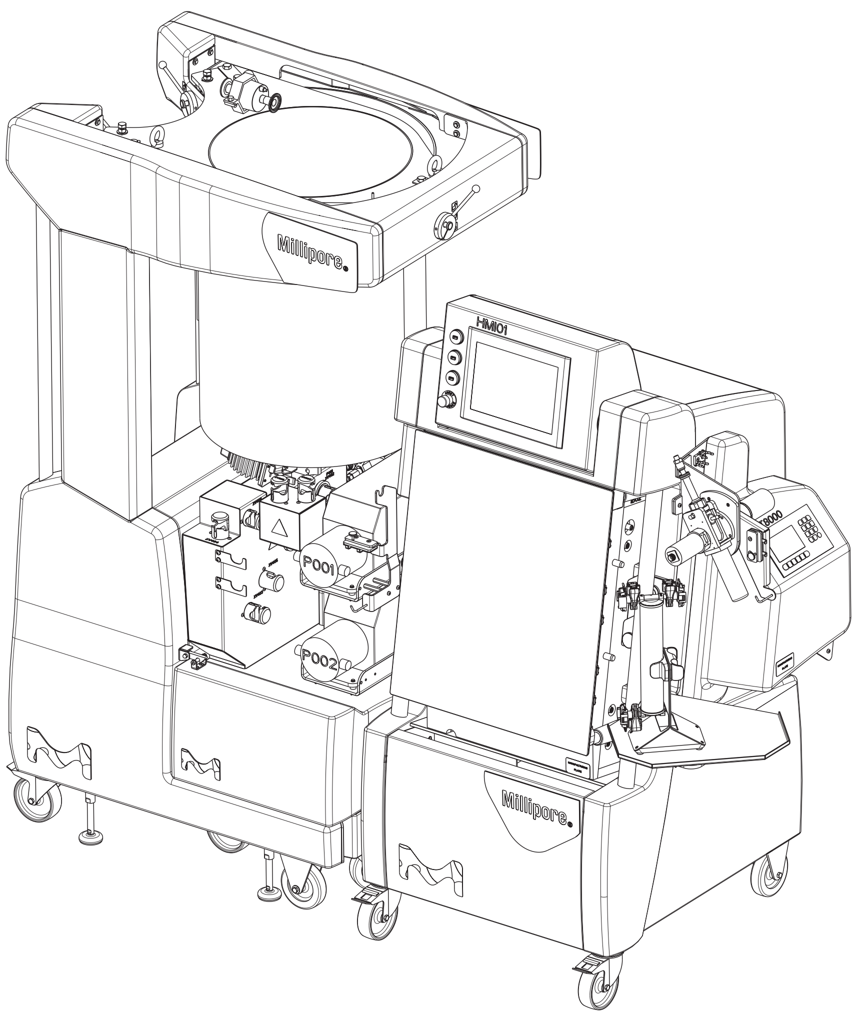
Millipore®

Preparation, Separation,
Filtration & Monitoring Products

Performance Guide

Closed TFF Processing, Application Testing using Mobius® FlexReady Solution (TF2S) with Smart Flexware® Assemblies for TFF with Closed Enabled Configuration



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How to Use this Guide

This Performance Guide is a reference document that provides highlights of key performance aspects of the Mobius® FlexReady Solution with Smart Flexware® Assemblies for Tangential Flow Filtration (TFF) used with a closed-enabled flowpath. It complements the Performance Guide reference TB2029EN (Mobius® FlexReady Solution with Smart Flexware® Assemblies for Tangential Flow Filtration—Performance Guide) and focuses on compiling data specific to a closed-enabled TF2S system.

The results included in this guide summarize outcomes and observations obtained in studies conducted using particular model feed streams and experimental conditions run on a system equipped with a closed-enabled flowpath. Therefore, all test results should be confirmed by the end user using feed stream and process conditions representative of the user's application. It is important to note that results are intended as general examples and should not be construed as product claims or specifications.

Introduction

The Mobius® FlexReady Solution with Smart Flexware® Assemblies for TFF is a fully automated system designed to enable the development and clinical-scale operation of TFF processes for the concentration and diafiltration of mAbs, vaccines, plasma, and therapeutic proteins. The system has the same functionality as traditional TFF systems, and by incorporating a completely single-use flowpath it provides operational flexibility while eliminating concerns of carryover or cross-contamination.

The Mobius® FlexReady Solution with Smart Flexware® Assemblies for TFF with closed-enabled flowpath is available for TF2S only. It uses the same Smart cart, tank cart, and membrane holder as the rest of the Mobius® FlexReady solutions. A specific pump cart, a Pellicon® Capsule support plate*, and sampling supports have been developed to enable closed processing. In addition to feed pump, retentate valve, and transfer pump control loops, all systems also include an automated flow control valve on the filtrate line to enable open UF and MF operations. The maximum reachable flow rate during processing may change, mainly due to the membrane area and product viscosity. A variety of recycle vessel configurations are available to provide maximum process capability. Vessel volumes of 50 L and 100 L are available for the TF2S system. In addition, all sizes are available with a plastic carrier or a double-jacketed carrier, depending on whether temperature control is required. All recycle vessels come with integrated load cells, bottom mounted mixer, and temperature sensor. An optional 0–600 kg capacity floor scale connects easily to the systems and allows for tracking of filtrate volume as well as calculation of filtrate flow rate, flux, concentration factor, diavolumes, and membrane permeability. This is a highly recommended option. Conductivity, UV instrumentation is available as an option for the TFF filtrate line. The UV cell measures dual wavelengths of 280 nm and 300 nm (as standard) with an optical path length of 10 nm. The instrumentation is in single-use format. All performance data included in this guide have been generated using Mobius® FlexReady TF2S with Smart Flexware® Assemblies for closed processing.

* For fully closed processing

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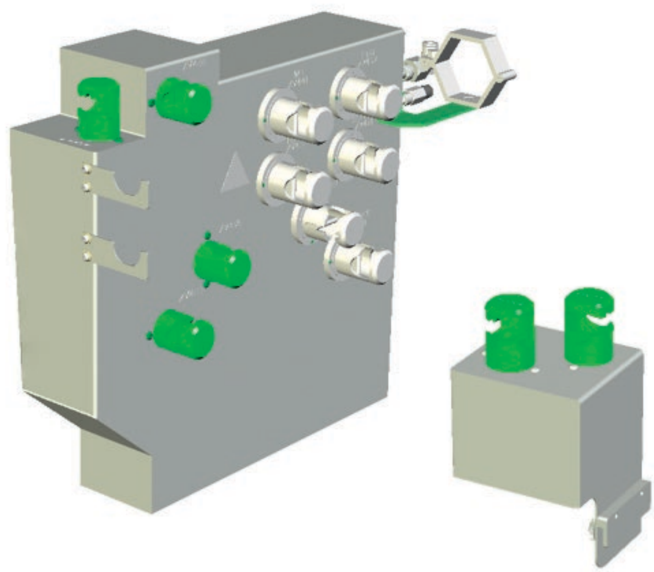
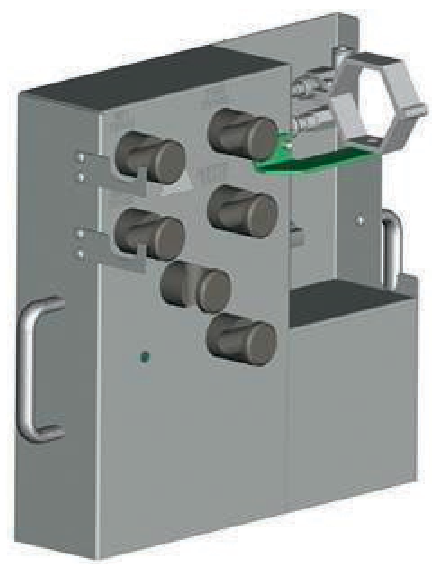
1 Differences between Mobius® TF2S and Mobius® TF2S for Closed Processing

The major difference between a Mobius® TF2S and a Mobius® TF2S for closed processing resides in the manifold of the pump cart. Closed processing TF2S systems have additional valves located on the transfer manifold and on a separate box. They also contain a spacer shaft and an extension fork to create a space between the Smart cart and the pump cart, allowing closed Flexware® assemblies to connect. Two sampling supports allow users to install the NovaSeptum® sterile sampling solution, embedded in filtrate and retentate sampling lines. An optional stand is available to support Pellicon® Capsule.

Manifold for Closed TFF Processing



Manifold for Standard TFF Processing



There are also some differences between the Flexware® assemblies for closed processing and for processing in which a closed flowpath is not required. Flexware® assemblies for closed processing contain aseptic connectors and embedded accessory lines creating closed flowpaths in non- or low-classified environment.

Pellicon® Capsule manifolds are also equipped with aseptic connectors enabling easy connectivity with the Flexware® assemblies and creating a flowpath adapted for fully closed processing. Pellicon® 2 and 3 devices can also be used for functionally closed processing mode.

Mobius® TF2S System: P&ID for Flexware® Assemblies without aseptic connectors and for Flexware® Assemblies with aseptic connectors to enable closed processing.

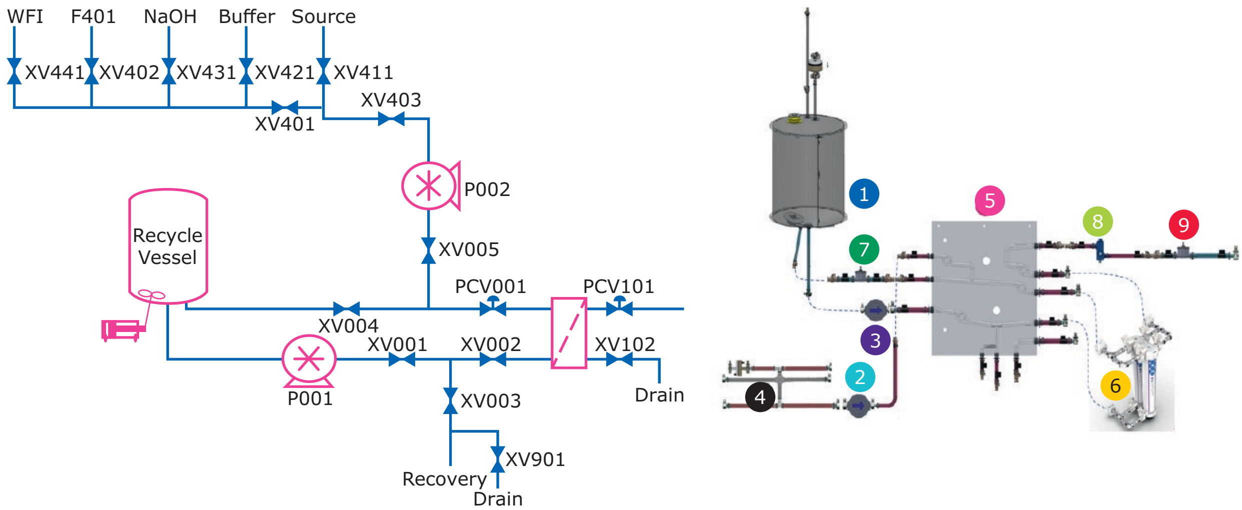


Figure 1.
P&ID and Standard Flexware® Assemblies.

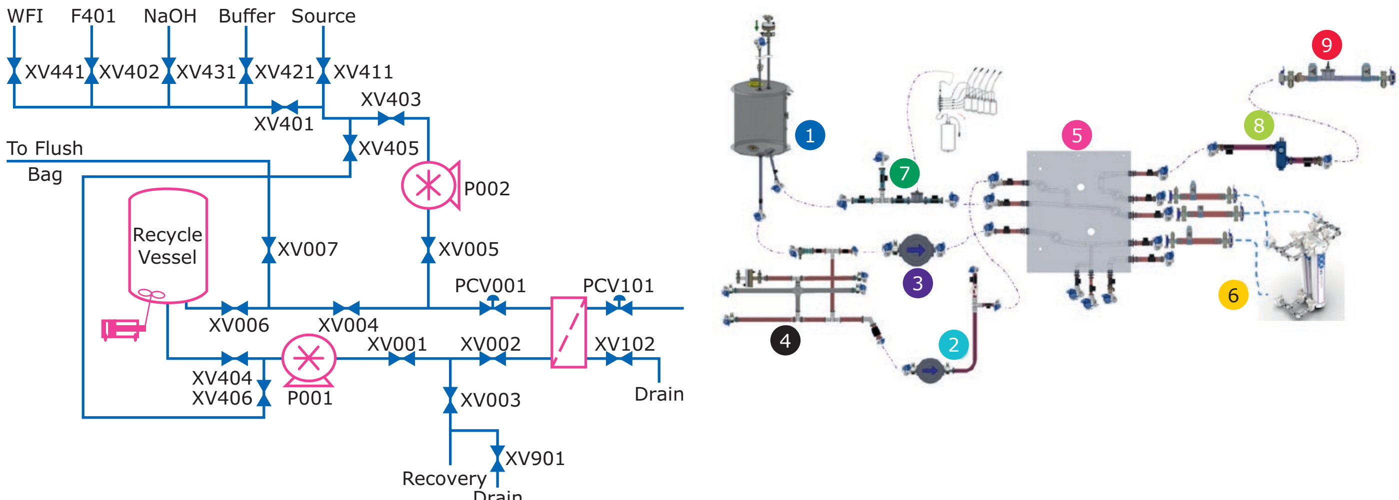


Figure 2.
P&ID and Flexware® Assemblies for Fully Closed Processing.

1. Recycle Vessel 100 L
2. Transfer Pump Assembly
3. Feed Pump Assembly
4. Transfer Pump Manifold Assembly
5. SMART Flowpath Assembly
6. Pellicon® Capsule Assembly
7. Retentate Sampling Port Assembly
8. Permeate Assembly (with Single-Use Sensor Flow Cell)
9. Permeate Sampling Port Assembly

2 Minimum Working Volume in Flexware® Assemblies for Closed Processing

2.1 Background and Objective

The Flexware® assemblies for functionally and fully closed mode are designed so that no disconnection/reconnection of the recycle bag is needed for membrane preparation. The main difference to enable closed processing is the addition of aseptic connectors. Flexware® assemblies for functionally closed processing with Pellicon® 2 or 3 cassettes include less aseptic connections as the flowpath is closed by a sanitization step. Flexware® assemblies for fully closed processing with Pellicon® Capsule include aseptic connectors to each inlet/outlet of the recycle bag, transfer manifold, feed and transfer pump assemblies, Smart flowpath, single-use sensor flow cell assembly, retentate, and permeate sampling assemblies. With the addition of aseptic connectors there is a need to evaluate their effect on hold up volume and the resulting pressure drop in the assemblies.

Understanding the minimum working volume (MWV) within a system is critical to ensure that air is not entrained into the outlet of the recycle bag. The system hold up volume is important for recovery, whereas the MWV, which may vary with the crossflow rate, product viscosity and mixing rate, is important for processing. Testing performed on a Mobius® TF2S system for closed processing with fully closed processing Flexware® assemblies in a range of flow rates from 0.5–22 L/min, will elucidate how low a solution can be concentrated volumetrically at a given flow rate.

2.2 Material and Methods

No filtration device was installed in the Mobius® TF2S system during testing to decouple the hold up volume of the recycle loop from the hold up volume of the device. Instead, a piece of tubing was installed to connect the feed and the retentate side of the SMART flowpath. The hold up volume of the tubing was determined prior to installation on the system and was 146.6 mL. Minimum working volume was determined by following the reverse method of measurement also known as subtractive method:

- Inflate the recycle bag prior to testing to minimize any wrinkles that could contribute to higher minimum working volume.
- Ensure the bottom of the bag is closed on both feed and return lines, to prevent any passage of water into the rest of the Flexware® assembly.
- Tare the load cells on the feed tank cart.
- Using a funnel with an attached dip tube, add 3 L of purified water to the recycle bag through the top bung port. Avoid water splashing on the walls of the tank.
- Verify the mass of the material within the recycle bag via the HMI.
- In a manual mode, set the recycle flowpath and verify that there is an eligible flowpath through the valve block in the transfer manifold.
- Open both lines on the bottom of the bag, and with the mixer off, turn on the pump and slowly ramp up to maximum pumping flow rate of 22 L/min. Completely fill the recirculation loop.
- Slowly bleed off water through the retentate sample port until the minimum working volume is reached at 22 L/min. Proceed in small increments and monitor air entrainment. Measure the collected mass of water and calculate the remaining amount in the system. The minimum working volume is defined as: the volume at which no air is entrained into the system and the removal of additional water would lead to immediate air entrainment.
- Lower the flow rate and repeat the measurements described above at the new flow rate set point. Repeat for several flow rates in the entire range of the pump. Repeat again in full range of the pump's flow rate range with 24 cP and 49 cP fluid (mixture of glycerin and water). Collect the samples and verify the viscosity of both solutions.

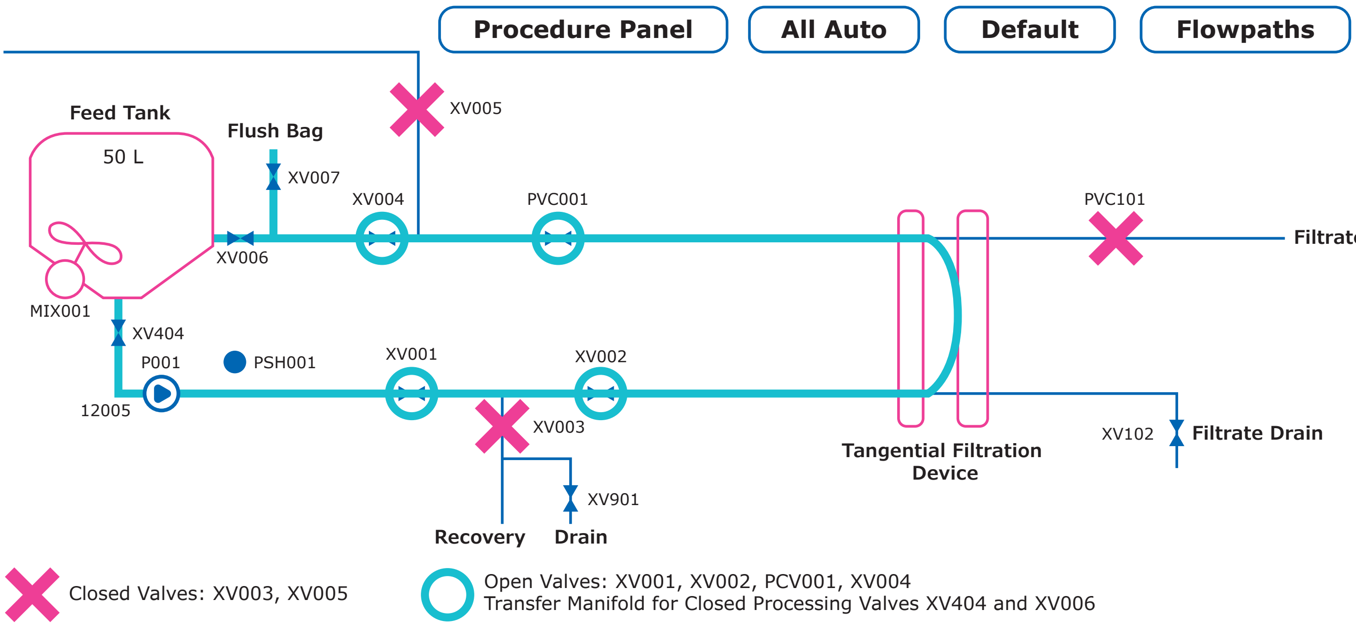


Figure 3.
Illustration of Closed/Open Valves during Minimum Working Volume Measurements.

2.3 Results and Conclusions

The measurements were performed for flow rates between 0.5–22 L/min and viscosities of 1 cP, 24 cP, and 49 cP. The results of testing are summarized in **Figure 4**.

The results in **Figure 4** summarize the minimum working volume in the recycle loop of Mobius® TF2S System with Flexware® Assemblies designed for closed processing. The results do not include the hold up volume of the devices. To get total minimum working volume, the hold up volume of the installed devices must be added to the hold up volume of the recycle loop at a given flow rate.

The minimum working volume in the Flexware® Assembly for closed processing is comparable with the minimum working volume using Flexware® Assemblies with non-aseptic connectors which indicates no significant increase of minimum working volume due to added aseptic connectors.

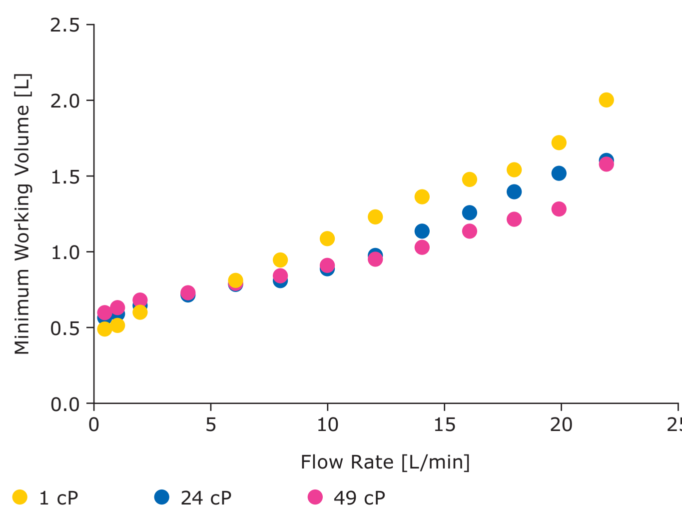


Figure 4.
Minimum Working Volume in a Recycle Loop of Mobius® TF2S System. Hold Up Volume of the Devices is not Included.

3 Pressure Drop Characterization, Flexware® Assemblies for Closed Processing

3.1 Background and Objective

Two pressure drops were measured during pressure drop testing, total pressure drop of the system and retentate pressure drop. Total pressure drop of the system is defined as the line drop from the feed pump discharge through the feed lines, and retentate line back to the recycle vessel and it is measured using the feed pressure sensor (PT-001). Retentate pressure drop is the line drop from the outlet of the device through the retentate line back to the recycle vessel and is measured using the retentate pressure sensor (PT-002). During the measurements, the filtration device was not installed. Instead, the feed and retentate lines of the SMART flowpath were connected with a piece of tubing to enable recirculation of fluid through the recycle loop.

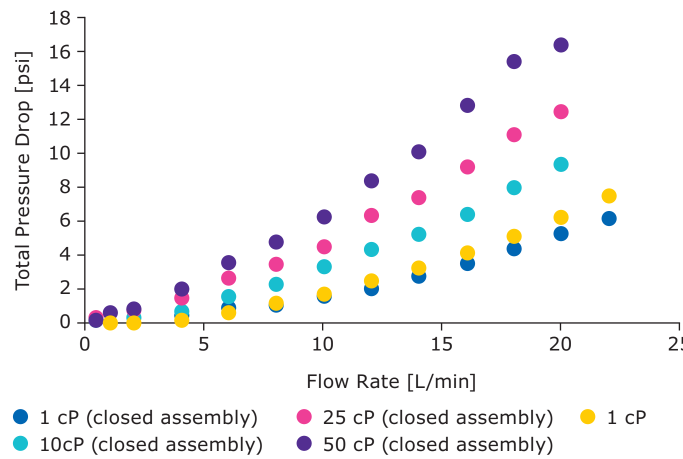
3.2 Materials and Methods

Pressure drop was measured for four viscosities in a full range of the feed pump flow rate, 0.5 to 22 L/min, but not exceeding the pressure rating of the system (60 psi) applicable for higher viscosity solutions. Purified water was used as a model solution to generate data for 1 cP viscosity, higher viscosity solutions were prepared by mixing purified water with glycerin. Before the pressure drop measurements at each viscosity, the solution was recycled in the system for 5–10 minutes to ensure homogeneity and sample collected through the retentate sample port to measure effective viscosity.

3.3 Results and Conclusions

The pressure drop in the recycle loop of Mobius® TF2S system increases with increasing flow rate and viscosity and the comparison of the results between the Flexware® assembly without aseptic connectors and the Flexware® assembly with aseptic connectors for closed processing at 1 cP viscosity illustrate very similar pressure profiles. The results summarized in **Figure 5** do not indicate pressure drop increase due to addition of aseptic connectors.

5a Mobius® TF2S System Total Pressure Drop



5b Mobius® TF2S System Retentate Line Pressure Drop

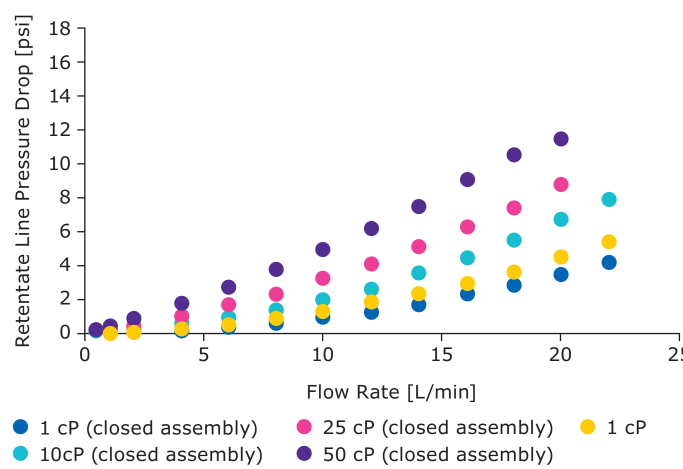


Figure 5a, 5b.

Total and Retentate Pressure Drop in Mobius® TF2S System for two configurations of Flexware® Assemblies (device not included).

In this study, the pressure drop of the filtration device was not included since the pressure drop varies with the type of the device installed into the system. The pressure drop of the device needs to be added to the pressure drop of the system to get an overall pressure drop of the system.

4 Demonstration of Protein UF/DF Step and Optimization of Product Recovery

4.1 Materials and Methods

Flexware® assemblies for closed processing and 1 m^2 Pellicon® Capsule manifold were installed in the system. Pellicon® Capsule manifold consisted of two 0.5 m^2 Pellicon® Capsules with 30 kD Ultracel® membrane, C screen. Mobius® bags containing buffer and the protein feed stream were connected to the transfer manifold via XV421 and XV411 respectively. Recipe for device equilibration was uploaded and initiated through HMI of Mobius® TF2S System. Devices were equilibrated with 30 L/m^2 of buffer (10 mM PBS, pH 7.2) at a flow rate of 5 L/min/m^2 (LMM) and TMP of 15 psi in a single pass mode of operation with the valve XV007 open. After the equilibration step and with the recycle loop still filled with buffer, the system was ready for introduction of protein solution.

A total of 14 L of polyclonal human immunoglobulin (IgG) solution at a concentration of 20 g/L was formulated in 10 mM PBS Buffer, pH 7.2. It was sterile filtered through Opticap® XL 600 Capsule with Millipore Express® SHC membrane prior to transfer into a recycle bag of Mobius® TF2S System. 60 L of diafiltration buffer, 50 mM sodium acetate, pH 5.5, was prepared and connected to the XV421 inlet. The “recycle bag fill” flowpath was engaged to transfer protein feed stream into the recycle bag using the transfer pump. The mixer is enabled in auto mode. Using a pre-programmed recipe, the following processes were executed: a concentration from 20 g/L to 60 g/L, 8 diavolumes diafiltration, and a final concentration from 60 g/L to 200 g/L. The feed pump flow control was set to 5 L/min and the TMP was set to 15 psi. For the final concentration step, the following methodology was programed into the recipe:

1. Feed flowrate control set to 5 LMM, TMP Control set to 15 psi until the retentate pressure decreases to 10 psi.
2. When the retentate pressure equals 10 psi, process switches to Retentate Pressure Control by setting the retentate pressure to 10 psi. The feed flow rate remains set to 5 LMM. As a result, the feed pressure increases with the progression of the concentration.
3. When the feed pressure reaches 50 psi, the system switches to Pressure Drop (dP) Control, setting dP to 40 psi. As a result, the feed flow rate starts to decrease with increasing concentration of the protein.
4. The process is completed when the target concentration is reached.

After the completion of the final concentration step, the system switched to depolarization step during which the permeate valve (PCV101) is fully closed, the retentate valve (PCV001) fully open and the protein is recirculated at 3% feed pump speed (~0.6 L/min). Since the level of the protein is very low, mixing is switched off. Next, the product recovery recipe was downloaded into the system, and the protein was recovered from the system using buffer flush, consisting of 4 steps. An additional buffer flush was used to recover any remaining protein from the system and complete the protein mass balance.

Figure 6 summarizes the steps during process run in the automated mode of operation.

Step	Component	Amount
Equilibration	10 mM PBS Buffer, pH 7.2	30 L/m^2
Transfer into Mobius® TF2S Recycle Bag, Recirculation	Human Plasma IgG	14.3 L
Concentration 1	Human Plasma IgG	20 g/L –60 g/L
Diafiltration	50 mM Sodium Acetate Buffer, pH 5.5	8 Diavolumes
Final Concentration	Human Plasma IgG	60 g/L –200 g/L
Product Recovery	Following 4 Recovery Steps	

Figure 6.

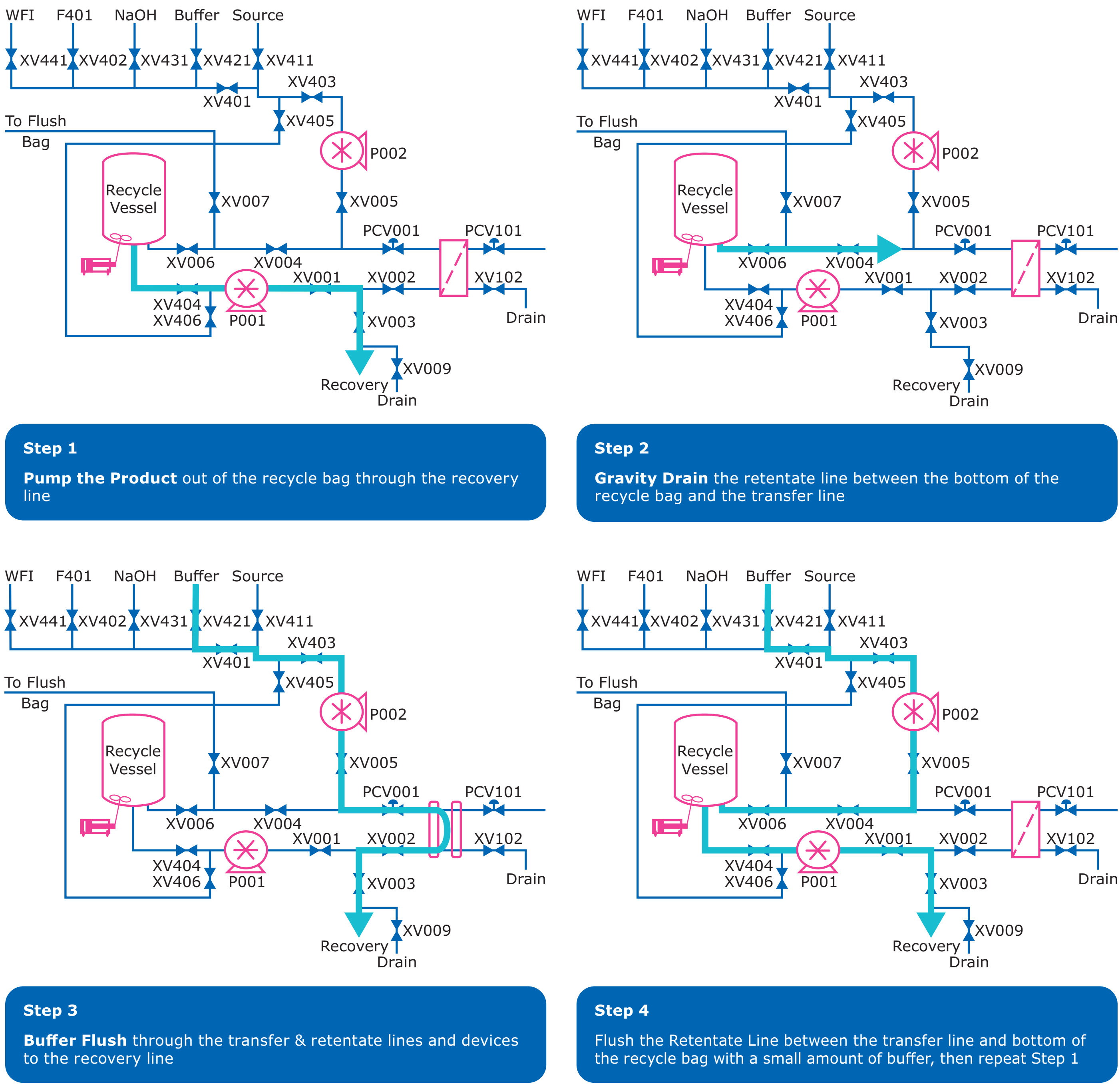
Process Outline during Process Simulation in Automated Mode of Operation.

4.2 Product Recovery Optimization

Product recovery was optimized for low and elevated viscosity applications using human plasma IgG as a model feed stream at the concentrations of 50 g/L (1.5 cP) and 200 g/L (13 cP) respectively. The optimization was first performed in a manual mode of operation and once the optimized method was determined, recipes were written to automate the recovery steps. Recovery step sequence illustrated in **Figure 7** was followed.

During the manual mode of operation, the protein from Step 1 and Step 2 were collected into one pool. During Step 3, ten 50 mL aliquots were collected into collection containers resulting in total volume of displacement buffer to be approximately 500 mL. During recovery Step 4, two 100 mL aliquots were collected.

The automated mode of operation was very similar to the manual mode of operation, except the product from recovery Step 1 and Step 2 were collected separately and the aliquots during Step 3 were six 100 mL amounts. Step 4 was the same as in the manual mode of operation, collecting two 100 mL aliquots.



Step 1

Pump the Product out of the recycle bag through the recovery line

Step 2

Gravity Drain the retentate line between the bottom of the recycle bag and the transfer line

Step 3

Buffer Flush through the transfer & retentate lines and devices to the recovery line

Step 4

Flush the Retentate Line between the transfer line and bottom of the recycle bag with a small amount of buffer, then repeat Step 1

Figure 7.
Product Recovery Procedure.

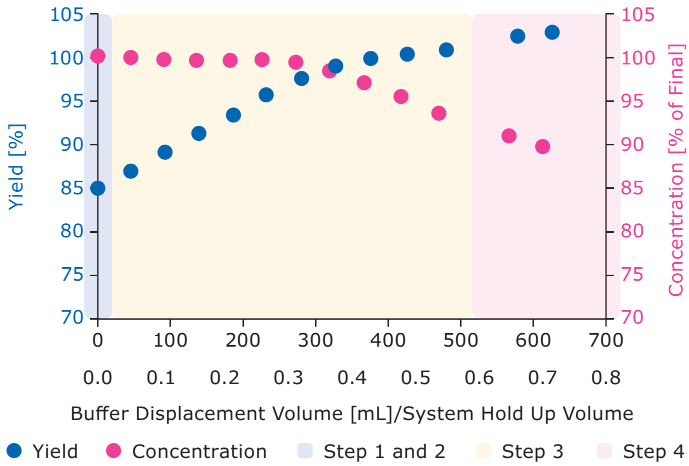
4.3 Results and Conclusions

Product Recovery Optimization

The results of overall product concentration and yield are summarized in **Figure 8**a, b, and c.

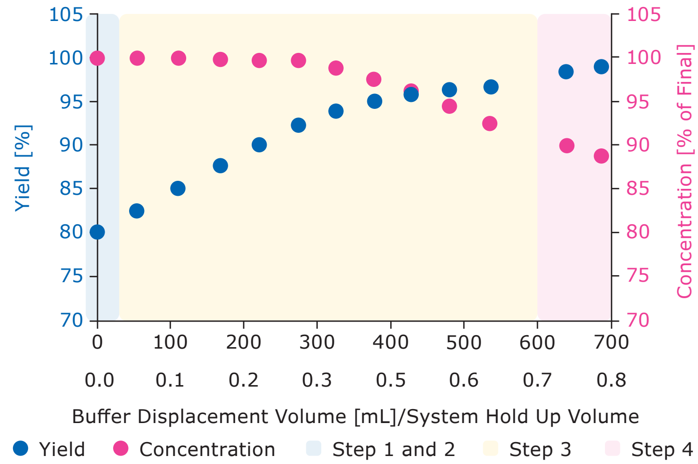
The results are comparable between the two modes of operation, process yield is above 95% and product dilution between 10 and 15%.

8a Low Viscosity Application, Manual Mode of Operation



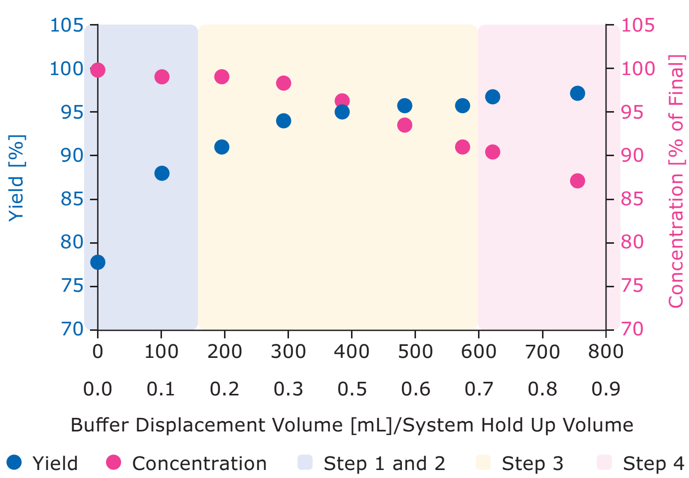
Protein Recovery Summary	
Conc, Pre-Recovery [g/L]	53
Viscosity [cP]	1.6
Yield [%]	102.9
Final Conc [g/L]	47

8c Elevated Viscosity Application, Manual Mode of Operation



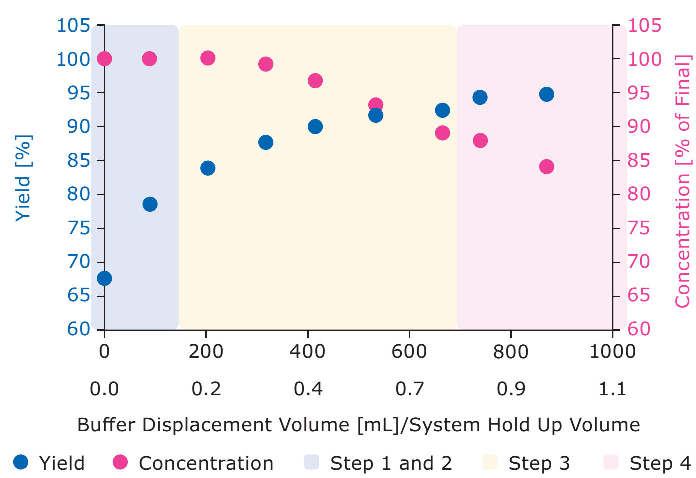
Protein Recovery Summary	
Conc, Pre-Recovery [g/L]	186
Viscosity [cP]	10
Yield [%]	99.0
Final Conc [g/L]	165

8b Low Viscosity Application, Automated Mode of Operation



Protein Recovery Summary	
Conc, Pre-Recovery [g/L]	51
Viscosity [cP]	1.5
Yield [%]	97.6
Final Conc [g/L]	44

8d Elevated Viscosity Application, Automated Mode of Operation



Protein Recovery Summary	
Conc, Pre-Recovery [g/L]	202
Viscosity [cP]	14.5
Yield [%]	94.9
Final Conc [g/L]	170

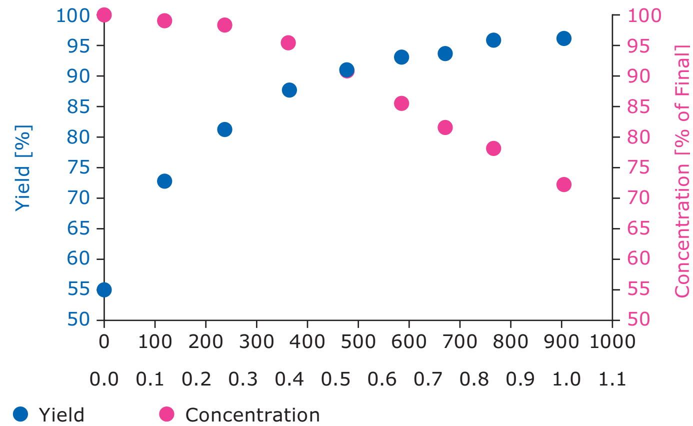
Figure 8a, b, c.
Overall Product Yield and Concentration for Low and Elevated Viscosity Applications using Mobius® TF2S System in a Manual and Automated Mode of Operation.

Process Simulation in Automated Mode of Operation

Recovery procedure was performed as part of a fully automated run. Overall yield was 96.4% and the results are summarized in **Figure 9**.

Graphs of product yield/concentration as a function of buffer displacement volume help to understand the relationship between the final product concentration, yield and buffer displacement volume and may help to determine the optimum for a process. Graphs illustrate that with increasing buffer displacement volume, the product concentration decreases, but at the same time process yield increases. The total volume of the buffer necessary to recover product from the system depends on process requirements for the final concentration, yield and allowance for product dilution during recovery.

Buffer Displacement Volume [mL]/ System Hold Up Volume



Protein Recovery Summary	
Pre-Recovery Conc [g/L]	217
Yield [%]	96.4
Final Conc [g/L]	157

Figure 9.
Overall Product Yield and Concentration for Elevated Viscosity Applications using Mobius® TF2S System in an Automated Mode of Operation.

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