APPLICATION NOTE

Rapid Cycling using the GORE® Protein Capture Device with Protein A, 58 mL for Clinical Applications

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Objective

Quantify the performance and productivity gains of the 58mL, GORE Protein Capture Device (PROA201) during non-loading steps using a representative harvest.

Purpose

Similar to the smaller scale GORE Protein Capture Devices that can be operated at faster flow rates (see Application Note PB8433, Increasing the Productivity of mAb Purification using the GORE Protein Capture Devices with Protein A), this evaluation demonstrates the capability to perform non-loading steps at flow rates up to 348 mL/min equivalent to 10 seconds residence time.

Materials and Equipment

- AKTA Pilot 600s LC System
- PROA201 GORE Protein Capture Device (58 mL)
- Chemicals outlined in protocols below
- Clarified CHO cell harvest (CCH): IgG1 with a titer of 4 g/L

Procedure

The initial cycling of the PROA201 was performed per Table 1. The table indicates starting column volumes for each step. The CIP step used an initial 3 CV of 0.2 M NaOH to fill the column followed by a pause of the flow and holding for 3 minutes. The equilibration post CIP employed a watch condition and was varied such that the conductivity returned to < 20 mS/cm and pH < 8±0.5 until stable for 0.5 minutes.

Table 1. CCH Cycling Protocol Method

Method Step	Solution/Buffer	Column Volumes (CV)	Flow Rate (mL/min)	Time (min)
Equilibrate	Tris Buffered Saline (TBS)	5	348	0.83
Sample Load	Trastuzumab CCH titer 4.0 g/l	7.5 (435 mL)	116	3.75
Equilibration Wash	TBS	5	348	0.83
High Salt Wash	TBS + 1M NaCl	5	348	0.83
Equilibration Wash	TBS	5	348	0.83
Elution	100mM Acetate, pH 3.1	100-100 mAU cutoff	348	0.83
Acid Strip	100mM Citric Acid, pH 2.0	5	348	0.667
Equilibration Wash	TBS	5	348	0.83
CIP wash	0.2 M NaOH	3	348	0.5
CIP Hold	0.2 M NaOH	N/A	0	3
Equilibration Wash	TBS	(conductivity <20mS/cm & pH< 8) +/- 0.5, stable for 0.5 min	348	1.307

* TBS Tris-buffered saline (50 mM Tris, 150 mM NaCl, pH 7.4)

ELISAs for Purity

The amount of Protein A leached from the device was evaluated using a Protein A ELISA kit from Repligen Bioprocessing, P/N9000-1 (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.

HCP data was collected form elution pools obtained during purification cycles. Cygnus kit # F550-1 (Cygnus Technologies, Southport, NC 28461) was used following manufacturer protocols.

Elution Width

Elution width was determined from the chromatogram using 100 mAu – 100 mAu as the cutoff.

Yield

Yield was determined from fractions. Concentration of the mAb in the fractions was calculated by measuring the absorption at a wavelength of 280 nm using the Little Lunatic UV/Vis Reader (Unchained Labs, 6870 Koll Center Parkway, Pleasanton, CA 94566) and an extinction coefficient of the IgG1 of 1.47 mL g^{-1} cm^{-1}.

Pressure

Pressure was determined from the chromatograms at the re-equilibration step post CIP treatment.

Results

The cycling protocol was amended during the initial runs since the transitions were complete, allowing for a reduction in CV and time. The overall CV and time per the amended protocol is shown in Table 2. The ability to run at 10srt reduced the overall cycle time from approximately 20 minutes to about 12.6 minutes, saving 38% more time. The device did not leak or burst during the 100 cycles under the cycling protocol method.

Table 2. Total cycle time with all but load step at 10srt.

Process Step	CV's	Flow Rate (mL/min)	Time (min)
Sample Load	7.5	116	3.750
Wash	4	348	0.667
Wash	4	348	0.667
Wash	4	348	0.667
Elution	5	348	0.833
Acid Strip	4	348	0.667
Wash	4	348	0.667
CIP Fill	2	348	0.333
CIP 3 MCT	0	0	3.000
Equilibration	7.8	348	1.307
		Total Run Time	**12.557**

Table 3 shows summarizes elution CV, yield, pressure, Protein A leaching and HCP LRV during the course of cycling.

Table 3. Results of CCH Cycling at various cycles

Cycle #	Elution CV's	Yield %	Pre Column (MPa)	Delta Column (MPa)	Protein A (ppm)	HCP LRV
4	2.6	94%	0.149	0.124	0.12	2.23
20	2.4	93%	0.156	0.132	0.22	2.25
39	2.5	94%	0.173	0.148	0.63	2.21
60	2.5	93%	0.172	0.147	0.35	2.15
81	2.5	94%	0.173	0.148	1.09	2.05
90	2.4	93%	0.177	0.151	0.50	2.04
100	2.4	94%	0.178	0.153	0.53	2.06

Figure 1 shows the overlaid chromatograms at cycle 10, 25, 50, 75 and 100. The absorbance difference in the harvest load portion reflects when lots of harvest changed during the cycling. The small uptick in UV at the end of the load is a result of not accounting for the up-stream hold-up volume in the system as part of the load volume.

There was no change in overall appearance of the elution across the 100 cycles to suggest loss of integrity also noted by the consistent yields (Figure 2). The overlaid cycles of the elution peak showing elution widths of approximately 2.5 CV that remain consistent through cycling (Figure 3). The slight shifts reflect optimizing wash conditions.

Figure 1. Overlay of chromatograms of cycle 10, 25, 50, 75 and 100.

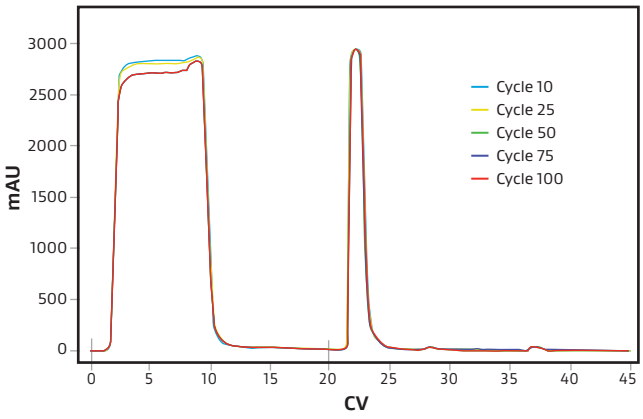


Figure 2. Percent yield over cycling

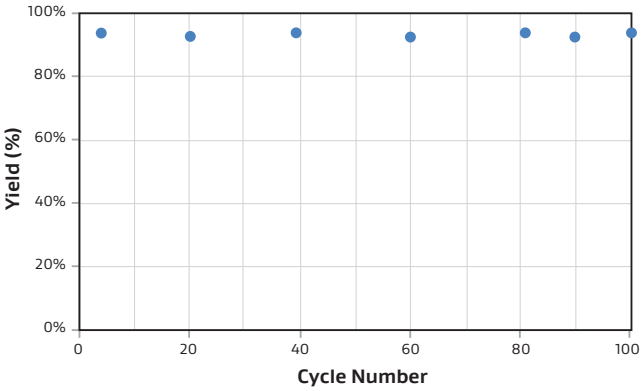
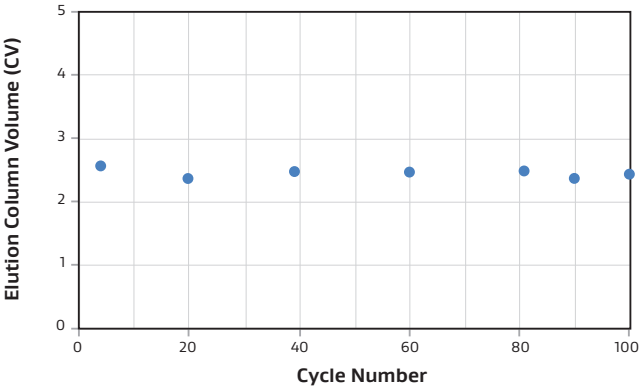


Figure 3. Column volume of elutions over cycling



Cycle 10 and cycle 100 were overlaid along with dP and conductivity traces in Figure 4. The chromatograms show no observable difference other than the noted difference in harvest. The conductivity traces show no changes other than slight shifts from optimizing the washes per the protocol. The dP traces show the slight increase in pressure that typically occurs during cycling and shows that dP is < 0.4 MPa at cycle 100. Figure 5 shows change in delta column pressure over cycling.

Figure 4. Overlay of chromatograms, pressure (dP), and conductivity traces for cycles 10 & 100

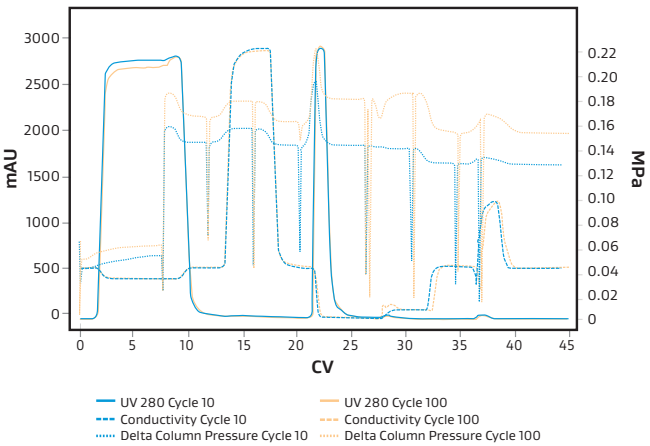
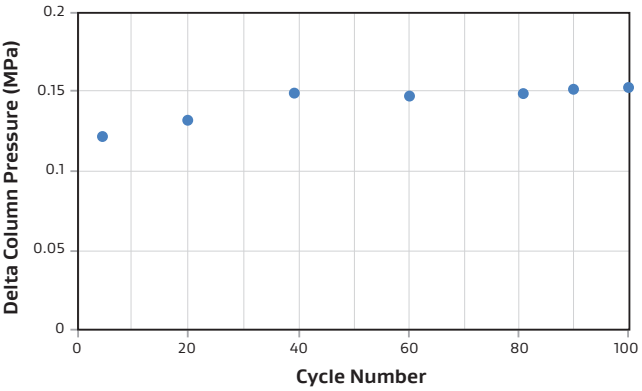


Figure 5. Delta column pressure through cycling



Purity was checked through Protein A leaching (Figure 6) and the reduction of HCP as measured via log reduction (Figure 7). The Protein A leaching was consistent below 2 ppm throughout the course of cycling while HCP remained > 2 LRV.

Figure 6. Protein A leaching over cycling

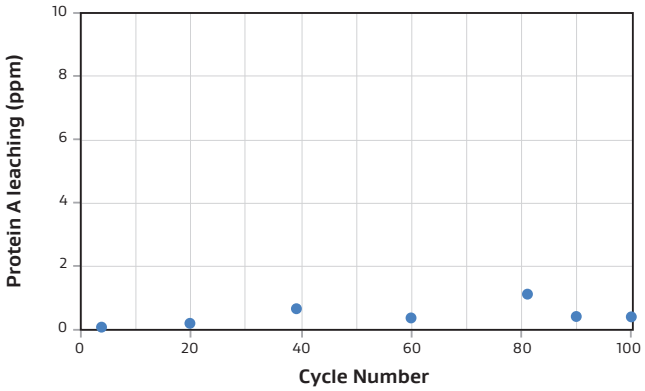
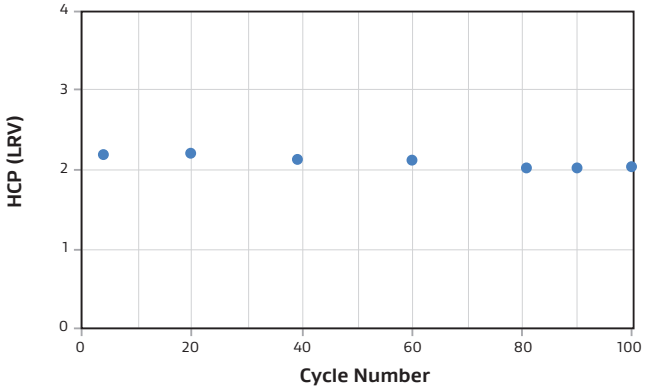


Figure 7. HCP reduction over cycling



Conclusions

The purpose of this application note was to determine if a shorter residence time could be used for more rapid cycling without impacting elution width, yield, Protein A leaching or HCP removal. In this cycling study, all but the loading steps were run at 10 srt and fractions analyzed for yield, elution width, Protein A leaching, and HCP LRV. The data indicates that the increased speed did not impact product quality or yield.

The application of this to other samples may vary as harvest may add pressure over time due to particulates and aggregates, however the use of a ramp in the program to slow flow to avoid getting close to burst pressure can be incorporated into the method.

The alteration from the original protocol resulted in a time savings of 38% and demonstrated that the 58 mL (PROA201) are capable of higher flow rates, with lower pressure rise without impacting typical product metrics. The productivity increased from approximately 88 g L^{-1} h^{-1} to 143 g L^{-1} h^{-1}.

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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