APPLICATION NOTE

Delivering Consistent Protein A Membrane Scalability: GORE® Protein Capture Devices with Protein A

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Objective

This application note demonstrates the scalability across multiple GORE Protein Capture Device sizes through dynamic binding capacity, CHO cell harvest, and purification experiments as demonstrated with a partially purified monoclonal antibody.

Purpose

GORE Protein Capture Devices are designed to scale with residence time. As such, purification methods can be easily transferred between device sizes from the 1.0 mL (PROA101) size through the 250 mL (PROA301) size. The purpose of this application note is to demonstrate scalability of purification methods across the commercially available device sizes.

Materials and Equipment

- Cytiva AKTA Pilot 600S Liquid Chromatography System
- Cytiva AKTA 150 Liquid Chromatography System
- 1 mL Gore Protein Capture Device (PROA101)
- 9 mL Gore Protein Capture Device (PROA103)
- 58 mL Gore Protein Capture Device (PROA201)
- 116 mL Gore Protein Capture Device (PROA202)
- 250 mL Gore Protein Capture Device (PROA301)
- Trastuzumab Biosimilar Clarified CHO Cell Harvest with 3.4 g/L titer
- Partially purified Trastuzumab Biosimilar Clarified CHO Cell Harvest with 3.0 g/L titer
- Chemicals outlined in protocols below

Elution Width

Elution widths were determined from chromatograms using 100 mAU – 100 mAU as the cutoff.

Yield

Yield was determined from concentration of mAb in the elution fractions, which was calculated by measuring the absorption at a wavelength of 280 nm using a Little Lunatic UV/Vis Spectrophotometer (Unchained Labs, 6870 Koll Center Parkway, Pleasanton, CA 94566) and an extinction coefficient of the IgG1 of 1.47 mL g-1 cm-1.

Pressure

The delta column pressure was determined from the chromatograms at the equilibration step.

Procedure

Scaling Assessment

GORE Protein Capture Devices were assessed across four sizes (9 mL, 58 mL, 116 mL, and 250 mL) for performance through $DBC_{10\%}$ and bind and elute experiments by characterizing elution widths using a partially purified antibody per each section below.

Dynamic Binding Capacity to 10% Breakthrough ($DBC_{10\%}$) Protocol

Purified trastuzumab was diluted to 3.0 g/L in Tris buffered saline and used to determine $DBC_{10\%}$. Purified material was analyzed to obtain the full absorbance (neat absorbance). The material was then run through the column to ensure the absorbance measured greater than 10% of the neat absorbance before stopping.

$DBC_{10\%}$ was calculated from the resulting chromatogram as follows in equations 1 – 4.

Equations:

Eq-01: $Absorbance\ at\ 10\%_{BT} = ((Neat\ abs - Plateau\ abs) \times 0.1) + Plateau\ abs)$

Eq-02: $Volume\ at\ 10\%_{BT} = (Volume\ at\ Absorbance\ at\ 10\%_{BT} - Volume\ at\ Sample\ Injection)$

Eq-03: $HoldUp\ Volume = (Volume\ at\ conductivity\ breakthrough\ during\ transition\ volume\ evaluation)$

Eq-04: $$DBC_{10\%} = \frac{((Volume\ at\ 10\%_{BT} - HoldUp\ Volume) \times Feed\ titer)}{(Bed\ Volume)}$$

Sample load volume

The $DBC_{10\%}$ was then used to determine the sample load volume (Eq-05), at 80% of 10% breakthrough for partially purified trastuzumab for bind and elute experiments.

Eq-05: $$Sample\ Load\ Volume = \frac{((DBC_{10\%}) \times 0.8 \times Bed\ Volume)}{Feed\ titer}$$

Bind and Elute Protocol

The bind and elute assessment was performed per Table 1. The loading residence time was kept constant at 30 SRT. A residence time of 20 SRT was used for non-loading steps per Table 1.

Table 1: Bind and Elute Protocol

Method Step	Solution/Buffer	Column Volumes (CV's)	Residence time (SRT)
Equilibrate	Tris Buffered Saline (TBS)	5	20 SRT
Sample Load	Trastuzumab partially purified 3.0 g/L	Varied by device size	30 SRT
Equilibration Wash	TBS	5	20 SRT
Elution	100 mM Acetate pH 3.1 +/- 0.1	6	20 SRT
Equilibration Wash	TBS	5	20 SRT
CIP wash	0.2 M NaOH	3	20 SRT
CIP Hold	0.2 M NaOH	3 Minutes	0 SRT
Equilibration Wash	TBS	(conductivity <20 mS/cm & pH< 8) +/- 0.5, stable for 0.5 min	20 SRT
Storage	ETOH/H_2O 20/80	5	–

Method Transfer

The ability to understand the impact of system and hold-up volume to method development is important. The system plumbing and sensor locations can impact the ability to overlay chromatograms and optimize buffer both from a transition perspective as well as from a buffer consumption perspective. The systems were optimized at column inlet/outlet and incorporation/elimination of pump washes were employed to achieve as close as possible overlays in UV, conductivity, and pH across three sizes, 1.0 mL, 9.0 mL, and 58 mL on two different AKTA systems.

The protocol used to assess scaling across the device sizes is shown in Table 2. The pump wash (PW) volumes are shown as well as inlets for solution designated by A or B. The pump washes are excluded in the 9 mL and 58 mL methods except during the elution step. Pump washes are included for all steps except the load and CIP hold for the 1 mL method. This was done to account for the relative system to device hold-up volume differences for each device size.

Table 2. Protocol for Bind and Elute with Clarified CHO Cell Harvest Across Device Sizes

Includes valve (AKTA Pure 150 and AKTA Pilot 600s)	1.0 mL			9.0 mL			58 mL		
	Volume (ml)	Column Volumes (CV)	PW (mL)	Volume (ml)	Column Volumes (CV)	PW (mL)	Volume (ml)	Column Volumes (CV)	PW (mL)
Load	9.5	9.5	None	85	9.4	None	545	9.4	None
Wash 1 (A1)	4	4	50	36	4	None	232	4	None
Wash 2 HS (A5)	4	4	50	36	4	None	232	4	None
Wash 3 (A1)	4	4	50	36	4	None	232	4	None
Elution (B1)	5	5	50	45	5	50	348	6	100
Acid Strip (B2)	4	4	50	36	4	None	232	4	None
Wash 4 (A1)	4	4	50	36	4	None	232	4	None
CIP fill (A4)	2	2	50	18	2	None	116	2	None
CIP flow (A4)	3	3	None	27	3	None	174	3	None
Re-Equilibration (A1)	7.18	7.18	50	65.7	7.3	None	463.8	8.0	None

System Hold-Up Volumes comparison

Considering the impact of hold-up and system volumes, the AKTA Pure 150 was used for the 1.0 and 9.0 mL devices, and it was plumbed with tan PEEK tubing. The column inlet/outlet for the 1.0 mL device used green PEEK tubing, and for the 9.0 mL device the column inlet/outlet was plumbed with 3mm i.d. clear tubing.

The AKTA Pure 150 System hold-up volumes were determined with and without pump washes and noted in Table 3. C1 denotes column position 1 for the 9.0 mL with the 3 mm ID clear tubing in the inlet and outlet positions, while C3 indicates column position 3 for the 1.0 mL with green PEEK (0.75 mm ID) tubing in the inlet and outlet positions.

Table 3: System Hold-Up Volumes for 1.0 mL and 9.0 mL Devices with AKTA Pure 150 LC System

Column position	Pump Wash (Yes or No)	Hold-Up Volume (mL)
C1	No	22.49
C3	No	21.13
C1	Yes	6.34
C3	Yes	2.44

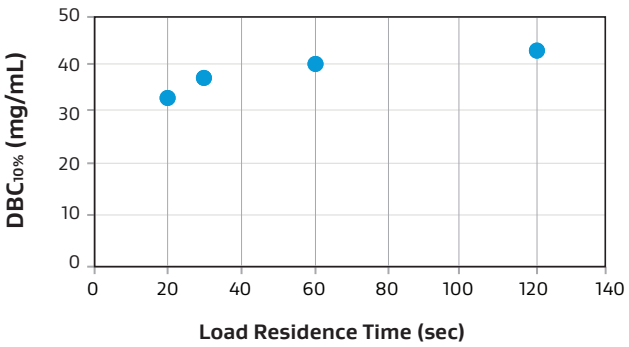
The AKTA Pilot 600S was used for the 58 mL device; the system tubing and column inlets/outlets were primarily 3 mm i.d. clear tubing. The system hold-up volume was measured at 45 mL.

Results

DBC Results

An initial $DBC_{10\%}$ sweep was conducted using a 58 mL device to assess the binding capacity of the partially purified trastuzumab antibody. This DBC sweep was conducted to determine the impact of residence time on binding capacity. Figure 1 shows the $DBC_{10\%}$ versus residence time for trastuzumab. The results show that trastuzumab has a $DBC_{10\%}$ of about 36 g/L at 30 SRT.

Figure 1. DBC of trastuzumab versus residence time on a 58 mL



The $DBC_{10\%}$ at both 20 SRT and 30 SRT are within 10% across the device sizes with individual measurements shown in Table 4.

Minimum and maximum DBC measurements were within 10% of group means for both 20SRT and 30SRT methods. Differences within 10% are likely within the sensitivity of the method.

Table 4. Results of $DBC_{10\%}$ Analysis at 20SRT and 30SRT across sizes

Device Size	$DBC_{10\%}$ 20 SRT (mg/mL)	$DBC_{10\%}$ 30 SRT (mg/mL)
9.0 mL	35.7	37.8
58 mL	33.4	37.3
116 mL	31.0	36.0
250 mL	34.8	38.6

Bind and Elute Results

Partially purified antibody was loaded at 80% of $DBC_{10\%}$ using the method set forth in Table 1. Figure 2 shows the individual elution chromatograms of the 9.0 mL, 58 mL, 116 mL, and 250 mL devices. For the purposes of the scaling analysis, the focus is on the elution width where sharp peaks and similar elution widths were observed in each device size.

Table 5 shows the elution width in column volumes (CV) being on average 2.5 CV, which is well within the ≤ 3.5 CV range desired. The specific flow rates shown in Table 6 were for non-loading steps including elution. The elution width in column volumes (CV), yield, and column dP (MPa) are shown for each device.

Figure 2. Elution profile of each device. A) 9.0 mL (PROA103); B) 58 mL (PROA201); C) 116 mL (PROA202); and D) 250 mL (PROA301).

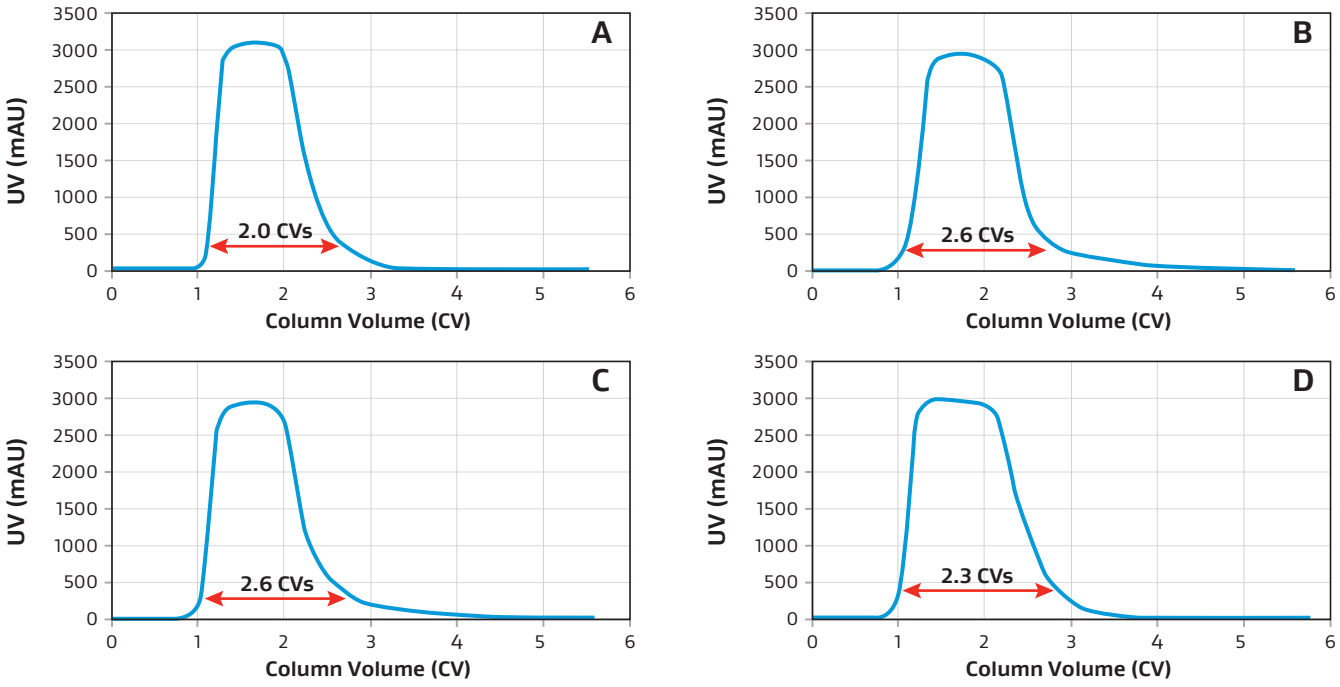


Table 5. Elution characteristics across device size

Device Volume (mL)	Flow Rate (mL/min)	Residence Time (sec)	Elution Width (CV)	Yield (%)	dP (MPa)
PROA103 (9 mL)	27	20	2.0	98.7	0.12*‡
PROA201 (58 mL)	174	20	2.6	95.4	0.06*
PROA202 (116 mL)	348	20	2.6	97.6	0.08*
PROA301 (250 mL)	750	20	2.3	97.6	0.08*

*20 SRT; pressure values are not corrected for system influence on dP.

‡ Relatively high volumetric flow rates with comparatively small inlet/outlet tubing for this device size and chromatography system result in a significant system dP contribution. This system contribution was measured to be approximately 0.05 MPa at the 20 SRT measurement condition for the PROA103.

Method Transfer Results Across 1.0 mL, 9.0 mL, and 58 mL Using Clarified CHO Cell Harvest

When performing the clarified CHO cell harvest bind and elute using the protocol set forth in Table 2, all wash steps except the CIP were performed at 10 SRT while the loading step was performed at 30 SRT. Figure 3 shows the overlay of 1 mL, 9 mL, and 58 mL for UV and conductivity while Figure 4 shows the overlay of UV and pH.

Figure 3. Chromatogram overlay of 1mL (blue), 9mL (yellow) and 58mL (red) in UV and Conductivity

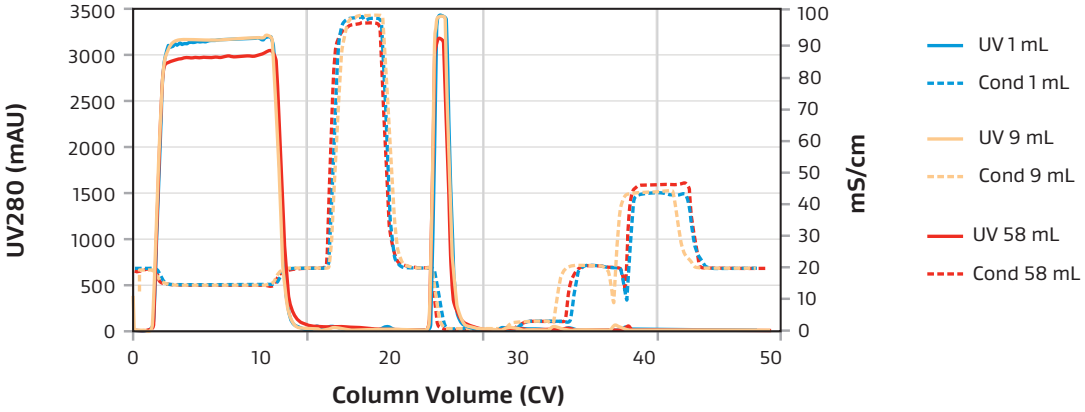
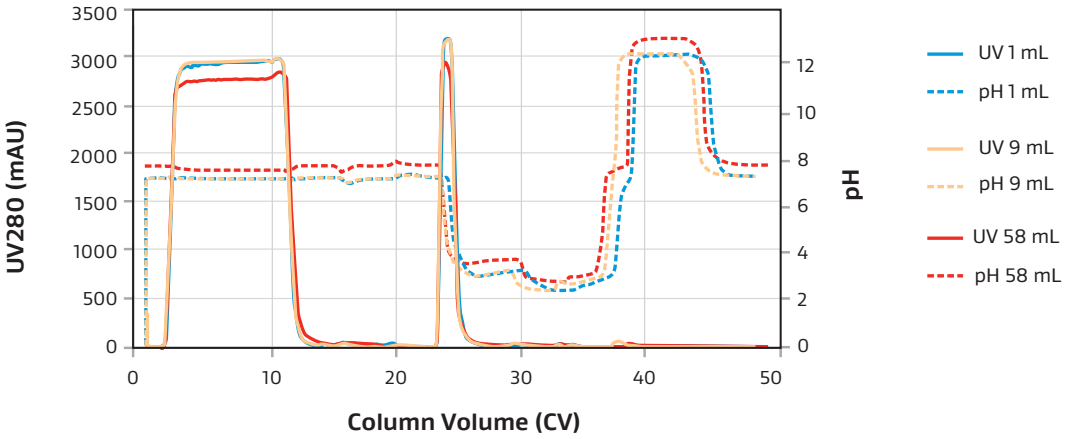


Figure 4. Chromatogram overlay of 1.0 mL (blue), 9.0 mL (yellow) and 58 mL (red) in UV and pH



The UV traces overlay well across sizes as the systems become more matched by hold-up volume. The differences between 58 mL (red), 9.0 mL (yellow), and 1.0 mL (blue) in UV of the load step reflects differences in the detectors between the two AKTA systems.

Conclusions

The objective of this application note was to demonstrate consistent scalability across GORE Protein Capture Device sizes, using residence time. The results demonstrate that DBC scales well across sizes (9 mL, 58 mL, 116 mL, and 250 mL), being within 10% across two residence times. The elution volumes are consistent across sizes and <3.5 CV. In addition, the pressure drop across the larger sizes is consistent and < 0.4 MPa when run at 20 seconds residence time.

The clarified CHO cell harvest bind and elute chromatograms across the 1.0 mL, 9.0 mL, and 58 mL sizes show that when the system hold-up volume and plumbing are considered, one can optimize to have the chromatograms overlay more consistently across sizes and LC systems. As the devices scale, one may be able to use the hold-up volumes of the device and LC system in a way to reduce the need for pump washes. This can be done when focusing on solution placement and assessing the hold-up volume impact. The results showed that pump washes were eliminated in all but one instance.

GORE Protein Capture Devices are designed to scale consistently by residence time. The data indicates the process can be transferred easily across LC systems to opitmize purification and method transfer.

Gore PharmBIO Products

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PBI2223-R5-TEC-US-AUG23