Savillex Technical Note

Freeze Drop Testing of Bulk Drug Substance Bottles

Summary

Frozen storage of bulk drug substance (BDS) at temperatures at or below -70°C is common in the bioprocess industry. Storage at temperatures below -80°C can lead to spontaneous failure of typical BDS containers, particularly if container temperature is reduced rapidly (also called flash freezing).

Most containers used to store BDS have glass transition temperatures well above -196°C; many containers structurally fail during the rapid descent through glass transition. Even worse, these failures are rarely detected until after the container is thawed, which can be days if not weeks or months after the freezing is completed.

Fluoropolymer materials, on the other hand, typically do not change structurally when flash frozen. Therefore, a container system manufactured from fluoropolymers has the potential to not only survive flash freezing but to continue to retain the same functionality it had when at room temperature.

Savillex Purillex® ETFE and PFA 1 L Bottles

Containers frozen to cryogenic temperatures are particularly susceptible to damage during handling. Because the containers become slippery, they are also easily dropped. Whether the container is dropped onto a surface from only a few inches or off a counter (typically 3 ft (1 m)), damage can be catastrophic, leading to breach of bottle integrity and product loss. Again, these failures are sometimes not detected until after the container is thawed.

This technical note outlines a study performed to test the integrity of common BDS bottles after subjecting them to freezing down to cryogenic temperatures followed by a drop from 3 feet (1 m). Bottles are observed to ensure structural integrity after freezing and then dropped from a typical countertop height to see if bottle integrity is maintained. Finally, they are inspected post-thaw for any leaks or other structural damage that is undetectable when frozen.

Bottle Material is Key to Cold-Temperature Performance

Material of construction plays a crucial part in performance at cryogenic temperatures. For example, containers made from fluoropolymers like PFA and ETFE naturally perform better below -70°C than other more common materials like PETG, PC, PE and PP. This is because fluoropolymers are innately more temperature stable and suitable for continuous use down to -200°C.

Another item to consider is the bottle closure system (i.e., the cap). Some bottles are manufactured using a different material of construction for the closure, sometimes even using closure inserts to ensure container integrity. This can be very problematic, as different materials behave differently at cryogenic temperatures. Also, bottles made from materials suitable for cold temperature applications could have closures made from materials that are not.

The container closure system of Purillex® fluoropolymer bottles has a superior design, which ensures a better seal and better protection of contents even under blast freeze conditions. The identical fluoropolymer resin type and grade are used for both bottle and closure, and both bottle thread and closure are injection molded, ensuring precise fit and superior seal integrity without the need for a closure insert. This means that only a single material is in contact with the product.

Bottle Freeze Drop Test Procedure

Seven 1,000 mL (1 L) of each of the following bottles were selected for testing from the same bottle lots:

- Savillex Purillex PFA bottles
- Savillex ETFE bottles
- Commercially available PETG bottles
- Commercially available PC bottles

All bottles were pre-sterilized or purchased sterilized from the manufacturer, prior to testing. Gamma sterilization was used for all bottles except PFA, which was autoclave sterilized. Each test bottle was filled with room temperature purified water to the minimum working volume of 1000 mL.

Bottle closures were tightened to the manufacturer's recommended torque settings using a calibrated dial torque wrench. Each closure was brought to the proper torque and held at the value for 10 seconds.

All 28 test bottles were frozen to -85°C for a minimum of 24 hours prior to testing. This temperature was chosen as it is the lowest temperature setting of commercially available, upright freezers that do not use liquid nitrogen as a cooling source.

Drop Test Procedure

Bottles were selected in random order for drops. The bottles were dropped from a height of 36" (1 m) onto a hard, concrete surface. Bottles were dropped to impact on either the bottom or side, which are common impact orientations for bottles dropped while being moved around manually or from being knocked off lab countertops. It was also common for bottles to bounce and rotate after the initial impact, striking other surfaces during the drops.

Each bottle was inspected for damage immediately after the drop and again 24 hours later after completely thawed. The second inspection was performed since minor damage that cannot be detected on frozen bottles due to frost buildup on the bottle surface may occur. Bottles were also inspected for paneling after thawing. Paneling is the inward deformation of sidewalls caused by a slight vacuum that occurs in the bottle on thawing. This occurs if the closure leaks during freezing, allowing air to escape as bottle contents expand, but remains sealed during thawing. If the sidewalls are not strong enough, the small vacuum that is formed inside the bottle can cause the sidewalls to deform inwards.

Results

All 28 bottles survived freezing to -85°C without any visible damage or structural failure. Therefore, all bottles moved on to the drop procedure. The results of the study are summarized in Table 1.

All seven PETG bottles failed at initial impact with significant structural failure. One bottle ejected the entire top portion of the bottle after impact (Figure 1).

Six of the seven PC bottles survived drops with no damage. One bottle, after the initial flat-on-bottom drop, fell to the side, hitting the closure on the cement floor, causing it to shatter (Figure 2). It was discovered later that the closure was made from polypropylene and was not suitable for use at cryogenic temperatures.

All seven PFA and seven ETFE bottles survived drops with no damage and no breaches.

Drop Test Results

Bottle	Material	Results	Notes
1	PFA	No leaking; no paneling after thaw	
2	PFA	No leaking; no paneling after thaw	
3	PFA	No leaking; no paneling after thaw	
4	PFA	No leaking; no paneling after thaw	
5	PFA	No leaking; no paneling after thaw	
6	PFA	No leaking; no paneling after thaw	
7	PFA	No leaking; no paneling after thaw	
1	ETFE	No leaking; no paneling after thaw	
2	ETFE	No leaking; no paneling after thaw	
3	ETFE	No leaking; no paneling after thaw	
4	ETFE	No leaking; no paneling after thaw	
5	ETFE	No leaking; no paneling after thaw	
6	ETFE	No leaking; no paneling after thaw	
7	ETFE	No leaking; no paneling after thaw	
1	PETG	**Cracked from bottom corner to side**	
2	PETG	**Bottom side cracked – piece fell out**	
3	PETG	**Cracked on bottom corner – piece fell out**	
4	PETG	**Cracked on bottom corner**	
5	PETG	**Top completely broke off; crack on bottom**	**See Figure 1**
6	PETG	**Crack on side to top**	
7	PETG	**Crack from bottom corner to side to top**	
1	PC	No leaking; no paneling after thaw	
2	PC	No leaking; no paneling after thaw	
3	PC	**PP closure shattered during flat-on-bottom drop**	**See Figure 2**
4	PC	No leaking; no paneling after thaw	
5	PC	No leaking; no paneling after thaw	
6	PC	No leaking; no paneling after thaw	
7	PC	No leaking; no paneling after thaw	

Table 1: Drop Test Results

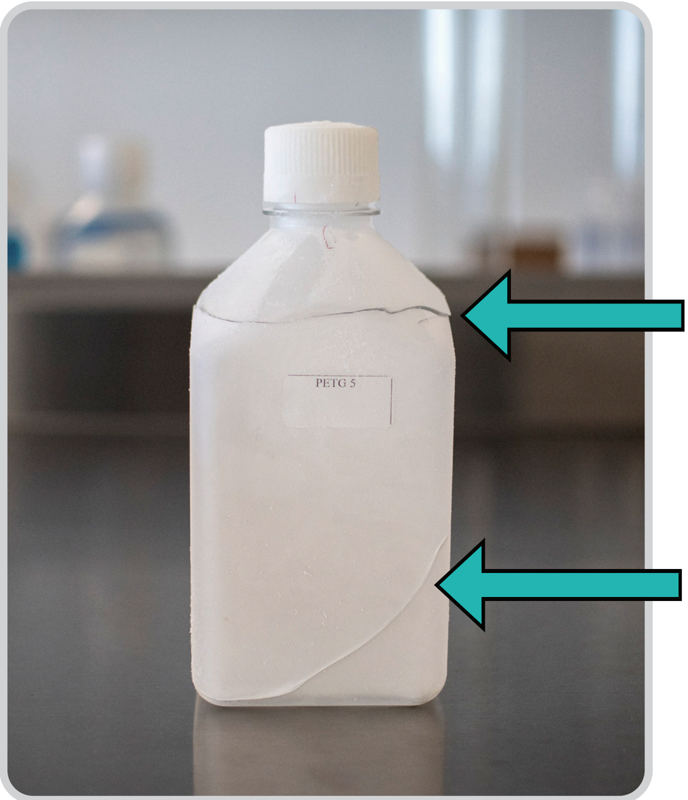

Figure 1: PETG Bottle Damage – Flat-on-Side Drop. The top is completely separated from the rest of the bottle and a crack at the bottom.

Figure 2: PC Closure Damage – Flat-on-Bottom Drop

Conclusions

Evidence from this test protocol indicates that the Savillex 1 L PFA and ETFE bottles are suitable for freezing to -85°C and can withstand a drop from 36" onto concrete with no bottle damage and no loss of bottle integrity. This is a testament to the structural durability of fluoropolymer materials when exposed to cryogenic temperatures. It is also illustrative of the strength, reliability and seal integrity of the Purillex bottle.

Conversely, all PETG bottles failed the drop test, and one of six PC bottles failed due to an incompatible closure made from a different material. Because of these failures, we do not recommend using commercially available PETG nor PC bottles for cryogenic storage of critical materials like BDS.

Savillex
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Savillex
10321 West 70th St. | Eden Prairie, MN 55344-3446 USA | Phone: 952.935.4100
Email: info@savillex.com | **www.savillex.com**