Life Sciences

Validation Guide

USTR 2196

Validation Guide for 'Pall' Disposable Mustang® S Cation Exchange Chromatography Units

*Filtration. Separation. Solution.*SM

CONTENTS

11. Overview

1.1 Introduction

Pall Mustang S capsules and cartridges are designed to capture positively charged biomolecules by cation exchange in downstream bioprocessing applications. **Mustang** S products are typically used for the initial capture of target biomolecules in a purification process or for the removal of positively charged contaminants during product purification.

Mustang S units 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** S capsules can be found in the product data sheets.

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

- **Determination of lysozyme dynamic binding capacities**
- **Determination of flow characteristics**
- **Endurance to autoclave sterilization**
- **Compatibility with sodium hydroxide**
- **Extractables testing**
- **Effects of preconditioning on total organic carbon in flush volumes**
- **Biological reactivity tests on Mustang S membrane**

1.2 Summary of conclusions

Determination of lysozyme dynamic binding capacities

Dynamic binding capacity tests have been performed with **Mustang** S units using lysozyme. Lysozyme was used for the tests as it has a high isoelectric point and is therefore a good model for proteins that bind to cation exchangers.

The tests performed demonstrate that **Mustang** S capsules exhibit extremely high lysozyme binding capacities, and are therefore suitable for downstream bioprocessing applications for the capture of biomolecules by cation exchange adsorption.

Different flow rates and the presence of other contaminants may influence the performance, and it is therefore recommended that the user evaluate **Mustang** S units using specific process fluids under standard operating conditions.

Average lysozyme dynamic binding capacities for Mustang S capsules

Pall Mustang S part number (number of samples tested)	Average lysozyme dynamic binding capacity (standard deviation)
CLM05MSTGSP1 (n = 7)	685 mg (14 mg)
CL3MSTGSP1 (n = 10)	4608 mg (674 mg)
NP6MSTGSP1 (n = 4)	14225 mg (2084 mg)

Determination of flow characteristics

The flow rate of 10mM MES buffer at different applied pressures was measured using typical **Mustang** S capsules. The results can be used to assist the user in sizing systems that employ **Mustang** S capsules when used with process fluids of similar viscosities.

Endurance to autoclave sterilization

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

Effect of autoclaving on lysozyme dynamic binding capacities for Mustang S capsules

Mustang S part number	Autoclave conditions	Average dynamic lysozyme binding capacity
CLM05MSTGSP1	Autoclaved 121°C for 30 minutes	821 mg
	No autoclave cycle	685 mg
CL3MSTGSP1	Autoclaved 121°C for 30 minutes	4900 mg
	No autoclave cycle	4608 mg

Compatibility with sodium hydroxide

Dynamic lysozyme binding capacity tests have been performed to demonstrate that **Mustang** S capsules can withstand exposure to 1N NaOH at 20°C for one hour.

Extractables testing

The levels of aqueous extractables determined for preconditioned autoclaved and non-autoclaved **Mustang** S capsules have been determined. Actual service will impose different conditions, such as 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** S cartridge (part number AB1MSTGS7PH4), the standard recommended preconditioning procedure was found to reduce the TOC in the flush volume by > 99%.

USP Biological reactivity tests on the Mustang S membrane

Mustang S membrane met the requirements of the USP Biological Reactivity Tests *(in vivo)* for Class VI-50°C Plastics. The tests included the systemic injection test, the intracutaneous test and the implantation test. Prior to performing the biological reactivity tests, the sample had been preconditioned by flushing with 1N NaOH, 1N NaCl and 18 MΩ water.

2. Determination of lysozyme dynamic binding capacities

2.1 Introduction

The aim of this series of tests was to determine the lysozyme dynamic binding capacities of typical **Mustang** S capsules. Lysozyme was used for the tests as it has a high isoelectric point and is therefore a good model for proteins that bind to cation exchangers.

2.2 Summary of methods

Typical **Mustang** S capsules from standard production lots were used for the tests (part numbers CLM05MSTGSP1, CL3MSTGSP1 and NP6MSTGSP1). Prior to performing the dynamic binding capacity tests the capsules were preconditioned according to the standard Pall recommended procedure shown in Table 2-1.

Table 2-1. Recommended preconditioning flush volumes for Mustang S capsules

Pall part number	Flow rate	Flush volumes for 1N NaOH	Flush volumes for 1N NaCl
CLM05MSTGSP1	200 ml/min	> 600 ml	> 600 ml
CL3MSTGSP1	300 ml/min	> 600 ml	> 600 ml
NP6MSTGSP1	1500 ml/min	> 3000 ml	> 3000 ml

Following preconditioning, the **Mustang** S capsules were flushed with 10mM MES buffer (pH 5.5) at the following flow rates: 250 ± 20 ml/min for the CLM05MSTGSP1 capsule; 1000 ± 100 ml/min for the CL3MSTGSP1 capsule, and 3000 ± 300 ml/min for the NP6MSTGSP1 capsule. The fluid collected on the downstream side was fed to a UV/visible spectrophotometer (Optek). After the flow rates had been adjusted, the UV baseline at 280nm was set to zero.

A solution of lysozyme at a concentration of 2 mg/ml in 10mM MES (pH 5.5), prefiltered through a 0.2µm-rated membrane, was then pumped through the **Mustang** S capsules at the flow rates used above. As soon as the lysozyme solution began to be pumped through the capsule the UV absorbence at 280nm was continually monitored on the downstream side and the flow-through volume was collected. The flow-through of lysozyme solution was maintained until a point where a steady increase in the absorbence readings in the fluid collected on the downstream side was observed to occur. The dynamic lysozyme binding capacity was then calculated as follows:

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

A further fraction of the lysozyme flow-through volume was collected in a separate container until the downstream absorbency readings began to plateau. The feed lysozyme solution was then exchanged with 10mM MES buffer at pH5.5 until the UV absorbency readings returned to the original baseline reading. The bound lysozyme fraction on the capsule was subsequently eluted with a solution of 1M NaCl in 10mM MES buffer at pH5.5. The absorbency of the eluted fraction was measured at 280nm and the amount of lysozyme bound was determined from a standard curve.

2.3 Results

The results of the lysozyme dynamic binding capacities for **Mustang** S capsules (part numbers CLM05MSTGSP1, CL3MSTGSP1 and NP6MSTGSP1) are shown in Figures 2-1 to 2-3. A summary of the average lysozyme dynamic binding capacities (from Figures 2-1 to 2-3) is shown in Table 2-2.

Table 2-2. Average lysozyme dynamic binding capacities for Mustang S capsules

Pall Mustang S part number (number of samples tested)	Average lysozyme dynamic binding capacity (standard deviation)
CLM05MSTGSP1 (n = 7)	685 mg (14 mg)
CL3MSTGSP1 (n = 10)	4608 mg (674 mg)
NP6MSTGSP1 (n = 4)	14225 mg (2084 mg)

Figure 2-1. Dynamic lysozyme binding capacity for Mustang S capsules, part number CLM05MSTGSP1

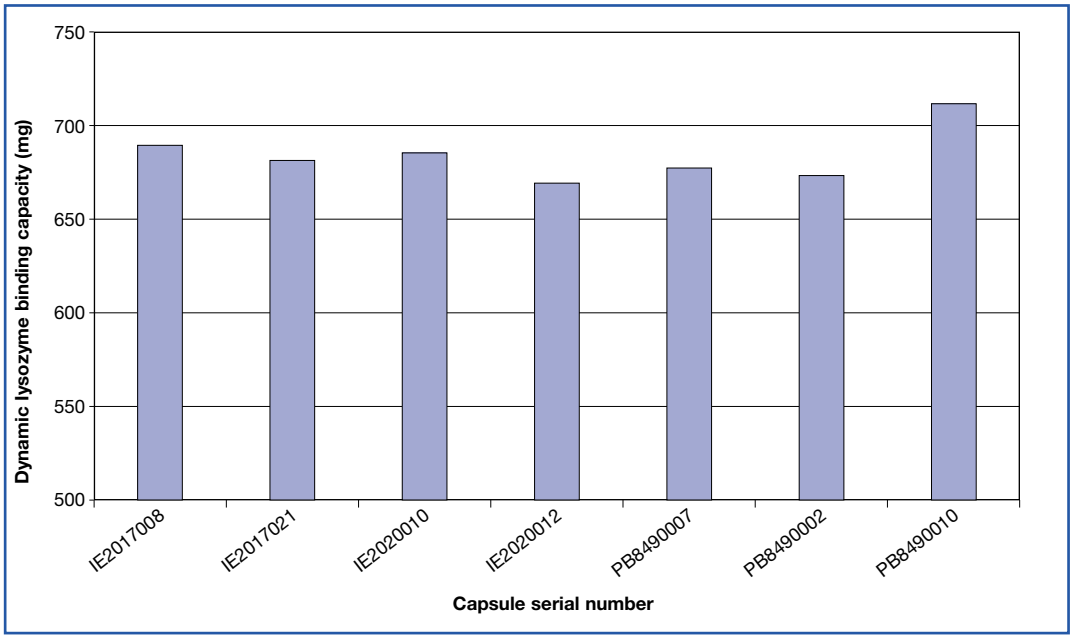


Figure 2-2. Dynamic lysozyme binding capacity for Mustang S capsules, part number CL3MSTGSP1

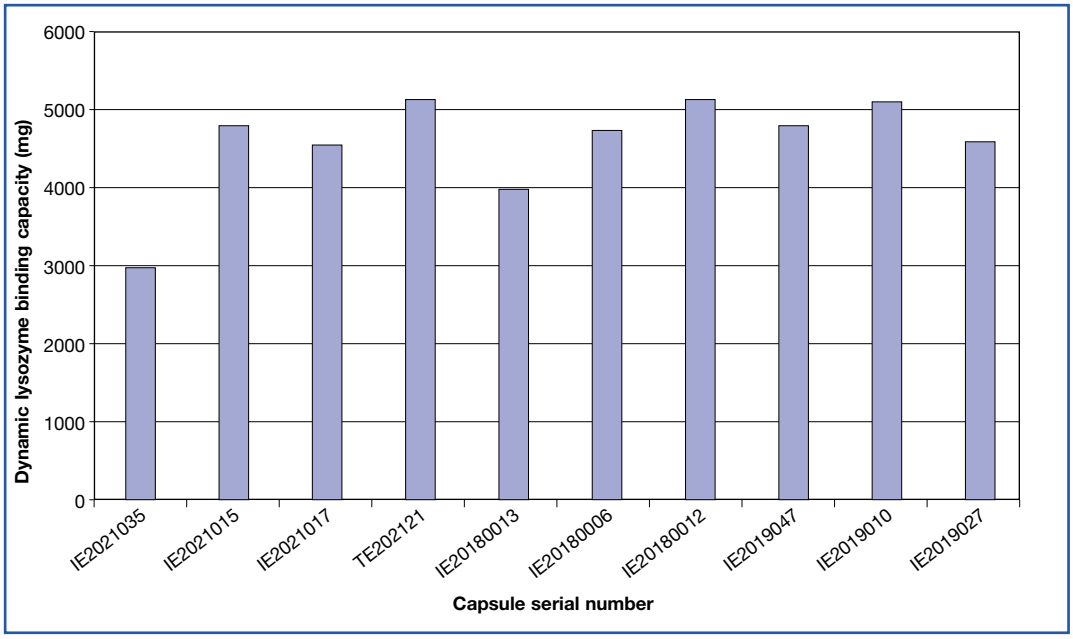
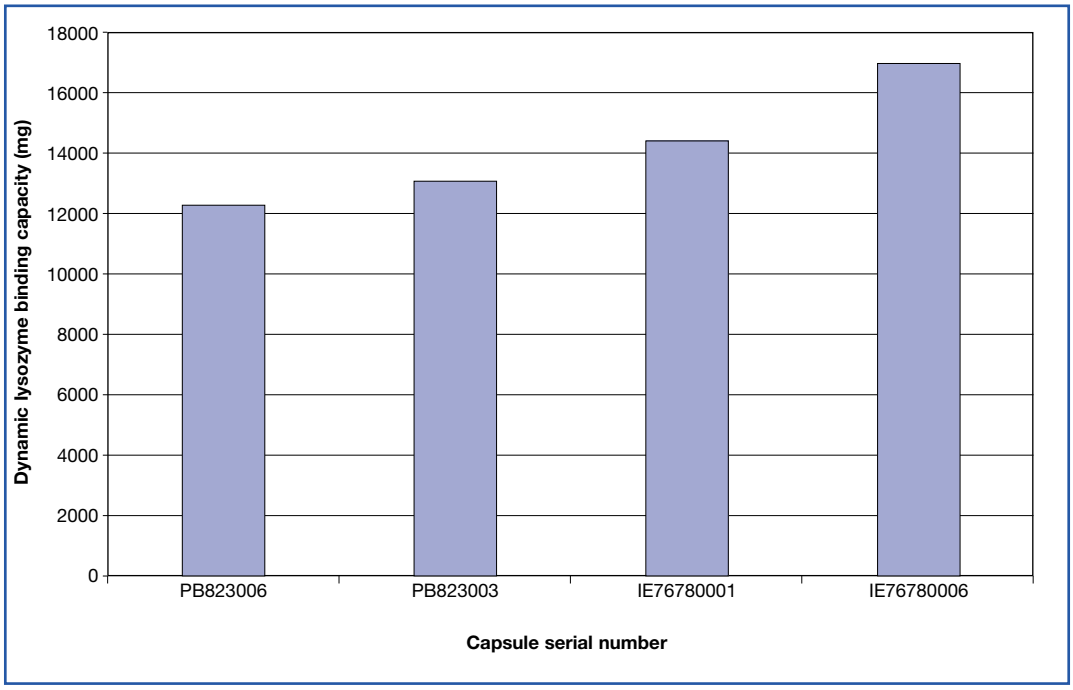


Figure 2-3. Dynamic lysozyme binding capacity for Mustang S capsules, part number NP6MSTGSP1



2.4 Conclusions

The studies reported here demonstrate that **Mustang** S capsules exhibit extremely high lysozyme dynamic binding capacities and are therefore suitable for downstream bioprocessing applications for the capture of biomolecules or removal of contaminants by cation exchange adsorption.

Different flow rates and the presence of other contaminants may influence the performance, and it is therefore recommended that the user evaluate **Mustang** S 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** S capsules at different applied upstream pressures using an aqueous test fluid.

3.2 Summary of methods

Typical **Mustang** S capsules from standard production lots were used for the tests (part numbers CLM05MSTGSP1, CL3MSTGSP1 and NP6MSTGSP1). The test capsules were initially subjected to the Pall recommended preconditioning procedure described previously in section 2.2.

The test fluid used to determine the flow characteristics of the **Mustang** S capsules was 10mM MES buffer (pH 5.5) at 20°C ± 5°C. The fluid was pumped through the test capsules at set upstream pressures and during the tests the capsule outlets were 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 rate of 10mM MES buffer versus applied upstream pressure are shown in Figures 3-1 to 3-3. Each of the points plotted on the graphs represent an average value obtained from testing a minimum of five different **Mustang** S capsules.

Figure 3-1. Flow of 10mM MES buffer versus applied upstream pressure for Mustang S capsules, part number CLM05MSTGSP1

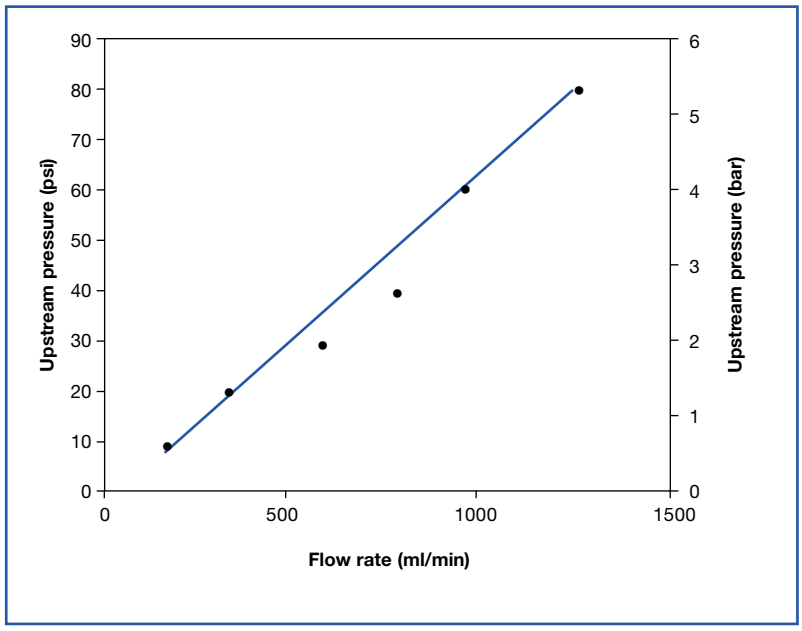


Figure 3-2. Flow of 10mM MES buffer versus applied upstream pressure for Mustang S capsules, part number CL3MSTGSP1

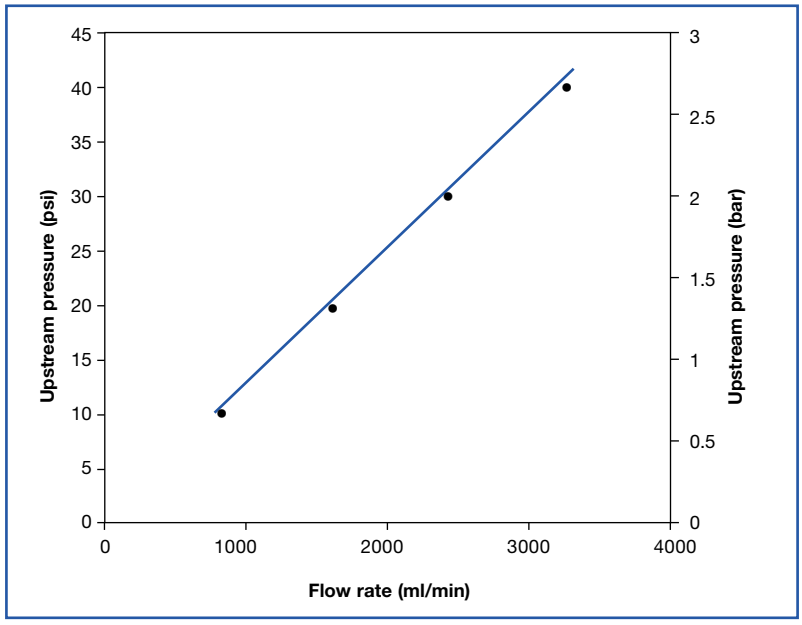
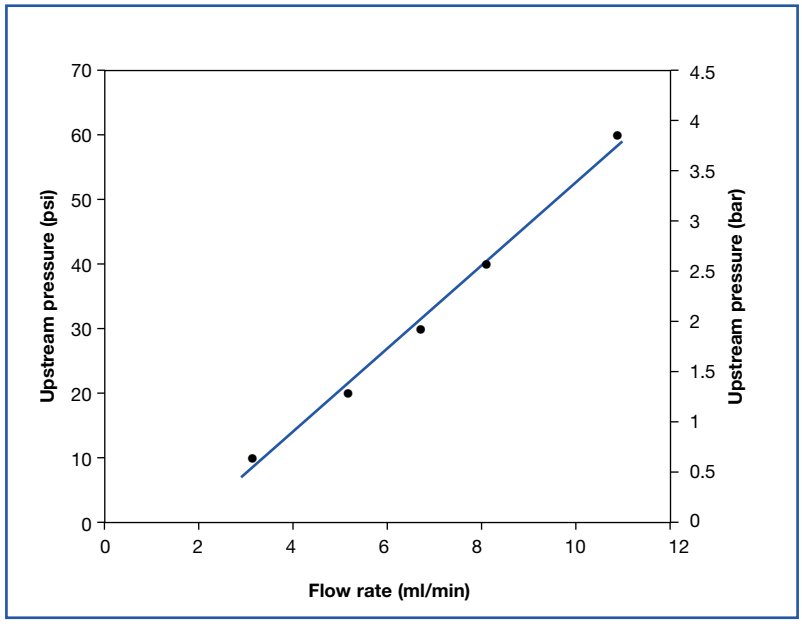


Figure 3-3. Flow of 10mM MES buffer versus applied upstream pressure for Mustang S capsules, part number NP6MSTGSP1



3.4 Conclusions

The flow characteristics quoted in this report can be used to assist in sizing systems employing **Mustang** S capsules when used with process fluids of similar viscosities to 10 mM MES buffer.

4. Endurance to autoclave sterilization

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** S capsules, as determined using a lysozyme dynamic binding capacity test.

4.2 Summary of methods

Typical **Mustang** S capsules from production were used for these tests (part numbers CLM05MSTGSP1 and CL3MSTGSP1). Samples were removed from their packaging, the inlet and outlet connections loosely wrapped with aluminium foil and the capsules were then autoclaved at 121°C for 30 minutes. Following autoclaving, the cooled capsules were tested for lysozyme dynamic binding capacity according to the procedure described previously in section 2.2. The results were compared with previous tests performed on non-autoclaved capsules.

4.3 Results

The results are shown in Table 4-1. It was found that the average amount of lysozyme 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 lysozyme dynamic binding capacities of **Mustang** S capsules.

Table 4-1. Effect of autoclaving at 121°C for 30 minutes on lysozyme dynamic binding capacities for Mustang S capsules

Mustang S part number	Capsule serial number	Dynamic lysozyme binding capacity following one autoclave cycle
CLM05MSTGSP1	IE20170009	816 mg
	IE20200002	958 mg
	PB8490012	690 mg
	Average binding for autoclaved samples	**821 mg**
	Average binding for non-autoclaved samples	**685 mg**
CL3MSTGSP1	IE2021008	4400 mg
	IE20180017	5400 mg
	Average binding for autoclaved samples	**4900 mg**
	Average binding for non-autoclaved samples	**4608 mg**

4.4 Conclusions

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

5. Compatibility with sodium hydroxide

5.1 Introduction

The purpose of these tests was to demonstrate that exposure to 1N NaOH solution for one hour would not influence the performance of **Mustang** S capsules, as determined using a lysozyme dynamic binding capacity test.

5.2 Summary of methods

A typical **Mustang** S capsule from production was used for this test, part number CLM05MSTGSP1. The capsule was initially subjected to the standard Pall recommended preconditioning procedure (described previously in section 2.2) and then flushed with 50 ml of 1N NaOH. Flow of the NaOH solution was stopped and the capsule was exposed to the NaOH solution for one hour at 20°C ± 5°C. After this time the NaOH was flushed out of the capsule using 10mM MEM buffer (pH 5.5). The lysozyme dynamic binding capacity was then determined according to the procedure described previously in section 2.2.

5.3 Results

The results of the dynamic lysozyme binding capacity tests shown in Table 5-1 demonstrate that exposure to 1M NaOH for one hour at 20°C had no significant effect on membrane performance.

Table 5-1. Effect of exposure to 1N NaOH solution for one hour on the lysozyme dynamic binding capacity of a Mustang S capsule, part number CLM05MSTGSP1

Dynamic lysozyme binding capacities for Mustang S capsules, part number CLM05MSTGSP1	
Average binding value for CL05 capsules	
Following exposure to 1N NaOH solution for one hour	Not exposed to NaOH
706 mg	685 mg

5.4 Conclusions

Mustang S capsules can withstand exposure to 1N NaOH at 20°C for one hour, as demonstrated using a lysozyme dynamic binding capacity test.

6. Extractables testing

6.1 Introduction

The purpose of this series of tests was to quantify the amount of material that can be extracted from a typical **Mustang** S unit by water at ambient temperature (20°C ± 5°C).

6.2 Summary of methods

Typical **Mustang** S capsules were used for the extraction tests, part number NP6MSTGSP1. Prior to the extraction procedure the cartridges were subjected to the standard Pall recommended preconditioning procedure described previously in section 2.2. The cartridges were then flushed with 15l of 18 MΩ water until the downstream pH and conductivity measurements were the same as the upstream measurements.

Following preconditioning, the extraction procedure was performed by recirculating approximately 1500 ml of 18 M water (20°C ± 5°C) through the cartridge for two hours at a flow rate of 400 ml/min. After the extraction period the entire volume of extraction water was drained from the system and carefully measured.

A sample of the extraction water 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.

The above was performed on an autoclaved and non-autoclaved capsule.

6.3 Results

The amount of extractables obtained from typical **Mustang** S capsules are shown in Table 6-3.

Table 6-3. Non-volatile aqueous extractables obtained using typical Mustang S capsules, part number NP6MSTGSP1

Treatment prior to extraction	Cartridge serial number	Non-volatile residue
Preconditioning	PB823001 IE76780005	98 mg 80 mg
Autoclave and preconditioning	PB823009 IE76780004	353 mg 261 mg

6.4 Conclusions

The levels of aqueous extractables determined for preconditioned autoclaved and non-autoclaved **Mustang** S capsules have been determined. 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 for Mustang S cartridges

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** S cartridge.

7.2 Summary of methods

A typical **Mustang** S cartridge (part number AB1MSTGS7PH4) was preconditioned by flushing with the following fluids at a flow rate of 1 L/min:

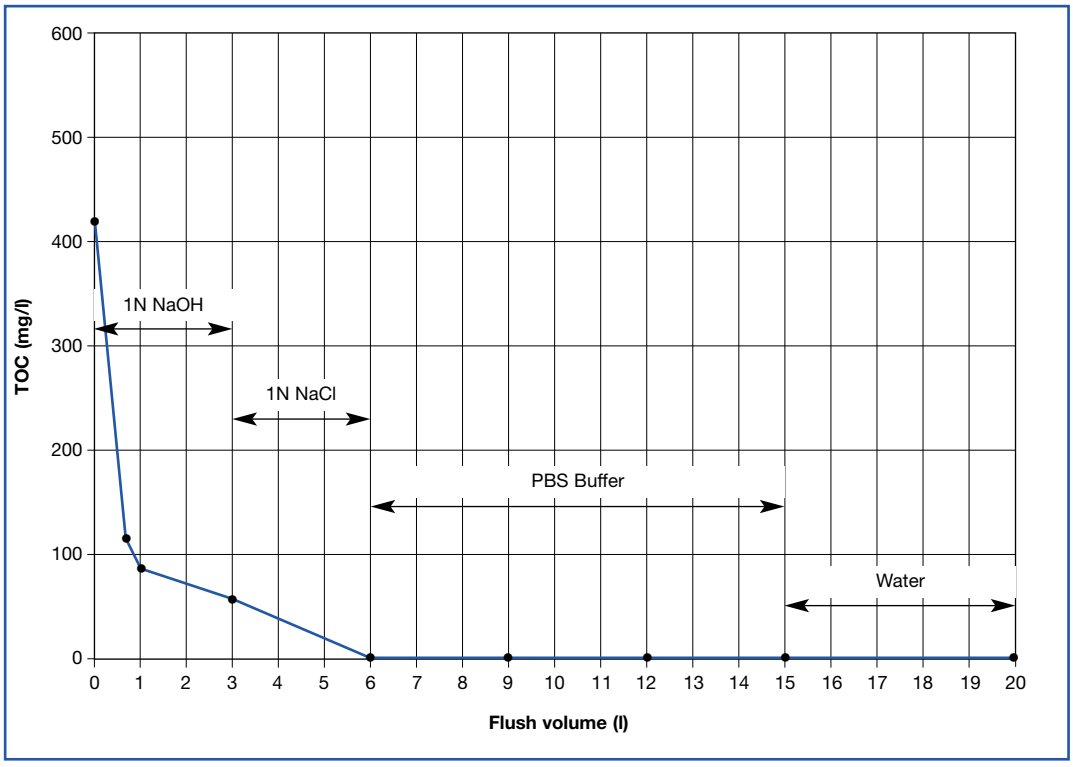
- **Three litres 1N NaOH**
- **Three litres 1N NaCl**
- **Nine litres PBS buffer**
- **Five litres water**

At intervals during the preconditioning and water flush, downstream samples were taken and analyzed for TOC.

7.3 Results

The levels of TOC determined downstream of a typical **Mustang** S cartridge during preconditioning flushes are shown in Figure 7-1. 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 7-1. Effects of preconditioning on TOC in flush volumes from a Mustang S cartridge, part number AB1MSTGS7PH4



7.4 Conclusions

Using a **typical** Mustang S cartridge (part number AB1MSTGS7PH4), the standard recommended preconditioning procedure was found to reduce the TOC in the flush volume by > 99.6%.

8. USP Biological reactivity tests on the Mustang S membrane

8.1 Introduction

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

8.2 Summary of methods

A typical **Mustang** S capsule was used for the tests, part number NP6MSTGSP1. The capsule was initially pre-conditioned by subjecting it to the standard Pall recommended preconditioning procedure described previously in section 2.2. The capsule was then flushed with 15l of 18 MΩ water until the downstream pH and conductivity measurements were the same as the upstream measurements.

The **Mustang** S 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** S 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** S membrane passed all of the tests specified.

8.4 Conclusions

Mustang S membrane met the requirements of the USP Biological Reactivity Tests *(in vivo)* for Class VI Plastics (50%). The tests included the systemic injection test, the intracutaneous test and the implantation test. Prior to performing the biological reactivity tests, the sample had been preconditioned by flushing with 1N NaOH, 1N NaCl and 18 MΩ water.

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