Particulate Contamination Levels in Single-Use Systems

Aramus™ bag assemblies and AccuSizer® SPOS systems

INTRODUCTION

The utilization of single-use systems (SUS) continues to grow in pharmaceutical and biopharmaceutical industries. With the growing adoption of SUS products more scrutiny has been given to the purity concerns of single-use components and their potential impact on biomanufacturing, storage, and transportation of high value final products. Aramus™ single-use bag assemblies are made of a single-layer, high-grade, gamma-stable fluoropolymer and provide high purity, extremely low extractable and leachable (E&I) profile, exceptional chemical compatibility, and increased safety for critical process fluids and final products. Specific attention is paid to minimize particulate contamination through ISO Class 5 cleanroom manufacturing and quality control (QC) testing for contamination with the AccuSizer® liquid particle counter.

Reasons for concern with particulate levels include:[1]

- Risk to patient from possible micro blood vessel obstruction
- Cell growth interference
- Drug product quality, toxicity, and safety
- Processing interference

For these reasons, Aramus bags are manufactured to the highest degree of cleanliness and tested for particulate contamination. Because Entegris manufactures both the Aramus fluoropolymer bag assemblies and the AccuSizer liquid particle counters used for QC testing, they are uniquely qualified to supply single-use systems that minimize particulate contamination. Subvisible particulate testing of Aramus bags is based on the USP <788> standard[2] and is well documented in other Entegris application notes.[3,4] These tests focus on the USP <788> limits of fewer than 25 particles/mL ≥10 µm and fewer than 3 particles/mL ≥25 µm. One hundred percent of all Aramus bags tested meet the USP <788> standard.

ADSORPTION STUDY OF mRNA ENCAPSULATED LIPID NANOPARTICLES

Entegris collaborated with Precision Nanosystems, Inc. (PNI)[5] to perform a study using model mRNA-encapsulated lipid nanoparticles (LNPs) to investigate material adsorption of mRNA-encapsulated LNP over seven-day storage time comparing the Aramus fluoropolymer bag to commonly used glass vials and polypropylene (PP) cryotubes. mRNA-encapsulated LNPs solution were evaluated for physicochemical properties after incubation at different conditions to establish initial data on stability of mRNA-LNPs in contact with these three enclosure materials. Model mRNA (EPO) were formulated and encapsulated into LNPs using PNI's proprietary lipid mix and formulation system, aliquoted into the enclosures stored at room temperature, and then analyzed at different timepoints, time zero (T0) right after formulation, after 24 hours, and after 168 hours storage for mRNA-LNP concentration, Figure 1, mRNA concentration, Figure 2, mRNA-LNP mean size, Figure 3.

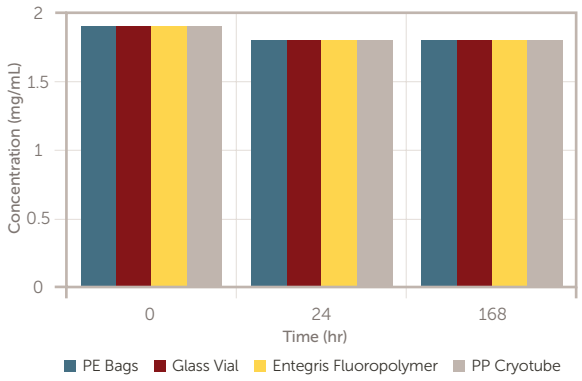

Figure 1. LNP concentration versus time.

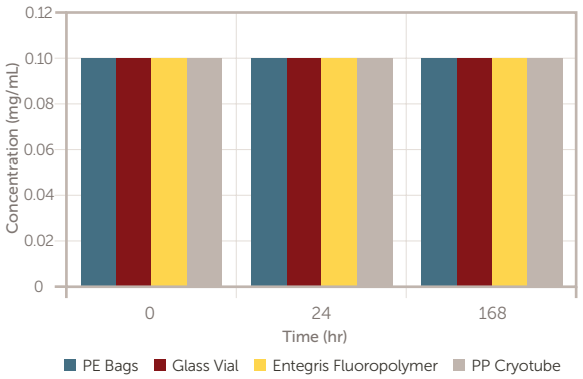

Figure 2. mRNA concentration versus time.

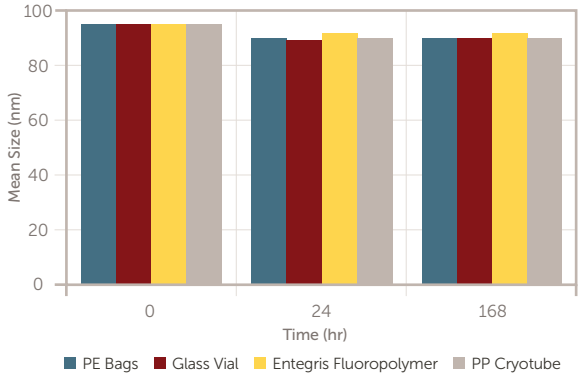

Figure 3. mRNA-LNP mean size versus time.

As shown in Figures 1 to 3, after 168 hours of storage at room temperature, model mRNA-encapsulated LNP has maintained almost identical lipid nanoparticle concentration, mRNA concentation, encapsulation efficiency, and mean size with the industrial standard container of glass vial, PP cryotube, and PE bags, indicating that Aramus fluoropolymer bags are suitable for pharmaceutical use.

The enclosures containing the LNP suspensions were then shipped to the Entegris Goleta, CA facility for additional testing using both the Nicomp® DLS and AccuSizer SPOS systems. The Nicomp system data showed an increase in the LNP size from 96 to 134 nm, most likely due to aggregation with time. The contents of the bags were then tested on the AccuSizer SIS system to measure particle size and concentration from 0.5 – 400 µm. The results shown in Figure 4 plot particle counts/mL versus size for the Aramus fluoropolymer bags versus the polyethylene (PE) bags at T0 and after 168 hours.

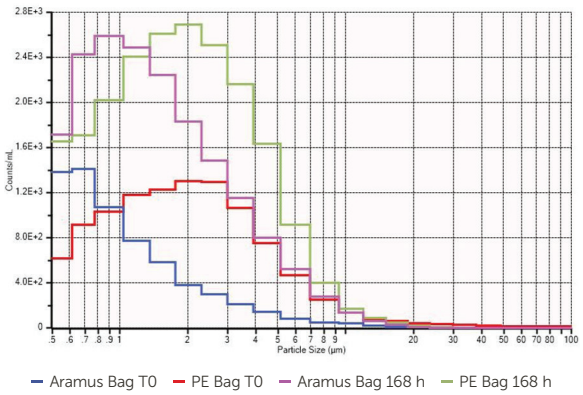

Figure 4. Particle concentration/mL in different single-use enclosures.

The results in Figure 4 indicate significantly more large particles in the PE bag both at both T0 and after 168 hours. This possibly indicates a higher degree of LNP aggregation rather than more contamination in the PE bags but cannot be confirmed because SPOS is not specific to particle chemistry. But the size distribution shown here is more consistent with intrinsic aggregated proteins than with extrinsic contamination.[6]

While reviewing these results, Entegris decided to investigate particulate contamination in Aramus bag assemblies versus other SU bag assemblies in the submicron region. The drive to achieve the highest degree of cleanliness with respect to particulate contamination could include measuring to sizes below 10 and 25 µm.

BAG CLEANLINESS COMPARISON TESTING

Several commercially available SUS bags were purchased, and a particulate contamination comparison study was performed.

Procedure:

- Test the Milli-Q water to establish background baseline
- Fill 250 mL bag assemblies with the above Milli-Q water at a surface area-to-volume ratio of SA/V = 6 cm²/mL (following BPOG guidance)
- Agitate bag on an orbital shaker at 40 RPM for 2 minutes
- Transfer water from bag to clean beaker
- Run liquid particle counting test for the water in the beaker

AccuSizer SPOS System Protocol:

Sensor mode = summation

Sample volume = 5 mL

Number of replicates = 4

Flow rate = 30 mL/min

A comparison of the Aramus bag versus one type of EVA bag from brand A is shown in Figure 5.

Size	EVA bag	Aramus bag
0.5 – 400 µm	6871.3	1455.3
1 – 400 µm	1222.3	488.3
2 – 400 µm	167.3	109.7
5 – 400 µm	42	22
10 – 400 µm	8.7	1.7
25 – 400 µm	0.3	0.3

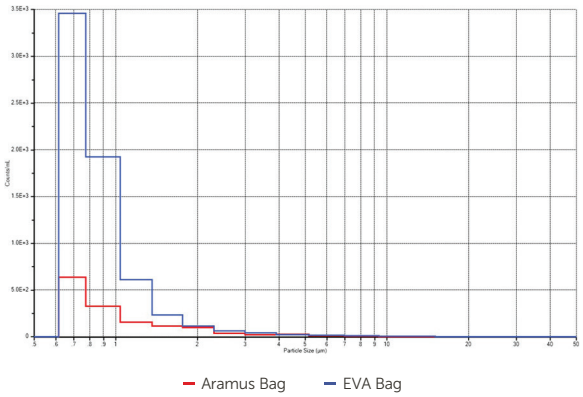

Figure 5. Particle concentration/mL, Aramus bag versus EVA bag.

These results indicate lower particulate contamination levels in the Aramus fluoropolymer bags than the EVA material bags. After reviewing this data, additional tests continued in the Entegris Billerica, MA facility comparing Aramus fluoropolymer to two EVA type bags. The same procedure and protocol as described above was used in the Billerica, MA studies.

As shown in Figure 6, two types of 250 mL EVA bag assemblies were compared to 250 mL Aramus bag assemblies for cleanliness level. Aramus bag assemblies consistently meet the USP <788> standard for less than three counts of particles/mL larger than 25 µm and less than 25 counts of particles larger than 10 µm, while the two types of EVA bags sometimes exceeded the limits set by USP <788>.

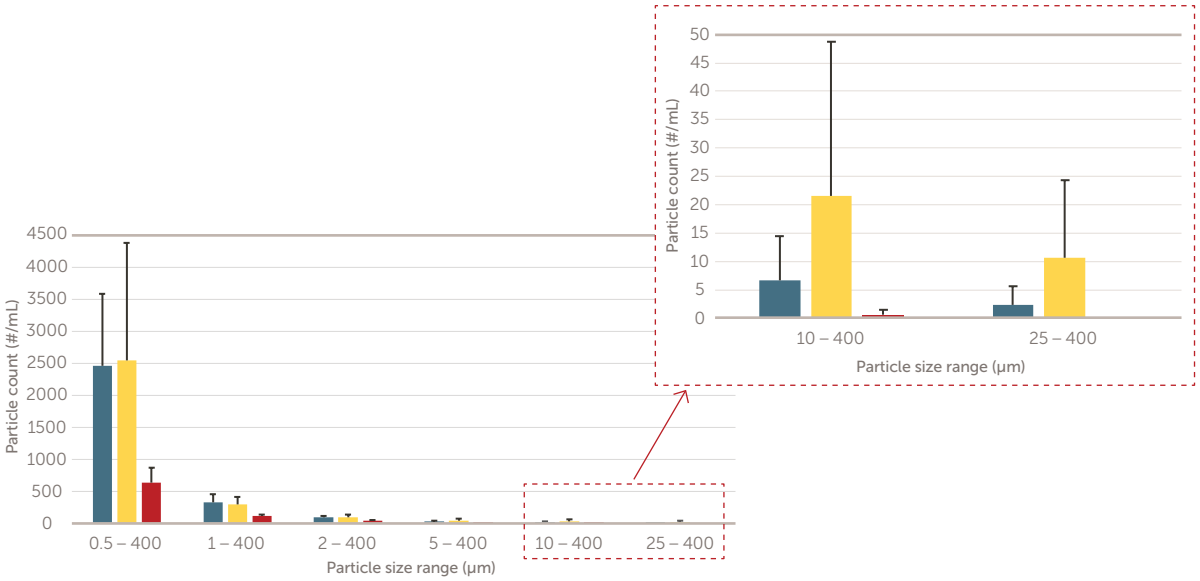

Figure 6. Entegris (red), Brand B (blue), Brand C (yellow) particles concentration/mL versus size (µm).

The results shown in Figure 7 also suggested that the Aramus bag assemblies had the lowest particulate contamination level at >0.5 µm range, which is approximately half of brand B and one third of brand C. Aramus bag assemblies also demonstrated a much more consistent cleanliness level from assembly to assembly compared to EVA brands B and C.

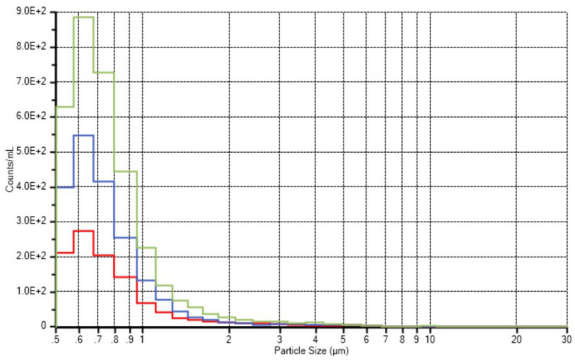

Figure 7. Entegris (red), Brand B (blue), Brand C (green) particles concentration/mL versus size (µm).

CONCLUSIONS

This study suggests that Aramus fluoropolymer bag assemblies are much cleaner than bags from competitors. Aramus bag assemblies consistently meet the USP<788> standard, while competitors' cryobags sometimes do not meet the standard. Aramus bag assemblies offer a more consistent performance in between different bags, giving customers quality assurance. We also observed that Aramus bag assemblies are two to three times cleaner in submicron particle range compared to competitors' EVA bags, indicating that Aramus bag assemblies can be a great single-use system for biopharmaceutical industry applications.

References

[1] *Recommendations for Testing, Evaluation, and Control of Particulates from Single-Use Process Equipment*, BPSA 2014 report

[2] USP <788>, *Particulate Matter in Injections*

[3] Entegris Application Note, *Aramus Single-Use Bag Particle Testing*, November 2018

[4] Entegris Application Note, *Monitoring Particulate Contamination in Medical Devices*, March 2021

[5] Precision Nanosystems, Inc., www.precisionnanosystems.com

[6] Chou, K. and Bumiller, M., *Opportunities and Pitfalls in the Analysis of Subvisible Particles during Biologics Product Development and Quality Control*, American Pharmaceutical Review, August 2020

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