Savillex
Your Fluoropolymer Solutions Partner

Savillex Technical Note
Freeze Cycle Testing

Summary
Storing frozen bulk drug substances (BDS) – including bioprocess solutions, vaccines, blood components, and other delicate process fluids – is common practice in the bioprocess industry.

1 L Savillex Fluoropolymer Bottles

Vessels used to store these fluids must not only be capable of withstanding long-term storage in frigid temperatures (e.g., -85°C or -196°C), but they must also maintain integrity after repeated thawing and subsequent re-freeze. Bottles are a container of choice for freezing due to their durability and convenience, compatibility with standard laboratory equipment, shelving and racking, and standard shipping containers.

Bottles are also advantageous for freezing as they can be easily integrity tested during manufacture by utilizing pressure decay test methods. Bottles also feature container closure systems that are ideal for torquing. Most bottles and other containers manufactured for bioprocess applications come with validated torque specifications, often with values and methods unique to each closure size and style.

When utilizing standard laboratory bottles for freeze/thaw applications, one risk is sidewall paneling. Bottles panel for several reasons. Paneling can be due to bottle material selection, inadequate sidewall thickness, product design (cubical vs. round vs. oval shape), and, more nefariously, air egress due to poor closure/seal design or inadequate closure application during use. In our experience, air egress is the most common cause of paneling in bioprocess flash-freezing applications.

Fluoropolymer bottles are an excellent choice for freeze/thaw processes as the structure does not change when flash-frozen. Therefore, containers manufactured from fluoropolymers not only have the potential to survive flash-freezing but retain the same functionality as at room temperature.

This technical note outlines a study performed to characterize the performance of fluoropolymer bottles with standard and two-piece closure systems after multiple freeze/thaw cycles, with a re-torque step added after each freeze cycle.

Summary of Freeze and Thaw Procedure
Savillex 1 L fluoropolymer bottles, filled to nominal volume, were frozen at -85°C for a minimum of 24 hours, the closure systems re-torqued, and then placed in a 37°C water bath until completely thawed. Per the procedure outlined below, visual inspection and integrity testing were the criteria by which the bottles were measured. Failure was defined as bottle material damage, paneling, or failure of integrity testing.

Fluoropolymer Abbreviations
- ETFE – ethylenetetrafluoroethylene
- PFA – perfluoroalkoxy

Equipment Used:

- Upright -85°C freezer
- 19 L laboratory water bath

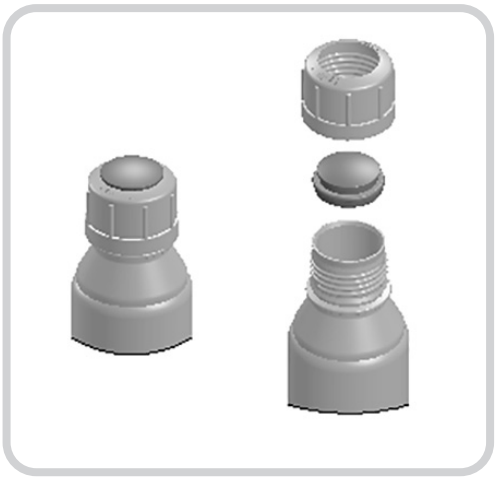
Bottle Assemblies tested:

- 1000 mL PFA bottle and standard PFA closure (PFA)
- 1000 mL PFA bottle and two-piece PFA closure (PFA2)
- 1000 mL ETFE bottle and two-piece ETFE closure (ETFE)

All bottle assemblies tested were manufactured by Savillex using stretch blow molding technology. With stretch blow molding, an injection molded preform is blown into the final bottle shape in a two-step process. Since the bottle threads and sealing surface are injection molded (during the preform molding), much greater precision and reproducibility of the bottle seal are attained.

The closures used during testing included the standard one-piece closure and a two-piece closure designed for flash freezing applications. The two-piece closure has a floating insert integral to the design that allows more precise sealing under challenging conditions. The two-piece closure is standard on the 1000 mL ETFE bottle.

Standard one-piece closure



Two-piece closure

Freeze Procedure

Note: Each bottle assembly type was tested in triplicate

1. Fill bottles to 1000 mL with tap water
2. Torque each bottle closure system to 45 inch-pounds
3. Place bottle in -85°C freezer allowing at least ½" of space between bottles
4. Allow the bottles to freeze for at least 24 hours

Thaw Procedure

1. Remove bottles from freezer
2. Prior to placing in water bath, re-torque each bottle to 45 in-lb
3. Inspect each bottle for paneling, damage and leaks
4. Place bottles in 37°C preheated recirculating water bath until completely thawed

Bottle Integrity Testing

Once the bottles reach the maximum number of freeze/thaw cycles per Table 1, they were integrity tested per the following pressure decay test method.

Note: Water remained in bottles during integrity testing

1. Drill and tap fitting into closure system and attach a pressure supply line
2. Support bottle in an inverted position
3. Pressurize to 2 PSI
4. After 5-minute period, observe threaded area using back light to observe for any water droplets
5. Pressurize to 15 PSI
6. After 5-minute period, observe threaded area using back light to observe for any water droplets

Pass Criteria: No water droplets observed in the threaded area of the bottle closure system during the integrity test protocol described above.

Results

All three bottle types passed integrity testing after up to 15 freeze thaw cycles. No bottle damage was observed and none of the bottles paneled post-thaw during the entire study.

See Table 1 for a summary of the results.

Configuration	Bottle	Number of Cycles	Freeze Damage (Y/N)	Paneling Post Thaw (Y/N)	Integrity Test
PFA	1	5	N	N	Pass
PFA	2	5	N	N	Pass
PFA	3	5	N	N	Pass
PFA	4	10	N	N	Pass
PFA	5	10	N	N	Pass
PFA	6	10	N	N	Pass
PFA	7	15	N	N	Pass
PFA	8	15	N	N	Pass
PFA	9	15	N	N	Pass
PFA2	1	5	N	N	Pass
PFA2	2	5	N	N	Pass
PFA2	3	5	N	N	Pass
PFA2	4	10	N	N	Pass
PFA2	5	10	N	N	Pass
PFA2	6	10	N	N	Pass
PFA2	7	15	N	N	Pass
PFA2	8	15	N	N	Pass
PFA2	9	15	N	N	Pass
ETFE	1	5	N	N	Pass
ETFE	2	5	N	N	Pass
ETFE	3	5	N	N	Pass
ETFE	4	10	N	N	Pass
ETFE	5	10	N	N	Pass
ETFE	6	10	N	N	Pass
ETFE	7	15	N	N	Pass
ETFE	8	15	N	N	Pass
ETFE	9	15	N	N	Pass

Table 1: Summary Test Results for Freeze/Thaw Cycling of 1000 mL Purillex® Bottles

Conclusions

Results indicate that both PFA and ETFE bottles are suitable for multiple flash freeze/thaw cycles down to -85°C with no visible material damage, leaks, paneling, headspace air egress, or loss of pressure integrity. Both the one-piece and two-piece closures performed identically during the study. The greater seal integrity of stretch blow molded bottles is a significant factor in preventing leaks and bottle paneling during freeze/thaw cycles. It is postulated that re-torque of the closure system after freezing is also a contributing factor in eliminating paneling and headspace air egress, as both have been observed periodically during previous studies where closure re-torque was not applied.

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