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Container Closure Integrity Issues in Cell & Gene Therapy Products

In recent years, the development of new modalities, cell and gene therapy (CGT) products, has been accelerated. CGT products are innovative therapeutic products that use cells and genes as "drugs" to cure diseases that are difficult to be treated with conventional drugs. It is expected to not only suppress symptoms but also cure the causes of genetic diseases and intractable cancers from the root and greatly contribute to improving the quality of life of patients. Cell therapy products process and administer living cells to restore lost function and are also known as part of regenerative medicine. Gene therapy products administer normal genes (or "vectors" that carry genes) directly into body to compensate for abnormal genes or suppress their function. Hybrid cell and gene therapy products are a method of genetically modifying cells taken from a patient's body and returning them to the body (e.g., CAR-T cell therapy for cancer treatment).

The CGT product market is expected to grow at a very high rate of around 20-30% per year over the next 10 years. Increasing number of approvals, expansion of target diseases, accelerated investment and innovation, and advent of aging population are the key factors for the growth.

Cell and gene therapy products (CGT) are "living drugs" and require much stricter ultra-low and cryogenic temperature control than the conventional drugs. The table below shows an example of the storage temperature, shelf life, thawing conditions, and storage conditions after thawing of CGT products approved in Europe and the United States. Cell therapy products are mainly stored at cryogenic temperatures in the liquid nitrogen vapor phase (below -150C). Dry shippers are mainly used for transportation and are temporarily stored with dry ice in some cases. Gene therapy products are mainly stored at ultra-low temperatures (below -60C) and transported with dry ice. The CGT products are stored and transported in harsh environments, so choice of appropriate containers is crucial.

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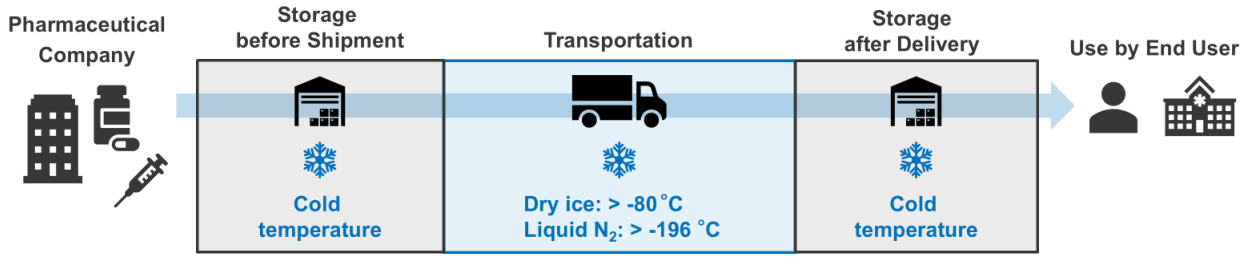
Typical Storage Condition of CGT Products

*Source of information:
www.fda.gov or www.ema.europa.eu

Product	Company	Category	Fill Volume	Primary Container	Shelf Life	Storage/Transportation Condition before Thawing	Thawing Condition	Storage Condition after Thawing	pH
		GT	8mL	COP 10mL Vial	36M	≤ -60°C	MAX 10 hours at RT (up to 25°C)	up to 3 days at 2 to 8°C	6.9 to 7.8
		GT	5.5 or 8.3mL	COP 10mL Vial	24M	≤ -60°C (transportation) 14 days at 2 to 8°C (after arrival)	12 or 16 hours at 2 to 8°C 4 or 6 hours at 20 to 25°C	8 hours	8.0
		GT	10mL	COP 10mL Vial	12M	≤ -60°C (transportation) 14 days at 2 to 8°C (after arrival)	2 hours at RT (up to 25°C)	24 hours at RT (up to 25°C)	8.0±0.3
		GT	0.5mL	COP 2mL Vial	36M	≤-65 °C	?	Less than 4 hours at RT (below 25°C)	7.3
		GT	20mL	Glass 20mL Vial	18M	≤ -60°C	4 to 5 hours at up to 8°C 3 to 5 hours at up to 25°C	24 hours at RT 7 days at 2 to 8°C	?
		GT	0.5mL	Glass 2mL Vial	?	≤-65 °C	15 min at RT	6 hours at RT	?
		GT	1mL	Plastic Vial	36M	-100 to -60°C	1 hour at 15 to 30°C	24 hours at 2 to 8°C	6.8 to 7.8
		GT	1mL	COP Vial	48M	-90 to -70°C	30 to 70 min at 20 to 25°C	7 days at 2 to 8°C 24 hours at RT	7.4
		CT	1.5 to 20 mL	COC Vial	24M	≤ -135°C	10 to 15 min. at 37°C using water bath	Within 20 min.	?
		CT	4.6mL	Cryo Vial	13M	≤ -130°C	15 to 25 min	2 hours at 15 to 25°C	?
		CT	3.8mL	COP Vial	5Y	≤ -135°C	5-8min at 37°C	5 hours	?

Typical Storage & Transportation Condition of CGT Products



Risks at Deep Cold / Cryogenic Temperature

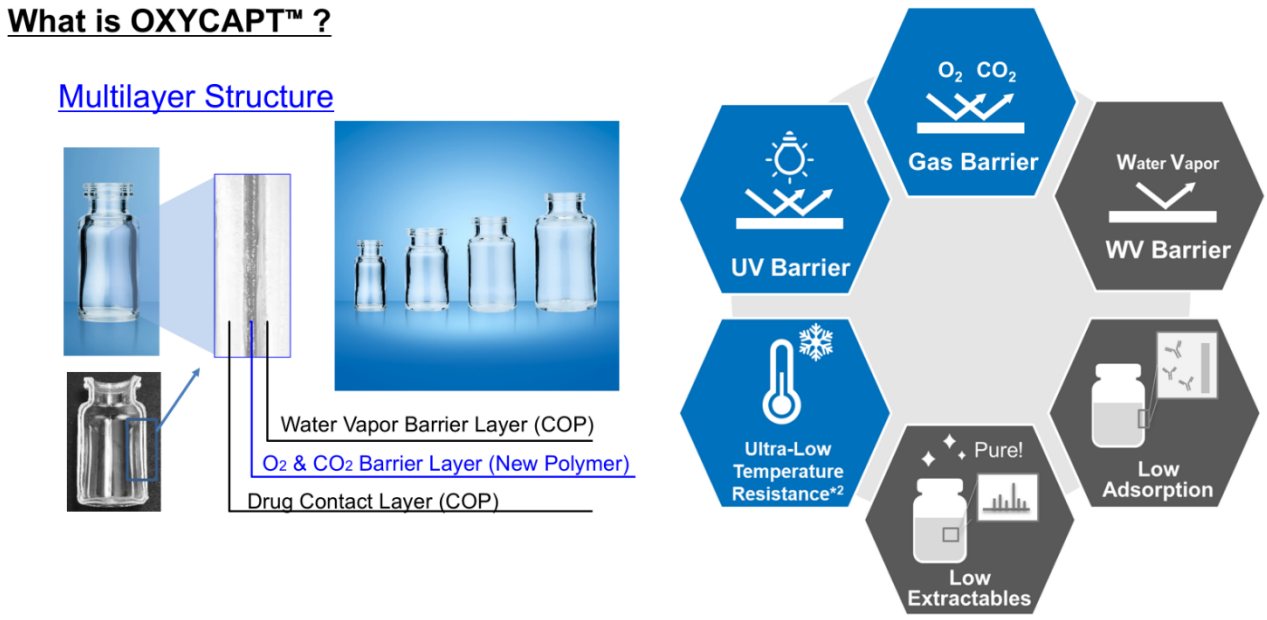
- ✓ Impact- & Temperature-induced breakage
- ✓ Loss of container closure integrity (CCI)
- ✓ CO_2 permeation after dry ice storage
- ✓ Drug destabilization due to pH shift

Conventional small molecule drugs and biopharmaceuticals have mainly adopted glass containers. Glass containers have excellent heat resistance, do not allow gas and water vapor to permeate, and have optimal characteristics for drugs terminally sterilized and stored at low to room temperatures. However, under freezing conditions, the pharmaceutical companies tend to refrain from using them due to cracking issues by drug

expansion and heat cycle stress. In addition, since the coefficient of thermal expansion (CTE) of glass and rubber is about 10 times different, which creates small gaps between them at freezing temperatures, failure of container closure integrity (CCI) has been reported.

As an alternative to glass containers, cycloolefin polymer (COP) or cycloolefin copolymer (COC) containers are mainly used for the CGT products stored at ultra-low to cryogenic temperatures. Although COP and COC have excellent freeze resistance and a similar coefficient of thermal expansion (CTE) with rubber, and generally overcome the drawbacks of glass, they have a disadvantage of poor gas barrier properties. For this reason, it has been confirmed that carbon dioxide generated during dry ice storage dissolves into the polymers and diffuses into CGT products during the thawing process. This can cause a pH shift in the drug and prevent it from providing its intended potency.

Under these circumstances, we have developed OXYCAPT multilayer plastic vial made of COP and high gas-barrier polymer. Since the drug-contact layer and the outer layer are composed of COP, it has excellent characteristics derived from COP, such as low extractables, breakage resistance, and low protein adsorption, while the gas barrier polymer in the middle layer prevents the diffusion of carbon dioxide and pH shift.



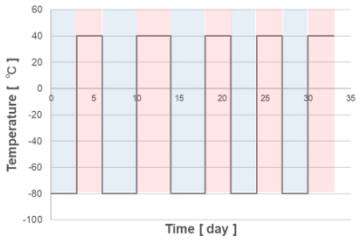
Our experimental data is shown below. First, to compare the breakage resistance of OXYCAPT and glass, we conducted a heat cycle stress test using mannitol solutions, which are frequently used in cell and gene therapy (CGT) products, as an osmotic adjuster. Aqueous mannitol solutions are known to expand

significantly when frozen, which can damage the container. Each 10 mL vial was filled with 8 mL of mannitol solutions and stored at -80°C and +40°C five times before being visually inspected for cracks and leaks. While 5 out of 20 glass vials broke in the first cycle and all 20 vials broke in the fourth cycle, OXYCAPT maintained a clean appearance with no cracks and leaks even after the fifth cycles.

Resistance to Heat Cycle Stress | Using Mannitol aq.

<Procedure>

1. 10mL OXYCAPT and conventional glass vials were filled with 8 mL of 15 w/v% aqueous mannitol solution.
2. Each vial was stored at -80℃ and 40℃ for 3 or 4 days.
3. Each vial was inspected visually by naked eyes at each time point.
4. These steps were repeated 5 times to evaluate heat cycle stress.



<Result>

Vial	Total Number of Breakage					Total Number of Layer-Separation				
	Cycle1	Cycle2	Cycle3	Cycle4	Cycle5	Cycle1	Cycle2	Cycle3	Cycle4	Cycle5
OXYCAPT™-P V10 RT	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
Competitor's Glass Vial(10mL)	5/20	10/20	14/20	20/20	-	N/A				

Broken Glass Vial

OXYCAPT Vial after 5 cycles

Third, we conducted a container closure integrity (CCI) test using OXYCAPT and COP vials for dry-ice transportation. Each 10 mL vial was crimped with rubber stopper and aluminum cap in a nitrogen chamber and stored with dry ice for 7 days before being stored at 23°C and 5°C for the thawing process. The carbon dioxide concentration in both vials was 0% immediately after removal from dry ice storage, but that in the COP vials gradually increased, rising to a concentration of more than 1. 0 % after a few days. On the other hand, the carbon dioxide concentration in the OXYCAPT vial did not rise for 30 days, confirming the high gas barrier properties of OXYCAPT. Under similar conditions, a pH shift test using ion-exchange water was also performed. The pH in the OXYCAPT vial did not change after dry ice storage, while the pH in the COP vial decreased by more than 1.0 after 1 day and by more than 1.5 after 4 days.

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CO_2 Permeation after Freeze & Thaw Process | Sample & Procedure

Sample

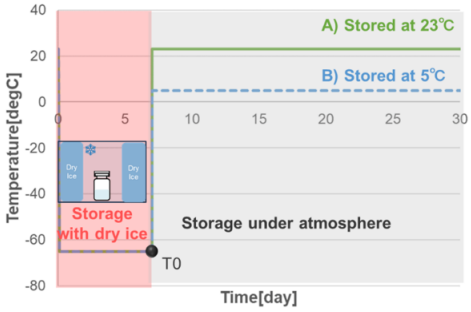
Entry	Vial	Closure	Quantity (For each conditions)
1	OXYCAPT-P (10mL)	V9025, DW* + Aluminum Cap	5
2	Commercially available COP (10mL)	V9025, DW* + Aluminum Cap	5

*The rubber closure was made of bromo-butyl rubber without coating.

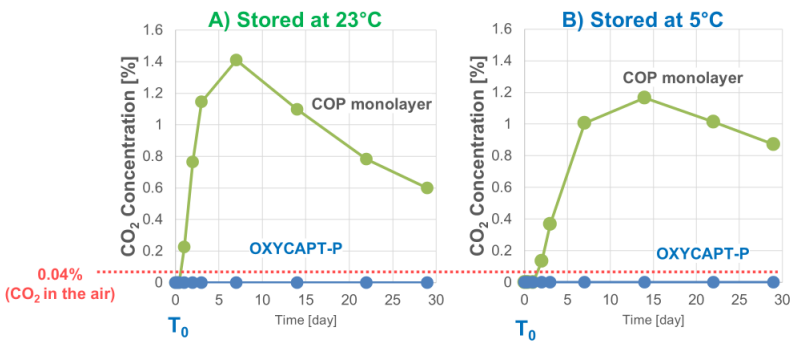
Procedure

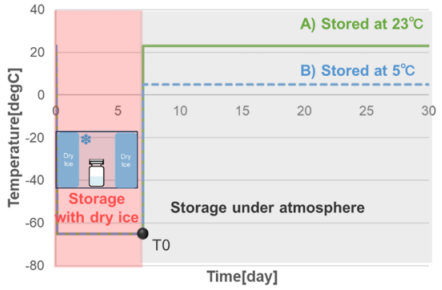
Each vial was;

1. filled with N_2 in a chamber.
2. sealed with the closures and aluminum cap by an auto-crimper.
3. put into insulation box with dry ice and stored for 7 days.
4. taken out to the atmosphere and initial CO_2 concentration was measured (**T0**).
5. stored under condition A (at 23℃) or B (at 5℃).
6. CO_2 concentration was measured at each measurement point.



CO_2 Permeation after Freeze & Thaw Process | Results





- ✓ CO_2 concentration at T0 was 0% in both COP and OXYCAPT vials.
- ✓ However, **CO_2 permeation was observed in the COP vials after 1 day at 23°C and 2 days at 5°C even in the atmospheric condition(CO2≒0.04%),**
- ✓ On the other hand, **no CO_2 permeation was observed in OXYCAPT vials** for 22 days at both 23 and 5℃.

Mitsubishi Gas Chemical Company, Inc. | Mitsubishi Building, 5-2 Marunouchi 2, Chiyoda-ku | Tokyo 100-8324, Japan
T: +81 3 3283 4913 | W: https://www.mgc.co.jp/eng/products/abd/oxycapt.html

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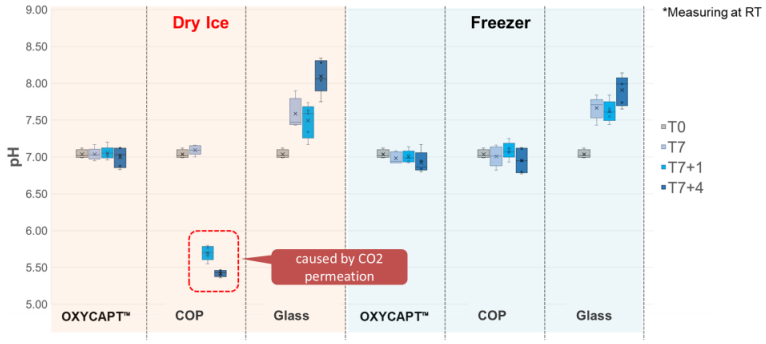
CO_2 Permeation after Freeze & Thaw Process | pH Stability

◆ Result

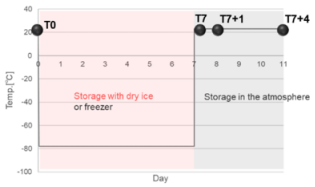


✓ No pH shift was observed in OXYCAPT vials even after dry-ice storage.

✓ pH shift was observed in COP monolayer vials after dry-ice storage because dissolved CO2 during dry-ice storage permeates into headspace.

■ **T0**: Before dry ice or freezer storage
■ **T7**: After 7days storage and thawing*
■ **T7+1**: After thawing, 1 day at 23°C under atmosphere
■ **T7+4**: After thawing, 4 days at 23°C under atmosphere

*T7 was measured 8 hours after thawing at 23°C .



◆ Materials
- Vial: 10mL COP Monolayer, OXYCAPT™, Glass
- Rubber closure: V9025, DW sealed with Al Cap
- Sample Number: 5

◆ Test Conditions
- 5 mL of deionized water treated to remove residual carbon dioxide (CO_2).
- Filled and sealed under 100% N_2 environment
- pH meter: pH-22B, HORIBA (Japan)
- Storage: Deep freezer (-80 °C) or in dry ice

The gas permeability of polymer is determined by the combination of dissolution and diffusion. Carbon dioxide is known as an adherent gas and has a higher solubility when frozen than at room temperature, but no permeation of carbon dioxide in the vial was observed because it does not diffuse under freezing. On the other hand, when the temperature was returned from freezing to low or room temperatures, the diffusion started rapidly, and the carbon dioxide dissolved in the COP permeated and the concentration in the vial increased. As OXYCAPT has a high gas barrier layer, it prevents the diffusion of carbon dioxide and the associated pH shift during storage at low to room temperatures.

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Theory of CO_2 Permeation | Steps of Gas Permeation

2 phenomena of gas molecule permeation through plastics:

1. **Dissolution**
2. **Diffusion**

Permeation Scheme

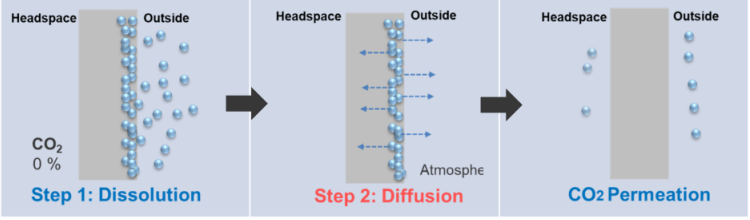
$$P = P_0 \exp(-\frac{E_P}{RT})$$

T: Temperature

$$P = S \cdot D$$

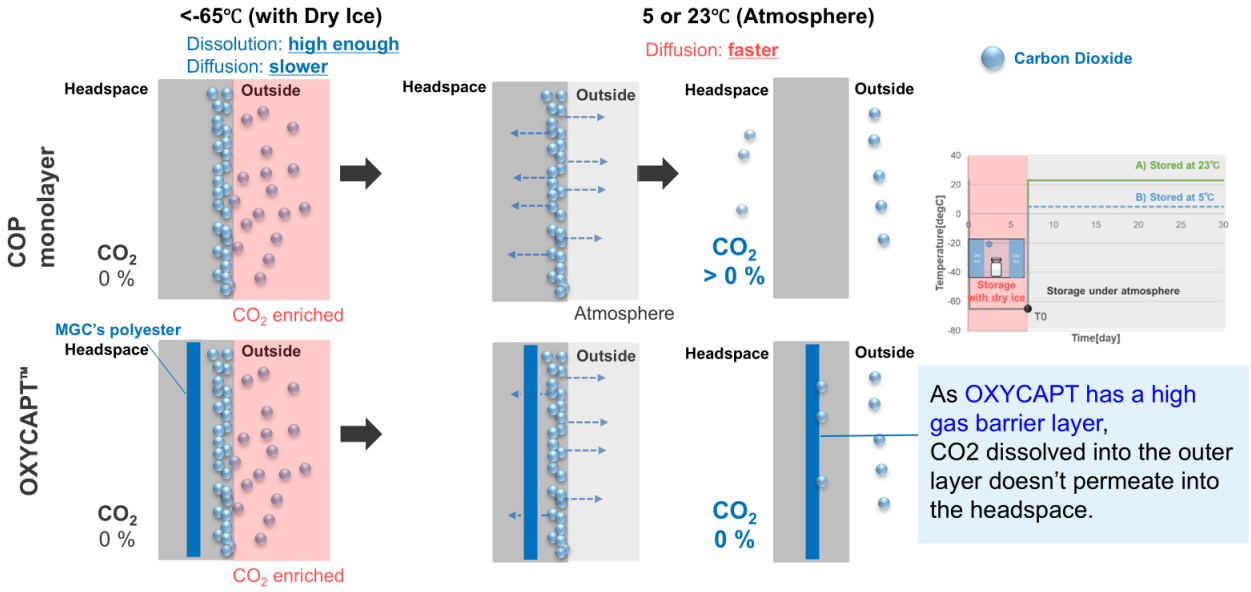
S: Solubility coefficient
D: Diffusion coefficient
P: Permeability coefficient

	-80℃ (Storage)	23℃ (Thawing)	23℃ (Before Injection)
Dissolution	Higher	High	
Diffusion	Slower	Faster	



- Diffusion speed at -80℃ is much slower but dissolution coefficient is high.
- CO2 dissolved into polymer at -80℃ permeates into headspace during thawing process.

Theory of CO_2 Permeation | Comparison between OXCAPT & COP



As OXYCAPT has a high gas barrier layer, CO2 dissolved into the outer layer doesn't permeate into the headspace.

Finally, we conducted a container closure integrity (CCI) test using OXYCAPT and glass vials in liquid nitrogen vapor phase. Since we had obtained information that the test results differ depending on the type of residual seal force (RSF) and rubber stoppers through prior hearings and surveys, we prepared samples with multiple residual seal forces (10 to 160N) and conducted CCI tests by combining them with two types of rubber stoppers (non-coated or ETFE coated).

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Each 10 mL vial was crimped with a rubber stopper and aluminum cap under the atmosphere and stored in the liquid nitrogen vapor phase for 7 to 30 days, after which the oxygen concentration in the vial was measured at room temperature.

In the samples using non-coated rubber stoppers, we confirmed that glass vials even with very strong RSF (70~100 N) could not maintain CCI, while OXYCAPT vials with normal RSF (40~70 N) could maintain CCI. In the samples using ETFE-coated rubber stoppers, glass vials showed a significant decrease in oxygen concentration and OXYCAPT vials also showed a slight decrease, even though they had a normal (40~70N) to high (80~160N) RSF.

CCIT in Liquid N_2 | How combinations of vial, closures and RSF affect CCI ?

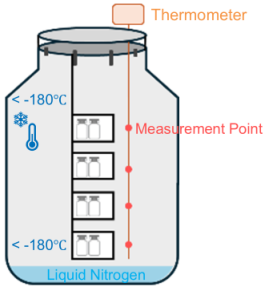
Vial
- **10R Glass Vial**, Competitor S
- **OXYCAPT-P V10**
- OXYCAPT-P V10 Positive Control[1)]

Closure
- **Non-Coated**, V9025 Datwyler
- **Full-Coated**, Supplier B

RSF (Residual Seal Force)
- Range from 10 to 160 N

1. All the vials were sealed with aluminum caps[2)] using the hand-held automatic capping machine under atmosphere (O_2 : 20.9%) .
2. Initial O_2 % was measured.
3. All the vials were placed into separate compartments.
4. The compartments were then placed in a LN_2 vessel as shown in the right figure.
5. After storage for 7 days and 30 days, the vials were taken out from the compartments.
6. The vials were kept at room temp until the internal temperature returned to room temperature, and then the O_2% in the headspace of the vials was measured with FMS-760.

1) Vial with a 5μm fine hole on the side made by Lenox Lazer
2) Aluminum cap : CTO020-101, Datwyler



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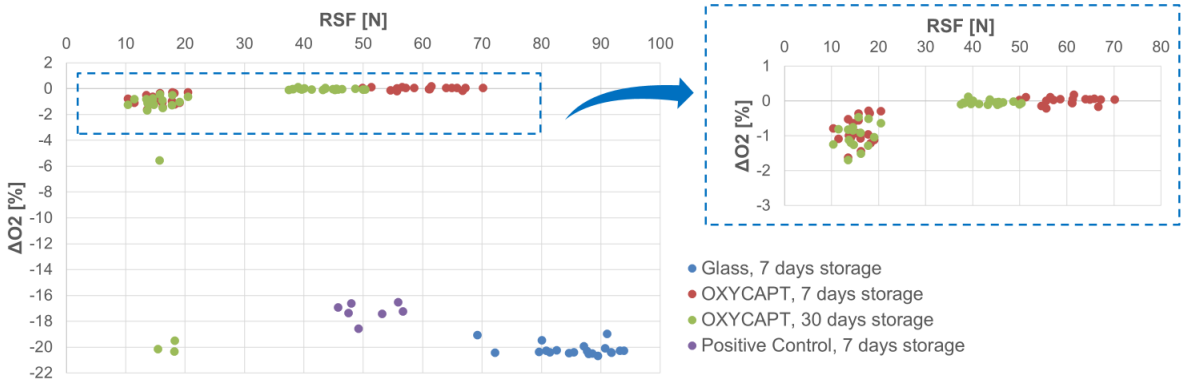
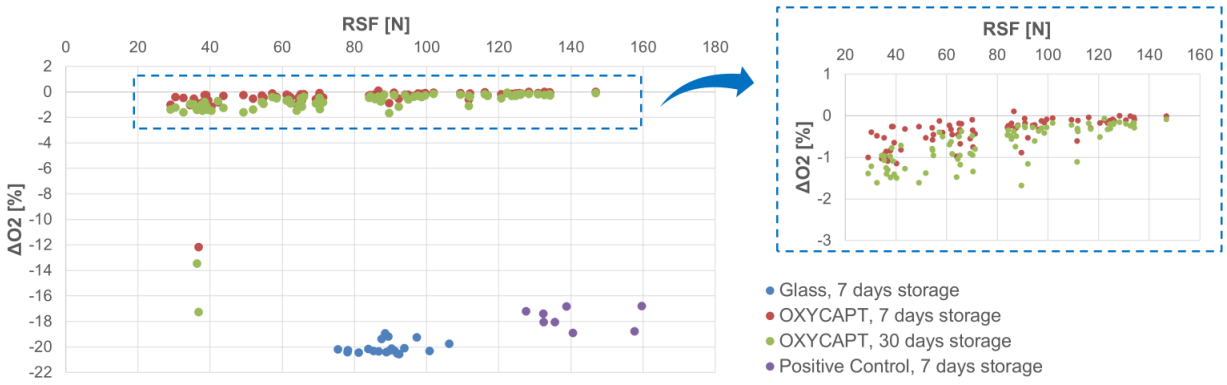
CCI in LN_2 Vapor Phase | Result of Non-Coated Stopper



- Alls glass vials could not maintain the initial O_2% even at sufficiently high RSF(70–100N), resulting in loss of CCI.
- OXYCAPT with lower RSF(10–20N): O2 concentration significantly decreased in some samples and slightly decreased in all other samples.
- OXYCAPT with moderate RSF(40–70N): OXYCAPT could maintain the initial O_2% for 7 and 30 days.

CCI in LN_2 Vapor Phase | Result of Full-Coated Stopper



- All glass vials could not maintain the initial O_2% even at sufficiently high RSF(70–110N), resulting in loss of CCI.
- OXYCAPT with lower RSF (below 40N): O2 concentration significantly decreased in some samples.
- OXYCAPT with moderate and higher RSF: O2 concentration slightly decreased in almost all samples.
- The decrease rate was larger at lower RSF and longer stored samples.

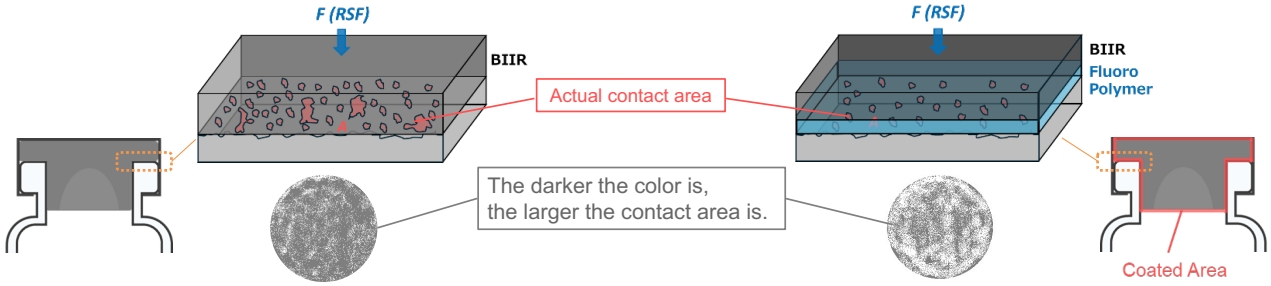
The coefficient of thermal expansion (CTE) of bromobutyl rubber (BBR) is similar to that of COP and OXYCAPT vials, but there is a large difference in the Young's modulus (E) between BBR and ETFE (about 5 to 600 at room temperature), and the difference is even greater at ultra-low to cryogenic temperatures. We believe that a small amount of nitrogen permeated into the vial because ETFE could not completely seal the microscopic surface gaps between the BBR and the flange top of COP & OXYCAPT vial. This is attributed to

the high Young's modulus of ETFE and its insufficient conformability to the microscopic surface gaps. We presume that the same result as the uncoated stopper will be obtained in the half-coated stopper, because its contact area to the vial is composed of BBR with very low Young's modulus (E).

CCI in LN_2 Vapor Phase | Conclusion

- BBR's coefficient of thermal expansion (CTE): is similar to COP and a factor of 10 bigger than glass.
- Young's Modulus (E): ETFE: approx. 600 MPa[1], BBR: approx. 5 MPa[2] at room temperature(RT).
  Much bigger at ultra-low & cryogenic temperature than RT.
- Invisible microscopic-gaps between rubber and vial flange:
  to fill invisible microscopic-gaps and create wider contact areas, softer & more flexible material is required.
- Conclusion: glass vials and ETFE full-coated stoppers are not suitable for CGT products stored at ultra-low or cryogenic temperatures due to their CTE and stiffness.

1) https://tefcap.com/etfe-properties-fluoropolymer-tubing/
2) https://astlettrubber.com/pdf/sr/biir.pdf



As mentioned above, in recent years, the development of new modalities, cell and gene therapy (CGT) products, has been accelerated. Since the CGT products are "living drugs", they require much stricter ultra-low or cryogenic temperature management than the conventional drugs. Conventional glass vials have problems with cracking due to drug expansion under freezing and heat cycle stress. Also, since the coefficient of thermal expansion (CTE) of glass and rubber is about 10 times different, which creates small gaps between them at freezing temperatures, failure of container closure integrity (CCI) has been reported. On the other hand, cycloolefin polymer (COP) and cycloolefin copolymer (COC) containers used in the CGT products, as an alternative to glass, have the disadvantage of poor gas barrier properties. It has been confirmed that carbon dioxide generated during dry ice storage dissolves into polymers and diffuses into CGT products during the thawing process, causing pH shift.

OXYCAPT developed by our company is a multi-layer plastic vial made of COP and high gas barrier polymer. Since the drug-contact and outer layers are composed of COP, it possesses excellent characteristics of COP, such as low extractables, breakage resistance, and low protein adsorption, while the gas barrier polymer in the middle layer prevents the diffusion of carbon dioxide and the pH shift of CGT products. OXYCAPT is a unique vial that can contribute to the shelf-stability of CGT products stored in very harsh environments.