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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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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.

Experimental

Clarified Harvest and Protein A Affinity Capture

500 L of a mAb expressing CHO cell culture harvest was processed in an N-stage 2000 L single use bioreactor. This harvest was then clarified using a two-stage cartridge depth filter followed by a 0.2 µm capsule sterile filtration. The mAb titer was 1.72 g/L. The harvest lot was aliquoted for separate purification using laboratory scale Protein A affinity capture products as follows:

- 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.

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.

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. 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.

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

Results (continued)

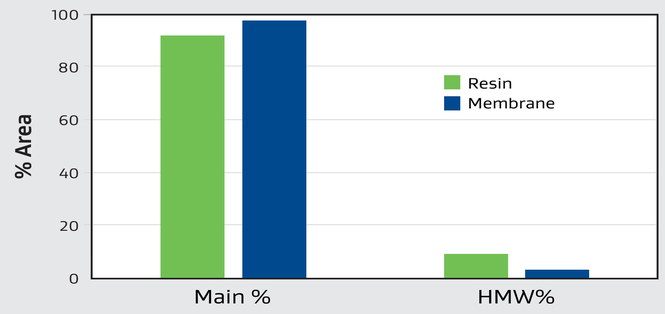

Figure 1: Eluent Average SEC Product Quality distributions for laboratory scale Protein A resin column and Gore Protein A membrane device.

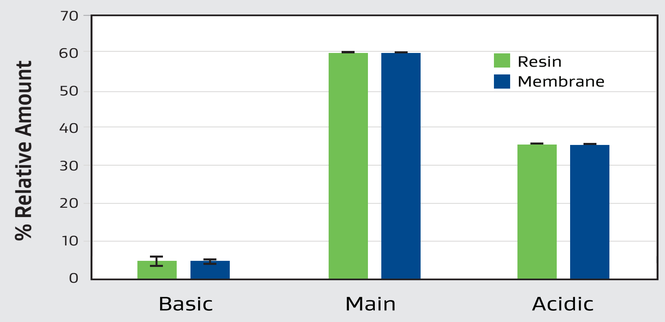

Figure 2: Eluent Charge Variant distributions for laboratory scale Protein A resin column and Gore Protein A membrane device. Error bars represent relative standard deviation on replicate preparations.

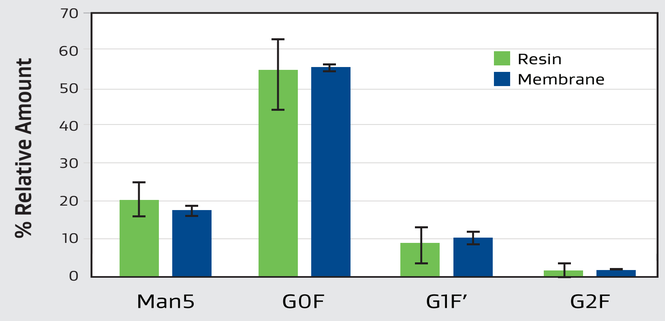

Figure 3: Eluent n-glycan distributions for laboratory scale Protein A resin column and Gore Protein A membrane device. Error bars represent relative standard deviation on replicate preparations.

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. The chromatograms indicate acceptable phase transitions, consistent performance, and sharp elutions over cycling for both 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 4: UV280 Overlays for lab scale Protein A resin column (left) and Gore Protein A membrane device (right).

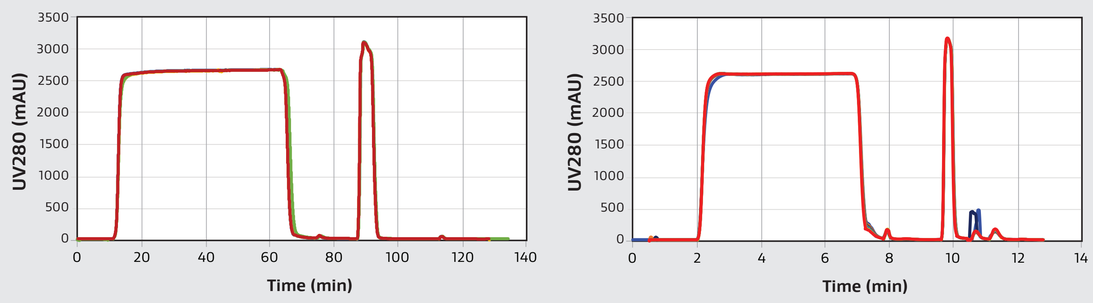

Figure 5: Elution Yields (left) and Productivity (right) for lab scale Protein A resin column and Gore Protein A membrane device over cycling.

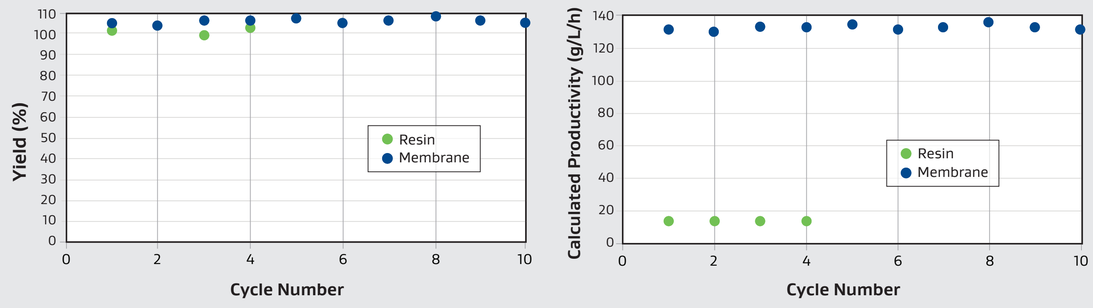

Table 3: Laboratory scale Protein A resin column and Gore Protein A membrane device chromatographic process performance results comparison.

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 [100-100 mAU cutoff]	2.72	1.84

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 consistent 10X increase in productivity for the Gore membrane device compared to the resin column.
- A collaborative approach between a CDMO and a technology company to demonstrate similarity between an incumbent and a new, intensified technology for antibody-based therapeutics.
- Evidence that barriers to adoption of new process technology can be addressed through thoughtful design and experimentation.
- 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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