

A Functional Suitability Assessment of Aseptically Reprocessed Bromobutyl Parenteral Packaging Components

By

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**A Functional Suitability Assessment of Aseptically Reprocessed Bromobutyl
Parenteral Packaging Components**

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Abstract

As the pharmaceutical industry seeks to improve sustainability and operational efficiency, the potential recycling of unused aseptically processed elastomeric packaging components presents a compelling opportunity. This thesis investigates the impact of repeated ATEC vessel processing bromobutyl serum stoppers in the context of functional suitability and intrinsic subvisible particulate matter burden. This study evaluates critical functional performance attributes of primary elastomeric closure components in accordance with USP <382>, including container closure integrity, needle penetration force and fragmentation. In parallel, subvisible particulate matter was assessed through USP <788> methods, as well as one orthogonal characterization technique: HIAC light obscuration, membrane microscopy, and Micro Flow Imaging, respectively. Visual inspection and dimensional analysis were also evaluated using vendor specifications for the components.

Results demonstrated that reprocessed stoppers retained their shape, size, and functional integrity, and met all compendial acceptance criteria. Particulate levels remained below 1/3 of the USP <788> threshold, a benchmark commonly used in industry for individual components; however, MFI analysis revealed a high prevalence of silicone oil droplets in reprocessed stoppers, likely attributable to repeated silicone exposure during the second ATEC processing cycle. This finding suggests that while reprocessing is viable, further optimization of the ATEC recipe may be warranted to minimize particulate risk.

Dedication

This thesis is dedicated to the memory of my beloved granny, Elaine Kneece, and my dear friend, Stephanie Blair. Though they are no longer here to see me cross the finish line, I continue to carry their strength and spirit with me every day. I hope this accomplishment makes my loudest cheerleaders proud, wherever they are.

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configured the empty and WFI containing units required for functionality testing. Shawn Astabraghpour and his team supported my project by executing the container closure integrity and penetration force testing. I recognize and appreciate the contributions made by the individuals listed, and anyone I may have inadvertently left out.

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Introduction and Background

Elastomeric stoppers are an integral primary packaging component for vial and bottle drug delivery systems containing parenteral therapeutics. As part of the container closure system, elastomeric stoppers preserve the quality of the finished drug product while simultaneously protecting the end user who prepares and administers the pharmaceutical. This is achieved in part by the secure placement of an elastomeric closure, which serves to provide an accessible barrier between the packaging system and the drug itself. As with any container and closure component utilized in the aseptic manufacturing of pharmaceutical products within the United States, elastomeric stoppers must adhere to the guidelines set forth by The FDA *Code of Federal Regulations (CFR)* part 21 211.94:

§ 211.94 Drug product containers and closures.

(a) Drug product containers and closures shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug beyond the official or established requirements.

(b) Container closure systems shall provide adequate protection against foreseeable external factors in storage and use that can cause deterioration or contamination of the drug product.

(c) Drug product containers and closures shall be clean and, where indicated by the nature of the drug, sterilized and processed to remove pyrogenic properties to assure that they are suitable for their intended use. Such depyrogenation processes shall be validated.

(d) Standards or specifications, methods of testing, and, where indicated, methods of cleaning, sterilizing, and processing to remove pyrogenic properties shall be written and

followed for drug product containers and closures¹ (Food and Drug Administration [FDA], 2025).

In the context of parenteral pharmaceutical packaging, the nature of the drug is such that container closure system sterility and depyrogenation are of utmost importance. Sterility refers to the complete absence of all viable microorganisms such as bacteria, fungi, and viruses from the drug product and its packaging system². Depyrogenation specifies the removal or inactivation of toxic components sourced from the cell walls of gram-negative bacteria, known as endotoxins³. The distinction between sterility and depyrogenation is important because endotoxins are heat stable and can remain present even after the sterilization process in the form of leftover bacterial fragments, thus posing a risk to patient safety. In addition to the biological reactivity requirements, elastomeric closure components for sterile injectable pharmaceuticals are subject to physicochemical testing and functional suitability assessments as outlined per USP <382> *Elastomeric Component Functional Suitability in Parenteral Packaging/Delivery Systems*. Designed to supersede USP <381> *Elastomeric Closures for Injections*, this shift in container closure evaluation allows for a functional evaluation of the entire container closure system of the drug delivery device. A non-exhaustive list of this testing includes penetrability, fragmentation, container closure integrity, and self-sealing capacity.

An abundant variety of elastomeric stopper formulations are available to pharmaceutical manufacturers, and they are sold as ready-to-sterilize (RTS), ready-to-use (RTU) and raw components that must be washed, depyrogenated, siliconized and sterilized by the end user⁴. Components that are received as RTS are washed and treated with silicone by the elastomer supplier, but must be sterilized by the pharmaceutical manufacturer prior to its use in the aseptic fill-finish process for a drug product. On the other hand, stopper components that are received as

RTU are both treated and sterilized by the elastomer manufacturer and can be used in aseptic environments without further processing. Many factors must be considered by the pharmaceutical manufacturer when choosing to purchase RTS or RTU stoppers for their operational needs; as the utilization of RTU components saves time and manpower, they also come at a greater financial burden compared to RTS components. For the sake of cost savings and overall flexibility, many sterile injectable pharmaceutical manufacturing operations choose to purchase RTS stoppers and process them onsite, using a system such as that provided by ATEC Pharmatechnik GmbH.

The ATEC Pharmatechnik Vessel is a stopper processing system that utilizes a rotating detachable vessel for optimal washing, siliconization, sterilization, drying, and cooling of the components. It is equipped with an Allen Bradley ControlLogix PLC and a Panelview 1500 Human Machine Interface (HMI)⁵, allowing for computer software operation and auditing capabilities that are compliant with regulatory requirements. Additionally, the system has the capability to process unrelated components with different recipes simultaneously or independently. Onsite at Pfizer McPherson, KS, unprocessed raw or RTS stopper components are loaded into a 150L or 250L vessel at the loading station in a dedicated component preparation area, which is classified as an ISO class 8 cleanroom environment (Figure 1). The vessel is then transported to the component processing system where the vessel is securely docked for processing (Figure 2). Once stoppers are loaded into an ATEC vessel, they remain in that same vessel even upon completion of the component processing activity, until they are transferred to the aseptic filling line for use. Alternatively, they may be aseptically transferred into sterile disposable bags and staged for production use.

The ATEC component processor executes programmed cycles to prepare the stoppers for use as primary packaging components in the production of parenteral drug products. The ATEC vessel is designed to wash, siliconize, sterilize, and dry elastomeric packaging components. Once the stoppers have been processed and the filling area requests the processed components, the vessel is transferred to the vessel positioning device within the filling area where the stoppers are consumed. The stoppers must then be used within their predetermined expiration date in alignment with manufacturing procedures or they will be discarded. The final filling and component preparation schedules are subject to unforeseen changes, which can result in wasted materials, time, and manpower. Disposing of stoppers before use can significantly impact a pharmaceutical manufacturer's finances and ability to meet market demands. Repeating the component processing procedure on expired stoppers could remedy this, however, it is important to anticipate the potential impact on the container closure system that reprocessed stoppers could have, as the altered treatment of a primary packaging component might prove incompatible with the finished drug product. Thus, a feasibility study was performed in which an elastomeric stopper was chosen for the experimental group, processed twice with the same recipe in an ATEC component processing vessel, and evaluated using USP <382> functional suitability testing requirements and the chosen elastomer material monograph as guides.

Figure 1

ATEC Pharmatechnik Component Processing Vessel



Figure 2

Operator unloading components into ATEC Vessel



Materials

Elastomer

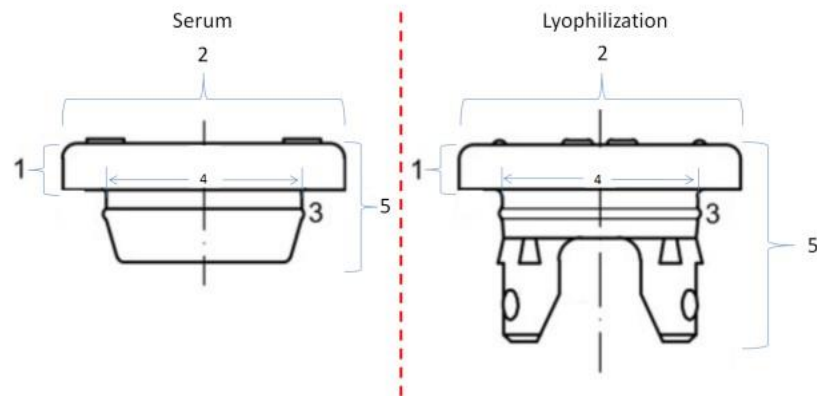
When selecting serum stoppers for parenteral drug packaging, the choice between bromobutyl and chlorobutyl elastomers involves several critical considerations related to chemical compatibility, gas permeability, and regulatory compliance. Bromobutyl stoppers are generally favored for their lower permeability to oxygen and moisture, which enhances product stability; particularly for oxygen sensitive formulations such as biologics and vaccines. They also tend to offer superior chemical resistance and reduced reactivity, resulting in a lower risk of extractables and leachables that could compromise drug integrity. Chlorobutyl elastomers, while also chemically resistant, typically exhibit slightly higher gas permeability and may have a greater tendency to interact with certain drug products; although advancements in formulation have narrowed this gap⁴. From a processing standpoint, both materials can be sterilized using standard methods such as steam autoclaving or gamma irradiation, but formulation specific compatibility must still be evaluated. Regulatory acceptance is strong for both materials, though bromobutyl stoppers may be preferred in highly regulated applications due to their broader history of use in sterile injectable products. Ultimately, the choice between bromobutyl and chlorobutyl stoppers should be guided by the specific drug formulation, intended shelf life, storage conditions, and extractables and leachables risk profile.

The primary elastomeric closure component selected for this study was a West 4405/50 formulation bromobutyl stopper (Figure 4). This material was chosen due to its availability onsite, along with its cost effectiveness in terms of base price and the operational expenses associated with component processing. The West 4405/50 gray stopper is a 13 mm serum vial closure manufactured from a bromobutyl-based elastomer specifically designed for use in

parenteral drug packaging. This formulation offers a combination of low permeability to gases and moisture, high chemical resistance, and excellent resealability, making it suitable for both single and multidose applications. With a hardness rating of approximately 50 Shore A, the stopper maintains an optimal balance between elasticity and compression resistance. The 4405/50 stopper exhibits a low extractables and leachables profile, supporting drug product stability and minimizing risk of contamination. It is compatible with a variety of pharmaceutical compounds, including vaccines, biologics, and aqueous injectables. This elastomeric formulation is also compatible with lyophilized drug products, however, the 4405/50 gray stopper is molded to be used with serum products (Figure 3). West provides this stopper in various RTU, RTS, and raw component formats, ensuring high levels of cleanliness and sterility. Additionally, the stopper meets international pharmacopeial standards (USP, EP, JP) which is supported by extractables and leachables data to aid regulatory compliance. Overall, the West 4405/50 gray stopper is a reliable and versatile closure system for demanding injectable product requirements⁶.

Figure 3

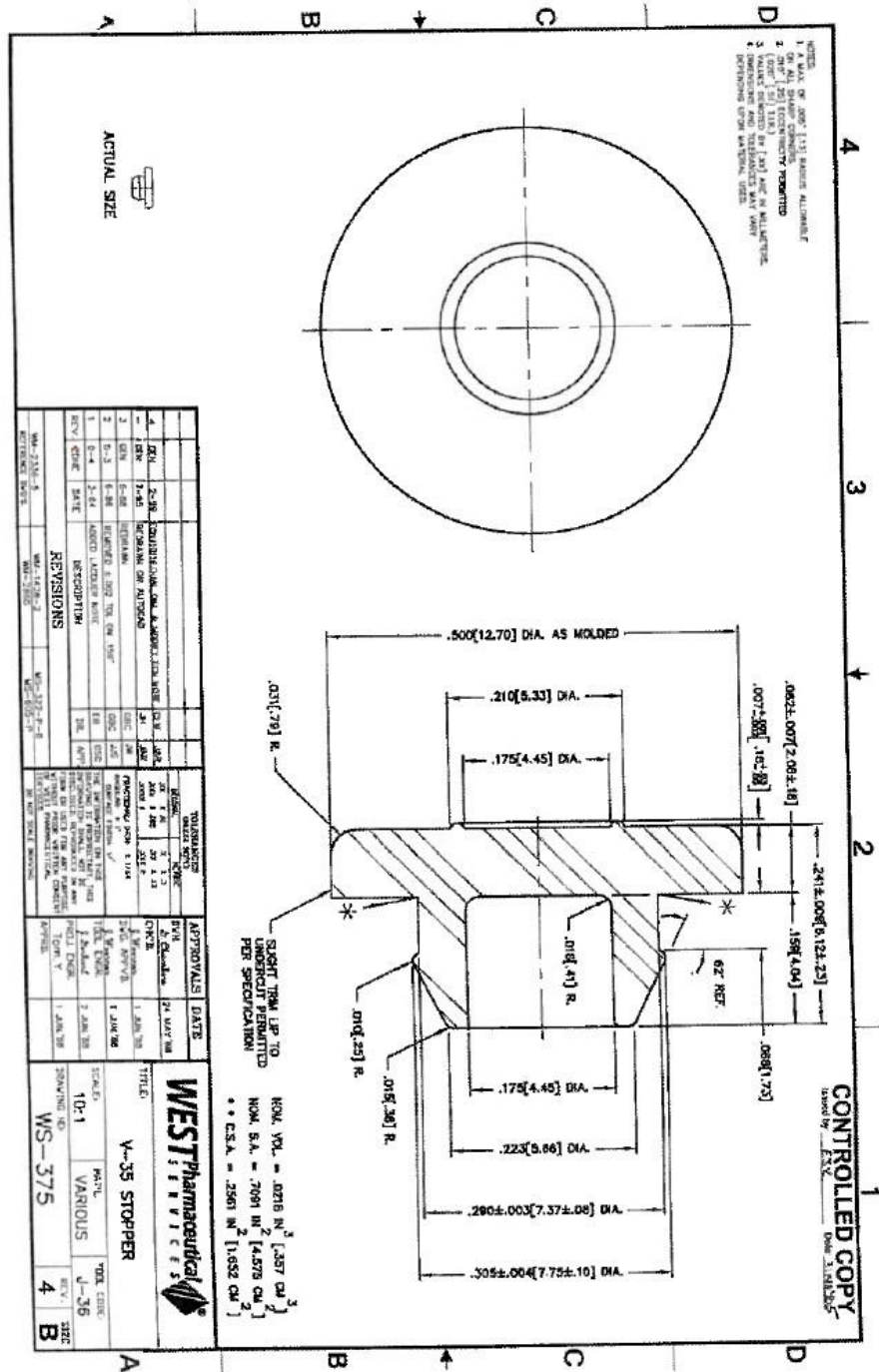
Dimensional schematics of a 13 mm serum stopper and a 13mm lyophilization stopper illustrating key features: (1) Flange thickness, (2) Flange diameter, (3) Tapered plug/skirt, (4) Plug diameter, and (5) Overall stopper height



These dimensions are critical for ensuring compatibility with vial geometry, secure sealing, and performance under processing and storage conditions.

Figure 4

Official drawing of 4405/50 Gray West stopper used in ATEC Vessel reprocessing feasibility study



ATEC Pharmatechnik Component Processing Vessel

The 150-liter ATEC Pharmatechnik vessel is a high-performance stainless-steel container designed for use in aseptic pharmaceutical manufacturing processes, including the preparation, storage, and transfer of buffers, media, or intermediate drug products. Constructed from AISI 316L stainless steel with an electropolished surface finish, the vessel ensures a hygienic, corrosion-resistant interior that supports clean-in-place (CIP) and steam-in-place (SIP) protocols⁷. Its cylindrical body with a dished bottom facilitates complete drainage and minimizes carryover, which is critical for maintaining batch integrity and reducing product loss. The vessel is typically mounted on a mobile or stationary frame with integrated lifting or transport features to support ergonomic handling within cleanroom environments. It is equipped with multiple aseptic Tri-Clamp ports for instrumentation, sterile filter connections, and process integration, as well as a manway for inspection or manual cleaning. Additional features often include aseptic sampling valves, spray balls for automated cleaning, and pressure/vacuum relief systems. The 150L ATEC vessel is fully compatible with Pharmatechnik's automation platforms, enabling precise control, real-time monitoring, and seamless integration into GMP-compliant production workflows. The ATEC processing cycle consists of multiple phases that can be selected from different recipes for individual component processing requirements. The phase descriptions are as follows:

1. **Vacuum leak test**: The vacuum leak test is conducted before the process begins. A vacuum is drawn to a predetermined value, and after a set period, the pressure is measured and compared to the initial pressure. Based on this comparison, the test is marked as either passed or failed. After completing the vacuum test, the vessel is vented with sterile air.

2. Component cleaning: The system features an automated detergent injection during processing to assist in cleaning the components. The vessel is filled with Water for Injection (WFI) from the bottom up. During the filling process, 6% sodium lauryl sulfate detergent is metered into the WFI-filled vessel via the dosing pump. Once the maximum fill level is reached, the wash timer is activated. Throughout the washing period, the vessel is continuously overfilled with WFI via the separator. When the wash timer expires, the WFI flow is turned off.
3. Siliconization: The emulsion tank is filled with WFI as Dow Corning 360 Medical Grade Fluid silicone is dosed into the tank simultaneously. Once the fill level in the emulsion tank is reached, the WFI supply is turned off. The system then signals for the component processor vessel to begin filling with WFI from the bottom up. As the component processor vessel is being filled with WFI, the emulsion tank is pressurized with sterile air forcing the WFI / silicone solution into the component vessel. The component vessel starts to rotate and is over pressured with sterile air via the emulsion vessel. After expiration of the siliconization timer, the supply of sterile air is stopped and the valve to the emulsion vessel is closed. The vessel is over pressurized with air and the line to the drainage system for the emptying of the vessel is opened.
4. Final rinse: The system requests WFI and begins filling the component vessel from the bottom up providing a constant overflow of WFI via the separator. As the vessel starts to rotate, filtered air is supplied to the WFI flow for a programmed period of time. After expiration of the final rinse timer, the WFI flow and filtered air are stopped. The vessel is then over pressurized with air and the line to the drain is

opened to empty the WFI from the vessel. The conductivity value is measured after the last rinse cycle is complete. If the conductivity value is too high, a message is displayed on the HMI screen, prompting the operator to acknowledge the alarm and determine whether or not to proceed with processing.

5. Sterilization: During the sterilization cycle, both the actual temperature of the clean steam, measured at each steam trap and the pressure within the system, is continually measured. Prior to exposure, the system alternates between vacuums and steam injections. After the fractionated vacuum, the system is filled with clean steam up to the sterilization pressure that corresponds to the selected sterilization temperature. The sterilization pressure is automatically calculated by the control system. When all temperature probes reach the corresponding sterilization temperature, the stabilization timer starts. When the timer for the dwell time is elapsed, the clean steam supply is closed. The vacuum pump starts and the steam is evacuated from the system until a defined pressure is reached. The system is filled with sterile air until a defined pressure is reached.
6. Vacuum drying: Filtered air is blown from the top of the vessel to the bottom using heated, dry, compressed air that has been filtered through a 0.2 μ m filtration apparatus. The vacuum pump starts and the system is continuously pulled under vacuum. After the drying timer is elapsed, the supply of process air is stopped, the vacuum pump is switched off and the system is pressurized with sterile air up to a defined pressure.
7. Pressure drying: Sterile air is blown from the bottom of the vessel to the top until the system pressure is more than 1.050 mbar. Once the system pressure is achieved, the

air leaves the system at the process via the separator. After the drying timer is elapsed the supply of process air is stopped.

8. Cooling: Sterile air is blown from the bottom of the vessel to the top. Once the system pressure is more than 1.050 mbar, the air leaves the system at the process air separator. After the cooling timer is elapsed the supply of process air is stopped.

The ATEC vessel component processing recipe selected for the feasibility study was the validated recipe for the selected 13mm bromobutyl serum stopper. This recipe was validated onsite by Pfizer McPherson, KS component preparation personnel, and is requalified for verification purposes for every component on a regular basis. No modifications were made to the recipe for the initial or reprocessing operations, even though it could be reasonably anticipated that siliconization during the reprocessing run could be unnecessary or even problematic to the machinability and particulate matter burden of the stoppers. The minimum load of 1000 stoppers each was loaded into a dual system of 150L ATEC vessels. The stoppers in each vessel were then processed according to the following recipe in table 1:

Table 1

ATEC Vessel Component Preparation Parameters – Validated Recipe

Phase	Parameter	Value
Leak Test	Vacuum Leak Test Performed	Before
	Max Pressure Increase (psia)	0.19
	Test Vacuum (psia)	2.8
	Duration of Test (min)	5
	Rotation	On
Component Cleaning Cycle	Cleaning Agent Volume (mL)	50
	Duration of Cycle (min)	10
	Number of Cycles	2
	Rotation	On
Siliconization	Silicone Volume (mL)	25
	Duration of Cycle (min)	10
	Number of Cycles	2
	Rotation	On
Final Rinse	Maximum Conductivity ($\mu\text{S}/\text{cm}$)	5.0
	Duration of Cycle (min)	5
	Number of Cycles	2
	Rotation	On
Sterilization	Filter Sterilization	On
	Initial Vacuum (psia)	1.45
	Intermediate Pressure (psia)	29.00
	Intermediate Vacuum (psia)	2.00
	Number of Cycles	5
	Temperature Timer Start ($^{\circ}\text{C}$)	122
	Temperature Setpoint ($^{\circ}\text{C}$)	123
	Stabilization Time (min)	1
	Dwell Time (min)	28
	Rotation	On
Vacuum/Pressure Drying	Number of Pulses	1
	Temperature ($^{\circ}\text{C}$)	90
	Pressure Phase #1 (psia)	5.80
	Duration Phase #1 (min)	15
	Pressure Phase #2 (psia)	18.55
	Duration Phase #2 (min)	45
	Rotation	On
Final Cool Down	Pressure (psia)	18.85
	Final Temperature ($^{\circ}\text{C}$)	25
	Pressure (psia)	19.5

Upon completion of the initial component processing, the stoppers were staged and quarantined within each ATEC vessel in the component preparation area for 21 days, in order to exceed the procedurally driven expiration/hold time of the processed stoppers. After the 21 day hold time was exceeded, the stoppers from one ATEC vessel were released and transferred out of the staging area to prepare for various testing as the control group. Great care was taken to identify the control group components as non-GMP experimental use only to eliminate the risk of mistakenly using expired components in a GMP batch of drug product. Additionally, some of the testing for this study was only performed on the reprocessed experimental group components rather than both populations. Data for such tests had previously been generated and supplied by the component supplier and onsite Pfizer McPherson, KS quality laboratories for the stoppers after the validated ATEC vessel preparation process.

Still contained in the second ATEC vessel of the twin system after exceeding the 21 day hold time, the experimental group of stoppers was reprocessed using the same recipe as outlined in table 1. These components were released and transferred out of the staging area within 24 hours of being reprocessed and were prepared for various testing as described.

Functional Suitability Analysis

Container Closure Integrity Testing – Lighthouse CO₂ Headspace

Container closure integrity is a critical quality attribute for injectable drug products, ensuring that the packaging system maintains sterility and prevents contamination throughout the product's shelf life. USP <382> establishes specific performance criteria for elastomeric

closures, including their ability to maintain an effective seal under simulated conditions of use and stress. According to USP <382>, elastomeric closures must demonstrate adequate integrity through validated test methods that are deterministic and capable of detecting leaks down to defined sensitivity limits. Acceptable methods include helium leak detection, high-voltage leak detection, and laser-based headspace analysis, among others. The test method must be sensitive enough to detect breaches that could compromise microbiological sterility or physicochemical stability of the drug product. Importantly, container closure integrity testing must be performed under worst case conditions, such as elevated temperature, humidity, or mechanical stress, to reflect real world storage and handling scenarios. The chapter emphasizes that closures must not allow ingress of contaminants or leakage of product. As stated in USP <382>, “the packaging/delivery system is acceptable if the results for all test samples conform to the maximum allowable leakage limit demanded of the product to ensure that there is no risk to product microbiological quality and no impact, or inconsequential impact, on product physicochemical quality attributes⁸” (United States Pharmacopeial Convention, 2023).

Lighthouse Instruments’ carbon dioxide (CO₂) headspace analysis is a non-destructive and deterministic method used for container closure integrity testing (CCIT) of sealed pharmaceutical containers, including vials, cartridges, and syringes. This technique relies on the detection and quantification of CO₂ within the headspace of a sealed container to identify potential leaks or breaches in integrity. The method operates using tunable diode laser absorption spectroscopy. A near-infrared laser beam is directed through the container’s headspace, where CO₂ molecules absorb light at specific wavelengths. The amount of laser light absorbed is directly proportional to the partial pressure of CO₂, as described by the Beer-Lambert Law, allowing for precise quantification in millibar units. During CCIT, containers are either exposed

to elevated CO₂ or evaluated under ambient conditions. A low CO₂ reading, typically below 10 millibar, indicates an intact seal, while elevated or anomalous readings suggest a potential leak. The system automatically compares each measurement against predefined acceptance criteria, classifying the sample as Accept, Reject, or Indeterminate. Each measurement takes approximately five seconds and is entirely non-destructive, preserving the container for further use⁹. Key advantages of the Lighthouse CO₂ headspace method include its deterministic nature, compliance with USP <1207>¹⁰, and compatibility with high-throughput production environments. It offers high sensitivity capable of detecting micron-scale leaks and meets regulatory expectations for electronic records and data integrity under 21 CFR Part 11. Due to its precision and speed, this technique is especially useful in validating sterile barrier systems, detecting microleaks, and supporting pharmaceutical product stability program.

A headspace CO₂ analysis was conducted on a batch of 2 mL clear tubing vials, each containing 1mL of water for injection (WFI) and prepared with the reprocessed stoppers and a 13mm aluminum crimp seal closure component, using a Lighthouse Instruments Model FMS-CO₂ Analyzer (Serial No. 795). This assessment aimed to evaluate the container closure integrity (CCI) at time zero for the reprocessed stoppers. The test was performed under GxP-compliant conditions using seven calibration standards (0-966 millibar CO₂), and a pass/fail threshold of <10 millibar was applied to determine integrity. The analyzer was configured with a 5-second measurement time per sample. A total of 100 vials, 50 pre-CO₂ and 50 post-CO₂ exposure, were analyzed. All 100 vials returned CO₂ partial pressures of 0.00 millibar and were classified as acceptable. No samples were rejected, indicating robust container integrity at the time of testing¹¹. This CCIT evaluation was performed in the same manner after the 21 day hold time had been exceeded a second time for the reprocessed stoppers, and demonstrated an

identical outcome¹². These results were compliant with the acceptance criteria for packaging/delivery system integrity testing outlined in USP <382>.

Penetration Force - Instron

USP <382> outlines mechanical performance criteria for elastomeric closures, including the measurement of penetration force, which is critical for ensuring functional compatibility with hypodermic needles during drug product withdrawal. Penetration force is defined as the amount of force required for a standard needle to pierce the closure under controlled test conditions. This parameter directly impacts usability and patient safety, as excessive force can lead to difficulty during injection or vial access, while insufficient resistance may indicate material degradation or potential seal compromise. According to USP <382>, the maximum allowable penetration force for elastomeric closures is 10 Newtons (N) when tested with a hypodermic needle conforming to ISO 7864, which is typically a 21-gauge needle. The test must be performed under standardized conditions, including specified insertion speed and angle, to ensure consistency and reproducibility of results. Closures must not exhibit coring or tearing during testing, as such defects can compromise the sterility or integrity of the drug product. Meeting penetration force requirements ensures that closures can be reliably pierced during normal use without causing undue strain or mechanical failure. Additionally, compliance with this standard helps support compatibility with various drug delivery systems, such as prefilled syringes or transfer devices. Instron mechanical testing systems are commonly used to evaluate the penetration force of elastomeric closures, as specified in USP <382>. This test measures the force required to pierce a rubber stopper or closure using a standardized hypodermic needle under controlled conditions. The purpose is to ensure closures can be reliably and safely pierced during drug administration.

In a typical Instron test setup, the elastomeric closure is securely mounted in a fixed position on the lower platen of the Instron system. A hypodermic needle is attached to the upper crosshead. The Instron's load cell is calibrated to detect small force changes, and the test is run in compression mode. The crosshead descends at a constant speed, commonly 100 mm/min, and drives the needle into the closure perpendicularly. The software records a force vs. displacement curve in real time. The peak force value on the curve corresponds to the maximum penetration force. The test may also be configured to detect additional failure modes such as coring, which is rubber fragments being removed by the needle, or incomplete resealability, both of which can compromise container integrity.

A needle penetration force test was conducted on two sets of samples constructed of 2 mL clear tubing vials, each containing 1mL of WFI and prepared with the reprocessed stoppers and a 13mm aluminum crimp seal closure component. Testing was performed on one set of samples using a BD 21G x 1" PrecisionGlide needle and an Instron Model 68TM-R universal testing system. The crosshead speed was set to 200 mm/min, and data were collected at a frequency of 100 Hz using Bluehill 3 software. Ten stoppers were tested to determine the maximum force required for needle penetration. The mean maximum load recorded was 2.32 N, with a standard deviation of 0.19 N. The lowest measured force was 1.99 N, and the highest was 2.55 N. The average displacement at maximum load was 4.02 mm, while the maximum displacement remained consistent across all samples at approximately 12.70 mm¹³.

The second set of samples were tested utilizing a BD 18G x 1" PrecisionGlide needle and was performed on the same Instron Model 68TM-R universal testing machine. The crosshead speed was set to 200 mm/min, and data acquisition was configured at 100 Hz. Ten stoppers were evaluated to determine the maximum force required for needle penetration. The mean maximum

load recorded was 3.08 N, with a standard deviation of 0.24 N. The maximum recorded force was 3.56 N, and the minimum was 2.82 N. The average displacement at maximum load was 4.28 mm, and the maximum displacement remained consistent at approximately 12.70 mm across all samples¹⁴. In both cases, these results confirm that the reprocessed elastomeric closures tested meet USP <382> requirements for needle penetration force and exhibit consistent mechanical performance.

Fragmentation

Fragmentation testing is a critical performance requirement for elastomeric closures used in injectable drug products as defined in USP <382>. The purpose of the fragmentation test is to ensure that closures do not shed visible particles such as rubber fragments, also known as stopper coring, when pierced by a hypodermic needle. Fragmentation poses a significant risk to patient safety, especially in parenteral products, as particles may lead to embolic, inflammatory, or immunologic reactions if administered intravenously¹⁵ (World Health Organization, 2019). According to USP <382>, fragmentation testing must be performed using a hypodermic needle conforming to ISO 7864 under standardized conditions. The total number of punctures per closure should match the intended use conditions of the final product, but no fewer than four piercings must be performed per closure. After puncturing the closures, the product is visually and microscopically examined for the presence of stopper fragments. Microscopic examination is performed by following the particle count procedure according to USP <788>, *Particulate Matter in Injections*, Method 2: Microscopic Particle Count Test. The packaging system closure is acceptable if no more than five elastomeric closure particles >150 µm in diameter are observed per twelve containers tested. To pass the test, closures must demonstrate both material resilience and surface integrity. Factors that influence fragmentation performance include rubber

formulation, lubrication, siliconization, and processing conditions such as sterilization or aging. Failure to meet USP <382> fragmentation limits may indicate material incompatibility or inadequate process control, and must be addressed through formulation optimization or manufacturing adjustments.

Twelve closures were evaluated for the purposes of fragmentation testing in accordance with USP <382>. The units were prepared using 2 mL clear tubing vials with each containing 1mL of WFI, and stoppered with the reprocessed stoppers and a 13mm aluminum crimp seal closure component. Each stopper was pierced four times using a BD 21G x 1” PrecisionGlide needle. To prevent blunting, a new needle was used for each sample. The piercing process involved injecting and withdrawing 1 mL of WFI per insertion. Following the punctures, the WFI from each vial was vacuum filtered with a gridded 0.45µm mixed cellulose ester membrane, then immediately analyzed for particulate contamination using USP <788>, Method 2: Microscopic Particle Count Test. The test results showed that no stopper fragments were observed in any of the twelve tested closures¹⁶. This outcome is well within the USP <382> acceptance criterion of not more than five fragments across twelve closures, confirming that the stoppers demonstrate excellent resistance to fragmentation under test conditions.

Conclusions

The comprehensive functional suitability assessments conducted in this study demonstrated that the reprocessed stoppers performed equivalently to their freshly prepared counterparts or remained well within acceptable thresholds. Lighthouse CO₂ headspace analysis confirmed that container closure integrity was preserved, with no detected leaks at initial testing or following an extended hold period. Instron mechanical testing showed that both 21G and 18G needle penetration forces were significantly below the USP <382> maximum limit of 10 N,

ensuring ease of use and functional compatibility. Fragmentation analysis yielded zero visible stopper fragments across all twelve tested units, satisfying USP requirements and indicating excellent puncture resilience.

Visual Inspection

Appearance and Dimensional Analysis

A visual inspection was conducted on a batch of the reprocessed elastomeric closures to assess the presence of cosmetic defects and visible particulate contamination. The inspection criteria were based on internal quality specifications aligned with industry standards and regulatory expectations. Each closure was examined under appropriate lighting conditions by trained personnel for the presence of surface defects such as discoloration, tearing, cracks, embedded particles, and gross deformation. A total of 600 stoppers were inspected, and all 600 closures passed visual inspection with zero observed defects¹⁷. This outcome demonstrates a high level of manufacturing consistency and surface quality, supporting the suitability of these reprocessed closures for use in parenteral packaging applications.

A dimensional analysis was conducted on 30 reprocessed stoppers to verify conformance with physical specifications defined for stopper compatibility and performance. Key dimensions evaluated included overall height, flange diameter, plug diameter, and skirt depth. Measurements were taken using calibrated Mitutoyo digital caliper equipment under controlled laboratory conditions. All tested stoppers were found to be within the specified tolerance limits outlined in the component design specification. No dimensions were observed to be out of the defined specifications, and the measured values showed low variability across samples. This indicated that reprocessing stoppers in the ATEC vessel did not impact the physical dimensions of the components, and maintained consistent molding and finishing processes¹⁸. The results confirm

that the reprocessed stoppers meet dimensional criteria for use with 2 mL vials and 13mm crimp seals, ensuring proper fit, seal integrity, and functional compatibility during stopper insertion and device assembly.

Conclusions

Visual inspection of reprocessed elastomeric closures revealed no cosmetic or particulate defects, while dimensional analysis demonstrated tight adherence to design specifications, thereby confirming consistency in component geometry and manufacturing integrity.

Subvisible Particulate Matter

Introduction

To date, there are no specific regulatory requirements for the particle load caused by primary packaging components used in sterile injectable pharmaceuticals. When performing particulate matter testing, there are internal limits in place to consider the burden that each primary packaging component might have on particle load, which generally assumes that up to 1/3 of the USP <788> specification limit¹⁹ could be caused by a component other than the final drug product. For individual containers with over 100 mL of drug product, the limits are 25 particles/mL for $\geq 10 \mu\text{m}$ and 3 particles/mL for $\geq 25 \mu\text{m}$. For containers with under 100 mL of drug product, the limits are 6000 particles/container for $\geq 10 \mu\text{m}$ and 600 particles/container for $\geq 25 \mu\text{m}$. Per USP <788>, there are two methods used to test for particulate matter in injections: Method I, which utilizes Light Obscuration technique with a HIAC, and Method 2, known as the Microscopic Particle Count Test. Informational chapter USP <1788>, *Methods for the determination of subvisible particulate matter*, discusses an additional method for particulate testing called Flow Imaging that can be used as a complimentary means for particle

characterization, particularly in the testing of biologic drug products²⁰. Three methods of particulate analysis were used for this feasibility study to evaluate the intrinsic subvisible particulate matter load sourced from reprocessed stopper components, with a special focus on evaluating silicone-complex particles that are known to form in the presence of commonly used surfactants such as Polysorbate, otherwise known as Tween solution.

Particulate Surface Extraction Technique

Unlike the system-wide functional suitability testing executed per USP <382>, subvisible particulate matter evaluation is done on a component prior to its assembly in the finished product. Due to the lack of regulatory requirements for particulates released by primary packaging components, the testing preparation and execution can vary between each pharmaceutical manufacturer. Using ISO 8871-3:2003 *Elastomeric parts for parenterals and for devices for pharmaceutical use*²¹, as a guide, subvisible particulate matter test samples were prepared with WFI and 0.03% v/v polysorbate 80²² extraction solutions as follows:

1. Component specification information from the packaging material monograph and vendor supplied drawing was used to calculate the number of stoppers needed to obtain approximately 100 cm² (total surface area) of reprocessed components and control samples. The calculated result was rounded up to the nearest whole integer:

$$\frac{100\text{cm}^2}{\text{Component Surface Area}} = \frac{100\text{cm}^2}{4.575\text{cm}^2} = \underline{\underline{22 \text{ Components Per Extraction Preparation}}}$$

2. In a horizontal laminar flow hood, the calculated value of 22 reprocessed components or 22 control samples from the previous step were placed into an empty 250 mL Erlenmeyer flask that had been prerinsed with WFI.

3. For the WFI extraction preparation, 150mL of WFI was added to each flask to just cover the stoppers, and then placed on a Thermo Scientific Compact Digital Mini Rotator at a speed of 325 rpm for 20 seconds. The WFI solution extractions were prepared in triplicate for each method of analysis for the reprocessed stoppers and control group.
4. For the polysorbate 80 extraction preparation, 150mL of 0.03% v/v Polysorbate 80; J.T. Baker; USP Grade, was added to each flask to just cover the stoppers, and then placed on a Thermo Scientific Compact Digital Mini Rotator at a speed of 325 rpm for 20 seconds. The PS80 solution extractions were prepared in triplicate for each method of analysis for both the reprocessed stoppers and control group.

HIAC Particulate Analysis

Introduction

The HIAC (High Accuracy) liquid particle counter is the preferred system commonly used to perform Method 1, the light obscuration test. It operates on the principle of light blockage, where a sample of the liquid drug product is passed through a flow cell illuminated by a laser or focused light beam. As particles pass through the light path, they interrupt the beam and cause a measurable drop in light intensity, which is detected by a photodiode. The magnitude of the light obscuration is directly related to the particle size, allowing the instrument to categorize and count particles in predefined size ranges²³. HIAC systems report the number of particles per milliliter or per container, and its software automatically compares results to USP acceptance criteria. These systems provide a rapid, sensitive, and reproducible method for

quantifying particulate contamination in injectable products and are widely used in both quality control and release testing environments.

Analysis and Results

Particulate matter analysis utilizing HIAC ROYCO 9703+ and its validated pharmspec software was performed in triplicate on WFI and 0.03% v/v PS80 extraction solutions prepared with the reprocessed and control group stoppers. Daily system suitability checks were performed for the instrument, and all solvents and containers used in extraction solution preparations were tested prior to sample preparation. The solvent and container quality control checks were upheld to the USP <788> environment blank acceptance criteria. Additionally, the particle counting accuracy of the instrument was verified prior to sample analysis by testing the concentration of a PHARM-TROL Particle Count Control Standard: 15 μ m NIST polystyrene beads, which produced results matching the acceptance criteria for the standard listed on its COA. The HIAC instrument was configured according to table 2, with four channels set to monitor particles $\geq 2\mu$ m, $\geq 5\mu$ m, $\geq 10\mu$ m, and $\geq 25\mu$ m in size. Each extraction solution was pooled in 50mL aliquots, which was allowed to degas in its sample container at room temperature for no less than one minute prior to analysis. Results were recorded in units of particles/mL. HIAC data was unable to be obtained for concentration of particle sizes $\geq 2\mu$ m and $\geq 5\mu$ m due to these values in the PS80 extractions exceeding the instrument concentration limit of 10,000 particles/mL. This was likely due to the presence of air bubbles rather than truly intrinsic particulate matter, but cannot be characterized by HIAC analysis alone. This demonstrates the importance of orthogonal test methods utilized for subvisible particulate matter analysis in this feasibility study. Graphical representations of the HIAC particle concentration results for particle sizes $\geq 10\mu$ m and $\geq 25\mu$ m converted from particles/mL to a logarithmic scale are found in figures 5 and 6.

Table 2

HIAC Configuration for Sample Analysis

Sample Volume (mL)	5
No. of Runs	5
Dilution Factor	1
Tare Volume (mL)	0.2
Multi Stroke Tare (mL)	0.2
Discard 1 st Run	Check box
Show this screen at beginning of each test	Check box

Table 3

HIAC WFI Extractions Concentration Particles/mL

	$\geq 10\mu\text{m}$	$\geq 25\mu\text{m}$
Control Group WFI 1	542	21
Control Group WFI 2	704	30
Control Group WFI 3	418	21
Reprocessed WFI 1	1556	127
Reprocessed WFI 2	1572	112
Reprocessed WFI 3	1209	98

Figure 5

Control group and reprocessed WFI extraction particle concentration results for particle sizes $\geq 10\mu\text{m}$ and $\geq 25\mu\text{m}$, transformed to a logarithmic scale

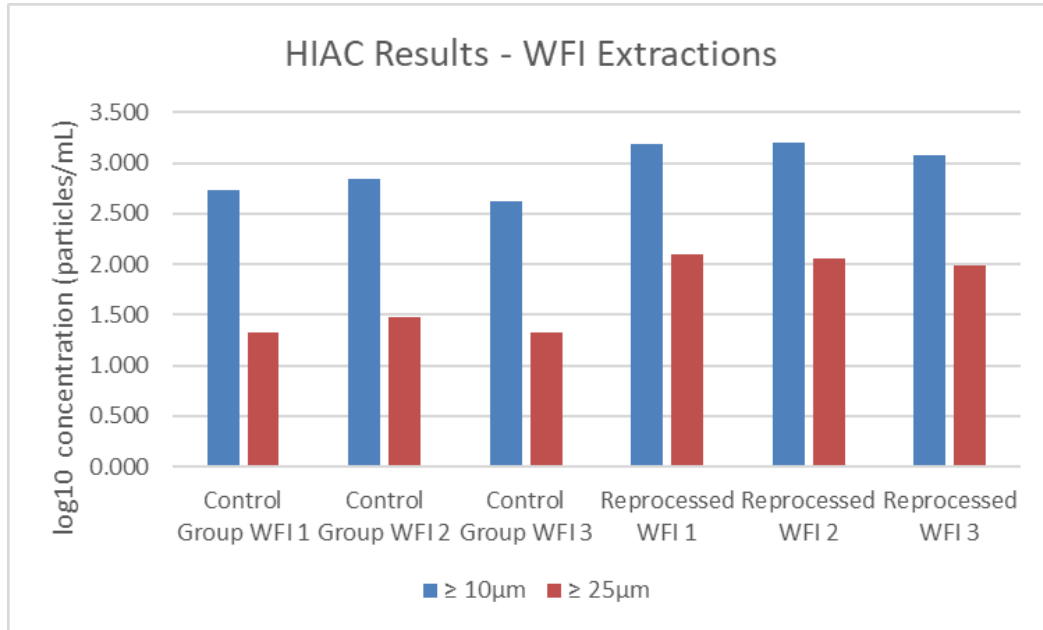


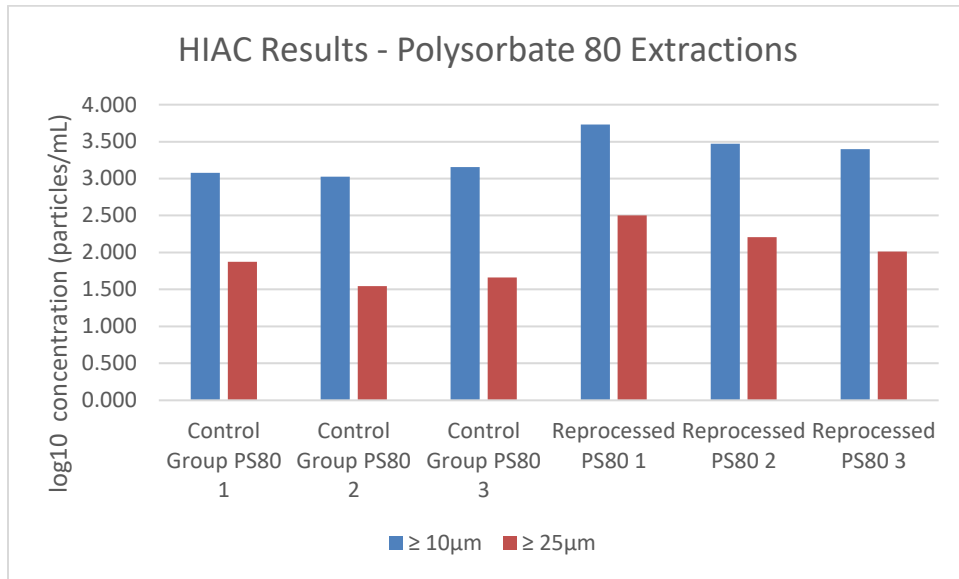
Table 4

HIAC 0.03% v/v Polysorbate 80 Extractions Concentration Particles/mL

	$\geq 10\mu\text{m}$	$\geq 25\mu\text{m}$
Control Group PS80 1	1192	75
Control Group PS80 2	1066	35
Control Group PS80 3	1430	46
Reprocessed PS80 1	5417	317
Reprocessed PS80 2	2955	161
Reprocessed PS80 3	2508	103

Figure 6

Control group and reprocessed 0.03% v/v polysorbate 80 extraction particle concentration results for particle sizes $\geq 10\mu\text{m}$ and $\geq 25\mu\text{m}$, transformed to a logarithmic scale



Membrane Microscopy: Method 2 Particulate Analysis

Introduction

The Microscopic Particle Count Test (Method 2), is an alternative to the preferred Method 1 Light Obscuration technique, and is typically used when samples are unsuitable for optical instruments. Sterile injectable drug products that must utilize membrane microscopy for particulate matter testing include liquids such as viscous solutions, opaque emulsions, or suspensions. In this method, a measured volume of the injection, or multiple containers pooled to obtain a specific volume, is filtered through a membrane filter with a pore size no greater than 0.45 μm . The filter retains subvisible particulate matter present in the solution. The membrane is then dried and placed on a mechanical stage under a binocular microscope with 100x magnification for inspection. One eyepiece in the microscope must contain an ocular micrometer with calibrated graticules containing reference circles and scales for particles $\geq 10 \mu\text{m}$ and $\geq 25 \mu\text{m}$ in size (figure 6). Episcopic brightfield illumination and reflected oblique illumination sources are required in order to accurately distinguish between true three-dimensional particles and filter membrane artifacts such as ink splotches. A defined central area on the filter is scanned using an optical microscope, and all particles $\geq 10 \mu\text{m}$ and $\geq 25 \mu\text{m}$ in size are counted and recorded. This method requires trained analysts and is more labor-intensive than light obscuration, but offers a direct visual assessment of particle size and shape, which is valuable when verifying atypical or unexpected results. This method eliminates the inclusion of air bubbles in particle matter results, allowing for more accurate characterization in particulate matter analysis. On the other hand, this method is incapable of accurately detecting and quantifying silicone oil droplets in injectable drug product packaging/delivery systems. Silicone

oil droplets pass through the membrane filter used in the method, resulting in an underestimation of the total particle concentration²⁴.

Analysis and Results

Microscope particle count/method 2 particulate matter analysis was executed in triplicate on WFI and 0.03% v/v PS80 extraction solutions prepared with the reprocessed and control group stoppers. Prior to vacuum filtration of the extraction solutions, a system suitability check of the vacuum filtration apparatus and each extraction solvent solution was performed to confirm compliance with USP <788> method 2 environment blank requirements. After the quality control checks had passed, 50mL of each extraction solution sample preparation was vacuum filtered onto a gridded 0.45µm mixed cellulose ester membrane. Once each filter membrane dried under horizontal laminar flow conditions, analysis of the target area on all filter surfaces was performed using an Olympus BX53 microscope configured with the required ocular scale, stage, and lighting attachments.

Table 5

USP <788> Method 2 WFI Extractions Concentration Particles/mL

	$\geq 10\mu\text{m}$	$\geq 25\mu\text{m}$
Control Group WFI 1	2	1
Control Group WFI 2	2	2
Control Group WFI 3	2	2
Reprocessed WFI 1	2	1
Reprocessed WFI 2	2	1
Reprocessed WFI 3	2	1

Figure 8

Control group and reprocessed WFI extraction particle concentration results for particle sizes $\geq 10\mu\text{m}$ and $\geq 25\mu\text{m}$

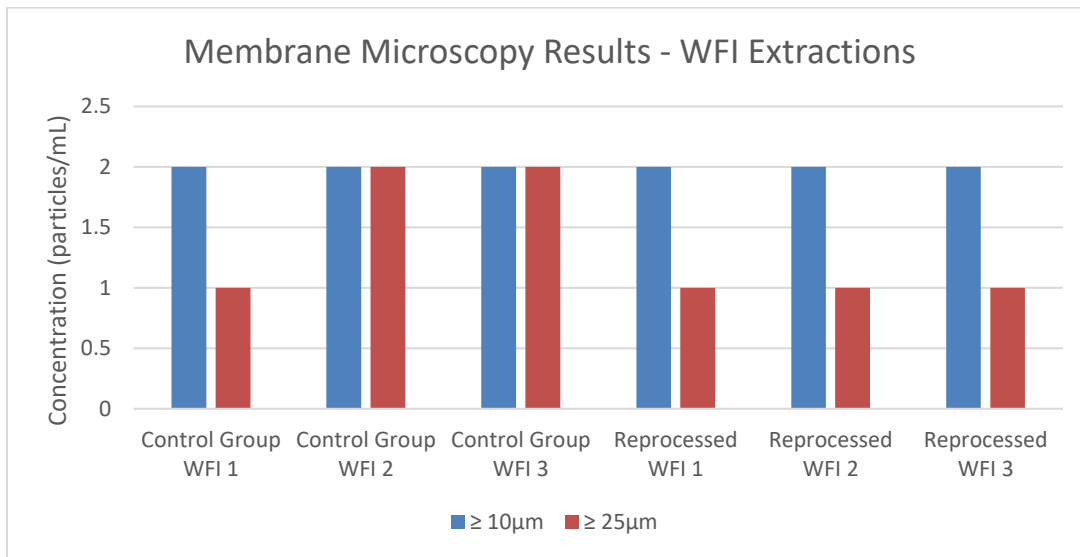


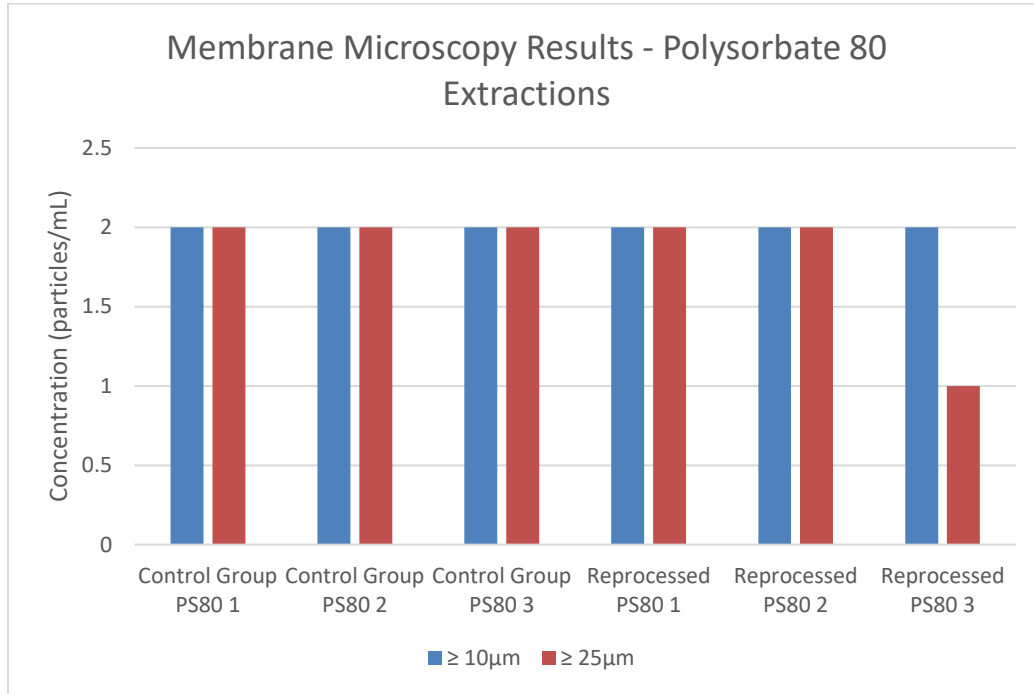
Table 6

USP <788> Method 2 0.03% v/v Polysorbate 80 Extractions Concentration Particles/mL

	$\geq 10\mu\text{m}$	$\geq 25\mu\text{m}$
Control Group PS80 1	2	2
Control Group PS80 2	2	2
Control Group PS80 3	2	2
Reprocessed PS80 1	2	2
Reprocessed PS80 2	2	2
Reprocessed PS80 3	2	1

Figure 9

Control group and reprocessed 0.03% v/v PS80 extraction particle concentration results for particle sizes $\geq 10\mu\text{m}$ and $\geq 25\mu\text{m}$



Flow Imaging Particulate Analysis

Introduction

Micro Flow Imaging is an analytical system that can be used to detect, count, and morphologically characterize subvisible particulate matter in pharmaceutical formulations. Unlike traditional light obscuration or membrane microscopy methods described in USP <788>, MFI provides both quantitative and qualitative data, including particle size, shape, and transparency. This makes it a powerful orthogonal method in assessing particulate contamination, especially for complex particle populations such as protein aggregates, silicone oil droplets, or elastomeric fragments from primary packaging components. Its characterization capabilities are especially useful in the subvisible particulate matter analysis of biologic drug products due its ability to readily differentiate between particles of intrinsic, extrinsic, and inherent sources of origin. MFI systems operate by circulating a liquid sample through a narrow flow cell positioned between a light source and a high-resolution camera, allowing images of each particle to be captured in real time. These images are then processed to calculate size and morphology metrics including equivalent circular diameter (ECD), aspect ratio, and circularity²⁵. This analytical technique is particularly advantageous for evaluating particles in biologic therapeutic formulations, due to the tendency of surfactants commonly found in such formulations to transform into silicone-complexes that may interfere with light obscuration measurements. An inquiry into the presence of such silicone-complexes was of great interest for the purposes of this study, as the ATEC recipe used in the reprocessing of the experimental group was not modified in any way. This resulted in the reprocessed stoppers being exposed to medical grade silicone a second time and was the greatest cause of concern when anticipating possible outcomes of repeated exposure to processing cycles without recipe modification.

Analysis and Results

Micro Flow Imaging analysis was conducted using a ProteinSimple MFI 5200 instrument with its validated MFI view system software, which was configured according to table 7. Similar to the previous particulate matter analyses, this technique was performed in triplicate on the WFI and 0.03% v/v PS80 extraction solutions prepared with the reprocessed and control group stoppers. Additionally, both solvents used for the extraction solution preparations were tested with each MFI run for baseline comparison. The instrument's particle quantifying and characterization capabilities were demonstrated for each batch by testing the mean ECD, concentration, and standard deviation of COUNT-CAL Particle Count Precision Standards 2 μm , 5 μm , 10 μm , and 25 μm in size. The calibration acceptance criteria were verified against the COA for each standard. In a 96-well plate, 2mL of each extraction solution, solvent blank, and particle count standards was transferred and subjected to vacuum degassing at 10 inches Hg for a minimum of one hour to eliminate the presence of air bubbles that could interfere with imaging or result in false particle counts. Individual solvent reservoirs containing WFI and 1% PS20 cleaning solutions used throughout the run were degassed in the same manner. All particle images obtained were examined for characterization purposes, but the quantified concentration result was only reported for particle sizes $\geq 10\mu\text{m}$ and $\geq 25\mu\text{m}$ to align with the data obtained by HIAC and membrane microscopy methods. Graphical representations of the MFI particle results converted to a logarithmic scale are found in figures 10 and 11.

Table 7

MFI Configuration for Sample Analysis

Sequence#	Action	Liquid Volume	Rate (mL/min)
1	Water flush	0.9 mL	6
2	1% PS20 flush	0.9 mL	6
3	Water flush	0.9 mL	6
4	Dry System	N/A	N/A
5	Water flush	0.9 mL	6
6	Optimize Illumination	0.22 μ L	N/A
7	Baseline	N/A	N/A
8	1% PS20 flush	0.9 mL	6
9	Water flush	0.9 mL	6
10	Dry System	N/A	N/A
11	Water flush	0.9 mL	6
12	Stir Sample	0.8 mL	N/A
13	Optimize Illumination	0.22 μ L	N/A
14	Sample Analysis	0.9 mL	6

Table 8

MFI WFI Extractions Concentration Particles/mL

	$\geq 10\mu\text{m}$	$\geq 25\mu\text{m}$
Control Group WFI 1	86	2
Control Group WFI 2	413	5
Control Group WFI 3	380	5
Reprocessed WFI 1	1604	105
Reprocessed WFI 2	592	10
Reprocessed WFI 3	498	10

Figure 10

Control group and reprocessed WFI extraction particle concentration results for particle sizes $\geq 10\mu\text{m}$ and $\geq 25\mu\text{m}$, transformed to a logarithmic scale

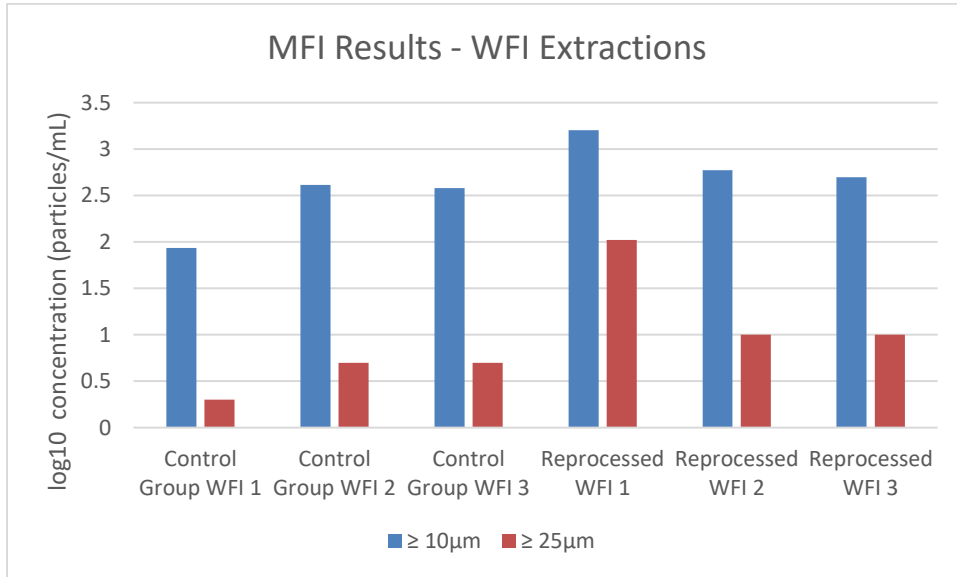


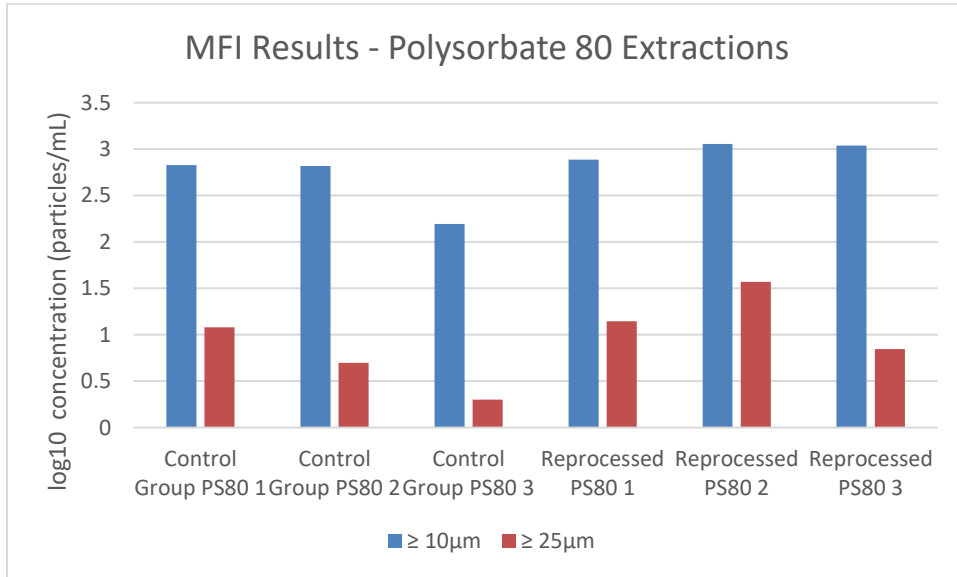
Table 9

MFI PS80 Extractions Concentration Particles/mL

	$\geq 10\mu\text{m}$	$\geq 25\mu\text{m}$
Control Group PS80 1	672	12
Control Group PS80 2	659	5
Control Group PS80 3	156	2
Reprocessed PS80 1	770	14
Reprocessed PS80 2	1137	37
Reprocessed PS80 3	1091	7

Figure 11

Control group and reprocessed PS80 extraction particle concentration results for particle sizes $\geq 10\mu\text{m}$ and $\geq 25\mu\text{m}$, transformed to a logarithmic scale



Conclusions

Traditional particulate matter testing conducted in this study demonstrated that while the overall subvisible particle load from reprocessed stoppers remained within acceptable limits, an orthogonal test method revealed an important process-specific consideration. MFI analysis found that the majority of subvisible particles detected in the reprocessed stopper extraction solutions were morphologically consistent with silicone oil droplets. This finding was particularly pronounced in the polysorbate 80 extractions, where the interaction between the surfactant and residual silicone may have promoted emulsification and droplet formation. The presence of silicone oil and silicone-complex particles strongly suggests that repeated siliconization of the stoppers during additional ATEC cycles can be an intrinsic source of particulate matter in a manner that may not remain compliant in the context of a finished drug product; especially in the case of a protein-based therapeutic.

Industry Prospects

This study briefly evaluated the functional suitability impact and intrinsic subvisible particulate matter burden caused by aseptically reprocessing bromobutyl elastomeric closures using ATEC vessels. Through comprehensive testing aligned with USP <382> and <788> guidelines, this work demonstrated promising results wherein the reprocessed stoppers maintained their critical quality attributes and met predefined acceptance criteria for use in parenteral drug packaging systems. While subvisible particle counts remained within conservative internal thresholds, further particle characterization evaluation revealed an increased presence of silicone oil droplets in reprocessed closures. This observation suggests that although the experimental stoppers remained functionally sound, the ATEC recipe used for the reprocessing cycle may warrant modification to reduce unnecessary silicone exposure.

From an industry perspective, this work contributes to the constantly evolving conversation around sustainability, resource conservation, and process efficiency in pharmaceutical manufacturing. As sterile injectable manufacturing becomes increasingly complex and cost sensitive, the ability to successfully reprocess critical unit components such as elastomeric stoppers could offer operational and economic advantages. Using risk-based decision making, the next steps in continuing this study involve elimination of the siliconization step in the reprocessing cycle. Functional suitability and particulate matter analysis would be executed in the same manner described throughout this thesis to determine influence on machinability and particulate matter. After the ATEC recipe for the reprocessing cycle has been successfully amended, further pursuit to validate and implement this practice in industry could be obtained through additional engineering trials. This would likely entail activities such as media fills and accelerated stability studies. Supplemental to the analytical methods included within the scope of this study, validation of this process would likely require additional suitability testing to evaluate variables such as extractables and leachables, and sterility.

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