Uttaranchal Journal of Applied and Life Sciences
U. J. App. Life. Sci. Vol: 3 (1): 1-13 **Dec 2022**

The effect of Lyophilization technique on the stability and durability of protein molecule: A Review

Umeh Chisom Theresa[1*], Balveer Singh[2], Yogesh Kumar Awasthi[2], Prashasti Raj[2]

[1]Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka, Nigeria.

[2]School of Applied and Life Sciences (SALS) Uttaranchal University Dehradun, Uttarakhand-248007 (India).

***Corresponding author: umehchi871@gmail.com**

Received: (8 August 2022) Revised: (10 Sept 2022) Accepted: (1 Dec 2022)

Abstract: Lyophilization is the process where moisture is removed from the perishable material and helps in extending their shed life and making the substance more portable. Lyophilization is otherwise called Crystallisation, used in the pharmaceutical industry to increase the stability and shelf life of the pharmaceutical industry. The Lyophilization of protein is removing the moisture from it, so that it could be stored for a longer period of time. The potency of frozen protein products is far greater than that of liquid formulations. The primary consideration during lyophilization is the long durability of protein compositions, which is correlated with water content. Small-molecule pharmaceuticals can typically be formulated without the addition of additives, or simply by adding a bulking agent or pH modifier to produce a liquid formulation that is rigid enough to withstand the time required before it can be freeze-dried. The primary consideration during lyophilization is the long durability of protein compositions, which is correlated with water content. Small-molecule pharmaceuticals can typically be formulated without the addition of additives, or simply by adding a bulking agent or pH modifier to produce a liquid formulation that is rigid enough to withstand the time required before it can be freeze-dried. The paper reviews different articles and journals to provide a critical analysis of the process of Lyophilization of protein and discuss its detailed process. The paper would review the strength and limitations of the Lyophilization process.

Key words: Lyophilization, Protein, Ice drying, shelf-life

INTRODUCTION

The terms "lyophilization", and freeze-drying, are interchangeable. Lyophilization is a moisture removal method that is commonly used to preserve perishable materials, extend shelf life, or make the fabric more portable. Lyophilization entails cooling the material, then lowering the pressure and raising the temperature to enable the water ice inside the cloth to dissolve. Lyophilization, also known as recrystallization, is a method commonly used to increase the stability as well as the shelf life of pharmaceutical products (Matejtschuk, 2007). Whilst also liquid formulas are generally preferred for injectable therapeutic proteins (in terms of convenience for the end user and ease of guidelines for the producer), this form isn't always viable due to proteins' receptivity to denatured proteins and accumulation beneath stresses such as heating, freezing, pH small tweaks, and agitation, all of which may cause denaturation and aggregation. Furthermore, water modulates hydrolysis and deamidation; under aqueous conditions, these reactions, as well as many other chemical degradation paths such as combustion, are accelerated, rendering the formulation inappropriate for long-term storage (Carpenter et al., 2020)

The frozen Protein

Lyophilization is frequently used to improve the stability profiles. "Frozen protein products are much more potent than liquid formulations,". Another advantage is that allocation is simplified because cold-chain issues and excitations strains during the period of shipment are reduced. Furthermore, contamination from substances and leachables from of the field closure is significantly less of an issue for lyophilized merchandise because the system only comes into contact with the closure for brief periods of time rather than the entire shelf lifestyle. The risks of Lyophilization, on the other hand, also involve a lengthy and complicated manufacturing process, higher average manufacturing prices, and a larger capital investment required for production (Morita et al., 2000). Lyophilization's primary goal is to impart desirable properties into the product, such as long-term balance, short reconstitution time, retention of the original liquid method's characteristics upon reconstitution (e.g., solution properties, isotonicity, protein structures or conformation, and particle-length distribution for suspensions), and fashionable cake appearance. Developing a freeze-drying cycle for protein formulations isn't without its difficulties. (Nail et al., 2002).

Characteristics of the Lyophilized Proteins

- The product's long-term stability.
- Fast reconstitution time.
- A beautiful cake appearance.
- After reconstitution, retains initial dosage form characteristics such as solution properties, pharmaceutical product structure and conformation, proteins, and particle-size diffusion in absences.
- The isotonicity of the product is preserved after reconstitution.

Advantages and Disadvantages of Lyophilization

Advantages-

- Decomposition of chemicals is minimised.
- Water is extracted from the product without excessive heating.
- Keeping the product dry improves its stability.
- Reconstituted products dissolve quickly.
- Water can be removed from thermosensitive materials without affecting the molecule's properties.
- A partially purified product with a large specific surface area that aids in the rapid and complete absorption of water of a solid.
- Freeze-dried delivery systems are packed as a solution in vials, which allows for easier and more precise having to fill than powder in vials.

Disadvantages -

- The need for sterile diluents for product reconstitution.
- Many pharmaceutical and biological products, such as inoculations, liposomes, and proteins, are harmed by freeze-drying during the manufacturing process.
- A drug's solid-state stability is primarily determined by the physical state of its molecules.
- Processing and handling take longer.
- Using the vacuum method, volatiles were deleted from the product.
- Processing time, equipment cost, and complexity have all increased.

Related Studies

According to Wang and Ohtake, (2019) The effective freeze-drying strategies for proteins. Laboratory services are available on a small scale, such as the purifying of a protein alternate

as part of a protein's explanatory portrayal, and on a medium scale, such as the pilot advancement of the lyophilized protein benchmark fabric for use in bioassay as well as immunoassay. There is also information on prevalent freeze-drying of proteins.

Starciuc et al. (2020) stated that using a controlled humidity mini-freeze-dryer, the stresses all through the secondary drying degree of lyophilization had been investigated. RH had a minor effect on lactate dehydrogenase (LDH) balance, however drying temperature and length had a good-sized impact. The healing of protein pastime become studied as a feature of relative humidity (RH), product temperature, and drying time. Various concentrations of sucrose, sorbitol, and poly (vinyl pyrrolidone) have been also tested (PVP).

Butreddy et al. (2020) suggested that Freeze drying, also known as lyophilization, is a common way to process heat sensitive items that need to be stored at temperature changes above freezing for an extended period of time. It is simple to achieve a sterile, particle-free, and precisely dosed remedy that can be administrated parenterally by restoring the lyophile with sterile water.

Active pharmaceutical substances that are thermolabile and moisture sensitive in nature degrade in atmospheric situations, resulting in decreased balance and self-life. Lyophilization is one of the strategies used to effectively enhance any such critical situation. It is a cutting-edge-day rising technology this is used inside the production of several antibiotics (e.g., chloramphenicol, doxycycline) and anti-most cancers drugs (e.g., doxorubicin, epirubicin). This technique correctly used the phenomenon of sublimation to reap number one dried product, observed by way of moisture removal via warmness modulation. This method not most effective extends the drug's shelf life however additionally permits for quick reconstitution and lowers garage and delivery expenses. This evaluation article discusses the principles of lyophilization, the steps worried, and the method aspects (Kulkarni, 2018). This method not only extends the drug's shelf life but also allows for quick reconstitution and lowers storage and shipping costs. This review article explained the principles of lyophilization, the overall conceptual, formulation aspects, the importance of lyophilization, and the recognition of the end state in lyophilization, as well as recent advancements.

Wang, (2019), provided an outline of the effect of protein process parameters on different phases of the protein cold process, as well as recommendations for selecting critical process conditions all through protein freeze-drying process development, optimization, and scale-up. Practical considerations, such as large-scale systems integration, scale-up obstacles, and concerns with less traditional freeze-drying delivery methods, such as double syringes, are

given special attention. The paper includes extensive research from a research article and journals on the stages of lyophilization processing. Here, the researcher has used a descriptive and exploratory research design to analyze and critique the research findings. The paper provides a literature review of the related articles and provides an analysis of the stability of lyophilization, the stages involved in the process, the cyclic process, quality, and technology used in the process.

The Importance of Stability

According to the views of Butreddy et al. (2020), the lengthy durability of protein compositions, which is correlated with water content, is the primary consideration during lyophilization. Small-molecule pharmaceuticals can typically be formulated except for additives, or simply by adding a bulking ingredient or pH modifier, to produce a liquid formulation that is quite rigid to withstand the time required before it can be freeze-dried, suggested Mutukuri et al. (2022). The goods typically have a low enough moisture level to guarantee the long-term stability of the composition. As proteins are fragile molecules, the issue is more difficult for preproteins. stability depends on the amount of water in the composition, while at the same time, a protein's active component depends on its conformational form, which requires some water to prevent dissociation reactions. Optimizing the formulation and maintaining proper process control can prevent such stability problems. optimizing the formulation and maintaining proper production process, such stability problems can be prevented. Thermodynamic consistency can be thought of as a new stability notion. "The situation is further compounded because although a protein can show thermodynamically unstable after freeze-drying & unfold if no irreparable events (e.g., aggregation) happen while preservation or restoration, the reconstituted protein may rearrange entirely within seconds and demonstrate excellent pharmaceutical stable," says the study. This consistency is the equilibrium point as the point of equilibrium among native and expanded helices.

Preston and Randolph, (2021) stated that the relationships among a specific protein, the preparation, and the lyophilization cycle are intricate and incompletely known. For instance, a new study shows that the freezing and annealing procedures had a significant impact on the rates of aggregate, oxidizing, and deamidation over the course of long-term stability and that the percentage of proteins at the solid-gas boundaries throughout the very porous lyophilized

cake was the most crucial element. It's interesting to observe that the protein degradation was only impacted by the composition of the protein near the contact said (Fang et al., 2020).

Stages of the lyophilization process

Phase of Freezing

There are several approaches to freeze the product. Freezing may be completed in a freezer, a chilled tub (shell freezer), or on a shelf in the freeze dryer. Cooling the material below its triple point reasons sublimation as opposed to dissolution. This preserves its physical form. Lyophilization is most honest whilst massive ice crystals are used, which may be produced via modern freezing or annealing. However, with regards to organic substances, too-large crystals will break the mobile dividers, resulting in less-than-perfect freeze-drying consequences. To keep away from the aforementioned, the freezing is finished abruptly. Annealing may be used on precipitating substances.

Primary Drying (Sublimation)

The main drying (sublimation) phase of lyophilization occurs when the pressure decreases and heat is added to the substance to allow water to dissolve. Sublimation is accelerated by the vacuum. The chilly compressor acts as an exterior on which water vapour can adhere and solidify. The condenser also protects the vacuum pump from water vapour. Roughly 95% of the fluid in the material is eliminated during this phase. Main drying can be a lengthy process. Too much heat can change the structure of a material (Wang, 2019).

Secondary Drying Phase (Adsorption)

Secondary drying (adsorption) is the final phase of lyophilization, which removes the electrolyte water molecules. By raising the temperature above those of the main drying phase, the bonds between the material and the water molecules are cracked. Freeze-dried materials possess their porous structure. Following the completion of the lyophilization process, the suction can be damaged with an inert carrier gas before the substance is sealed. Most components can be dehydrated to a residual moisture level of 1-5% (Butreddy et al., 2020).

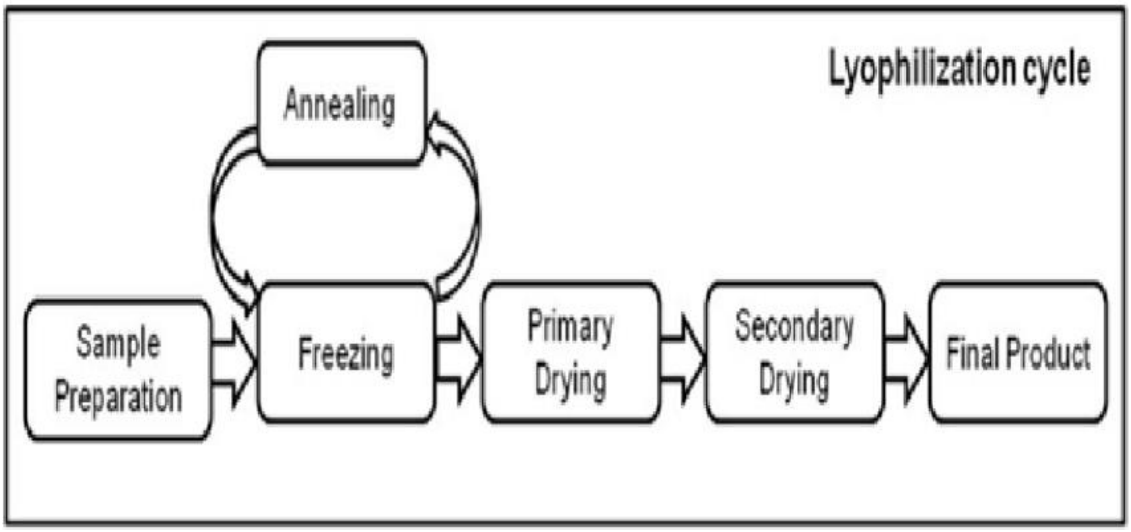

Figure 1: Unit operations in Lyophilization cycle (Yoshioka et al., 1999)

The cycle of the developmental process

Researchers operating on the "University of Bristol" inside the United Kingdom are growing medicinal lyophilized cycles for protein therapeutics. One of the most tough demanding situations is growing a freeze-dry cycle for a poorly designed formulation. Horn et al. (2018) viewed that "A properly designed system considers and corrects any problems with associated freezing and drying strain." Protein freeze-drying cycles ought to be optimised to be as quick as viable at the same time as keeping a sufficient protection factor to keep away from manner deviation when confronted with fluctuations in gadget performance. "The worst-case state of affairs is an attempt to layout a cycle around a formulation advanced by someone without knowledge of lyophilization," as per the views of (Ohari et al., 2018). When constructing a lyophilization loop for protein formulations, the most important step is freezing. The commodity should be frozen at a low enough temperature to completely solidify. Recognizing the physical manifestation of the solute (whether crystalline as well as amorphous) after cooling is important for drying characteristics and final product appearance. Based on protein folding factors and ice crystal size, there is an optimized freezing rate variety for a given formulation. Enhanced ice surface may have an impact on protein stability. Because residual humidity can accelerate degradation reactions during storage period, it should be kept to a minimum, typically below 3% (Garidel, 2019).

Quality by Design

The QBD approach represents a leap forward toward a greater systematic and green technique of growing new protein formulations and tactics. Carpenter, (2020) adds that the critical procedure parameters (CPPs) can be decided previous to the start of the cycle development. Temperature and strain are the important technique parameters for the freeze-drying method, both throughout freezing and drying. There are numerous CQAs for freeze-drying protein formulations, and their relative importance depends at the product type so as to be advanced. Protein-specific CQA to do not forget encompass aggregation, pH, water content material, native shape, and other substances that seem due to degradation reactions which include deamidation, oxidation, and so forth. A problem in the course of primary drying will first affect the cake elegance, causing collapse, which may cause higher moisture content material. If a high temperature is encountered for an extended period of time in the course of secondary drying, it could have an effect no longer best on moisture content material but additionally on product integrity (Pretson, 2020). The final results of this sort of deviation ought to be highlighted by the procedure boundary described in the course of the development method. An examination of the protein's behaviour (in phrases of aggregation, the equilibrium of the folded and unfolded shape, the outcomes of pH shifts, and the degradation reactions) is needed. A strategy like this lets in for exams at extraordinary CPP tiers to improve the cycle and outline the layout area. “When implemented over the loops, we could indeed characterise the control method by performing a principal component evaluation on the work products” (Wang, 2019).

Technology for analysis of the Process

To ensure that the product complies with the preferred quality attributes, advanced processing techniques must be employed to supervise CPPs during lyophilization. Wireless temperature experiments, drying-rate measurement systems, and endpoint detection tools are some of the most significant advancements in this field. "Key PAT breakthroughs for lyophilization involve wireless suitable thermal, drying-rate measuring systems, as well as endpoint detection instruments," according to the report (Fang, 2020). Connectivity temperature probes used during non-GMP design batches can provide item temperature changes in instantaneously packed lyophilizers, enabling comparison to laboratory advancements that need to run for this critical parameter. A pressure and heat release test gives a complete picture of the truck full within the freeze-dryer. "More sophisticated tools, such as near-

infrared spectroscopy (NIR), can be used during the process to assess the evolution and quality of the species that fade away (water) as well as show up (dry commodities). PAT techniques for freeze-drying can help you learn more about the process. "The correct strategy is to employ a tool that offers global load surveillance." "The microbalance scheme provides useful information, but it is focused on one vial." Starting with the given model, a QbD method for protein lyophilization would then boost understanding of the product and process (Horn, 2018). The selection of a PAT tool must be made with execution in mind, as it can be challenging to justify its existence or absence throughout the audit.

Heating v/s Lyophilisation

Most chemistry course teaches that heat starts to break protein, whether it's the heat from the a pan attempting to break down the proteins in eggs or even the heat from a detangling iron breaking beneath the protein in tresses. Heat changes the structure and feature of proteins in meals in a number of ways (Wang, 2019). Many people worry about whether or not high-protein foods lose their nutritional value when they are heated. So, heating your protein changes the shape of the protein complexes, but it doesn't change the protein's nutritional value or make it harmful. In reality, our bodies take in the amino acid residues from proteins in the same way whether or not they are heated.

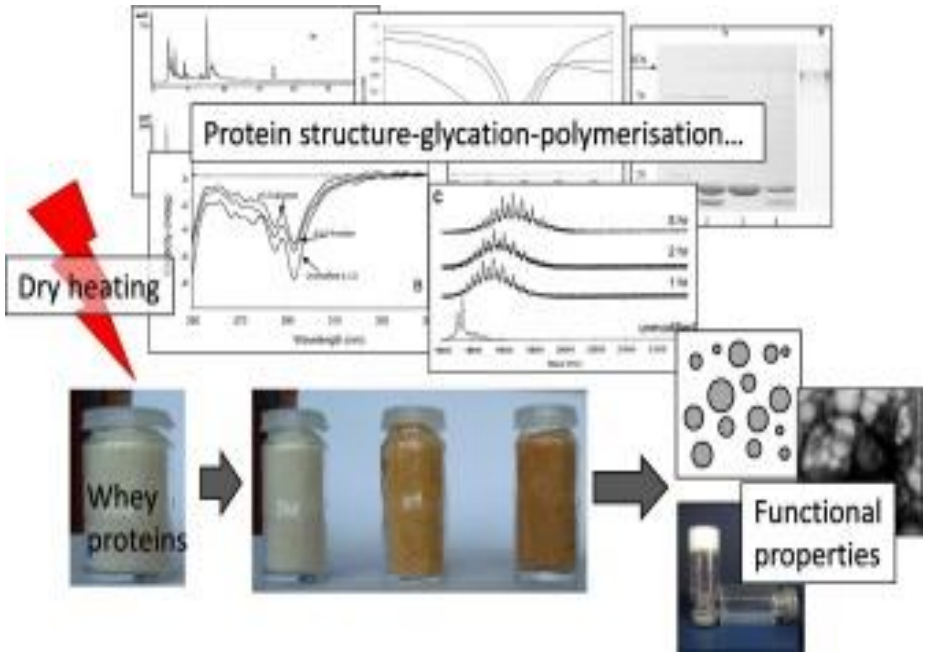

Figure 2: Heating Whey Protein (Wang et al., 2019)

Unfortunately, the process of lyophilization includes both freezing and drying, which can damage proteins in different ways. Even if a protein stabilizer is used to make lyophilization work well, proteins in stable state might not be stable for long-term storage. Freeze-drying removes water from protein solubility, which include water from the surface of the protein, which is an important part of the structure of the protein and often causes damage. Scientists often make the mistake of assuming that proteins don't change when they are stored. But the truth is that freezing, long-term storage, or even freeze-thaw cycles can cause proteins to lose their function and change their structure.

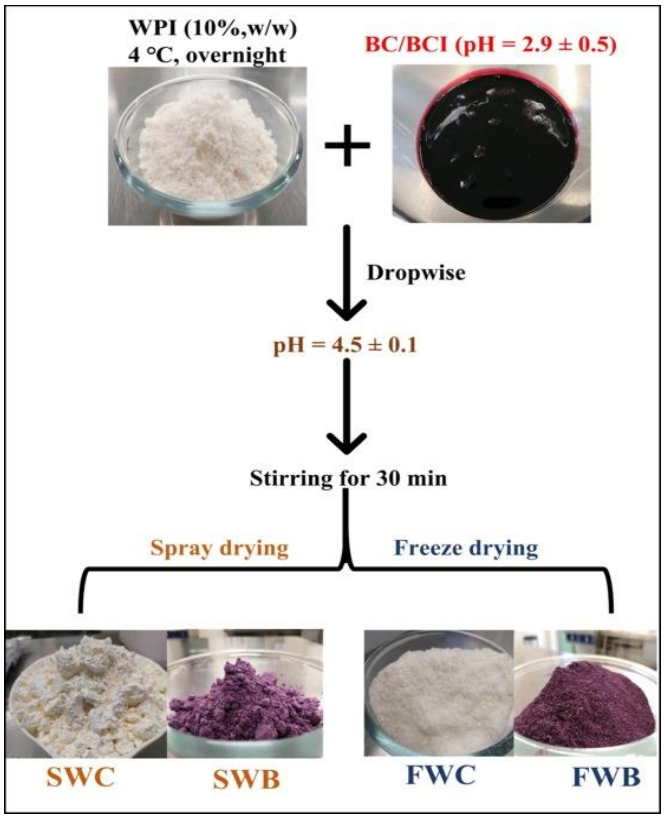

Figure 3: Effect of spray drying and freeze drying on Whey protein

Also, freezing whey protein doesn't change its nutritional value. Because the freezing process keeps most of the nutrients, there's only a small loss of protein content. This means that you can get more protein from the frozen process. This is very important because protein helps the body grow and repair itself.

CONCLUSION

Frozen protein products have far greater potency than liquid formulations. The long durability of protein compositions, which is correlated with water content, is the primary consideration during lyophilization. Small-molecule pharmaceuticals are typically formulated without the use of additives, or with the addition of only a bulking agent or pH modifier. Lyophilization, also known as recrystallization, is a common method for increasing the stability and shelf life of pharmaceutical products. Because of proteins' receptivity to denatured proteins and accumulation beneath stresses such as heating, freezing, pH small tweaks, and agitation, liquid form isn't always viable. Creating a freeze-drying cycle for protein formulations is not without challenges. Protein healing has been studied as a function of relative humidity (RH), product temperature, and drying time. Sucrose, sorbitol, and poly (vinyl pyrrolidone) concentrations were also tested (PVP). Freeze drying is a common method for preserving heat sensitive items that must be kept above freezing for an extended period of time. Lyophilization is a cutting-edge rising technology used in the production of several antibiotics (for example, chloramphenicol and doxycycline) and anti-cancer drugs. This technique successfully used the phenomenon of sublimation to produce the best dried product, as measured by moisture removal via warmness modulation. This method not only increases the drug's shelf life, but it also allows for quick reconstitution and lowers storage and shipping costs.

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