Application Note

Gasket material and seal integrity in Thermo Scientific™ 2D Barcoded Storage Tubes

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Key Words
Cryostorage, liquid nitrogen, gasket, silicone, TPV, seal integrity, caps, long-term storage, o-ring cap.

Goal

Compare the sealing performance of storage tubes with silicone O-ring-gasketed cap versus tubes with molded-in TPV seal material cap.

Abstract

A critical aspect of successful sample storage across a range of temperatures is the preservation of sample material over the storage lifetime. The seal integrity of the storage tube is critical in this regard, as it must maintain the sample contents in the tube as well as prevent contamination from outside the tube. For such applications, a soft-material gasket is often used to ensure a proper seal between the tube and closure. While this gasket has typically been constructed of a silicone rubber O-ring, thermoplastic vulcanizate (TPV) materials have several advantages over silicone in gasket components. Here we examine the performance of different closures, made with gaskets of both silicone rubber and TPV used with identical tubes, and show the improved performance of TPV over silicone for storage applications.

Introduction

Seal integrity is critical when maintaining samples in storage in order to prevent contamination and maintain the integrity of the sample being stored. This is especially true in cryopreservation applications, where materials are stored for long periods of time at temperatures as low as -196°C. For tubes used in cryostorage applications, a soft-material gasket is normally used between the tube and the closure to maintain the integrity of the seal at extremely low temperatures. The soft material's properties allow it to compress and conform its shape to the mating surfaces of the tube and closure, which when tightened eliminates any gaps. Typically, this gasket has been 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. While silicone O-rings do provide an effective seal, there are weaknesses inherent in the material and design of the gasket. Because the gasket material is not physically attached to either surface, there are two sets of mating surfaces for each tube: one at the gasket/tube interface and one at the gasket/closure interface. This increases the likelihood of leakage around the gasket, as the proper compression of the gasket is responsible for the seal at both interfaces.

The loose nature of the gasket can also lead to problems if closures are overtightened. Due to the simultaneous compressive force and torsion experienced by the gasket from the turning of the closure threads, the flexible gasket material will extrude out from between the closure and tube surface if an excessive amount of force is applied. This can leave an area where the gasket no longer contacts the mating surfaces, compromising the seal integrity. Finally, the chemical structure of the silicon-based polymer

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chain backbone in the silicone rubber material used for such gaskets contributes to greater gas permeability at room temperature, especially for water vapor, compared to other elastomers[1]. This can lead to evaporation of water from the sample if left at this temperature for long periods of time.

Increasingly, the thermoplastic vulcanizate materials (TPVs) have been used in place of silicone as a gasket material for use in closure seals. Thermo Scientific tubes have featured TPV gaskets since 2006, and these tubes have been successfully used in cryostorage conditions, at temperatures down to -196°C in the vapor phase of liquid nitrogen, since that time. TPVs, a class of thermoplastic elastomer (TPE), have the elastic and compressive properties of a rubber material while preserving the melt and molding characteristics of a thermoplastic material. This allows the rubbery TPV to be molded in the same way as a standard plastic part, or molded in combination with pre-formed plastic parts to form rubber portions directly on the part. In tube closure applications, this means that the sealing gasket can be molded directly onto the tube closure to produce a one-piece closure and gasket. This arrangement has several advantages over the two-piece O-ring/closure. Since the gasket is molded directly to the plastic of the closure, any gaps between them are filled in while the gasket is still in the liquid phase, eliminating the need for gasket compression to seal that interface. As a result, only one compression-dependent mating surface exists when the tube is closed, reducing the possibility of leaks. Since the gasket is physically attached to the closure, the gasket cannot extrude out when torque is applied to the closure. This significantly reduces the risks associated with overtightening tube closures. TPV is also less gas-permeable than silicone rubber, reducing the capacity for water evaporation of samples left at temperatures above freezing.

For this study, we examined the seal integrity of TPV-sealed tubes, using silicone rubber O-rings as controls for comparison. We tested four different tube sizes that utilize two different closure styles. Tubes were tested for leakage and for evaporation, and both new and accelerated shelf-life aged tubes were examined. All tubes were frozen at least once to liquid nitrogen temperature (approximately -196°C) to mimic typical applications for cryostorage tubes. This was achieved by submerging tubes in liquid nitrogen, which would maximize stress on the closure gasket system and exceed the products' specifications for use in the vapor phase of liquid nitrogen only. The intent of this study was to subject the tubes to a worst-case scenario condition – submerging in liquid nitrogen – to induce failures where they would not occur under normal use.

Submerging tubes in liquid nitrogen is not recommended without additional precautions.

Experimental details

Freeze/Thaw Cycling

Each tube was filled to the full nominal volume – 0.2mL in 3748, 0.5 mL in 3744, 1.0 mL in 3741, 1.8 mL in 374500 tubes.

Sample sizes for 0.5 and 1.0 mL tubes included 60 TPV gasket closures, 30 each of two different styles (each designed for a specific torque tool), and 10 silicone O-ring closures.

200 µL tubes included 30 TPV closures and 30 silicone O-ring closures. 30 1.8 mL Externally Threaded tubes were capped with TPV gasket closures; no equivalent silicone gasket closure is available for this tube.

All tubes were capped using the respective Matrix or Nunc style Thermo Scientific 8 Channel Handheld Decapper to maintain consistent closure torque. Tubes were then leaked tested to examine seal integrity using a vacuum leak testing apparatus with 5 inches of mercury (inHg) vacuum pressure. All tubes were inverted and subjected to partial vacuum for 30 minutes during each test, and the presence of any water outside the gasket seal represented a failed test for that tube. Tubes were then frozen overnight in the liquid phase of liquid nitrogen to push the limits of the specified tube usage conditions. Tubes were removed the following day and allowed to thaw overnight. Approximately 24 hours later, tubes were leak tested (30 min at 5 inHg vacuum), then placed back into liquid nitrogen. This process was repeated for 10 total freeze/thaw cycles. Failures were recorded at each cycle, and if possible failed tubes were re-tightened and returned to freeze/thaw testing.

Evaporation

Each tube was filled with water to the intended volume (see volumes and sample sizes above). Tubes were leak tested (15 min at 5 inHg vacuum, see Freeze/Thaw Cycling) to screen seal integrity before freezing, and then weighed. Tubes were then placed in the liquid phase of liquid nitrogen overnight. Tubes were removed the following day (Day 0). Tubes were then weighed every five days (±1 day) thereafter for 30 days.

Accelerated Aging

New tubes were placed in aging oven for 10 weeks to simulate 5 years of shelf life. All evaporation and freeze/thaw testing was then repeated on aged tubes (as above). Testing on aged external thread tubes included 25 samples due to limited availability of aged tubes.

Data Analysis

Once weights were measured over the thirty-day period, the final weight of each tube at day 30 was subtracted from the initial weight at day 0. The difference represented the weight of sample lost to evaporation from each tube over the time period. The resulting calculated weight loss was averaged over the entire sample group to determine mean weight loss in milligrams. From the mean weight loss, the percent lost was calculated using $\frac{\text{mass lost}}{\text{total mass}} \times 100$, where *total mass* is the mass of water initially in the filled tube. Graphical representations of evaporation over the 30-day time period were determined by averaging actual measured weights for all tubes at each time point.

Results and discussion

Freeze/Thaw Cycling

Tubes that were capped with a TPV gasket closure showed very few failures during freeze/thaw testing in comparison to those with silicone gaskets. In new tubes, only one tube (0.5 mL) of all TPV tubes tested showed any leakage. On further examination, the failure was caused by a loose closure, not leakage around a closed gasket. It is unknown whether the closure was not properly torqued prior to testing or if the closure backed off during the freeze/thaw cycle. However, following this failure, the closure was re-tightened and returned to testing with no subsequent leaks. In 0.5 mL and 1.0 mL tubes with silicone gaskets (and 2 aged tubes with the TPV gasket), all but one of the failures seen were due to breakage of the closure or tube neck during freezing. These failures were noted upon thaw and discarded without leak testing. Such failures occurred due to excessive pressure on the closure, either due to freezing expansion of residual water at the closure or liquid phase nitrogen entering the tube during storage and expanding upon thaw. In the former case, the tubes must be inverted to put water near the gasket during leak testing to make any leaks observable. If water is retained near the threads during freezing cycles, ice expansion could cause the tube or closure to fracture. Under standard use conditions, it is recommended to keep tubes upright, and ensure that liquids are at the bottom of the tube prior to cryostorage. In the latter case, the tubes for this study were submerged in liquid nitrogen in order to maximize stress on the system for testing purposes. If some liquid nitrogen entered tubes during storage, the seal may prevent the gas from escaping during thawing. Trapped nitrogen gas could create enough pressure as it expands to fracture the tube. **For this reason, the tubes tested in this study are not recommended to be submerged in liquid nitrogen, and instead be kept in the vapor phase.**

In 200 µL tubes, none of the TPV gasket tubes failed testing. Several tubes with silicone gaskets (4 new tubes and 1 aged tube) failed leak testing, and in all cases the failure was due to extrusions of the gasket out of the closure mating area. In 1.8 mL tubes, 1 aged tube with TPV gasket failed leak testing with undetermined cause. The closure was re-tightened and returned to testing with no subsequent leaks. Results for all tubes can be seen in Table 1.

	New Tubes		Aged Tubes	
Tube	**TPV**	**Silicone**	**TPV**	**Silicone**
200 µL	0%	13%	0%	3.3%
0.5 mL	1.6%†	50%*	0%	90%*
1.0 mL	0%	0%	3.3%*	10%*
1.8 mL	0%	--	3.3%‡	--

Table1. Percentage of failures in freeze/thaw leak testing for each type of tube. *Nearly all tubes marked failed due to breakage, not seal failure (see paragraph above). †One closure loosened during one cycle; re-tightened with no subsequent failures. ‡One failure observed on one freeze/thaw cycle; closure re-tightened with no subsequent failures.

Evaporation

The limited gas permeability of TPV makes it a superior gasket material to silicone rubber in terms of preventing room-temperature evaporation. In all three tubes tested with both silicone and TPV gaskets, the TPV gaskets retained a significantly higher percentage of the sample than silicone gasket tubes (Student's T-test, $p<0.01$). See Table 2 and Figures 1 through 4 for results. It is important to note that the evaporation test followed a freeze/thaw cycle to stress the seal, but was conducted at room temperature to speed sample evaporation. Similar evaporation in silicone gasket tubes would not be expected during cryostorage due to both the properties of evaporation at cryostorage temperatures and the material properties of the gaskets at these temperatures.

It is also important to note the possibility that not all of the sample lost from each tube evaporated through the closure. All plastic resins, including the polypropylene these tubes are constructed of, are permeable to gases at some level. While this permeability is limited, some evaporation should be expected even with a perfectly sealed closure. The goal of the closure seal is to limit this as much as possible.

		New Tubes		Aged Tubes	
Tube	**Gasket**	**Average Calculated Weight Loss (mg)**	**Average Percent Sample Loss**	**Average Calculated Weight Loss (mg)**	**Average Percent Sample Loss**
200 µL	**TPV**	1.86	0.93%	0.86	0.43%
	Silicone	14.57	7.28%	13.60	6.80%
0.5 mL	**TPV**	0.30	0.06%	1.47	0.29%
	Silicone	16.20	3.24%	15.30	3.06%
1.0 mL	**TPV**	0.77	0.08%	1.47	0.15%
	Silicone	14.20	1.42%	10.20	1.02%
1.8 mL	**TPV**	0.50	0.03%	1.96	0.11%

Table 2. Sample evaporation (by weight) over 30 days.

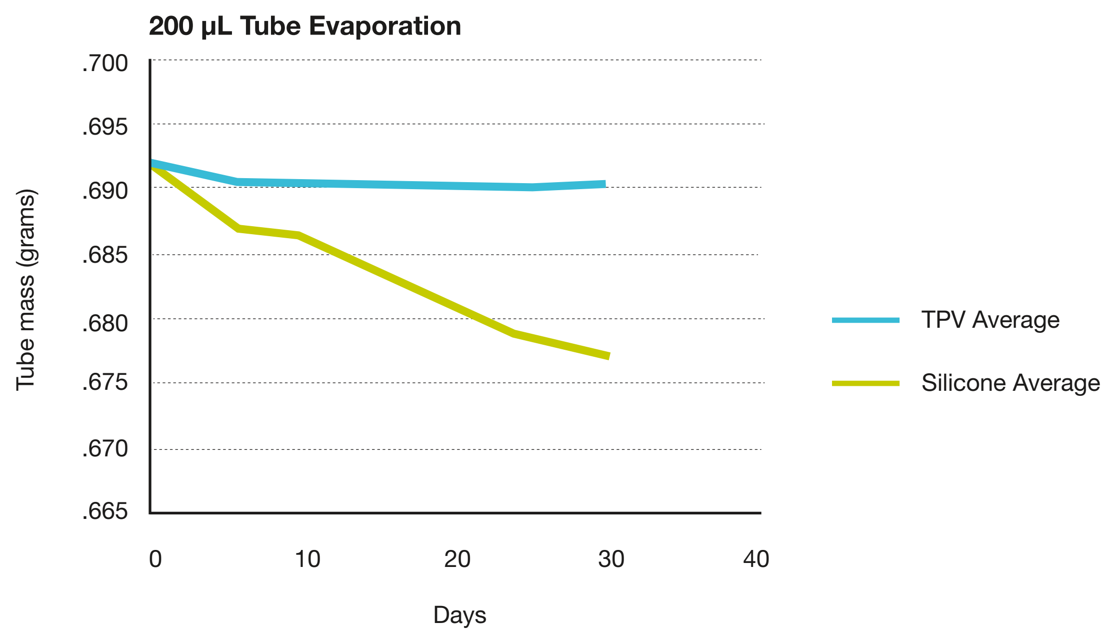

Figure 1.
Sample evaporation (by mass) in 200 µL tubes over 30 days at room temperature.

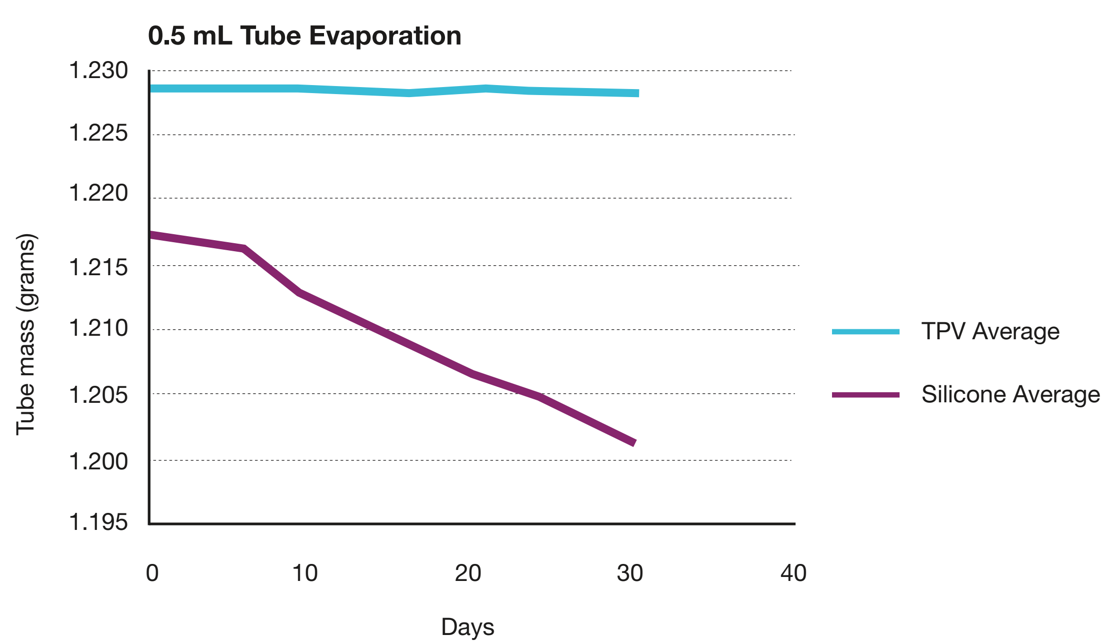

Figure 2.
Sample evaporation (by mass) in 0.5 mL tubes over 30 days at room temperature. Tube mass represents the mass of the tube, closure, and contents; increased TPV mass at Day 0 is due to greater mass of closure.

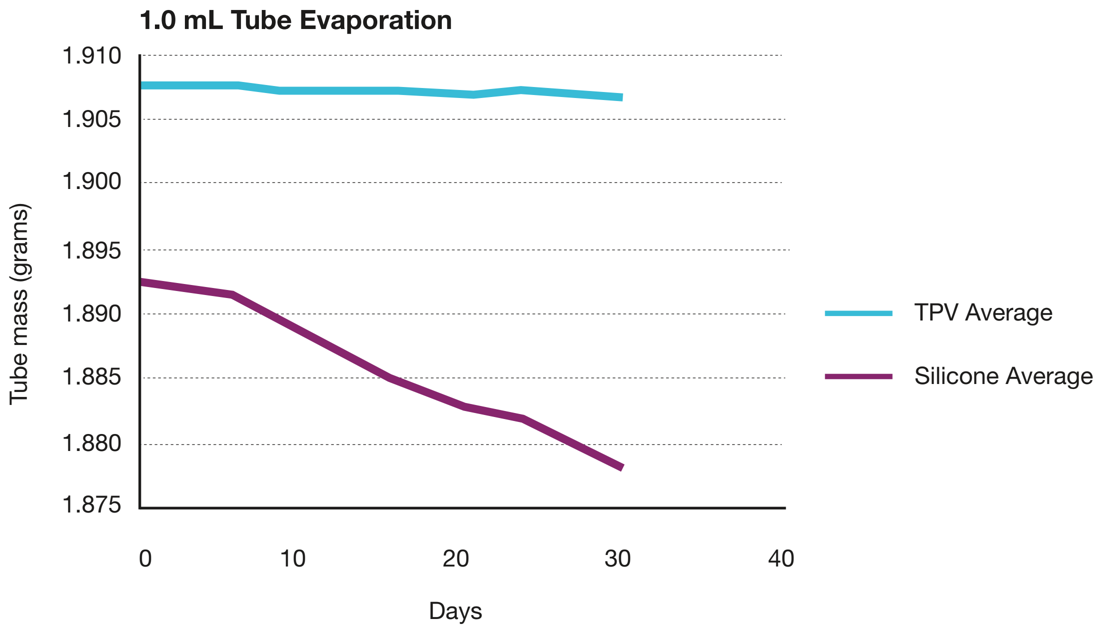

Figure 3.
Sample evaporation (by mass) in 1.0 mL tubes over 30 days at room temperature. Tube mass represents the mass of the tube, closure, and contents; increased TPV mass at Day 0 is due to greater mass of closure.

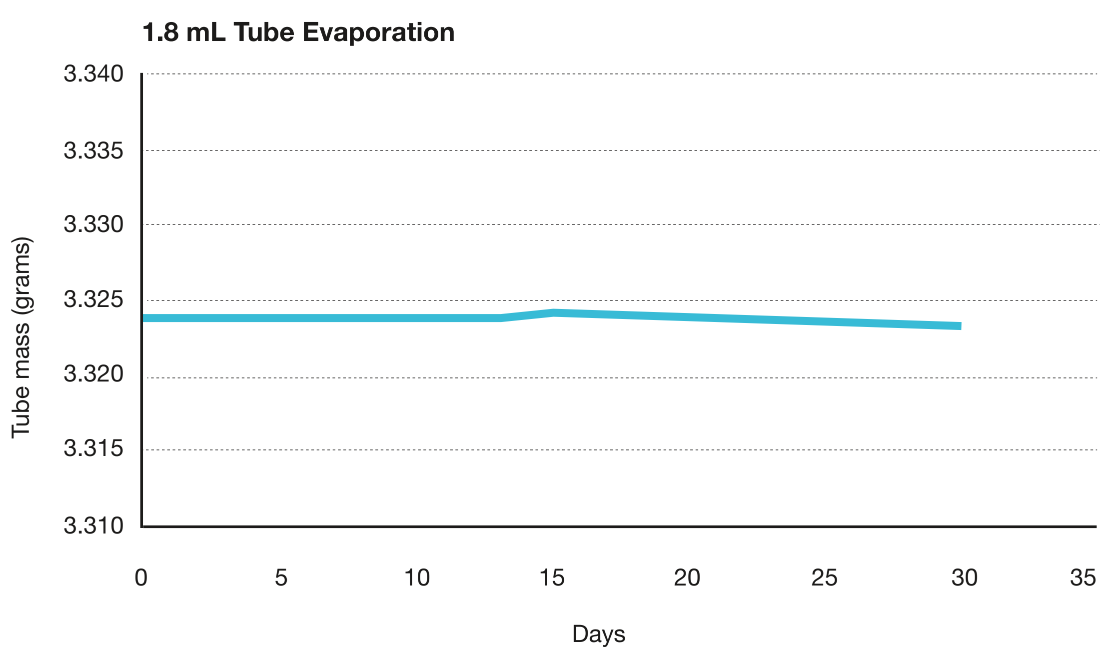

Figure 4.
Sample evaporation (by mass) in 1.8 mL tubes over 30 days at room temperature.

Conclusion

- Molded-on design of TPV gaskets prevents leaks due to gasket extrusion from overtightening and other causes.
- The lower gas permeability of TPV prevents sample loss from evaporation at room temperature.

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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