Selection of Containers/Closures for Use in Lyophilization Applications: Possibilities and Limitations

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Abstract

The unit operation of freeze-drying is commonly employed in the pharmaceutical industry to enhance the storage stability of relatively fragile biopharmaceuticals. Yet without a suitable container closure system, the advantages of freeze-drying a biopharmaceutical product cannot be fully realized and appreciated. Primary packaging provides the first line of defense for all pharmaceutical products by maintaining the critical quality attributes (CQAs) throughout the product shelf life. Although primary packaging components are intended to provide a stable environment for the pharmaceutical products, without proper understanding they can affect the product adversely by adsorption, absorption, leaching and permeation. For biologic products, proper selection of container and closure (c/c) components is even more important as they are more sensitive compared to other small molecule pharmaceuticals. Additionally, c/c also impacts the lyophilization process development and ultimately the drug product characteristics. The composition and processing history of the packaging components can play significant role as the impurities and residuals can induce destabilization and alter the drug product characteristics. It is critical, therefore, to understand and address all of these concerns related to c/c selection for successfully developing a stable biologic product to avoid potential product incompatibilities.

Introduction

Development of a stable lyophilized biopharmaceutical hinges on the proper selection of formulation, lyophilization process, container/closure and diluent for reconstitution. Although the significance of formulation design and lyophilization process has been given due consideration in the past years, selection of containers and closures is often overlooked aspect, at least, until the late stages despite their significant impact on the freeze-drying process, safety and efficacy of the product, and in establishing the shelf-life of the product. Lyophilization and formulation development, the focus of our previous article [1], has shown the influence of process parameters and formulation design on the stability of the high concentration antibody formulation. Packaging components play an important role in safety

and stability aspect influencing the product properties such as storage condition (temperature, % RH etc.), and impact of light exposure. The current article reviews the impact of packaging components not only on the effectiveness of freeze-drying process but also on final product quality and pharmaceutical elegance over the shelf-life of the product.

A container closure system is defined as the sum of both primary packaging components (i.e. a component that is in direct contact with the dosage form) and the secondary packaging component (i.e. a component that will not be in direct contact with the dosage form) that are required to contain and to safeguard the drug product against factors that can promote degradation over the shelf-life of the product [2]. Most of the lyophilized products are injectables and the container closure system must meet the pharmacopeial standards for injectable products of the respective market regions. For detailed requirements and test methods for proper selection of containers/closures for biopharmaceuticals, the reader is referred to sections in US, European and Japan pharmacopoeias. This paper will outline the various container/closure (c/c) commonly used as pharmaceutical packaging for biopharmaceuticals with special considerations, requirements and case studies describing the unique parameters involved in the production, storage, and use of freeze-dried products.

Consideration for Liquid vs. Lyophilized Formulation

Given the intrinsic differences and product requirements for solid-dosage versus liquid formulations, the container closure considerations are significantly different for each dosage form. Surface adsorption of the active drug substance to the containers/closures or extractable from the container/closures, for example, can be a significant concern mostly in a liquid protein formulation. It should be noted that surface adsorption (e.g. polysorbate, gelatin etc.) upon direct contact with the hydrophobic surfaces can be limited by adding surface-active agents to the formulation or through the use of specialty glass coatings to reduce the glass-protein interactions [3,4]. Leaching of extractable from the containers such as metal ions (especially at extreme acidic or alkaline pH conditions) or closures (such as silicone oils) into the liquid formulation during storage can catalyze many degradation reactions (e.g. oxidation, aggregation, particulate formation etc.). Similarly, moisture/oxygen ingress during product storage, presence of moisture in stoppers, and/or vial breakage during manufacturing, in contrast to liquid formulation, are important considerations for the freeze-dried product while maintaining seal integrity is relevant to both lyophilized and liquid-dosage forms. It should be noted that usually lyophilized containers are often sealed under vacuum to ensure that the stopper remain closed until the crimping stage, however, the likelihood of moisture/oxygen ingress or volatilized moisture (or an extractable) from stopper into the hygroscopic lyophilized cake might also increase impacting the product quality during long-term storage. Therefore, it is recommended that suitable container closure for a lyophilized product can be chosen/designed once their impact on the lyophilization process itself and the desired properties of the container/closure systems such as levels and nature of extractable

present, the durability of coatings etc. required to avoid potential product incompatibilities are known. The possibilities and limitations of some commonly used primary packaging (containers/closures) systems available for single-dose lyophilized pharmaceutical products and for bulk storage are discussed below.

Container Closure for Freeze-dried Products

Suitability of a proposed packaging system is defined by various factors such as: (a) ability of the packaging system to adequately protect the dosage form against factors that can promote degradation over the shelf-life of the product, (b) compatibility with the product, (c) material of construction that is considered safe for the intended use of drug, and (d) proper functionality of the container/device (if any) [2]. It should be noted that although the risk of interaction between the container surface and the lyophilized drug is generally recognized to be small due to a relatively short contact time compared to a liquid formulation; adverse affects during the pre-lyophilization, and/or post-reconstitution stage cannot be overlooked. In addition to these concerns, other factors involved in product manufacturing, handling and storage such as the impact of lyophilization process and formulation on the container itself and vice versa, product throughput, storage conditions (%RH, light exposure etc.) etc. illustrates the fact that the product containment are no less important than the contents [5]. Thus, careful selection of a container closure with careful examination of potential interaction between the drug and the c/c is required to mitigate any potential product incompatibilities.

Containers

Ampoules and **vials** represent the major class of containers for small-volume freeze-drying. Historically, the fused glass ampoules, were used as the container of choice for biological standards and other reference materials due to concerns of (a) obtaining an imperfect seal and/or (b) detrimental effect of stoppers on the product in the vial/stopper combination. A study by National Institute for Biological Standards & Control, United Kingdom compared ampoules with vials for lyophilized albumin and reported that while the gas and moisture contents of ampoules do not change even under stress conditions, but detectable changes in moisture and some oxygen ingress were observed during storage in vials [6]. The use of ampoules may allow the storage of the international reference standards for indefinite periods of time. Consequently, a continuous glass sealed envelope in the ampoule was preferred over the vial/stopper configuration for enhanced stability of biological standards [7,8]. The limitations of using ampoules for lyophilization include filling procedure, speed of filling, and ampoule sealing and product reconstitution. Most of the lyophilizers are equipped for vial stoppering, ampoule sealing needs special handling and equipment. An additional concern is

the introduction of small glass pieces into the product when breaking the ampoule seal before use.

Technological advancement in the procedures for vial filling and capping resulting in higher throughput, greater acceptability to end user due to ease of sampling aided with literature evidence providing comparability of vials to ampoules encouraged the use of vials as an alternative to ampoules. Ford and Dawson, for example, found that the use of vials with treated stoppers was comparable to DIN ampoules for the freeze-drying and storage of alkaline phosphatase under a range of temperatures and humidities [9]. The majority of lyophilized biopharmaceutical products use glass vials as a primary container. It should be noted that glass vials are preferred, in general, over plastic vials due to the standard operations of washing, depyrogenation, and possibly terminal autoclaving (usually not applicable for biologics) for which glass vials are better suited. Additionally, phthalates in plastics serve as a source for extractable and leachates in the finished product and may cause allergic reaction and/or immunogenicity [10].

Freeze-drying, in general, is affected not only by the dimension of the vial and the protein-container interactions but also by the chemical and physical properties of the vials. Type I (A and B) tubing glass vials, for example, are typically used (not always though and USP type II or type III may also be used) over molded glass vials due to superior dimensional tolerance consistency. Additionally, a standard EU blow back (European Style blow back) style is preferred over an US blow back (American style blow back). Greater variability from vendor to vendor is observed for the US blowback style while the EU blowback has additional benefits with line performance and vacuum retention for lyophilized products. Vials intended for use in freeze-drying should have a low coefficient of expansion for the glass type with uniform thickness on the heat transfer surfaces i.e. the bottom and the sides of the containers. Because molded vials usually fail to meet these characteristics, the remaining discussion will focus on tubing vials. It should be noted that for some parenterals (e.g. oncology drugs that are larger volume products) where the standard tubing glass vials do not easily meet the products' clinical needs, molded glass vials may be required. In general, the overall wall thickness of a tubing vial should be thin with a bottom thickness ~ 60±5 % of the wall thickness to allow less resistance to heat transfer (greater conductivity) during the freeze-drying process resulting in greater chamber throughput; but thick enough to provide the desired strength and unit integrity to prevent "ring-out" or vial breakage [11]. Pikal et. al. reported an approx. 4% relative standard deviation for the vial heat transfer coefficient (sensitive to geometry of vial and has contributions from physical contact between glass and shelf, radiative heat transfer and conduction through vapor phase) for vials from the same lot [12]. Other factors such as an optimal rounded bottom outside diameter, bottom inside diameter and bottom flatness are required to alleviate the potential stress points that might result in vial breakage or inefficient heat transfer during the lyophilization cycle [11,13].

The amount of fill in the container, glass surface interaction with the formulation components, and leaching and dissolution of glass are some additional factors that should be considered to

create the fewest processing problems during lyophilization. Crystallization of formulation containing sodium dibasic phosphate, for example, resulted in ampoule breakage during freeze-drying while the heating of the frozen solution [14]. Similarly, vial breakage for mannitol and even sodium chloride-sucrose has been reported for processes where excipients and amorphous water crystallizes after initial freezing [15,16]. This is attributed to an increase in the volume of water (by 9% due to lower density of ice compared to water) upon freezing causing additional strain on the container. Generally, a maximum fill volume of usually 35% of the vial's capacity is recommended. Additionally, highly acidic or basic pH can lead to leaching or dissolution of glass surface during the pre-lyophilization or post-reconstitution stage. Furthermore, adsorption to the hydrophobic glass surface can be avoided by the use of surface active agents such as polysorbates or gelatins or using specialty glass coatings [3,4]. Presence of chemical coatings such as silicone oil (frequently used to prevent protein binding on surface), however, has also been shown to have implication in inducing protein aggregation and therefore the exact type of container must be empirically determined on a case-by-case basis [17].

Syringes and **Dual chamber devices** are a subject of more and more development process and focus of pharmaceutical industry due to fast reconstitution time (with or without of the use of a reconstitution device), containment in the "ready-to-use" device and possibility of self-administration resulting in an increased convenience to end user. Additional factors such as differentiation (especially in case of biosimilars) and improved drug delivery accuracy and precision thereby avoiding the need for an overfill (as drug is contained in final delivery device and drug transfer between vial and syringe is not required) are added advantages of using syringes and dual chamber devices. Because these devices share some common attributes present in the commonly used containers and closure type by the virtue of similar composition (glass type, rubber etc.); only special considerations associated with the use of these device in freeze-drying application is discussed here. Septa stoppers used in dual chamber cartridges and devices, for example, are typically composed of elastomeric materials. A detail description of the closure/dosage form interaction is present in the next section.

Development of a freeze-dried product in a syringe or dual chamber device is a challenging task due to concerns ranging from (a) materials compatibility, (b) freeze-drying cycle, (c) need for special equipments to (d) device performance. The choice of contact packaging materials (during the pre-lyo or post-reconstitution or in the diluent chamber) and their influence on the drug product quality throughout the shelf life of a product is critical [18]. It should be noted that plastic syringes are less preferred than glass syringes due to potential interaction with EtO sterilizing agent, however, formulation intolerances with silicone oil lubricant (used to coat syringe barrel to reduce the break-free and glide force of pistons can mediate protein denaturation) [17,19] may demand the use of plastic syringe over glass syringe. Alternatively, baked-silicone and polymeric syringes may be used to substitute the standard level siliconized syringes.

Similarly, additional challenges are posed in the freeze-drying step as the product in the device is usually not in intimate contact with the shelf. Consequently, heat transfer is

dominated by convection and therefore the influence of chamber walls is greatly enhanced. Given these differences compared to lyophilization in vials (conduction dominated mostly) the cycle needs to be optimized for syringe geometry, fill and size to achieve uniform total heat input to each cartridge. Vials for example, freeze and dry faster while syringes show a lag resulting in longer freeze-drying time in syringes. Similarly, moisture mapping studies are required to assure that product from all locations on the shelf consistently meet all the predetermined specifications and quality attributes. Equipment challenges including handling issues and the need for special processing components such as stopper holder to accommodate stopper loading, stopper insertion devices, device holding apparatus etc. poses additional constraints for the development of such products.

Performance of the device refers to its ability to function for the intended use [2]. A comprehensive study to evaluate the performance of the device requires an evaluation of not only the functionality of the device but also the ability to deliver the intended dosage form. Faulty manufacturing design, improper assembly, misuse or wear and tear during usage might compromise the performance of the device for the particular dosage form, route of administration and the design feature.

Bulk containers for use in freeze-drying applications should meet the same requirements for protection of dosage form, safety as well as compatibility as any container closure system. Additionally, long-term storage of drug product in bulk containers requires not only the description (such as composition, inner liners, desiccant (if any), inner seal etc.) but also justification for the use of container closure systems with the established stability studies [2]. Stainless steel, glass bottles and recently commercially available trays and containers with membranes and pores represent the widely used method of large-scale bulk freeze-drying [20,21]. Gassler and Rey reviewed some advantages of such trays over the conventional stainless steel freeze-drying approaches and documented improved containment, reduction in the risk of contamination through the use of ePTFE (expanded polytetrafluoroethylene) membrane and reduced cleanup of the lyophilizer enabling faster dryer turnaround [21]. Similar studies comparing freeze-drying in glass bottles and trays demonstrated more homogenous cake morphology in trays (due to lower fill height), slight resistance to mass transfer in trays (due to presence of semi-permeable membrane), and improved heat transfer (due to lower thickness of plastic membrane in trays) [20]. It should be noted that long-term storage in such trays requires storage in sealed foil pouches that prevent water vapor ingress through the semi-permeable membrane. It is recommended that careful process development and compatibility studies should be performed with the desired container type (including real-time stability studies or simulation studies with smaller versions) to evaluate the container suitability for freeze-drying process.

Closures

Closures (screw caps, stoppers, etc.) must satisfy all the functions required of the container (i.e. protection, compatibility, safety, and performance), plus a number of additional requirements such as seal integrity and reseal properties (during multiple penetration by hypodermic needle) and low surface tackiness during processing [11]. Such requirements demand a unique combination of resilience and elasticity and therefore elastomeric closures (composed predominately of a propriety rubber formulation) are used for lyophilization. General requirements for closures used in lyophilization are same as those described for stoppers and therefore the remaining discussion will focus on stoppers.

Stoppers for freeze-drying application must not only provide the necessary vent/slot through partial insertion in the container thereby allowing outgassing of water vapor during the drying step but should also provide the closure function upon complete stoppering post-lyophilization. It should be noted that the stoppers' resistance to vapor flow depends on the size and depth of the slot. The resistance of the dried layer to vapor flow (which depends on the nature of product and cake thickness), however, is significantly higher than the stopper resistance even for the 13 mm (slot diameter 0.2 cm) finish stopper (except for very dilute formulations) [12,3]. Furthermore, the igloo stoppers (i.e. single-slotted stoppers) are usually preferred over two-legged slotted or fluted stoppers, despite similar performances in freeze-drying application, due to better machinability and handling properties during filling operation on commercial scale. Additionally, commercially available vial isolators might be used to contain and enable aseptic transfer and freeze-drying processing during the early phases (safety assessment, stability studies etc.) of development programs. Care must be taken, however, to optimize the cycle parameter to account for the increased resistance to the flow of water vapor especially for dilute formulation. The glass transition temperature of certain finished rubber formulations ranges from -75 oC to -55 oC [11,24] and even though the elasticity is regained by bringing the stopper back to normal temperature; additional care must be taken during storage of the freeze-dried product at temperature below the glass transition point. Similarly, subjecting rubber matrix to drying conditions especially during sterilization might result in increased tackiness, "over-curing" or a change in molecular composition of the stopper [25]. Other factors such as vial type and dimensions, optimal lubrication on stopper and stoppering process are critical when choosing the closure configuration to ensure complete stoppering during freeze-drying. To minimize incomplete stoppering or pop-out problem, for example, it is recommended that excessive lubrication should be avoided although a coating should be included on top of the stopper to avoid sticking of stopper to itself or to the shelf.

Besides physical attributes of stoppers, care should be taken in terms of its chemical properties, extractables and leachables, gas and water-vapor transmission while keeping in

mind their specific functionality within the packaging system (vials or dual cartridge device). According to FDA's guidance, extractables are compounds that can be extracted from individual components of a packaging system under stressed condition with various solvents while leachable (a sub-class) are extractable that migrate from the packaging system into the drug product during storage conditions [2]. The volatile extractable present in the stoppers used for freeze-drying can contaminate the product either during lyophilization (under high vacuum) or long-term storage conditions. Formation of haze, for example, in certain freeze-dried parenterals was associated with the presence of unsaturated and aromatic hydrocarbons from halobutyl stoppers [26,27]. Similarly particulate generation was observed when the TNF (tumor necrosis factor) formulation made contact with the siliconized stopper [28]. Leaching of allergans (e.g. latex from natural rubber) [29], and extraction of phenolic derivative by polysorbate 80 resulting in PRCA [30] (pure red cell aplasia) are some other examples for product incompatibility with the stopper. It should be noted, however, the use of coated rubber stopper with Flurotec prevented the leaching of impurities into product [31]. Inverted vials can be placed on accelerated stability to magnify such incompatibilities, and a comprehensive extraction study is required to study the type and concentration of migrated species and the corresponding toxicological impact [2,32].

In addition to extractables, volatized moisture and/or oxygen can ingress triggering cake collapse and compromising the product quality during storage. An optimal moisture level is usually desired for freeze-dried product. Freeze-dried *S. Cerevisiae*, for example, lost ≥ 60% of the invertase activity at water activity above monolayer moisture content [33]. Similarly, a decrease in the stability of bovine somatotropin and lysozyme was observed with increasing moisture content [34]. It should be noted that besides the residual moisture in cake, moisture in the lyophilized drug may increase due to absorption of moisture present in the stopper or moisture ingress through the stopper. Therefore, it is desired that the moisture vapor transmission (MVT), a measure of vapor permeability through stopper, should be low enough to prevent moisture ingress during the shelf-life of the product. Thus, butyl stoppers (halogenated as well as non-halogenated) are usually recommended for lyophilization. Also, a direct relationship was found between the initial stopper moisture (i.e. pre-sealing) and the amount transferred to the product during long-term storage of a freeze-dried sucrose formulation [35].

Seal integrity is another area of challenge that requires multiple features to come together for satisfactory outcome. As compared to liquid products, maintaining seal integrity is a bigger challenge for lyophilized product due to longer lag time between stoppering and application of aluminium overseal (crimping). Incompatibility of closure with the container, closure coating, closure formulation (hard vs. elastic) and/or packaging component tolerances under the given storage condition are some of the factors that may adversely influence seal integrity eventually leading to ingress of contamination, oxygen and/or moisture or egress of gases or vacuum present in the container headspace. Proper evaluation of seal integrity (a mandatory

part of stability studies) [2] should be carried out by an adequate and validated procedures such as live bacterial challenge [36], helium leakage test [37] and potentially water vapor ingress test [38] for the given container/closure combination.

Summary

This article underscores the significance of understanding, evaluating and addressing the many facets of container/closure system in assuring the safety and efficacy of the drug and establishing the shelf-life of the product when developing freeze-dried biopharmaceuticals. These seemingly minor aspects of container/closure system, if overlooked, can directly or indirectly influence the performance of product and may even lead to adverse clinical effects. Therefore, a careful study of potential interaction of the drug product and container/closure system and their impact on the freeze-drying process and the shelf-life of the product should be performed on a case-by-case basis to adequately protect the freeze-dried biopharmaceuticals from deleterious effect of improper container/closure selections and preparation. **APR**

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