PALL Life Sciences

Application Note USTR 2486

Contaminant Removal by Mustang® Q Membrane Chromatography from a Protein A Purified Monoclonal Antibody

*Filtration. Separation. Solution.*SM

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Introduction

Monoclonal antibodies (mAb) are expected to dominate the biopharmaceutical landscape in the future.[1] Although chromatography is the mainstay of mAb downstream purification processes, more efficient and cost effective chromatography technologies are needed in order to address increasing mAb titers in mammalian cell culture supernatants.

Ion-exchange membrane chromatography has been demonstrated to capture large biomolecules such as viruses and plasmid DNA with high dynamic binding capacity compared to ion exchange beaded column chromatography.[2],[3] Recently, anion-exchange membrane chromatography has been viewed as a viable alternative to column chromatography for trace contaminant removal from a protein A-purified mAb.[4],[5]

In this application note, a 0.35 mL membrane volume (MV) Mustang® Q coin was evaluated for Chinese Hamster Ovary (CHO) host cell protein (HCP) and CHO host cell DNA removal from a protein A purified mAb. Spiking a protein A-purified mAb with CHO DNA showed more than 4.9 log removal at pH 8.0 and 4 mS/cm. It was found that the best HCP clearance was accomplished at pH 8.0 and 4 mS/cm conductivity when the HCP levels were reduced from 208 ng/mg of mAb to 4 ng/mg of mAb.

Materials

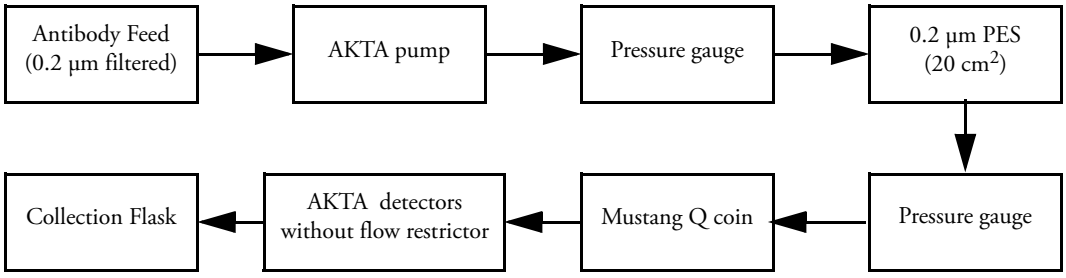
All flow through chromatography testing with Mustang Q coins (Pall Life Sciences, MSTG18Q16) in a coin housing (Pall Life Sciences, MSTG18H16) was performed using an AKTA* Explorer 100 (GE Healthcare). The load material was a recombinant humanized antibody produced in CHO cell supernatant and purified by protein A column chromatography. CHO DNA was isolated from a CHO cell line using tangential flow filtration. The antibody concentration was received at either 3.1 mg/mL or 2.0 mg/mL in 3 mM citrate, 25 mM Tris pH 7.5 and 0.1 M NaCl with a measured (Thermo Orion, model number 130A) conductivity of 11 mS/cm at 20 °C. The Mustang Q membranes used in this study were in the coin (0.35 mL membrane volume) format. To reduce the risk of feedstock aggregation, the high flow rate capability of Mustang membranes was utilized with a flow rate of 10.5 mL/min (30 MV/min) to load the antibody process feed through the Mustang Q coin in its housing. A slower flow rate of 10 MV/min may be used. In-line pre-filtration upstream of the Mustang Q coin was performed using a 47 mm, 0.2 µm Supor® polyethersulfone (PES) membrane (Pall Life Sciences, part number 60301) in a stainless steel housing (FTK 200, Pall Industrial). For large volume feedstreams (> 50 mL), an alternative 0.2 µm in-line pre-filter, Mini Kleenpak™ (part number, KM5EDFP2S) with 20 cm^2 may be used. Pre-filtration of the mAb feedstreams was also performed prior to loading as shown in Figure 1 with either a 25 mm 0.2 µm Supor Acrodisc® (Part number 4612) for volumes of 40 mL or less or using a 0.2 µm Supor Acrocap® (Part number 4480; alternatively, KM5EKVP2S) for volumes of 100 – 200 mL.

The following loading buffers were tested:

1. 25 mM BisTris hydrochloride [Bis(2-hydroxyethyl)amino-tris(hydroxymethyl) methane] pH 6.5 with 4 mS/cm conductivity,
2. 25 mM BisTris hydrochloride pH 6.5 with 11 mS/cm conductivity adjusted with 5 M NaCl solution,
3. 25 mM Tris pH 8.0 with 4 mS/cm conductivity, and
4. 25 mM Tris pH 8.0 with 11 mS/cm conductivity.

All conductivities were adjusted using 5 M NaCl solution.

Figure 1: Experimental Setup



Methods

The CHO protein levels were measured by an enzyme-linked immunosorbent assay (ELISA) with a high sensitivity commercial kit from Cygnus Technologies (Southport, NC, USA), CHO HCP ELISA kit, catalog number F015. According to the manufacturer, the lower limit of detection with this kit was < 100 pg/mL while the lower limit of quantitation was ~0.7 ng/mL. The CHO DNA levels were measured by quantitative PCR at Cogenics, Inc. (Houston, TX, USA). Commercially available CHO DNA (Cogenics) as well as DNA that was isolated from a CHO cell line was used by Cogenics to obtain calibration curves for different samples. The mAb concentration was calculated from A_{280} readings using the conversion factor 1.5 mg mL^{-1} cm^{-1}.

The Mustang Q membrane coin was pre-conditioned with 10 MV of 1M NaOH, 10 MV of 1 M NaCl and finally with the loading buffer until the effluent pH and conductivity was same as that of the loading buffer prior to loading the sample. A 3 M BisTris solution or a 3 M Tris base solution was added to the mAb solution in order to either reduce pH to 6.5 or increase pH to 8.0, respectively, before loading on the Mustang Q coin. In cases where reduction in conductivity to 4 mS/cm was necessary, the mAb feedstream was diluted with water and the buffer concentration was adjusted with the required volume of either 3 M BisTris for pH 6.5 or 3 M Tris for pH 8.0 base solution. All mAb feedstreams were filtered prior to loading on the Mustang Q membrane using either a 25 mm 0.2 µm Supor Acrodisc for volumes of 40 mL or less or using a 0.2 µm Supor Acrocap for volumes of 100 – 200 mL.

The mAb feedstream that was pH adjusted to 8.0 and diluted to 4 mS/cm was spiked with 7 mg of CHO DNA (0.7 mg/mL) before processing through a Mustang Q coin in order to measure the CHO DNA removal from a protein A-purified mAb.

The Mustang Q coin was washed following the sample load with a loading buffer at the appropriate pH and conductivity until the A_{280} reading reached the baseline. The Mustang Q coin wash was combined with the flowthrough and this combined fraction was called the flowthrough fraction. A 4 mL aliquot of the feedstream and the Mustang Q flowthrough were saved for HCP and CHO DNA assay. Following completion of loading and loading buffer wash, the Mustang Q coin was cleaned with 20 MV of 1 M NaCl in the appropriate loading buffer at
10.5 mL/min (30 MV/min) followed by 20 MV of 1 M NaOH.

Results and Discussion

The residual impurity clearance capacity in an antibody solution from a chromatography resin or a membrane is typically determined from spiking experiments. Table 1 shows that when the antibody sample at pH 8.0 and 4 mS/cm was spiked with 7.0 mg CHO DNA (324 ng/mL DNA conc.) and processed through the Mustang Q membrane, the amount of DNA removed by the membrane corresponded to 4.9 log. This may not necessarily be an upper limit of DNA clearance as the spike load was limited by the amount of CHO DNA that was available. In order to validate the upper limit of DNA clearance a higher spike load may be used.

Table 1: Quantitative PCR Analysis of Residual CHO cell DNA on CHO DNA Spiked Antibody Sample Loaded on Mustang Q Membrane Coin

	IgG conc. mg/mL	pH	Conductivity (mS/cm)	pg DNA/mg mAb	Log reduction
Load (non-spiked)	2.0	8.0	4	760 (1)	
Load (spiked with 7.0 µg CHO DNA)	0.34	8.0	4	1.12×10^6 (2)	
Mustang Q FT	0.1	8.0	4	15 (1)	4.9

(1) Based on QPCR analysis

(2) Based on an estimation of 0.7 µg/µL from A_{260} readings.

The residual CHO protein impurities in the Mustang Q membrane processed samples were measured by ELISA assay. Table 2 shows that without any pH or conductivity adjustments (pH 6.5 and 11 mS/cm) approximately 7-fold reduction in CHO proteins was observed in the antibody solution. However, when the conductivity was lowered to 4 mS/cm while the pH was held constant, a 52-fold CHO protein reduction was observed. The antibody recovery was calculated from the Mustang Q membrane in the flowthrough and wash fractions based on A_{280} measurements.

Table 2: CHO Cell Protein Analysis in Mustang Q Coin Flow through (FT) and Wash

Sample description	IgG concentration mg/mL	ng HCP/mg Mab
Load	3.1	208
Mustang Q loading pH 6.5, conductivity 11 mS/cm	2.8	32
Mustang Q loading pH 6.5, conductivity 4 mS/cm	0.9	19
Mustang Q loading pH 8.0, conductivity 11 mS/cm	2.8	16
Mustang Q loading pH 8.0, conductivity 4 mS/cm	0.9	4

(1) Cubic spline calibration method.

The CHO protein clearance was significantly higher at pH 8.0, 16 ng/mg and 4 ng/mg (ppm) at 11 mS/cm and 4 mS/cm conductivities respectively. Thus, Table 2 suggests that the optimum loading conditions for CHO protein clearance with Mustang Q membranes is at pH 8.0 and 4 mS/cm conductivity.

Since the contaminant levels are much lower (by more than 3 orders of magnitude for both DNA and proteins) than the dynamic binding capacity of the membrane, efficient use of the membrane for binding these contaminants can be made by scaling-up based on the antibody throughput (grams of protein processed per unit membrane volume and time). Based on results in this report, the next step would be to develop a scaled-down flowthrough process on a Mustang Q coin that will provide a maximum throughput in terms of grams of mAb per liter of membrane at pH 8.0 and 4 mS/cm. An adequate safety margin may be defined for further scale-up. Furthermore, depending on the eventual scale, the flow rate may need to be optimized because some chromatgraphy systems may not be able to support flow rates of 30 MV/min with larger scale devices. Once the throughput has been determined, an appropriate Mustang Q membrane capsule size may be selected in order to process a given antibody batch size that will be generated from the protein A chromatography step.

Conclusion

- Mustang Q membrane loading at pH 8.0 with 4 mS/cm (3-fold dilution of the sample) in 25 mM Tris buffer resulted in reduction of CHO cell proteins to 4 ppm.
- Purification of a CHO DNA spiked mAb solution using a Mustang Q coin in the flowthrough mode at pH 8.0 and 4 mS/cm conductivity showed a 4.9 log reduction in CHO DNA.

References

(1) Low, D., O'Leary, R. and Pujar N.S. Future of antibody purification. *J. Chromatogr. B.* 2007, 848 48-63.

(2) Lajmi, A., Kutner, R., Reiser, J. A membrane chromatography application: A rapid, high capacity gene therapy vector purification tool. In *Process Scale Bioseparations for the Biopharmaceutical Industry;* Shukla, A., Etzel, M., Gadam, S., Eds.; Taylor & Francis, Boca Raton, FL, 2006.

(3) Zhang, S., Krivosheyeva, A., Nochumson, S. *Biotechnol. Appl. Biochem.* 2003, 37, 245-249.

(4) Zhou, J.X., Tressel, T. Membrane chromatography as a robust purification system for large-scale antibody production. *Bioprocess Int.* 2005, 3(9), Suppl. 32-37.

(5) Zhou, J.X., Tressel, T. Basic concepts in Q membrane chromatography as a robust purification unit for large-scale antibody production. *Biotechnol. Prog.* 2006, 22, 341 – 349.

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