Savillex Technical Note

Freeze Cycle Testing on Purillex® Fluoropolymer Bottles

Summary

Storing frozen bulk drug substances (BDS) – including solutions, vaccines, blood components, and other process fluids – is common practice in life science applications.

1 L Purillex® 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 applications for a variety of reasons, including:

- Durability and convenience
- Compatibility with standard lab equipment
- Shelving, racking, and standard shipping containers
- Can be easily integrity tested during manufacture via pressure decay test methods
- They feature container closure systems that are ideal for torquing
- Most 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, including 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 life science flash-freezing applications.

Purillex® fluoropolymer bottles are an excellent choice for freeze/thaw processes as the structure does not change when flash-frozen. Therefore, they not only have the potential to survive flash-freezing but also 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.

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Summary of Freeze and Thaw Procedure

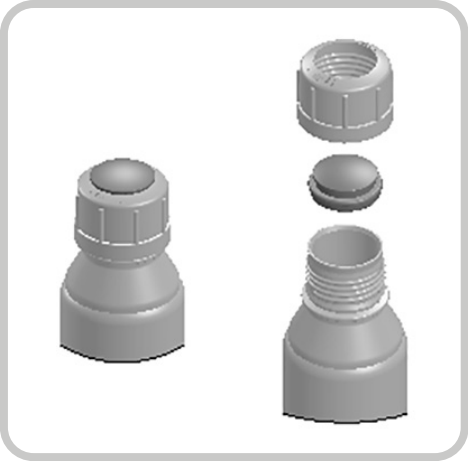
Purillex 1 L fluoropolymer bottles, filled to nominal volume, were frozen at -85°C for a minimum of 24 hours, the closure systems re-torqued, and then placed in a 37°C water bath until completely thawed. Per the procedure outlined below, visual inspection and integrity testing were the criteria by which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

Equipment Used	Bottle Assemblies Used
Upright -85°C freezer	1000 mL PFA bottle and standard PFA closure (PFA)
19 L laboratory water bath	1000 mL PFA bottle and two-piece PFA closure (PFA2)
	1000 mL ETFE bottle and two-piece ETFE closure (ETFE)
**Fluoropolymer abbreviations: EFTE (ethylenetetrafluoroethylene), PFA (perfluoroalkoxy)*	

All bottle assemblies tested were manufactured by Savillex using stretch blow molding technology, in which 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 for 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

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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, use back light to observe threaded area for any water droplets
5. Pressurize to 15 psi
6. After 5-minute period, use back light to observe threaded area 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 below for a summary of the results.

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Configuration	Bottle	# 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

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Conclusions

Results indicate that both PFA and ETFE Purillex 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.

The Purillex family of PFA Bottles

Click here to learn more about Savillex Purillex Bottles on savillex.com.

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