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For Lyophilization, Excipients Really Do Matter

Excipient selection strongly influences lyophilization performance for biologic drugs.

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Lyophilization, or freeze drying, is a well-recognized method for obtaining stable biologic drug products that have short shelf-lives in solution form. Lyophilized products are easy to store and ship. Because freeze-drying is a low-temperature process, thermal degradation is minimized. Furthermore, the preparation of high concentration formulations is readily achievable using lyophilized material.

The three-step process—freezing, often with annealing; primary drying via sublimation; and secondary drying via desorption—exposes biologic drug substances to harsh conditions and stresses that can affect their structure and function. The lyophilization process is also costly and can be time consuming.

Excipients not only affect the stability of proteins and other biologics, they also determine the appropriate operating parameters for the lyophilization process. They are, therefore, crucial to the development of cost-effective, freeze-drying operations that afford solidified products with desirable appearance and performance properties.

Destabilizing forces

The bioactivity and function of a biomolecule is closely related to its higher order structure. Lyophilization subjects a protein to conditions such as freezing, temperature ramps, vacuum, and dehydration, all of which can disrupt the fragile protein structure leading to loss of activity, according to Anjali Joshi, director of formulation development with Recipharm. “The role of excipients is to ensure that this disruption is minimized,” she says.

An additional goal of the formulator for all molecules is to keep the formulation as simple as possible while achieving acceptable chemical, physical, and microbiological stability, according to Gregory A. Sacha, senior research scientist, BioPharma Solutions, Baxter Healthcare. That can become challenging when working with biologics that will be lyophilized because the formulation and process are intimately connected.

Sacha notes that biologics, in particular, can be sensitive to interfacial interactions, including interactions at the air/liquid interface, the interface of the liquid and the primary packaging, and at the solution/ice interface. “Some large molecules can unfold at these interfaces, which can lead to irreversible aggregation. Aggregation can lead to loss of the activity of the molecule, may lead to hypersensitivity reactions in the patient, and can make future doses of the medication ineffective if the patient develops antibodies against the active protein,” he explains.

Another challenge for large molecules is the phenomenon known as freeze concentration, in which the solutes become concentrated as ice forms. “The concentration of other excipients in the formulation then occurs, which allows the large molecules to come sufficiently close to interact with one another,” Sacha observes. Depending on the contents of the formulation, freeze concentration can lead to changes in pH and increases in ionic strength.

Removing water from a formulation (drying) to create a freeze-dried solid also presents a challenge to the stability of some large molecules. “Protein structure and activity in solution is largely determined by its interactions with the surrounding water. During lyophilization, removal of water can therefore cause destabilization of the protein structure,” comments Joshi, referencing a review by Ohtake *et al* (1).

Excipients as stabilizers

Cryoprotectants protect proteins from damage during freezing, while lyoprotectants provide protection against the stresses that occur during drying. Typical chemicals used as excipients for lyophilized biologics include sugars or polyols, polymers, surfactants, buffers, amino acids, chelating complexes, and inorganic salts. Because the conditions during freezing and drying are different, the stresses on biologic drug substances are different, and combinations of excipients are often required to provide the protection and stabilization required for a given protein, monoclonal antibody, etc.

The most commonly used excipients are protectants, surfactants, buffers, and bulking agents, according to Alan Fites, a senior pharmaceutical scientist with Singota Solutions. Stabilizers include both cryo- and lyoprotectants. “These excipients are most critical to the structure and ultimately the function of freeze-dried biologic drug products. They not only stabilize/protect the protein structure during freezing, primary drying, and secondary drying, but often also reduce the primary drying time. Many also

act as bulking agents," Fites says.

Some amorphous sugars can also offset the effects of dehydration because they can hydrogen bond to the dried protein to replace the lost water, according to Joshi. Research studies conducted by Michael Pikal at the University of Connecticut School of Pharmacy suggest that including small quantities of sorbitol may improve the stability of some large molecules (2), notes Sacha. "These results may seem counterintuitive, because sorbitol can decrease the glass transition temperature of the frozen solute (Tg') and decrease the glass transition of the dried solid," he comments.

In addition, Joshi notes that during storage in the freeze-dried state, the high glass transition temperature of sugars imparts stability by maintaining the protein in a glassy matrix in which molecular mobility is restricted (1).

Examples of common stabilizers include sucrose, trehalose, mannitol, polyvinylpyrrolidone (PVP), dextrose, and glycine. They can be used in combination, such as sucrose and mannitol, to produce both an amorphous and crystalline structure.

Surfactants such as polysorbates 80 and 20 are used with large molecules that exhibit sensitivity to interfacial interactions, according to Sacha. These excipients preferentially coat surfaces and prevent proteins from unfolding at the surfaces. "Specifically, surfactants can protect proteins against surface-induced aggregation by competing with the protein for adsorption sites on surfaces, by binding to hydrophobic regions on the protein surface to decrease protein-protein interactions, by coating the protein and preventing unfolding, and/or by increasing the free energy of protein unfolding," adds Joshi, referencing a study by Agarkhed *et al* (3).

Minimization of adsorption to the container material is also critical in low-dose formulations in which small percentages of protein adsorption can greatly alter the overall concentration of the formulation, according to Fites.

Bulking agents provide a desirable structure and appearance to the product. "They are particularly important for formulations with low concentrations of drug substance," Fites notes. In this case, not only can the appearance of the dried product be improved, loss of the drying powder due to ejection from the vial during the drying process can be prevented, according to Sacha. Fites adds that use of an amorphous excipient (e.g., a non-reducing sugar) in a formulation containing an active ingredient that is subject to hydrolysis can enhance product stability by adsorbing residual moisture. Some stabilizers can also serve as bulking agents, reducing the number of required excipients, according to Joshi. She adds that the most commonly used crystalline bulking agents, mannitol and glycine, crystallize during freezing and provide an elegant lyophilized cake structure (4).

Buffers help to maintain pH during freezing and after reconstitution, which is important because proteins contain multiple ionizable groups and their conformational integrity and biological activity are critically linked to pH in the aqueous state, according to Joshi. She adds that in the lyophilized state, proteins have 'pH memory', which means they retain the stability properties corresponding to the aqueous solution prior to drying (5).

Other stabilizers used in lyophilized biopharmaceutical formulations include antioxidants. Proteins can oxidize at specific amino acids, namely methionine (Met), cysteine (Cys), histidine, tryptophan, and tyrosine, says Joshi. "The mechanisms for oxidation in proteins are complex and varied and antioxidant choices must be made based on the oxidation mechanism. A widespread practice to minimize oxidation is to include nitrogen gas in the vial headspace. If oxidation is catalyzed by trace metal ions, chelating agents (e.g., EDTA, citrate) can act as antioxidants by complexing with the metal ion. Oxidizable amino acids (e.g., Met, Cys) that are preferentially oxidized can also be added as excipients, thereby protecting the protein from oxidative stress," she says. Sacha asserts, however, that it is most desirable to eliminate such catalysts rather than add other excipients.

Balancing the chemistry

While excipients do stabilize biomolecules during the lyophilization process, some can negatively affect the stability of proteins. In general, according to Sacha, other excipients can most affect the performance of the formulation during the freezing step.

“Care should be taken to not increase the salt content of the formulation because salts can increase the ionic strength during freeze concentration and change the thermal behavior of the formulation, making it difficult to freeze dry,” he notes. Fites points to phosphate buffers, and particularly sodium phosphate, which can undergo pH shifts during freezing. “If a formulation requires the use of buffers, two options are to use a phosphate buffer at low concentration or a non-phosphate buffer that undergoes minimal pH shifts during freezing, such as Tris, citrate, or histidine,” he says.

Some excipients can prevent or delay the crystallization of components that should crystallize in the formulation. Salts can cause this type of issue, too, for instance by preventing or delaying the crystallization of mannitol, which is often included to provide structure for an acceptable-appearing dried solid. The mannitol may then crystallize in the sealed vial after lyophilization, resulting in the release of moisture into the micro-environment of the dried solid,” Sacha notes.

Certain sugars may interact with proteins and lead to browning of the dried solid via the Maillard reaction, which occurs between proteins and reducing sugars, such as lactose.

Trace impurities, such as metals in sugars and peroxides in polysorbates, can also be problematic. “In such cases,” says Joshi, “studies are needed to understand the impact of the trace impurities on protein degradation/oxidation. They may also justify working with excipient vendors to tighten specifications for any impurities that cause problems,” she states.

Narrow choice

The use of excipients depends completely on the behavior of the molecule. For instance, Sacha notes that there are peptides that are self-buffering and do not require any excipients at all. “In general, large molecules typically need protection from interfacial interactions and from over-drying. That means using a surfactant or human serum albumin and an amorphous disaccharide, such as sucrose or trehalose,” he observes.

There are, however, a limited number of excipients that have been used in marketed protein biopharmaceuticals (6). “The selection of excipients is empirical, and pharmaceutical scientists first evaluate excipients that have a precedent in marketed products. Using a novel excipient that has no precedent requires additional safety data and the cost and risk associated with this path make it an undesirable first choice,” asserts Joshi.

In addition to the desired performance characteristics of an excipient, there are other factors that should be considered when selecting excipients for design of experiment studies, according to Tammy Thompson-Madsen, a senior pharmaceutical scientist at Singota. These factors include previous regulatory acceptance, purity, supply chain availability, cost, ethical acceptability in the target market, and others. “During early-stage development, novel or experimental excipients may be tested, but when moving closer to commercialization, the factors mentioned above must be weighed more heavily in final decisions for the biologic lyophilized formulation,” she comments.

Process considerations

“The goal when developing formulations for lyophilized biologic products is not only to achieve a stable, elegant product, but also develop a short and efficient freeze-drying cycle,” Joshi states. That can be challenging because there is no way to separate formulation development from process development when creating a lyophilized solid, according to Sacha. “The excipients and active ingredient affect the thermal behavior of the formulation. Consequently, formulators require knowledge of the thermal behavior of the individual compounds. Those excipients that require temperatures below approximately -37 ˚C to create an acceptable dried solid will be challenging to transfer to full-scale manufacturing,” he explains.

Conducting pre-formulation studies in the liquid state can help reduce this complexity before any cycle development begins. Forced degradation studies using acid, base, heat, peroxide, and agitation can identify the most likely conditions to cause instability and how the instability manifests itself (aggregation, oxidation, etc.). Aqueous formulation studies can identify the baseline environment of pH and buffer in which the instability is minimized. “Once these studies are completed, formulation and lyophilization studies can be conducted to choose the optimal excipients,” says Joshi.

Evaluating the options

Because there is significant potential for undesirable interactions between excipients and the biologic drug substance, studies must be designed to understand the behavior and sensitivities of each large molecule, according to Sacha. In addition to the studies mentioned above, freeze/thaw and agitation studies intentionally expose the large molecule to interfacial interactions and thus help identify excipients that prevent aggregation. Studies should also be conducted to determine if the molecule requires a buffer to maintain a specific pH range.

“Accelerated stability studies provide critical information regarding excipient selection,” agrees Thompson-Madsen. She also points to quick studies, such as removing the stoppers from lyophilized products and exposing them to various humidity/temperature combinations, followed by analytical testing to identify acceptable drug excipient combinations that do not lead to degradation.

Thermal analysis techniques are invaluable for evaluating the impact of excipients on the folding/unfolding of proteins (7). When a protein is heated to above its Tm, it undergoes a conformational change that exposes previously hidden areas, which can lead to aggregation and/or degradation, according to Joshi. “Excipients should therefore be chosen based on their ability to maximize the unfolding temperature, which is best determined using thermal analysis techniques, most commonly differential scanning calorimetry (DSC),” she observes. DSC studies can be performed on various formulation matrices and the excipients affording the highest Tm can be selected (7).

Data generated about the glass transition temperature using both DSC and freeze-dry microscopy (FDM) can help to predict or rule out various excipients or combinations of excipients, adds Thompson-Madsen. FDM enables the identification of temperatures at which visible changes occur in samples.

Solution formulations can also be evaluated using second derivative Fourier transform infrared spectroscopy, according to Sacha. In this approach, spectra of different formulations are compared to determine if any changes to the secondary structure of the molecule have occurred.

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