SARTORIUS

Application Note

August, 2024

Keywords or phrases:
Adeno-associated virus (AAV), Gene therapy; Tangential flow filtration (TFF), Sartoflow® Smart, Ambr® Crossflow, Sartocon®

A High-Throughput and Automated Method for AAV Tangential Flow Filtration Process Development

Marc Noverraz[1], Franziska Bollmann[2,*], Piergiuseppe Nestola[1]

[1]Sartorius Stedim Switzerland AG, 8317 Tagelswangen, Switzerland
[2]Sartorius Stedim Biotech GmbH, 37079 Göttingen, Germany

*Correspondence
Email: franziska.bollmann@sartorius.com

Abstract

Adeno-associated viruses (AAVs) are versatile and commonly used delivery vectors for gene therapies. Faster and more efficient solutions for the development of their manufacturing processes are required to rapidly establish a reliable supply of those next-generation therapies to the patients.

Ultrafiltration and diafiltration (UF|DF) is a key process step for adapting the AAV solution concentration and its buffer composition into an optimized composition while removing impurities , enabling high downstream yields. High-performing filters must be identified early in development; however, process scientists are under significant time pressures, and feed material is often scarce.

This application note presents a strategy for rapidly establishing a high-performing AAV Tangential Flow Filtration (TFF) step. We evaluated different molecular weight cut-offs (MWCOs) and membrane materials to establish a TFF process from small-scale screening experiments to a large-volume operation.

Introduction

Adeno-associated viruses are a common gene delivery vehicle in next-generation therapies. As a relatively new modality, their manufacturing requires more efficient and cost-effective solutions to keep up with increasing demands for gene therapies. Ideally, the stream volume should be kept low in order to limit the volume entering the purification process (and, therefore, avoid the expenditure associated with equipment, materials, and footprint required for large volumes).

Tangential flow filtration is a valuable intermediate process step that can be employed for volume reduction and buffer exchange. Ultrafiltration is typically the method of choice for concentration and diafiltration (buffer exchange) of AAV while removing low molecular weight impurities and reducing product volume. It is a relatively low-cost technique and usually leads to high recovery. However, finding a high-performing filter—i.e., the correct membrane material and molecular weight cut-off—for the TFF step can require multiple experiments to identify and optimize the critical process parameters.[1] Such process development activities require time and AAV material—both of which are usually scarce when developing a new product. High throughput, scaled-down systems can help process development scientists quickly screen potential technologies and conditions with a small amount of product material.[2,3]

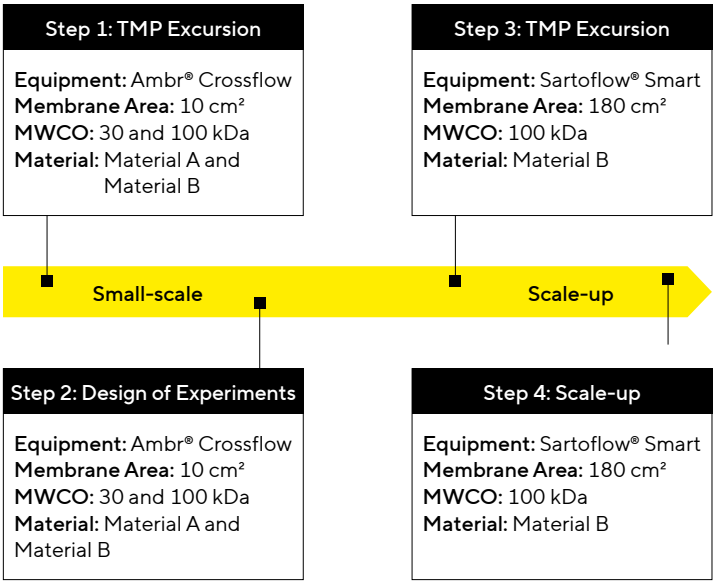
The Ambr® Crossflow system is a tool for high throughput development of the ultrafiltration | diafiltration operation. Its small scale reduces material requirements, and its high throughput capacity significantly increases the number of possible experimental runs (the system can operate with as little as 5 mL circulation volume and can process up to 16 runs in parallel, requiring the same amount of starting material of one 1 L bench-scale process). When used in combination with Design of Experiments (DoE) methods, its screening capabilities are elevated even further.[4-6]

We utilized the Ambr® Crossflow system to evaluate two ultrafiltration membrane materials, for the UF|DF of AAV8-containing clarified lysate. To test their performance, we analyzed the AAV total particle recovery, permeate flux behavior during the TFF concentration and diafiltration process, and the removal of impurities (total protein and DNA).
To test the scalability of the results , we transferred the best-performing membrane to a larger volume TFF system (Sartoflow® Smart with Sartocon® Slice 200 cassette).

Materials and Methods

The full materials and methods can be found in Mendes et al (2024)[7] and are summarized below. The experimental setup is summarized in Figure 1.

Figure 1: *Study Workflow*



AAV8 Production

AAV8 was produced in a 10 L stirred-tank bioreactor (STB). Suspension HEK293T cells were triple transfected with pRC8 (produced in-house from an Escherichia coli clone and purified using ZymoPURE II™ Plasmid GigaPrep [Zymo Research]), pAAV-GFP and pHelper plasmids (PlasmidFactory).
At 72 hours post-transfection, cells were lysed, subject to nuclease treatment, harvested, and clarified.

High-Throughput TFF Process Development Using the Ambr® Crossflow System

The Ambr® Crossflow system comprises a high-throughput TFF processing unit that enables parallel operation with four independent crossflow channels per module, with up to four modules managed by one control station. A predefined recipe was run for each experiment.

A full factorial DoE was designed with MODDE® and performed using Ambr® Crossflow at a feed flow rate (QFeed) from 50 to 75 mL/min, and TMP from 600 to 900 mbar for 30 kDa devices and from 300 to 600 mbar for 100 kDa devices. All experiments were conducted using the same initial total loading volume of 70 mL (67.7 mL of feed AAV8 stock + 2.3 mL of buffer within the system hold-up) followed by a 10-fold concentration (final retentate volume of 7 ml) and five volumes diafiltration (with buffer 10 mM Bis-Tris propane, 700 mM NaCl, 0.003% poloxamer 188, 1% sucrose, pH 6.9 ± 0.2).

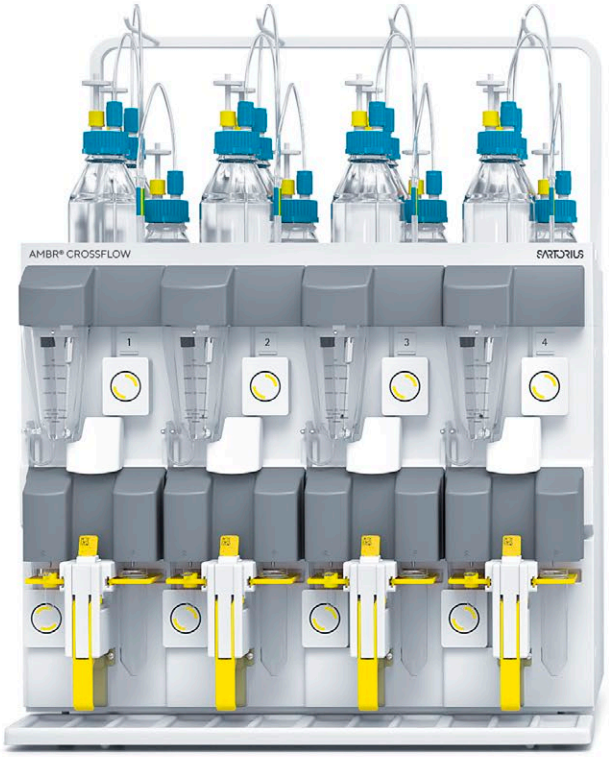
Transfer to the Sartoflow® Smart

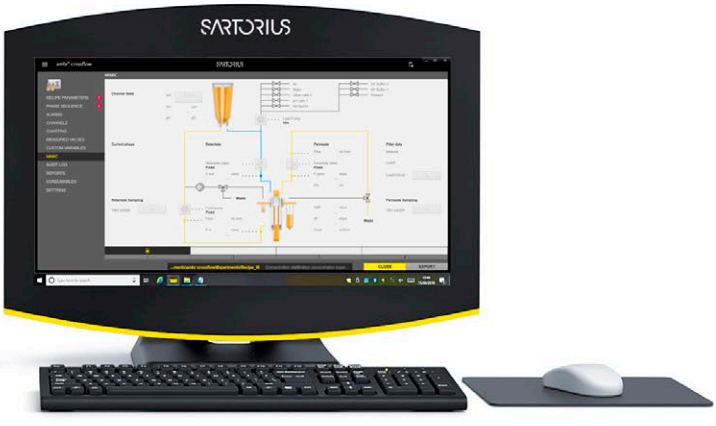
The best performing TFF cassette (100 kDa Material B Sartocon® Slice 200 E-screen with an effective filter area of 180 cm²) was evaluated on the larger scale Sartoflow® Smart TFF system.

The initial feed volume was 1 L for 100 kDa Material B Sartocon® Slice 200 cassette. After UF | DF with a 10-fold concentration and with five diafiltration volumes of buffer was performed to increase product recovery.

Analytical Methods

The determination of total AAV particle concentration (TP) was carried out with a conformational AAV ELISA assay (Progen Biotechnik GMBH) according to the manufacturer's instructions. The total protein content was quantified using a BCA Protein Assay Kit (Thermo Fisher Scientific), and total ds-DNA was quantified with a Quant-iT™ Picogreen® dsDNA assay kit (Invitrogen).





Results and Discussion

Small-Scale Screening Experiments

The Ambr® Crossflow was used to perform high-throughput screening of TFF conditions using Material A and Material B (with MWCO of 30 and 100 kDa) Ambr® crossflow filters of 10 cm² membrane area. We first defined the feed flow rate and optimal TMP values to maximize permeate flux without causing excess polarization or fouling. The results were used to design and perform a full factorial DoE. Seven experiments were performed for each device. Total particle recovery, average permeate flux, total protein removal, and total DNA removal were evaluated at the end of the TFF operation (Table 1).

The results for each MWCO are described below.

30 kDa Flat Sheet Filter

Overall, the Material B membrane performed better than Material A membrane for total particle recovery and DNA removal at any combination of TMP and QFeed, but permeate flux was 2-fold higher in Material A than the Material B membrane. Total protein removal was similar in both membrane types.

The highest total particle recovery was achieved with the Material B membrane (97%) at 75 mL/min QFeed (high) and 600 mbar TMP (low).

100 kDa Flat Sheet Filter

The performance of both membranes is similar for total particle recovery, permeate flux, and total protein removal. However, the Material B membrane achieved a slight increase in DNA removal compared to the Material A membrane (Table 1).

The highest total particle recovery was obtained with the Material B membrane (93%) at 75 mL/min QFeed (high) and 300 mbar TMP (low).

The DoE data was further analyzed with MODDE® using the membrane type (Material A and Material B) and the MWCO (30 kDa and 100 kDa) as additional factors (Figure 2). The results show that the best performance in terms of protein and DNA removal and average flux was achieved with 100 kDa Material B membrane at low TMP and high QFeed ranges. For total protein removal, the best condition was with the 30 kDa Material B membrane at mid-low TMP.

Table 1: *DoE Results for Small-Scale Experiments*

			Material B				Material A			
MWCO [kDa]	QFeed [mL/min]	TMP [mbar]	TP recov. [%]	Average permeate flux [LMH]	Protein removv. [%]	DNA removv. [%]	TP recov. [%]	Average permeate flux [LMH]	Protein removv. [%]	DNA removv. [%]
30	50	600	89	23	74	33	86	49	81	13
	50	900	86	26	77	35	74	36	82	19
	75	600	97	27	75	22	69	55	81	25
	75	900	85	32	78	30	80	54	81	18
	62.5	**750**	**95**	**23**	**78**	**35**	**82**	**46**	**80**	**19**
	62.5	**750**	**92**	**27**	**101**	**31**	**88**	**54**	**81**	**23**
	62.5	**750**	**93**	**25**	**78**	**23**	**91**	**56**	**73**	**37**
100	50	300	85	46	90	69	84	50	84	53
	50	600	77	30	83	41	79	36	81	42
	75	300	93	63	88	72	85	66	84	57
	75	600	80	38	87	52	86	43	77	37
	62.5	**450**	**83**	**50**	**83**	**43**	**86**	**45**	**79**	**49**
	62.5	**450**	**80**	**52**	**86**	**57**	**82**	**41**	**77**	**39**
	62.5	**450**	**88**	**46**	**86**	**55**	**77**	**45**	**79**	**47**

Note. Parameters were total particle recovery (TP recovery), average permeate flux, total protein removal, and total DNA removal using the 30 and 100 kDa Material A and Material B flat sheet membranes with TMP and QFeed as factors. Center point values are highlighted in bold.

However, we found that the total protein removal model was poorly fitted, likely because removal levels higher than ~70% were observed for all conditions tested. As a result, this response was not considered when deciding on the best-performing membrane and condition. Based on these findings, we selected the 100 kDa MWCO Material B flat sheet membrane, 75 mL/min QFeed, and 300 mbar TMP as the best combination of factors for further development and transfer to larger volume processes.

Transfer of Optimized TFF Operations to the Sartoflow® Smart

The developed TFF process was transferred to the Sartoflow® Smart TFF system to verify the optimal cassette identified during small-scale experiments. Through flux characterization experiments, we identified optimal QFeed and TMP to continue further.

Our findings identified the following parameters:

- 100 kDa Material B Sartocon® Slice 200 E-screen flat sheet cassette–QFeed = 260 mL/min, and TMP = 600 mbar

We then performed three independent larger-scale experiments on the Sartoflow® Smart. Figure 3 shows the permeate flow rate, TMP, volume of retentate, and permeate plotted against process time (concentration and diafiltration phases). When analyzing functional read-outs, reproducible results were obtained, with Coefficient of Variation (CV) between 1 and 6% regardless of the parameters evaluated. The only exception was DNA removal, which had a higher CV than observed in small-scale experiments (i.e., 18% CV).

Performance results achieved in the 180 cm² flat sheet cassette were TP recovery of 91 ± 2%, permeate flux of 37 ± 1 LMH, total protein removal of 79 ± 1%, and dsDNA removal of 36 ± 6%.

Figure 2: *Contour Plots of (A) Total Particle Recovery, (B) Average Flux, and (C) DNA Removal*

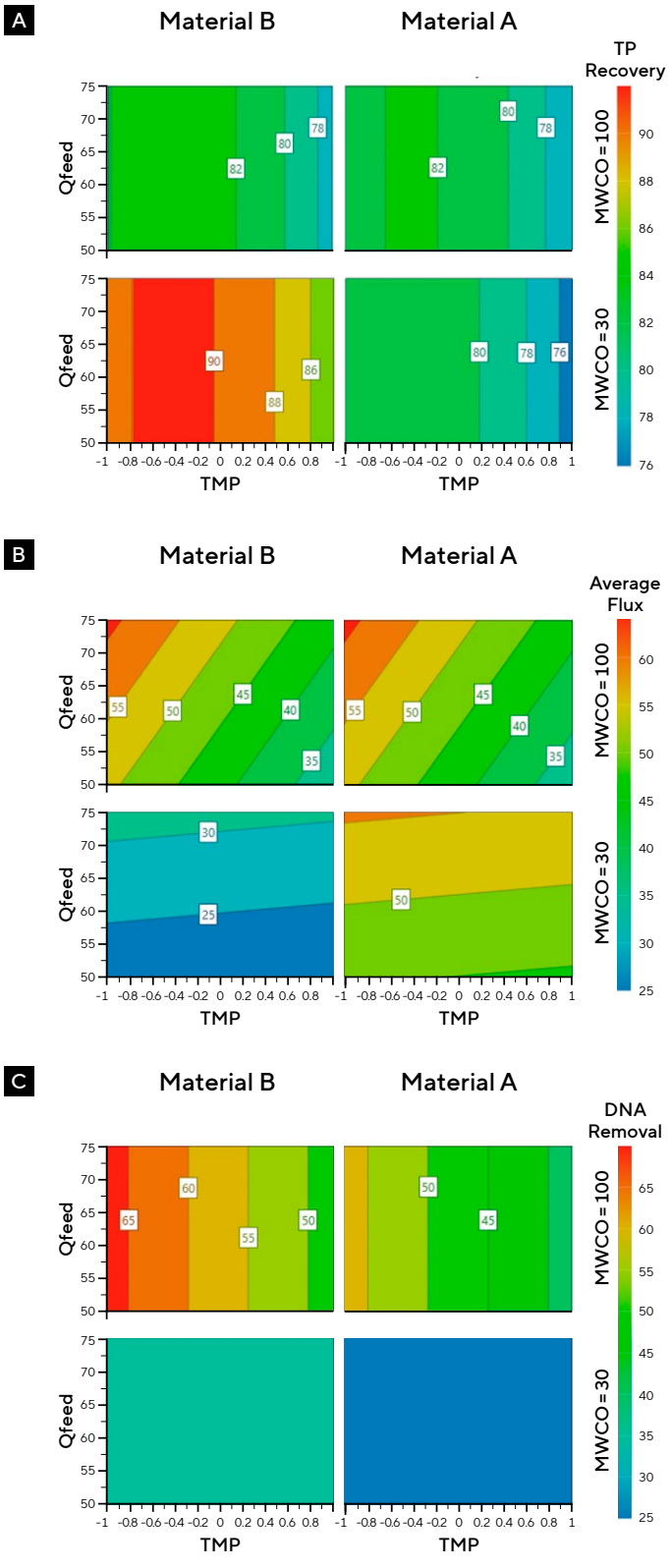
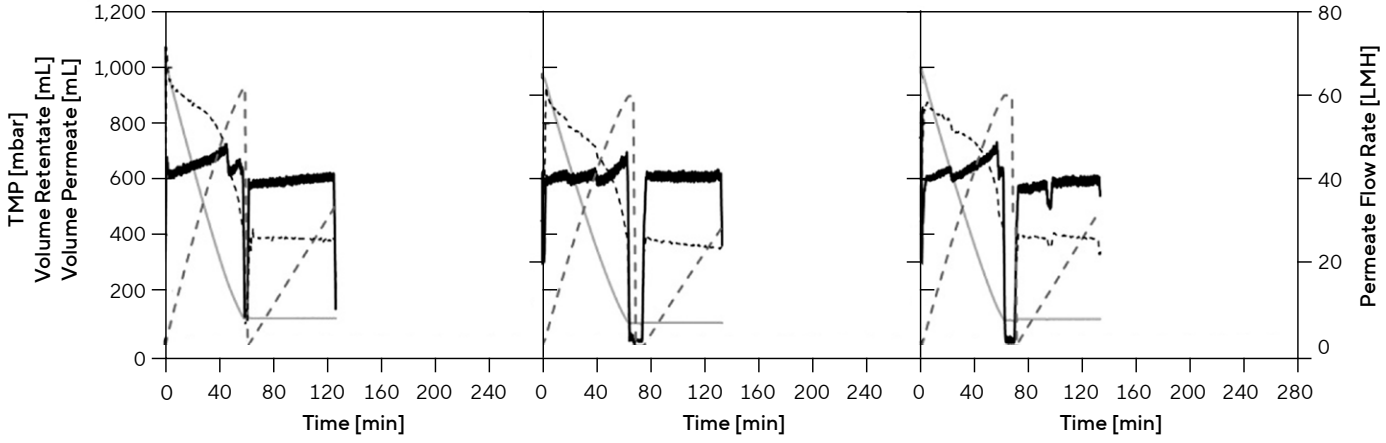


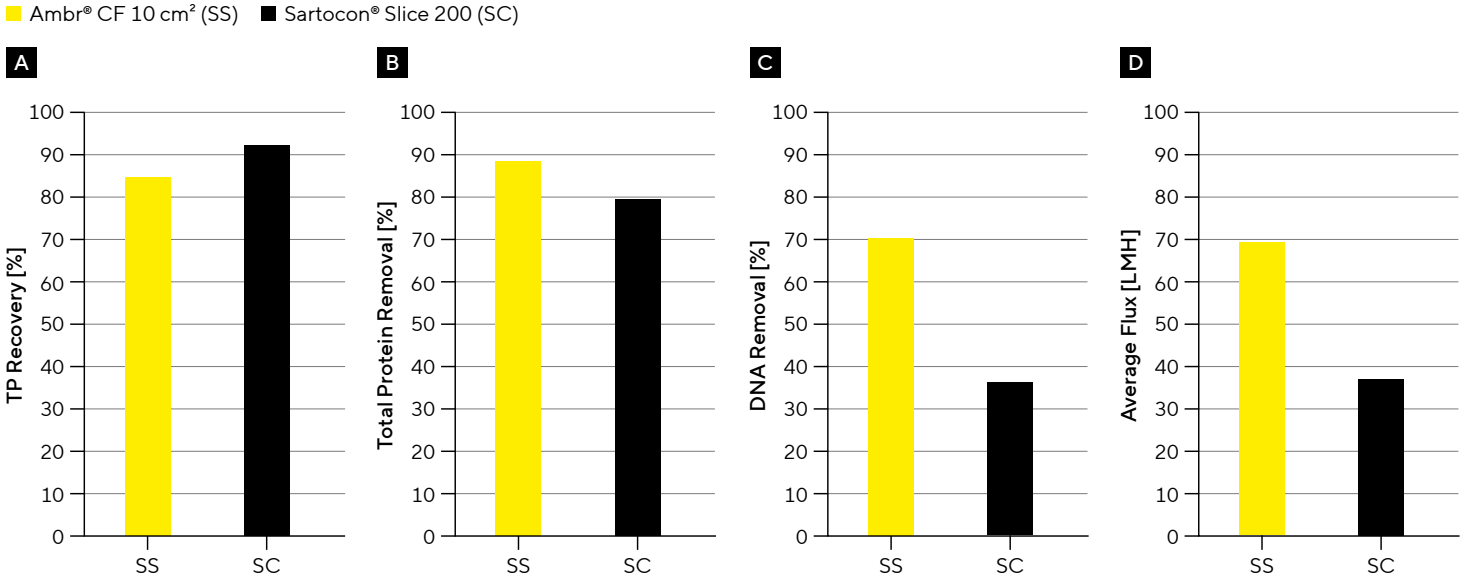
Figure 4 shows a comparison of the performance results between the 100kDa Material B membrane across all devices and scales evaluated performing three independent replicates each. The small-scale Ambr® Crossflow device performs similarly to the 100 kDa Material B Sartocon® Slice 200 in terms of total particle recovery and total protein removal. However, both total dsDNA removal and average permeate flux were significantly lower for the 100 kDa Material B Sartocon® Slice 200, indicating a possibility for further development of the operation at a larger scale.

Figure 3: *Profiles of Permeate Flow Rate, TMP, Volume of Retentate, and Volume of Permeate as a Function of Process Time for 100 kDa Material B Sartocon® Slice 200 Flat Sheet Cassette*



Note. Three independent experiments were performed. Permeate flow rate (black dashed line), TMP (full black line), volume of retentate (grey full line) and volume of permeate (grey dashed line)

Figure 4: *Comparison of Parameters Across Devices and Scales*



Note. **(A)** Total particle recovery, **(B)** total protein removal, **(C)** dsDNA removal, and **(D)** average permeate flux for 100 kDa Material B Sartocon® flat sheet cassettes.

Conclusion

The data presented here demonstrate that the Ambr® Crossflow system can be successfully used in the development of ultrafiltration and diafiltration steps for AAV processing. Its advanced multi-parallel processing capabilities, supported by MODDE® software, enabled the fast and simple evaluation of the impact of different membrane materials and MWCO on AAV recovery, process time, and impurity removal. Only small volumes of material are needed to perform screening experiments, avoiding the depletion of expensive and often limited product.

As AAV feed streams can vary based on the serotype used and the upstream production method applied, optimal TFF conditions, like filter membrane material and cut-off need to be identified for each AAV product. We present a simple, time- and material-efficient method, that combines high-throughput TFF system capabilities with advanced data analytics software to rapidly develop the UF|DF step of AAV.

Crucially, the transfer of the best-performing membrane candidate from the Ambr® Crossflow system to the larger bench-top crossflow filtration system Sartoflow® Smart showed consistent results. Overall, this TFF process development strategy enabled the fast selection of reliable ultrafiltration consumables at an early stage, which contributes to faster AAV process development timelines.

For more information about End-To-End AAV Gene Therapy Solutions, visit
sartorius.com/en/applications/cell-and-gene-therapy/gene-therapy/aav-gene-therapy

References

1. Arunkumar, A., & Singh, N. (2021). Ultrafiltration behavior of recombinant adeno associated viral vectors used in gene therapy. Journal of Membrane Science, 620, 118812. https://doi.org/10.1016/J.MEMSCI.2020.118812
2. Fernandez-Cerezo, L., Rayat, A. C. M. E., Chatel, A., Pollard, J. M., Lye, G. J., & Hoare, M. (2019). An ultra scale-down method to investigate monoclonal antibody processing during tangential flow filtration using ultrafiltration membranes. Biotechnology and Bioengineering, 116(3), 581–590. https://doi.org/10.1002/bit.26859
3. Fernandez-Cerezo, L., Wismer, M. K., Han, I. K., & Pollard, J. M. (2020). High throughput screening of ultrafiltration and diafiltration processing of monoclonal antibodies via the Ambr® crossflow system. Biotechnology Progress, 36(2). https://doi.org/10.1002/btpr.2929
4. Borujeni, E., Betts, J., Jenke, M., & Pollard, J. (2019). Applications of an Automated High Throughput Crossflow System for Ultrafiltration Process Development to Assess Manufacturability of Monoclonal Antibodies. Sartorius.
5. Fernandez-Cerezo, L., Benner, S. W., & Pollard, J. M. (2021). Streamlining process characterization efforts using the high throughput ambr® crossflow system for ultrafiltration and diafiltration processing of monoclonal antibodies. Biotechnology Progress, 37(3). https://doi.org/10.1002/btpr.3118
6. Gibson, D., Betts, J., Jenke, M., & Lewis, W. (2019). Optimizing Diafiltration Parameters for Maximum Recovery of a Bispecific Antibody Using Low Process Volumes and an Automated High Throughput Crossflow System. Sartorius.
7. Mendes, S.; Faria, T.Q.; Nascimento, A.; Noverraz, M.; Bollmann, F.; Nestola, P.; Roldão, A.; Peixoto, C.; Silva, R.J.S. Accelerated Development of AAV Purification Process Using a High-Throughput and Automated Crossflow System. Separations 2024, 11, 73. https://doi.org/10.3390/separations11030073

The authors would like to thank Sónia Mendes, Tiago Q. Faria, André Nascimento, António Roldão, Cristina Peixoto, Ricardo J.S. Silva from iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2780-901, Oeiras, Portugal **for carrying out these experiments.**

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
sartorius.com/en/applications/cell-and-gene-therapy/gene-therapy/aav-gene-therapy

Status: 10 | 2024