



Life Sciences

Validation Guide

USTR 2101

Validation Guide for Pall® Mustang™ Q Disposable Chromatography Units

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1. Overview

1.1 Introduction

Pall **Mustang Q** capsules are designed to capture biomolecules or remove contaminants such as DNA by anion exchange adsorption in downstream bioprocessing applications. **Mustang Q** membranes are constructed from an anion exchange support with pendant quaternary amine groups in a crosslinked polymeric coating that allows a high dynamic DNA binding capacity, especially large molecules such as DNA. **Mustang Q** units contain 16 layers of pleated membrane and are available either as cartridges for use in stainless steel housings or as fully disposable capsules housed within either a polyetherimide or polypropylene shell. Further details about **Mustang Q** capsules can be found in the product data sheets, reference Pall publication USD 2039 and USD 2077.

The purpose of this report is to summarise the tests that were performed to qualify the performance of **Mustang Q** units under standard conditions. This testing program included:

- Determination of BSA and DNA dynamic binding capacities
- Determination of flow characteristics at different applied pressures
- Endurance to autoclave sterilisation
- Endurance to sanitisation using sodium hydroxide
- Extractables testing
- Effects of preconditioning on Total Organic Carbon in flush volumes
- Biological reactivity tests on Mustang Q membrane

1.2 Summary of conclusions

Determination of BSA and DNA dynamic binding capacities

The tests performed demonstrate that **Mustang Q** capsules exhibit extremely high BSA and DNA binding capacities, and are therefore suitable for downstream bioprocessing applications for the capture of biomolecules or removal of contaminants such as DNA.

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** capsules using specific process fluids under standard operating conditions.

Table1: Average BSA and DNA dynamic binding capacities for Mustang Q capsules

Mustang Q part number	Average BSA dynamic binding capacity (mg)	Average DNA dynamic binding capacity (mg)
CLM05MSTGQP	595	266
CL3MSTGQP	4,350	1,871
NP6MSTGQP	15,350	8,700

Determination of flow characteristics

The flow rate of Tris-HCl buffer at different applied pressures was measured using typical **Mustang Q** capsules. The results can be used to assist the user in sizing systems that employ **Mustang Q** capsules when used with process fluids of similar viscosities.

Endurance to autoclave sterilisation

BSA dynamic binding capacity tests have been performed to demonstrate that autoclave sterilisation for 30 minutes at 121°C does not influence the performance of **Mustang Q** capsules.

Table2: Effect of autoclaving on BSA dynamic binding capacity of Mustang Q capsules

Mustang Q part number	Average BSA dynamic binding capacities on non-autoclaved controls	Average BSA dynamic binding capacities on samples autoclaved for 30 minutes at 121°C
CLM05MSTGQP1	595 mg	545 mg
CL3MSTGQP	4,400 mg	4,300 mg
NP6MSTGQP	15,300 mg	13,800 mg

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. Fluids that should not be used include cleaning agents and fluids containing organic solvents such as alcohol.

Compatibility with sodium hydroxide

Soak tests performed with **Mustang Q** capsules in 1M NaOH solution for 30 minutes at 20°C demonstrated that no micro-cracking was observed in the cartridge hardware and the DNA dynamic binding capacity of the membrane was also found to be unaffected.

Extractables testing

The amount of non-volatile residue extracted from preconditioned **Mustang Q** cartridges was found to be extremely low.

Table3: Non-volatile aqueous extractables obtained using typical Mustang Q cartridges, part number AB1MSTGQ7PH4

Treatment prior to extraction	Cartridge serial number	Non-volatile residue
Autoclave and flush with NaOH, NaCl 18 MΩ water	IE4568007	20.3 mg
Flush with NaOH, NaCl and 18 MΩ water	IE4568021	15.2 mg

Actual service will impose different conditions, different exposure times, temperature, liquid purity etc. Evaluation under process conditions is therefore also recommended.

Effects of preconditioning on total organic carbon in flush volumes

Using a typical **Mustang Q** cartridge (part number AB1MSTGQ7PH4), the standard recommended preconditioning procedure was found to reduce the TOC in the flush volume by > 99.6%.

Biological reactivity tests on the Mustang Q membrane

Mustang Q membrane met the requirements of the USP for Class VI (50°C) Plastics (*in vivo*). Prior to performing the biological reactivity tests, the sample had been preconditioned by flushing with 1M NaOH, 1M NaCl and 18 MΩ water.

2. Determination of BSA and DNA dynamic binding capacities

2.1 Introduction

The aim of this series of tests was to determine the BSA and DNA dynamic binding capacities of typical **Mustang Q** capsules.

2.2 Summary of methods

Typical **Mustang Q** capsules from standard production lots were used for the tests (part numbers CLM05MSTGQP1, CL3MSTGQP1 and NP6MSTGQP1). Determinations of the dynamic binding capacities for BSA and DNA were performed as described below.

Determination of BSA dynamic binding capacities

The **Mustang Q** capsules were initially flushed with 25mM Tris-HCl buffer at pH 8.0 at the following flow rates: 250 ± 20 ml/min for the CLM05MSTGQP1 capsule; 1000 ± 100 ml/min for the CL3MSTGQP1 capsule, and 3000 ± 300 ml/min for the NP6MSTGQP1 capsule. The fluid collected on the downstream side was fed to a UV/visible spectrophotometer (Pharmacia Biotech, model Ultrospec 1000). After the flow rates had been adjusted, the UV baseline at 280nm was set to zero.

A solution of bovine serum albumin (BSA) at a concentration of 2 mg/ml in 25mM Tris-HCl buffer, prefiltered through a 0.2µm-rated membrane, was then pumped through the **Mustang Q** capsules at the same flow rate. As soon as the BSA solution began to be pumped through the capsule the UV absorbance at 280nm was continually monitored on the downstream side and the flow-through volume was collected. The flow-through of BSA solution was maintained until a point where a steady increase in the absorbance readings in the fluid collected on the downstream side was observed to occur. The dynamic BSA binding capacity was then calculated as follows:

$$\text{Dynamic BSA binding capacity} = \text{Total flow-through volume} \times \text{BSA concentration in feed solution}$$

A further fraction of the BSA flow-through volume was collected in a separate container until the downstream absorbance readings began to plateau. The feed BSA solution was then exchanged with 25mM Tris-HCl buffer at pH 8.0 until the UV absorbance readings returned to the original baseline reading. The bound BSA fraction on the capsule was subsequently eluted with a solution of 1M NaCl buffer. The absorbance of the eluted fraction was measured at 280nm and the amount of BSA bound was determined from a standard curve.

Determination of DNA dynamic binding capacities

The **Mustang Q** capsules were initially flushed with 25mM Tris-HCl buffer at pH 8.0 at the following flow rates: 250 ± 20 ml/min for the CLM05MSTGQP1 capsule; 1000 ± 100 ml/min for the CL3MSTGQP1 capsule, and 3000 ± 300 ml/min for the NP6MSTGQP1 capsule. The fluid collected on the downstream side was fed to a UV/visible spectrophotometer (Pharmacia Biotech, model Ultrospec 1000). After the flow rates had been adjusted, the UV baseline at 260nm was set to zero and in the case of the CLM05MSTGQP1 capsules only, the buffer was completely drained from the capsule housing.

A solution of herring sperm DNA at a concentration of between 100 - 200 µg/ml in 25mM Tris-HCl buffer, prefiltered through a 0.2µm-rated membrane, was then pumped through the **Mustang Q** capsules at the same flow rate. As soon as the DNA solution began to be pumped through the capsule the UV absorbance at 260nm was continually monitored on the downstream side and the flow-through volume was collected. The flow-through of DNA solution was maintained until a point where a steady increase in the absorbance readings on the fluid collected on the downstream side was observed to occur. The dynamic DNA binding capacity was then calculated as follows:

$$\text{Dynamic binding capacity} = \text{Total flow-through volume} \times \text{DNA concentration in feed solution}$$

A further fraction of the DNA flow-through volume was collected in a separate container until the downstream absorbance readings began to plateau. The feed DNA solution was then exchanged with 25mM Tris-HCl buffer at pH 8.0 until the UV absorbance readings returned to the original baseline reading. The bound DNA fraction on the capsule was subsequently eluted in 1M NaCl in 0.1N NaOH elution buffer. The sample of eluted DNA was adjusted to pH 7 with dilute HCl and the absorbance at 260nm was measured in the eluted sample in order to determine the amount of DNA bound from a standard curve.

2.3 Results

The results of the BSA and DNA dynamic binding capacities for **Mustang Q** capsules (part numbers CLM05MSTGQP1, CL3MSTGQP1 and NP6MSTGQP1) are shown in Figures 1 to 6. Figure 7 shows a set of typical DNA breakthrough curves for **Mustang Q** capsules. These curves demonstrate the very sharp breakthrough points typically exhibited by **Mustang Q** capsules.

A summary of the averaged BSA and DNA dynamic binding capacities (from Figures 1 to 6) is shown in Table 4.

Table 4. Average BSA and DNA dynamic binding capacities for Mustang Q capsules

Mustang Q part number	Average BSA dynamic binding capacity (mg)	Average DNA dynamic binding capacity (mg)
CLM05MSTGQP1	595	266
CL3MSTGQP1	4,350	1,871
NP6MSTGQP1	15,350	8,700

Fig 1. BSA dynamic binding capacities for Mustang Q capsules, part number CLM05MSTGQP1

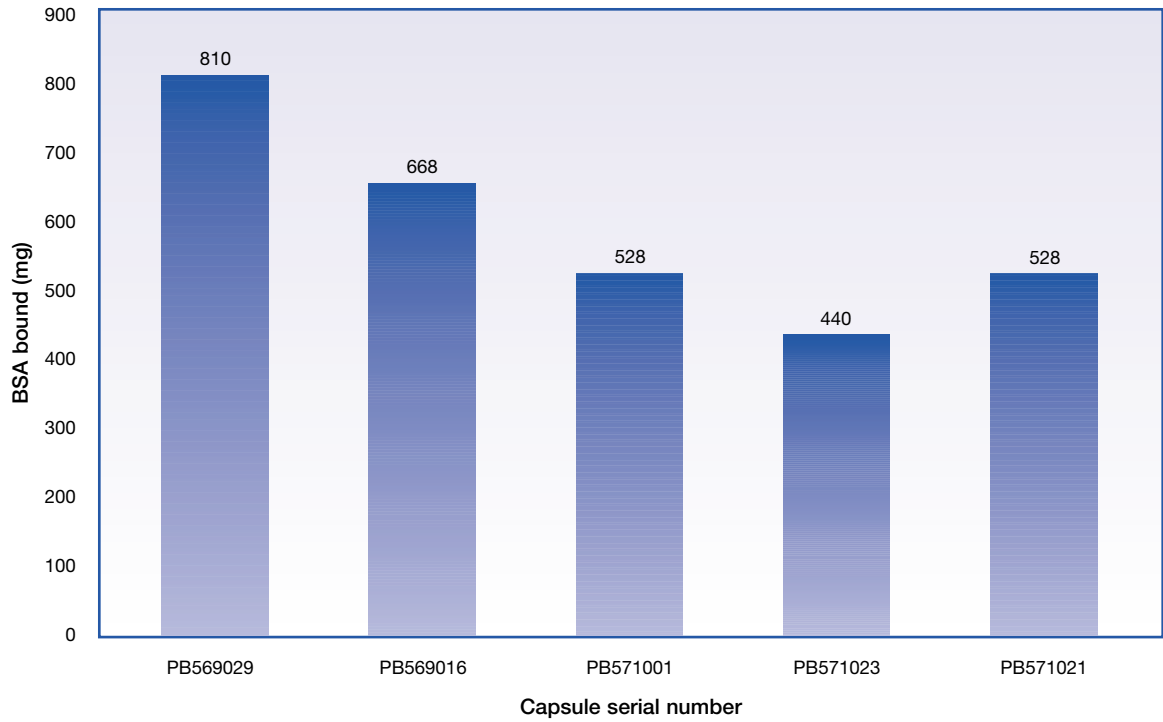


Fig 2. BSA dynamic binding capacities for Mustang Q capsules, part number CL3MSTGQP1

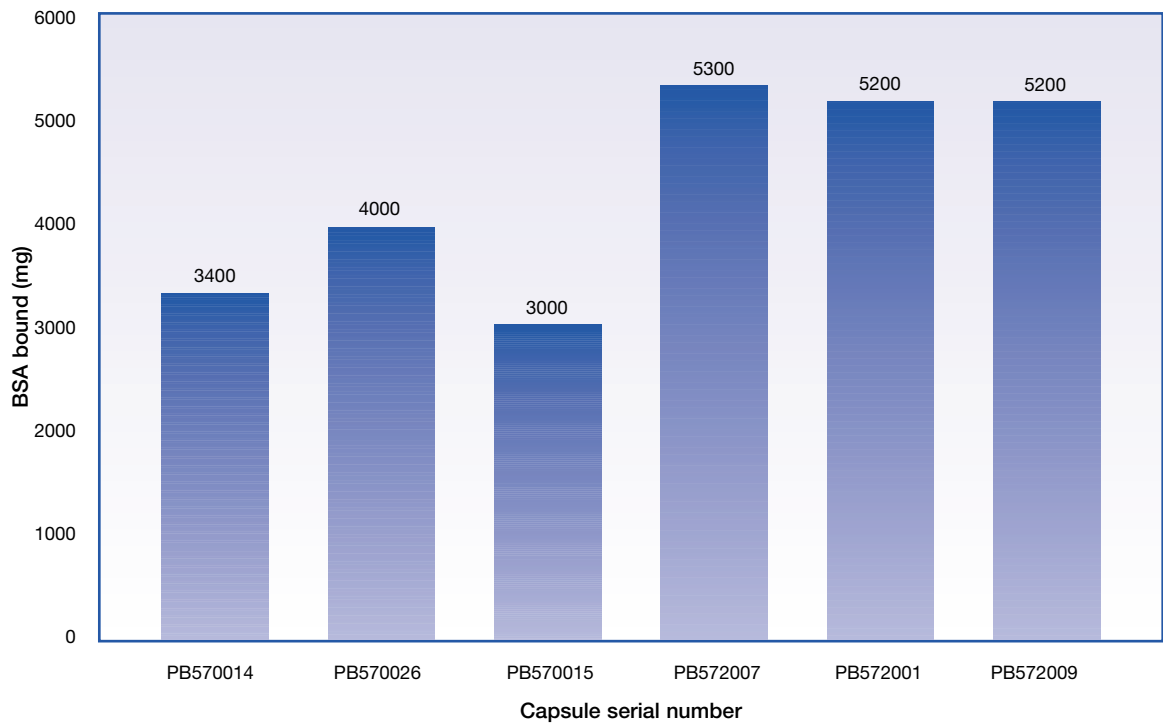


Fig 3. BSA dynamic binding capacities for Mustang Q capsules, part number NP6MSTGQP1

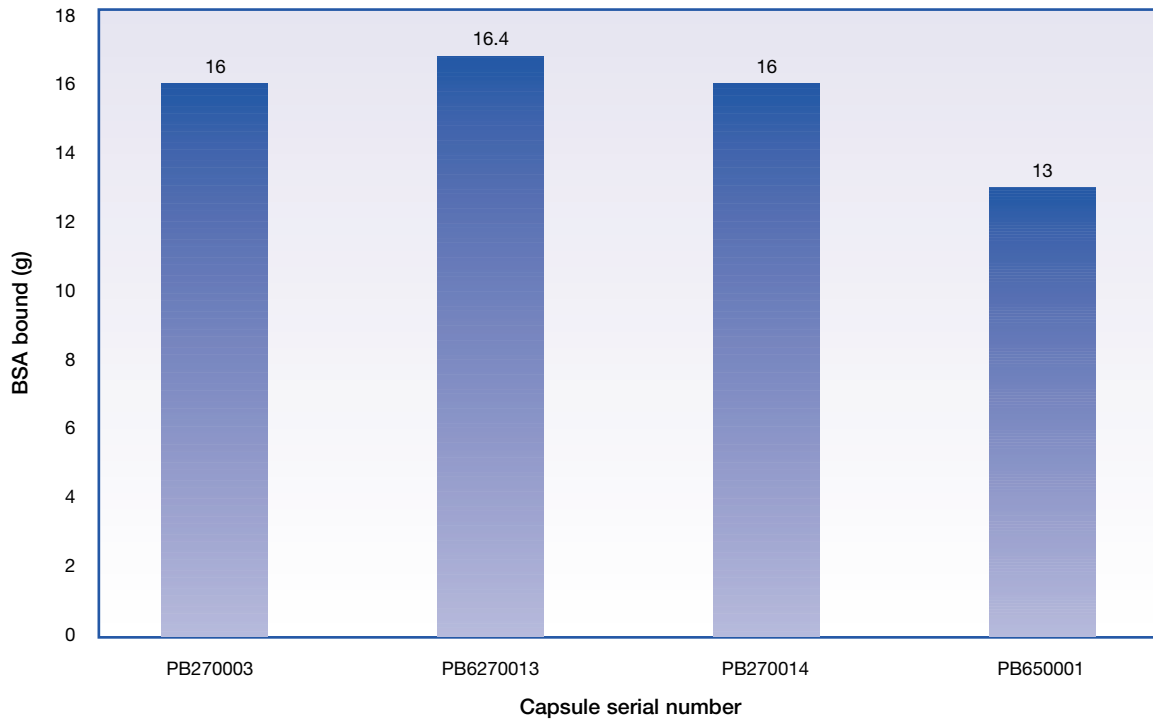


Fig 4. DNA dynamic binding capacities for Mustang Q capsules, part number CLM05MSTGQP1

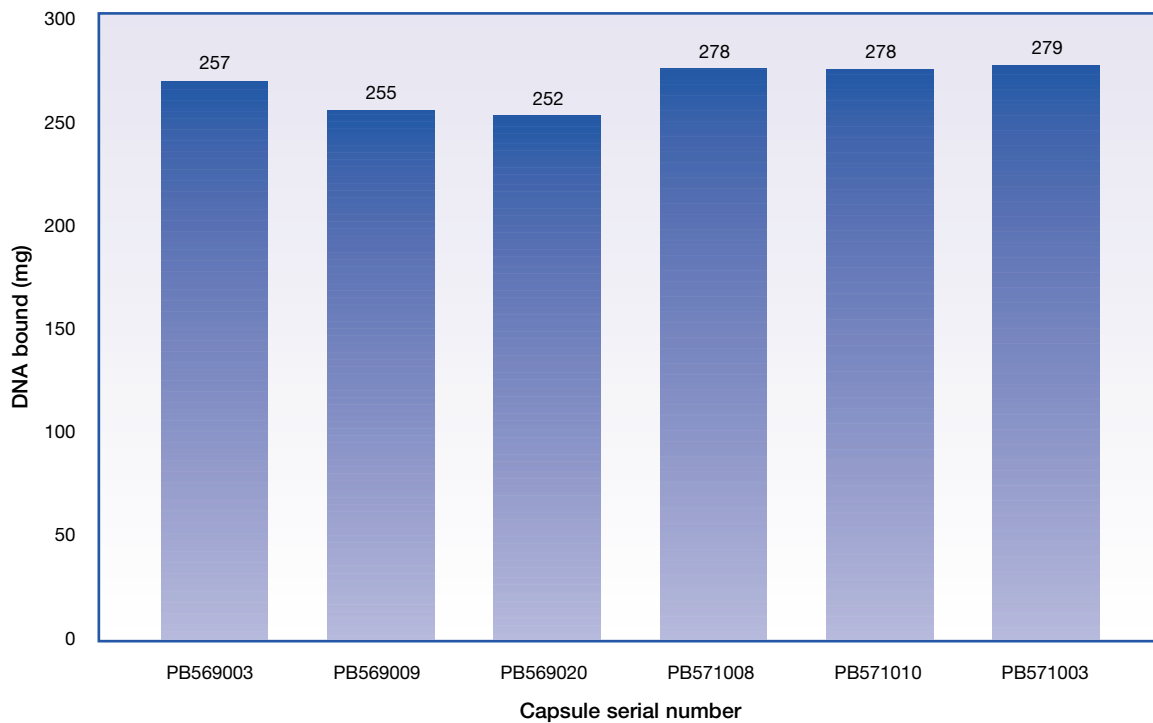


Fig 5. DNA dynamic binding capacities for Mustang Q capsules, part number CL3MSTGQP1

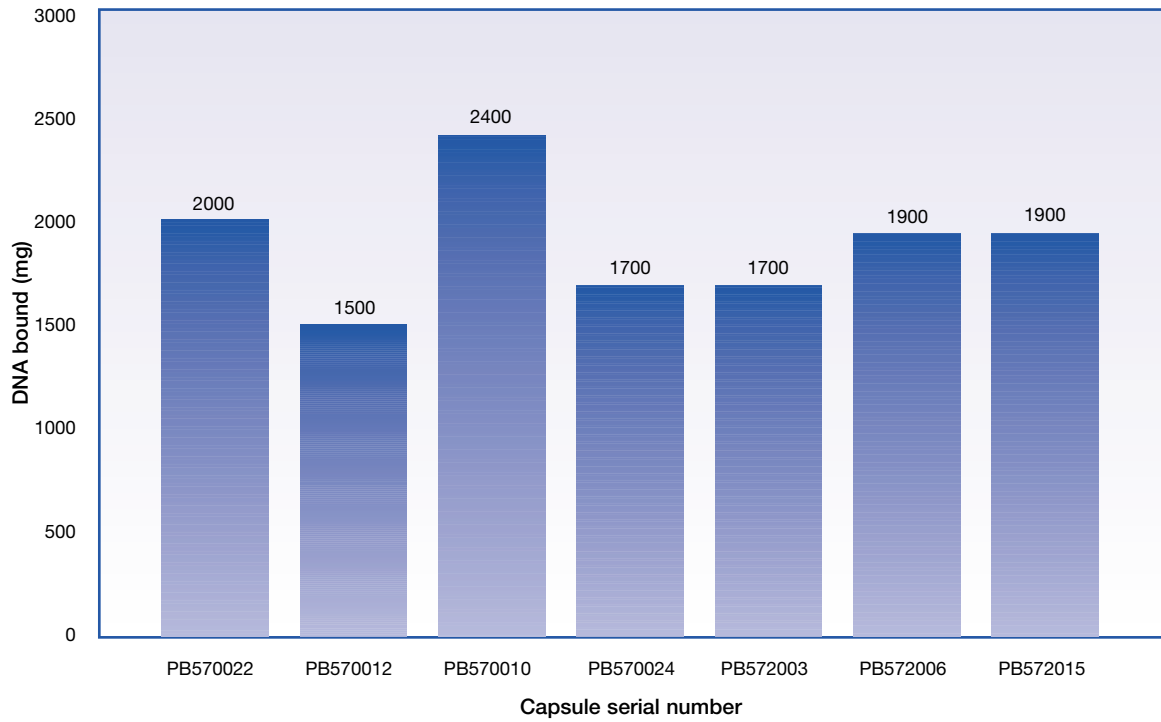


Fig 6. DNA dynamic binding capacities for Mustang Q capsules, part number NP6MSTGQP1

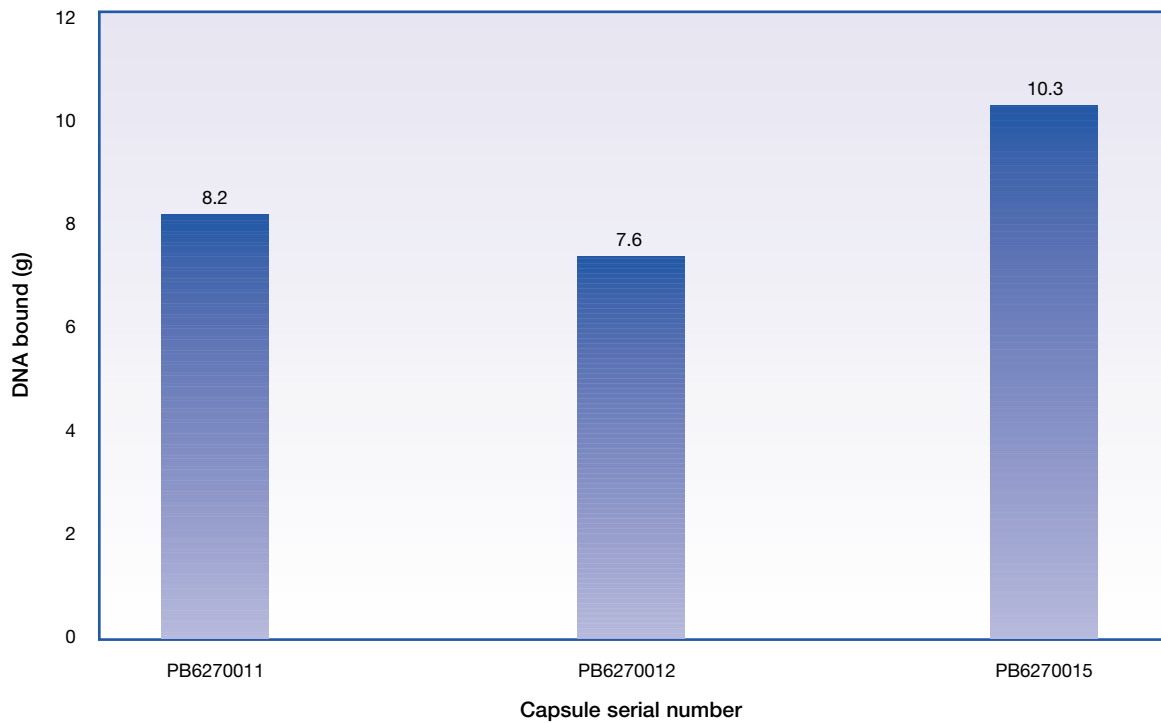
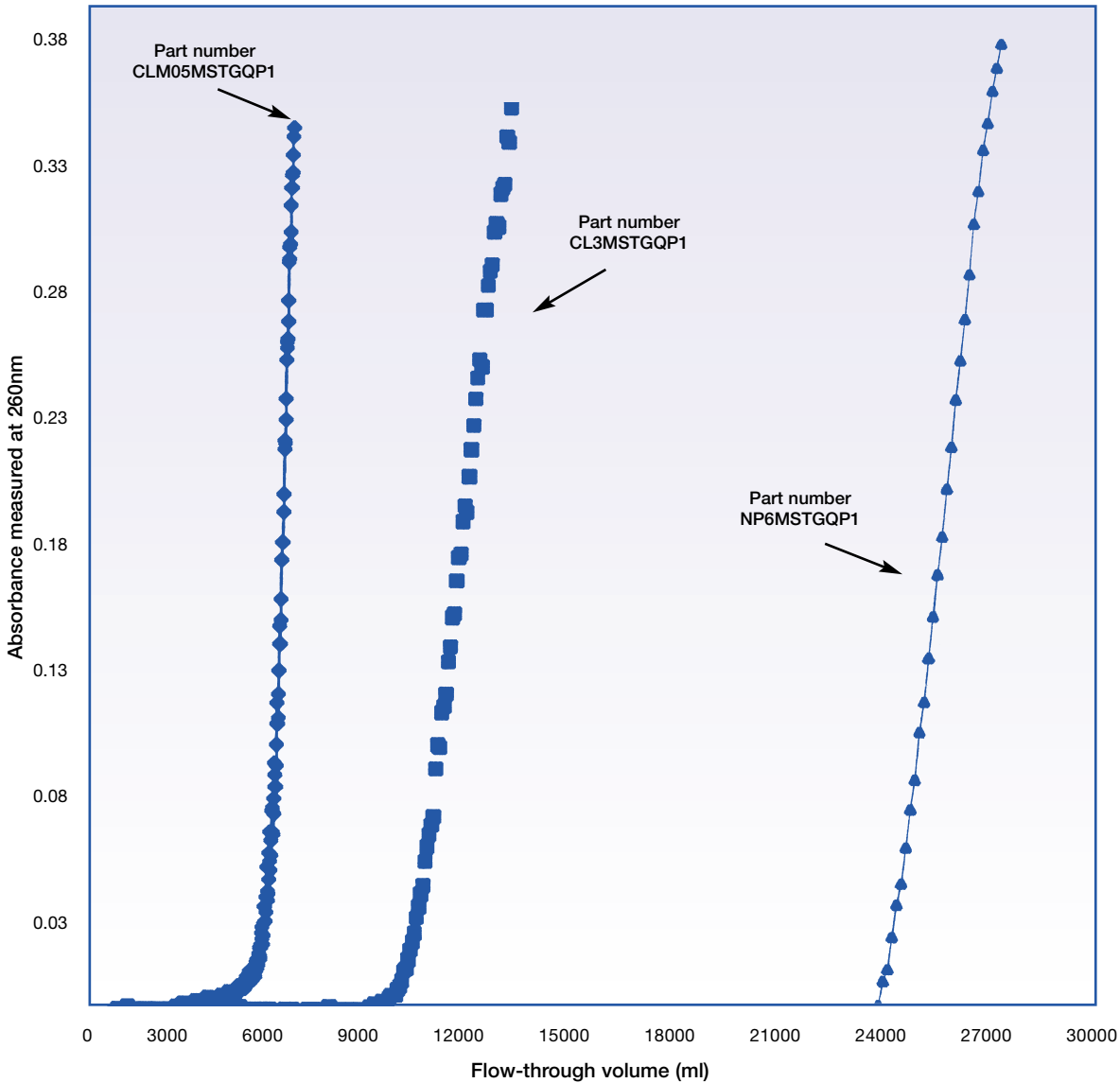


Fig 7. Typical DNA breakthrough curves for Mustang Q capsules



2.4 Conclusions

The studies reported here demonstrate that **Mustang Q** capsules exhibit extremely high BSA and DNA dynamic binding capacities and are therefore suitable for downstream bioprocessing applications for the capture of biomolecules or the removal of contaminants such as DNA.

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** capsules using specific process fluids under standard operating conditions by the user.

3. Determination of flow characteristics

3.1 Introduction

The aim of this series of tests was to determine the flow characteristics of typical **Mustang Q** capsules at different applied upstream pressures using an aqueous test fluid.

3.2 Summary of methods

Typical **Mustang Q** capsules from production were used for the tests (part numbers CLM05MSTGQP1, CL3MSTGQP1 and NP6MSTGQP1). The test fluid used was 25mM Tris-HCl buffer at pH 8, temperature 20°C. The fluid was pumped through the test capsules at set upstream pressures. Throughout each test the capsule outlets were maintained at atmospheric pressure. At each set pressure the flow rate on the downstream side of the capsule was measured over a one-minute interval.

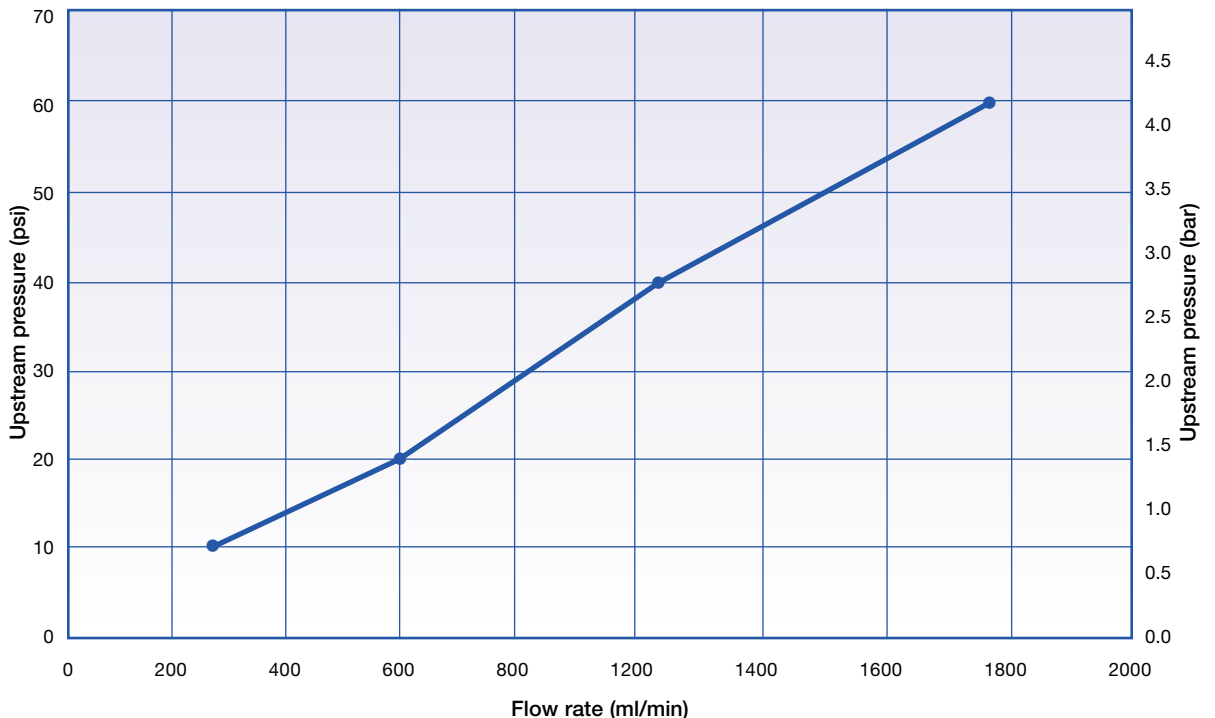
3.3 Results

Graphs of flow of Tris-HCl buffer versus applied upstream pressure are shown in Figures 8 to 10. Each of the points plotted on the graphs represent an average value obtained from testing a minimum of five different **Mustang Q** capsules.

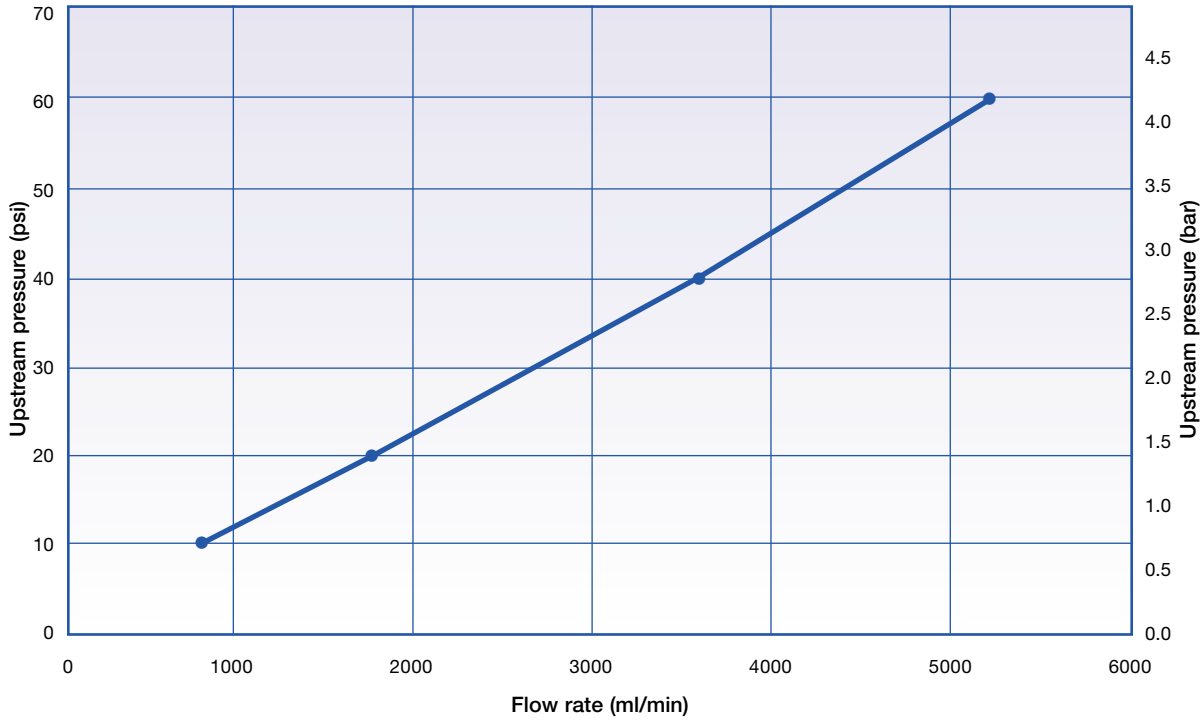
3.4 Conclusions

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

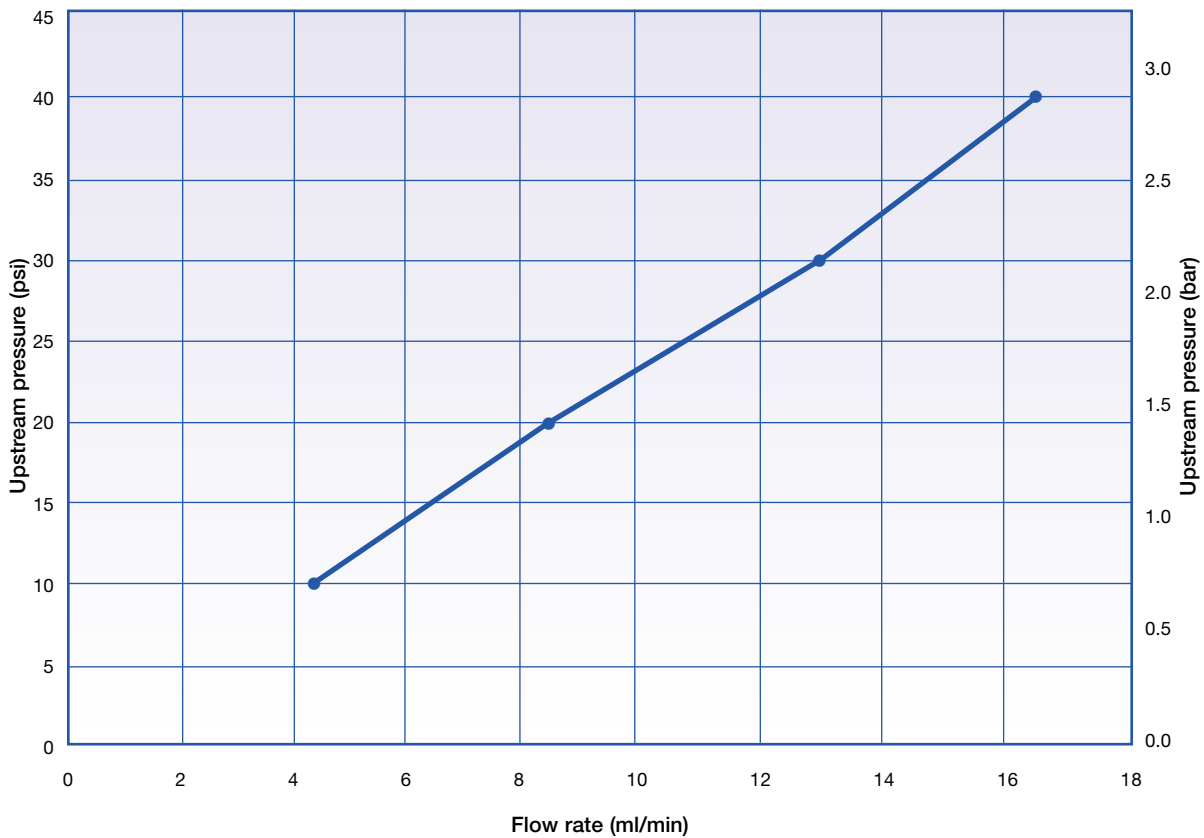
Fig 8. Flow versus differential pressure for Mustang Q capsules, part number CLM05MSTGQP1



**Fig 9. Flow versus differential pressure for Mustang Q capsules,
part number CL3MSTGQP1**



**Fig 10. Flow versus differential pressure for Mustang Q capsules,
part number NP6MSTGQP1**



4. Endurance to autoclave sterilisation

4.1 Introduction

The purpose of these tests was to demonstrate that a 30-minute autoclave cycle at 121°C would not influence the performance of **Mustang Q** capsules, as determined using a BSA dynamic binding capacity test.

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. Fluids that should not be used include cleaning agents and fluids containing organic solvents such as alcohol.

4.2 Summary of methods

Typical **Mustang Q** capsules from production were used for these tests (part numbers CLM05MSTGQP1, CL3MSTGQP1 and NP6MSTGQP1). Samples were removed from their packaging, the inlet and outlet connections loosely wrapped, and then the capsules were autoclaved at 121°C for 30 minutes. Following autoclave, the cooled capsules were tested for BSA dynamic binding capacity according to the procedure described previously in 2.2. Non-autoclaved samples were also tested as a control.

4.3 Results

The results are shown in Table 5. It was found that the BSA bound on autoclaved and non-autoclaved samples was very similar, indicating that a 30 minute autoclave cycle at 121°C has very little effect on the BSA dynamic binding capacities of **Mustang Q** capsules.

Table 5. Effect of autoclaving on BSA dynamic binding capacity of Mustang Q capsules

Mustang Q part number	Average BSA dynamic binding capacities on non-autoclaved controls	Average BSA dynamic binding capacities on samples autoclaved for 30 minutes at 121°C
CLM05MSTGQP1	595 mg	545 mg
CL3MSTGQP1	4,400 mg	4,300 mg
NP6MSTGQP1	15,300 mg	13,800 mg

4.4 Conclusions

Mustang Q capsules can be autoclaved at 121°C for 30 minutes without the performance of the capsule being influenced, as demonstrated using a BSA dynamic binding capacity test.

5. Compatibility with sodium hydroxide

5.1 Introduction

Users of **Mustang Q** capsules may wish to use sodium hydroxide for sanitisation purposes or for preconditioning the units using the protocol recommended by Pall. The aim of these tests was to determine if **Mustang Q** capsules are compatible with 1M NaOH for 30 minutes at 20°C. There were two aspects to this testing as follows:

- Test to determine if 1M NaOH caused any visible damage to the cartridge hardware
- Test to determine if exposure to 1M NaOH influenced the dynamic DNA binding capacity of the **Mustang Q** membrane

5.2 Summary of methods

Examination of cartridge hardware following exposure to 1M NaOH

The Pall standard membrane cartridge component normally used within **Mustang Q** capsules (part number CL3MSTGQP1) were used for these tests without the inclusion of the polyetherimide housing.

Typical samples from production were used for the tests. The sample cartridges were soaked in 1M NaOH at 20°C. At intervals during the soak test, the cartridges were removed from the NaOH solution and flushed with water prior to being carefully examined for any appearance of stress cracking within the cartridge hardware.

Determination of dynamic DNA binding capacity before and after exposure to 1M NaOH

Samples of **Mustang Q** membrane discs (18 mm diameter) were used for these tests (part number MSTG18Q16). The DNA dynamic binding capacity was determined on samples before and after exposure to 1M NaOH solution for 30 minutes at 20°C. The method used was a scaled down version of that described previously in section 2.2.

5.3 Results

The results of the physical checks for micro-cracking following exposure to 1M NaOH are shown in Table 6. Out of the six **Mustang Q** cartridge samples tested no evidence of micro-cracking was observed after exposure to 1M NaOH for 30 minutes at 20°C.

The results of the dynamic DNA binding capacity tests shown in Table 7 demonstrate that exposure to 1M NaOH for 30 minutes at 20°C had no significant effect on membrane performance.

Table 6. Results of checks for micro-cracking performed on Mustang Q cartridges after soaking in 1M NaOH

Pall capsule reference number	Results of visual inspection for micro-cracking		
	Initial observations	After 15 minutes exposure to 1M NaOH at 20°C	After 30 minutes exposure to 1M NaOH at 20°C
PB570 F	No micro-cracking	No micro-cracking	No micro-cracking
PB570 I	No micro-cracking	No micro-cracking	No micro-cracking
PB570 J	No micro-cracking	No micro-cracking	No micro-cracking
PB572 B	No micro-cracking	No micro-cracking	No micro-cracking
PB572 E	No micro-cracking	No micro-cracking	No micro-cracking
PB572 I	No micro-cracking	No micro-cracking	No micro-cracking

Table 7. Results of DNA dynamic binding capacity tests on Mustang Q membrane before and after exposure to 1M NaOH solution at 20°C

Membrane lot number	DNA dynamic binding before exposure to 1M NaOH	DNA dynamic binding capacity following exposure to 1M NaOH for 30 minutes at 20°C
08683	33.0 mg/ml of membrane	31.0 mg/ml of membrane
99180	29.6 mg/ml of membrane	29.5 mg/ml of membrane

5.4 Conclusions

Soak tests performed with Mustang Q capsules in 1M NaOH solution for 30 minutes at 20°C demonstrated that no micro-cracking was observed in the cartridge hardware and the DNA dynamic binding capacity of the membrane was also found to be unaffected.

6. Extractables testing

6.1 Introduction

The purpose of this series of tests was to quantify and analyse the amount of material that can be extracted from **Mustang Q** units by water at ambient temperature ($20 \pm 5^\circ\text{C}$).

6.2 Summary of methods

Typical 25cm **Mustang Q** cartridges were used for the extraction tests, part number AB1MSTGQ7PH4. Prior to the extraction the cartridges were preconditioned by flushing, at a flow rate of 1500 ml/min, with:

- 1M NaOH (minimum 3000 ml)
- 1M NaCl (minimum 3000 ml)
- 18 M Ω water (until the downstream pH and conductivity measurements were the same as the upstream measurements)

Following preconditioning, the extraction procedure was performed by recirculating 1500 ml of 18 M Ω water through the cartridge for two hours at a flow rate of 400 ml/min. After the extraction period the sample was concentrated to approximately 100 ml using a rotoevaporator. A volume of the sample was then evaporated to dryness and the non-volatile residue (NVR) was determined gravimetrically. The results were corrected to express the NVR for the entire extraction volume used.

A sample of the NVR was analysed by Fourier Transform Infra Red Spectroscopy (FTIR).

The above procedure was performed on both an autoclaved and non-autoclaved **Mustang Q** cartridge.

6.3 Results

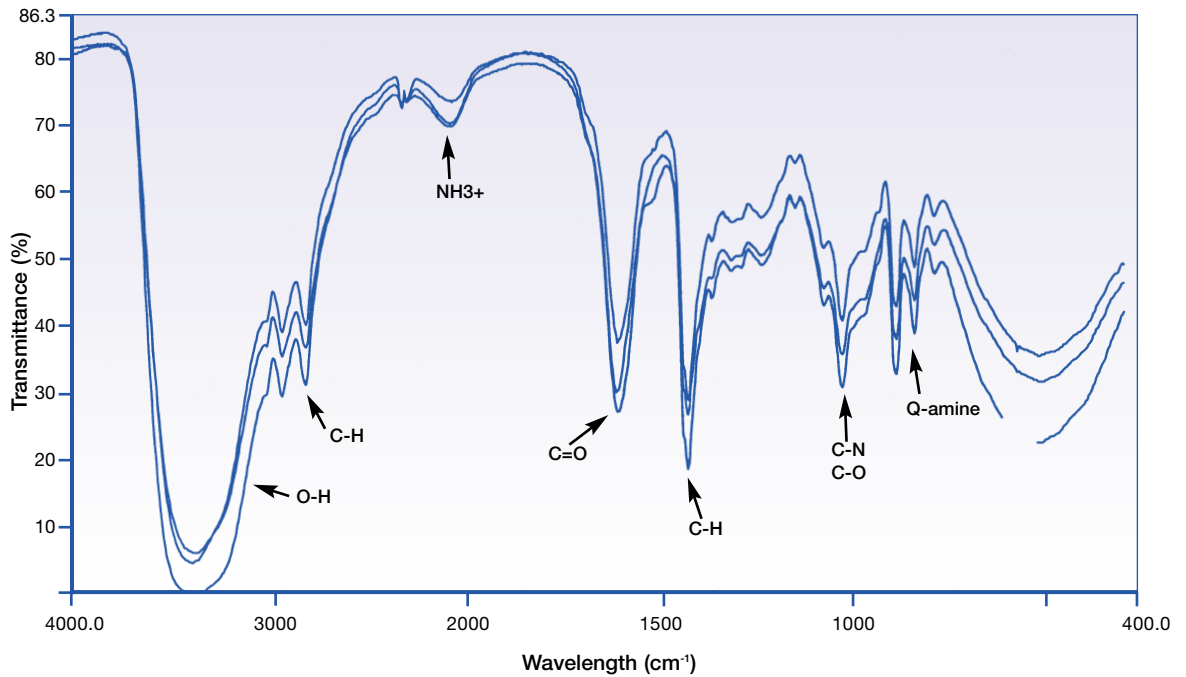
The amount of extractables obtained from typical **Mustang Q** cartridges are shown in Table 8.

An infra red spectrum obtained from a sample of the aqueous extractables obtained from the non-autoclaved sample is shown in Figure 11. Analysis of the infra red spectrum indicates the presence of residual materials from the membrane grafting process, for example amine, hydroxyl and hydrocarbon groups.

Table 8. Non-volatile aqueous extractables obtained using typical Mustang Q cartridges, part number AB1MSTGQ7PH4

Treatment prior to extraction	Cartridge serial number	Non-volatile residue
Autoclave and flush with 5 litres NaOH, 5 litres NaCl and 20 litres 18 M Ω water	IE4568007	20.3 mg
Flush with 5.7 litres NaOH, 5 litres NaCl and 8.5 litres 18 M Ω water	IE4568021	15.2 mg

Figure 11. Infra red spectrum of an aqueous extract from a Mustang Q cartridge, part number AB1MSTGQ7PH4



6.4 Conclusions

The levels of aqueous extractables determined for preconditioned Mustang Q cartridges were found to be extremely low. The levels measured for typical cartridges, part number AB1MSTGQ7PH4, were 20.3 mg for the autoclaved sample and 15.2 for the non-autoclaved sample.

Actual service will impose different conditions, such as different exposure times, temperature, liquid purity etc. Evaluation under process conditions is therefore also recommended.

7. Effects of preconditioning on total organic carbon in flush volumes

7.1 Introduction

The aim of this test was to determine the effects of the recommended preconditioning procedure on the amount of Total Organic Carbon (TOC) in the flush water using a typical Mustang Q cartridge.

7.2 Summary of methods

A typical **Mustang Q** cartridge (part number AB1MSTGQ7PH4) was subjected to the standard Pall recommended preconditioning procedure as follows:

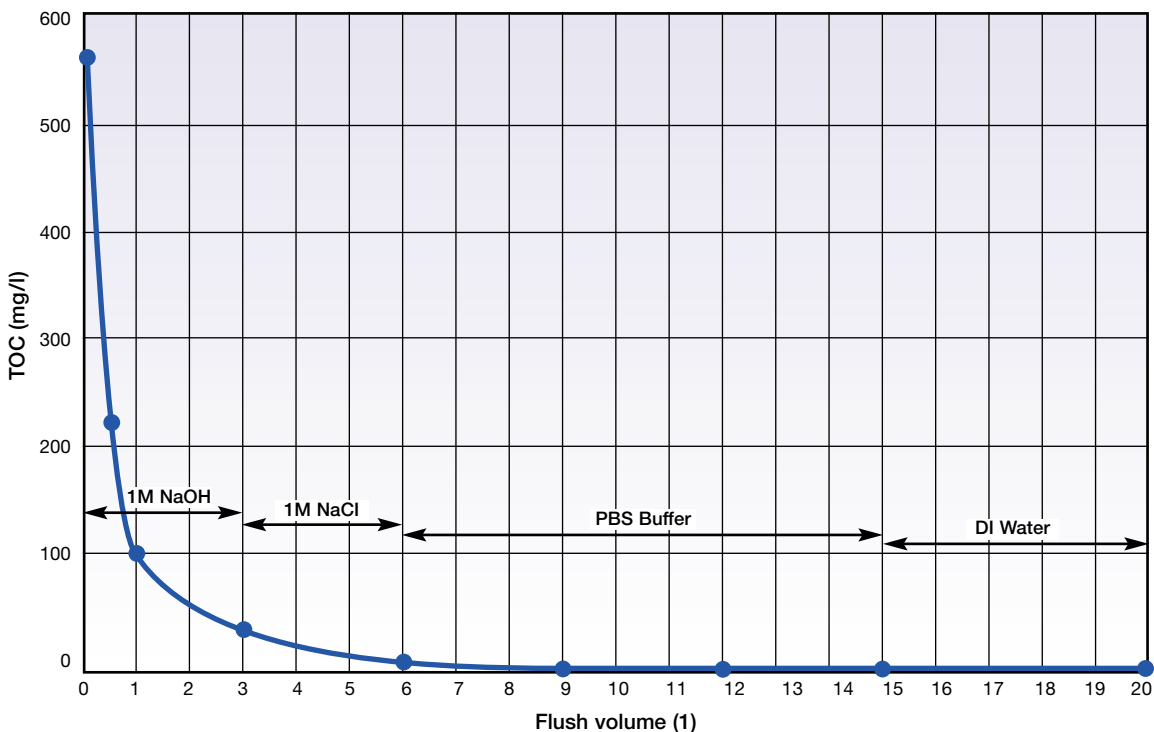
- Flush with 3 litres 1M NaOH
- Flush with 3 litres 1M NaCl
- Flush with 9 litres PBS buffer

The **Mustang Q** module was then flushed with DI water. At intervals during the preconditioning and water flushes, downstream samples were taken and analysed for TOC.

7.3 Results

The levels of TOC determined downstream of a typical **Mustang Q** cartridge during standard preconditioning flushes are shown in Figure 12. Following the flush with PBS buffer the TOC in the downstream sample had reduced to < 0.4% of the value at the start of the preconditioning step.

Figure 12. Effects of preconditioning on TOC in flush volumes from Mustang Q cartridges, part number AB1MSTGQ7PH4



7.4 Conclusions

Using a typical **Mustang Q** cartridge (part number AB1MSTGQ7PH4), the standard recommended preconditioning procedure was found to reduce the TOC in the flush volume by > 99.6%.

8. Biological reactivity tests on the Mustang Q membrane

8.1 Introduction

The purpose of this study was to evaluate the biological suitability of the membrane used in Mustang Q units.

8.2 Summary of methods

A typical Mustang Q capsule was used for the tests, part number NP6MSTGQP1. The capsule was initially pre-conditioned by flushing at 1500 ml/min with:

- 1M NaOH (3 litres)
- 1M NaCl (3 litres)
- 0.85% NaCl in water (10 litres)

The Mustang Q membrane was then cut from the capsule and the Biological Reactivity Tests were performed on the preconditioned membrane.

The tests were performed in accordance with the Biological Reactivity Tests *in vivo* for Class VI Plastics (50°C) as described in the current *United States Pharmacopeia*. The tests were conducted by Toxikon Corporation, Bedford, USA.

The testing procedures described in the USP include:

- Injection of extracts of the test article
- Implantation of the test article into animal tissue.

The four extracting media listed in the USP simulate parenteral solutions and body fluids. These include:

- Sodium Chloride for Injection
- 1 in 20 Solution of Alcohol in Sodium Chloride Injection
- Polyethylene Glycol 400
- Vegetable Oil (sesame or cottonseed oil).

The USP states that extracts may be prepared at one of three standard conditions: 50°C for 72 hours, 70°C for 24 hours, or 121°C for 1 hour. The Mustang Q membrane was tested at 50°C for 72 hours.

Acute Systemic Injection Tests

An Acute Systemic Injection Test was performed to evaluate the potential of a single injection of an extract to produce systemic toxicity. Sodium Chloride Injection and 1 in 20 Solution of Alcohol in Sodium Chloride Injection were injected intravenously. Vegetable oil extract and Polyethylene Glycol 400 extract were injected intraperitoneally.

Intracutaneous Tests

An Intracutaneous Test was performed to evaluate the potential of a single injection of an extract to produce tissue irritation. All four of the extracts listed above were used for these tests.

Implantation Tests

Implantation tests were also performed, in order to subject the **Mustang Q** membrane to the most stringent conditions included in the USP.

8.3 Results

No biological response was observed in any of the tests performed and therefore the preconditioned **Mustang Q** membrane passed all of the tests specified.

8.4 Conclusions

Mustang Q membrane met the requirements of the USP for Class VI (50°C) Plastics (*in vivo*). Prior to performing the biological reactivity tests, the sample had been preconditioned by flushing with 1N NaOH, 1M NaCl and 18 M Ω water.



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