Overcoming Steam Challenges with Single-Use Assemblies

Functionally closed systems deliver process flexibility and reduce risks

OVERVIEW

For more than a century, steam sterilization has been a critical process used in the biopharmaceutical and biotech industries to effectively sterilize raw materials and components utilized in the drug manufacturing process. The process of autoclaving is a sterilization method that uses high pressure steam to kill harmful bacteria and viruses. This usually requires the use of high temperatures of about 121° to 132°C (250° to 270°F) for about 30 minutes per cycle.

Over the last 15 to 20 years, biomanufacturers developing and manufacturing drugs have been using more polymer-based, single-use processing systems and components that are pre-assembled and pre-sterilized typically by ionizing radiation such as gamma before being shipped to a biomanufacturer. Therefore, many single-use component materials are required to demonstrate compatibility with the rigors of sterilization by gamma irradiation but not steam sterilization.

INTRODUCTION

The preference for pre-sterilized and pre-assembled single-use processing has emerged from industry's desire to increase process flexibility, reduce capital cost, and reduce contamination risk via utilization of functionally closed systems. The utilization of pre-sterilized, single-use process technology works because raw materials, intermediates, bulk, and final products are typically capable of being sterilized via filtration, hence alleviating much of the need for sterilization in situ of a fluid container and thus relying on the sterile filtration of a fluid into a closed, pre-sterilized, single-use container.

Utilization of pre-sterilized, irradiation-compatible, single-use assemblies is effective at serving most bioprocessing requirements. There are key exceptions where the ability to steam sterilize a fluid in situ within a single-use assembly helps achieve process flexibility and risk reduction. The most common case for sterilizing a fluid in situ of a container is that the fluid is not effectively filter sterilized. In such cases having the ability to sterilize in a single-use assembly that is designed as a functionally closed system delivers process flexibility and reduces risk.

Specific examples where bioprocessing raw materials could require steam sterilization:

- Antifoams used for addition to bioreactors
- Adjuvants such as aluminum hydroxide used in vaccine formulations
- Raw materials that pose a biological contamination risk even after sterile filtration, such as risk from mycoplasma and virus or glucose stock solutions and animal derived serum
- Liposomal and other particle-based drug delivery technologies

Specific examples where process efficiency and economics could be drivers of single-use bag assembles for autoclave sterilization:

- Reduction in space requirements for warehousing empty bottles and carboys

- Closing open processes where run rate contaminations have been "tolerated" with corrective actions that "retrain the operator"
- Customers who use pre-sterilized process fluids desire a single-use technology (SUT) assembly that is functionally closed to reduce contamination risk

What process tools can biomanufacturers utilize when they primarily manufacture using single-use closed systems and are required to steam sterilize a critical raw material?

Rigid bottles with no autoclaving integrity issues have been used, but those present challenges because not all bottles are capable of being adapted for use as a closed system, which is desired.

Alternatively, fluoropolymer single-use Aramus™ bags have broad temperature compatibility and can be successfully used to autoclave liquids in situ, allowing process flexibility and reducing risk in the most critical operations. From the cryogenic freezer to the autoclave, Aramus 2D bags remain an effective integral closed processing system that can meet the most challenging process objectives.

DEMONSTRATION: FEASIBILITY OF AUTOCLAVING FLUIDS IN SITU USING ARAMUS 2D SINGLE-USE ASSEMBLIES

The most important factors of sterilizing a material within its container are ensuring that under sterilization conditions the closed system remains integral and protects the sterility of its contents, and the container is physically and chemically compatible with process and the fluid it contains.

To better understand the capability of our Aramus 2D single-use assemblies to meet this challenge, we undertook a feasibility study. The purpose was to determine the feasibility to develop an effective autoclave cycle to sterilize a liquid in the Aramus bag while ensuring the single-use bag system remain integral and withstand the various thermal and differential pressure stresses that occur in the autoclave.

Testing was conducted with a 121°C (250°F) autoclave cycle for 30 minutes. The impacts on and from the bag chamber, tubing-retainer configurations, and autoclave cycle exhaust rates were considered.

Phase 1: Feasibility Study

For this study, a preliminary autoclave cycle was developed, with only the exhaust rate modified from a standard liquid cycle to prevent a fluid "boil-over" effect that is known to occur in steam sterilized flexible containers. This is at the end of the autoclave cycle when the sterilization chamber is cooling down and releasing pressure but brings the pressure down before the fluid temperature is below its boiling point, which causes a rapid expansion of the container to the point of bursting and leaking. A slower exhaust of the pressure can prevent this from happening.

Aramus 500 mL and 5L bags, at two different fill volumes, were used to evaluate the integrity of the bag assembly post autoclaving. In addition, the tubing-hose barb junction integrity was evaluated to determine optimal tubing and retainer configuration.

Test Conditions (Materials and Method)

- All bags were non-gamma irradiated
- Bags were initially integrity tested by pressure decay method to 30 µm detection limit
- DI water fluid was used
- 0.5 psi/min exhaust rate
- Bag integrity was tested post autoclaving by pressure decay to 30 µm detection limit
- Tubing-retainer Integrity was tested via pressure decay and bubble leak test
- A vent filter was used on each bag

Fill Volume Test

The bags were filled with DI water to 65% and 100% fill volume, 350 mL, and 500 mL for the 500 mL bags, and 3.5 L and 5 L for the 5 L bags. After autoclaving, the integrity of the bags was assessed visually as well as through pressure decay testing yielding the following results:

	SUCCESS RATE	
Percent fill	**500 mL bags**	**5 L bags**
65%	7/7 (100%)	3/4 (75%)
100%	13/14 (93%)	1/4 (25%)

Tubing-Hose Barb Joint Integrity Test

The tubing-hose barb joint is defined as the combination of tubing, bag hose barb, and retainer that forms the joint connection. Not every combination of tubing, hose barb, and retainer were available for evaluation.

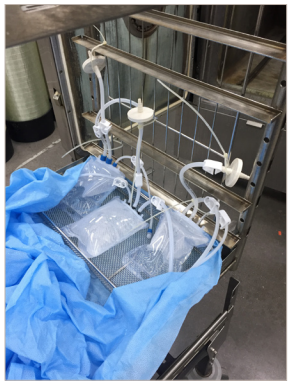
Test methods:

- Pressure Decay – Pressurized connection to 3.5 psi and measured decay at 30 µm detection limit
- Bubble Test – Pressurized connection to 15 psi, submerged underwater, and observed for bubbles

Table 1.

HOSE BARB ID	TUBING TYPE	RETAINER	PRESSURE DECAY	BUBBLE TEST
¼"	Silicone	Oetiker®	Pass	Pass
	Silicone	BarbLock®	Pass	Pass
	TPE	Oetiker	Pass	Fail
	TPE	BarbLock	Pass	N/A
⅜"	Silicone	Oetiker	Pass	N/A
	Silicone	BarbLock	N/A	N/A
	TPE	Oetiker	Fail	Fail
	TPE	BarbLock	N/A	N/A

Discussion



In the initial feasibility assessment, high success was seen at the smaller bag size (500 mL) and fluid volumes but not at the larger size (5 L) and volumes. We believe this was due to a not fully optimized autoclave cycle because only one parameter was changed from the standard liquid cycles used with rigid containers. The success of the smaller sizes was an indication that the Aramus bags can perform in this application with the proper steam sterilization cycle.

For the tubing-hose barb joints, failures were observed at both hose barb IDs for the TPE tubing and Oetiker retainer connections. We believe this was due to the thermoplastic nature of the tubing that may have allowed it to deform around the hose barb under the autoclave conditions. Because we did not evaluate all the TPE configurations it is possible some may still work as evidenced from one example of it passing the pressure decay test. TPE connections will need to be investigated further for autoclave applications. The silicone tubing was successful in tested configurations particularly coupled with the Oetiker retainer. Both components utilize materials that are stable at high temperatures, which may be the reason for their success (silicone and stainless steel).

For our next steps, a fully optimized cycle was developed to confirm whether the performance observed at the smaller sizes could be replicated in larger bags. In addition, silicone tubing with Oetiker retainers was preferred for future configurations due to the positive results and high-temperature compatibility of these components. These findings further prove that we could be successful in developing an autoclaving solution under the recommended guidelines for use.

Recommended Autoclave Cycle Design Parameters

- Consider the following to prevent fluid boil-over:
  - A liquid cycle with a slow exhaust rate ~0.5 psi/min
  - A fast liquid cooling exhaust step
  - Air-over-pressure cycle exhaust
  - Steam-air-mixture cycle
  - Dedicated line for venting vapor built up in bag
  - Silicone tubing with Oetiker retainers is preferred for connections
  - DO NOT fill bags more than nominal volume before or after autoclaving
  - To have a scalable process, during sterilizer qualification determine maximum autoclave chamber liquid loads

Case Study to Develop an Autoclave-Capable, Single-Use Bag Assembly for Adjuvant Sterilization

One client encountered this challenge. While filing a process for regulatory approval they desired to reduce the contamination risk to their final bulk drug formulation by making process improvements to a sterile addition of an aluminum hydroxide adjuvant solution that could not be sterile filtered due to a particle size larger than nominal pore size.

The therapeutic manufacturer had been utilizing a carboy-type container to autoclave the adjuvant fluid and make an aseptic transfer to their bulk drug vessel. The process improvement objective was to reduce contamination risk by using closed system processing with single-use connectors. The desired solution was a pre-sterilized container that could contain the fluid and be sterilized as a closed system within an autoclave and facilitate the sterile addition of the heat-sterilized adjuvant solution to the final bulk drug formulation via aseptic connectors.

Despite this process improvement need being arrived at in preparing for regulatory filing the improved process workflow and associated process tools and raw materials would also require qualification and validation. The filing date deadline meant the studies required to support this process improvement needed to be completed in approximately six months.

The client relied on Entegris to help implement the process-improving solution in the following key areas:

1. Bag assembly design
2. Design and verification of effective autoclave cycle
3. Evaluation of Extractable and Leachable (E&L) substances

Phase 2: Design, verify, and qualify autoclave-compatible, single-use assembly for adjuvant sterilization and dispensing

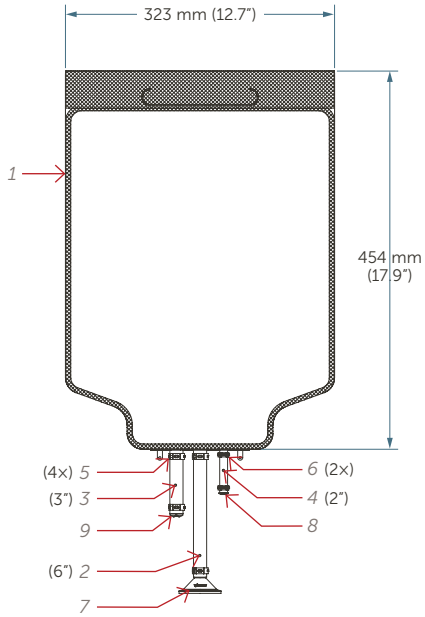
The goal of this second phase of the study was to further assess the compatibility of the Aramus bag and evaluate any E&L observed.

Test materials were prepared for autoclaving by filling 5 L Aramus Assemblies with selected fluids to model the customer formulation and ensure chemical compatibility as well as relevant model solvents for E&L analysis.

The samples were subsequently autoclaved, and assemblies were incubated as received. After incubation, the the plug was removed from the tubing the assemblies were drained into a certified clean, container. The solvent extracts were then analyzed.

Outcomes and Results Phases 1 and 2

1. **Assembly Design Optimization:** key design features based on customer need and our assembly recommendation learned from feasibility.



1	Aramus subassembly, 5 L
2	Silicone tubing ⅜" x ⅝"
3	Silicone tubing ⅜" x ⅝"
4	Silicone tubing ¼" x ⅜"
5	Oetiker clamp, 17 mm
6	Oetiker clamp, 11.3 mm
7	Sanitary connector 1.5", PVDF ⅜" barb
8	Press-in plug ¼" PVDF
9	Press-in plug ⅜" PVDF

In designing the bag assembly, components were selected per the customer's requirements for integration into their process while special care was taken to ensure the materials would support the autoclave process. Silicone tubing with Oetiker retainment along with PVDF plugs and sanitary connectors were recommended.

2. **Autoclave Cycle Design and Verification:**

In collaboration with a steam sterilizer and the client's provided current autoclave cycle inputs, a similar cycle was developed using parameters as close as possible to the client's parameters with slight differences. Because the load was liquid inside of a flexible container, with known tendency to boil over, an air overpressure cycle was selected. This cycle injects compressed air into the autoclave chamber during the sterilization phase to maintain pressure during cool down and prevent boil over.

Table 2. Air-Over-Pressure Moist Heat Steam Autoclave Cycle Parameters

Loop	Phase	Set point	Rate (psia/min)	Hold time (min:sec)	Jacket temp
0	Heat Up*	121.8°C (251.2°F)	2.0°C	05:00	120.0°C (248.0°F)
1	Heat Up*	121.5°C (250.7°F)	0.5°C	01:00	120.0°C (248.0°F)
0	Sterilization*	121.5°C (250.7°F)	0.0	25:00	120.0°C (248.0°F)
0	Air Pressurization Drying	49 to 49.5 psia	N/A	30:00**	<120.0°C (<248.0°F)
0	Exhaust	14.7 psia	2.0	00:00	<120.0°C (<248.0°F)
0	Equalization	ambient	1.0	N/A	<125.0°C (<257.0°F)

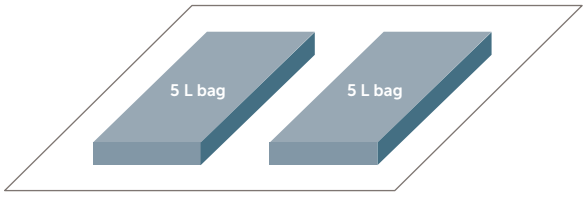
**AOP cycle with steam/air mixture enabled, 19 psia support pressure, controlled with load probe in 2 L bottle. Jacket water enabled for air pressurization drying.*

***Final pressurization temperature of 70°C (158°F).*

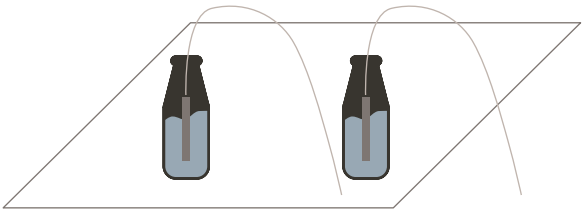
During this phase of study, six Aramus bags were sent to be steam autoclaved:

- Two, 5 L bags with DI water
- Two, 5 L bags with 0.1 M H_3PO_4
- Two, 5 L bags with 0.5 N NaOH

Configuration on Shelf



Load Probes



The steam sterilizer verified the cycle by using temperature probes in 2 L bottles of water, along with the main load of bags, to actively control the cycle. Bags were placed on a cart with three shelves, two bags stored on each shelf, and placed into the autoclave chamber. After cycling, all six, 5 L bags were inspected and found to be intact and without damage or leaks.

3. Evaluation of Extractable and Leachable Substances

The adjuvant fluid being heat sterilized by our client was an aqueous solution of aluminum hydroxide because aluminum hydroxide and is a weak base but can be amphoteric in nature and behave as both a weak base and weak acid. Therefore, it was decided that a worst-case approach should be taken to extractable substance model solvent selection. From this perspective it was decided to utilize the appropriate modeling solvents that are recommended in the BPOG (Biophorum Operations Group) recommended E&L protocol. Therefore, the acid, base, and water model solvents were selected for use. Because the formulation did not contain solvent or solubilizers the polysorbate or alcohol model solvents proposed in the BPOG E&L protocol were not selected for this E&L simulation study. The other advantage to selecting BPOG E&L model solvents, analytic techniques, and methods was to enable comparison of E&L results to the standard E&L package of pre-irradiated and extracted Aramus bags that did not receive subsequent Autoclave treatment and could therefore allow some comparison of the impacts of the additional sterilization of materials by heat.

The parameters and conditions of the BPOG test are listed below in Tables 3 and 4.

Table 3. Model Solvents and Time Points Summary

SOLVENT	VOLUME	TIME INTERVALS AND CONDITIONS
0.5 N NaOH	500 mL	1 day 50 rpm at 40°C (104°F) 35 days 50 rpm at 40°C (104°F)
0.1 M H_3PO_4		
WFI		

Table 4. Analytical Method and Model Solvent Summary

	HPLC-DAD/MS							
SOLVENT	**ESI (±)**	**APCI (±)**	**DI-GC/MS**	**HS-GC/MS**	**ICP/MS***	**TOC**	**PH**	**NVR**
WFI	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
0.5 N NaOH	Yes	Yes	Yes	Yes	No	Yes	No	No
0.1 M H_3PO_4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No

**Reference included in full report.*

While the BPOG approach and recommendations were used as a basis for the E&L study, certain test conditions were modified to better simulate the customer's process. Thereby making this a simulation study versus a worst-case E&L assessment, which is the objective of the specific BPOG test conditions.

Table 5 shows modifications made to the BPOG standard approach for this simulation study and the supporting rationale.

Table 5.

BPOG E&L study requirement	Simulation study modification	Rationale to support modification
Extraction solutions	The simulation study will utilize model solvent streams relevant to customer formulation and not utilize the 50% ethanol stream	Product formulation aqueous aluminum hydroxide solution can behave as weak acid/ weak base and selected solvents represent worst case
Extraction temperature: 40°C (104°F)	No modification 40°C (104°F) extraction temperature will be used	Selected temperature and timepoints are worst case versus process requirement
Extraction duration: 1 day 21 days 70 days	1 day and 35 days	Based on product expiry 35 days was selected, 1 day was utilized to have a comparison, and ensure that more volatile species can be present
Fill volume/SA/V	0.47 cm^2/mL instead of 6 cm^2/mL	0.47 cm^2/mL based on actual use of 5 L of fill volume in 2315 cm^2 SA container
Analytical methods	Not modified	Process conditions were modified for simulation not analytics
Sterilization pre-treatment	Gamma within 10 kGy maximum dose or autoclave	Bags not pre-sterilized by gamma, only filled bags sterilized by autoclave

The results of the E&L simulation study are summarized in Table 6. The details of this E&L report and background data remain on file and available to Entegris customers requiring E&L risk assessments for autoclave applications.

Table 6

ANALYTICAL METHOD	0.5 N NAOH	0.1 M H_3PO_4	WFI
HPLC-DAD/MS			
1 day	<DL	<DL	<DL
35 days	<DL	<DL	<DL
GC/MS			
1 day	<DL	<DL	<DL
35 days	<DL	<DL	<DL
HS-GC/MS			
1 day	<DL	<DL	<DL
70 days	<1 ppm	<1 ppm	<1 ppm

ANALYTICAL METHOD	0.5 N NAOH	0.1 M H_3PO_4	WFI
ICP-MS			
1 day	N/A	<DL	<0.5 ppm
35 days	N/A	<DL	<0.5 ppm
NVR			
1 day	N/A	N/A	<0.5 ppm
35 days	N/A	N/A	<0.5 ppm

DL = Detection Limit

Extractable and leachable compounds are facts of life when using polymer materials for pharmaceutical processing. It is desired to choose materials that are well suited for their application such as pre-treatments, customer use, and in further sterilization operations. It is due to the inert and robust nature of Aramus assembly's fluoropolymer film that results in a rather unremarkable outcome for this testing where no volatile or semi-volatile compounds were noted above reporting limits in GC-MS DI and HS testing.

Non-volatile extracted compounds were detected in the LC-MS testing at levels below 1 ppm and were mostly attributed to extractables associated with nylon-6. This is not unexpected due to not using nylon component parts in the system design. However, other components used in the filling of the bags potentially incorporated nylon elements.

The only compound detected via the ICP/MS analysis was silicone and most likely a result of extraction from the tubing included on the assembly.

Our client used this study to carry forward their own patient safety risk assessment and implement Aramus single-use bag assemblies into their process.

Summary and Conclusions

Aramus single-use bag assemblies can be used successfully to autoclave fluids in situ and help overcome single-use processing workflow challenges with heat sterilization, giving end users added flexibility to continue using single-use processing technology to close processes and further reduce contamination risks.

Aramus fluoropolymer film is an inherently robust and inert material with demonstrated capability to be pre-sterilized by gamma irradiation and used for packaging fluids and further autoclave sterilization.

- Use a liquid cycle with a slow exhaust rate ~ 0.5 psi/min, a fast liquid cooling exhaust step, air-over-pressure cycle exhaust, steam-air mixture, or other step to prevent fluid boil-over
- Silicone tubing with Oetiker retainers is the preferred connection because of the temperature compatibility of both components
- DO NOT fill bags more than nominal volume before or after autoclaving
- Entegris can consult on single-use product design and autoclave cycle development

The additional heat treatment and sterilization process does not pose unacceptable E&L risk to process and patient when the pre-gamma sterilized Aramus assemblies are utilized to autoclave fluid in situ, even in the case of this bulk drug product example.

- Existing BPOG E&L results are available at Entegris for gamma only
- Modified BPOG E&L results are available at Entegris for autoclave application
- Entegris can assist end users with application-specific risk assessments for SUT autoclave applications

Entegris' application engineering and science professionals can help you achieve your process objectives and define and execute a project to deliver an SUT autoclave-capable process solution. Project timeline:

- Customer needs assessment and process review (1 – 2 weeks)
  - Determine if existing E&L data can be utilized or if testing is required
- Design concept proposal (1 – 2 weeks)
- Engineered design for customer approval (1 – 2 weeks)
- Autoclave cycle design and verification by third party (6 weeks)
- E&L risk assessment based on existing data (2 weeks)

  Or

- Commence E&L testing and risk assessment (20 weeks)

Clearly, utilizing existing E&L data versus commencing a study can have significant impact on timeline. We encourage customers to provide the process details in the first stage of interaction so we can determine the most efficient project path.

FOR MORE INFORMATION

Please call your Regional Customer Service Center today to learn what Entegris can do for you. Visit entegris.com and select the Contact Us link to find the customer service center nearest you.

TERMS AND CONDITIONS OF SALE

All purchases are subject to Entegris' Terms and Conditions of Sale. To view and print this information, visit entegris.com and select the Terms & Conditions link in the footer.

Entegris

Corporate Headquarters
129 Concord Road
Billerica, MA 01821
USA

Customer Service
Tel +1 952 556 4181
Fax +1 952 556 8022
Toll Free 800 394 4083