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Ashutosh Sharma, Pierre Cazade, Ambrose Hayden, Dikshitkumar Khamar, Damien Thompson, Helen Hughes

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On the role of excipients in biopharmaceuticals manufacture: Modelling-guided formulation identifies the protective effect of arginine hydrochloride excipient on spray-dried Olipudase alfa recombinant protein.

Ashutosh Sharma[1], Pierre Cazade[2], Ambrose Hayden[1], Dikshitkumar Khamar[3], Damien Thompson[2*], Helen Hughes[1*]

[1]Pharmaceutical and Molecular Biotechnology Research Centre (PMBRC), South East Technological University (SETU), Main Campus, Cork Road, Waterford, Ireland, X91K0EK.

[2]Department of Physics, Bernal Institute, University of Limerick, Limerick, Ireland, V94 T9PX

[3]Sanofi, Manufacturing Science, Analytics and Technology (MSAT), IDA Industrial Park Waterford, Ireland, X91TP27.

*Corresponding authors. Email: helen.hughes@setu.ie; damien.thompson@ul.ie

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Abstract

Biopharmaceuticals are labile biomolecules that must be safeguarded to ensure the safety, quality, and efficacy of the product. Batch freeze-drying is an established means of manufacturing solid

biopharmaceuticals but alternative technologies such as spray-drying may be more suitable for continuous manufacturing of inhalable biopharmaceuticals. Here we assessed the feasibility of spray-drying Olipudase alfa, a novel parenteral therapeutic enzyme, by evaluating some of its critical quality attributes (CQAs) in a range of excipients, namely, trehalose, arginine (Arg), and arginine hydrochloride (Arg-HCl) in the sucrose/methionine base formulation. The Arg-HCl excipient produced the best gain in CQAs of spray-dried Olipudase with a 63% reduction in reconstitution time and 83% reduction in the optical density of the solution. Molecular dynamics simulations revealed the atomic-scale mechanism of the protein–excipient interactions, substantiating the experimental results. The Arg-HCl effect was explained by the calculated thermal stability and structural order of the protein wherein Arg-HCl acted as a crowding agent to suppress protein aggregation and promote stabilization of Olipudase post-spray-drying. Therefore, by rational selection of appropriate excipients, our experimental and modelling dataset confirms spray-drying is a promising technology for the manufacture of Olipudase and demonstrates the potential to accelerate development of continuous manufacturing of parenteral biopharmaceuticals.

1 Introduction

Biopharmaceuticals provide new opportunities and challenges for manufacturing complex medicines that have massive potential to treat currently incurable diseases. For injectables, aqueous biopharmaceutical formulations are preferred but protein–water interactions can destabilize the protein, triggering biochemical degradation pathways such as hydrolysis, deamination, oxidation and aggregation of proteins (Carpenter et al., 2002). Hence, the controlled removal of water confers important benefits including improved stability, prolonged shelf-life, ease of storage, and reduced cost of transportation at ambient temperatures (Langford et al., 2017; Moeller and Jorgensen, 2008; Shalaev et al., 2008; Tang and Pikal, 2004b). To date, freeze-drying is the gold standard for manufacturing biopharmaceuticals but drawbacks include high operational and maintenance costs, reduced efficiency, challenges with respect to heat and mass transfer, scale-up and inability to produce free-flowing powder (Salnikova et al., 2015; Tang and Pikal, 2004a, 2004b). In order to overcome the disadvantages associated with conventional shelf-based freeze-drying, alternative drying technologies must be evaluated to develop safe and cost-effective ways

to manufacture biologics (Sharma et al., 2021). Spray-drying is a promising candidate for the biopharmaceutical industry as it is cost-effective, continuous, and improves production efficiency (Roser, 1991; Santivarangkna et al., 2007; Walters H. et al., 2014). This technique is particularly suited to thermolabile products as the evaporation process is very fast (taking from milliseconds to a few seconds) thereby minimizing exposure to high temperatures (Celik and Wendell, 2010). The production of millions of small droplets provides a large surface area for heat and mass transfer allowing rapid evaporation. However, factors such as uncontrolled dehydration, large increases in temperature, shear and protein adsorption at interfaces have been reported to affect the stability of proteins (Rajan et al., 2021).

To protect and preserve the stability of proteins during freeze-drying and spray-drying, a wide range of excipients such as amorphous saccharides, polyols, amino acids and surfactants have been explored (Narhi et al., 2012). Spray-drying technology has recently been successfully applied to antibody-based formulations (Duran et al., 2021; Fiedler et al., 2021; Massant et al., 2020; Pan et al., 2022; Shepard et al., 2022, 2021; Tejasvi Mutukuri et al., 2021). Four main mechanisms have been reported that partly explain the stabilizing effect of different excipients during protein dehydration (Ajmera and Scherließ, 2014; Elversson and Millqvist-Fureby, 2005). The first proposed mechanism is the "water replacement hypothesis" which states that stabilizers such as sucrose and trehalose form hydrogen bonds at specific sites on the surface of the protein during dehydration. As water is removed from the protein solution, the hydrogen bonds between the protein and water molecules are disrupted. The hydrogen bonds formed between the stabilizer and protein create a water-like environment that stabilizes the native structure of the protein (Allison et al., 1999; Carpenter et al., 1994; Kreilgaard et al., 1999; Starciuc et al., 2020). Protein–protein interactions are decreased upon addition of sugars, reducing protein aggregation (Costantino et al., 1994). The alternative "glass dynamics mechanism" proposes that amorphous stabilizers such as sucrose, trehalose, glucose and raffinose form a rigid, inert matrix around protein molecules wherein the motion of the protein is coupled to the motion of the matrix, thereby limiting its structural relaxation and preserving the tertiary structure (Chang et al., 2005; Green and Angell, 1989; Ying at al., 2012; Zhao et al., 2018). By contrast, the theory of "preferential exclusion/interaction" states that excipients such as sucrose, trehalose, mannitol and sorbitol in solution are preferentially excluded from the surface of proteins, thereby stabilizing the native structure of the protein as a result of increased chemical potential and interaction with water molecules (Arakawa and Timasheff, 1982; Carpenter et al., 1994; Lerbret et al., 2008; Sudrik et al., 2019). The final alternative is the "reducing surface adsorption hypothesis" which states that protein adsorption at the surface of the drying layer is reduced in the presence of surfactants such as polysorbate 20 and polysorbate 80, thereby preventing protein denaturation at the air-liquid interface (Arsiccio and Pisano, 2018; Maa et al., 1998).

In the present study, we characterise the stability of a spray-dried enzyme-based formulation and compare our measurements with atomic-resolution models generated from molecular dynamics (MD) computer simulations. By mapping between the experimental measurements and the model predictions, we identify the protein–excipient interaction networks created with various excipients. The enzyme formulation we study is Olipudase alfa (Sanofi, 2022; Zhou et al., 2016). Sold under the brand name Xenpozyme™, it is a therapeutic sucrose-based formulated drug substance (FDA, 2022). The Active Pharmaceutical Ingredient (API) is a copy of the normal acid sphingomyelinase enzyme, and is the first disease-modifying treatment of non-central nervous system manifestations of acid sphingomyelinase deficiency (ASMD) type A/B or type B. Also known as acid sphingomyelinase-deficient Niemann-Pick disease, ASMD is a rare progressive genetic disorder linked to deficiency of the enzyme acid sphingomyelinase and hence inability to metabolise the sphingomyelin lipid. Here we evaluate the potential protective effect of sucrose, trehalose, arginine and arginine hydrochloride excipients, which are among the most commonly used stabilizers in freeze-dried and spray-dried protein formulations (Sharma et al., 2021) yet little is known

about their mechanisms of interaction with proteins during freeze-drying and spray-drying (Bjelošević et al., 2020; Pinto et al., 2021). Here, the critical quality attributes (CQAs) of Olipudase were evaluated in terms of its reconstitution properties as measured by UV-Vis spectroscopy and Size Exclusion Chromatography (SEC) and supported by atomically-detailed MD simulations of the protein–excipient interface in aqueous solution. To the best of our knowledge, the stability of spray-dried Olipudase has not been studied before. Here we resolve its protein–excipient interaction networks and demonstrate the importance of selecting the most appropriate formulation components in seeking to protect fragile biologics during processing.

2 Materials and Methods

2.1 Preparation of enzyme formulations

Trehalose, L-arginine (Arg) and L-arginine hydrochloride (Arg-HCl) were purchased from Sigma Aldrich, Ireland and used without further purification. Formulated liquid and lyophilized Olipudase with 5 % (w/v) sucrose and 100 mM methionine in sodium phosphate buffer at pH 6.5 was used as received from Sanofi, Waterford, Ireland. 5 % (w/v) each of trehalose, Arg and Arg-HCl were separately added to make the alternative formulations.

2.2 Spray-drying

Olipudase was spray-dried using the Procept 4M8-Trix Spray Dryer installed with a cyclone-based separator at two different inlet temperatures (60 °C and 120 °C) and corresponding outlet temperatures (35 °C and 60 °C) to assess the impact of near-ambient as well as accelerated inlet/outlet temperatures. The spray rate, air flow rate, feed flow rate and nozzle size were kept constant at 0.3 m^3/min, 80 L/min, 1 mL/min and 0.4 mm, respectively. 25 mL of each formulation was spray-dried, and the dry powder was transferred into 20 mL SCHOTT glass vials inside a glove bag purged with nitrogen gas. The vials were then rubber stoppered, crimped, and stored at 5 ± 3 °C. To assess the impact of shear stress on the enzyme, the formulated drug substance was sprayed through the spray nozzle with nozzle diameters of 0.4 mm and 1.2 mm, at a nozzle pressure of 0 bar and 4 bar and feed flow rates of 1 mL/min and 4 mL/min without drying. The sprayed liquid was collected in a glass beaker.

2.3 Reconstitution time, concentration, turbidity analyses

All dried formulations were reconstituted with 5 mL Milli-Q® ultrapure water and the glass vials were gently rotated at an angle of 45 °C. The reconstitution time was determined using a stop watch and the reconstitution end-point was recorded when the dissolution process was complete. The concentration and turbidity (optical density) were measured in a 1x1 cm transparent glass cuvette using a UV/Vis spectrometer (Spectro Star nano from BMG Labtech) at 280 nm and 350 nm, respectively. The molar extinction coefficient of Olipudase is 2.41 mL mg^{-1} cm^{-1}. Sub-visible particle size analysis was out of scope of this study and is recommended to be included as a stability indicating test method in future studies.

2.4 Size Exclusion Chromatography

The aggregation profile of Olipudase was determined using High-Performance Liquid Chromatography (HPLC 1260, Agilent Technologies) with a UV-Vis detector at 280 nm, at a flow rate of 0.5 mL/min through a TSK gel 3000 SWXL column with an injection volume of 20 µL. The mobile phase contained 20 mM sodium phosphate dibasic and 200 mM sodium chloride at pH 6.5. All data were analysed on the Empower Chromatography Data System (Waters™).

2.5 Molecular dynamics computer simulations

MD simulations were performed on Olipudase to understand the influence of excipient interactions on protein stability during spray-drying. To simulate the high-temperature environment of spray-drying, the enzyme was modelled at three different temperatures, 300 K (27 °C), 340 K (67 °C) and 380 K (107 °C) in water solution in the presence of the base formulation (sucrose/methionine) alone, the base formulation with added Arg-HCl, and the base formulation with added trehalose. The simulations were performed for two different excipient concentrations. The first used the starting concentrations of experimental solutions prior to spray-drying (labelled 1×) to study the protein in its original solution. The second set of simulations used ten times the starting concentrations (labelled 10×) to study the protein in its partially dried state as the high local effective concentration of bound excipients form more large-area, specific interactions with the surface of the protein. The data were analyzed in terms of excipient-induced changes in the protein structure. These changes were monitored through the root mean square deviation (RMSD) of protein residues away from their starting positions (from X-ray crystal structure PDB ID 4WLD), the fraction of native contacts between amino acid residues, the root mean square fluctuation (RMSF) with respect to the time-averaged structure as a measure of the flexibility of the protein, and the preservation of the secondary structure. MD simulations were carried out using the Gromacs 2018.4 package (Van Der Spoel et al., 2005) with a time step of 2 fs using the Leapfrog integrator (Hockney et al., 1974; Verlet, 1967). Bond lengths to hydrogen were constrained using the LINCS algorithm (Hess, 2007). Long-range electrostatics were treated by the Particle mesh Ewald (PME) method (Darden et al., 1998). Protein and solvent molecules were coupled separately to an external heat bath (300 K) with a coupling time constant of 1 ps using the velocity rescaling method (Bussi et al., 2007). CHARMM force field parameters (Guvench et al., 2011; Huang et al., 2016) were used for the sucrose/methionine base formulation, the added Arg-HCl and trehalose excipient molecules, the enzyme, and water. All systems were minimized for 100 ps and equilibrated for 500 ps in a constant volume – constant temperature ensemble. One microsecond (1 µs) of equilibrated, unconstrained molecular dynamics was then performed at constant pressure – constant temperature for each formulation.

3 Results and Discussion

3.1 Reconstitution properties

Figure 1 shows the reconstitution behavior of freeze-dried and spray-dried Olipudase formulations. A clear solution was obtained within 1 min of reconstitution of the freeze-dried cake (Figure 1 (a)). The

spray-dried samples containing sucrose took 15 min to reconstitute and appeared highly turbid due to the presence of large amounts of insoluble particles. The concentration values at 280 nm and the optical density (O.D.) values at 350 nm of the spray-dried solutions (4.47 – 5.56 mg/mL, O.D. > 0.450) were higher than their freeze-dried counterparts (4.15 mg/mL, O.D. = 0.074 ± 0.05). It is important to note that Olipudase showed poor reconstitution properties for samples spray-dried at high as well as at low inlet/outlet temperatures (Table 1). Possible reasons for the presence of insoluble agglomerates causing turbidity may be increased aggregation promoted by temperature, shear, and adsorption at liquid-air interfaces. Shear forces (Andrews et al., 2008; Bee et al., 2009) and protein adsorption at the liquid-air interface have been reported to be the major reason for aggregation in spray-dried recombinant growth hormone (rhGH), tissue-type plasminogen activator (tPA) and other proteins (Kumar et al., 2009; Maa and Hsu, 1997; Mukherjee et al., 2009; Mumenthaler et al., 1994). Furthermore, the sprayed enzyme solution through the spray nozzle (without drying) exhibited aggregate levels of <2% and O.D. of <0.1 which were comparable to the reconstituted freeze-dried product. This showed that temperature significantly affected the stability of Olipudase, rather than shear alone. The impact of other excipients on the stability of spray-dried Olipudase was also evaluated (Figure 1) and compared to their pre-dried counterparts (Table 1). The reconstituted spray-dried Olipudase formulations containing sucrose/trehalose, sucrose/Arg and sucrose/Arg-HCl are shown in Figure 1 (c – e), respectively. The turbidity of reconstituted spray-dried Olipudase was significantly improved in the presence of sucrose/trehalose, sucrose/Arg and sucrose/Arg-HCl, compared to the turbidity of the sucrose formulation by itself (Figure 1 (b)), resulting in optically clearer solutions.

A significant improvement in the reconstitution behaviour of spray-dried Olipudase sucrose/Arg and sucrose/Arg-HCl was observed and was consistent with the results obtained for the O.D. in the light scattering region (Figure 2), reconstitution time and concentration as shown in Table 1. As shown in Figure 2, a reduction in the O.D. of the spray-dried enzyme formulation with sucrose/trehalose was also observed while the O.D. of the reconstituted formulation containing sucrose/Arg and sucrose/Arg-HCl was significantly lower than that of sucrose and sucrose/trehalose. Moreover, a significant reduction in the reconstitution time was observed for sucrose/Arg (8 min) and sucrose/Arg-HCl (5.5 min) but the formulation containing sucrose/trehalose did not show any reduction in the reconstitution time (15 min) (Table 1). Also, the enzyme concentrations obtained for the formulations containing sucrose/trehalose, sucrose/Arg and sucrose/Arg-HCl were consistent within the specification range of the product (4.0 ± 0.5 mg/mL).

Table 1: Reconstitution time and concentration analyses of Olipudase formulations.

Sample	Reconstitution Time (min)	Protein Concentration (mg/mL)
Freeze-dried Sucrose (control)[a]	1	4.15 ± 0.05
Spray-dried Sucrose (60 °C)	15	4.47 ± 0.17
Spray-dried Sucrose (120 °C)	15	5.56 ± 2.44

Pre-dried Sucrose/Trehalose	-	4.01 ± 0.02
Spray-dried Sucrose/Trehalose (60 °C)	15	3.81 ± 0.01
Spray-dried Sucrose/Trehalose (120 °C)	15	4.00 ± 0.01
Pre-dried Sucrose/Arginine	-	3.88 ± 0.06
Spray-dried Sucrose/Arginine (60 °C)	8	3.43 ± 0.01
Spray-dried Sucrose/Arginine (120 °C)	8	3.93 ± 0.01
Pre-dried Sucrose/Arg-HCl	-	4.38 ± 0.03
Spray-dried Sucrose/Arg-HCl (60 °C)	5.5	3.97 ± 0.05
Spray-dried Sucrose/Arg-HCl (120 °C)	5.5	4.01 ± 0.01

[a] The attributes of pre-dried Olipudase sucrose were comparable to its freeze-dried counterpart.

3.2 Size Exclusion Chromatography

Figure 3 shows the relative percentage loss of monomer in the different pre- and post-spray-dried Olipudase formulations. All SEC aggregation profiles of spray-dried Olipudase formulations are provided in Figure S1-S3 (supplementary material). The formulation containing sucrose/Arg exhibited maximum loss of monomer due to a pH shift from 6.5 to 9.8. Due to the presence of a positively charged guanidium group, arginine is basic amino which resulted in a pH shift from 6.5 to 9.8. The large shift in the pH resulted in significant dimerization (> 50 %) of the enzyme, thereby leading to a maximum loss of the monomer (supplementary material, Figure S3). Efforts to titrate the pH back from 9.8 to 6.5 did not improve the stability of the protein to its native state but resulted in a significant increase of aggregates (87 %) and a significant reduction of the monomer (6 %) due to irreversible aggregation at pH 9.8 (supplementary material, Figure S3). The loss of monomer in formulations containing sucrose, sucrose/trehalose and sucrose/Arg-HCl were comparable, with on average 5 % higher loss of monomer in sucrose/Arg-HCl (Figure 3). This showed that even though Arg-HCl played a significant role in suppressing insoluble aggregation (Figure 2), it resulted in fragmentation of the monomer as observed through low molecular weight species (LMWS) in the SEC aggregation profile (supplementary material, Figure S2). Loss of monomer due to both increased fragmentation and aggregation has been reported to influence protein activity and stability (Ma at al., 2020; Moussa et al., 2016). However, in some cases, dimerization and oligomerization can be integral to protein activity (Marianayagam et al., 2004).

Several theories have been proposed to explain the mechanism of stabilization of proteins by arginine. According to preferential interaction, arginine preferentially excludes itself from the surface of lysozyme, BSA and α-chymotrypsinogen A, particularly at high concentrations, leading to an increase in the transfer free energy, thereby stabilizing the protein against unfolding and aggregation (Kita et al., 1994; Schneider and Trout, 2009; Shukla and Trout, 2011a). An alternative explanation based on surface tension posits that at high arginine concentrations, the rate of increase in the surface tension is low which suggests that arginine forms molecular clusters that interact with the hydrophobic surface of proteins, thereby suppressing protein aggregation (Arakawa et al., 2007; Das et al., 2007; Tsumoto et al., 2005). A third theory, based on the solubility of amino acids, proposes that arginine increases the solubility of amino acids by weakening both hydrophobic and polar interactions, thus, suppressing protein aggregation (Arakawa et al., 2007; Das et al., 2007). Finally, the excluded-volume effect, also known as the gap effect, states that the presence of excipients such as arginine creates an energy barrier between protein molecules that decreases the rate of protein aggregation (Baynes et al., 2005; Baynes and Trout, 2004; Shukla and Trout, 2010). In spite of the reported mechanisms for protein stabilization by arginine, protein destabilization has also been reported in the presence of arginine (Kim et al., 2016). The guanidinium group present in arginine contributes to the destabilizing effect. Guanidine hydrochloride (GdnHCl) is a well-known chaotrope, and the destabilizing effect of GdnHCl is lower than the stabilizing effect of arginine itself (Ishibashi et al., 2005) which is in agreement with the results obtained. In addition, previous studies have reported lesser positive and negative k_d (protein-protein interaction parameter) values at an acidic pH away from the isoelectric point of IgG attributed to the increase in the ionic strength of the solution in the presence of arginine derivates resulting in reduced repulsive intermolecular interactions, thereby, increasing the propensity for protein aggregation (Hada et al., 2023; Kim et al., 2021). Moreover, other mechanisms of destabilization by arginine have been reported. Increase in the molar concentrations of arginine and its derivates resulted in inhibition of protein activity by affecting substrate binding and a decrease in protein denaturation temperatures due to interactions with the amide group in the α-carbon chain of arginine (Ishibashi et al., 2005; Kim et al., 2021; Kim et al., 2016).

3.3 Molecular dynamics simulations

The RMSD and the secondary structure distribution of the Olipudase formulations (supplementary material, Figure S4 and S5, respectively) and the comparison of all computed properties at low and high concentrations of excipients (supplementary material, Figure S6 – S9) shows that the macromolecular crowding of the excipients on the protein surface (Figure 4) tends to stiffen the protein structure, offsetting the natural entropic effect of elevated temperature and which may also protect against other external factors such as shear stress. At high excipient concentrations, similar to the high effective concentration in the local environment of the protein in a droplet as drying takes place, the differential effects of each excipient on the protein structure were clearly identified in the molecular models. Figure 5 shows the fraction of native contacts that were conserved during the simulation. This not only captures changes affecting the secondary structure but also changes in the tertiary structure. At a near-ambient temperature of 27 °C (300 K) and at a temperature of 67 °C (340K) reached during the spray-drying process, both sucrose (Figure 5 (a)) and the sucrose/Arg-HCl (Figure 5 (b)) mixture conserve a large fraction of the native contacts, suggesting preservation of the protein structure with only minimal change. Furthermore, in both cases, the fraction converges towards an equilibrium value > 0.85. The fraction conserved was also slightly larger by ~0.05 for sucrose/Arg-HCl (Figure 5 (b)), in good agreement with the experimental results. On the other hand, the sucrose/trehalose (Figure 5 (c)) formulation conserved the protein structure at room temperature but not at the higher temperature of 67 °C (340K) of operation of the spray-dryer.

This reduced ability of sucrose/trehalose to protect the protein structure is in good agreement with the experimental results. 107 °C (380K) was used as a control for the upper bound of the operating temperature of the spray-dryer. For all excipients, we observe a loss of native contacts at 107 °C (380 K) with sucrose and sucrose/Arg-HCl reaching a new equilibrium and, therefore, a new conformation. Sucrose/trehalose leads again to a large change in the protein structure. These results support the findings from spray-drying experiments, as do the RMSD and the secondary structure analysis (Supplementary Figures S4 and S5).

The RMSF, plotted in Figure 6, shows the fluctuations of each residue during the one microsecond of molecular dynamics. Sucrose maintains very low fluctuations at 27 °C (300 K) and 67 °C (340 K) (Figure 6 (a)), as does Arg-HCl (Figure 6 (b)), although the domains 100-110 and 145-155 show slightly larger values. At 107 °C (380 K) however, Arg-HCl dampens protein dynamics compared to sucrose alone, in particular around the termini. The presence of trehalose leads again to very large fluctuations at temperatures ≥67 °C (≥ 340 K) (Figure 6 (c)). The near-terminal sequence regions 0-100 and 450-528 are particularly affected and explain the loss of native contacts. These results corroborate the experimental findings that the combination of sucrose and Arg-HCl is the most suitable for Olipudase during spray-drying.

Figure 7 (a) and (b) show the total number of excipient molecules bound to the protein and the number of protein amino acid residues coordinating these bound excipient molecules at 27 °C (300 K) and 107 °C (380 K), respectively. This allows us to distinguish between pairwise 1:1 binding and multivalent binding of excipients to more than one protein residue at a time. While sucrose and trehalose interact in a similar fashion via a few hydrogen bonds (Figure 7 (a) and (b)), Arg-HCl molecules are present in a significantly larger number at both ambient and elevated temperatures, compared with sucrose and trehalose, and Arg-HCl also binds more often to multiple protein residues, via its three charged sites of $-NH_3^+$, $-COO^-$, and -guanidinium$^+$ (Figure 7 (a) and (b)). By contrast, the number of sucrose and trehalose molecules is equal to that of the bound protein residues, suggesting a 1:1 binding process and therefore the formation of a single H-bond between the glycan excipients and the protein. A detailed representation of the types of protein residue that each excipient binds (Figure 7 (c) and (d)) revealed that the major population of Arg-HCl bound with charged residues on the surface of Olipudase. Arg-HCl preferentially binds with negatively-charged glutamate (Glu) and aspartate (Asp) residues. It has been shown that the inclusion of Arg and Glu increases protein solubility, and stability and prevents aggregation (Golovanov et al., 2004). Moreover, the polar group of positively-charged Arg interacts with Asp and Glu residues via hydrogen bonds present on the surface of E3 ubiquitin-protein ligase while the other charged groups interact with other Arg and Glu molecules in the solution (Shukla and Trout, 2011b). Such increased interactions lead to a crowding effect on the surface of the protein, thereby, suppressing protein-protein associations. These results are consistent with the excluded-volume mechanism discussed above. Furthermore, large populations of Arg-Arg and Arg-Lys excipient-protein interactions are observed at both 27 °C (300 K) and 107 °C (380 K). The plots show that Arg also interacts with polar uncharged, aromatic, and aliphatic residues of the enzyme but to a lesser extent, most notably proline (Pro), tyrosine (Tyr) and glutamine (Gln). Non-specific interactions of Arg with hydrophobic and aromatic residues have also been reported to improve solubility and reduce the development of aggregation-prone intermediate states (Li et al., 2010; Shah et al., 2012).

4 Conclusion

The combined experimental and modelling dataset demonstrated the feasibility and mechanism of spray-drying as an alternative to freeze-drying for the manufacture of a parenteral biopharmaceutical. In seeking to optimize the excipient sucrose/methionine base formulation, it was found that the inclusion of Arg-HCl significantly reduced the reconstitution time of spray-dried Olipudase by 66 % and reduced its turbidity by 83 % and was capable of acting as the main stabilizer, while an alternative additive trehalose did not improve the reconstitution time. In terms of protein aggregation, only the sucrose/Arg and sucrose/Arg-HCl-based formulations were able to suppress the formation of insoluble aggregates, but Arg by itself resulted in significant dimerization of Olipudase which further resulted in a maximum loss of monomer. However, while Arg-HCl also promoted fragmentation which resulted in significant loss of monomer, its protein aggregation suppressing effect dominated its destabilizing effect. A good agreement between the experimental results and molecular dynamics simulations provided further insights on protein–excipient interactions. The simulations revealed that while sucrose and trehalose interacted weakly with the protein and showed the least improvement in protein stability as evident from structural analysis, Arg-HCl interacted strongly with negatively-charged Glu and Asp residues on the surface of Olipudase. This excipient-mediated electrostatic stabilization is consistent with the excluded-volume effect hypothesis wherein Arg acts as a crowder to suppress protein aggregation and thereby stabilize Olipudase by Arg-HCl post-spray-drying. Our findings provide a further demonstration of the importance of selecting the most appropriate formulation components in seeking to protect fragile biologics during processing, in this case, spray-drying. Product-specific characterization coupled with modelling and data analytics can provide the molecular-level insights required for integrated product–process co-development and optimization in biopharmaceutical manufacture.

CrediT authorship statement

Conceptualization, Ashutosh Sharma, Dikshitkumar Khamar, Pierre Cazade, Damien Thompson; Data curation, Ashutosh Sharma, Pierre Cazade; Formal analysis, Ashutosh Sharma, Pierre Cazade; Funding acquisition, Ashutosh Sharma, Helen Hughes, Damien Thompson; Methodology, Ashutosh Sharma, Pierre Cazade; Project administration, Helen Hughes, Dikshitkumar Khamar, Damien Thompson; Resources, Dikshitkumar Khamar, Helen Hughes, Damien Thompson; Software, Ashutosh Sharma and Pierre Cazade; Supervision, Dikshitkumar Khamar, Ambrose Hayden, Helen Hughes, Damien Thompson; Validation, Dikshitkumar Khamar, Helen Hughes, Damien Thompson; Writing – original draft, Ashutosh Sharma, Pierre Cazade; Writing – review & editing, Ashutosh Sharma, Pierre Cazade, Dikshitkumar Khamar, Ambrose Hayden, Helen Hughes, Damien Thompson.

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References

Ajmera, A., Scherließ, R., 2014. Stabilisation of proteins via mixtures of amino acids during spray drying. Int. J. Pharm. 463, 98–107. https://doi.org/10.1016/j.ijpharm.2014.01.002

Allison, S.D., Chang, B., Randolph, T.W., Carpenter, J.F., 1999. Hydrogen Bonding between Sugar and Protein Is Responsible for Inhibition of Dehydration-Induced Protein Unfolding.

Andrews, J.M., Weiss IV, W.F., Roberts, C.J., 2008. Nucleation, growth, and activation energies for seeded and unseeded aggregation of α-chymotrypsinogen A. Biochemistry 47, 2397–2403. https://doi.org/10.1021/bi7019244

Arakawa, T., Ejima, D., Tsumoto, K., Obeyama, N., Tanaka, Y., Kita, Y., Timasheff, S.N., 2007. Suppression of protein interactions by arginine: A proposed mechanism of the arginine effects. Biophys. Chem. 127, 1–8. https://doi.org/10.1016/J.BPC.2006.12.007

Arakawa, T., Timasheff, S.N., 1982. Stabilization of Protein Structure by Sugars. Biochemistry 21, 6536–6544. https://doi.org/10.1021/bi00268a033

Arsiccio, A., Pisano, R., 2018. Surfactants as stabilizers for biopharmaceuticals: An insight into the molecular mechanisms for inhibition of protein aggregation. Eur. J. Pharm. Biopharm. 128. https://doi.org/10.1016/j.ejpb.2018.04.005

Baynes, B.M., Trout, B.L., 2004. Rational Design of Solution Additives for the Prevention of Protein Aggregation. Biophys. J. 87, 1631–1639. https://doi.org/10.1529/BIOPHYSJ.104.042473

Baynes, B.M., Wang, D.I.C., Trout, B.L., 2005. Role of arginine in the stabilization of proteins against aggregation. Biochemistry 44, 4919–4925. https://doi.org/10.1021/BI047528R/ASSET/IMAGES/MEDIUM/BI047528RN00001.GIF

Bee, J.S., Chiu, D., Sawicki, S., Stevenson, J.L., Chatterjee, K., Freund, E., Carpenter, J.F., Randolph, T.W., 2009. Monoclonal antibody interactions with micro- and nanoparticles: Adsorption, aggregation, and accelerated stress studies. J. Pharm. Sci. 98, 3218–3238. https://doi.org/10.1002/jps.21768

Bjelošević, M., Zvonar Pobirk, A., Planinšek, O., Ahlin Grabnar, P., 2020. Excipients in freeze-dried biopharmaceuticals: Contributions toward formulation stability and lyophilisation cycle optimisation. Int. J. Pharm. https://doi.org/10.1016/j.ijpharm.2020.119029

Bussi, G., Donadio, D., Parrinello, M., 2007. Canonical sampling through velocity rescaling. J. Chem. Phys. 126, 014101. https://doi.org/10.1063/1.2408420

Carpenter, J.F., Prestrelski, S.J., Anchordoguy, T.J., Arakawa, T., 1994. Interactions of Stabilizers with Proteins During Freezing and Drying, in: Cleland, J.L., Langer, R. (Eds.), Formulation and Delivery of Proteins and Peptides. American Chemical Society (ACS), pp. 134–147. https://doi.org/10.1021/bk-1994-0567.ch009

Carpenter, John F, Chang, B.S., Garzon-Rodriguez, W., Randolph, T.W., 2002. Rationale design of stable lyophilized protein formulations: Theory and Practice., in: Carpenter, J.F., Manning, M.C. (Eds.), Rational Design of Stable Protein Formulations - Theory and Practice. Kluwer Academic Publishers-Plenum Publishers, New York, pp. 109–133.

Celik, M., Wendell, S.C., 2010. Spray Drying and Pharmaceutical Applications, in: Parikh, D.M. (Ed.), Handbook of Pharmaceutical Granulation Technology. Taylor and Francis, pp. 98–125. https://doi.org/10.1201/9780849354953.ch5

Chang, L., Shepherd, D., Sun, J., Ouellette, D., Grant, K.L., Tang, X., Pikal, M.J., 2005. Mechanism of protein stabilization by sugars during freeze-drying and storage: Native structure preservation, specific interaction, and/or immobilization in a glassy matrix? J. Pharm. Sci. 94, 1427–1444. https://doi.org/10.1002/jps.20364

Costantino, H.R., Langer, R., Klibanov, A.M., 1994. Solid-Phase Aggregation of Proteins under Pharmaceutically Relevant Conditions†. J. Pharm. Sci. 83, 1662–1669. https://doi.org/10.1002/jps.2600831205

Darden, T., York, D., Pedersen, L., 1998. Particle mesh Ewald: An N·log(N) method for Ewald sums in large systems. J. Chem. Phys. 98, 10089. https://doi.org/10.1063/1.464397

Das, U., Hariprasad, G., Ethayathulla, A.S., Manral, P., Das, T.K., Pasha, S., Mann, A., Ganguli, M., Verma, A.K., Bhat, R., Chandrayan, S.K., Ahmed, S., Sharma, S., Kaur, P., Singh, T.P., Srinivasan, A., 2007. Inhibition of Protein Aggregation: Supramolecular Assemblies of Arginine Hold the Key. PLoS One. https://doi.org/10.1371/journal.pone.0001176

Duran, T., Minatovicz, B., Bai, J., Shin, D., Mohammadiarani, H., Chaudhuri, B., 2021. Molecular Dynamics Simulation to Uncover the Mechanisms of Protein Instability During Freezing. J. Pharm. Sci. 110, 2457–2471. https://doi.org/10.1016/J.XPHS.2021.01.002

Elversson, J., Millqvist-Fureby, A., 2005. Aqueous two-phase systems as a formulation concept for spray-dried protein. Int. J. Pharm. 294, 73–87. https://doi.org/10.1016/j.ijpharm.2005.01.015

FDA, 2022. HIGHLIGHTS OF PRESCRIBING INFORMATION. XENPOZYME (olipudase alfa-rpcp) for injection, for intravenous useInitial U.S. Approval: 2022.

Fiedler, D., Hartl, S., Gerlza, T., Trojacher, C., Kungl, A., Khinast, J., Roblegg, E., 2021. Comparing freeze drying and spray drying of interleukins using model protein CXCL8 and its variants. Eur. J. Pharm. Biopharm. 168, 152–165. https://doi.org/10.1016/J.EJPB.2021.08.006

Golovanov, A.P., Hautbergue, G.M., Wilson, S.A., Lian, L.Y., 2004. A simple method for improving protein solubility and long-term stability. J. Am. Chem. Soc. 126, 8933–8939. https://doi.org/10.1021/JA049297H/ASSET/IMAGES/MEDIUM/JA049297HN00001.GIF

Green, J.L., Angell, C.A., 1989. Phase relations and vitrification in saccharide-water solutions and the trehalose anomaly. J. Phys. Chem. 93, 2880–2882. https://doi.org/10.1021/j100345a006

Guvench, O., Mallajosyula, S.S., Raman, E.P., Hatcher, E., Vanommeslaeghe, K., Foster, T.J., Jamison, F.W., MacKerell, A.D., 2011. CHARMM additive all-atom force field for carbohydrate derivatives

and its utility in polysaccharide and carbohydrate-protein modeling. J. Chem. Theory Comput. 7, 3162–3180. https://doi.org/10.1021/CT200328P/SUPPL_FILE/CT200328P_SI_001.PDF

Hada, S., Burlakoti, U., Kim, K.H., Han, J.S., Kim, M.J., Kim, N.A., Jeong, S.H., 2023. A comprehensive evaluation of arginine and its derivatives as protein formulation stabilizers. Int. J. Pharm. 647, 123545. https://doi.org/https://doi.org/10.1016/j.ijpharm.2023.123545

Hess, B., 2007. P-LINCS: A Parallel Linear Constraint Solver for Molecular Simulation. J. Chem. Theory Comput. 4, 116–122. https://doi.org/10.1021/CT700200B

Hockney, R.W., Goel, S.P., Eastwood, J.W., 1974. Quiet high-resolution computer models of a plasma. J. Comput. Phys. 14, 148–158. https://doi.org/10.1016/0021-9991(74)90010-2

Huang, J., Rauscher, S., Nawrocki, G., Ran, T., Feig, M., De Groot, B.L., Grubmüller, H., MacKerell, A.D., 2016. CHARMM36m: an improved force field for folded and intrinsically disordered proteins. Nat. Methods 2016 141 14, 71–73. https://doi.org/10.1038/nmeth.4067

Ishibashi, M., Tsumoto, K., Tokunaga, M., Ejima, D., Kita, Y., Arakawa, T., 2005. Is arginine a protein-denaturant? Protein Expr. Purif. 42, 1–6. https://doi.org/10.1016/J.PEP.2005.03.028

Kim, N.A., Hada, S., Jeong, S.H., 2021. N-Acetylated-L-arginine (NALA) is an enhanced protein aggregation suppressor under interfacial stresses and elevated temperature for protein liquid formulations. Int. J. Biol. Macromol. 166, 654–664. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2020.10.223

Kim, N.A., Hada, S., Thapa, R., Jeong, S.H., 2016. Arginine as a protein stabilizer and destabilizer in liquid formulations. Int. J. Pharm. 513, 26–37. https://doi.org/10.1016/J.IJPHARM.2016.09.003

Kita, Y., Arakawa, T., Lin, T. yin, Timasheff, S.N., 1994. Contribution of the Surface Free Energy Perturbation to Protein-Solvent Interactions. Biochemistry 33, 15178–15189. https://doi.org/10.1021/BI00254A029/ASSET/BI00254A029.FP.PNG_V03

Kreilgaard, L., Frokjaer, S., Flink, J.M., Randolph, T.W., Carpenter, J.F., 1999. Effects of Additives on the Stability of Humicola Lanuginosa Lipase During Freeze-Drying and Storage in the Dried Solid. J. Pharm. Sci. 88, 281–290. https://doi.org/10.1021/js980399d

Kumar, V., Sharma, V.K., Kalonia, D.S., 2009. In situ precipitation and vacuum drying of interferon alpha-2a: Development of a single-step process for obtaining dry, stable protein formulation. Int. J. Pharm. 366, 88–98. https://doi.org/10.1016/j.ijpharm.2008.09.001

Langford, A., Bhatnagar, B., Walters, R., Tchessalov, S., Ohtake, S., 2017. Drying of biopharmaceuticals: Recent developments, new technologies and future direction. Dry. Technol. 19, 15–25. https://doi.org/10.11301/jsfe.18514

Lerbret, A., Affouard, F., Bordat, P., Hédoux, A., Guinet, Y., Descamps, M., 2008. Molecular dynamics simulations of lysozyme in water/sugar solutions. Chem. Phys. 345, 267–274. https://doi.org/10.1016/j.chemphys.2007.09.011

Li, J., Garg, M., Shah, D., Rajagopalan, R., 2010. Solubilization of aromatic and hydrophobic moieties by

arginine in aqueous solutions. J. Chem. Phys. 133, 054902. https://doi.org/10.1063/1.3469790

Ma, H., Ó'Fágáin, C., O'Kennedy, R., 2020. Antibody stability: A key to performance - Analysis, influences and improvement. Biochimie 177, 213–225. https://doi.org/https://doi.org/10.1016/j.biochi.2020.08.019

Maa, Y.-F., Hsu, C.C., 1997. Protein Denaturation by Combined Effect of Shear and Air-Liquid Interface.

Maa, Y.-F., Nguyen, P.-A.T., Hsu, S.W., 1998. Spray-Drying of Air–Liquid Interface Sensitive Recombinant Human Growth Hormone. J. Pharm. Sci. 87, 152–159. https://doi.org/10.1021/js970308x

Marianayagam, N.J., Sunde, M., Matthews, J.M., 2004. The power of two: protein dimerization in biology. https://doi.org/10.1016/j.tibs.2004.09.006

Massant, J., Fleurime, S., Batens, M., Vanhaerents, H., Van den Mooter, G., 2020. Formulating monoclonal antibodies as powders for reconstitution at high concentration using spray-drying: Trehalose/amino acid combinations as reconstitution time reducing and stability improving formulations. Eur. J. Pharm. Biopharm. 156, 131–142. https://doi.org/10.1016/j.ejpb.2020.08.019

Moeller, E.H., Jorgensen, L., 2008. Alternative routes of administration for systemic delivery of protein pharmaceuticals. Drug Discov. Today Technol. 5, e89–e94. https://doi.org/10.1016/J.DDTEC.2008.11.005

Moussa, E.M., Panchal, J.P., Moorthy, B.S., Blum, J.S., Joubert, M.K., Narhi, L.O., Topp, E.M., 2016. Immunogenicity of Therapeutic Protein Aggregates. J. Pharm. Sci. https://doi.org/10.1016/j.xphs.2015.11.002

Mukherjee, S., Chowdhury, P., Gai, F., 2009. Effect of dehydration on the aggregation kinetics of two amyloid peptides. J. Phys. Chem. B 113, 531–535. https://doi.org/10.1021/jp809817s

Mumenthaler, M., Hsu, C.C., Pearlman, R., 1994. Feasibility Study on Spray-Drying Protein Pharmaceuticals: Recombinant Human Growth Hormone and Tissue-Type Plasminogen Activator. Pharm. Res. An Off. J. Am. Assoc. Pharm. Sci. 11, 12–20. https://doi.org/10.1023/A:1018929224005

Mutukuri, T.T., Wilson, N.E., Taylor, L.S., Topp, E.M., Zhou, Q.T., 2021. Effects of drying method and excipient on the structure and physical stability of protein solids: Freeze drying vs. spray freeze drying. Int. J. Pharm. 594, 120169. https://doi.org/10.1016/j.ijpharm.2020.120169

Narhi, L.O., Schmit, J., Bechtold-Peters, K., Sharma, D., 2012. Classification of Protein Aggregates. J. Pharm. Sci. 101, 493–498. https://doi.org/10.1002/JPS.22790

Pan, H.W., Seow, H.C., Lo, J.C.K., Guo, J., Zhu, L., Leung, S.W.S., Zhang, C., Lam, J.K.W., 2022. Spray-Dried and Spray-Freeze-Dried Powder Formulations of an Anti-Interleukin-4Rα Antibody for Pulmonary Delivery. Pharm. Res. 39, 2291–2304. https://doi.org/10.1007/S11095-022-03331-W

Pinto, J.T., Faulhammer, E., Dieplinger, J., Dekner, M., Makert, C., Nieder, M., Paudel, A., 2021. Progress in spray-drying of protein pharmaceuticals: Literature analysis of trends in formulation and process attributes. Dry. Technol. 1–32. https://doi.org/10.1080/07373937.2021.1903032

Rajan, R., Ahmed, S., Sharma, N., Kumar, N., Debas, A., Matsumura, K., 2021. Review of the current state of protein aggregation inhibition from a materials chemistry perspective: special focus on polymeric materials. Mater. Adv. 2, 1139–1176. https://doi.org/10.1039/D0MA00760A

Roser, B., 1991. Trehalose, a new approach to premium dried foods. Trends Food Sci. Technol. 2, 166–169. https://doi.org/10.1016/0924-2244(91)90671-5

Salnikova, M., Varshney, D., Shalaev, E., 2015. Heterogeneity of Protein Environments in Frozen Solutions and in the Dried State, in: Lyophilized Biologics and Vaccines. Springer New York, pp. 11–24. https://doi.org/10.1007/978-1-4939-2383-0_2

Sanofi, 2022. XENPOZYME™ (olipudase alfa-rpcp) for ASMD treatment (non-CNS manifestations) [WWW Document]. URL https://www.xenpozyme.com (accessed 9.17.23).

Santivarangkna, C., Kulozik, U., Foerst, P., 2007. Alternative Drying Processes for the Industrial Preservation of Lactic Acid Starter Cultures. Biotechnol. Prog. 23, 302–315. https://doi.org/10.1021/bp060268f

Schneider, C.P., Trout, B.L., 2009. Investigation of Cosolute−Protein Preferential Interaction Coefficients: New Insight into the Mechanism by Which Arginine Inhibits Aggregation. J. Phys. Chem. B 113, 2050–2058. https://doi.org/10.1021/JP808042W

Shah, D., Li, J., Shaikh, A.R., Rajagopalan, R., 2012. Arginine-aromatic interactions and their effects on arginine-induced solubilization of aromatic solutes and suppression of protein aggregation. Biotechnol. Prog. 28, 223–231. https://doi.org/10.1002/btpr.710

Shalaev, E., Gatlin, L.A., Auffret, T., Speaker, S.M., 2008. Freeze-drying concepts: the basics.

Sharma, A., Khamar, D., Cullen, S., Hayden, A., Hughes, H., 2021. Innovative Drying Technologies for Biopharmaceuticals. Int. J. Pharm. 121115. https://doi.org/https://doi.org/10.1016/j.ijpharm.2021.121115

Shepard, K.B., Pluntze, A.M., Vodak, D.T., 2022. Simultaneous Spray Drying for Combination Dry Powder Inhaler Formulations. Pharm. 2022, Vol. 14, Page 1130 14, 1130. https://doi.org/10.3390/PHARMACEUTICS14061130

Shepard, K.B., Vodak, D.T., Kuehl, P.J., Revelli, D., Zhou, Y., Pluntze, A.M., Adam, M.S., Oddo, J.C., Switala, L., Cape, J.L., Baumann, J.M., Banks, M., 2021. Local Treatment of Non-small Cell Lung Cancer with a Spray-Dried Bevacizumab Formulation. AAPS PharmSciTech 22, 1–11. https://doi.org/10.1208/S12249-021-02095-7/FIGURES/5

Shukla, D., Trout, B.L., 2011a. Preferential interaction coefficients of proteins in aqueous arginine solutions and their molecular origins. J. Phys. Chem. B 115, 1243–1253. https://doi.org/10.1021/JP108586B/ASSET/IMAGES/MEDIUM/JP-2010-08586B_0011.GIF

Shukla, D., Trout, B.L., 2011b. Understanding the synergistic effect of arginine and glutamic acid mixtures on protein solubility. J. Phys. Chem. B 115, 11831–11839. https://doi.org/10.1021/jp204462t

Shukla, D., Trout, B.L., 2010. Interaction of arginine with proteins and the mechanism by which it inhibits aggregation. J. Phys. Chem. B 114, 13426–13438. https://doi.org/10.1021/JP108399G/SUPPL_FILE/JP108399G_SI_001.PDF

Sudrik, C.M., Cloutier, T., Mody, N., Sathish, H.A., Trout, B.L., 2019. Understanding the Role of Preferential Exclusion of Sugars and Polyols from Native State IgG1 Monoclonal Antibodies and its Effect on Aggregation and Reversible Self-Association. Pharm. Res. 2019 368 36, 1–12. https://doi.org/10.1007/S11095-019-2642-3

Starciuc, T., Malfait, B., Danede, F., Paccou, L., Guinet, Y., Correia, N.T., Hedoux, A., 2020. Trehalose or Sucrose: Which of the Two Should be Used for Stabilizing Proteins in the Solid State? A Dilemma Investigated by In Situ Micro-Raman and Dielectric Relaxation Spectroscopies During and After Freeze-Drying. J. Pharm. Sci. 109, 496–504. https://doi.org/10.1016/j.xphs.2019.10.055

Tang, X., Pikal, M.J., 2004a. Design of Freeze-Drying Processes for Pharmaceuticals: Practical Advice. Pharm. Res. https://doi.org/10.1023/B:PHAM.0000016234.73023.75

Tang, X., Pikal, M.J., 2004b. Design of freeze-drying processes for pharmaceuticals: practical advice. Pharm. Res. 21, 191–200.

Tejasvi Mutukuri, T., Maa, Y.-F., Gikanga, B., Sakhnovsky, R., Tony Zhou, Q., 2021. Electrostatic Spray Drying for Monoclonal Antibody Formulation. Int. J. Pharm. 120942. https://doi.org/10.1016/J.IJPHARM.2021.120942

Tsumoto, K., Ejima, D., Kita, Y., Arakawa, T., 2005. Review: Why is arginine effective in suppressing aggregation? Protein Pept. Lett. 12, 613–619. https://doi.org/10.2174/0929866054696109

Van Der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A.E., Berendsen, H.J.C., 2005. GROMACS: Fast, flexible, and free. J. Comput. Chem. 26, 1701–1718. https://doi.org/10.1002/JCC.20291

Verlet, L., 1967. Computer "Experiments" on Classical Fluids. I. Thermodynamical Properties of Lennard-Jones Molecules. Phys. Rev. 159, 98. https://doi.org/10.1103/PhysRev.159.98

Walters H., R., Bhatnagar, B., Tchessalov, S., Iztutsu, K.-I., Tsumoto, K., Ohtake, S., 2014. Next-Generation Drying Technologies for Pharmaceutical Applications. J. Pharm. Sci. 44, 91–93. https://doi.org/10.1002/jps.23998

Ying, D., Sun, J., Sanguansri, L., Weerakkody, R., Augustin, M.A., 2012. Enhanced survival of spray-dried microencapsulated Lactobacillus rhamnosus GG in the presence of glucose. J. Food Eng. 109, 597–602. https://doi.org/10.1016/j.jfoodeng.2011.10.017

Zhou, Y.F., Metcalf, M.C., Garman, S.C., Edmunds, T., Qiu, H., Wei, R.R., 2016. Human acid sphingomyelinase structures provide insight to molecular basis of Niemann-Pick disease. Nat. Commun. 7. https://doi.org/10.1038/ncomms13082

Zhao, Z., Huang, Z., Zhang, X., Huang, Y., Cui, Y., Ma, C., Wang, G., Freeman, T., Lu, X. yun, Pan, X., Wu, C., 2018. Low density, good flowability cyclodextrin-raffinose binary carrier for dry powder inhaler: anti-hygroscopicity and aerosolization performance enhancement. Expert Opin. Drug Deliv.

15, 443–457. https://doi.org/10.1080/17425247.2018.1450865

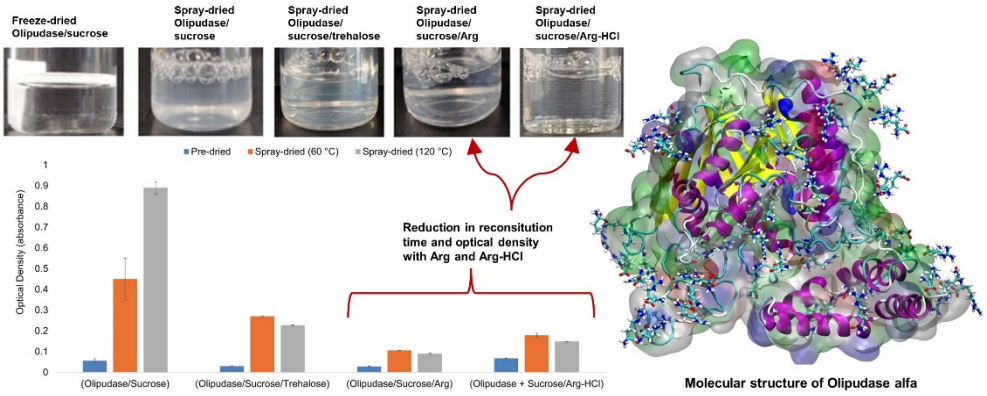


Figure Captions

Figure 1: Reconstituted Olipudase (a) freeze-dried with sucrose (control), (b) spray-dried with sucrose, (c) spray-dried with sucrose/trehalose, (d) spray-dried with sucrose/Arg, (e) spray-dried with sucrose/Arg-HCl.

Figure 2: Optical density of Olipudase formulations.

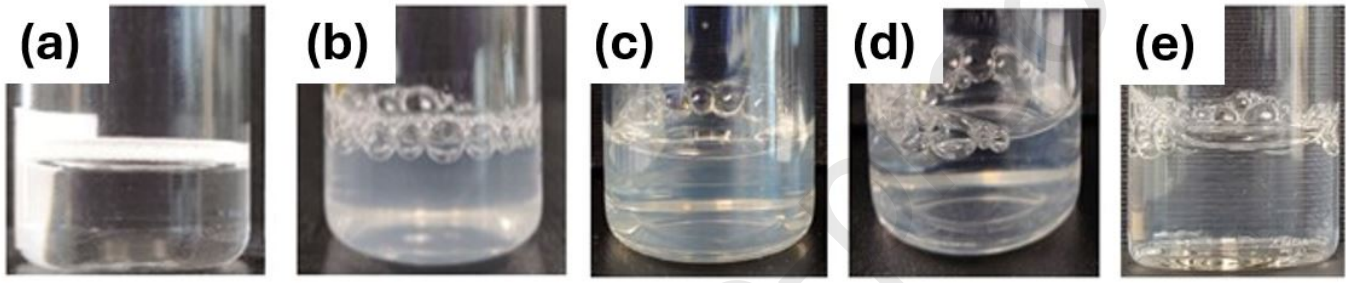
Figure 3: Relative loss of monomer in Olipudase formulations by SEC.

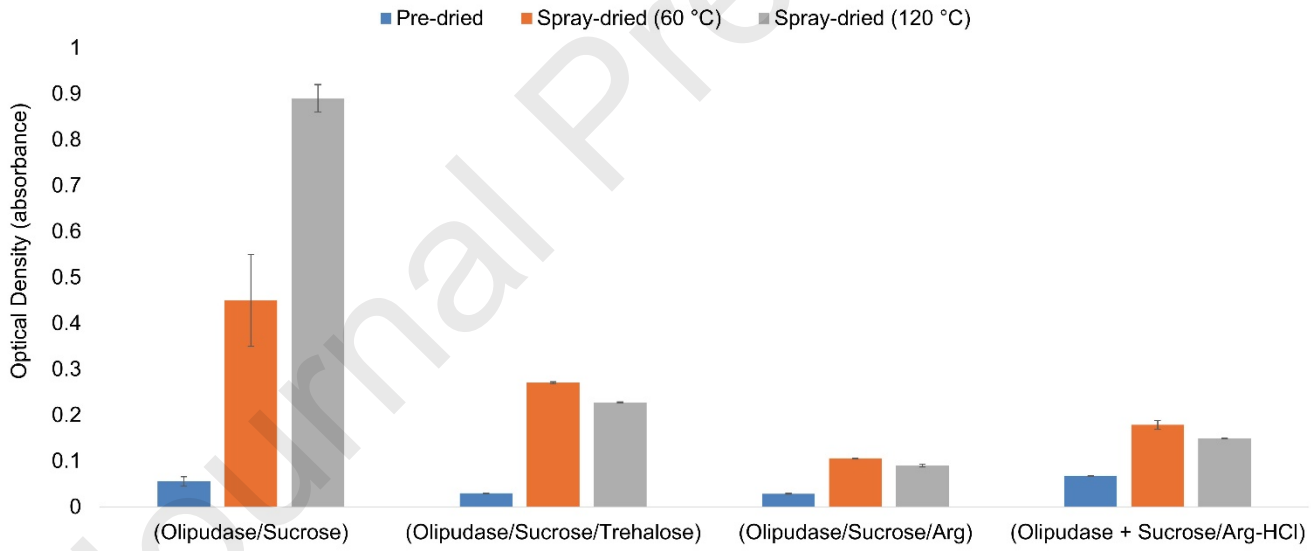
Figure 4: Representative MD structure showing arginine excipients adsorbed on the surface of Olipudase. Arginine molecules are shown as sticks with carbon atoms coloured cyan, oxygen atoms are red, nitrogens are blue, and hydrogen atoms are coloured white. The Olipudase alfa protein structure is shown in ribbon representation with colours reflecting the protein secondary structure: purple for □-helices, yellow for □-sheets, cyan for turns, and white for random coils. The overlaid semi-transparent surface is coloured according to the surface hydropathy: green for polar residues, white for non-polar residues, red for acidic residues, and blue for basic residues. The encompassing water medium (water, sucrose, and ions) is omitted for clarity.

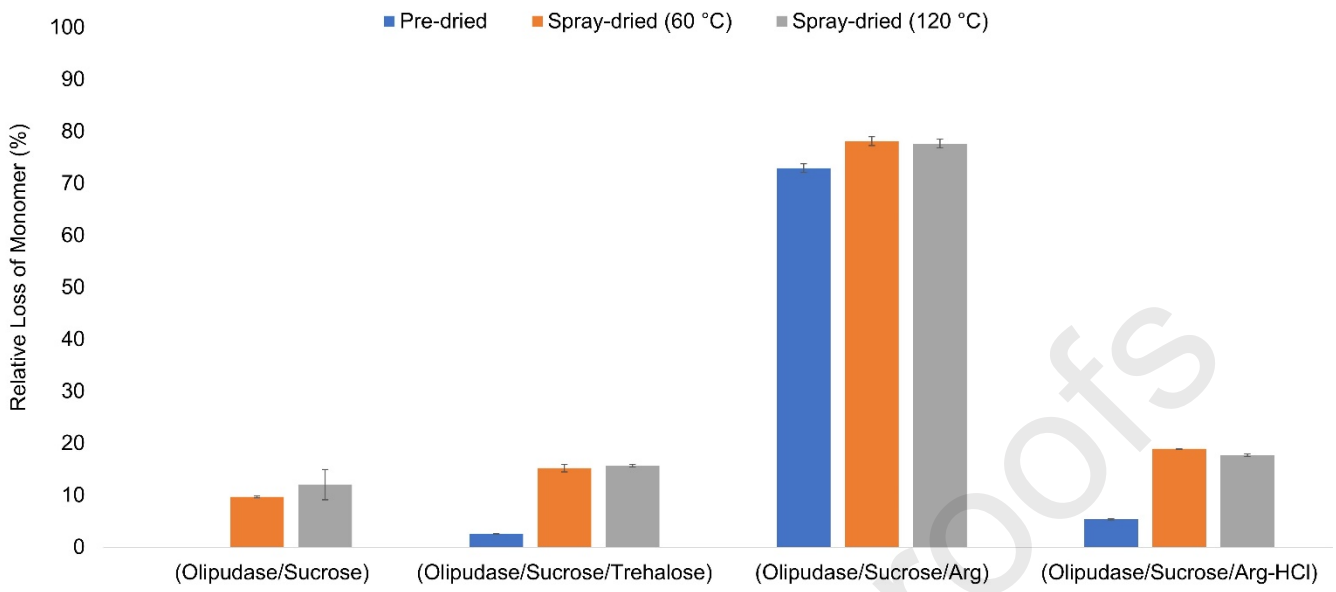
Figure 5: Plot of the fraction of native contacts of Olipudase with 10× concentration of (a) Sucrose, (b) Sucrose/Arg-HCl and (c) Sucrose/Trehalose, at 300, 340 and 380 K.

Figure 6: Plot of the RMSF of Olipudase with 10× concentration of (a) Sucrose, (b) Sucrose/Arg-HCl and (c) Sucrose/Trehalose, at 300, 340 and 380 K.

Figure 7: (a, b) Number of excipient molecules (sucrose, arginine, and trehalose) binding to the protein is shown in black, while the corresponding number of coordinating protein amino acid residues (AA) is depicted in red. (c, d) Number of sucrose (black), arginine (red) and trehalose (green) molecules bound to the protein as a function of the protein residue amino acid type. (a, c) Data obtained in simulations at 300 K and (b, d) for simulations at 380 K.






Pre-dried
Spray-dried (60 °C)
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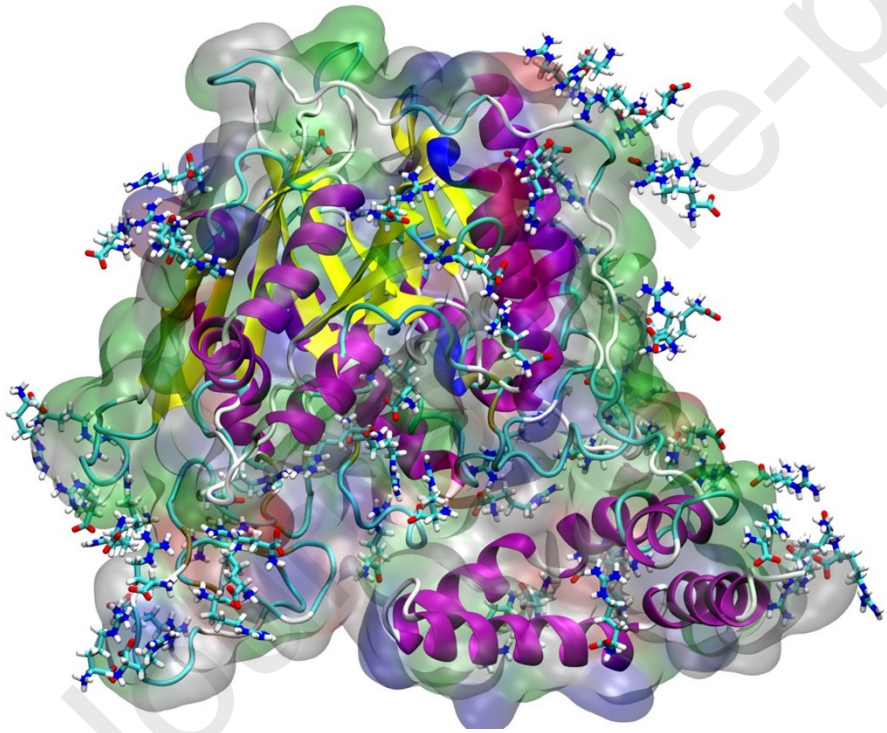
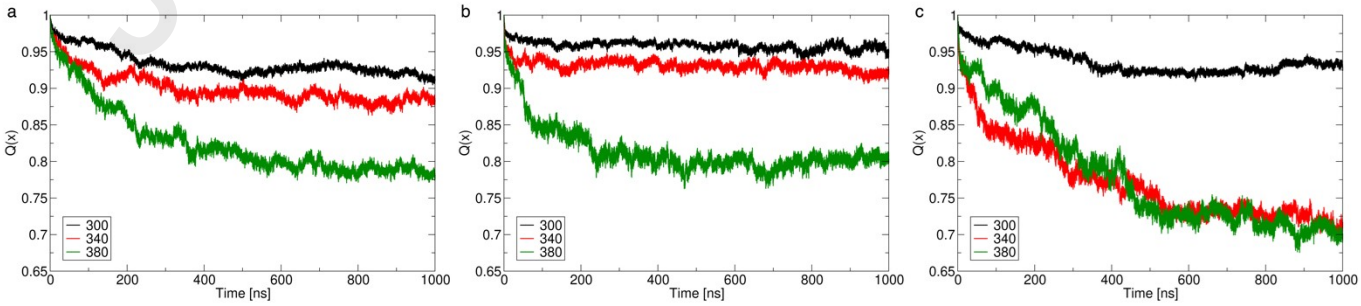
Relative Loss of Monomer (%)
100
90
80
70
60
50
40
30
20
10
0
(Olipudase/Sucrose)
(Olipudase/Sucrose/Trehalose)
(Olipudase/Sucrose/Arg)
(Olipudase/Sucrose/Arg-HCl)




a
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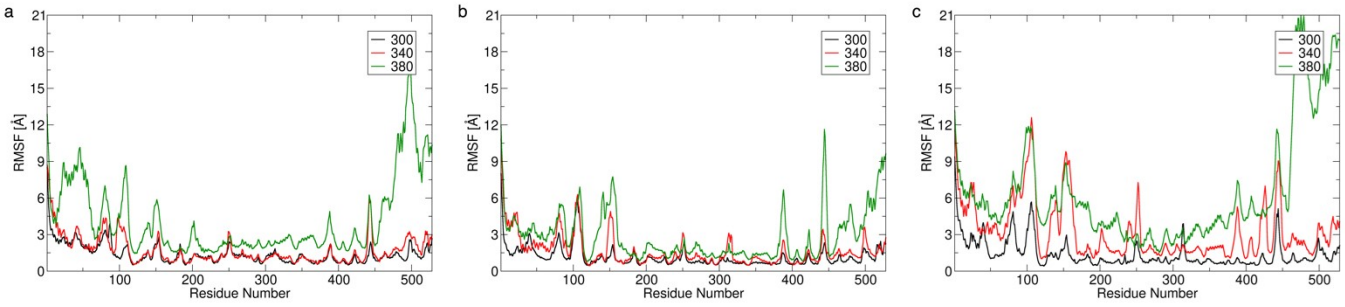
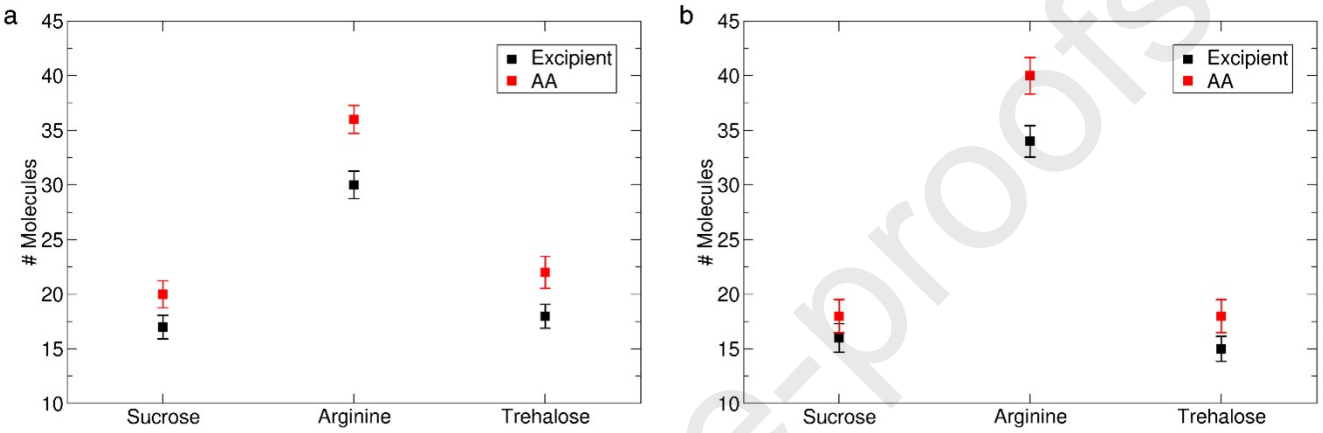
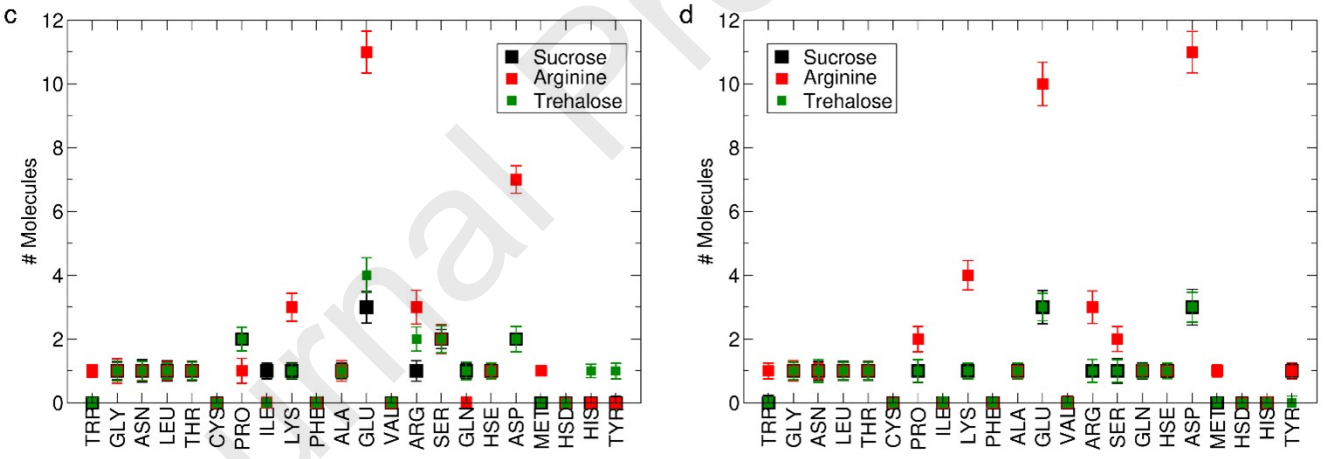
c
Q(x)
Time [ns]
300
340
380







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.