Life Sciences

Validation Guide

USTR 2597

Mustang® Q XT140 Membrane Chromatography Capsules

*Filtration. Separation. Solution.*SM

Contents

1. Overview

1.1 Introduction

Pall's Mustang Q XT140 capsules (Part Number XT140MSTGQP05) are designed as a scale-down companion to the Mustang Q XT 5000 capsules (Part Number XT5000MSTGQP1) and as a scale-up companion to the Mustang Q XT5 capsule (Part Number XT5MSTGQPM6). All three Mustang XT capsules are designed to simplify and reduce the operational requirements of the production processes in the manufacture of biopolymers. The mechanism involved is rapid surface anion exchange adsorption within the convective pores of the membrane. Mustang Q membranes are manufactured from a hydrophilic, low protein binding polyethersulfone (PES) 0.8 µm membrane. The membrane is coated with a polymer containing pendant quaternary amine groups; the polymer is irreversibly cross-linked, resulting in high dynamic binding capacities at very high flow rates. The Mustang Q XT140 capsule contains 16 layers of membrane in a unique lay-over pleat (LOP) wrapped around a cylinder to yield a radial flow configuration which maximizes the amount of membrane packaged within its housing. The total membrane bed volume is nominally 140 mL, and the polypropylene capsule has been designed to improve flow dynamics and reduce hold-up volume. Additional details about Mustang Q XT140 capsules can be found in the product literature (USD 2599 and USD 2600) and in the Instructions for Use (USD 2510).

1.2 Purpose

This Validation Guide is a record that summarizes the test results performed to qualify the performance of Mustang Q XT140 capsules under standard conditions. This testing program included:

- Extractables testing before and after preconditioning
- Determination of flow characteristics
- Integrity and dispersion testing
- 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 Extractables Testing Before and After Preconditioning

Extractables were measured in water flushes before and after preconditioning. The amounts of non-volatile residue (NVR) and total organic carbon (TOC) from preconditioned Mustang Q XT140 capsules were found to be very low. Preconditioning procedures are effective in reducing NVR up to 17-fold and TOC up to 43-fold. Actual service will impose different conditions such as different exposure times, temperature, liquid purity, etc. Evaluation under process conditions is therefore also recommended.

1.3.2 Determination of Flow Characteristics

The Mustang Q XT140 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 plotted against the flow rate. Over the flow rate range of 0.070 – 2.8 L/min (0.5 – 20 membrane volumes/min), the pressure drop was linear, and the inlet pressure did not exceed the pressure tolerance for the capsule. The results can be used to assist the user when sizing systems that employ Mustang Q XT140 capsules when used with process fluids of similar viscosities.

1.3.3 Integrity and Dispersion Tests

Adenosine monophosphate (AMP) is used as a marker for integrity and dispersion. For integrity, 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 units 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 total dispersion, AMP is injected again under non-binding conditions, and absorbance at 260 nm is also monitored. The AMP should pass through the unit unimpeded and emerge as a single peak with tailing. All of the units that were included in the validation program exhibited dispersion with measurable tailing of the peak resulting in a 2.1 average asymmetry value.

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

The tests performed indicate that Mustang Q XT140 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 endotoxin.

After equilibration with 25 mM Tris, pH 8.0, the Mustang Q XT140 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 20 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 70 mg of BSA per mL of membrane volume.

1.3.5 Sanitization and Storage

Sanitization is performed using 1 M NaOH. The capsule storage solution is 0.1 M NaOH in 1 M NaCl.

1.3.6 Biological Reactivity Tests on the Materials of Construction

Prior to performing the biological reactivity tests, Mustang XT140 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 XT membrane all met the requirements of the USP for Class VI (50 °C) Plastics *(in vivo).*

2. Extractable Testing Before and After Preconditioning

2.1 Introduction

The purpose of this series of tests was to analyze and quantify the amount of material that can be extracted from new Mustang Q XT140 capsules by water at ambient temperature (20 °C ± 5 °C) before and after preconditioning.

2.2 Extractable Testing Before and After Preconditioning

Typical Mustang Q XT140 capsules were first evaluated for extractables as new, unused capsules. Equipment used in these tests (pump, tubing, connectors) was sanitized by exposure to 1 M NaOH for 30 minutes and rinsed with deionized water prior to connecting to the Mustang Q XT140 capsule.

The Mustang Q XT140 capsule was connected to the pump, and 600 mL of deionized water was recirculated through the capsule at a flow rate of 300 mL/min for a period of 30 minutes. The pump was stopped, and samples of the recirculated water were taken for TOC (total organic carbon) and NVR (non-volatile residue) analysis.

The Mustang Q XT140 capsule was preconditioned at 300 mL/min with:

- 750 mL of 1 M NaOH
- 750 mL of 25 mM H_3PO_4/1 M NaCl

Following preconditioning, the Mustang Q XT140 was equilibrated to neutral pH using 750 mL of 20 mM sodium phosphate buffer, pH 7.0, and then flushed with 5 L of deionized water to reduce conductivity.

Following the last step, 300 mL of deionized water was recirculated through the capsule at a flow rate of 300 mL/min for a period of 30 minutes. The pump was stopped, and samples of the recirculated water were taken for TOC and NVR analysis.

2.3 Results

The amount of extractables (NVR and TOC) obtained from Mustang Q XT140 capsules is shown in Table 1. Results shown are the average of six capsules from three different membrane lots.

Table 1

Non-volatile Aqueous Extractables and TOC Measurements Obtained Using Five Mustang Q XT 140 Capsules, Part Number XT140MSTGQP1

TOC Before Preconditioning (mg/L of extract)	TOC After Preconditioning (mg/L of extract)	NVR Before Preconditioning (mg/L of extract)	NVR After Preconditioning (mg/L of extract)
116.3	2.7	271.2	16.2

2.4 Conclusions

The preconditioning procedure reduces the levels of aqueous extractables to an extremely low level. TOC levels were reduced approximately 43-fold, and NVR levels were reduced 17-fold.

It is essential that end users follow the two-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, liquid purity etc. Evaluation under process conditions is therefore also recommended.

3. Flow vs. Differential Pressure

3.1 Introduction

The aim of this series of tests was to determine the pressure drop across the Mustang Q XT140 capsules at different flow rates using an aqueous test fluid.

3.2 Summary of Methods

The Mustang Q XT140 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. At that point, the test was run in recirculation mode.

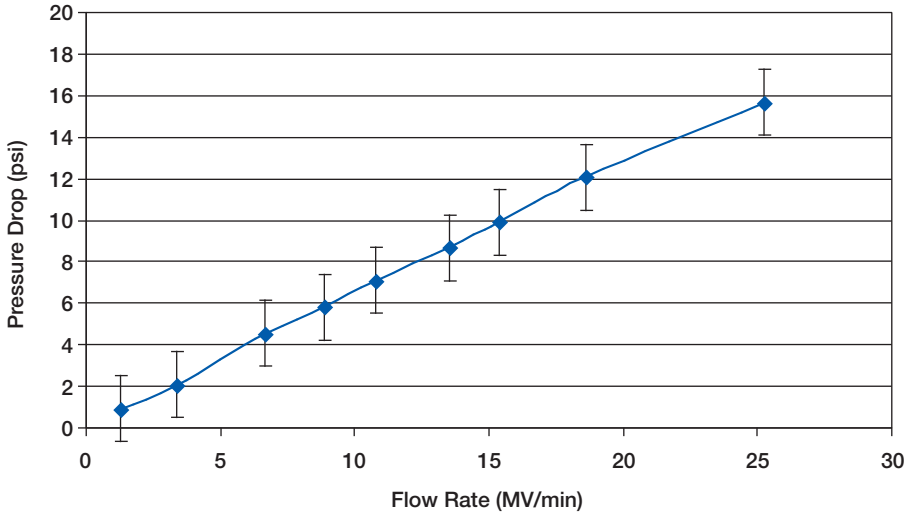
The flow rate was set at 140 mL/min (1 membrane volumes/minute), and the pressure was measured on both the inlet and outlet sides of the device. The flow rate was then raised in increments to a final flow rate of 2.8 L/min (20 membrane volumes/minute). At each flow rate, inlet and outlet pressures were measured. For each flow rate, the pressure drop was calculated by subtracting the outlet pressure from the inlet pressure.

3.3 Results

A graph of the flow of the Tris buffer versus pressure drop is shown in the following Figure 1. Nine capsules (three each from three different lots) were used in performance of the test.

Figure 1

Flow vs. Differential Pressure for Mustang Q XT140 Capsules, Part Number XT140MSTGQP05



3.4 Conclusions

The Mustang Q XT140 capsule exhibited a linear pressure drop throughout the entire range of flow rates tested (1 – 25 membrane volumes/minute), 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.

The flow characteristics described in this report can be used to assist in sizing systems employing Mustang Q XT140 capsules when used with process fluids of similar viscosities.

4. Integrity and Dispersion

4.1 Introduction

The Mustang Q XT140 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 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 to give 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.

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

4.2 Summary of Methods

Integrity

For the integrity test, AMP is introduced into the Mustang Q XT140 capsule under binding conditions. The capsule was first equilibrated with 20 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 20 mL of that solution was manually injected into the injection loop of the chromatography system. The system pump was then started at 1.4 L/min, and the AMP is swept onto the Mustang Q XT140 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 a single step of 1 M NaCl in 20 mM Tris, pH 8.0. Absorbance at 260 nm continued to be monitored, and AMP should elute as a single sharp peak.

Dispersion

For the dispersion test, AMP is introduced into the Mustang Q XT140 capsule under non-binding conditions. The test is run immediately following the integrity test because 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 20 mL of that solution was manually injected into the injection loop of the chromatography system. The system pump was then started at 1.4 L/min, and the AMP was swept into the Mustang Q XT140 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 on the trailing edge. This indicates the flow pattern under nonbinding conditions. Under bind and elute conditions, the elution peak is very sharp and concentrated as seen in Figure 2.

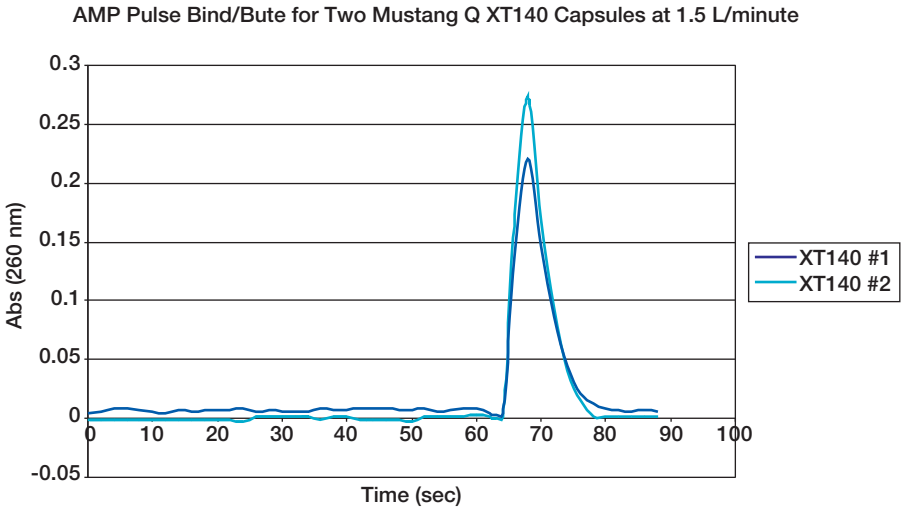
4.3 Results

Integrity

All of the 9 capsules tested in this study proved to be integral. There was no evidence of breakthrough during the injection step, and the AMP eluted as a single sharp peak. An overlay of the integrity curves for two of these capsules can be seen in Figure 2. All other capsules showed a similar integrity profile.

Figure 2

Integrity Curves for Mustang Q XT140 Disposable Chromatography Capsules

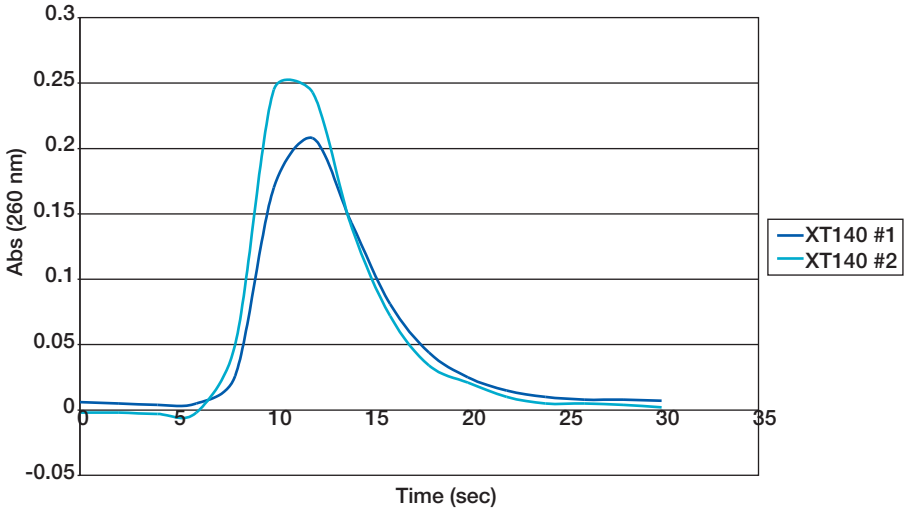


Dispersion

Each of the nine 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. An overly of the dispersion curves for two of these capsules can be seen in Figure 3. All other capsules exhibited a similar dispersion pattern.

Figure 3

Dispersion Patterns for Mustang Q XT140 Disposable Chromatography Capsules



4.4 Conclusions

AMP can be a useful marker to demonstrate both integrity and fluid dispersion in the Mustang Q XT140 capsule. In addition, it also identifies the membrane within the capsule as an anion exchanger, because the anionic 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.

5. Determination of BSA Dynamic Binding Capacity

5.1 Introduction

The purpose of this series of tests was to determine the BSA dynamic binding capacity of the Mustang Q XT140 capsule.

5.2 Summary of Methods

Typical Mustang Q XT140 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 1.4 L/min [10 membrane volumes (MV/min)].

The Mustang Q XT140 capsule was equilibrated with a sufficient volume (3L) of buffer (25 mM Tris, pH 8.0) such that the pH recorded at the outlet was equal to the original pH of the buffer, and remained constant for an additional two 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. The BSA loading process continued until the UV absorbance reached a plateau and was continued until 80% of breakthrough was achieved.

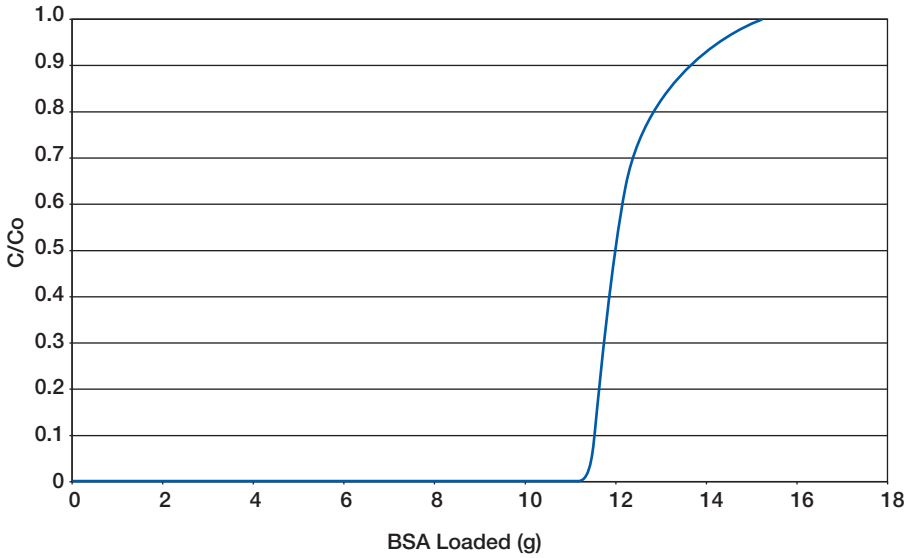
Once the loading was completed, unbound BSA was washed out until the UV absorbance returned to baseline and held there for two membrane volumes.

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 in the elution fraction by 0.14 L gives the binding capacity of the Mustang Q XT140 capsule for BSA in units of grams/liter of membrane.

5.3 Results

Figure 4

Binding Capacity Curve for Mustang Q XT140 Capsule, Part Number XT140MSTGQP05

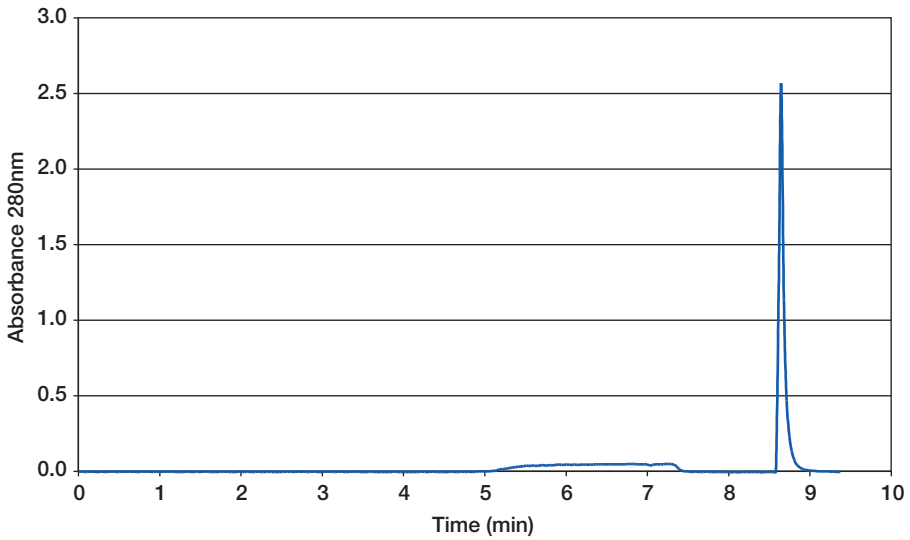


The ratio C/C0 is the ratio of the BSA concentration in the effluent and in the original feed plotted against the mass of BSA loaded onto the capsule Mustang Q membrane. Initial breakthrough occurs after 11.6 g of BSA has been loaded.

In Figure 5, the complete bind/elute breakthrough chromatogram from the same capsule can be seen. Arrows on the chromatogram indicate where the load, wash, and elute steps take place. Initial breakthrough occurs shortly after 5 minutes have elapsed, and full saturation is achieved in less than 8 minutes at a flow rate of 1.5 L/min. Wash and elution takes only 2 minutes. These three steps together take place in less than 10 minutes, and approximately 10 g of BSA were bound and eluted in total.

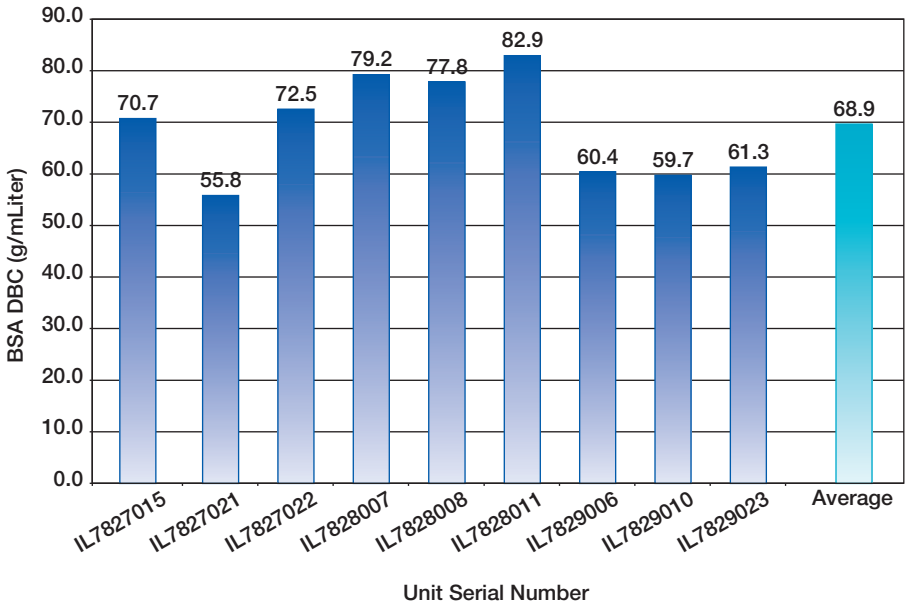
Figure 5

Typical Dynamic Binding and Elution Curve for Mustang Q XT140 Capsule, Part Number XT140MSTGQP05



Dynamic binding capacities for nine Mustang Q XT140 capsules are shown in Figure 6. The average binding capacity is 70 mg of BSA per mL of membrane volume.

Figure 6
BSA Dynamic Binding Capacities at 80% Breakthrough for Mustang Q XT140 Capsules, Part Number XT140MSTGQP05



5.4 Conclusions

Mustang Q XT140 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 XT140 capsule.

Other target molecules will have different binding capacities when run on Mustang Q 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 XT140 capsules using specific process fluids under standard operating conditions.

6. Sanitization and Storage

6.1 Introduction

Users of Mustang Q XT140 capsules may wish to use sodium hydroxide for sanitization purposes. Conditions for sanitization and storage of Mustang Q XT140 capsules have been established that have no effect on extractables, differential pressure, and dynamic binding capacity. To ensure reliable performance, users should follow these procedures exactly as described in this section.

6.2 Summary of Methods

Sanitization with 1 M NaOH

Three liters of 1 M NaOH was prepared, and 5 bed volumes (700 mL) were pumped through the Mustang Q XT140 capsule at a flow rate of 280 mL/min. The pump was stopped, and the capsule was held for 30 minutes.

Preparation for Storage

Three liters of 0.1 M NaOH/1 M NaCl were prepared, and 5 bed volumes (700 mL) were pumped through the Mustang Q XT140 capsule at a flow rate of 280 mL/min. The pump was stopped, and the capsule positioned over a drain. The lines attached to the outlet side and inlet

side of the capsule were disconnected, and the drainage vent on the bottom of the capsule was opened to allow the capsule to drain out completely. The capsule was inverted over the floor drain to allow complete drainage of the upstream side. Plastic blind end caps were fastened to the outlet and inlet sides of the device using a silicone gasket and clamp, and both vent valves were completely closed. The capsule was stored on its side at room temperature.

Determination of Extractables, Pressure Differential, and Dynamic Binding Capacity after Sanitization and Storage

Mustang Q XT140 capsules were sanitized and prepared for storage as described above. At intervals of 3, 6, 9, and 12-month time points, each capsule will be taken out of storage, preconditioned, and reevaluated for extractables, differential pressure, integrity, dispersion, and BSA dynamic binding capacity. Results after storage are then compared with the results that were generated when the capsules were first tested.

6.3 Results

Extractables

At 0, 6, and 12 month time points, the nine capsules that were originally tested as new were taken out of wet storage, conditioned using the protocol in Section 2.2, and were retested for TOC and NVR.

Table 2

Non-volatile Aqueous Extractables and TOC Measurements Obtained Using Typical Mustang Q XT140 Capsules, Part Number XT140MSTGQP05, Under Conditions of Prolonged Wet Storage

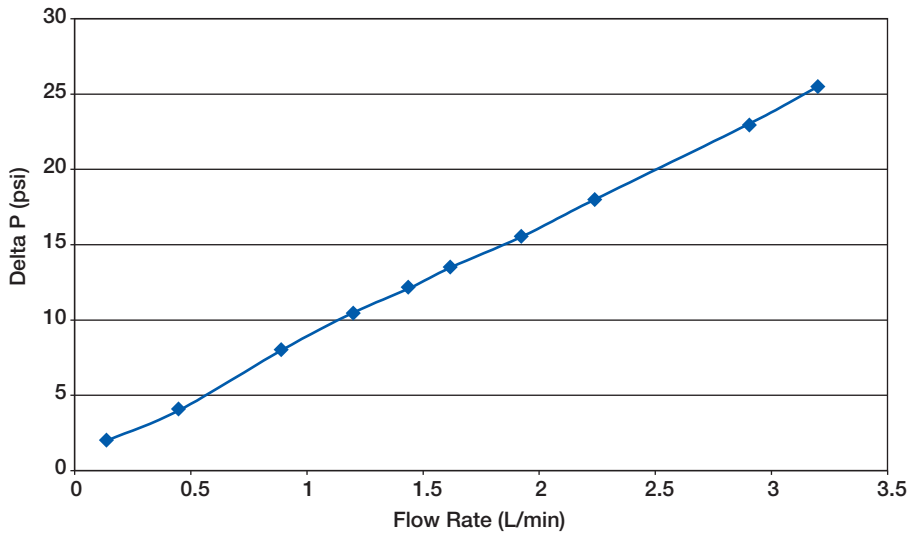
Time Point	TOC After Preconditioning (mg/L of extract)	NVR After Preconditioning (mg/L of extract)
New capsules	2.7	16.2
6 months	1.6	25.0
12 months	TBD	TBD

Flow vs. Pressure Drop

Following the test for extractables, the nine capsules in the validation study that were originally tested as new were then tested for flow vs. pressure drop using the same procedure as described in section 3.2. Figure 7 shows a typical pressure drop for a Mustang Q XT140 capsule after six months in wet storage at room temperature. Although there is a slight increase in pressure drop over time during wet storage, the pressure limits of the membrane and the capsule were never exceeded.

Figure 7

Typical Flow vs. Pressure Drop Curve for a Mustang XT140 Capsule in Wet Storage for Six Months

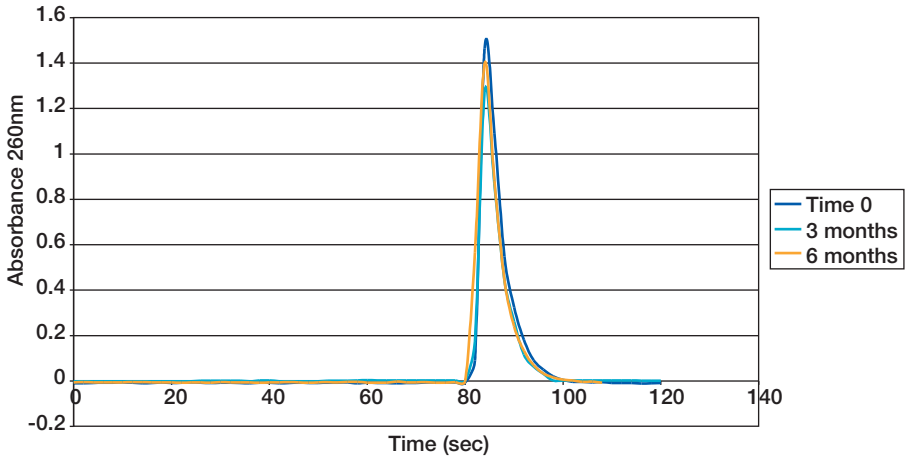


Integrity

Capsules in the wet storage study were tested for integrity using the same protocol as described in section 4.2, and the results were compared to when the capsules were originally tested as new. Figure 8 shows the integrity test curves at first test, and after 3 and 6 months of wet storage. Each curve represents one run from the nine capsules tested at each time point. There is no evidence of integrity loss over the course of six months of wet storage, indicating that the integrity of the membrane and the capsule is not affected by long-term wet storage using the recommended storage solution.

Figure 8

Integrity after Prolonged Wet Storage

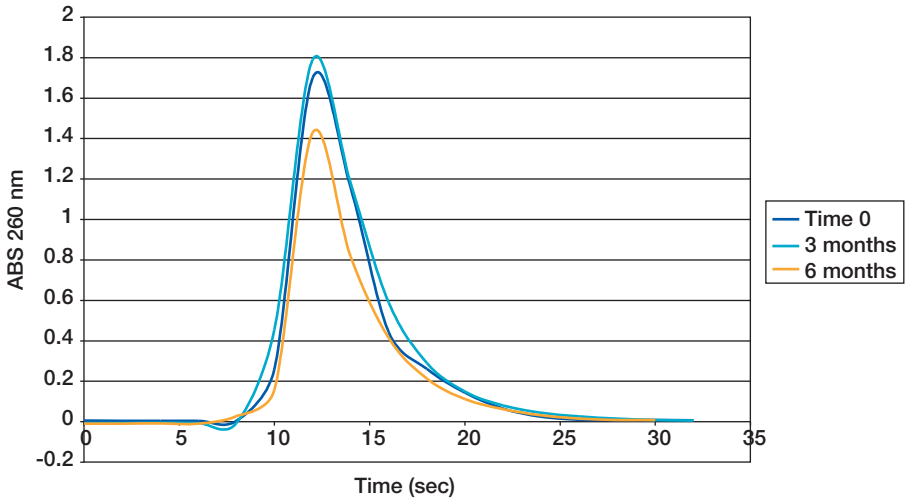


Dispersion

Capsules in the wet storage study were tested for dispersion using the same protocol as described in section 4.2, and the results were compared to when the capsules were originally tested as new. Figure 9 shows the dispersion curves at first test, and after 3 and 6 months of wet storage. Each curve represents one run from the nine capsules tested at each time point. There is no evidence of changes in dispersion over the course of six months of wet storage, indicating that the flow characteristics of the membrane and the capsule are not affected by long-term wet storage using the recommended storage solution.

Figure 9

Dispersion after Prolonged Wet Storage

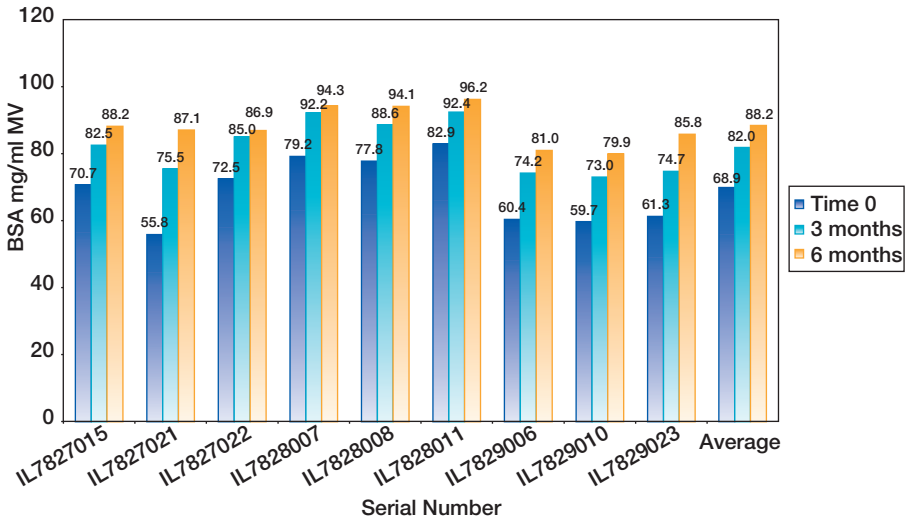


BSA Dynamic Binding Capacity

Capsules in the wet storage study were tested for dynamic binding capacity using the same protocol as described in Section 5.2, and the results were compared to when the capsules were originally tested as new. Figure 10 shows the DBC values for each capsule at first test, and after 3 and 6 months of wet storage. The average for all capsules over the course of the study is also shown. Although there is a slight increase in DBC with prolonged wet storage, it is stable over time and is not decreasing, indicating that the binding capacity of the membrane is not adversely affected by long-term wet storage using the recommended storage solution.

Figure 10

BSA DBC after Prolonged Wet Storage



6.4 Conclusions

Sanitization with 1 M NaOH and long-term storage in 0.1 M NaOH/1M NaCl is shown to have minimal effect on the performance of the Mustang Q XT140 capsule over the course of 6 months. Measures of performance for individual capsules as well as the average of all nine capsules remain within specification after as long as 6 months of storage in the storage solution. End users can, therefore, use Mustang Q XT140 membrane chromatography capsules multiple times with periods of storage in between uses and expect no deterioration in performance associated with prolonged wet storage.

7. Biological Reactivity Tests on the Materials of Construction

7.1 Introduction

The materials of construction for the Mustang Q XT 140 capsules are identical to those in the Mustang Q XT 5000 capsule, and therefore the data can be found in the Mustang Q XT5000 Validation Guide (USTR 2500).

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