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Evaluation of an Automated High Throughput Crossflow System as a Small-Scale Model of Bench Top Crossflow Filtration in Formulation Studies of Monoclonal Antibodies

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Abstract

Benchtop tangential flow filtration (TFF) is commonly used as a scaledown model for large scale UF | DF purification processes. While TFF can efficiently buffer exchange large volumes at one time, most systems are single-channel and require rigorous preparation, e.g. setup, cleaning between uses, etc. This limits the efficiency of the TFF system when assessing a variety of parameters or producing many discrete formulations, such as is necessary when preparing formulation robustness studies. Therefore, there is a need for a high throughput TFF system that can utilize several, small amounts of material. Initial assessments of the Ambr® Crossflow system, including water flux tests, flux scouting studies, and other UF | DF process studies, show the system's core functionalities, such as protein behavior (i.e. flux recovery during DF), and process characteristics (i.e. flux, process time, and pressure drop), to be comparable to the traditional TFF.
After confirming the acceptability of these core functions, formulation robustness was evaluated using a set of monoclonal antibodies (mAbs) of differing shear sensitivities, aggregation potentials, and susceptibilities to the Donnan Effect. The results show that these molecular attributes have a major impact on TFF processing. In addition, the system processing parameters and components were critical in protecting product quality throughout the process, and that the pH of the buffer chosen is important in performing Donnan Effect studies to determine the feasibility of implementing these systems for future formulation robustness studies.

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

Introduction

Biopharmaceutical manufacturers are required by regulatory agencies, such as the FDA, to demonstrate product robustness and deliver product quality within specified ranges for the claimed shelf-life period of all their drugs. Typically, real time GMP stability studies are conducted within the product development process at the intended target manufacturing and formulation conditions. However, there is a further requirement to generate robust data over an extended range of conditions to back up the product quality and efficacy claims, as submitted within the commercial licence, to ensure that the product can be supported for any given process excursion that might occur over the lifetime of manufacturing the product. By developing the correct design of experiments (DoE) approach scientists can study factors that have an impact on formulation stability, then data analysis can be applied to create predictions of product robustness.

With the formulation of mAb based therapeutics, various factors including pH, product concentration and buffer formulation can be assessed to determine the critical quality attributes. To do this, formulation scientists must prepare a range of different product formulations to determine which provides optimum stability over time. Although using crossflow filtration is more efficient and requires less buffer than methods such as dialysis for formulation preparation, generating each formulation in a traditional bench top crossflow system can still take several weeks. This is because only one formulation can be prepared at a time and systems require rigorous cleaning between different product formulations to prevent cross-contamination. The lack of automation associated with these traditional systems means they require significant time and manual processing efforts from scientists.

Moreover, these systems often have a minimum material requirement greater than 200 mL and typically some 10s of grams of purified product per formulation, so scientists can only perform formulation studies when enough material is available.

In recent years, miniaturized single-use bioreactors such as the Ambr® technology have revolutionised upstream bioprocess optimization, allowing scientists to screen, select and develop optimal cell culture conditions and scale-up strategies in weeks instead of months. However, the ability to concentrate and predict how a biological will behave in small-scale formulation studies is currently limited.

To address this issue, Sartorius Stedim Biotech (SSB) has developed the Ambr® Crossflow system to assist process development scientists in assessing the formulation robustness of biologics. The 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. 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 a major biopharmaceutical company used Ambr® Crossflow to automate parallel crossflow filtration experiments to validate this as a suitable scale-down method to concentrate mAb-based biologics and model core filtration and diafiltration processes for formulation studies of mAbs. The Ambr® Crossflow is designed for this application with multiple, small scale channels; each fully equipped to be a mimic of a traditional bench scale crossflow filtration set up such as the Sartoflow® Smart system. 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 final product volume.

In addition, the Ambr® CF single-use filter 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 buffer type and protein concentration is allowing these scientists to determine if the formulation will affect product quality even at a very early stage in development.

Materials and Methods

In this case study, two sets of experiments were performed using the Ambr® Crossflow system. Experiment one was to determine how effectively the Ambr® Crossflow system could concentrate two different mAbs, designated (B and C) to high product concentrations and the impact the system had on product quality. The mAbs were run on two channels of the Ambr® Crossflow system simultaneously and both had different initial load and final target concentrations, mAbs B and C had target concentrations of 130 and 200 g/L respectively. After each run, final product quality was measured in terms of High Molecular Weight Species (HMWS) content by Size Exclusion Chromatography (SEC).

In experiment two, the ability of the Ambr® Crossflow system to model filtration and diafiltration processes at small scale was tested. This was achieved by determining if Ambr® Crossflow system was able to recreate the Donnan effect; a processing characteristic which is experienced in traditional bench and production scale crossflow systems. In this study, all experiments used mAb C (10 mL) at 100 g/L protein concentration. Three channels of the Ambr® Crossflow system were used to run the experiment in parallel and experimental design parameters are shown in Table 1.

Two different buffers were used for the diafiltration experiments. Buffer 1 is a positive control and Buffer 2 a negative control for the Donnan Effect. Typically, it is expected that after diafiltration the final pH should match that of the diafiltration buffer. However, if the Donnan effect is observed, then there is an expected difference between final pH and the diafiltration buffer pH.

Channel	mAb	Protein concen-tration [mg/mL]	Volume [mL]	Buffer	Expected final pH	TMP [bar]
1	C	100	10	Buffer 1 pH 4.8	5.1*	2.0
2	C	100	10	Buffer 1 pH 4.8	5.1*	3.0
3	C	100	10	Buffer 2 pH 5.4	5.4	2.0

Table 1: Experimental design to assess the diafiltration performance of the Ambr® Crossflow system. A 2 bar TMP is the standard target parameter and a 3 bar TMP is used as an high value as a worst case processing scenario.

*Calculated theoretically with experimentally confirmed model.

Results

The results from experiment one showed that using ultrafiltration, the Ambr® Crossflow system was able to concentrate both mAbs to within 10 % of their target concentrations (Table 2) and high concentrations of greater than 200 mg/mL, (a 36-fold concentration) were achieved with an automated protocol. Most importantly, for formulation studies, the changes in HMWS values were as expected when compared to bench scale data; the increase in aggregate levels for mAb C is less than 0.2 % so is within assay variability and for mAb B there is an increase of 0.46 %, however, this mAb is known to be shear sensitive and therefore this increase is considered typical for this product.

Channel	mAb	Starting protein concen-tration [mg/mL]	Target protein concen-tration [mg/mL]	Final protein concen-tration [mg/mL]	Variation from target
1	B	8.5	130	142.4	9.5%
2	C	5.9	200	212.4	6.2%

Table 2: The effect on product concentration of mAb molecules B and C after crossflow filtration with the Ambr® Crossflow system

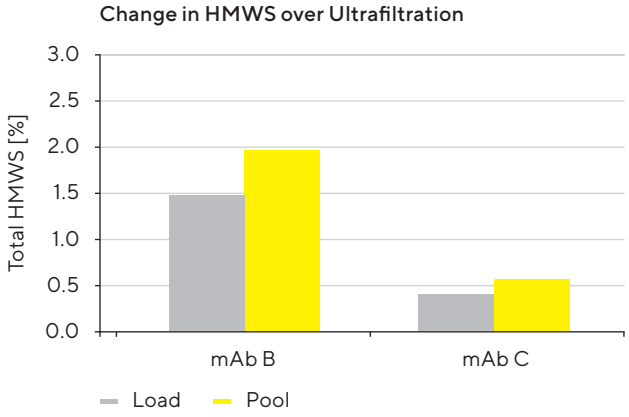

Figure 1: The effect on product quality of mAbs B and C after crossflow filtration with the Ambr® Crossflow system

The results from experiment two (Figures 2 and 3) demonstrate that for the negative control, the final pH of channel 3 with Buffer 2 was consistent both with the expected final pH and data previously collected in a bench scale system, where the final pH matched that of the diafiltration buffer pH. However, for channels 1 and 2, the positive test case, the final pH did not match that of the diafiltration buffer pH. Therefore, the Ambr® Crossflow system is modelling a small-scale crossflow system, with the Donnan effect in action as would be seen in a bench scale system.

The final pH after diafiltration was pH 5.0, lower than calculated, but as expected is significantly different than the diafiltration buffer pH because of the Donnan effect operating even in this small-scale system using a 10 cm^2 filter cassette. Operating the diafiltration with Buffer 1 at a higher TMP did not affect the final pH achieved. In all cases, data shown in Figure 3 illustrates that there is no impact on product quality, as measured by the change in HMWS, in any of the tests performed on the Ambr® Crossflow system, which is as expected from the traditional bench scale system data.

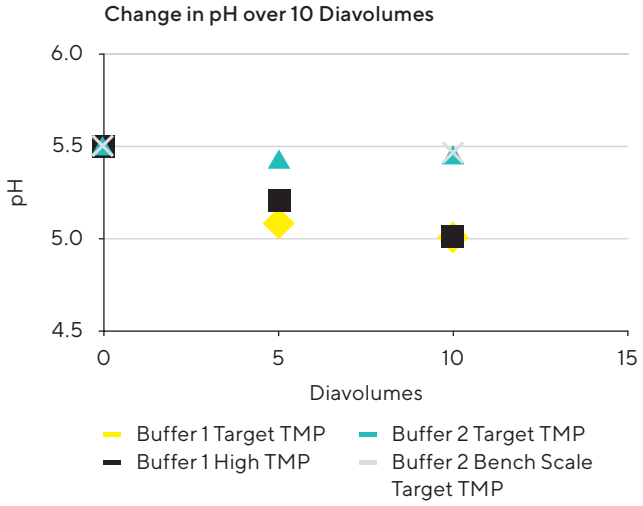

Figure 2: The effect on pH of diafiltration performed in the Ambr® Crossflow system

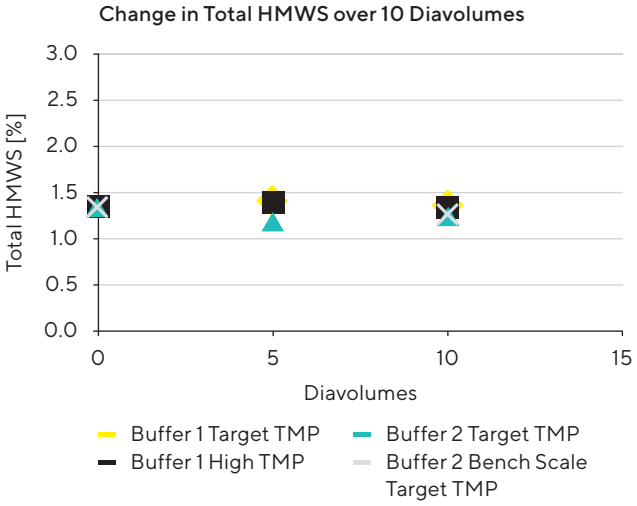

Figure 3: The effect on HMWS of diafiltration performed in the Ambr® Crossflow system

Conclusion

The Ambr® Crossflow is an automated high throughput solution for parallel screening of crossflow conditions. This study shows that the Ambr® Crossflow system can be used to accurately concentrate protein formulations of mAbs without affecting product quality. It also found that diafiltration experiments could be performed in the system in parallel, with very low volumes of product solution (starting with just 10 mL) and could be used in diafiltration with different buffers under different conditions to achieve comparable results to those usually seen at bench scale. In summary, this indicates that with minimal manual intervention, formulation scientists could use the Ambr® Crossflow system as a high-throughput preparation method and as a small-scale model of bench top crossflow filtration functionality to accelerate formulation studies of their promising mAb-based product candidates.

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