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Efficient Performance of the Sartoflow® Expert SU in ADC Processes

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Abstract

Antibody-drug conjugates (ADCs) represent a significant area of growth in the biopharmaceutical industry. Currently, downstream processing steps are typically carried out as individual unit operations. However, manufacturers require more integrated solutions to make their ADC process more robust.

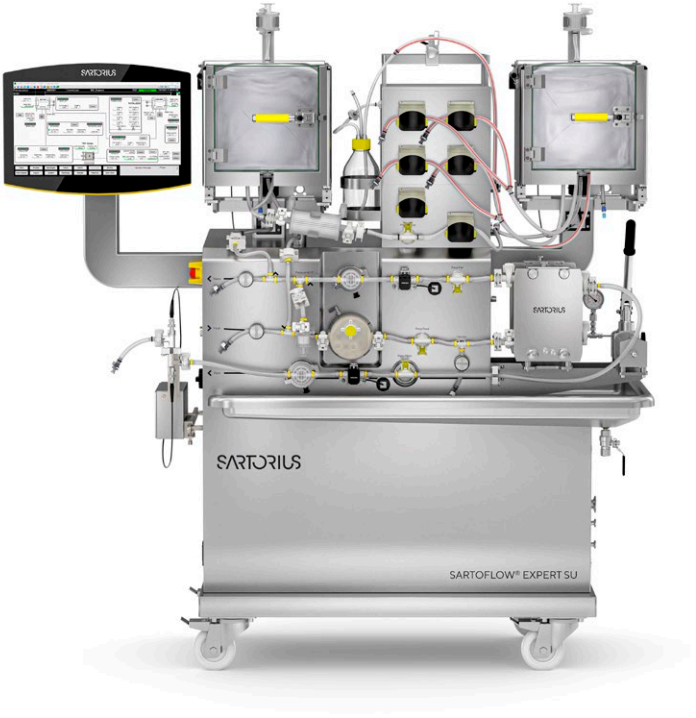
Here, we demonstrate the capabilities of the Sartoflow® Expert SU, which can be configured to perform the incubation, purification, and concentration steps of ADC synthesis. Our results highlight that the Sartoflow® Expert SU represents a highly scalable, productive platform to streamline ADC production processes.

Introduction

Antibody-drug conjugation (ADC) represents a new class of biopharmaceuticals in which monoclonal antibodies (mAbs) are chemically bound to potent anti-cancer agents. Currently, ADC synthesis, precipitate removal, and subsequent TFF are carried out as separate unit operations, requiring time-consuming transfer steps that expose the process to increased risks.

The Sartoflow® Expert SU is a fully automated, single-use (SU) tangential flow filtration (TFF) system with a closed single-use loop. As well as a state-of-the-art TFF system, the Sartoflow® Expert SU can be used as a fully-integrated system for ADC manufacturing. Incubation, purification, and concentration steps of clinical material can be carried out in a closed environment, securing safety for both the operator and the product.

In this application note, we demonstrate the successful performance of the Sartoflow® Expert SU in ADC processes.



Materials

Table 1: *Equipment Used in This Study*

Name	Manufacturer	Lot | Batch No.
Zeba Desalt Spin Columns	Thermo Scientific	89890
Sartorius Sartoflow® Expert SU	Sartorius	

Table 2: *Chemicals Used in This Study*

Name	Manufacturer	Concentration
IgG stock solution from Prot-A eluate	Sartorius	
Feed buffer KPi	Self-manufactured using Roth chemicals	50 mM Kpi, pH 7.0
Diafiltration buffer PBS buffer	Self-manufactured using of Roth chemicals	20 mM sodium-phosphate-buffer, 0.15 M sodium-chloride, pH 7.2
DMSO	Thermo Scientific	
NHS-Fluorescein (46409)		
Quencher: Glycine buffer	Thermo Scientific	200 g/L, pH 8.5

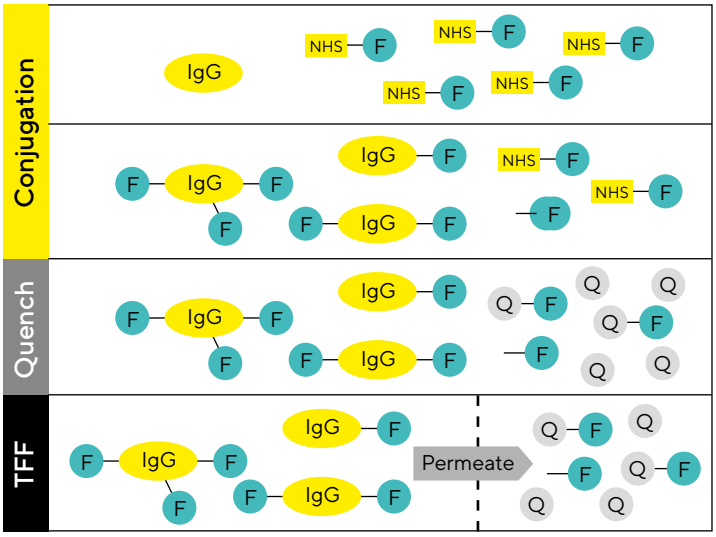
Table 3: *Consumables Used in This Study*

Name	Manufacturer	Part No.
Recirculation - single-use bag	Sartorius	Prototype (DR4196)
Conjugation – single-use bag	Sartorius	Prototype (DR4196)
Feed single-use flow kit	Raumedic AG	Prototype (of SFE-TUB-FEED)
Permeate single-use flow kit	Raumedic AG	Prototype (of SFE-TUB-PERM-RET)
Retentate single-use flow kit	Raumedic AG	Prototype (of SFE-TUB-PERM-RET)
Precipitate filtration single-use flow kit	Raumedic AG	Prototype (of SFE-TUB-PRECIFIL)
Hydrosart® (30 kDa) Self-contained units for TFF (0.28 m^2)	Sartorius	08103123 – test cassette
Sartolon® Size 9 – 0.2 m^2, 0.2 µm	Sartorius	5105307H9-FF-A

Methods

This application note describes the typical use case of ADC-mAb processing. We accomplished this using a surrogate system with NHS-fluorescein as the linker-payload and a mAb as a carrier. Process conditions were parametrized according to customer process data. During the test, ~40 g of mAb were processed in a conjugation volume of 3.8 L.

Figure 1: *ADC Manufacturing Process*



Scale up to Sartoflow® Expert SU

Conjugation, quenching, sterilizing-grade filtration during transfer, concentration, diafiltration, and final concentration were performed at a larger scale on the Sartoflow® Expert SU.

The IgG solution was transferred into the conjugation tank, and the dye was then transferred to initiate the conjugation. Samples were collected every 15 mins for 1 hour. The reaction was stopped by adding quencher solution. It was then transferred to the recirculation bag through a Sartolon® size 9 sterile-grade filter (0.2 µm) capsule for precipitate removal.

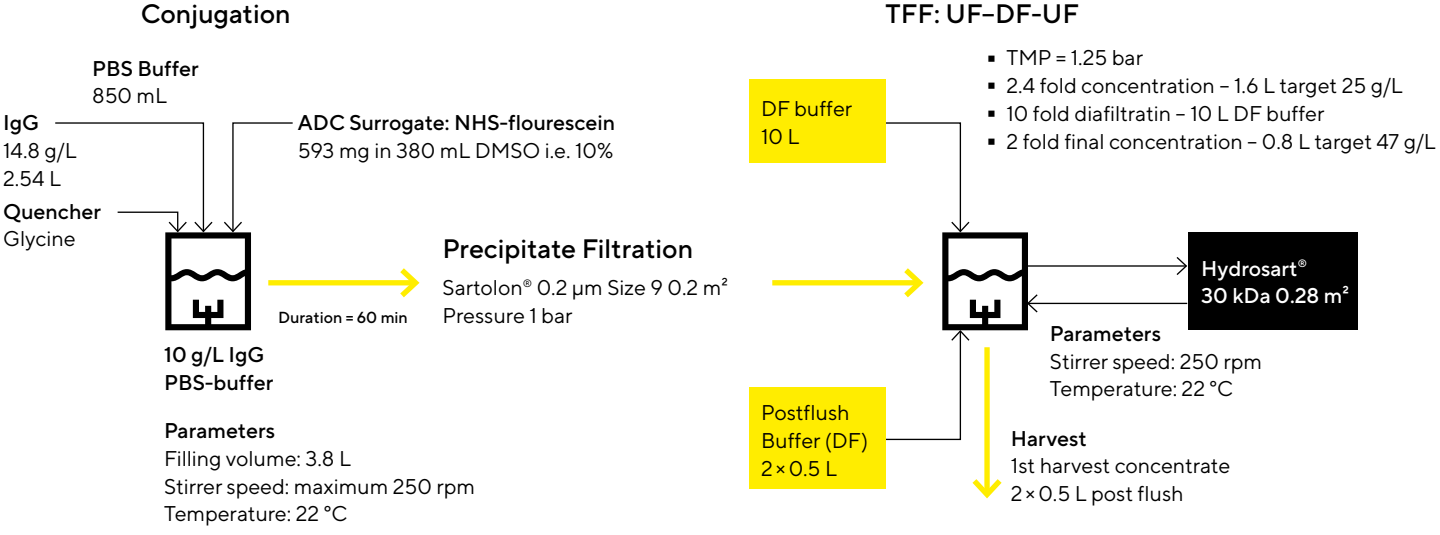
The subsequent TFF purification was performed using a Hydrosart® 30 kDa TFF cassette with a 0.28 m² filter area. During the initial concentration, the volume was reduced approximately 4×, followed by a 10×diafiltration and a final 2× concentration. Two post flushes and harvest steps | phases were performed.

The solute dynamics were examined using pH, conductivity, A280, and A493 measurements of the permeate stream during TFF filtration. The protein solution, harvest pool, and post flush pool facilitated the calculation of recoveries and final dilution factors.

Temporal Progression of the Degree of Labeling (Small Scale Experiments)

To establish the temporal progression of the dye labeling reaction, we performed a small-scale conjugation using IgG and NHS-fluorescein. The test solution was prepared with a 5:1 molar dye: protein ratio, and the degree of labeling was measured at 0, 15, 30, 45, and 60 min.

Figure 2: *Schematic Illustration of the Experiment on the Sartoflow® Expert SU System.*



Results

Investigation of Buffer Exchange Rate During Sartoflow® Expert SU Diafiltration

Dissociation of the payload – mAb bond during diafiltration is often hard to distinguish from apparent microsolute retention. We performed the following test to evaluate the course of buffer exchange during diafiltration independent of this effect.

A 5 g/L BSA solution was prepared with a volume of 3.7 L. Instead of the dye NHS-fluorescein, 200 g NaCl were added directly to the protein solution. Further, 0.5 L DMSO and 0.8 L quencher solution were added directly to the protein solution, resulting in a final volume of 5 L.

The test solution was then pumped into the conjugation bag of the Sartoflow® Expert SU and transferred through the Sartolon® size 9 sterile-grade filter capsule to the recirculation bag.

The subsequent TFF was performed using a Hydrosart® 30 kDa TFF cassette, with a filter area of 0.28 m². During the initial concentration, the volumes were reduced approximately 4×, followed by a 10× diafiltration. Determination of the permeate stream conductivity at regular intervals facilitated precise tracking of the retention-free buffer exchange rate.

Conjugation Analytics

To remove the non-reacted dye, the quenched sample and PBS were slowly added to a prepared and equilibrated SEC spin column (Thermo Scientific Zebra Desalt Spin Columns 89890). After purification, as per the manufacturer's instructions, the samples were diluted 1:20 before photometric determination of A280 and A493.

The following equation was used to calculate the protein concentration:

$$\text{Protein concentration (M)} = \frac{A_{280} - (A_{493} \times 0.3)}{\varepsilon_{protein}} \times \text{dilution factor}$$

To calculate the degree of labeling, we used the following equation:

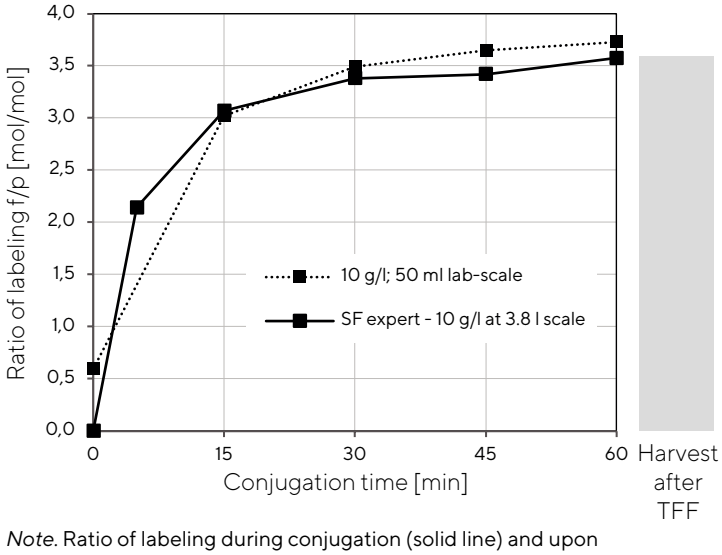
$$\text{Mol dye per mol protein (M)} = \frac{A_{493}}{\varepsilon_{dye} \times \text{Protein concentration (M)}} \times \text{dilution factor}$$

Temporal Progression of Labelling

We first measured the degree of labeling over time by performing the conjugation on the Sartoflow® Expert SU at two different scales (50 mL and 3.8 L). NHS reactivity is time limited and influenced by various factors. Therefore, controlling the reaction conditions and molar ratios is critical for scale-up reproducibility.

The degree of labeling achieved during the scaled-up experiment is shown in Figure 3.

Figure 3: *Progression of the Degree of Labeling in Mol Dye per Mol Protein During the 60 Min Conjugation Reaction Between NHS-Fluorescein and HAS.*



Note. Ratio of labeling during conjugation (solid line) and upon final harvest (bar chart) on Sartoflow (SF) expert 3.8 L scale and in a scaled-down experiment (dashed line).

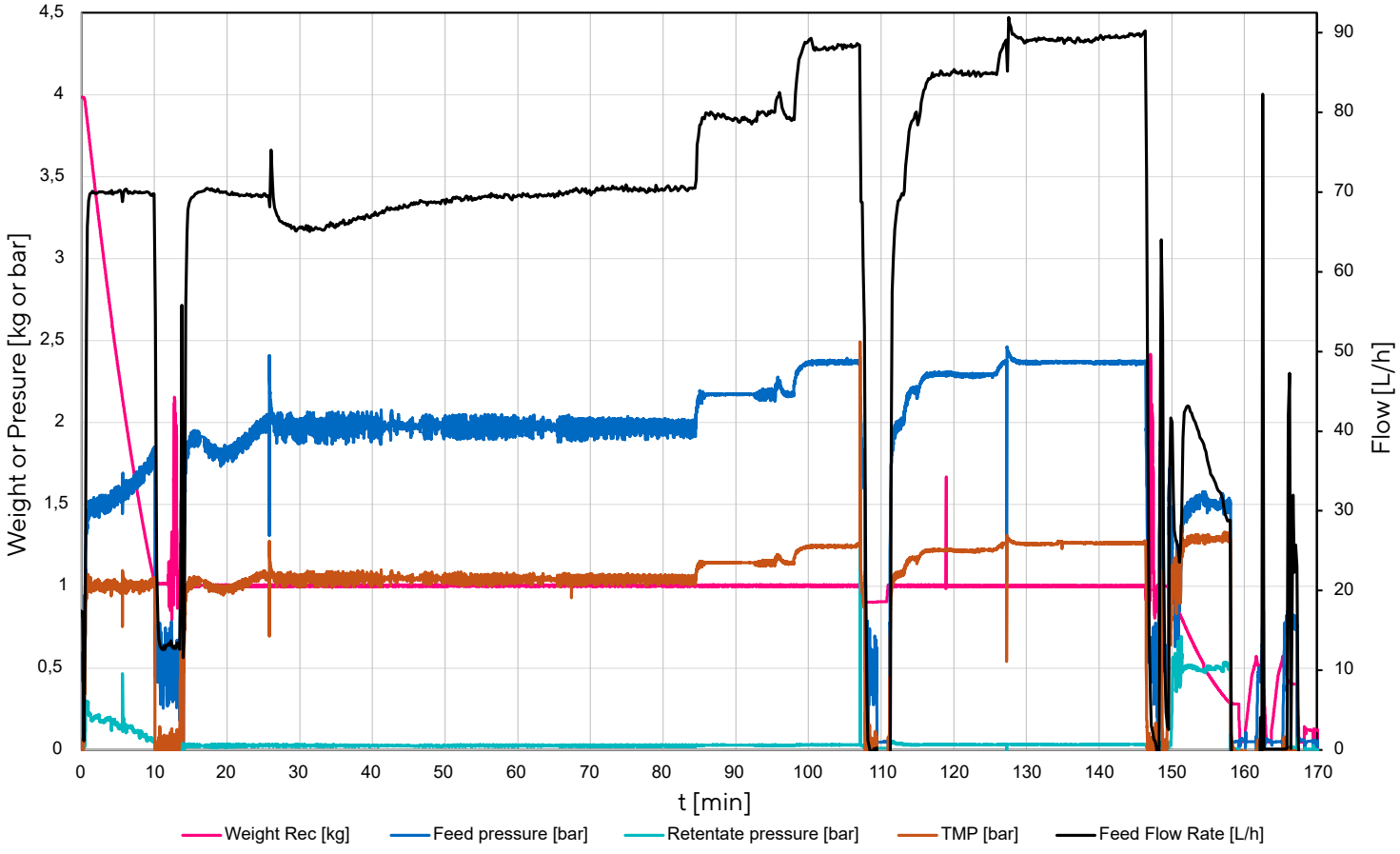
The progression of the scaled-up conjugation reaction using the Sartoflow® Expert SU was almost identical to lab-scale experiments (Figure 3), demonstrating a successful transition between scales.

After quenching the reaction at 60 min, the ratio of labeling was 3.6 (mol/mol). This remained unchanged during the subsequent concentration and purification steps, yielding 3.6 mol/mol in the final harvest.

TFF Performance on Sartoflow® Expert SU

To evaluate the performance of the downstream TFF process, we determined volumetric flow rates and pressures during the concentration, diafiltration, and final concentration steps (Figure 4).

Figure 4: *Relevant Volumetric Flow Rates and Pressures During TFF Processing on Sartoflow® Expert SU.*



Note. The start of the first concentration step was set as the 0 min time point. The harvest step included two 500 mL post flushes.

During the initial concentration step from 4 L to 1 L, the feed flow was controlled at 70 L/h. The retentate valve was set to TMP control with a setpoint of 1 bar. As shown in Figure 4, the TMP was successfully kept at the target level. As the protein concentration increased, the backpressure also increased, causing the feed pressure to increase slightly from 1.5 bar to 1.8 bar. As a result – to keep the TMP constant - the retentate valve opened fully, leading to the drop of pressure from 0.25 bar to atmospheric pressure.

The protein concentrations increased from 9 g/L to 25 g/L, representing a 2.8× concentration factor. Factoring in the estimated TFF loop void volume of ~0.6 L, the estimated actual concentration factor by weight was 2.9×, closely matching the 2.8× protein concentration factor.

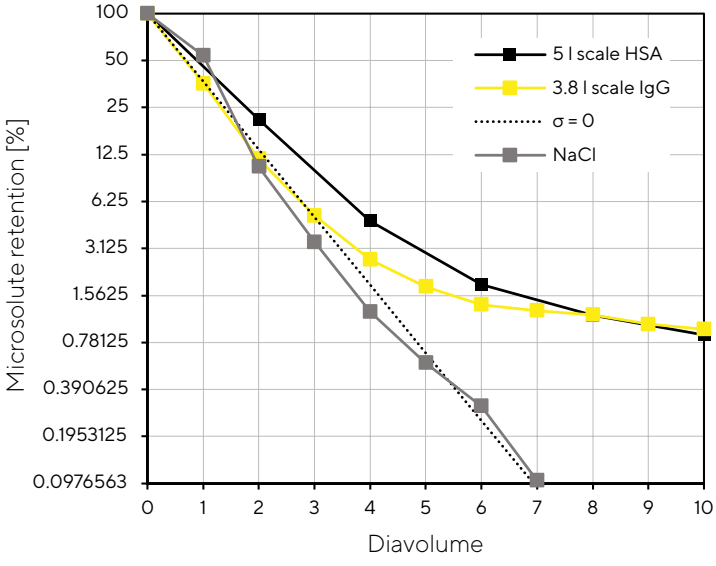
The process parameters of the initial concentration steps were also adopted for the diafiltration. As feed pressure reached (but did not exceed) the setpoint limit of 2 bar, the feed pump only temporarily reduced its flow rate setpoint of 70 L/h at ~30 min.

The diafiltration performance indicated further capabilities. Therefore, we incrementally increased the setpoint feed pressure to 2.4 bar after the 85 min mark to allow more feed flow, and increased the feed rate setpoint to 90 L/h.

At the 108 min time point, we tested the error response to insufficient diafiltration buffer addition rate. By limiting the max-addition rate of the diafiltration buffer below the permeate flow rate, the net-retentate volume decreased gradually. The resulting deviation from the filling setpoint led to the automatic closure of the permeate valve and gentle recirculation at 0.5 bar feed pressure. In this standby state, the operator was prompted to acknowledge the automated remediation. Once confirmed, the filling level was automatically restored by adding diafiltration buffer, and the diafiltration was resumed as expected. Recirculation during the standby state prevented an irreversible membrane fouling, confirmed by the unchanged flows and pressures before and after the event.

Conjugated IgG and free dye were monitored during the concentration and diafiltration steps. Figure 5 shows the free dye percentage present in the retentate as a function of permeate stream A493 over the course of the diafiltration, where we observed gradual breakage of the protein-dye bond.

Figure 5: *Percentage Free Dye Retention*



Note. Percentage free dye retention over the course of the diafiltration for the IgG (yellow), the course of a previous experiment with conjugated HSA and NHS-fluorescein under identical molar ratios and 5 L scale (black), solute retention control experiment by tracking conductivity in NaCl containing buffer (grey). The dotted line represents the ideal microsolute washout for constant volume diafiltration with the apparent rejection coefficient σ = 0.

For constant volume diafiltration (CVD), the solute concentration can be calculated according to the following equation (Foley G. (2016) Diafiltration Factor. In: Drioli E., Giorno L. (eds) Encyclopedia of Membranes. Springer, Berlin, Heidelberg):

$$c = c_0 e^{-(1-\sigma)Vb/Vs}$$

For a given solute with the apparent rejection coefficient σ ranging from 0 to 1, the retentate concentration can be calculated from the initial concentration c_0 for any given point during the diafiltration. The diavolume (DV) or diafiltration factor is described as V_b/V_s, with Vb being the volume of diluent (buffer) and V_s the volume of the solution (retentate).

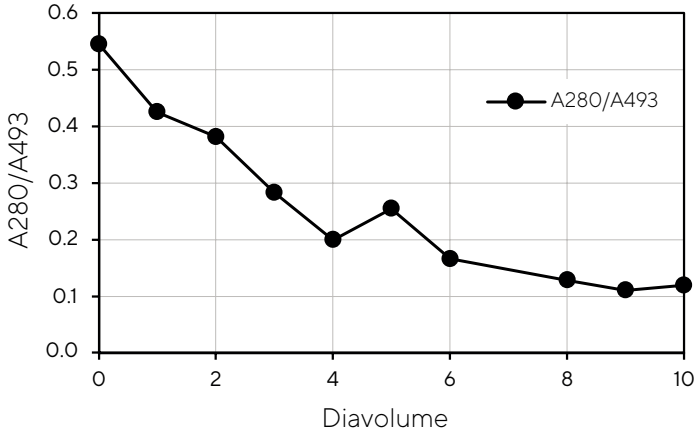
As fluorescein has a molecule size far below the MWCO of the filter, we can assume there is no apparent retention, i.e., σ = 0. This would result in a free dye reduction to ~0.1% after 7 DV and 0.004% after 10 DV.

During our experiments, residual relative retentate concentrations of ~0.8% were observed by the permeate stream A493 after 10 DV for the IgG and HSA experiments. The flattening course of the free dye retention would indicate an increasing apparent retention coefficient that is commonly caused by a decrease in effective pore size due to blockage. However, this was unlikely since retention of the small dye molecules would require such a drastic pore size reduction that the resulting solvent permeability decrease would be clearly noticeable.

The most likely explanation is a gradual breakage of the protein-dye bond. The constant influx of free dye would increase the apparent retention coefficient as the total free dye retentate concentration decreases throughout the diafiltration. To exclude other causes of deviation from the ideal diafiltration course related to the system composition, we further investigated the diafiltration buffer exchange. As shown in Figure 5, no apparent microsolute retention was observed for NaCl. The decreasing percentage of free dye retention closely matched the theoretical course with σ = 0. Hence, fluid dynamic causes like insufficient mixing or diffusive void volume areas (dead legs) can be excluded.

The absorption ratio of 280 nm/493 nm plotted over the diavolumes is shown in Figure 6.

Figure 6: *A280/A493 of the diafiltration permeate stream*



Note. A280/A493 of the diafiltration permeate stream over the course of the 10× diafiltration during the IgG experiment on the Sartoflow® Expert SU

Unconjugated NHS-fluorescein and fluorescein absorb at 280 nm and 493 nm. A280 of the permeate stream signifies clearance of small proteins, protein fragments, and NHS groups that absorb at 280 nm. As these microsolutes are washed out through the permeate during diafiltration, the ratio should theoretically stay constant as it can be assumed that they all demonstrate no apparent retention, i.e., $\sigma = 0$.

However, the ratio of A280/A493 decreased over the course of the diafiltration and approached 0.1 (Figure 6). A decreasing ratio is evidence of changing ratios between dye and substances absorbing at 280 nm. The observed decreasing value that settles at ~0.11 indicates a constant ingress of fluorescein with no NHS group. As the A280/A493 ratio of pure fluorescein is 0.11, we assume that towards the end of the diafiltration, the only detected microsolute present in the permeate stream was fluorescein. Together with the observed apparent free dye retention indicated in Figure 5, these results again speak for a continuous ´leaching´ of previously IgG-conjugated fluorescein by cleaving of the conjugation bond. Therefore, the observed apparent limited purification performance during diafiltration can be solely attributed to the chosen chemical system.

After the purification step by diafiltration, a final 2× concentration step was carried out. In contrast to the previous steps, the feed pump speed was controlled to maintain a constant feed pressure of 1.5 bar. A constant retentate pressure of 0.5 bar was established by retentate valve control.

As seen in Figure 4, the initial maximum feed flow rate of 42 L/h continuously reduced during the concentration to 30 L/h to maintain 1.5 bar feed pressure. After 9 min, the target volume of 800 mL was reached, and the phase was completed. The IgG concentration factor was 2.1×, closely matching the target of 2×.

The initial harvest and post flush fractions were collected separately and weighed to calculate the respective and total yields (Table 4).

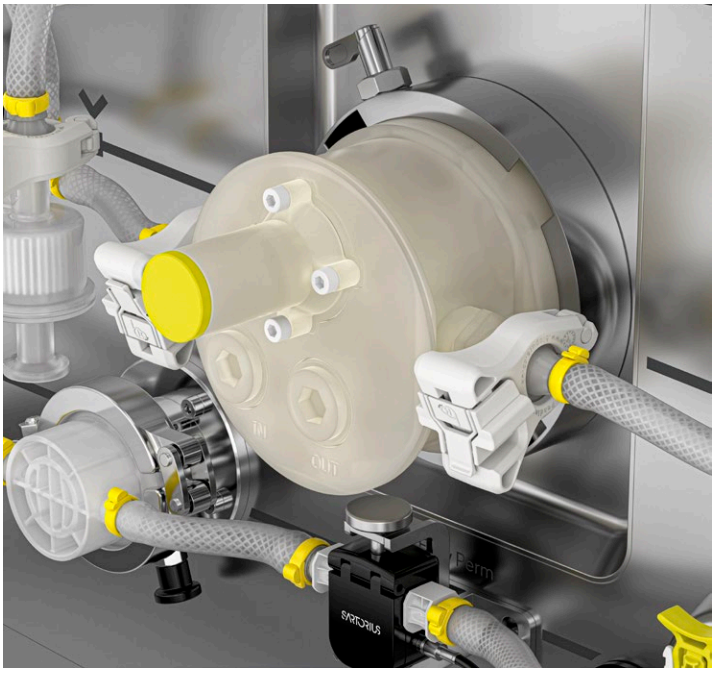
The IgG yield was 81.5% of the initial protein mass, which is relatively low. Previous conjugation experiments using HSA revealed a significant impact of the batch size on the yield. At a 20 L scale, the yield was significantly higher at 97%; at a 5 L scale, the yield decreased to 94%. In this experiment, a final concentration to a total product volume of just 800 mL was performed. Therefore, a blow-down would be advisable. The Sartoflow® Expert SU supports blow-down capability with internally supplied pressured air controlled at 0.25 bar. Thus emptying the retentate line and feed side of the cassette into the recovery port can be carried out conveniently and safely. In a separate experiment, with a total filling volume of 2 L, a yield of 97 % was achieved by the following recovery order: (1) recovery, (2) blow-down, (3) buffer flush, and (4) blow-down.

Table 4: *Yields (Displayed as Percentage Recovery) Calculated From Initial Feed, Harvest, and Post Flush Samples.*

	Volume [mL]	Yield [%]	Concentration [g/L]
Harvest	406	51.5	45.4
Post Flush 1	500.8	23.2	16.6
Post Flush 2	537.3	6.8	4.5
Calculated Total*	**1444.1**	**81.5**	**20.2**

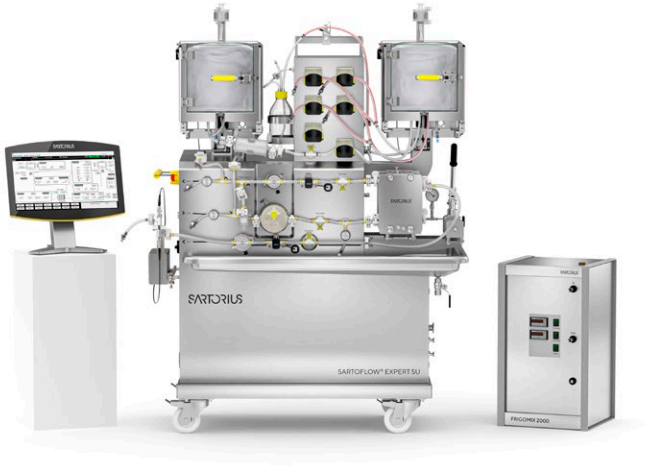
*Total values representing pool of harvest and post flushes calculated

Conclusion



Here, we demonstrate that the Sartoflow® Expert SU enables highly efficient yet flexible TFF performance, supporting stable control of pump speed and reliable error handling to ensure safety, consistency, and traceability. Our results highlight the excellent scalability of the system, which supports a wide range of working volumes (from ~1 L to 20 L) and filter areas (0.14 – 1.4 m^2) and enables the recovery with high yields (~97% for 2 L) aided by blow-down capability.

The ability to carry out mixing and sterilizing-grade filtration on one instrument, as well as the reliable error handling and mitigation capabilities, promote highly safe processing and reduced occupation of floor space. These features represent the core of the risk mitigation concept.



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