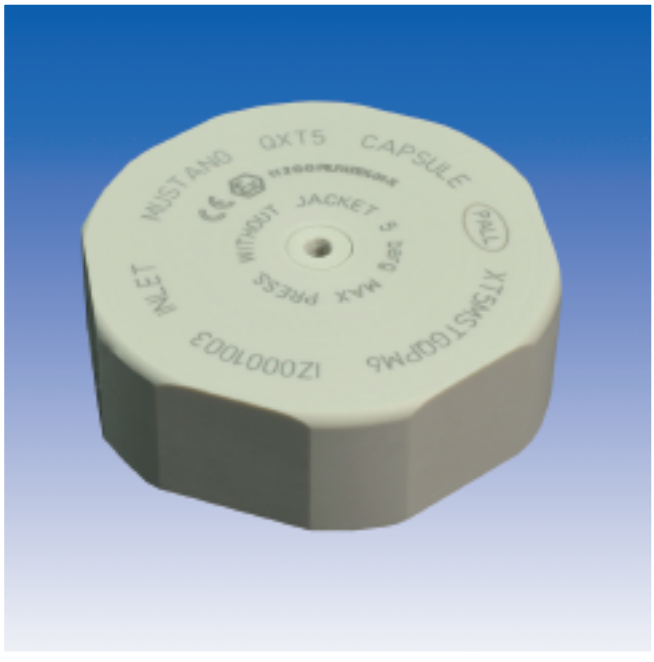
PALL Life Sciences

Validation Guide

USTR 2490a

Mustang® Q XT5 Membrane Chromatography Capsules



*Filtration. Separation. Solution.*SM

Contents

1. Overview

1.1 Introduction

The Pall Mustang Q XT5 capsule (Part Number XT5MSTGQPM6) is an anion-exchange membrane chromatography capsule designed for use in the purification of biopharmaceuticals. The Mustang Q XT5 capsule has been designed as a scaled-down partner of both the Mustang Q XT140 (Part Number XT140MSTGQP05) and the XT5000 (Part Number XT5000MSTGQP1) capsules. It can be used either as a stand-alone chromatographic device, or to assist with scale-down optimization studies for both the Mustang Q XT140 and XT5000 capsules.

1.2 Purpose

The purpose of this guide is to summarize the tests that were performed to qualify the performance of Mustang Q XT5 membrane chromatography capsules under standard conditions. This testing program included:

- Installation and preconditioning procedures
- Determination of flow characteristics
- Determination of integrity and dispersion
- Determination of BSA (bovine serum albumin) dynamic binding capacity
- Sanitization and storage
- Biological reactivity tests on the materials of construction

Warning: Mustang Q products should not be used with fluids that are incompatible with the materials of construction. Incompatible fluids are those that chemically attack, soften, stress crack or adversely affect the materials of construction in any way.

1.3 Summary of Protocols

1.3.1 Installation and Preconditioning Procedures

The validation protocols were carried out on a chromatography system of a design that consistently produces high system backpressures at the flow rates typically used for membrane chromatography (ÄKTAexplorer* 100, GE Healthcare). In order to avoid a breach in membrane integrity, the Mustang Q XT5 membrane chromatography capsule was installed in a special pressure-resistant housing (13 barg, part number XT5MSTGJKT). In addition, the system flow restrictor was removed. These two steps effectively allow the capsule to be run at pressures higher than the limits that are specified for operating the capsule alone despite high pressures inherent in such a system. For a more detailed installation procedure, see the Mustang Q XT5 Assembly and Installation Procedures (USTR 2432).

The preconditioning protocol is run first on every capsule in order to reduce the levels of extractables from the materials of construction. Since the materials of construction are identical to those found in the Mustang Q XT5000 chromatography capsule, it is assumed that the levels of extractables before and after preconditioning of the Mustang Q XT5 membrane chromatography capsule will be reduced to the same low levels as was seen for the Mustang Q XT5000 chromatography capsule. The amount of non-volatile residue (NVR) and total organic carbon (TOC) extracted from preconditioned Mustang Q XT5000 chromatography capsules was found to be very low (see Mustang Q XT5000 membrane chromatography capsule validation guide, USTR 2500). Preconditioning procedures are effective in reducing non-volatile residuals up to 7-fold and total organic carbon up to 20-fold. Actual service will impose different conditions, such as different exposure times, temperature, and liquid purity. Evaluation under process conditions is therefore also recommended.

1.3.2 Determination of Flow Characteristics

The Mustang Q XT5 membrane chromatography capsule was equilibrated with 25 mM Tris, pH 8.0 and run over a range of flow rates. At each flow rate, the inlet and outlet pressures were measured, and the pressure drop across the capsule was calculated. The pressure drop was then plotted against the flow rate. The pressure drop was linear over the flow rate range of 10 – 50 mL/min (2 – 10 membrane volumes/min), and the use of the pressure-resistant housing extended the pressure tolerance for the capsule. These results can be used to assist the user when sizing systems and processes that employ Mustang Q XT5 membrane chromatography capsules with process fluids of similar viscosities.

1.3.3 Determination of Integrity and Dispersion

Adenosine monophosphate (AMP) is used as a marker for determining both the integrity and dispersion of the Mustang Q XT5 membrane chromatography capsule. For integrity, a pulse of AMP is injected under binding conditions, and the absorbance at 260 nm is monitored. Any breach in integrity will be detected in the absorbance trace during the injection step. All of the capsules included in the validation program were integral, and subsequent elution of the bound AMP indicated low dispersion on the downstream side of the membrane. For dispersion, AMP is injected again but under non-binding conditions, and absorbance at 260 nm is also monitored. The AMP should pass through the capsule unimpeded and emerge as a single peak with only minor tailing. All of the capsules that were included in the validation program exhibited low and uniform dispersion.

1.3.4 Determination of BSA (Bovine Serum Albumin) Dynamic Binding Capacity

The tests performed indicate that Mustang Q XT5 membrane chromatography capsules exhibit extremely high BSA dynamic binding capacity and are therefore suitable for downstream processing applications for the capture of biomolecules or removal of contaminants such as DNA, viruses, host cell proteins, and endotoxins.

After equilibration with 25 mM Tris, pH 8.0, the Mustang Q XT5 membrane chromatography capsule was loaded to saturation with a solution of 1.5 mg/mL BSA in equilibration buffer. After loading was complete, any unbound BSA was washed out, and bound BSA was eluted with a single step of 1 M NaCl in 25 mM Tris, pH 8.0. Dynamic binding capacity was determined from the amount of BSA that was present in the elution fraction. Typical binding capacities for each capsule were approximately 80 – 85 grams of BSA per liter of membrane volume.

1.3.5 Sanitization and Storage

Sanitization is performed using 1 M NaOH. The capsule can then be stored in 0.1 M NaOH plus 1 M NaCl.

1.3.6 Biological Reactivity Tests on the Materials of Construction

The materials of construction for the Mustang Q XT5 membrane chromatography capsule are identical to those used in the Mustang Q XT5000 chromatography capsules, and those materials have previously been tested for biological reactivity. Prior to performing the biological reactivity tests, Mustang Q XT5000 chromatography capsule materials of construction were conditioned using recommended procedures. The materials used in the construction of the polypropylene capsule housing, polypropylene end cap, the polypropylene support and drainage nets, and the Mustang Q XT5 membrane chromatography capsule all met the requirements of the United States Pharmacopeia (USP) Class VI 50 °C Biological Reactivity Tests, *in vivo,* for Plastics.

2. Preconditioning and Determination of Flow vs. Differential Pressure

2.1 Introduction

The aim of this series of tests was to determine the pressure drop across the Mustang Q XT5 membrane chromatography capsule at different flow rates using an aqueous test fluid.

2.2 Summary of Methods

- Mustang Q XT5 membrane chromatography capsules were evaluated as new unused capsules.
- The Mustang Q XT5 membrane chromatography capsule was assembled into the pressure-resistant housing, connected to the chromatography system, and preconditioned at 10 mL/min with:
- 25 mL of 1 M NaOH
- 25 mL of 25 mM H_3PO_4/1 M NaCl

⚠ *Warning: It is essential that end users follow the 2-step preconditioning protocol prior to first use, between cycles, and after prolonged storage. Preconditioning ensures low levels of extractables prior to equilibration with the appropriate buffer. Actual service will impose different conditions, such as different exposure times, temperature, and liquid purity. Evaluation under process conditions is therefore also recommended.*

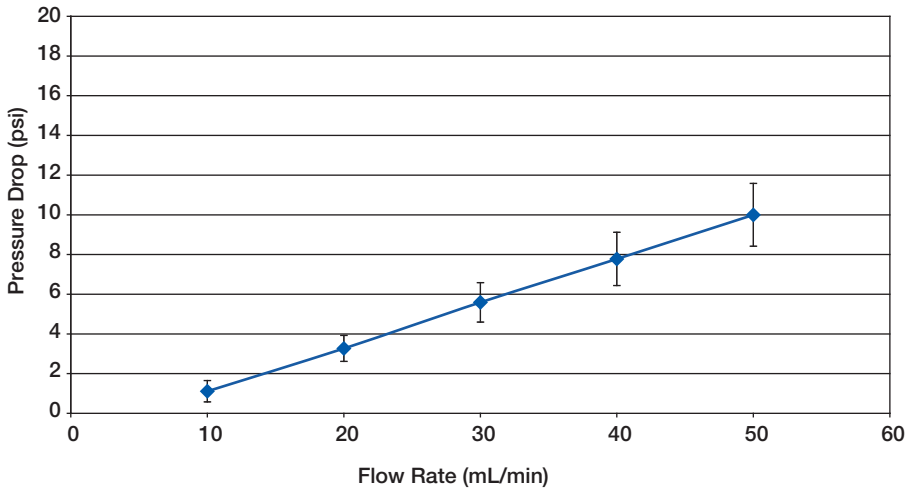
The Mustang Q XT5 membrane chromatography capsule was first flushed with 25 mM Tris pH 8.0 at 50 mL/min for the purpose of displacing air trapped in the device. The Mustang Q XT5 membrane chromatography capsule was then equilibrated with 25 mM Tris, pH 8.0 until the pH and conductivity of the effluent were equal to that of the buffer in the reservoir. The flow rate was set at 10 mL/min (2 membrane volumes/minute), and the pressure was measured on both the inlet and outlet sides of the device. The flow rate was then raised in increments to a final flow rate of 50 mL/min (10 membrane volumes/min). At each flow rate, inlet and outlet pressures were measured. For each flow rate, the pressure drop was calculated by subtracting the outlet pressure from the inlet pressure.

2.3 Results

A graph of flow rate versus differential pressure is shown in Figure 1. Nine capsules (three each from three different lots) were used in performance of the test. Each point represents the mean ± S.D. for all nine capsules.

Figure 1

Flow vs. Differential Pressure for Mustang Q XT5 Capsules, Part Number XT5MSTGQPM6



2.4 Conclusions

The Mustang Q XT5 membrane chromatography capsule exhibited a linear pressure drop throughout the entire range of flow rates tested (10 – 50 mL/min), and the inlet pressure did not exceed the pressure tolerance for the capsule. The pressure curves were consistent from capsule to capsule and from lot to lot, and are directly scalable to the Mustang Q XT5000 process-scale membrane chromatography capsule.

The flow characteristics described in this report can be used to assist in sizing systems employing Mustang Q XT5 membrane chromatography capsules when used with process fluids of similar viscosities.

3. Integrity and Dispersion

3.1 Introduction

The Mustang Q XT5 membrane chromatography capsule comes ready to run without the need for column packing and qualification associated with conventional chromatography resins. However, because the configuration of the capsule is much like that of a filter, a test is necessary to demonstrate that the capsule is integral without any defects in the membrane or other components that would allow bypass of the membrane. In addition, because the capsule is a chromatography device, a test is also necessary that gives the end user an assessment of the fluid flow characteristics of the capsule as a whole. For both of these purposes, a test has been developed that utilizes a common non-toxic negatively charged molecule — adenosine monophosphate (AMP) — to determine both the integrity of the capsule and its dispersion characteristics. In addition, because AMP carries a negative charge, the test will also confirm the identity of the positively-charged Mustang Q XT5 membrane chromatography capsule within the capsule.

A total of 9 capsules have been tested, 3 each from 3 different membrane lots.

3.2 Summary of Methods

3.2.1 Integrity

For the integrity test, AMP is introduced into the Mustang Q XT5 membrane chromatography capsule under binding conditions. The capsule was first equilibrated with 25 mM Tris, pH 8.0 until the pH and conductivity of the effluent were equal to that of the buffer in the tank.

A solution of 3 mg/mL AMP in 25 mM Tris, pH 8.0 was prepared, and 300 µL of that solution was manually injected into the sample loop of the chromatography system. The system pump was then started at 50 mL/min, and the AMP is swept onto the Mustang Q XT5 membrane chromatography capsule. Absorbance at 260 nm was monitored. A capsule that has no breach in integrity will show no change in UV absorbance during the injection step — all of the AMP should bind to the membrane.

Following the injection, the AMP was eluted with 1 M NaCl in 25 mM Tris, pH 8.0. Absorbance at 260 nm continued to be monitored, and AMP should elute as a single sharp peak. The AMP integrity test also serves as an identity test to confirm that the chemistry on the membrane is an anion exchanger.

3.2.2 Dispersion

For the dispersion test, AMP is introduced into the Mustang Q XT5 membrane chromatography capsule under non-binding conditions. The test is run immediately following the integrity test since the capsule is already in 1 M NaCl in 25 mM Tris, pH 8.0 at the end of that test.

A solution of 3 mg/mL AMP was prepared in 1 M NaCl in 25 mM Tris, pH 8.0, and 300 µL of that solution was manually injected into the sample loop of the chromatography system. The system pump was then started at 50 mL/min, and the AMP was swept onto the Mustang Q XT5 membrane chromatography capsule. Absorbance at 260 nm was monitored.

Under these conditions, AMP should not bind and should emerge from the capsule as a single peak following the injection step with no shouldering and minimal tailing. This indicates uniform flow distribution through the entire capsule.

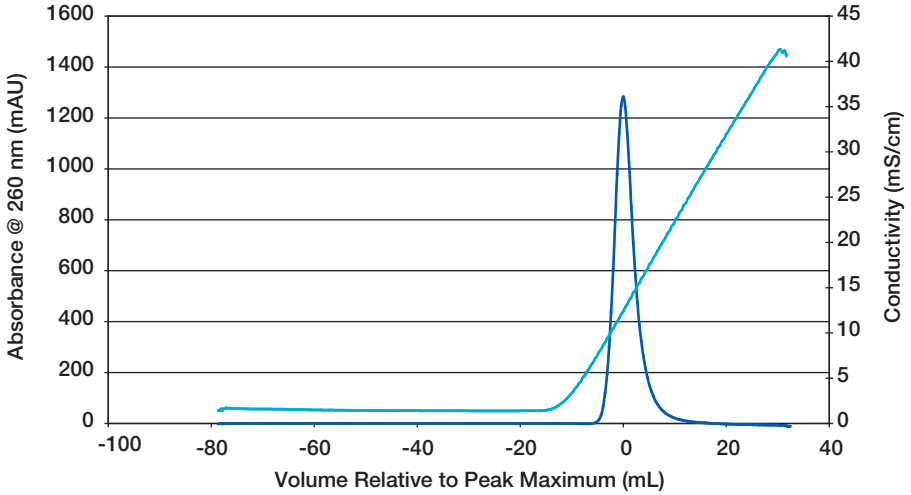
3.3 Results

3.3.1 Integrity

All of the 9 capsules tested in this study proved to be integral, and gave results that were comparable to those obtained with the Mustang Q XT5000 process-scale membrane chromatography capsule. There was no evidence of breakthrough during the injection step, and the AMP eluted as a single sharp peak. Figure 2 shows the average of all 9 integrity curves obtained during the validation.

Figure 2

Integrity Curves for Mustang Q XT5 Disposable Chromatography Capsules

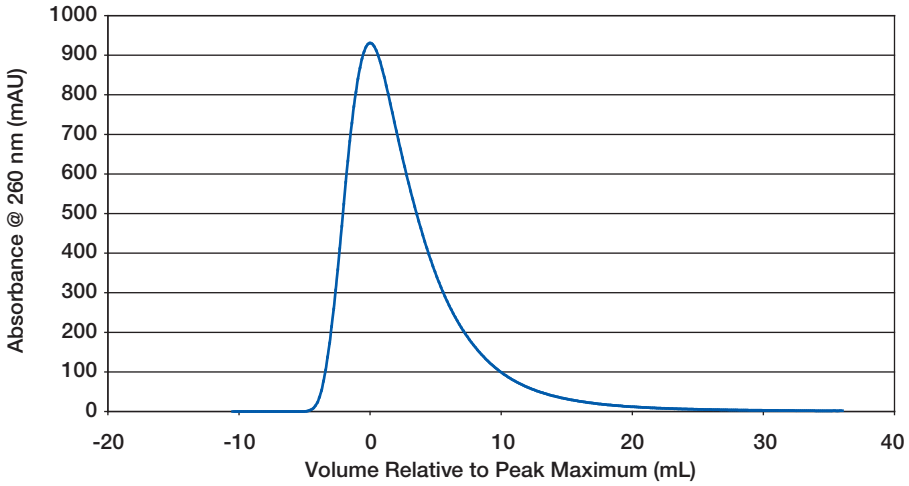


3.3.2 Dispersion

Each of the 9 capsules tested in this study showed similar dispersion patterns. The AMP peak emerged from the capsule with a sharp leading edge, no shouldering, and minimal tailing on the trailing edge. Figure 3 shows the average of the dispersion curves obtained from all 9 capsules. This is the same dispersion pattern that was seen with the Mustang Q XT5000 process-scale membrane chromatography capsule.

Figure 3

Dispersion Pattern for Mustang Q XT5 Disposable Chromatography Capsules



3.4 Conclusions

AMP is an extremely useful marker to demonstrate both integrity and fluid dispersion in the Mustang Q XT5 membrane chromatography capsule. In addition, it also identifies the membrane within the capsule as an anion exchanger, since AMP binds under conditions of low conductivity as seen in the integrity test. These two tests give the end user assurance that the capsule is integral and shows relatively uniform flow distribution before committing it to the purification of high value products. Each of these tests can be completed in a matter of minutes.

A patent application for this technique is pending.

4. Determination of BSA Dynamic Binding Capacity

4.1 Introduction

The aim of this series of tests was to determine the BSA dynamic binding capacity of the Mustang Q XT5 membrane chromatography capsule.

4.2 Summary of Methods

Typical Mustang Q XT5 membrane chromatography capsules from standard production lots were tested. A total of 9 capsules were tested, 3 each from 3 different membrane lots. The flow rate throughout every run was 50 mL/min (10 membrane volumes/min).

The Mustang Q XT5 membrane chromatography capsule was equilibrated with a sufficient volume of buffer (25 mM Tris, pH 8.0) such that the pH recorded at the outlet is equal to the original pH of the buffer, and remained constant for an additional 2 membrane volumes. At this point, the UV detector was zeroed to establish baseline.

A solution of 1.5 mg/mL BSA in 25 mM Tris, pH 8.0 was loaded, and the UV absorbance of the fluid collected on the downstream side was monitored at 280 nm. The point at which the absorbance rises to 0.1 AU (absorbance units) above baseline is considered the breakthrough point. BSA loading continued until the UV absorbance reached a plateau and was continued for another 2 MV to assure complete saturation of the membrane.

Once the loading was completed, unbound BSA was washed out until the UV absorbance returned to baseline and held there for 2 membrane volumes.

Bound BSA was eluted with a single step of 1 M NaCl in 25 mM Tris, pH 8.0. The elution peak was collected from the moment that the UV absorbance began to rise to the moment it returned back to baseline. The volume of the elution peak was measured, the BSA concentration was determined, and the mass of BSA in the elution fraction was calculated.

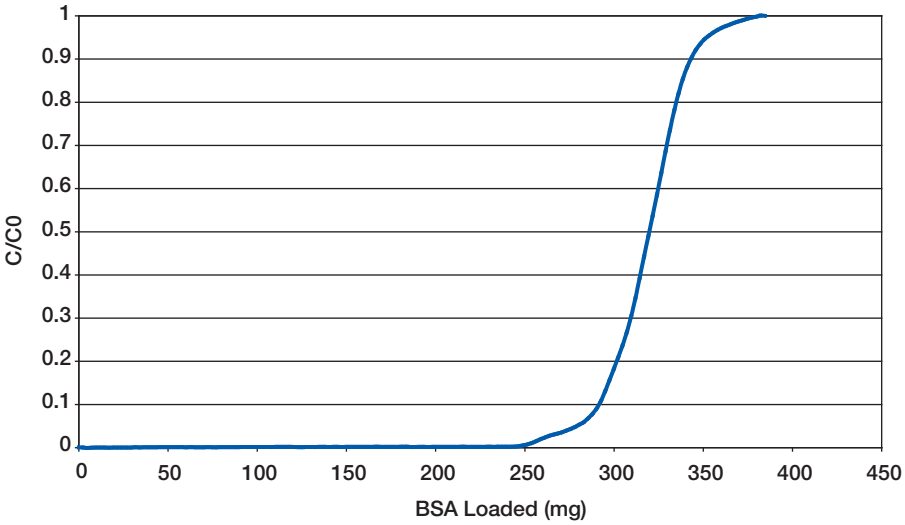
Dividing the mass of BSA (mg) in the elution fraction by 5 mL gives the binding capacity of the Mustang Q XT5 membrane chromatography capsule for BSA in units of mg/mL or grams/liter of membrane.

4.3 Results

Figure 4 shows a typical dynamic binding curve from capsule 483109-1. The ratio of the BSA concentration in the effluent and the concentration in the feed is determined and plotted against the mass of BSA loaded. Initial breakthrough occurs after 250 mg of BSA have been loaded.

Figure 4

Binding Capacity Curve for Mustang Q XT5 Capsule, Part Number XT5MSTGQPM6

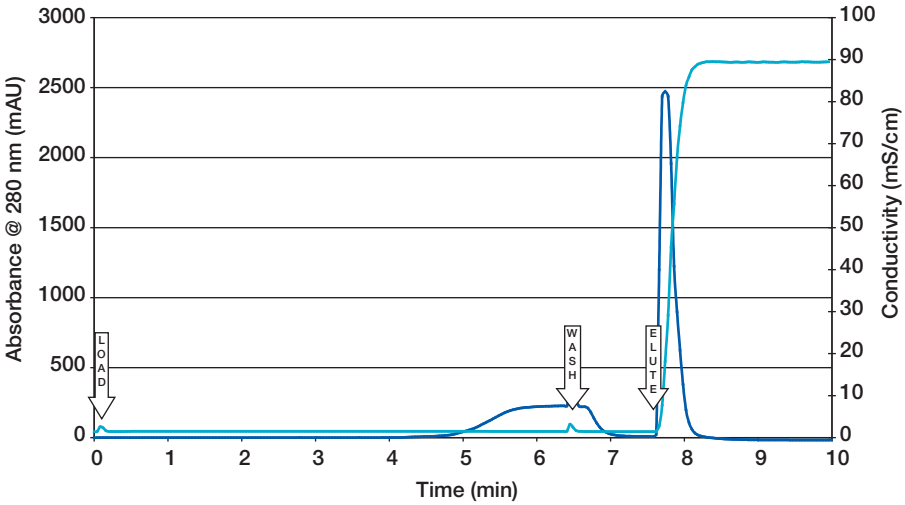


C/C0 is the ratio of the BSA concentration in the effluent and in the original feed.

Figure 5 shows the full chromatogram from the same capsule. Arrows on the chromatogram indicate where the load, wash, and elute steps take place. Initial breakthrough does not occur until after 4 minutes have elapsed, and full saturation is achieved in just over 6 minutes. Wash and elution take only 2 minutes. These three steps together take place in less than 9 minutes, and approximately 400 mg of BSA were bound and eluted in total.

Figure 5

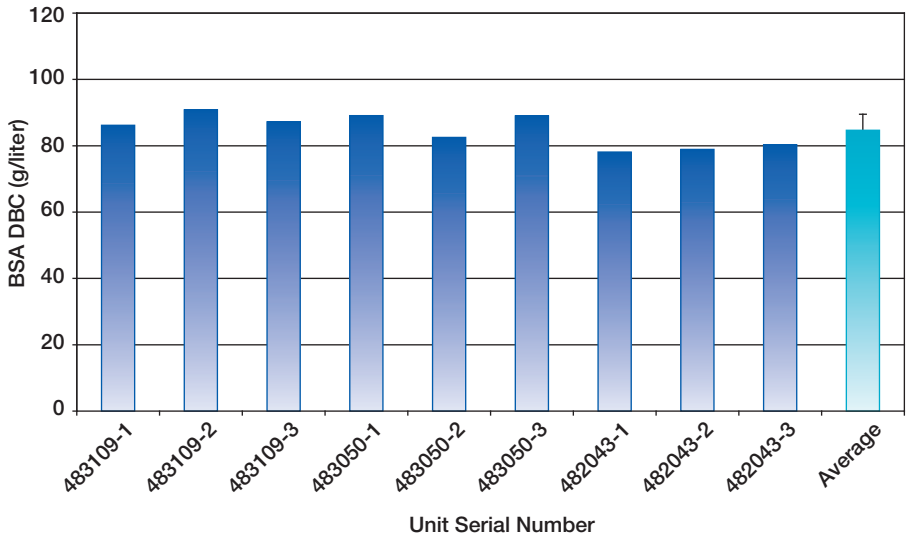
Typical Dynamic Binding and Elution Curve for Mustang Q XT5 Capsule, Part Number XT5MSTGQPM6



Dynamic binding capacities for nine Mustang Q XT5 capsules are shown in Figure 6. The average binding capacity is 85 ± 5 grams of BSA per liter of membrane volume, and is directly scalable to the Mustang Q XT5000 process-scale membrane chromatography capsule.

Figure 6

BSA Dynamic Binding Capacities at Saturation for Mustang Q XT5 Capsule, Part Number XT5MSTGQPM6



4.4 Conclusions

Mustang Q XT5 membrane chromatography capsules exhibit high BSA dynamic binding capacities and are therefore suitable for downstream bioprocessing applications for the capture and elution of negatively charged target molecules. This test demonstrates both the high speed and high capacity of the Mustang Q XT5 membrane chromatography capsule.

Other target molecules will have different binding capacities when run on Mustang Q XT5 membrane. Also, different flow rates and the presence of host cell protein or other contaminants may influence the performance, and it is therefore recommended that the user evaluate Mustang Q XT5 membrane chromatography capsules using specific process fluids under standard operating conditions.

5. Sanitization and Storage

5.1 Introduction

Users of Mustang Q XT5 membrane chromatography capsules may wish to use sodium hydroxide for sanitization purposes. Conditions for sanitization and storage of Mustang Q XT5 membrane chromatography capsules have been established that have little or no effect on integrity, dispersion, differential pressure, and dynamic binding capacity. To ensure reliable performance, users should follow these procedures exactly as described in this section.

5.2 Summary of Methods

5.2.1 Sanitization with 1 M NaOH

A sufficient quantity of 1 M NaOH was prepared, and 5 bed volumes (25 mL) were pumped through the Mustang Q XT5 membrane chromatography capsule at a flow rate of 10 mL/min. The pump was stopped, and the capsule was held for 30 minutes.

5.2.2 Preparation for Storage

A sufficient quantity of 0.1 M NaOH/1 M NaCl was prepared, and 5 bed volumes (25 mL) were pumped through the Mustang Q XT5 membrane chromatography capsule at a flow rate of 10 mL/min. The pump was stopped, and the lines attached to the outlet side and inlet side of the capsule were disconnected. Plastic blind end caps were fastened to the outlet and inlet sides of the capsule, and the capsule was stored between 4 and 20 °C.

5.3 Conclusions

Sanitization with 1 M NaOH and long-term storage in 0.1 M NaOH/1M NaCl is expected to have minimal effect on the performance of the Mustang Q XT5 membrane chromatography capsule. The materials of construction of the Mustang XT5 membrane chromatography capsule are identical to those used for the Mustang XT5000 and Mustang XT140 chromatography capsules. Please refer to USTR 2500 and USTR 2597 for additional information on sanitization and storage of Mustang XT capsules.

6. Biological Reactivity Tests on the Materials of Construction

The materials of construction of the Mustang Q XT5 membrane chromatography capsule are identical to those used for the Mustang Q XT5000 chromatography capsule. All of the materials of construction have previously passed USP Class VI 50 °C Biological Reactivity Tests, *in vivo,* for Plastics during the validation of the Mustang Q XT5000 chromatography capsule. Please refer to the Mustang Q XT5000 chromatography capsule validation guide (USTR 2500) for further information.

7. Extractables Testing

The materials of construction of the Mustang Q XT5 membrane chromatography capsule are identical to those used for the Mustang Q XT5000 chromatography capsule. For information regarding extractables testing, please refer to the Mustang Q XT5000 validation guide (USTR 2500).

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