



KEY CONCERNS IN LYOPHILIZATION CYCLE DEVELOPMENT

Lyophilization (freeze-drying) continues to grow in popularity as a method for processing active biopharmaceutical ingredients and excipients into stable drug products. However, the process remains complex and costly, demanding efficient solutions; particularly at large scale.

A lyophilization cycle consists of three processing steps: freezing, in which the product's temperature is rapidly lowered; primary drying, in which ice formed during freezing is removed under vacuum; and secondary drying, in which residual moisture is removed by adsorption. Finally, the freeze-dried product is stoppered under vacuum.

Risks of failure abound throughout each of these stages. Excessively rapid supercooling can cause the formation of a greater-than-expected number of ice crystals, damaging the delicate organic molecules of the active pharmaceutical



ingredient (API). Inadequate moisture during drying can reduce the water content of the formulation, destabilizing it and rendering it useless.

Excipients such as stabilizers, buffers, pH adjusters, tonicity modifiers and bulking agents, meanwhile, must be chosen carefully and added in the correct amounts, to prevent destructive fluctuations in the formulation's pH or cake structure. In addition, even subtle variations in the diluent can significantly impact the final product's solubility and rate of dissolution.

Thus, throughout all stages of the lyophilization cycle, experts must carefully monitor the formulation via an array of modern analytical techniques, in order to ensure minimal variability and maximum viable product. X-ray powder diffraction is needed to track the stability of pharmaceutical molecules, while Karl-Fischer titration helps ensure that residual moisture remains within acceptable parameters, and differential scanning calorimetry (DSC) informs delicate adjustments in the amount of heat used to raise the product's temperature.

The following are key concerns in the development of any pharmaceutical lyophilization cycle, addressing common issues in each of the stages.

TESTING A VARIETY OF FORMULATIONS WILL HELP CREATE SIGNIFICANT SAVINGS ON LONG-TERM COSTS.

In order to ensure that the lyophilization process results in products of ideal composition, stability and pharmaceutical efficacy, it is important to perform a testing round (or, if necessary, several such rounds) of lyophilization on a variety of formulations. Just as freezing speeds, drying temperatures, and degrees of vacuum should be optimized through trials, variations in formulation will provide equally useful parameters for larger-scale production.

First, the concentration of the active biological pharmaceutical ingredient should be varied. Many proteins can cause dramatic shifts in pH during the freezing process, especially when buffers such as sodium phosphate are used. Thus, it is also useful to test the formulation with several different buffers, such as potassium phosphate, citrate and/or histidine, in a range of concentrations, in order to establish workable parameters for pH fluctuations.



A variety of excipients should also be tested in a range of concentrations. Crystalline bulking agents, while known to produce well-formed cakes, also have a tendency to destabilize emulsions, proteins and liposomes. Thus, it may sometimes be more practical to use a disaccharide bulking agent such as sucrose, which offers the added benefit of forming an amorphous sugar glass that helps stabilize liposomes and proteins.

The presence of stabilizers is crucial throughout both drying stages. Stabilizers prevent the accidental removal of the hydration shell, and the destabilization of the formulation's protein structure. This protection is necessary because lyophilization places proteins under two types of denaturation stresses: freezing stress and drying stress. While some proteins remain stable under freezing stress, but denature during the drying phases, others exhibit precisely the reverse vulnerability; or a combination of both.

When working with API proteins that denature during the freezing phase, cryoprotectants such as surfactants, sugars, salts, amines and non-aqueous solvents may serve as ideal stabilizers. For proteins that denature during the drying stages, on the other hand, lyoprotectants like polymers, sugars and amino acids will prove more useful. Although many non-aqueous solvents exhibit a strong tendency

to its original condition



to denature proteins, rendering them ineffectual, some non-aqueous solvents can actually serve as cryoprotectant shields during the freezing stage, preventing proteins from becoming desiccated. Polyethylene glycol, ethylene glycol, glycerol and dimethyl sulfoxide (DMSO) have all been shown to protect certain proteins from freezing-related damage.

Finally, in the case of isotonic formulations, it may also be necessary to add one or more tonicity adjusters, such as mannitol, sucrose, glycine, glycerol and sodium chloride, to the diluent rather than to the formulation itself. Many isotonic lyophilized formulations may require tonicity adjusters in order to further improve the solubility and absorption that have proven to be such significant selling points for lyophilized drug products. The choice of tonicity adjuster may be governed by the formulation's intended route of administration, or by the stability requirements of the bulk solution.

THE FREEZING STAGE MUST TAKE PLACE AT PRECISELY THE CORRECT RATE.

As the formulation cools, pure crystalline ice forms within it, creating a freeze concentration of non-frozen liquid, which transforms into a more viscous state rather than crystallizing. Ideally, this concentrated, viscous solution will solidify into a further phase, which may be amorphous, crystalline, or amorphous-crystalline.

However, if supercooling takes place too quickly, excessively rapid nucleation can cause a large number of small ice crystals to develop. When proteins come into contact with this large ice-water interface, they can become desaturated, denaturing

the formulation by disrupting the structure of the liposome bilayer, and rendering the product useless in the earliest stage of the lyophilization cycle.

Thus, it is crucial to freeze the solution in a stepwise manner, carefully monitoring the temperature within the freezing chamber, as well as the rate at which the formulation itself is cooling. To pinpoint the correct temperature and rate of cooling, a variety of formulations must be tested under a range of freezing conditions, then assayed afterward to determine the resulting structural properties of their APIs.

PRIMARY DRYING MUST TAKE PLACE AT THE RIGHT RATE, AND BE TERMINATED AT THE RIGHT TIME.

In the primary drying stage, ice formed during freezing is removed through the use of sublimation at subambient temperatures, within a vacuum; typically at chamber pressures of 40 to 400 Torr, and shelf temperatures from -30° to -10°C. Ideal primary drying time is typically 10 hours, with the possible addition of another hour or two in order to ensure complete drying.

In order to dry the product while retaining the structure established in the freezing stage, the product is maintained in a solid state below its eutectic melting point or collapse temperature. The primary drying temperature should be kept several degrees below this temperature, in order to avoid melting the frozen cake, resulting in its collapse and improper drying.

Differential scanning calorimetry (DSC) can provide useful insight into the eutectic melting temperature of a frozen solution, providing clear



parameters for the temperatures to be used in both primary and secondary drying. This measurement is obtained by taking a neutral sample (such as an aluminum pan) for reference, equilibrating the sample and freezing it in a stepwise manner until it reaches -50°C, then heating it at at a rate of 10°C per minute, and noting the precise temperature at which melting begins.

During primary drying, the temperature of both product and shelf within the drying chamber must be carefully monitored in order to identify the point at which the product's temperature equals the shelf temperature, signaling the end of this phase. The degree of vacuum in the primary drying chamber must also be carefully watched, as it determines the rate of effective heat transfer between the shelf and the vials containing the product. If the vacuum can be increased moderately without causing harm to the API, a primary drying phase under greater vacuum will allow more effective heat transfer, speeding up drying and resulting in a more costeffective process.

PROPER MOISTURE AND TEMPERATURE CONTROL ARE ESSENTIAL DURING THE SECONDARY DRYING PHASE.

The secondary drying stage removes the relatively minor amount of bound water remaining in the matrix, via desorption, while the shelf and product temperature are both raised in tandem. Thus, the rate at which the chamber temperature rises must facilitate adequate desorption rates, while simultaneously producing a good level of residual moisture.

Secondary drying is often carried out at a higher temperature than primary drying; sometimes 30°C or more; for a significantly longer time; often seven hours or more. The higher the secondary drying temperature, the more rapidly water will desorb; and the less time this phase will take. However, the temperature and rate of drying are limited by the stability of the API protein. Some proteins are highly heat-labile, requiring secondary drying temperatures lower than 25°C; while other, more stable proteins allow for a secondary drying temperature of 35°C or even 50°C, with no damage to the biological molecules of the API.

The amount of residual moisture in the final product determines the time necessary to complete the secondary drying phase. It is often counterproductive to remove all residual moisture, since protein molecules require some level of hydration in order to remain stable. A higher degree of vacuum in the secondary drying phase, relative to the vacuum used in primary drying, can facilitate more rapid water desorption, and speed up the secondary drying process.

However, the amount of residual moisture requires varies from one protein to another, necessitating careful tests of secondary drying time and temperature. Some proteins whose quaternary structure survives the freezing phase may lose their stability and efficacy if they lose their hydration shell and become desiccated



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during the drying phases. This is of particular concern during the secondary drying phase, when temperatures are high and moisture is at its lowest.

Thus, throughout the secondary drying phase, it is useful to remove the vials from the lyophilizer and assess their residual moisture content via infrared scans. As soon as the moisture content of the cake has drops below excessive levels, while retaining enough moisture to enable the protein molecules to remain stable, the secondary drying phase is complete.

BULKING AGENTS AND BUFFERS CAN HELP PRODUCE A MORE IDEAL CAKE COMPOSITION.

After freezing, primary drying, secondary drying and stoppering, the time has come to inspect lyophilized samples in terms of appearance and activity. If the cakes appear diffuse, this is due to low solid content, which can be counteracted by the addition of a bulking agent to the formulation. In terms of bulking agents, disaccharides are typically a better choice than crystalline agents, because (as mentioned above) many crystalline bulking agents destabilize proteins, while disaccharides form an amorphous sugar glass that helps stabilize them.

The exact reason for disaccharides' stabilizing effect has not been proven conclusively, but two main hypotheses have been put forward to explain it. According to the water replacement hypothesis, disaccharides interact with liposomes and proteins by hydrogen-bonding in a similar way to the water they replace. The vitrification hypothesis, on the other hand, posits that disaccharides form sugar glasses of extremely high viscosity, which immobilize the drug and water molecules, creating the extremely high activation energies necessary for reactions to occur.

A solution's pH also exerts a significant effect on the activity of API proteins. This relationship may follow a variety of curves, depending on a given protein's biochemical properties; but in any case, the activity of many enzymes is highly pHdependent. In addition, many lyophilized formulations undergo significant pH shifts during the freezing process, necessitating a buffer that undergoes minimal pH change, such citrate or histidine; or the use of a buffer that can maintain a steady pH at low concentrations.

ALL LYOPHILIZATION EQUIPMENT MUST BE STERILIZED AFTER EACH CYCLE.

Once the freezing, primary drying, secondary drying and stoppering phases are complete, the lyophilization chamber, along with all related equipment, must be fully sterilized before a new batch of product can be processed. Many biologics are highly hazardous in their active forms; yet many facilities have recently come under scrutiny for improper sterilization. Thus, the sterilization phase must be strongly emphasized in any list of key concerns in lyophilization.

One popular sterilization technique is to pump moist steam into the lyophilization chamber under high pressure, simulating the effect of an autoclave on the lyophilizer's interior surfaces. Effective as this technique might seem, the fact is that many facilities use older lyophilizers, which exhibit poor tolerance for steam under pressure, allowing much of the steam to escape. In addition, this technique requires two independent temperature-sensing systems: one to monitor overall temperatures throughout the steaming cycle, and a second to track the temperature of the coldest spot within the chamber, to ensure that even that point is fully sterilized.

Another common technique is simply to manually wipe down the lyophilizer's interior surfaces with a chemical agent. However, this technique, too, produces highly uneven results, because manual application of a cleaning agent often fails to reach the backfill piping used to introduce inert gas and sterile air into the chamber. Proper sterilization also requires thorough cleaning of all vent filters, shelf support rods, and other components, many of which are not attended by manual cleaning. Thus, the next lyophilization cycle may re-introduce contaminants accrued during the previous cycle, unless complete sterilization procedures are used.

STORAGE AND VIAL FILLING OPERATIONS MUST ALWAYS FOLLOW GOOD MANUFACTURING PRACTICES.

Lack of sterilization is only one significant risk for a lyophilization facility. An inspection by the US Food and Drug Administration (FDA), or another regulatory agency, can result in a citation for failure to follow good laboratory practices (GLP) or good manufacturing practices (GMP), risking a letter of warning at best, and an outright product ban or facility shutdown at worst.

GLP and GMP failures can emerge at every stage of the development and production processes, from the initial formulation of a solution, to the filling of vials, to the validation of fills, to the testing an packing of the final product. One common failure is unexpected instability in the final package product; a strange problem, given that stability is precisely the reason why lyophilization is used in the manufacture of so many biologic drugs. However, some lyophilized dosage forms actually lose their antibacterial effect in solution, minimizing their bioburden, and creating different requirements in the sterilization and fill phases in order to maintain stability.



Concerns like these necessitate careful monitoring of microbiological levels at all times prior to final sterilization, to prevent the development of endotoxins at an accelerated rate. GLP dictates that prior to sterilization, all lyophilized products must be batched in a highly controlled environment, within sealed tanks. In addition, variations in stoppering equipment may result in improperly placed stoppers, negating all previous efforts at sterilization before the lyophilization process even begins. Thus, it's crucial to validate each filling operation with media fills, and to sample critical surfaces and air throughout the active filling stage. Within the lyophilizer itself, partially stoppered vials should always be transferred and handled under proper barriers, such as laminar hoods.

Assurance of fill volumes presents another common risk. A low fill will result in a vial whose potency falls below standard, potentially invalidating an entire batch of product. And unlike a powder or liquid fill, a low fill of a lyophilized vial may not become obvious until after the entire costly cycle has been completed. Thus, the volume of each fill should be monitored on an ongoing basis; particularly in areas of the filling operation where high or low fills have been noticed in previous tests.

EACH BATCH OF DOSES MUST BE TESTED FOR STERILITY, STABILITY AND PHARMACOKINETIC ACTIVITY.

Before a batch of lyophilized doses is complete, it must be tested for uniformity, sterility, and stability; particular if it represents an aged batch of API that has been reconstituted. In some cases, the lyophilized product may consist only of an API, while in most cases it will contain some excipients as well. In any case, unexpected variations in weight can signal a lack of potency.

Another common issue is a lack of proper testing under varied reconstitution procedures. Despite the fact that many lyophilized drug products are tagged for reconstitution with Bacteriostatic Water for Injection (BWFI), some hospitals only use Sterile Water for Injection (WFI), due to the toxicity risks associated with BWFI. The use of WFI, however, may actually kill some of the vegetative cells that are present as contaminants; a fact that necessitates testing for reconstitution with Sterile WFI, and proper labeling if its use presents a problem.



Once each batch has been weighed, tested, and successfully reconstituted, the final step is to critically review all data on the product's stability. This data, combined with data on the batch's highest moisture content, and on the stability of the least and most reconstituted solutions, will enable the product to be labeled with a conservative expiration date. In addition, stability data should factor in any relevant findings from assays of aged samples, as well as their reconstitution at any point up to their existing expiration data.

CONCLUSION

As this survey demonstrates, key concerns in lyophilization cycle development extend far beyond the lyophilization cycle itself, into the areas of excipient selection, equipment sterility, facility management, and testing, labeling and packaging of lyophilized drug products. All these factors exert significant impact on the composition and efficacy of the final formulation.

At any point in the cycle, a seemingly minor mistake in in freezing temperature, drying speed, degree of vacuum, pH buffer, stoppering or dosage measuring can create significant hazards for the developer and patient alike. For all these reasons, it is crucial to take a view of the lyophilization cycle within its complete context, in order to ensure that small variations do not add up into wasted batches and unmarketable products, but can be counteracted in time.

