Scale-Up Case Study for Long Term Storage of a Process Intermediate in Bags

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Summary

A scalable frozen process intermediate step was introduced following cell culture and harvest to allow for long-term storage in a protected environment prior to purification to drug substance. Process scalability from bench-scale models to the large-scale system was demonstrated. The bench-scale models were subsequently used for additional investigation and characterization. The process intermediate containers are single-use disposable bags in which product stability and safety was demonstrated for approximately 2 years of frozen storage. Validation, successful regulatory approval, and subsequent commercial experience with this system demonstrated repeatability in performance and low failure rates.

Introduction

The development and transfer of new processes provides the opportunity to introduce new technologies to the current platforms of commercial biopharmaceutical production. This case study examines improvements made to freezing and thawing operations on process intermediates upon scale up of a process. The principles of this case study also apply to the freezing of drug substance.

The previous platform for freezing process intermediates required filling of individual, small volume bottles (typically less than 1 liter) and subsequent transfer of the bottles to a freezer. The thawing operations required transferring the bottles to a roller rack to thaw and subsequent manual pooling of the bottle contents. The bottle fill operation required approximately 30 hours of manual operations (three operators for 10 hours). The amount of time required to completely freeze and thaw the material in the bottles was an additional 6 to 9 hours for each step.

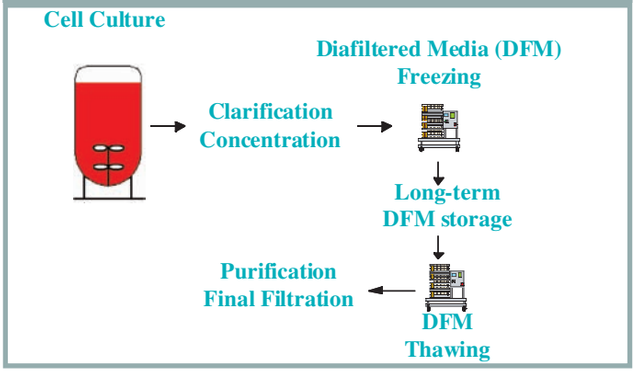
The scale up of the process included almost a doubling of the process intermediate volume to be frozen for long term storage. The demands of time and labor to complete the filling, freezing and thawing operations for the previous platform combined with the doubling of the process intermediate volume created an opportunity to evaluate potential process improvements. The team was also assigned the task to improve the consistency of the fill, freeze and thaw process relative the previous platform in order to minimize potential for product pool stability issues prior to freezing.

Amgen evaluated and implemented a customized version of commercially available bags for the storage of the process intermediate combined with a commercially available freezing system. The results, including an evaluation of the impact to operational costs and throughput and measured freeze and thaw profiles, are presented in this report. The approach for evaluation of leachables is described herein. Leachables are the components and concentration of components caused by leaching from the bag or other process contact surface under actual process conditions. The leachables are assessed for potential impact to any of the "safety, identity, strength, or purity of the drug product beyond the official or other established limits" per 21 CFR 211.65(a). The rational for demonstrating product safety are also included in this report.

Equipment and Technology

A general process flow diagram for the case study discussed in this report is shown in Figure 1. The cell culture process starts with culture expansion in disposable flasks and culture bags followed by a series of instrumented bioreactors of increasing scale. The cell culture process ends with a suspension culture production bioreactor. The cell culture process is followed by harvest processing that includes clarification, filtration and product concentration and diafiltration. The resulting clarified, concentrated and diafiltered medium is the process intermediate that undergoes freezing for long term storage.

Figure 1.

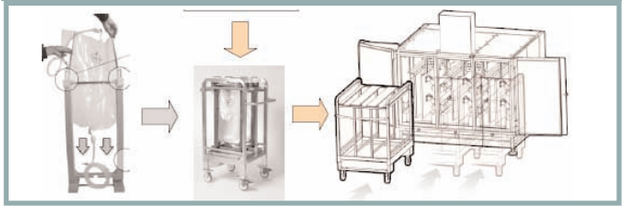


Overall Process Flow Diagram.

The long term storage allows for decoupling of the cell culture and harvest operations discussed above from the downstream purification process. The decoupling of the process provides operational flexibility for manufacturing between the cell culture and purification areas.

The freeze and thaw process also provides the benefit of improved process control for downstream operations. In traditional biopharmaceutical operations, the total of the cell culture harvest is forwarded to the purification suite for processing to drug substance. The load to the downstream process varies in terms of the variance of grams of product produced in the cell culture and harvest operations. As bioreactor titers and harvest recovery yields vary, this variance is passed on to the purification process. One example of the impact of variance is that it impacts the range of column load as measured in terms of grams of protein loaded per liter of resin for the downstream chromatography steps. The freezing and thawing of the clarified, concentrated and diafiltered medium allows for thawing a precise amount of protein and for improved control over the product load for chromatography steps.

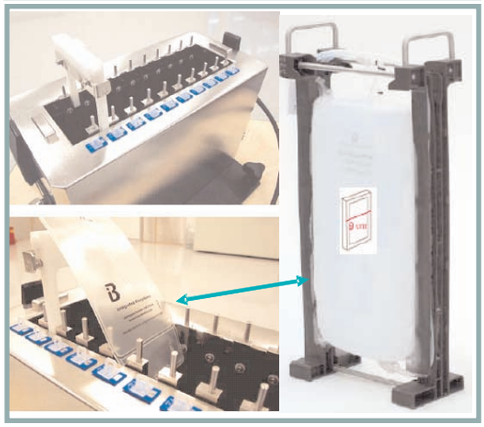
Figure 2.



Operational Flow for Freezing DFI.

The general process flow for filling and freezing the process intermediate is shown in Figure 2. For this case study, Amgen evaluated a customized version of commercially available bags for the storage of the process intermediate combined with a commercially available freezing system. The concentrated diafiltered medium is distributed into a customized version of a commercially available tri-layer storage bag. The empty bags are manually inserted onto a protective framing device. The frame containing the empty bag is then placed onto a bag filling station. An air overlay is applied to each bag to ensure proper contact between the bag surface and the freezing plates prior to freezing and potentially prevent disfiguring of the bag that may complicate the thaw cycle performance due to variable bag thickness. The bags filled with the process intermediate are transferred from a cart, which is maneuvered in front of the freeze unit for loading purposes. Two bags are placed in each of the three freezing bays of the freeze unit. The freeze cycle is activated and the chilled heat transfer fluid is introduced around the plates of the freeze units. The frozen bags are transported to a storage freezer in the storage modules following the freezing process. The frozen bags remain in the storage modules until the material is thawed. The thawing process is essentially the reverse of the freezing operation.

Figure 3.



Scale-Down Model for Freeze and Thaw Operations.

The fundamental principle of operation for the system is heat transfer perpendicular to the flat interface between the metal plates and the disposable bags that should be consistent throughout the height and width of the bags with minor edge effects. A scale-down model of the freezing and thaw procedures was developed using the commercially available system shown in Figure 3. The scale-down model establishes a similar heat transfer process. In the scale-down model, the width of the bags represents the thickness across which the heat transfer process occurs for the full-scale process, thereby maintaining the freezing pathlength constant.

The temperature at which the metal walls are maintained throughout the scale-down model process can beprogrammed to better match the freeze and thaw profiles of the full-scale process. Table 1 shows a comparison of the scale-down model and case study storage containers and scale-down parameters.

Table 1.

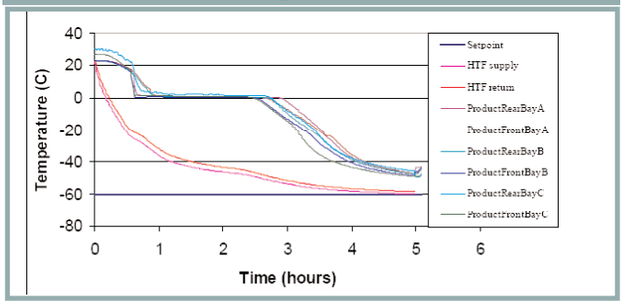
Property	Bench Scale Stability Study Conditions	Case Study Conditions
Product Contact Layer	Medical grade EVAM	Medical grade EVAM
Gas and Moisture Barrier Layer	EVA / EVOH / EVA	EVA / EVOH / EVA
Fill and Drain Ports	EVA	EVA
Film Surface Area (cm^2)	275	4,826
Fill Volume (mL)	100	16,000
Surface Contact Area to Volume (cm^2/mL)	2.75	0.302
Freezing path length (cm)	8.4	8.4

Conditions for scale-down model and commercial process.

Results and Discussion

A series of measurements were performed using water as a surrogate for the concentrated diafiltered medium, given that the process intermediate has similar heat transfer properties to the process intermediate. The results of the surrogate testing demonstrated uniformity and reproducibility of the freeze and thaw process while implementing the new technology. In Figure 4, the temperature profile is shown to be independent of the location of the bag within the freezing unit.

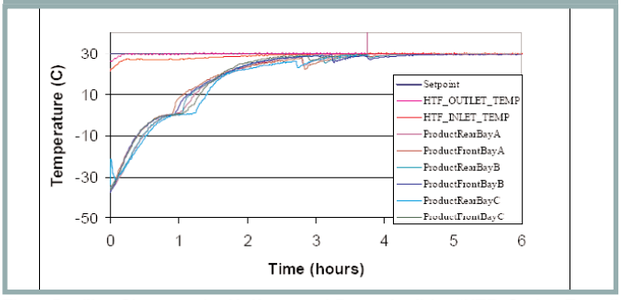
Figure 4.



Freeze Profiles Shown to be Independent of Bag Placement. "HTF supply" and "HTF return" denotes the temperature of the heat transfer fluid when entering and exiting the freeze units. "ProductRearBayA" denotes the freeze profile for the bag placed in the front section of bay A. All other labels follow the same naming system.

Figure 5 also demonstrates uniformity and reproducibility of the thaw profiles. The freeze and thaw profiles can also be controlled or modified by adjusting the temperature set points or profiles of the heat transfer fluid.

Figure 5.



Thaw Profiles Shown to be Uniform and Reproducible. "HTF_Outlet_Temp" and "HTF_Inlet_Temp" denotes the temperature of the heat transfer fluid when entering and exiting the freeze units. "Setpoint shows the target setpoint of the heat transfer fluid. "ProductRearBayA" denotes the freeze profile for the bag placed in the front section of bay A. All other labels follow the same naming system.

Implementation of the new technology resulted in the anticipated increased throughput and reduced operational costs. The original process resulted in approximately 1000 containers filled with process intermediate in a process that required 30 man-hours (three operators for 10 hours) plus six to nine hours each for the freeze and thaw process. The new process requires 14 man-hours (seven operators for 2 hours) in addition to six hours each for freeze and thaw. The new process is also required to handle a 40% volumetric increase of process intermediate for each cell culture and harvest lot. The resulting improvement is a 30% reduction in man-hours required per unit volume of process intermediate produced and a 3.4-fold increase in throughput (liters of process intermediate per hour of operation). The number of containers per lot was reduced to less than 50, thus increasing the amount of material stored in each container to 16 L from less than 1 L for the original process. Based on current testing, the rate of failure of the new process has been below 0.4% after processing over 9000 L of the process intermediate. This is key indicator of the successful implementation of the application given that other bench-mark applications have had signifi cantly higher scrap levels for process intermediate storage in bags due to issues with the freeze, thaw, storage, or container closure reliability.

The vendor of the storage bags provided physicochemical and biocompatibility data supporting USP class VI plastic classification. As part of the USP Class VI testing, extractable levels were measured by

performing extractions with water for injection (WFI), high pH, low pH and organic solutions. The non-aqueous extraction tests included non-volatile residue, residue on ignition, turbidity, and UV absorption. Test results of the aqueous extraction showed that the extractables levels were below limits required to meet USP <661>. Specifically, non-volatile residue was less than 15 mg, residue on ignition was less than 5 mg, heavy metals concentration was less than 1 ppm, and buffering capacity was less than 10.0 mL per USP test methods. Testing for extractables was performed under conditions that offer a greater potential to extract compounds relative to the specific process conditions of this case study. The extractables data, the length of liquid contact time and the temperature for the relevant solvents tested were used to establish a maximum concentration of leachables that could be present in the process intermediate when stored in the disposable bags. The level of worst case leachables was calculated using vendor provided concentrations of extractables and applying the appropriate volume to area ratio for the bag surface area and minimum process intermediate volume to be processed per bag. The resulting estimate of the level of leachables was assessed and found to be well within safe limits.

Product purity and stability were supported with in-house studies during long-term storage. Specifically, product stability was demonstrated with long-term frozen hold studies in which the process intermediate was thawed at 12 month intervals and processed through the first purification step using a qualified scaled down model. The product was tested for product concentration by reverse-phase high-pressure liquid chromatography (RP-HPLC). Anion-exchange HPLC, capillary zone electrophoresis, isoelectric focusing, size-exclusion HPLC, RP-HPLC, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) techniques were used to assess product purity. Test results were compared to pre-approved acceptance criteria documented in the stability test protocol. No significant trends were observed, and all time points met the pre-determined acceptance criteria. The test results demonstrated frozen process intermediate stability to beyond two years using the fill, freeze, and thaw process described in this case study.

Conclusion

A scalable frozen intermediate step allows for long-term storage, decoupling of upstream and downstream operations, and tighter control of input for downstream process operations. The freeze and thawing process described in this case study is scalable from the bench to the commercial scale. The successful use of the bench model allows for confidence in scale up and usefulness in troubleshooting and technical support during routine operations. The disposable storage containers introduced are safe and maintain product stability. The implemented process resulted in a 30% cost reduction and a 3.4-fold increase in throughput.

About Amgen Manufacturing Limited

Amgen Manufacturing Limited is a subsidiary of Amgen Inc. located in Juncos, Puerto Rico. AML has been operating for over 15 years. The AML campus currently includes state of the art manufacturing plants for bulk production of biologicals and fill/finish facilities.

Rich Reynolds is a purification scientist in the Process Development group at Amgen's Puerto Rico campus. He has 8 years of experience in biotechnology including positions held in Bayer Biological Products and Amgen.

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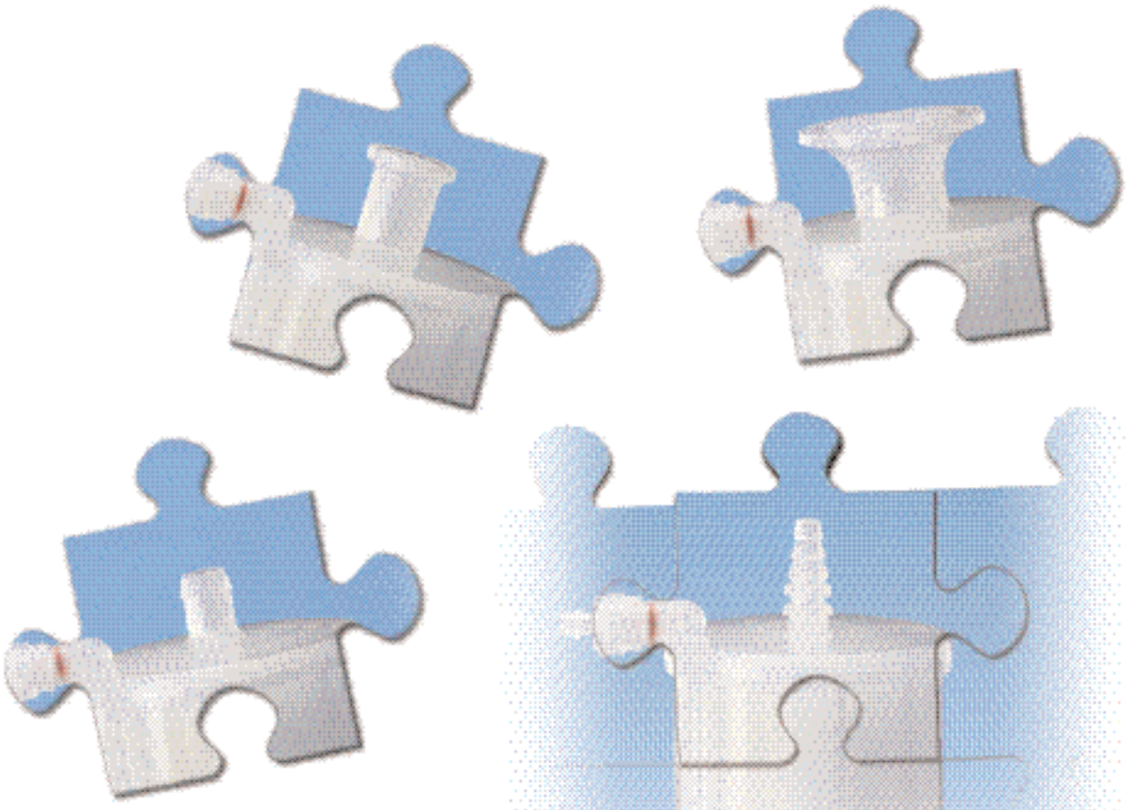

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