Evaluation of Dynamic Binding Capacity and Residence Time of Membrane-Based GORE® Protein Capture Device with Protein A

Chao Zheng[1], Fei Han[1], Chelsea Chen[2], Rachel Kroe-Barrett[1], Andrew Nixon[1]

1. Department of Biotherapeutics Discovery, Boehringer Ingelheim, Ridgefield, CT 06877 USA;
2. University of Connecticut, CT 06269 USA

OBJECTIVE

To evaluate the Dynamic Binding Capacity (DBC10*) and residence time of the pre-packed GORE Protein Capture Device with Protein A for purification of therapeutic antibodies.

BACKGROUND & PURPOSE

Protein A purification is part of the standard workflow in downstream processing of therapeutic monoclonal antibodies. Traditional antibody purification processes can be slow due to long sample loading time. Also, additional steps may be required to concentrate the purified material. A new technology couples Protein A ligands to a proprietary membrane matrix, offering the potential for high dynamic binding capacity with a shorter loading time.

The purpose of this note is to assess the DBC10 and residence time of the membrane-based GORE Protein Capture Device as compared with an agarose-based Protein A column.

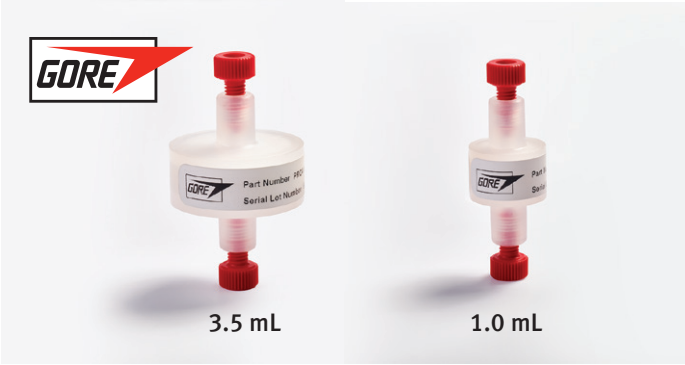

Image courtesy of Gore & Associates

MATERIALS/COLUMNS

- 1.0 mL GORE Protein Capture Device- Part number PROA101
- Agarose-based Protein A column
- ACQUITY UPLC® Protein BEH200 SEC Column
- SEPAX PROTEOMIC® HIC Butyl NP2 Column

METHODS

A monoclonal human IgG1 was cultured in CHO cell expression systems. To determine the DBC10, one-step Protein A purified material was diluted 1:10 (v/v) with phosphate-buffered solution (PBS) (to about 0.34 mg/mL) before loading.

The DBC10 was determined by running the purified sample on either the agarose-based Protein A column or the GORE Protein Capture Device with Protein A at 20-second, 30-second, 60-second, or 120-second residence time, corresponding to 0.5 mL/min, 1.0 mL/min, 2.0 mL/min, and 3.0 mL/min flow rate for the 1.0 mL column. Samples were eluted and neutralized, then subject to analysis by ACQUITY UPLC BEH200 column (aSEC†) and SEPAX PROTEOMIX HIC butyl NP2 column (aHIC††).

*Dynamic binding capacity calculated at 10% breakthrough
†Analytical size exclusion chromatography
††Analytical hydrophobic interaction chromatography

RESULTS

The GORE Protein Capture Device with Protein A showed significantly higher dynamic binding capacity and allowed Protein A purification with much shorter residence time. The antibodies purified by the Protein A column from Gore and the agarose-based Protein A column had similar qualities as assessed by aSEC and aHIC.

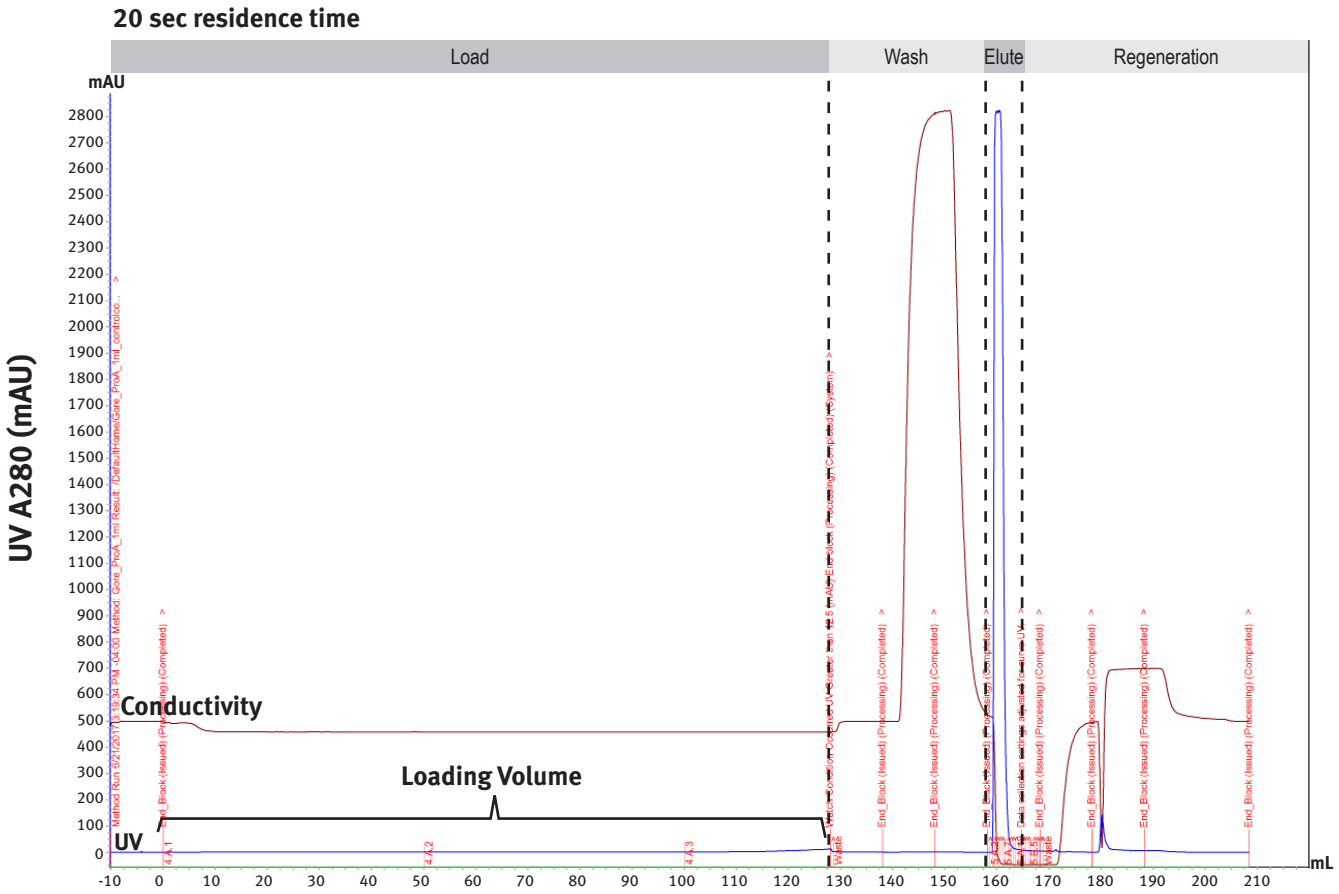

FIGURE 1: An example of the purification process, including the steps for load, wash, elute, and column regeneration. The sample loading was stopped automatically when the UV A280 reading reached 10% breakthrough levels.

The DBC10 of the agarose-based Protein A column was determined to be 25.0 mg/mL at 120 sec residence time, similar to the typical yield from this type of column in the standard work-flow. In comparison, the GORE Protein Capture Device with Protein A showed significantly higher DBC10. Even at 20 sec residence time, the GORE column achieved 38.8 mg/mL DBC10. At 120 sec residence time, the DBC10 of the GORE column was 68.8 mg/mL. In addition, the actual Protein A yield of the pooled elution fractions was comparable to the DBC10. The higher dynamic binding capacity also translated into a higher elution concentration when the samples were loaded at the same residence time.

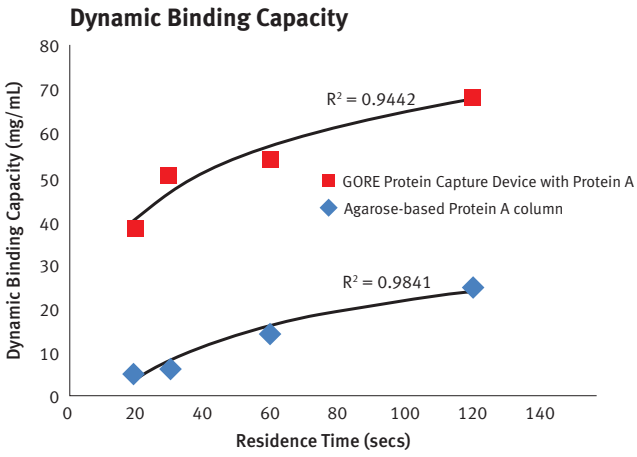

FIGURE 2: Dynamic Binding Capacity determined for GORE Protein Capture Device with Protein A vs. Agarose-based Protein A column at four different residence time: 20 sec, 30 sec, 60 sec and 120 sec.

DBC10 vs Protein A Yield

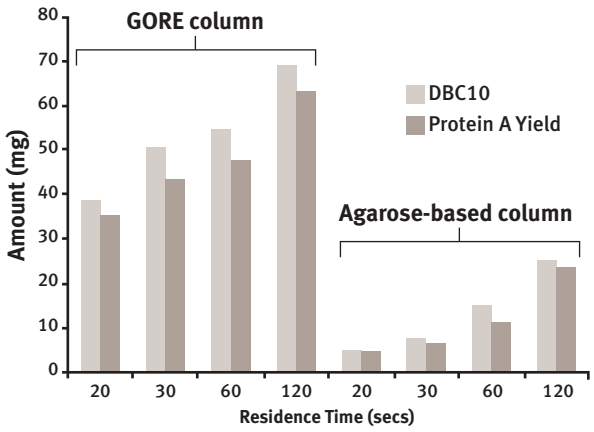

FIGURE 3: The dynamic binding capacity is in good agreement with the actual amount of Protein A recovered after pooling the elution fractions

TABLE 1: Data Summary of dynamic binding capacities, actual yield and concentrations of the pooled elution fractions

	Residence Time (sec)	Flow Rate (mL/min)	DBC10 (mg)	Actual Yield (mg)	Concentration (mg/mL)
GORE Protein Capture Device	120	0.5	68.8	63.3	13.2
	60	1	54.6	48.0	5.3
	30	2	50.5	43.3	5.0
	20	3	38.8	35.2	4.2
Agarose-based column	120	0.5	25.0	23.5	4.5
	60	1	15.2	11.1	2.3
	30	2	7.7	6.5	2.2
	20	3	5.0	4.6	1.2

Protein A Purification from Cell Culture

Column	Flow Rate (mL/mL)	% Monomer	% Recovery
Agrose-based	0.5	96.1	88.5
Gore column	2.0	97.1	88.6

Analytical Characterization

aSEC

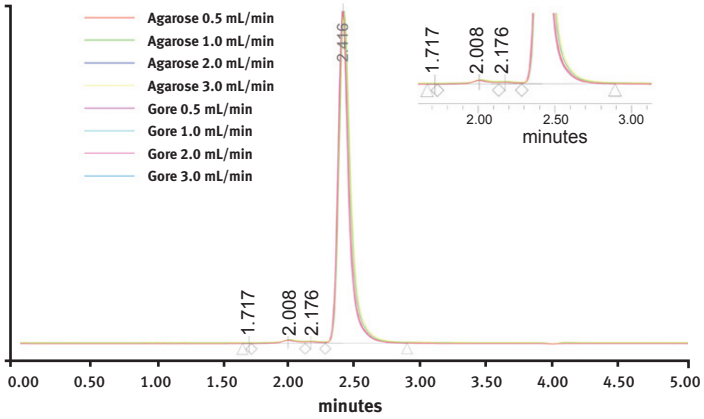

FIGURE 4: Samples after purification by either agarose-based Protein A column or the GORE Protein Capture Device showed similar size distribution profiles on aSEC

aHIC

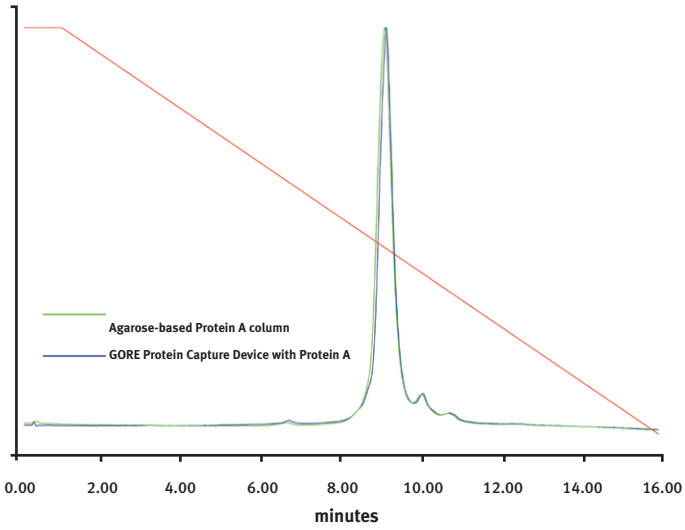

FIGURE 5: Samples after purification by either agarose-based Protein A column or the GORE Protein Capture Device showed similar profiles on aHIC

To confirm the finding based on the purified IgG1, the same molecule was purified from the cell culture supernatant with the agarose-based Protein A column and the GORE Protein Capture Device with Protein A at a flow rate of 0.5 mL/min (i.e. 120 seconds residence time) and 2.0 mL/min (i.e. 30 seconds residence time), respectively. The percent monomer and the percent recovery by the two columns were comparable.

CONCLUSION

Membrane-based technology such as the GORE Protein Capture Device with Protein A offers the advantages of high dynamic binding capacity and short residence time. Together with high titers, the GORE column can reduce overall process time and may help increase yields in monoclonal antibody purification by eliminating the need for additional downstream concentration steps.

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

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