Non-reducing sugars as excipients in spray-dried powders for inhalation

A brief introduction and discussion of several case studies

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Introduction

Generally, dry powder inhaler (DPI) formulations consist of either micronized drug solid-state particles (usually 1-5 µm) alone or physically mixed (blended) with large sugar carrier particles, usually 80-100 µm in size [1]. Spray drying is one of the most common techniques for engineering dry powders of drug/excipient mixtures as solid-state particles with the desired properties for efficient lung delivery as inhalable powders [2, 3].

The continuous spray-drying process involves the transformation of a liquid feed (a solution, suspension or emulsion) into a solid dry powder by passing an atomized spray of fine droplets through two steps: primary drying and secondary drying [4]. Manipulation of particle size, surface morphology, density and internal structure of the dry powder particles can be achieved by controlling multiple spray-drying parameters and feedstock properties such as the liquid feed type, concentration or rate, inlet air temperature, surface tension, flow of atomization, gas type and particle residence time [5].

In spray-dried formulations, various excipients or carriers are incorporated to improve the aerosolization behavior, stability and mechanical properties of active pharmaceutical ingredients (APIs) as well as to modify the pharmacokinetics and pharmacodynamics of APIs. The excipients used in spray-dried powder formulations are categorized into sugars, polyols, amino acids, salts, surfactants and polymers. Among them, sugars are the most common excipients used as diluents and flow enhancers. In addition, sugars can provide hydrogen bonding and act as product stabilizers when water is removed.

While formulating the inhalation powder and selecting a sugar excipient, the ability of the sugar to be reduced with the API must be considered. This characteristic affects the stability of the API in the finished product, rather than the morphology or behavior of the powder in the dry powder formulation. In fact, the Maillard reaction between reducing sugars and the amine groups in APIs can cause a brown discoloration and a decrease in API stability. Not all carbohydrates are reducing sugars. However, nearly all monosaccharides and some oligosaccharides (such as lactose) are reducing sugars [6].

Among the sugars, lactose is the most commonly used excipient in marketed dry powder inhaler products. However, due to its reducing nature (via the Maillard reaction), it is not suitable for stabilization of spray-dried powder formulations containing biological drugs with amines. Therefore, non-reducing sugars are recommended as stabilizers in spray-dried inhalation formulations.

This article describes non-reducing sugars used as excipients in spray-dried powders for inhalation and their mechanism for stabilization and provides examples of spray-dried powder formulations composed of non-reducing sugars.

Non-reducing sugars

The non-reducing sugars commonly used in dry powders for inhalation include mannitol, trehalose dihydrate, xylitol, sorbitol and raffinose. Their chemical structures are depicted in Figure 1.

Mannitol

Mannitol ($C_6H_{14}O_6$, molecular weight 182.17 g/mol), a sugar alcohol, is a non-reducing sugar with low hygroscopicity [7]. It has crystalline polymorphic forms, namely anhydrous α-, β- and δ-mannitol, and mannitol hemihydrate, a stoichiometric hydrate form. Mannitol is compatible with APIs that are moisture-sensitive and contain amine groups. It increases mucus clearance through its hyperosmotic effect and improves lung function in patients suffering from lung diseases such as cystic fibrosis [8]. Under certain spray-drying conditions, spray-dried mannitol has a very low glass transition temperature, below room temperature (T_g ~11 °C), rendering it in an amorphous, rubbery state with high molecular mobility that can lead to crystallization after spray drying [9]. Mannitol in combination with other excipients can increase the glass transition temperature, which is favorable for stabilization [10].

Trehalose dihydrate

Trehalose dihydrate (α,D-glucopyrannosyl-1,1-α,D-glucopyrannoside dihydrate, $C_{12}H_{26}O_{13}$, molecular weight 378.33 g/mol) is a non-reducing disaccharide composed of two glucose units joined by an α-1,1-glycosidic linkage. It exists as a stoichiometric crystalline hydrate. It was first isolated from the cocoon of the parasitic beetle *Trehala manna* in 1858. The glycosidic linkage between the two glucose moieties of trehalose makes it highly resistant to acid hydrolysis and very stable in aqueous solutions [11]. Upon spray drying, trehalose dihydrate produces an amorphous glass non-crystalline trehalose matrix with a glass transition temperature of 50 °C [12] and is suitable as a stabilizer in spray-dried formulations. A potential limitation when using higher amounts of trehalose (> 50% w/w) is hygroscopicity, which can lead to particle agglomeration and poor aerosolization. However, hygroscopicity can be addressed by using hydrophobic amino acid excipients such as leucine and trileucine. [2].

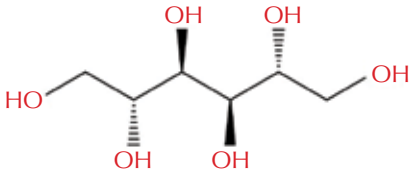
Xylitol

Xylitol ($C_5H_{12}O_5$, molecular weight 152.15 g/mol) is a polyol of pentitol type. Found in fruits, vegetables and mushrooms in very small amounts, it is produced commercially from lignocellulosic biomass [13]. It has been used as an alternative sugar to lactose in producing dry powder formulations [6, 14]. However, the major challenge in using xylitol as an excipient in spray-dried formulations is its hygroscopicity [6].

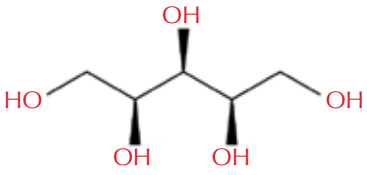
Morgan, et al. [15] evaluated the ability of three binary excipient blends (mannitol/dextran, lactose/

Figure 1

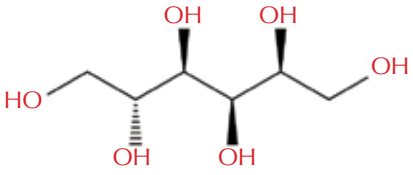
Chemical structures of non-reducing sugars used in spray-dried formulations. (Drawn using ChemDraw® Version 20.1.1.125, CambridgeSoft, Cambridge, MA, US.)



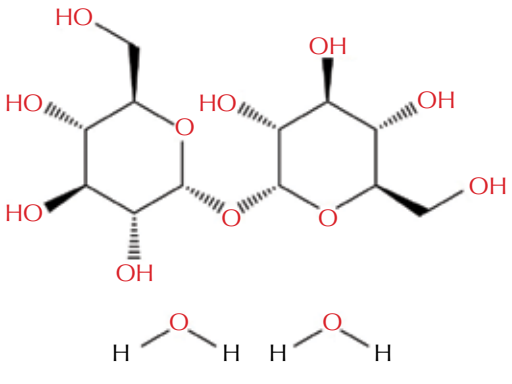
Mannitol



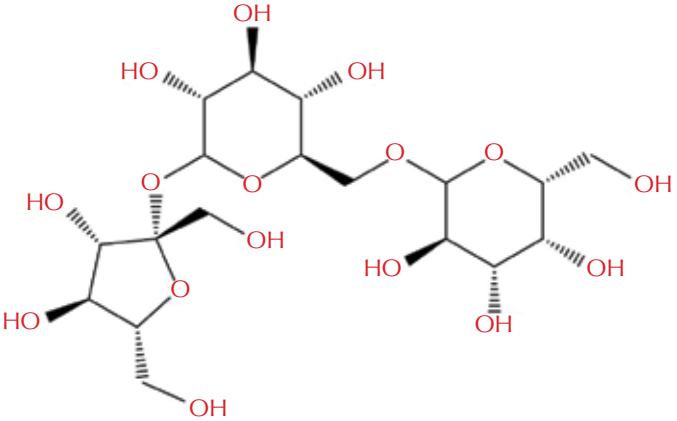
Xylitol



Sorbitol



Trehalose dihydrate



Raffinose

trehalose, and xylitol/dextran) to stabilize a human type 5 adenovirus. In the study, they produced dry powders using spray drying and single-droplet drying via acoustic levitation ("slow motion" spray drying). Spray drying of adenovirus using lactose/trehalose and mannitol/dextran showed a larger activity loss of viral vector. However, the xylitol/dextran combination showed excellent thermal stability and retention of viral activity for both spray-dried and levitated powders.

Sorbitol

Sorbitol (D-glucitol, $C_6H_{14}O$, molecular weight 182.17 g/mol) is a polyol, naturally found in fruits such as apples, cherries, pears and plums. It is produced commercially from glucose and corn syrup. Sorbitol may crystallize in two hydrated forms (hydrates I and II) and five anhydrous forms (A, B, Γ, Δ and E). Sorbitol is used as an inert carrier in DPI formulations and can improve formulation stability [16]. Like xylitol, it is hygroscopic in nature and must be incorporated with other excipients.

Raffinose

Raffinose ($C_{18}H_{32}O_{16}$, molecular weight 504.42 g/mol) is a trisaccharide composed of galactose, glucose and fructose. Naturally found in vegetables and whole grains, this non-reducing sugar is used as an excipient in spray-dried formulations to stabilize biomolecules [17].

Ógáin, et al. [17] produced spray-dried, nonporous microparticles (NPMPs) using hydrophilic excipients such as trehalose and raffinose and investigated the feasibility of incorporating a model protein, lysozyme, into the sugar-based NPMPs. The high glass transition temperatures of these sugar NPMPs (124 °C and 120 °C for trehalose and raffinose, respectively) indicate good stability and potential to be used as a protein stabilizer. The spray-dried composite NPMP particles composed of lysozyme with sugar (trehalose/raffinose) at a mass ratio of 1:4 were spherical in shape with pores < 90 nm in size. Both trehalose and raffinose containing composite NPMPs powders showed good aerosolization with high fine particle fraction (FPF) and similar lysozyme activity compared to the control (unprocessed lysozyme).

Mechanism of formulation stabilization by non-reducing sugars

The mechanism of stabilization of biopharmaceuticals by non-reducing sugars in spray-dried formulations is described by the water replacement theory and the vitrification theory [18]. The water replacement theory explains the stabilization of protein structure from a thermodynamic viewpoint. During spray drying, the hydroxyl groups of the stabilizing sugar molecules form hydrogen bonds with the protein/peptide, thereby replacing the hydrogen bonds between water molecules and the protein amino acid residues. Through this replacement, the native confirmation of the protein/peptide can be maintained in the dry powder form [19, 20]. The vitrification process involves amorphous, glass-forming sugar molecules that stabilize the protein/peptide molecules in a rigid, amorphous glassy matrix with a high glass transition temperature. The amorphous glassy matrix reduces molecular mobility and thereby, improves physical and chemical stability.

Studies of non-reducing sugars as excipients in spray-dried powder formulations

Mannitol study 1

D-mannitol, a known osmotic agent and non-reducing sugar alcohol, was employed as a sugar carrier in a dry powder for inhalation. When dry powder mannitol is inhaled, it forms an osmotic gradient that drives water molecules from the surrounding tissue into the viscous mucus layer in the airway lumen of cystic fibrotic lungs. This raises the water content, which reduces the viscosity of the thick viscous mucus, which is then removed by the mucociliary escalator.

Li, et al, [21] designed and characterized inhalable microparticulate/nanoparticulate dry powders of mannitol that had the particle properties needed for targeted dry powder inhaled delivery in cystic fibrosis mucolytic treatment. The study reported the interactions among advanced spray-drying conditions, mannitol crystalline polymorphic interconversion behavior and solid-state physicochemical properties, and their influence on *in vitro* aerosol dispersion deposition patterns. The spray-dried mannitol particles had favorable particle and surface characteristics to reduce interparticulate interactions and provide improved aerosol performance. There was a positive linear correlation with spray-drying pump rate and aerosol dispersion parameters, i.e., FPF and respirable fraction (RF) increased with pump rate.

The spray-dried mannitol crystalline powders were comprised of polymorphs (stability order: beta > alpha > delta). The stable alpha and beta polymorphic form content decreased with increasing unstable delta polymorphic form content as the pump rate increased. In addition, the aerosol dispersion performance of the spray-dried mannitol particles was influenced by the various spray-drying pump rates from organic solution in closed-mode.

Mannitol study 2

Lactose is the only carrier approved by the United States Food and Drug Administration (FDA) for use in dry powder inhaler formulations. However, lactose-free inhaled formulations could benefit asthmatic patients who are allergic to lactose. Muralidharan, et al. [22] used advanced organic solution spray drying in closed-mode to molecularly combine two drugs (fluticasone propionate and salmeterol) and

mannitol as an excipient to produce lactose-free, co-spray-dried dry powder formulations.

The resulting powders were solid-state nanoparticles and microparticles that had the physicochemical and aerosol properties necessary for inhaled delivery and would be expected to effectively target the mid-lung and deep-lung small airway regions (Figure 2). All the powders readily aerosolized and had excellent dispersion properties. Among the three spray drying conditions, the 100% pump rate produced improved particles with lower mass median aerodynamic diameter (MMAD) and greater FPF. Therefore, these DPI formulations could potentially be used to treat patients who have asthma or COPD as well as lactose allergy.

Trehalose study 1

The peptide hormone angiotensin (1–7) is a central agonist of the Mas receptor and a vital peripheral component of the renin-angiotensin system (RAS). The Ang (1–7)/Mas receptor system is a unique therapeutic strategy for the treatment of numerous vascular and neurological disorders, due to activation of this receptor in the central nervous system (CNS), which then stimulates a variety of biological

Figure 2

NGI stage deposition of co-spray-dried mannitol particles at 25%, 50% and 100% pump rate and Advair® Diskus® particles. (Reproduced from Reference 21 with permission from the Royal Society of Chemistry.)

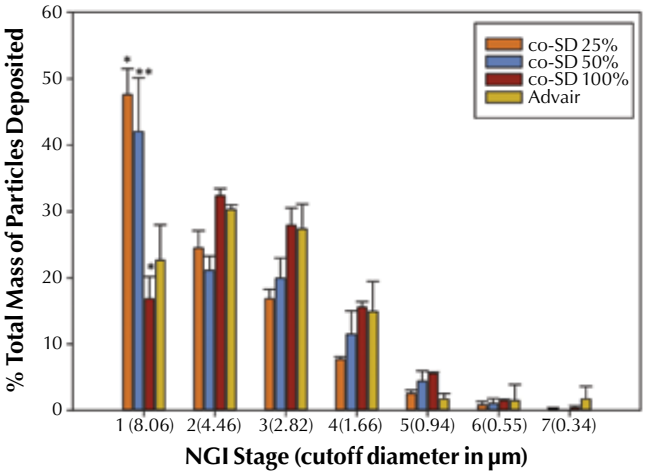
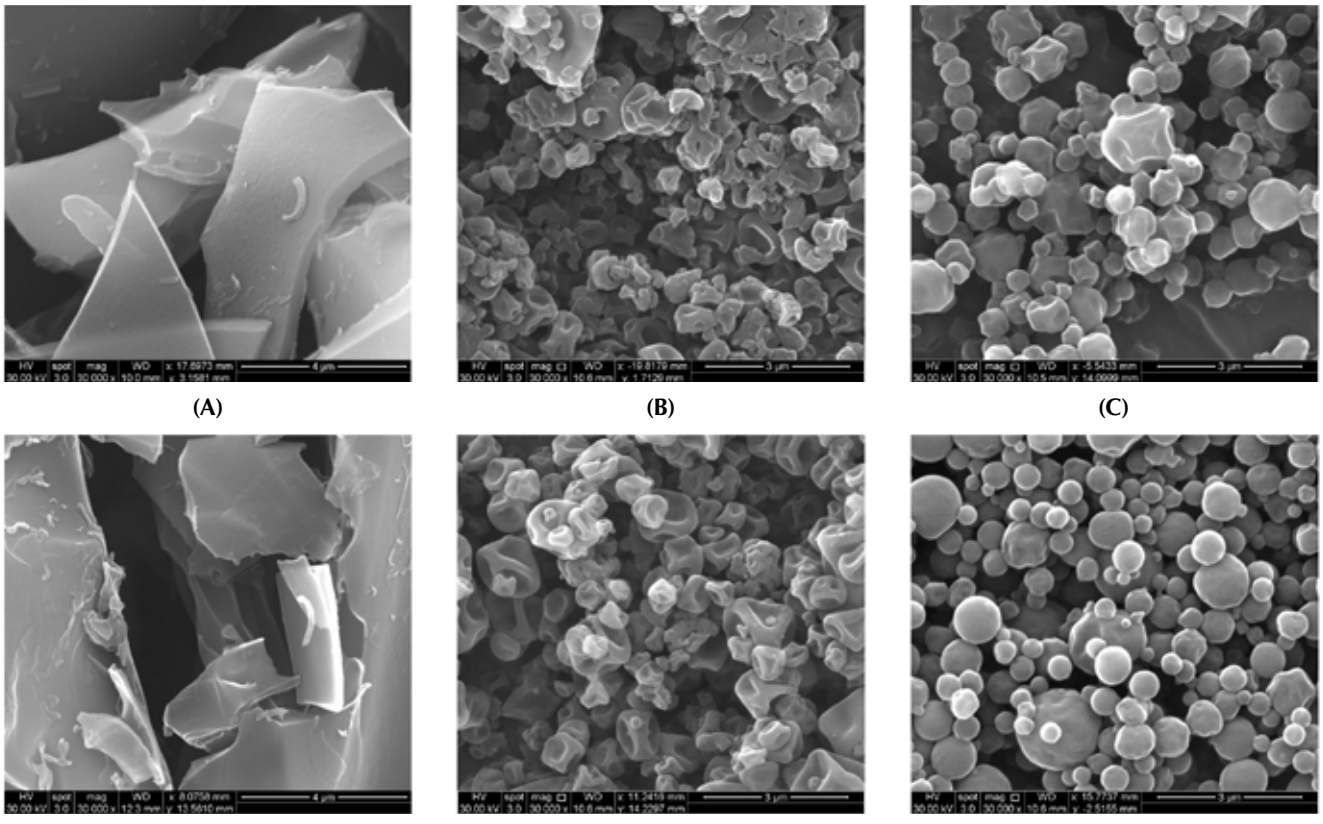


Figure 3

SEM micrographs (30,000×) of (A) Raw Ang (1–7) (unprocessed), (B) Spray-dried Ang (1–7), (C) Co-spray-dried Ang (1–7):Trehalose (25:75), (D) Raw PNA5 (unprocessed), (E) Spray-dried PNA5 and (F) Co-spray-dried Ang PNA5:Trehalose (25:75). (Reproduced from Reference 22 with permission from the MDPI.)



(A) (B) (C)

(D) (E) (F)

functions. When generating peptide particles for dry powder inhalation with the physicochemical properties needed for respiratory delivery, creating solid-state particles by spray drying offers several advantages. These include engineering of particles of desired size, shape, morphology and surface texture for better aerosolization performance, and large-scale production of powders with high batch-to-batch reproducibility.

Alabsi, et al. [23] used trehalose as an excipient at various sugar:peptide ratios in co-spray-dried systems of the Ang (1–7) peptide and PNA5, a glycosylated Ang (1–7) peptide derivative. The study demonstrated that Ang (1–7) and PNA5 co-spray-dried with trehalose formed small, smooth, spherical particles at a low pump rate. The favorable hydrogen interaction between the trehalose and peptide molecules in the solid state and the trehalose:peptide ratio affected the morphology of the particles, as shown in Figure 3.

Following organic solution advanced spray drying, physicochemical characterization showed retention of an amorphous glassy state with high glass transition and melting temperatures. Furthermore, the use of trehalose resulted in low residual water content in the powders (which reduced interparticulate interactions) and high aerosol dispersion performance as DPI formulations when tested with FDA-approved inhaler devices.

Trehalose study 2

Gomez, et al. [24] produced a spray-dried formulation of an adjuvanted tuberculosis vaccine (D93 + GLA-SE) using a trileucine/trehalose excipient combination. The spray-dried vaccine powder was stored at various temperatures (-20, 5, 25, 40 and 50 °C) over a one-year period and evaluated for stability of the powder, adjuvant and antigen. The spray-dried powder showed excellent physical stability with no significant change in *in vitro* aerosolization behavior after long-term storage at a temperature as high as 50 °C. The antigen in the control formulation was completely degraded after 7 months of storage at 40 °C. However, the spray-dried formulation containing trehalose/trileucine showed the presence of 45% antigen, even after one year of storage at 50 °C. This indicated the combination of trehalose with trileucine is a promising system for the development of thermostable, inhalable dry powder formulations of biological drugs.

Conclusion

This article has described the potential use of several non-reducing sugars as excipients to generate spray-dried powders and presented several case studies. Among the various non-reducing sugars, mannitol may be an alternative to lactose in dry powder inhaled formulations and is listed as a safe excipient for inhalation by the US FDA. It has already been approved as an active ingredient in Bronchitol® (Pharmaxis, New South Wales, Australia) and was an excipient in Exubera® (Pfizer, New York, NY, US). In the last several years, trehalose has been studied as an excipient to improve the stability of spray-dried formulations. The non-reducing nature and low chemical reactivity of these sugars offer advantages in spray-dried inhalable formulations. However, their potential drawback is hygroscopicity, which may require the addition of hydrophobic moisture protectors such as hydrophobic amino acids.

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