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Application Note

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Optimizing Diafiltration Parameters for Maximum Recovery of a Bispecific Antibody Using Low Process Volumes and an Automated High Throughput Crossflow System

Dan Gibson[1], John Betts[2], Marc Jenke[3]*, Will Lewis[1]

1. GlaxoSmithKline, UK, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, United Kingdom
2. Sartorius Stedim Biotech, York Way, Royston, Hertfordshire, SG8 5WY, United Kingdom
3. Sartorius Stedim Biotech GmbH, August-Spindler-Strasse 11, 37079 Goettingen, Germany

* Correspondence
E-Mail: Marc.Jenke@sartorius-stedim.com

Abstract

Manufacturability of therapeutic proteins like bispecific antibodies has become more important in recent years as the streamlining of process development has become a primary target for time to market optimization. Challenges like buffer screening, as well as optimizing concentration and diafiltration steps to achieve final product concentration targets can be time consuming and requires large quantities of valuable product solution.

The ability to study optimal crossflow conditions using an automated high throughput system for parallel screening with smallest volumes of product needed, accelerates downstream development productivity of promising drug candidates.

Identifying the specific behavior of therapeutic proteins under stress conditions, different buffer conditions and formulations at the earliest stage will support optimizing of the downstream development process.

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

Introduction

With higher 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 profitability and return on investment on these drugs, biopharmaceutical companies are evaluating strategies to improve productivity of their product development and manufacturing activities and lower 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 when there is often limited material available with which to perform experiments.

One new class of biological molecules being developed is bispecific antibodies (bsAbs) which are designed to bind to two different antigens or epitopes simultaneously. These have the advantage over traditional monoclonal antibodies (mAbs) of potentially targeting therapies to specific tissues and being able to activate additional arms of the immune system at a tumor site. They also have the potential to induce more than one immunotherapy mechanism simultaneously and could offer cost-saving alternatives to combination therapy.

In recent years, miniaturized single-use bioreactors such as the Ambr® technology 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, on the downstream purification, the ability to predict how a biological will behave during ultra-filtration and diafiltration at scale is currently limited, yet these processes also have a major 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 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 pharmaceutical company used Ambr® Crossflow to automate parallel crossflow filtration experiments to select the optimal buffer and protein set point conditions to concentrate bsAbs. The Ambr® Crossflow is designed for this application with multiple, small scale channels; each fully equipped to be similar to any traditional bench scale crossflow filtration set up. The system is fully automated, but each channel is independently controlled in terms of input product | buffer streams and process conditions, such as recirculation rate, pressure, load volume, diafiltration set point and 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 buffer type and protein setpoints is allowing these scientists to determine if the bsAbs can be formulated at a high concentration for more cost-effective production, and whether shear forces will affect the protein structure and product quality.

Materials and Methods

In this case study, two sets of experiments were performed using the Ambr® Crossflow system. Both used a bsAb solution at an initial loading concentration of 16.7 g/L which had a starting monomer content of 98.9%. A normalized water permeability (NWP) test was performed prior to the crossflow steps to establish a baseline for all the Ambr® CF Filter cassettes and ensure they met standard operating performance criteria. All studies were conducted prior to product launch and therefore used prototype Ambr® CF Filter cassettes. During both experimental runs, the bsAb solution was concentrated to a pre-defined target concentration for diafiltration. The diafiltration was then performed and a final concentration step executed to meet final target product concentration. Both experiments were run with an inlet pressure (PIN) of 1.5 bar, a transmembrane pressure (TMP) of 1.0 bar and the 30kDa Hydrosart® (regenerated cellulose) Ambr® CF Filter cassette. Final product quality was measured in terms of monomer content by SEC-HPLC in both experiments.

In experiment one, to investigate the effect of target diafiltration concentration and diafiltration buffer composition on process performance, four crossflow runs were performed simultaneously using the Ambr® Crossflow system. These were used to compare two different proprietary diafiltration buffers, (designated buffer Acetate and buffer Histidine and two target diafiltration set points (20 g/L or 45 g/L). Following diafiltration, a final concentration step was performed to achieve a target product concentration of 60 g/L.

In experiment two, four crossflow runs were performed simultaneously using the Ambr® Crossflow system to investigate the effect of target diafiltration concentration on process performance. Histidine diafiltration buffer and two diafiltration set points (60 g/L or 80 g/L) were used. A final concentration step was performed after diafiltration to reach a target product concentration of 130 g/L.

Results

The results from experiment one (Table 1) show that the Ambr® Crossflow system was able to operate with low retentate volumes (minimum 5 mL) with all final harvest retentate volumes within 10% of the 60g/L product concentration target. The decrease in the proportion of monomer present following crossflow operations was minimal for both buffers, indicating that neither the process environment of the Ambr® Crossflow system nor the buffer composition negatively affected the shear sensitivity of the molecule.

With regards to process performance, the diafiltration flux was higher at a lower diafiltration concentration set point, however, this had minimal impact on overall process time (data not shown). Therefore, the higher diafiltration concentration set point would be preferred from a manufacturing perspective as this would require the least amount of buffer and would therefore be more cost-effective.

Channel	Buffer	Starting protein set point concentration [g/L]	C1 max flux [LMH]	DF mid flux [LMH]	C2 min flux [LMH]	Retentate volume [mL]	Retentate concentration [g/L]	Final % Monomer
1	Acetate	20	52	32	13	6.59	55.6	98.3
2	Acetate	45	42	20	14	5.85	62.6	98.3
3	Histidine	20	76	56	19	6.91	56.3	98.3
4	Histidine	45	40	20	15	7.09	57.1	98.1

Table 1: The effect of two different diafiltration buffer compositions and diafiltration concentration set points on final product concentration and quality of bsAbs after cross flow filtration with the Ambr® Crossflow system.

The results from the second experiment (Table 2) show the Ambr® Crossflow can operate with low retentate volumes with consistent performance, as seen in terms of flux across duplicate channels, and final harvest volumes within 10 – 15% of the target harvest product concentration of 130 g/L. The decrease in the proportion of monomer present following crossflow operations was again insignificant at approximately 1% across all channels.

The higher diafiltration concentration set point does not significantly impact on flux values, and therefore overall processing times can be reduced. The higher set point would therefore again be preferred for manufacturing, as it would require less buffer and be more cost-effective.

Channel	Buffer	Starting protein set point concentration [g/L]	C1 max flux [LMH]	DF mid flux [LMH]	C2 min flux [LMH]	Retentate volume [mL]	Retentate concentration [g/L]	Final % Monomer
1	Histidine	60	48	18	4	4.1	133.6	97.8
2	Histidine	60	38	12	2.5	3.4	139.7	97.8
3	Histidine	80	47	10	3.5	3.7	136.8	98.1
4	HIstidine	80	52	12	3.5	3.5	151.4	98.0

Table 2: The effect of two different diafiltration set points on final product concentration and quality of bsAbs after cross flow filtration with 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 assess the manufacturability of a bsAb therapeutic protein by performing buffer screening, as well as optimizing concentration and diafiltration steps to achieve final product concentration targets with very low volumes of product solution. This indicates that the Ambr® Crossflow system could enable purification development scientists to use a Quality by Design (QbD) approach to perform Design of Experiments (DoE) studies with minimal manual intervention to accelerate downstream development productivity of their promising bsAb product candidates.

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 further contacts, visit
www.sartorius.com

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