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Flexible Freeze Container

Preventing Gas Ingress in Single-Layer Fluoropolymer Bags

By Joe Cintavey, Gore PharmBIO Products

For many emerging cell and gene therapies, their increasing sensitivity and how that may impact stability have made their storage and transport uniquely challenging. This complexity has led to pioneering new container technologies designed for increasingly complex biologics; while the space had been historically dominated by bottles, closed multilayer bag assemblies and, more recently, single-layer fluoropolymer bags have become the more attractive choice for companies with colder storage and transport temperature requirements.

While bags have afforded the industry a number of advantages over bottles, including aseptic closed processing and improved space utilization, their fragility in the face of ultra-cold chain applications has created a new obstacle for suppliers to overcome. As many advanced therapies, monoclonal antibodies, and precursors become more temperature sensitive, the need for container solutions that can withstand freeze/thaw processes without integrity issues will continue to grow and evolve. To this end, single-layer fluoropolymer bags have been found to outperform more traditional multilayer assemblies at colder temperatures and have begun to see more widespread adoption as a result.

Yet this recent innovation has created the potential for a new issue – the permeability of the films used in single-layer assemblies can result in oxygen and carbon dioxide ingress, which may impact the pH of a drug substance and affect its overall stability and efficacy. While barrier films in bags can be useful for cold chain applications, they do not fully protect against gas ingress and ignore other sources of permeability, such as tubing. This is particularly true of smaller bags with larger surface-to-volume ratios (SA/V), which are frequently used for bespoke cell and gene therapies.

Barrier wraps that encompass the entirety of a bag system, including any tubing, connectors, can consistently mitigate concerning pH shifts. By combining the robustness of fluoropolymer bags in cold chain operations with the additional protection of a comprehensive barrier wrap, companies can minimize gas ingress to an extremely low level and better protect these sensitive, valuable therapeutics.

Revisiting Container Solutions for Sensitive Biologics

Historically, multilayer non-fluoropolymer bags have dominated the biologics market, representing a key innovation transferred from other industries. But as storage requirements for advanced therapies have increasingly dipped below the glass transition temperature of conventional films, incumbent bag assemblies have become suboptimal for these applications, as these containers are prone to cracking at extreme cold temperatures. Fluoropolymer bags have emerged as an alternative to more brittle bag assemblies, but the relative novelty of both these bag assemblies and the drugs stored within them has created a learning curve for the industry to overcome as fluoropolymers are typically more permeable than conventional films containing a barrier layer. Exposure to dry ice during the freezing and shipping process has the potential to introduce gases such as O_2 or CO_2 that may result in a pH change in the product.

Gas ingress and subsequent pH shifts are a possibility for certain cold chain applications; evaluating an application for potential gas ingress is key to preserving the efficacy and safety of a drug product. To do so may require internal studies, as well as an

understanding of the factors that may influence gas ingress, such as the size and surface area of the bag assembly. For larger bags, gas ingress is less worrisome, as it is less likely to engender a meaningful pH shift for a product due to the SA/V. Smaller bags, however, can experience more dramatic shifts, as the larger SA/V creates more potential for gas to penetrate parts of the bag over a smaller volume of liquid. This consideration is especially crucial because smaller bags are more often employed for bespoke therapeutics, such as gene therapies, which are tailored to a smaller patient population and often represent a critical intervention that is both expensive and time sensitive. Other variables should also be considered as they are hypothesized to impact gas ingress, such as tubing material and sizing, and product life cycle variables such as buffers, headspace volume, pre-freezing, shipping simulation, and storage duration.

Barrier Wraps to Prevent Gas Ingress

Addressing the potential for gas ingress will be an important consideration for companies pursuing sensitive, advanced therapies. W. L. Gore, a global materials science company, has developed a solution to gas ingress, validated through rigorous testing, that represents an improvement over standard barrier films. After conducting a multi-phase study , Gore determined that a barrier overwrap that encompasses the entire bag system, including tubing, connectors, and bag film was found to be the most effective way to consistently mitigate a pH shift of concern.

With the understanding of film permeability and surface area to volume ratio being critical, the study utilized bags with conventional films containing a barrier layer and the GORE STA-PURE Flexible Freeze Containers (FFCs), which are single-use fluoropolymer bags designed to store and transport bulk drug substance at temperatures as low as -86° C. The goal of the study was to understand which life cycle and product parameters were most impactful as it relates to gas ingress, as well as how those various parameters interacted to influence the pH of a product after exposure to dry ice for 10 days

The initial phases of Gore's study found that factors such as volume of headspace within the bag, pre-freezing in a freezer prior to shipping simulation, and tube path or tortuosity had no significant impact on gas ingress. However, in the absence of a barrier wrap, the amount of exposed tubing (regardless of size or material) was found to be significant, indicating that ingress through tubing needs to be considered. Just as important as the bag properties is choosing an appropriate buffer. The evaluation found buffer selection is critical for minimizing pH shifts of concern and should be considered.

Based on the findings from the initial phase, a final phase of the study was conducted to further explore the impact of using a barrier overwrap, evaluate impact of exposed tubing length, and further evaluate when SA/V is a significant factor. In addition, a second buffer preparation was included to capture any variation that may be inherent in buffer batches. A custom design of experiment was created, resulting in 70 samples. A model was generated to evaluate the main effects and two-way interactions. This analysis found that bag size in combination with whether the bag was wrapped or not was the most significant factor. While wraps provide protection for small bags, it is hypothesized that there is likely an SA/V for a larger bag at which the wrap provides minimal benefit. To better understand the more minor effects, data was split by bags wrapped and not wrapped, and an analysis rerun. It was found when not wrapped, bag size in combination with exposed tubing length was found to be significant. This suggests that there is an impact related to exposed tubing on smaller bags with a larger SA/V. It also indicates that barrier layers in a bag are not as impactful as a barrier wrap since the gas ingress will still occur through the tubing.

Conclusion

The permeability of the films used in single-layer assemblies can result in oxygen and carbon dioxide ingress, which may impact the pH of a drug substance and affect its overall stability and efficacy. While barrier films in bags can be useful for cold chain applications, they do not fully protect against gas ingress and ignore other sources of permeability, such as tubing. This is particularly true of smaller bags with larger surface-to-volume ratios (SA/V), which are frequently used for bespoke cell and gene therapies

Several hypotheses related to bag design, materials, and lifecycle parameters were tested throughout the execution of this study, identifying factors that were found to impact pH shifts after 10 days of storage on dry ice. Factors found to be not impactful included volume of headspace within the bag, pre-freezing in a freezer prior to shipping simulation, tubing material (within the material sets studied), and tube size. Factors found to be impactful included film permeability, the surface area to volume ratio of the bag, whether the bag, tubing, and connectors were covered with a barrier wrap, buffer type and preparation, and exposed tubing length.

Choosing an appropriate buffer and using bags with a lower surface are to volume ratio is critical for minimizing pH shifts of concern and should be considered. However, neither a barrier film in the body of the bag nor minimizing the length of exposed tubing may be enough to mitigate a meaningful shift, especially in bags that have a larger SA/V. Ultimately, while a number of factors play a part in influencing the impact of exposure to high concentrations of carbon dioxide, the use of a barrier overwrap that encompasses the entire bag system, including tubing, connectors, and bag film was found to be the only reliable means of consistently mitigating a pH shift of concern.

As cell and gene therapies continue to gain a larger share of the biopharmaceutical market, the need for container solutions that adequately safeguard these fragile modalities will grow apace. Therapeutics like autologous cell therapies, which utilize a patient's own cells and are manufactured with limited materials, are often a last resort for critically ill patients. Minimizing the potential for error when freezing and transporting these drugs is crucial to safeguarding patients, meeting stringent timelines, and avoiding the exorbitant costs associated with rework.

About Gore

W. L. Gore & Associates is a global materials science company dedicated to transforming industries and improving lives. Since 1958, Gore has solved complex technical challenges in demanding environments — from outer space to the world's highest peaks to the inner workings of the human body. For the pharmaceutical and biopharmaceutical industry, Gore is helping those in drug discovery and manufacturing compete in this increasingly regulated landscape with reliable solutions for purifying, transferring, storing, filtering, processing, and packaging. These solutions include durable, multi-use process components and single-use products that enable researchers and manufacturers to control costs through process improvements.

Gore PharmBIO Products

Our technologies, capabilities, and competencies in fluoropolymer science are focused on satisfying the evolving product, regulatory, and quality needs of pharmaceutical and bioprocessing customers, and medical device manufacturers. GORE STA-PURE Flexible Freeze Container, like all products in the Gore PharmBIO Products portfolio, are tested and manufactured under stringent quality systems. These high-performance products provide creative solutions to our customers' design, manufacturing, and performance-in-use needs.

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Americas | W. L. Gore & Associates, Inc.
402 Vieve's Way • Elkton, MD 21921 • USA
Phone: +1 410 506 1715 • Toll-free (US): 1 800 294 4673
Email: pharmbio@wlgore.com

Europe | W. L. Gore & Associates, GmbH
Wernher-von-Braun-Strasse 18 • 85640 Putzbrunn, Germany
Phone: +49 89 4612 3456 • Toll free: 0 800 4612 3456
Email: pharmbio_eu@wlgore.com

gore.com/pharmbio

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