Where the periphery matters: Protective excipients investigated in dry powder formulations of inhaled biomacromolecules

Appreciation of excipient properties and multifunctional roles is essential in formulation development

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Introduction

Biological drugs, also known as biologics and biopharmaceuticals, have become an indispensable therapeutic modality in the treatment of a wide variety of diseases. In contrast to small molecule drugs, biologics are derived from living organisms and encompass a broad spectrum of complex macromolecules ranging from blood components and antitoxins to vaccines and allergenics [1]. In the context of this article, biologics are defined as drugs with peptidic backbones. Prominent members of this large protein/peptide family are monoclonal antibodies (mAbs) in their array of formats, while non-mAb members include enzymes, hormones, and peptides [2].

Between 2016 and 2020, about a quarter of the novel drugs approved by the United States Food and Drug Administration (US FDA) were such biologics [3]. The majority of biopharmaceuticals are formulated for parenteral administration, primarily via the intravenous, subcutaneous and intramuscular routes. Notwithstanding the excellent bioavailability granted by this invasive means of administration, other methods of drug delivery that may be more suitable and acceptable do exist.

Oral inhalation is one such alternative and is a feasible route for delivering biologics [4]. As a non-invasive route, oral inhalation presents several advantages, including deposition on an extensive pulmonary vasculature and ease of administration [5]. Furthermore, inhalation allows the drug to be delivered directly to the target site in respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD).

This article provides an overview of the considerations for the formulation development of inhaled biologics and the various excipients that are routinely investigated for use in dry powder formulations of biologics intended for oral inhalation, with particular emphasis on protein stabilization.

Formulation development of inhaled biologics

Biological macromolecules, or biomacromolecules, can be inhaled into the lungs as liquid or powder aerosol. Liquid aerosol may be generated from metered dose inhalers (MDIs), soft mist inhalers or nebulizers, the latter of which include jet, mesh and ultrasonic types [6, 7]. However, these delivery platforms may not be practical because prolonged storage of biomacromolecules in aqueous media can compromise physical stability [8]. MDIs also contain a propellant, hydrofluoroalkane, which could potentially denature proteins [9]. On the other hand, biomacromolecules delivered as powder aerosol dispersed from a dry powder inhaler (DPI) are in the solid state, a phase that promotes stability [10]. A significant challenge in the manufacture and storage of biologics is protein instability, in particular, protein aggregation, which may result in loss of pharmacological activity and increased risk of immunogenicity [11]. Indeed, proteins in the liquid state are less complicated to process and undergo faster development [12]. However, formulating proteins in solutions is still problematic considering important degradation mechanisms, including aggregation, fragmentation, deamidation and oxidation, occur hydrolytically [13]. Moreover, storing and transporting proteins in liquid form often demands the cold chain, a formidable obstacle in medicines supply recently thrust into the international spotlight by COVID-19 vaccines.

Table 1

Potential excipients for inhaled dry powder formulations of biomacromolecules

Class	Example	Mechanism of Stabilization	Remarks
Monosaccharides	Glucose	• Water replacement • Vitrification	Reducing sugar
Disaccharides	Lactose		Reducing sugar; FDA-approved inactive ingredient for inhalation
	Trehalose		High Tg
Oligosaccharides	β-cyclodextrins		Also displays surfactant-like effects
	Inulin		Weak water replacer
Sugar Alcohols (Polyols)	Mannitol		Low Tg; FDA-approved inactive ingredient for inhalation
	Sorbitol		Low Tg
Amino Acids	Arginine Cysteine Glycine Leucine Lysine Phenylalanine	• Water replacement	Only glycine is FDA-approved inactive ingredient for inhalation
Surfactants	Polysorbate 20 Polysorbate 80	• Inhibition of protein unfolding at interfaces	Only polysorbate 80 is FDA-approved inactive ingredient for inhalation

Tg: glass transition temperature

Three popular technologies employed for drying inhaled biopharmaceuticals are spray-drying, spray-freeze-drying and supercritical fluid drying [14]. Each of these are accompanied by their pros and cons. For instance, all three methods subject labile biomacromolecules to shear during atomization when the feed solution is pumped through the nozzle. The mechanical damage combined with the propensity of proteins as amphiphilic molecules to adsorb at the expansive air/liquid interface created during atomization may alter protein conformation [15]. Spray-drying exposes proteins to heat, since the solvent is removed by evaporation, which can lead to thermal denaturation [16]. Spray-freeze-drying is associated with freezing stress that causes cold denaturation. The impact on the protein molecules is further aggravated by a rise in the solute concentration and a pH shift in the buffer as the solution freezes [17].

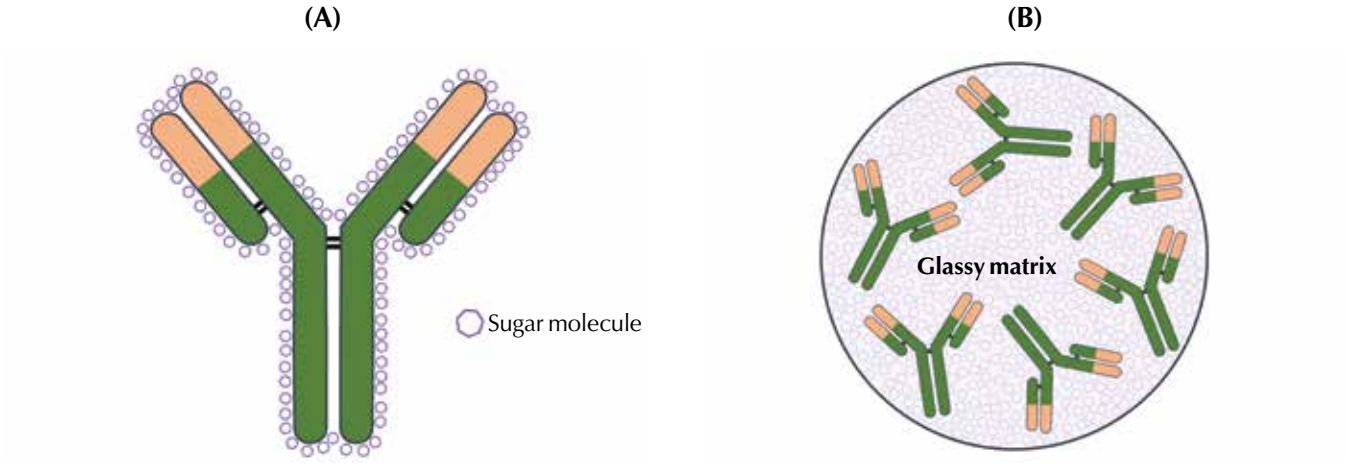
A common strategy to tackle the various stresses experienced by biomacromolecules as they become dehydrated is to incorporate at least one protein protectant (or more precisely referred to as stabilizer) into the formulation as an excipient (Table 1). On top of serving their unique role to shield protein molecules from stresses, the excipients in inhaled biopharmaceutical products can function to increase bulk and therefore facilitate handling of potent active ingredients, where quantities involved are relatively small [18]. Since excipients in inhaled dry powder formulations double up as the bulking agent, their inhalation-specific characteristics, namely, particle aerosolization performance, aerodynamic diameter, surface morphology and hygroscopicity, would substantially influence pulmonary administration efficiency [19]. In addition, universally desired attributes expected of excipients, such as inertness, compatibility with the active drug and packaging, physical and chemical stability, and low cost [20], apply to inhaled excipients as well. Of note, inhalation toxicology data represent the key barrier to the development of excipients in inhaled therapeutics [18]. The challenges of manufacturing technologies and of characterization methods for inhaled biologics have been extensively reviewed [21].

Carbohydrates

The carbohydrates frequently utilized as protein stabilizers in inhaled powder formulations containing biologics are broadly classified into three groups: simple sugars, complex sugars and sugar alcohols. Simple sugars consist of monosaccharides and disaccharides, while complex sugars are made up of oligosaccharides (3 to 10 monosaccharide units joined together via glycosidic linkages) and polysaccharides. Sugar alcohols, as the name implies, are alcohols. They are derived from monosaccharides through the reduction of the carbonyl functional

Figure 1

Schematic depiction of water replacement (A) and vitrification (B) mechanisms of stabilization by a sugar, using an immunoglobulin G as a model protein (not drawn to scale). In (A), the sugar molecules interact with the protein and reduce local mobility, whereas in (B), global mobility is hindered as the macromolecules are embedded in a glassy matrix formed with the sugar.



group to a hydroxyl group. Therefore, they are also termed polyols and bear close resemblance to sugars in regard to biological, chemical and physical aspects [22]. In fact, some sugar alcohols even taste sweet, a feature that may be exploited to assess whether a patient is able to execute the correct inhalation technique for deep lung delivery [18].

There are two longstanding classic theories on the mechanism of stabilization by sugars on proteins in the solid state: water replacement and vitrification (Figure 1). The water replacement theory involves the formation of hydrogen bonds between the protein and the hydroxyl groups of the sugar or polyol. Since this substitutes the pre-existing hydrogen bonds between the protein and water molecules, the conformation of the protein remains unchanged, even in the absence of water after drying [10]. In contrast, the vitrification hypothesis encompasses the immobilization of the protein within a restrictive amorphous matrix. By limiting global movement of the protein, detrimental processes such as unfolding and degradation are profoundly impeded [23]. In short, both mechanisms preserve native protein structure, but the water replacement theory is based on thermodynamics (equilibrium) and inhibiting alterations to the structure of the protein, while the vitrification theory is related to reaction kinetics and reduction in molecular mobility [24]. Although a protein-to-sugar mass ratio between 1:1 and 1:5 is generally considered adequate to confer stability, the optimal ratio is protein-specific and must be determined [25].

In order for the sugar to sustain long-term stabilization during storage, it is imperative that the storage temperature is below the glass transition temperature (Tg) of the powder [25]. Tg is a property of sugar glasses, above which the immobilizing and stabilizing ability of the sugar is drastically attenuated [26]. Besides, at temperatures higher than the Tg, the sugar is in the rubbery phase and may crystallize. Crystallization is destructive to stabilized proteins because the proteins would suffer from shear stress and a loss of protein/carbohydrate interactions [27]. Amorphous solids with a weak predisposition to crystallize are thus preferred in stabilizing dehydrated biologics [24]. At storage temperatures more than 20 °C below the Tg, water replacement dominates, provided that satisfactory vitrification is achieved. However, at temperatures near or over the Tg, proteins are heavily reliant on vitrification for stability [28]. A high inherent Tg that would translate to a Tg decently above the storage temperature in the formulation is therefore favorable. In addition, sufficient drying (e.g., < 1% residual moisture) is vital as the Tg is dramatically decreased by water [10, 29].

Simple sugars

Among the licensed DPIs, the most frequently encountered excipient is lactose [18], a disaccharide comprising the monosaccharides galactose and glucose, linked by a glycosidic bond [30]. The rationale for using lactose is that it may be conveniently and inexpensively obtained, and has evident safety whether inhaled or swallowed [31]. A drawback of using reducing sugars like lactose and glucose is Maillard browning, which is directly relevant to peptidic and proteinaceous molecules, as it involves a reaction between the carbonyl of a sugar and the amino group of a protein [32]. The concerns with this glycation of the protein are that it synthesizes a new chemical entity, generates water which lowers the Tg and may exacerbate aggregation [10].

A worthy disaccharide successor to lactose is trehalose. Being non-reducing, it is not susceptible to

Maillard reaction [32]. Trehalose possesses several other advantages such as high Tg [33], low hygroscopicity [34] and dual cryoprotective/lyoprotective roles [32]. It has been investigated as a protein stabilizer in spray-dried formulations of infliximab with cysteine [35], in spray-freeze-dried formulations of IgG alone and in combination with other carbohydrates [36]. In these studies, trehalose demonstrated the capacity to stabilize mAbs and produce powders of acceptable aerosolization. In spite of its seemingly innocuous chemical composition of two glucose monomers, trehalose is not yet an approved excipient for the respiratory route [37].

Complex sugars

In general, small carbohydrates can attain more intimate hydrogen bonding with the protein, while using larger carbohydrates will improve vitrification [38]. If a small sugar has an undesirably low Tg, an oligosaccharide or a combination of simple and complex sugars may be considered [39]. Compared to simple sugars, complex sugars are less extensively studied for use in dry powder formulations of inhaled biologics. Inulin is a polysaccharide that has been investigated in a spray-dried formulation of an influenza subunit vaccine containing a glycoprotein. The fine particle fraction (FPF; aerodynamic diameter < 5 μm) was 37%, and the powder formulation was physically stable for longer than three years at 20 °C. Moreover, the immunoglobulin G (IgG) titers elicited by pulmonary immunization were higher than those by intramuscular immunizations in mice [40].

More recently, cyclodextrins have been explored for inhaled biotherapeutic formulations. They are oligosaccharides with a cyclic structure; the interior space is hydrophobic while the exterior surface is hydrophilic. Cyclodextrins are known to form complexes with drugs, including proteins, through noncovalent interactions in the hydrophobic core. Three types of cyclodextrins are utilized in pharmaceutical formulations based on the number of glucopyranose rings: α (6), β (7) and γ (8) [30, 41]. β-cyclodextrin and its derivatives are notably attractive because the size of their cavity is exceptionally suited to aromatic amino acids [41].

A distinctive feature of cyclodextrins that gives them an edge over other protein stabilizers is that, while they embody cryoprotective and lyoprotective capabilities typical of sugar-based stabilizers, some cyclodextrins could also exhibit a non-ionic, surfactant-like behavior at very low concentrations (e.g., 0.1-1%.) [42]. The amphiphilic quality of such cyclodextrins, for instance, hydroxypropyl-β-cyclodextrin, supports their adsorption at interfaces, which might facilitate the displacement of proteins from the interfaces, thereby preventing denaturation and subsequent aggregation. Furthermore, they normally exist as amorphous glasses [41].

There appear to be differences between the various forms of cyclodextrins with respect to lung toxicity, with natural γ-cyclodextrin and hydroxypropyl-β-cyclodextrin demonstrating tolerability in mice [43] and human bronchial cells [44]. Although no cyclodextrin has been approved for the inhalation route, β-cyclodextrins seem to be preferred for pulmonary delivery development given that a number of these have already received US FDA approval for the intravenous, subcutaneous, intramuscular, oral or topical routes [45]. Published studies of inhaled β-cyclodextrins relate mostly to small molecule drugs in the pre-clinical stage [46-48]. Nonetheless, pulmonary delivery of recombinant human growth hormone [49], insulin [50] and salmon calcitonin [51] with β-cyclodextrins has been studied in rats. A study investigating spray-freeze-dried IgG co-formulated with hydroxypropyl-β-cyclodextrin plus trehalose yielded spherical and immensely porous particles and an FPF (cut-off diameter < 6.4 μm) as high as 56% for one of the formulations [52].

Sugar alcohols

Sugar alcohols share many similarities with simple sugars, such as their mechanisms of stabilization, being proficient lyoprotectants and the ability to modulate against multiple causes of protein instability (i.e., aggregation, chemical degradation, denaturation) with a single agent [53]. Their metabolism is slower compared with simple sugars and they only exist as straight chains [22]. A sugar alcohol that has shown promise in the formulation of dry powder inhaled biological drugs is mannitol, having been incorporated into an approved powder form of insulin in the past. A non-reducing polyol [30], mannitol powder is approved by the FDA as an inactive ingredient for the respiratory route with a maximum daily exposure (MDE) of 6 mg [45].

Interestingly, beyond functioning as an excipient, mannitol is also used therapeutically and diagnostically. Mannitol inhalation powder is indicated to improve pulmonary function in patients with cystic fibrosis as an add-on maintenance treatment [54] by increasing mucociliary clearance [55]. As a diagnostic tool, mannitol inhalation powder is approved to identify bronchial hyper-responsiveness in a challenge test kit [56]. Mannitol is contraindicated in people who failed a tolerance test, and patients who have passed the test may experience bronchospasm during maintenance therapy. This necessitates premedication with an inhaled short-acting bronchodilator [54]. In view of the risk of bronchospasm with chronic inhalation of mannitol, the risk/benefit of its use as an excipient in inhaled formulations should be taken into consideration. Sorbitol, an isomer of mannitol, is less favored, as it is more hygroscopic and therefore more sensitive to humidity [18, 30]. A limitation of sugar alcohols is low Tg, which heightens the tendency to

crystallize especially when concentrated or exposed to high temperatures and moisture levels [53].

Amino acids

Next to carbohydrates, amino acids are another important class of excipients that are often evaluated as protein stabilizers in solid dosage forms. Among the numerous amino acids, only glycine, in powder form, has received FDA approval for the respiratory route, with an MDE of 2 mg [45]. Like mannitol, glycine is part of the cocktail of excipients present in an approved powder insulin for oral inhalation [57]. The mechanism of long-term protein stabilization by amino acids is the water substitution hypothesis described above [10]. They stabilize proteins from denaturation and thermal degradation pathways [58]. As an excipient in DPIs, certain amino acids, such as leucine and trileucine, are known to enhance aerosol performance of particles through the reduction of hygroscopicity and surface tension [59-61]. In solid dosage forms, amino acids (e.g., alanine) can act to inhibit crystallization that is detrimental to both the protein and its aerosol performance [62]. Since amino acids are endogenous compounds, there is less concern in relation to safety when inhaled. Nevertheless, despite the vigorous research into amino acids as protein stabilizers, the toxicity and pharmacokinetics of inhaled amino acids remain poorly understood [18].

A study compared the effects of arginine, cysteine, glycine, leucine, lysine and phenylalanine individually on the molecular/thermodynamic stability and aerosolization of spray-dried IgG [63]. The formulation containing 50% w/w phenylalanine exhibited the most inhibition of antibody aggregation (4.1% soluble aggregates vs. 13.6% for pure IgG; at 2 months, 45 °C), coupled with quite an impressive FPF (cut-off diameter < 6.4 µm) of 62%. The best aerosol performance was, however, accomplished by 50% w/w cysteine (around 71% FPF), which also produced the lowest aggregation rate. By contrast, arginine at both concentrations (20% and 50% w/w) invoked structural perturbation to the native antibody conformation and yielded the highest aggregation rate. With the encouraging aerosolization profile and the assumed pulmonary safety, amino acids are promising candidates as excipients for the delivery of biological drugs via the respiratory tract [30].

Surfactants

So far, stresses from the drying process that carbohydrates are reasonably effective in protecting proteins against—dehydration, thermal and freezing [64]—have been highlighted. Unfortunately, these are not all-inclusive. Spray-drying and spray-freeze-drying introduce air/liquid and solid/liquid interfacial stresses to protein molecules, respectively, and in both techniques the atomization is associated with shear stress [15]. Carbohydrates and amino acids do not offer proteins adequate protection from interfacial and shear stresses. To counter these stresses, surfactants are recruited [65]. They work by competing with proteins for adsorption at interfaces, thereby inhibiting protein unfolding and the ensuing aggregation [25, 41]. Polysorbates (Tweens) and sorbitan esters (Spans) are widely employed surfactants in inhaled liquid formulations, but are less appropriate in inhaled dry powder formulations, since surfactants tend to have a low melting point and exist as a liquid or semi-solid at room temperature [18]. The effective concentrations of surfactants are remarkably low (e.g., < 0.5% w/v); this would also ensure the Tg is not lowered by the surfactants [32].

Polysorbates, or polyoxyethylene sorbitan fatty acid esters, are non-anionic surfactants that have gained widespread utility in pharmaceutical formulations [18]. Four polysorbates are approved inactive ingredients according to the FDA—polysorbates 20, 40, 60 and 80—of which only polysorbate 80 suspension is listed for the respiratory route at a maximum potency per unit dose of 0.02% w/v [45]. For instance, approved products containing polysorbate 80 (polyoxyethylene 20 sorbitan monooleate) include an inhalation suspension of budesonide [66] and an inhalation powder of insulin [67]. Although not an excipient in inhaled therapeutic products, polysorbate 20 (polyoxyethylene 20 sorbitan monolaurate) is incorporated into dry powder preparations of biological drugs meant for reconstitution for injection. Lyophilized powders of the mAbs trastuzumab and omalizumab are such examples [68-69].

Conclusions

In dry powder formulations, excipients should constitute as little as possible in an inhaled product to avoid tolerability and safety problems and increased burden of therapy by having to inhale larger amounts of materials. Where sensitive biologics are concerned, the choice of protein stabilizer and drying method are crucial factors affecting stability of the dehydrated biomacromolecules. The lack of toxicity data may account for the scarce options in approved inactive ingredients for the inhalation route, a gap that is further widened by the rigmarole of securing a marketing authorization for the excipient. The pool of viable candidates is confined to substances that are biocompatible with and readily cleared from the respiratory system. With such a limited number of approved inhaled biologic products available in the market and no mAbs on this list, there is a pressing need to contemplate patient-oriented research to garner beneficial clinical and commercial outcomes.

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