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LYOPHILIZATION TECHNOLOGY SERVES AS A STRATEGIC CHOICE FOR DEVELOPING STABLE BIOLOGICALS

Lyophilization, also known as freeze-drying, is a widely used technology in the pharmaceutical industry for developing stable injectable products, particularly those containing peptides, proteins, mRNAs, monoclonal antibodies (mABs), antibody-drug conjugates (ADCs) and other biologicals. This process involves the removal of water from a frozen solution through sublimation, resulting in a dry and stable product that can be reconstituted before administration of the product to the patient. Lyophilization technology plays a crucial role in ensuring the long-term stability and preservation of these sensitive biological molecules, which are often prone to degradation in aqueous solutions. This white paper provides a comprehensive overview of lyophilization technology, focusing on formulation development, process optimization, and regulatory considerations for the successful development and manufacture of biological injectable products.

Key words: Freeze-drying, lyophilization, formulation development, active pharmaceutical ingredient, biological, excipient, injectable, process optimization, heat transfer coefficient, regulatory

Introduction

Biological molecules or biologicals such as peptides, proteins, mRNAs, monoclonal antibodies (mABs), antibody-drug conjugates (ADCs) and other biologicals are increasingly important therapeutic modalities in modern medicine, offering targeted and effective treatments for various diseases. However, these biological molecules are inherently unstable in aqueous solutions, susceptible to various degradation pathways such as aggregation, denaturation, and chemical modifications. Lyophilization technology offers a robust solution to address these stability challenges, enabling the development of stable, long-term storage formulations for injectable products. As the demand for peptide and protein-based therapeutics continues to rise, the importance of lyophilization technology in enabling their long-term stability and effective delivery has become increasingly significant, often serving as a conservative approach until superior drug development paths are identified.

Formulation Development

Formulation development is a critical aspect of lyophilization technology, as it involves identifying and optimizing the appropriate excipients and their concentrations to ensure the stability of the active pharmaceutical ingredient (API) during the lyophilization process and subsequent storage.

The following factors should be considered during formulation development, especially, to provide stability to the molecule of interest –

Buffers with different pHs: The pH of the formulation plays a crucial role in maintaining the solubility and stability of the API. Buffers, such as citrate, acetate, and phosphate, are commonly used to control the pH of the formulation matrix to solubilize the drug substance as well as to maintain the stability of the drug during the shelf-life. However, these buffers can undergo pH shifts during the freezing process, potentially destabilizing the API. It is essential to carefully select and optimize the buffer system to minimize these effects.

Non-reducing Disaccharides: Excipients such as sucrose and trehalose are often included in lyophilized formulations as stabilizers during the freezing and drying processes. These non-reducing disaccharides form an amorphous matrix around the API, providing protection against denaturation and aggregation.

Sugar Alcohols: Sugar alcohols, including glycerol, sorbitol, and mannitol, play a stabilizing role for proteins in solution as well as during lyophilization and subsequent storage. Like trehalose and sucrose (non-reducing disaccharides), these sugar alcohols primarily act as osmolytes, protecting the structural integrity of the protein. They

achieve this by modulating the protein-solvent interactions and by effectively replacing water molecules during the lyophilization process.

Bulking Agents: Bulking agents, such as mannitol and glycine, are used to provide structural support to the lyophilized cake, preventing shrinkage and collapse. These excipients crystallize during the freeze-drying process, contributing to the cake's stability and appearance.

Surfactants: Surfactants, such as polysorbates, are added to lyophilized formulations to prevent the formation of aggregates during various stages, including filtration, filling, freezing, and freeze-drying.

Lyophilization Cycle Development

Lyophilization cycle development is a critical aspect of ensuring the quality and stability of freeze-dried pharmaceutical products during the process and storage. During early development stages, many companies use a generic lyophilization cycle until a science-based lyophilization cycle is established. However, this generic cycle may not maintain product stability during clinical manufacturing and/ or throughout the duration of the clinical study. Our scientific team at UI Pharmaceuticals understands the importance of selecting the right parameters for the lyophilization cycle, and we follow an evidence-based approach from the beginning. Every new molecular entity is unique, and selecting a tailored lyophilization cycle is as important as selecting the right formulation composition. At the University of Iowa Pharmaceuticals (UIP), our formulation experts employ thermal analysis techniques such as **Differential Scanning Calorimetry (DSC)** and **Freeze-Drying Microscopy (FDM)** to determine critical temperatures and design the product in a scientific manner that ensures the stability of your product is uncompromised.

DSC helps determine critical temperatures, such as the glass transition temperature (T_g) and eutectic temperature (T_{eu}). FDM provides real-time visual insights during freezing, allowing us to observe ice crystal formation and the product's structure. By determining the need for annealing during freezing and understanding the collapse temperature (T_c), we can design robust lyophilization cycles that prevent product failures. By understanding these nuances, we ensure both scientific rigor and seamless technology transfer as we move toward advanced manufacturing stages.

Process Optimization

Optimizing the lyophilization process is crucial for ensuring the quality and stability of the final product. The following steps are involved in process optimization:

After selecting the lyophilized formulation composition, the cycle is optimized using a design space approach. This involves understanding the edge of failure for the cycle, heat transfer rate, heat transfer coefficient of the vial (K_v), and the resistance of the dried product layer (R_p), which is product specific. By considering these factors, a safe zone of operation for primary drying is established, allowing for the development of the fastest possible cycle, thereby reducing production time as well as manufacturing costs.

Secondary Drying Optimization: Secondary drying conditions are established by creating samples with varying moisture levels and utilizing techniques such as Karl Fischer moisture titration to determine the optimal moisture content for long-term stability.

Confirmation Batch and Stability Studies: Following the establishment of optimized lyophilization cycle conditions, a confirmation batch is produced, and samples are placed on accelerated and long-term stability studies to validate the process and formulation. The stability data helps both the selection of lead candidate meaning, lead composition as well as suitable lyophilization parameters.

Technology Transfer and Scale-up

While different lyophilizers may have subtle variations in their design and performance, maintaining functional equivalency through Critical Process Parameters (CPPs) is crucial for ensuring the lyophile's quality remains consistent regardless of the equipment used.



Bench top R&D unit used for development



Unit used for Clinical/ Commercial

It is essential to consider equipment functional equivalency during technology transfer by comparing the lyophilizers used in the development lab and the one that will be used for manufacturing the clinical/commercial batch. Any differences in equipment design, such as the arrangement of temperature-controlled shelves, vacuum systems, and condenser configurations, can affect the overall process. Establishing the functional equivalency and scale-up factor will help ensure seamless process transfer.

Regulatory Considerations

Developing lyophilized injectable products requires adherence to regulatory guidelines and standards to ensure product quality, safety, and efficacy. Some of the key regulatory considerations include, but are not limited to –

Good Manufacturing Practices (GMP): Lyophilization processes must be conducted in compliance with GMP regulations, ensuring proper documentation, process validation, and quality control measures.

Analytical Methods: Appropriate analytical methods such as Karl Fischer titration, Powder X-Ray Diffraction (PXRD) are employed to characterize and monitor the lyophilized product's critical quality attributes, including moisture content, crystallinity, and reconstitution behavior. Compliance with stability study guidelines ensures patient safety and product efficacy.

Stability Studies: Comprehensive stability studies, including accelerated and long-term studies, are required to demonstrate the product's stability over its intended shelf-life and to support expiration dating. The need for stability studies depends on various factors such as the product development stage, to understand process impact on stability and any changes in the composition, container closure system (vial type or vial size) and process impacting the stability should be evaluated.

Conclusion

Lyophilization technology plays a vital role in the development of stable injectable pharmaceutical products, particularly those containing peptides, proteins, mABs, and ADCs. By optimizing formulations and lyophilization processes, and adhering to regulatory guidelines, pharmaceutical companies can ensure the long-term stability and quality of these sensitive drug molecules. Ongoing research and advancements in lyophilization technology will continue to support the development of innovative and effective injectable therapeutics, contributing to improved patient outcomes and healthcare.

UI Pharmaceuticals is the largest and most experienced university-affiliated, FDA-registered pharmaceutical manufacturing facility in the United States that produces both sterile and non-sterile dosage forms. Our team at UI Pharmaceuticals offers evidence-based scientific solutions to unique needs of our clients. We have been in the industry for 50+ years, serving small, virtual, big players in the market, including government bodies, universities, KOLs in the industry, by solving their problem statements addressing unmet medical needs. Please visit our website to find the services we offer, and do not hesitate to contact us for further information.

UI Pharmaceuticals is the longest running FDA registered CDMO, affiliated with the College of Pharmacy at the University of Iowa.

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