APPLICATION NOTE

Extending the Lifetime of GORE™ Protein Capture Devices through Alternative Cycling Parameters

Jared Clinger, B.S. and William C. Barrett, PhD. W. L. Gore & Associates, Inc.

Objective

Provide an alternative cycling method to extend the useable lifetime of a given GORE Protein Capture Device.

Purpose

The GORE Protein Capture Device enables increased productivity for affinity protein A chromatography by leveraging higher binding capacities (>30mg/mL) at shorter residence times (20 seconds residence time) over commercially available agarose technology. The combination of high capacity and short residence time may result in reaching the lifetime significantly faster than incumbent protein A resin columns. This evaluation demonstrates a method to extend the lifetime of a GORE Protein Capture Device from 20 to 50 cycles while maintaining capacity as assessed by Dynamic Binding Capacity (DBC) at 10% breakthrough, yield, and purity.

Materials/Equipment

- Liquid Chromatography System (LC System)
- PROA101 Device
- Chemicals outlined in protocols (footnotes)
- CHO Cell Harvest: IgG1 mAb with a titer of 1.8 g/L

Procedure

The method outlined in Table 1 was used to perform 50 purification cycles of a mAb expressing CHO Cell Harvest with a titer of 1.8 g/L. Table 2 outlines the sequence of purifications for the PROA101 device used which included three incremental sanitization exposures using 0.1M NaOH for 15 minute contact time. The differences in the method outlined in Table 1 when compared to the current recommended operating instructions are the addition of a high salt wash prior to eluting the antibody and a change in Clean In Place (CIP) step from 0.1M sodium hydroxide for 15 minutes contact time per cycle to 0.2M sodium hydroxide for 3 minutes of contact time per cycle.

TABLE 1: Method Used to Perform 50 CHO Cell Harvest Purifications

Step	Solution	Column Volumes	Device Flow Rate (mL/min)	Time (min)
Equilibration	PBS*	5	3	1.7
Load	Feed Stock	n/a	3	5.2
High Salt Wash	PBS + 1M NaCl	6	3	2.0
Equilibration	PBS	6	3	2.0
Elution	Citrate**	4	3	1.3
Equilibration	PBS	4	3	1.3
(CIP)	0.20 M NaOH	3.6	1.2	3.0
Equilibration	PBS	8	3	2.7

* PBS - Phosphate-buffered saline (150mM NaCl, 50mM Phosphate, pH 7.4)
** 100mM Sodium Citrate dihydrate/Citric Acid pH 3.4

TABLE 2: Sequence of Purifications for the PROA101 Device

Device Purification Schedule
10% DBC using polyclonal IgG with a 15 minute sanitization using 0.1M NaOH
25 Feed purification cycles following Table 1 method
10% DBC using polyclonal IgG with a 15 minute sanitization using 0.1M NaOH
25 Feed purification cycles following Table 1 method
10% DBC using polyclonal IgG with a 15 minute sanitization using 0.1M NaOH

Elution fractions were captured from every 5th cycle to evaluate for yield and cycles 1 and 50 were captured and evaluated for Host Cell Protein (HCP) contamination and impurities using Size Exclusion Chromatography (SEC).

GORE
Creative Technologies Worldwide

Results

The method used in Table 1 allowed for the successful completion of 50 purification cycles of a CHO Cell Harvest with a titer of 1.8 g/L. Figure 1 displays the yields across the 50 purification cycles. The yield decreased 8.2% from cycle 1 to cycle 50.

The DBC evaluations performed during the 50 purification cycles met the specification of ≥ 30 mg/mL. The pressure rise of the device can be observed in Figure 2 and stayed well below the maximum operating pressure of 0.40 MPa.

Figure 1. PROA101 mAb Yields across 50 Purification Cycles

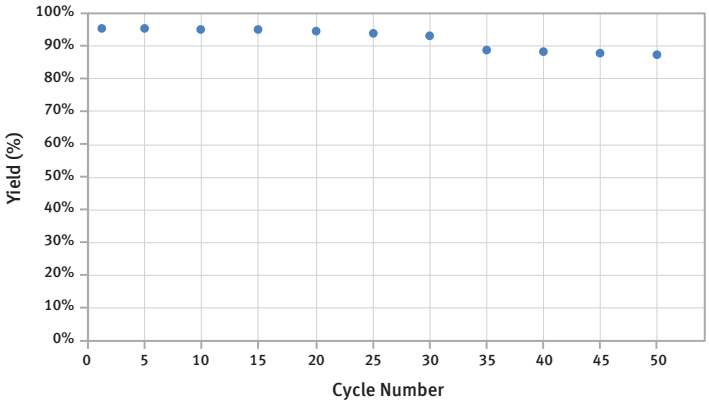
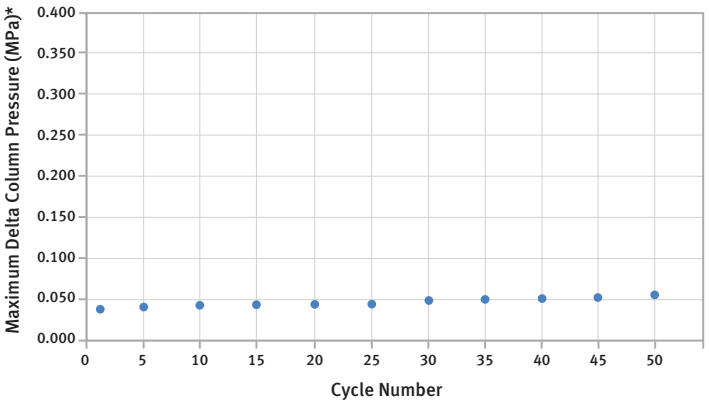


Figure 2. PROA101 Delta Column Pressure across 50 Purification Cycles*



* Per the operating instructions, the maximum operating delta column pressure is ≤ 0.40 MPa.

Table 3 displays the total mass processed by the PROA101 device, the time taken to perform the 50 purification cycles, the total productivity of the PROA101 device, as well as the HCP and SEC metrics to assess purity.

Table 3. Purification Productivity and Performance over 50 Bind/Elute/CIP Cycles

Device	Total Mass Processed (g)	Device Size (mL)	Cycle 1 DBC (mg/mL)	Cycle 50 DBC (mg/mL)	Total Process Time (hours)	Productivity (g/L/h)	Neat HCP in Harvest (ppm)	Cycle 1 [HCP] (ppm)	Cycle 50 [HCP] (ppm)	Cycle 1 Monomer - SEC (%)	Cycle 50 Monomer - SEC (%)
PROA101	1.3	1.0	35.1	31.0	16.0	81.0	136,000	644.2	688.9	96.2	96.9

Conclusion

The methodology outlined in this application note allowed for the successful extension of lifetime for a GORE Protein Capture Device from 20 to 50 cycles. The 50 purification cycles displayed acceptable product quality and acceptable impurity profiles. This method can be employed to increase the number of purification cycles of a GORE Protein Capture Device and continue to leverage the increased capacity at shorter residence times.

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, 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.

Europe | W. L. Gore & Associates GmbH
Phone: +49 89 4612 3456 • Toll Free (EU): 0 800 4612 3456
Email: pharmbio_eu@wlgore.com

Americas | W. L. Gore & Associates, Inc.
402 Vieve's Way • Elkton, MD 21921 • USA
Phone: +1 410 506 1715 • Toll-free (US): 1 800 294 4673
Email: pharmbio@wlgore.com

gore.com/pharmbio

PB8850_Rev1_EN_OCT18