APPLICATION NOTE

Evaluation of 3.5 mL GORE® Protein Capture Devices Used In-Series for Monoclonal Antibody Purification

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Objective

To evaluate the yield and concentration factor of two 3.5mL GORE Protein Capture Devices (PROA102) connected in-series for purification of monoclonal antibodies.

Purpose

The GORE Protein Capture Device provides increased purification capacity at a short residence time, thus allowing the purification of over 30 to 105 mg of antibody in less than 40 minutes per purification cycle. Two 3.5 mL GORE Devices can be connected in-series, which doubles the column capacity by providing a 7.0 mL bed volume and allows capture of at least 200 mg of monoclonal antibody within 60 minutes.

Materials/Equipment

- 3.5 mL GORE Protein Capture Device (PROA102), 2 devices
- Liquid chromatography system (LC system)
- UV/Vis spectrophotometer
- IDEX, Coupler PEEK Male/Male – ½"

Procedure

Two 3.5 mL Gore Protein Capture Devices were connected in-series using an IDEX, PEEK Male/Male ½" fitting (Figure 1). When used in-series, the GORE Devices create a 7.0 mL bed volume. Similar to other chromatography devices, the overall device pressure will increase as the path length increases. To stay within the recommended pressure settings, the in-series devices were operated at a residence time of 30 seconds (flow rate of 14 mL/min). The cycling conditions, including buffers and volumes are listed in Table 1.

Two separate purification cycles were run using two different collection methods. During the first cycle, bound antibody was collected in 4 column volumes (CV). During the second cycle, the elution peak from 100–100 mAu was collected.

Table 1. Buffers used for each purification method

Buffer	Column volumes (CV)	Flow Rate (mL/min)	Volume (mL)	Step process
DI H2O	10	14	70 mL	Storage rinse
PBS*	10	14	70 mL	Equilibrate
CHO harvest	21.5	14	151 mL	Load
PBS	10	14	70 mL	Wash
Citrate**	10	14	70 mL	Elution***
PBS	4	14	28 mL	pH adjustment
0.1 M NaOH	2.1	1	15 mL	CIP
PBS	10	14	70 mL	pH adjustment
DI H2O	5	14	35 mL	Salt removal
20% Ethanol	5	7	35 mL	Storage

* PBS Phosphate-buffered saline (150 mM NaCl, 50 mM phosphate, pH 7.4)
** Citrate (100 mM citrate, pH 3.4)
*** Two cycles were performed, one collecting a 4 CV elution and one collecting the elution peak from 100-100 mAU.

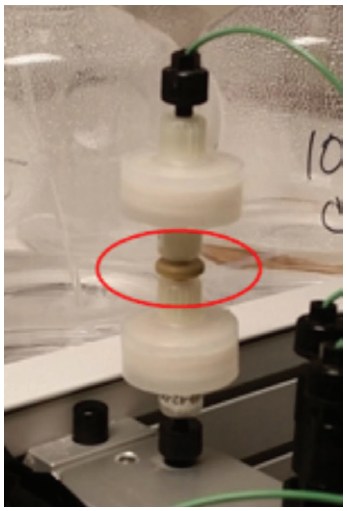


Figure 1. GORE Protein Capture Devices connected in-series with a PEEK fitting.

Two 3.5 mL GORE Protein Capture Devices (PROA102) connected in-series were used to perform affinity purification of a clarified CHO cell harvest expressing human IgG1 monoclonal antibody. The DBC of the in-series devices was assumed as being ≥ 30 mg/mL. For both purification cycles, the first device in the series was loaded with 206 mg of the CHO cell harvest at a concentration of 1.36 mg/mL.

The first purification cycle followed the cycling conditions listed in Table 1, and elution fractions were collected in 4 CV (28 mL) of citrate buffer and neutralized with 8.4 mL of 1.0 M Tris pH 9.0 for a total fraction volume of 36.4 mL. The second purification cycle followed the same purifcation conditions, but the elution peak from 100–100 mAU was collected in 10 mL of citrate buffer. The eluted fraction was neutralized with 3.0 mL of 1.0M Tris pH 9.0 for a total fraction volume of 13 mL.

Results

Results for the purification of human IgG1 monoclonal antibody using Gore in-series devices are shown in Table 2. The two cycles performed with the in-series devices were completed in 60 minutes due to the reduction in flow rate to a residence time of 30 seconds. A representative chromatogram from one of the purification cycles is shown in Figure 2.

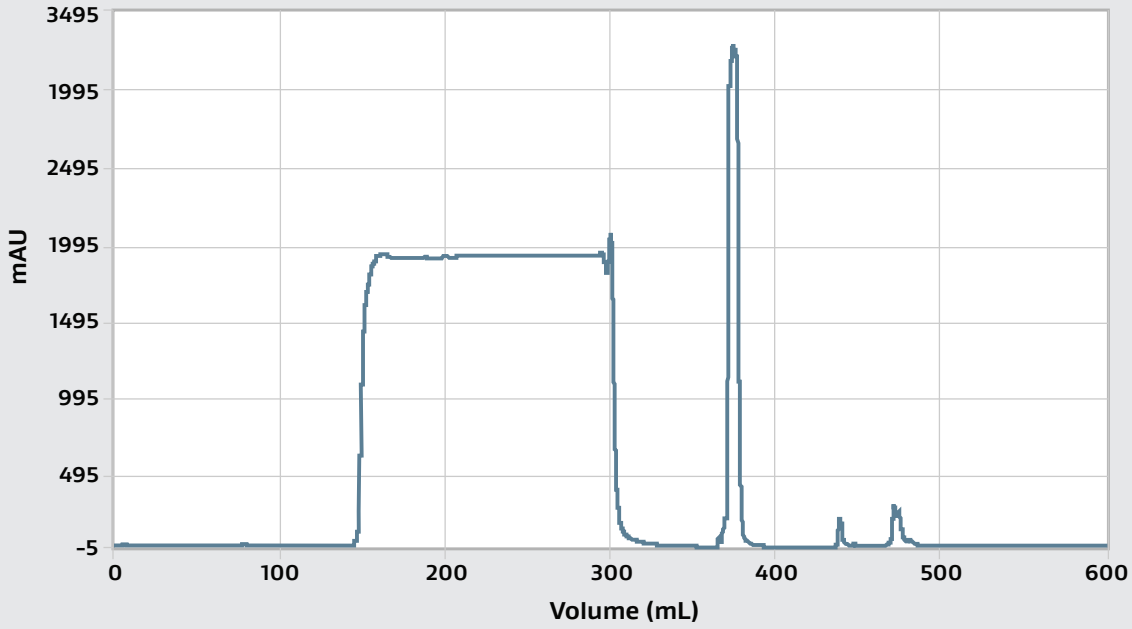
The elution obtained by collecting 4 CV concentrated the titer of the monoclonal antibody to 5.63 mg/mL (including elution and neutralizing buffer), thus yielding at least a four-fold increase in concentration compared to the initial titer of the CHO cell harvest (1.36 mg/mL). The elution obtained by collecting the elution peak from 100–100 mAU concentrated the titer of the monoclonal antibody to 15.7 mg/mL (including elution and neutralizing buffer), thus yielding at least a 11.6-fold increase in concentration compared to the initial titer of the CHO cell harvest.

Table 2. Purification results for the purification cycles using two GORE Protein Capture Devices in-series

Output	4 CV Elution	100-100 mAU Elution
Feed titer (mg/mL)	1.36	1.36
Elution fraction (mL)	28	10
Tris buffer added (mL)	8.4	3
Elution volume (mL)	36.4	13
Elution concentration (mg/mL)	5.6	15.7
Feed loaded (mg)	205.4	205.4
mAb collected (mg)	204.9	204.6
Yield (%)	99.8	99.6
Concentration factor	4.1	11.6

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

Figure 2. Sample chromatogram from a purification cycle performed using two GORE Protein Capture Devices in-series



Conclusion

Monoclonal antibody purification using two 3.5 mL GORE Protein Capture Devices (PROA102) connected in-series provided over 200 mg of purified antibody within one hour and resulted in a concentrated elution pool. The concentration factor was between 4 to 11 times the initial antibody titer. The antibody yield after each purification cycle was greater than 99%, thus indicating that most of the antibody loaded onto the in-series devices is recovered in the elution pool.

The antibody purification method used in this study indicates that it is possible to use two 3.5 mL GORE Protein Capture Devices connected in-series to provide a 7.0 mL bed volume that increases DBC capacity and allows capture of ≥ 200 mg of purified antibody within one hour. Although the clarified CHO cell harvest used in this study allowed operation at a residence time of 30 seconds, the operating residence time may vary depending on the initial feed. For example, the residence time might need further adjustment to ensure operation within the maximum operating pressure of the device. The maximum pressure recommended for operation in the in-series devices is 0.6 MPa. A pressure monitor warning should be set for this limit, and flow rates should be adjusted if the operating pressure reaches this limit. If in-series devices are used, it is recommended to alternate the direction of flow after each purification cycle to mitigate any pressure effects resulting from connecting the devices in-series.

Gore PharmBIO Products

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