APPLICATION NOTE

Alternative Method for mAb Purification: Rapid Purification without a Liquid Chromatography Unit using a GORE® Protein Capture Device

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

Objective

To assess the speed, purity, and yield performance of the GORE Protein Capture Device with Protein A to purify antibodies using alternative methods to liquid chromatography (LC) units.

Background

Liquid chromatography units provide exceptional process monitoring controls and automation for the purification of biomolecules, including monoclonal antibodies. However, many labs perform purifications without an LC unit, using methods such as a syringe or a peristaltic pump system. The GORE Protein Capture Device with Protein A can be used to rapidly purify monoclonal antibodies without an LC unit as demonstrated in three different manual/semi-automated operations.

Methods Evaluated:

1. Manual Syringe Purification
2. Syringe Pump Purification
3. Peristaltic Pump Purification

Materials:

Table 1. Materials required to execute the methods outlined

Materials	Part Number
1.0 mL GORE Protein Capture Device	PROA101
HSW NORM-JECT® Single-use Syringe 30 mL	4830003000
GE Healthcare Life Sciences Connector 1/16" Male/Luer female	28-9858-12
Nordson Medical Male Luer Integral Lock Ring to 200 Series Barb, 1/16"	MTLL210-6005
FALCON Centrifuge Tubes 15 mL	352096
MASTERFLEX® TYGON® Chemical Tubing, L/S 13	06475-13
New Era Pump Systems, Inc. NE-300 JUST INFUSION Syringe Pump	NE-300
MASTERFLEX® L/S Variable-Speed Console Drive	EW-77521-40
MASTERFLEX® L/S Easy-Load II Head	HV-77201-60

Procedure

The same buffers were used for consistency between the three purification methods. The purification process including buffers and wash are shown in Table 2. Purified monoclonal antibody (IgG_1) was used for each method of purification to demonstrate capability and capture yield values with minimal equipment requirements. A UV/Vis spectrophotometer was used to evaluate antibody concentrations, all evaluated at an absorbance of 280nm.

Table 2. Buffers used for each purification method

Buffer	Column Volumes (CV)	Volume (mL)	Step Process
DI Water	10 CV	10 mL	Rinse
PBS	10 CV	10 mL	Equilibrate
MAb	10 CV	10 mL	Load
PBS	10 CV	10 mL	Wash
Citrate	4 CV	4 mL	Elution
Citrate	6 CV	6 mL	Wash
PBS	5 CV	5 mL	pH adjustment
NaOH	10 CV	10 mL	CIP
PBS	8 CV	8 mL	pH adjustment
DI Water	5 CV	5 mL	Salt removal
Ethanol 20%	5 CV	5 mL	Storage

DI = Deionized water
PBS= Phosphate-buffered saline
MAb= Monoclonal antibodies
NaOH=Sodium hydroxide
CV= Column volumes

Method 1: Manual Syringe Purification

Purification Method 1 used a 30 mL luer adapted syringe attached to a 1.0 mL GORE® Protein Capture Device (PROA101) using a GE 1/16" male/luer female connector (Figure 1). The buffer order and volume dispensed per step followed the recipe outlined in Table 2. The flow-through by-products from the monoclonal antibody loading step were collected to evaluate any unbound antibody that passed through the GORE Device. A nominal Dynamic Binding Capacity (DBC) of 30 mg/mL was assumed for loading, and the GORE Device was loaded to approximately 80-90% of maximum DBC.

Fractions of 4.0 mL were collected during the elution step and neutralized using 1.0 mL of 1 Molar (M) Tris at 9.0 pH. The CIP step for this process followed Table 2 with a pause for 3 to 5 minutes after loading the first 5 CV of 0.1M sodium hydroxide to ensure sanitization of the GORE Device. A total of three purifications (bind-elute) were performed for this method using the same GORE Device, with an average total time of 28 minutes and 40 seconds, not including the CIP hold time.

Method 2: Syringe Pump Purification

Purification Method 2 follows the same steps and procedures as the manual syringe purification with the aid of a syringe pump (Figure 2), operated at 20 seconds residence time for the GORE Device (3.0 mL/min; see Operating Instructions for details). Loading and elution steps as well as neutralization of fractions for the purification followed the steps in the first method. The same GORE Device was used for both Method 1 and Method 2. Total time for this semi-automated purification method was 29 minutes and 20 seconds, not including the CIP hold time.

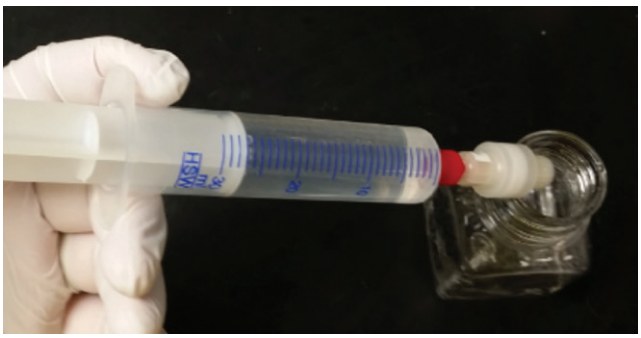

Figure 1. Manual purification using a 30 mL syringe attached to a 1.0 mL GORE Protein Capture Device

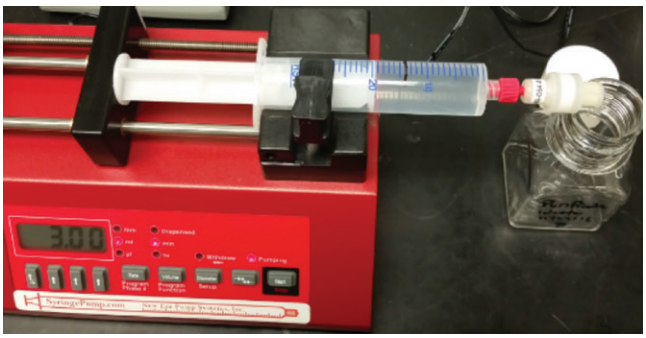

Figure 2. Semi-automated purification using a syringe pump and a 30 mL syringe attached to a 1.0 mL GORE Protein Capture Device

Method 3: Peristaltic Pump Purification

Purification Method 3 followed the same steps and procedures as the syringe purification methods with an additional priming step between each buffer/antibody transfer (Figure 3). This method also used a second 1/16" male luer/200 barb fitting to attach the pump tubing to the GE connector. The priming step for each solution transfer was performed by capping the outlet of the GORE Protein Capture Device, detaching the connector from the device, priming the new solution through the lines, then reattaching the device to the connector, and removing the cap on the outlet side to start the next step. All other steps were the same as the first two methods. The total time for this semi-automated purification method was 28 minutes and 37 seconds, not including the CIP hold time.

Figure 3. Semi-automated purification method using a peristaltic pump, attached to a 1.0 mL GORE Protein Capture Device

Results

Results for the three methods outlined above can be seen in Table 3. All purification methods fell within the specifications for the GORE Device.

Table 3. Purification Results for three methods with GORE Protein Capture Device

Method	MAb Feed Conc. (mg/mL)	MAb Loaded (mg)	Elution Pool Conc. (mg/mL)	Yield (%)	Leached Protein A (ppm)
Method 1: Manual Syringe Purification	2.66	26.6	5.19	97.5	13.7
Method 2: Syringe Pump Purification	2.64	26.4	5.07	97.9	8.1
Method 3: Peristaltic Pump Purification	2.68	26.8	5.25	98.1	7.5

Conclusion

The three purification methods outlined in this application note provide alternative options to successfully purify a monoclonal antibody in under 30 minutes with yields greater than 95%. These methods provide the ability to perform affinity purifications without the use of an LC unit. Though these methods are feasible options for purification, individualized feed stocks purification results will vary. The method and buffers should be optimized based on the target antibody and specific feed stock. Further optimization of the protocol may be explored to fit the needs of a given lab.

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.

All technical information and advice given here is based on our previous experiences and/or test results. We give this information to the best of our knowledge, but assume no legal responsibility. Customers are asked to check the suitability and usability of our products in the specific applications, since the performance of the product can only be judged when all necessary operating data is available. Gore's terms and conditions of sales apply to the purchase and sale of the product.

NORM-JECT is a registered trademark of Henke-Sass, Wolf GmbH, FALCON is a registered trademark of Corning, Inc., MASTERFLEX and TYGON are registered trademarks of Cole-Parmer., JUST INFUSION is a trademark of New Era Pump Systems, Inc.

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

PB6956_Rev2_MA_EN_MAR20