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Application Note

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Applications of an Automated High Throughput Crossflow System for Ultrafiltration Process Development to Assess Manufacturability of Monoclonal Antibodies

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Abstract

The biopharmaceutical industry is currently facing a number of challenges. In recent years, companies have experienced a decline in peak revenue sales from their newly launched products while the cost of developing a new biological drug continues to increase. The industry's pipeline of products is becoming increasingly diverse and requires production platforms that are more adaptable and flexible than ever before.

To address this issue, the Ambr® Crossflow system was developed to assist downstream process development scientists in assessing the manufacturability of biologics. The system is an automated high throughput solution for the parallel screening of ultra- and diafiltration conditions at lowest process volumes. Ambr® Crossflow allows significantly faster data generation and uses a fraction of the starting material compared with conventional benchtop crossflow systems.

Find out more: www.sartorius.com/ambr-crossflow

Introduction

With increasing numbers of biologics and biosimilar drugs becoming commercialized each year, average peak drug sales are declining while the cost of developing these drugs continues to rise. To maximize return on investment, biopharmaceutical companies are evaluating strategies to increase the efficiency of the of their product development pipelines, improve productivity in manufacturing and reduce production costs. This has led biomanufacturers to assess and develop production processes to optimize the manufacturability of an increasing array of biologicals during the earliest phases of development, often when there is limited material available with which to perform experiments.

In recent years, miniaturized single-use bioreactors such as the Ambr® 15 and 250 technologies have revolutionized upstream bioprocess optimization, allowing scientists to screen, select and develop optimal cell culture conditions and scale-up strategies in weeks instead of months. However, for downstream processing, a significant investment of time and resources is needed to generate sufficient material for ultrafiltration studies because of the quantities of product required for current systems. This means the ability to predict how a potential therapeutic will behave during ultrafiltration and diafiltration at scale is currently limited, yet these processes also have a major impact on molecular stability, final protein concentration, formulation and ultimately, therapeutic efficiency.

To address this issue, Sartorius Stedim Biotech (SSB) has developed the Ambr® Crossflow system to assist down-stream process development scientists in assessing the manufacturability of biologics. The system is an automated high throughput solution for the parallel screening of crossflow conditions and works with Ambr® CF Filter single-use cassettes with a membrane area of 10 cm^2. The system uses low process volumes with a minimum 5 mL recirculation volume. Scientists can expand the system to match their research demands with 4, 8, 12 or 16 channels allowing them to perform up to 16 crossflow trials simultaneously.

Case Study

This application note describes how scientists at MSD Research Laboratories, Kenilworth, NJ used Ambr® Crossflow to automate parallel crossflow filtration experiments to assess the manufacturability of two mAb-based biopharmaceutical drug candidates. The three studies described have allowed scientists to understand how these mAbs behave with different process parameters, buffers and pH conditions, as well as to compare the performance of the ambr crossflow system with another commercial scale-down crossflow system.

The Ambr® Crossflow has multiple, small scale channels; each fully equipped to be similar to any traditional bench scale crossflow filtration set up. The system is fully automated, with each channel independently controlled in terms of input product | buffer streams and process conditions, such as recirculation rate, pressure, load volume, diafiltration set point and final product volume. In addition, the Ambr® CF Filter single-use cassettes 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 concentration is allowing these scientists to determine if the mAbs can be formulated for more cost-effective production and to match desired delivery options to the patient.

Materials and Methods

In this case study, three sets of experiments were performed using the Ambr® Crossflow system. In experiment one, a flux characterization study was carried out with a mAb molecule, (designated mAb B) at 5 mg/mL and 30 mg/mL concentrations, four different transmembrane pressures (TMP) ranging from 5 – 20 psi and five crossflow rates between 4 – 20 L/minute/m^2 (LMM).

In experiment two, the Ambr® Crossflow system was used to study the effect of buffer composition and pH on diafiltration flux rates and protein stability. Four runs were performed using the Ambr® Crossflow system with mAb B at 40 mg/mL concentration and four different buffers (10 mM sodium acetate pH 6, 10 mM histidine pH 6, 10 mM sodium acetate pH 5.5 and 10 mM histidine pH 5.5). To assess product stability after crossflow, the turbidity (A350nm) of mAb B in each buffer solution was measured with a spectrophotometer.

In the final study, experiment three, the ability of the Ambr® Crossflow system to concentrate a mAb was investigated. A mAb molecule (designated mAb C) was loaded at an initial concentration of 3.55 g/L onto either the Ambr® Crossflow system (which uses a 10 cm^2, 0.001 m^2 membrane) or another commercial scale-down crossflow device (system X) using either a 0.088 m^2 or a 0.57 m^2 membrane. In all experiments, systems were operated at 1 bar TMP and a crossflow rate of 4 LMM to a target concentration set-point of 150 g/L. The changes in flux during the runs were measured for all three membranes and after each run, final product quality was measured in terms of High Molecular Weight Species (HMWS) content by Size Exclusion Chromatography (SEC).

Results

The results from experiment one (Figure 1) shows that using the Ambr® Crossflow system, at higher mAb concentrations, the rate of flux is in the range of 10 – 30 LMH, which is much lower than the rate of flux at the lower mAb concentration. Additionally, doubling the TMP from 5 to 10 psi increases the rate of flux at both mAb concentrations. Beyond a TMP of 10 psi, the rate of flux increases as crossflow rate increases but remains relatively constant for each crossflow rate beyond 10 psi. This type of crossflow performance is typical of what would be seen in a benchtop system, indicating that the Ambr® Crossflow system could produce predictive data compared to traditional TFF systems. The major advantages of using the Ambr® Crossflow system for this study is that the system was able to rapidly evaluate these 45 different process conditions and the entire study used just 0.21 g of mAb B because of the low volumes the system requires. When compared to their historical data, the pharmaceutical company that performed this study estimated that carrying out an equivalent study on a standard benchtop crossflow system would have increased the time taken to perform the study by five-fold and required 10 times more product starting material. These results indicate that the Ambr® Crossflow could be used as a cost-effective scale down model for rapidly determining optimum process parameters for mAb manufacturability.

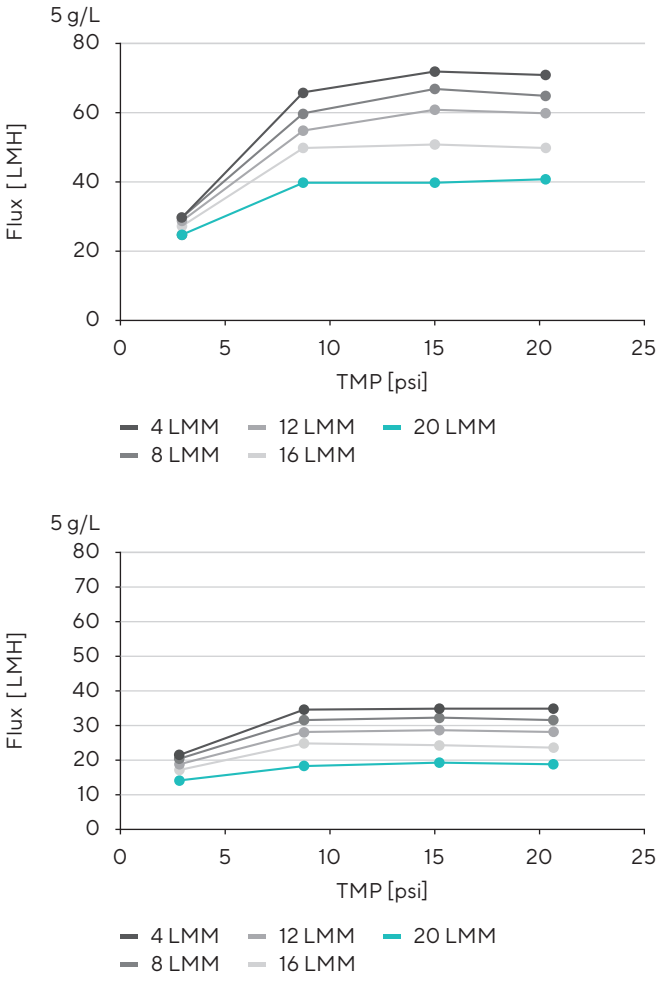

Figure 1: Characterization of flux with the Ambr® Crossflow using different mAb concentrations, flux and TMP parameters.

The results from experiment two (Figure 2) demonstrate that the two buffers at the lower pH (5.5) produce the highest flux rates. Additionally, the turbidity measurements (Table 1) show that mAb B in histidine buffers (at either pH) have the lowest turbidity values, indicating that this mAb is more stable in this buffer type than in acetate buffers with the best buffer choice being 10mM histidine pH 5.5. These results are again consistent with data from benchtop crossflow systems produced by this pharmaceutical company, suggesting that the Ambr® Crossflow could be used as a scale down model for the relative ranking of buffers for optimum diafiltration flux and product stability of mAbs.

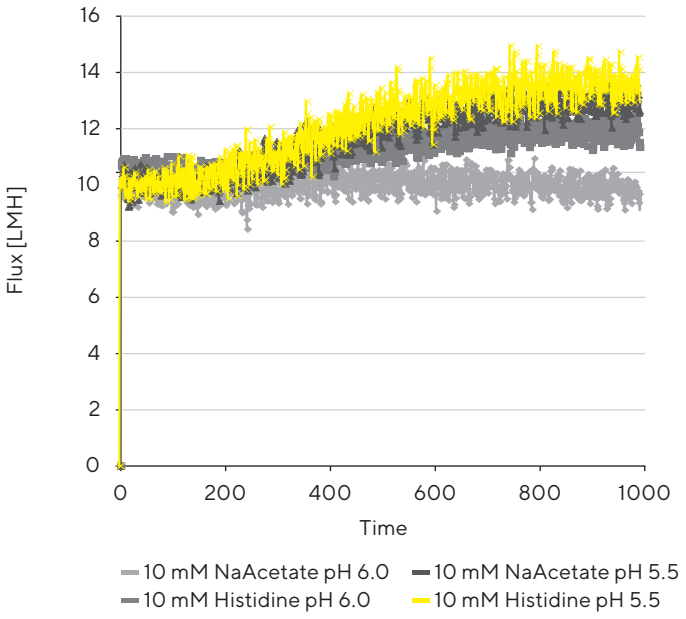

Figure 2: The effect of four diafiltration buffers on the flux profiles of a mAb during crossflow filtration with the Ambr® Crossflow system

Buffer	Turbidity (A350)
10 mM NaAcetate pH 6	0.286
10 mM Histidine pH 6	0.152
10 mM NaAcetate pH 5.5	0.122
10 mM Histidine pH 5.5	0.089

Table 1: The effect of four different buffers on the turbidity of a mAb after crossflow filtration with the Ambr® Crossflow system

System	Membrane area m^2	UFP concentration g/L	Recovery %	Increase of HMWS %
Ambr® Crossflow	0.001	147	105	0.45
System X	0.088	148	109	0.62
System X	0.57	135	98.2	0.58

Table 2: The effect on product concentration and quality of mAb C after cross flow filtration with different scale-down crossflow systems.

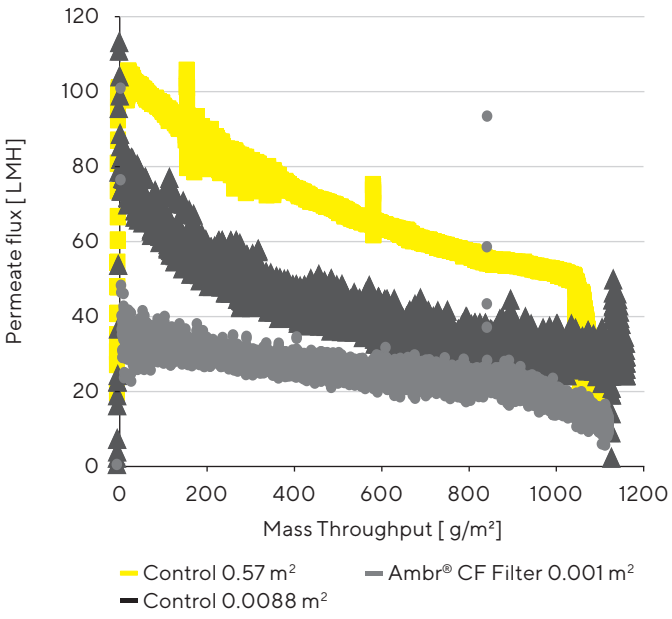

Figure 3: Changes in flux rate during product concentration of mAb C with different scale-down crossflow systems.

The results of experiment three (Table 2) demonstrate that the Ambr® Crossflow system can concentrate mAb C to within 2 % of its target concentration, without affecting product quality as there is a change in HMWS of less than 1 %. These results are comparable to the performance of the 0.088 m^2 membrane and show a better performance than the 0.57 m^2 membrane. Since the system design is comparable to bench scale systems and the membrane area is smaller in the Ambr® Crossflow system, the amount of mAb required for each run is 88 or 570 times less (depending on which membrane is used) than a run in system X. The flux data (Figure 3) also shows that the Ambr® Crossflow membrane has the smallest flux rate and the lowest flux decline. These results demonstrate that with significantly less starting material the Ambr® Crossflow system can accurately concentrate a mAb without adversely affecting product quality, indicating that the system is suitable for assessing the manufacturability of mAb-based drug candidates.

Conclusion

The Ambr® Crossflow provides a unique combination of single use membrane technology and high throughput automation for parallel screening of crossflow conditions with the minimum of material. This study shows that the Ambr® Crossflow system can generate consistent, reliable data which can be correlated to laboratory scale for determining optimal process conditions and performing buffer ranking studies with a mAb-based therapeutic protein. The system allows significantly faster data generation and uses a fraction of the starting material compared with conventional benchtop crossflow systems.

This study also demonstrates that using very low volumes of product candidate, the Ambr® Crossflow system can optimize mAb concentration and diafiltration steps to accurately achieve final mAb concentration targets, with similar process performance characteristics as compared to another commercial scale-down crossflow device without affecting product quality. This indicates that purification development scientists could use the Ambr® Crossflow system as a small-scale model of bench top crossflow filtration functionality to perform Design of Experiments (DoE) studies, accelerating early assessment of manufacturability and ensuring that only the most promising mAb-based product candidates progress through the development pipeline.

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