Membrane, Resin, and Impact to Product:

Overcoming Barriers to Adopting Membrane Technology by Demonstrating Protein A Affinity Capture Step Product Quality and Process Characteristic Similarity

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Shunsuke Shiina, Ph.D., Neha Saxena, Ph.D., and Rodrigo Gonzalez, Ph.D., AGC Biologics, Inc.
Jeffrey Cassel, Eric Van Voorhees, and William C. Barrett, Ph.D., W.L. Gore & Associates, Inc.

Abstract

Comparability in both product quality and chromatographic process characteristics between a Protein A affinity capture membrane and a packed-bed resin reference was demonstrated using a set of proposed criteria. Product quality attributes and process impurities from a purified IgG1 monoclonal antibody cell culture harvest were comparable between Protein A resin and membrane affinity stationary phases, with the membrane demonstrating order-of-magnitude higher chromatographic productivity. When referenced to previously published manufacturing-scale data, these laboratory-scale results suggest that membrane and resin Protein A media can be used interchangeably and that they can be concurrently validated across lab scale, process development, clinical bioprocessing, and manufacturing scale bioprocessing.

Introduction

Biopharmaceutical manufacturing is the process of producing therapeutic products using living systems, such as bacteria, yeast, or animal cells. Biopharmaceuticals are often complex proteins that can treat a variety of diseases, such as cancer, diabetes, and autoimmune disorders. Biopharmaceutical manufacturing has benefited from advancements in new product and process technologies; however, biopharmaceutical manufacturing also faces many challenges and barriers associated with adopting new technologies, across the areas of business, regulatory, technical, and people and/or culture.[1]

The preceding citation highlighted business barriers including costs, risk to supply chain interruption, timelines, and portability of processes. The regulatory barriers were noted as real or perceived, though the technology being adopted may influence regulatory aspects. The technical barriers included inflexibility of manufacturing operations to change predictably performing incumbent technologies and uncertainty regarding whether the new technology impacts the process or product quality. Lastly, people and/or culture barriers are largely demonstrated by the industry and regulators reacting or hesitating to implement, operate, or review something new, as well as the often-stated fear of being first. Therefore, driving adoption of new technologies in biopharmaceutical manufacturing requires innovation, collaboration, education, and demonstration.

Biopharmaceutical manufacturers are constantly seeking to improve the efficiency, effectiveness, flexibility, and quality of their operations. New manufacturing technologies such as membrane-based separation technologies can address these desires. However, a lack of comparability data as well as hesitancy to implement small scale studies demonstrating comparability that could supplement regulatory filings limit their adoption.

Single-use and membrane Protein A affinity capture technologies are benefiting the downstream processing of antibody-based therapeutics by increasing productivity, reducing labor intensive cleaning processes, decreasing product changeover times in multi-product facilities, reducing cross-contamination risks and minimizing bioburden.[2,3] For example, membrane-based Protein A affinity capture devices have demonstrated order-of-magnitude productivity improvement over traditional Protein A resin columns, across manufacturing and laboratory scales.[2,4]

Despite an increasing amount of membrane affinity capture options available,[5,6] there is a scarcity of quality attribute data comparing affinity purification from membrane and traditional resin columns. Moreover, there is a similar lack of information comparing purification process outputs between the two methods. This absence of comparability evidence can lead to hesitation in evaluating or adopting intensified process technologies such as Protein A membranes.

Ideally, the critical quality targets of the molecule being purified should be met, regardless of the affinity capture medium. A recent approach providing an example technique for addressing regulatory expectations of comparability indicates that materials pre- and post-process change are expected to be highly similar, but they are not expected to be identical.[7]

The objective of this study was to demonstrate highly similar product quality and process performance outputs between a high productivity Protein A membrane capture and a reference resin capture using a matching purification protocol. This study demonstrates a collaborative approach between a CDMO and a technology company on demonstrating equivalency in the Protein A capture step for antibody-based therapeutics.

Experimental

Clarified Harvest and Protein A Affinity Capture

A target volume of approximately 500 L of a monoclonal antibody (mAb) expressing CHO cell culture harvest was processed in an N-stage 2000 L bioreactor. This harvest was then clarified using a two-stage cartridge depth filter followed by a 0.2 µm capsule sterile filtration. Of note, a single lot of clarified harvest was interrogated in this study. Multiple lots may also be considered for comparability studies.[7] The harvest lot was aliquoted for separate purification using laboratory scale Protein A affinity capture products set forth below:

- A 5 mL HiTrap MabSelect SuRe™ Protein A resin column (Cytiva P/N 11003493), cycled four times using the purification protocol in Table 1.
- A 3.5 mL GORE® Protein Capture Device with Protein A (Gore P/N PROA102) membrane device, cycled ten times using the purification protocol in Table 1.

Laboratory scale Protein A affinity purification cycling was performed on an AKTA avant 25 chromatography system, using the purification protocol in Table 1.

Table 1. Chromatography Method for Laboratory Scale Protein A Affinity Capture device cycling.

Step	Buffer	Resin Step Duration (Column Volumes (CV))	Membrane Step Duration (Membrane Volumes (MV))	Resin Residence Time (min)	Membrane Residence Time (min)	Resin Step Time (min)	Membrane Step Time (min)
Equilibration	Tris-HCl buffer	3.00	3.00	3.0	0.2	9.0	0.60
Load	Cell Culture Harvest Fluid	17.50	11.60	3.0	0.4	52.4	4.66
Wash 1	Tris-HCl Buffer	3.00	1.43	3.0	0.4	9.0	0.57
Wash 2	Tris-HCl + NaCl buffer	3.00	3.00	3.0	0.2	9.0	0.60
Pre-elution wash	Tris-HCl buffer	2.00	3.00	3.0	0.2	6.0	0.60
Elution	Sodium Acetate buffer	5.00	3.75	3.0	0.2	15.0	0.75
Wash 3	Tris-HCl Buffer	3.00	N/A	3.0	N/A	9.0	N/A
Clean in Place (CIP)	0.1N NaOH	3.00	3.00	3.0	0.4	9.0	1.20
Re-Equilibration	Tris-HCl Buffer	5.00	3.00	3.0	0.2	15.0	0.60
Total time per cycle (min)						Resin 133.4	Membrane 9.58

The target load was set at 30 mg/mL at 3 minutes (180 seconds) residence time for the resin column and 20 mg/mL at 0.4 minutes (24 seconds) residence time for the membrane device. Load volumes were determined based on prior work with this molecule (AGC Biologics unpublished data); loading was performed to 80% of $DBC_{10\%}$ for both resin and membrane.

Analytical Characterization of Protein A Affinity Capture Eluates for Critical Quality Attributes

- Cell culture harvest mAb concentration was quantified using titer assay via Ultra Pressure Liquid Chromatography (UPLC). Elution mAb concentrations were measured with a NanoDrop One (Thermo Fisher Scientific).
- After neutralization of resin and membrane eluates to pH 6-7, product related impurities were quantified using Size Exclusion Chromatography (SEC) on an Agilent 1100 HPLC platform. Products were isocratically separated at a 0.3 ml/min flow rate on a TSKgel® SuperSW mAB HTP column coupled with a Diode Array Detector. Peaks were analyzed to quantify the percent of high molecular weight species in relation to the target mAb main peak.
- Clearance of Host Cell Proteins (HCP) during the Protein A affinity capture process was quantified using automated ELISA (Cygnus kit #F550-1 with ProteinSimple Ella™ cartridges) following manufacturer protocols. HCP concentrations were normalized to mAb concentration and expressed as ng/mg (ppm). Log Reduction Value (LRV) was subsequently determined in relation to cell culture harvest HCP values.
- Leached Protein A was quantified using a Protein A ELISA kit from Repligen Bioprocessing (p/n 9000-1). The ELISA was performed using the "Dilute and Go" extraction according to the manufacturer's instructions. Concentrations were normalized to mAb concentrations and LRV's calculated from cell culture harvest values.
- Host cell DNA were quantified using qPCR technology. The residual DNA concentration was normalized to mAb concentration as pg/mg and removal efficiencies were evaluated by Log Reduction Value (LRV).
- Charge variant distribution was quantified in terms of percent relative area. Separation of acidic and basic variants from the main peak was based on capillary zone electrophoresis using Revvity Labchip platform.
- N-Glycan distribution was quantified by chip based CGE technique via LIF detection using the Revvity Labchip platform. Relative amount was evaluated by alignment of detected peaks across the ladder run and normalization with a spiked internal standard.

Recent guidance indicates that specific understanding of the product influences characterization methods employed in assessing similarity or comparability.[7] In this study, the product quality attributes of percent monomer, percent high molecular weight (HMW) species, charge variant distribution, and n-glycan distribution were selected based on understanding of the IgG1 mAb interrogated in this study and a risk assessment (AGC Biologics unpublished data) indicating that similarity within the Protein A capture step could be adequately assessed by measuring these attributes. Residual HCP and DNA concentrations were characterized to ensure that these process impurities were cleared to similar levels across both resin and membrane capture media.

Results

Product Quality & Process Impurities Results

Table 2 summarizes average SEC product quality, charge variant distribution, n-glycan distribution, HCP, DNA, and leached Protein A data in eluates from both the 5 mL Protein A resin column and the 3.5 mL Gore membrane device. HCP and DNA data are presented as log reduction values relative to the clarified cell culture harvest. Figures 1 through 3 graphically depict average SEC product quality, charge variant, and n-glycan distributions, respectively, demonstrating the similarity between resin and membrane. Regarding the average SEC product quality results, note that low molecular weight peak fractions that are indicative of protein fragmentation were typically < 0.04 area percent in all cases and are therefore neither tabulated nor plotted.

Table 2: Laboratory scale Protein A resin column and Gore Protein A membrane device product quality and process impurities results comparison.

Attribute	5 mL Protein A Resin Column	3.5 mL Gore Membrane Device with Protein A
Average SEC Product Quality (% main / % HMW)	91.83 / 8.17	97.59 / 2.41
Charge Variant Distribution (% basic / % main / % acidic)	5.05 / 59.50 / 35.50	5.05 / 59.34 / 35.46
N-Glycan Distribution (% Man5 / % G0F / % G1F' / % G2F)	20.7 / 54.2 / 8.6 / 1.33	17.4 / 55.1 / 10.27 / 1.36
Elution HCP (LRV)	2.03	2.17
Elution DNA (LRV)	3.49	3.62
Elution Protein A (ppm)	4.28	5.25

Figure 1: Eluent Average SEC Product Quality distributions for Laboratory scale Protein A resin column and Gore Protein A membrane device.

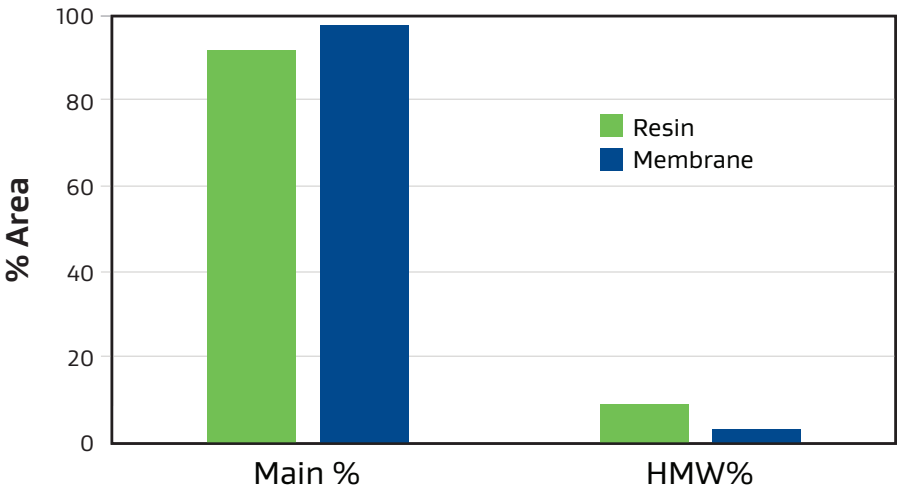

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Figure 2: Eluent Charge Variant distributions for Laboratory scale Protein A resin column and Gore Protein A membrane device. Error bars represent relative standard deviation on replicate preparations.

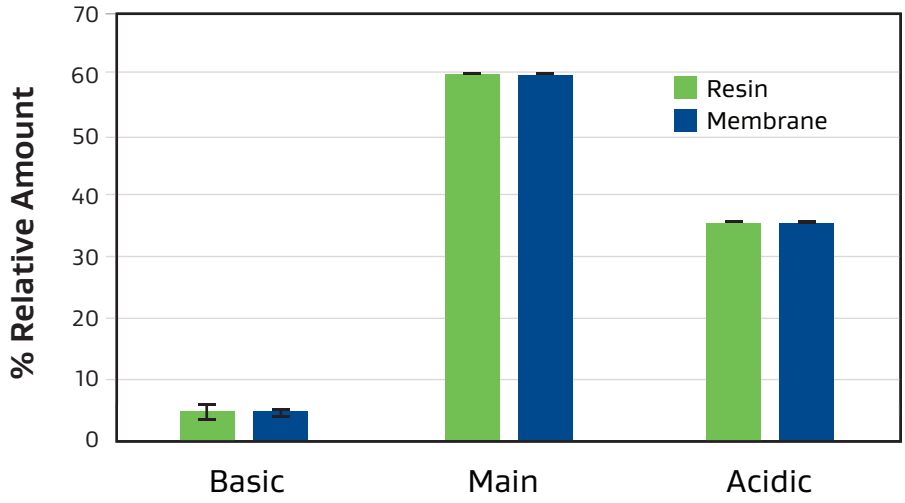
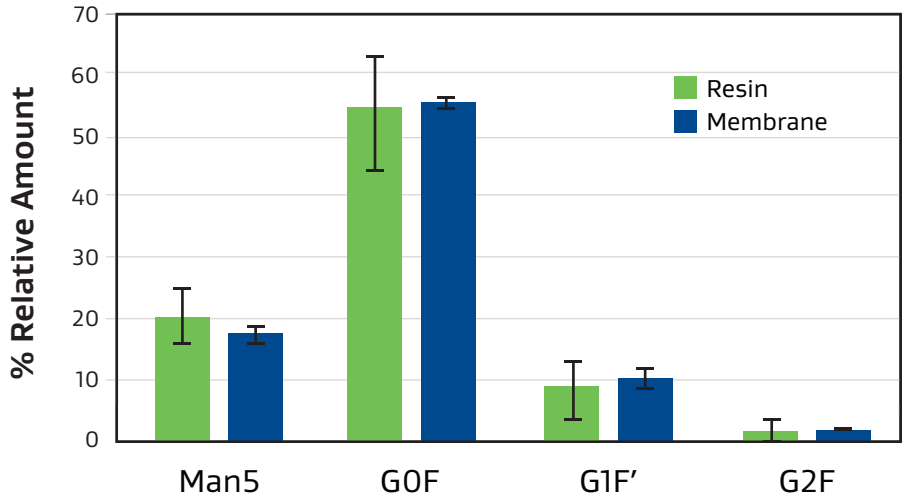


Figure 3: Eluent n-glycan distributions for Laboratory scale Protein A resin column and Gore Protein A membrane device. Error bars represent relative standard deviation on replicate preparations.

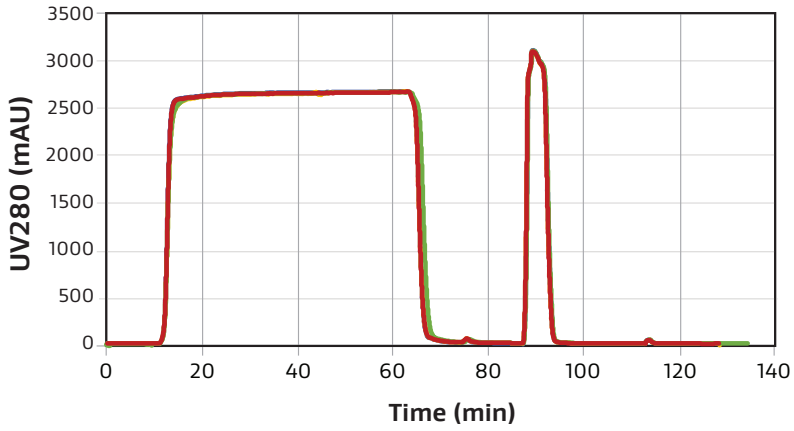


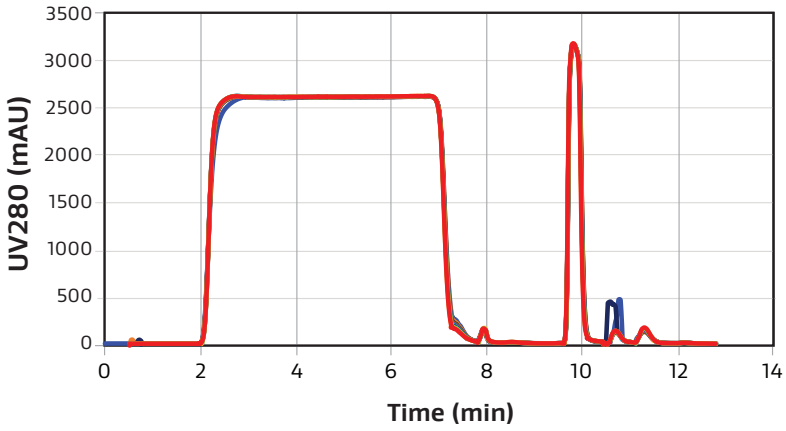
The data in Table 2 and Figures 1 through 3 suggest highly similar product quality and process impurities demonstrating the comparability between the two Protein A affinity chromatographic modes.

Chromatographic Process Performance Results

Figure 4 shows UV280 chromatogram overlays for both the 5 mL Protein A resin column and the 3.5 mL Gore membrane device, using the cycling protocols in Table 1.

Figure 4: UV280 Chromatogram Overlays for Laboratory scale Protein A resin column (top) and Gore Protein A membrane device (bottom)





The chromatograms indicate acceptable phase transitions, consistent performance, and sharp elutions over cycling for both Protein A affinity chromatographic modes. The small peaks between 10.5 and 11.5 minutes in the membrane chromatograms were attributed to LC system flushes before the sanitization step; since these flushes did not flow through the membrane device, the chromatogram time is longer than the actual process time.

Figure 5 exhibits elution yields, as calculated from load titer and elution concentration data, and productivity over cycling for both the resin column and the membrane device. Table 3 summarizes calculated productivity, average yield, and average elution volume for both chromatographic modes.

Figure 5: Elution Yields (top) and Calculated Productivity (bottom) for Laboratory scale Protein A resin column and Gore Protein A membrane device over cycling

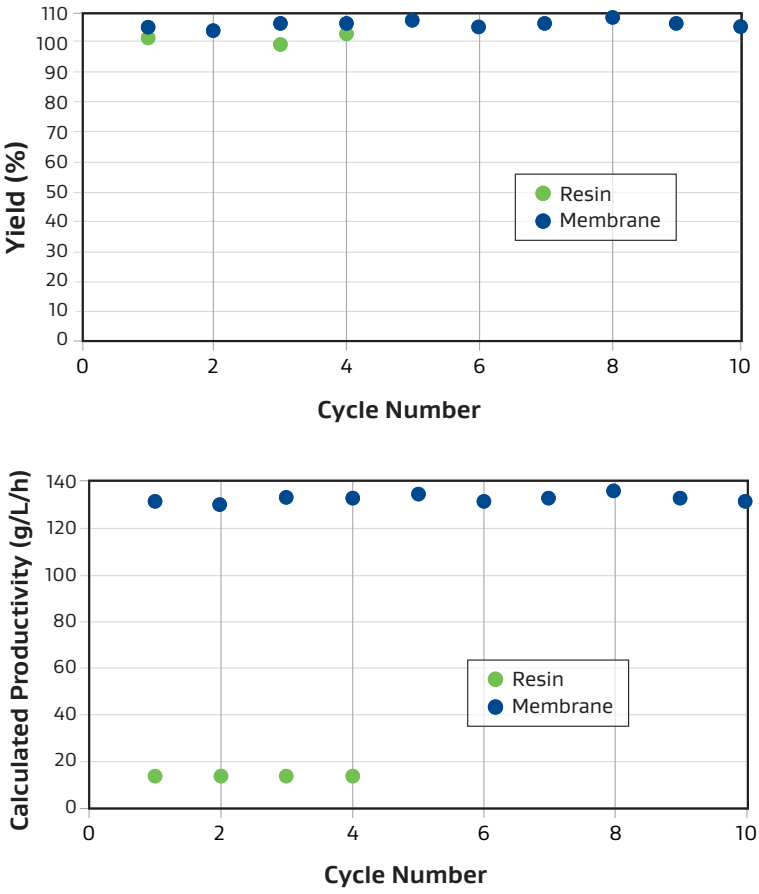


Table 3: Laboratory scale Protein A resin column and Gore Protein A membrane device chromatographic process performance results comparison.

Attribute	5 mL Protein A Resin Column	3.5 mL Gore Membrane Device with Protein A
Productivity (g/L/h)	13.8	132.8
Average Yield (wt %)	102.0 %	106.1 %
Average Elution Volume (Column Volumes, CV) [100-100 mAU cutoff]	2.72	1.84

Consistent yields were observed for both chromatographic modes over the course of cycling. Calculated yields over 100% are likely a measurement artifact of the two different analytical methods used to determine product concentration in purified eluate vs. clarified harvest fluid. Productivity calculations indicate a consistent 10X increase in productivity for the Gore Protein A membrane device compared to the resin column. Sharp and consistent elutions were observed for both chromatographic modes, with elution volume for the membrane device consistent with or better than the resin column.

Discussion

The results from the empirical approach employed in this study suggest comparable critical quality attributes from a mAb cell culture harvest through the Protein A affinity capture step using both conventional Protein A resin and Protein A membrane stationary phases. Laboratory scale Protein A affinity capture was performed on the same clarified CHO cell culture harvest with both a 5 mL Protein A resin column and 3.5 mL Gore Protein A membrane device. Both affinity chromatographic capture modes demonstrated highly similar performance in terms of product quality (HPLC-SEC % main and % HMW, charge variant distribution, n-glycan distribution), process impurities (HCP, DNA, and leached Protein A), and chromatographic process performance (A280 chromatograms, mAb yield, elution volume). Performance of both chromatographic modes was stable over multiple purification cycles. The Gore Protein A membrane device demonstrated 10 times higher productivity compared to the resin column. Consistent with recent guidance[7], the aforementioned product attributes were selected based on understanding of the particular mAb interrogated in this study and a risk assessment (AGC Biologics unpublished data) indicating that comparability within the Protein A capture step could be adequately assessed by measuring only these attributes.

The performance equivalency demonstrated herein between Protein A resin and Gore Protein A membrane can be applied to process development, clinical bioprocessing, and manufacturing scales as well, since Gore membranes scale consistently using residence time.[2,8] This means that regardless of scale of intended use, similarity or comparability assessment can be done at laboratory scale, applying multiple lot replication[7] if desired, resulting in significant time and cost savings compared to conducting comparability trials at large scale.

Conclusions

The findings herein established Protein A capture step similarity at laboratory scale representative of manufacturing scale between a 5 mL Protein A resin column and a 3.5 mL Gore Protein A membrane device.

This study shows a collaborative approach between a CDMO and a technology company to demonstrate similarity between an incumbent and a new, intensified technology in the Protein A step for antibody-based therapeutics. This study provides evidence that barriers to adoption of new process technology can be addressed through thoughtful experimental design and collaboration. The findings exemplify the cooperative benefit gained when Biopharma companies and process technology companies work together to develop, assess, and adopt new manufacturing technologies.

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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

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

gore.com/pharmbio

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