APPLICATION NOTE

Increasing the Productivity of mAb Purification using the GORE® Protein Capture Devices with Protein A

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

Objective

Evaluate the impact of increasing operational flow rate steps (e.g. equilibrations, washes, and clean-in-place (CIP)) on the overall productivity (mg mAb/hour) of GORE Protein Capture Devices (PROA101 & PROA102).

Purpose

The GORE Protein Capture Device can perform at higher pressure drops during operation than that of agarose resins without loss of performance from bed compression. Agarose columns experience bed compression when exposed to high pressures (high flow rates) which can impact overall column performance and may require repacking the matrix or discarding the column depending on the severity of the pressure exposure event. This evaluation demonstrates the benefits that GORE Protein Capture Devices offer by increasing operational flow rate steps to increase productivity without impacting the stability of the membrane bed.

Materials/Equipment

- Liquid chromatography system (LC system)
- 1.0 mL GORE Protein Capture Device (PROA101) and 3.5 mL GORE Protein Capture Device (PROA102)
- Chemicals outlined in protocols (footnotes) below
- GORE Protein Capture Devices for Drug Discovery Applications Operating Instructions (1.0 mL and 3.5 mL)
- CHO Cell Harvest: IgG1 mAb with a titer range of 1.5–1.9 mg/mL

Procedure

GORE Protein Capture Devices were cycled 20 times with CHO Cell Harvest (feed stock), using either a PROA101 (1.0 mL) or a PROA102 (3.5 mL) device following the cycling protocols which employed increased wash flow rates and decreased sodium hydroxide exposure times. The flow rates for each experiment were optimized to decrease the overall purification time per cycle resulting in an increase in productivity for the amount of mAb captured. GORE Device dynamic binding capacity (DBC) was evaluated before and after purifications to ensure the device did not exhibit any loss in performance over the course of the 20 purification cycles. Antibody yield was evaluated throughout cycling to ensure that no product loss was being observed with the increased flow rate exposures. Each purification cycle loaded 90% of 0% breakthrough with the IgG1 CHO Cell Harvest.

Table 1 outlines the 1.0 mL GORE Device (PROA101) increased flow rate protocol used per cycle. Table 2 outlines the 3.5 mL GORE Device (PROA102) increased flow rate protocol used per cycle. The flow rates for each size were governed by the differing geometries between the PROA101 and PROA102 devices.

Table 1. PROA101 (1.0 mL) Increased Flow Rate Protocol

Step	Solution	CV	Residence Time (seconds)	Device Flow Rate (mL/min)	Time (min.)
Equilibration	PBS*	5	10	6.0	0.83
Load	CHO harvest	n/a	20	3.0	7.07
Load wash	PBS	10	10	6.0	1.67
Elution	Citrate**	4	20	3.0	1.33
Re-equilibration	PBS	5	10	6.0	0.83
CIP	0.1M NaOH	10	10	6.0	1.67
Equilibration	PBS	8	10	6.0	1.33

* PBS Phosphate-buffered saline (150 mM NaCl, 50 mM Phosphate, pH 7.4)

** Citrate (100 mM Citrate, pH 3.4)

Table 2: PROA102 (3.5 mL) Increased Flow Rate Protocol

Step	Solution	CV	Residence Time (seconds)	Device Flow Rate (mL/min)	Time (min.)
Equilibration	PBS*	5	15	14.0	1.25
Load	CHO harvest	n/a	20	10.5	8.50
Load wash	PBS	10	15	14.0	2.50
Elution	Citrate**	4	20	10.5	1.33
Re-equilibration	PBS	5	15	14.0	1.25
CIP	0.1M NaOH	10	20	10.5	3.33
Equilibration	PBS	8	15	14.0	2.00

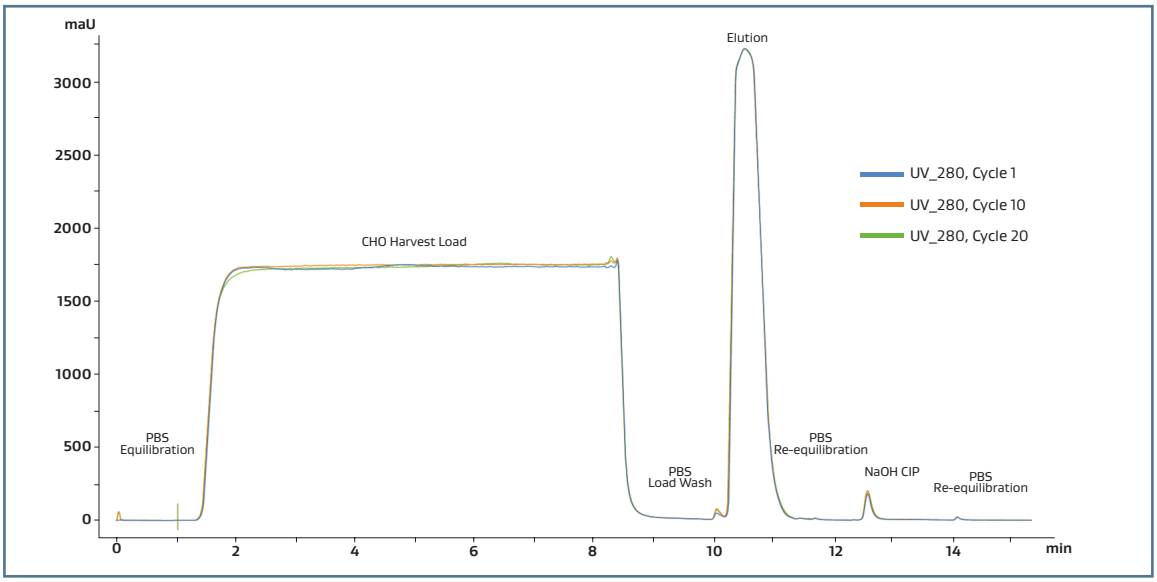
Results

1.0 mL GORE Device (PROA101) Increased Flow Rate Protocol

The purification protocol outlined in Table 1 was used to perform 20 cycles using a PROA101 device. The DBC value after purification remained unchanged and the device yield was constant at 98.6%, capturing 38.5mg of antibody per purification cycle. The overall purification time for each cycle was 14.7 minutes which translates to a mAb capture rate of approximately 157 mg of antibody per hour.

Figure 1 shows a UV absorbance chromatogram overlay of cycles 1, 10 and 20 demonstrating consistent purification performance over the course of cycling with the purification protocol outlined in Table 1.

Figure 1: PROA101 Purification Performance across 20 Purification Cycles using the Increased Flow Rate Protocol

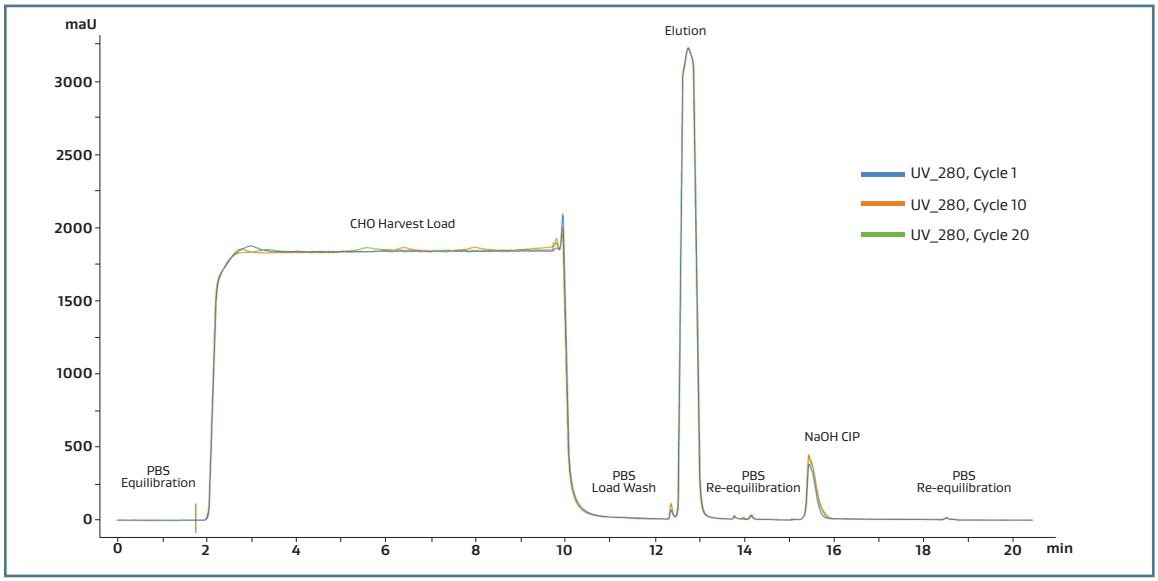


3.5 mL GORE Device (PROA102) Increased Flow Rate Protocol

The purification protocol outlined in Table 2 was used to perform 20 cycles using a PROA102 device. The DBC value after purification was within 5% of the starting DBC, yields were consistent at 102% and 130.1 mg of antibody captured per purification cycle. The overall purification time for each cycle was 20.2 minutes which translates to a mAb capture rate of approximately 387 mg of antibody per hour with a 3.5 mL bed volume.

Figure 2 shows a UV absorbance chromatogram overlay of Cycles 1, 10 and 20 which display a consistent purification performance over the course of cycling with the purification protocol outlined in Table 2.

Figure 2. PROA102 Purification Performance across 20 Purification Cycles using the Increased Flow Rate Protocol



Overall, the increased flow rate protocols improved the amount of antibody captured in a given hour and therefore improved the productivity of the columns. Table 3 indicates the productivity increase of these two increased flow rate protocols when compared to the recommended operating instructions for GORE® Protein Capture Devices.

Table 3. Productivity Comparison between Increased Flow Rate Protocols and Current Operating Instructions

Purification Process	Process Time (minutes)	Mass Captured Per Cycle (mg)	mAb Captured per hour (mg)	Productivity Increase (%)	Mass Captured in 20 Purification Cycles (mg)	Time to Perform 20 Cycles (hours)	Time Savings Over 20 Purification Cycles (hours)
Standard PROA101 procedure	35.07	38.5	66	—		11.7	—
Increased productivity PROA101 procedure	14.73	38.5	157	238%	770	4.9	6.8
Standard PROA102 procedure	35.07	130.1	223	—		11.7	—
Increased productivity PROA102 procedure	20.17	130.1	387	174%	2602	6.7	5.0

Conclusions

The increased flow rate protocols effectively improved the productivity of a given GORE Protein Capture Device when measuring mass of antibody captured per hour. The increase in productivity was higher for the PROA101 device due to the faster flow rate exposures possible for that column geometry. The corresponding productivity increase for the PROA101 device was approximately 238% when compared to the standard operating procedure recommended for a PROA101 device. The overall productivity increase for the PROA102 device was approximately 174% when compared to the standard operating procedure recommended for the PROA102. Translating the increased productivity into time savings over 20 cycles equated to 6.8 hours of saved time for the 1.0 mL PROA101 device and 5.0 hours of saved time for the 3.5 mL PROA102 device, both which finished 20 purification cycles within an 8 hour work day.

These experimental results provide the opportunity to optimize operational flow rates to improve productivity. In conjunction with the increased capacity at short residence times and the concentrated elution pools that the GORE Protein Capture Devices offer, overall productivity is greatly improved when compared to the current capabilities of resin-based protein A chromatography columns.

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.

Europe | W. L. Gore & Associates, GmbH
Wernher-von-Braun-Strasse 18 • 85640 Putzbrunn, Germany
Phone: +49 89 4612 3456 • Toll free: 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

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