SARTORIUS

Application Note

April 10, 2022

Keywords or phrases
Tangential flow filtration, TFF, crossflow, UF | DF, ultrafiltration, diafiltration, scale-down system, high throughput, multi-channel, parallel screening, monoclonal antibodies, mAb, antibody-drug conjugates, ADC

Evaluation of Sartocon® Slice 50 Ambr® CF Adapter for Ambr® Crossflow Using mAbs and ADCs

Thomas Prouzeau[1,*], Benjamin Ha[2], Ludivine Profit[2]
[1] Sartorius Stedim France S.A.S, ZI des Paluds, Avenue de Jouques, 13781 Aubagne, France
[2] Purification Process Development, Sanofi, 1 Impasse des Ateliers, 94400 Vitry-sur-seine, France

* Correspondence
Email: thomas.prouzeau@sartorius.com

Abstract

Crossflow, or tangential flow filtration (TFF), is an important process used for the ultrafiltration (UF) and diafiltration (DF) of biologics, such as monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs). This technique is used to purify and concentrate mAbs and ADCs, and it is an important method for ensuring both the safety and efficacy of these drug classes.

To efficiently determine the optimum buffer combinations and process control conditions for mAb and ADC manufacturing, benchtop TFF systems are commonly used as a scale-down model for UF | DF. However, these systems are single channel. This limits their efficiency for assessing a variety of parameters or different buffer types, which are required for robust results in formulation studies.

Therefore, multi-channel systems such as the Ambr® Crossflow are becoming more popular for downstream development work, as they offer early development stage information on how an mAb, or ADC will behave at scale.

To determine how effectively a multi-channel scale-down TFF model would perform with mAbs and ADCs, the Purification Process Development Group at Sanofi evaluated the Sartocon® Slice 50 Ambr® CF Adapter Kit with the Ambr® Crossflow system.

For more information, visit
www.sartorius.com

This application note describes the experimental protocol, and includes a comparison of the Sartocon® Slice 50 filter with an Ambr® CF Filter Hydrosart® (10 cm² cassette), as well as the results from transmembrane pressure (TMP) optimization studies and concentration | diafiltrations runs performed with two different mAbs and one ADC.

The results of these studies show the benefits of using the Sartocon® Slice 50 adapter, which include being able to process more product with the Ambr® Crossflow, by taking advantage of the automation and miniaturization of a multi-channel crossflow system. Additionally, the run time is significantly shorter because higher permeate flux is obtained than when using the 10 cm² cassette format, due to the difference in channel geometry. Analytical results also show that the final concentrated product is of high quality. Finally, using the Sartocon® Slice 50 with ECO-Screen or E-Screen allows for control of the process via DP and TMP setpoints, which is a better representation of large-scale processing and could provide more robust results to help save time with scale-up.

Introduction

Ambr® Crossflow

Benchtop tangential flow filtration (TFF) systems are commonly used as a scale-down model for ultrafiltration (UF) and diafiltration (DF) purification processes. Most of these systems are single-channel and have relatively large recirculation volumes. This limits the efficiency and robustness of results when assessing a variety of parameters or buffer formulations and can mean that the monoclonal antibody (mAb) or antibody-drug conjugate (ADC) candidate chosen is sub-optimal and/or the process is not transferrable at scale. It also means that a greater number of candidate molecules can be assessed, and thus reduces the possibility of excluding a candidate molecule based on resource limitations alone. Therefore, multi-channel systems such as the Ambr® Crossflow, with its low recirculation volume, are becoming more commonly used. These systems can offer early development stage information on how an mAb, or ADC will behave at scale in relation to viscosity, buffer composition, shear stress, and process performance. This enables important criteria for mAb or ADC candidate selection and can significantly speed up the process while reducing costs per experiment.

The Ambr® Crossflow has 4, 8, 12, or 16, small-scale channels. Each is fully equipped to act like any traditional bench-scale TFF filtration set-up. The system is fully automated, with each channel independently controlled in terms of product input, buffer streams, and process conditions such as recirculation rate, pressure, load volume, diafiltration set point, and final product volume.

The Ambr® Crossflow has a minimum recirculation volume of 5 mL and works with Ambr® CF single-use filter cassettes with a membrane area of 10 cm² or with Sartocon® Slice 50 module with a membrane area of 50 cm².

Being able to study the impact of process parameters, buffer types, and protein concentration with an automated, small-scale, high-throughput process allows scientists to determine if it is possible to formulate their biologics more cost-effectively.

Ambr® Crossflow Software

Ambr® Crossflow software is designed to provide scientists with a user-friendly way to set up multi-parallel experiments. Recipe design is intuitive, with pre-programmed, flexible phases that allow researchers to design their own phases and recipes from scratch. Each channel operates independently, allowing individual control of process conditions, set points, and control strategy. The software provides a range of pre-programmed methods to enable the system to perform routine tasks independently, such as flux assessment or identification of the optimal DF point, to save time and ensure the best product recovery. Additionally, Ambr® Crossflow software can be installed on researchers' PCs so they can write process sequences at their desks before moving to the control system.

Data is automatically collected and stored in an experiment folder and can be viewed on the control system as well as from a user's PC with Ambr® Crossflow software installed.

Alternatively, data can be exported as a .csv file and viewed using third-party data analysis packages (OPC control and data collection incorporated).

Visualization of multiple experiments in "result viewer" allows scientists to look at data from different trials simultaneously to enable a better analysis of the impact of buffer type and protein concentration. MODDE® software can also be used to create and analyze DoE experiments for the Ambr® Crossflow system.

Ambr® CF Filter

The Ambr® CF single-use filter cassette (Figure 1) has been designed to study the impact of buffer type and protein concentration, allowing researchers to explore a large experimental design space even at small-scale operation. This supports scientists in determining at an early stage of development if their formulations will affect product quality.

Figure 1: *Ambr® CF Filter.*

The embedded spacer structure is designed to achieve a high-mass transfer and low-pressure drop over the flow field, which is beneficial for processing highly-viscous solutions. The properties of the flow field prevent protein damage and maintain a constant shear rate at the entire membrane surface. To reduce void volume and avoid edge effects a single-layer membrane is integrated. The total membrane area is 10 cm².

Sartoflow® Smart

The Sartoflow® Smart is a modular, flexible small-scale bench-top crossflow system optimized for UF and DF applications used in downstream processes, such as purification of vaccines, mAbs, and recombinant proteins. The system is suitable for use in laboratory environments, process development, and clinical trials, as well as in cGMP environments.

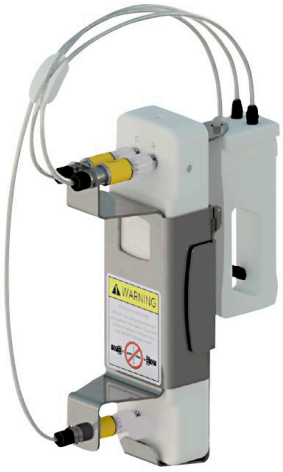
A low shear four-piston membrane pump provides a wide range of flow rates, making it possible to use membrane areas from as small as 50 cm² to as large as 0.14 m².

Sartocon® Slice 50 and Sartocon® Slice 50 Adapter

The Sartocon® Slice 50 is a ready-to-use, 50 cm² single-use crossflow module that is ideally suited for membrane screening and process development. It is available in two flow channel geometry versions. E-Screen filters are usually used for protein concentrations above 20%, and viscosity conditions greater than 3 cP. ECO-Screen devices are most used for water-based protein solutions below 20% protein concentration and less than 3 cP viscosity.

The Sartocon® Slice 50 can easily be interfaced with the Sartoflow® Smart system, and a range of other laboratory-scale crossflow systems. It can now also be connected to an Ambr® Crossflow using the new Sartocon® Slice 50 Adapter (Figure 2).

Figure 2: *Sartocon® Slice 50 Adapter for Ambr® Crossflow.*



The adapter connects to an Ambr® Crossflow module in the same way as a standard Ambr® Crossflow filter – via three ports that link to the feed, and permeate and retentate ports. The tubing provided with this accessory connects each of these ports to its corresponding port on the filter. The filter holder allows the filter to be attached and securely connected to the adapter via custom Luer fittings.

The filtration process is unchanged for either UF or DF when using this device with the Ambr® Crossflow module. Instead of flowing directly into the standard filter, the fluid flows from the adapter feed port into the corresponding port on the Sartocon® Slice 50 filter. Then, the fluid flows out from the Sartocon® Slice 50 filter via the retentate and/or permeate ports, back into the adapter's corresponding ports, and into the Ambr® Crossflow module.

Case Study

The Purification Process Development team wanted to access all the benefits of the Ambr® Crossflow automation, with its multi-channel screening capability and low minimum working volume. This equipment is already used as a powerful screening tool, but its potential as a representative scale-down model has not been fully evaluated yet.

Until 2021, the system was only compatible with Ambr® CF 10 cm² cassettes, whose wide recirculation channels allow for high protein concentrations but only generates low differential pressure, even at high recirculation flow rates. These features prevent cassettes from being used to mimic large- scale process parameters.

The team assessed the Sartocon® Slice 50 Adapter for Sartocon® Slice 50 cassettes. The cassette structure embeds spacers, making it available in E-Screen and ECO-Screens, so the channel geometry is representative of larger-scale cassettes. This allows for generation of a significant differential pressure, which can be used as a control parameter.

This application note shows the results of experiments performed to characterize differences between 10 cm² and 50 cm² cassettes, through processes piloted on the Ambr® Crossflow. These experiments involved three test molecules.

Materials

Test Molecules

Polished intermediates of two mAbs (designated "A" and "B" in this application note), as well as one ADC (designated "C"), were used for these experiments.

Equipment and Consumables

Most of the experiments were run on the Ambr® Crossflow. A run with Sartoflow® Smart was also performed to provide comparative results for molecule C. For the consumables, a range of cassettes containing 30 kD membrane were used. These were the Sartocon® Slice 50 Hydrosart 30 kD ECO-Screen and E-Screen, Ambr® CF Hydrosart 30 kD, and Ambr® CF PESU 30 kD.

Table 1: *List of scale-down models used for the trials*

Molecule	Type	Initial Protein Concentration	Equipment + Consumable Combination	Model #ID
A	mAb	6.3 g/L	Ambr® Crossflow + Ambr® CF Hydrosart 30 kD	#1
			Ambr® Crossflow + Sartocon® Slice 50 Hydrosart 30 kD ECO	#2
B	mAb	3.6 g/L	Ambr® Crossflow + Sartocon® Slice 50 Hydrosart 30 kD E	#3
C	ADC	2.3 g/L	Ambr® Crossflow + Ambr® CF PESU 30 kD	#4
			Ambr® Crossflow + Sartocon® Slice 50 Hydrosart 30 kD ECO	#6
			Sartoflow® Smart + Sartocon® Slice 50 Hydrosart 30 kD ECO	#6

Methods

Product Characterization

Before and after each run, initial and final product were analyzed by UV 280 nm protein titration and SEC-HPLC for high molecular weight (HMW) species titration. Final products from the tests with ADC C were further analyzed by dynamic light scattering (DLS) and free drugs quantification (a critical quality attribute for ADCs).

TMP Optimization Studies With Ambr® Crossflow

A first transmembrane pressure (TMP) optimization study was conducted using mAb A and a 10 cm² Ambr® CF cassette (model #1), four different TMP values ranging from 0.2 bar to 0.8 bar, and five feed flowrates in the range 20 - 40 mL/min, corresponding to 1,200 - 2,400 L/hour/m² (LMH).

Another assessment was performed with Ambr® Crossflow, the same product, and a 50 cm² cassette (model #2). The TMP ranged from 0.4 - 1.6 bar and the feed flowrate from 20 - 50 mL/min, corresponding to 240 - 600 LMH. The trial, first performed with mAb A at low concentration (initial titer of 6.3 g/L), was repeated at higher concentration (titer of 40 g/L). The 40 g/L mAb intermediate was generated using a crossflow ultrafiltration step without exchanging the buffer.

Concentration | Diafiltration Runs

Six experiments were performed – one for each model. During the process, pressure parameters, feed flowrate, permeate flux, conductivity, and retentate volume were monitored.

The test conditions were selected based on the results of the TMP optimization studies or based on the customer's experience with the test molecules. The experimental conditions used are summarized in Table 2.

Table 2: *Process Parameters for Concentration | Diafiltrations Runs.*

Run | Model #ID	Feed Control Parameter	Retentate Control Parameter	Protein Loading	Process
#1	Feed Flow 40 mL/min	TMP 1.0 bar	1044 g/m²*	Concentration – Diafiltration – Concentration
#2	Feed Flow 40 mL/min	TMP 1.0 bar	800 g/m²*	Concentration – Diafiltration – Concentration
#3	DP 1.5 bars	TMP 1.0 bar	400 g/m²	Concentration – Diafiltration – Concentration
#4	Feed Flow 15 mL/min	TMP 0.35 bar	220 g/m²	Concentration – Diafiltration
#5	Feed Flow 25 mL/min	TMP 0.8 bar	220 g/m²	Concentration – Diafiltration
#6	Feed Flow 25 mL/min	TMP 0.8 bar	220 g/m²	Concentration – Diafiltration

*Protein loading for Run #2 had to be decreased compared to Run #1. The diafiltration setpoint was 40 g/L. Limited by the maximum retentate vessel volume of 100 mL, the maximum loaded product quantity was therefore 4 g, corresponding to 800 g/m² with a 50 cm² cassette.

Results

Studies With mAb A

Figure 3 shows that using a Sartocon® Slice 50 cassette improves the permeate flux compared to 10 cm² filter at equivalent feed flowrate. Therefore, the ratio of permeate flux over recirculation flux is significantly higher for a Sartocon® Slice 50 cassette, so it is more representative of process performance at a larger scale.

With the Sartocon® Slice 50, higher TMP values are reached, which increases permeate flux without causing a plateau effect.

As expected, the flux decreases when mAb A is more concentrated (as shown in Figure 4). This type of experiment is easily performed with Ambr® Crossflow and generates process and product knowledge for a wide range of product concentrations. It can, for example, be used to predict the evolution of permeate flux during a full process with control parameters kept constant.

The permeate flux results obtained in the optimization experiments were confirmed in the full Con-Di-Con runs, leading to a 57% lower run duration with the Sartocon® Slice 50 cassette (Table 3). Part of this difference is due to the lower capacity evaluated for Model #2.

For both models, no HMW species were generated during the runs indicating that protein aggregation has not occurred during processing.

Figure 3: *TMP | Feed Flowrate Optimization, and Superposition of the Results Obtained With Models #1 and #2.*

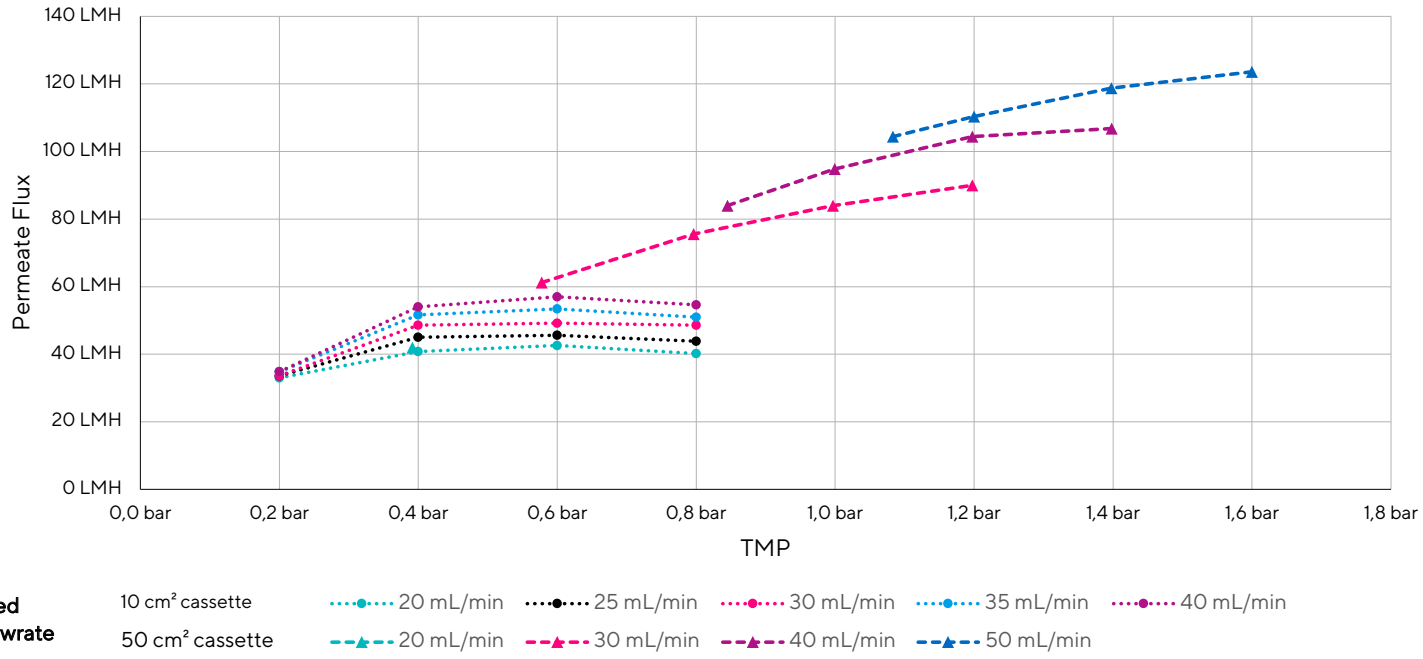
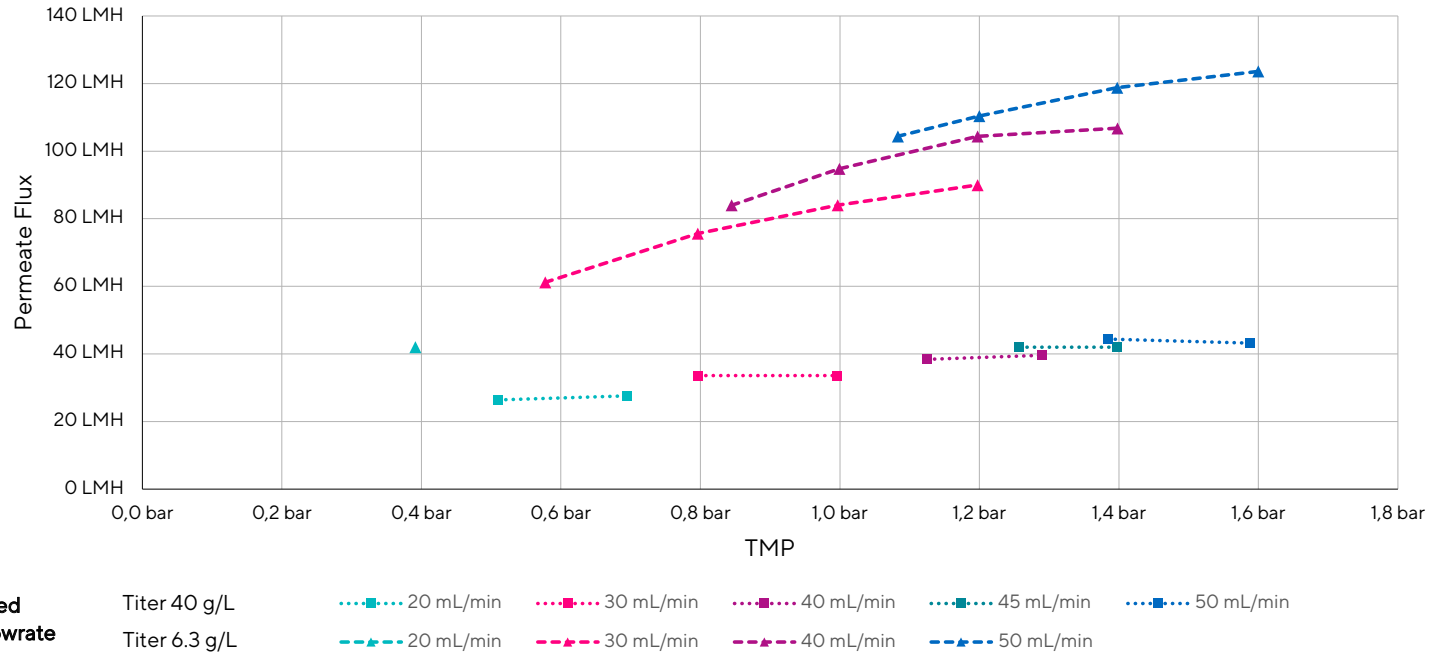


Table 3: *Con-Di-Con Run Results on Ambr® Crossflow With mAb A, Model #1 (Ambr® CF Filter) and Model #2 (Slice 50 Cassette).*

Run | Model #ID	Starting Titer and Aggregates Level	Intermediate Titer	Number of Diavolumes	Final Titer and Aggregates Level	Total Run Duration	Recovery*
#1	6.3 g/L; 1.0% HMW	38 g/L	7	169 g/L; 1.1% HMW	14.5 h	81%
#2	6.3 g/L; 1.0% HMW	43 g/L	7	179 g/L; 1.1% HMW	6.3 h	85%

*The protein recovery was calculated based on UV280 nm titers (initial and final), initial volume, and final recovered volume. It does not include the protein recovered in the flush.

Figure 4: *TMP | Feed Flowrate Optimization and Superposition of the Results Obtained With Model #2 at Two mAb Concentrations.*



Studies With mAb B

The Con-Di-Con run performed with mAb B was controlled with a differential pressure (DP) setpoint. The results (Figure 5) show that the feed pump flow rate is continuously alternating to reach or maintain the 1.5 bars DP setpoint: it increases at the start of Concentration 1 and Diafiltration phases to reach the setpoint; during the Concentration 2 phase, it progressively decreases as the product reaches high concentrations, and thus becomes increasingly viscous.

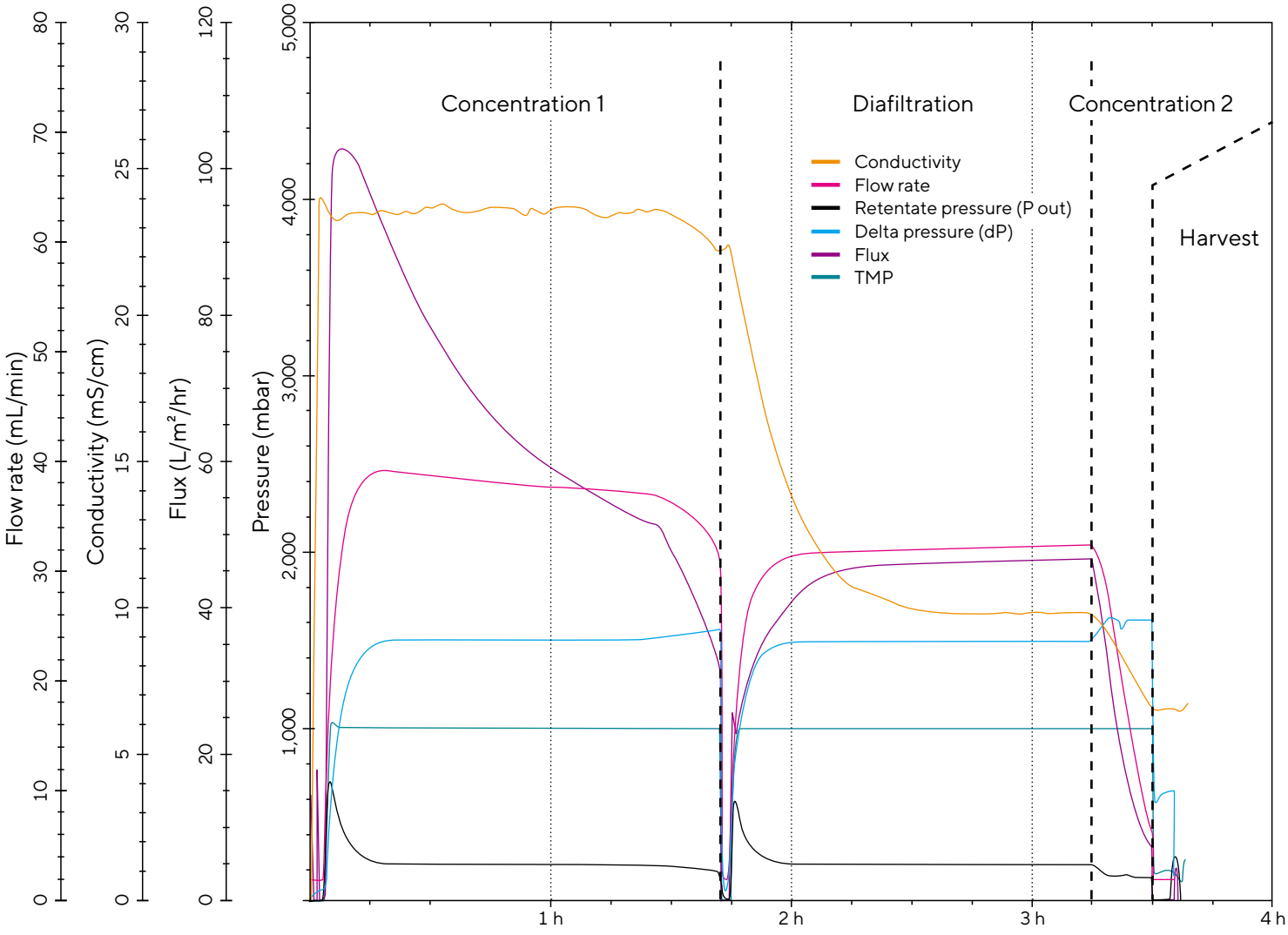
These results indicate that this type of process control, often used on pilot and large-scale systems, is now enabled on Ambr® Crossflow by the Sartocon® Slice 50 adapter.

The mAb was concentrated to approximately 50 g/L before diafiltration and was then concentrated up to 180 g/L (Table 5). The run duration was 3.2 hours for a membrane capacity of 400 g/m². The increase in HMW content is molecule-dependent and has also been observed on other small-scale runs with this project.

Table 4: *Con-Di-Con Run #3 Results.*

Run | Model #ID	Starting Titer and Aggregates Level	Intermediate Titer	Number of Diavolumes	Final Titer and Aggregates Level	Total Run Duration	Recovery
#3	3.6 g/L; 0.2% HMW	52 g/L	8	180 g/L; 3.9% HMW	3.2 h	>100%

Figure 5: *Process Parameters Over Time for Run #3.*



Studies with ADC

A comparison of three small-scale models (see Table 1 and Table 2 for details) was performed maintaining the same sequence of steps for all runs: first a concentration to 15 g/L, then a diafiltration with 10 DV of buffer, and a final harvest with 1:1 dilution in pre-formulation buffer. The results are summarized in Table 5.

It is again observed that the use of a Sartocon® Slice 50 cassette significantly reduces the total run duration on Ambr® Crossflow (3.3 hours instead of 9.2 hours).

Runs on Ambr Crossflow and Sartoflow Smart were compared and equivalent results were observed, with 100% product recovery and no increase in HMW species.

Process parameters over time for Run #5 are also detailed in Figure 6.

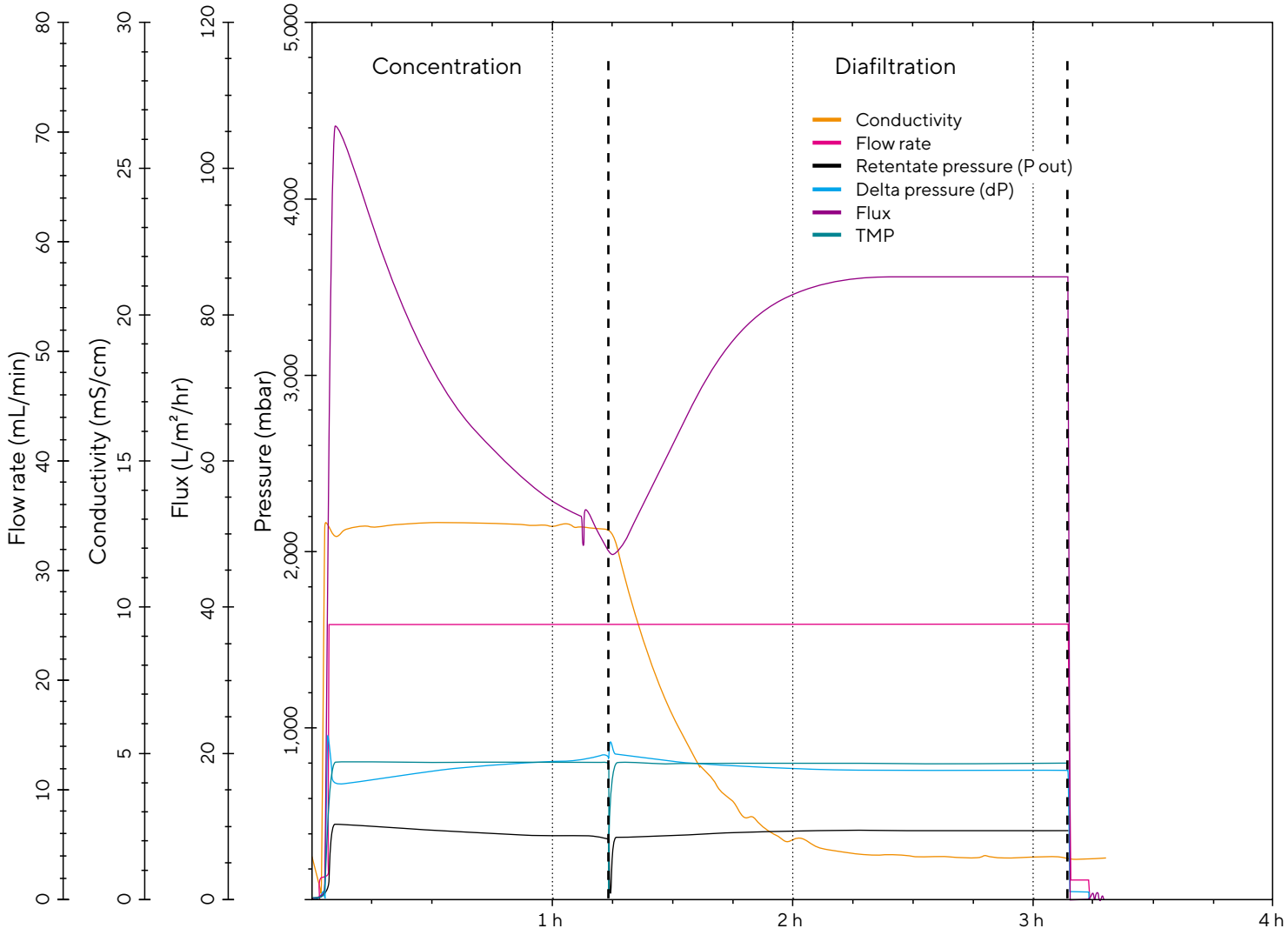
Table 5: *Con-Di-Con Runs #4, #5 and #6 Results.*

Run | Model #ID	Starting Titer and Aggregates Level	Intermediate Titer	Number of Diavolumes	Final Titer and Aggregates Level	Total Run Duration	Recovery*
#4	2.3 g/L; 3.7% HMW	15 g/L	10	7.4 g/L; 3.5% HMW	9.2 h	95%
#5	2.3 g/L; 3.7% HMW	15 g/L	10	7.7 g/L; 3.6%* HMW	3.3 h	>100%
#6	2.3 g/L; 3.7% HMW	15 g/L	10	10.4 g/L; 3.6%* HMW	2.7 h	>100%

*DLS analysis showed the presence of a minor submicronical population generated with Model #5.
This phenomenon is usually observed with other TFF systems using peristaltic pump.

Further analysis showed no impact on free drugs content for all models, which is an important quality attribute for ADC.

Figure 6: *Process Parameters Over Time for Run #5.*



Conclusion

The objective of this study was to evaluate the Sartocon® Slice 50 adapter on an Ambr® Crossflow system for testing mAb and ADC molecules, as well as a range of process conditions. The study demonstrates the benefits of enabling the connection of a Sartocon® Slice 50 to an automated, multi-channel, high-throughput crossflow system. The screen channel of the cassettes is more representative of large-scale cassettes. Therefore, the differential pressure is similar and can even be a control setpoint for the process.

This study shows that it is possible to process mAbs or ADCs using Ambr® Crossflow with Sartocon® Slice 50 to achieve high quality, representative concentrated products. Compared to the 10 cm^2 cassette screening format, it significantly enhances permeate flux for lower viscosity feeds thus reducing process duration.

Additionally, using the Ambr® Crossflow, optimizing TMP | feed flowrate can be easily performed to rapidly provide critical process knowledge for a more efficient and robust scale-up.

Acknowledgments

Sartorius would like to thank Benjamin Fabre, Damien Girard, and Jérôme Pezzini for their contribution to this study.

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For more information, visit
www.sartorius.com