APPLICATION NOTE

Extended Cycling Durability, High Productivity Membrane-Based Affinity Purification using GORE® Protein Capture Devices with Protein A

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Objective

This application note provides data demonstrating high productivity, extended cycling durability performance of membrane-based GORE® Protein Capture Devices with Protein A across two rapid Clean-in-Place methods bracketing caustic contact time and concentration. These data were then combined with results from a manufacturing-scale demonstration to extrapolate protein capture capabilities at a 2000 L batch bioreactor size across CIP methods.

Purpose

PB12180 *GORE Protein Capture Devices with Protein A: Increased Productivity at Multiple Scales with Rapid CIP* demonstrates the performance of Gore devices using a high productivity cycling protocol incorporating fast residence times and a rapid CIP out to 100 cycles. Critical quality attribute targets, including eluate endotoxin and bioburden, were achieved in the study across several device sizes. The success of this method influenced the desire to extend cycling duration to 200 cycles and demonstrate performance with a longer duration, lower caustic concentration CIP approach.

Moreover, PB12793 *Building a Fully Single-Use Process: High Productivity Protein A Membrane Devices that Complement Disposable Upstream Technology* demonstrates the capability to manifold commercially available 1 L Gore devices and then purify at a manufacturing scale with performance that scaled from smaller devices.

It was then ultimately desired to combine the extended durability results from this study with the results from the previous work to enable a range of capability projections for the purification of 2000 L bioreactor outputs across a range of titers.

Materials and Equipment

- AKTA pure™ 150 Liquid Chromatography System
- Trastuzumab Biosimilar Clarified CHO Cell Harvest at ~ 4 g/L titer
- 1 mL GORE Protein Capture Devices (PROA101)

Dynamic Binding Capacity at 10% Breakthrough ($DBC_{10\%}$) Calculation:

Eq-01: *10% Breakthrough Absorbance (mAU) = (0.1 × (Absorbance (mAU) of feed - Absorbance (mAU) of loading plateau)) + Absorbance (mAU) of the loading plateau*

Eq-02: *Hold Up Volume (mL) = Device hold up volume (mL) + System hold up volume (mL)*

Eq-03: *$DBC_{10\%}$ (mg/mL) = (Volume at 10% Breakthrough Absorbance (mL) – Hold up volume (mL)) × Harvest Titer (mg/mL)/ Device volume (mL)*

Procedure

Table 1 summarizes the harvest cycling protocol applied to two separate 1 mL Gore devices over 200 cycles. One device incorporated a 2-minute duration, 0.2M NaOH CIP while the other a 0.5 minute duration, 0.5M NaOH CIP. The method includes a short post-load equilibration at the loading flow rate to allow complete binding of the mAb within the column and system/tubing holdup volume. Cycle times for the two protocols are shown in Table 2, reflecting longer duration pump washes used for the 0.5 minute CIP.

Table 1: Cycling protocol for 1 mL Gore devices, delineating two CIP approaches

	Step	Buffer or Harvest	Column Volumes (CV)	Residence Time in seconds (SRT)	Flow Rate (mL/min)
1	Equilibration	Tris Buffered Saline (pH=7.4)	2	7	8.6
2	Load	Trastuzumab cell harvest	80% of $DBC_{10\%}$	15	4
3	Equilibration	Tris Buffered Saline (pH=7.4)	1	15	4
4	High Salt Wash	Tris Buffered Saline + 1M NaCl	4	7	8.6
5	Equilibration	Tris Buffered Saline (pH=7.4)	4	7	8.6
6	Elution	100 mM Acetic Acid (pH=3.0)	6	7	8.6
7	Acid Strip	Citric Acid (pH =2.0)	4	7	8.6
8a	2 min. CIP	*0.2M NaOH*	8	15	4
8b	0.5 min. CIP	*0.5M NaOH*	3	10	6
9	Equilibration	Tris Buffered Saline (pH=7.4)	5	7	8.6

Pump washes were applied for steps 4-9

Table 2. Overall cycle times for the respective protocols

Cycling Protocol	Cycle Time (mins)
2 min. CIP	9.5
0.5 min. CIP	8.7

Longer duration pump washes used for 0.5 min CIP

ELISAs for Purity

The amount of Protein A leached from the devices was evaluated using a Protein A ELISA kit from Repligen Bioprocessing, P/N 9000-1 (Repligen Corporation, 41 Seyon Street, Building 1 Suite 100, Waltham, MA 02453). The ELISA was performed using the "Dilute and Go" extraction according to the manufacturer's instructions. Host Cell Protein (HCP) concentration in elution pools obtained during purification cycles was determined using Cygnus kit #F550-1 (Cygnus Technologies, Southport, NC 28461) following manufacturer protocols.

Elution Width

Elution widths were determined from chromatograms using 100 mAU - 100 mAU as the cutoff criterion.

Yield

Yield was determined from the concentration of the mAb in the elution fractions. This was calculated by measuring the 280 nm absorption using a Little Lunatic UV/Vis Spectrophotometer (Unchained Labs, 6870 Koll Center Parkway, Pleasanton, CA 94566) and using an extinction coefficient of 1.47 mL mg^{-1} cm^{-1}.

Pressure

Delta column pressures were determined from the chromatograms at both the equilibration step and the maximum within the elution step.

Endotoxin

Endotoxin was determined from a kinetic-turbidimetric limulus amebocyte lysate (LAL) assay of the elution fractions following standards established in USP <85>, USP <161> and ANSI/AAMI ST72.

Bioburden

Bioburden was determined from analysis of elution fractions following guidance from USP <1231>.

Results

Demonstration of 200 Cycle Performance Using Increased Productivity Protocols

Figure 1 summarizes overlayed UV280 chromatograms at selected cycling increments for the respective devices over the 200 cycles.

Figure 1: UV280 chromatograms at selected cycling increments for 2 min. CIP (left) and 0.5 min. CIP (right)

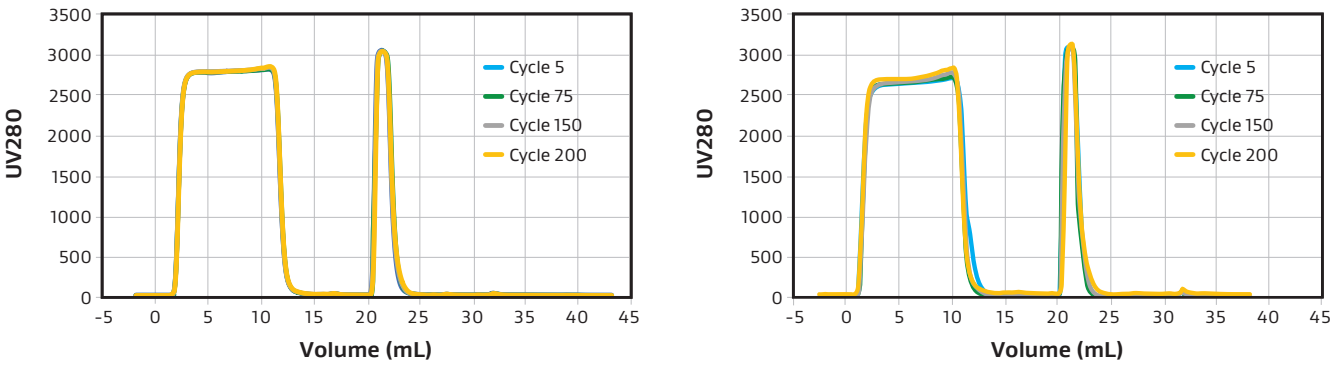

Figure 2 plots delta column pressure at equilibration and elution spike for the respective devices. The data indicate a modest pressure rise over the 200 cycles, well within the acceptance limit of 0.4 MPa.

Figure 2: Column ΔP data over 200 cycles for 2 min. CIP (left) and 0.5 min. CIP (right)

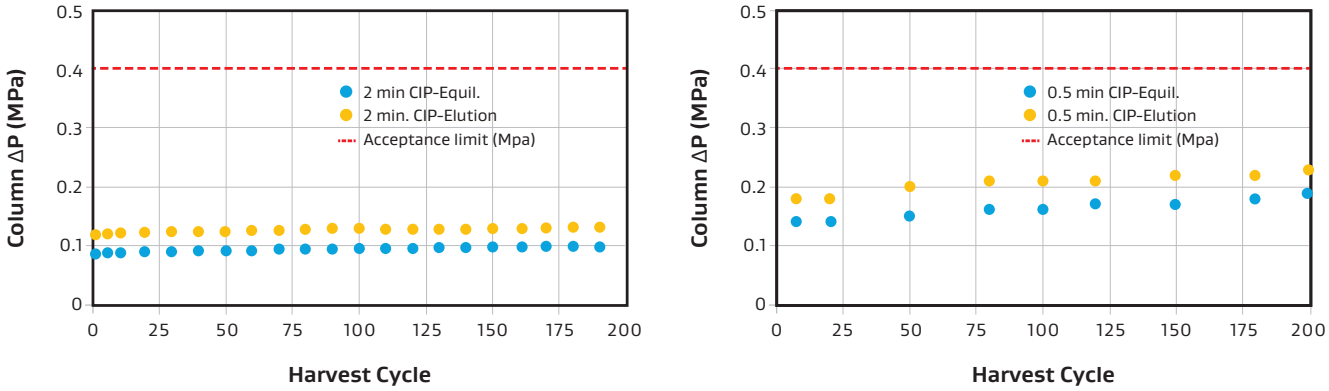

Figure 3 plots elution yield and elution volume data for the respective devices. Yield data remain above 90% for both devices over the 200 cycles. Elution volumes range between 2.5 and 3.1 column volumes.

Figure 3. Yield (left) and elution volume (right) (100–100 mAU cutoff) data for respective devices over 200 cycles.

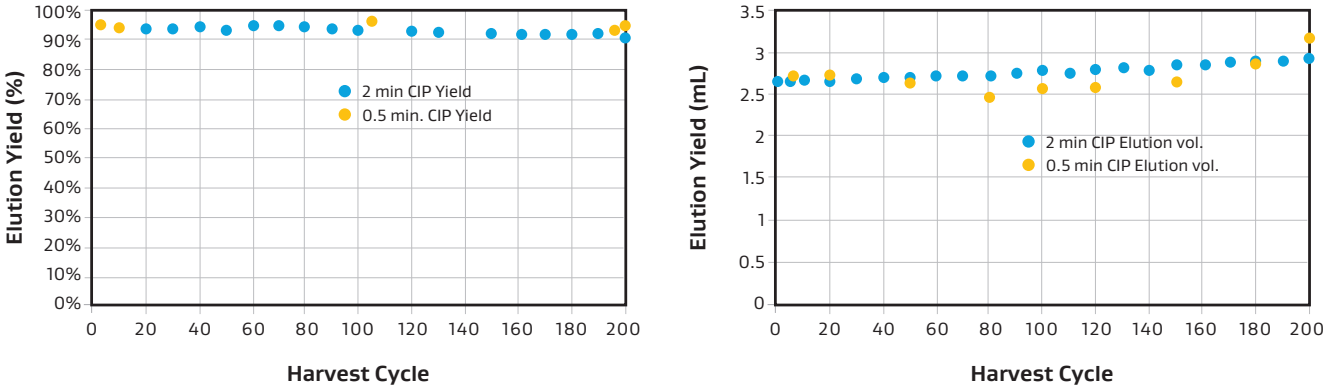

Table 3 summarizes productivity calculations and process impurity data for the respective devices. Productivity calculations incorporate elution yields and are averaged over the 200 cycles. HCP and leached Protein A data showed little trending over the course of the 200 cycles and are also presented as averages. Starting HCP values in the harvest were measured to calculate log reduction values.

Table 3. Average yielded productivity and process impurity data for respective devices over 200 cycles.

	Cycling protocol	
Attribute	**0.5 min CIP**	**2 min CIP**
Average yielded productivity (g/L/h)	211	191
Average HCP (LRV)[1,2]	1.83	2.1
Average Leached Protein A (ppm)[1]	0.95	0.75

1. Little to no trending over cycling
2. Protocols not optimized for HCP clearance

Table 4 summarizes bioburden and endotoxin levels from the initial devices and select elution pools sampled during cycling, indicating acceptance criteria limits of <10 CFU/mL and < 5 EU/mL, respectively, were met for both devices at all sampling points.

Table 4. Bioburden and Endotoxin results

	2 min CIP		0.5 min CIP	
Sampling interval	**Bioburden (CFU/mL)**	**Endotoxin (EU/mL)**	**Bioburden (CFU/mL)**	**Endotoxin (EU/mL)**
Post sanitization	0	<0.1	0	0.037
Pooled elutions cycles 1-6	0	<0.1	0	<0.1
Pooled elutions cycles 98-103	0	<0.1	1	<0.1
Pooled elutions cycles 128-133	0	<0.1	N/A	N/A
Pooled elutions cycles 148-153	0	<0.1	N/A	N/A
Pooled elutions cycles 173-178	1	<0.1	N/A	N/A
Pooled elutions cycles 195-200	0	<0.1	1	4.163

Applying Demonstrated Results to Forecast Manufacturing Scale Capability

The high productivity, extended cycling durability data presented here can be combined with a) the previously referenced size scaling performance and b) the demonstration of parallel-manifolded 1 L commercial devices to calculate projected capability at the 2000 L bioreactor scale. As outlined in Table 5, these extrapolated values suggest the potential to clear harvest titers ranging from 1.5 to 10 g/L, across a range of CIP protocols, in 27 hours or less.

Table 5: 2000 L Culture Harvest Fluid Protein A Purification Scenarios[1] Using GORE Protein Capture Devices with Protein A

Bioreactor Batch Size (L)	Titer (g/L)	Gore Protein A Membrane Device Configuration	Cycles[2] Needed to Clear Bioreactor	Total Time per Cycle[3] (min)	CIP Time per Cycle (min)	Total Bioreactor Clearance Time (h)	Productivity (g/L/h)[4]
2000	1.5	1 L	94	14.8	0.5	23.2	130
				16.3	2	25.5	118
				17.3	3	27.1	111
2000	3	2 x 1 L parallel manifold	94	9.5	0.5	14.8	203
				11.0	2	17.2	175
				12.0	3	18.7	160
2000	6.4	4 x 1 L parallel manifold	100	6.7	0.5	11.1	289
				8.2	2	13.6	236
				9.2	3	15.3	210
2000	10	4 x 1 L parallel manifold	157	5.8	0.5	15.0	334
				7.3	2	18.9	265
				8.3	3	21.5	233
				10.3	3	26.7	187

1. Assumes $DBC_{10\%}$ of 40 g/L at 30 SRT, loading to 80% of $DBC_{10\%}$ at 30 SRT, non-loading steps at 7 SRT.
2. GORE Protein Capture Devices with Protein A are validated to 100 cycles and demonstrated to 200 cycles
3. Range of total time per cycle is inherently higher for lower titers due to increased time needed for loading low titer harvest to 80% of $DBC_{10\%}$
4. The Gore Productivity Calculator (Gore document PB11711) can be used to model a wide range of additional scenarios.

Conclusions

High productivity affinity capture of a mAb cell culture harvest is demonstrated to 200 cycles across two CIP methods. Chromatographic performance data (yield, elution volume, column pressure) and process related impurity data (Host Cell Protein and leached Protein A) are shown to be consistent and acceptable. Endotoxin and bioburden data are also shown to meet acceptance criteria for the duration of both cycling protocols. When integrated into a productivity calculator applied at manufacturing scale, extrapolated data suggest high productivity affinity capture capability across a range of harvest titers and CIP preferences using commercially available Gore Protein A membrane devices.

Gore PharmBIO Products

Our technologies, capabilities, and competencies in fluoropolymer science are focused on satisfying the evolving product, regulatory, and quality needs of pharmaceutical and bioprocessing customers, and medical device manufacturers. GORE® Protein Capture Devices with Protein A, like all products in the Gore PharmBIO Products portfolio, are tested and manufactured under stringent quality systems. These high-performance products provide creative solutions to our customers' design, manufacturing, and performance-in-use needs

NOT INTENDED FOR USE in medical device or food contact applications or with radiation sterilization. GORE Protein Capture Devices are intended for research use only and should not be used for clinical or diagnostic procedures.

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