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Application Note

Closed Tangential Flow Filtration Processing at Benchtop Scale with Mobius® Assemblies

Summary

Closed processing technologies isolate biopharmaceutical products from the environment and prevent contaminants from entering the product flow path. Single-use technologies are an increasingly important component of bioprocessing closed system design particularly for antibody drug conjugate (ADC), viral gene therapy (VGT), cell and gene therapy (CGT) and monoclonal antibody (mAb) manufacturing as well as continuous processing applications.

Mobius® assemblies offer a customizable, closed processing option for tangential flow filtration (TFF) operations. Customized benchtop Mobius® TFF assemblies can accomodate different modes of diafiltration and product recovery, offering flexibility for process development. The goal of this study was to evaluate product yield and recovery from benchtop Mobius® assemblies designed for different modes of diafiltration.

Efficient diafiltration was achieved in three diafiltration modes: through the transfer manifold, through the diafiltration port, or vacuum diafiltration. In addition, equivalent product recovery was observed with recovery assemblies located on the feed or retentate lines, however slightly lower recovery was observed in closed systems due to hold-up in the sterile filter assembly.

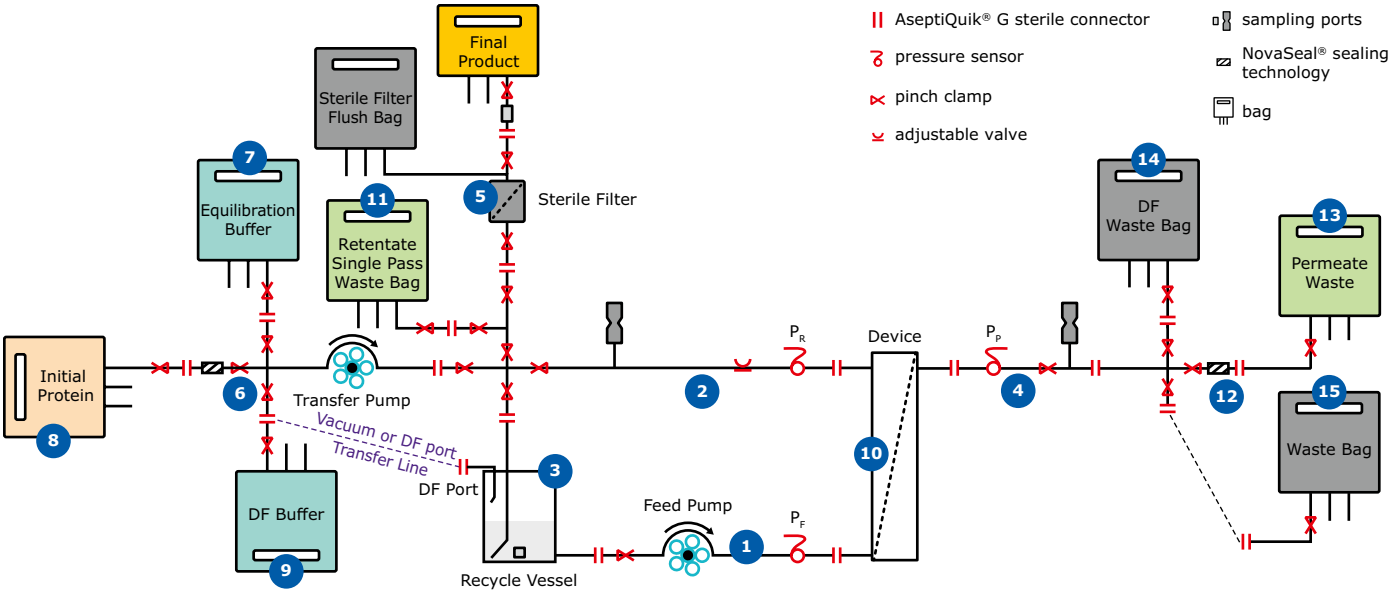
Introduction

A single-use (SU) benchtop scale closed TFF system was established using Mobius® assemblies. Using the definition provided by the BioPhorum, a closed system "isolates the process zone from its manufacturing environment and prevents ingress of environmental contaminants during product contact". By contrast, open systems are "subject to contaminants in the manufacturing environment."[1]

Single-use Mobius® assemblies offer a customizable option for diverse TFF needs across multiple modalities and process volumes. Customization of TFF can accommodate different modes of diafiltration and product recovery within the same Mobius® assembly, while enabling closed operations. The goal of the study was to evaluate product yield and recovery from Mobius® assemblies tailored for three different modes of diafiltration. In addition, the impact on performance of two vessels of different materials of construction was assessed:

- Polycarbonate vessel: designed for mAb and VGT applications.
- Polypropylene vessel: designed for ADC applications as polypropylene can withstand exposure to the chemicals commonly used in ADC manufacturing.

Testing was completed using Mobius® assemblies for closed processing that consisted of a Pellicon® Capsule with 30 kDa Ultracel® membrane and C Screen, 2000 mL recycle vessel, and the product recovery assembly located on the retentate line or the feed line (not shown), Figure 1.



1. Feed Line Assembly; **2.** Retentate Line Assembly; **3.** Recycle Vessel; **4.** Permeate Line Assembly; **5.** Sterile Filter Assembly; **6.** Transfer Manifold; **7.** Equilibration Buffer Single-use Bag; **8.** Single-use Bag containing Protein; **9.** Diafiltration Single-use Buffer Bag; **10.** Pellicon® Capsule Filtration Device; **11.** Retentate Waste Single-use Bag; **12.** Permeate Manifold; **13.** Permeate Waste Single-use Bag; **14.** Diafiltration Waste Single-use Bag; **15.** Waste Single-use Bag (Device Flush).

Figure 1.

Mobius® assemblies for closed TFF processing. Grayed out assemblies are optional.

Study Design

Mobius® assemblies are highly customizable enabling the end-user to select the mode of diafiltration and product recovery that best suits their process. Additionally, if desired, these assemblies can accommodate a sterile filter assembly, consisting of a filter and a flush bag. The permeate waste stream has an option for up to three bags: a bag to collect waste during device flushing, a bag for permeate collection during concentration, and a bag to collect permeate during diafiltration.

During the characterization of the system, the minimum working volume (MWV), hold-up volume (HV) and pressure drop of the system were measured in an “open” system where liquids were in re-usable containers open to the environment. Purified water (1 cP) and mixtures of glycerin in water with viscosities of 18 cP and 25 cP, were used as feed solutions to mimic increasing viscosity when protein is concentrated.

Understanding the MWV of the system is critical to ensure that air is not entrained in the outlet of the recycle vessel. MWV varies with cross flow rate and testing performed at flow rates from 100–600 mL/min (1–6 L/min/m^2, LMM) will elucidate volumetric concentration limits for any given flow rate.

Understanding the pressure drop of the system within the operating flow rate range is important to determine its capabilities. Selecting an appropriate tubing diameter for the flow path ensures sufficient flow to avoid excessive pressure drop and hold up volume, which would limit the maximum achievable concentration. Pressure drop is dependent on flow rate, viscosity, and tubing diameter; flow rates from 100–600 mL/min were tested.

System characterization was followed by process run simulations, which were first performed in an “open” mode, followed by testing in a “closed” mode where all liquid streams were in single use bags and protected from the environment.

The model feed for the process runs was a 20 g/L protein solution of human plasma gamma globulin (IgG) in phosphate buffered saline buffer (PBS) pH 7.2. Feed was concentrated using a Pellicon® Capsule with 30 kDa Ultracel® membrane and C Screen. A sample of the feed was collected to measure the initial protein concentration. The feed was then transferred into a single-use bag using a peristaltic pump, Figure 1.

A typical test run consisted of the following steps:

1. Flush with 20 L/m^2 RO Water and water permeability (NWP) measurement.
2. Equilibration with 10 L/m^2 PBS Buffer.
3. Initial Concentration at Feed Flow of 5 LMM to 60 g/L, Target transmembrane pressure (TMP) 11–12 psi.
4. Diafiltration with 50 mM Sodium Acetate at 60 g/L, Target TMP 11–12 psi.
5. Final Concentration to Target Concentration ≥200 g/L. The following process control strategy was followed as the concentration of the protein solution increased:
   - Maintain TMP by adjusting retentate valve.
   - Maintain feed flow rate until feed pressure reaches 30 psig.
   - Ramp down the feed pump to maintain feed pressure around 30 psig.
   - End the process when the feed flow rate reaches approximately 1 LMM, air bubbles start to enter the feed tubing or target final concentration is reached.
6. Depolarization of the membrane; 10–15 min, total recirculation at low feed flow rate with retentate valve fully opened and permeate line completely closed.
7. Protein Recovery with buffer using one mode of operation:
   - Recovery Line located on the Feed Line.
   - Recovery Line located on the Retentate Line.
8. Optional 20 L/m^2 Flush with 50 mM Sodium Acetate Buffer.
9. Mobius® assemblies disposal.

The benchtop scale TFF system with Mobius® assemblies enables three different modes of diafiltration: diafiltration through the transfer manifold, through the diafiltration port, or vacuum diafiltration.

Diafiltration through the transfer manifold

Figure 2 illustrates a diafiltration through a transfer manifold. The diafiltration buffer bag (DF Buffer) is attached to the transfer manifold through an aseptic connection and the buffer is pumped into the recycle vessel using a transfer pump.

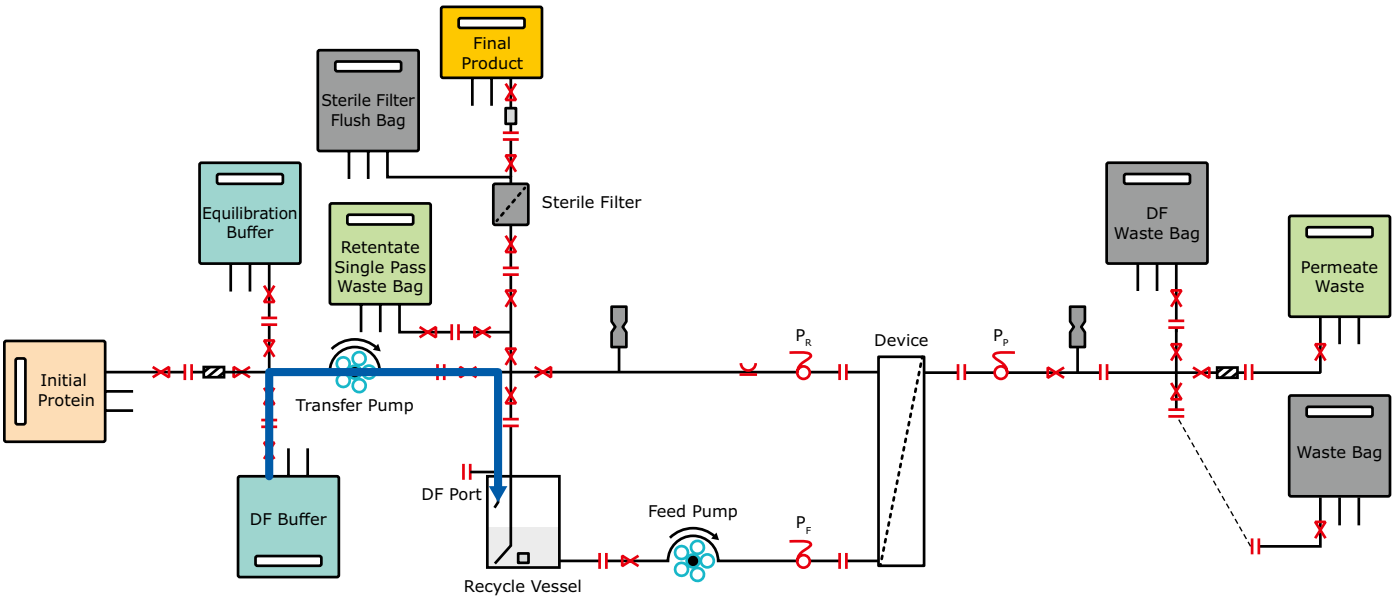


Figure 2.

Diafiltration through the transfer manifold. Blue arrow illustrates buffer transfer from the bag to the recycle vessel.

Vacuum diafiltration or diafiltration through a diafiltration port

Figure 3 illustrates vacuum diafiltration or diafiltration through a diafiltration port. The diafiltration buffer bag (DF Buffer) is attached to the diafiltration port located on the recycle vessel through an aseptic connection. The buffer is transferred into the recycle vessel either by vacuum, or by a transfer pump.

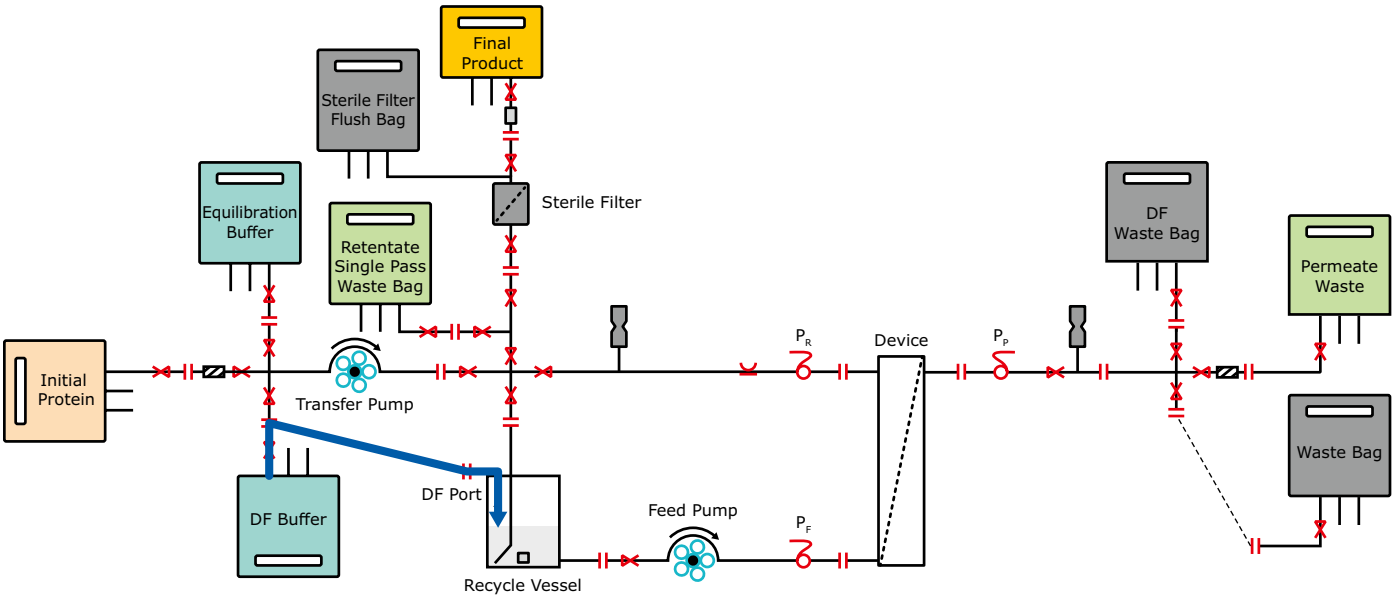


Figure 3.

Vacuum diafiltration or diafiltration through a diafiltration port using a vacuum or offline transfer pump. Blue arrow illustrates a transfer through a transfer line connecting the bag and the diafiltration port located on the recycle vessel.

Two modes of product recovery were tested:

- Recovery assembly located on the feed line (feed line product recovery).
- Recovery assembly located on the retentate line (retentate line product recovery).

Product recovery through the recovery assembly located on the feed line

Product recovery through the recovery assembly located on the feed line, Figure 4, involves three steps:

1. Recycle vessel emptied through the feed line and the recovery line.
2. Retentate line installed into a transfer pump and product pumped out of the system in reverse through the retentate line, Pellicon® Capsule and through the recovery line into the product bag.
3. 12 mL of DF buffer added to recycle vessel through DF port and Step 1 repeated.

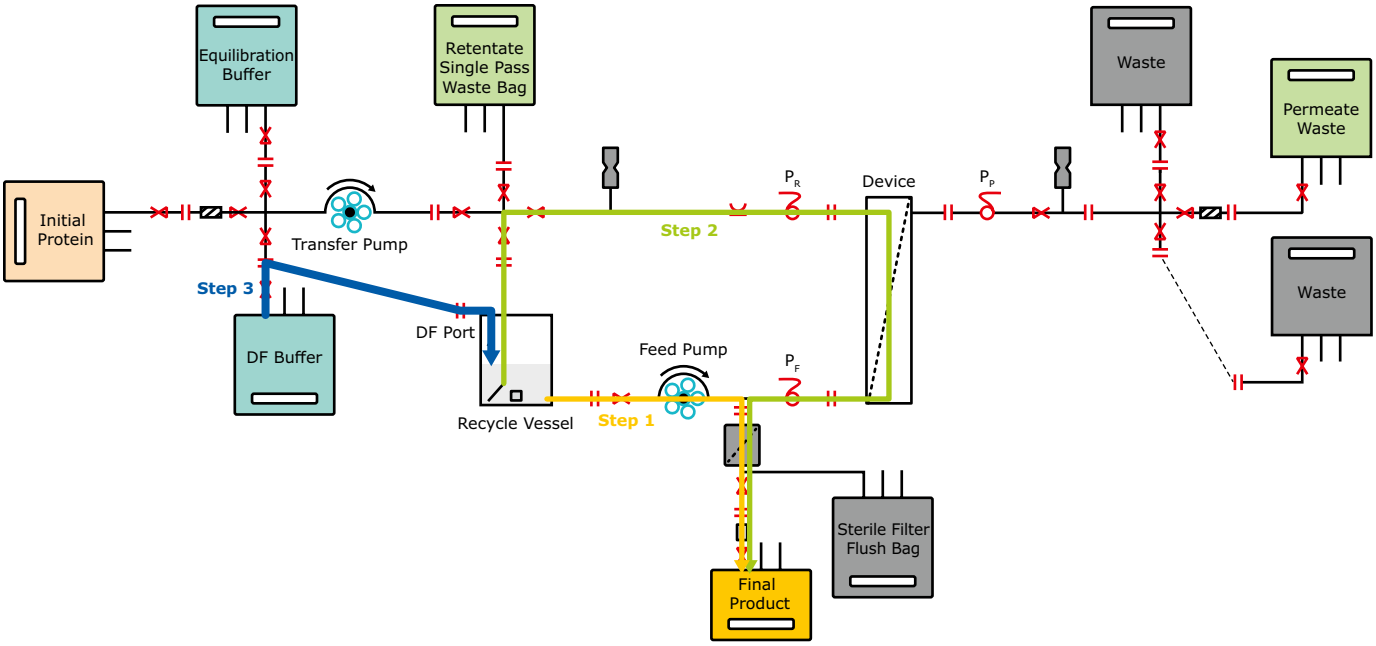


Figure 4.

Product recovery through the recovery assembly located on the feed Line.

Product recovery through the recovery assembly located on the retentate line

Product recovery through the recovery assembly located on the feed line, Figure 4, involves three steps:

1. Recycle vessel emptied through the feed line, Pellicon® Capsule device, retentate line and recovery line.
2. 12 mL of DF buffer added to the recycle vessel through DF port and Step 1 repeated.

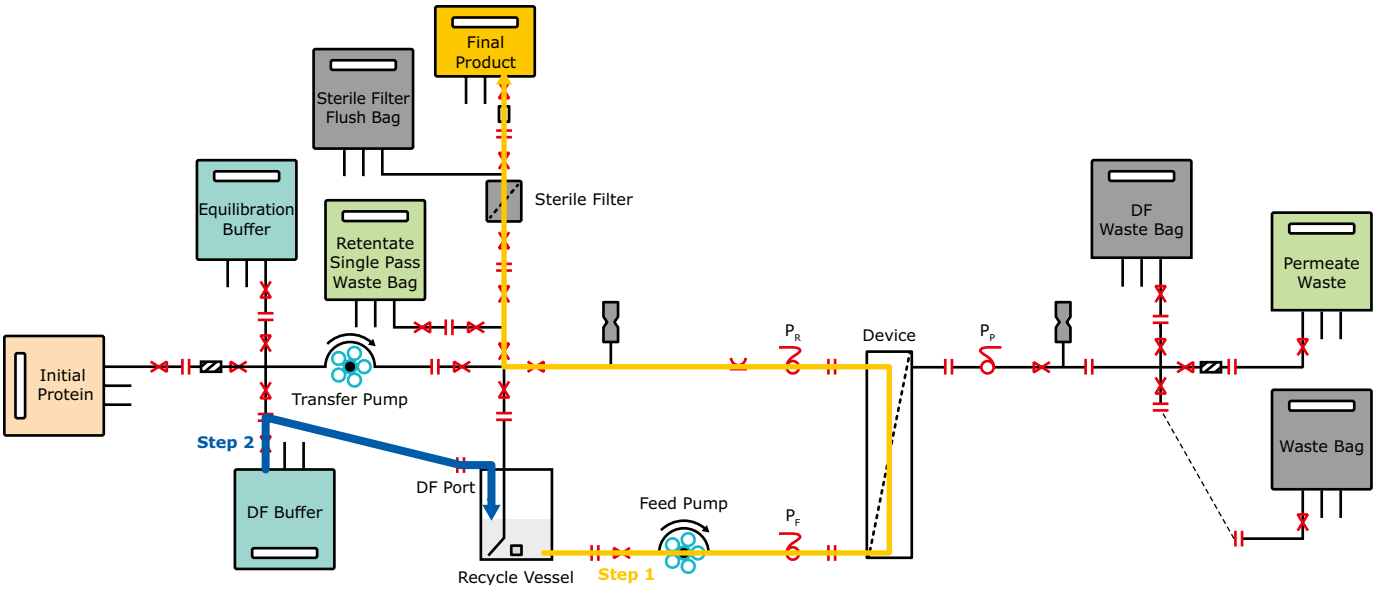


Figure 5.

Product recovery through the recovery assembly located on the retentate line.

Results

Closed processing systems are an integral technology for maintaining product integrity by preventing environmental contamination entering the product flow path while improving safety for operators by minimizing risk of exposure to harmful chemicals. Closed processing systems are becoming a critical element of bioprocessing system design particularly for ADC, VGT, CGT and mAb manufacturing as well as continuous processing applications.

System characterization

Minimum working volume/hold-up volume

The minimum working volume in the system as a function of flow rate was tested in the flow range of 1–6 LMM at three different viscosities, Figure 6. For this part of the testing, the Pellicon® Capsule was not installed into the system to decouple the hold-up volume of the system from the hold-up volume of the device.

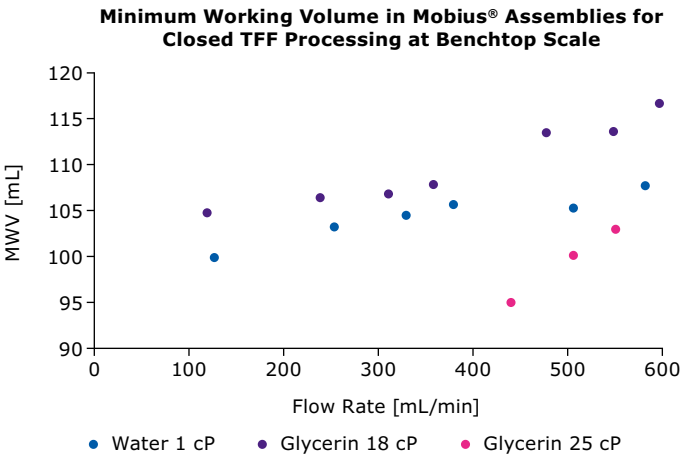


Figure 6.

Minimum Working Volume in Mobius® Assemblies using purified water and glycerin solutions to mimic increased viscosity observed during protein concentration. The data in the graph do not include the hold-up volume of the device. The hold-up volume of the feed channel for 0.1 m^2 Pellicon® Capsule is 26 mL.

Hold-up volume

The system hold-up volume is important for product recovery. At the end of the minimum working volume testing at minimum flow rate, the remaining amount of water in the recycle vessel and assemblies is equal to the hold-up volume of the system. The results of hold-up volume measurements are listed in Table 1.

Table 1.

Hold-up volume of the benchtop system for closed TFF processing. Polycarbonate vessel used for mAb/VGT applications; polypropylene vessel used for ADC applications.

Hold-Up Volume [mL]	Vessel	
	Polycarbonate	Polypropylene
Recycle Loop and Feed Channel of the Device	100	100
Vessel	200	100
Total	**300**	**200**

Results suggest that the hold-up volume of the polycarbonate vessel designed for mAb/VGT processing is two times higher than the hold-up volume of the polypropylene vessel designed for ADC processing, due to the design and shape of the vessel. Therefore, the maximum achievable concentration with the polycarbonate vessel will be lower compared to the Mobius® assemblies containing the polypropylene vessel designed for ADC processing.

Pressure drop of the system

Pressure drop measurements for the three viscosity solutions were performed with the device not installed in the system. Additionally, measurements with water were repeated after installing the 0.1 m^2 Pellicon® Capsule into the system, Figure 7.

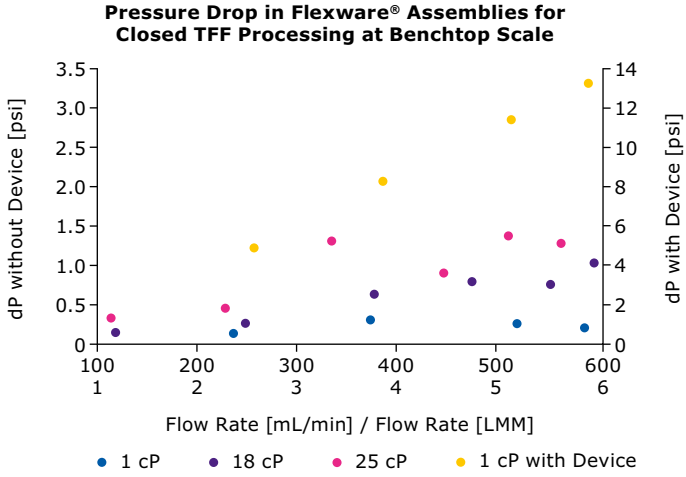


Figure 7.

Pressure Drop in Mobius® Assemblies using purified water and glycerin solutions with and without the device installed into the system.

The pressure drop in the system with Pellicon® Capsule installed was between 5–14 psi in the feed flow range of 250–600 mL/min (2.5–6 LMM).

Process simulations

Four process runs were completed for each vessel where the target concentration was ≥200 g/L. During the diafiltration step, the DF buffer was connected to a DF port located on the recycle vessel and the buffer was transferred using a transfer pump. Process runs 1 and 2 were completed as an open system while runs 3 and 4 were completed as a closed system with single-use process bag, a sterile filter and product recovery assemblies. The results in Table 2 show that Mobius® assemblies containing the polypropylene vessel achieved higher final concentration, due to the lower minimum working volume.

Table 2.

Overview of concentration run results.

Polycarbonate Vessel for mAb/VGT Processing				
Process Run Recovery Location/System	**Run 1** Feed, Open	**Run 2** Retentate, Open	**Run 3** Feed, Closed	**Run 4** Retentate, Closed
Overall Recovery (%)	94	99	90	98
Concentration Pre-Recovery (g/L)	185	207	194	185
Final Concentration (g/L)	190	179	181	151
Polypropylene Vessel for ADC Processing				
Process Run Recovery Location/System	**Run 1** Feed, Open	**Run 2** Retentate, Open	**Run 3** Feed, Closed	**Run 4** Retentate, Closed
Overall Recovery (%)	92	99	90	90
Concentration Pre-Recovery (g/L)	204	198	224	194
Final Concentration (g/L)	198	204	186	178

Comparison of recovery: Retentate line versus feed line recovery

Protein was recovered through the recovery line located on the feed line during runs 1 and 3. The results in Table 2 illustrate that the product recovery was similar for both open and closed systems.

During runs 2 and 4 protein was recovered through the recovery line located on the retentate line. Recovery of 99% was reached in an open system during run 2 with polycarbonate and polypropylene vessels. However, with the closed system which includes the sterile filter assembly, higher system hold-up volumes are observed which can account for ~5% product loss.

The target concentration was not met during run 1 and run 4 using the polycarbonate vessel for mAb/VGT applications. During run 4 excess buffer was added to the feed vessel during recovery which resulted in a higher product recovery and higher product dilution. This illustrates the importance of optimizing the product recovery procedure without compromising product concentration.

Conclusions

Mobius® assemblies for closed TFF at benchtop scale are a simple solution for small scale processing of bioprocessing feed streams that need to be isolated from the environment. These small-scale, single-use assemblies provide flexibility to end-users to efficiently meet different processing needs:

- Efficient diafiltration was achieved in all three diafiltration modes, offering flexibility based on lab settings and process requirements: diafiltration through the transfer manifold, through the diafiltration port, or vacuum diafiltration provided equivalent results.
- Product recovery was not impacted by the position of recovery assemblies on the system: no differences were observed between connection via feed or retentate line.
- Slightly reduced yields in closed manifolds due to increased hold-up volumes and limitation to recover downstream volumes from sterile filters without exceeding bubble point.
- Mindfulness of material compatibility when selecting the recirculation vessel for given modality, solvents, and buffer systems. Two vessels that differed in composition and geometry were tested and resulted in different minimum working volume and hold-up volume. The geometry of the vessel affected product recovery and maximum achievable protein concentration.

The assemblies designed for this study use readily available development laboratory equipment, such as peristaltic pump and balance. Further designs could accommodate specific configurations and constraints, while maintaining the closed system and the same general working principles. When scaling up, similar characterization of larger scale single-use set-up would be beneficial. An example of such a characterization of single-use, automated production system can be found in the Performance Guide of Mobius® FlexReady Solution available at sigmaaldrich.com.

Additional Information

1. https://www.biophorum.com/download/closure-playbook-glossary-of-key-terms-abbreviations-and-acronyms/
2. Mobius® FlexReady Solution with Smart Flexware Assemblies for Tangential Flow Filtration, TB2029 Ver 1.0.
3. Performance Guide, Closed TFF Processing, Application Testing using Mobius® FlexReady Solution (TF2S) with Smart Flexware® Assemblies for TFF with Closed Enabled Configuration. PG8413EN Ver. 2.0.
4. Compatibility of a Mobius® Single-use Solution for ADC Processing, WP4737EN.
5. Pellicon® Capsules with Ultracel® Membrane, Performance Guide PG4104EN Ver. 2.0.
6. Pellicon® Capsules with Ultracel® Membrane, DS1285EN Ver 7.0.
7. Pellicon® Capsules for Ultrafiltration/Diafiltration in the Antibody Drug Conjugate Manufacturing Process. AN1524EN Ver. 2.0.

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Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

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