BioPharma Solutions

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Best Practices in Formulation and Lyophilization Development

Proteins, mAbs and ADCs

Baxter

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Best Practices in Formulation and Lyophilization Development

The ultimate goal of formulation development is a stable product. In the case of a protein product, that can be defined as delivering the correct dose, in the native secondary and tertiary structure, without unintended chemical modifications, and free of extrinsic and intrinsic particles. In many cases, a lyophilized formulation can provide the highest probability of technical success. A well-designed product development plan can develop a phase 1 product quickly, while laying the foundation for commercial product success.

Development Approach

In new product development for proteins, monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs), it is common for the phase 1 and final commercial product presentation to be undetermined. Liquid, frozen liquid and freeze-dried solid formats may all be under consideration pending stability outcomes. Despite this uncertainty, clinical trials should be performed with a formulation representative of the final product. An appropriate freeze-dried formulation is always suitable for a frozen liquid and liquid formulation, but the inverse is not true. Therefore, end-in-mind development of a formulation that can be lyophilized, even if a liquid is used for phase 1 studies, results in overall faster timelines and lower risk.

Analytical Toolbox

Formulation development of biomolecules requires a broad tool box of analytical methods to understand the impact of formulation and process on stability. Typically, Size-Exclusion Chromatography (SEC) and charged-based methods such as imaging capillary electrophoresis (iCE) are the most stability-indicating methods for mAbs and ADCs, but Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) may be required for proteins. Monitoring of subvisible particulates using light obscuration and flow imaging (Flow Cam) is critical throughout formulation and process development.

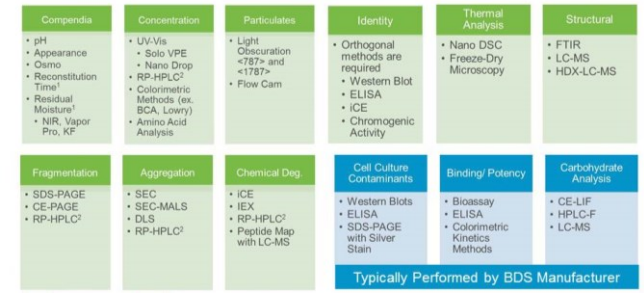

Figure 1. Analytical Methods for Protein Formulation Development

Formulation Development

Formulation development of protein products typically begins with characterization of the biomolecule to identify critical quality attributes of its physical and chemical stability. These may include determination of isoelectric point (pI) and assessment of physical stability after concentration, aggregation and freeze-thaw.

Studies to understand the effect of pH, ionic strength and surfactants on solubility and stability can provide insights to suitable formulation components for screening studies.

Many common solution components used in the production and formulation of proteins, mAbs, and ADCs can destabilize the molecules when used in a frozen or freeze-dried formulation. Potentially destabilizing components are shown in orange in Table 1.

Table 1. Common Formulation Components.

Component	Function	Examples
Tonicity Agent	Isotonicity	NaCl, Mannitol, and Glycine
Non-ionic surfactant	Inhibit formation of aggregates	Polysorbate 20 or Polysorbate 80
Buffer	pH Control	Sodium phosphate, potassium phosphate, citrate, histidine, and tris
Disaccharide	Stabilizer	Sucrose, Trehalose, Maltose, Lactose, Sorbitol
Crystalizing Excipient	Provide structure to cake	Mannitol or Glycine

Sodium chloride increases the osmotic strength of the remaining solution during the freezing process, which can influence aggregation and chemical stability. Sodium phosphate buffers are commonly used in solution formulations. However, during freezing, one component of the buffer can precipitate, shifting the equilibrium and result in a pH shift. The presence of phosphate buffers, such as sodium phosphate, will not always cause a pH shift. This occurs only in the event that one component precipitates. Figure 2 illustrates this change in pH that can occur during freezing through the inclusion of pH indicator in the buffer solution.

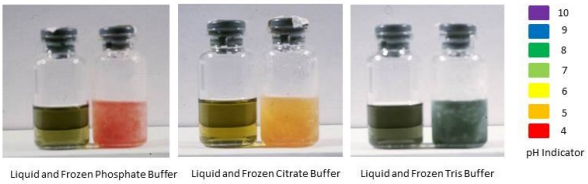

Figure 2. pH shift in between liquid and frozen states for phosphate, citrate, and Tris buffers.

Regardless of buffer selection, the concentration of buffer used in freeze-dried and frozen systems should be reduced to the minimum amount needed to control pH in order to prevent instability resulting from concentrated regions during the freeze-dried process.

Non-reducing disaccharides are used to stabilize proteins during the freezing and drying process. Reducing disaccharides such as maltose and lactose should be avoided because, at elevated temperatures, protein degradation can occur via the Maillard reaction between the carbonyls of the sugar and the free amino groups in the protein. Common non-reducing disaccharides are sucrose and trehalose. Protection during freezing is dependent on the concentration of the sugar and can require up to 5% (w/v) while protection during drying depends on the ratio between the sugar and the protein and can require ratios of sugar to protein of 1:1 or greater.

Surfactants, commonly polysorbates, are used to protect against the formation of aggregates during filtration, filling, freezing and freeze-drying. The need for a surfactant is typically determined using agitation, freeze-thaw, and accelerated stability

studies with monitoring by SEC, Dynamic Light Scattering (DLS), and/or Flow Imaging. Polysorbates are typically used at concentrations of 0.01% (v/v) or less.

Bulking agents, such as mannitol and glycine, are utilized in freeze-dried formulations to provide structure to the lyophilized cake, preventing appearance problems known as shrinkage (Figure 3) and collapse. Bulking agents are not required in solution or liquid formulations but may be added to increase the tonicity of the solution. Crystallizing excipients, such as mannitol and glycine, do not provide protection to the protein so, typically, combinations of non-reducing disaccharides and bulking agents are used.

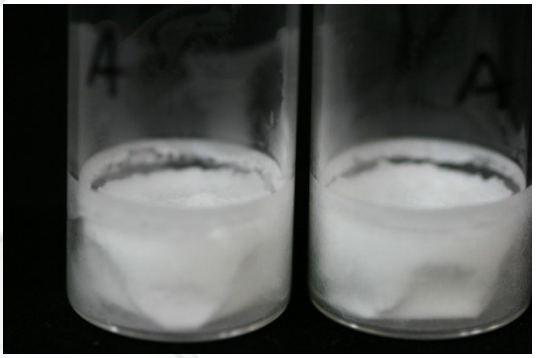

Figure 3. A Lyophilized ADC-Sucrose Formulation Showing Shrinkage of Cake

When using mannitol, it is essential to ensure that it is fully crystallized. If mannitol crystallizes post-lyophilization, it can release the water associated with it back into the cake, potentially accelerating destabilization of the product. Annealing is a step in the freeze-drying process which can be used to promote mannitol crystallization. In the annealing step, the frozen product is warmed to a temperature greater than the Tg' but not to melting, and then cooled back to freezing temperatures. Differential Scanning Calorimetry (DSC) is used to optimize annealing conditions, and X-Ray Powder Diffraction (XRPD) (Figure 4) can be used to determine the crystalline state of mannitol in a freeze-dried solid.

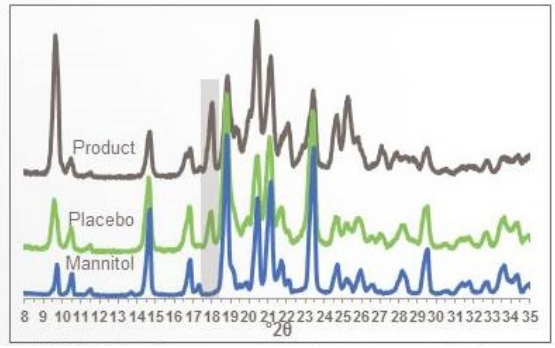

Figure 4. XRPD with area of mannitol hydrate form highlighted. On stability, mannitol hydrate can release moisture back into the freeze-dried solid causing instability.

Screening studies comparing relative ratios of non-reducing disaccharides and crystalizing excipients are performed with liquid and lyophilized samples evaluated for both appearance and stability under accelerated conditions.

Establishment of a Conservative Lyophilization Cycle

In non-crystalline frozen systems, the glass transition temperature (Tg') of the freeze-concentrated solute determines the collapse temperature (Tc) during freeze-drying. Above the Tg', the freeze-concentrated

material undergoes viscous flow after the supporting ice structure is gone, resulting in collapse. Below the Tg', the freeze-concentrated material is rigid enough to support its own weight after the ice has sublimed away. Freeze-dry microscopy can be used to screen multiple formulations. A few microliters are placed on freeze-drying stage and visually monitored during the freezing and primary drying process. The Tg' provides a preliminary upper limit of product temperature during primary drying which can be used to design a conservative cycle suitable for phase 1 manufacturing.

Development Stability Studies

The conservative lyophilization cycle can be utilized to produce development stability samples. Liquid, frozen liquid (if desired), and lyophilized samples can be placed on stability simultaneously, reducing cost. Data used from this study can be used to make a decision regarding final product format.

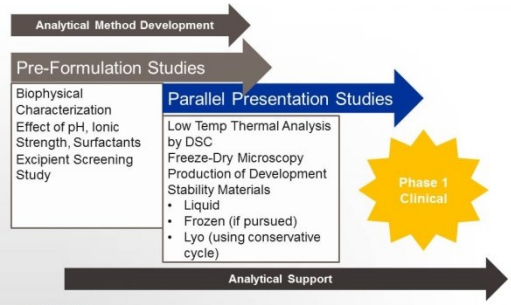

Figure 5. Development Pathway to Phase 1 Clinical Manufacturing

Lyophilization Cycle Optimization

Following a decision to use a lyophilized presentation, the cycle may be optimized. Using a design space approach, a safe zone of operation for primary drying is identified. Within the safe zone, conditions resulting in a minimum time cycle are identified which can significantly reduce manufacturing cost. The first step to developing the safe zone is to identify the edge of failure for the cycle, since the Tg' does not always represent the maximum product temperature which will result in a stable product with acceptable appearance. The failure point is established through three or four lyophilization cycles at increasing primary drying product temperature. The remainder of the design space is based on three factors: the equipment capability, which should be known by the drug manufacturer for each piece of equipment, the heat transfer coefficient of the vial (Kv), and the resistance of the dried product layer (Rp). The Kv can be measured with liquid-filled vials, and once known for a vial does not need to be re-established. Only the Rp value determination requires the use of product. Therefore, the primary design space can be developed quickly, typically within 4-5 weeks.

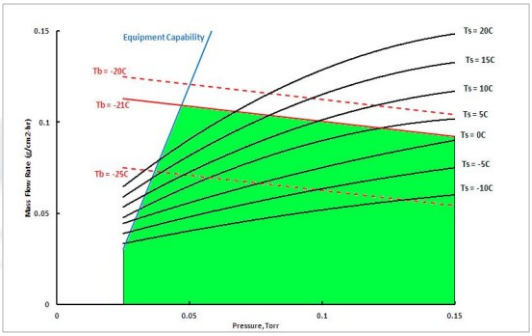

Figure 6. Primary drying design space. The solid red line represents the maximum product temperature based on edge-of-failure studies. The fastest possible primary cycle is obtained by the conditions represented in the top left corner of the green area.

Secondary drying conditions can be established by first creating samples at a variety of moisture levels, using a sample thief during secondary drying, then using the samples to create a calibration curve of moisture vs. absorbance by NIR and Karl Fischer moisture titration. A second freeze-drying run can be used to remove large groups of samples at specific points during the secondary drying step. The moisture levels of the samples are determined non-destructively using NIR, and the samples are placed on accelerated stability. Once the optimum moisture level for the product is selected, the secondary drying time used to generate the sample can be identified and utilized for future production.

Following establishment of optimized lyophilization cycle conditions, a confirmation batch is produced with samples placed on accelerated and long term stability.

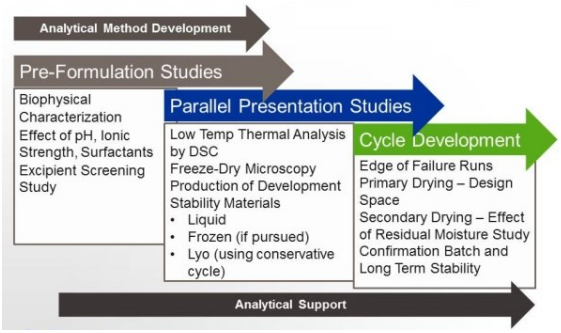

Figure 7. Full Formulation and Lyophilization Development Process

Conclusions

For protein, mAb, and ADC products, lyophilized formulations provide the greatest probability of technical success. Multi-path development, exploring liquid, frozen liquid and lyophilized presentations early in the development process, can reduce the time required to reach critical clinical milestones while minimizing risk of failure. However, formulation development must be conducted with the end point in mind. While the optimum lyophilized formulation will always be the optimal frozen formulation, the opposite is not true. The formulation development considerations are the same for frozen liquid and lyophilized product, and development of a conservative lyophilization cycle requires knowledge of the Tg' and Tc only. The use of an optimized lyophilized formulation in early trials, whether using solution, frozen solution or lyophilized final presentation, provides the option to move forward with a lyophilized presentation if needed, further optimize the lyophilization cycle, and have the best chance for ensuring a stable commercial product.

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