Freeze Drying of Vaccines – Challenges and Concepts in Formulation and Process Development

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Vaccination prevents millions of deaths from infectious diseases per year; however, there are still millions of people who die from these diseases due to weak thermostability demanding cold chains, long-term stability issues and distribution challenges. All these limitations can be overcome by creating a dry product through lyophilization. With the urgency to develop a coronavirus vaccine, it is a crucial time to discuss the process of freeze-drying vaccines.

Recently, Ms. Julia Kosan, a PhD candidate from Friedrich Alexander University, Erlangen, Germany presented a webinar that discussed the suitability of freeze-drying vaccines to increase their thermostability. This tech note summarizes the webinar and includes a selection of questions from the Q&A session.

Impact of Lyophilization on Vaccines

There are many types of vaccines all of which trigger specific immune responses. Several coronavirus vaccines under development are based on recombinant vector vaccine which uses coronavirus genomic material packaged into a viral vector.

Freeze-drying a vaccine provides a significant advantage but there are several obstacles that need to be overcome. Complex formulations, especially vaccines that are made up of several antigens or multiple strains can result in challenging critical formulation temperatures and complicated freeze-drying processes. Freezing and drying can stress the vaccine whereby the extent of this process sensitivity will vary between different vaccines. Internal ice formation and direct damage to a component of the vaccine e.g. the lipid membrane, proteins or nucleic acids can act as stress factors.

During freezing, intraviral ice crystals can form which will increase the volume of the product and may damage the lipid bilayer (Figure 1). The ice also creates new interfaces between liquid and ice and increases the risk of surface induced aggregation.

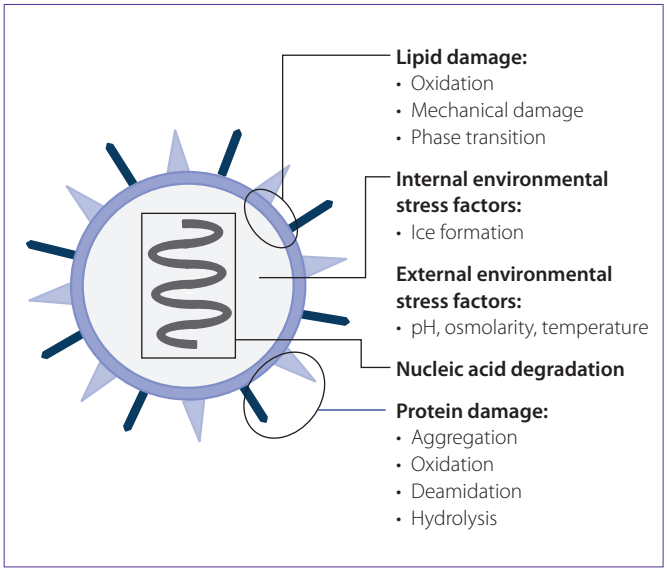


Figure 1. Stress Factors During Freeze Drying

In the primary drying step of the lyophilization cycle, drying above the critical formulation temperature results in increased mobility of the amorphous phase which allows protein interactions and can increase membrane permeability.

As part of the secondary drying stage where the hydration shell of each is removed, protein aggregation and inactivation can occur, and in the case of phospholipids, the change in thermotropic phase transition can also increase membrane permeability. Secondary drying directly affects residual moisture levels which can impact long term stability.

Required Characteristics of Vaccine Formulations

Optimally, vaccines need to be stable in both liquid state for at least 24 hours and dry state for long term storage. To achieve this, vaccines need to be developed with appropriate formulation and processes.

Stabilizers (cryo- or lyo-protectants) play a huge role in developing a stable vaccine formulation. During freezing, the amorphous cryoprotectants, such as saccharides and sugar alcohols thermodynamically stabilize through preferential exclusion of the cryoprotectant and hydration of the protein (Figure 2). They also provide kinetic stabilization through vitrification which decelerates aggregation of proteins and lipid membrane. Some cryoprotectants, such as dextran, do not penetrate the compound but can inhibit internal ice

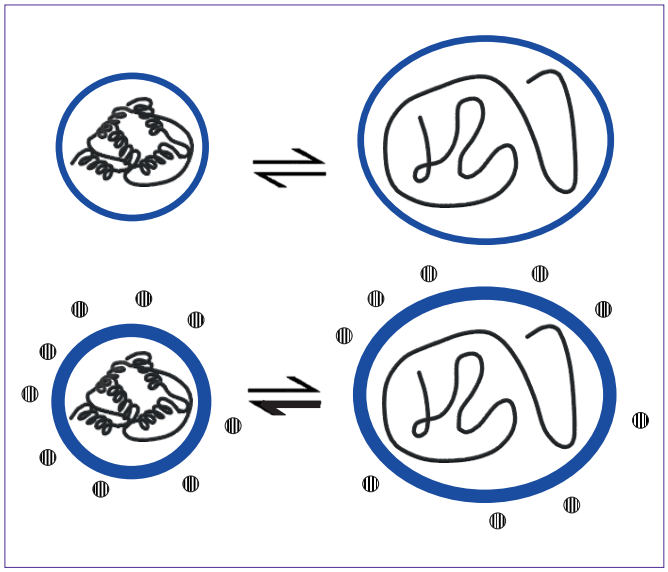


Figure 2. Preferential Exclusion Theory

formation by increasing the osmotic gradient. Lyoprotectants work during the drying phase of the freeze-drying cycle by replacing hydrogen bonds between water and protein or phospholipid (Figure 3). As with cryoprotectants, kinetic stabilization can be achieved through vitrification allowing mobility of proteins and the lipid membrane and therefore, stabilization of conformation and structure.

Other excipients can be added to the formulation to increase the stabilization of the vaccine, include buffers, surfactants that minimize surface induced destabilization and less frequently used excipients, such as bulking agents, organic co-solvents and tonicity adjustment agents.

Case Study – Developing a Thermostable Lyophilized Polio Vaccine with Three Inactivated Serotypes

Different formulations of the polio vaccine were tested with various excipients using a Design of Experiment (DoE) approach and the stability of serotypes was investigated. A basic screening with a limited number of excipients didn't reveal a stable product so an extensive screening was performed which was successful in identifying a stabilization agent. Optimization with the best candidates led to a final formulation that achieved high thermostability compared to the liquid formulations and other marketed formulations of the polio vaccines.

Process Development

Freezing has major impact on product characteristics which influence product stability (Figure 4). Slow freezing leads to the formation of a small number of large crystals and this can be detrimental to the membrane. However, fast freezing reduces the time for osmotic water release, which creates a higher risk of internal ice formation. The choice between slow or fast freezing is not easy but will be influenced by the vaccine formulation and sensitivity. It is, therefore, important to investigate the impact of the freezing rate on stability during lyophilization cycle development.

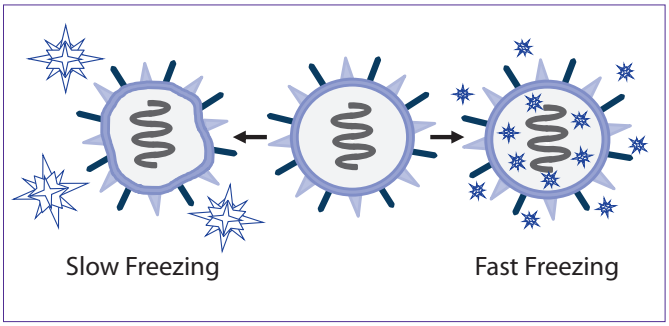


Figure 4. Impact of Freezing Rate

During the primary drying step, product temperature is important and impacts the sublimation rate, drying time and stability. It is worth considering the cost efficiency of reducing the drying time versus the product stability when optimizing the primary drying parameters for vaccines.

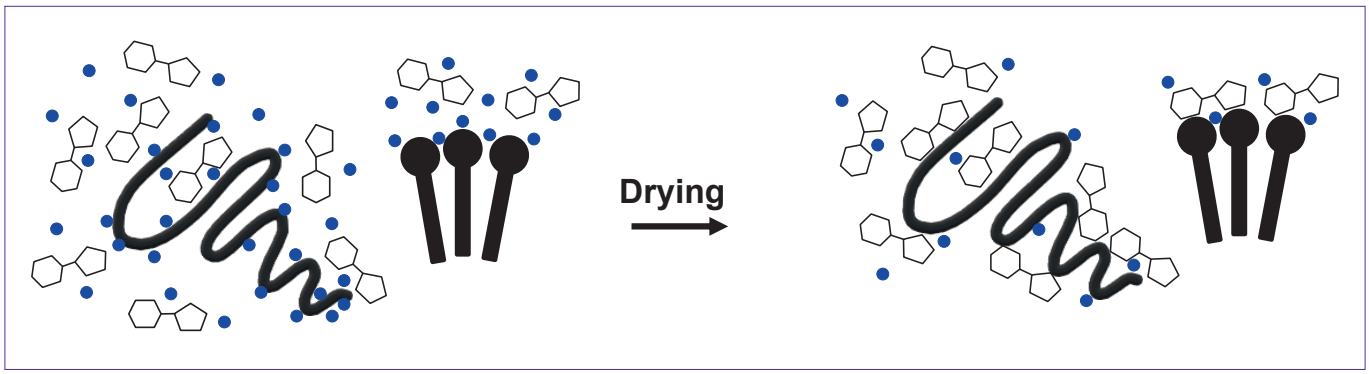


Figure 3. Water Replacement Theory

Removal of the hydration shell during secondary drying can reduce product stability. In addition, increased residual moisture can cause collapse, degradation and aggregation. Therefore, optimal secondary drying conditions and residual moisture levels should also be part of the development phase.

Case Study - The Importance of Product Temperature during Primary Drying for Long Term Stability

In an example of a bacterial vaccine investigated, three different cycles were tested based on product temperature (T_p) and product characteristics were analyzed in respect to stability.

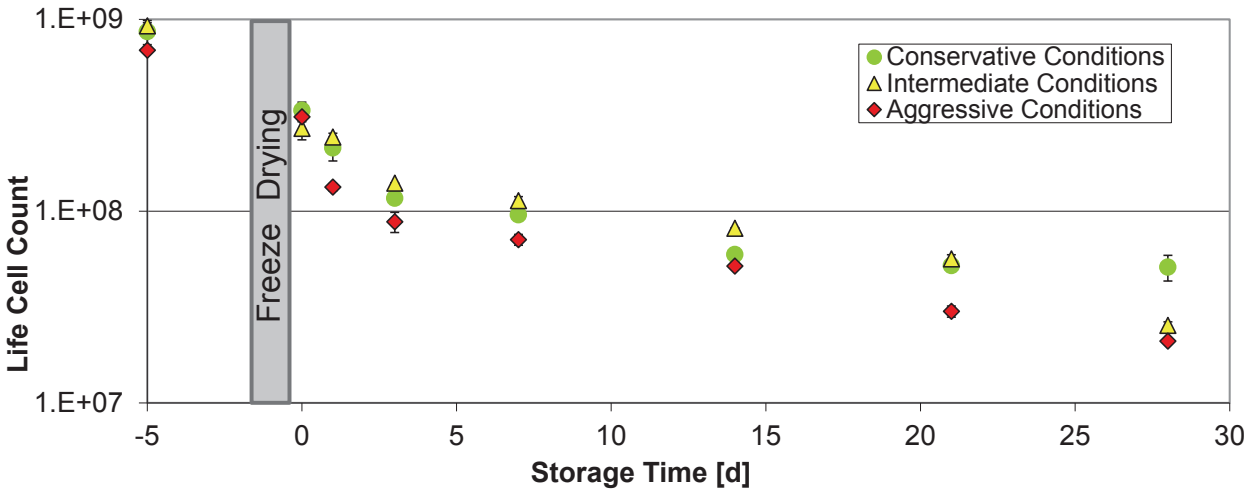
Stability was measured by comparing live cell count of a live bacterial vaccine after freeze drying. Immediately after freeze drying there is no difference between conservative (T_p well below the collapse temperature (T_c) but above glass transition temperature (T_g')) and aggressive cycles (T_p above T_c). After a few days, the aggressive cycle did not perform as well and after 1 month the intermediate (T_p at T_c) and aggressive cycles were not as good as the conservative cycle (Figure 5). It is recommended to start the drying cycle with conservative conditions but for some vaccine formulations primary drying above T_c might not be related to loss of stability.

Summary

It is evident that freeze drying is a suitable method to increase the thermostability of a vaccine. Vaccine formulation development should look at the impact of selection of lyo- and cryoprotectants, other stabilizing excipients and the freeze drying protocols during lyophilization to prevent damage to the vaccines.

Impact on process conditions and how it can affect product quality attributes should be considered during the development projects based on formulation and process issues but through understanding the underlaying mechanisms of these, rational development can be realized to obtain long-term stability.

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



Life cell count of a life bacterial vaccine after freeze drying with conservative ($T_p < T_{oc}$, triangles), intermediate ($T_p = T_{oc}$, circles) and aggressive ($T_p > T_{oc}$, squares) conditions. Storage conditions: 40°C, 75% rH.

Figure 5. Relevance of Critical Formulation Temperature (CFT) and Impact of Freeze Drying Conditions on Vaccine Stability

Q&A Session

1. **What techniques may be applied to monitor pH change during different steps of freeze-drying?**

   Observation of pH changes can be tricky, since there is no method to directly measure pH in a freeze dryer. Specific freezing tests (e.g. special setup that allows pH measurement during cooling or use of pH indicators) might be an opportunity to monitor pH changes.

2. **How will amino acids be selected for a freezing process? Does histidine, arginine or lysine can be used as well?**

   Amino acids used for stabilization need to remain amorphous to allow interaction with the vaccine. Therefore, physicochemical state is an important criterion for selection. Furthermore, adequate stabilization needs to be achieved and can be investigated by stability studies after freeze-drying.

3. **Did you try controlled nucleation and if yes, did you notice any major improvement in the freeze-drying process and stability of your vaccines?**

   So far, there is no published data on the use of controlled nucleation for vaccines. The relevance of certain freezing stresses will vary for different vaccines; therefore, the impact of controlled nucleation on the stability of vaccine formulations is expected to vary. Nevertheless, if stability is not compromised controlled nucleation would be beneficial in terms of reduced process duration and increased batch homogeneity.

4. **No "one fits it all," but when developing a new formulation, what are the most important points to address first in your opinion?**

   Since process sensitivity and long-term stability are major concerns for the development of freeze-dried vaccines, optimization in terms of stability is crucial. Investigation of different excipients and their stabilizing effect could be performed using conservative freeze drying conditions. With the most promising formulations process development can be performed afterwards to increase cost efficiency.

5. **What is the optimal cooling rate of most vaccines?**

   Sensitivity to freezing will vary in different vaccines, whereby optimal cooling rates vary. Most vaccines are freeze-dried using fast freezing rates or pre-cooled shelves.

6. **Is there a possibility to monitor and to check products quality during drying procedure?**

   Use of a sample thief is beneficial, especially for secondary drying development. It allows the extraction of product vials during freeze-drying, that can be analyzed (e.g. in respect of stability) afterwards. Regarding process development product temperature is critical and can be monitored conservatively (e.g. thermocouples, RTDs) or using innovative PAT tools (e.g. MTM).

7. **Do you see / anticipate any vaccine-specific challenges in the scale-up process to commercial scale processing?**

   Scale-up can be especially challenging, since vaccines show an inherent variability within the product due to the biological manufacturing processes and vaccine formulations are typically highly complex. Formulations frequently exhibit low critical formulation temperatures and therefore require relatively low product temperatures during primary drying. Differences in heat input that might arise during technical transfer or scale-up can be especially relevant here.

8. **What are typical moisture content in a vaccine formulation?**

   Optimal residual moisture levels will vary for different vaccines. Residual moisture levels might affect long-term stability and optimal values should be investigated during development.

9. **Should be amorphous for stabilization, is there a danger for crystallization and how could you determine that?**

   If excipients are used that tend to crystallize (e.g. mannitol) crystallization during drying or storage could occur causing batch inhomogeneity and possible vaccine stability issues. This can be analyzed using XRD analysis after freeze-drying or in accelerated storage tests.

10. **Have you any suggestions for excipients or process parameters to use to prevent thermotropic phase transition of the lipid layer e.g. for virus-like particles?**

    Sugars (e.g. sucrose) are known to prevent thermotropic phase transitions of the lipid layer (e.g. due to replacement of hydrogen bonds) and can stabilize lipid membranes.

11. **Can Histidine (neutral buffer) be kept amorphous during lyophilization? What issues to look out for?**

    Histidine typically remains amorphous during freeze-drying, but pH dependent crystallization can occur, especially in acidic environment.

12. **Could you explain how you could develop optimum secondary drying?**

    For secondary drying development, a sample thief is highly beneficial and allows extracting vials during the process. This way optimal residual moisture contents can be determined in respect of long-term stability.

13. **What sample amount is needed for FDM measurements?**

    A typical FDM measurement uses 2 µL of product solution.

14. **In your case, was the stability difference between aggressive and conservative cases related in RM%?**

    Yes, as expected there were differences between aggressively and conservatively dried products. Aggressive conditions in primary drying resulted in 1.8% and conservative conditions in 1.3% residual moisture.

15. **Regarding initial formulation development, if cycle development occurs after the formulation choice, what lyophilization cycle parameters are used (and what is the justification for using them) when freeze-drying the formulation candidates? If crystalline excipients are considered, is annealing applied at this point?**

    For formulation development, I would suggest starting with conservative conditions in primary drying to reduce the risk of stability impairment. Later on, applicability of higher product temperatures should be investigated to achieve optimum processes. If crystalline excipients are considered application of an annealing step should be tested as well.

16. **Which stabilizer can add to vaccine/alum formulation to prevent alum aggregation?**

    Mainly sugars (e.g. sucrose, trehalose) are known to prevent aluminum aggregation. Though, use of aluminum salts as adjuvants in freeze-dried vaccines is challenging and might demand increased development efforts.

17. **How can we avoid a too dry product?**

    Too dry products can be avoided by decreasing secondary drying temperature and time.

18. **To focus on impact of freezing rate - will the thawing process have a confounding influence, or can it be controlled to not influence the conclusions on freezing rate?**

    The impact of freezing rates would ideally be studied by freeze-drying, but to reduce the development time freeze-thaw studies can give a first indication.

19. **What are the major differences (formulation design and Lyo process) between typical protein/Anti-body based drugs and Vaccines?**

    A freeze-dried vaccine can consist of a variety of antigens (whole-pathogens, nucleic acids, proteins, polysaccharides, alum-precipitated toxoids). Therefore, requirements regarding formulation and process significantly vary. Furthermore, we typically see combinations of antigens and complex formulations, that challenge the freeze-drying process.

20. **Any annealing benefit in freezing process for vaccine?**

    Annealing is beneficial in terms of ensuring crystallization of excipients (e.g. mannitol). If such excipients do not crystallize during freezing, there is a risk of crystallization during drying or storage, which results in batch inhomogeneity and might negatively affect vaccine stability. Furthermore, annealing causes crystal growth and can therefore reduce product resistance and drying time. On the other hand, annealing could also negatively impact vaccine stability. Therefore, the applicability of an annealing step should be studied.

21. Do you have experiences of mixed system in vaccination FD formulations? For example, crystallizing NaCl as major component with stabilizer like sucrose?

When using sodium chloride crystallization should be assured, otherwise amorphous sodium chloride will depress collapse temperatures and therefore will complicate the freeze-drying process. Here the application of an annealing step can be beneficial.

22. In your second case study, it looks like the product is losing stability even for a conservative cycle after 25 days. What are the typical shelf life requirement and how would you set-up a stability study (5, 25 and 40°C storage?) to establish desired shelf life.

Ideally, a freeze-dried vaccine would be stable at 2 - 8°C for 24 – 36 months. For stability studies accelerated but also long-term testing at intended storage temperatures should be performed to evaluate long-term stability, e.g. an accelerated test was performed at 40°C in the case study presented.

23. Do you suggest the need for evaluating the glass transition of the solid state?

Analysis of the glass transition temperatures in solid state (e.g. using DSC) can be used to define storage temperatures and predict long-term stability and is especially useful along with (accelerated) storage testing. It can also be used as a complementary tool to XRPD to characterize the physicochemical state of the product.