Vial Containment Systems for Gene Therapies

Margaret A. Craig
Manager, Scientific Communications

Rui Li, Ph.D.
Senior Scientist, R&D, Cryobiology

Vidya Murthy, Ph.D.
Director, Strategic Marketing, Advanced Therapies

T. Page McAndrew, Ph.D.
Director, Scientific Communications

CHAPTER 1

Introduction

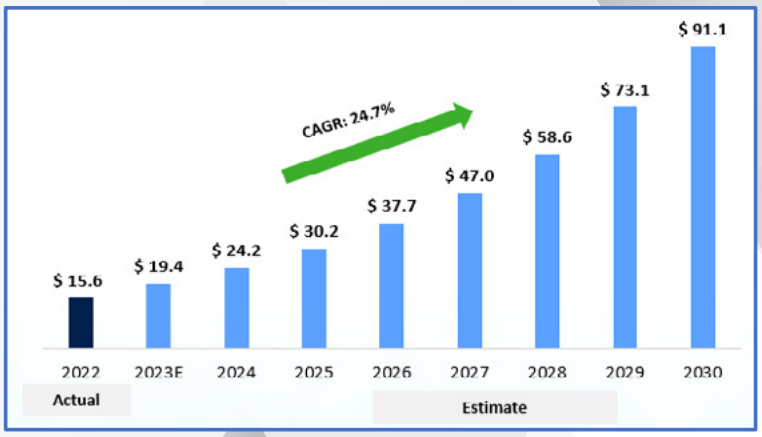
Gene therapy is an approach to treat or cure selected cancers, genetic diseases, and infectious diseases by addressing the underlying genetic causes. It operates by the modification and/or manipulation of biological properties of living cells via:[1]

- replacing a disease-causing gene with a healthy copy
- inactivating a disease-causing gene
- introducing a new or modified gene

One of the principal ways in which gene therapies are delivered is through viral vectors. Viral vectors are viruses that can efficiently deliver genetic material into the cells they infect – a process known as *transduction*.[2]

Development in this area is increasing rapidly. The combined gene and cell therapy markets are expected to grow at a combined annual growth rate (CAGR) of approximately 25% from 2022 to 2030 – reaching approximately $90 billion in sales.[3] See Figure 1 for anticipated trends.

FIGURE 1 *Growth of Cell and Gene Therapies. Dollar values in billions. Data past 2022 are estimates.*



This growth is driven by:[4]

- approximately 1,400 companies involved world-wide
- approximately 2,220 clinical trials underway (254 new in 2022, 202 in Phase 3)

Leading companies and the number of their therapies in the pipeline are:[5]

- 64 Moderna, Inc.
- 47 Alnylam Pharmaceuticals, Inc.
- 37 Novartis AG
- 35 Pfizer Inc.
- 34 Bristol-Myers Squibb
- 31 Hoffmann-La Roche AG
- 29 Sarepta Therapeutics, Inc.
- 22 Johnson & Johnson
- 22 CSIPR Therapeutics AG
- 21 Sangamo Therapeutics, Inc.

Factors enabling this growth are that these therapies often treat diseases that are rare, have very high unmet needs, or have limited treatment options. As such, they often receive accelerated pathway designations such as *Fast Track* or *Breakthrough*, leading to fast development cycles and expedited regulatory approvals. For example, on average, *Breakthrough* drug products have around two years shorter pre-market development times and are approved about three months ahead of the *Prescription Drug User Fee Act* (PDUFA) goal date.

With advances in gene editing and viral vector manufacturing, the FDA projects 10-20 approvals per year starting in 2025 and 30 approvals per year by 2030.[6] This rate is 10-fold higher than in 2017. As in any area, there are also obstacles to growth. The primary one is cost. This results in inaccessibility for those not insured or without financing solutions.

At present, 12 gene therapies have been approved or are projected to be approved by the US Food and Drug Administration and/or the European Medicines Agency.[7] See the Table for details.

TABLE *Approved Gene Therapies*

Product (Viral Vector*)	Company	Indication	FDA Approval
IMYLGIC® (HSV - 1)	Amgen, Inc.	nodal lesions in melanoma patients post-surgery	2016
LUXTURNA® (AAV – 2)	Spark Therapeutics, Inc.	retinal dystrophy	2017
ZOLGENSMA® (AAV-9)	Novartis AG	spinal muscular atrophy	2019
ADSTILADRIN® (AAV)	Ferring B.V.	Bacillus Calmette-Guerin (BCG) unresponsive non-muscle invasive bladder cancer	2022
HEMGENIX® (AAV-5)	CSL Behring LLC	hemophilia B	2022
UPSTAZATM (AAV)	PTC Therapeutics, Inc.	severe aromatic L-amino acid decarboxylase (AADC) deficiency	2022
ZYNTEGLO® (lentivirus)	Bluebird Bio, Inc.	adult and pediatric ß-thalassemia	2022
ROCTAVIANTM (AAV-5)	BioMarin Pharmaceuticals, Inc.	hemophilia A	2022-EMA 2023
ELEVIDYS® (AAV)	Sarepta Therapeutics, Inc.	Duchenne muscular dystrophy	2023
VYJUVEKTM (HSV-1)	Krystal Biotech, Inc.	dystrophic epidermolysis bullosa	2023
SPK-8011 (AAV-LK03)	Spark Therapeutics, Inc.	hemophilia A	estimated 2023/24
SB-525 (AAV-6)	Sangamo Therapeutics, Inc. and Pfizer Inc.	hemophilia A	estimated 2023/24

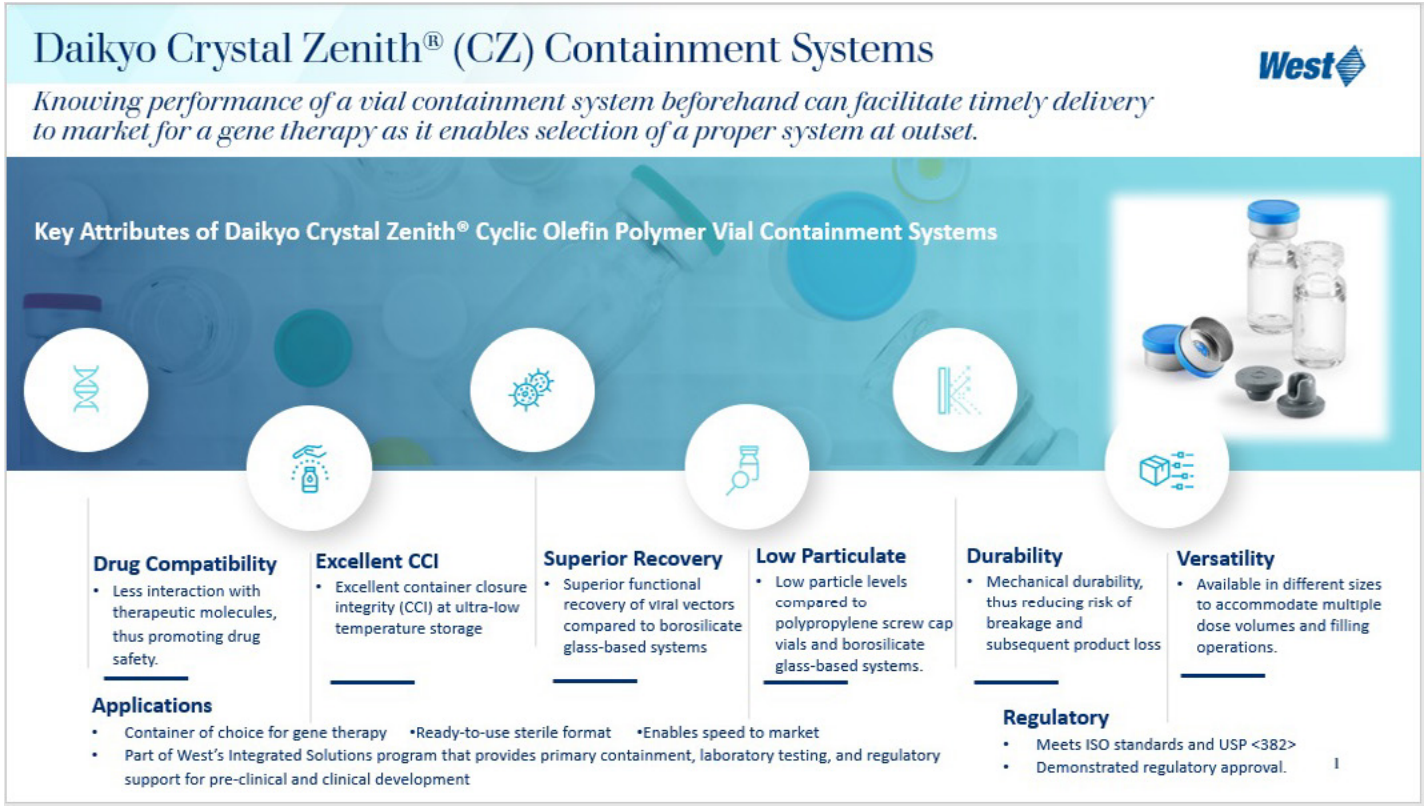
It is essential that the viral-vector-based gene therapy be delivered without loss of efficacy to the clinician for administration to the patient – a point amplified by the cost. This involves, *inter alia*, minimizing risk of loss resultant from typical storage/transport at ultra-low temperature (approximately -80°C or vapor of dry ice at -78°C). To achieve this, selection of the proper vial containment system is essential. *This book discusses the utility of vial containment systems comprising components from West Pharmaceutical Services, Inc. and Daikyo-Seiko, Ltd. and how said systems may minimize risk of loss of efficacy of a gene therapy, vis-à-vis borosilicate glass-based and polypropylene-based containment systems.*

Components examined were:

- Daikyo Seiko Crystal Zenith® cyclic olefin polymer (CZ) vials
- West NovaPure® elastomer serum stoppers and Daikyo Seiko elastomer serum stoppers – both with fluoropolymer barrier film facing drug product
- West Flip-Off® seals

Chapters 2-5 present summaries of selected key aspects of research done either by, or under contract for, West. Copies of full reports of said research are embedded. Chapter summaries are given below. In Figure 2 are listed briefly the key points of this book.

FIGURE 2 *Key Points of CZ Containment Systems*



2 CHAPTER 2

Interactions with Glass, Polymer, and Elastomer

Based on studies with proteins, it was demonstrated that less interaction occurs with CZ vials than with borosilicate glass vials. It was demonstrated also that less interaction occurs with FluroTec™ fluoropolymer barrier film-laminated elastomer stoppers than with non-laminated elastomer stoppers. These data strongly suggest less interaction with protein-based materials, such as viral vectors.

3 CHAPTER 3

Materials Considerations

Storage of any vial containment system at ultra-low temperature presents risks not encountered at ambient temperature – resulting from mis-matched components, breakage, over-filling, or improper sealing. Material (thermal expansion, mechanical, particle level) benefits of CZ vials are presented along with discussion of risks of over-filling and improper sealing.

4 CHAPTER 4

Container Closure Integrity

Vial containment systems comprising CZ vials and West NovaPure and Daikyo Seiko, Ltd. elastomer stoppers were evaluated for container closure integrity (CCI) performance over: (a) two years at -80°C and (b) two weeks in a dry ice container (-78°C). Excellent performance was observed. No sample was breached. These data enable judgment of whether the performance meets the maximum allow leakage limit (MALL) of the gene therapy.

5 CHAPTER 5

Viral Vector Viability

Studies were conducted with selected lentivirus vectors, adenovirus vectors, and adeno-associated virus vectors. After storage at -80°C and subsequent thaw, compared to standard borosilicate glass-based and polypropylene-based vial systems, all vectors showed equivalent or better functional recovery with CZ-based vial systems. These data strongly suggest general utility of CZ-based vial systems for preserving viral vector function.

6 CHAPTER 6

Summary

Knowing performance of a vial containment system beforehand can facilitate timely delivery to market for a gene therapy as it enables selection of a proper system at outset. All factors considered, performance of CZ-based vial containment systems exceeds or matches those based on borosilicate glass or polypropylene, and thus, they appear potentially to be an excellent choice for gene therapies.

References

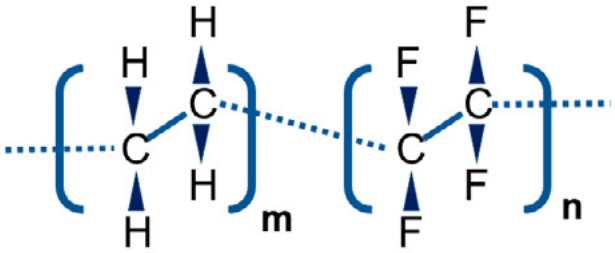
1. (a) https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/what-gene-therapy (accessed 4/10/23) (b) https://patienteducation.asgct.org/gene-therapy-101/gene-therapy-approaches#:~:text=Gene%20Therapy%20Approaches-Gene%20Therapy%20Approaches,RNA%20therapy%20and%20cell%20therapy (accessed 7/26/23)
2. https://www.cancer.gov/publications/dictionaries/cancer-terms/def/viral-vector (accessed 4/10/23)
3. compiled from information available at: https://www.coherentmarketinsights.com/market-insight/cell-and-gene-therapy-market-2475 (accessed 7/26/23)
4. compiled from information available at (all accessed 7/26/23): (a) https://www.fda.gov/drugs/development-approval-process-drugs/drug-approvals-and-databases, (b) http://alliancerm.org/wp-content/uploads/2023/01/SOTI-2023-Slides.pdf (c) https://www2.deloitte.com/content/dam/insights/us/articles/6642-cell-and-gene-therapies/DI_Cell-and-gene-therapies.pdf
5. compiled from information available at pharma circle.com e.g., https://www.pharmacircle.com/info/wp-content/uploads/2022/09/PharmaCircle_Pipeline_Dynamics_2022_05_30_Update.pdf (accessed 7/26/23)
6. https://www.fda.gov/news-events/press-announcements/statement-fda-commissioner-scott-gottlieb-md-and-peter-marks-md-phd-director-center-biologics (accessed 7/26/23)
7. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products (accessed 7-26-23)

Elastomer Stoppers with and without Fluoropolymer Barrier Films

Protein Interaction

Studies with proteins were conducted to evaluate interaction with two different types of elastomer stoppers (both based on bromobutyl elastomer) – bare stoppers and stoppers with a FluroTec™ barrier film laminated onto surfaces that face drug product.[1,2] FluroTec film is based on poly(ethene tetrafluoroethene) (ETFE) (Figure 1) which, like fluoropolymers in general, has low surface energy (~ 25 dyne/cm) and thus low levels of interaction with other compounds as compared to, for example, typical elastomers (~ 42 dyne/cm).[3]

FIGURE 1 *Structure of Poly(ethene tetrafluoroethene) (ETFE)*



Four proteins were evaluated for interaction with elastomer stoppers by placing 3 ml of aqueous solution of protein (1 mg/ml) into 5 ml borosilicate glass vials, capping with 20 mm elastomer stoppers (with and without FluroTec film) and agitating by end-over-end rotation (40 rpm) at room temperature for:

- 2 hours and 6 hours – particle count was measured by dynamic fluid imaging
- 24 hours – protein recovery was measured by size exclusion high performance liquid chromatography; turbidity was measured by light absorbance (350 nm)

Level of interaction was determined by particle count, turbidity, and recovery of protein after said agitation.[1,2] Results are shown in Table 1.

TABLE 1 *Levels of Particles, Turbidity, and Recovery for Selected Proteins Resultant from Interaction with Elastomer Stoppers with and without FluroTec Film. Values for particles are in thousands. Parenthetical numbers are standard deviations.*

FluroTec Film	Particles per ml (1-10 µm)		Turbidity	Recovery
	at 2 hrs	at 6 hrs	at 24 hrs (a)	at 24 hrs (%)
β-Lactoglobulin				
with	24.5 (6.0)	58.2 (25.3)	0.07 (0.005)	99.3 (0.13)
without	87.7 (20.7)	181.7 (29.7)	0.10 (0.006)	98.9 (0.08)
Immunoglobulin				
with	23.5 (7.9)	44.0 (16.0)	0.01 (0.002)	98.2 (0.13)
without	94.6 (28.8)	289.6 (172.2)	0.04 (0.004)	95.6 (0.26)
Abatacept (fusion protein)				
with	12.8 (11.0)	41.0 (11.2)	0.02 (0.001)	99.1 (0.3)
without	14.3 (7.2)	64.2 (29.2)	0.03 (0.005)	97.3 (0.3)
human serum albumin – recovery at 21 days (%) (b)			with	98.6 (0.3)
			without	78.6 (0.4)

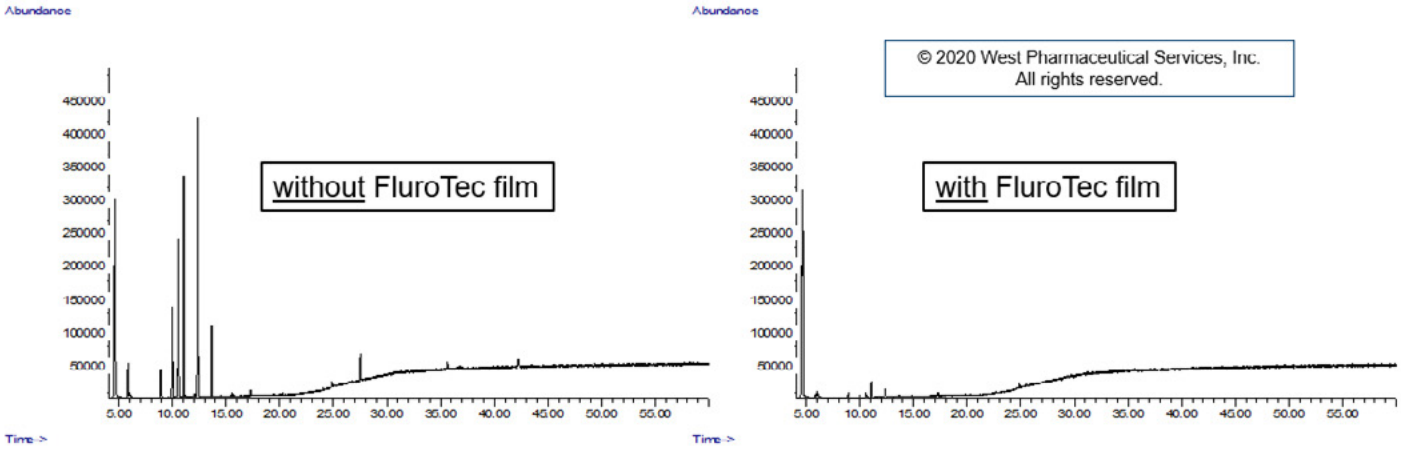
a. absorbance at 350 nm
b. not agitated, stored quiescently at room temperature

In all cases, the use of stoppers with FluroTec film resulted in fewer particles, lower turbidity, and higher protein recovery. That is, stoppers with FluroTec film had less interaction with the proteins tested than stoppers without.

Migration from Elastomer Component

Studies were conducted to evaluate the potential migration of compounds from elastomer components into drug product. This was with the same two types of components just discussed – bromobutyl elastomer components, bare and with FluroTec barrier film laminated onto surfaces that face drug product. Elastomer lined seals, with and without FluroTec film, were crimped onto empty 10 ml borosilicate glass vials and stored up to six months at room temperature.[4] Headspace gas chromatography with mass spectrometry were performed. Results are shown in Figure 2.

FIGURE 2 *Headspace Gas Chromatography with Mass Spectrometry of Elastomer Components. Data are at six months.*



A large number of compounds were observed for the system without FluroTec film, virtually none for the system with FluroTec film. Mitigation of migration was achieved. Stoppers with FluroTec film thus should more effectively protect gene therapies from leachables from elastomer.

Daikyo Seiko Crystal Zenith® Cyclic Olefin Polymer Vials and Borosilicate Glass Vials

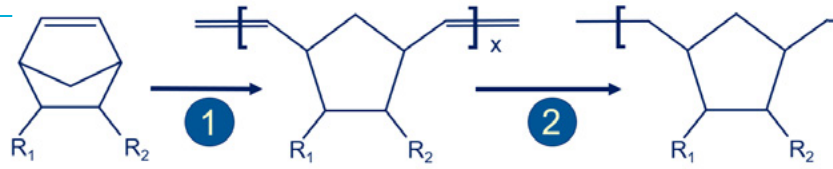
Polymers, in general, have lower surface energies than borosilicate glass:

- borosilicate glass (typical): ~ 250 dyne/cm[3]
- Crytal Zenith cyclic olefin polymer (CZ): ~ 70 dyne/cm[5]

Any compound, in particular a protein-based compound, is more likely to be attracted to, interact with, and adhere to, higher-surface-energy materials. That is, a protein-based compound is more likely to interact with borosilicate glass than CZ.

Synthesis of cyclic olefin polymer, shown in Figure 3, employs the ring opening metathesis polymerization method. This was discovered in the 1970s and was the basis of the 2005 Nobel Prize in chemistry. Synthesis requires two steps, polymerization followed by hydrogenation.[6]

FIGURE 3 *Synthesis of Cyclic Olefin Polymer from Norbornene.*
1. ring-opening metathesis polymerization.
2. hydrogenation



Interactions of proteins with borosilicate glass and CZ were examined.[7,8] Five protein-based compounds were evaluated for level of interaction by placing solutions of them (as received from suppliers replicating simulated potential drug products) into 2 ml vials (CZ and glass), capping with 13 mm elastomer stoppers with FluroTec film, and agitating on an orbital shaker (200 rpm) at room temperature for four days. Particle count was measured by dynamic fluid imaging, protein recovery was measured by size exclusion high performance liquid chromatography, and turbidity was measured by light absorbance (350 nm). Results are shown in Table 2.

TABLE 2 *Levels of Particles, Turbidity, and Recovery for Selected Proteins Resultant from Interaction with Borosilicate Glass and CZ Vials. Values for particles are in thousands.*

	mAb – 1		mAb – 2		mAb – 3		mAb – 4		IgG	
	Glass	CZ	Glass	CZ	Glass	CZ	Glass	CZ	Glass	CZ
Particles (per ml)	36	18	0.8	0.7	325	165	9	1	9	4
Turbidity	1.4	0.4	0.05	0.003	2.4	1.5	0.48	- 0 -	0.21	0.01
Recovery (%)	75	95	90	100	10	68	90	100	90	98

In all cases, CZ vials had fewer particles, lower turbidity, and higher protein recovery. That is, CZ vials had less interaction with proteins than borosilicate glass vials.

CHAPTER 2

Interactions with Glass, Polymer, and Elastomer

1
2
3
4
5
6

Discussion

Lower levels of protein interaction were observed for elastomer stoppers with FluroTec film and CZ vials, as compared to elastomer stoppers without FluroTec film and borosilicate glass vials, respectively. This is to be expected based upon differences in surface energy. Components with lower surface energy will have less interaction. As noted above, ETFE (the basis of FluroTec film) has a lower surface energy than a typical elastomer. And CZ has a lower surface energy than borosilicate glass. Since virus capsids comprise proteins, it may be expected that lentivirus vectors, adenovirus vectors, and adeno-associated virus vectors likewise will have less interaction and thus be more stable in vial-stopper-seal containment systems comprising an elastomer stopper with FluroTec film and a CZ vial. Further, FluroTec film mitigates migration of extractables from elastomer stopper. This should provide an added layer of protection to viral vectors.

The overall result of lower level of interaction and reduced level of extractables is reduced potential risk of: (a) virus adsorption (and dosage reduction), (b) unwanted chemical change, and (c) particle formation resulting in immunogenic effects (these phenomena are reported).[9-14]

References

1. R. Singh, L. Waxman, L. Fang, C. Zhao. *An Investigation to Examine the Effect of the Elastomeric Surface Treatment on Protein Stability.* PDA Journal of Pharmaceutical Science and Technology, 75, 230-244 (2021)
2. R. Singh, W. Garzon-Rodriguez, C. Zhao, P. Frandolig, P. McAndrew. *Elastomer Stoppers with FluroTec® Film: The Right Choice for SARS-CoV-2 Vaccines* (published online – May 22, 2020) **(attached)**
3. https://www.3m.com/3M/en_US/bonding-and-assembly-us/resources/science-of-adhesion/categorizing-surface-energy/ (accessed 4/11/2023) https://www.atlanticgasket.com/3M-adhesion-basics.html (accessed 4/11/2023)
4. *FluroTec® Products Protecting Drug Product Quality and Safety.* West Pharmaceutical Services, Inc. Technical Report 2019/210 (2019) **(attached)**
5. J.Y. Shin, et al. *Chemical Structure and Physical Properties of Cyclic Olefin Polymers.* Pure and Applied Chemistry, 77 (5), 801-814 (2005)
6. Sutthasup, S., et al. *Recent Advances in Ring-Opening Metathesis Polymerization, and Application to Synthesis of Functional Materials.* Polymer Journal, 42, 905-915 (2010)
7. R. Singh and L. Waxman. *Effect of Container Surface on Adsorption and Aggregation of Protein Therapeutics.* 2018 PDA Universe of Pre-Filled Syringes and Injection Devices, Orlando, FL (August 8, 2018) **(attached)**
8. D. DeCou, W. Garzon-Rodriguez, M. Gehrmann, P. Frandolig, P. McAndrew. *Alternative for SARS-CoV-2 Vaccine Primary Package Systems: Daikyo Crystal Zenith® Cyclic Olefin Polymer Vials* (published online – September 2, 2020) **(attached)**
9. Barnard, J.G., et al. *Characterization and Quantitation of Aggregates and Particles in Interferon-b Products: Potential Links between Product Quality Attributes and Immunogenicity.* Journal of Pharmaceutical Sciences, 102 (3), 915-928 (2013)
10. Rosenberg, A. *Effect of Protein Aggregates: An Immunologic Perspective.* The AAPS Journal, 8 (3), E501-E507 (2006)
11. Philo, J. and Arakawa, T. *Mechanism of Protein Aggregation.* Current Pharmaceutical Biotechnology, 10, 348-351 (2009)
12. Gerhardt, A., et al. *Protein Aggregation and Particle Formation in Prefilled Glass Syringes.* Journal of Pharmaceutical Sciences, 103, 1601-1612 (2014)
13. Qadry, S.S., et al. *Evaluation of CZ-resin Vials for Packaging Protein-Based Parenteral Formulations.* International Journal of Pharmaceutics, 252, 207–212 (2003)
14. Eu, B., et al. *Direct Visualization of Protein Adsorption to Primary Containers by Gold Nanoparticles.* Journal of Pharmaceutical Sciences, 100 (5), 1663-1670 (May 2011)

Ultra-low temperature storage of vial-stopper-seal containment systems for gene therapies presents unique issues. These issues, each of which is discussed, are:

- differences in coefficients of thermal expansion among components – unequal rates of volume reduction may cause a system breach and loss of sterility
- overfilling – expansion on freezing of a water-based drug product may cause a system breach and loss of sterility
- improper assembly – beyond loss of sterility, over-pressurization may result

Issues related to mechanical durability and particles are discussed also.

Coefficients of Thermal Expansion

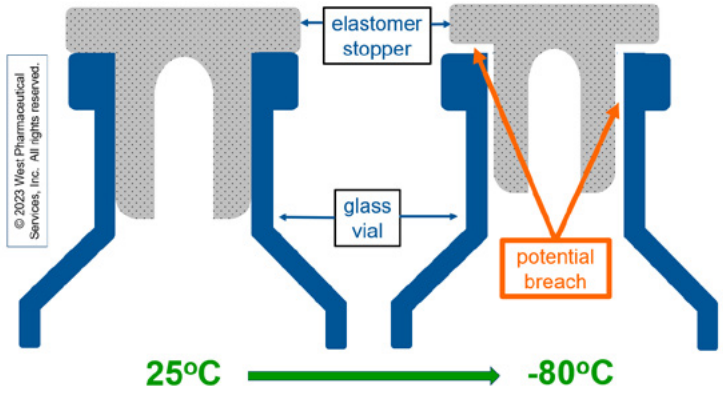
Potential effects of coefficients of thermal expansion (CTE) of components must be considered for any vial-stopper-seal containment system, whether based on a Daikyo Crystal Zenith® cyclic olefin polymer (CZ) vial or a borosilicate glass vial, upon assembly at room temperature and cooling to -80°C. See Table 1.

TABLE 1 *Coefficients of Thermal Expansion and Volume Changes*[1,2]

	Coefficient of Thermal Expansion (10^{-6} cm /cm-K)	Volume Reduction (%) (25°C) → (-80°C)
borosilicate glass	4	0.12
typical elastomer	77	2.4
CZ	70	2.2

Components are designed with dimensions to provide proper fit at room temperature, so for vial-stopper-seal containment systems storing drug products at room temperature, CTE is not a concern. However, upon assembly and cooling to -80°C, component volume reduction occurs. As seen in Table 1, volume reduction occurs to only a small extent with borosilicate glass, but to a larger extent with elastomer and CZ. For a system based on a borosilicate glass vial and elastomer stopper, CTE difference is significant; the elastomer stopper volume reduction is significantly greater than that of borosilicate glass. This presents a risk of a system breach. This is depicted schematically in Figure 1. For a system based on a CZ vial, CTE difference in the two materials is small; elastomer and CZ volume reductions are comparable. Thus, the risk of a system breach is lower as compared to a borosilicate glass vial system.

FIGURE 1 *Schematic Diagram (not to scale) Illustrating Breach Potential Resultant from Unequal Volume Reduction of Vial and Stopper upon Cooling from 25°C to -80°C*



Overfilling

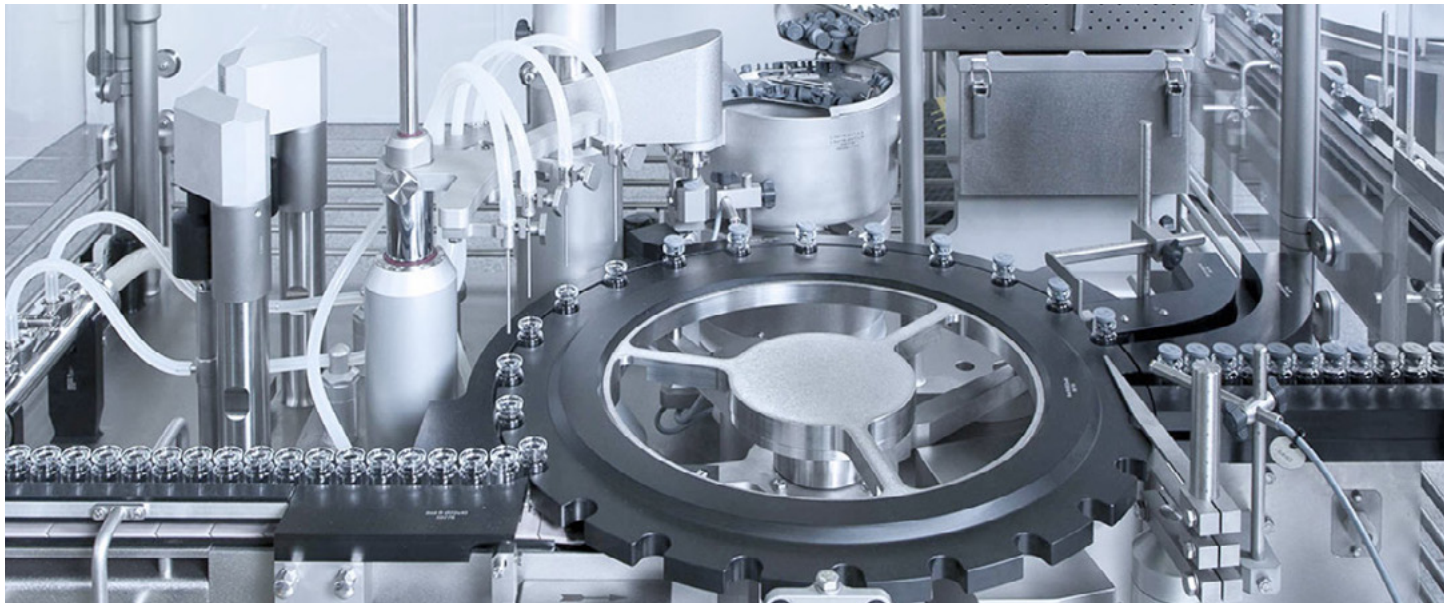
Vials typically are described in terms of nominal volumetric capacity. However, description may not represent the maximum amount of liquid that a vial-stopper-seal containment system can accommodate. Upon freezing, a water-based drug product can expand up to 109% of its liquid volume. Absent knowledge of maximum fill volume, liquid filling and subsequent expansion on freezing may lead to system breach and loss of sterility. Recommended maximum fill volumes have been quantified for CZ-based vial stopper-seal containment systems:[3] See Table 2.

TABLE 2 *Maximum Fill Volumes of CZ Vials*

CZ Vial Description	2 ml	5 ml	10 ml
Maximum Fill Volume	1.7 ml	5.0 ml	10.0 ml

Improper Assembly

Beyond the issue of loss of sterility, proper assembly of vial-stopper-seal containment systems is essential to avoid the issue of over-pressurization. Systems are assembled at room temperature and atmospheric pressure (i.e., 1 atm). Upon exposure to low temperature, there is a reduction of the interior pressure of the system, while the refrigerating chamber remains at atmospheric pressure. In the event of improper sealing or other breach, the resultant pressure differential will drive refrigerated chamber air into the vial containment system until it reaches equilibrium at atmospheric pressure. Upon removal from the refrigerated chamber and return to room temperature, the vial system is thus over-pressurized. In the case of storage at -80°C, or on dry ice, vial system internal pressure can reach 1.5 atm, which may present a handling or safety issue.[4]

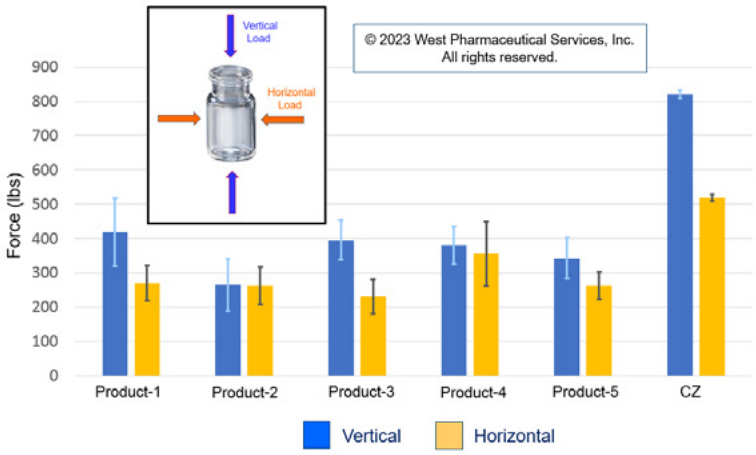


Fill-Finish Operation

Mechanical Durability

The resistance to fracture of 2 ml CZ vials was compared to 2R borosilicate glass vials from several suppliers.[5] Results are shown in Figure 2. Whether load force was applied vertically or horizontally, all borosilicate glass vials fractured at a lower force than CZ vials. This greater durability of CZ vials is an advantage for handling before low temperature storage, and after thaw and administration. Reduced risk of fracture reduces both risk of drug product loss and production delay resultant from cleaning.

FIGURE 2 *Fracture Resistance of 2R Borosilicate Glass Vials and 2 ml CZ Vials. Shown is force required to cause fracture. Error bars are standard deviation – 20 samples.*



Particles

The presence of particles is a concern for any parenteral drug product; for example, damage to blood vessels and immunogenic effects can result.[6-9] A natural concern for vial-stopper-seal containment systems for gene therapies is whether storage at -80°C and subsequent thaw increases particles levels. The five systems shown in Table 3 were evaluated.[10]

TABLE 3 *Vial-Stopper-Seal Systems for Particle Testing*

Vial	13 mm Elastomer Serum Stopper	13 mm Seal
2 ml CZ	NovaPure® 4023/50 Gray Article 1358	Article 5417 Flip-Off® seal
2 ml CZ	D21-7S Article S2-F451	Article 5417 Flip-Off® seal
2R borosilicate glass	NovaPure® 4023/50 Gray Article 1358	Article 5417 Flip-Off® seal
2R borosilicate glass	D21-7S Article S2-F451	Article 5417 Flip-Off® seal
2.0 ml polypropylene cryogenic vial with screw-top cap		

Samples of each were filled with 1 ml of a filtered phosphate buffered saline (PBS) solution in an ISO 5 clean room. Selected samples of each were tested for particle level by the validated West method of inverting 10 times, capturing particles on a 0.8 mm filter and counting by membrane microscopy according to USP Chapter <788> Method 2.[11] Different selected samples were stored at -80°C for seven days and thawed to room temperature. These were tested for particle level in the exact same way.

Results are shown in Figures 3 and 4. In all cases, CZ-based systems had levels either slightly better than, or comparable to, borosilicate glass-based systems. Most notable though is the substantially better particle levels of CZ-based systems as compared to the 2.0 ml polypropylene cryogenic vial. Further analysis of CZ and borosilicate glass systems indicated that: (a) no particles were glass or CZ, and (b) no surface change resulted from freeze/thaw.

FIGURE 3 *Particle Counts ≥ 10 mm of Vial Containment Systems Before/After Seven Days at -80°C. Error bars are standard deviation – 5 samples*

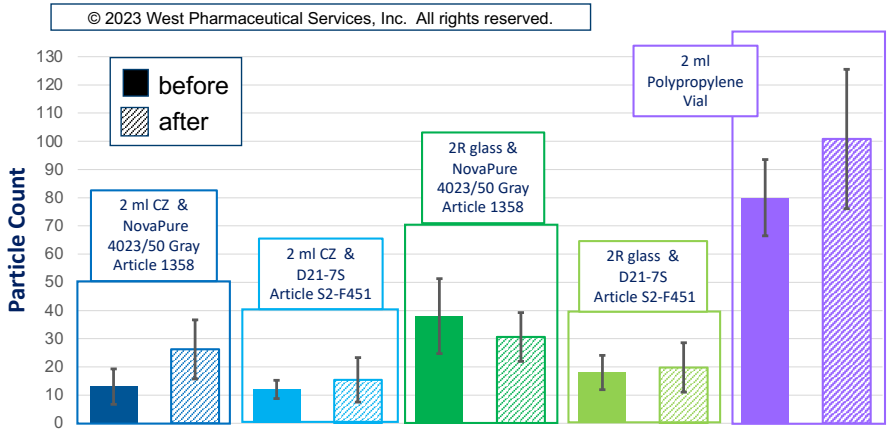
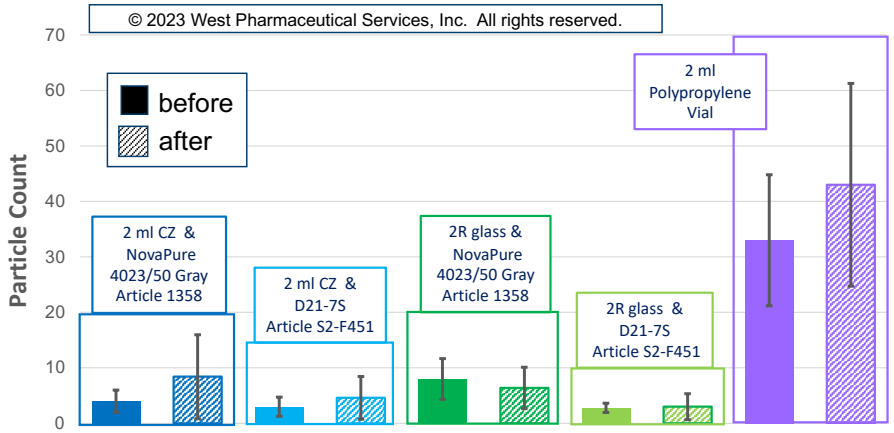


FIGURE 4 *Particle Counts ≥ 25 mm of Vial Containment Systems Before/After Seven Days at -80°C. Error bars are standard deviation – 5 samples*



Discussion

Based on consideration of materials properties, vial containment systems comprising CZ vials and halobutyl elastomers (with FluroTec™ barrier film facing drug product) may be excellent choices for gene therapies. Coefficients of thermal expansion are close, reducing risk of breach during ultra-low temperature storage. This point is amplified by the good CCI performance discussed in Chapter 4. Compared to borosilicate glass vials, CZ vials have better mechanical durability, thus reducing risk of breakage and subsequent product loss.

Regarding particles, CZ-based systems perform better than polypropylene cryogenic vial systems and equivalent to or better than borosilicate glass-based systems. Further for CZ systems, maximum fill volume and issues of improper sealing have been quantified. Taken altogether, this information can aid manufacturers of gene therapies in proper selection of vial-stopper-seal containment systems.

References

1. http://www.engineeringtoolbox.com/linear-expansion-coefficients-d_95.html (accessed 4/19/23)
2. *Characteristics of Daikyo Resin CZ*. Daikyo Seiko, Ltd. Technical Report DS-CZ-E017 (January 2022)
3. *Volumetric Capacity of Daikyo Crystal Zenith® Vials Stored in Freezing Conditions*. West Pharmaceutical Services, Inc. Technical Report 2022-039 **(attached)**
4. *Potential Over-Pressurization Issues with Ultra-Low Temperature or Cryogenic Storage of Improperly Sealed Vial/Stopper Primary Package Systems*. West Pharmaceutical Services, Inc. Technical Service Bulletin 2020-072 **(attached)**
5. Unpublished Observation. West Pharmaceutical Services, Inc. (2019)
6. Barnard, J.G., et al. *Characterization and Quantitation of Aggregates and Particles in Interferon-β Products: Potential Links between Product Quality Attributes and Immunogenicity*. Journal of Pharmaceutical Sciences, 102 (3), 915-928 (2013)
7. Rosenberg, A. *Effect of Protein Aggregates: An Immunologic Perspective*. The AAPS Journal, 8 (3), E501-E507 (2006)
8. Philo, J. and Arakawa, T. *Mechanism of Protein Aggregation*. Current Pharmaceutical Biotechnology, 10, 348-351 (2009)
9. Gerhardt, A., et al. *Protein Aggregation and Particle Formation in Prefilled Glass Syringes*. Journal of Pharmaceutical Sciences, 103, 1601-1612 (2014)
10. *Comparison of Particles in Daikyo Crystal Zenith® Vials, Glass Vials, Polypropylene Screw Top Vials and Cryogenic Bags*. Daikyo Crystal Zenith® Technical Report 2022/038 **(attached)**
11. United States Pharmacopeia Chapter <788> *Particulate Matter in Injections*

CHAPTER 4

Container Closure Integrity

1 2 3 4 5 6

Vial-stopper-seal containment systems for gene therapies must protect quality and efficacy through shelf life. One aspect of this is that they must maintain container closure integrity. According to United States Pharmacopeia Convention (USP) Chapter <1207>: container closure integrity (CCI) is demonstrated when a container system meets the maximum allowable leakage limit (MALL) – the smallest gap or leak rate that puts product quality at risk – established to meet product quality attributes for sterility and physiochemical stability through expiration date.[1,2]

Making this a challenge for gene therapies is that ordinarily they are stored and shipped at approximately -80°C – what is termed *ultra-low* temperature (as distinguished from *cryogenic* temperature which is ≤ -130°C, i.e., vapor of liquid nitrogen). This can be either in a refrigerator or in a container with dry ice (i.e., solid CO_2 with vapor temperature of -78°C).

Storage at -80°C

Container closure integrity of the two vial-stopper-seal containment systems (empty) comprising Daikyo Crystal Zenith® cyclic olefin polymer (CZ) vials shown in Table 1 was evaluated over two years stored in a refrigerator at -80°C:[3]

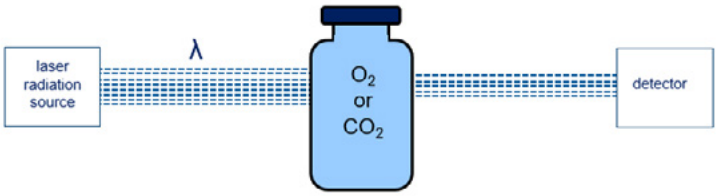
TABLE 1 *Vial-Stopper-Seal Systems Tested at -80°C*

2 ml Vial	13 mm Elastomer Serum Stopper	13 mm Seal
CZ	NovaPure® 4023/50 Gray Article 1358	Article 5417 Flip-Off® CCS
CZ	D21-7S Article S2-F451	Article 5417 Flip-Off® CCS

Samples were assembled in a non-sterile environment in a N_2-filled glove bag so that upon capping the sample atmosphere comprised substantially 100% N_2. Seals were applied and capping was performed immediately upon removal from said bag. Capping was performed to an average stopper compression of approximately 40%, which corresponded to an average residual seal force of 14-19 lbs.

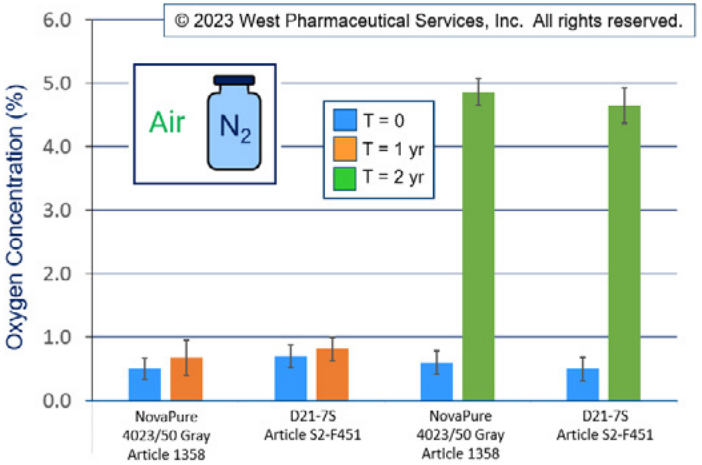
Container closure integrity was evaluated by laser-based gas headspace analysis. This method, which is endorsed in USP Chapter <1207>, is based upon frequency modulated spectroscopy.[1] The instrument near infrared radiation laser source is set to match an absorption resonance frequency of the gas of interest (O_2 or CO_2). Upon sample irradiation, the amount of radiation absorbed is measured and correlated to concentration of gas in the sample.[4] This is depicted in Figure 1. The instrument employed was the FMS-760 by Lighthouse Instruments, LLC.

FIGURE 1 *Laser-Based Gas Headspace Analysis.*



Oxygen concentration was measured for all samples at T = 0, after which they were placed directly into the refrigerator at -80°C. Pre-selected samples were removed after 1 year and allowed to thaw to room temperature before O_2 concentration was measured. These samples were discarded. Likewise pre-selected samples were tested after 2 years. Results are shown in Figure 2.

FIGURE 2 *Oxygen Headspace Concentration of Systems Stored FIGURE 2 Maximum Fill Volumes ofat -80°C. Components: 2 ml COP CZ vials, stoppers as Various CZ Vial Sizes listed, Article 5417 Flip-Off CCS seals, capped to approximately 40% stopper compression*



Note that all T = 0 values of O_2 concentration are approximately 0.5%, not 0%, resultant from small experimental error (calibration standards for the FMS-760 are based on glass vials, not CZ vials) and that glove bag assembly of samples cannot provide quantitative 100% N_2 atmosphere. Nevertheless, after 1 year, there was no appreciable increase in O_2 concentration. After 2 years, there was an increase to approximately 5% O_2. Through 1 year or 2 years, there was no breach of any sample (e.g., by failure at vial/stopper interface or fracture of vial or stopper). Since the refrigerator atmosphere was 21% O_2 (i.e., the O_2 concentration of air), had there been a breach, the O_2 concentration in a sample would have changed to an equilibrium value of 21% O_2. This was not observed for any sample. Amplifying this is an analysis according to Gay-Lussac's Law that relates pressure and temperature in a closed system. When samples capped under atmospheric conditions at room temperature (i.e., 1 atm and 298 K) are placed in a -80°C refrigerator (i.e., 1 atm and 193 K), sample internal pressure decreases to 0.65 atm.

$$\frac{P_1}{T_1} = \frac{P_2}{T_2} \Longrightarrow P_2 = \frac{P_1 \times T_2}{T_1} \Longrightarrow P_2 = \frac{1 \text{ atm} \times 193 \text{ K}}{298 \text{ K}} = 0.65 \text{ atm}$$

Since the refrigerator is at 1 atm, there is a 0.35 atm pressure difference that would promote air ingress if there was a breach. As stated, for no sample was this observed.

The increase in O_2 concentration no doubt is due to slow diffusion of O_2 through CZ. This is not completely unexpected, as all polymers are known to be gas permeable.[5] This increase was not due to any significant extent to diffusion through elastomer stopper, as they have been established to have low permeability (in large measure based on thickness).[6, 7]

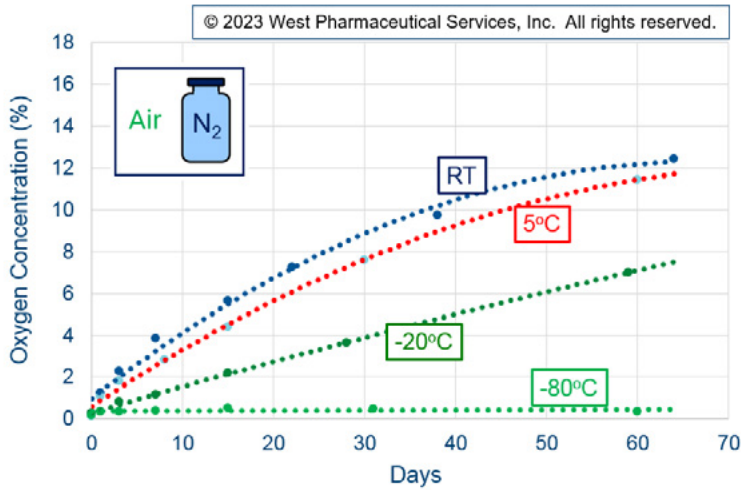
For CZ-based containment systems (empty), CCI has been examined not just at -80oC, but across temperatures. The vial-stopper-seal containment system evaluated through 60 days is shown in Table 2.[8, 9]

TABLE 2 *Vial-Stopper-Seal System Tested at Various Temperatures*

2 ml Vial	13 mm Elastomer Serum Stopper	13 mm Seal
CZ	NovaPure® 4023/50 Gray Article 1358	13-10T LQ

Samples were prepared in a manner essentially identical to that for samples whose performance is shown in Figure 2. Oxygen concentration was measured for all samples at T = 0, after which samples were placed directly into a refrigerator or stored at room temperature. Results are shown in Figure 3. As expected, permeability decreases with temperature, and at -80°C it is practically nil through 60 days. These observations are entirely consistent with those shown in Figure 2. While borosilicate glass is essentially impermeable to all gas ingress, and borosilicate glass-based vial-stopper-seal containment systems may be better suited to maintain CCI at higher temperatures, at temperatures needed for gene therapies, CZ-based systems appear quite effective.

FIGURE 3 *Oxygen Headspace Concentration of Systems at Various Temperatures. Components: 2 ml CZ vial, NovaPure 4023/50 Gray Article 1358 stopper, 13-10T LQ seal, hand capped*



Storage in Container with Dry Ice (-78°C)

Shipment or storage of gene therapies may be in a container filled with dry ice, i.e., solid CO_2. At atmospheric pressure, solid CO_2 converts from solid phase to gas phase with no intermediate liquid phase (sublimation) at -78°C; a container filled with solid CO_2 thus obviously is filled with CO_2 gas at -78°C.

Container closure integrity of a CZ-based vial containment system was evaluated stored in a container filled with dry ice. The (empty) system shown in Table 3 was evaluated after 1-, 3-, and 7-day storage in a container (YETI TUNDRA® 35 hard cooler) filled with dry ice (routinely replenished) and subsequent removal and post-storage in air at room temperature up to 14 days.[8,9] Samples were prepared in a manner comparable to that for samples whose performance is shown in Figures 2 and 3, except assembly was not in a N_2-filled glove bag, i.e., sample atmosphere was air.

TABLE 3 *Vial-Stopper-Seal System Tested Stored with Dry Ice*

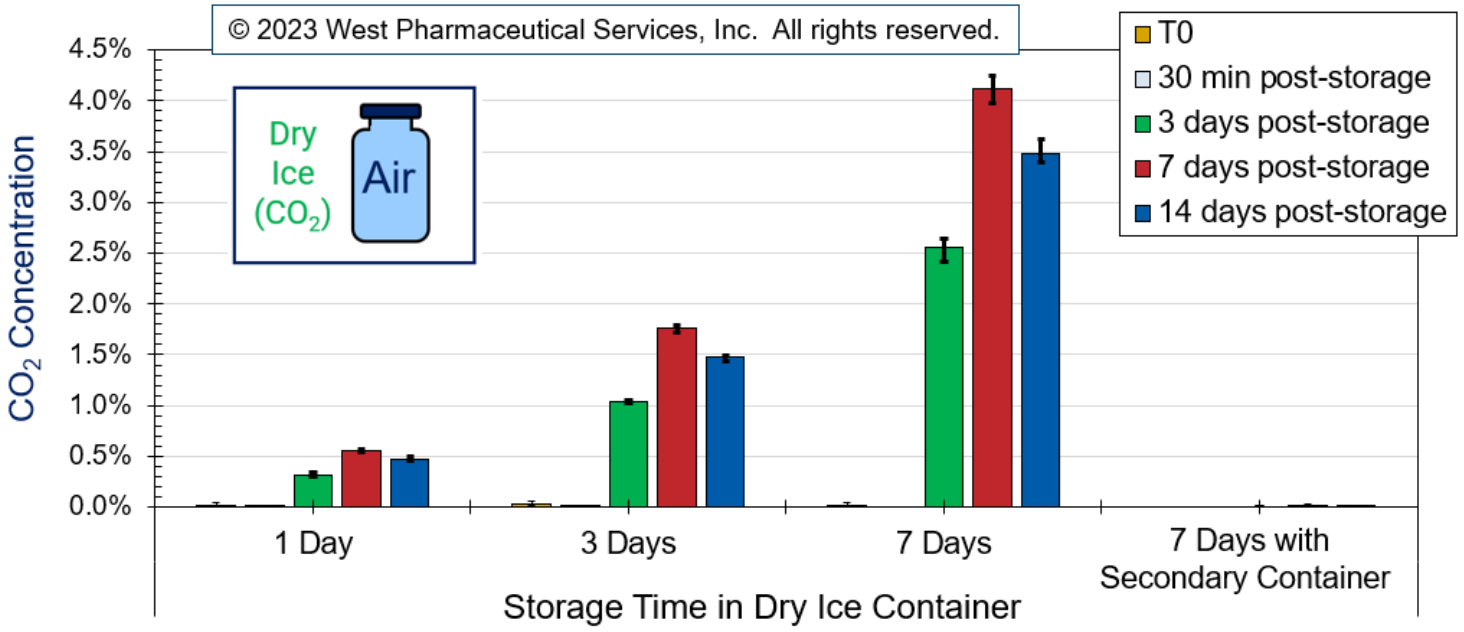
5 ml Vial	20 mm Elastomer Serum Stopper	20 mm Seal
CZ	NovaPure® 4023/50 Gray Article 1343	20 FO LQ TE (6B)

Carbon dioxide concentration was measured for all samples at T = 0, after which all samples were placed directly into a container. Note that one set of samples was first placed in a secondary container – a poly(ethylene terephthalate)-based heat-sealed bag such as commonly used for food storage. Results are shown in Figure 4.

With increased storage time in a dry ice container and increased time at room temperature, higher concentrations of CO_2 were observed. In all cases, essentially no CO_2 was observed after 30 minutes (a time selected to estimate typical time of removal from storage, thaw, and administration). Note that samples stored in a secondary container showed essentially no CO_2, even after 7 days storage and 14 days at room temperature.

Evidently, some CO_2 dissolves in CZ, then diffuses into the system upon storage at room temperature. A concern with CO_2 ingress is reaction with water in a drug product formulation to produce carbonic acid and potentially change the drug product pH. The first point to note is that the level of CO_2 ingress is quantified so a judgement regarding MALL can be made for the gene therapy in question. The second point to note is that use of an appropriate secondary container essentially stops CO_2 ingress completely.

FIGURE 4 *Carbon Dioxide Headspace Concentration of Systems Stored with Dry Ice. Components: 5 ml CZ vial, NovaPure 4023/50 Gray Article 1343 stopper, 20 FO LQ TE (6B) seal, hand capped. Post storage was at room temperature. Samples in secondary container were removed from secondary container prior to post storage.*



CHAPTER 4

Container Closure Integrity

1 2 3 4 5 6

Summary

For vial-stopper-seal containment systems based on CZ vials, CCI performance at -80°C has been quantified. Through 1 year there was no ingress of O_2, and through 2 years only a small ingress of O_2. No breach of any sample was observed. Also quantified was CCI performance stored in a dry ice container (-78°C) through 2 weeks. Small ingress of CO_2 was observed over several days, but essentially none over the 30 minutes typical for thaw and administration. Moreover, CO_2 ingress essentially can be stopped by use of a secondary container. These data are instructive in aiding determination of whether a vial-stopper-seal containment system based on CZ vials can meet MALL for a gene therapy.

References

1. United States Pharmacopeia Chapter <1207> *Sterile Product Packaging – Integrity Evaluation*
2. F. DeGrazio. *Holistic Considerations in Optimizing a Sterile Product Package to Ensure Container Closure Integrity*. PDA Journal of Pharmaceutical Science and Technology, 72 (1), 15-34 (2018)
3. *Long-Term Container Closure Integrity Testing of Vial-Stopper-Seal Combinations Comprising Daikyo Crystal Zenith® Vials at Ultra-Low and Cryogenic Temperatures*. West Pharmaceuticals Services, Inc. Technical Report 2023/261 **(attached)**
4. K.G. Victor, et al. *Method Development for Container Closure Integrity Evaluation via Headspace Gas Ingress by Using Frequency Modulation Spectroscopy*. PDA Journal of Pharmaceutical Science and Technology, 71 (6), 429-453 (2017)
5. L.M. Robeson. *Correlation of Separation Factor Versus Permeability for Polymeric Membranes*. Journal of Membrane Science, 62, 165-185 (1991).
6. M.R. Gehrmann and T.P. McAndrew. *Long-Term Study of Container Closure Integrity of Rubber-Glass Vial Systems by Multiple Methods*. PDA Journal of Pharmaceutical Science and Technology, 74 (1), 147-161 (2020)
7. *Container Closure Integrity of Rubber-Glass Vial Systems*. West Pharmaceutical Services, Inc. Technical Report 2018/199 **(attached)**
8. *Ingress of Gases into Cyclic Olefin Polymer Vial-Based Container Closure Systems at -80°C*. West Pharmaceutical Services, Inc. Technical Report 2019/028 **(attached)**
9. M. Gehrmann, O. Laskina, L. Fang, P. McAndrew. *Challenges in Low-Temperature Storage of Cell Therapy Drug Products.* PepTalk 2020, San Diego, CA, January 20, 2020 **(attached)**

CHAPTER 5

Recovery of Viral Vectors

Essential for any vial containment system for a viral vector-based gene therapy is the ability to maintain the viability of the viral vectors throughout storage at ultra-low temperatures and subsequent thaw. Specifically, what must be maintained is the quantity of viral vectors and the effectiveness of those viral vectors to transduce cells. Examples of viral vector-based gene therapies are shown in Table 1.

Select examples of the three main classes of viral vectors used in gene therapies were evaluated in various vial containment systems comprising:

- Daikyo Seiko Crystal Zenith® cyclic olefin polymer (CZ) vials
- borosilicate glass vials
- bromobutyl and chlorobutyl elastomer stoppers with FluroTec™ poly(ethene tetrafluoroethene) (ETFE) barrier film facing drug product
- aluminum Flip-Off® seals
- polypropylene cryogenic vials with screw caps

TABLE 1 *Vial-Vector Gene Therapy Examples*

Class	Serotype	Gene Therapy Examples	Indication
Adeno-associated virus	AAV2	voretigene neparvovec-rzyl (LUXTURNA®) (a)	Leber congenital amaurosis (biallelic RPE65-mediated inherited retinal disease)
Adenovirus	Ad5, Ad5. F35	nadofaragene firadenovec (ADSTILADRIN®) (b)	high-grade Bacillus Calmette-Guérin (BCG)-unresponsive non-muscle-invasive bladder cancer
Lentivirus	NA	elivaldogene autotemcel (SKYSONA™) (c)	cerebral adrenoleukodystrophy

a. LUXTURNA is a registered trademark of Spark Therapeutics, Inc.
b. ADSTILADRIN is a registered trademark of Ferring, B.V.
c. SKYSONA is a trademark of bluebird bio, Inc.

Adeno-Associated Virus

Recovery of the adeno-associated viral vector AAV2-CMV-EGFP (abbreviated as AAV2), carrying an enhanced green fluorescent protein (eGFP) reporter gene, from each of the vial containment systems shown in Table 2 was evaluated after three weeks storage at -80°C.[1]

TABLE 2 *Vial-Stopper-Seal Systems Evaluated for Recovery of Viral Vector AAV2*

Vial	Stopper	Seal/Cap
2 ml CZ	13 mm NovaPure® 4432/50	13 mm Flip-Off® CCS
2R Borosilicate Glass	13 mm NovaPure® 4432/50	13 mm Flip-Off® CCS
1.8 ml Polypropylene (PP)	NA	Screw cap

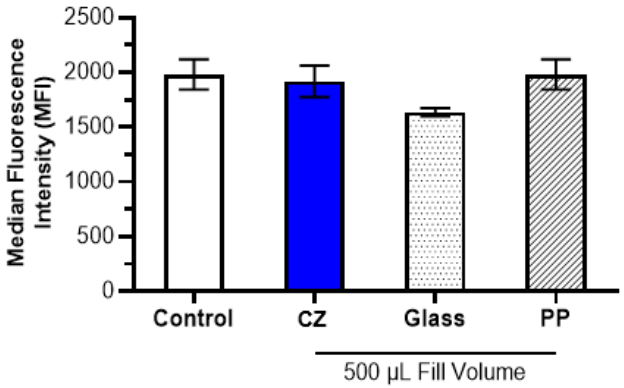
CHAPTER 5

Recovery of Viral Vectors

1 2 3 4 5 6

The AAV2 was formulated in a 0.001% Poloxamer 188 in Dulbecco's phosphate-buffered saline solution to a measured titer of 1 x 10^{10} genomic copies per milliliter (GC/ml). The samples were frozen in an uncontrolled fashion in a -80°C refrigerator and, at the end of storage time, thawed at room temperature for one hour protected from light. The AAV2 recovered from said storage/thaw was assessed for its ability to transduce HEK-293 cells (human embryonic kidney 293 cells) and deliver the eGFP payload. The AAV2 dosing concentration (estimated 12,000 GC/cell) was selected to target a sub-saturating multiplicity of infection (MOI) with sensitivity to potential differences in transduction function of the AAV2 recovered from different sample conditions. Functional titer of the AAV2 was measured using a standard flow cytometer in the Fluorescein-5-isothiocyanate (FITC) channel in terms of the median fluorescence intensity (MFI) of HEK-293 cells transduced by the AAV2 and expressing eGFP. Control samples were measured before freezing. Results are shown in Figures 1 and 2.

FIGURE 1 *Recovery of AAV2 after Three Weeks at -80°C in CZ, Borosilicate Glass, and Polypropylene (PP) Vial Containment Systems. Measurement was by flow cytometry post-thaw in terms of eGFP fluorescence of HEK-293 cells transduced. Control was measured post-formulation and pre-freeze. Fill volume was held constant at 500 µL. Error bars are standard deviation (n = 6).*

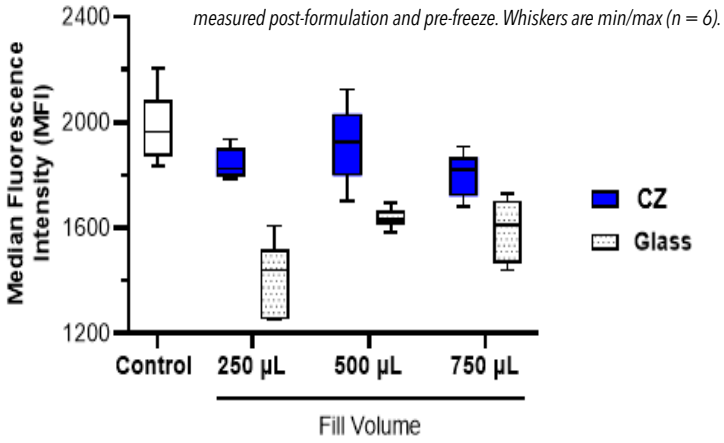


Note from Figure 1 that the CZ system was comparable to the PP system in ability to preserve the functional AAV2 titer level to that of the control. With the borosilicate glass system, however, a loss of functional AAV2 titer level was observed.

In Figure 2, further comparison of the CZ and borosilicate glass systems is shown. For the lower fill volumes at 250 µL and 500 µL, the CZ system enabled significantly higher recovery of functional AAV2 titer level as compared to the borosilicate glass system; at 750 µL the difference was less pronounced. This may be explained by the higher surface energy of borosilicate glass as compared to CZ, as previously highlighted in Chapter 2. The lower the fill volume, the greater the surface-area-to-volume ratio, and consequently the greater the exposure of viral vectors to container system surface. The higher energy glass surface would be expected to interact more strongly and potentially adsorb viral vectors, or otherwise affect performance.

In preliminary work (to be published in 2024), similar enhanced performance of CZ systems vis-à-vis borosilicate glass systems likewise appears to be observed for select examples of AAV8 and AAV9 serotypes.

FIGURE 2 *Recovery of AAV2 after Three Weeks at -80°C in CZ and Borosilicate Glass Vial Containment Systems as a Function of Varying Fill Volumes. Formulated titer was held constant at 1 x 10^{10} GC/ml. Measurement was by flow cytometry post-thaw in terms of eGFP fluorescence of HEK-293 cells transduced. Control was measured post-formulation and pre-freeze. Whiskers are min/max (n = 6).*



Adenovirus

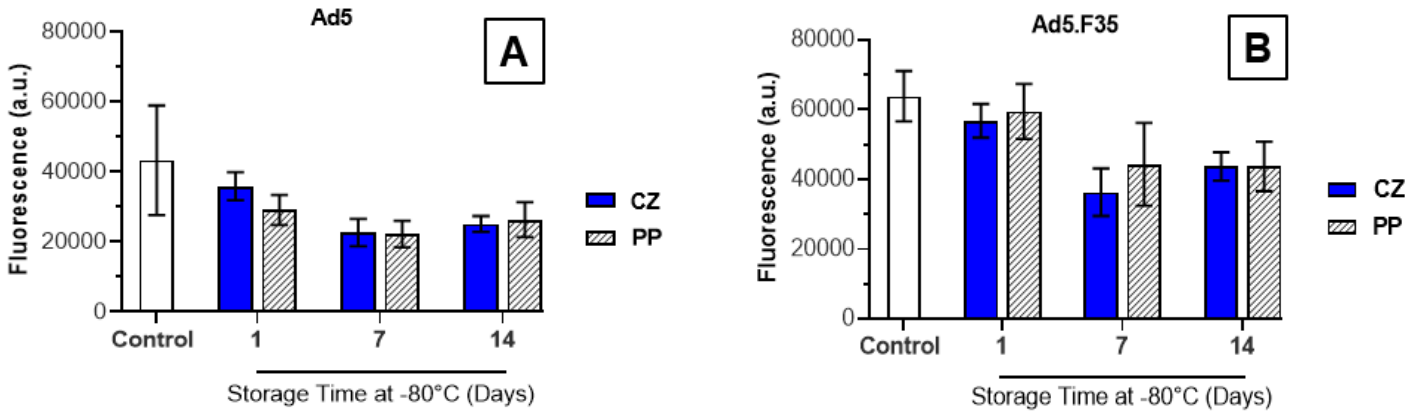
Recovery of two adeno viral vectors carrying the eGFP reporter gene, Ad5-CMV-eGFP (abbreviated as Ad5) and Ad5.F35-CMV-eGFP (abbreviated as Ad5.F35) from each of the vial containment systems shown in Table 3 was evaluated after varying times storage at -80°C.[2]

TABLE 3 *Vial-Stopper-Seal Systems Evaluated for Recovery of Viral Vectors Ad5 and Ad5.F35*

Vial	Stopper	Seal/Cap
2 ml CZ	13 mm NovaPure® 4023/50	13 mm Flip-Off® CCS
1.8 ml Polypropylene (PP)	NA	Screw cap

Ad5 and Ad5.F35 were formulated in 2.5% glycerol in 20 mM tris-HCl buffer solutions to a measured titer of 1 x 10^{11} viral particles per milliliter (vp/ml). Samples were stored in a -80°C freezer for 1 to 14 days and subsequently thawed on wet ice. Recovered Ad5 and Ad5.F35 samples from said storage/thaw were assessed for ability to transduce A549 cells (adenocarcinomic human alveolar epithelial cells) and deliver the eGFP payload. Functional titer was measured using a standard microplate reader at 480 nm excitation / 520 nm emission in terms of fluorescence value of A549 cells transduced and expressing eGFP. Control samples were measured before freezing. Results are shown in Figure 3.

FIGURE 3 *Recovery of (A) Ad5 and (B) Ad5.F35 after Varying Times at -80°C in CZ and Polypropylene (PP) Vial Containment Systems. Functional titer was measured by a microplate reader at 480 nm excitation / 520 nm emission in terms of eGFP fluorescence (a.u., arbitrary units) of transduced A549 cells. Control was measured post-formulation and pre-freeze. Error bars are standard deviation (n = 9).*



For Ad5 and Ad5.F35, across all times, CZ and PP systems were comparable in ability to maintain functional titer level. After one day storage, titer level in both systems was comparable to the control, but with longer storage, titer levels in both systems decreased. This loss suggests suboptimal storage/preservation in this first phase of research. Emphasized is that CZ and PP systems performed comparably.

Lentivirus

Recovery of Lenti-GFP (lentiviral vector with GFP reporter gene) from vial containment systems shown in Table 4 was evaluated after: (a) 48 hours at -80°C followed by 48 hours at 4°C, (b) 48 hours in a container with dry ice (-78°C) followed by 48 hours at 4°C, and (c) 48 hours at 4°C (no freezing).[3]

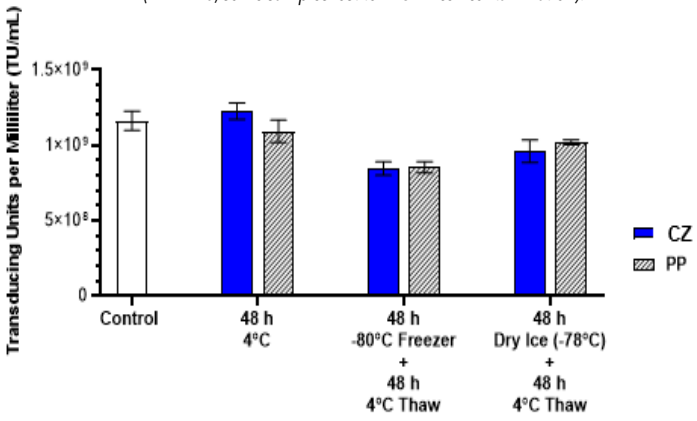
TABLE 4 *Vial-Stopper-Seal Systems Evaluated for Recovery of Viral Vector Lenti-GFP*

Vial	Stopper	Seal/Cap
2 ml CZ	13 mm NovaPure® 4023/50	13 mm Flip-Off® CCS
1.8 ml Polypropylene (PP)	NA	Screw cap

Lenti-GFP was formulated in a 10% lactose, 25 nM proline in 10 mM tris buffer solution to an approximate titer above 1 x 10^9 transducing units per milliliter (TU/ml). As noted above, samples were stored in either a -80°C freezer or dry ice container (-78°C) for 48 hours – to imitate common short-term ultra-cold storage and shipment conditions. The frozen samples were subsequently thawed in a 4°C refrigerator for 48 hours – to imitate a common post-freeze storage condition for viral vector-based gene therapies at the point of care, or during manufacture. A selected set of samples was stored at 4°C for 48 hours – to imitate commercial handling and to identify potential viral vector interactions with containment systems. Control samples were measured before low temperature exposure.

Lenti-GFP recovered from said storage/thaw was assessed for ability to transduce 293LTV cells (embryonic human kidney transformed with human adenovirus type 5 DNA cells) and to deliver GFP payload. Functional titer of the Lenti-GFP was measured using a standard flow cytometer in the FITC channel, in terms of transduced cells (i.e., number of 293LTV cells transduced by the viral vector and expressing GFP) per volume of viral vectors dosed. The dosing of recovered Lenti-GFP onto 293LTV culture was titrated to select a titration where 10% to 20% of the cells were GFP-positive, so that the multiplicity of infection (MOI) was sufficiently low to yield a predominantly single integration event per transduced cell. Results are shown in Figure 4.

FIGURE 4 *Recovery of Lenti-GFP after Storage in CZ and Polypropylene (PP) Vial Containment Systems. Functional titer was measured by a flow cytometer in the FITC channel, in terms of GFP-positive transduced 293LTV cells per volume of viral vector used. Control was measured post-formulation and before low temperature exposure. The 4°C – 48 hr samples were liquid phase only. Error bars are standard deviation (n = 4-10, some samples lost to 293LTV cell contamination).*



The CZ and PP systems were comparable in ability to maintain functional titer level for -80°C and dry ice storage. For 4°C storage (i.e., liquid phase only, never frozen), the CZ system appeared slightly better, and equivalent to control, suggesting less loss of Lenti-GFP vector with the CZ system. This may be indicative of a difference in how Lenti-GFP vectors interact with PP, vis-à-vis CZ. It suggests greater stability of lentiviral vectors in CZ systems during ambient processing steps before ultra-low temperature storage/shipment. Also note that at -80°C and in dry ice storage, there was some loss of functional viral vectors from both CZ and PP systems. Similar to the adenoviral vector results described above, this suggests suboptimal storage/preservation in this first phase of research.

Summary

For the viral vectors evaluated, at ultra-low temperature storage, CZ vial containment systems performed well in preserving functional performance – comparable to the current industry standard polypropylene cryogenic screw cap vial containment systems and better than borosilicate glass vial containment systems. CZ systems would appear to be excellent candidates for gene therapies.

References
1. *Functional Assessment of Adeno-Associated Virus Stored in Daikyo Crystal Zenith Vials at Ultra-Cold Temperatures*. Daikyo Crystal Zenith® Technical Report 2021/033 **(attached)**
2. *Evaluation of Adenoviral Vector Recovery from Ultra-Low Storage in Daikyo Crystal Zenith® Vials*. West Pharmaceutical Services, Inc. Technical Report 2022/245 **(attached)**
3. *Evaluation of Daikyo Crystal Zenith® Vials for Ultra-Cold Storage and Shipment of Lentiviral Vectors*. West Pharmaceutical Services, Inc. Technical Report 2022/247 **(attached)**

CHAPTER 6
Summary

Each manufacturer of a viral-vector-based gene therapy must make their own decision regarding the suitability of the vial-stopper-seal containment system selected. That is, evaluation must be made to demonstrate the ability of the containment system to maintain quality and efficacy from manufacture through storage at ultra-low temperature, thaw, and administration. This evaluation comprises many aspects. Among them are:

1. the ability to maintain virus transduction ability
2. the absence of any deleterious interaction
3. the ability to maintain container closure integrity below maximum allowable leakage limit (MALL),
4. physical performance (mechanical durability and particle levels)

This e-book has reviewed research conducted by, or performed for, West Pharmaceutical Services, Inc. that evaluated these four aspects for vial-stopper-seal containment systems comprising:

- Daikyo Seiko Crystal Zenith® cyclic olefin polymer (CZ) vials
- West NovaPure® elastomer serum stoppers and Daikyo Seiko, elastomer serum stoppers – both with fluoropolymer laminate barrier film facing drug product
- West Flip-Off® seals

Comparisons were made to borosilicate glass-based and polypropylene-based containment systems.

For selected examples of each of the three major types of viral vectors for gene therapies (adeno-associated virus, adenovirus, lentivirus), CZ-based systems provided for maintenance of performance through ultra-cold storage as well as (industry-standard) polypropylene-based systems, and better than borosilicate glass-based systems.
This suggests general utility of CZ-based systems.

Using selected proteins as models, it was demonstrated there is less interaction with CZ-based systems than with borosilicate glass-based systems. Moreover, it was demonstrated that the presence of fluoropolymer laminate barrier film reduces risk of permeation of compounds from stopper. These data suggest potential very good suitability.

Container closure integrity for CZ-based systems was evaluated for both refrigerated storage and storage on dry ice. Levels of oxygen and carbon dioxide permeation were quantified and demonstrated to be low. These data inform a judgment if MALL can be met.

Regarding mechanical durability, CZ-based systems exceed borosilicate glass-based systems as being more fracture resistant. Regarding particle levels, CZ-based systems exceed polypropylene systems and are equivalent to borosilicate glass-based systems.

Knowing performance of a vial containment system beforehand can facilitate timely delivery to market for a gene therapy as it enables selection of a proper system at outset. *All factors considered, performance of CZ-based vial containment systems exceed or match those based on borosilicate glass or polypropylene, and thus appear potentially to be an excellent choice for gene therapies.*

Acknowledgements

Recognition goes to the following for the research on viral vectors presented herein:

- Thomas Jefferson University: A. Snook, J. Singh, T. Baybutt
- Advanced Bioprocess Services, Ltd. and Loughborough University Centre for Biological Engineering: K. Glen, J. Harriman, R Thomas

Recognition goes to the following members of the West Pharmaceuticals Services, Inc. scientific staff for the materials, chemistry, and CCI research presented:

- Alex Lyness, Olga Laskina, Amy Kim, Amy Gindhart, Le Ho

West Pharmaceutical Services, Inc.
530 Herman O. West Drive, Exton, PA 19341 USA
610.594.2900 www.westpharma.com

Elastomer Stoppers with FluroTec® Film: The Right Choice for SARS-CoV-2 Vaccines

Ranjana Singh, Ph.D.
William Garzon-Rodriguez, Ph.D.
Peggy Frandolig
Cathy Zhao, Ph.D.
T. Page McAndrew, Ph.D.

Abstract

For primary package systems for SARS-CoV-2 vaccines, elastomer stoppers with FluroTec® film enable the lowest risk. Employing the chemical properties of poly(ethylene tetrafluoroethylene), FluroTec film: (1) reduces migration of leachables into drug product, (2) reduces interaction of drug product with stopper, and (3) enables excellent container closure integrity down to low temperatures. This article discusses how these features mitigate package system risks with the six platforms of SARS-CoV-2 vaccines in development – especially important in view of greatly accelerated timelines that do not permit standard evaluations. Components with FluroTec film are globally available and market accepted – over 125 drugs, including three vaccines and 30 novel drugs, are FDA/EMA approved.

Background

Development and distribution of a vaccine for SARS-CoV-2 presents challenges that are, without hint of exaggeration, unprecedented. One of these challenges concerns storage, namely selection of vial/stopper primary package systems that guarantee quality and safety of the vaccine from manufacture through delivery. This selection is complicated greatly by the accelerated timelines for vaccine approval.

Two aspects comprise the challenge in package system selection:

1. The first concerns the vaccine platform. Six platforms are now considered; they are listed with their proposed vehicles in Table 1. (1) Noteworthy is that two of them (RNA, DNA) are new. Ordinarily, there would be no difficulty in selecting a package system for any of the platforms, since ample time would be available for evaluation of compatibility with both the vaccine and the vehicle. But, for a SARS-CoV-2 vaccine, this is not the case, since approval timelines are accelerated. So, whether the vaccine platform is extant or new, selection of the package system must be made quickly. This creates a risk.
2. The second concerns stability during storage and distribution. A vaccine platform and package system may be identified, but other factors must be considered, such as:
   - form: serum or lyophilized
   - delivery: multi- or single-dose
   - temperature: room (25°C), refrigerated (2-8°C), or low (-80°C)
   - availability of package system components

Lack of certainty regarding whether a package system is available, and can accommodate the format needed, creates a risk.

Table 1. Potential Vaccines for SARS-CoV-2 (1)

Vaccine Platform	Chemical Composition	Vehicle	Existing, Licensed Human Vaccine
RNA	nucleotides (ribose groups, amino/amide groups, charged phosphate groups)	encapsulated in lipid in non-polar liquid	No
DNA	nucleotides (ribose groups, amino/amide groups, charged phosphate groups)	aqueous (saline) solution, encapsulated in lipid in non-polar liquid	No
Recombinant Protein	polypeptides (amino acid groups)	aqueous	Yes (baculovirus and yeast expression)
Viral Vector Based	virus shell comprises proteins (i.e., polypeptide: amino acid groups)	aqueous	Yes (vesicular stomatitis virus)
Live Attenuated	virus shell comprises proteins (i.e., polypeptide: amino acid groups)	aqueous	Yes
Inactivated	virus shell comprises proteins (i.e., polypeptide: amino acid groups)	aqueous	Yes

An approach that addresses the risks of both aspects is use of the highest performing elastomer stopper platform, namely elastomer stoppers with FluroTec® film. See Figure 1.

1. FluroTec film has been demonstrated to reduce migration of leachables from the stopper, reduce interaction of proteins with the stopper, and as part of vial systems, enable excellent container closure integrity down to -80°C. These features indicate enhanced preservation of drug product quality and safety, as compared to stoppers without film.
2. Elastomer stoppers with FluroTec film are available worldwide, in serum and lyophilized configurations, in 13 mm and 20 mm sizes, and are compatible with vial systems down to -80°C.

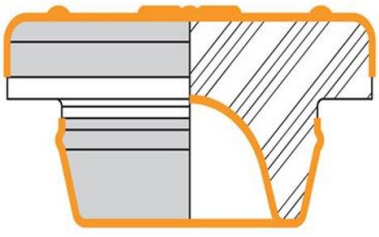


Figure 1. Schematic of FluroTec Film on an Elastomer Stopper

Timelines for SARS-CoV-2 vaccines are accelerated; ordinary evaluations cannot be performed. The package systems must be selected quickly, and this creates risks. These risks can be addressed by selection of the highest performing elastomer stoppers, namely those with FluroTec film. They have been shown to have lowest potential interaction with drug product, and

hence are the best option to maintain quality and safety. This article discusses FluroTec film in detail: market presence, chemistry, and the experimental work that demonstrates performance.

FluroTec Film

Market Acceptance

Elastomer components with FluroTec film have gained global acceptance. Based upon West analyses, they are used on over 125 approved drug products, 30 of which are novel drugs, and at least three of which are vaccines. See Table 2. Concentrations of active pharmaceutical ingredients range from approximately 0.1 mg/ml to 600 mg/ml. Packaging in vial/stopper and syringe/cartridge systems include, but are not limited to, the administration routes of intravenous, subcutaneous, and intramuscular.

Table 2. Drug Products Approved with Components Comprising FluroTec Film

Type	FDA Only	FDA and EMA	EMA Only	Total
Small Molecule	44	14	2	60
Monoclonal Antibody	7	19	3	29
Protein	6	17	---	23
Peptide	6	2	---	8
Protein Small Molecule	2	2	---	4
Oligonucleotide	---	2	---	2
Carbohydrate	1	---	---	1
Total	66	56	5	**127**

Novel drugs, as defined by FDA, are those that are not yet approved, but serve unmet needs or advance patient treatments, either new molecular entities or new therapeutic biologics. (2) Novel drugs typically are classified as first-in-class, rare diseases, expedited development, and review pathways. The expedited designation includes fast track, breakthrough therapy, priority review, and accelerated approval. These 30 novel drugs comprise the range of types: small molecules, proteins, peptides, monoclonal antibodies, and oligonucleotides. Since 2015, one-third of drug approvals using West and Daikyo Seiko, Ltd. components with FluroTec film were novel drugs. Where new molecules are involved and rapid decisions are needed, components with FluroTec film are a frequent choice.

Chemistry

Because of the electronegativity of fluorine and the strength of carbon-fluorine bonds, fluoropolymers are chemically inert. This feature has resulted in a vast array of applications – one of which is films for elastomer components in drug product package/systems. Among the many fluoropolymers known, West and Daikyo Seiko, Ltd. use poly(ethylene tetrafluoroethylene) (ETFE) for its FluroTec film. This is based on:

- moldability
- adhesion to elastomers (either bromo- or chloro-butyl)
- translucency
- compatibility with sterilization by either autoclave or gamma irradiation

The structure of ETFE is shown in Figure 2.

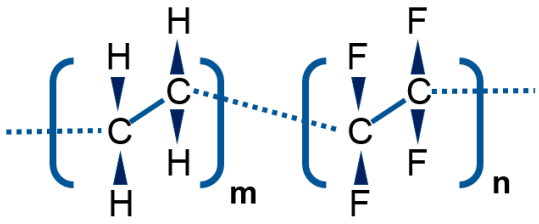


Figure 2. Structure of Poly(ethylene tetrafluoroethylene) (ETFE)

Performance of FluroTec Film

Discussed is published research at West that demonstrates the performance of FluroTec film, namely the ability to:

- reduce interaction with drug product
- reduce migration of leachables
- enable good container closure integrity of a vial/stopper package system

and explains why stoppers with FluroTec film are the lowest risk option for the six current platforms of SARS-CoV-2 vaccines. (3-6)

Interaction with Drug Product

Because of very low surface energy, fluoropolymers such as ETFE have very low levels of interactions with other compounds. Interactions of drug products with package/delivery system components may have deleterious effects, such as immunogenicity due to formation of particles. (7) Interaction was evaluated by measurement of particles, turbidity, and recovery of drug products (simulated/commercial) under agitated/stressed conditions. (3)

Four protein-based products were evaluated. This was done by placing 3 ml of aqueous solution (1 mg/ml) into 5 ml glass vials, then capping with 20 mm elastomer stoppers, with and without FluroTec film. Vials were agitated end-over-end (room temperature at 40 revolutions/minute):

- for 2 hours and 6 hours – particle level was measured by dynamic fluid imaging
- for 24 hours – protein recovery was measured by size exclusion high performance liquid chromatography; turbidity was measured by light absorbance (350 nm)

Results are shown in Table 2. For a variety of proteins, stoppers with FluroTec film resulted in lower levels of particle formation, lower turbidity, and higher protein recovery. These results demonstrate reduced interaction with drug product and mitigation of the effect of elastomer.

Referring to Table 1, four vaccine platforms are protein-based; one is a recombinant protein, and three are virus–based (the outer shell of a virus comprises proteins). This research indicates that use of stoppers with FluroTec film would result in less interaction with these platforms, and concomitant better stability. Reduced risk comprises not only lower levels of particles, but reduced possibility of unwanted changes resultant from interaction. For the other vaccine platforms, RNA and DNA, a similar analysis applies. FluroTec film, based on chemical inertness, would be expected to interact less with these platforms that comprise not only amino/amide groups (building blocks of proteins), but ribose groups and charged phosphate groups.

Table 2. Levels of Particles, Turbidity, and Recovery Resultant for Protein-Based Products, with and without FluroTec Film. Values for particles are in thousands. Parenthetical numbers are standard deviations. (3)

	Particles per ml (1-10 µm)		Turbidity at 24 hrs (a)	Recovery at 24 hrs (%)
	at 2 hrs	at 6 hrs		
β-Lactoglobulin				
with	24.5 (6.0)	58.2 (25.3)	0.07 (0.005)	99.3 (0.13)
without	87.7 (20.7)	181.7 (29.7)	0.10 (0.006)	98.9 (0.08)
Immunoglobulin				
with	23.5 (7.9)	44.0 (16.0)	0.01 (0.002)	98.2 (0.13)
without	94.6 (28.8)	289.6 (172.2)	0.04 (0.004)	95.6 (0.26)
Abatacept (fusion protein)				
with	12.8 (11.0)	41.0 (11.2)	0.02 (0.001)	99.1 (0.3)
without	14.3 (7.2)	64.2 (29.2)	0.03 (0.005)	97.3 (0.3)
human serum albumin – recovery at 21 days (%) (b)			with	98.6 (0.3)
			without	78.6 (0.4)

a. absorbance at 350 nm
b. not agitated, stored quiescently at room temperature

Leachables

Based upon chemical inertness, hydrophobicity, and dense packing of chains, fluoropolymers such as ETFE can mitigate leaching (migration of compounds/elements from elastomer component into drug product). This is accomplished by acting as a barrier that prevents transport of compounds/elements. In other words, ETFE prevents the two processes that comprise permeability: (a) diffusion (movement of leachables from elastomer) and (b) partitioning (drug product excipient migrating into elastomer and withdrawing leachables).

In one study, bromobutyl elastomer lined seals, with and without FluroTec film, were crimped onto empty 10 ml glass vials and stored up to six months at room temperature. (4) Headspace gas chromatography and mass spectrometry were performed. See Figure 3.

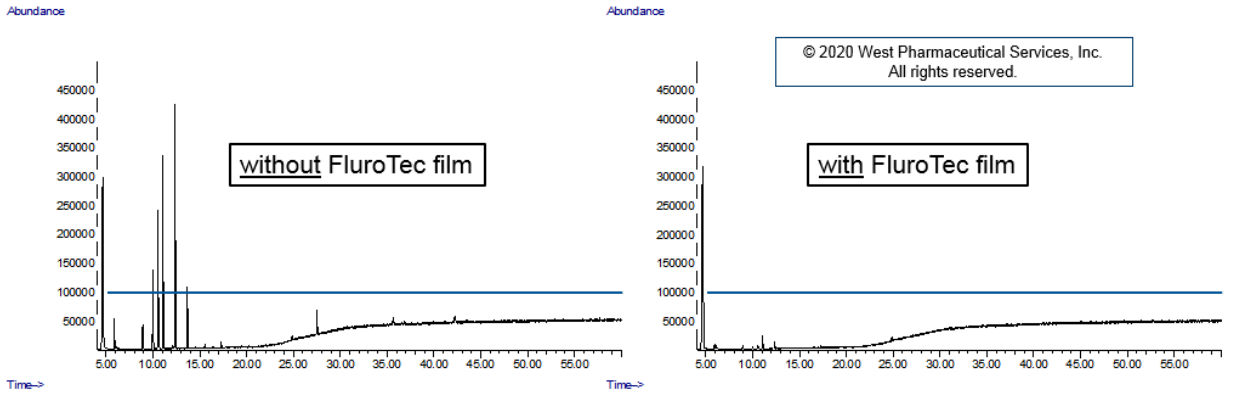


Figure 3. Headspace Gas Chromatography and Mass Spectrometry of Lined Seals, with and without FluroTec Film. Data are at six months. Blue line indicates an estimated identification threshold of 0.5 µg/unit. (4)

A large number of compounds were observed for the system without film, virtually none for the system with film. The drawn blue line indicates an estimated identification threshold of 0.5 µg/unit, which is lower than the Product Quality Research Institute recommended safety concern threshold for parenteral drug products. (8) Mitigation of leachables was achieved. Similar results were observed with chlorobutyl elastomers.

Referring to Table 1, vaccine vehicles can be polar (aqueous) or non-polar (lipid-based); moreover they may contain numerous ingredients (aluminum salts, oil-in-water emulsions, antibiotics, formaldehyde, sugars, amino acids, buffering agents, surfactants). (9) Leachables, whether inorganic (e.g., metal ions/salts) or organic (e.g., oligomers, antioxidants) pose a risk since interaction with vehicle or ingredient may affect quality or safety. For example, a metal salt may affect the lipid system encapsulating an RNA vaccine. Leachables also pose a risk to the vaccine itself. For example, a metal ion or organic compound could interact with DNA or a recombinant protein, alter the configuration, and render it less effective. The possibilities are numerous and very difficult to predict; use of a FluroTec film mitigates them.

Container Closure Integrity

Good container closure integrity (CCI) performance is essential; it demonstrates that a package system can meet the requirements of the maximum allowable leakage limit (MALL) of a drug product. MALL is discussed in detail in United States Pharmacopeia Chapter <1207> *Package Integrity Evaluation – Sterile Products* (2016). Stoppers were examined, with and without FluroTec film. (5, 6)

In one study, 20 mm stoppers were capped onto 6R glass vials under air at varying compression levels. Deterministic evaluation methods (endorsed by USP <1207>) were:

- tracer gas leak detection (with helium) (i.e., He-Leak): stored in air at room temperature
- frequency modulated spectroscopy headspace analysis (with oxygen) (i.e., OHS): stored in nitrogen at room temperature

Evaluation was made over two years. See Figures 4 and 5. (5)

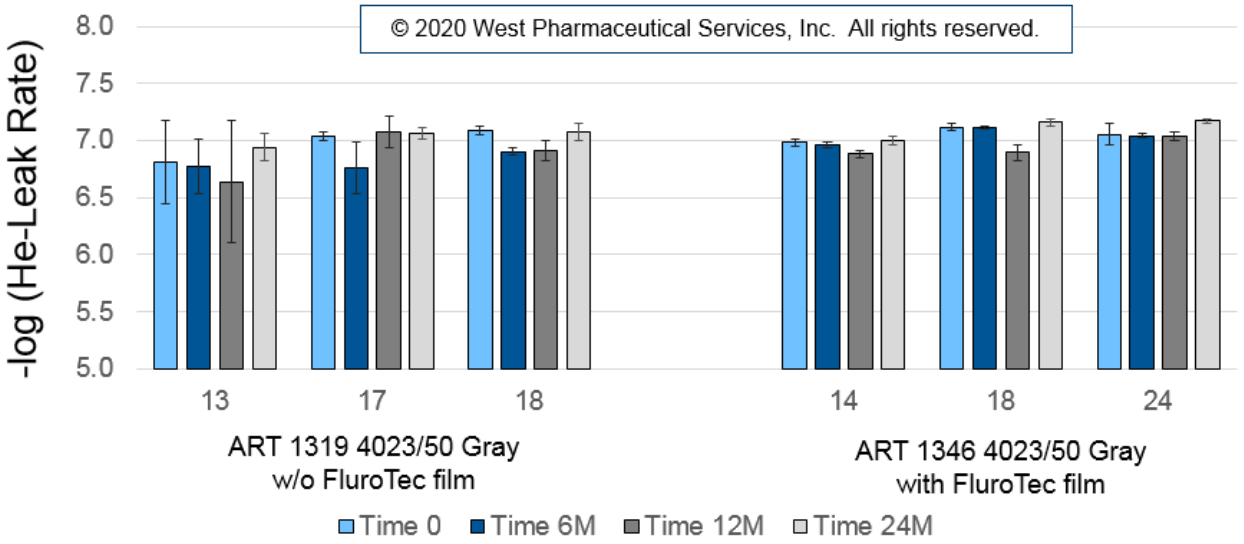


Figure 4. He-Leak Performance over Two Years. Data are reported as [-log of helium leak rate (cm^3/s)]. Initial values of compression are given (e.g., 13%). Error bars are standard deviation. (5)

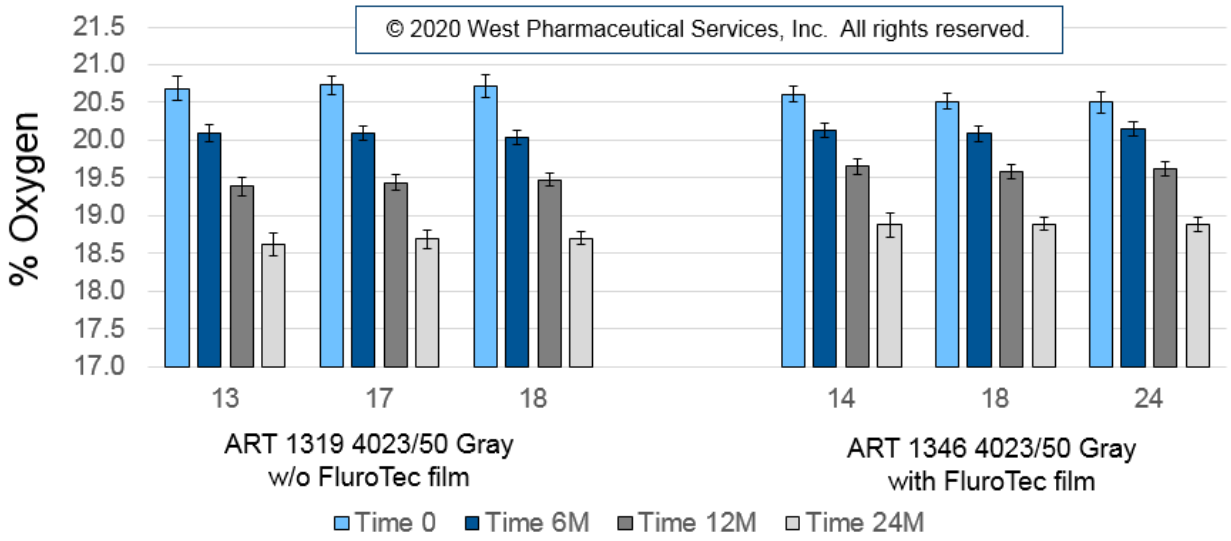


Figure 5. OHS Performance over Two Years. Initial values of compression are given (e.g., 13%). Error bars are standard deviation. (5)

For He-Leak, performance of systems with and without film was similar. Values that exceed 6.6 would correspond, per the well-known Kirsch study (10), to 0% risk of microbial ingress. For OHS, performance of systems with and without film was likewise similar. There is some egress of oxygen over time, a very small amount. This was expected; it is well known that elastomers are gas permeable. Stoppers with FluroTec film enable excellent CCI, there is no loss of performance resultant from presence of FluroTec film.

In another study, 13 mm stoppers with FluroTec film were capped onto 2R glass vials under nitrogen and stored in an air-filled freezer at -80ºC for 60 days. (6) There was essentially no ingress of oxygen observed by OHS. This indicates excellent CCI at -80ºC.

Summary

Elastomer stoppers with FluroTec film offer best performance. They mitigate migration of leachables from elastomer and minimize elastomer interaction with drug product. Moreover, they enable excellent package system container closure integrity. They are globally available, market accepted, and available in varying sizes and configurations (13 mm, 20 mm, serum, lyophilization). For these reasons, for the six platforms considered for SARS-CoV-2 vaccines, they offer the lowest risk. Reduced risk is essential since accelerated timelines do not permit standard evaluation of drug product with package system.

References

1. Amanat, F. and Krammer, F. *SARS-CoV-2 Vaccines: Status Report*. Immunity, 52, 583-589 (April 14, 2020)
2. *New Drugs at FDA: CDER's New Molecular Entities and New Therapeutic Biological Products.* https://www.fda.gov/drugs/development-approval-process-drugs/new-drugs-fda-cders-new-molecular-entities-and-new-therapeutic-biological-products (5/14/20)
3. Singh, R., et al. *An Investigation to Examine the Effect of the Elastomeric Surface Treatment on Protein Stability*. Journal of Pharmaceutical Sciences, in preparation (2020)
4. *FluroTec® Products Protecting Drug Product Quality and Safety*. West Pharmaceutical Services, Inc. Technical Report 2019/210 (2019)

5. M. Gehrmann and P. McAndrew. *Long-Term Study of Container Closure Integrity of Rubber-Glass Vial Systems by Multiple Methods*. PDA Journal of Pharmaceutical Science and Technology, 74 (1), 147-161 (2020)
6. *Ingress of Gases into Cyclic Olefin Polymer Vial-Based Container Closure Systems at -80°C*. West Pharmaceutical Services, Inc. Technical Report 2019-028 (2019)
7. Barnard, J.G., et al. *Characterization and Quantitation of Aggregates and Particles in Interferon-β Products: Potential Links between Product Quality Attributes and Immunogenicity*. Journal of Pharmaceutical Sciences, 102 (3), 915-928 (2013)
8. *PQRI-PODP Working Group Recommendations*. http://pqri.org/wp-content/uploads/2017/02/CombinedPQRI-PODPSlides_PQRI-FDA_22Mar2017.pdf (5/14/20)
9. *Common Ingredients in U.S. Licensed Vaccines.* https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/common-ingredients-us-licensed-vaccines (5/14/20)
10. Kirsch, L.E., et al. *Pharmaceutical Container/Closure Integrity II: The Relationship between Microbial Ingress and Helium Leak Rates in Rubber-Stoppered Vials.* PDA Journal of Pharmaceutical Science & Technology, 51 (5), 195-202 (1997)

FluroTec® Products Protecting Drug Product Quality and Safety

I. Background

FluroTec® is the brand name for various West proprietary ethylene tetrafluoroethylene (ETFE) copolymer films that are laminated onto elastomeric components including stoppers and plungers to create a protective barrier between the drug product and the elastomer[1]. The purpose for creating a barrier is to reduce the risk of chemical migration from elastomers into drug products (leachables) which can potentially compromise drug product quality, stability and/or safety. Protein products are especially susceptible to leachables causing quality issues associated with degradation, aggregation and impurities arising from leachables.[2] This is a dynamic process involving permeation, diffusion and partitioning, the mechanisms are explained herein along with experimental data. This information is applicable to the laminated family of products including Daikyo Flurotec® film coated elastomers.

Permeation is the migration of chemicals through the thickness of a polymer via volatile liquid transport. Chemicals can permeate through an elastomer to the surface based on the free volume within the polymer, mobility of polymer chains and solubility of the chemical migrant. A protective barrier such as FluroTec® can block or diminish migration of chemicals and prevent partitioning (leaching) into a drug product. The type and amount of leachables will depend on the component configuration, type of contact material, extraction propensity of the drug product and other environmental factors. This is a dynamic process involving permeation, diffusion and partitioning as described below:[3]

- **Permeation:** Mass transport of a penetrant across the film at a rate = $\frac{cc\ x\ mil}{m^2\ x\ day\ x\ atm}$
    - Soluble penetrant molecules will diffuse through the film depending on the permeant concentrations and temperature.
    - Permeation is influenced by forces holding together polymer chains.
        - Diffusivity is lower with tightly packed polymer chains
        - Increased temperature will expand polymer free volume
    - Film **P**ermeability = **S**olubility x **D**iffusion
        - The greater solubility of the permeant in the film the greater permeability
        - The greater the diffusion rate the greater the permeability
- **Diffusion:** Penetrant molecules will move inside the bulk polymer at a rate expressed as $\frac{cm^2}{sec}$
    - The diffusion process involves molecular flux through a unit area that is proportional to the concentration gradient.
        - Diffusion rate will be variable based on temperature, contact duration, material configurations (contact area, thickness, mass), penetrant concentration and solubility in the drug product.
        - The mass diffusivity of a migrant molecule is indicated by its molar volume/size and diameter/shape.

[1] TSB 2009/033: Overview of FluroTec® and B2-Coating

[2] Wei Wang et.al. Impact of Residual Impurities and Contaminants on Protein Stability, Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23931y,

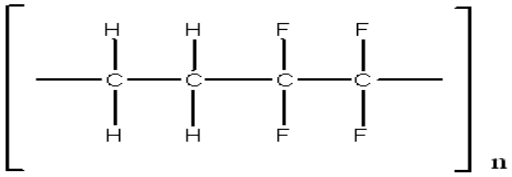
[3] Pringer O.G. Plastic Packaging: Interactions with Food and Pharmaceuticals, SBN: 978-3-527-31455-3

- The mechanism is based on desorption of diffused molecules from the material surface and sorption at the packaging-product interface, then drug product partitioning (leachables).

- **Partitioning**: Movement of molecules (migrants) from one phase to another.
  - The partition coefficient (K) is the ratio of the concentration of a migrant in the polymer and in drug product at equilibrium.
  - The type and amount of leachables will depend on the material's resistivity to chemical attack, extraction propensity of the drug product formulation and solubility of the migrants.

Some chemical species readily migrate from the bulk elastomer into a drug product, but leachables can be mitigated by applying a barrier film such as FluroTec film. A barrier film can delay, diminish or even block chemical migration. FluroTec film is a barrier film that provides an advantage over other types of coatings because ETFE is comprised of closely packed fluorine and carbon backbone which is very strong and resistant to chemical attack. FluroTec laminated products have a unique surface treatment to achieve the best conformity during molding process and provides a strong and long-lasting adhesion with the elastomer components. This added layer of FluroTec film will reduce the number and amounts of migrants originating from the elastomer.

ETFE Backbone



Application of FluroTec film has been shown to reduce leachables based on both clinical use as well as empirical data, demonstrating reduction of gas permeation into vial headspace and minimal to no migration into liquid when challenged with strong solvents and harsh conditions.

- Clinical examples demonstrating protective barrier properties include:
  - Eprex® packaged using FluroTec laminated stoppers reduced the incidence of pure red cell aplasia (PRCA) by creating a barrier to a rubber accelerator leaching from the elastomer See Figure 1. Incidence of PRCA before and after FluroTec[4] film
  - Metal ion induced degradation of epinephrine in Lidocaine-Epinephrine Injection resulting in lidocaine ineffectiveness was mitigated with FluroTec laminated stoppers.[5] See Figure 2. Aluminum Complex formed with Epinephrine

[4] Boven, K, et.al., The increased incidence of pure red cell aplasia with an Eprex formulation in uncoated rubber stopper syringes, Kidney Int. 2005 Jun;67(6):2346-53.
[5] Milano E.A. et.al., Aluminum Catalysis of Epinephrine Degradation in Lidocaine Hydrochloride with Epinephrine Solutions. J Parenter Sci Technol. 1982 Nov-Dec;36(6):232-6.

Figure 1. Incidence of PRCA before and after FluroTec® Film (adapted from [4])

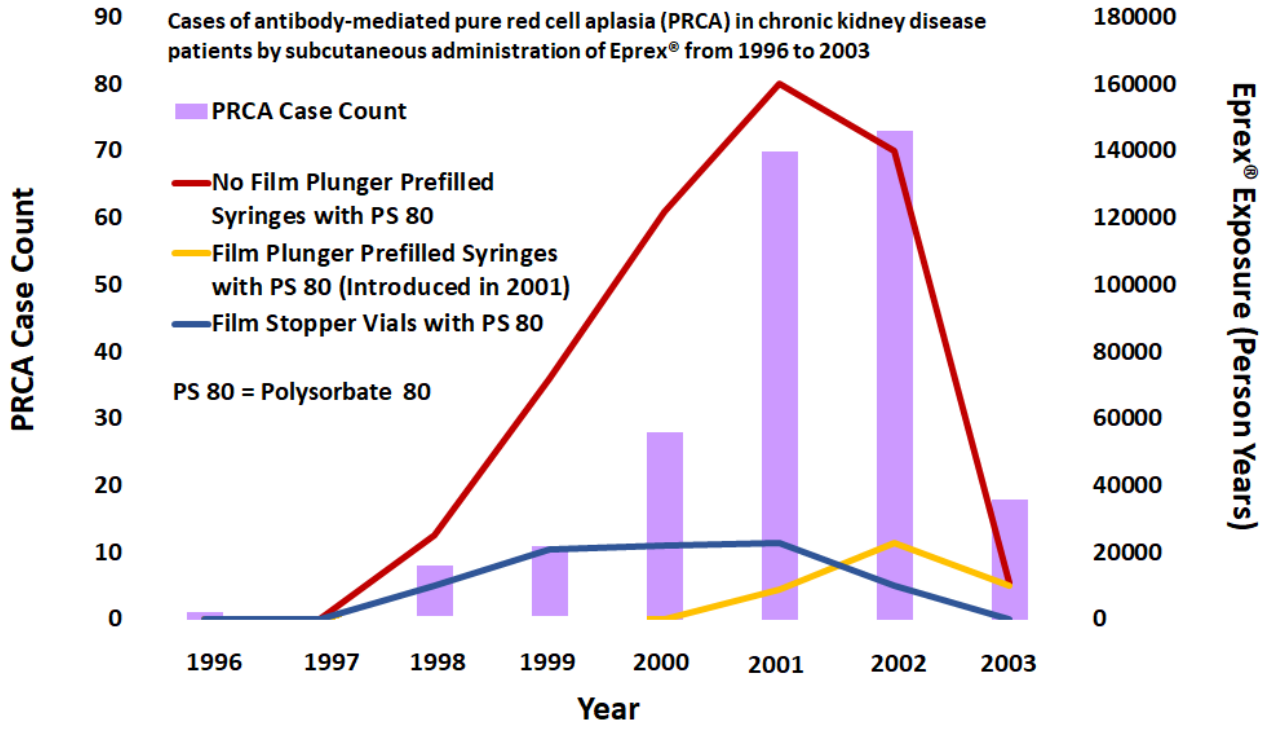
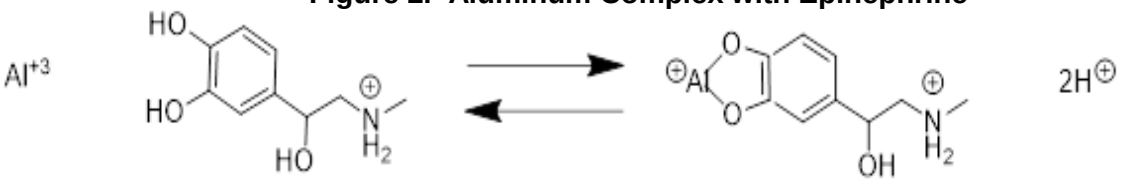


Figure 2. Aluminum Complex with Epinephrine



The maximum amount of migrant that can leach will depend upon the concentration in the elastomer, conditions of use and equilibrium between the drug product and migrant. Volatile organic compounds (VOCs) are more likely to permeate due to the small size and simple structures of the permeants. The VOC methods are highly sensitive and often reported in units of ng/mL (ppb) compared to semi volatile compounds (SVOCs) in liquids that would be at µg/mL (ppm) concentrations. SVOC are larger and the structures can be hindered affecting migrant diffusion and solubility.

The type of migrants that could permeate through the FluroTec film will depend upon the individual drug product, the elastomer formulation, component contact area and conditions of use. There is a wide variety of interrelated factors to be considered. To understand the type of compounds that can be blocked, experiments were conducted under different conditions to demonstrate: i) the potential for VOCs to permeate through FluroTec film, and ii) FluroTec barrier effectiveness with SVOCs and the resistance to chemical attack when stressed at elevated temperatures and strong solvent overtime.

II. Experimental

The predominant elastomers used in primary container systems for long-term storage of injectable delivery systems are bromobutyls and chlorobutyls, which are the subjects of this study. The elastomers were prepared in the form of seals and plaques to maximize the sample contact area.

Analysis	Sample Description
VOC	Bromobutyl Elastomer Discs Inserted into Seals
VOC	FluroTec® Laminated Bromobutyl Elastomer Discs Inserted into Seals
VOC	Chlorobutyl Elastomer Discs Inserted into Seals
VOC	FluroTec Laminated Chlorobutyl Elastomer Discs Inserted into Seals
SVOC	Spiked Bromobutyl Plaque
SVOC	FluroTec Laminated Spiked Bromobutyl Plaque

i) VOC Permeants in vial headspace[6]

a. The rubber lined seals with and without FluroTec® film were capped onto 10mL vials filled with ambient air, using pneumatic crimper. Blanks were prepared by capping with Gerstel seals. All prepared vial samples were stored in upright positions, at 25 ˚C ± 2 ˚C/60% RH ± 5% and tested at T=0, 3M and 6M. The samples were analyzed using headspace gas chromatography/ mass spectrometry (GC/MS-03) for VOCs.
b. The VOCs estimated >0.5µg/unit were identified and the identified compounds in the chromatograms of elastomers with and without film were compared to semiquantitative indicate the effectiveness of FluroTec film.

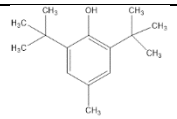
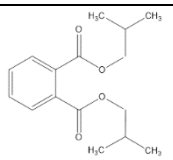
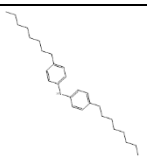
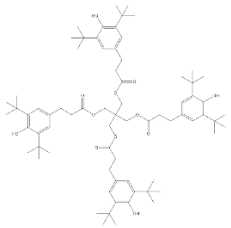
ii) SVOC Migrants into liquid[7]

a. Bromobutyl plaques were spiked with known compounds to determine effectiveness of barrier properties. The spiked compounds, illustrated in Figure 3, were chosen independent of the formulation ingredients to represent a range of molecular weight and structural configurations that could potentially permeate the film and those not likely to permeate. FluroTec laminated and non-FluroTec laminated plaques were prepared to minimize thickness (diffusion distance) and analyzed after exposure of the laminated and non-laminated surfaces in 100% 2-propanol. The conditions of exposure were intentionally stressed to demonstrate that FluroTec film was robust enough to withstand harsh conditions.
b. The sample vials were inverted and were tested under 40 °C, 60 °C and 80 °C up to 15 days. Temperatures at 60 and 80 °C are above normal accelerated conditions but were used to challenge the barrier properties to show robustness.

[6] West Analytical Service report :2017001192 Rev. 2 Extractables and Leachables Evaluation of Barrier Property of FluroTec® Laminated Rubber Lined Seals
[7] Data adapted form Paskiet D. Biopharmaceutical Quality: Factoring Risk Into the Extractables Equation: presented at Peptalk 2015

c. The solutions were analyzed for the spiked target compounds using reversed phase HPLC-DAD. The data from the laminated and non-laminated samples were compared to evaluate the barrier effectiveness. The migration of spiked compounds from non-laminated and FluroTec film-coated samples were calculated as µg/cm^2 and converted to % migrated compared to the % blocked with FluroTec film.

Figure 3. Spiked Compounds Independent of Formulation Ingredients

Potential Migrant	Spike parts/100parts	MW g/mol	Shape/Structure
BHT	0.10	220	
DIIBP Diisobutyl phthalate	0.10	278	
DODP Dioctyldiphenlyamine	0.08	393	
Irganox® 1010	2.6	1178	

III. Results & Discussions

VOC

The non-laminated elastomers showed approximately eight VOC peaks estimated to be approximately >0.5µg/unit at all time points. Elastomers with FluroTec® film did not show any peaks >0.5µg/unit, the effectiveness of the FluroTec film was demonstrated even for the most volatile compounds. The chromatograms of bulk and FluroTec film-coated chlorobutyl and bromobutyl elastomers Figures- 4-9, illustrates suppression of VOCs. These chromatograms are semiquantitative and the results expected to be variable. A line is drawn across the chromatogram to indicate an estimated identification threshold of 0.5µg/unit. This is below the PQRI recommended safety concern threshold for parenteral products.[8] Observations of the peaks showed increasing

[8] PQRI-PODP Working Group Recommendations; http://pqri.org/wp-content/uploads/2017/02/CombinedPQRI-PODPSlides_PQRI-FDA_22Mar2017.pdf

levels of VOC over time as indicated by higher abundance (y axis). The chromatograms are scaled appropriately to the same level for each time point to allow for peak comparison. This data is indicative of the experimental system analyzed and would need to be verified for the intend systems for the drug product.

Figure 4. Chlorobutyl - T0

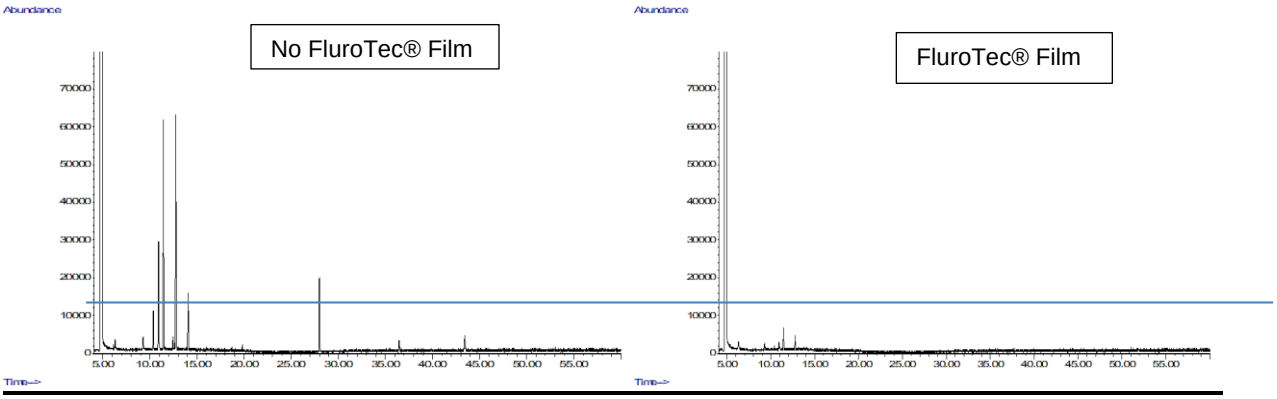


Figure 5. Chlorobutyl - T3

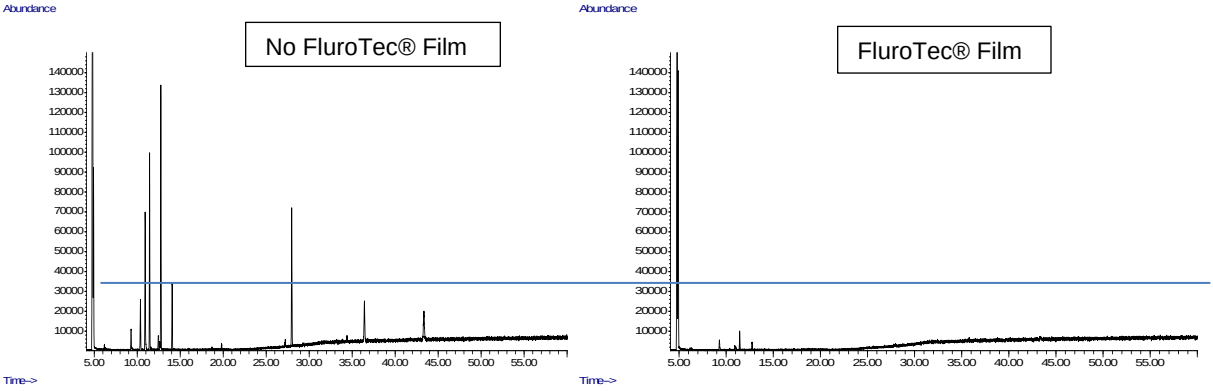


Figure 6. Chlorobutyl - T6

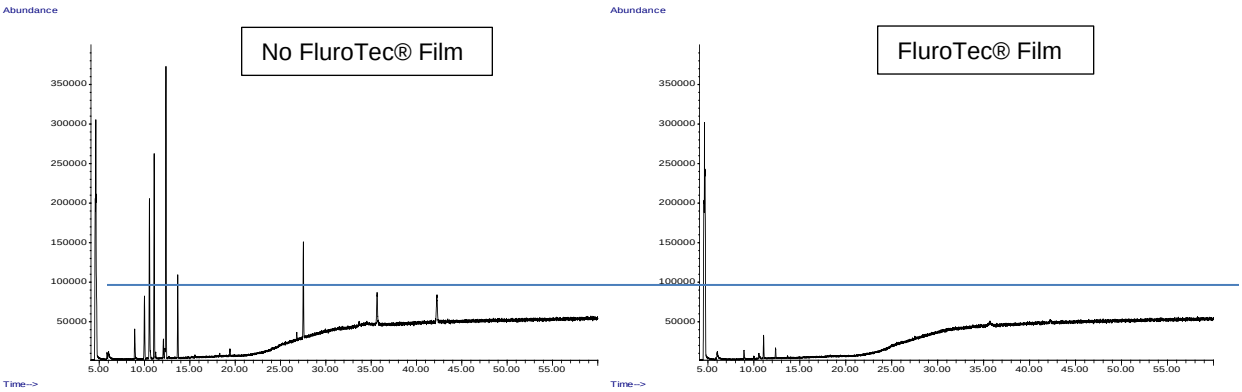


Figure 7. Bromobutyl -T0

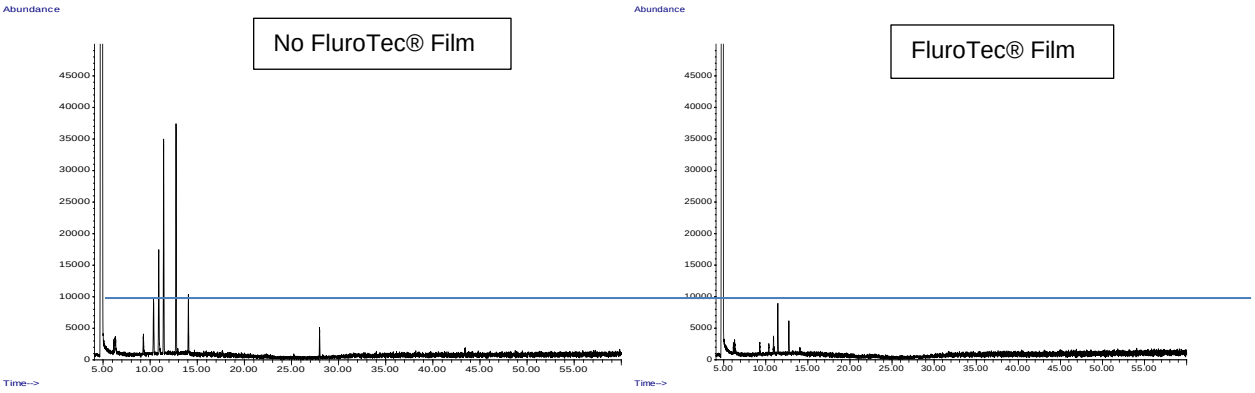


Figure 8. Bromobutyl -T3

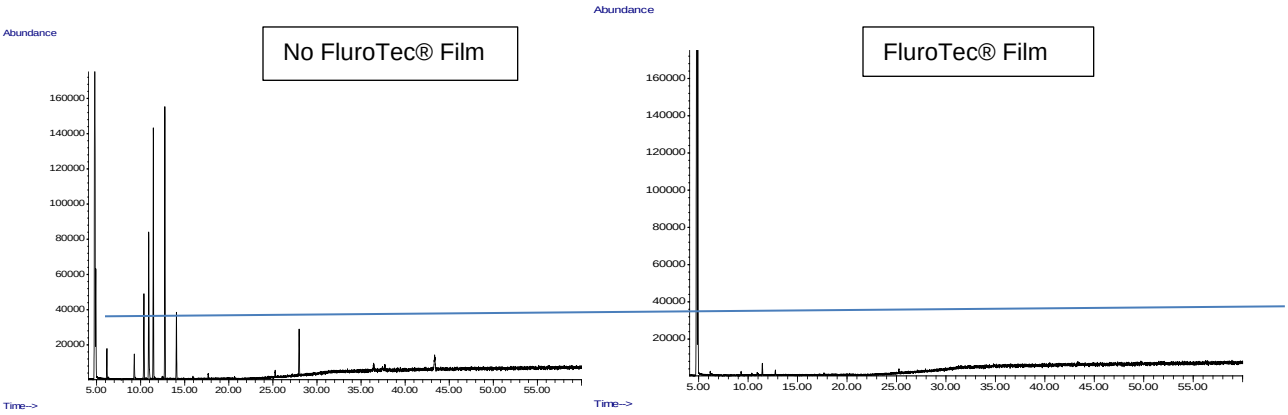
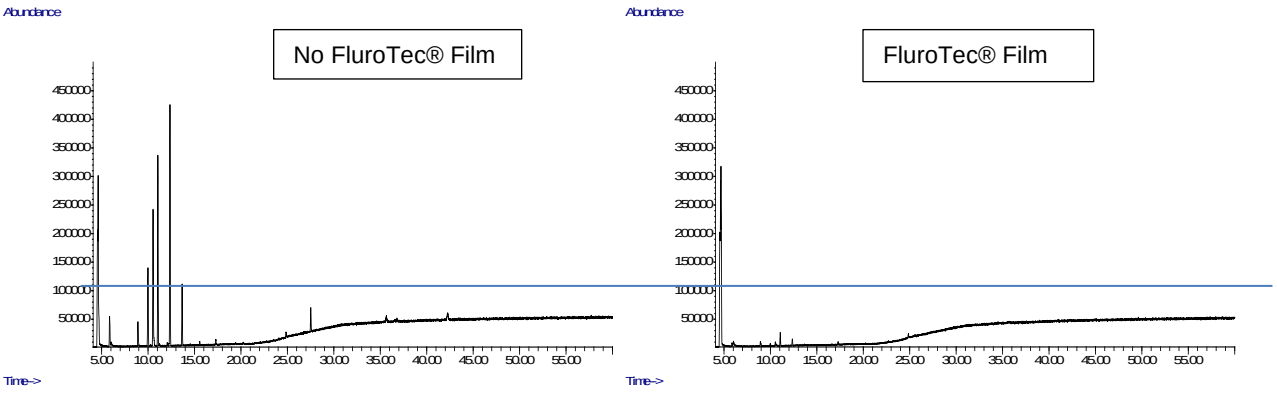


Figure 9. Bromobutyl -T6



SVOC

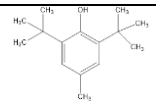
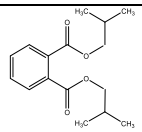
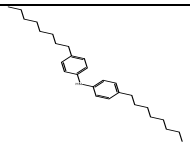
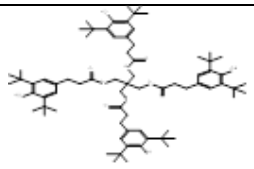
The barrier effectiveness of FluroTec® film is illustrated based on four target compounds that were spiked into the elastomer formulation and extracted with IPA. The targets were measured and calculated as % migrated vs % blocked. Given the polar nature of BHT and DIDP and molecular weights <350 Daltons these compounds can readily migrate. The data showed however, even under harsh conditions the BHT and DIDP were 99% blocked at 40 °C and 60 °C. At 80 °C high levels of BHT and DIDP migrated from the bulk, that would allow for a higher accumulation at the elastomer-film interface. Minimal BHT and DIDP was seen at 80 °C but considered negligible compared to the amount spiked.

Irganox® 1010 and DODPA were 99% blocked at all three temperatures. Irganox 1010 was spiked at very high concentration with greater potential to accumulate at the elastomer-film interface. Considering the atypical conditions of extraction, the blockage of >99% demonstrated the effectiveness and robustness of FluroTec film.

This data does demonstrate that FluroTec film is an effective barrier at accelerated and exaggerated conditions, but the actual drug product system would need to be tested over real time to verify the occurrence of leachables. Typically, accelerated exposure would not reflect actual rate of migration especially when temperatures are greater than 40 °C. The boiling point of 2-propanol is 82 °C which means the laminated stopper surface was stressed to near boiling at 80 °C for 15 days and showed only minimal migration.

A wide range of target migrant solubility was investigated. 2-propanol is relatively polar and the octanol/water solubility coefficients (LogP) of the migrants were between 5.3 (polar) to 18.8 (nonpolar). The % Migrated in Bulk vs % Blocked by FluroTec film, associated Log Ps, structures and molecular weights (MW) are shown n Table 2. The 40 °C and 80 °C chromatograms are shown in Figures 10 and 11.

Table 2. Bulk % Migrated vs % Blocked by FluroTec® Film

TEMP °C	40 °C	60 °C*	80 °C*	Observations
Est. Years Accelerated	**0.15**	**0.6**	**2.5**	
BHT				**MW 220g/mol LogP 5.3** 
Bulk % Migrated	6.9	14.8	36.2	The concentration of BHT increased with higher temperatures but the film essentially blocked BHT at 40 and 60 °C. Penetration of BHT through the film depends on the temperature which influences free volume; BHT is at high risk for penetration because it is relatively small molecule and soluble in IPA. At 80 °C the diffusion of BHT in the bulk elastomer was increased resulting in a high accumulation at the elastomer-film interface, yet > 80% of BHT was blocked.
FluroTec® Film % Blocked	99.9	99.6	83.1	
DIBP				**MW 278 9/mol LogP 4.4** 
Bulk % Migrated	4.3	10.4	27.3	The diffusion of DIDP increased with temperature and essentially blocked by the film at 40 °C and 60 °C. DIBP is a polar compound and soluble in IPA resulting at higher accumulation at the elastomer film interface. At 80 °C the DIDP concentration was higher yet blocked > than 90%. This illustrates that even under harsh atypical conditions FluroTec® film will minimize migration even for molecules that are at high risk for migration.
FluroTec® Film % Blocked	99.8	99.6	90.8	
DODPA				**MW 393g/mol LogP 11.6** 
Bulk % Migrated	0.9	1.6	2.7	DODPA did not readily diffuse through the bulk polymer, it is a non-polar compound and at the harshest conditions DODPA was essentially blocked at all temperatures with FluroTec® film.
FluroTec® Film % Blocked	99.4	99.9	99.7	
Irganox® 1010				**MW 1178g/mol Log P 18.8** 
Bulk % Migrated	5.4	15.5	32.2	Irganox 1010 is a larger nonpolar molecule, it was spiked at a high concentration compared to the other compounds. Although diffusion occurred in the bulk polymer, FluroTec® film essentially blocked Irganox 1010 at all temperatures.
FluroTec® Film % Blocked	99.6	99.8	99.8	

***Extreme Accelerated Conditions**

Figure 10. Chromatograms at 40 °C

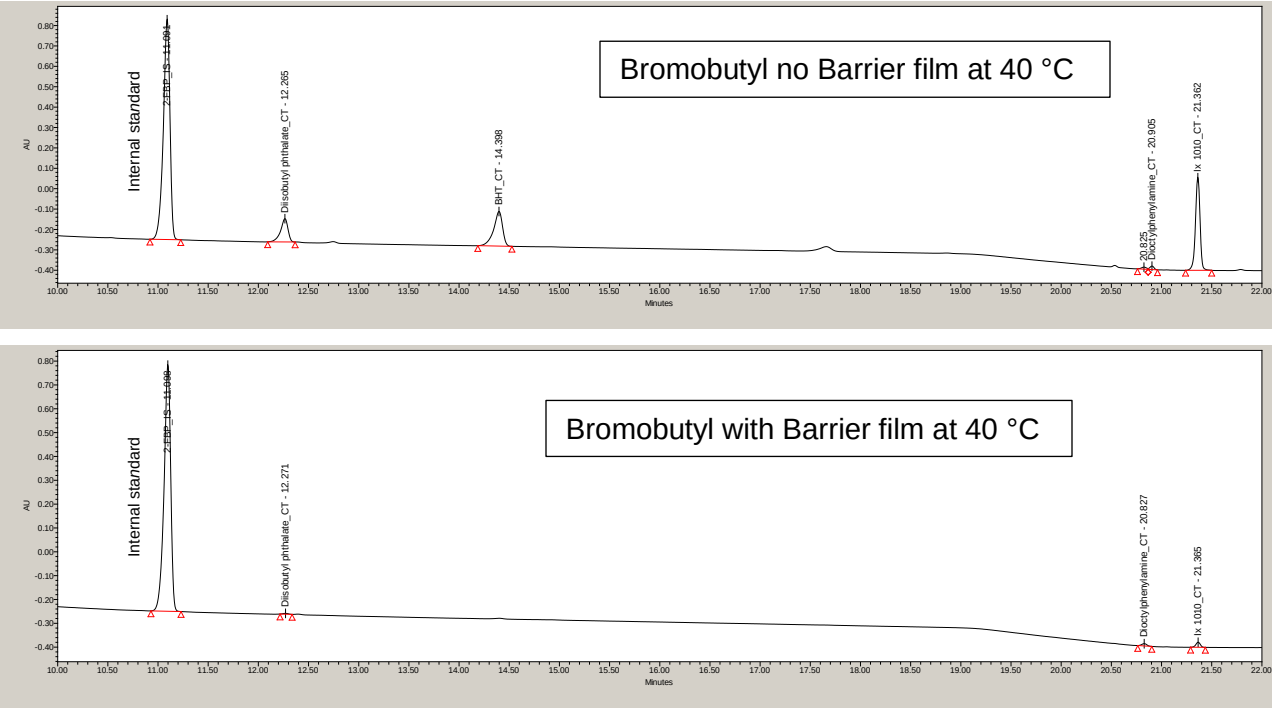
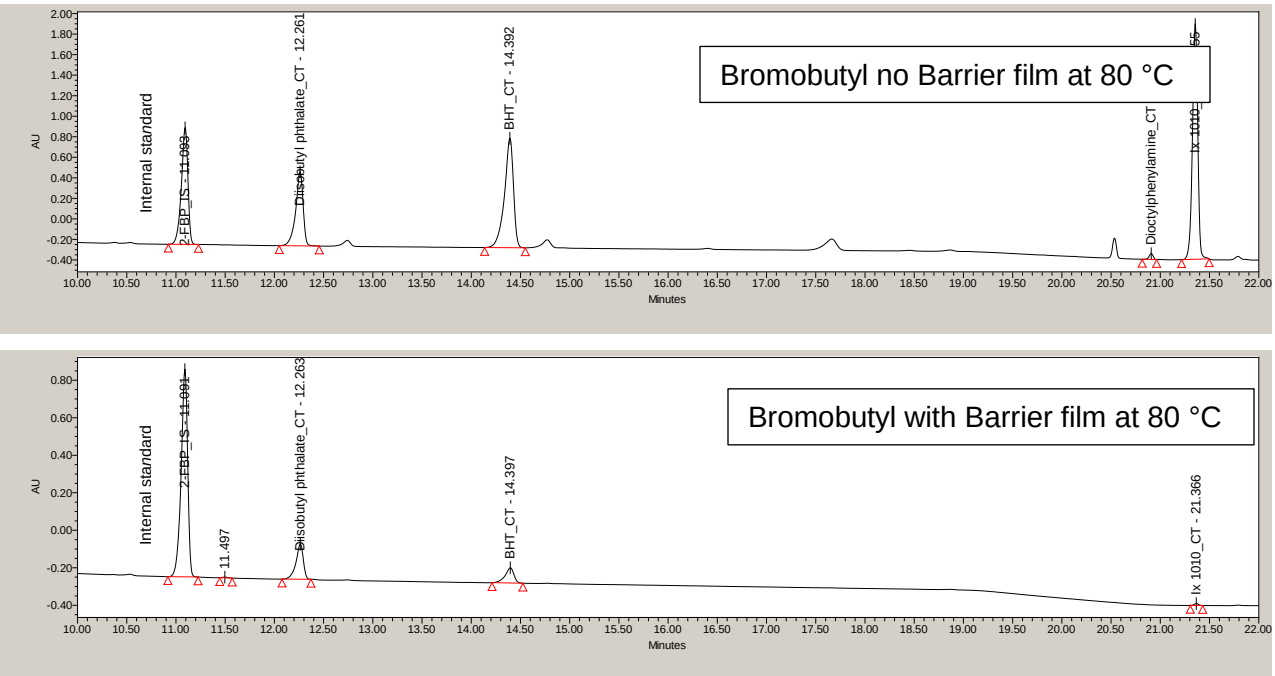


Figure 11. Chromatograms at 80 °C



IV. Conclusions

FluroTec® film does mitigate the risk for leachables even under extremely harsh conditions. The FluroTec film is resistant to chemical attack, has a high degree of chemical and physical stability, low toxicity and reactivity.[9] The film is compliant with biocompatibility for hemocompatibility, systemic toxicity and cytotoxicity.[10]

Leachables depend upon various interrelated factors associated with the drug product and its environment. The diffusion rate of extractables in bulk polymer is a critical factor for leachables accumulation over time and the permeation rate of barrier film is critical to blocking leachables. Extractables data will provide the basis for understanding the type and amount of potential leachables and risk to drug product quality. Extractables and leachables will have a high degree of variability based on the component chemistry, migrant equilibrium constants, component surface contact area, drug formulation, conditions of exposure and duration.

FluroTec barrier film has been on the market since the 1990s and used with small and biologic molecule drug products. It has been proven to successfully reduce the risk of chemicals that can migrate from elastomers based on clinical experience and migration studies.

West's products are sold on the basis that it is the customer's responsibility to evaluate and test the West product to determine its compatibility with other materials and fitness for any end use.

This *Technical Report* dated 18 November 2019, supersedes any other previously versions of this *report*.

[9] Chemical Compatibility Guide https://www.researchgate.net/.../Chemical-Resistance-Chart-Detail.pdf Guide

[10] Compliance Bulletin

Effect of Container Surface on Protein Aggregation

Ranjana Singh, Cathy Zhao and Lloyd Waxman
West Pharmaceutical Services, Inc.

Return to Chapter 2

Background

USP chapters 787 and 1787 provide guidance to measure and characterize subvisible particles in therapeutic protein drugs. These particles, if present, can induce life threatening immunological reactions in patients (Rosenberg 2006, Rosenberg, et al. 2012). Interactions with the surface material of primary container systems during storage and shipment can induce subvisible aggregates of the therapeutic protein itself. While glass primary containers are predominantly used in the industry, new cyclic olefin polymer (COP) resin offerings may provide reduced protein aggregation of drug product due to the unique surface properties of the COP material. Therefore, our study focused on examining the subvisible particles induced in protein solutions contained in primary containers made of COP resin compared to primary containers made of glass.

Objectives

To compare the stability of IgG and monoclonal antibodies (mAbs) in vials/syringes made of glass and COP subjected to various forms of agitation.

Materials and Methods

- *Vials and Stoppers:* Pre-sterilized 2 mL silicone free vials made of glass or COP, and 13 mm non-siliconized stoppers laminated with FluroTec™ laminate were used in this study. 1 mL long siliconized glass syringes and 1mL long COP syringes were also used.

- *Proteins*
  - IgG and monoclonal antibodies (mAbs) were used in this study.
  - IgG was purchased from Sigma.
  - Late stage investigational drugs (Pro1, Pro2 & Pro3), mAbs were obtained from a partnering pharmaceutical company.
  - Therapeutic protein (Pro4), a mAb was purchased.

- *Buffers*
  - Epo buffer: 20 mM sodium phosphate, 2.7 mM sodium citrate, and 100 mM NaCl (pH 6.9)
  - SE-HPLC elution buffer: 20 mM sodium phosphate and 150 mM NaCl (pH 6.8)

- *Agitation:* The COP or glass vials/syringes containing proteins solubilized in Epo buffer were agitated using either an orbital shaker (OS) at 200 rpm for 4 days or an end over end (EOE) rotator at 15 rpm for 10 days. The end over end mode of agitation was adopted and modified from Teska, et al. 2016.

- *Turbidity:* The baseline and endpoints were measured as absorbance at 350 nm.

- *Flow Imaging:* The baseline and endpoint measures for the protein aggregates were performed on a FlowCam™ 8000 device. The protein aggregates of the estimated spherical diameter (ESD) ranging between 1-100 µm were measured, and reported as particles/mL.

- *SE-HPLC:* It was carried out on an Agilent 1260 liquid chromatography system using a Tosoh TSKgel™ G3000SWxl 7.8 mm x 30 cm column. The protein elution was monitored at 214 nm and 280 nm. The resulting peaks were integrated to calculate protein recovery.

- *Statistical Analysis:* The data is reported as mean and analyzed using a Student's t-test, 2 tailed.

Results

Figure 1: *IgG and mAbs aggregated less in vials made of COP when vigorously agitated using an orbital shaker (OS)*

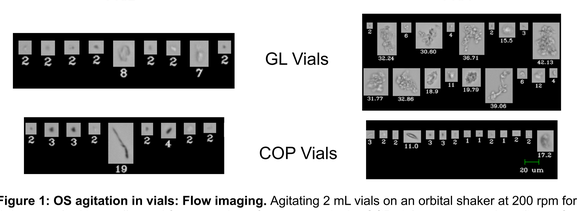


Figure 1: OS agitation in vials: Flow imaging. Agitating 2 mL vials on an orbital shaker at 200 rpm for 4 days resulted in smaller and fewer number of aggregates in the COP vials as compared to silicone free glass vials, for four out of five proteins tested. Pro2 did not show difference between two surfaces. Pro2: Late stage investigational drug, mAb; Pro4: Marketed drug, mAb.

Figure 2: *IgG and mAbs aggregated less in vials made of COP when vigorously agitated using an orbital shaker (OS)*

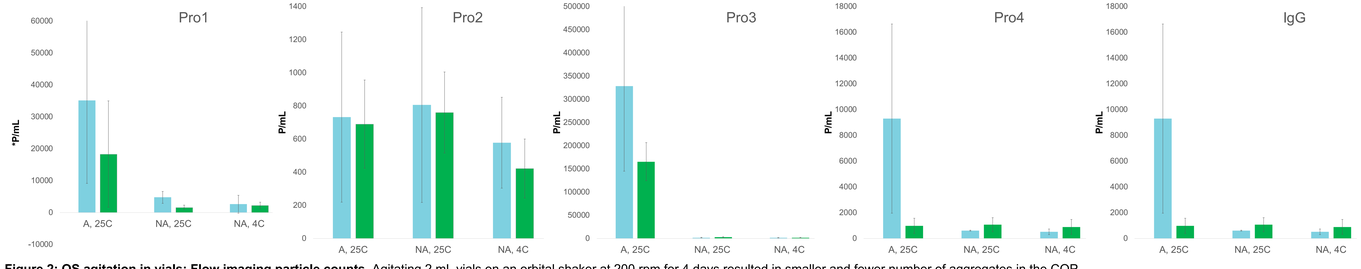


Figure 2: OS agitation in vials: Flow imaging particle counts. Agitating 2 mL vials on an orbital shaker at 200 rpm for 4 days resulted in smaller and fewer number of aggregates in the COP vials as compare to silicone free glass vials, for four out of five proteins tested. Pro2 did not show difference between two surfaces. Pro1, 2, 3: Late stage investigational drugs, mAb; Pro4: Marketed drug, mAb. The data is reported as mean±SD. *P/mL: Particles/mL. A (Agitation), NA (Non Agitation)

Figure 3: *IgG and mAbs aggregate less in vials made of COP when vigorously agitated using an orbital shaker (OS)*

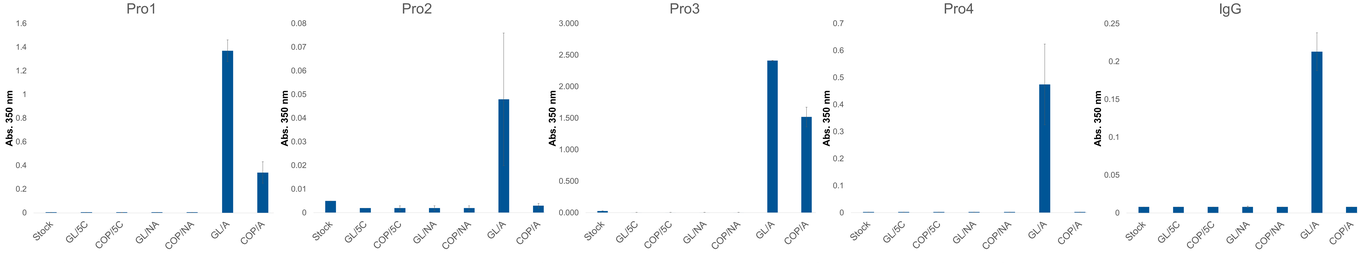


Figure 3: OS agitation in vials: Turbidity. Agitating 2 mL vials on an orbital shaker at 200 rpm for 4 days resulted in less turbid solution in the COP vials as compared to silicone free glass vials. Pro1, 2, 3: Late stage investigational drugs, mAb; Pro4: Marketed drug, mAb. The data is reported as mean±SD. A (Agitation), NA (Non Agitation), GL (Glass)

Figure 4: *IgG and mAbs aggregated less in vials made of COP when vigorously agitated using an orbital shaker (OS)*

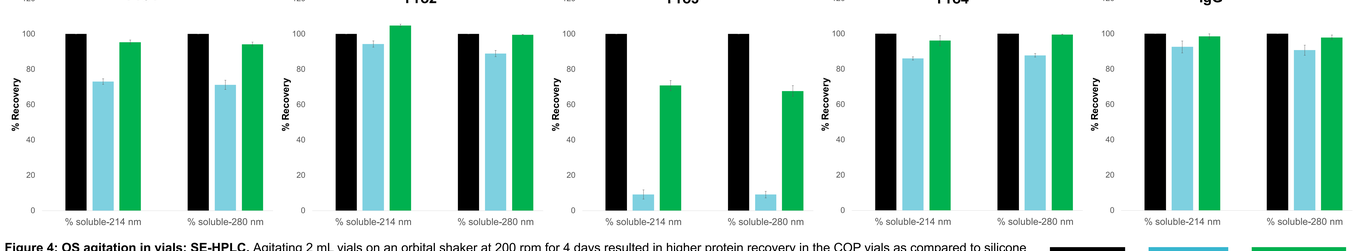


Figure 4: OS agitation in vials: SE-HPLC. Agitating 2 mL vials on an orbital shaker at 200 rpm for 4 days resulted in higher protein recovery in the COP vials as compared to silicone free glass vials. Pro1, 2, 3: Late stage investigational drugs, mAb, Pro4: Marketed drug, mAb. The data is shown as mean±SD.

Figure 5: *IgG and an mAb also aggregated less in syringes made of COP when agitated using an end over end rotation (EOE)*

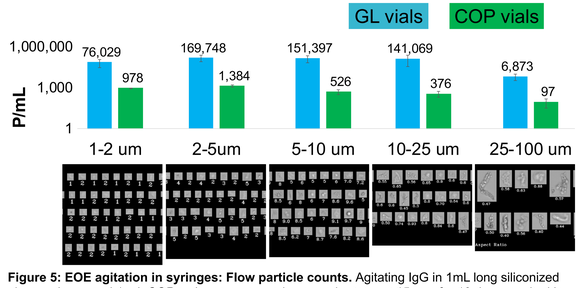


Figure 5: EOE agitation in syringes: Flow particle counts. Agitating IgG in 1mL long siliconized glass syringes and 1 mL COP syringes on an end over end rotator at 15 rpm for 10 days resulted in fewer number of aggregates in the COP syringes. Similar results were obtained using Pro4, a mAb. The data is presented as mean±SD. P/mL: Particles/mL

Figure 6: *mAb was more stable in COP vials since the COP surface does not carry any charge*

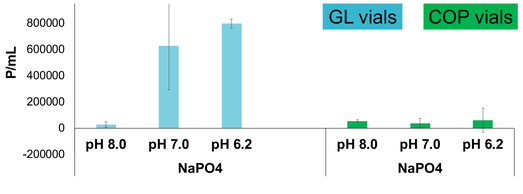


Figure 6: Effect of charge on protein aggregation: Pro4 was agitated in 2 mL vials containing buffers of varying pHs (6.2- 8.0). Isoelectric point of Pro4 is 8.4. Therefore, Pro4 carries greater positive charge when the pH is decreased to 6.2. Pro4 aggregation was higher at lower pH as compared to pH close to the isoelectric point, in glass vials. Glass surface carries negative charge, which may contribute to frequent adsorption and desorption of the positively charged proteins on agitation, subsequently leading to aggregation. However, in COP vials, there was no increase in Pro4 aggregation. Pro4 aggregation was independent of the pH of the buffer/ charge on the protein in the COP vials. COP surface does not carry any charge. Pro4: Marketed drug, mAb. The data is presented as mean±SD. P/mL: Particles/mL

Conclusions

- IgG and monoclonal antibodies are generally more stable in COP vials/syringes than in ones made of glass, suggesting surface characteristics play a role in protein stability.

- Protein stability depends in part on protein properties, e.g., Pro2 is stable in both COP and glass vials.

- We suggest that proteins are more stable in COP containers since the COP surface does not carry a charge. On the other hand, the negative charge on the glass surface contributes to frequent adsorption and desorption of the positively charged proteins with each rotation due to movement of the air bubble, leading to partial unfolding, and onset and formation of aggregates. This mechanism needs to be tested with other classes of proteins/mAbs.

References

- ***Teska BM et al.*** Aggregation and particle formation of therapeutic proteins in contact with a novel fluoropolymer surface versus siliconized surfaces: Effects of agitation in vials and in prefilled syringes. *Journal of Pharmaceutical Sciences 2016; 105: 2053-2065*
- ***Rosenberg AS et al.*** Managing uncertainty: a perspective on risk pertaining to product quality attributes as they bear on immunogenicity of therapeutic proteins. *J Pharm Sci 2012; 10: 3560–3567*
- ***Rosenberg AS.*** Effects of protein aggregates: An immunologic perspective. *AAPS J 2006;8(3):E501–7*

Alternative for SARS-CoV-2 Vaccine Primary Package Systems: Daikyo Crystal Zenith® Cyclic Olefin Polymer Vials

Donald F. DeCou, Ph.D.
William Garzon-Rodriguez, Ph.D.
Matthew R. Gehrmann
Peggy Frandolig
T. Page McAndrew, Ph.D.

Abstract

For primary package systems for SARS-CoV-2 vaccines, vials based on Daikyo Crystal Zenith® cyclic olefin polymer (COP) are a potential alternative to glass vials. COP vials have low levels of extractables, potential low levels of interaction with vaccines, and very good resistance to breakage. Permeability of oxygen and carbon dioxide has been quantified from room temperature through cryogenic temperature – enabling risk assessment and judgment if a COP-based system can meet the maximum allowable leakage limit (MALL) for a vaccine. COP vials are compatible with elastomer stoppers with FluroTec® film. They are approved world-wide for drug products comprising monoclonal antibodies, proteins, peptides, small molecules, and gene therapies.

Background

A challenge in the distribution of a SARS-CoV-2 vaccine concerns storage, namely selection of a vial/stopper primary package system that guarantees quality and safety from manufacture through delivery. This selection challenge, which is complicated by accelerated timelines for vaccine approval, results from:

1. Vaccine Platform. Six platforms are considered; they are listed with their proposed vehicles in Table 1. (1) Noteworthy is that two (RNA, DNA) are new. Ordinarily, there would be no difficulty in selecting a package system for any of the platforms, since ample time would be available for evaluation of compatibility with both vaccine and vehicle. But, for a SARS-CoV-2 vaccine this is not the case, since approval timelines are accelerated. So, whether the vaccine platform is extant or new, selection of the package system must be made quickly.
2. Suitability and Availability. Once a package system is demonstrated compatible with a vaccine and vehicle, other factors must be considered, such as:
    - stopper design/performance
    - storage temperature: room (25°C), refrigerated (2-8°C), ultra-low (-80°C), or cryogenic (-180°C)
    - component availability

The issues of stopper design/performance and storage temperature have been discussed prior. (2) This article considers the issue of component availability as it relates to vials. Typically, primary package systems for vaccines employ glass vials. But, with the increased demand resultant from

the SARS-CoV-2 pandemic, potential glass vial shortages and unacceptable lead times must be anticipated. In view of this, polymer vials as an alternative should be considered.

Table 1. Potential Vaccines for SARS-CoV-2 (1)

Vaccine Platform	Chemical Composition	Vehicle	Existing, Licensed Human Vaccine
RNA	nucleotides (ribose groups, amino/amide groups, charged phosphate groups)	encapsulated in lipid in non-polar liquid	No
DNA	nucleotides (ribose groups, amino/amide groups, charged phosphate groups)	aqueous (saline) solution, encapsulated in lipid in non-polar liquid	No
Recombinant Protein	polypeptides (amino acid groups)	aqueous	Yes (baculovirus and yeast expression)
Viral Vector Based	virus shell comprises proteins (i.e., polypeptide: amino acid groups)	aqueous	Yes (vesicular stomatitis virus)
Live Attenuated	virus shell comprises proteins (i.e., polypeptide: amino acid groups)	aqueous	Yes
Inactivated	virus shell comprises proteins (i.e., polypeptide: amino acid groups)	aqueous	Yes

Cyclic Olefin Polymer Vials

A primary package system must be fit-for-purpose, i.e., compatible with drug product and able to provide protection through shelf life. A key requirement of a vial (glass or polymer) for such a system is transparency, so drug product may be inspected. There are many commercially-available transparent polymers, such as poly(ethylene terephthalate) (e.g., beverage bottles). Among them, the best choice for a vial is Daikyo Crystal Zenith® cyclic olefin polymer (COP). COP has the best overall combination of properties, namely resistance to permeation (air/water) and potential compatibility (i.e., inertness toward) with drug products (3).

COP vials and syringes have become widely accepted over the past 20 years. They are approved by regulatory bodies for drug products comprising monoclonal antibodies, proteins, peptides, small molecules, and gene therapies.

This article discusses the performance of Daikyo Crystal Zenith® COP vials and offers why they can be an alternative to glass vials for primary package systems for vaccines.

COP Chemistry

Synthesis of COP, shown in Figure 1, employs a novel polymerization method: ring opening metathesis polymerization. This method was discovered in the 1970's and was the basis of the 2005 Nobel Prize in chemistry. (4) Synthesis of COP requires two steps, polymerization followed by hydrogenation.

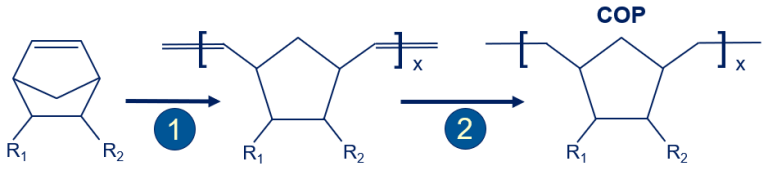


Figure 1. Synthesis of Cyclic Olefin Polymer (COP) from Norbornene. 1. ring-opening metathesis polymerization. 2. hydrogenation

Extractables and Leachables

An area where Daikyo Crystal Zenith® COP has inherently good performance is extractables and leachables (E&L). An E&L study is performed on a package system component to identify elements/compounds that may migrate from component into drug product, in particular those that may put patient safety at risk. The first phase is the extractables evaluation. This employs accelerated conditions to cause migration of any compound that possibly could appear in the drug product. Results inform the subsequent leachables study.

For glass and COP vials, the extractables evaluation process consisted of sectioning the samples (to maximize surface area), immersion in a selected liquid media at elevated temperature for a fixed time, and analysis of resultant media by chromatographic and mass spectrometric methods. As anticipated, extractables analysis of glass and COP vials revealed differences. Results for various Type 1B glass vials revealed that numerous elements can be observed at levels ≥ 0.01 μg per g sample: e.g., B, Ca, As, and Ba. Results for COP vials revealed only the presence of a small number of low molecular volatile organic compounds, such as 2-ethyl-1-hexene, at levels ≥ 0.01 μg per g sample. No inorganic element was observed. This was expected; COP comprises essentially only carbon and hydrogen.

With few extractables, and those extractables being present at low levels, COP vials present a low risk for leachables that might interact with vaccine and put patient safety at risk. Thus, they can be considered a potential alternative to glass vials.

Interaction with Drug Product

Polymers, in general, have a much lower surface energy than glass or silicon dioxide (5,6):

- glass (typical): ~ 80 mJ/m^2
- cyclic olefin polymer: ~ 40 mJ/m^2
- SiO_2 ~ 280 mJ/m^2

A drug product is more likely to be attracted to, interact with, and adhere to, high-surface-energy materials. This can cause: (a) adsorption of the drug product to the container, thereby reducing dosage, (b) unwanted chemical change in the drug product, or (c) formation of particles, possibly causing immunogenetic effects. (7,8) These phenomena have been reported. (9-12)

Interactions with glass and Daikyo Crystal Zenith® COP have been examined at West – with a focus on formation of particles. (13) Vials (2 mL), filled with solutions of simulated drug products, were subjected to agitation (orbital shaker, 200 rpm, 4 days, room temperature). Analyses of resultant solutions are given in Table 2. For each, COP vials showed fewer particles, lower levels of turbidity, and better product recovery. Data clearly indicate less interaction of simulated drug product with COP.

Table 2. Levels of Particles, Turbidity, and Recovery Resultant for Simulated Drug Products after Agitation. Particle level was measured by dynamic fluid imaging, protein recovery was measured by size exclusion high performance liquid chromatography, and turbidity was measured by light obstruction (350 nm). (13)

	mAb – 1		mAb – 2		mAb – 3		mAb – 4		mAb-5	
	Glass	COP	Glass	COP	Glass	COP	Glass	COP	Glass	COP
Particles (per ml)	36 K	18 K	750	700	325 K	165 K	9 K	1 K	9 K	4 K
Turbidity	1.4	0.4	0.05	0.003	2.4	1.5	0.48	- 0 -	0.21	0.01
Recovery (%)	75	95	90	100	10	68	90	100	90	98

Based on both the literature reports noted above and this work, risk of interaction with vaccine could well be lower for COP vials than for glass vials. Thus, COP vials can be considered a very good potential alternative to glass vials.

Note that since SiO_2 has a much higher surface energy than glass, under similar conditions interactions with vaccines might be much higher than observed with glass. Thus, vials comprising SiO_2 may not be a good alternative.

Mechanical Properties

Almost all polymers perform better than glass in terms of fracture resistance. This is common knowledge, and certainly true in the case of Daikyo Crystal Zenith® COP. In Figure 2 is shown the fracture resistance of COP vials and several commercial glass vials. (14) In this regard, COP vials are a better option than glass vials, and may be suited to lyophilized vaccines. (15)

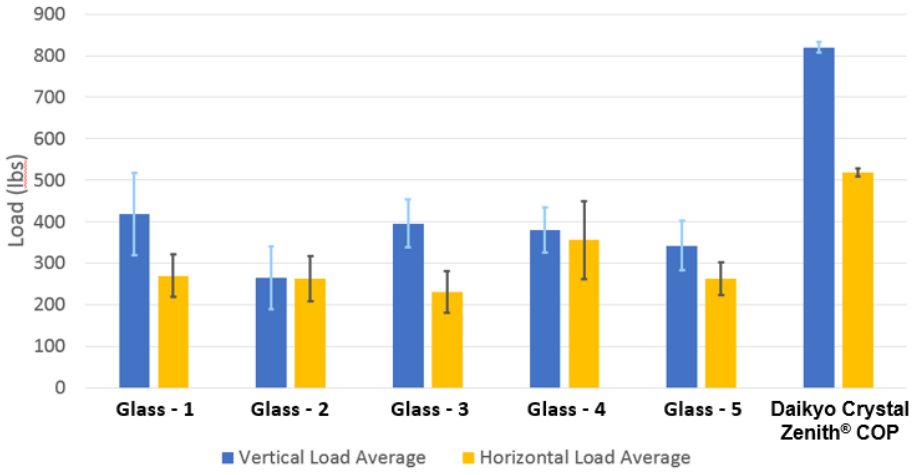


Figure 2. Load Required to Cause Fracture of 2 mL Glass and COP Vials (14)

Container Closure Integrity and Permeability

An area where glass exceeds all polymers in performance is permeability. All polymers are permeable; glass is not. (16) This point relates to the primary package system's ability to provide container closure integrity (CCI), in other words the ability to meet the requirements of the maximum allowable leakage limit (MALL) for the vaccine. MALL is discussed in detail in United States Pharmacopeia Chapter <1207>. (17) Even though Daikyo Crystal Zenith® COP vials do not have the same resistance to permeation as glass vials, that does not necessarily mean unsuitability. Each situation must be assessed in view of the MALL for the vaccine. Quantification of permeability enables this assessment, and determination if a COP vial package system of a given size (matching stoppers/seals are established) is suitable.

In Figure 3, the permeability of COP vials to oxygen is shown. (14) Package systems were filled with nitrogen and stored in air. Rate of permeability of oxygen decreases with temperature, as expected. In fact, the permeability rate at -80°C is very similar to that observed for glass vials. From these data, rates of oxygen ingress can be determined. For example, at room temperature, rate of oxygen ingress is only approximately 0.04% per hour.

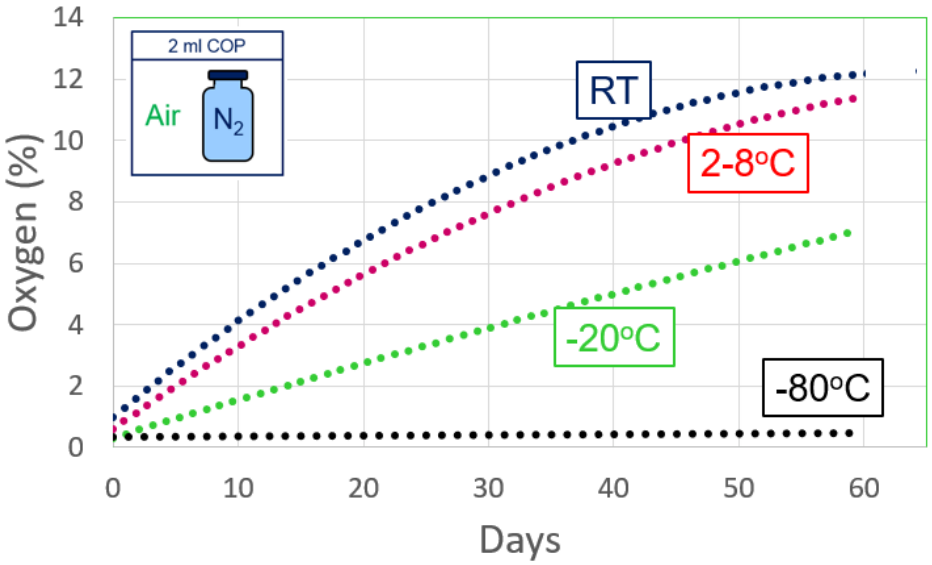


Figure 3. Oxygen Concentration vs Time and Temperature for 2 mL Daikyo Crystal Zenith® COP Vial Primary Package Systems. Elastomer stoppers were NovaPure® 1358 / 4023/50 Gray (i.e., with FluroTec® barrier film). Measurement was by frequency modulated spectroscopy headspace analysis. (14)

In Figure 4, the permeability of COP vials to oxygen at cryogenic temperature (-180°C, i.e., vapor of liquid nitrogen) is shown. Package systems were filled with air and stored in the vapor of liquid nitrogen. COP vials show excellent performance, no gas exchange at all, as evidenced by no change in oxygen level.

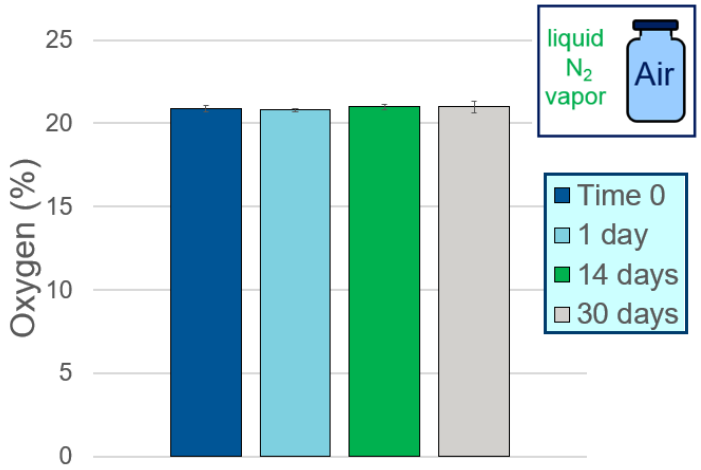


Figure 4. Oxygen Concentration vs Time for 2 mL Daikyo Crystal Zenith® COP Vial Primary Package Systems at -180°C. Elastomer stoppers were NovaPure® 1358 / 4023/50 Gray (i.e., with FluroTec® barrier film). Measurement was by frequency modulated spectroscopy headspace analysis.

Another aspect to consider is ingress of carbon dioxide resultant from storage/shipment on dry ice (i.e., solid carbon dioxide, -78°C). It has been reported that COP vials can absorb some carbon dioxide during dry ice storage exposure, and that this absorbed carbon dioxide can desorb into the package system upon warming.

As was the case with oxygen, the presence of carbon dioxide does not necessarily mean that a COP vial package system is unsuitable. The key is to quantify the permeability, so that a risk assessment can be made. See Figure 5, where 5 mL COP vial package systems, filled with air, were stored in dry ice for up to seven days. Upon removal and storage at room temperature, levels of carbon dioxide were measured. Note there is only a very small amount of ingress. For example, after three days (common for shipment), and 30 minutes at room temperature, there is no observable carbon dioxide, and after three days only 1% carbon dioxide (a rate of 0.01% per hour). Moreover, systems stored in a secondary container (heat-sealed, three-layer polyester-based bag, common for food storage) showed no ingress; this should provide a solution for a vaccine that cannot tolerate carbon dioxide exposure. (14)

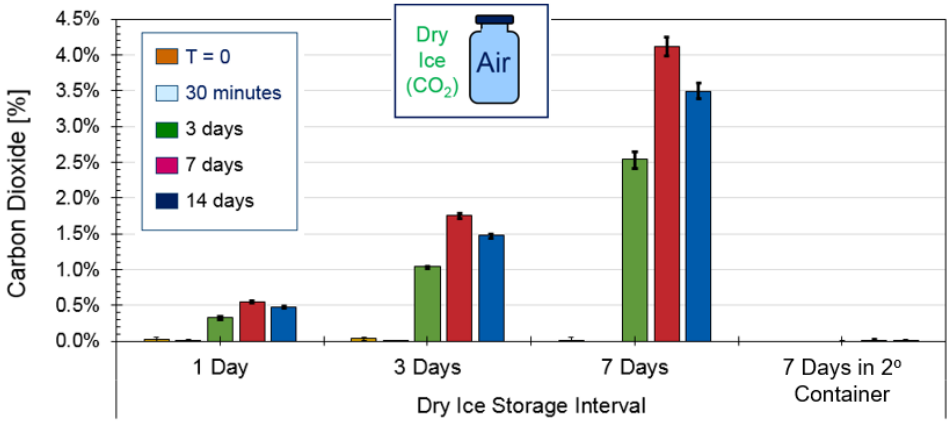


Figure 5. Carbon Dioxide Concentration vs Time for 5 mL Daikyo Crystal Zenith® COP Vial Containment Systems after Storage in Dry Ice (-78°C) and Storage in Air (room temperature). Elastomer stoppers were 20mm NovaPure® 1343 / 4023/50 Gray (i.e., with FluroTec® barrier film). Measurement was by frequency modulated spectroscopy headspace analysis. (14)

Knowing the rate of ingress of gas versus time and temperature enables the risk assessment needed to determine if a Daikyo Crystal Zenith® COP vial package system is suitable for a vaccine.

Summary

Daikyo Crystal Zenith® COP vials are potentially a good alternative to glass vials for primary package systems for SARS-CoV-2 vaccines. Levels of extractables are low, potential interaction with vaccines could well be low, and fracture resistance compared to glass is better. Permeability by oxygen and carbon dioxide, from room temperature through cryogenic temperature, has been quantified. This enables a risk assessment and judgment if a COP-based system can meet the MALL for a vaccine. COP vials are compatible with elastomer stoppers with FluroTec® film.

References

1. Amanat, F. and Krammer, F. *SARS-CoV-2 Vaccines: Status Report*. Immunity, 52 (4), 583-589 (April 14, 2020). https://www.sciencedirect.com/science/article/pii/S1074761320301205 (8/17/20)
2. Singh, R., et al. *Elastomer Stoppers with FluroTec® Film: The Right Choice for SARS-CoV-2 Vaccines* (2020) – https://www.westpharma.com/en/blog/2020/May/sars-cov2-vaccines-primary-packaging-challenges

3. *Cyclic Olefin Polymer – Advantages versus Glass for Parenteral Drug Packaging/Delivery – a Detailed Analysis.* West Pharmaceutical Services, Inc. Technical Report 2018-028 (2018) - https://www.westpharma.com/-/media/WestPharma/Files/Knowledge-Center-Files/ Customer Level Technical Reports/TR CZ2018026 COP Advantages for Parenteral Drug Pkg
4. Sutthasup, S., et al. *Recent Advances in Ring-Opening Metathesis Polymerization, and Application to Synthesis of Functional Materials*. Polymer Journal, 42, 905-915 (2010)
5. Rhee, S.K. *Surface Energies of Silicate Glasses Calculated from Their Wettability Data. Journal of Materials Science, **12** (4) 823–824 (1977)*
6. https://www.twi-global.com/technical-knowledge/faqs/faq-what-are-the-typical-values-of-surface-energy-for-materials-and-adhesives (7/21/20)
   Shin, J.Y., et al. *Chemical Structure and Physical Properties of Cyclic Olefin Polymers*. Pure and Applied *Chemistry*, 77 (5), 801-814 (2005)
7. Barnard, J.G., et al. *Characterization and Quantitation of Aggregates and Particles in Interferon-β Products: Potential Links between Product Quality Attributes and Immunogenicity*. Journal of Pharmaceutical Sciences, 102 (3), 915-928 (2013)
8. Rosenberg, A. *Effect of Protein Aggregates*: *An Immunologic Perspective.* The AAPS Journal, 8 (3), E501-E507 (2006)
9. Philo, J. and Arakawa, T. *Mechanism of Protein Aggregation*. Current Pharmaceutical Biotechnology, 10, 348-351 (2009)
10. Gerhardt, A., et al. *Protein Aggregation and Particle Formation in Prefilled Glass Syringes*. Journal of Pharmaceutical Sciences, 103, 1601-1612 (2014)
11. Qadry, S.S., et al. *Evaluation of CZ-resin Vials for Packaging Protein-Based Parenteral Formulations*. International Journal of Pharmaceutics, 252, 207–212 (2003)
12. Eu, B., et al. *Direct Visualization of Protein Adsorption to Primary Containers by Gold Nanoparticles*. Journal of Pharmaceutical Sciences, 100 (5), 1663-1670 (May 2011)
13. Singh, R. and Waxman, L. *Effect of Container Surface on Adsorption and Aggregation of Protein Therapeutics*. 2018 PDA Universe of Pre-Filled Syringes and Injection Devices, Orlando, FL (August 8, 2018)
    https://www.westpharma.com/Site-Repository/Knowledge-Articles/Registered/ka-01531
14. McAndrew, P. *How to Address Challenges in Oncology Drug Containment. P. McAndrew*. Pharmaceutical Online (May 2020)
    https://www.pharmaceuticalonline.com/doc/how-to-address-challenges-in-oncology-drug-containment-0001
15. DeGrazio, F. *Closure and Container Considerations in Lyophilization*. Freeze-Drying/Lyophilization of Pharmaceutical and Biological Products. (ISBN: 9780429151859). L. Rey and J.C. May, Eds., Ed., CRC Press, 396-412 (2010)
16. Robeson. L. *Correlation of Separation Factor versus Permeability for Polymeric Membranes*. Journal of Membrane Science, 62, 165-185 (1991)
17. United States Pharmacopeial Convention (USP) Chapter <1207> *Sterile Product – Package Integrity Evaluation* (1207.1, Section 3.9)

Volumetric Capacity of Daikyo Crystal Zenith® Vials Stored in Freezing Conditons

Introduction

Vial-based container systems are increasingly being evaluated for advanced therapies products, all of which need to be stored frozen and stored at cryogenic conditions to maintain stability and efficacy.[1,2] However, there is little industry guidance or standard tests to evaluate how filled, frozen containers will perform during cold chain handling, where low temperatures bring unique challenges for a container system, including, but not limited to, liquid volume expansion as ice is formed.

To better understand a product's robustness to freeze-thaw stress and to choose an appropriate fill volume with the representative head space, it is important to create reliable small-scale models for freeze-thaw processes that mimic, as closely as possible, the processes and conditions that will occur at larger scale.[3]

At normal atmospheric pressure, water (H_2O) expands 9% of the initial liquid volume as it crystallizes at 0°C to form ice.[4] Since vials stored at ambient temperature are frequently filled in excess of the required volume to accommodate for loss of drug during transfer and administration steps, this expansion must be considered as it concerns the integrity, safety, and efficacy of containment systems for biologics. In this study, vials were filled with Water for Injection (WFI) to simulate the worst-case scenario possible for aqueous drug products that requires cryogenic storage to expand in volume during freezing. They were frozen to -20°C as another worst-case scenario of volume expansion for advanced therapy products. Typically, these drug products are formulated in cryoprotective agents, which cause ice crystals to grow during freezing as temperature decreases, and lower storage temperatures would result in greater volume expansion.[5] However, for pure water, ice crystals fully form upon freezing and contract as temperature decreases, where -20°C results in greatest volume expansion among the varying storage conditions of freezing.

The focus of this *Technical Report* is to investigate the effect of the expansion due to nucleation on acceptable fill volumes for CZ (Daikyo Crystal Zenith®) vials stored in freezing conditions and provide a guidance on working fill volumes to avoid container closure integrity (CCI) failures due to overfill.

Objectives

Evaluate the volumetric capacity of CZ and glass vials to suggest guidance fill limits considering ice expansion.
Vial size: 2mL, 5mL, 10 mL CZ vials; 2mL, 5mL and 10 mL glass vials.

- Fill liquid: Water for Injection (WFI) with food coloring.

1. Materials & Methods

1.1. Materials and Equipment

Materials with their catalog numbers and equipment information used are outlined in Table 1.

Table 1. List of materials and equipment used in this study.

Materials & Equipment	Product Description
2 mL CZ Vials	Daikyo Crystal Zenith® 2mL Vial, 13 mm E-Beam Sterilized 15.50 mm OD
5 mL CZ Vials	Daikyo Crystal Zenith® 5mL Vial, 20 mm E-Beam Sterilized 19.50 mm (about 0.77 in) OD
10 mL CZ Vials	Daikyo Crystal Zenith® 10mL Vial, 20 mm E-Beam Sterilized 24.00 mm OD
2 mL Glass Vials	Schott 2R StandardLine Fiolax® 16.0 mm OD
5 mL Glass Vials	ACS Pharma 5 ml Sterile Injection Vial 21.50 mm OD
10 mL Glass Vials	Schott 10R TopLine Fiolax® 24.3 mm OD
13 mm Aluminum Seals	West 5417 13 mm Aluminum Seal
20 mm Aluminum Seals	West 5415 20 mm Aluminum Seal
13 mm 4023 Stoppers	4023/50G 13 mm Serum NovaPure® 1358 (Plug Height = 4.20 ±0.25 mm)
20 mm 4023 Stoppers	4023/50G 20 mm Serum NovaPure® 1343 (Plug Height = 5.20 ±0.25 mm)
Hand crimper	Genesis (13 mm C.138F.NS and 20 mm C.205F.NS)
Analytical Balance	Mettler Toledo Accuracy ≤ 0.10% Cal Due: 30 Jun 2022
WFI	Millipore OmniPur WFI CAS 7732-18-5 Exp: 07 Jun 2024
Food Color	McCormick Culinary Blue Food Color Exp: 07 Apr 2025

1.2. Container Systems Used and Instructions to Fill

The volumetric capacity was measured for 2 mL, 5 mL, and 10 mL CZ vials, 2 mL, 5 mL, and 10 mL glass vials. Five vials of each size were sealed with 13mm and 20mm 4023 Stoppers and 13 mm and 20 mm Aluminum seals respectively. The cap of the aluminum seal was removed, and each container was weighed. The vials were then filled with colored WFI (0.5 mL blue food coloring/500 mL WFI), using a 3 mL syringe and a venting method shown in

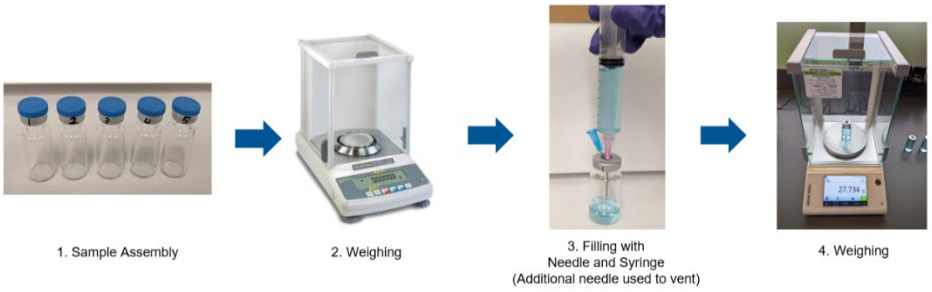


Figure 1: Experimental method and vial filling by venting. An additional needle which was placed in the lower end of the stopper cavity was used to allow air to be expelled out as it was displaced by WFI. Vials were continuously filled to the stopper leg (visually inspected by means of a mirror and stopped when the liquid touches the vent needle tip).

After filling, 5 sets of vials were weighed again, and the WFI volume was calculated (density of WFI = 0.9982 g/mL at 20 °C).[4] Vial volumetric capacity to plug height, which is an average value for each vial set was calculated and used to build the comparison graphs. The samples were then frozen in Norlake Scientific -20°C Freezer overnight.

1.3. Important parameters and Calculations

As seen in Figure 2, vials were manually filled to a "measured" fill volume, reaching the plug height with WFI, and fill volume changes at sub-zero temperatures were predicted. Measured fill volume is equivalent to 100 % fill volume for this study, which is already lower than the volume of a brim-full vial without stopper. A 90% fill was considered to be close enough that the drug product might touch the stopper when the liquid content is frozen, factoring 10% overfill. Ideally, 80 % fill would mitigate these issues.

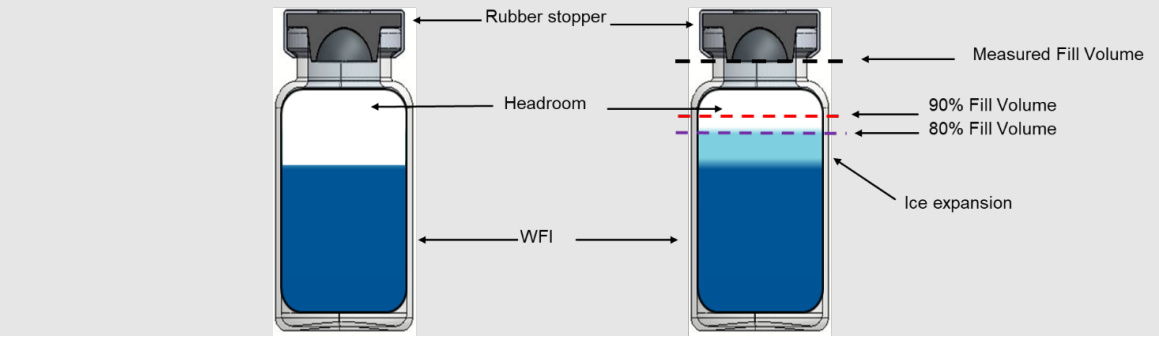


Figure 2: ***Important parameters of the study design.*** Each vial system tested in this study was comprised of three commonly used, glass and CZ vial sizes (2 mL, 5 mL, and 10 mL) paired with 13 mm and 20 mm serum stoppers and corresponding aluminum seals.

Volumetric capacity values and related parameters were calculated by Equations [1-3]:

$$\Delta \text{ Mass (g)} = \text{Mass of Water (g)} = \text{Volume } H_2O \text{ (mL)} \quad [1]$$

$$(\text{Vial 1} + \text{Vial 2} + \ldots + \text{Vial 5}) / 5 = \text{Volumetric Capacity (mL)} \quad [2]$$

$$\text{Marketed Fill (mL)} / \text{Volumetric Capacity (mL)} = \text{Headroom (\%)} \quad [3]$$

2. Results

In the field of advanced therapies, many of the fillings are done manually given the small-scale nature of these therapies, therefore overfilling accidentally or intentionally is a common issue. This overfill risk should be accounted for when determining the operating window.

As seen from Figure 3, when filled to their marketed volume, 2 mL CZ vials have only 4% headroom remaining of volumetric capacity if the liquid content is frozen. All glass vials and 5 mL CZ vials have significant amount of headroom available. A 2.0 mL liquid fill of 2 mL CZ vials could compromise the container integrity at freezing conditions as shown in Figure 4B, therefore it is recommended that a 1.7 mL maximum fill volume is used as shown in Figure 4C.

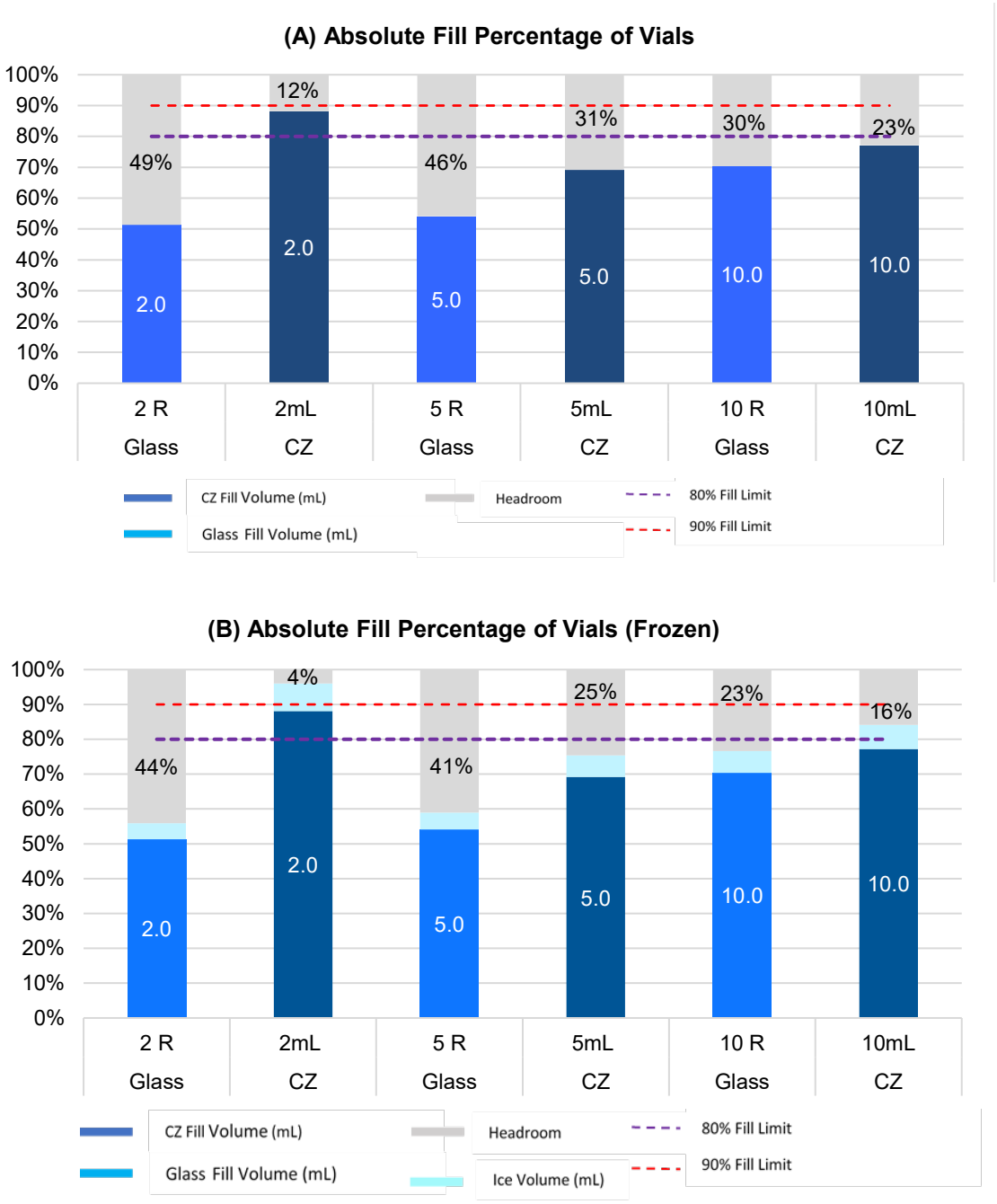


Figure 3: Absolute Fill percentage of vials (A) Room Temperature, (B) Frozen. 2 mL CZ vials are above their working limits, particularly when factoring in 10% overfill, when the liquid content is frozen. Glass, 5 mL CZ and 10 mL CZ vials represent less risk to container integrity.

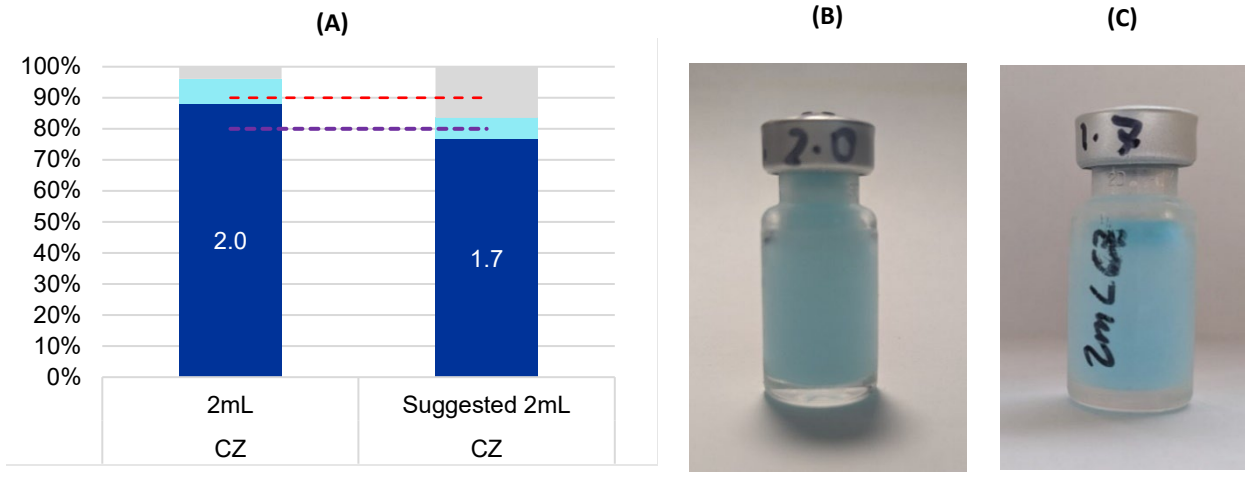


Figure 4: Marketed vs recommended fill volume for 2 mL CZ vial in freezing conditions. (A) Graphical representation of the suggested fill volume, (B) 2.0 mL liquid fill at freezing conditions, (C) 1.7 mL liquid fill at freezing conditions. All empirical data was collected using WFI alone. Colored solution was used for illustrative purpose only.

Figures 3 and 4 indicate the need for customers to avoid using fill volumes that cause CCI or Extractables and Leachables issues, and to mitigate the impact of overfill vial to account for dead volume (+10 %) storing in freezing conditions. Contact or displacement of the elastomer stopper may lead to cause CCI and/or Extractables and Leachables issues as evidenced in Figure 4B. Although freezing decelerates chemical degradation considering temperature dependency of chemical reaction kinetics, interactions with stoppers and other container constituents can significantly influence drug substance and drug product stability during freeze-thaw cycles. Table 2 provides marketed fill (name of the vial) along with the suggested maximum amount that can fit in that vial to reduce any guesswork for the customer and would help to establish safe working volumes.

Table 2**: **Marketed vs. recommended fill volumes. **Recommended fill volume for freezing application is lower than marketed fill volume.

Material	**CZ**			**Glass**		
Vial Type	2mL	5 mL	10.0 mL	2mL	5 mL	10.0 mL
Marketed Fill (mL)	2.0	5.0	10.0	2.0	5.0	10.0
Recommended Fill (mL)	1.7**	5.0	10.0	2.0	5.0	10.0
Image in Table 2*	(A)	(B)	(C)	(D)	(E)	(F)

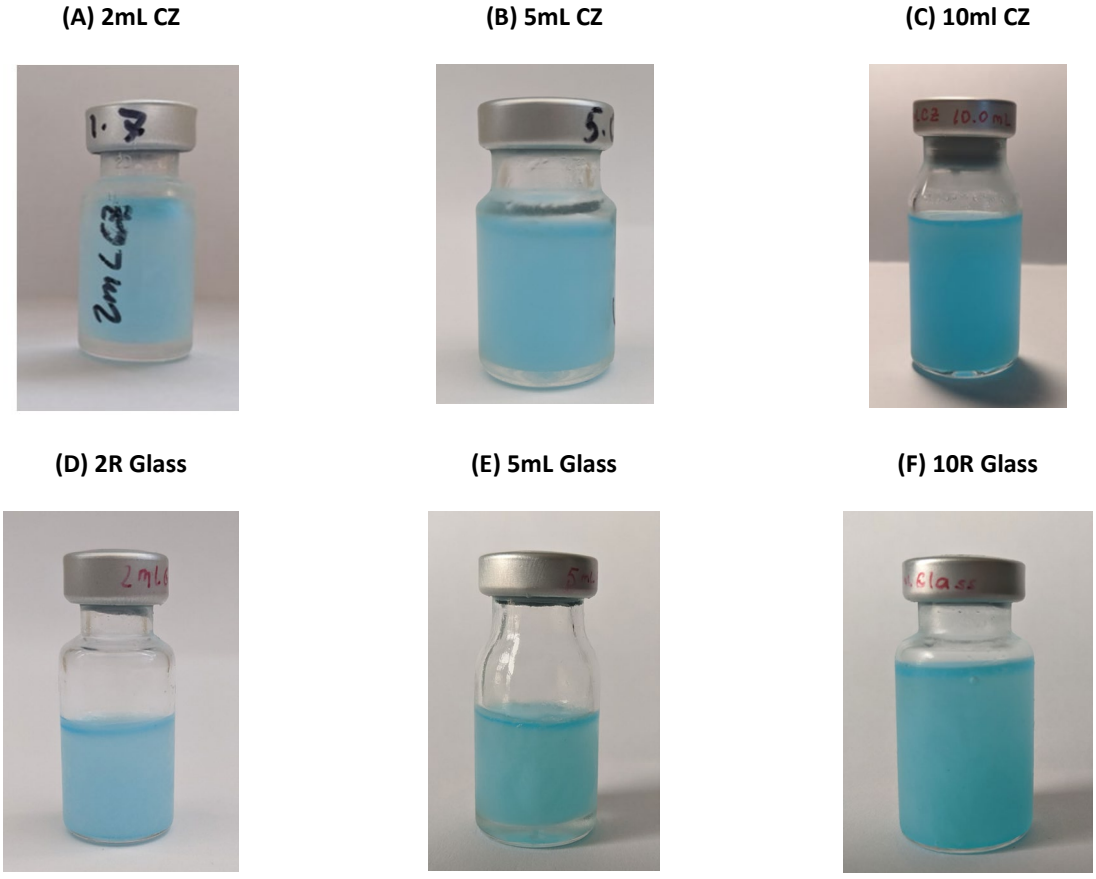


Figure 5: Recommended fill volume for CZ and glass vials in freezing conditions. Pictures show the recommended fill volumes at freezing conditions. Images not to scale.

3. Conclusions

This study provides information leading to the filling optimization of CZ vials for storage of advanced therapeutics and other products stored in freezing conditions. All glass vials had enough headroom left to mitigate the risk for CCI and the impact of overfill. Glass can be stored down to temperatures as low as -80°C, but often cannot be used on applications colder than this as the glass elastomer interface can lose container closure integrity (CCI), owing to differences in coefficient in thermal expansion between the different materials. Glass vials also carry a greater risk of fracture.[6,7] The data also showed that the 5mL and 10 mL CZ vials perform well. A maximum fill volume of 1.7mL is recommended for low temperature application without creating any risks to container closure integrity (CCI) failure.

WFI used in this study represents the worst-case scenario for aqueous drug products that need to be cryopreserved since the media used in advanced therapeutics generally act to suppress the quantity of ice crystal formation compared to pure water. This data is provided for guidance only and the integrity of all package offerings must be confirmed by

the customer using the intended drug product formulation. Some statements are forward-looking and are subject to evolve as further experiments are performed and more data is generated for different stopper-vial combinations.

References

1. *Daikyo TR CZ2021033 Functional Assessment of Adeno-Associated Virus Stored in Crystal Zenith® Vials at Ultra-cold Temperatures.*
2. *Daikyo TR CZ2021034 Evaluation of Daikyo Crystal Zenith® Vials versus Ethylene Vinyl Acetate Cryobags for the Cryopreservation of Therapeutically Relevant T Cells.*
3. *Puri et al, BioProcess Int., 13(1) Jan 2015.*
4. *Density Anomalies of Water (https://water.lsbu.ac.uk)*
5. *Whaley D, Damyar K, Witek RP, Mendoza A, Alexander M, Lakey JR. Cryopreservation: An Overview of Principles and Cell-Specific Considerations. Cell Transplant. 2021;30:963689721999617. doi:10.1177/0963689721999617.*
6. *Correlating Vial Seal Tightness to Container Closure Integrity at Various Storage Temperatures*. PDA Annual Meeting (March 2015). https://www.pharmaceuticalonline.com/doc/correlating-vial-seal-tightness-to-container-closure-integrity-at-various-storage-temperatures-0001.
7. *Performance of Daikyo Crystal Zenith® 2 mL Vials at Cryogenic Temperatures: Container Closure Integrity and Cell Preservation West Pharmaceutical Services, Inc. Technical Report 2020-032, 6 pp. (2020).*

Acknowledgements

The author is grateful to A. Lyness, J. Cicarelli and S. Molina for their support study design and interpretation.

This Technical Report dated 15 June 2022, is the first release version of this report.

Potential Over-pressurization Issues with Ultra-Low Temperature or Cryogenic Storage of Improperly Sealed Vial/Stopper Primary Package Systems

I. Introduction

This bulletin addresses the over-pressurization issues that can develop if a vial/stopper/seal primary package system is stored at ultra-low or cryogenic temperatures without appropriate container closure integrity (CCI).

It is imperative that a vial/stopper/seal primary package system for a parenteral drug product provides CCI. This is achieved by:

- selection of compatible components
- establishment of proper assembly
- demonstration of CCI experimentally

A process for achieving CCI has been discussed by DeGrazio. (1) West offers this as a service, through its Integrated Solutions platform.

With the growing importance of gene and cell therapies, CCI at -80 °C and -180 °C has become a necessity. Achieving CCI at these temperatures is more challenging than at room temperature. Filling and assembly of a package system is performed at room temperature. The package system comprises a rubber stopper and (glass or polymer) vial – materials with different coefficients of thermal expansion. This results in different amounts of shrinkage upon decreasing to ultra-low or cryogenic temperatures. This may result in gaps, which consequently result in loss of CCI. As discussed by Gehrmann, et al. (2, 3), when properly done, CCI can be achieved at these temperatures. Failure to achieve CCI can result in serious issues. The first two are loss of drug product sterility and efficacy. The third, which is the subject of this bulletin, is over-pressurization of the package system.

For purpose of discussions, each case considers the vial/stopper/seal primary package system depicted schematically in the Figure. This comprises a 6 mL vial filled with 1 mL of drug product; so, there is 5 mL of empty space. Atmospheric pressure inside the package system at capping is 1 atm.

Figure. Schematic of 6 mL Vial/Stopper/Seal Package System with 1 mL Drug Product

Technical Support Bulletin 2020/072

08 Apr 2020

Case 1. Loss of CCI – Freezer at -80 °C (193 K) – Gene Therapy

Upon placement in the freezer, a system will come to thermal equilibrium at -80 °C (193 K). As a result, the interior pressure of the system will decrease to 0.65 atm, according to the equation (Gay-Lussac's Law for a vessel at constant volume):

$$\frac{P_1}{T_1} = \frac{P_2}{T_2} \rightarrow P_2 = \frac{P_1 \, x \, T_2}{T_1} \rightarrow P_2 = \frac{1 \, atm \, x \, 193 \, K}{298 \, K} = 0.65 \, atm$$

As a freezer is maintained at 1 atm, there is a pressure gradient of 0.35 atm (i.e., 1 minus 0.65) promoting movement of air at -80 °C into the system. With a loss of CCI, air will enter and bring the interior pressure of the system to 1 atm. Upon removal from the freezer, the system will see an increase in pressure to 1.5 atm as the air rises in temperature from -80°C to room temperature, according to the equation:

$$P_2 = \frac{P_1 \, x \, T_2}{T_1} \rightarrow P_2 = \frac{1 \, atm \, x \, 298 \, K}{193 \, K} = 1.5 \, atm$$

For reference, a soda bottle has an interior pressure of 2.7 to 3.7 atm. (4)

Case 2. Loss of CCI – Vapor of Liquid Nitrogen at -180 °C (93 K) – Cell Therapy

Upon placement in the vapor of a cryo-freezer, a system will come to thermal equilibrium at -180 °C (93 K). As a result, the interior pressure of the system will decrease to 0.31 atm, according to the equation (Gay-Lussac's Law for a vessel at constant volume):

$$\frac{P_1}{T_1} = \frac{P_2}{T_2} \rightarrow P_2 = \frac{P_1 \, x \, T_2}{T_1} \rightarrow P_2 = \frac{1 \, atm \, x \, 93 \, K}{298 \, K} = 0.31 \, atm$$

As a cryo-freezer is maintained at 1 atm, there is a pressure gradient of 0.69 atm (i.e., 1 minus 0.31) promoting movement of nitrogen gas at -180 °C into the system. With a loss of CCI, nitrogen gas will enter and bring the interior pressure of the system to 1 atm. Upon removal from the cryo-freezer, the system will see an increase in pressure to 3.2 atm as the nitrogen gas rises in temperature from -80 °C to room temperature, according to the equation:

$$P_2 = \frac{P_1 \, x \, T_2}{T_1} \rightarrow P_2 = \frac{1 \, atm \, x \, 298 \, K}{93 \, K} = 3.2 \, atm$$

For reference, a soda bottle has an interior pressure of 2.7 to 3.7 atm. (4)

Case 3. Loss of CCI – Immersion in Liquid Nitrogen at -196 °C (77 K) – Cell Therapy

Upon immersion in liquid nitrogen (LN2), system will come to thermal equilibrium at -196 °C (77 K). As a result, the interior pressure of the system will decrease to 0.26 atm, according to the equation (Gay-Lussac's Law for a vessel at constant volume):

$$\frac{P_1}{T_1} = \frac{P_2}{T_2} \rightarrow P_2 = \frac{P_1 \, x \, T_2}{T_1} \rightarrow P_2 = \frac{1 \, atm \, x \, 77 \, K}{298 \, K} = 0.26 \, atm$$

As a cryo-freezer is maintained at 1 atm, there is a pressure gradient of 0.74 atm (i.e., 1 minus 0.26) promoting movement of LN2 into the system. With a loss of CCI, LN2 will enter and fill the available 5 mL of empty space.

The specific gravity of LN2 is 0.8 g / cm^3. Thus 5 mL of LN2 is:

5 mL x 0.8 g / cm^3 = 4 g LN2 x 1 mole / 28 g = 0.14 mole N_2 (1 mL = 1 cm^3)

Upon removal from the cryo-freezer and warming to room temperature, LN2 becomes nitrogen gas. According to the ideal gas law, 0.14 mole N_2 in a 5 mL container at room temperature has a pressure of 685 atm (i.e., 10,000 lbs / in^2):

$$P\,V = n\,R\,T \;\rightarrow P = \frac{n\,R\,T}{V}$$

$$P = \frac{(0.14\ mol\ N_2)(0.0821\ \frac{l \cdot atm}{m \cdot K})\ (298\ K)}{0.005\ l} = 685\ atm$$

This can result in stopper popping, system cracking, or explosion. Even if a smaller amount of LN2 enters, it is still an issue. See below. As little as 0.1 mL LN2 results in a pressure of 220 lbs / in^2 or 15 atm..

Amount of LN2			Pressure	
mL	grams	moles	atm	lbs / in^2
5	4	0.14	685	10,000
4	3.2	0.11	538	7,900
3	2.4	0.09	440	6,500
2	1.6	0.07	342	5,000
1	0.8	0.03	147	2,200
0.5	0.4	0.014	69	1,000
0.1	0.08	0.003	15	220

II. Summary

CCI of a vial/stopper/seal drug product primary package system must be demonstrated for the temperature of storage. Apart from loss of drug product sterility/efficacy, if CCI is not achieved, over-pressurization can occur during storage at -80 °C (gene therapy) or vapor of liquid nitrogen at -180 °C (cell therapy). Storage immersed in liquid nitrogen (cell therapy) at -196 °C can result in gross over-pressurization.

III. References

1. F. DeGrazio. A Holistic Strategy for Container Closure Integrity: Selecting and Evaluating an Integral Packaging System. PDA Journal of Pharmaceutical Science and Technology, 72 (1), 15-34 (2018)
2. M. Gehrmann and A. Lyness. Performance of Daikyo Crystal Zenith® Vials at Cryogenic Temperatures: Container Closure Integrity and Cell Preservation. West Pharmaceutical Services, Inc. Technical Report 2018/027 (2018)
3. M. Gehrmann, O. Laskina, L. Fang. Ingress of Gases into Cyclic Olefin Polymer Vial-Based Container Closure Systems at -80 °C. West Pharmaceutical Services, Inc. Technical Report 2019/028 (2019)
4. https://education.seattlepi.com/much-pressure-can-two-liter-bottle-handle-6313.html#:~:text=At%20room%20temperature%2C%20the%20pressure,100%20pounds%20per%20square%20inch (6/12/20)

West's products are sold on the basis that it is the customer's responsibility to evaluate and test the West product to determine its compatibility with other materials and fitness for any end use.

This *Technical Bulletin* dated 8 April 2020, is the first release version of this *bulletin*.

Comparison Of Particles in Daikyo Crystal Zenith® Vials, Glass Vials, Polypropylene Screw Top Vials and Cryogenic Bags

I. Background

There is clear guidance provided by regulatory bodies for injectable medicines on particulate enumeration and USP provides particle limits.[1-5] Packaging components are considered one of the potential sources of particulate matter and should be prepared in a way to minimize the introduction of particles to the pharmaceutical products.[6]

Particles present in IV infusions might result in inflammation and infection if the particle is non-sterile. Injected particles can travel through the body and damage different tissues or organs, depending on their size. Larger particles can damage the vein. Smaller particles can get trapped in small vessels or capillaries, including pulmonary capillaries, resulting in impaired oxygen transfer and compromised respiratory function. The smallest particles can be deposited in spleen, liver, kidney, or other organs.[7]

Cell and gene therapies require cold and cryogenic (cryo) storage to preserve the drug product and some therapies may intentionally contain particles (e.g., particles as drug delivery vehicles such as lipid nanoparticles, exosomes, extracellular vesicles containing protein, DNA and RNA, or cells). In the study outlined in this technical report we evaluated particle load from Daikyo Crystal Zenith® (CZ) vials that are manufactured from CZ, a cyclic olefin polymer (COP) and compared it to glass and plastic vials as well as plastic cryo bags to understand the baseline particle load in these different packaging components. Particle load was compared before and after freezing to understand the effects of freeze-thaw cycle. Particle analysis was also done to determine the origin of the observed particles. Finally, the surface of the vials was examined for any visual damage post freeze-thaw cycle.

II. Experimental

Packaging components of two sizes were used in this study: 2 mL components (1 mL fill) and 50 mL components (20 mL fill). Components used in this study as well as filling media and volume are shown in Table 1.

Table 1. Component description

Vial/Bag	Stopper	Seal	Fill volume and media
2 mL CZ vial	ART 1358 formulation 4023/50 S2-F451 formulation D21-7S	ART 5417	1 mL PBS buffer
ISO standard 2R glass vial	ART 1358 formulation 4023/50 S2-F451 formulation D21-7S	ART 5417	
2 mL polypropylene cryo vial with screw top	NA – vial comes with screw top		
50 mL CZ vial	ART 1343 formulation 4023/50 S10-F451 formulation D21-7S	ART 5465	20 mL WFI
50 mL bag, ethylene vinyl acetate (EVA)	NA		
50 mL bag, fluorinated ethylene propylene (FEP)			

PBS buffer solution (180 mM sodium chloride, 10 mM sodium phosphate and 0.001% poloxamer 188) was filtered through a 0.2 µm filter. All samples were prepared in replicates of five. Filling was done in an ISO 5 cleanroom. Glassware that was used for sample filling and filtering was tested for cleanliness according to USP <788> Method 2 and tested against USP criteria (no more than 20 particles ≥ 10 µm and 5 particles ≥ 25 µm).

Storage conditions for samples in 2 mL containers were designed to mimic storage of gene therapy products: uncontrolled-rate freezing and thawing with storage at -80°C. Storage conditions for 50 mL containers were designed to mimic storage of cell therapies: controlled-rate freezing, and rapid thawing and storage in vapor phase of liquid nitrogen.

Half of all filled containers were analyzed shortly after filling. Another half of samples in 2 mL containers was placed in -80°C freezer and stored for seven days. At the end of seven days, samples were thawed at room temperature and analyzed. The other half of samples in 50 mL containers was frozen to -120°C in a controlled-rate freezer, then transferred to vapor phase of liquid nitrogen and stored there for seven to nine days. After the vapor phase of liquid nitrogen storage, the samples were thawed in a 37°C water bath and analyzed.

All components were visually examined for particles and defects. After visual inspection, vials were inverted 10 times and contents of the vials were filtered through a 0.8 µm filter and particles on the filter were counted according to USP <788> Method 2.

Selected representative particles on the filters were analyzed by FTIR and SEM/EDX to determine their composition. Samples from 50 mL containers were additionally tested with flow imaging technique. Surface of the vials was analyzed using light microscopy after freeze-thaw cycle to look for freeze-thaw triggered defects.

III. Results

2 mL containers.

1. Visual inspection

Visual inspection resulted in two polypropylene cryo vials having scratches/cracks before freeze-thaw cycle. One polypropylene cryo vial had a fiber. One polypropylene cryo vial had external scratches after the freeze-thaw cycle. The CZ and glass vials did not have any abnormalities upon visual inspection.

2. Microscopic particle count test

Figure 1 shows particle numbers for vials that were not frozen (marked as “before”) and vials after freeze-thaw cycle (marked as “after”). The left panel shows all particles ≥10 µm. The panel on the right shows all particles ≥25 µm. Results are displayed as average values from five samples with standard deviation.

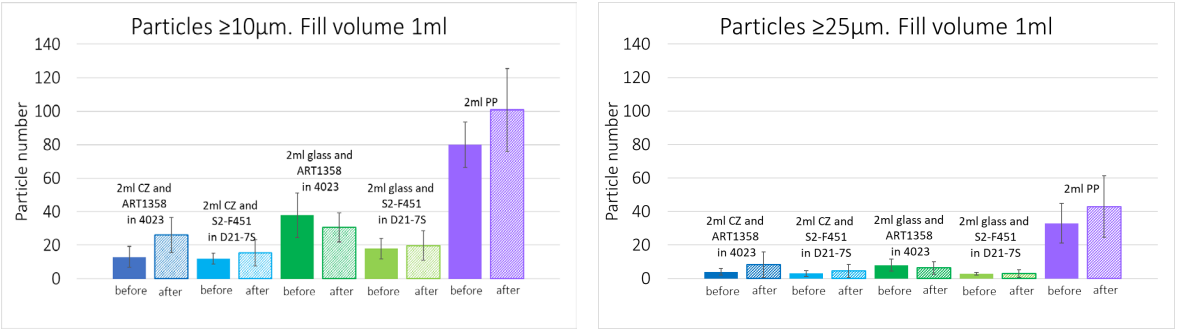


Figure 1. Microscopic particle count test results.

Further analysis was performed on this data to establish significance of difference between vials, stoppers and samples before and after freezing. Figures 2 and 3 display these differences. Symbols represent amount of particulates found. Each symbol represents individual measurement. Horizontal lines represent average value. Standard deviation is shown with vertical lines. Lines above the data points represent correlation between each

sample type. And stars above these lines represent statistical significance. The level of statistical significance is shown as a p-value and is denoted as as * for p≤0.05, ** for p≤0.01, *** for p≤0.001; **** for p<0.0001, and ns for p≥0.05 by two-way Analysis of variance (ANOVA) with Tukey's MCT (multiple comparison test). A p-value less than 0.05 (typically ≤ 0.05) is statistically significant and lower p value means greater statistical significance, meaning greater difference between the data.

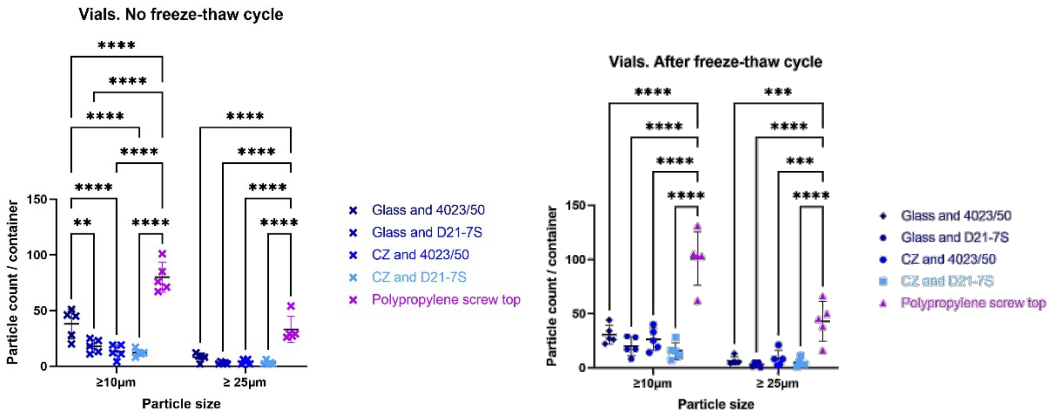


Figure 2. Microscopic particle count test results. Effect of vial material and stopper formulation.

Figure 2 compares particle numbers in samples that were stored in vials of different material and, where applicable, with different stoppers. The left panel shows particle numbers in vials that were not subject to freeze-thaw cycle and the right panel shows vials that underwent a freeze-thaw cycle. Due to many comparison pairs, only pairs with significant differences are shown. As can be seen from here, polypropylene screw top cryo vials have consistently significantly higher particle load than CZ or glass vials either with or without freeze-thaw cycle and regardless of particle sizes. Glass vials with 4023/50 stoppers have higher loads of particles ≥10 µm without freeze-thaw cycle, but this difference was insignificant for larger particles and for vials that went through a freeze-thaw cycle.

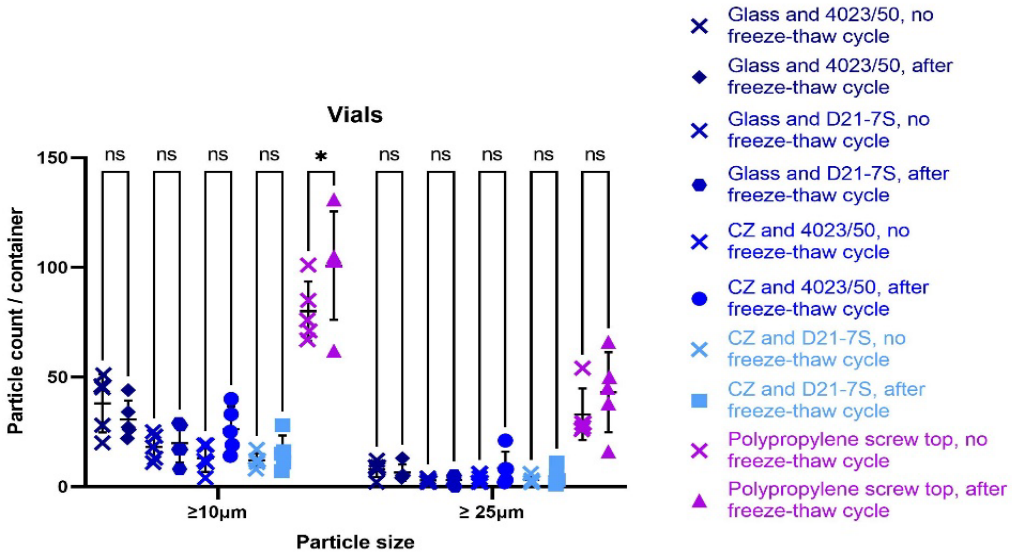


Figure 3. Microscopic particle count test results. Effect of freeze-thaw cycle.

Effect of freeze-thaw cycle on particle count is shown in Figure 3. Only polypropylene screw top cryo vials show a higher particle load after freeze-thaw cycle and only for smaller particles. The freeze-thaw cycle does not have an effect on particle number in other vials.

3. Particle analysis

Some selected particles were photographed and analyzed with FTIR and SEM/EDX after counting. Pictures of some representative particles are shown in Figure 4.

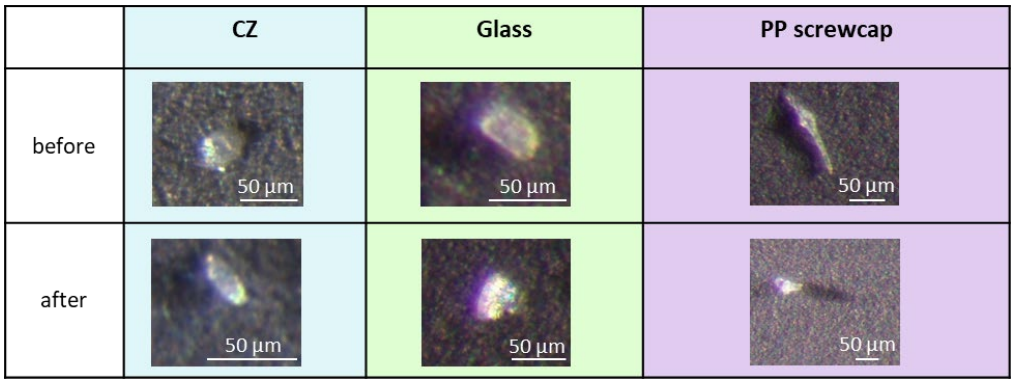


Figure 4. Microphotographs of representative particles. Samples that were not subject to freeze-thaw cycle are marked as “before” and those that we subjected to freeze-thaw cycle are marked as “after.”.

CZ vials with 4023/50 stoppers not subjected to freeze-thaw cycle show the presence of many protein particles. Individual particles of acrylic material, calcium carbonate, aluminum silicate, a styrene, and a carbonyl compound, as well as cellulose and polyester were found. Some inclusions of stainless steel were identified as well. CZ vials with 4023/50 stoppers after a freeze-thaw cycle show the presence of many cellulose particles as well as individual particles of amide, calcium or zinc stearate, polypropylene, polystyrene, and protein.

CZ vials with D21-7S stopper not subjected to a freeze-thaw cycle show the presence of many protein particles and individual particles of cellulose, calcium stearate, and polypropylene. Vials after a freeze-thaw cycle also show many protein particles, cellulose and silicate compound.

Glass vials with 4023/50 stopper not subjected to a freeze-thaw cycle have only cellulose and protein particles. After the freeze-thaw cycle, many protein particles were present, as well as one cellulose and one polystyrene particle.

Glass vials with D21-7S stopper not subjected to a freeze-thaw cycle show mainly cellulose particles and one protein particle. After the freeze-thaw cycle, there were mainly protein and cellulose particles, as well as one mixed particle with inclusions of butyl, polyethylene, and inorganic fillers with minor addition of titanium dioxide. It should be noted that protein and cellulose particles were observed in all samples and can be linked to environmental contamination during sample handling.

Polypropylene vials have mostly polypropylene particles with small addition of cellulose and protein particles, regardless of exposure to a freeze-thaw cycle.

4. Surface analysis

Surface analysis was performed using light microspcopy on these vials to monitor for signs of surface damage triggered by a freeze-thaw cycle. Selected images of vial surface with (“after”) and without (“before”) a freeze-thaw cycle are shown in Figure 5. No signs of damage or delamination, were observed.

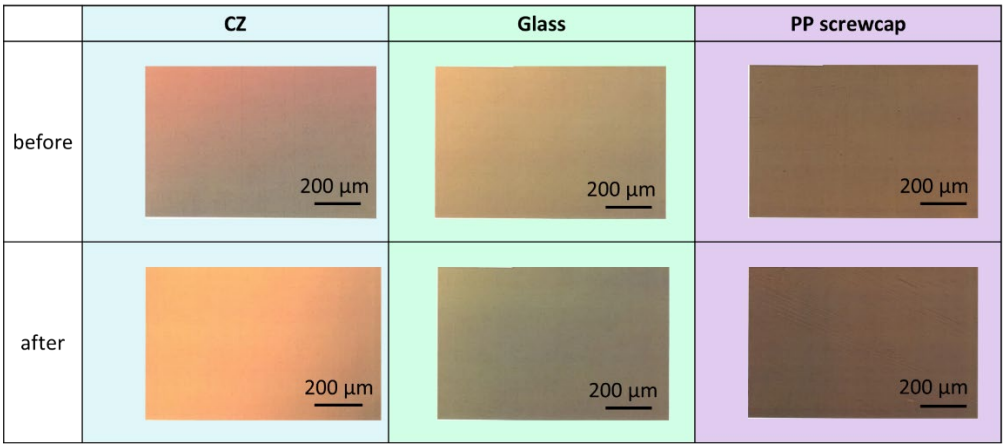


Figure 5. Surface analysis. Samples that were not subject to a freeze-thaw cycle are marked as "before" and those that we subjected to freeze-thaw cycle are marked as "after".

50 mL containers.

1. Visual inspection

Visual inspection of 50 mL CZ vials did not show any particles or defects either before or after the freeze-thaw cycle. Visual inspection of the bags that didn't undergo a freeze-thaw cycle resulted in one EVA bag being scuffed and two FEP bags having visible particles/fibers in them. After the freeze-thaw cycle, two EVA bags broke upon thawing and samples were lost. Another bag had a pinhole leak from tubing and appoximately half of the sample was lost. Two remaining bags didn't show any particles or defects. None of the FEP bags had defects or particles after the freeze-thaw cycle.

2. Microscopic particle count test

Figure 6 shows particle numbers for vials and bags that were not frozen (marked as "before") and vials and bags after the freeze-thaw cycle (marked as "after"). Left panel shows all particles ≥10 µm. Panel on the right shows all particles ≥25 µm. Results are displayed as average values from five samples with standard deviation. All particles in a given container were counted.

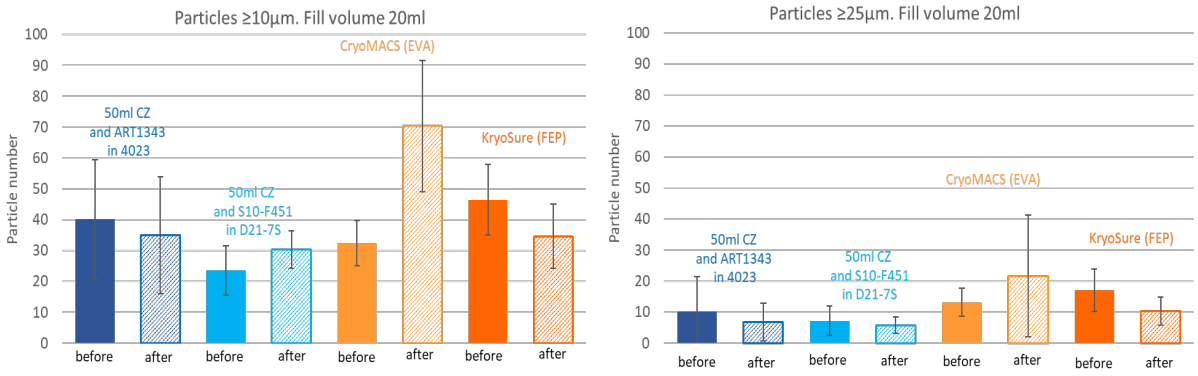


Figure 6. Microscopic particle count test results.

Further analysis was performed on this data to establish significance of difference between vials with different stoppers and bags before and after freezing. Figures 7-10 display these differences.

Figure 7 shows the effect of stoppers after thaw within one freeze-thaw cycle. Once thawed, the vials were inverted to collect any defects from the elastomer formualtions tested.

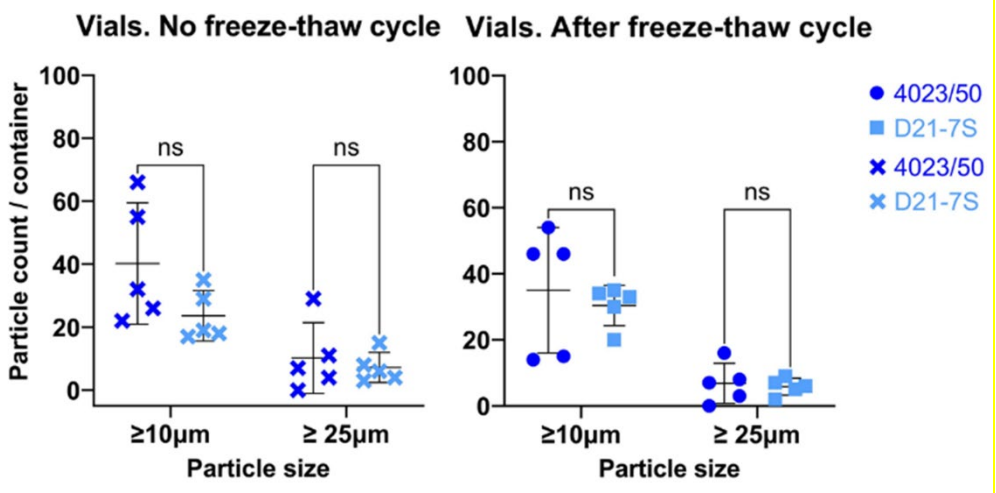


Figure 7. Microscopic particle count test results from the efect of stopper formulation.

As can be seen in Figure 7, no statistically-significant difference was observed between samples in CZ vials closed with different stoppers neither with or without a freeze-thaw cycle and regardless of the particle size.

Figure 8 compares different bags.

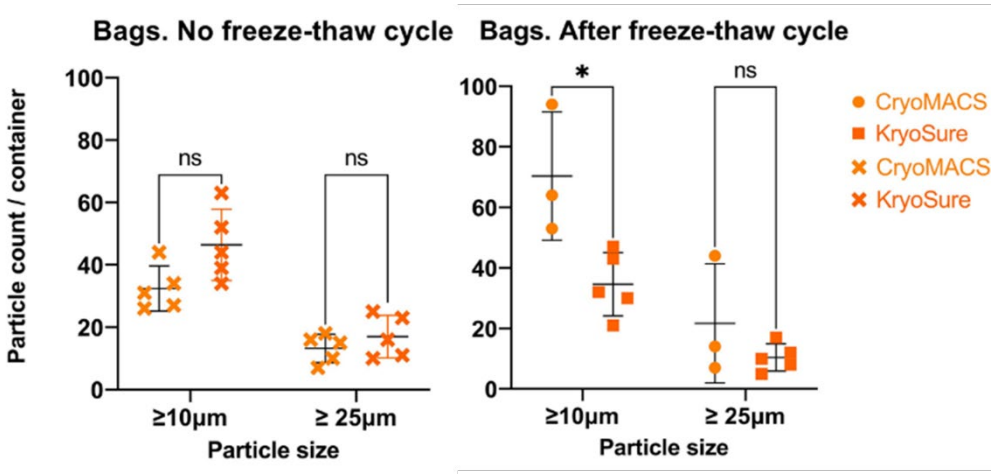


Figure 8. Microscopic particle count test results. Effect of bag material.

As can be seen in Figure 8, no statistically-significant difference was observed between samples in different bags without a freeze thaw cycle. After the freeze-thaw cycle CryoMACS® (EVA) bags had more particles ≥10 µm than KryoSure® (FEP) bags. No statistically-significant difference was observed for larger ≥25 µm particles.

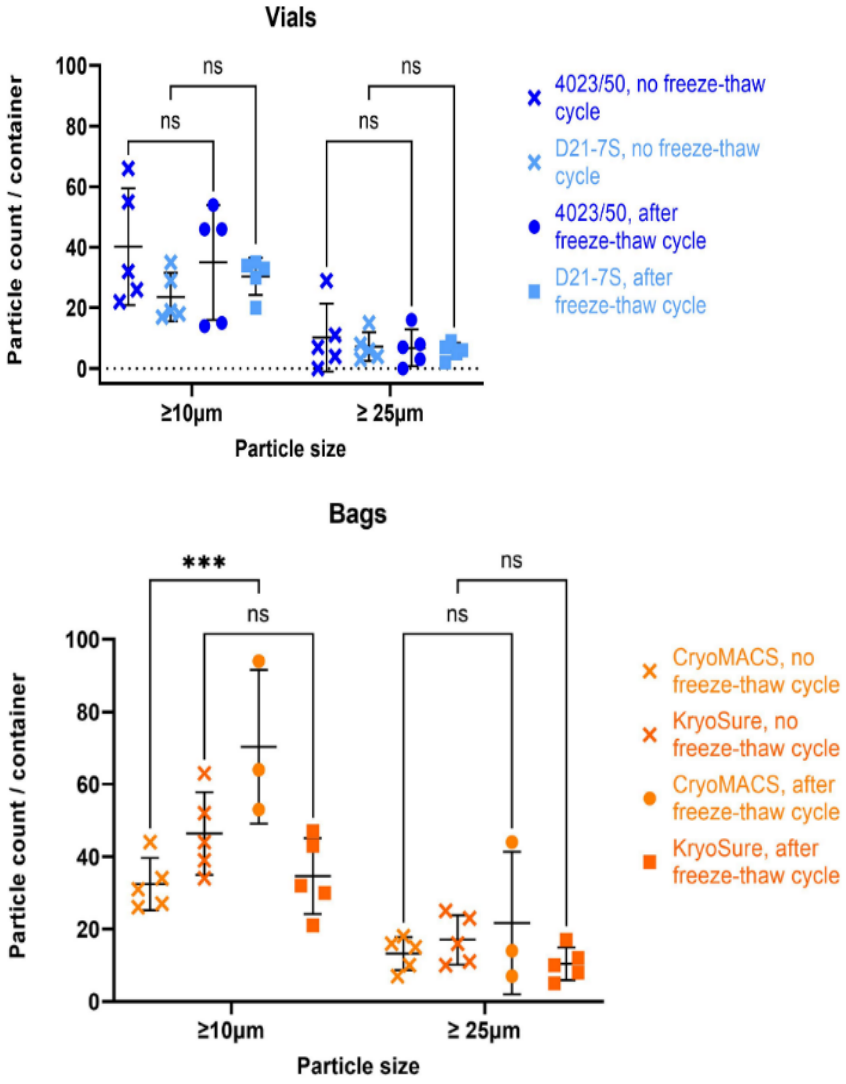


Figure 9. Microscopic particle count test results. Effect of freeze-thaw cycle.

Figure 9 shows the effect of a freeze-thaw cycle on particle numbers in CZ vials and bags. There was no change in particle load in 50 mL CZ vials after a freeze-thaw cycle, regardless of particle size or stopper used. The only change observed for bags was the increase of ≥10 µm particles in CryoMACS (EVA) bags after the freeze-thaw cycle. Larger particles and the other type of bag didn't show any statistically-significant difference.

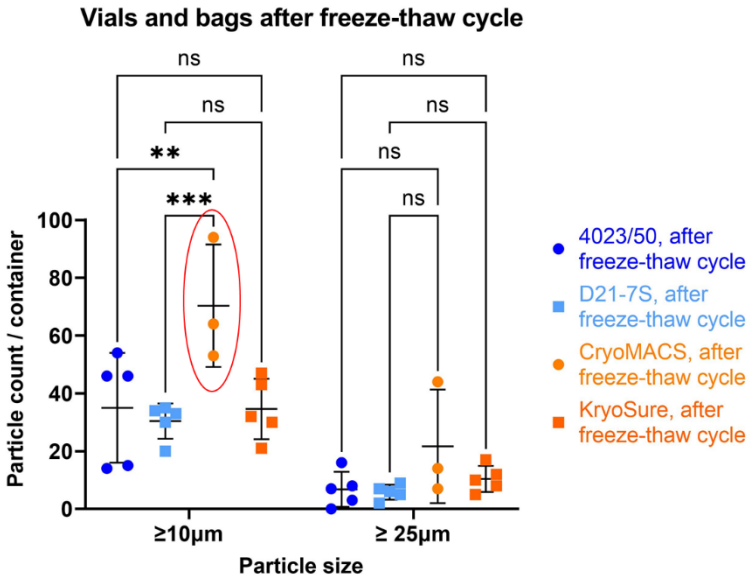


Figure 10. Microscopic particle count test results. Effect of container type after freeze-thaw cycle.

Figure 10 compares particle loads in CZ vials with different stoppers and cryo bags, all after subjected to a freeze-thaw cycle. As can be seen in Figure 10, one of the bag types, CryoMACS (EVA), has more ≥10 µm particles than the vials. The other bag type, KryoSure (FEP), has the same particle load. And there is no statistically-significant difference between these packaging components for larger particles ≥25 µm.

3. Flow imaging

An alternative particle counting and imaging techique was used for 50 mL CZ vials and bags, flow imaging. Flow imaging results are shown below for 50 mL containers.

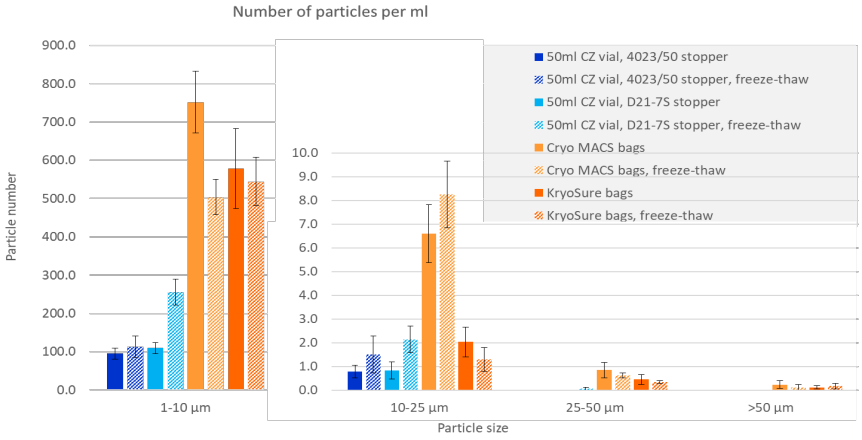


Figure 11. Flow imaging results.

Figure 11 shows particle numbers for vials and bags that were not frozen (solid bars) and vials and bags after freeze-thaw cycle (patterned bars). Here, particles are divided into classes of 1-10 µm, 10-25 µm, 25-50 µm, and all particles >50 µm. Data is displayed as particles per mL of sample. Sample size for this techique is 1 mL. Seven separate measurements were done on each of the vial/bag. Results are displayed as average values from five vials/bags with standard deviation. Note different scales for 1-10 µm and other particles.

Further analysis was performed on this data to establish significance of difference between vials with different stoppers and bags before and after freezing. Figures 12-15 display these differences for this data.

Figure 12 shows the effect of stoppers.

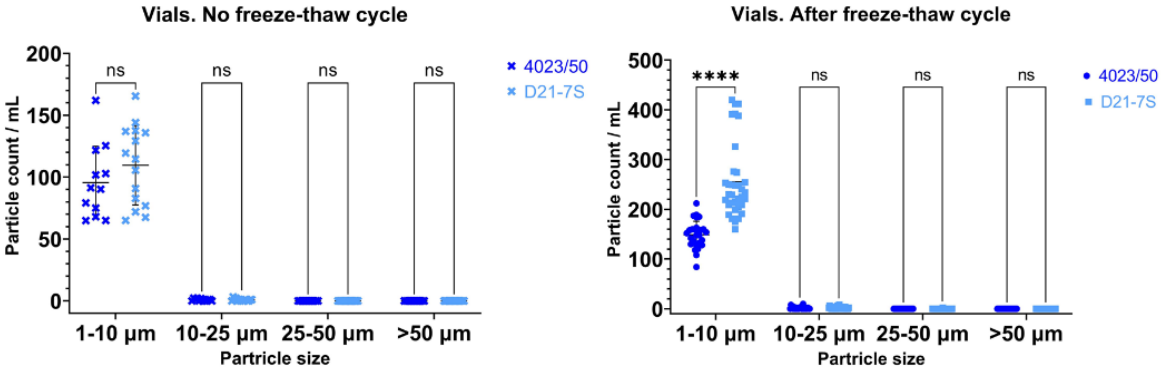


Figure 12. Flow imaging results. Effect of stopper formulation.

As can be seen in Figure 12, no statistically-significant difference was observed between samples in CZ vials closed with different stoppers without a freeze-thaw cycle and regardless of the particle size. After the freeze-thaw cycle, samples that used stopper S10-F451 in D21-7S formulation have more small (1-10 µm) particles than samples with the other stopper.

Figure 13 compares different bags.

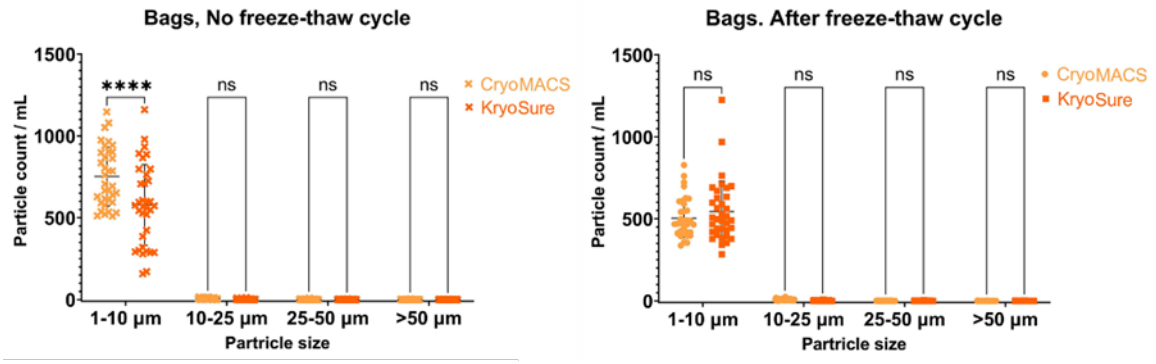
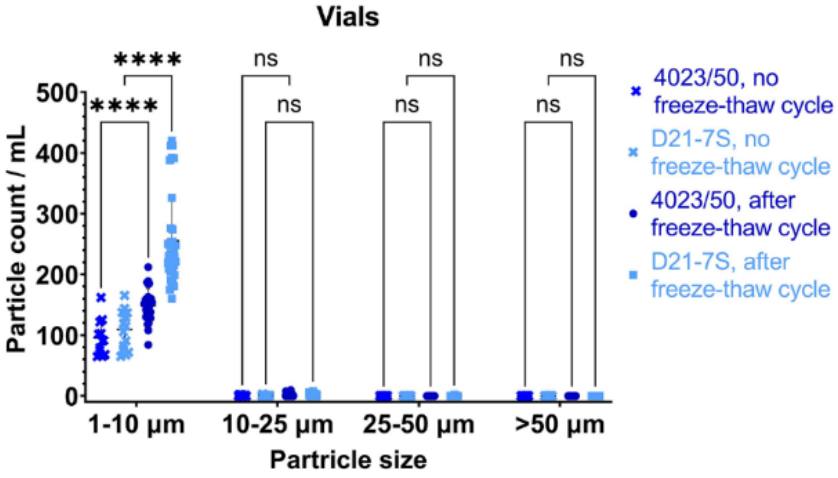


Figure 13. Flow imaging results. Effect of bag material.

As can be seen, the only difference observed is between small particles (1-10 µm) in different bag types without freeze-thaw cycle. After freeze-thaw cycle no statistically-significant differences were observed for these two bag types.



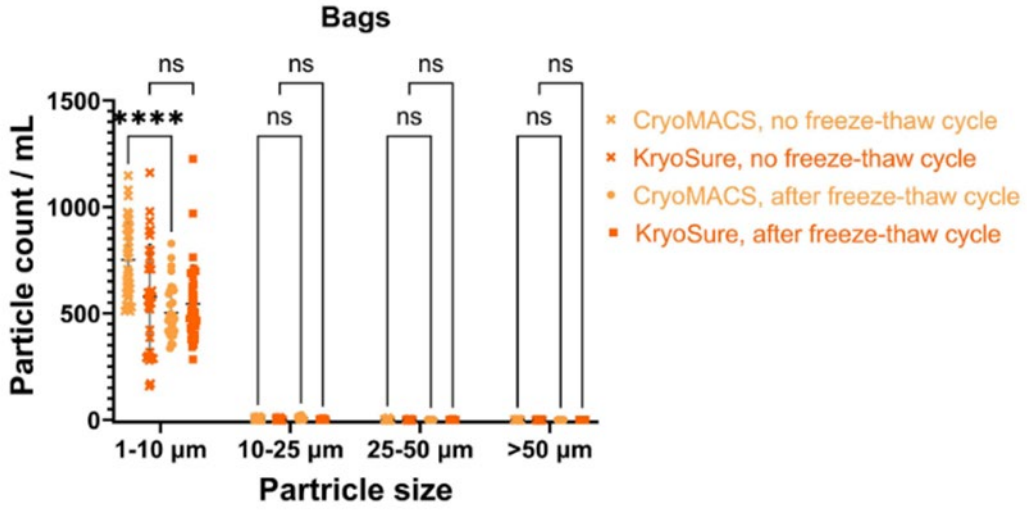


Figure 14. Flow imaging results. Effect of freeze-thaw cycle.

Figure 14 shows the effect of freeze-thaw cycle on particle numbers in CZ vials and bags. Samples with both stoppers show an increase in small (1-10 µm) particle numbers after a freeze-thaw cycle. The only difference observed for bags was for 1-10 µm particles in CryoMACS (EVA) bags after the freeze-thaw cycle. Larger particles and the KryoSure bag didn't show any difference.

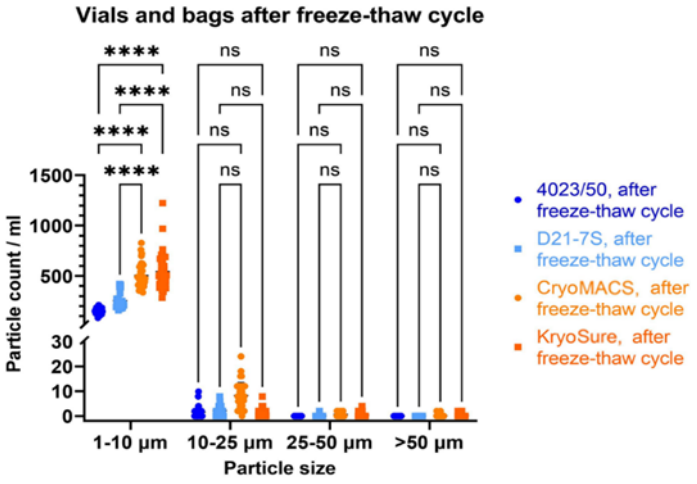


Figure 15. Flow imaging results. Effect of container type after a freeze-thaw cycle.

Figure 15 compares particle loads in CZ vials with different stoppers and cryo bags, all after subjected to a freeze-thaw cycle. As can be seen in Figure 15, bags have more small (1-10 µm) particles than vials. There is no significant difference between these packaging components for larger particles ≥10 µm.

4. Particle analysis

Some selected particles were photographed and analyzed with FTIR and SEM/EDX after counting on the filters. Pictures of some representative particles are shown in Figure 16.

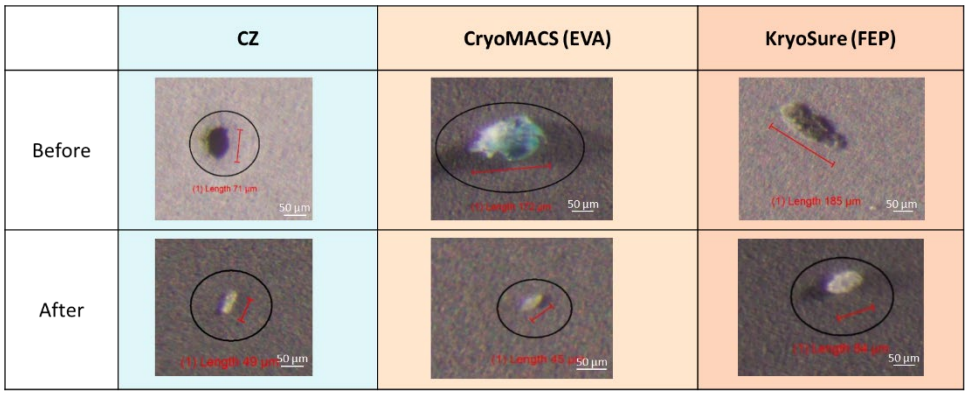


Figure 16. Microphotographs of representative particles. Samples that were not subject to freeze-thaw cycle are marked as "Before" and those that we subjected to freeze-thaw cycle are marked as "After".

50 mL CZ vials with 4023/50 stopper not subjected to a freeze-thaw cycle show the presence of many cellulose particles. Individual particles of protein, magnesium silicate and calcium carbonate were also found. 50 mL CZ vials with 4023/50 stoppers after a freeze-thaw cycle show the presence of several fluorocarbon particles as well as one cellulose and one calcium stearate particle.

50 mL CZ vials with D21-7S stopper not subjected to a freeze-thaw cycle had several silicone particles, and several calcium carbonate particles mixed with polyolefin. After the freeze-thaw cycle there were several cellulose, several protein and one polyolefin particle.

EVA bags that didn't undergo a freeze-thaw cycle had two cellulose, two fluorocarbon, two protein, and two steel particles, and one silicone dioxide particle mixed with magnesium silicate particle. After the freeze-thaw cycle, most of the particles were EVA. There was also one particle each of cellulose, protein, and calcium stearate/carbonate mix. FEP bags had multiple cellulose and fluorocarbon particles both with and without a freeze-thaw cycle.

5. Surface analysis

The surface of the CZ vials was inspected using light microscopy after the freeze-thaw cycle for signs of temperature induced damage and none were obeserved.

IV. Conclusions

Cyclic olefin polymer vials were extensively studied previously for cold temperature and cryo temperature applications. Previous work conducted at West Pharmaceutical Services, Inc includes suitability of CZ vials for cryopreservation of various cell types[8,9,10], suitability for AAV2 storage[11] and CCI during cold storage[9]. In this work we investigated particle load of different types of vials and cryo bags and its dependence on freeze-thaw cycle. Vial materials used here include borosilicate glass, cyclic olefin polymer CZ and polypropylene. Bag materials used here were ethylene vinyl acetate and fluorinated ethylene propylene. 2ml containers were filled at 50% of nominal capacity (1ml fill in a 2ml vial); 50ml containers were filled at 40% of nominal capacity (20ml in 50ml container). Polypropylene cryo vials with screw tops have higher particle load than glass and CZ vials. A freeze-thaw cycle (to -80°C) only increases the number of smaller particles in these vials. CZ vials and glass vials have a similar particle load. The majority of the particles found in polypropylene cryo vials with screw top either with or without a freeze-thaw cycle are polypropylene, the rest being cellulose and protein. The majority of the particles found in CZ and glass vials are cellulose and protein with some other individual particles that can be related to environment contamination or stopper-related materials. There were no glass or COP particles found in these vials. Surface analysis doesn't show any temperature-related damage on these vials.

Two particle-counting techniques used in this study showed a generally comparable number of particles in vials, regardless of the stopper being used. Both bags tested here were also generally comparable in particle load. The freeze-thaw cycle increases the number of small (1-10 µm) particles but doesn't generally affect the number of particles >10 µm. Several bags were lost after a freeze-thaw cycle due to damages. Comparison of the containers used in this study shows that bags have a greater number of small (1-10 µm) particles than vials do, and there is no significant difference between these packaging components for particles >10 µm. 50 mL CZ vials didn't have any specific trends of particle composition, although protein and cellulose were common in most samples. EVA bags showed presence of EVA particles after a freeze-thaw cycle that can be an indicator of the temperature-related stress these bags experience. FEP bags show the presence of fluorocarbon particles that might also indicate integrity issues. Surface analysis doesn't show any temperature-related damage on these vials.

V. Acknowledgements

Thanks are extended to West Analytical Labs for support in sample assembly and testing; and to S. Molina for for his support in performing cryo studies, data interpretation and critical review of this report.

VI. References

1. USP 43 – NF 38. General Chapters: <1> Injections and Implanted Drug Products (Parenterals)—Product Quality Tests (2020), US Pharmacopeia/National Formulary
2. USP 43 – NF 38. General Chapters: USP <790> Visible Particulates in Injections (2016), US Pharmacopeia/National Formulary
3. USP 43 – NF 38. General Chapters: <788> Particulate Matter in Injections (2013), US Pharmacopeia/National Formulary
4. USP 43 – NF 38. General Chapters: <787> Subvisible Particulate Matter in Therapeutic Protein Injections (2021), US Pharmacopeia/National Formulary
5. USP 43 – NF 38. General Chapters: <789> Particulate Matter in Ophthalmic Solutions (2015), US Pharmacopeia/National Formulary
6. USP 43 – NF 38. General Chapters: <1790> Visual Inspection of Injections (2021), US Pharmacopeia/National Formulary
7. Bukofzer, S., Ayres, J., Chavez, A. Industry Perspective on the Medical Risk of Visible Particles in Injectable Drug Products. PDA J Pharm Sci and Tech, 2015, 69, 123-139
8. West Technical Report TR 2020/030. Evaluation of Daikyo Crystal Zenith® Vials for the Cryopreservation of Therapeutically Relevant Human Cell Types.
9. West Technical Report TR 2020/032. Performance of Daikyo Crystal Zenith® 2mL Vials at Cryogenic Temperatures: Container Closure Integrity and Cell Preservation.
10. West Technical Report TR 2021/034. Evaluation of Daikyo Crystal Zenith® Vials versus Ethylene Vinyl Acetate (EVA) Cryobags for the Cryopreservation of Therapeutically Relevant T Cells.
11. West Technical Report TR 2021/033. Functional Assessment of Adeno-Associated Virus Stored in Daikyo Crystal Zenith Vials at Ultra-Cold Temperatures.

This Technical Report dated 21 July 2022, is the first release version of this report.

Long-Term Container Closure Integrity Testing of Vial-Stopper-Seal Combinations Comprising Daikyo Crystal Zenith® Vials at Ultra-Low and Cryogenic Temperatures

I. Background

Gene and cell therapies require storage at -80°C (ultra-low) and below -130°C (cryogenic), respectively. The vial-stopper-seal combinations selected for storage of these must be able to maintain container closure integrity (CCI) at said temperatures. The objective of this study is to evaluate CCI over 24 months at ambient, ultra-low, and cryogenic temperatures for NovaPure® and Daikyo elastomer stopper designs (13 mm Serum NovaPure® RP ART 1358 4023/50G, 13 mm Lyo NovaPure® RP ART 1356 4023/50G, 13 mm Serum Daikyo S2-F451 D21-7S RB2-40 RUV, and 13 mm Lyo Daikyo V2-F451W D21-7S RB2-TR RUV) with gamma-sterilized Flip-Off® CCS (clean, certified, sterilized) seals (13 mm CCS Seal 5922-1120) and Daikyo Crystal Zenith® (CZ) vials (2 mL). These components are available as part of West's Ready Pack™ containment solution offering.

II. Executive Summary

The study was designed to investigate CCI over a period of 24 months at ambient, ultra-low (-80°C), and cryogenic (≤ -130°C) temperatures for serum-stopper samples and at ambient temperature for lyo-stopper samples for the combinations of Ready-to-Use CZ vials, stoppers, and seals described in further detail below. At each time point, vial-stopper-seal combinations were tested for CCI using helium leak detection and laser-based gas headspace analysis. The samples evaluated over 24 months maintained CCI. Results indicate that NovaPure® Serum/Lyo 4023/50 stoppers or Daikyo Serum/Lyo D21-7S stoppers, in combination with Daikyo CZ vials and West's gamma-sterilized Flip-Off® CCS seals, offer viable CCI when used under these study conditions for up to 24 months. As such, they are potentially viable candidates for storage of gene and cell therapies. This is the first experimental demonstration of long-term ambient, ultra-low and cryogenic temperature CCI performance of CZ-based-vial-stopper-seal combinations.

III. Experimental

Table 1: Vial-Stopper-Seal Combinations

Vial description	Stopper description	Seal description
2 mL 13 mm Daikyo CZ RU vial	13 mm Serum stopper (NovaPure® RP article 1358, formula 4023/50 with FluroTec® film coating)	13 mm (ART 5417) Flip-Off® CCS Seal 13 mm
	13 mm Lyo stopper (NovaPure® RP article 1356, formula 4023/50 with FluroTec® film coating)	
	13 mm Serum stopper (Daikyo article S2-F451, formula D21-7S with RB2-40 coating RUV)	
	13 mm Lyo stopper (Daikyo article V2-F451W, formula D21-7S with RB2-TR coating RUV)	

Technical Report 2023/261

20 MAR 2023

Table 2: Sample Configurations with Combinations at Medium Compression

Sample Configuration	Vial	Stopper	Seal	Average Compression (%)*
1	2 mL 13 mm Daikyo CZ RU vial	13 mm Serum NovaPure® RP ART 1358 4023/50G	13 mm Flip-Off® CCS 5417 Seal	41%
2		13 mm Lyo NovaPure® RP ART 1356 4023/50G		
3		13 mm Serum Daikyo S2-F451 D21-7S RB2-40 RUV		
4		13 mm Lyo Daikyo V2-F451W D21-7S RB2-TR RUV		

* Serum and Lyo stoppers are different configurations so compressions cannot be directly compared. West customers may cap CZ vials under varying conditions and with a range of different equipment; thus, compression/RSF values may differ based on customer's capping parameters.

All benchtop capping was performed using pre-set, nominal conditions of the Bausch + Strobel HWM 4610 Closing Machine. For vial-stopper-seal combinations to be tested at ambient and cryogenic temperatures, assembly was done in atmospheric air. For combinations to be tested at -80°C, assembly was done in a N_2-filled glove bag (i.e., ca. 100% N_2 atmosphere inside combination), after which samples were removed from the glove bag and benchtop capping was performed. Those samples were stored under nitrogen until the samples were set down on stability at the various storage conditions.

The Residual Seal Force (RSF) was measured within 48 hours of capping using the Genesis Model AWG RSF Tester. RSF is a measurement of the seal tightness of the stopper against the vial in the vial-stopper-seal combination resulting from the sealing process. This involves indirectly measuring the force exerted by the stopper on a vial's land surface. The sealed vial is placed into the sample holder along with an appropriately sized cap anvil on top of the vial. As pressure is applied to the sample, the machine calculates the force (pounds-force) required to dislodge the crimp seal from the underside of the vial crown.

Helium leak measurements at ambient temperature, -80°C and -180°C were performed with the Leak Detection Associates (LDA)/Packaging Technologies and Inspection (PTI) 1284+ Seal Integrity Monitoring System (SIMS). See Figure 1. Ambient temperature helium leak measurements were made with a continuous helium flow adaptation comprising a fitting enabling isolation and measurement of the vial/stopper seal; continuous helium flow was facilitated through a drilled opening in the vial. Ultra-low and cryogenic temperature helium leak measurements were made with a West-designed vacuum chamber.

Figure 1. Photo of the SIMS 1284+ helium leak detector.

Laser-based headspace analysis (i.e., O_2 headspace) was performed with the Oxygen Headspace Analyzer, FMS-760, manufactured by Lighthouse Instruments. See Figure 2. To prepare for testing, ultra-low temperature storage (-80°C) was performed in an air-filled freezer. For cryogenic storage, samples were first placed in a -80°C freezer for approximately 4 hours, then removed and placed directly into a cryogenic freezer for continued storage. Details of removal from storage and CCI measurement are given below. Oxygen headspace analysis was performed only on serum stopper-based combinations as combinations with lyo stoppers are not generally intended for ultra-low or cryogenic storage.

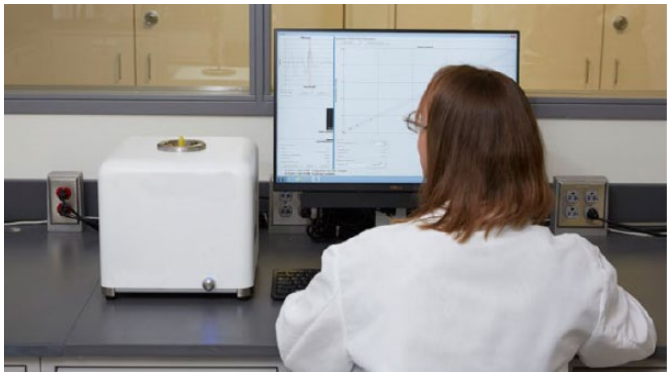


Figure 2. Photo of the FMS-760, Oxygen Headspace Analyzer.

IV. Results

Results presented herein demonstrate 24 months CCI data to guide recommendations for customer use. To meet the USP <1207> recommendation for validation, the customer will need to perform validation on the final drug product and vial-stopper-seal combination.

Residual Seal Force

Residual seal force (RSF) was tested per RSF-01 to measure the seal tightness in the vial-stopper-seal combination resulting from each capping process.[1] This testing was done using 10 samples of each vial-stopper-seal combination at T=0. Average initial RSF values can be seen in Figure 3.

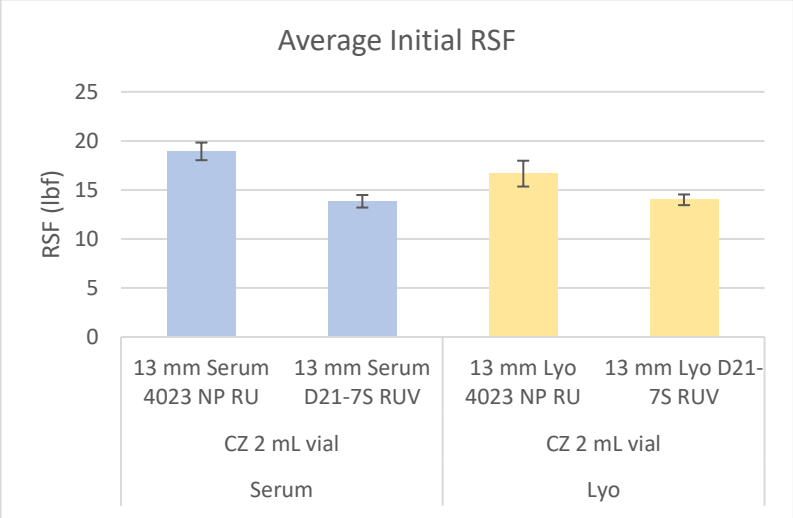


Figure 3. Average Initial RSF (10 samples per combination). Error bars represent standard deviation.

Helium Leak Results

Data were generated on air-filled (RT and -180°C) or nitrogen filled (-80°C) vial-stopper-seal combinations.

Note: Work by Kirsch, *et al*.[2] has been used generally in evaluating helium leak data. Kirsch uses std*cc/s for helium leak testing reporting units whereas West uses mbar L/s. The conversion factor between mbar L/s and std*cc/s is 1.013 mbar L/s = 1 std*cc/s. The 0.013 difference creates an **~1.3%** difference in the value reported.

i) Ambient Temperature Results (helium leak detection using 100% helium flow)

Testing was performed according to an established West test method.[3] Data are given in Figure 4. During this testing, the CCI of a vial-stopper-seal combination is assessed by inserting into a specially designed fixture that isolates the stopper-vial interface, applying 100% helium flow to the back of that interface, and monitoring the combination under vacuum for the leak rate of helium across the interface. Helium flows into the configuration through a hole placed in the vial. As this test is non-destructive to the stopper-vial interface, the same samples can be evaluated over time, which was the case in the present work. This method directly measures the performance of the vial-stopper interface. Sample helium leak rate values

were seen to be below the Kirsch Limit (≤ $6x10^{-6}$ cm^3/s, i.e. ≥ 5.2 on a -log_{10} scale). The results shown are the average of 20 samples for each combination. Results are represented as -log values (as opposed to exponential) of the helium leak rate (mbar L/s) for easier comparison and in order to clearly show sample helium leak values on the same scale as the Kirsch limit. Moreover, according to the Kirsch work, the data reported correspond to no microbial ingress (i.e., 0%).

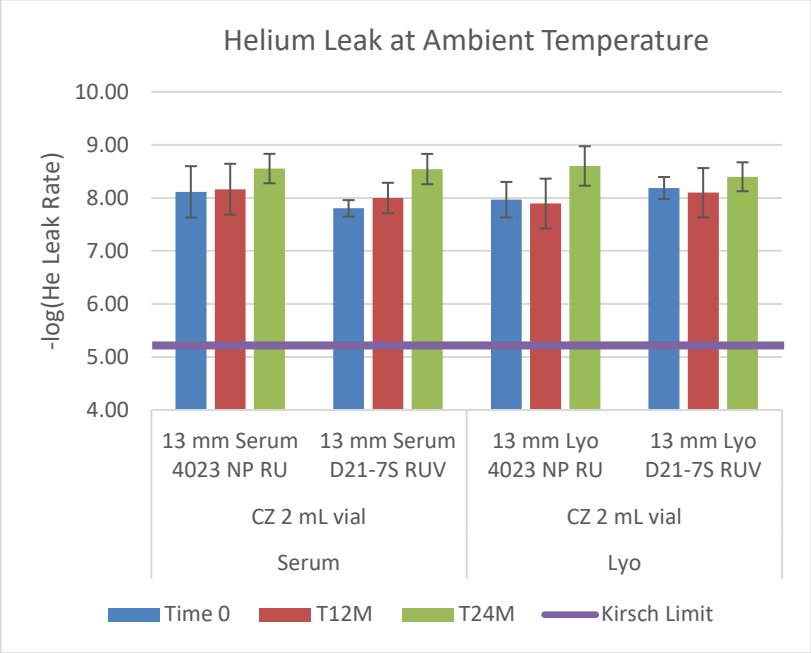


Figure 4. Average helium leak for 13 mm Combinations. Error bars represent standard deviation.

ii) Temperature -80°C Results (helium leak detection using headspace gas exchange)

Testing was performed according to an established West test method.[4] Samples evaluated were originally capped under N_2 before being stored at -80°C in a freezer for 12 and 24 months. Representative samples were tested at Time 0. The samples were removed from the freezer at their respective time points and transferred to the benchtop to equilibrate to ambient temperature prior to filling with helium. Once filled, the sealed vials were put into a -80°C freezer for a minimum of 3 hours prior to analysis. The samples were then placed in a chiller that is maintained at -80°C and analyzed.

During this testing, the CCI of a vial-stopper-seal combination is assessed by filling the vial-stopper-seal combination with helium and then monitoring under vacuum for the leak rate of helium at -80°C conditions (*i.e.*, the entire combination is in the vacuum chamber at said temperature). See Figure 5. For all tested samples, the helium leak rate values were below the Kirsch Limit. The results shown are the average of 20 samples for each combination. Results are represented as -log values (as opposed to exponential) of the helium Leak rate (mbar L/s) for easier comparison and to clearly show sample helium leak values on the same scale as the accepted Kirsch standard. Note that there is some helium leak. It could be through the

vial and stopper as helium is the most permeating gas known.[5] It is also important to mention that it appears the vial-stopper-seal combination maintained integrity throughout the thaw/freeze cycle.

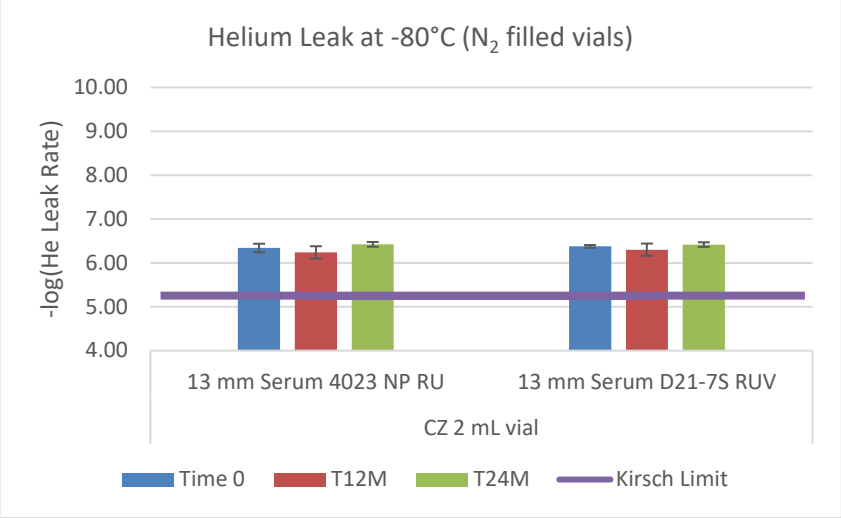


Figure 5. Average helium leak for 13 mm Systems at -80°C. Error bars represent standard deviation.

iii) Cryogenic Temperature Results (helium leak detection using headspace gas exchange)

Testing was performed according to an established West test method.[6] Samples evaluated were capped under standard atmospheric conditions. The samples were placed into a -80°C freezer for a minimum of 4 hours before being cooled down to cryogenic temperatures (≤ -130°C, vapor of liquid N_2 atmosphere) for 12 and 24 months. The samples were removed at their respective time points and transferred to the benchtop to equilibrate to ambient temperature prior to filling with helium. The sealed samples were subsequently put back into a freezer and brought back down to -80°C for a minimum of 4 hours, then transferred to a dewar containing liquid nitrogen for a minimum of 1 hour. The samples were then placed inside a fixture capable of maintaining a minimum temperature of -180°C and analyzed.

During this testing, the CCI of the vial-stopper-seal combination is assessed by filling the vial-stopper-seal combination with helium and then monitoring under vacuum for the leak rate of helium at -180°C (*i.e.*, entire combination is in vacuum chamber at said temperature). See Figure 6. Sample helium leak rate values were below the Kirsch Limit. The results shown are the average of 20 samples for each combination. Results are represented as -log values (as opposed to exponential) of the helium leak rate (mbar L/s) for easier comparison and to show clearly sample helium leak values on the same scale as the accepted Kirsch standard. It is important to note that it appears the vial-stopper-seal combination maintained integrity through the thaw/freeze cycle.

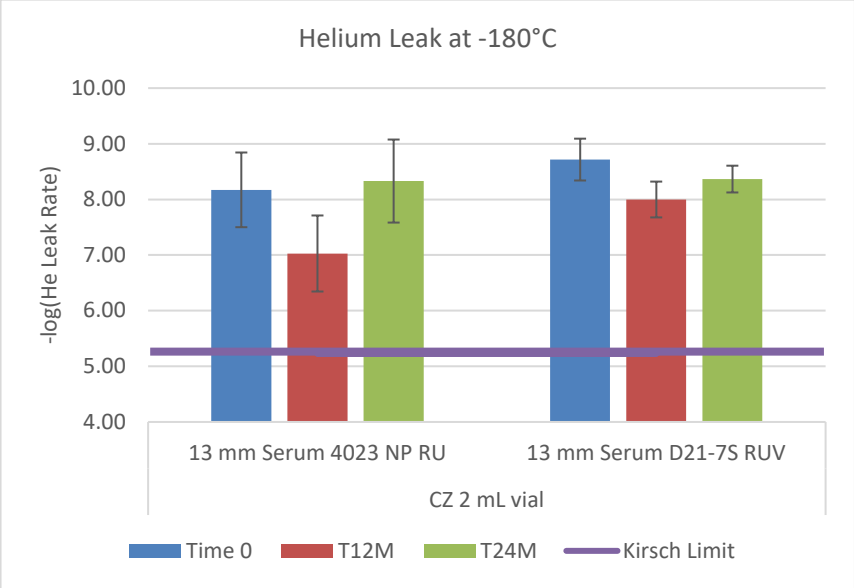


Figure 6. Average helium leak for 13 mm configurations at -180°C

Oxygen Headspace Results

Laser-based headspace testing (i.e., O_2 headspace) was performed according to an established West test method.[7] During this testing, the oxygen in the headspace of the sealed combination is monitored. Light from a near-infrared laser is passed through the container in the region above the product and below the stopper-seal. The laser frequency is tuned to match an internal absorption frequency of the oxygen molecule. The amount of light absorbed while passing through the container is measured and then related to the oxygen concentration in each sample using a series of prior-established standards. T=0 testing was done for each sample to be tested prior to storage at -80°C (freezer – air atmosphere) and ≤ -130°C (vapor of liquid N_2 atmosphere) conditions for both the 12- and 24-months samples. Samples were pulled from storage conditions -80°C and ≤ -130°C and placed on the benchtop at ambient temperature to thaw to ambient temperature. Samples were tested within 4 hours of removal from the storage conditions. The results are for the average of 20 samples for each combination. Testing was done using the same samples for Time 0 and 12 months and the same samples for Time 0 and 24 months. That is, 12-month samples were not reused for 24-month measurements.

i) Oxygen headspace results – Initial vs. 12-24 months at -80°C Temperature Storage

These samples were capped under N_2 conditions. Testing results represent the amount of oxygen that permeated into the vials after 12- and 24-month storage conditions. Samples were brought to ambient temperature and tested within 4 hours of removal from storage. There was slight permeation of oxygen, not unexpected in polymer vials, at 24 months. Oxygen permeation was negligible at 12 months. Breach of container-closure would have resulted in the container-closure atmosphere coming into equilibrium with the outside

atmosphere which would have resulted in ca. 21% O_2. Thus, even though small ingress of O_2 was seen at 24 months, the container-closure did not fail.

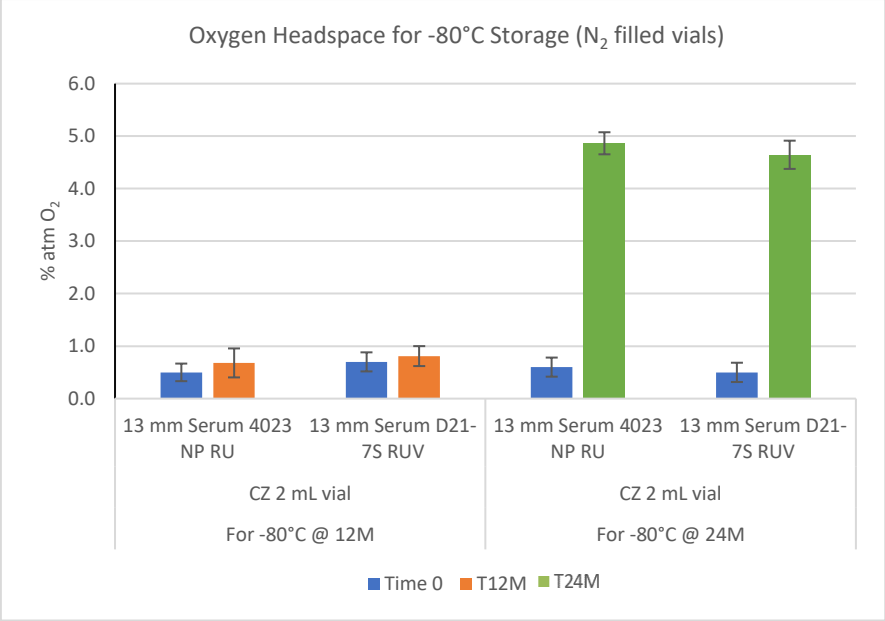


Figure 7. Average oxygen headspace for 13 mm configurations at -80°C.

ii) Oxygen headspace results – Initial vs. 12-24 months at ≤ -130°C Temperature Storage

These samples were capped under air atmosphere. Testing results represent the amount of nitrogen that permeated into the vials after 12- and 24-months cryogenic (≤ -130°C, vapor of liquid nitrogen) storage conditions at 1 atm. Samples were brought to ambient temperature and tested within 4 hours of removal from storage. Permeation of both O_2 and N_2 was determined to be negligible and consistent at ≤ -130°C storage conditions over 24 months. That is to say, no O_2 exited and no N_2 entered the samples – which would have been represented by a decrease in O_2 level. The data indicate that at ≤ -130°C, the temperature is low enough to slow permeation, as opposed to -80°C where permeation does occur. Considering that the atmosphere in the cryogenic freezer was approximately 100% N_2, had there been a breach or failure of any vial-stopper-seal combination, the interior of container-closure would have reached equilibrium at about 100% N_2 as indicated by O_2 concentrations less than 1%.

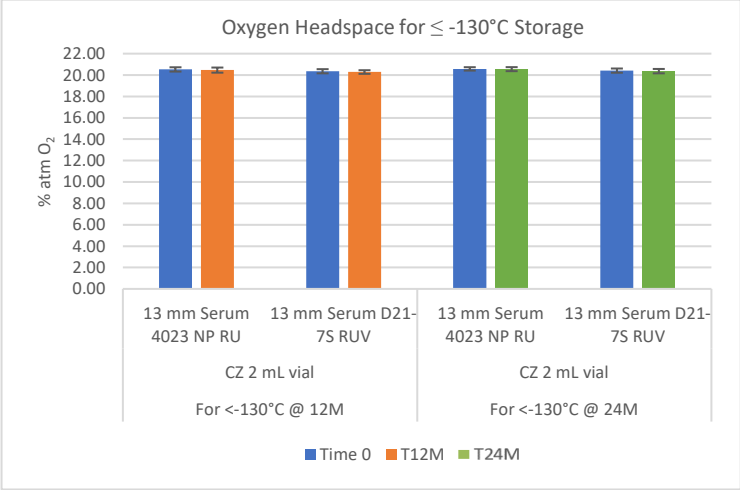


Figure 8. Average oxygen headspace for 13 mm configurations at ≤ -130°C.

V. Discussion of results

The present study examined CCI performance over 24 months of samples stored at ambient temperature, -80°C, and ≤ -130°C for combinations of 2 ml CZ vials with the following NovaPure and Daikyo elastomer stoppers:

- 13 mm Serum NovaPure® ART 1358 4023/50G
- 13 mm Serum Daikyo S2-F451 D21-7S RB2-40 RUV
- 13 mm Lyo NovaPure® ART 1356 4023/50G
- 13 mm Lyo Daikyo V2-F451W D21-7S RB2-TR RUV

CCI measurements by helium leak (100% helium flow) for all vial-stopper-seal combinations at ambient temperature over 24 months showed no change in performance, giving indication of the durability of the vial-stopper interface when properly capped.

CCI measurements by helium leak for serum stopper combinations after 24 months exposure to ultra-low (-80°C) and cryogenic (≤ -130°C) temperatures showed no change in performance, even after a subsequent thaw/freeze cycle, giving indication of the durability of these combinations. Note that, as expected, helium leak values were higher at -80°C than at -180°C. Permeation is a thermally-activated process, so a higher rate is expected at the higher temperature. Note also that these low-temperature helium leak measurements exposed the entire vial-stopper-seal combination to vacuum, whereas the ambient temperature 100% helium flow method which exposed only the seal portion of the container-closure. Both methods provide instructive information, but the data are not directly comparable.

CCI measurements by O_2 headspace measurements for serum stopper combinations over 24 months exposure to ultra-low (-80°C) temperature showed no change in headspace after 12 months and only a small increase in O_2 level to 5% after 24 months. Considering the atmosphere in the freezer was comprised of air (i.e., 21% O_2), had there been a breach or failure of any combination, the interior of the container-closure

would have reached equilibrium at 21% O_2. The fact that it did not, shows the durability of serum stopper combinations. This increase in oxygen concentration likely resulted from the permeation of O_2 through the CZ vial. Permeation is not unexpected, as all polymers are known to be gas permeable.[7] It appears, the combinations maintained integrity.

CCI measurements by O_2-headspace measurements for serum stopper combinations after 24 months exposure to cryogenic (≤ -130°C) temperature showed no change after 24 months. Considering that the atmosphere in the cryogenic freezer was approximately 100% N_2, had there been a breach or failure of any vial-stopper-seal combination, the interior of container-closure would have reached equilibrium at about 100% N_2 as indicated by O_2 concentrations less than 1%. These data, show the durability of the serum stopper combinations at this temperature. When contrasted to the -80°C observations, the lower temperature of ≤ -130°C was sufficient to suppress any significant level of permeation.

Helium leak data shows all configurations maintained CCI over a period of 24 months ambient temperature and cold storage conditions based on averaged data. Oxygen headspace data show that there was slight oxygen permeation into nitrogen filled vials in air at -80°C. Permeation by oxygen is not unexpected for polymer vials in oxygen headspace gas exchange experiments and therefore is not necessarily indicative of a true leak. During cold storage, the cooling of the vial causes a change in the internal pressure of the vial. The pressure on the outside of the vial is 1 atm while the pressure inside the vial is ~0.65 atm. Therefore, if there was a failure seen, the pressure gradient would increase, and a level near 21% oxygen would have been expected. The ingress of nitrogen in this instance is minimal in comparison to failure levels. Oxygen egress out of vials stored in liquid nitrogen vapor phase at ≤ -130°C is negligible.

VI. Conclusions

This study indicates that a viable container closure combination, potentially suitable for gene and cell therapies, can be created using NovaPure® Serum/Lyo 4023/50 stoppers, Daikyo Serum/Lyo D21-7S stoppers, Daikyo CZ vials, and West's gamma-sterilized Flip-Off® CCS seals when used as they were in this study. These components are available as part of West's Ready Pack™ containment solution offering. All tests over the 24 months, at ambient temperature, ultra-low temperature, and cryogenic temperature, demonstrated durability of these combinations and maintenance of CCI with proper capping.

VII. References

1) West Standard Laboratory Test Method, RSF-01, Determination of Residual Seal Force, current version.
2) L. E. Kirsch, et al. (University of Iowa) *Pharmaceutical Container/Closure Integrity II: The Relationship between Microbial Ingress and Helium Leak Rates in Rubber-Stoppered Vials.* PDA Journal of Pharmaceutical Science & Technology, 51 (5), 195-202 (1997).
3) West Standard Laboratory Test Method, Selblty-34, Helium Leak Testing on Pharmaceutical Packaging using Fixtures and 100% Helium Flow, current version.
4) West Standard Laboratory Test Method, Selblty-28, Helium Leak Testing on Pharmaceutical Packaging at -80° C (DMF), current version.

5) Robeson, L.M. Correlation of separation factor versus permeability for polymeric membranes Journal of Membrane Science, 62 (1991) 165-185.
6) West Standard Laboratory Test Method, Selblty-32, Helium leak at -180C, current version.
7) West Standard Laboratory Test Method, Selblty-31, Measurement of Oxygen in Headspace of Sealed Containers, current version.

VIII. Legal

This document is for informational purposes only. West's products and solutions are sold on the basis that it is the customer's responsibility to evaluate and test the West product or solution to determine its compatibility with other materials and fitness for any end use. WEST MAKES NO WARRANTIES, WHETHER EXPRESS, IMPLIED OR STATUTORY, INCLUDING, WITHOUT LIMITATION, THE WARRANTIES OF MERCHANTABILITY AND FITNESS FOR PARTICULAR PURPOSE, RELATING TO THE INFORMATION IN THIS DOCUMENT.

This technical report dated 20 March 2023 is the first release version of this report.

Container Closure Integrity of Rubber-Glass Vial Systems

I. Summary

A two-year study to establish the container closure integrity (CCI) performance landscape of systems comprising rubber stoppers and glass vials was completed. It was based upon a design of experiments to consider the full range of: (a) stopper formulations, sizes, and configurations; (b) vial sizes, types, and suppliers; and (c) compressions. Evaluation was based upon measurement of residual seal force and CCI (with the USP-endorsed methods of tracer gas leak detection and frequency modulated spectroscopy). All systems showed good performance – no decrease in CCI with time. Formulations 4023, 4432 and 4031 showed comparable results. However, low compressions tended in some cases to give slightly lower performance and wider scatter in results, and thus should not be recommended. A small amount of oxygen exchange through the stoppers was observed for all systems; this was to be expected since rubber is known to be gas permeable. Residual seal force decreased initially, then remained constant – consistent with models.

II. Background

Container closure integrity (CCI) is an essential element of a drug product container system. Without the ability to provide performance over the drug product's shelf life, at a level below the maximum allowable leak limit (MALL), the system has no value. This is discussed in detail in the recent USP guidance: *Chapter <1207> Package Integrity Evaluation – Sterile Products*. This guidance describes methods to evaluate CCI; it strongly endorses deterministic methods (e.g., tracer gas leak detection) where possible, vis-a-vis probabilistic methods (e.g., tracer liquids). West has published a Technical Service Bulletin commenting on this guidance. (1)

An aspect not yet evaluated in detail is the CCI performance provided by systems comprising West rubber stoppers with glass vials – as a function of time. Prior research had examined the systems given in Table 1, at initial time. (2) Systems represented a variety of formulations, sizes, and configurations. All systems were observed to have good CCI as determined by tracer helium gas leak detection (hereafter called He-leak). The standard was a value lower than 6.0×10^{-6} cm^3/s (sometimes called *Kirsch level*) – typical values were $< 2 \times 10^{-7}$ cm^3/s for 20 mm stoppers and $< 7 \times 10^{-8}$ cm^3/s for 13 mm stoppers. (3) Clearly this indicates an *initial* robustness of West products. Evaluation of product performance versus time needs to be the next objective.

Technical Report 2018/199

23 Sep 2019

Table 1. Configurations for Time = 0 Study. Compression targets were low, medium, and high – corresponding to approximately 10-15%, 20-35%, and 25-40% for 20 mm stoppers – and 20-30%, 45-55%, and 50-60% for 13 mm stoppers, respectively. These corresponded to residual seal force values of 5-18 lbs. F-Tec indicates stoppers had a FluroTec® laminate. (2)

Formulation	**Stopper Size** (mm)/**Configuration**	**Glass Vial** (blowback)
Serum		
4023/50	13 (ART 1104)	None
	20 (ART 1071)	None
	20 (ART 1343, F-Tec)	US
4432/50	13 (S2-F451, F-Tec)	US
	13 (V-35)	EU
	20 (S-127)	EU
Lyophilization		
4023/50	13 (ART 1079)	None
	13 (ART 1356, F-Tec)	US
	20 (ART 1319)	None
	20 (ART 1346, F-Tec)	US
4432/50	13 (V2-F451W, F-Tec)	EU
	20 (V10-F597, F-Tec)	EU
Seals: 13 mm stoppers: 13 FO LQ Long TE (6-B) 3767 Red Matte 20 mm stoppers: 20 FO LQ TE (6-B) 3767 Red Matte		

Performance versus Time

This study versus time is necessarily more involved since a new rubber formulation has been introduced to serve the generics market – 4031/45 Gray (AccelTRA® brand components). Thus, in practical terms, the variables to be considered are shown in Table 2.

Table 2. Variables in Vial/Stopper Systems

Stopper			**Vial**		
Formulation	Configuration	Size	Size	Configuration (blowback)	Vendor
4023/50	Serum	13 mm	2R	None	Schott
4432/50	Serum – NovaPure® component	20 mm	6R	European	Ompi
4031/45	Lyophilization	---	---	US	---
---	Lyophilization – NovaPure® component	---	---	---	---

From the elements in Table 2, there are 144 permutations of stoppers/vials. Consider Table 3. This describes the number of samples that are needed for a 2-year study, considering evaluation by both He-leak and measurement of oxygen headspace (hereafter called OHS) concentration by frequency modulated spectroscopy.

Table 3. Sample Count for 2-Year Study for One Stopper/Vial System. He-leak samples are discarded after measurement. OHS samples are reused.

	He Leak					OHS	Total
Time (months)	0	3	6	12	24		
Low	20	20	20	20	20	30	130
Medium	20	20	20	20	20	30	130
High	20	20	20	20	20	30	130
							~ 400

For 144 permutations, at ~ 400 vials per, 58,000 stopper/vial samples are needed; clearly this is impractical. As such, a design of experiments must be developed to establish the landscape of performance while employing a manageable number of samples.

III. Design of Experiments

The design of experiments (DOE) focuses on alternating variables – selecting stopper/vial combinations such that each variable is considered with the highest number of other variables that is practical. The DOE is shown in Table 4. Consider for example the yellow rows: It is seen that formulation 4432/50 is considered in both configurations (serum and lyophilization) and both sizes (13 mm and 20 mm), with two vial blowback configurations (EU and Non), and two vendors (Schott and Ompi). Note that each combination was reviewed with a team comprising persons from Technical Customer Support and Product Management to verify that those selected were reasonable.

Table 4. Design of Experiments

Formulation	Stopper Size	Configuration	Vial Blowback	Vendor	Nominal Interference (%)	Interference Min / Max (%)
Serum						
4432/50	20 mm	S-127	EU	Schott	2.8	0.2 / 5.5
4023/50	13 mm	NP 1358	Non	Schott	6.4	1.4 / 11.8
4031/45	13 mm	ART 1104	Non	Ompi	7.1	2.8 / 11.8
4031/45	13 mm	V-35	US	Ompi	5.3	0.6 / 10.3
Lyophilization						
4023/50	20 mm	ART 1319	EU	Schott	4.8	2.3 / 7.3
4432/50	13 mm	NP V2-F451-W (F-Tec)	Non	Ompi	9.6	3.7 / 15.7
4031/45	20 mm	S-87-I	EU	Schott	4.8	2.1 / 7.5
4023/50	20 mm	ART 1346 (F-Tec)	US	Ompi	6.3	2.7 / 10.1
interference fit = 100 {[(stopper plug diameter) – (vial inner neck diameter)] / (stopper plug diameter)}						

In Table 5, it is shown how many of the possible combinations are evaluated. For example, consider "row 4023." It can be seen that the DOE enables this formulation to be considered with every other variable. The result of such a study is that a performance landscape is developed so that trends of performance can be understood. For this DOE, ~3,200 vial/stopper samples are needed (8 combinations x ~400 stopper/vial samples per) – a large number – but manageable, as compared to 58,000.

Table 5. Combinations Achieved with DOE. Red boxes indicate those not addressed.

	Formulation			**Stopper Type**		**Stopper Size** (mm)		**Vial Type** (Blowback)			**Vendor**	
	4023	4432	4031	Serum	Lyo	13	20	Non	EU	US	Schott	Ompi
4023				•	•	•	•	•	•	•	•	•
4432				•	•	•	•	•	•		•	•
4031				•	•	•	•	•	•	•	•	•
Serum	•	•	•			•	•	•	•	•	•	•
Lyo	•	•	•			•	•	•	•	•	•	•
13 mm	•	•	•	•	•			•		•	•	•
20 mm	•	•	•	•	•				•	•	•	•
No BB	•	•	•	•	•	•					•	•
EU BB	•	•	•	•	•		•				•	
US BB	•		•	•	•	•	•					•
Schott	•	•	•	•	•	•	•	•	•			
Ompi	•	•	•	•	•	•	•	•		•		

Note that 9% of boxes in Table 6 are red, indicating said combination is not addressed. For example, consider "row 4432" – this formulation is not addressed with a vial having US blowback. Yet, 91% of combinations are addressed. Capturing every red box increases number of samples substantially, and thus is impractical.

IV. Experimental

Components used are listed in Table 6. Capping was performed in air at Genesis Packaging Technologies (GPT, Exton, PA). Residual seal force (RSF) measurements were also made at GPT. Samples were stored at ambient conditions (23 ± 3 °C) and sent to GPT for RSF measurements at appointed times. Subsequently, He-leak measurements (LDA SIMS 1284+) were made on the exact same samples, after which they were discarded. Helium Leak results are reported as the -log of their leak rate (mbar·L/s) for ease of comparison. Samples for measurement of oxygen headspace concentration by frequency modulated spectroscopy (Lighthouse Oxygen Analyzer FMS-760) were stored in a nitrogen-filled glove box (100% nitrogen, continuous flow) at ambient temperature (20 ± 5 °C) and returned immediately after measurement (i.e., re-used). Three compression levels were targeted for each combination – low, medium, and high. He-leak, OHS, and RSF measurements were made at times: 0, 3 months, 6 months, 12 months, and 24 months.

Table 6. Component Product Names

Formulation	Stopper Size	Stopper Product Name	Vial Product Name	Seal
Serum				
4432/50	20 mm	S-127 4432/50 GRY Westar® RS	Schott 6R EBB TopLine Fiolax®	20FO LQ TE (6-B) 3768 Green Matte Top
4023/50	13 mm	NovaPure® RS 1358 4023/50G SQS	Schott 2R NBB StandardLine Fiolax®	5135 FOS 13 MM AFAL/IWAB Orange
4031/45	13 mm	1104 4031/45 Grey Westar® RS SIL 3 SQS	Ompi 2R NBB	5135 FOS 13 MM AFAL/IWAB Orange
4031/45	13 mm	V-35 4031/45 GRY DC1000 SIL 3 Westar® RS	Ompi 2R USBB	5135 FOS 13 MM AFAL/IWAB Orange
Lyophilization				
4023/50	20 mm	1319 4023/50/Grey B2-42 Westar® RS SQS	Schott 6R EBB TopLine Fiolax®	20FO LQ TE (6-B) 3768 Green Matte Top
4432/50	13 mm	NovaPure® RP V2-F451W 4432/50 G	Ompi 2R NBB	5135 FOS 13 MM AFAL/IWAB Orange
4031/45	20 mm	S-87-I 4031/45 GRY DC1000 SIL3 Westar® RS	Schott 6R EBB TopLine Fiolax®	20FO LQ TE (6-B) 3768 Green Matte Top
4023/50	20 mm	1346 4023/50/Grey B2-TR Westar® RS SQS	Ompi 6R USBB	20FO LQ TE (6-B) 3768 Green Matte Top

V. Results and Discussion

Compression and initial RSF values are given in Table 7. Compression level was measured immediately after capping (i.e., time = 0) only. He-leak data are averages of 20 samples and are reported as: - log [measured value], so that higher numbers correspond to lower leak rates. OHS data are averages of 30 measurements and are reported as percent O_2. Both He-leak and OHS are deterministic methods endorsed in *USP Chapter <1207>*. RSF data are averages of 20 measurements and reported as lbs. Standard deviations are reported for each.

Table 7. Compression Levels and Initial RSF Values.

Formulation	Stopper Size	Configuration	Vial Blowback	Compression (%)		Initial RSF (lbs) (a)
Serum						
4432/50	20 mm	S-127	EU	Low	16	6.8 (1.8)
				Medium	20	15.4 (3.0)
				High	22	14.7 (3.4)
4023/50	13 mm	NP 1358	Non	Low	18	5.0 (1.0)
				Medium	25	11.4 (1.9)
				High	30	20.4 (1.3)
4031/45	13 mm	ART 1104	Non	Low	27	4.9 (1.2)
				Medium	40	13.7 (2.1)
				High	42	22.7 (1.8)
4031/45	13 mm	V-35	US	Low	22	6.0 (2.5)
				Medium	28	12.1 (4.0)
				High	29	22.9 (3.2)
Lyophilization						
4023/50	20 mm	ART 1319	EU	Low	13	6.3 (2.5)
				Medium	17	11.8 (4.1)
				High	18	18.5 (3.4)
4432/50	13 mm	NP V2-F451-W (F-Tec)	Non	Low	29	4.1 (1.4)
				Medium	35	12.7 (1.2)
				High	41	20.8 (3.3)
4031/45	20 mm	S-87-I	EU	Low	16	8.7 (2.7)
				Medium	20	11.2 (2.4)
				High	20	14.0 (1.9)
4023/50	20 mm	ART 1346 (F-Tec)	US	Low	14	6.7 (1.9)
				Medium	18	13.2 (1.7)
				High	24	19.9 (2.8)

a. parenthetical number is standard deviation

He Leak

Consider Figure 1. All 20 mm systems show good performance. However, at Low compression, 4432 (S-127), 4023 (ART 1319), and 4031 (S-87-I) show a higher level of scatter, and slightly higher leak rates. At Medium and High compression, performance of these is equivalent to other systems/compressions. For this reason, Low compression is not recommended, in general. No trend of decrease of performance with time is observed.

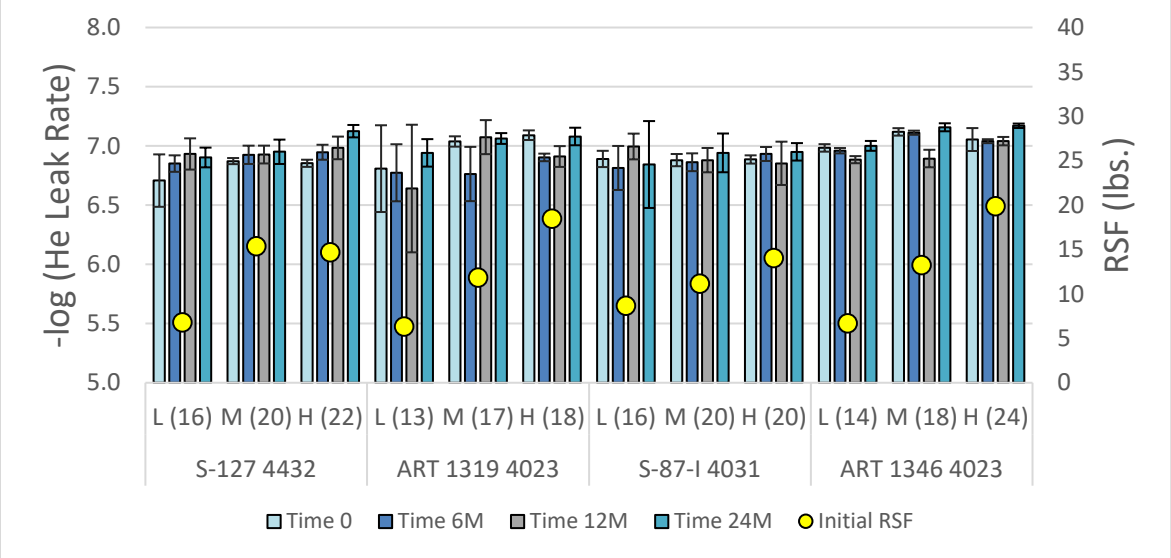


Figure 1. He-Leak Performance of 20 mm Stoppers over Two Years. Initial values of compression [e.g., L (16%)] and RSF are given. Data at 3 months are not shown. Error bars represent standard deviation. Yellow dots are initial RSF values.

Consider Figure 2. A similar trend is observed for 13 mm systems, all show good performance. However, at Low compression, 4031 (ART 1104 and V-35) show a higher level of scatter, and slightly higher leak rates. At Medium and High compression, performance of these is equivalent to other systems/compressions. For this reason, Low compression is not recommended, in general. No trend of decrease of performance with time is observed.

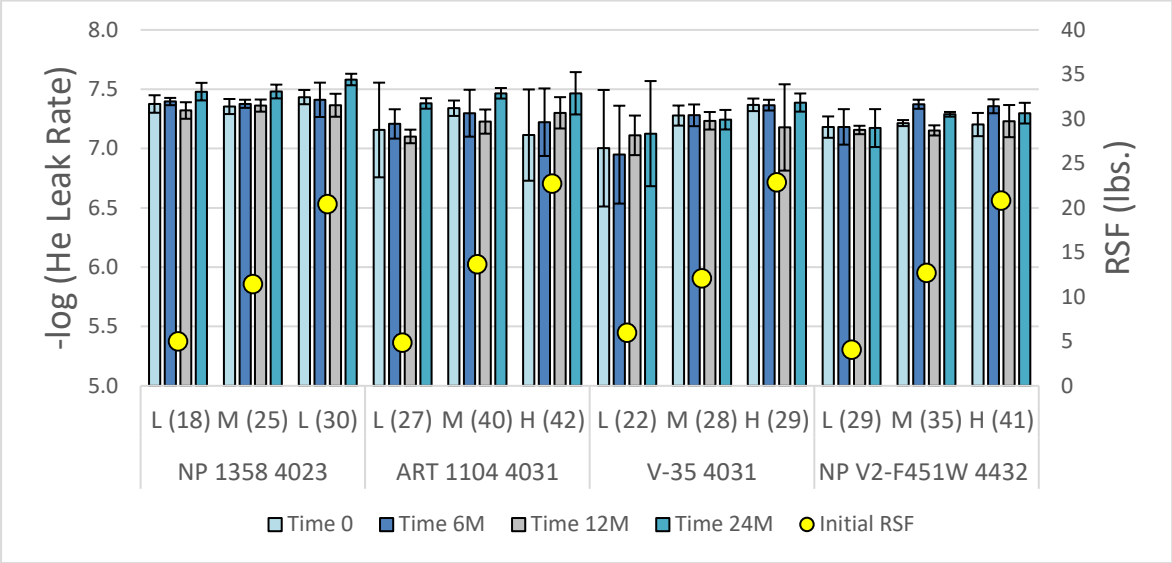


Figure 2. He-Leak Performance of 13 mm Stoppers over Two Years. Initial values of compression [e.g., L (18%)] and RSF are given. Data at 3 months are not shown. Error bars represent standard deviation. Yellow dots are initial RSF values.

Note that for 20 mm systems, the overall average of all data points is ~ (-6.9), whereas for 13 mm systems it is ~ (-7.3) – indicating on average that more He is detected leaking from 20 mm stoppers. This is an experimental artifact only. The area available through which He can diffuse through rubber (i.e., vial opening area) is larger for a 20 mm stopper (1.2 cm^2), than for a 13 mm stopper (0.38 cm^2).

OHS

Consider Figures 3 and 4. These show the level of gas exchange for air-filled stopper/vial systems prepared in air and stored under nitrogen. O_2 levels decrease with time from ~ 20.7% to ~ 18.8% for 20mm systems and ~ 19.2% for 13mm systems (4432 and 4023). 4031 samples showed a slightly greater decrease to ~ 18.0%, but as a practical matter, this difference is imperceptible. Clearly, O_2 can permeate through rubber. This is not surprising, rubber is known to be gas permeable. (4) Considered from the other direction, these data indicate that a stopper/vial system assembled in nitrogen and stored in air will see about 2 - 2.5% O_2 after 2 years.

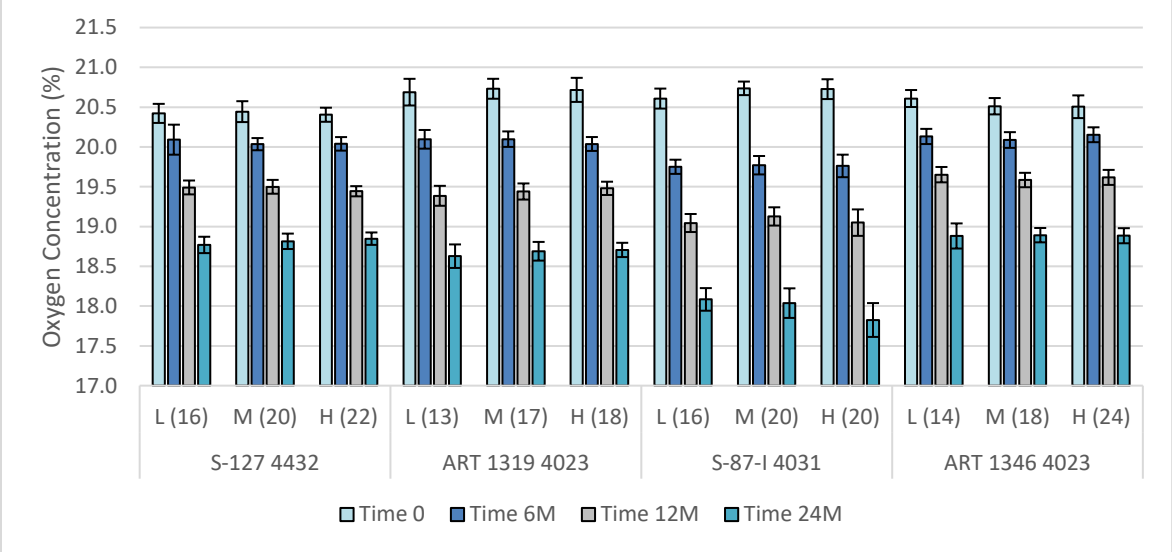


Figure 3. OHS Performance of 20 mm Stoppers over Two Years. Initial values of compression [e.g., L (16%)] are given. Data at 3 months are not shown. Error bars represent standard deviation.

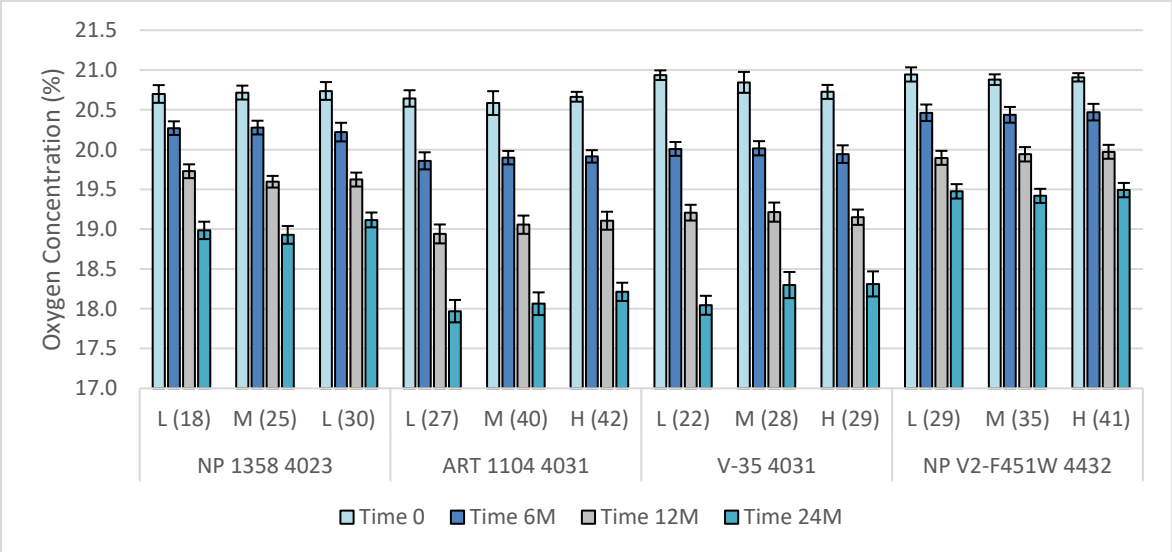


Figure 4. OHS Performance of 13 mm Stoppers over Two Years. Initial values of compression [e.g., L (18%)] are given. Data at 3 months are not shown. Error bars represent standard deviation.

RSF

Consider Figures 5 and 6. For all systems, RSF decreases within the first three months, then is essentially constant. This is consistent with models. (5) Note: anomalously high results and standard deviation were seen for 4023 (ART 1319) at 24 months, the cause of which is likely due to an undefined experimental error. The general decrease in RSF appears to have no effect on He leak or OHS observations.

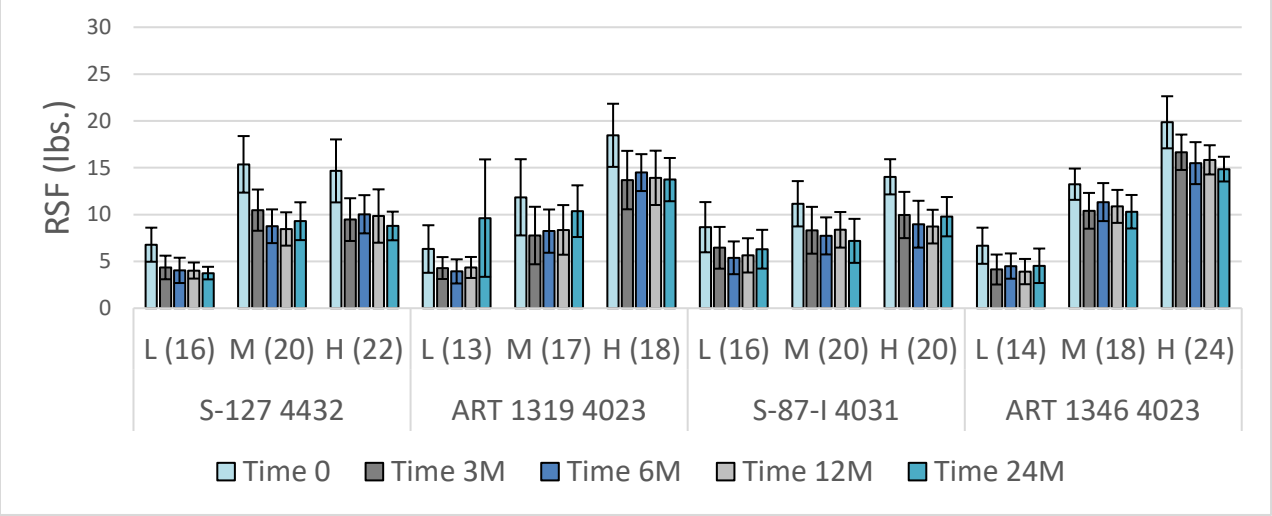


Figure 5. RSF Values of 20 mm Stoppers over Two Years. Initial values of compression [e.g., L (16%)] are given. Error bars represent standard deviation.

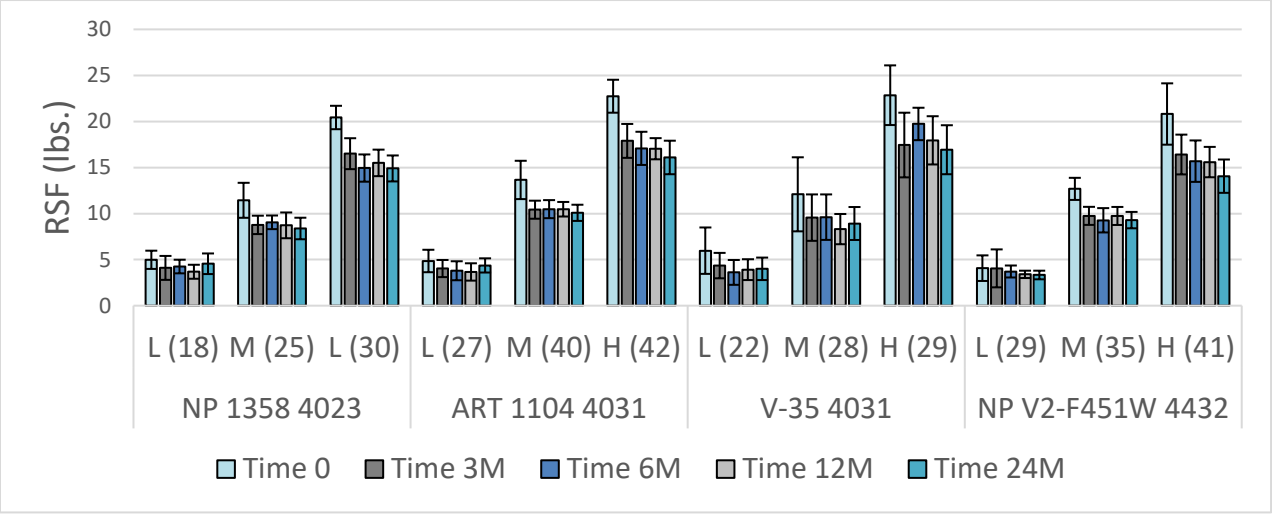


Figure 6. RSF Values of 13 mm Stoppers over Two Years. Initial values of compression [e.g., L (18%)] are given. Error bars represent standard deviation.

Consider Figure 7. Note that in general, as compression increases, RSF increases, as expected. In some cases, this increase is linear.

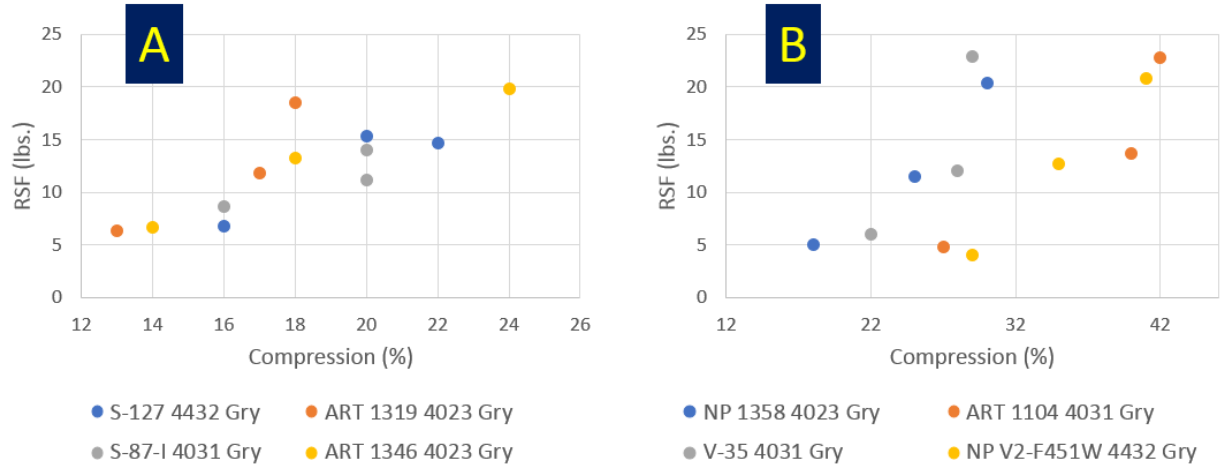


Figure 7. RSF versus Compression for: A. 20 mm Stoppers and B. 13 mm Stoppers

VI. Summary

A two-year study to establish the container closure integrity (CCI) performance landscape of systems comprising rubber stoppers and glass vials was completed. It was based upon a design of experiments strategy to consider the full range of: (a) stopper formulations, sizes, and configurations; (b) vial sizes, types, and suppliers; and (c) compressions. Evaluation was based upon measurement of residual seal force and CCI (with the USP-endorsed methods of tracer gas (helium) leak detection and frequency modulated spectroscopy). All systems showed good performance – no decrease in CCI. Formulations 4023, 4432 and 4031 showed comparable results. This clearly demonstrates the robustness of the West stoppers. However, low compressions tended in some cases to give slightly lower performance and wider scatter in results, and thus should not be recommended. A small amount of oxygen exchange through the stopper was observed for all systems; this was to be expected since rubber is known to be gas permeable. Residual seal force decreased initially, then remained constant – consistent with models.

Appendix – Comment on Kirsch Study (3)

Data by Kirsch, et al. often is used as a standard for determining the performance of a stopper/vial system based upon He leak data. (3) In summary, Kirsch, et al., measured the He leak performance of systems in which a hole of known size was introduced. Said systems were then evaluated by immersion in a microbe-containing broth and measurement of the frequency with which microbes entered. A failure rate of 10% was considered acceptable. Data by Kirsch, et al. is recapitulated in Table 8. It is noted that all He-leak data in the present report is > 6.6 (0% failure), except for selected lower, and one medium, compression system(s).

Table 8. Correlation of He-Leak Rate to Risk of Microbial Failure per Kirsch, et al. (3). This Table is a reproduction based upon data reported.

Hole Diameter (μ)	**He Leak Rate** (cm^3/s @ STP)	**- log (He Leak Rate)**	**Microbial Ingress Failure Rate** (%)
2	1.0×10^{-3}	3.0	70
0.7	2.0×10^{-4}	3.7	65
0.4	9.0×10^{-6}	5.0	11
Kirsch acceptable level			
---	6.0×10^{-6}	5.2	8 - 10
0.3	2.0×10^{-6}	5.7	7
0.2	2.2×10^{-7}	6.6	0
0.1	1.0×10^{-7}	7.0	0

VII. References

1. J. Mangus. *Summary of "Package Integrity Evaluation – Sterile Products" USP <1207>*. West Pharmaceutical Services, Inc. Technical Service Bulletin 2016/056
2. K. Davidson and J. Mangus. *Selecting and Verifying Integral Packaging Systems for Injectable Drug Products*. PDA Annual Meeting, Las Vegas, NV (March 16, 2015)
3. L.E. Kirsch, et al. (University of Iowa). *Pharmaceutical Container/Closure Integrity II: The Relationship between Microbial Ingress and Helium Leak Rates in Rubber-Stoppered Vials*. PDA Journal of Pharmaceutical Science & Technology, 51 (5), 195-202 (1997)
4. L. Robeson. *Correlation of Separation Factor versus Permeability for Polymeric Membranes*. Journal of Membrane Science, 62, 165-185 (1991)
5. Q. Zeng and X. Zhao. *Time-Dependent Testing Evaluation and Modeling for Rubber Stopper Seal Performance.* PDA Journal of Pharmaceutical Science & Technology, 72, 134-148 (2018)

Acknowledgements

Thanks are extended to the Genesis Packaging Technologies team for their consultation and capping services: R. Asselta and C. Flores-Crespo.

Thanks are extended to the West Analytical Services, LLC team for performance of He-leak and OHS measurements: J. McCaw, B. Jacobs, A. McClean, S. Vuong.

West's products are sold on the basis that it is the customer's responsibility to evaluate and test the West product to determine its compatibility with other materials and fitness for any end use.

This *technical report* dated 23 September 2019, supersedes any other previously released versions of this *report*.

Ingress of Gases into Cyclic Olefin Polymer Vial-Based Container Closure Systems at -80 °C

I. Executive Summary

The container closure integrity (CCI) performance of cyclic olefin polymer (COP)-based container closure systems (CCS) at -80 °C was evaluated. When stored in air, using headspace analysis, it was demonstrated that performance is equivalent to that of glass through 60 days, i.e., essentially no O_2 ingress. When stored over dry ice, using both headspace analysis and pH measurements, it was demonstrated that there is a small amount of CO_2 ingress over time. This is quantifiable, enabling customers to make a risk assessment. In cases where no CO_2 ingress can be permitted, poly(ethylene terephthalate) (PET)-based secondary packages provide an effective, convenient, and low-cost solution.

II. Background

Gene therapy (i.e., nucleic-acid based) drug products typically require storage and transport at -80 °C. This presents a challenge for container closure systems (CCS) to maintain container closure integrity (CCI) under these conditions. For stopper/vial-based CCS which comprise different materials (glass or polymer for vials, and elastomer for stoppers), a potential concern at -80 °C is a breach of integrity at the vial/stopper interface. Since these materials have different coefficients of thermal expansion, and will consequently shrink at different rates upon cooling, gaps in the seal may form. Thus, loss of CCI must be considered at such low temperatures. Additionally, permeation of gas through the individual components of CCS must be considered, especially for polymer-based CCS whose materials of construction are gas-permeable. In this study, polymer-based CCS comprising Daikyo's Crystal Zenith® cyclic olefin polymer (COP) vials and halobutyl elastomeric stoppers were evaluated. For storage in air, oxygen is a potential contaminant. For transport over solid carbon dioxide (i.e., dry ice), CO_2 is a potential contaminant.

A concern as it relates to carbon dioxide ingress of CCS is the maintenance of drug product pH. It is known that dissolution of carbon dioxide in water exists in equilibrium with carbonic acid per the reaction:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \quad K = 1.7 \times 10^{-3} \text{ (in pure water)}$$

Since many gene therapy drug products typically require shipment over dry ice, potential ingress of CO_2 into the headspace of a vial should be understood and mitigated through control strategies, if required. And though gene therapy drug products are usually in buffered solutions, the potential change in pH presents a risk to drug product stability.

It is noted that gas ingress at low temperature is affected by three factors: pressure gradient, solubility, and diffusivity. Each contributes to the total ingress.

Pressure Gradient. CCS typically are filled with drug product at room temperature (23 °C, i.e., 296 K) and at one atm. Upon cooling to -80 °C (i.e., 193 K), the internal pressure deceases to 0.65 atm, according to Charles's Law:

$$\frac{P_1}{T_1} = \frac{P_2}{T_2} \rightarrow \frac{1\ atm}{296\ K} = \frac{x}{193\ K} \rightarrow x = 0.65\ atm$$

Thus, there is a 0.35 atm pressure gradient promoting gas ingress.

Solubility. As temperature decreases, the solubility of any gas in a liquid or solid matrix increases. Thus at -80 °C, the absorption of gas in a polymeric vial is promoted. Conversely, at elevated temperatures, desorption of gas is promoted.

Diffusivity. As temperature decreases, diffusion rate also decreases. Thus at -80 °C, the diffusivity of gas through a polymeric vial is impeded. Conversely, at elevated temperatures, diffusion of gas is accelerated.

The purpose of the present study was to quantify the ingress of O_2 and CO_2 into CCS at -80 °C – the temperature required for storage of gene therapy drug products.

III. Experimental

Permeability

Components and parameters for permeability studies are given in Table 1. Glass vials were Schott non-blowback (NBB) ISO vials. COP vials were Daikyo's Crystal Zenith® products. Stoppers and seals were West Pharmaceutical Services, Inc. products.

Table 1. Components and Parameters for Permeability Studies

Study	O_2 Permeability	O_2 Permeability	CO_2 Permeability	CO_2 Permeability
Vial Material	Glass	COP	COP	
Vial Size	2R	2 mL	5 mL	
Stopper Formulation	4023/50 Gray		4023/50 Gray	
Stopper Size (mm)	13		20	
Stopper Configuration	Serum – NovaPure® 1358		Serum – NovaPure® 1343	
Seal	All-Aluminum (13-10T LQ)		ReadyPack® (20FO LQ TE (6-B))	
Compression	Hand-crimped		Pneumatically-crimped (RSF ~5-6 lbs)	
Initial Headspace	100% N_2 (1 atm)		Air (1 atm)	
Storage Atmosphere	Air		Dry Ice	
Secondary Package	No		No	Yes

The secondary package was a poly(ethylene terephthalate) (PET)-based product, Fairly Odd Treasures, LLC Dry-Packs 4.3 mils 1-Quart, 8" x 8" Mylar® Moisture & Static Shielding Bag (MB8x8-100PK) – sourced through Amazon. Heat sealing was performed using a Packworld USA PW4424V heat sealer at 178 °C for 1.8 seconds. See Figure 1.

Figure 1. Secondary Package and Heat Sealing Equipment

For O_2 permeability, vials were capped in a glove bag with 100% nitrogen (N_2) flow. CCS were stored both in air at room temperature and in a freezer at -80 °C. Since gene therapy drug products are typically thawed in air, understanding the intrinsic permeability of COP-based CCS at room temperature was critical to assessing permeability at -80 °C. CCS which had been stored at -80 °C were removed and allowed to reach room temperature for approximately 30 minutes in air before headspace oxygen concentration was measured with a Lighthouse Instruments, LLC (LHI) FMS-760 Oxygen Headspace Analyzer (a frequency-modulated spectroscopy method). CCS which had been stored at -80 °C were discarded after measurement, CCS which had been stored at room temperature were re-used (i.e., same CCS were used for entirety of study). Data was collected versus time through 60 days. Data reported are an average of 5 vials. All work was performed at West Analytical Services, LLC in Exton, PA.

For CO_2 permeability, vials were capped in air. Five CCS were subsequently heat sealed in a PET-based secondary package. All vials were sent to LHI (Charlottesville, VA) where their initial CO_2 concentration was measured (with the exception of those in the PET-based secondary package) before being placed in a commercial cooler filled with dry ice. See Figure 2. It was ensured that the cooler stayed filled during the duration of storage. CCS were removed from the cooler at their designated time points and allowed to reach room temperature in air. CCS sealed in a secondary package were removed from their package upon being removed from the cooler. Headspace CO_2 concentration was measured with an LHI FMS-Carbon Dioxide Headspace Analyzer. Data was collected versus time (30 minutes, 3 days, 7 days, and 14 days) post-removal from storage. Data reported are an average of 5 vials.

Figure 2. Commercial Cooler

pH Measurements

Components and parameters for pH studies are given in Table 2. COP vials were Daikyo's Crystal Zenith® products. Stoppers and seals were West Pharmaceutical Services, Inc. products.

Table 2. Components and Parameters for pH Studies

Study	pH	
Vial Material	COP	
Vial Size	10 mL	
Stopper Formulation	4432/50 Gray	
Stopper Size (mm)	20	
Stopper Configuration	Serum – S10-F451	
Seal	20-I LQ	
Compression	Hand-crimped	
Storage Atmosphere	Dry Ice	Air

Four 10 mL COP vials were filled with 10 mL air-equilibrated Milli-Q® water and capped in air by hand-crimping with 20 mm S10-F451 FluroTec® barrier film laminated 4432/50 Gray serum stoppers and 20-I LQ aluminum seals. These water-filled CCS were stored over dry ice for both 7 and 14 days – two CCS per storage duration. CCS were removed at their designated time points and allowed to return to room temperature in air. The seals were removed, and pH was measured versus time. Hand-crimped CCS comprising just-named vials/stoppers/seals, but without water (i.e., empty), were also stored over dry ice for both 7 and 14 days – two CCS per storage duration. Upon removal from storage, these CCS were allowed to return to room temperature in air, the seals were removed, the vials filled with 10 mL air-equilibrated Milli-Q water, and pH was measured versus time. For each experiment, i.e., stored with and without water, controls that were never exposed to dry ice were examined.

IV. Results and Discussion

O_2 permeability data are shown in Figure 3. Shown are concentrations after storage at both room temperature and -80 °C for both COP- and glass-based CCS. At room temperature, O_2 is observed to permeate through the COP-based CCS, in contrast to those assembled with glass, as expected. All polymers have some intrinsic permeability to gases at room temperature. With this in mind, vials were measured 30 minutes post-removal from -80 °C storage to minimize any permeation contributions. Compared to the results at room temperature, the permeability results of COP-based CCS that were stored at -80 °C show no signs of O_2 ingress and performance is essentially equivalent to that of glass-based CCS through 60 days. An expanded view of these systems is shown in Figure 4. Slightly higher values for COP are likely due to calibration differences (LHI Analyzer is calibrated based upon glass standards) as well as permeation of oxygen during the 30-minute hold at room temperature. Though both COP- and glass-based CCS provide CCI at -80 °C, the COP-based system has the advantage of better fracture resistance.

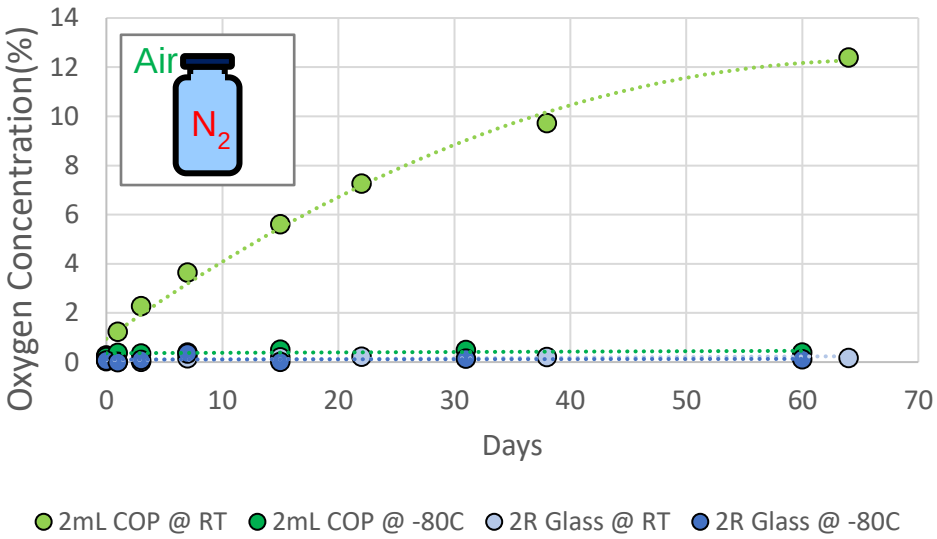


Figure 3. O_2 Permeability

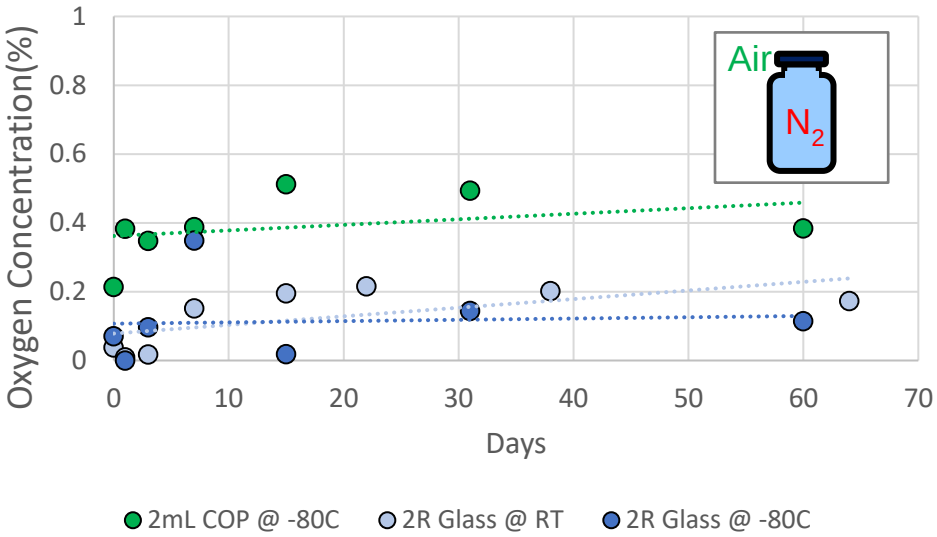


Figure 4. Expanded O_2 Permeability

CO_2 permeability data are shown in Figure 5. Shown are concentrations after storage on dry ice and subsequent times after removal and holding at room temperature. Note that CO_2 gradually permeates through COP over time at ambient conditions, the degree of which depends on the duration of storage. Additionally, CO_2 is not detected in the vial headspace 30 minutes post removal, which signifies that sealing integrity is maintained. These observations suggest CO_2 dissolves within COP over dry ice at -80 °C (the sublimation temperature of dry ice is -78.5 °C) and only diffuses into the headspace of the vial once the temperature rises – whereby molecular motion and desorption is promoted. By 14 days, CO_2 has already begun to permeate out of the CCS, as measured concentration decreases. Moreover, use of the PET-based secondary package stops CO_2 permeation completely.

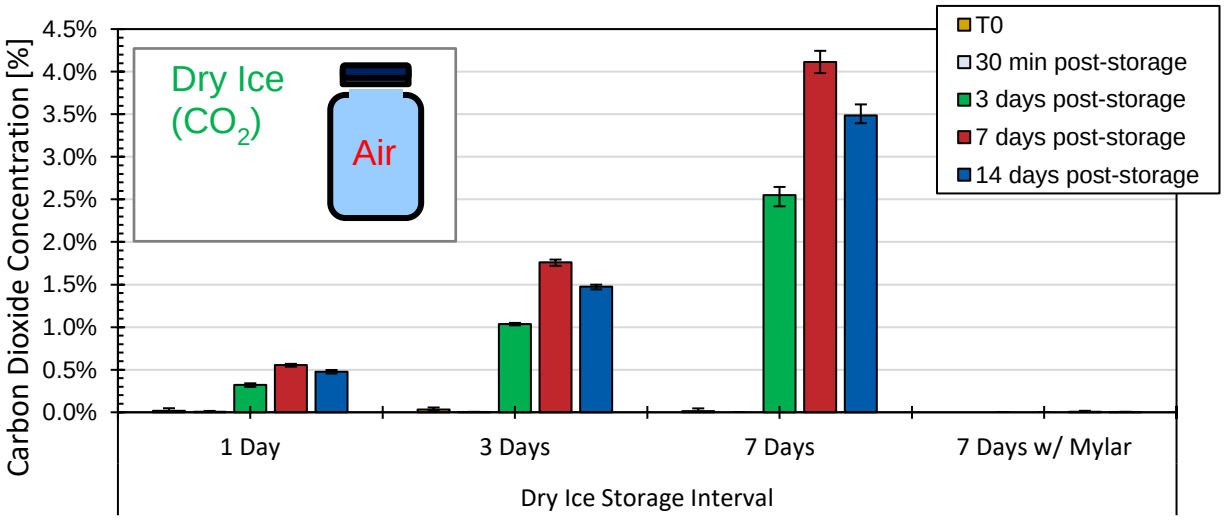


Figure 5. CO_2 Permeability

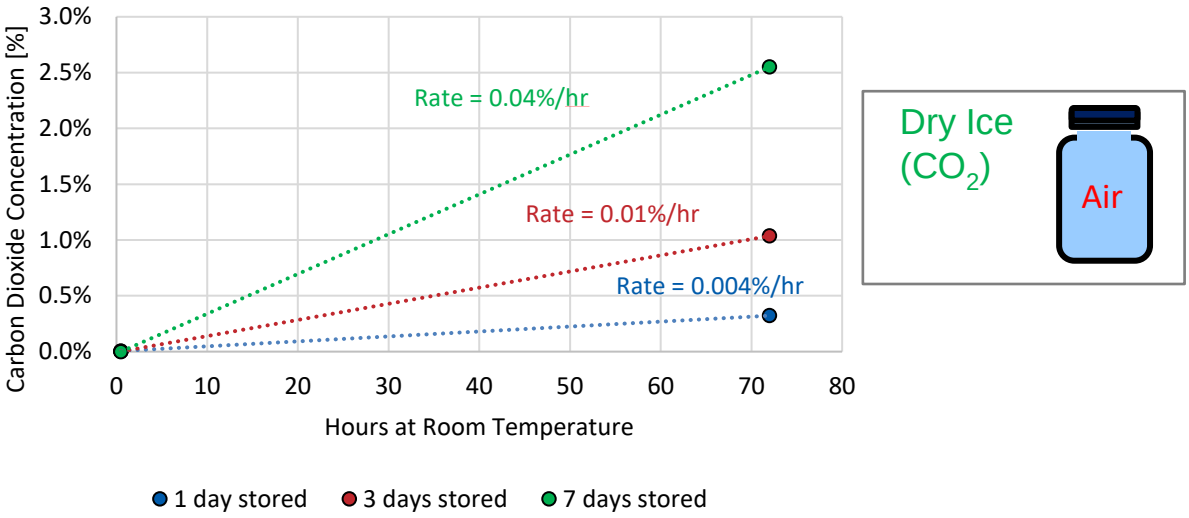


Figure 6. CO_2 Permeability through Three Days at Room Temperature

Considering Figure 6, note that though COP-based CCS are not impervious to CO_2 permeation, the rate is low and has been quantified. Upon thawing, even after seven days on dry ice, the rate is only 0.04%/hr. Using drug product within hours of thaw can reduce the risk substantially. Where there is concern of any CO_2 permeation – the PET-based secondary package is demonstrated to be a very effective, convenient, and low-cost solution.

Permeability measurements are consistent with pH measurements. Figures 7 and 8 show results from 7- and 14-day dry ice exposures with COP-based CCS. As CO_2 permeates, carbonic acid is formed and pH gradually decreases over a span of days, then slowly increases as CO_2 outgasses. Note that regardless of being stored filled or empty, pH gradually decreases over time at room temperature, further demonstrating that CO_2 dissolves within COP when stored over dry ice.

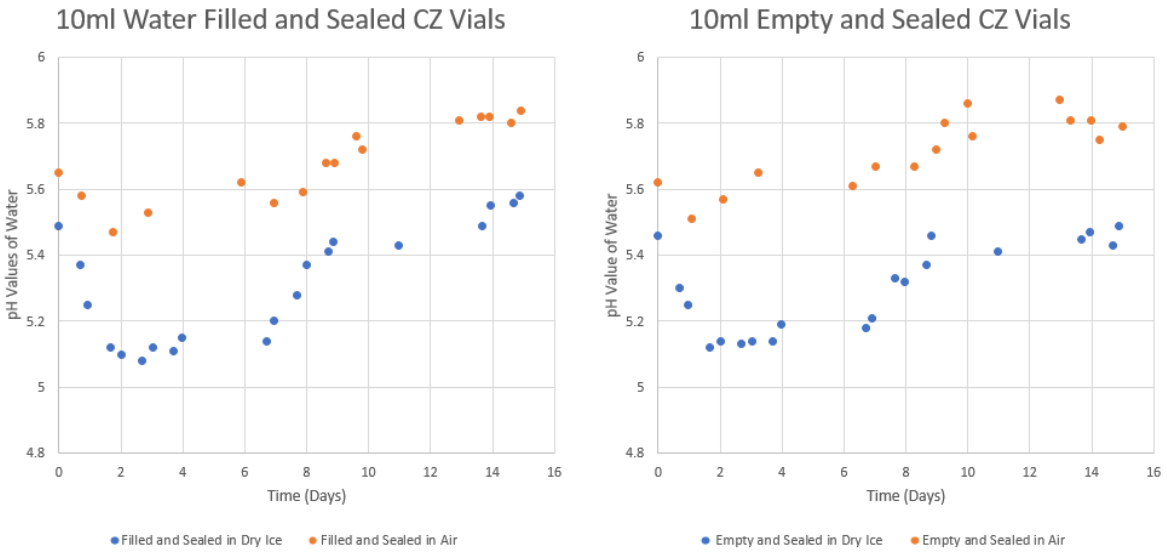


Figure 7. pH Results – 7 Days on Dry Ice. Left graph is CCS filled with water before dry ice storage, right graph is CCS filled with water after dry ice storage. Orange points are controls (no dry ice exposure). Blue dots are for CO_2 exposure. Data represent one experiment only, but duplicate experiments gave comparable results.

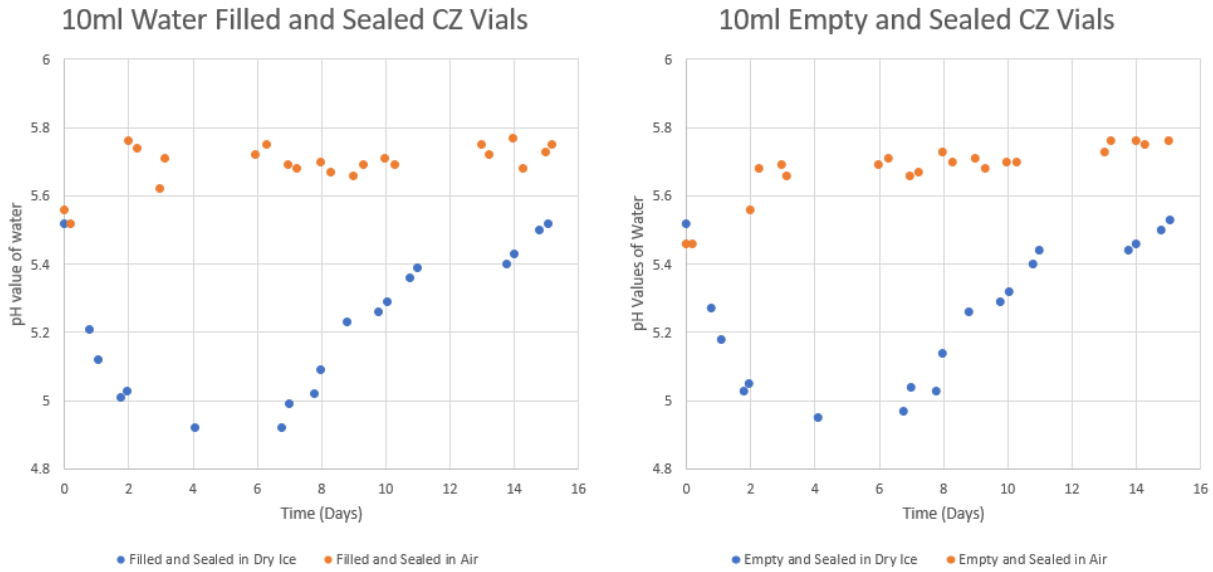


Figure 8. pH Results – 14 Days on Dry Ice. Left graph is CCS filled with water before dry ice storage, right graph is CCS filled with water after dry ice storage. Orange points are controls (no dry ice exposure). Blue dots are for CO_2 exposure. Data represent one experiment only, but duplicate experiments gave comparable results.

V. Summary

Examination was made to quantify ingress of O_2 and CO_2 into Crystal Zenith® cyclic olefin polymer (COP) vial-based container closure systems (CCS) stored at -80 °C. Ingress of gas could have occurred by two distinct pathways: 1) through potential sealing gaps formed at the stopper/vial interface and/or 2) by way of permeation through the individual components. CCS stored in air at -80 °C showed essentially no ingress of O_2, consistent with O_2 ingress rates seen for glass-based CCS. When COP-container closure systems were stored over dry ice (-80 °C), sealing integrity was maintained, but ingress of CO_2 was only detected as a function of time after removal from storage. This fact suggests CO_2 dissolved within COP during dry ice storage and gradually diffused over time at room temperature. The rate is low, is proportional to the storage duration, and has been quantified (0.04%/hr at room temperature; 7 days of dry ice exposure). Ingress was shown to be blocked completely with the use of a poly(ethylene terephthalate) PET-based secondary package. By rigorously measuring and adopting mitigation strategies for gas ingress, it is demonstrated that COP-based CCS can serve as a suitable containment option for gene therapy drug products.

West's products are sold on the basis that it is the customer's responsibility to evaluate and test the West product to determine its compatibility with other materials and fitness for any end use.

This *Technical Report* dated 4 December 2019, is the first release version of this *report*.

Return to Chapter 4

Challenges in Low-Temperature Storage of Cell Therapy Drug Products

T. Page McAndrew, Ph.D.
Director, Scientific Communications
West Pharmaceutical Services, Inc.
Exton, PA

PepTalk 2020
Formulation & Stability - Optimizing Biologics Formulation Development
San Diego, January 20, 2020

Abstract

Low-temperature storage of cell therapy drug products presents challenges to achieving good packaging system container closure integrity (CCI). These challenges result from (1) differences in thermal expansion coefficients of components and (2) permeability of gases. This presentation considers the fundamentals of these challenges and how they may be overcome, as well as differences between glass- and polymer-based systems.

Abstract in Two Words

Why Polymers ?

Outline

- polymers vs. glass
- the right polymer
- permeability and container closure integrity (CCI)
  - room temperature
  - - 80°C
  - - 180°C

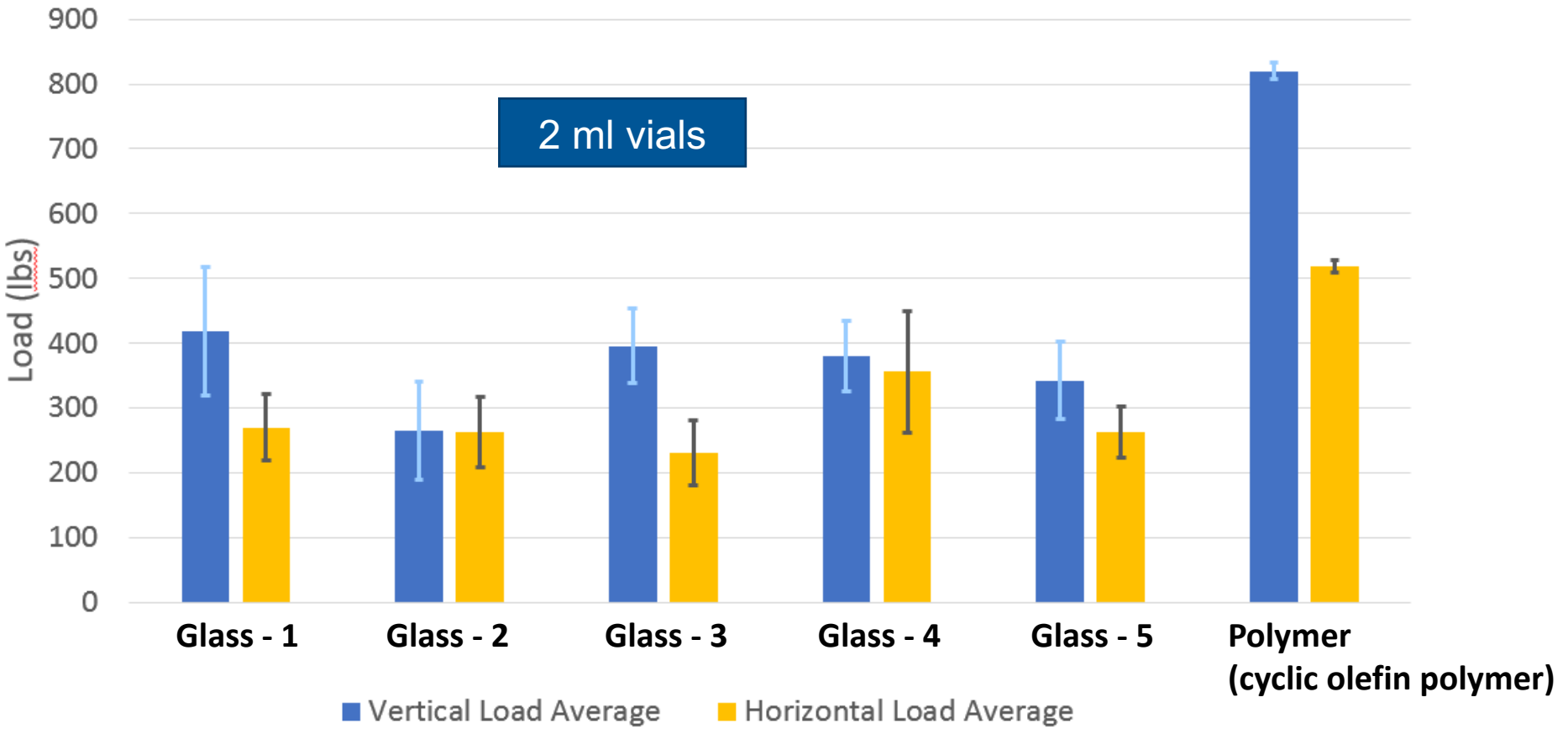
Polymers vs. Glass: Chemical

- lower surface energy
  - glass: 0.31 N/m
  - polymer: ~0.03 N/m
  - lower risk of interaction with drug product
- reduced risk of pitting or delamination
- fewer elements
  - lower risk of leachables

	Typical (weight %)	
	Glass	**Polymer**
SiO_2	70-82	-----
B_2O_3	5-13	-----
Al_2O_3	2-7	-----
CaO / MgO	0-7	-----
Na_2O / K_2O	4-12	-----
As / Sb / Ba	trace possible	-----
C	-----	~ 70 – 95
O	-----	~ 0 – 20
H	-----	~ 5 – 10
catalyst	-----	trace

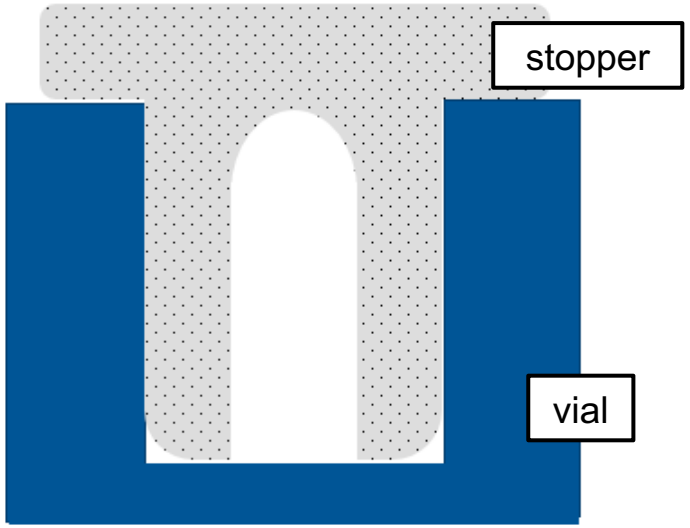
Polymers vs. Glass: Mechanical

- more fracture resistant



Polymers vs. Glass: Vial-Stopper Fit

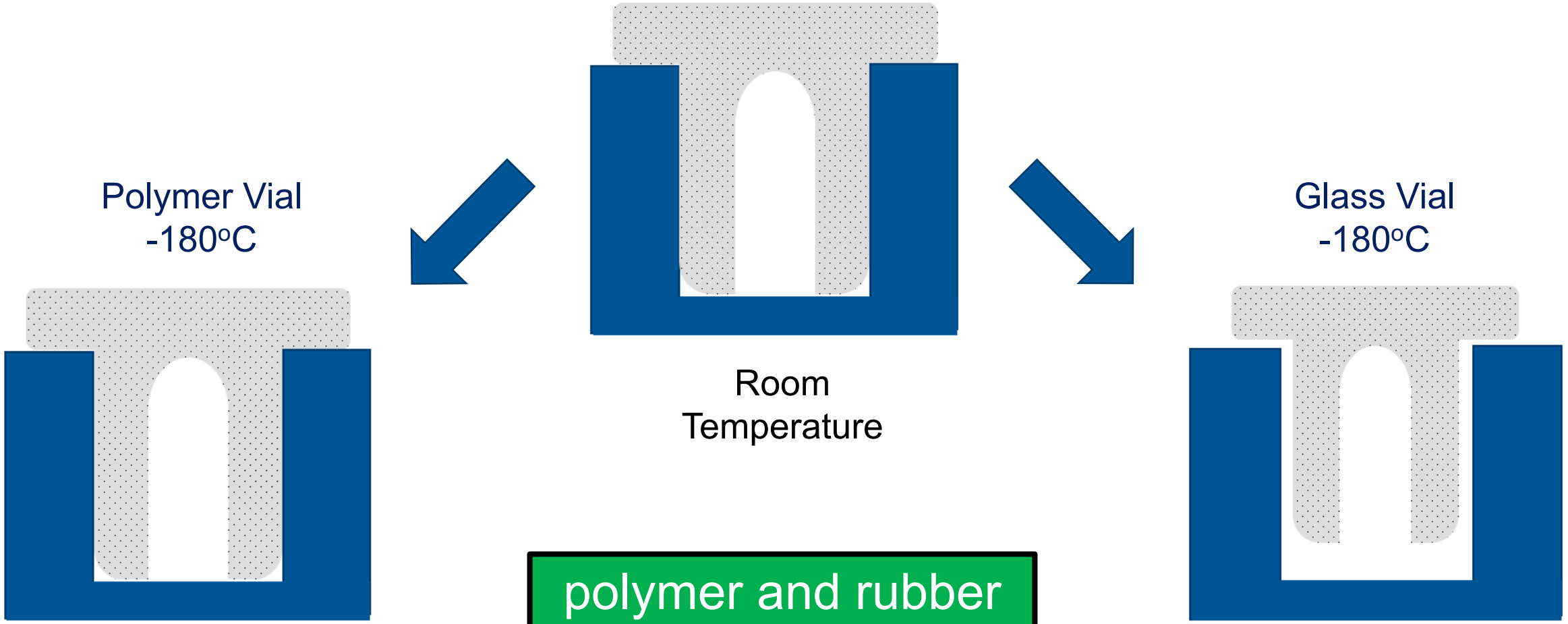
Room Temperature



	Coefficient of Thermal Expansion (10^{-6} cm / cm – K)	Volume Shrinkage (%) (room temperature to -180°C)
glass	4	0.2
rubber	77	4.5
polymer	70	4.1

West

Polymers vs. Glass: Vial-Stopper Fit



polymer and rubber shrink at ~ same rate

Polymers vs. Glass: Vial-Stopper Fit

Literature

- *Evaluation of Container Closure System Integrity for Storage of Frozen Drug Products: Impact of Capping Force and Transportation*. PDA Journal of Pharmaceutical Science and Technology, 72, 544-552 (2018)
- *When Glass Vials Fail at Low Temperatures, Consider A Cyclic Olefin Polymer System,* (2017) https://www.pharmaceuticalonline.com/doc/when-glass-vials-fail-at-low-temperatures-consider-a-cyclic-olefin-polymer-system-0001?immediate=true
- *Correlating Vial Seal Tightness to Container Closure Integrity at Various Storage Temperatures*. PDA Annual Meeting (March 2015). https://www.pharmaceuticalonline.com/doc/correlating-vial-seal-tightness-to-container-closure-integrity-at-various-storage-temperatures-0001
- Container Closure Integrity Testing and the Identification of a Suitable Vial/Stopper Combination for Low-Temperature Storage at -80ºC. PDA Journal of Pharmaceutical Science and Technology, 66, 453-465 (2012)

Customers ask !!!

The Right Polymer: Requirements

no exception

- transparency
- good resistance to permeation
  - O_2
  - H_2O
- compatibility with drug product

Transparent Polymers

	Polymer	O_2 Permeability (a)	H_2O Vapor Transmission (b)	Comment
1	cyclic olefin polymer (COP)	1.2	< 10	only carbon and hydrogen – minimal risk of interaction – minimal extractables
2	polyethylene terephthalate	~ 0.04	~ 120	concern with release of phthalate compounds (endocrine disruptors) from hydrolysis during autoclave sterilization
3	polycarbonate	1.4	> 400	contains bisphenol-A groups
4	poly(methyl methacrylate)	1.2	> 900	concern regarding release of methanol from hydrolysis during autoclave sterilization
5	poly(vinylidene chloride)	0.005	~ 10	at temperatures of autoclave sterilization (~ 120°C) – can undergo degradation to discolor and release HCl
6	low density polyethylene	2.2	~ 80	lower performance than COP
7	polypropylene	1.2	~ 30	translucent
8	polyamide blend (PA-11 and PA-12)	NA	NA	amide groups (CONH) and residual acid catalyst pose risk via interaction with protein
9	cyclic olefin copolymer (COC)	~ 1.2	~ 10	brittle (elongation-at-break < 5%), not suitable for multiple sterilizations

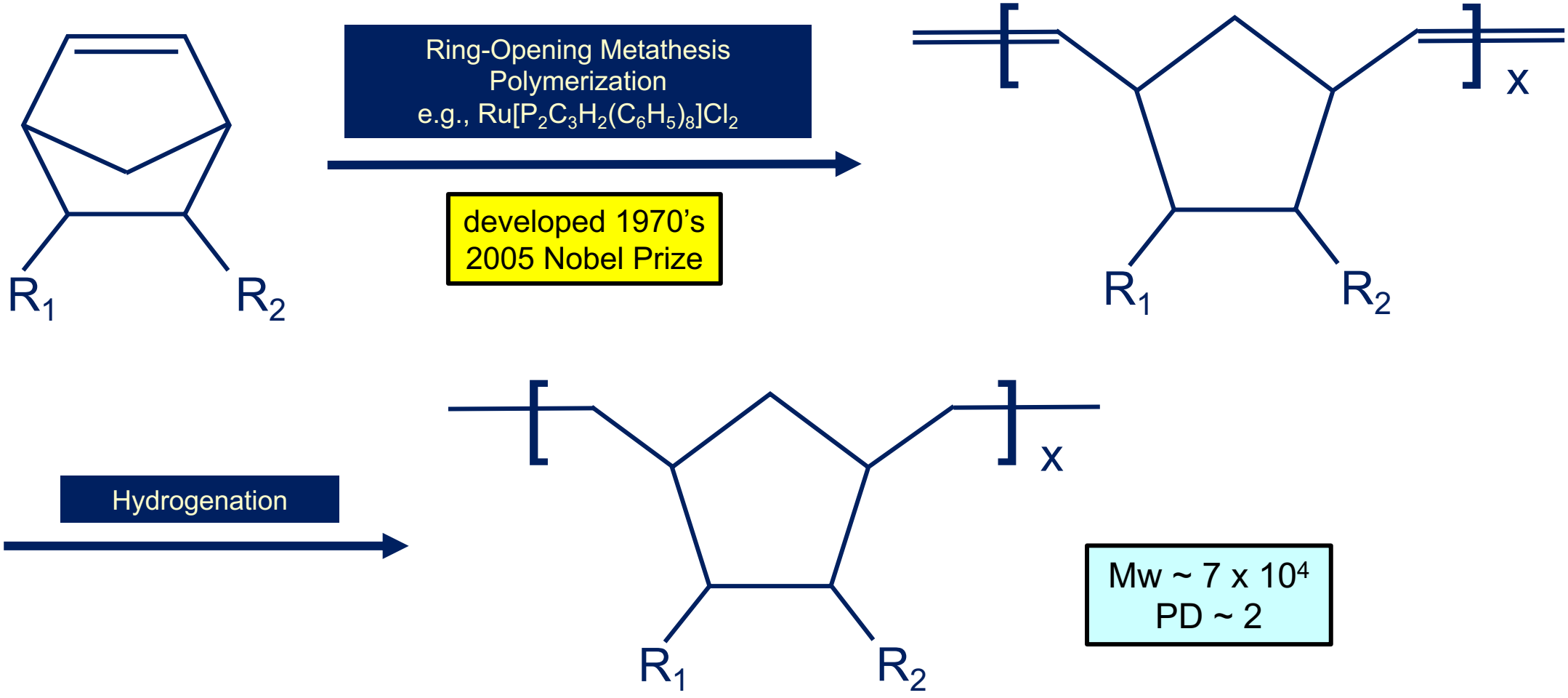
a. Barrer = [10^{-11} x (cm^3 (STP)) x cm] / [(cm^2) x (seconds) x (torr)]
b. (grams – mm) / (m^2 – day) – some values estimated

Transparent Polymers

	Polymer	O_2 Permeability (a)	H_2O Vapor Transmission (b)	Comment
1	cyclic olefin polymer (COP)	1.2	< 10	only carbon and hydrogen – minimal risk of interaction – minimal extractables
2	polyethylene terephthalate	~ 0.04	~ 120	concern with release of phthalate compounds (endocrine disruptors) from hydrolysis during autoclave sterilization
3	polycarbonate	1.4	> 400	contains bisphenol-A groups
4	poly(methyl methacrylate)	1.2	> 900	concern regarding release of methanol from hydrolysis during autoclave sterilization
5	poly(vinylidene chloride)	0.005	~ 10	at temperatures of autoclave sterilization (~ 120°C) – can undergo degradation to discolor and release HCl
6	low density polyethylene	2.2	~ 80	lower performance than COP
7	polypropylene	1.2	~ 30	translucent
8	polyamide blend (PA-11 and PA-12)	NA	NA	amide groups (CONH) and residual acid catalyst pose risk via interaction with protein
9	cyclic olefin copolymer (COC)	~ 1.2	~ 10	brittle (elongation-at-break < 5%), not suitable for multiple sterilizations

a. Barrer = $[10^{-11} \times (cm^3\ (STP)) \times cm] / [(cm^2) \times (seconds) \times (torr)]$
b. (grams – mm) / (m^2 – day) – some values estimated

Cyclic Olefin Polymer



Polymers vs. Glass: Permeability

all polymers are gas permeable

---

glass is not gas permeable

challenge

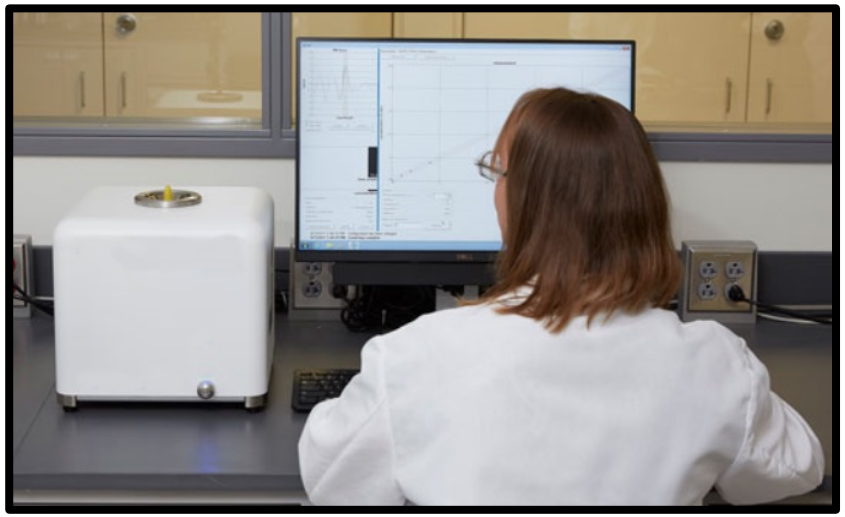
- measure permeability of gases through vial
  - enable risk assessment
    - maximum allowable leak limit (**MALL**)

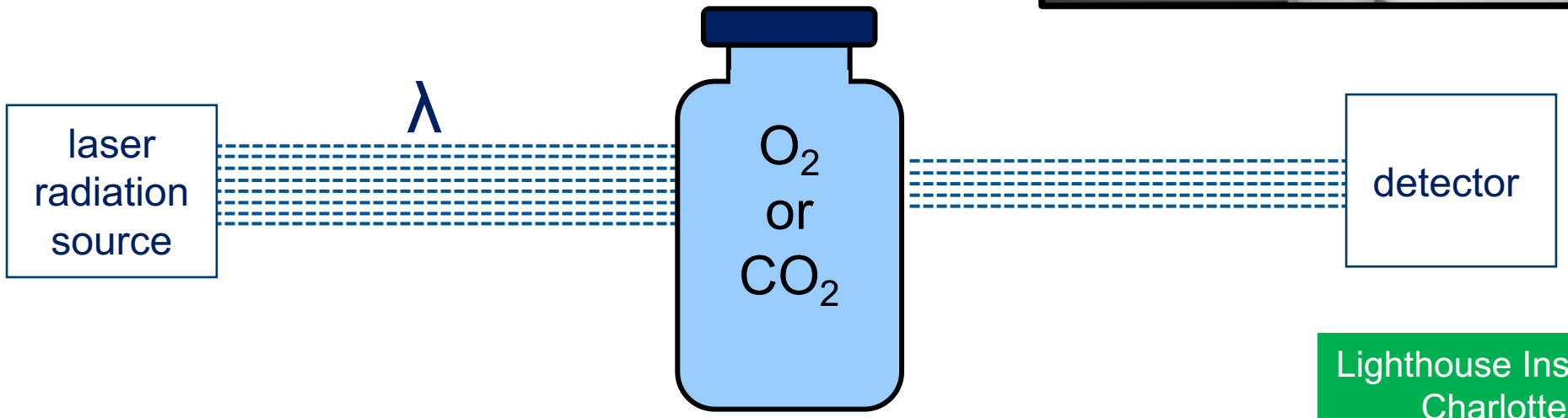
MALL

- MALL: Maximum Allowable Leak Limit
  - USP Chapter <1207> *Package Integrity Evaluation – Sterile Products* (2016)
  - smallest gap, or leak rate, that places drug product quality at risk – established to maintain drug product quality attributes for sterility and physio-chemical stability through expiry
  - varies with drug product
- essential to understand permeability

Permeability: Measurement

- frequency modulated spectroscopy headspace analysis
  - deterministic method endorsed by USP <1207>
  - near-IR radiation wavelength that is absorbed by gas of interest
    - amount absorbed correlates to concentration

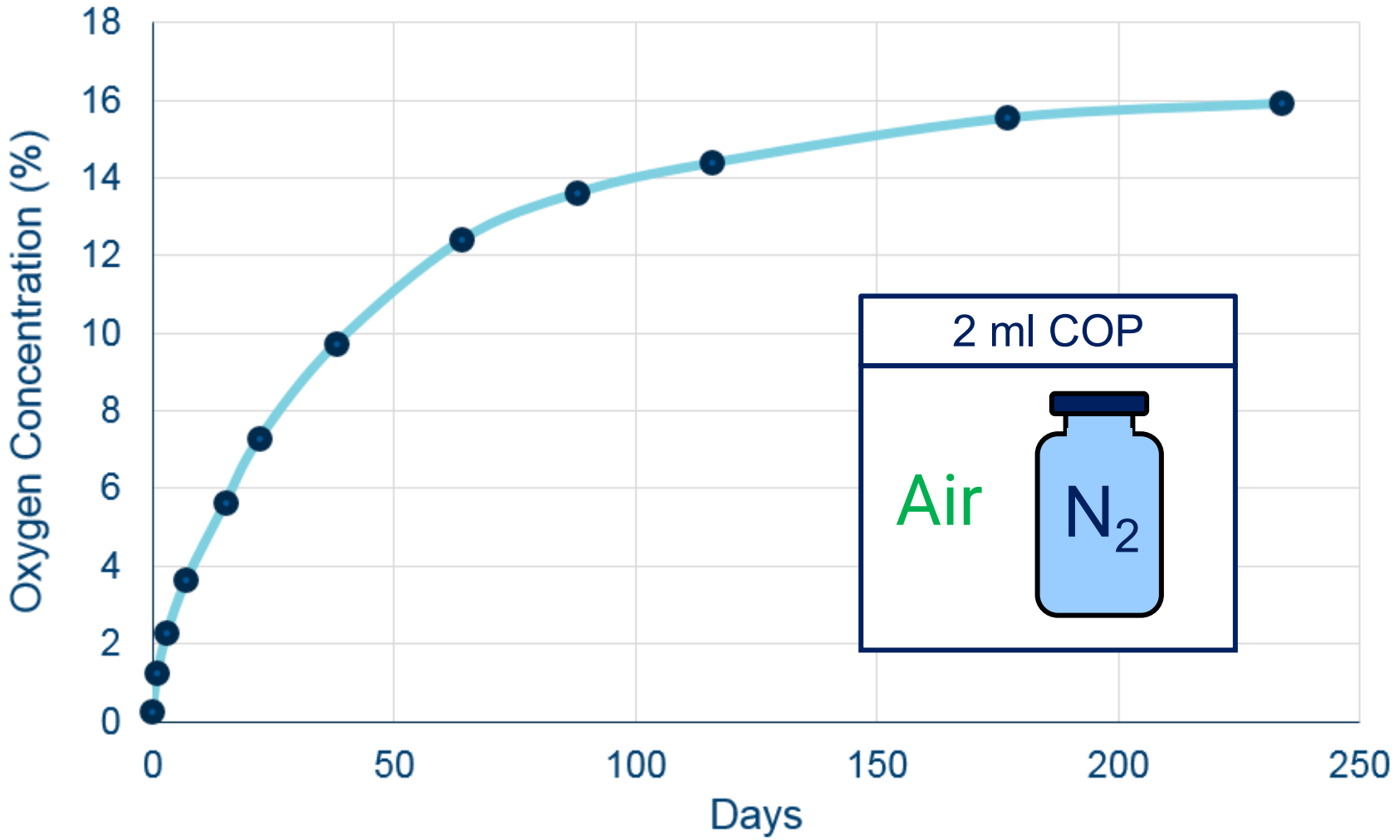




Lighthouse Instruments, LLC
Charlottesville, VA

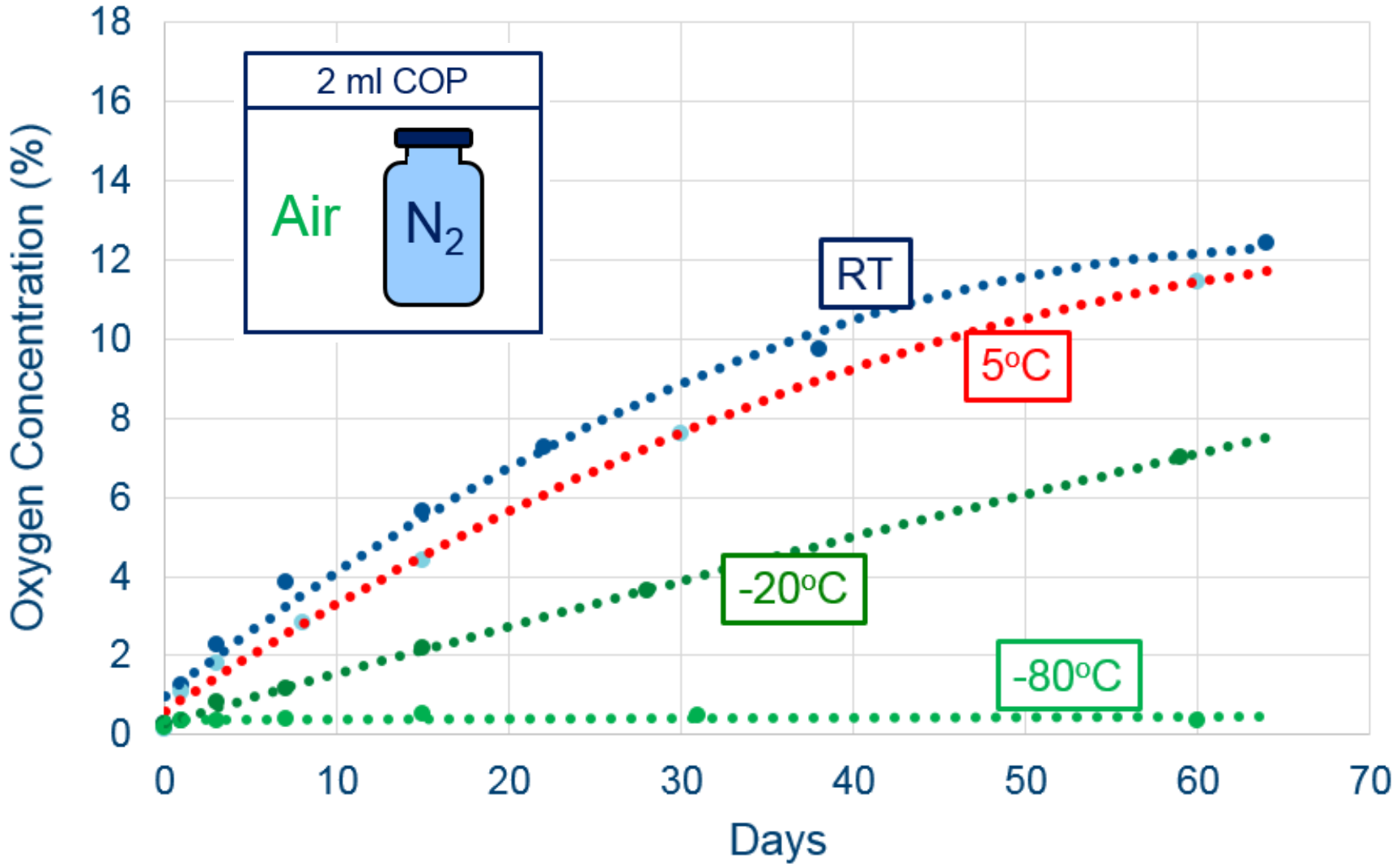
Permeability: O_2 at Room Temperature

- cyclic olefin polymer (COP) vial
- rate quantified
  - 0.02% O_2 / hr



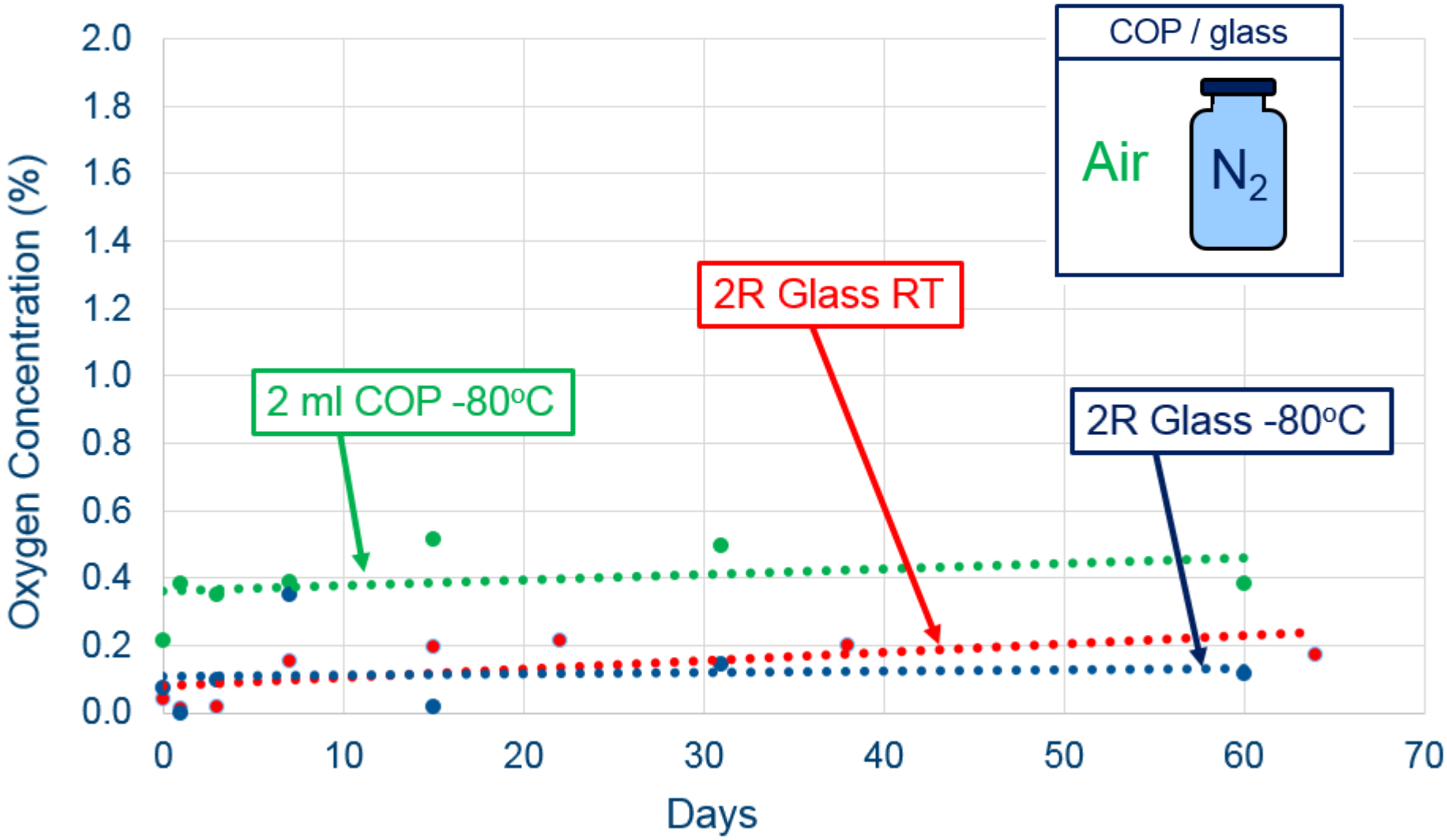
Permeability: O_2 at Low Temperature

- COP vial
- rate decreases with T
  - expected



Permeability: O_2 at -80°C

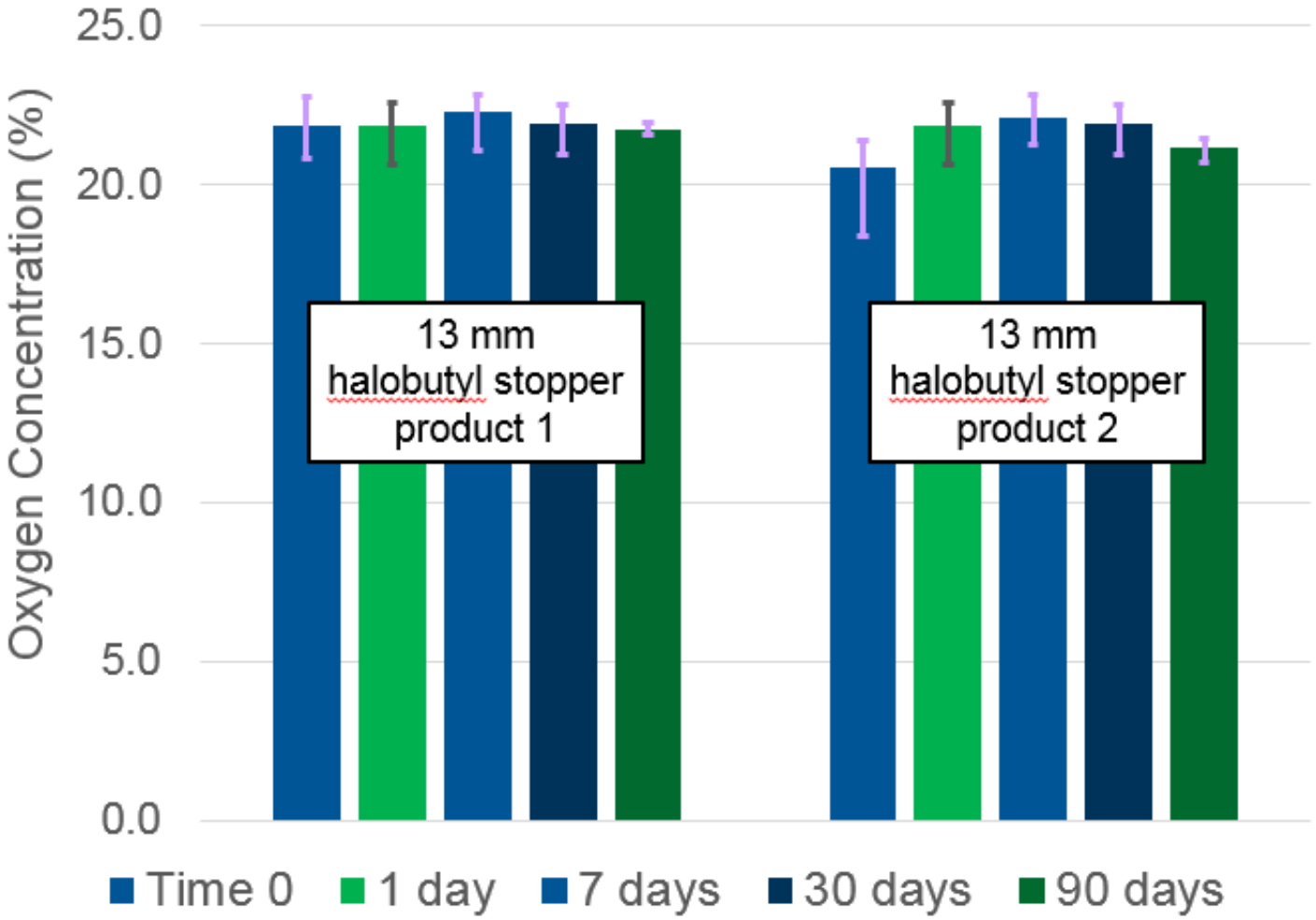
- COP vial
- equivalent to glass



West

Permeability: O_2 at -180°C

- COP **0.5 ml vial**
- excellent CCI thru 90 days

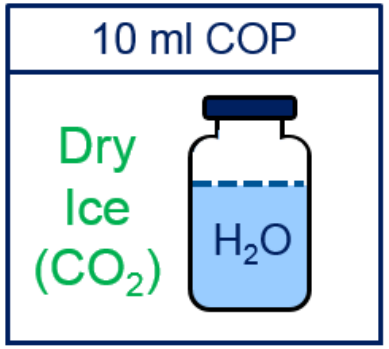


Permeability: CO_2 on Dry Ice (-78°C)

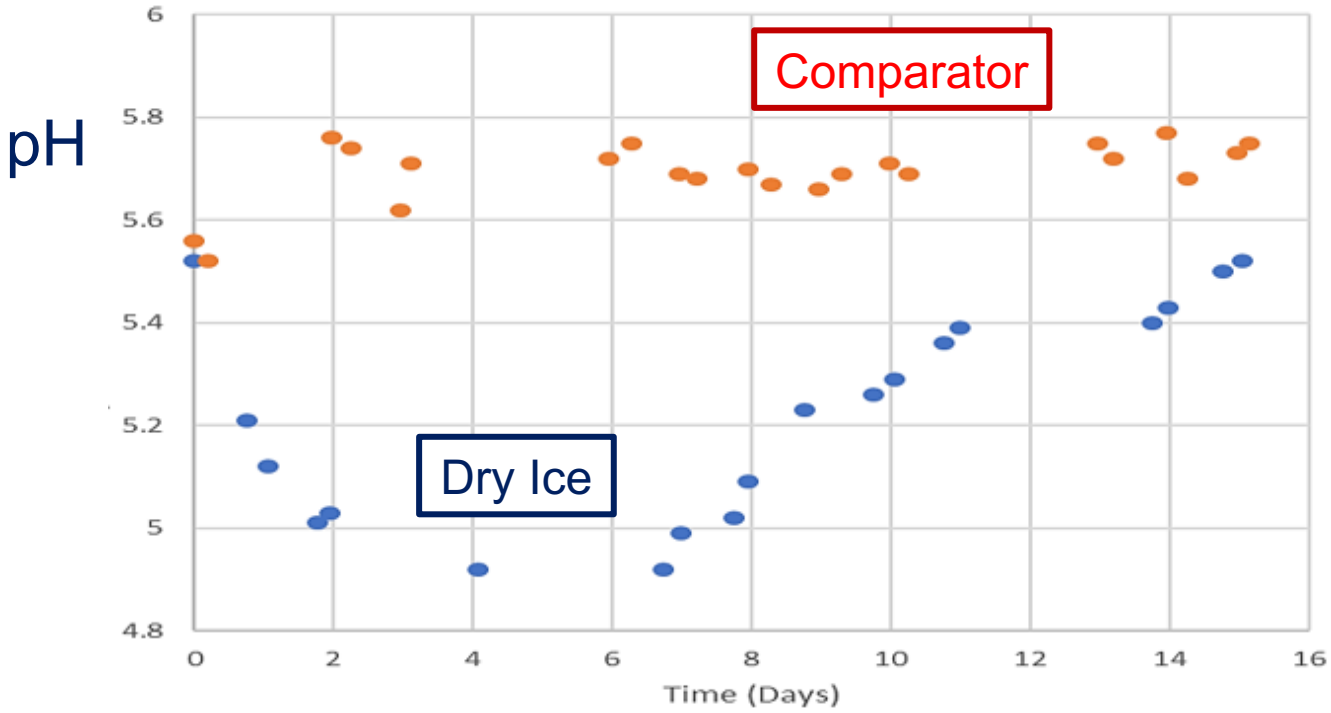
industry observation

drug product stored on dry ice in COP container – slight decrease in pH with time after warming

Experiment
- 10 ml COP vial
- filled with water
- stored on dry ice 14 days
- pH vs time after removal

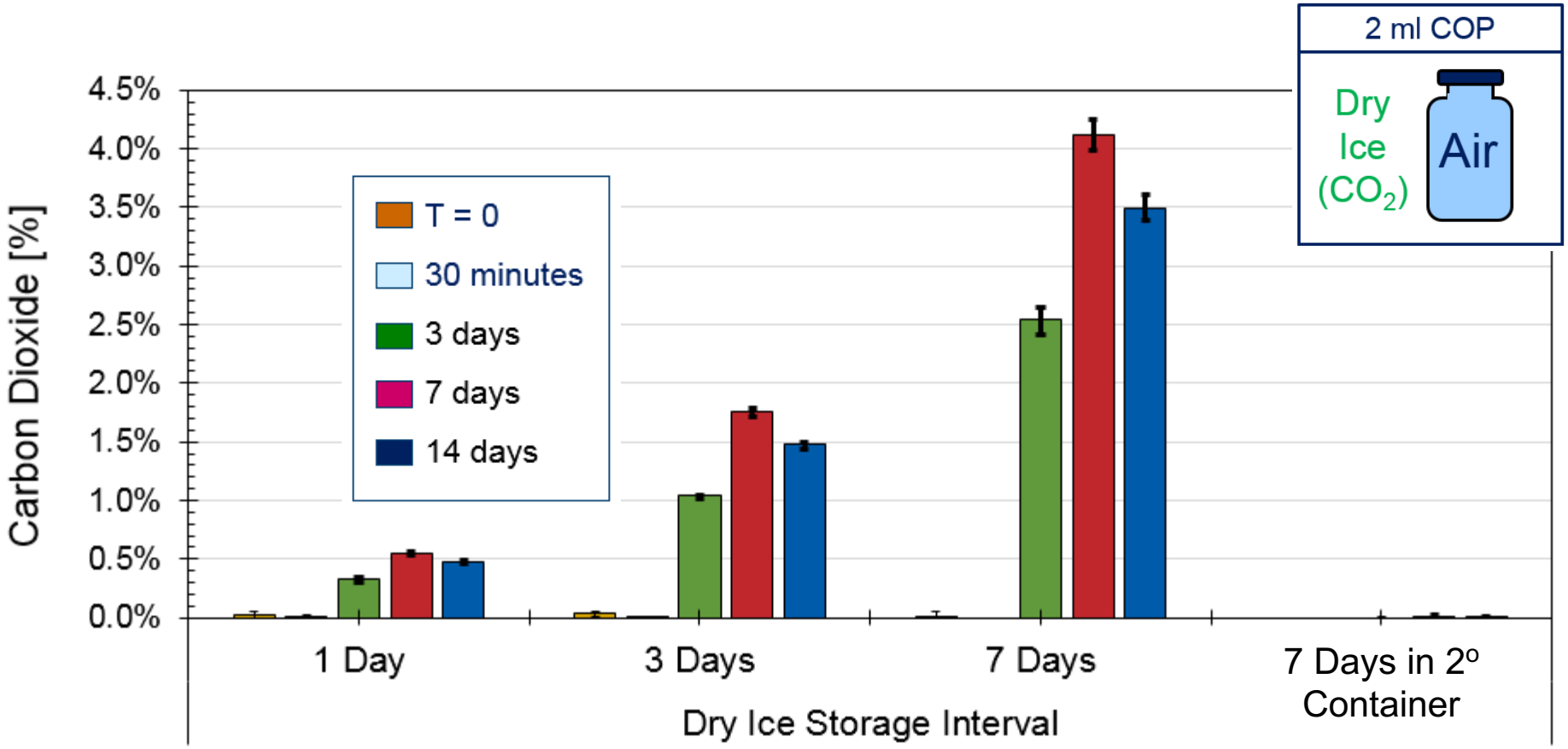


- CO_2 absorbs into COP vials – desorbs slowly – causes small decrease in pH per reaction
  - $CO_2 + H_2O \leftrightarrow H_2CO_3$
    - $K = 1.7 \times 10^{-3}$



Permeability: CO_2 on Dry Ice (-78°C)

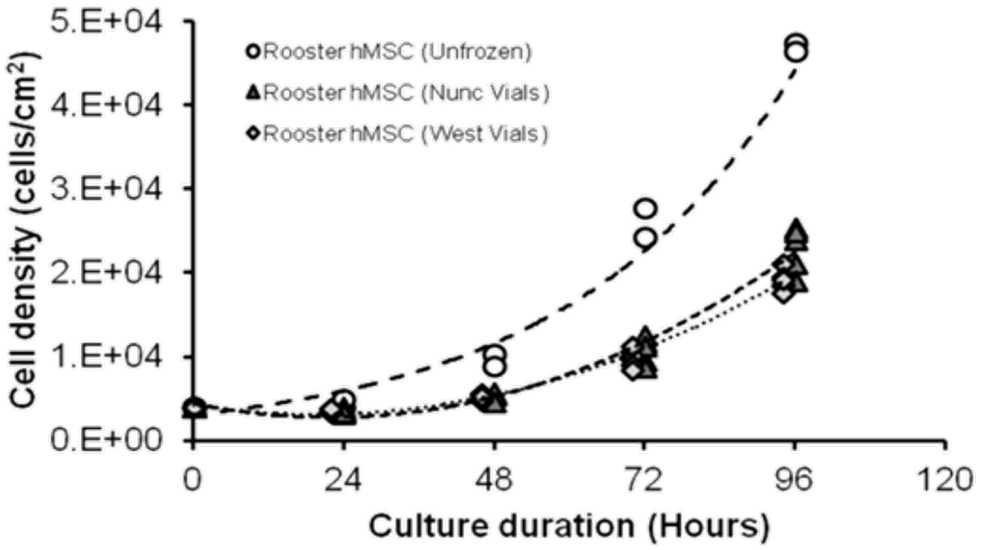
- CO_2 absorbs into COP vials – desorbs slowly
- none observed within 30 minutes
- rate quantified
  - 0.04% CO_2 / hr
- prevented <u>completely</u> with heat-sealed MYLAR® poly(ethylene terephthalate) bag secondary container



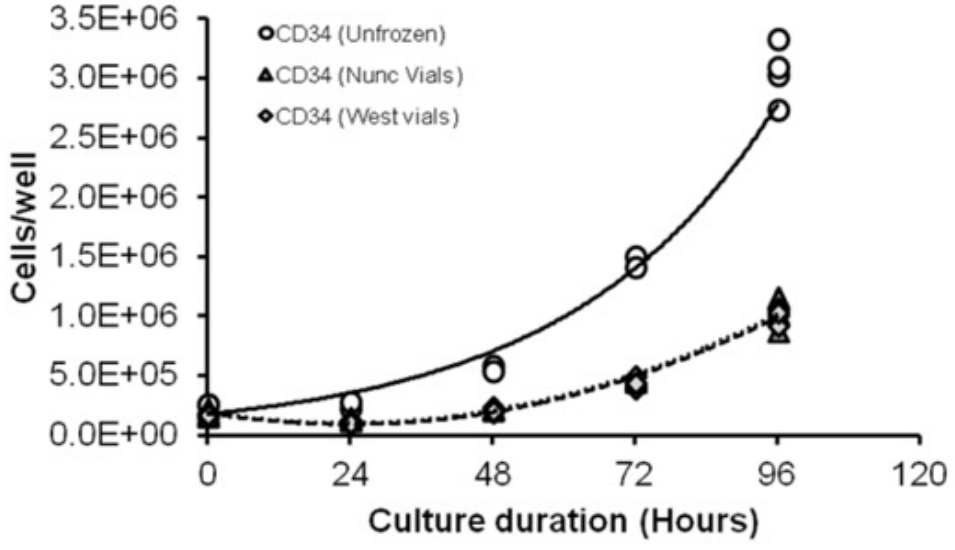
One More Thing: Cell Viability

- equivalent growth after -180°C
  - COP vials vs. Nunc™ polypropylene vials
  - M. Gehrmann and A. Lyness. *Low-Temperature Storage Preservation of Cell- and Gene- Based Drug Products*. PDA Biopharma Week, Long Beach, CA (5/6/19)

mesenchymal stem cells



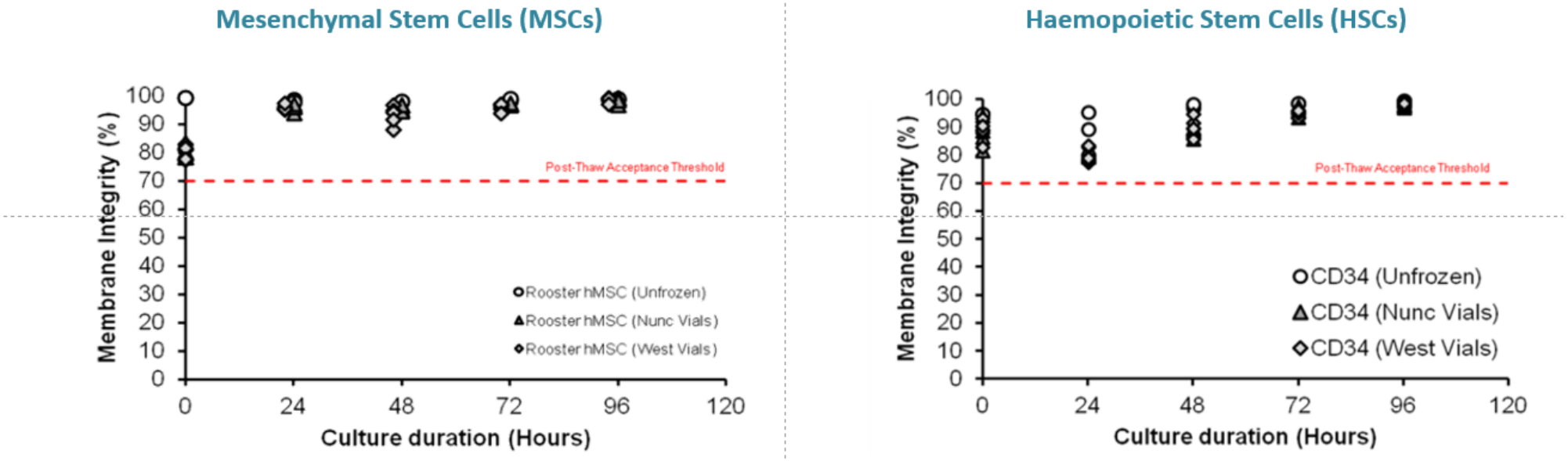
haemopoietic stem cells



West

One More Thing: Cell Viability

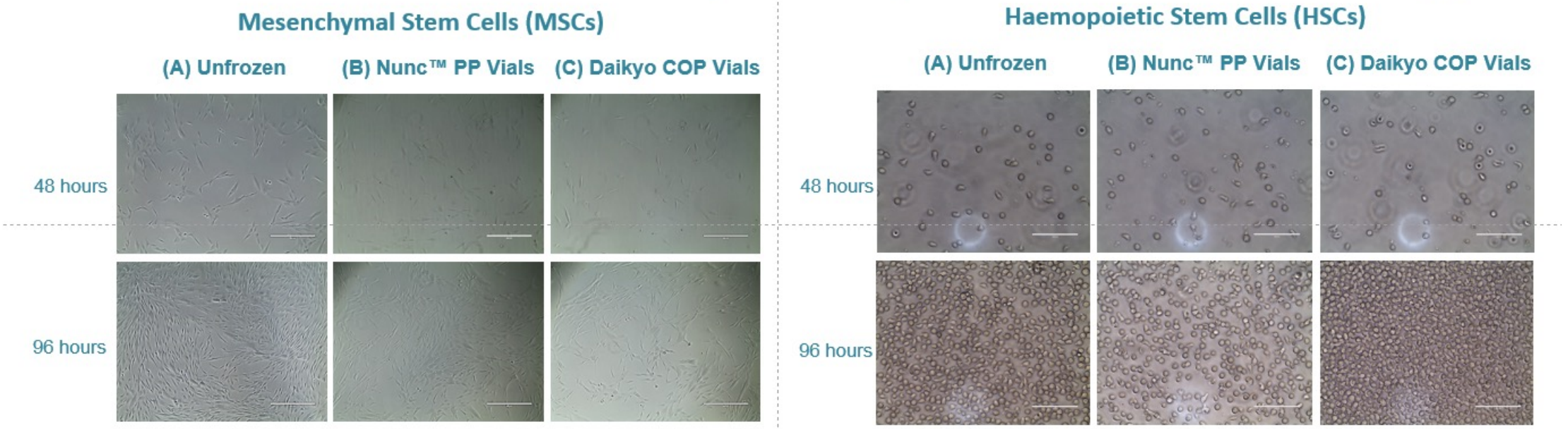
Stem Cell Compatibility Study – Cell Viability



- The recovery of membrane integrity (48 hours post-thaw) remained above 90% for both cell types

One More Thing: Cell Viability

- Stem Cell Compatibility Study – Cell Morphology



- MSCs cells that proliferated after the first 24 hours retained the classical mesenchymal morphology
- HSCs cells which proliferated after the first 24 hours retained their rounded morphology

Summary – Enabling Risk Assessment

- polymers offer advantage versus glass at the temperatures of storage/ transport of cell and gene therapy drug products
- -180°C: cyclic olefin polymer (COP) vials provide excellent CCI
  - literature reports issues with glass vials
- O_2 permeability of COP vials quantified
  - -80°C – equivalent to glass
- CO_2 permeability of COP vials quantified
  - with heat-sealed MYLAR® bag secondary container – CO_2 permeability stopped completely through 14 days

Recognitions

- **Experimental Work**
  - Matthew Gehrmann
    - Senior Scientist, Scientific Communications
  - Liang Fang, Ph.D.
    - Principal Scientist, Scientific Insights Laboratory
  - Olga Laskina, Ph.D.
    - Senior Technical Account Specialist, Technical Customer Support
- Lighthouse Instruments, LLC
    - Charlottesville, VA
  - O_2 measurements at -180°C and CO_2 measurements at -80°C

FUNCTIONAL ASSESSMENT OF ADENO-ASSOCIATED VIRUS STORED IN DAIKYO CRYSTAL ZENITH® VIALS AT ULTRA-COLD TEMPERATURES

Executive Summary

This study from West Pharmaceutical Services, Inc. (West) provides evidence for functionally and statistically significant outperformance of Adeno-Associated Virus (AAV) viral vector, commonly used for gene therapies, stored in Daikyo Crystal Zenith® (CZ) vials versus glass vials. The study has also shown equivalency to polypropylene vials. However, for the many reasons cited in this report polypropylene vials are not suited for storage of gene therapies, leaving a CZ containment closure system as the optimal container solution from those tested.

Objectives

1) Investigate compatibility of a West container system, comprised of 2 mL CZ vials; 13 mm chlorobutyl stoppers; and aluminum seals, with the storage of commercial research use only (RUO)-grade Adeno-Associated Virus viral vector Serotype 2 (AAV2) at ultra-cold temperatures.
2) Demonstrate utility of CZ containment closure system for optimizing recovery and functionality of the contained AAV2 viral vector throughout the freeze/thaw life cycle of AAV-based therapeutics using a cell-based transduction test, pH testing, and recovery tests.

Observations

1) The functional integrity of AAV2 cryopreserved in CZ container closure system compared equally to AAV2 cryopreserved in glass or polypropylene (PP) vials using a post-thaw transduction assay.
2) Post-freeze/thaw recovery of transduction competent AAV2 was higher at lower fill volumes when compared to recovery of active vector from glass containers of similar size.
3) Cryopreserved AAV2 aliquots stored in CZ container systems were subjected to a 7-day hold on dry ice, followed by a 14-day hold at 2-8°C. Recoverable activity of the viral vector remained consistent after direct exposure to dry ice regardless of the dry ice hold time.
4) CZ container closure system is an excellent replacement for PP screwcap vials used in research and early Chemistry and Manufacturing Control (CMC) process development allowing the early adoption of CZ container closure system as a scalable alternative.

In conclusion, the 2 mL CZ container system is compatible with storage of AAV viral vectors that serve as a basis for gene therapies strongly suggesting CZ containment closure system could serve as a storage solution to help facilitate industrialization of gene therapies. Utilizing CZ containment closure system for fill and finish operations could help manufacturers achieve gene therapy drug product potency demands, which may lead to both time and cost savings.

1. Introduction

Adeno-associated virus serotype 2 (AAV2) was one of the first adenovirus serotypes evaluated as viral vector to deliver normal human gene for treating genetic disease and resulted in the first Food and Drug Administration (FDA) approved gene therapy drug. The new drug modality is challenging to manufacture and to store, requiring container closure integrity at -80°C. Previously, West demonstrated the utility of CZ container systems at cryogenic temperatures [1] as well as outlined the advantages of working with CZ vials for cell therapy storage [2, 3]. This report is focused on the utility of CZ vials for -80°C storage of AAV material and determining the relative performance of cyclic olefin polymer (COP) CZ vials relative to glass vials, and traditional PP cryovials for the cryopreservation of purified commercial research use only grade AAV2 at ultra-cold temperatures.

The utility of viral vectors used for both in-process manufacturing of cellular therapies and as final drug product drives a critical need for scalable container systems compatible with various viral vectors and their manufacturing processes. PP screwcap cryovials commonly used for research are not specified to operate at the -80°C required for long term storage of viral vectors. PP vials are not well suited for production considering all four gene therapies approved by the FDA are in pharmaceutical grade container systems aimed at controlling issues like the inherent risk for loss of container closure integrity and sterility [3, 4, 5]. The CZ container system has a low extractables profile, high optical clarity, and a rubber stopper-aluminum seal closure that provides a hermetic seal. This study investigates the suitability of a container system composed of the novel COP CZ as an alternative for the ultra-cold storage of therapeutically relevant AAV viral vectors.

West has collaborated with Advanced Bioprocess Services Ltd., working in partnership with Loughborough University at the Centre for Biological Engineering, an academic leader in process and product understanding of commercialization of cell and gene therapies, to conduct the following experimental work.

2. Materials & Methods Summary

Daikyo Crystal Zenith® (CZ) vials in a Ready-to-Use (RU) 2 mL format with 13 mm chlorobutyl serum stoppers and corresponding aluminum seals were used as the container closure system for all studies. Cryopreservation performance was compared to that of 1.8 mL PP screwcap and glass vials.

Table 1. Parameters tested in this study of AAV2 ultra-cold storage in Crystal Zenith® (CZ) vials.

Formulation	*0.001% Pluronic-F68 in 1x DPBS (-/-), pH 7.1*
Concentration	1E10 Genome Copies/mL, working
Vial	2 mL, 13 mm CZ vials
Elastomer	S2-F451 4432/50G 13 mm stoppers
Seal	ART 5117 13mm Aluminum Flip-Off
Freezing Method	Passive, cardboard box inside a -80°C mechanical freezer
Cell Line	HEK293 plated at 70,000 cells/well 24 hours prior to transduction
Cytometer	BD FACSCanto II

Vials containing AAV2 were manually filled and crimped in a biosafety cabinet. Freezing was achieved after the fill/finish process by placing vials into a cardboard box and then directly into a -80°C freezer.

To assess relative performance of 2mL CZ vials with the cryopreservation process, various effects of the polymer material that may impact or influence the functionality of the viral material cryopreserved inside were measured, such as fill concentration and volume related to surface area, pH following holds in an ultra-cold (-80°C) mechanical freezer, on dry ice (solid phase CO_2) and dry ice followed by a refrigerated hold at +4°C in a phosphate-buffered formulation. Functionality of AAV2 post-thaw was assessed by transduction of the HEK-293 cell line followed by flow cytometric detection of enhanced green fluorescent protein (eGFP) fluorescence 48 hours post-transduction.

3. Results

3.1. Impact of vial material on AAV functionality after ultra-cold storage

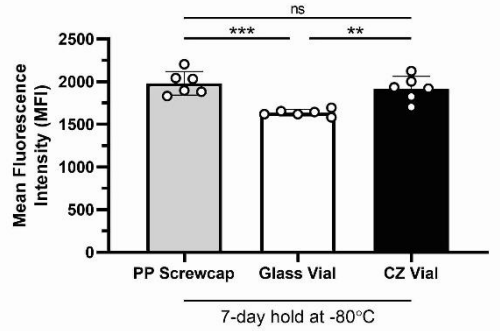


Figure 1: Infectivity is Maintained in CZ Vials After One Freeze/Thaw Cycle. *Formulated AAV2 was filled into various vials, frozen in cryoboxes within an ultra-cold freezer, and then stored for 3 weeks. Frozen vials were thawed in a pre-heated water bath, equal virus (according to the pre-freeze titer) were applied to growing HEK293 cells, and then assayed for mean eGFP fluorescence with a flow cytometer. Mean Fluorescence Intensity (MFI) is then interpreted as a measure of AAV2 transduction functional activity. n = 6 (3 trials, 2 replicates each) as Mean ± SD with *p≤0.1000, **p≤0.0100, ***p≤0.0010; ****p<0.0001, and ns=p≥0.5000 by Two-Way ANOVA with Tukey's MCT.*

A cell-based fluorescence assay was used to evaluate functional transduction performance of AAV2-CMV-eGFP (described here as AAV2) following a freeze-thaw process in CZ vials and compared to standard glass and PP vials. AAV2 was frozen to -80 °C in CZ, PP, and glass vials at a standardized concentration of 1E10 GC/mL and fill volume of 500 µL. **Figure 1** shows that CZ vials outperformed glass vials (MFI of 1919 ± 144.8 vs 1636 ± 38.14, CZ vs glass; p=0.0025) and performed equally as well as traditional PP screwcap vials (MFI of 1919 ± 144.8 vs 1982 ± 138.4, CZ vs PP; p=0.8602) in this functional test of recoverable transduction activity.

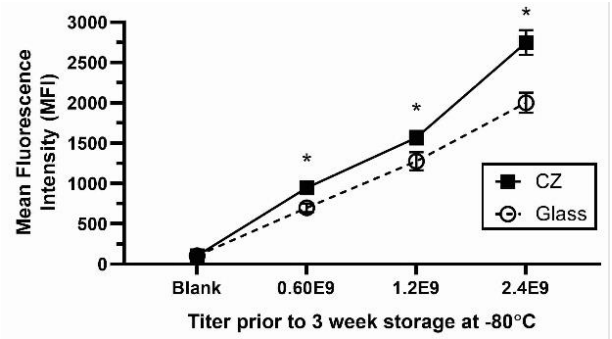


Figure 2: Increasing AAV2 Concentration Results in Higher Recoveries from Daikyo Crystal Zenith® (CZ) Vials Compared to Glass Vials. Vials were filled with 500 µL formulated AAV2 of increasing quantities of virus. Data interpreted from a transduction linearity test of cryopreserved AAV2 and presented as n = 6 for each point (3 trials, 2 technical replicates each), Mean ± SD with * to denote general significance by Two-Way ANOVA with Tukey's MCT. Both groups, except at the blank sample reading, are significantly different at the indicated concentration.

Overall recoverability of functional AAV2 was also increased when frozen in CZ vials versus glass at all concentrations tested (**Figure 2**). More AAV2 infectivity recovered from CZ material was consistently observed compared to glass vials filled with split dilutions from the same stock of 1E11 GC/mL virus concentrate. In turn, this may mean more formulated AAV2 can be recovered from CZ vials compared to glass vials, which could increase the storage efficiency of AAV2 and reduce loss volumes that impact the targeted dose. The results also indicate an acceptable linearity of the transduction method of recovered AAV2 used throughout this study.

CZ material differences may account for the higher recoverability of AAV2 viruses when compared to vials made of glass. The volume amount of filled liquid gives rise to the area of contact between the fill substance and the inner wall of the vial. This surface area should influence the vial material effect that may drive increased recoveries from CZ vials.

3.2. Impact of fill volume on AAV functionality after ultra-cold storage

Only a portion of the vial contacts the drug product which is based on the fill volume of the formulation. The surface area to volume ratio can be calculated and used to assess the effect of interaction between the vial and formulated AAV2. Vial material effect on drug product directly affects product recovery rates, where these rates can play an important role in the overall cost of goods and total manufacturing costs per batch.

Vial surface area to volume ratio was calculated using the formula:

$A_{TSF} = A_b + A_w$

and

Fill Contact Area: Volume = A_{TSF} / V_F (expressed as a fraction of whole)

Where Total Surface Area of Fill (A_{TSF}), Area of base (A_b), Area of walls (A_w), Vial Fill Volume (V_F), and where 1 cm^3 = 1 mL; realizing the geometry of a vial as a cylinder (Area = π * r^2) and the vial theoretically touching a constant plane of formulated AAV2 at the side walls (Area = h x w).

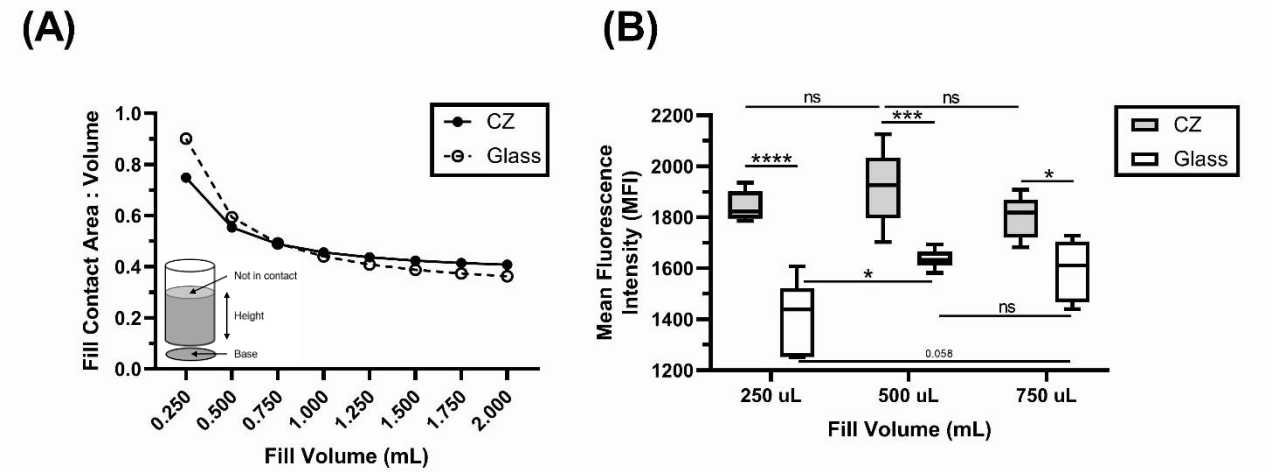


Figure 3: Fill Volume Does Not Influence Recovery of AAV2 Infectivity from CZ Vials Used in Ultra-cold Storage. A) Computed fill contact area to volume ratios for various fill volumes of a standard West 2mL CZ vial and standard glass vial. Inset: Generalized visual aid for vial surface area to volume ration calculation. **B)** MFI is interpreted as a measure of AAV2 transduction functional activity resulting from storage at the conditions noted. n = 6 (3 trials, 2 replicates each) as Mean ± SD with *p≤0.1000, **p≤0.0100, ***p≤0.0010; ****p<0.0001, and ns=p≥0.5000 by Two-Way ANOVA with Tukey's MCT.

Figure 3A visualizes the differences in the amount of vial surface area in contact with drug product at 0.25 mL increments utilizing the area calculations described above (inset visual aid in Figure 3A). The largest difference was at small fill volumes of 0.25 mL, where the difference in ratio between CZ (0.749) and glass (0.901) was ~15%, which implies more glass material in contact with formulated AAV2 than CZ. A reduction in contact between CZ and virus resulted in a noticeable recovery of active viral vector as shown in **Figure 3B**. Viral vector recovered from CZ resulted in a significant difference (p<0.0001) in mean fluorescence intensity (MFI) of 1843 ± 58.65 for material stored in CZ and an MFI of 1414 ± 139.9 for material stored in

glass vials at a volume of 0.25 mL in a 2 mL container, a ~30% increase in recovery. This increased recovery may be influenced by the base area of a CZ vial, which is 63% of a standard glass vial base.

In **Figure 3B**, it can be observed that the CZ material consistently recovered more AAV2 infectivity as the fill volume increased compared to glass vials. This means more formulated AAV2 can be recovered from CZ vials compared to glass vials, effectively increasing the storage efficiency and the ease of recoverability of AAV2 viral vector material potentially reducing costs.

3.3. Impact of ultra-cold storage on AAV formulation pH and subsequent function

pH fluctuation is a regulatory concern and a change in pH can occur when aqueous buffered products undergo the freeze/thaw process. A common phosphate-buffered formulation was utilized to assess changes in pH after one freeze/thaw cycle of formulated AAV2 in various vial materials.

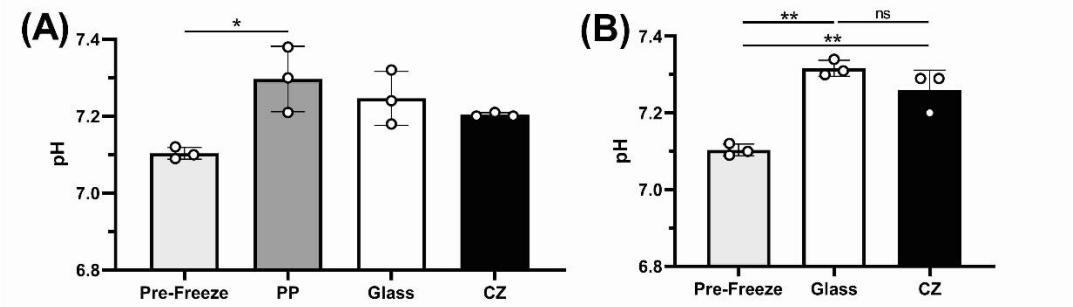


Figure 4: Impact of CZ and Glass on pH of AAV2 in Ultra-Cold Storage. A) pH of 500 µL fills of formulated AAV2 in various vial materials after one freeze/thaw cycle. **B)** pH of 750 µL fills after one freeze/thaw cycle in CZ or Glass vials. n = 3 for each group; Mean ± SD with *p≤0.1000, **p≤0.0100, and ns=p≥0.5000 by Two-Way ANOVA with Tukey's MCT.

There were no significant differences in pH between AAV2 recovered from the different vial materials after one freeze/thaw cycle filled at 500 µL (**Figure 4A**) compared to the unfrozen pre-freeze control sample likely due to high variation and low *n*. However, there were significant differences (**p≤0.0100) in pH between control (7.10 ± 0.015) and CZ (7.26 ± 0.052) and between control and glass (7.32 ± 0.012) at a fill volume of 750 µL (**Figure 4B**), but no differences were observed between CZ and glass. These results indicate the freeze/thaw process may influence pH of commonly stored and formulated AAV2 material, but the vial material has little effect on the resulting pH.

3.4. Impact of dry ice storage on AAV formulation pH and subsequent function upon thaw

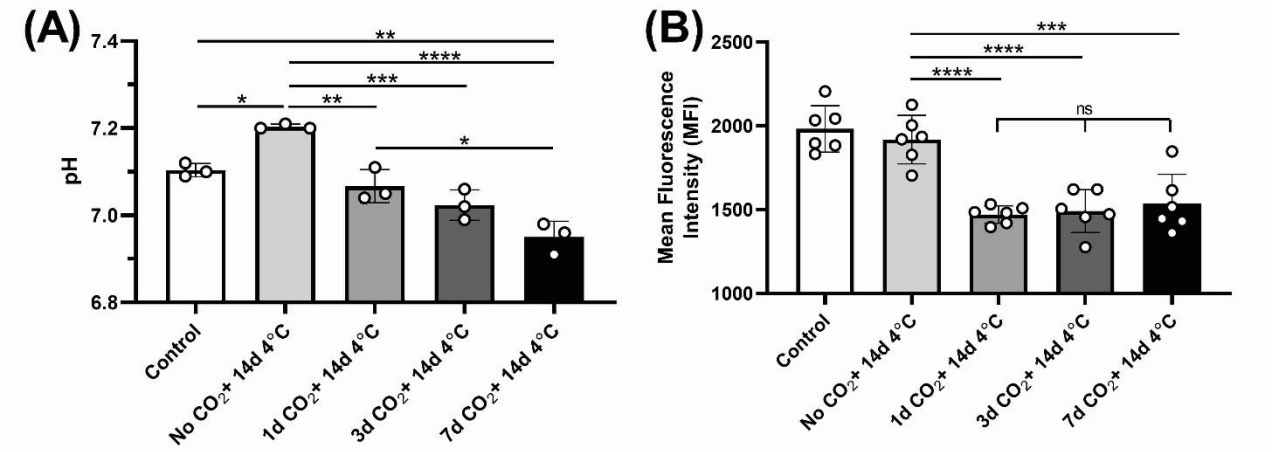


Figure 5: Effect of Dry Ice Storage Has Minimal Impact on 500 µL Fill of AAV pH and Functional Performance. A) Measured pH of CZ vials after either pre-freeze, no dry ice exposure (no CO_2), 1-day, 3-day, or 7-day holds in dry ice followed by a 14-day hold at 2-8°C. **B)** MFI as a readout of transduction functional activity of AAV2 stored in CZ vials on dry ice for the time indicated, same groups as **A**. CO_2 indicates dry ice storage condition; d indicates time in days; n = 6 (3 trials, 2 replicates each) as Mean ± SD with *p≤0.1000, **p≤0.0100, ***p≤0.0010; ****p<0.0001, and ns=p≥0.5000 by Two-Way ANOVA with Tukey's MCT.

To futher understand the impact of packaging and transport of formulated AAV2 on dry ice, CZ vials containing 500 µL of formulated AAV2 were stored on dry ice for 1, 3 or 7 days in order to replicate potential shipping conditions. After being held on dry ice, vials were thawed and stored in a mechanical refrigerator at 2-8°C for 2 weeks to simulate potential storage conditions post-shipment but pre-administration. All cryovials were measured at the same time for pH after the 2 weeks hold at 4°C (**Figure 5A**) and immediately used for transduction in the cell-based functional transduction test (**Figure 5B**). There was a noticable and significant (p≤0.0050) drop in pH in all dry ice time conditions (7.07 ± 0.038, 7.02 ± 0.035, and 6.95 ± 0.036 at 1-, 3-, and 7-day, respectively) compared to the post-freeze control vial (7.20 ± 0.0058) that was not subjected to any time on dry ice but was freeze/thawed and incubated at 2-8°C for 14-days (**Figure 5A**). In a prior study [1], the CCI at ultra-cold temperatures was tested and found to be intact. This suggests ingress of CO_2 through the vial walls to into the formulated AAV2 upon thaw [6].

The functional activity of the formulated AAV2 stored in dry ice was tested with the functional transduction activity assay to assess any effects of potential CO_2 ingress leading to pH changes (**Figure 5B**). Despite the change in pH due to the greater hold time to dry ice, infectivity (MFI) was not materially affected across 1-, 3-, and 7-day results. The AAV2 that had been held on dry ice did show a slightly reduced, but significant (p≤0.001), functionality relative to the nominally cryopreserved post-freeze control, however the lack of significance between the MFI of those vials with respect to dry ice hold time strongly indicates that this decline is associated with the exposure to dry ice and not the 2 weeks hold at 4°C. Therefore, the impact of dry ice storage on AAV2 function is minimal and characterizable.

4. Conclusions

This study investigated an existing container system commonly used for commercial drug products as an alternative to the current polypropylene (PP) screwcap cryovials used for cryopreservation of advanced therapies. The Daikyo Crystal Zenith® (CZ) vial system was composed of a commercially available CZ vial, a rubber stopper, and an aluminium seal. For comparison, AAV2-eGFP was stored at ultra-low temperature in various volumes and titers in CZ vials, glass vials, COC vials and PP screwcap cryovials. Test effects were measured by mean fluorescence intensities (MFI) from cells transduced functional assay as an indication of AAV2 function.

Table 2: Summarized Results of the Current Study. Performance of each vial type and condition tested in this research study, relative to each other.

	Glass	PP	Crystal Zenith
Freeze pH	+/-	+/-	+/-
Recovery	-/-	+/-	+/+
Dose Effect	-/-	Not Tested	+/+
Dry Ice Storage	Not Tested	Not Tested	+/-

CZ Proves Its Utility

The main findings of the study are that post-thaw AAV2 stored in 2mL CZ vials systems maintains equivalency to and can outperform AAV2 stored in glass vials in terms of recoverability (**Figure 1**). This outperformance is increased at higher titers (**Figure 2**) and lower storage volumes likely due to the inherent differences in the material properties and vial design (**Figure 3**).

CZ vials have been shown to functionally outperform glass for storing and recovering AAV2 viral vector material (**Figures 1, 2, and 3**). Viral vector recovery from CZ vials versus glass vials at a volume of 0.25 mL in a 2 mL container resulted in a ~30% increase in recovery. CZ vials outperformed glass best at 0.25 mL fill volumes likely due to the differences between vial design geometries (**Figure 3A**). More viral vector material was recovered from CZ vials compared to glass vials across three different fill volumes (**Figure 3B**). All three volumes were at or below 50% fill capacity of the vial, ensuring ample room for expansion of ice during cryopreservation. Inherent differences between vial designs, like the material properties of surface charge or degree of hydrophobicity or wettability, may aid the recovery of AAV2 viral vector material as demonstrated by this study. Overall, CZ is well suited as a storage solution for AAV viral vectors.

Considerations for Shipping on Dry Ice

Post-thaw potency and sterility, protected by CCI, are critical quality attributes (CQAs) of advanced therapies. Material properties did not seem to affect the observed increase in pH after a cryopreservation and thaw cycle where the pH increased ~0.2 pH units in either a 0.500 mL (**Figure 4A**) or 0.750 mL fill volume (**Figure 4B**). Further, evaluation of AAV from CZ vials after a hold period on dry ice showed a reduction in the pH of stored virus that tracked with length of hold time at 2-8°C (**Figure 5A**). However, neither the length of hold nor the pH of the vials correlated with the functionality of the recovered AAV2, which was consistent across all dry ice hold conditions (**Figure 5B**). Thus, while CZ vial material may have influenced the pH drop of AAV2 stored on dry ice, the resulting pH was not detrimental to AAV2 viral vector function. In addition, the present results suggest that the formulated AAV2 used in this study did not show signs of sterility issues or loss of function, lending indirect evidence of an in-tact container closure integrity (CCI) in cold storage temperatures. A separate study described in CZ TR 2020/032 [1] investigated the CCI of the same CZ container closure system during cryogenic storage. Results from that study demonstrated CCI was maintained for the duration of 90 days. The results of the current study lend evidence demonstrating the ability of West CZ cryo-containment systems to maintain both sterility and CCI without negatively affecting CQAs compatible with advanced therapy manufacturing requirements.

Considerations for Scalable Vial Solutions in Advanced Therapies

Screwcap cryovials carry inherent risks for loss of container closure integrity and thus loss of sterility and the therapies inside them [4]. Further, opaque polypropylene does not allow for easy visual inspection of the contents. Glass systems are quite brittle at cold storage temperatures and pose a cost liability and a safety hazard when frozen. Unlike PP, both Daikyo Crystal Zenith® and glass container closure systems (i.e., rubber stopper-aluminium seal closure) have greater CCI performance and excellent optical clarity, which assists inspection. The observed increases in the storage efficiency of AAV2 in CZ vials, and possibly other viral vectors, could help reduce fill volumes necessary to achieve the same targeted dose for those manufacturing gene therapies. With these considerations in recoverability, time, costs, and durability of sterility, CZ and West stoppers and seals make an excellent choice for the ultra-cold storage requirements of next generation advanced therapies.

References

1. “Performance of Daikyo Crystal Zenith® Vials at Cryogenic Temperatures: Container Closure Integrity and Cell Preservation.” West CZ Technical Report 2020/032.
2. “Evaluation of Daikyo Crystal Zenith® Vials for the Cryopreservation of Therapeutically Relevant Human Cell Types.” West CZ Technical Report 2020/030
3. Woods, E.J. et al. “Container system for enabling commercial production of cryopreserved cell therapy products.” *Regenerative Medicine.* 5(4), 659-667, 2010.
4. Contaminated liquid nitrogen vapour as a risk factor in pathogen transfer, B.W.W. Grout, G.J. Morris, Theriogenology 71 (2009) 1079-1082.
5. Hunt CJ. Technical Considerations in the Freezing, Low-Temperature Storage and Thawing of Stem Cells for Cellular Therapies. Transfus Med Hemother. 2019;46(3):134-150. doi:10.1159/000497289
6. “Ingress of Gases into Cyclic Olefin Polymer Vial-Based Container Closure Systems at -80°C” West CZ Technical Report 2019/028

Acknowledgements

Thanks are extended to the team at Advanced Bioprocess Services Ltd., working in partnership with Loughborough University at the Centre for Biological Engineering for their consultation services and performance of the AAV2 work: K. Glen, J. Harriman and R. Thomas.

Thanks are extended to the West Pharmaceutical Services team for study design, data analysis and report writing: S. Molina, C. Kraft. M Gehrmann and A. Lyness.

Daikyo's products are sold on the basis that it is the customer's responsibility to evaluate and test the Daikyo product to determine its compatibility with other materials and fitness for any end use.

This *technical report* dated 19 April 2021, supersedes any other previously released versions of this *report*.

Prepared by: S. Molina, Sr Scientist, Advanced Therapies, R&D

Evaluation of Adenoviral Vector Recovery from Ultra-Low Storage in Daikyo Crystal Zenith® Vials

I. Introduction

Adenoviral vectors are a major platform used to develop vaccines for infectious diseases as well as oncology indications. [1,2]

Adenoviral vectors are typically stored between room temperature and -80°C, with lower storage temperatures targeting longer storage durations, in line with the design considerations of the adenovirus vaccine supply chain. Ultra-cold storage of the viral vector can be helpful to ensure uninterrupted supply of vaccines in critical times such as a pandemic and in the case of off-the-shelf cancer therapies.

Polypropylene (PP) vials and tubes have been commonly used as the primary container in research, due to their low cost. However, they may be not suitable for clinical manufacturing of drug product, due to concerns related to container closure integrity of the snapcap- and screwcap-based systems, high particle generation from the PP material,[3] under characterized extractables profiles,[4] and loss of viral vectors to surface adsorption upon contact with the container.[5] Daikyo Crystal Zenith® (CZ) vials may be helpful to overcome these challenges and maintain better drug safety, purity and efficacy. In addition, CZ vials bring about a new modality of fluid transfer after storage and for the administration of the drug product, needleless and aseptically via a vial adapter, compared to PP screwcap or snapcap vial that require the uncap of the container and the use of a needle to effectively withdraw fluid.

In this study commissioned by West Pharma, two types of adenoviral vectors were used to test the cryopreservation performance of CZ vials and the ambient-temperature performance of CZ syringes. They are respectively Ad5, a common serotype used in transient delivery of transgenes for immunization against pathogen and tumor antigens, and Ad5.F35, a modified Ad5 where the fiber is replaced with that of the rare adenovirus serotype Ad35, which is effective at evading neutralization associated with immune systems pre-exposed to Ad5.

For ultra-cold storage, CZ vials with elastomer stoppers and aluminum seals were evaluated for its impact on the physical and functional recovery of adenoviral vectors in comparison to commercially available PP screwcap vials. For fluid transfer after storage and before administration, CZ Luer lock syringes were evaluated for their impact on the physical, functional recovery and volume retention of adenoviral vectors in comparison to commercially available PP Luer lock syringes.

II. Experimental Materials and Methods

Packaging components for two purposes were used in this study: vial components for ultra-cold storage of viral vectors, and syringe components for fluid transfer from storage vials before administration of viral vectors. These components, as well as materials and process parameters used in this study, are shown in Table 1.

Reporter adenoviral vectors, Ad5-CMV-eGFP (Ad5) and Ad5.F35-CMV-eGFP (Ad5.F35), were produced using stock vector obtained from Baylor College of Medicine (Houston, Texas), amplified in 293A cells, purified, and dialyzed into the storage buffer containing 2.5% glycerol, 25 mM NaCl, 20 mM Tris-HCl. After physical titer of the bulk volume was determined, viral vectors were diluted to 1E11 vp/mL before pre-freeze titration and ultra-cold storage or transfer testing.

Technical Report 2022/245

24 FEB 2023

Evaluation of Adenoviral Vector Recovery from Ultra-Low Storage in Daikyo Crystal Zenith® Vials

Table 1. Parameters tested in the study of adenoviral vector ultra-cold storage in CZ vials or room-temperature stability in and transfer from CZ syringes.

Viral Vector	Ad5-CMV-eGFP and Ad5.F35-CMV-eGFP
Concentration	1E11 "viral particles"/mL (vp/mL), at fill
Volume	1 mL, at fill
Storage Buffer	2.5% glycerol, 25 mM NaCl, 20 mM Tris-HCl, pH 8.0
Vial	2 mL Daikyo Crystal Zenith® (CZ) vials or 1.8 mL polypropylene (PP) screwcap vials
Elastomer	13 mm NovaPure® bromobutyl 4023/50 serum stoppers (for CZ vials)
Seal	13 mm aluminum Flip-Off® CCS (Clean, Certified, Sterilized) seals (for CZ vials)
Syringe	1 mL Daikyo Crystal Zenith® Luer Lock syringe (product discontinued at the time of this writing, 0.5 mL Daikyo Crystal Zenith® Luer Lock syringes still commercially available)
Vial Adaptor	13 mm West Vented Vial Adapters
Needle	23 gauge
Freezing	Passive, inside a fiberboard box inside a -80°C mechanical freezer for 1, 7 or 14 days
Thawing	Passive, on wet ice
Physical Titer	Spectrophotometer, absorbance, OD260
Transduction Assay	A549 cells at 1E6 cells/mL with serially diluted aliquots of viral vectors, fluorescence microplate reader

Part A – Ultra-Cold Storage in Vials

2 mL Daikyo Crystal Zenith® (CZ) vials with 13 mm NovaPure® bromobutyl serum stoppers and 13 mm aluminum Flip-Off® seals were used as the container closure system for ultra-cold storage experiments. The recovery of viral vectors from cryopreservation in CZ vials was compared to that in 1.8 mL polypropylene (PP) screwcap vials.

All vials were filled and crimped or capped manually inside a biosafety cabinet. A constant 1 mL fill volume was used. Freeze-thaw conditions for the CZ and PP vials were designed to mimic the typical storage of vaccine products: uncontrolled passive freezing in a secondary packaging (i.e., 5.25' x 5.25' x 2' fiberboard box) inside a -80°C freezer, for relatively short-term storage times between 1 and 14 days, and uncontrolled thawing on ice. 3 CZ vials and 3 PP vials were thawed for each of Ad5 and Ad5.F35 at each time point of 1, 7, or 14 days in storage.

Immediately after thawing, physical titer was determined, in 6 technical replicates for each vial, by lysing the viral particles in 0.2% sodium dodecyl sulfate (SDS), reading the absorbance at 260 nm and 280 nm via spectrophotometry, and quantifying the amount of DNA as a surrogate measurement for viral particles. In parallel, functional titer was determined, in 3 technical replicates for each vial, by transducing A549 cells with serial dilutions of the thawed viral vectors in 96-well plates, incubating the plate at 37°C for 48 hours, and measuring GFP fluorescence from the transduced cells via a microplate reader at 480 nm excitation/ 520 nm emission.

Part B – Fluid Transfer and Drug Interaction in Syringes

1 mL CZ Luer lock syringes were used as the container closure system for non-cryopreserved fluid transfer experiments in ambient conditions. The recovery of viral vectors from holding in CZ syringes was compared to that in 1 mL PP Luer lock syringes. The volume retention of viral vector in CZ syringes was also compared to that in the PP syringes.

Single-dose volumes (1 mL) of non-cryopreserved fresh Ad5 and Ad5.F35 were withdrawn from CZ vials into CZ syringes using vial adaptors, and from PP vials into PP syringes using 23G needles. After filling, syringes were held

at room temperature for 0, 1, 2, and 24 hours before being evaluated for physical and functional titers as described above for post-thaw measurements. Syringes were weighed before filling, after filling, and after dispensing at each time point. Vial-to-syringe transfer efficiency was calculated as the increase of mass in the syringe after filling compared to before filling. Retention in syringe was calculated as the increase of mass in the syringe after dispensing compared to before filling. Three syringes were measured for each sample condition.

III. Results and Discussion

Part A – Ultra-Cold Storage in Vials

1. Physical Titer

An OD_{260} spectrophotometer assay was used to measure the physical titer and evaluate the post-thaw recovery of adenoviral vectors, in terms of the quantity of DNA. Ad5 and Ad5.F35 were cryopreserved in CZ and PP vials, at a constant fill volume, starting concentration and in the same storage buffer formulation. The vials of viral vectors were stored at -80°C and thawing on ice after 1, 7, and 14 days of ultra-cold storage. As seen in Figure 1, the variability of this measurement was high in some sample groups, where the standard deviation ranged from 11% to 90% of the sample mean.

Compared to the control titer measured before freezing of fresh viral vectors, the effect of freeze-thaw on Ad5 was not statistically significant regardless of vial type and storage time, possibly due to the high variability of the measurement. For Ad5.F35, CZ vials had significantly better recovery than PP vials after the shortest term of ultra-cold storage at 1 day, while the difference between CZ and PP vials was not statistically significant upon longer durations in storage. There was a general trend that the physical recovery of Ad5 and Ad5.F35 from CZ vials declined gradually over time in ultra-cold storage, whereas the physical recovery from PP vials was relatively low throughout the course of ultra-cold storage. Considering both the intrinsic variability of this physical titer and its dissociation from the recovery of therapeutically efficacious viral vector units, it may be more relevant to evaluate the post-thaw recovery of Ad5 and Ad5.F35 in terms of the functionality of the recovered viral vectors.

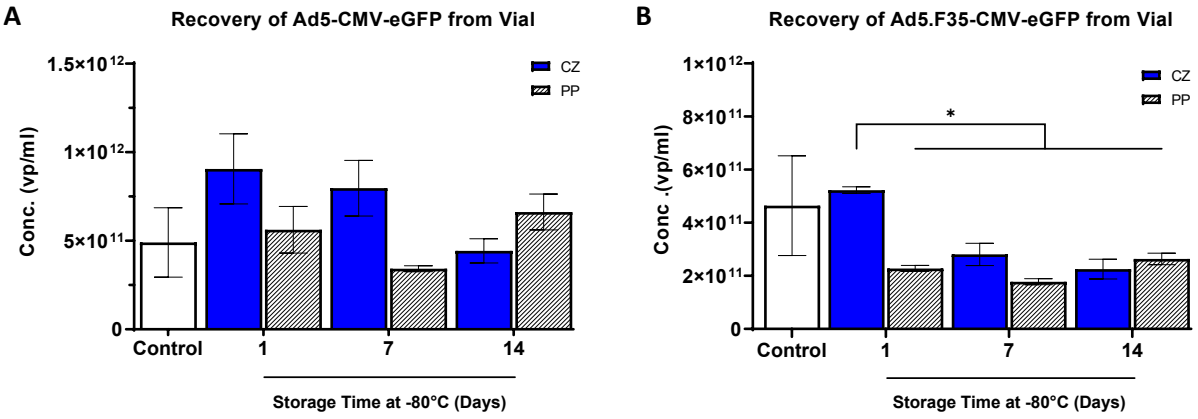


Figure 1. Recovery of (A) Ad5 and (B) Ad5.F35 viral vector DNA after ultra-cold storage in CZ versus PP vials for varying durations. Error bar: standard error. *: $p < 0.05$, two-way ANOVA corrected for multiple comparisons. $n = 3$.

2. Functional Titer

A GFP fluorescence microplate reader assay was used to measure the functional titer and evaluate the post-thaw recovery of adenoviral vectors, in terms of quantity of GFP reporter transgene transduced by the recovered viral vectors and expressed by the A549 cells. Ad5 and Ad5.F35 were stored in the CZ and PP vials at -80°C and thawing on ice after 1, 7, and 14 days of ultra-cold storage. Figure 2 shows that the functional recovery of Ad5 and Ad5.F35 was comparable between the two vial types. Compared to the control titer measured before freezing of fresh viral vectors, 1 day of ultra-cold storage did not have statistically-significant impact on the recovery of functional Ad5 or Ad5.F35 from either vial type. However, 7 and 14 days of ultra-cold storage significantly decreased the quantity of functional adenoviral vectors after thawing, regardless of adenovirus serotype and vial type.

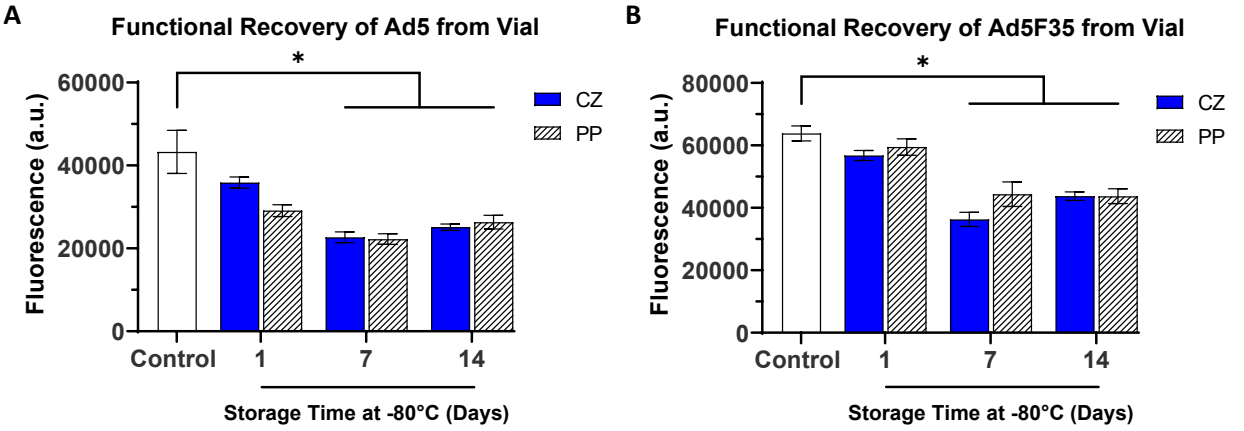


Figure 2. Recovery of functional (A) Ad5 and (B) Ad5.F35 viral vectors in terms of total GFP fluorescence of transduced cells after ultra-cold storage in CZ versus PP vials for varying durations. Error bar: standard error. *: $p < 0.05$, two-way ANOVA with corrected multiple comparison. $n = 9$.

It is noteworthy that the passive freezing and passive thawing methods tested in this study may not be optimized for minimizing cryoinjury in the viral vectors, resulting in more uncontrolled ice formation and osmotic stresses than needed to achieve higher functional recovery of the viral vectors. However, they represent the infrastructure and processes commonly used in the cancer vaccine cryopreservation practices. The observed significant loss of functional viral vectors may be reduced by optimizing the storage buffer formulation and improving its cryoprotective and biostabilizing effects on the viral vectors during the ultra-cold storage and the subsequent thawing process.

Part B – Fluid Transfer and Drug Interaction in Syringes

1. Vial-to-Syringe Transfer

CZ syringes and PP syringes were paired with CZ vials and PP vials to evaluate the efficiency of fluid transfer corresponding to the end of the adenoviral vector supply chain downstream of the ultra-cold storage, at the point of administering the drug product or testing it for quality purposes. CZ and PP vials containing Ad5 and Ad5.F35 equivalent to the pre-freeze samples in the ultra-cold storage experiments were connected to CZ and PP syringes via a vial adapter and a 23G needle, respectively, for filling. Figure 3 shows that, upon measuring the weight of the syringes before and after filling, significantly greater weight of the viral vector-containing fluid was transferred from the open PP screwcap vials using the PP syringe-needle configuration than from the sealed CZ vials using the CZ syringe-vial adapter configuration (i.e., 95% confidence interval of this difference is 107 +/- 26 mg). While the

vial adapter improved the usability of the vial-to-syringe transfer procedure, the fluid transfer may be improved by decreasing the dead volume of the vial adapter design.

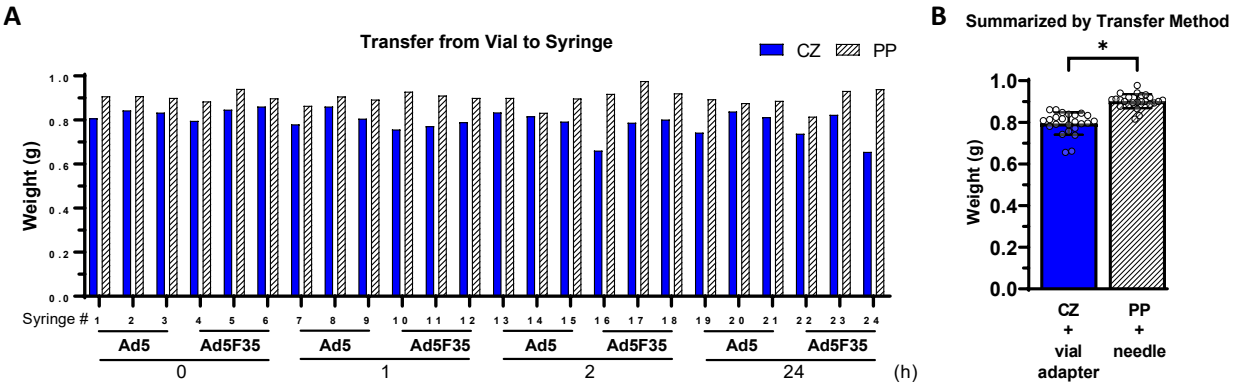


Figure 3. Weight of adenoviral vector fluid effectively transferred from CZ crimped vial to CZ syringe via a vial adapter versus from PP screwcap vial to PP syringe via a needle, shown (A) for individual vial-syringe pairs and (B) per transfer method. Error bar: standard error. *: $p < 0.05$, *t*-test.

2. Physical Titer

Similar to post-thaw physical titer, an OD_{260} spectrophotometer assay was used to measure the physical titer of fresh adenoviral vectors after being filled and held in CZ and PP syringes at room temperature, in terms of the quantity of DNA. Like Figure 1, Figure 4 shows a certain level of variability in this measurement with standard deviation ranging from 23% to 90% of the sample mean.

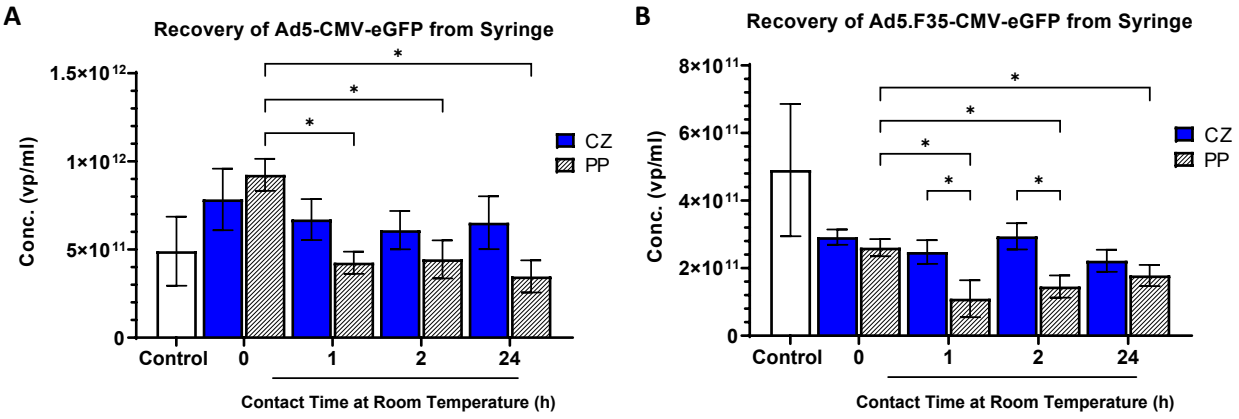


Figure 4. Recovery of (A) Ad5 and (B) Ad5.F35 viral vector DNA after temporary hold in CZ versus PP syringes for varying durations at room temperature. Error bar: standard error. *: $p < 0.05$, two-way ANOVA with corrected multiple comparison. $n = 12$.

The recovery of viral vector DNA content from syringes was not statistically significantly different from the initial control titer measured of the fresh viral vectors before filling into syringes, regardless of adenovirus serotype, syringe type and contact time with the syringe, likely due to the high variability of the measurement. However, the physical titer of both Ad5 and Ad5.F35 declined significantly in PP syringes after 1 hour and longer at room temperature, suggesting loss of viral vector material to the PP syringe possibly via surface adsorption. On the other

hand, the physical titer of Ad5 and Ad5.F35 was stable over time inside CZ syringes at room temperature, without statistically-significant difference. Considering both the intrinsic variability of this physical titer and its dissociation from the recovery of therapeutically-efficacious viral vector units, functional titer was also measured next to evaluate the stability of adenoviral vectors more directly in CZ and PP syringes.

3. Functional Titer

Similar to post-thaw functional titer, a GFP fluorescence microplate reader assay was used to measure the functional titer of fresh adenoviral vectors after being filled and held in CZ and PP syringes at room temperature, in terms of quantity of GFP reporter transgene transduced by the recovered viral vectors and expressed by the A549 cells. Figure 5 shows that the functional recovery of Ad5 and Ad5.F35 was comparable between the two syringe types, with the exception of Ad5 at 24 hours.

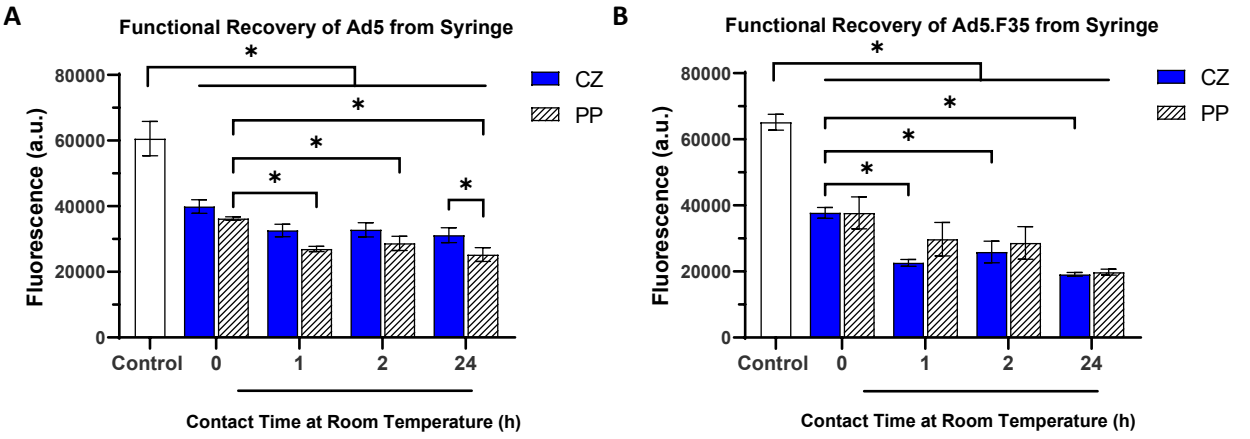


Figure 5. Recovery of functional (A) Ad5 and (B) Ad5.F35 viral vectors in terms of total GFP fluorescence of transduced cells after temporary hold in CZ versus PP syringes for varying durations at room temperature. Error bar: standard error. *: $p < 0.05$, two-way ANOVA with corrected multiple comparison. $n = 6$.

However, compared to the control titer measured before filling into the syringes, functional titer of the viral vectors decreased significantly immediately upon contact with the syringes, regardless of adenovirus serotype and syringe type. This loss of functional viral vectors persisted while the viral vectors were held inside the syringes at ambient conditions, showing a statistically-significant decline in the case of Ad5 in PP syringes and Ad5.F35 in CZ syringes after 1 hour of contact. A general trend of decline was also observed, although lacking statistical significance in the case of Ad5 in CZ syringes and Ad5.F35 in PP syringes. The loss of functional viral vectors upon contact with the CZ and PP syringes may point to a certain level of adverse interaction between the viral vectors and the drug contact surfaces of the syringes, the nature of which is to be determined, and upon which may a solution be provided to mitigate such functional loss.

4. Retention in Syringe

CZ syringes and PP syringes were also evaluated for fluid retention corresponding to the point of administration downstream of the vial-to-syringe fluid transfer and the temporary hold in the syringes. CZ and PP syringes containing Ad5 and Ad5.F35 equivalent to the 0-hour samples in the ambient stability experiments were tested for dispensing the viral vector material via a 23G needle. Figure 6 shows that, upon subtracting the weight of the

syringes after dispensing, significantly greater weight of the viral vector-containing fluid was retained in the PP syringe than in the CZ syringe (i.e., 95% confidence interval of this difference is 23 +/- 7 mg). This difference may be a combined effect of the difference in material properties between CZ and PP and the difference in geometrical design between the CZ and PP syringe barrels or that between the elastomeric pistons in the CZ and PP syringes.

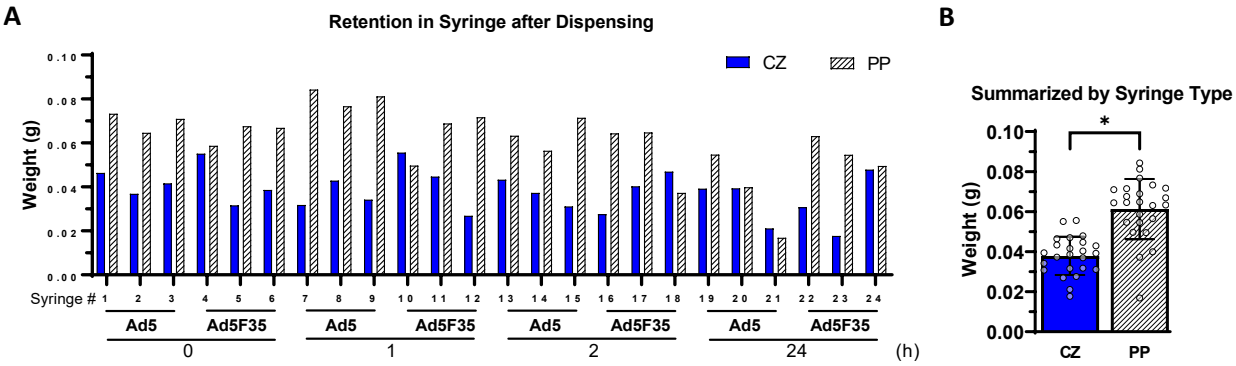


Figure 6. Weight of adenoviral vector fluid retained by CZ versus PP Luer lock syringes after dispensing via a 23G needle, shown (A) for individual syringes and (B) per syringe type. Error bar: standard error. *: $p < 0.05$, *t*-test.

IV. Conclusion

This study investigated an existing container closure system commonly used for commercial parenteral drug products, Daikyo Crystal Zenith® (CZ) vial systems, as an alternative to the conventional polypropylene (PP) screw cap vials for the cryopreservation of adenoviral vector-based vaccines and immuno-oncology therapies. A CZ vial-based container closure system consists of a CZ vial, an elastomer stopper, and an aluminum seal. The crimp closure of CZ vials can provide a sufficient container closure integrity withstanding the ultra-cold conditions.[6] The drug-contacting components of CZ vials have low particulate levels through cryogenic temperatures.[3] The materials of CZ vials have high break resistance at ambient through ultra-low temperatures.[7] The rigid form of CZ vials and their compatibility with automated fill-finish systems can allow effective scaling and high-throughput manufacturing of adenoviral vectors. Based on the results of this study, CZ vials showed suitability for the ultra-cold storage of adenoviral vectors, as an alternative to PP vials, without significant impact on the physical or functional recovery of the viral vectors. The physical and functional loss of the viral vectors in the longer-term storage in CZ and PP vials may be potentially mitigated by optimizing the storage buffer and cryoprotectant formulations. CZ syringes can also be a suitable alternative to PP syringes in the temporary containment of adenoviral vectors without significant impact on the physical or functional stability of the viral vectors during the hold time after transfer from the storage vial and before administration. With these considerations in the safety, durability, efficiency, and efficacy, CZ vials and the accompanying West stoppers and seals make an excellent choice for the containment and cold chain requirements of off-the-shelf cancer therapies and the regular as well as responsive immunization needs.

Note: Materials used in this study were procured before January 2021, and the study was executed to completion in May 2021.

V. Acknowledgements

Thanks are extended to the West Pharmaceutical Services team, S. Molina and A. Lyness, for experimental design, as well as the team at Thomas Jefferson University, A. Snook, J. Singh and T. Baybutt, for their consultation services, execution of the experiments, and data analysis.

VI. References

1. Chang, J. Adenovirus Vectors: Excellent Tools for Vaccine Development. Immune Netw. 21, e6 (2021).
2. Flickinger, J. C. et al. Chimeric adenoviral (Ad5.F35) and listeria vector prime-boost immunization is safe and effective for cancer immunotherapy. Npj Vaccines 7, 1–10 (2022).
3. "Comparison Of Particles in Daikyo Crystal Zenith® Vials, Glass Vials, Polypropylene Screw Top Vials and Cryogenic Bags". Daikyo CZ Technical Report 2022/038.
4. Jenke, D. Extractables Screening of Polypropylene Resins Used in Pharmaceutical Packaging for Safety Hazards. PDA J. Pharm. Sci. Technol. 71, 346–367 (2017).
5. Weikart, C. M. et al. Plasma-Treated Microplates with Enhanced Protein Recoveries and Minimized Extractables. SLAS Technol. Transl. Life Sci. Innov. 22, 98–105 (2017).
6. "Container Closure Integrity Testing on NovaPure® and Daikyo Closure Configurations for Ready Pack™ System with Daikyo CZ 2mL Vials". West CZ Technical Report 2021/242.
7. "Cyclic Olefin Polymer – Advantages versus Glass for Parenteral Drug Packaging/Delivery – a Brief Analysis". Daikyo CZ Technical Report 2017/021.

VII. Legal

This document is for informational purposes only. West's products and solutions are sold on the basis that it is the customer's responsibility to evaluate and test the West product or solution to determine its compatibility with other materials and fitness for any end use. WEST MAKES NO WARRANTIES, WHETHER EXPRESS, IMPLIED OR STATUTORY, INCLUDING, WITHOUT LIMITATION, THE WARRANTIES OF MERCHANTABILITY AND FITNESS FOR PARTICULAR PURPOSE, RELATING TO THE INFORMATION IN THIS DOCUMENT.

This Technical Report dated 24 February 2023, is the first release version of this report.

Evaluation of Daikyo Crystal Zenith® Vials for Ultra-Cold Storage and Shipment of Lentiviral Vectors

I. Introduction

Lentiviral vectors are a major platform used for ex vivo gene modification of cell-based therapies[1] including FDA-approved CAR-T cell therapy drug products KYMRIAH®, ABECMA®, BREYANZI® and CARVYKTI®. Recently lentiviral vectors have also gained regulatory approval for use in the *in vivo* gene therapy[2] drug products Zynteglo® for beta thalassemia and SKYSONA® for cerebral adrenoleukodystrophy.

Lentiviral vectors are typically stored between room temperature and -80°C, targeting varying storage durations in line with different supply chain use-case scenarios. Ultra-cold storage of these viral vectors can be helpful to ensure effective scaling of viral vector production and uninterrupted supply of the drug substance needed for a variety of immuno-oncology cell therapies.

Polypropylene (PP) vials and tubes are commonly used as the primary container in research settings and in upstream clinical manufacturing processes for the packaging of drug substances. However, they may not be suitable for clinical manufacturing of drug product due to concerns related to the integrity of the snap or screw cap closures, particle generation,[3] and extractables profile.[4] Daikyo Crystal Zenith® (CZ) vials may be helpful to overcome these challenges and maintain better cryogenic container closure integrity (CCI), low particle level, and low leachable risk for the safety and purity of the drug products down the line. As the industry is evolving to adopt more rigorous standards to ensure product safety and testing methods more appropriate for advanced therapies, particle, CCI, and extractables and leachables (E&L) considerations that have been required for traditional biologics may begin to take root in the practices and regulations for cell therapy drug products as well as drug substances, processing equipment and materials that contributed to the manufacturing of the drug products.

The impact of CZ vials on the functional recovery of lentiviral vectors is evaluated in comparison to PP vials. Storage in dry ice is also evaluated in addition to storage in a -80°C freezer, in lieu of potential concerns related to CO_2 ingress and pH excursions upon exposure to and removal from dry ice-based shipping conditions.

II. Experimental

Table 1. Parameters tested in the study of lentiviral vector ultra-cold storage in CZ vials.

Viral Vector	Lenti-GFP (plasmids: pCDH-EF1a-MCS-T2A-copGFP, pMDLg/pRRE, pRSV-Rev, pVSVg)
Concentration	1.16E9 transforming units (TU)/mL, at fill
Volume	1 mL, at fill
Storage Buffer	10% lactose, 25 nM proline, 10 mM Tris, pH 7.5
Vial	2 mL Daikyo Crystal Zenith® (CZ) vials or 1.8 mL polypropylene (PP) screwcap vials
Elastomer	13 mm NovaPure® bromobutyl 4023/50 serum stoppers (for CZ vials)
Seal	13 mm aluminum Flip-Off® CCS (Clean, Certified, Sterilized) seals (for CZ vials)
Freezing	Passive, inside a fiberboard box, in a -80°C mechanical freezer or dry ice, for 48 hours
Thawing	Passive, in a 4°C refrigerator, for 48 hours
Functional Titer	Flow cytometer, GFP-positive 293LTV cells per volume of viral vector used

2 mL Daikyo Crystal Zenith® (CZ) vials with 13 mm bromobutyl serum stoppers and 13 mm aluminum seals were used as the container closure system for this ultra-cold storage study, in comparison with 1.8 mL polypropylene (PP) screwcap vials. These components, as well as materials and process parameters used in this study, are shown in Table 1.

Evaluation of Daikyo Crystal Zenith® Vials for Ultra-Cold Storage and Shipment of Lentiviral Vectors

GFP-expressing, VSV-G pseudotyped lentiviral vectors, Lenti-GFP, were produced by transfecting HEK293T/17 cells, filtered, concentrated using PEG-8000, and resuspended in the lentiviral storage buffer in a polypropylene conical tube. All vials were filled with the lentiviral vectors and crimped or capped manually in a biosafety cabinet. A consistent 1 mL fill volume was used. Freeze-thaw conditions for the CZ and PP vials were designed to mimic a short-term storage and a typical shipment condition of lentiviral vectors as the final drug product in gene therapies and as a drug substance for gene-edited cell therapies.

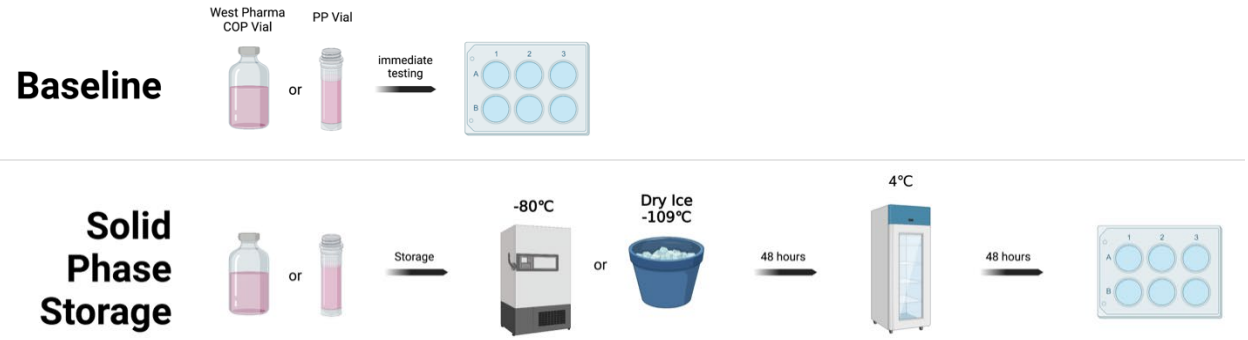


Figure 1. Lentiviral vector ultra-cold storage and analysis workflow.

As shown in Figure 1, baseline titers were established after briefly exposing the viral vectors to the CZ and PP vials. Ultra-cold storage performance was evaluated by measuring the viral vector functional titer after 48 hours of freezing in -80°C freezer versus dry ice, which has a surface temperature between -79°C and -109°C, and 48 hours of thawing at 4°C. Five vials of each vial type were sampled for each storage condition, and three technical replicates of functional titer were taken for each sample. To measure the functional titer, 293LTV cells were transduced with ten-fold serial diluted viral vector aliquots, trypsinized, counterstained with SYTOX™ AADvanced™ dead cell stain, and analyzed using a BS LSRII flow cytometer. Equal volume of undiluted viral vectors from all vials was used to seed the functional titer assay, without controlling the potential variable of physical titer recovered from each viral-vector sample. Titer calculation was based on the cell population and viral-vector dilution where 10% to 20% of the cells were GFP-positive.

III. Results

A GFP flow cytometry assay was used to measure the functional titer and evaluate the post-thaw recovery of lentiviral vectors cryopreserved in CZ and PP vials, in terms of the ratio of the number of GFP-expressing 293LTV cells effectively transduced by the recovered viral vectors to the volume of undiluted viral vectors used. Lenti-GFP was stored in the vials inside either a -80°C freezer or in dry ice and thawed in a 4°C refrigerator after 48 hours of ultra-cold storage. Figure 2 shows the post-thaw functional recovery of Lenti-GFP was comparable between the CZ and PP vial, after 48 hours of ultra-cold storage, whether in the freezer or in dry ice. For both CZ and PP vials, the functional titer of recovered lentiviral vectors was significantly higher after passive freezing in dry ice than in the -80°C freezer, which may be a result of the different heat transfer properties influencing the freezing process and cryobiological impact on the viral vectors, or the different temperatures influencing the phase properties and molecular stability of the frozen viral vector samples.

In addition, compared to the control titer measured from the stock viral vector before filling into the vials, the functional titer was lower after filling in and subsequently recovering from the PP vials with statistically significant

difference, whereas it was similar after filling in and subsequently recovering from CZ vials with no statistically-significant difference. The drop in functional titer after brief contact with the PP vial may be a result of interaction between the viral vectors and the container closure system. However, this interaction should be investigated more directly in the future. Comparison with the stock titer and the 0-hour titer also shows that the ultra-cold freeze-thaw resulted in significant loss in functional lentiviral vectors, suggesting a possibility to improve the functional recovery in general via more optimized cryopreservation methods. These methods may include a more optimized storage buffer formulation with more effective cryoprotective agents protecting the viral vectors from over-dehydration, ice recrystallization, solute precipitation, and more effective stabilizing agents protecting them from molecular and structural changes through the thermal and osmotic excursions over time.

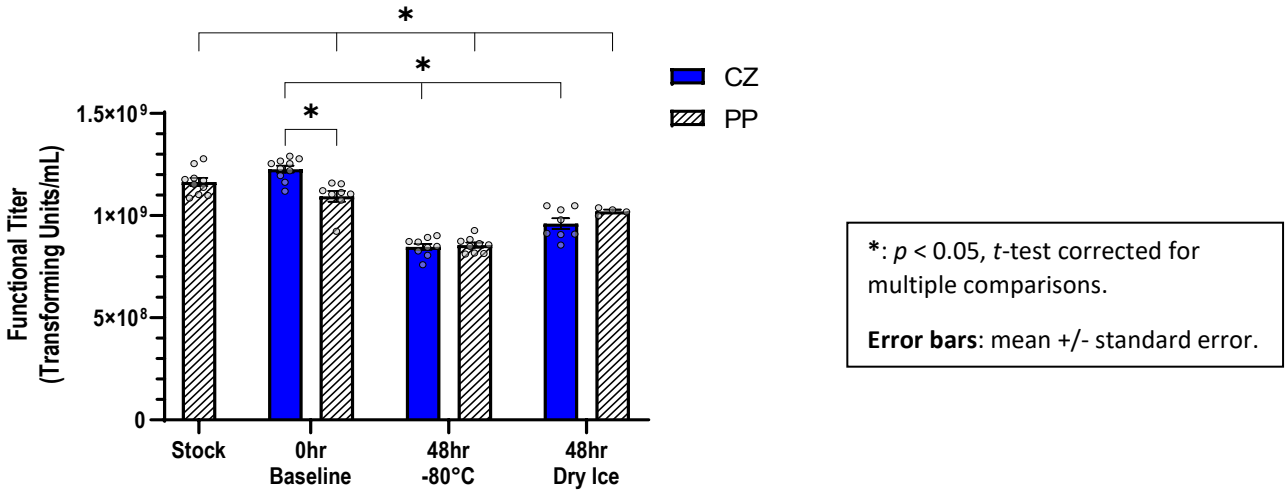


Figure 2. Functional lentiviral vector titers from freeze-thaw in CZ versus PP vials, from the freshly-formulated stock and from contact-only baseline condition as controls. Main effects model shown based on two-way ANOVA with Šidák correction. *n* = 4 to 10 with some replicates omitted due to the percentage of GFP-positive 293LTV population falling outside the 10% to 20% range.

IV. Conclusion

This study investigated an existing container closure system commonly used for commercial parenteral drug products, Daikyo Crystal Zenith® (CZ) vial systems, as an alternative to the conventional polypropylene (PP) screw cap vials for the cryopreservation of lentiviral vector-based advanced therapy drug substances and drug products. A CZ vial-based container closure system consists of a CZ vial, an elastomer stopper, and an aluminum seal. The crimp closure of CZ vials can provide a sufficient container closure integrity withstanding the ultra-cold conditions.[5] The drug-contacting components of CZ vials have low particulate levels through cryogenic temperatures.[3] The materials of CZ vials have high break resistance at ambient through ultra-low temperatures.[6] The rigid form of CZ vials and their compatibility with automated fill-finish systems can allow effective scaling and high-throughput manufacturing of lentiviral vectors. Based on the results of this study, CZ vials showed suitability for the ultra-cold storage and shipment of lentiviral vectors, as an alternative to PP vials, without significant impact on the functional recovery of the viral vectors. With these considerations in the safety, durability, efficiency, and efficacy, CZ vials

and the accompanying West stoppers and seals make an excellent choice for the containment and cold chain requirements of the growing generation of advanced therapies.

V. Acknowledgements

Thanks are extended to the West Pharmaceutical Services team, S. Molina and A. Lyness, for experimental design, as well as the team at Thomas Jefferson University, A. Snook, J. Singh and T. Baybutt, for their consultation services, execution of the experiments, and data analysis.

VI. References

1. Labbé, R. P., Vessillier, S. & Rafiq, Q. A. Lentiviral Vectors for T Cell Engineering: Clinical Applications, Bioprocessing and Future Perspectives. Viruses 13, 1528 (2021).
2. Bulcha, J. T., Wang, Y., Ma, H., Tai, P. W. L. & Gao, G. Viral vector platforms within the gene therapy landscape. Signal Transduct. Target. Ther. 6, 1–24 (2021).
3. "Comparison Of Particles in Daikyo Crystal Zenith® Vials, Glass Vials, Polypropylene Screw Top Vials and Cryogenic Bags". Daikyo CZ Technical Report 2022/038.
4. Jenke, D. Extractables Screening of Polypropylene Resins Used in Pharmaceutical Packaging for Safety Hazards. PDA J. Pharm. Sci. Technol. 71, 346–367 (2017).
5. "Container Closure Integrity Testing on NovaPure® and Daikyo Closure Configurations for Ready Pack™ System with Daikyo CZ 2mL Vials". West CZ Technical Report 2021/242.
6. "Cyclic Olefin Polymer – Advantages versus Glass for Parenteral Drug Packaging/Delivery – a Brief Analysis". Daikyo CZ Technical Report 2017/021.

VII. Legal

This Technical Report dated 14 March 2023, is the first release version of this report.