Interplay of Formulation Components on Excipient Functionality During Lyophilization

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February 25th, 2021

The overall goal of a pharmaceutical company is to produce a drug product that is safe, efficacious, and stable. Ideally, a lyophilized product should also look elegant, with a fluffy cake morphology, and be freeze-dried in a short time. These properties are influenced by the phase behavior of the formulation components.

Recently, Dr. Bhushan Munjal, Ph.D., University of Minnesota, USA presented a webinar describing the complex effects of combining excipients in a formulation and the importance of determining optimal concentrations and processing conditions for lyophilization of protein formulations. This tech note summarizes the webinar.

Interactive Effects of Components in Product Formulations

The overall product attributes of a lyophilized protein formulation are governed by the phase behavior of individual formulation components during the freezing and drying steps of the lyophilization process.

The freezing process exert multiple stresses that can destabilize the active protein. These include increased solute concentration and ionic strength in the freeze-concentrated matrix, alteration of the pH of the freeze-concentrated solution and formation of new interfaces. The removal of ice by sublimation during the drying phase may also lead to unwanted effects through desorption of unfrozen water and alterations of water content in the product.

As a result, protein formulations often contain multiple excipients to obtain the desired product attributes. For example, sugars, surfactants and buffers are added to improve the stability of the product, while bulking agents are added to improve the manufacturability, process efficiency and cake elegance (Figure 1).

The functionality of these excipients is often dependent upon their physical state in the formulation during processing and in the final dried product. For example, a lyoprotectant should remain amorphous throughout while a bulking agent (like mannitol) is desired in the crystalline state before initiation of primary drying. Crystallization of a lyoprotectant separates it from the protein phase, thus reducing its stabilization effect. On the other hand, amorphization of a crystalline bulking agent (like mannitol) would lower its critical temperature for lyophilization. However, the phase behavior of excipients is often affected by the presence of other components in the formulation and this can have an effect on the product performance.

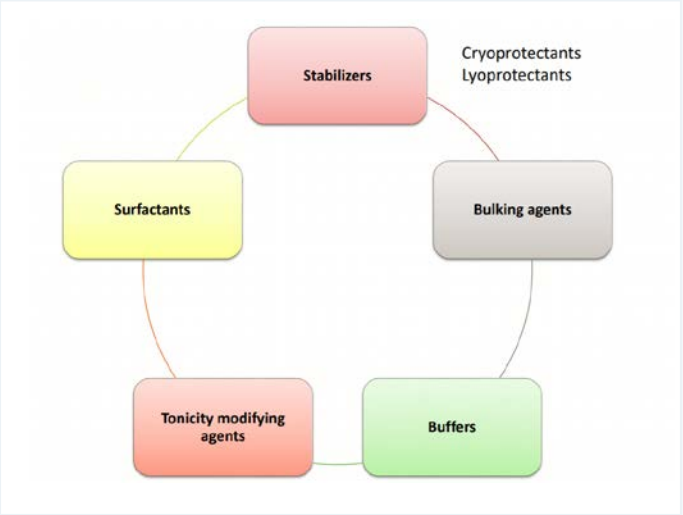

Figure 1: *Excipients in lyophilized protein formulations*

In the webinar, Dr. Munjal described several case studies taken from published literature to demonstrate how the interactions between formulation components can affect the freeze-drying process or product performance.

Case Studies

Sugars Affecting The Functionality of Bulking Agent and Vice Versa

Sugars e.g., sucrose and trehalose are the most popular lyoprotectants, however, the addition of sugars often tend to decrease the glass transition temperature (T_g') of the protein formulations, thus requiring relatively longer lyophilization cycle.

As a result, a bulking agent e.g., mannitol or glycine is often added to sugar based formulations. Bulking agents have high eutectic temperatures (T_{eu}), thus enabling faster lyophilization cycles whilst retaining good cake morphology. However, the effectiveness of this combination relies on the physical state of the two excipients during processing and storage, which in turn may be dependent

upon the ratio of the two components, active concentration, and processing conditions. The first set of case studies have been selected to highlight this aspect.

Case study 1: Sucrose with glycine

The first case study from Bai et al. 2004 demonstrated the importance of sucrose:glycine ratio on the phase behavior of the two components. At high sucrose to glycine ratios (4:1 and 9:1) the functionality of the bulking agent may be completely lost as it remained substantially non-crystalline, even after incorporation of an annealing step during lyophilization. In contrast, at low sucrose to glycine ratios (1:9, 1:4 and 2:3) glycine was substantially crystalline even without annealing (Figure 2).

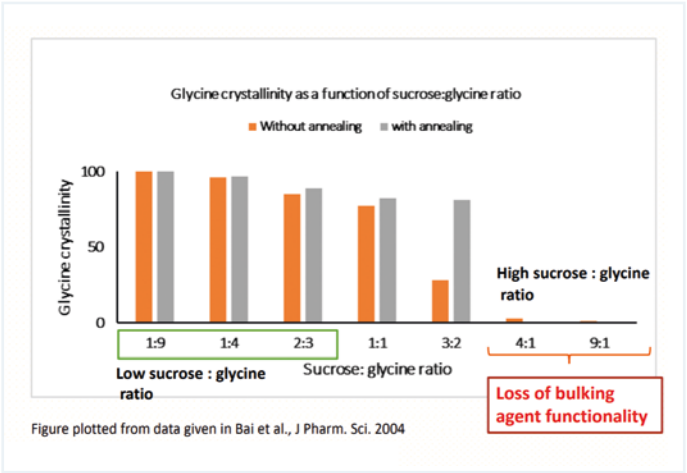

Figure 2: *Case study 1 - Sucrose with Glycine*

However, at these ratios, the low sugar concentration may not provide the desired level of lyoprotection. At intermediate ratios, glycine crystallinity could be significantly improved by adding an annealing step to the lyo recipe. This study reflected the importance of the ratio of the two excipients and processing parameters on the excipient functionality.

Case study 2: Trehalose with mannitol

The second case study dealt with the interplay of mannitol and trehalose. Sundaramurthi et al. 2010 had shown that mannitol can facilitate crystallization of trehalose dihydrate in the frozen solutions, while trehalose tends to inhibit mannitol crystallization. This was further studied in more details by Jena et al. 2016 and Jena et al. 2019, wherein solutions containing different ratios of trehalose:mannitol were lyophilized with a model protein e.g., bovine serum albumin, BSA. The physical state of the two excipients was found to be dependent upon the trehalose:mannitol ratio and protein:sugar ratio in the pre-lyo solution.

Case study 3: Sucrose with mannitol

Sugars can also alter the solid form of mannitol. The case study from Thakral et al. 2020 discussed the formation of mannitol hemihydrate (MHH) when used with sucrose in a ratio of 4:1. MHH tends to release water by dehydration during storage that can interact with other components and compromise product stability.

API/ Excipients Affecting the Functionality of Buffers

The second set of case studies discussed the impact of active and/or excipients on buffer functionality. Buffering agents are added to lyophilized products for maintaining the pH in the pre-lyo solution, during freeze-drying and in the reconstituted solution. Buffer selection for such products depends upon multiple factors including (but not limited to), the acid dissociation constant (pK_a) and crystallization propensity of buffer components during freezing.

Case study 4: Inhibition of buffer crystallization

Crystallization of a buffer component is undesirable as it can lead to 'pH-shifts' and impact product stability. This case study from Thorat et al. 2020 discussed about the 'pH shift' mediated aggregation of a model protein, BSA and its mitigation.

At high concentration (100 mM), the basic component of sodium phosphate buffer (dibasic hydrogen phosphate) crystallizes leading to a decrease in pH. This resulted in substantial protein aggregation upon freeze-thaw cycling. However, addition of cellobiose (5% w/w) inhibited the buffer crystallization and associated pH shift, thereby preventing protein aggregation. Lowering the buffer concentration (10 mM) also prevented protein aggregation. Here, the protein itself was in sufficient amount to inhibit the buffer crystallization.

Case study 5: Glycine with sodium phosphate buffer

The final case study from Pikal-Cleland et al. 2002 discussed about the impact of a bulking agent, glycine on functionality of phosphate buffer. Glycine tends to facilitate buffer crystallization and associated pH shift. Glycine, in a concentration dependent manner, showed a pronounced effect at low buffer concentration (10 mM).

Conclusions

The development of a stable freeze-dried product with desired attributes requires a judicious selection of excipient concentrations and optimization of processing conditions, to avoid loss of functionality of individual components.

To view the full webinar and download the slides, please go to the archived webinars on our website https://www.spscientific.com/Webinars/Archives/.

References

Bai, Shu Jun, et al. "Quantification of glycine crystallinity by near-infrared (NIR) spectroscopy." Journal of Pharmaceutical Sciences 93.10 (2004): 2439-2447.

Sundaramurthi, Prakash, and Raj Suryanarayanan. "Influence of crystallizing and non-crystallizing cosolutes on trehalose crystallization during freeze-drying." Pharmaceutical Research 27.11 (2010): 2384-2393.

Jena, Sampreeti, Raj Suryanarayanan, and Alptekin Aksan. "Mutual influence of mannitol and trehalose on crystallization behavior in frozen solutions." Pharmaceutical Research 33.6 (2016): 1413-1425.

Jena, Sampreeti, et al. "Stability of lyophilized albumin formulations: Role of excipient crystallinity and molecular mobility." International Journal of Pharmaceutics 569 (2019): 118568.

Thakral, Seema, Jayesh Sonje, and Raj Suryanarayanan. "Anomalous behavior of mannitol hemihydrate: Implications on sucrose crystallization in colyophilized systems." International Journal of Pharmaceutics 587 (2020): 119629. Jena et al. 2016 (3)

Thorat, Alpana A., et al. "Freezing-induced protein aggregation-Role of pH shift and potential mitigation strategies." Journal of Controlled Release 323 (2020): 591-599.

Pikal-Cleland, Katherine A., et al. "Effect of glycine on pH changes and protein stability during freeze–thawing in phosphate buffer systems." Journal of Pharmaceutical Sciences 91.9 (2002): 1969-1979.

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