PALL Life Sciences

Scale Up/Down Purification Study Using Mustang® XT Acrodisc® Membrane Adsorber

Aleksandar Cvetkovic[1], Iann Rancé[2], René Gantier[1] – 1. Pall Life Sciences, Northborough, MA, USA; 2. Cytheris SA, Issy Les Moulineaux, France

INTRODUCTION

Membrane chromatography is now routinely implemented in many large scale biotech processes to remove contaminating host cell proteins, DNA and viruses at high flow rate.

Pall developed the range of ready-to-use and scalable Mustang XT membrane chromatography capsules (5 to 5000 mL) for purification process development from lab to industrial scale. A new small scale device, the Mustang XT Acrodisc unit (0.86 mL) was recently introduced to complete that range of products.

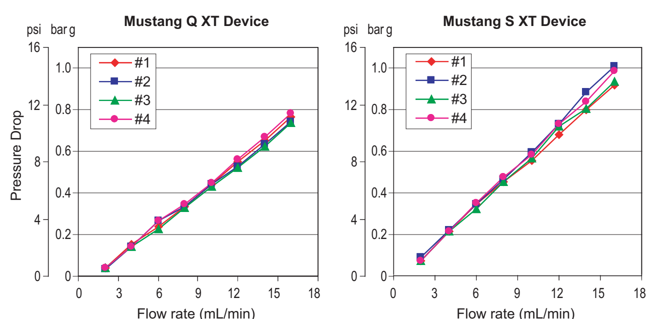
The performance evaluation (dynamic binding capacity, pressure drop, peak asymmetry and flow pattern) of the new Mustang XT Acrodisc unit is presented here. Additionally, a scalability study was conducted on various Mustang devices including the XT Acrodisc (0.86 mL), XT5 (5 mL) and XT140 (140 mL) capsules. A biotech process where Mustang Q membrane was used as an early contaminant removal step for the purification of a therapeutic recombinant protein was considered. The three different size capsules were used following a linear scalability rule. Almost identical chromatography elution, pressure drop, contaminant removal and product recovery patterns were obtained on all three capsules, which confirmed a solid scalability between the devices tested. This demonstrated that the new Mustang XT Acrodisc capsule can be used as a scalable device at the lab scale for early stage purification process development studies. The new device could therefore be considered for any scale down study, such as a viral clearance.

CHARACTERIZATION OF THE MUSTANG XT ACRODISC CAPSULE PERFORMANCE

The Mustang XT Acrodisc devices are developed in two chemistries: quaternary amine (Q) and sulfonic acid (S) respectively for anion and cation exchange chromatography. The membrane is enclosed in a polypropylene housing with an easy-to-use female luer lock inlet and outlet connectors. The devices are designed to withstand maximal operating pressure of 4 bar g and should generate less that 1 bar g of differential pressure at 10 mL/min flow rate.

Figure 1

Pressure Drop versus Flow Rate Using Mustang Q and S XT Acrodisc Devices



Four devices of each chemistry tested. Equilibration buffer: 25 mM Tris-HCl buffer, pH 8 for Mustang Q device, 10 mM MES, pH 5.5 for Mustang S device.

- **Pressure performance – ΔPressure drop vs. flow rate (Figure 1)**
  - Pressure drop far below 15 psi at 10 MV/min for both chemistries
  - High similarity between different devices with the same chemistry, small differences between devices with different chemistries
- **Flow, binding and recovery performance (Table 1)**
  - High dynamic binding capacity (DBC) for proteins and DNA at high flow rate (10 membrane volume (MV) per minute)
  - Robust product recovery
- **Separation performance – Peak asymmetry and resolution (Figure 2)**
  - Peak asymmetry of ~2.2 for Q and ~2.6 for S chemistry at 10 MV/min is inferior to one of resins, but similar or better than for other membrane devices
  - Resolution similar to that of rigid Q agarose beads and much better than cellulose Q membrane

CHARACTERIZATION OF THE MUSTANG XT ACRODISC CAPSULE PERFORMANCE *(continued)*

Table 1

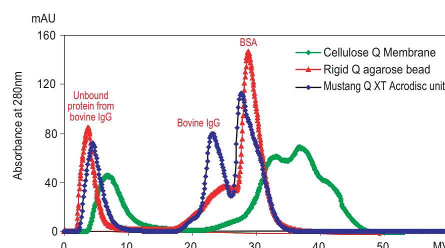
Dynamic Binding Capacity (DBC) and Recovery of Mustang Acrodisc XT Device

Ligand Chemistry	Biomolecules	DBC (mg/mL)	Recovery (%)
Q	BSA	90 ± 4	98 ± 2
S	Lysozyme	39 ± 4	102 ± 3
S	IgG	55 ± 5	99 ± 1
Q	Herring Sperm DNA	50.4	88

Sample: 1.5 g/L bovine serum albumin (BSA), IgG or lysozyme or 0.325 g/L of DNA in equilibration buffer; Equilibration buffer: 25 mM Tris-HCl buffer, pH 8 (Q chemistry) or 10 mM MES, pH 5.5 (S chemistry); Residence time: 0.1 min (or 10 MV/min).

Figure 2

Separation Performance of Mustang Q XT Acrodisc device, Rigid Q Agarose and Cellulose Q Membrane Measured by Resolution Model Proteins (Bovine IgG and BSA)



Sample: 2 mg of bovine IgG and 3 mg of BSA in equilibration buffer per mL of sorbent; Equilibration buffer: 25 mM Tris-HCl buffer, pH 8; Residence time: 4 min; Wash: 10 device volumes of equilibration buffer; Elution: 0 to 1 M NaCl in equilibration in 50 device volumes.

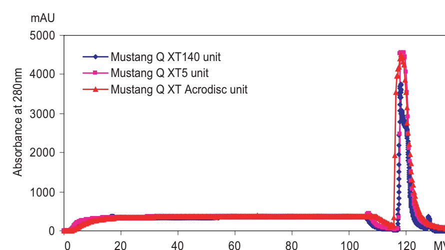
SCALABILITY OF THE MUSTANG XT PRODUCT LINE

A scale-up/down study was conducted to evaluate the scalability of Mustang Q XT products, namely XT Acrodisc units (0.86 mL), XT5 (5 mL) and XT140 (140 mL). The different capsules were used for early protein contaminant removal from a CHO cell culture supernatant, kindly provided by Cytheris (France), containing 0.1 mg/mL of recombinant human interleukin 7 (rhIL-7) in total protein concentration of 0.39 mg/mL. The performance of the different Mustang XT devices screened was evaluated regarding similarity of their chromatography UV280nm profile (Figure 3), contaminant removal and product recovery in the collected fractions (Figures 4 and 5).

Chromatograms on Figure 3 illustrate high level of similarity between the absorbance profiles obtained during chromatography runs using different size Mustang Q XT devices.

Figure 3

Scalability of Mustang Q XT Devices: Chromatography Profile Obtained Using XT Acrodisc, XT 5 and XT 140 Devices During Contaminant Capturing in the Initial Stage of rhIL-7 Purification from CHO CCS

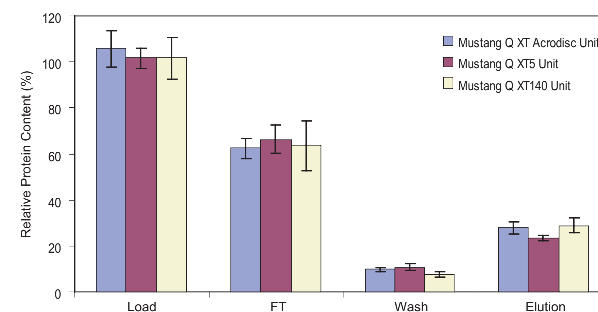


Absorbance at 280 nm profile during chromatography. Mustang Q devices: XT Acrodisc, XT5 and XT140; Sample: CHO CCS filtrate diluted 4 times with 10 mM Na phosphate, pH 7.5, conductivity 5.6 mS/cm; Load: 107 MV; Wash: 12 MV in equilibration buffer; Elute: 10 MV of 2 M NaCl in equilibration buffer; Equilibration buffer: 50 mM phosphate pH 7; Flow rate: 1.5 MV/min.

SCALABILITY OF THE MUSTANG XT PRODUCT LINE *(continued)*

Figure 4

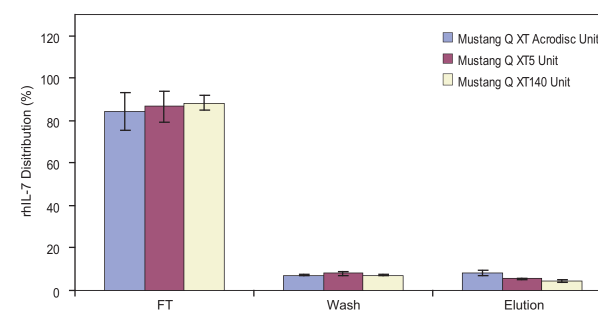
Relative Total Protein Content in Fractions Collected During Contaminant Capturing by Different Size Mustang Q XT Devices in the Initial Stage of rhIL-7 Purification from CHO CCS



Similar performance for different Mustang Q XT products supported by their chromatography profile is confirmed after determination of the total protein (Bradford assay) and product content (ELISA assay) of the collected fractions (Figures 4 and 5). Unbound rhIL-7 (flowthrough + wash) remains stable (>90%) for all capsules, as does the impurity content too. Mustang Q membrane helps removing 35% protein based impurities.

Figure 5

Relative rhIL-7 Content Fractions Collected During Contaminant Capturing by Different Size Mustang Q XT Devices in the Initial Stage of rhIL-7 Purification from CHO CCS.



CONCLUSION

- **Study has shown good scalability of Mustang XT devices, XT Acrodisc, XT5 and XT140:**
  - **Same chromatography profile**
  - **Same total protein distribution (25 to 30% total protein bound)**
  - **Same rhIL-7 distribution (>90% yield of recovery)**
- **A new addition to the Mustang XT product line, Mustang XT Acrodisc unit fills the missing link required for the successful and reliable scale up and scale down of the purification processes.**

Contact: +800.717.7255 (USA) • +41 (0)26 350 53 00 (Europe) • +65 6389 6500 (Asia/Pacific) • E-mail: biopharm@pall.com
Web: www.pall.com/biopharm

© 2013, Pall Corporation. PALL, Pall, Acrodisc and Mustang are trademarks of Pall Corporation.
Filtration.Separation.Solution. is a service mark of Pall Corporation. ® indicates a trademark registered in the USA. 3/13, GN 13.8524