Highly Efficient Process for the Purification of IgG

Sheldon Broedel, Jr.[1], Mike McManaway[2] and William Barrett[2]

1. Athena Enzyme System, AthenaES, 1450 S. Rolling Road, Baltimore, MD 21227
2. W. L. Gore & Associates, Inc., 402 Vieve's Way, Elkton, MD 21921

OBJECTIVE

Evaluate the GORE® Protein Capture Device with Protein A combined with a simulated moving bed chromatography technique, CaptureSMB™ to increase the productivity of IgG purification.

BACKGROUND & PURPOSE

Protein A affinity chromatography is widely used for the commercial purification of IgG antibodies. Protein A is a Staphococcal protein with an affinity for the Fc portion of IgG antibodies. The high degree of binding specificity of Protein A for IgG molecules has been exploited to develop chromatographic separations with high step yields and product purity. The Protein A is covalently attached to solid supports including agarose, polymethylacrylate, silica, CPG, polyacrylamide and polystyrene. Recombinant versions of the protein impart greater stability and specificity and enable the use of more rigorous cleaning in place protocols that help to further reduce contaminants.

Despite these advances, Protein A chromatography remains a bottleneck in downstream processing. There is often a tradeoff between resin capacity utilization and processing speed with the choice affecting the economics of the process. Due to the small pore sizes of traditional resins, mass transfer rates reduce the overall binding kinetics between Protein A and IgG. Consequently, slower flow rates are needed to ensure capture of the IgG molecule. In contrast, membrane matrices are macroporous and the overall binding kinetics is determined by the protein-protein kinetics and not on mass transfer. Thus, membrane chromatography can be run at higher flow rates.

W. L. Gore & Associates has developed a unique Protein A column, the GORE Protein Capture Device, using an expanded polytetrafluoroethylene membrane composite that offers high binding capacity at high flow rates. These devices have an 8-fold higher capacity than agarose resins at a 20s residence time. Therefore, they have the potential to greatly reduce the processing time and significantly increase productivity when used in conjunction with multicolumn chromatographic processes.

The objective of the research presented here was to evaluate the use of a GORE Protein Capture Device with Protein A in combination with a simulated moving bed chromatography technique, CaptureSMB™, as a means of further increasing the productivity of IgG purification.

MATERIALS/COLUMNS

- 3.5 mL GORE Protein Capture Device (PR0A102), 2 devices
- Agarose-based Protein A columns

PROCEDURE

The CaptureSMB process with GORE Protein A column included:

- CaptureSMB is a two column counter-current chromatography technique (FIGURE 1)
- Increases column capacity utilization by maximizing resin use
- Cyclical process enables continuous manufacturing (FIGURE 2)

FIGURE 1: Comparison of chromatography techniques

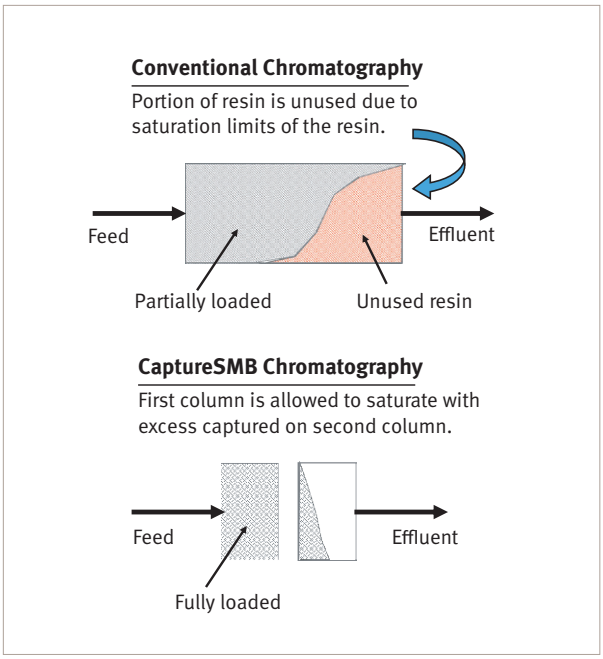
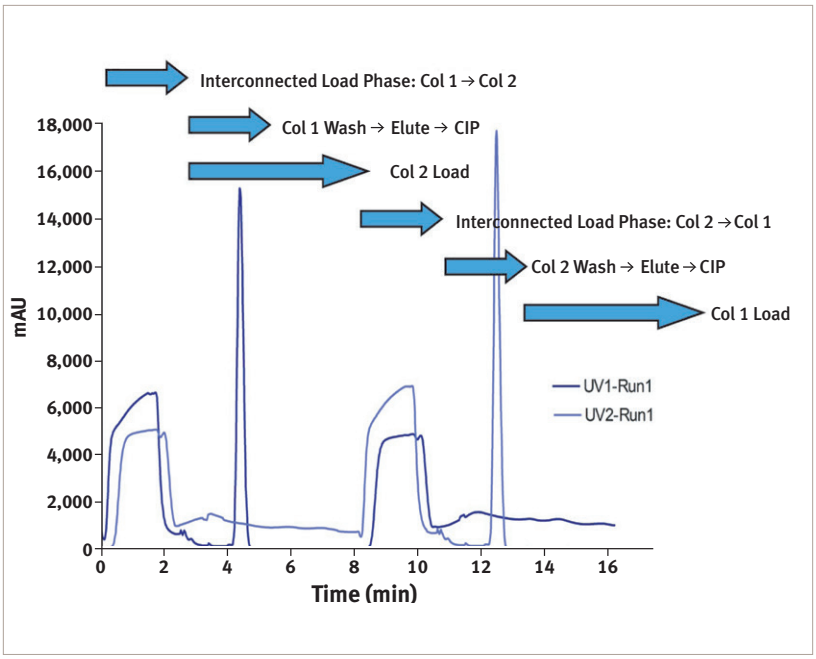


FIGURE 2: Single cycle CaptureSMB process



RESULTS

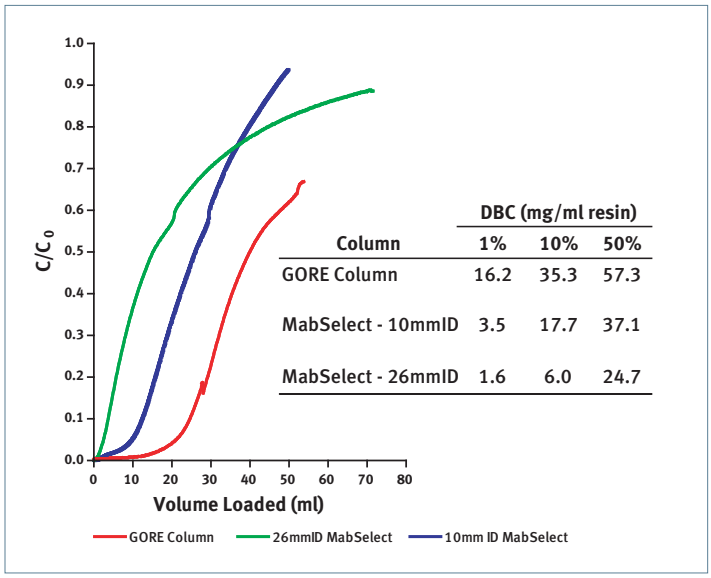
Dynamic Binding Capacity Comparison

The Dynamic Binding Capacity (DBC) was determined for the GORE® Protein Capture Device with Protein A and for two columns packed with MabSelect Protein A resin (GEHealthcare). All three columns contained 3.5 ml of matrix or resin. The column dimensions are shown in TABLE 1. One MabSelect column had the same dimensions as the GORE column whereas the second column had more conventional column dimensions. The DBC was determined from the break-through curves generated when loading feed at 100 cm/h. The feed was composed of bovine IgG in UV Transparent RPMI 1640 culture medium which permits direct calculation of the DBC from the chromatographic trace (FIGURE 3).

TABLE 1: Comparison of Column Dimensions

Column	ID (cm)	Bed Height (cm)
Gore Protein A (26 mm ID)	2.8	0.6
MabSelect (10 mm ID)	1.0	4.5
MabSelect (26 mm ID)	2.6	0.7

FIGURE 3: Dynamic Binding Capacity of the GORE Protein Capture Device is higher compared to conventional resin



Column	DBC (mg/ml resin) 1%	10%	50%
GORE Column	16.2	35.3	57.3
MabSelect - 10mmID	3.5	17.7	37.1
MabSelect - 26mmID	1.6	6.0	24.7

Cycle Comparison of GORE to Conventional Columns

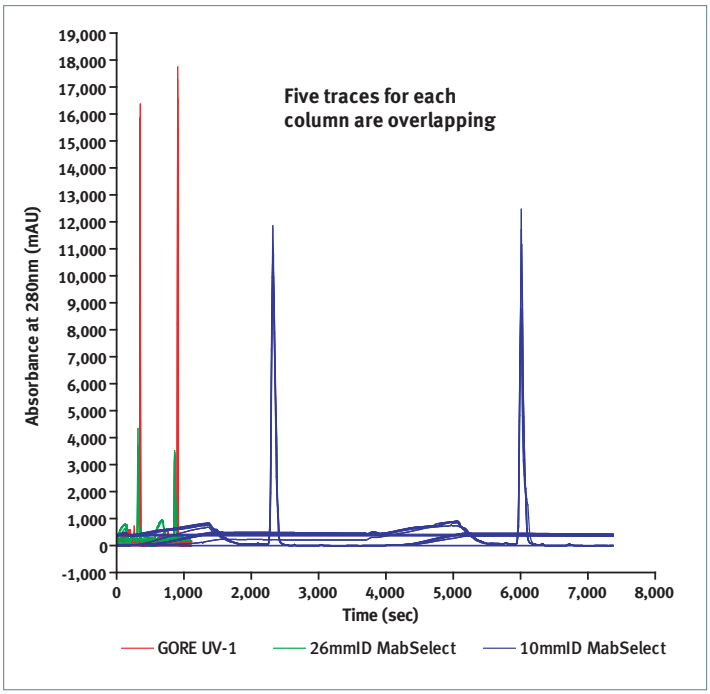
Comparison of the GORE Column to conventional columns (TABLE 2 and FIGURE 4). Experimental design included:

- DBC results used to design CaptureSMB process
- Column Dimensions: As for the DBC determination, 3.5 mL each
- Feed: IgG at 5 mg/ml in UV Transparent RPMI 1640
- Flow Rate: 100 cm/h Feed, 150 cm/h Wash, Elute and CIP

TABLE 2: Process performance: higher yield and productivity with lower buffer consumption

Process Parameter	GORE Column (26 mm ID)	MabSelect Column (10 mm ID)	MabSelect Column (26 mm ID)
Yield (%)	79.2	66.3	25.2
Concentration (g/L)	12.9	8.0	4.6
Mass balance (%)	93.2	91.1	40.1
Productivity (g/L/h)	122.14	11.89	44.69
Load (g/L)	52.1	39.8	58.9
Capacity utilization (%)	97	102	217
Buffer consumption (L/g)	0.53	0.81	1.47
Total process run time (min)	93	615	91

FIGURE 4: Chromatographic traces for 5-cycle CaptureSMB



Purification if IgG from Bovine Serum

Purification of IgG from Serum are show in FIGURES 5-7.

- Process conditions as for comparison model
- Feed: Bovine serum diluted 1:1 with 1x PBS pH 7.3
- 20-Cycles followed by a cleaning[1] and 20-Cycles repeated

FIGURE 5: 20-cycles serum IgG run 1

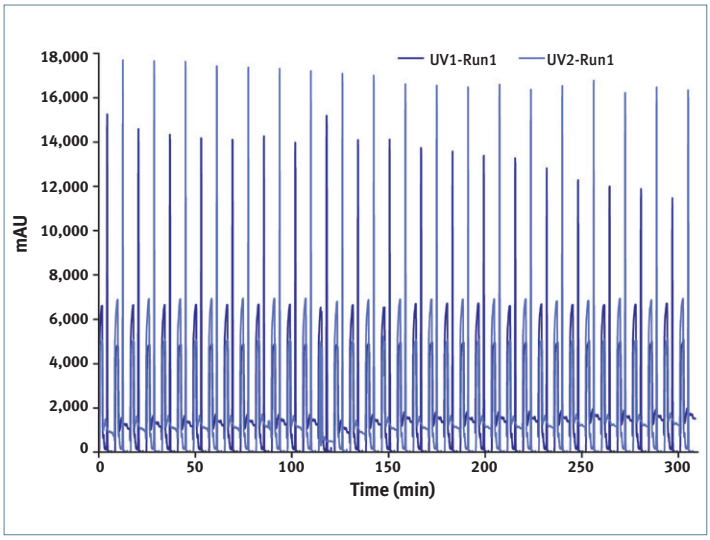
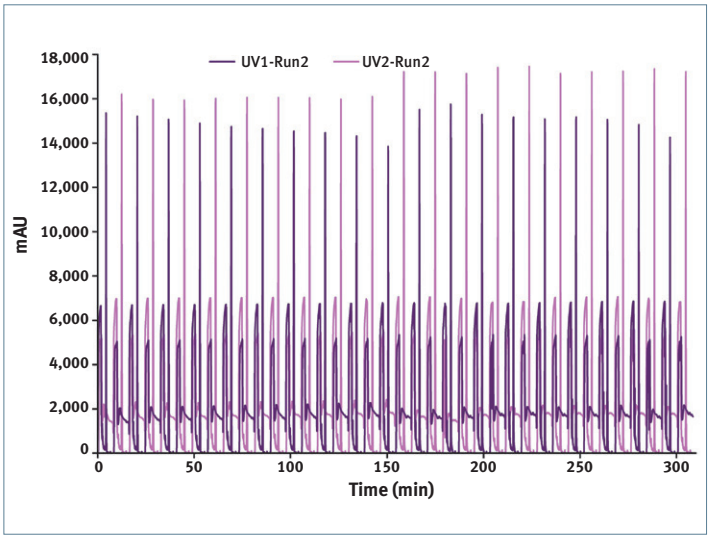


FIGURE 6: 20-cycles serum IgG run 2



[1] GORE Application Note #PB1502

FIGURE 7: Recovery over 20-cycles and 2 runs is consistent

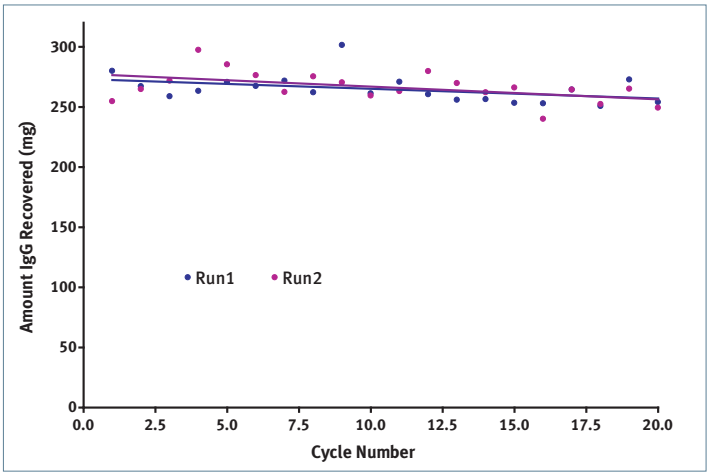


TABLE 3: Process performance: 5 g IgG in 5.5 h with two 3.5 mL columns

Process Parameter	Run 1	Run 2
Yield (%)	105.9	106.2
Concentration (g/L)	11.9	11.9
Mass balance (%)	117.9	118.0
Productivity (g/L/h)	130.63	130.99
Load (g/L)	34.2	34.2
Capacity utilization (%)	100	100
Buffer consumption (L/g)	0.55	0.55

CONCLUSION

The dynamic binding capacity of the GORE column was compared to conventional columns with the same bed volume. The Gore column had a higher DBC than either configuration of MabSelect at each linear velocity tested. The DBCs10% were, 270, 117 and 37 mg/ml for the GORE, 1.0cmID and 2.6cmID columns, respectively.

The DBC values were used to design a 5-cycle SMB process for each column. The Gore column was 27x higher relative to the MabSelect 26cm ID column and 10x higher relative to MabSelect 10cm ID in productivity. The productivity values were 122, 12 and 45 g/L/h for the GORE, 1.0cm ID and 2.6cm ID columns, respectively. The yield for the Gore column was 79%, while the highest productivity MabSelect (26cm ID) only yielded 25%. The lower productivity MabSelect (10cm ID) had a yield of 66%.

The SMB process was applied to the purification of IgG from bovine serum using the GORE columns. In two runs, the overall productivity was 130 g/L/h yielding 5 g of bovine IgG in 55 h using two 35 mL GORE Protein Capture Devices.

As noted in the SDS-PAGE gels, the purity was comparable among all the columns. The percent aggregation as determine by SEC for the Gore column was within expected levels for bovine IgG.

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
Phone: +49 89 4612 3456 • Toll Free (EU): 0 800 4612 3456
Email: pharmbio_eu@wlgore.com

gore.com/pharmbio

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