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Application Note

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Qualification of an automated, high throughput crossflow system as a scale-down model for ultrafiltration and diafiltration process development

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Abstract

R&D productivity and the associated high costs have been under intense scrutiny for several years now. Automated and high-throughput screening technologies can identify potential issues efficiently and early, and so inform candidate selection, process development decisions and selection of downstream operating parameters. Application areas for parallel small-scale crossflow screening can include formulation development, candidate screening, buffer comparison, protein stability and viscosity trials. Early access to manufacturability information or constraints saves time, streamlines process development and increases process understanding. This application note will present system design and software of a novel, high-throughput crossflow screening system that allows the exploration of candidate behaviour under "stress" conditions such as high concentration and viscosity, and within the design space of crossflow operations. Four different case studies using model protein solutions examine the potential of the screening system: Variation in flux and protein concentration, exploration of flux behavior at different transmembrane pressures, the preparation of a highly concentrated solution and the effect of diafiltration buffer on process performance. These studies demonstrate how these and similar results can contribute to increased R&D productivity through early stage characterization and optimization. Screening results identified, for example, the impact of buffer choice on flux, and showed a relative order of diafiltration flux and stability.

Introduction

Over the past decade, small scale, high throughput, single-use bioreactors such as the ambr® technology have revolutionized upstream bioprocess research and development. Such systems allow scientists to screen, select and develop optimal cell culture conditions and scale-up strategies in weeks instead of months. At present however, there are a limited number of systems available to bring the same benefits for downstream processing (DSP). In particular, as the crossflow filtration unit operation typically sits at the end of a DSP train, a significant investment of time and resources is needed to generate material for ultrafiltration | diafiltration studies. This means the ability to predict how a biological will behave during crossflow filtration at scale is currently limited, yet these processes also have a significant impact on molecular stability and bioprocessing efficiency.

To address this issue, Sartorius Stedim Biotech (SSB) has developed the ambr® crossflow system to assist downstream process development scientists in assessing the manufacturability of biologics. The system can be used as a ranking tool, to optimize process parameters such as buffer type, buffer volumes, pH and conductivity. The ambr® crossflow system can also be used to determine the effect that different process conditions including TMP or flowrate have on the final product formulation.

Manufacturability of a protein-based drug candidate is highly influenced by protein stability, which is itself affected by a number of factors, including buffer composition, conductivity and pH used during product formulation. Poor protein stability can result in a range of issues including fragmentation | aggregation and ultimately may impact on product quality, safety or efficacy. Ideally, to determine the manufacturability of a protein-based drug candidate these factors need to be assessed during the earliest phases of product development. However, at this stage, availability of material with which to perform these types of experiments can often be limited and therefore there is a constraint as to how much of this data can be determined experimentally.

System Design

The ambr® crossflow system is an automated, high throughput solution for the parallel screening of crossflow conditions and works with ambr® CF single-use filter cassettes with a membrane area of 10 cm^2. The system uses low process volumes with a minimum 5 mL recirculation volume. Each ambr® crossflow module consists of four independent crossflow channels which are fully integrated and controlled by simple to use software (Figure 1). Scientists can expand the system to match their research demands with four, eight, 12 or 16 channels allowing them to perform up to 16 crossflow trials simultaneously.

Figure 1: ambr® crossflow system of four independent crossflow channels and integrated software

The ambr® crossflow is designed with multiple, small scale channels; each fully equipped complete with feed bottles, pumps, stirrers, pH probes and a single-use ambr® CF filter to make the system similar to any traditional bench scale crossflow filtration set-up. (Figure 2). The system is fully automated, but each channel is controlled independently in terms of input product | buffer streams and process conditions, such as recirculation rate, pressure, load volume, diafiltration set point and harvest volume. In addition, the ambr® CF single-use cassette has been designed for high viscosity solutions, allowing researchers to explore a large experimental design space even at small scale operation. Being able to study the impact of process parameters, buffer types and protein set-points could for example, allow scientists to determine if their protein-based drug candidates can be formulated at a high concentration for more cost-effective production, and whether shear forces will affect the product quality.

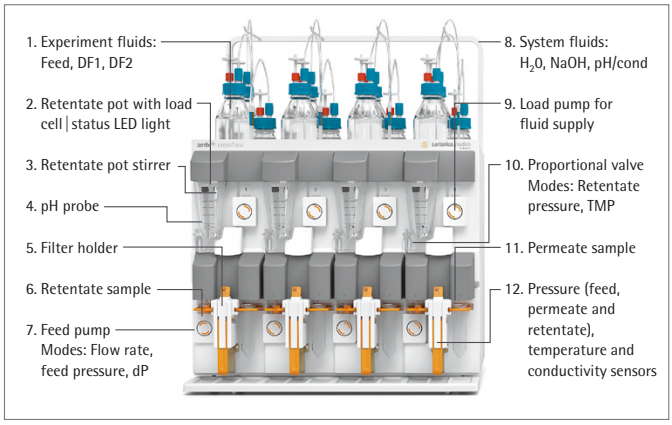

Figure 2: ambr® crossflow detailing the system's components

The ambr® CF filter sits inside a 10 cm^2 single-use cassette (Figure 3), which can be clipped into and out of a filter holder in seconds. Scientists can choose single-use cassettes in a range of pore sizes and either a Hydrosart® (regenerated cellulose) or Polyethersulfone (PESU) filter material, enabling them to compare membrane materials and molecular weight cut-off (MWCO) for their samples. With the single-use cassette, scientists do not need to perform laborious experimental set-up and cleaning processes, which can affect filter performance. Using a filter which is quality controlled and has not been used for a number of different runs, makes it easier to compare results of parallel trials and reduces development time. Additionally, staff require less training and support as the filters don't require cleaning and validation for each crossflow run.

Figure 3a: ambr® CF filter cassette and Figures 3b and c showing easy installation of the cassette into the ambr® crossflow system

System Software

The ambr® crossflow software makes it easy to set independent parameters for each crossflow channel by adding template phases of the process. Each phase consists of individual steps that have default settings but can also be configured by the user. Scientists simply drag and drop phases from the right-hand panel of the software screen (Figure 4) to build their automated set up sequence to include activities such as Integrity Testing the system; pH probe calibration; pump calibration and filter flushing plus water flux and air integrity testing.

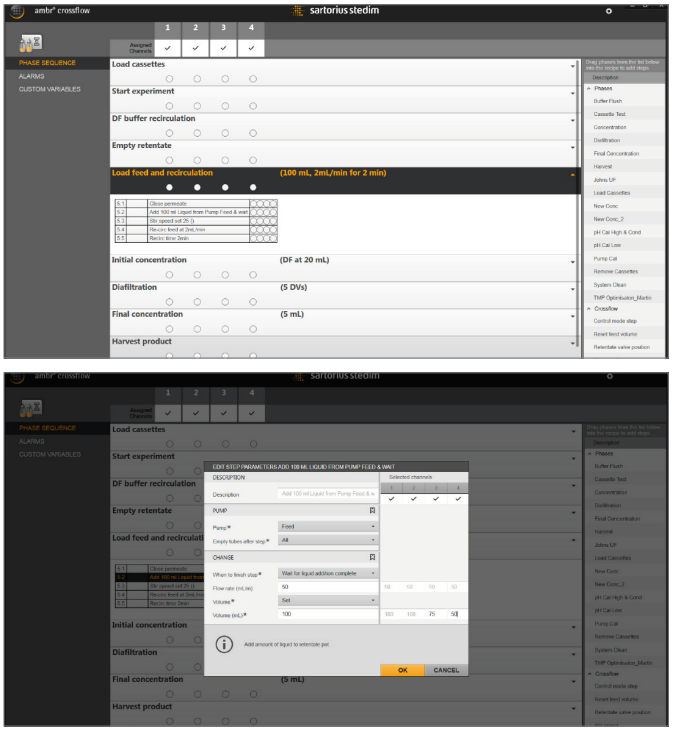

Figure 4: Phase editing screen of ambr® crossflow software with drag and drop menus

The software is pre-set to log data every five seconds from the ambr® crossflow system during a run and can automatically plot data from up to 16 channels simultaneously, allowing scientists to collect data on parameters such as retentate weight, volumes, flux, flowrate, TMP and pH (Figure 5) from each channel in parallel.

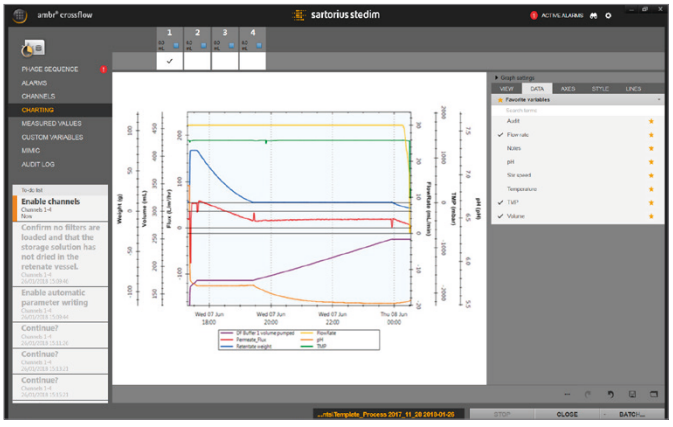

Figure 5: Data collection and charting screen of ambr® crossflow software

At the end of a crossflow run, the software can be set to automate activities including product harvest, buffer rinse and second harvest, and upon removal of the cassette, an automated system clean-in-place (CIP) procedure.

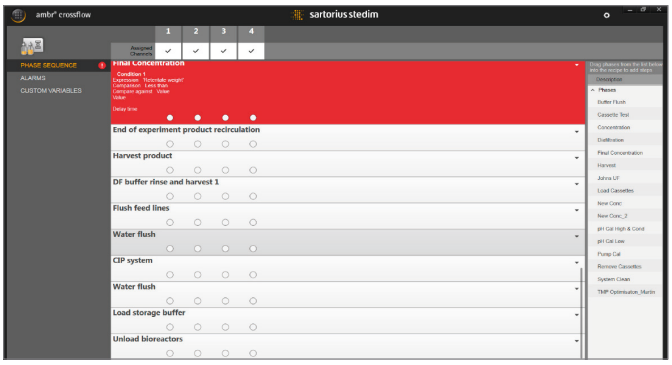

Figure 6: Phase sequence screen of the ambr® crossflow software displaying the standard end of experiment activities to flush and clean the system

System Performance

This section of the application note describes how scientists at SSB have tested the ambr® crossflow's ability to automate parallel crossflow filtration experiments to determine if the system is suitable for use as a scale-down method for assessing the manufacturability of protein-based biopharmaceutical candidates. The four studies described have tested channel-to-channel variability, flux characterization, protein concentration and buffer ranking capabilities of the ambr® crossflow.

Materials and Methods

In experiment one, a channel-to-channel variability study was carried out to determine the reproducibility of flux and protein concentration results across four different channels of the ambr® crossflow system. Four ambr® CF filter cassettes containing a 30 kDa Hydrosart® filter were loaded with a pre-filtered Bovine Serum Albumin (BSA) test protein at an initial loading concentration of 20 g | L. The system was run in parallel at a fixed feed rate (10 mL | min) and TMP 1.5 bar. The flux rate was monitored by the ambr® crossflow system and the final protein concentration was determined after the crossflow filtration by UV280 measurement.

In experiment two, a flux characterization study was performed with the ambr® crossflow system and ambr® CF filter cassettes containing a 10 kDa Hydrosart® filter. The filters were loaded with a BSA test protein at 20 g | L concentration. Five different TMPs ranging from 0.25 – 2 bar and three crossflow rates of between 2.5 – 10 mL | min were tested using the ambr® crossflow system. The flux rate data was collected and plotted automatically by the ambr® crossflow system.

The ability of the ambr® crossflow system to concentrate a BSA test protein to a final recirculation volume of 5 mL was investigated in experiment three. BSA was loaded at an initial concentration of 15 g | L on the ambr® crossflow system into four channels of ambr® CF filter cassettes containing a 10 kDa Hydrosart® filter. The system was run at a feed rate (10 mL | min) and TMP 1.5 bar. The final protein concentration from each channel was measured after the crossflow filtration by UV280.

In the final study, experiment four, the ambr® crossflow system was used to study the effect of buffer composition on protein stability, and thus on process performance. Four crossflow runs were performed with BSA at 20 g | L concentration loaded into four channels of ambr® CF filter cassettes each containing a 10kDa Hydrosart® filter. The system was run for 90 minutes with four different diafiltration buffers (designated A, B, C and D) at a feed rate of 10 mL | min and the TMP set at 1.5 bar. The retentate weight, feed pressure, flux and flow rates were analysed from each channel and automatically plotted by the ambr® crossflow system.

Results

From experiment one (Figure 7) the results show the final concentration of BSA ranges from 65 to 68 g | L with a 3 % coefficient of variation (CV) in final protein concentration and flux varies from 4.7 to 5.1 mL | min with a 4 % CV across the four different channels of the ambr® crossflow system. This demonstrates that the ambr® crossflow system can generate consistent results with less than 5 % variance across all four channels and could thus be used as a robust parallel screening method.

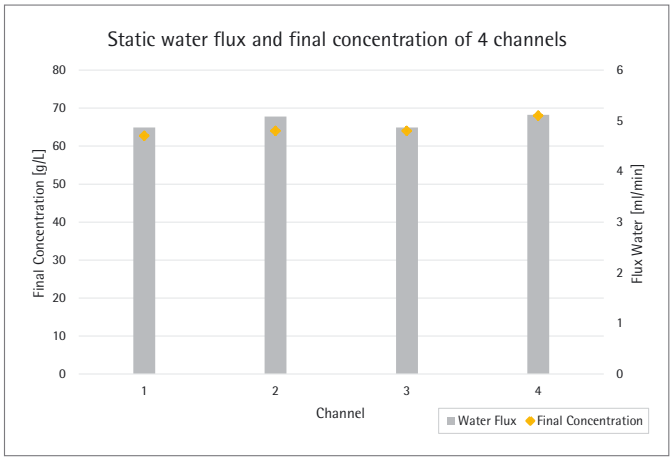

Figure 7: Variation in flux and protein concentration across four channels of the ambr® crossflow system using fixed protein concentration, feed and TMP parameters.

The results from experiment two (Figure 8) show that using the ambr® crossflow system at the two low flow rates, doubling the TMP from 0.25 to 0.5 and from 0.5 to 1 bar increases the rate of flux. At the lower flow rates, beyond a TMP of 1 bar, the rate of flux increases as crossflow rate increases but remains relatively constant for each crossflow rate beyond 1 bar. This type of crossflow filtration performance is as expected according to the literature, indicating that the ambr® crossflow system performance will be similar to larger scale crossflow systems. The major advantages of using the ambr® crossflow is that the system can rapidly evaluate many different process conditions, using very low test volumes. These results indicate that the ambr® crossflow could be

used as a cost-effective scale down model for rapidly determining optimum process parameters.

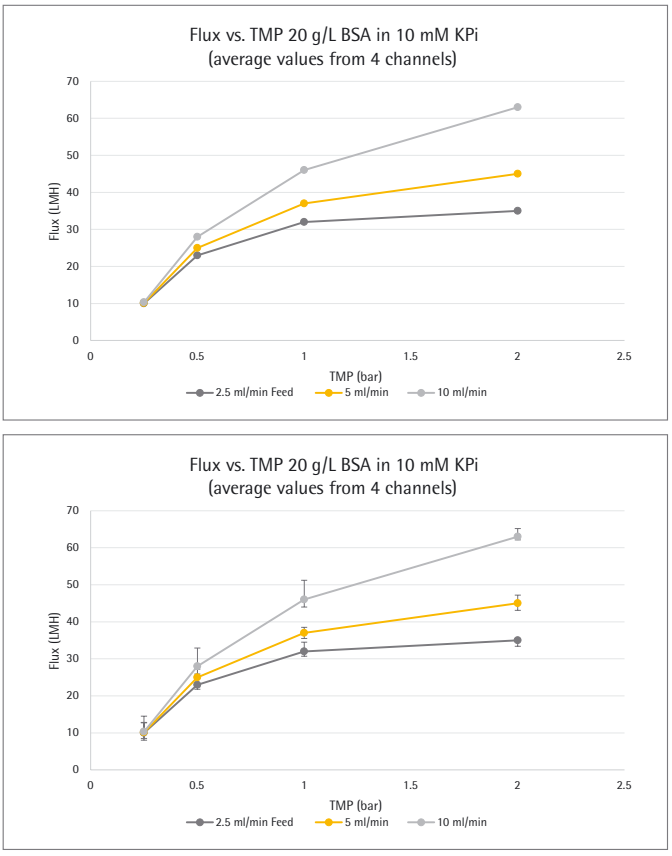

Figure 8: Assessing variations in flux with the ambr® crossflow using different feed and TMP parameters.

From experiment three (Table 1) the results demonstrate that the ambr® crossflow system can concentrate BSA protein to a highly concentrated solution, with only a 3 % variance across all four channels. Since the system design is comparable to bench scale systems and the membrane area is much smaller in the ambr® crossflow system, the amount of protein required for each run is significantly less than other benchtop system. Therefore, with small amounts of starting material the ambr® crossflow system can accurately concentrate a protein, with a high level of reproducibility across channels indicating that the system could be suitable for use in formulation studies to assess the manufacturability of protein-based drug candidates.

Channel	Load volume (mL)	Initial load concentration (g | L)	Harvest volume (mL)	Final harvest concentration (g | L)
1	63	15	5	170
2	63	15	5	172
3	63	15	5	166
4	63	15	5	169

Table 1: System consistency as illustrated by a product (BSA) concentration experiment using crossflow filtration with the ambr® crossflow system

The results from experiment four demonstrate that the choice of diafiltration buffer can have a significant effect on process performance. The experiments were run in parallel on the ambr® crossflow system, maintaining process set points automatically, as illustrated in Figure 9.

Difference in filtration performance is clearly visible from the data. While the process conditions have been maintained equal in all experiments, buffer B in channel 2 and buffer D in channel 4 display the slowest filtration rates, as illustrated by the rate of decline in retentate weight, shortly followed by buffer C in channel 3. Buffer A which was run on Channel 1 generates a significantly higher process performance; a high filtration rate is clearly illustrated by the sharpest reduction in retentate weight. This model data demonstrates how important the buffer composition is to the protein stability, as illustrated by process performance, and how this can be easily screened for at small scale using the ambr® crossflow system.

These results are comparable to data produced by benchtop crossflow systems, suggesting that the ambr® crossflow could be used as a scale down model for the relative ranking of buffers to determine diafiltration conditions for optimum protein stability and final formulation concentration.

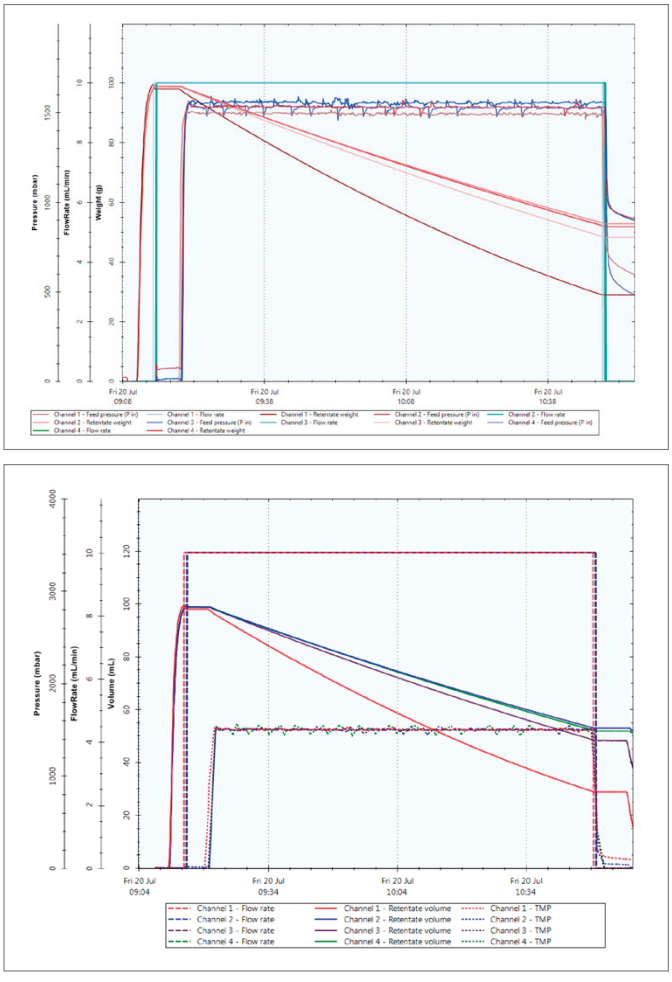

Figure 9: The effect of four diafiltration buffers on the process performance profiles of BSA during crossflow filtration with the ambr® crossflow system

Conclusion

The ambr® crossflow system provides a unique combination of membrane technology and high throughput automation for parallel screening of crossflow conditions. This study shows that the system can generate consistent, reliable data with less than 5 % variance across four channels, exhibiting flux performance comparable to laboratory scale crossflow systems and thus can be run in parallel to determine optimal process parameters such as feed rate and TMP.

This study also demonstrates that using low volumes, the ambr® crossflow system can accurately concentrate a protein, with a high level of reproducibility across channels and can help determine the effects of different buffers on protein stability and process performance so could be used for buffer ranking | formulation studies.

In summary, the ambr® crossflow system has the potential to be used as a small-scale model of bench top crossflow filtration functionality in Design of Experiments (DoE) and formulation studies to accelerate early assessment of manufacturability and ensure that the most promising protein-based product candidates progress rapidly through the development pipeline.

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