0.1; 0.5; 1; and 2% (w/w)). Dispersion studies with the Twincer® dry powder inhaler showed that the FPF of the formulation increased with an increase in magnesium stearate content. Hence, compared to jet-milled isoniazid, the highest FPF increase of approximately 65% was obtained with the formulation containing 2% (w/w) magnesium stearate. However, it should be noted that above 1% (w/w) magnesium stearate the FPF appeared to plateau. The results also showed that the addition of magnesium stearate (regardless of the content) significantly decreased powder retention in the inhaler, resulting in an improvement of the emission. Altogether, these results imply the potential of magnesium stearate for the preparation of high-dose inhalation powders via co-jet-milling. A content of 2-5% (w/w) magnesium stearate appears to be sufficient for maximum improvement of the aerosolization performance of co-jet-milled formulations.

Nonetheless, when using magnesium stearate as excipient, its compatibility with APIs should be carefully considered. Indeed, as thoroughly reviewed by Li & Wu [67], there are several things to consider when including magnesium stearate in a formulation, not only its compatibility with APIs, but also compatibility of common impurities found in magnesium stearate batches with APIs, increased alkalinity resulting in increased susceptibility of the API to degradation, and other metal ion-mediated degradation reactions. Some reported examples are interaction of ibuprofen with magnesium oxide, a

common magnesium stearate impurity [68], and magnesium ion-mediated degradation of fosinopril sodium that was tableted with magnesium stearate [69].

The amount of magnesium stearate in approved adhesive mixtures is relatively low, as the maximum content per unit dose for inhalation enlisted in the inactive ingredient list of the FDA is only 80 μg [8]. Given this maximum content, a total powder dose of only 1.6-2 mg could be administered if magnesium stearate were to be used at a content of 2-5% (w/w) in a marketed high-dose inhalation powder. Therefore, additional toxicology studies will likely have to be performed for the use of magnesium stearate in high-dose inhalation formulations. Toxicity concerns of magnesium stearate at higher doses are mainly due to its limited water solubility (40 μg/ml at 25 °C [70]), potentially resulting in lung accumulation upon chronic administration. However, Baritussio et al. [71] showed that the solubility of magnesium stearate in bronchoalveolar lavage fluid was approximately five times higher than in water (233.1 μg/ml and 48.9 μg/ml, respectively, after 24 h at 37 °C) due to its interaction with lung surfactant. Based on these results, and given that the volume of the alveolar lining layer is roughly estimated to be 20-40 ml [72], the alveolar lining layer could dissolve 4.66-9.32 mg magnesium stearate after 24 h. Therefore, the relatively low water solubility of magnesium stearate may not be a limiting factor for the use of higher doses of magnesium stearate in inhalation products. However, further research is required to confirm this.

4.2. Phospholipids

Phospholipids are a class of NBEs endogenous to the human body, present in all cell membranes, and a primary constituent of pulmonary surfactant. Phospholipids owe their amphipathic nature to two hydrophobic ‘tails’ which are derived from fatty-acids and bound by a glycerol group and to a phosphate group as hydrophilic ‘head’. The phosphate group may be conjugated with organic compounds, such as choline and glycerol. Over the last decades, phospholipids have been broadly explored as particle-engineering excipients, especially in the context of particle density control. The PulmoSphere^{TM} technology, which

is at the basis of many marketed dry powder inhalation formulations, exemplifies DPPC use in this context. For in-depth information regarding this application of phospholipids, we refer the reader to reviews by Vehring [6] and Healy et al.[34]. Furthermore, phospholipids have gained increasing attention as constituent of solid lipid nanoparticles, used as delivery systems for the current mRNA SARS-CoV-2 vaccines. Nevertheless, in DPI formulations, phospholipids have mainly been investigated as excipient for hollow lipid nanoparticles, particularly liposomes. Liposomes may generally be described as spherical vesicles composed of concentric phospholipid bilayers and an aqueous core (Fig. 4C). Liposomes can be generated from phospholipids endogenous to the lungs, hence showing good biocompatibility, and can be used to carry a wide range of drugs. The amphiphilic characteristics of a liposome enables encapsulation of hydrophilic APIs in its aqueous compartment(s), as well as incorporation of hydrophobic APIs in its phospholipid bilayer(s). In addition, liposomal formulations can improve API stability, reduce API toxicity, minimize pulmonary clearance, and increase API retention time [73]. For these reasons, liposomes have been extensively studied as pulmonary drug delivery systems. Indeed, over the past two years, several phospholipids were studied to formulate a variety of compounds into liposomal dry powders, such as flavonoids [73], antimicrobial drugs [74–76], and chemotherapeutics [77,78] .

For example, Yu et al. [74] developed several liposomal DPI formulations via ultrasonic spray-freeze-drying (USFD), for local co-delivery of ciprofloxacin and colistin. These two APIs show synergistic antimicrobial activity towards multi-drug resistant bacteria, such as *Pseudomonas aeruginosa*, a pathogen responsible for pulmonary infections, in particular in cystic fibrosis patients. The authors generated liposomes using cholesterol (Chol) and several phospholipids, namely hydrogenated soybean phosphatidylcholine (HSPC), 1,2-distearoyl-*sn*-glycero-3-phosphoglycerol sodium salt (denoted as DSPG), and *N*-(methylpolyoxyethyleneoxycarbonyl)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine sodium salt (denoted as PEG). A solution of HSPC:DSPG:PEG:Chol in chloroform with a mass ratio of 3:1:0.5:1.7 was used as basis, which was further processed into a liposomal suspension. To maintain liposomal integrity

during USFD, mannitol and sucrose were used as cryo- and lyoprotectants. Leucine was used to improve aerosolization performance. The optimal formulation yielded decent rehydrated encapsulation efficiency values of ciprofloxacin and colistin (44.9 ± 0.9% and 47.0 ± 0.6%, respectively), as well as satisfactory aerosolization performance (FPF of 45.8 ± 2.2% and 43.6 ± 1.6%, respectively; ED of 97.0 ± 0.5% and 95.0 ± 0.6%, respectively). Furthermore, there was no observed reduction in pulmonary cell viability due to free drugs, blank liposomes, or drug-loaded liposomes. Reconstituted liposomal ciprofloxacin/colistin formulations showed a slightly reduced antimicrobial activity in colistin-resistant *Pseudomonas aeruginosa* isolates, compared to a fresh ciprofloxacin/colistin solution. Notwithstanding, monotherapy of colistin showed no antimicrobial activity and monotherapy of ciprofloxacin was less effective than either fresh or liposomal combination therapy. With this, the authors demonstrated that a liposomal dry powder may function as a drug delivery system for co-administration of antibacterial drugs, as local treatment of drug-resistant pulmonary infections. Since designing and characterizing a liposomal DPI formulation was the aim, the authors did not compare 'simple' binary spray-dried colistin/ciprofloxacin powder blends with the USFD liposomal formulation. Comparing for example pulmonary toxicity, drug-retention, and anti-microbial activity between different types of powder formulations may have provided insight into potential advantages of liposomal powder formulations. In a similar study, Gomez et al. [76] developed liposomal formulations containing amphotericin B (AmB), an antifungal drug. Pulmonary fungal infections are common in susceptible patients, such as those suffering from cystic fibrosis. Generally, a problem associated with AmB is self-association. AmB as monomer mainly targets its therapeutic target, ergosterol, in fungal cell membranes. However, AmB agglomerates have increased affinity for cholesterol, which is found in mammalian host cell membranes. This leads to reduced activity as well as increased toxicity and severe side-effects. Including AmB in lipid-based dispersions has been shown to reduce toxicity and increase the therapeutic index due to reduced self-associations[79]. Indeed, currently all commercial AmB formulations are lipid-based dispersions for infusion. Therefore, the authors hypothesized that incorporation of AmB in liposomal dry powders may both reduce

AmB self-association and allow for local administration, in case of pulmonary fungal infections. Synthetic dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylglycerol (DPPG) were used as NBEs. Interestingly, natural DPPC as well as DPPG are endogenous to the lungs as main constituents of pulmonary surfactant. Co-spray-drying DPPC/DPPG (3:1 molar ratio) and AmB in a 9:1 molar ratio at varying pump rates yielded dry powders that were further investigated and compared to spray-dried AmB in methanol. Generally, spray drying at a high pump rate resulted in the best aerosolization performance for all formulations. Co-spray-dried AmB/DPPC/DPPG yielded a higher ED than spray-dried AmB (74.5 ± 9.9% and 66.6 ± 21.3%, respectively) while FPFs (46.8 ± 5.4% and 47.3 ± 20.0%, respectively) and MMADs (2.2 ± 0.1 µm and 2.0 ± 0.0 µm, respectively) were very similar. The authors concluded that incorporating AmB in a liposomal formulation reduced AmB self-association, compared to free AmB. This claim was substantiated by a trend of dose-dependent increased toxicity in H358 cells due to incubation with free AmB, which was not observed for liposomal formulations. However, it is worth noting that A549 cell viability was not impacted by free AmB, except in the highest concentration of 100 µM. Unfortunately, the authors did not assess antifungal activity, nor did they study the impact of empty DPPC/DPPG liposomes on cell viability.

Overall, short pulmonary retention times are a hurdle in pulmonary drug formulation. Indeed, as extensively reviewed by Wright & Clements [80], endogenous phospholipids are characterized by rapid metabolism and a high turnover (i.e., renewal) rate, for the alveolar regions as well as the whole lung. Besides phagocytosis, uptake and storage by pneumatic type II cells are thought to be primarily responsible for surfactant clearance and recycling. Pulmonary surfactant is constantly cleared and replaced, with turnover times of phosphatidylcholine, from type II cells into alveoli, estimated to be ~10 h, for several species. [81] Due to this constant renewal, short pulmonary retention time of exogenous phospholipids, and in extension liposomes, may be problematic. Nevertheless, DLPC and DPPC based liposomes were found to be relatively slowly cleared in healthy humans, with 79 and 83% found in the lungs, respectively, 24 h post-inhalation [82].

Interestingly, Yu et al. [73] found that DPPC administration elicited lung-protective effects in rats with induced acute lung injury. Total protein in bronchoalveolar fluid and pulmonary edema, which are both markers for acute lung injury, were significantly reduced. In addition, super oxide dismutase activity was upregulated in DPPC-treated rats, suggesting suppression of oxidative stress. These results indicate that DPPC, and possibly other phospholipids, may have intrinsic lung-protective properties during acute lung injury, making them very interesting as drug delivery systems. In spite of DPPC and DPPG being endogenous, neither are listed in the inactive ingredient list of the FDA for pulmonary use. This is remarkable, as DPPC is used as primary excipient (up to 8% w/w) in a recently FDA-approved levodopa inhalation powder (Inbrija®; CVT-301). Indeed, in a phase 3 clinical trial, treatments with CVT-301 were found to be safe and well tolerated, with daily DPPC exposure up to 4 mg [83]. Moreover, DPPC is also used as primary excipient in an approved liposomal nebuliser dispersion, which results in inhalation of roughly 275 mg DPPC per daily dose [84,85]. Distearoylphosphatidylcholine (DSPC), another frequently explored and endogenous phospholipid, is included in the inactive ingredient list with a maximum potency per unit dose of 6.4 mg, for respiratory use [8]. Long-term pulmonary safety of DSPC has been confirmed in cystic fibrosis patients, using the PulmoSphere™ based TOBI® Podhaler® [86]. It is worth noting that, while DPPC and DSPC may be considered inactive and relatively safe, when formulated as liposomes these compounds may still evoke an immune response, as reviewed by Weers [87].

5. Biodegradable polymers

Biodegradable polymers are used in inhalation dry powders in order to achieve sustained release (Fig. 5A) and/or targeted (Fig. 5B) formulations. Generally, these formulations consist of drug-loaded polymer-based microparticles, or nanoparticles that are embedded in a microparticulate matrix composed of other excipients (e.g., sugars) (Fig. 3C). Frequently used biodegradable polymers are poly(lactic-*co*-glycolic acid) (PLGA) and chitosan.

<Figure 5 here>

5.1. PLGA

PLGA is a synthetic biodegradable and biocompatible copolymer that is composed of lactic and glycolic acid monomers. The degradation rate of PLGA into lactic and glycolic acids by hydrolysis, and therefore the release rate of the incorporated drug(s), can easily be tuned by changing the lactic:glycolic acid ratio, the molecular weight, and the end-capping of the polymer. PLGA has been used as NBE to prepare drug-loaded microparticles [88]and drug-loaded nanoparticles embedded in a microparticulate matrix [89,90] for pulmonary delivery. For example, Nurbaeti et al. [90] prepared chloramphenicol- and thiamphenicol-containing PLGA nanoparticles using an emulsion-solvent evaporation method. In order to generate respirable particles, a suspension of the obtained drug-loaded PLGA nanoparticles was spray-dried with lactose as bulking agent and leucine as dispersion enhancer. This resulted in a drug load of 13.9 ± 3.0% and 21.0 ± 0.7% (w/w), an FPF of 27 ± 13% and 36 ± 10%, and an MMAD of 3.3 ± 0.5 μm and 2.8 ± 0.3 μm for chloramphenicol and thiamphenicol, respectively. *In vitro*, the formulations showed a sustained release profile with a cumulative release of approximately 90% after 14 days.

The sustained release from PLGA particles can be attributed to the relatively slow degradation rate of PLGA (Fig. 5A). However, this slow degradation may require long residence time of PLGA-based microparticles in the lungs. Consequently, lung clearance mechanisms may greatly limit efficacy of PLGA-based microparticles, as the particles may be cleared before embedded API can be released. Nevertheless, when PLGA was used as matrix for large porous particles (diameter > 5 μm and tapped density < 0.4 g/cm^3), phagocytosis was circumvented, resulting in several days of sustained insulin release following inhalation. [91]

Nevertheless, although PLGA has been used in several sustained-release drug products that have been approved by the FDA (e.g., Zoladex®; a goserelin-containing PLGA implant that is administered subcutaneously), it is yet to be used in an approved drug

product for inhalation. As a consequence, characterization of the toxicity profile of a PLGA-containing inhalation formulation is mandatory prior to potential market approval. It should be noted that PLGA degrades into acidic degradation products, i.e., lactic and glycolic acids, which may irritate and/or damage the lungs. As a matter of fact, an important cause of acute respiratory distress syndrome (a serious lung condition causing low blood oxygen) is acid aspiration-induced lung injury following gastric reflux [92]. It has been shown that acid aspiration-induced lung injury is primarily mediated by the recruitment of neutrophils to the lungs by interleukin-8 [92,93]. Therefore, the assessment of interleukin-8 levels may be of special interest in toxicity studies for inhalable PLGA-containing formulations.

5.2. Chitosan

Chitosan is a non-toxic biodegradable linear polysaccharide, manufactured by deacetylation of chitin. Chitin is abundantly found in nature, for example as a component of fungal cell walls and crustacean shells. Structurally, chitosan is composed of randomly distributed β-1,4-linked D-glucosamine and *N*-acetyl-D-glucosamine units. Due to its polycationic nature, chitosan is highly mucoadhesive (Fig. 5B). Furthermore, chitosan is known to interact with mannose receptors expressed on macrophages [94]. Therefore, chitosan may be used to increase pulmonary retention of inhalation formulations, making it an interesting NBE for targeted and controlled-release pulmonary drug delivery. Furthermore, owing to its positive charge, chitosan particles are prone to self-assemble with negatively charged compounds. Over the last two years, chitosan has been explored as NBE to facilitate targeting of alveolar macrophages for potential local treatment of tuberculosis (TB) [94,95], as DNA carrier for potential gene therapy [96], and as (nanoparticulate) inhalation formulation excipient in general [97,98].

The application of chitosan for pulmonary delivery of anti-TB drugs was demonstrated by Changsan & Sinsuebpol [94]. They showed that by adding the multivalent anion tripolyphosphate (TPP) to an isoniazid/pyrazinamide/chitosan solution, while

694 continuously homogenizing, a nanosuspension of drug-embedded particles spontaneously
695 formed. Subsequently, the nanosuspension was freeze-dried using a 10% mannitol (w/w)
696 matrix, generating a dry powder of drug-loaded chitosan nanoparticles. A formulation with
697 a 1:3 weight ratio of TPP:chitosan showed the best aerosolization performance. With an
698 MMAD of 3.37 ± 0.05 µm and 3.28 ± 0.07 µm, an FPF of 43.95 ± 1.34% and 41.03 ±
699 0.92%, and an ED of 93.28 ± 1.28% and 95.03 ± 0.23% for isoniazid and pyrazinamide,
700 respectively, this formulation was within the size range suitable for pulmonary delivery.
701 Unfortunately, the authors did not assess drug release or permeability *in vitro*. In a similar
702 study, Mukhtar et al. [95] generated and studied isoniazid-loaded hybrid nanoparticles.
703 Chitosan was hybridized with a negatively charged polysaccharide, i.e., hyaluronic acid
704 (HA), and subsequently loaded with isoniazid. HA is another biodegradable polymer and a
705 ligand for CD44 receptors. Since alveolar macrophages with overexpressed CD44 receptors
706 are more susceptible to *Mycobacterium tuberculosis* infection [99], the authors
707 hypothesized that HA may further improve targeted delivery of isoniazid. Isoniazid-loaded
708 chitosan/HA nanoparticles had an MMAD of 2.59 µm and FPFs of 61.53% (< 5 µm) and
709 46.86% (< 3 µm) from a Breezhaler®. In addition, incorporation of isoniazid into
710 chitosan/HA nanoparticles allowed for slow and controlled release. Non-thiolated isoniazid
711 nanoparticles released 63% of isoniazid after 48 h, whereas free isoniazid was nearly
712 completely dissolved after 5 h. Unfortunately, neither Changsan & Sinsuebpol nor Mukhtar
713 et al. studied alveolar phagocytosis, which would have been an interesting next
714 experimental step.

715 Changsan & Sinsuebpol [94] as well as Mukhtar et al. [95] found that subjecting
716 different respiratory tract cell lines to chitosan-based nanoparticles did not reduce cell
717 viability, nor did it induce proinflammatory cytokine expression. Notwithstanding, it was
718 shown that inhalation of chitosan microparticles resulted in a dose-dependent
719 proinflammatory response in rat lungs, as evidenced by significant increases in
720 bronchoalveolar lavage fluid protein content, lactate dehydrogenase activity, and leukocyte
721 migration to lung tissue [100]. Moreover, as chitosan is not included in the inactive

ingredient list of the FDA [8], toxicology studies will be required before incorporation in a marketed pulmonary formulation.

6. Discussion and conclusions

In this review, we have described four main groups of natural and bioinspired excipients. We focused on applied as well as fundamental research, on both established and experimental excipients, published over the past two years.

Amino acids, specifically leucine and the tripeptide trileucine, were mainly assessed to enhance powder dispersibility and provide protection against moisture uptake. Their effect on these formulations can be remarkable, even at a few weight percent. Therefore, these excipients will likely play an important role in future DPI developments. This will mostly be for particularly cohesive and hygroscopic drugs.

Sugars were broadly explored as drug-carrying diluent, as stabilizer, and as surface-enricher. Increasing fundamental understanding of lactose may reduce the need for additional dispersion enhancing excipients, such as magnesium stearate, in adhesive mixtures. Moreover, mannitol may be a viable alternative to lactose in such mixtures. Trehalose was mainly explored as stabilizing matrix for biopharmaceuticals. Similarly, pullulan was used as a stabilizer in spray-dried powders in which it enriches at particle surfaces during spray drying. With the fraction of marketed biological drugs continuously increasing, particles with a higher glass transition temperature towards the particle surface are desirable, as biologics typically tend to migrate to the surface during particle formation. This makes pullulan an interesting alternative to more conventional shell-formers like (tri)leucine. Notwithstanding, hygroscopicity of sugar-glass stabilized formulations may prove challenging.

Lipids and lipid-like excipients are abundantly found in marketed inhalation dry powders. Co-jet-milling API particles with small amounts of magnesium stearate (2-5% w/w) was shown to markedly reduce inhaler powder retention and increase FPFs, by reducing inter-particle adhesive forces. Phospholipids were studied as a constituent of dry

751 powder liposomes, which can be used to incorporate both hydro- and lipophilic drugs.
752 Additionally, incorporation into liposomes potentially reduces drug toxicity and may
753 improve drug stability and pulmonary retention time. However, practically, liposomal dry
754 powder formulations may still be far removed from commercialization, especially for
755 highly dosed drugs. For example, the final product as described by Yu et al., contains
756 ~4.8% ciprofloxacin hydrochloride (w/w), that is encapsulated for ~45%, following
757 ultrasonic spray-freeze-drying. This results in ~2.2% ciprofloxacin (w/w) encapsulated in
758 liposomes. To achieve a total daily lung dose similar to liposomal ciprofloxacin
759 hydrochloride (Lipoquin®) of 25 mg [87], the nominal dose must contain roughly 2.5 g of
760 dry powder, taking into consideration the reported ED of 97% and FPF of 45%. In practice,
761 this would not be feasible at all and would moreover result in high excipient exposure.
762 Comparatively, Bayer's ciprofloxacin dry powder, which incorporated ciprofloxacin in its
763 neutral form in Pulmospheres™, allows a BID regimen that results in a total lung dose of
764 33.8 mg/day, with a total excipient exposure of only 22.9 mg/day [87]. Nevertheless, we
765 believe liposomal dry powders may still be viable for highly potent drugs, as toxicity,
766 enzymatic degradation and a short pulmonary retention time remain major challenges in
767 pulmonary drug delivery, which liposomes may help to (partly) overcome.

768 A short pulmonary retention time may also be overcome by the use of
769 biodegradable polymers. To this end, PLGA has been mostly studied as matrix to facilitate
770 sustained drug release. Similarly, chitosan was explored in the context of alveolar
771 macrophage targeting and increasing pulmonary retention time, among other applications.
772 Nevertheless, pulmonary accumulation, and consequently toxicity, remains a major concern
773 when using polymers.

774 Indeed, pulmonary clearance is both a challenge and a risk to be overcome, for most
775 of the discussed excipients. As reviewed by Geiser [101], particles that are deposited in the
776 conducting (i.e., upper) airways are cleared through mucociliairy clearance in 24-48 h.
777 Particles that are within the range of 1 to 5 µm, and reach the respiratory (i.e., lower)
778 airways are primarily phagocytosed or absorbed by epithelial cells. Phagocytosis is a rapid
779 process, with 90% or more of particles thought to be phagocytosed within 10 h after

deposition. Subsequently, phagocytosed particles may translocate to the conducting airways, though the mechanisms of this 'mucociliairy escalator' remain poorly understood. [101] Nonetheless, phagocytosis can be circumvented by formulating API in large porous particles, to escape the 1 to 5 μm size range. [91] However, it should be noted that clearance of phagocytosed particles deposited deeply in alveolar regions may take weeks to months, when they are not moved by the mucociliairy escalator to the conducting airways. [101] Clearance may be similarly slow for particles that are deposited in the deep lung but are not absorbed nor phagocytosed there. Therefore, when studying new excipients, pulmonary clearance and potential bioaccumulation, and consequently toxicity, should be strongly considered. To some extent, this is true for established FDA-approved excipients as well, depending on their particle size distribution and consequential lung deposition pattern. Unfortunately, clearance is generally a neglected topic in fundamental and experimental small-scale pulmonary research, especially (and understandably so) when performing in vitro and ex vivo experiments, warranting more focus in future research.

Overall, by administering drugs through inhalation, common patient-related challenges, such as fear of needles or dysphagia, may be overcome. For manufacturers, stabilizing drugs in a dry solid state may help in circumventing the costly and logistically challenging cold-chain, thereby greatly improving sustainability, as well as increasing their reach and efficiency in global drug distribution. Importantly, by formulating drugs in an inhalation formulation, pulmonary diseases could be treated locally, concurrently increasing drug deposition in target tissue and reducing toxicity related to high systemic exposure. However, information on pulmonary toxicity of excipients is generally lacking. Whereas toxicity studies may be costly, increasing toxicological knowledge of excipients may greatly accelerate advancements in the field of pulmonary therapeutics; with toxicity data available, manufacturers may be incentivized to consider pulmonary drug delivery as potential route of administration. To achieve these goals, better mechanistic understanding of excipients, and when and how to combine them, is of paramount importance. Overall, we believe formulating drugs in an inhalation dry powder has great potential, from a patient's as well as from a manufacturer's perspective.

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In this study, the authors demonstrate that magnesium stearate and L-leucine improve the aerosolization performance of co-jet-milled DPI powders by different mechansims. With this, the fundamental understanding of the critical effects of magnesium stearate and L-leucine in DPI formulations is improved.

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The authors demonstrated that co-spray-drying trehalose with 30% (w/w) L-leucine protected trehalose from moisture-induced recrystallization, while concurrently reducing aerosolization performance. These results suggest careful consideration of physical stability and aerosolization peformance when including L-leucine in a DPI formulation.

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Figure captions

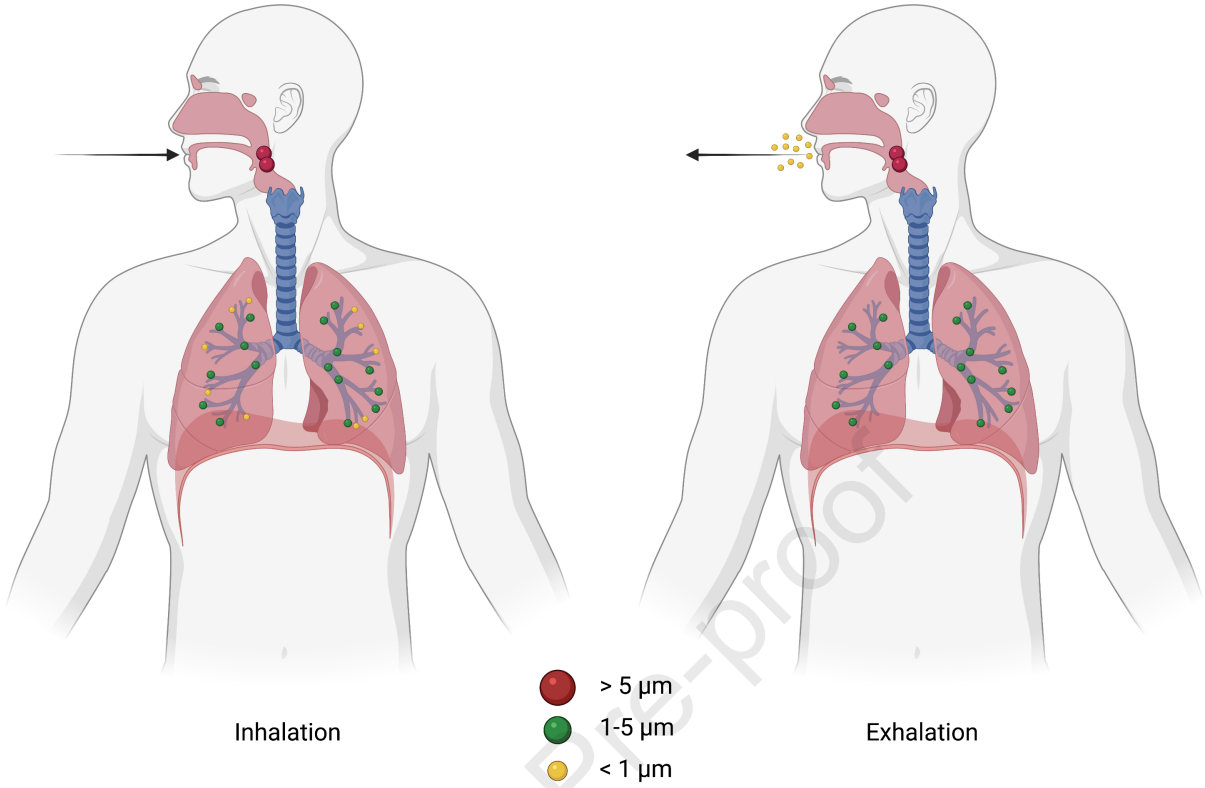
Figure 1. Deep lung deposition of particles with aerodynamic diameters of 1-5 μm (green). Generally, particles > 5 μm (red) impact on the oropharynx, while the bulk of particles < 1 μm (yellow) is exhaled.

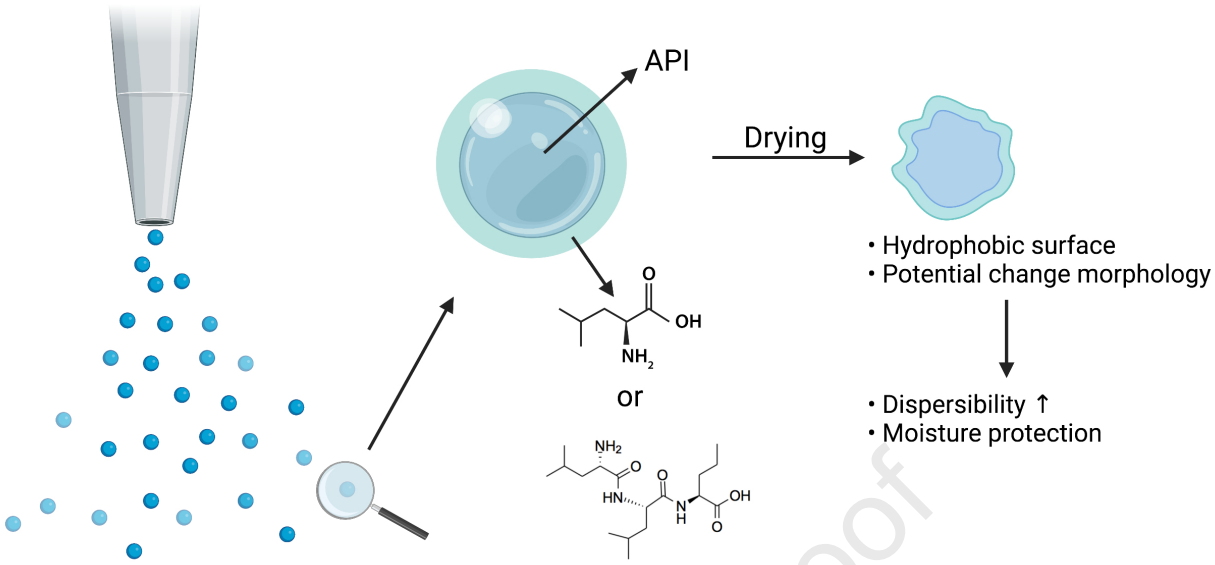
Figure 2. Use of (tri)leucine in spray-dried inhalation dry powders. Surface enrichment of (tri)leucine during spray-drying changes the surface composition and potentially the morphology of the resulting particles.

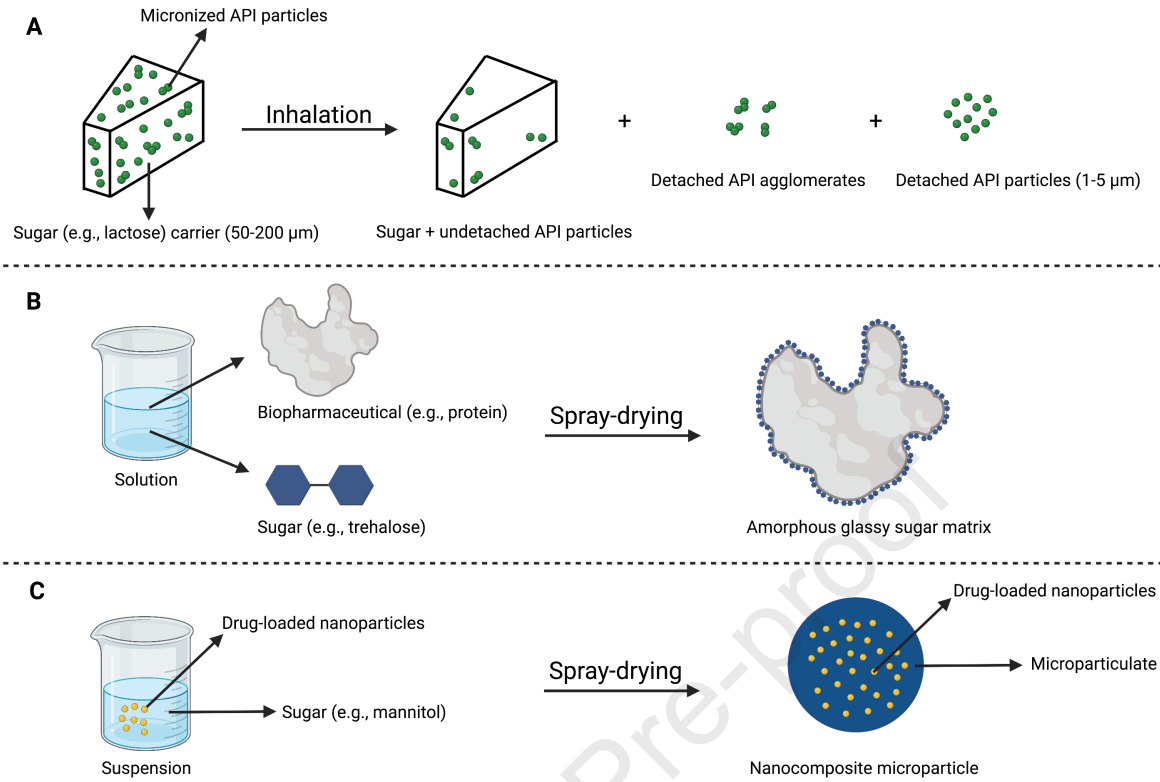
Figure 3. Use of sugars in inhalation dry powders. As diluent and powder flow enhancer for micronized API particles (A), as stabilizer for biopharmaceuticals in spray-dried inhalation powders (B), and as microparticulate matrix for nanoparticles (C).

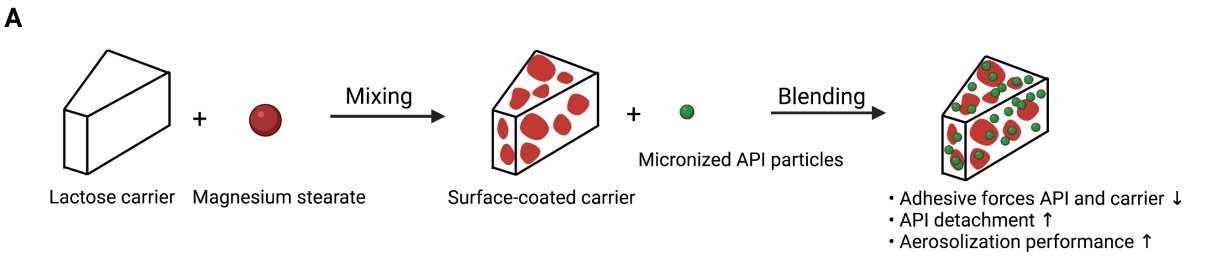
Figure 4. Use of lipids in inhalation dry powders. Magnesium stearate to partially dry-coat the surface of lactose carriers in adhesive mixtures via mixing (A) and API particles via co-jet-milling; adapted from [14] (B), and phospholipids to prepare liposomes (C).

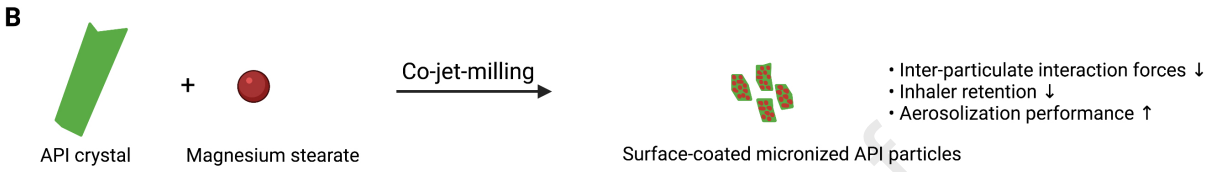
Figure 5. Use of biodegradable polymers in inhalation powders. Biodegradable polymers are used for sustained release (A) and targeted (B) micro- or nanoparticles.

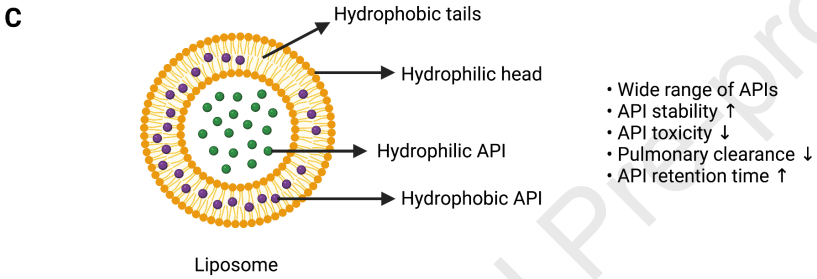


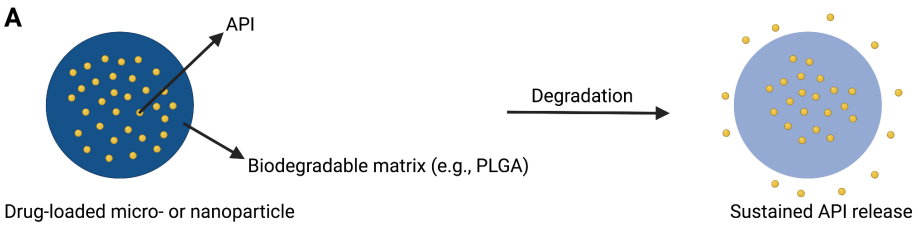


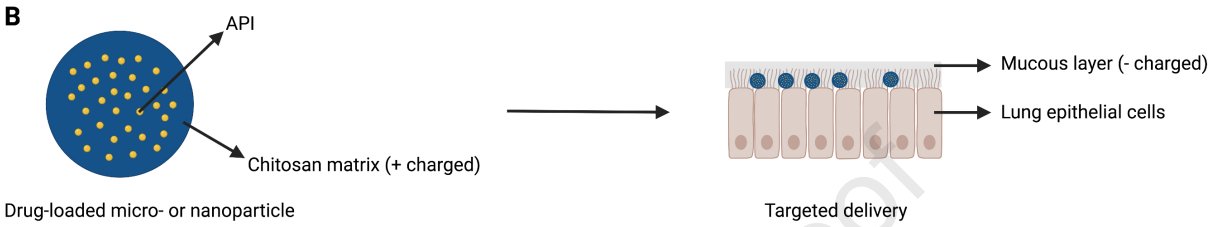












Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

PureIMS, the employer of F. Grasmeijer, is the manufacturer of the Twincer and Cyclops inhalers. The employer of D. Zillen, M. Beugeling, W. Hinrichs and H.W. Frijlink has a license agreement with PureIMS on the Twincer and Cyclops inhalers and is funded by DFE Pharma GmbH & Co. KG for D. Zillen's PhD track. Neither of both companies played a role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.