TECHNICAL NOTE

Reverse flow cycles at higher flow rates with the GORE® Protein Capture Device with Protein A

Objective

This technical note demonstrates that the GORE® Protein Capture Device (GPCD) PROA201 is capable of reverse flow cycles up to 30srt flow rates to facilitate air expulsion without significant impact to column operating pressure, flow characteristics and binding capacity.

Background

The primary design of the PROA201 GPCD is for the system to be operated in the forward flow direction (Down-Flow). However, the PROA201 GPCD is capable of limited reverse flow operation and is routinely run at 58 mL/min (60 seconds residence time) reverse flow rate as part of initial device hook-up to a liquid chromatography (LC) system.

During the initial attachment onto a LC system such as a Cytiva AKTA Pilot 600, some air may be introduced into the inlet of the device. If this occurs, then operating the system in the reverse flow direction (Up-Flow) may be performed to expel the introduced air from the system. In this situation it is desirable to flow at 80 to 100 mL/min to force the small air bubbles through the various valves and analytic instruments situated in the downstream flow path as well as flush the integrated air trap (IAT) of any bubbles for the PROA202 and PROA203 devices.

Materials and Equipment

- Cytiva AKTA Pilot 600 Liquid Chromatography System
- Four (4) PROA201 GORE Protein Capture Devices
- Chemicals outlined in Table 1
- Human Polyclonal Antibody (pIgG) with a titer of 3.0 g/L

Table 1. Chemicals utilized

Buffer/Solution	Composition
Phosphate Buffered Saline (PBS)	50 mM Phosphate 150 mM NaCl
Citrate	100 mM Citrate
H_2O	
Sodium hydroxide (NaOH)	0.2 M NaOH
20% Ethanol (EtOH)	20% EtOH/80% water (v/v)

Methods

Delta column pressure

Delta column pressure was measured at 348 mL/min (10 seconds residence time) on a control device to assess initial column pressure drop. The same measurement was taken after reverse flow testing to assess any impact to column pressure performance.

Transition Test

The transition volume from PBS to water at 348 mL/min (10 SRT) was measured on three replicate devices to assess flow front performance and device integrity associated with reverse flow testing. The transition volume testing was performed per Table 2. A conductivity threshold of 0.15 mS/cm was used to determine the final volume reported.

Table 2. Transition Analysis Protocol

Method Step	Solution/ Buffer	Column Volumes (CV's)	Seconds Residence time (srt)
Equilibration Wash I	Phosphate Buffered Saline (PBS)	10	10srt
H_2O Wash	H_2O	10	10srt
Equilibration Wash II	Phosphate Buffered Saline (PBS)	10	10srt

Dynamic Binding Capacity to 10% Breakthrough (DBC10%)

Dynamic Binding Capacity was measured at 20 SRT on the three replicate devices to assess impact to binding characteristics associated with reverse flow testing. The DBC method is outlined in Table 3 below.

Table 3. Dynamic binding capacity method

Method Step	Solution/Buffer	Column Volumes (CV's)	Seconds Residence time (srt)
Equilibration Wash I	Phosphate Buffered Saline (PBS)	4	10srt
Sample Application	pIgG 2.76 g/l	As Needed	20srt
Equilibration Wash II	Phosphate Buffered Saline (PBS)	4	10srt
Elution	011 mM Citrate pH-3.0	5	10srt
CIP Strip	0.2M NaOH	1	10srt
Re-Equilibration	Phosphate Buffered Saline (PBS)	~ 8	10srt

Reverse Flow Cycling

The GPCD PROA201 reverse flow capability was assessed by performing Reverse Flow, Transition test and DBC10% evaluation testing sequence as described in Table 4. The test sequence was repeated for three (3) PROA201 GPCD's. Since the 58 mL/min reverse flow rate is used routinely as part of device preparation, the transition volume and DBC data represent a starting baseline. The 116 mL/min reverse flow rate is the test condition representative of the 80-100 mL/min flow rates intended for use. Delta column pressures were also measured on the devices and compared against the control.

Table 4. Reverse flow cycling progression

Testing Sequence	Test Type
Test 1 58 mL/min (60 srt)	Reverse Flow
Post-Test 1	10 srt Transition
Post-Test 1	20 srt $DBC_{10\%}$
Test 2 116 mL/min (30 srt)	Reverse Flow
Post-Test 2	10 srt Transition
Post-Test 2	20 srt $DBC_{10\%}$
Post-Test 2	Delta Column Pressure

The GPCD must maintain ability to operate with delta column pressure (dP) ≤ 0.4 MPa. In addition, the transition volume should be ≤ 5 CV and DBC10% within 10% pre and post testing. In the reverse flow, the device should not leak or burst.

Results

Delta Column Pressure Reverse Flow Results

The reverse flow delta column pressures (dP) are provided in Table 5 for reference. All are well below 0.4MPa.

Table 5. Results of reverse flow dP measurements across devices 60 srt & 30 srt

Device	60 srt RF dP (MPa)	30 srt RF dP (MPa)
RD62219-02	0.02	0.04
RD62219-03	0.02	0.04
RD62219-04	0.02	0.04

Transition Results

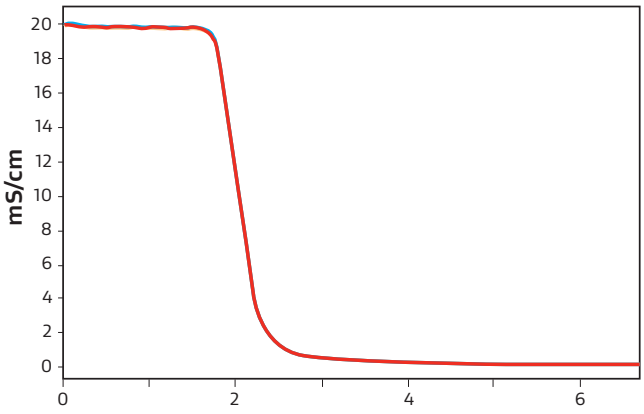
Transition volume and final column dP data for the three test devices are shown in Table 6 per the Table 2 test progression. The data suggest no significant impact of the 116 mL/min reverse flow testing to either transition volume or column dP as referenced by control data. All transitions are < 5 CV's.

Table 6. Results of reverse flow dP measurements across devices 60 srt & 30 srt

Device	Post 60 srt RF TV (CV)	Post 30 srt RF TV (CV)	Equilibration 10SRT Forward Flow dP (MPa)
RD62219-02	3.95	3.99	0.14
RD62219-03	3.86	3.95	0.15
RD62219-04	3.96	4.00	0.15
RD62219-05	Control	Control	0.17

Figure 1 shows the 10 SRT transitions curves for the control device, with no reverse flow, and the three devices after both the reverse flow of 60 srt and 30 srt with no deterioration of performance noted by the presence of shoulders or shifts in the curves.

Figure 1. Chromatogram overlay of all 6 transitions curves



DBC Results

The DBC10% at 20srt is within 10% when comparing the initial DBC10% post 60srt reverse flow versus the DBC10% post 30srt reverse flow across three devices with individual values shown in Table 7 and results relative to acceptance criteria are shown in Table 8.

Table 7. Results of DBC10% Testing at 20srt across devices post reverse flow at 60 srt & 30 srt

Device	Post 60 srt RF $DBC_{10\%}$ (g/L)	Post 30 srt RF $DBC_{10\%}$ (g/L)	$DBC_{10\%}$ Absolute Percent Difference (%)
RD62219-02	37.2	38.0	2%
RD62219-03	38.7	38.2	1%
RD62219-04	37.7	37.4	1%

Table 8. Acceptance Criteria and Summary of Results

Acceptance Criteria	Observations	Result
Transition volume ≤ 5 CV	All transition volumes ≤ 4.0 CV	Pass
No leaks or bursting of device	No leaks or failures	Pass
$DBC_{10\%}$ within 10% pre and post testing	All $DBC_{10\%}$ were within ± 2%	Pass
Delta column pressure (dP) ≤ 0.4 MPa	All devices < 0.4 MPa at 10srt	Pass

Conclusions

All devices operated at delta column pressure (dP) < 0.4 MPa at 10srt. No leaks or failures were observed in any devices. All transitions were ≤ 4.0 CV and the DBC10% were within ± 2% from initial test. The data indicate that the GPCD PROA201 is capable of operating in reverse flow up to 30 srt without loss of performance with respect to column pressure, transition volume or dynamic binding capacity. When discussing removal of air with customers, one can recommend using reverse flow at rates up to 30srt.

Gore PharmBIO Products

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