EVALUATION OF A NOVEL CYCLIC OLEFIN POLYMER CONTAINER SYSTEM FOR STORING ADENO-ASSOCIATED VIRUS

Rui Li[1], Samuel A. Molina[1], Katie Glen[2,3], Jon Harriman[2,3], Crystal Kraft[1], Vidya Murthy[1], Robert Thomas[2,3], Alexander Lyness[1]
Affiliations: 1. West Pharmaceutical Services, Inc., Exton, PA, USA, 2. Centre of Biological Engineering, Loughborough University, Leicestershire, UK, 3. Advanced Bioprocess Services Ltd., Loughborough, Leicestershire, UK

Contact Information
vidya.murthy@westpharma.com
www.westpharma.com

Introduction

One of the most effective vehicles for delivery of therapeutic nucleic acids are viral vectors, demonstrated by their utility in many commercial cell and gene therapy products. Despite their success, gaps remain in the optimization of viral vector storage. Traditional polypropylene snap- and screw-cap vials used in academic and research settings do not have the closure integrity or inert properties required for storage of a commercial drug product. Further, glass vials commonly used for biologic drug products have not been fully characterized in the context of viral vectors, which have a need for colder storage temperatures and an associated need for increased break resistance. In the present work, three vial types used for biologic product storage, cyclic olefin polymer (COP), polypropylene (PP), and glass, were evaluated for the preservation of adeno-associated virus (AAV) during ultra-low temperature storage.

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 Daikyo Crystal Zenith® (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 cyclic olefin polymer CZ vials for -80°C storage of AAV material and determining the relative performance of CZ vials relative to glass vials, and traditional PP cryovials for the preservation 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 screw-cap 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.

Materials & Methods

Vials made of cyclic olefin polymer (COP) 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.

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.

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® seal
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

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

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.

Results

Impact of vial material on AAV MFI after ultra-cold storage

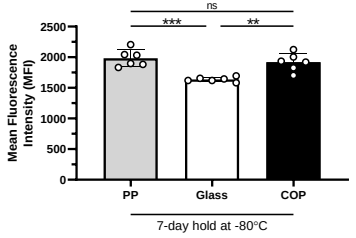

Figure 2: Infectivity is Maintained in CZ Vials After One Freeze/Thaw Cycle. *Formulated AAV2 was filled into various vials, frozen in cryoboxes within an ultra-cold freezer, and then stored for 3 weeks. Frozen vials were thawed in a pre-heated water bath, equal virus (according to the pre-freeze titer) 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.*

Impact of vial material on AAV formulation pH after ultra-cold storage

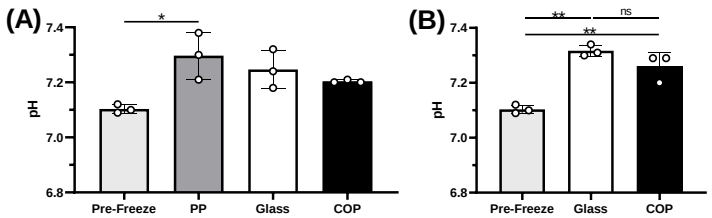

Figure 3: Impact of CZ and Glass on pH of AAV2 in Ultra-Cold Storage. *A) pH of 500 µL fills of formulated AAV2 in various vial materials after one freeze/thaw cycle. B) pH of 750 µL fills after one freeze/thaw cycle in CZ or Glass vials. n = 3 for each group; Mean ± SD with *p≤0.1000, **p≤0.0100, and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

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

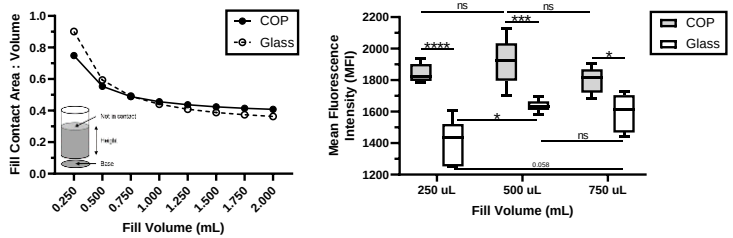

Figure 4: Fill Volume Does Not Influence Recovery of AAV2 Infectivity from CZ Vials Used in Ultra-cold Storage. *Left) Computed fill contact area to volume ratios for various fill volumes of a standard West 2mL CZ vial and standard glass vial. Inset: Generalized visual aid for vial surface area to volume ration calculation. Right) 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.*

Impact of dry ice hold time on AAV formulation pH and subsequent function

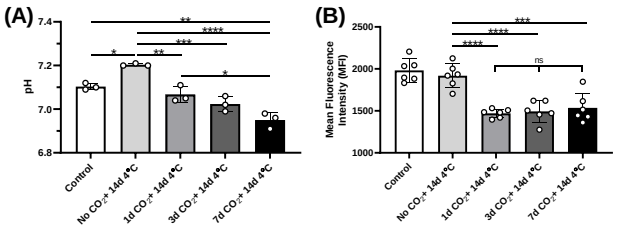

Figure 5: Effect of Dry Ice Storage Has Minimal Impact on 500 µL Fill of AAV pH and Functional Performance. *A) Measured pH of CZ vials after either pre-freeze, no dry ice exposure (no CO_2), 1-day, 3-day, or 7-day holds in dry ice followed by a 14-day hold at 2-8°C. B) MFI as a readout of transduction 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.*

Conclusions & Considerations

Adeno-associated virus (AAV) was evaluated with the AAV2-eGFP serotype expression vector stored frozen at -80°C in either COP, PP, or glass vials. Different storage volumes were investigated in COP and glass vial types to determine the effect of surface area-to-volume ratio on viral recovery. A quantitative viral activity assay was used to measure any loss of activity during the freeze-thaw process through a HEK293 transduction efficiency test coupled with flow cytometry.

Overall, viral recovery was greater as concentration increased, but improved recovery was observed in low storage volumes. Further, AAV recovery was greater after storage in COP in comparison to glass. These results suggest that surface area-to-volume ratio and material properties of the storage container may be an important consideration for the container choice of viral gene therapies. The results provide baseline data that indicate the compatibility of the storage container for a viral vector therapy can affect product quality and COP container systems may serve as a preferred container for commercial viral vector storage.

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