Application Note

A superior gasket material improves seal integrity in Thermo Scientific™ Storage Tubes

Key Words

Cryostorage, seal integrity, TPV gasket, O-ring gasket.

Abstract

A critical aspect of successful sample storage across a range of temperatures is the preservation of the sample material over the storage lifetime. The seal integrity of the storage tube is important in this regard, as it must maintain the sample contents in the tube as well as prevent contamination from outside the tube. Among the most widely used closures in storage tube applications are those that have separate soft silicone O-ring gaskets and those with solid one-piece closures utilizing thermoplastic vulcanizate (TPV) gaskets. Here we examine the performance of the closures with TPV gaskets for cryostorage applications.

Introduction

Seal integrity of the storage tube is crucial for preventing contamination and maintaining the integrity of the sample. This is especially true in cryopreservation applications, where materials are stored for long periods of time at temperatures as low as -196°C. In many cryostorage tubes, a soft-material gasket is used between the tube and the closure to maintain the integrity of the seal at extremely low temperatures. Typically, this gasket is made of silicone rubber material in a ring shape, or O-ring, that sits at the mating surface either inside (external thread) or outside (internal thread) the threads of the closure. On one hand, the soft silicone material allows it to compress and conform its shape to the mating surfaces of the tube and closure, which eliminates any gaps when properly tightened. On the other hand, the flexible silicone gasket will extrude out from between the closure and tube surface if an excessive amount of force is applied due to the simultaneous compressive force and torsion from the turning of the closure threads. This can leave an area where the gasket no longer contacts the mating surfaces, compromising the seal integrity. Hence, extra precautions need to be taken when tightening closures with silicone O-ring gaskets.

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TPV is an alternative gasket material that has become the new standard material for closure seals. Thermo Scientific storage tubes have featured TPV gaskets since 2006, and have been successfully used in long-term cryostorage conditions in the vapor phase of liquid nitrogen. TPV is a class of thermoplastic elastomer (TPE) with the elastic and compressive properties of a rubber material. Unlike silicone, TPV can be melted and molded in combination with a pre-formed plastic part to form an integral bond with the plastic. In tube closure applications, this means that the TPV gasket can be molded directly onto the cap to produce a one-piece closure and gasket seal. The bond between the TPV and the plastic closure prevents the gasket from extruding out during tightening. In addition, TPV material is less gas-permeable than silicone rubber, therefore, effectively reducing the evaporation of samples[1].

In this study, we tested four different storage tubes with the TPV gaskets, both aged and new, to examine sample leakage after multiple cycles of freeze and thaw as well as evaporation over time.

Experimental Details

Storage Tubes

The four types of storage tubes included in this study are Matrix 3748 (0.2mL), Matrix 3744 (0.5mL), Matrix 3741 (1.0mL), and Nunc 374500 (1.8mL). Aged tubes were generated by placing new tubes in an aging oven at 50°C for 10 weeks to simulate 5 years of shelf life.

Leak-Testing

All tubes were filled with proper volumes (above) of deionized water with dye and capped using the respective Matrix or Nunc style Thermo Scientific 8 Channel Handheld Decapper to maintain consistent closure torque. Tubes were inverted and subjected to a leak-test using a vacuum apparatus with 5 inches of mercury (inHg) vacuum pressure for 30 minutes. The presence of any water outside the gasket seal represented a failure of that tube due to leakage.

Freeze/Thaw Cycling

Properly filled tubes were frozen overnight in liquid nitrogen. Tubes were removed from liquid nitrogen the following day and allowed to thaw overnight. Approximately 24 hours later, tubes were leak-tested, and then placed back into liquid nitrogen. Leak failures were recorded at each cycle, and if possible failed tubes were re-capped and returned to liquid nitrogen. This process was repeated for 10 freeze/thaw cycles.

Evaporation Test

Properly filled tubes were leak tested and weighed before freezing. Tubes were placed in the liquid nitrogen overnight and removed the following day (Day 0). Tubes were then weighed every five days thereafter for 30 days. The weight of each tube at each time point was subtracted from the initial weight at day 0. The difference represented the liquid lost to evaporation from each tube over the time period.

Results and Discussion

Tube and closure integrity during freeze/thaw Cycling

Both new and aged tubes showed very few failures during freeze/thaw testing. In new tubes, only one 0.5mL Matrix tube showed any leakage caused by a loose closure. The loosened closure was re-tightened and the tube was returned to the testing cycle with no subsequent leaks. In aged tubes, one 1.0mL Matrix tube broke during one cycle, presumably due to freezing expansion of residual water that was left at the closure when the tube was inverted during leak testing. Under standard use conditions, it is recommended to keep tubes upright, and ensure that liquid samples are at the bottom of the tube prior to cryostorage. In 1.8mL tubes, one aged Nunc tube failed leak testing. The closure was re-tightened and the tube was returned to the testing cycle with no subsequent leaks. Results of all groups are summarized in Table 1.

Table 1. Number of tubes failed in 10 cycles of freeze/thaw

Tube Type	New	Aged†
Matrix 200µL	0	0
Matrix 0.5mL	1	0
Matrix 1.0mL	0	1
Nunc 1.8mL	0	1

n=60 for each Matrix testing group
n=30 for each Nunc testing group
†Simulated 5-year shelf life through accelerated aging process.

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Sample evaporation

The limited gas permeability of TPV makes it an ideal gasket material in preventing sample evaporation during storage. In tubes tested with either silicone or TPV gaskets, the TPV gaskets showed a clear advantage in minimizing sample evaporation over time. While tubes with silicone gaskets/closures performed very well with <14µL volume loss from each tube after 30-day storage period, tubes with TPV gaskets/closures demonstrated excellent sealing quality with <1µL volume loss from each tube under the same storage conditions (Figure 1). It is important to note that the evaporation test followed a freeze/thaw cycle to stress the seal, but was conducted at room temperature where sample evaporation is most likely to happen.

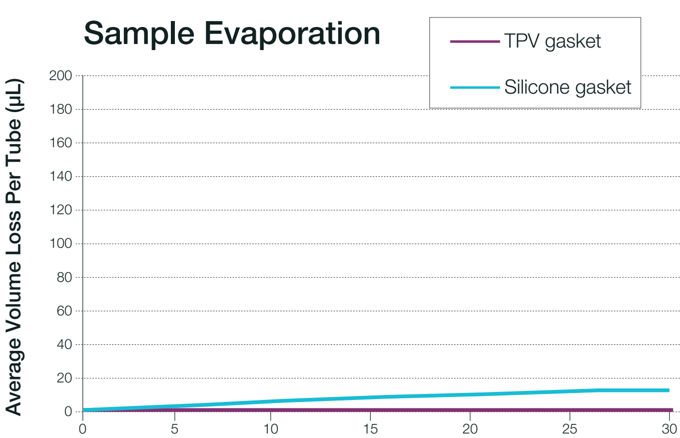

Figure 1. Average sample evaporation at room temperature in tubes with either TPV or silicone gaskets/closures.

Conclusions

- One piece molded-on design of TPV gaskets/closures protects sample integrity during repeated freeze/thaw cycles.
- The low gas permeability of TPV material minimizes sample loss from evaporation during storage.
- The innovative closure design coupled with superior gasket material make the Thermo Scientific storage tubes ideal for long-term sample storage.

References

[1]Velderrain, M. Moisture Permeability of Silicone Systems – Case Study #1: Water Vapor Transmission Rate as Influenced by Durometer, Silica, and Organic-Siloxane Group. NuSil Technology, Carpinteria, CA

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