APPLICATION NOTE

Cleaning Protocols to Reduce the Effects of Pressure Rise over the Lifetime of a GORE® Protein Capture Device

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Objective

Provide two cleaning protocols to implement in the event that a GORE® Protein Capture Device with Protein A experiences pressure rise over the course of the device lifetime.

Purpose

The GORE Protein Capture Device improves potential processing capabilities for affinity Protein A chromatography, thus shortening the time needed to purify a given monoclonal antibody. Increased speed and capacity allows for more purification cycles in a given amount of time. Therefore, the lifetime of a device might be reached more rapidly than Protein A resins with less capacity and slower speeds to purification. Two cleaning protocols outlined in this application note potentially extend the lifetime of a GORE Protein Capture Device in the event that pressure elevates over the expected lifetime of the device.

Table 1. Intra-Cycle Cleaning Protocol

Buffer	Column Volumes (CV)	Flow Rate (mL/min)	Volume (mL)	Step Process
PBS*	10.0	3.0	10.0	Equilibration
Feed stock	*****	3.0	*****	Load
PBS	10.0	3.0	10.0	Load wash
Citrate**	12.5	3.0	12.5	Elution
DTT cleaning buffer***	10.0	1.0	10.0	Enhanced clean
DTT hold****	—	—	—	10 minute hold step
PBS	10.0	3.0	10.0	Cleaning wash
0.2M NaOH	3.6	1.2	3.6	CIP
PBS	10.0	3.0	10.0	Equilibration

* PBS phosphate-buffered saline (150 mM NaCl, 50 mM phosphate, pH 7.4)

** Citrate (100 mM citrate, pH 3.4)

*** DTT cleaning buffer (50 mM tris base, 100 mM NaCl, 1% SDS, 10-15 mM 1,4-Dithiothreitol (DTT), pH 10.4, conductivity 11-12 mS/cm)

**** DTT hold (static reduction step)

***** Feed load dependent

Materials/Equipment

- Liquid Chromatography System (LC System)
- 1.0 mL GORE Protein Capture Device (PROA101), 3 devices
- Chemicals outlined in recommended protocols
- GORE Protein Capture Devices for Drug Discovery Applications 1.0 mL and 3.5 mL Operating Instructions

Protocols

Three GORE Protein Capture Devices (PROA101) were used to perform CHO Cell Harvest purifications until pressure rises were observed. Separate cleaning protocols were implemented for each device to reduce the observed pressure rise. Tables 1 and 2 outline the steps and buffers used for both cleaning protocols.

Table 2: Inter-Cycle Cleaning Protocol

Buffer	Column Volumes (CV)	Flow Rate (mL/min)	Volume (mL)	Step Process
L-Arginine HCl*	5.0	3.0	5.0	Acidic equilibration
DTT cleaning buffer**	10.0	1.0	10.0	Enhanced clean
DTT hold***	—	—	—	10 minute hold step
L-Arginine HCl	10.0	3.0	10.0	Acidic wash
0.2M NaOH	3.6	1.2	3.6	CIP
PBS****	10.0	3.0	10.0	Equilibration

* L-Arginine HCl (100 mM L-Arginine HCl, pH 2.3) Adjust pH using HCl to reach pH 2.3

** DTT cleaning buffer (50 mM tris base, 100 mM NaCl, 1% SDS, 10-15 mM DTT, pH 10.4, conductivity 11-12 mS/cm)

*** DTT hold (static reduction step)

**** PBS phosphate-buffered saline (150 mM NaCl, 50 mM phosphate, pH 7.4)

The first cleaning protocol outlined in Table 1, Intra-Cycle Cleaning Protocol, was implemented while performing feed cycling experiments during a mid-cycle Dynamic Binding Capacity (DBC) test as defined in the operating instructions.

The second cleaning protocol outlined in Table 2 was implemented as a standalone cycle, independent of any purification cycling or DBC tests as pressure approached the maximum recommended operating value after performing multiple cycles with a CHO cell harvest.

All CHO cell harvest purifications followed the operating instructions for GORE Protein Capture Devices. The metric used to observe column pressure rise over the course of use was delta column pressure (dP) (output from the LC unit). Specifically, maximum loading delta pressure in the presence of either human monoclonal antibody (CHO Cell Harvest), polyclonal human IgG (DBC evaluations), or initial PBS delta column pressure at the beginning of each purification cycle was used.

Results

Intra-Cycle Method

While performing multiple CHO cell harvest purifications with harvest titer ranges from ≤ 0.50 mg/mL to 2.0 mg/mL, significant pressure rise was observed for both devices. During the cycling, a DBC test was performed with polyclonal hIgG and implementation of the intra-cycle cleaning protocol.

Figure 1 indicates the delta column pressure (purple line) during the intra-cycle cleaning protocol performed during the DBC test from the PROA101 device. An observed spike in both pressure and UV (blue line) can be observed during the DTT cleaning step as well as the subsequent PBS wash after the 10 minute static-reduction step. The subsequent PBS equilibration performed at the end of this intra-cycle cleaning demonstrated a lower delta column pressure than the pressure at the beginning of the cycle.

Figure 2 shows a second DBC performed with the same device after the intra-cycle DTT cleaning protocol (orange line) compared to the run shown in Figure 1 prior to cleaning.

Figure 3 demonstrates a recovery in DBC after the intra-cycle DTT cleaning protocol (orange line) compared to DBC from Figure 1 prior to cleaning.

Figure 1: Intra-cycle (DTT) washing protocol with delta column pressure (MPa) in purple, and UV absorbance (280nm) in blue.

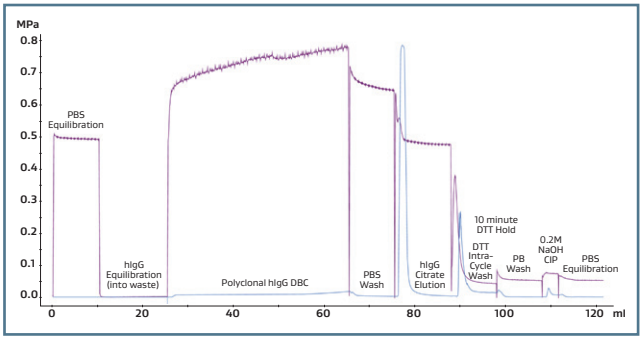


Figure 2: Pressure curves from the follow-up DBC (orange line) after performing intra-cycle (DTT) washing protocol (blue line).

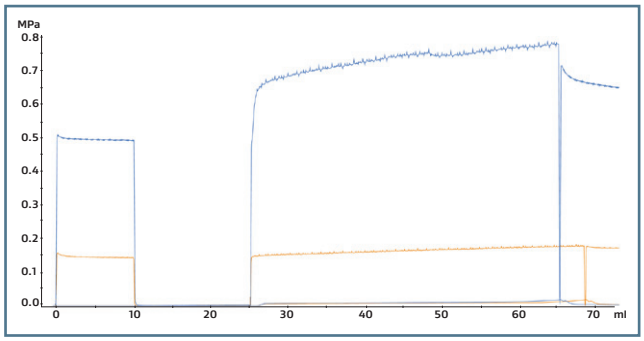
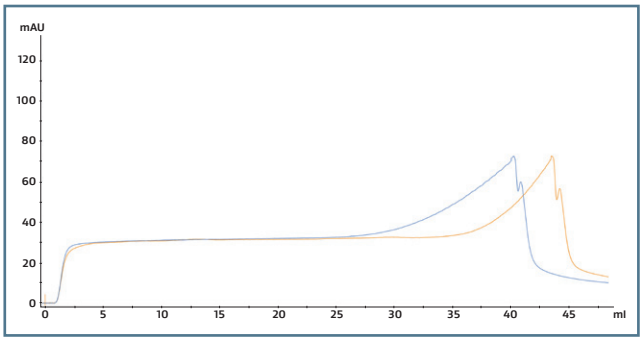


Figure 3: UV trace of DBC showing the difference before performing the intra-cycle (DTT) washing protocol (blue line) vs. after the washing protocol (orange line).



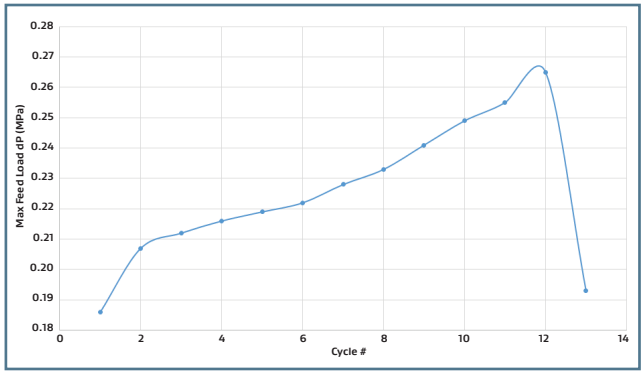
A second device was tested using the intra-cycle cleaning protocol as shown in Table 3. The maximum loading delta column pressure in the presence of either human monoclonal antibody (CHO cell harvest) or polyclonal human IgG (DBC evaluations) throughout cycling and including the implementation of the intra-cycle cleaning protocol at cycle 12 is shown in Table 3 and Figure 4.

Table 3: PROA101 maximum load delta column pressure over the course of CHO cell harvest cycling.

Protein purified	Cycle	Max load dP (MPa)
Polyclonal hIgG DBC	1	0.186
CHO harvest purification	2	0.207
CHO harvest purification	3	0.212
CHO harvest purification	4	0.216
CHO harvest purification	5	0.219
CHO harvest purification	6	0.222
CHO harvest purification	7	0.228
CHO harvest purification	8	0.233
CHO harvest purification	9	0.241
CHO harvest purification	10	0.249
CHO harvest purification	11	0.255
Polyclonal hIgG DBC*	12	0.265
Polyclonal hIgG DBC**	13	0.193

* Prior to intra-cycle DTT wash protocol
** DBC post intra-cycle DTT cleaning protocol

Figure 4: Maximum loading delta column pressure during CHO cell harvest purifications along with subsequent pressure recovery after implementing the intra-cycle DTT washing protocol.



Inter-Cycle Method

A third device was cycled with CHO cell feed until the delta column pressure rose to near maximum recommended operating values (0.4MPa). The device was subsequently cleaned using the inter-cycle cleaning protocol. This cleaning protocol was implemented independently of feed purifications and/or DBC testing.

Figure 5 shows the UV chromatogram (280nm) for the inter-cycle DTT wash protocol. A significant UV absorbance peak is observed when exposed to the DTT cleaning solution as well as when followed up with the acidic wash buffer. This inter-cycle DTT washing protocol recovered most of the pressure rise observed in the device over the course of cycling as shown in Figure 6.

Figure 5: UV trace from inter-cycle DTT washing protocol.

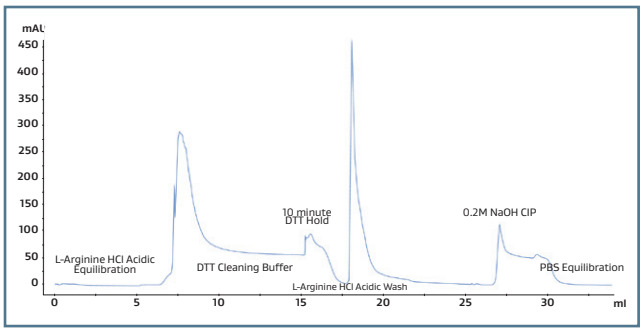


Figure 6. Maximum initial PBS equilibration delta column pressure during CHO cell harvest purifications along with subsequent pressure recovery after implementing the inter-cycle DTT washing protocol.

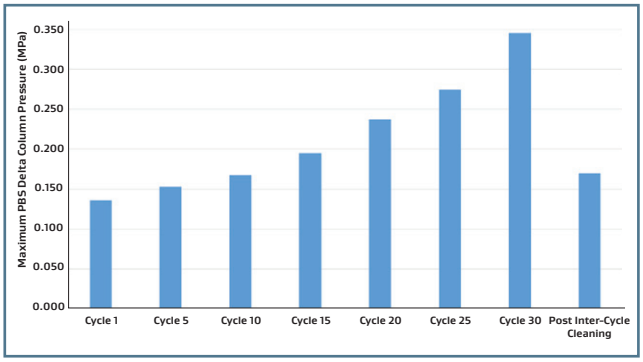


Figure 6 indicates the maximum delta column pressure during the initial PBS equilibration step for each purification cycle. The device underwent 30 purification cycles followed by the inter-cycle washing protocol. The inter-cycle cleaning method restored column pressure to levels well within the recommended operating pressure.

Conclusion

Two washing protocols were explored using DTT– both of which successfully reduced the delta column pressures of (3) PROA101 devices after delta column pressures had increased over the course of use.

The three PROA101 devices spanned CHO cell harvest titer ranges from ≤0.50 mg/mL to 2.0 mg/mL and all experienced different levels of pressure rise over the course of cycling. The pressure rise experienced for all three of the devices used were reduced once cleaned with one of the two DTT washing protocols.

These two cleaning protocols provide options for the effective recovery of unexpected delta column pressure rise during the use of a GORE Protein Capture Device.

Gore PharmBIO Products

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NOT INTENDED FOR USE in medical device or food contact applications or with radiation sterilization. GORE Protein Capture Devices are intended for research use only and should not be used for clinical or diagnostic procedures.

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