Validation Guide for Membrane Solutions Nylon Sterilizing Filter Cartridges

PolymidPure® Cartridge Filters

By Validation Service Center (2016)

CONTENTS

Part Ⅳ.   Extractables Testing

1. Introduction

2. Summary of methods

3. Test results

4. Conclusions

Part Ⅴ.   Biological Safety Testing

1. Introduction

2. Summary of methods

3. Conclusions

Part I .Introduction

PolymidPure Nylon 6,6 membrane pleated cartridge filter use a controlled porosity and naturally hydrophilic Nylon 6,6 membrane contains no wetting agents. High quality all-polypropylene components and high strength Nylon 6,6 membrane with polyester support layer provide excellent endurance versus thermal, hydraulic stress and meet the requirements of most applications.

Validation data contains: Bacterical Challenge、Extractable、Biological Safety Tests..ect. All of the Validation tests abide by ISO 9000 quality system standards.

This report is designed to assist the filter user in meeting the validation requirements of regulatory authorities within the pharmaceutical industry.

PartⅡ. Studies on Removal Efficiency

Microbial Validation using Brevundimonas diminuta Liquid Challenge Tests.

1. Introduction

The FDA guidelines on Sterile Products Produced by Aseptic Processing (1987) state, "A sterilizing filter is one which, when challenged with the micro-organism Pseudomonas diminuta (P. diminuta), at a minimum concentration of 10^7 organisms per cm^2 of filter surface, will produce a sterile effluent".

In order to meet the requirements of this guideline, liquid challenge tests using Brevundimonas (Pseudomonas) diminuta (ATCC 19146) were performed with PolymidPure Nylon (0.2μm) filter cartridges using a minimum of $1x10^7$ colony forming units (CFU)/cm^2 of effective filtration area.

The correlation between microbial retention and a non-destructive integrity test is also an important aspect of the validation of sterilizing grade filters. The FDA guideline further states, "After a filtration process is properly validated for a given product, process and filter, it is important to assure that identical filter replacements (membrane or cartridge) used in production runs will perform in the same manner. One way of achieving this is to correlate filter performance data with filter integrity testing data". The integrity tests used during this validation study were the Forward Flow and Bubble Point tests.

The Forward Flow Integrity Test

In the Forward Flow test, a filter is wetted with a suitable test liquid and a pre-determined gas pressure is applied to the upstream side of the filter assembly. After a stabilization period, the gas flow through the wetted membrane can be measured manually on the downstream side or on the upstream side, using sensitive flow measurement equipment such as the integrity test devices.

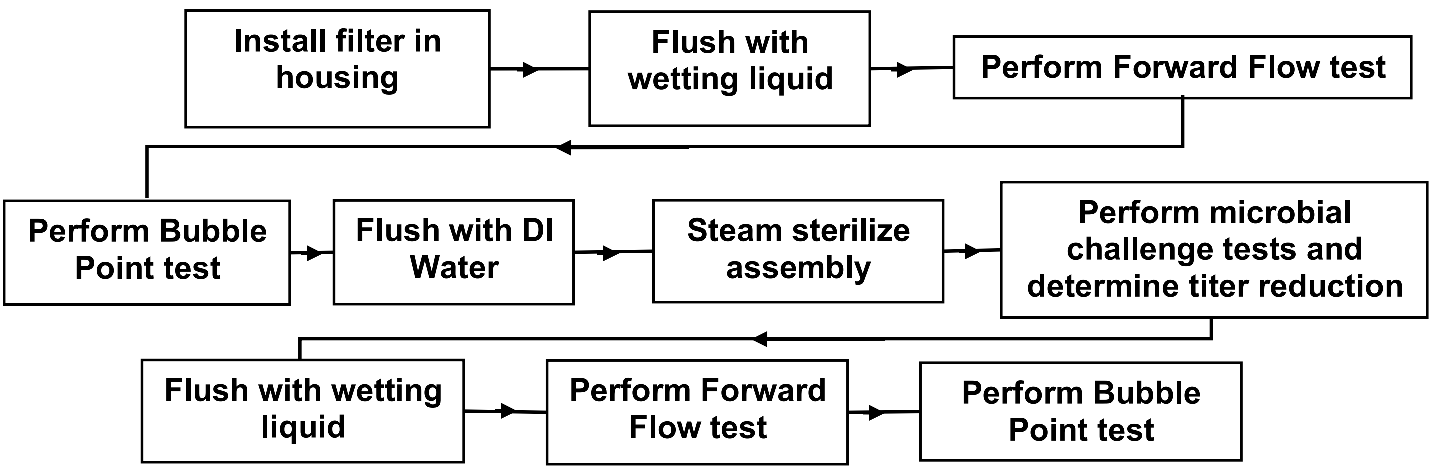
The Bubble Point Integrity Test

In the Bubble Point test, a filter is wetted with a suitable test liquid. After a stabilization period, increasing the gas pressure to the upstream side of the filter assembly, using sensitive flow measurement equipment such as the integrity test devices to test the change point of the gas flow rate.

The aims of this series of tests were to:

- Determine the microbial removal efficiency of PolymidPure Nylon (0.2µm) filter cartridges in liquid challenge tests using Brevundimonas diminuta (ATCC 19146)
- Correlate non-destructive Forward Flow and water intrusion integrity tests with destructive challenge tests
- Determine integrity test parameters

2. Summary of Methods



PolymidPure Nylon (0.2µm) filter cartridges(different S/N) with a range of Forward Flow and Bubble Point values were selected from manufacturing lots and subjected to microbial challenge tests using anaqueous suspension of Brevundimonas diminuta (ATCC 19146).

The filter sample was installed in a housing and tested for integrity by the Bubble Point and Forward Flow method, prior to being autoclaved at 121°C (250°F) for 30 minutes. The filter assembly was then aseptically connected to a pre-sterilized challenge apparatus.

An aqueous suspension of B. diminuta was passed through the filter to achieve a challenge level of $>1\times10^{7}$CFU colony forming units (CFU) per cm^2 of effective filtration area. A total challenge per filter (10inch, $0.62m^2$) of $> 6.2\times10^{10}$CFU was achieved in all tests. On completion of the challenge, a second Bubble Point and Forward Flow test was performed.

During the challenge test, the entire filter effluent was passed through a 0.2 µm-rated analysis disc on the downstream side of the test filter assembly. The filter disc was incubated on TSA, following incubation, the disc was examined to determine if bacteria had passed through the test filter during the challenge. The titer reduction (T_R) for each filter was determined as follows:

$$T_R = \frac{\text{Total number of organisms influent to the filter}}{\text{Number of colonies recorded on the downstream analysis disc}}$$

When no colonies were detected downstream, the titer reduction was expressed as:

>Total number of organisms influent to the filter (e.g.> 6.2×10^{10})

Please contact **PU** if a more detailed description of the test methods is required.

3. Test Results

Forward Flow Correlation

The Forward Flow and B. diminuta retention results are shown in Table (1), and presented graphically in Figure (1).

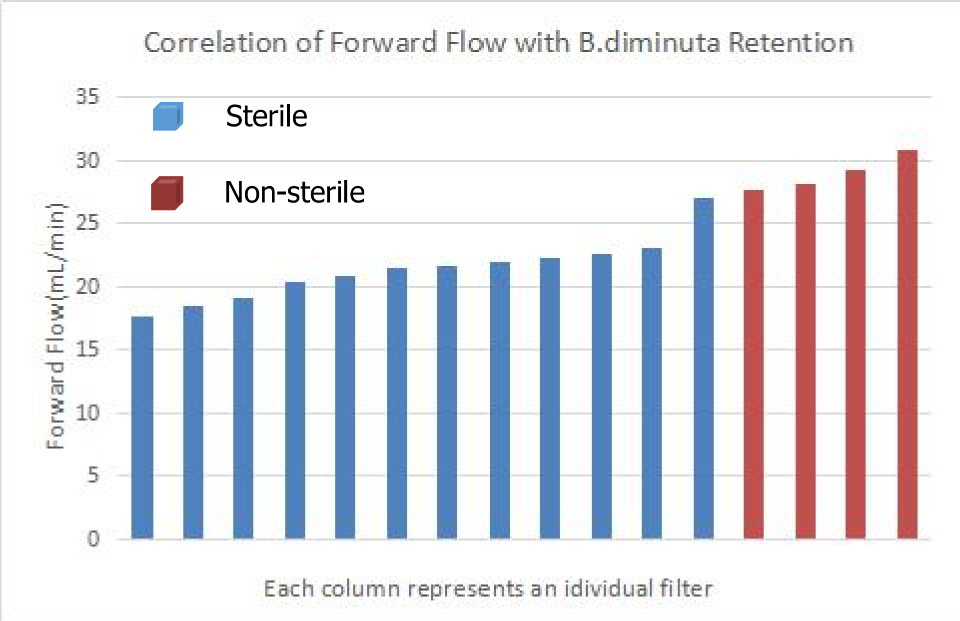
It was found that all of the 12 filters with Forward Flow values ≤27.0 ml/min gave sterile effluent when challenged with $>6.2\times10^{10}$ CFU of B.

diminuta per filter. Four filters with Forward Flow values between 27.7 ml/min to 30.8ml/min gave titer reductions between 3.1×10^{10} to 6.0×10^{10}.

Table (1)   Correlation of Forward Flow with B. diminuta Retention for MS PolymidPure Nylon (0.2µm) Filters

Filter Serial Number	Forward Flow (ml/min)	Sterile Effluent	Titer Reduction
16050807125	17.6	Yes	$> 6.2\times10^{10}$
16050501122	18.4	Yes	$> 6.2\times10^{10}$
16051301116	19.1	Yes	$> 6.2\times10^{10}$
16050908338	20.4	Yes	$> 6.2\times10^{10}$
16050312124	20.9	Yes	$> 6.2\times10^{10}$
16050512431	21.5	Yes	$> 6.2\times10^{10}$
16050501438	21.7	Yes	$> 6.2\times10^{10}$
16050511102	21.9	Yes	$> 6.2\times10^{10}$
16051003171	22.2	Yes	$> 6.2\times10^{10}$
16050811419	22.6	Yes	$> 6.2\times10^{10}$
16050210104	23.0	Yes	$> 6.2\times10^{10}$
16050202331	27.0	Yes	$> 6.2\times10^{10}$
16050513411	27.7	No	6.0×10^{10}
16050702121	28.2	No	5.8×10^{10}
16050404031	29.3	No	4.3×10^{10}
16050404222	30.8	No	3.1×10^{10}

Figure (1) Correlation of Forward Flow with B.diminuta Retention for PolymidPure Nylon (0.2µm) Filters



29 psig（2000 mbar）Forward Flow test by air, Wetting by DI water

Bubble Point Correlation

The Bubble Point and B. diminuta retention results are shown in Table (2), and presented graphically in Figure (2).

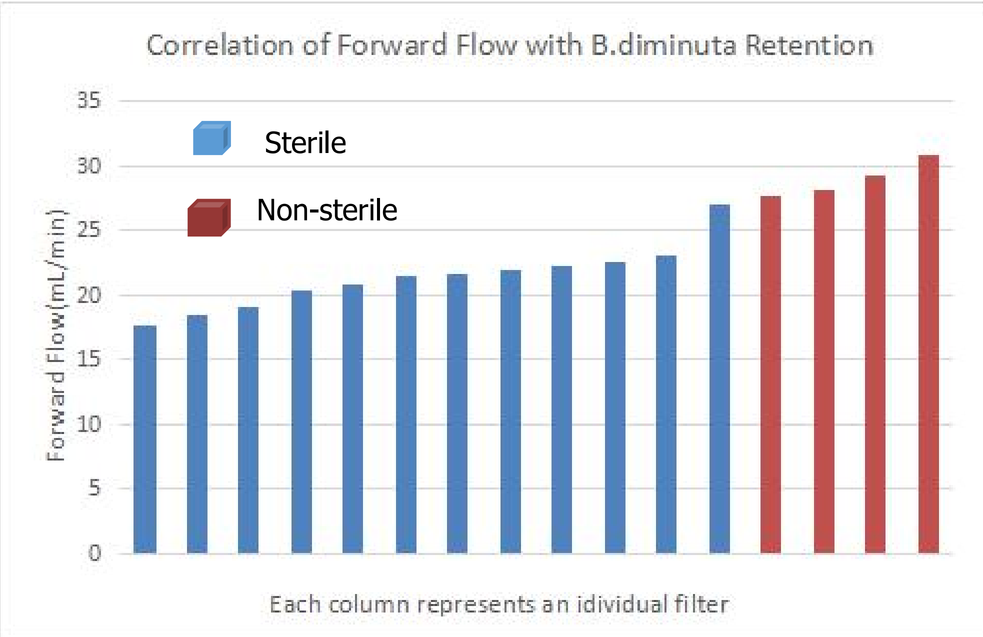
It was found that all of the 13 filters with Bubble Point values $\geq$30.5psig gave sterile effluent when challenged with ＞ 6.2×10^{10} CFU of B. diminuta per filter. Three filters with Bubble Point values between 24.6psig to 29.8psig gave titer reductions between 8.5×10^{9} to 5.2×10^{10}.

Table (2) Correlation of Bubble Point with B.diminuta Retention for PolymidPure Nylon (0.2μm) Filters

Filter Serial Number	Bubble Point (psig)	Sterile Effluent	Titer Reduction
16010305014	46.1	Yes	$> 6.2\times10^{10}$
16010204208	45.7	Yes	$> 6.2\times10^{10}$
16010504123	44.3	Yes	$> 6.2\times10^{10}$
16011206137	43.5	Yes	$> 6.2\times10^{10}$
16011101214	42.7	Yes	$> 6.2\times10^{10}$

16011122138	41.9	Yes	> 6.2×10^{10}
16011104074	40.7	Yes	> 6.2×10^{10}
16012102027	39.4	Yes	> 6.2×10^{10}
16012102184	38.8	Yes	> 6.2×10^{10}
16012112216	38.2	Yes	> 6.2×10^{10}
16012534109	37.1	Yes	> 6.2×10^{10}
16012502109	36.5	Yes	> 6.2×10^{10}
16012501623	30.5	Yes	> 6.2×10^{10}
16012709148	29.8	No	5.2×10^{10}
16012703311	26.2	No	9.3×10^{9}
16012703086	24.6	No	8.5×10^{9}

Figure (2) Correlation of Bubble Point with B.diminuta Retention for PolymidPure Nylon (0.2µm) Filters



Wetting by DI water

4. Conclusions

Based on the results of the validation study, both the Forward Flow and Bubble Point test methods were demonstrated to be suitable non-destructive integrity tests for PolymidPure Nylon (0.2µm) filter cartridges. Integrity test parameters for PolymidPure Nylon (0.2µm) filter cartridges were set as follows:

Forward Flow Integrity Test Parameters for PolymidPure Nylon (0.2µm) Cartridge Filters (10inch, 0.62m²)

Wetting liquid	DI Water
Test gas	Air
Test pressure	29psig (2000mbar)
Temperature	25℃±2℃
Maximum allowable Forward Flow limit	23.0ml/min

Bubble Point Integrity Test Parameters for PolymidPure Nylon (0.2µm) Cartridge Filters (10inch, 0.62m²)

Wetting liquid	DI Water
Test gas	Air
Temperature	25℃±2℃
Minimum allowable Bubble Point limit	36.5psig（2500mbar）

These Forward Flow and Bubble Point integrity test parameters:

- Incorporate a safety margin.
- Provide a high level of assurance of retention of Brevundimonas diminuta when challenged with＞1 x 10^7CFU/cm^2 of effective filtration area.
- Confirm that PolymidPure Nylon (0.2µm) filter cartridges satisfy the requirements of sterilizing grade filters as described in the FDA guidelines for aseptic processing (1987).

Please note: Integrity test values are continually reviewed and monitored during routine production tests. Test values are issued and controlled by Validation Services Center. Please contact **PU** for further details.

Part Ⅲ. Studies on Physical Characteristics

1. Resistance to Steam Sterilization

1.1 Introduction

The aim of these tests was to determine the effects of steam-in-place sterilization on the integrity of PolymidPure Nylon (0.2µm) filter cartridges.

1.2 Summary of Methods

During the tests, typical production filters（10inch, 0.62m^2）installed in a stainless steel housing were steamed in place using saturated condensate -free steam.

In each series of tests the following was performed:

- Steam pressure and flow were held constant during the sterilization period.
- Test filter cartridges were Forward Flow integrity tested at appropriate intervals.
- After each steam in place cycle the filters were cooled by passing dry compressed air through the test filter.

1.3 Test Results

During this study, filters were steamed using high initial differential pressures (1000 mbar (14.5 psig)) at 124°C (255°F). The tests were performed in 30 minutes cycles in the forward (out to in) direction. These tests were performed in order to simulate steam conditions where transient high differential pressures (> 300 mbar (4.3 psig)) may occur during the steam sterilization cycle. At appropriate intervals, the filters were Forward Flow integrity tested and the results are shown in Table (3).

All filters passed the Forward Flow integrity test after exposure to 30 minutes steam cycles 15 times.

Table(3) Effect of High initial Differential Pressures on Forward Flow Values

Filter Serial Number	Forward Flow(ml/min) after the following Number of 30 minutes Steam Cycles							
	0	1	2	4	8	10	12	15
16010904025	22.3	22.2	21.8	22.1	21.3	22.3	22.9	23.0
16010904171	22.2	21.7	21.4	21.8	21.7	21.9	22.3	22.9
16010904123	22.6	22.3	22.1	21.7	22.6	23.0	22.6	23.0
16010904178	22.2	22.6	21.6	22.1	22.9	22.7	22.4	22.6

Wetting by DI water before steam sterilizing, Limit value: ≤23.0 ml/min, 29 psig（2000 mbar), 25 ℃, DI water.

1.4 Conclusions

The data presented in this section support the following conclusions:

PolymidPure Nylon (0.2µm) filter cartridges are robust and capable of withstanding multiple steam sterilization cycles, where the differential pressure may exceed 300 mbar (4.3 psig) in the forward direction, as demonstrated by exposing filters to high transient differential pressures of up to 1000 mbar (14.5 psig) during steaming at 124°C (255°F).

2. Water Flow/Differential Pressure Measurements

2.1 Introduction

The aim of these tests was to determine the water flow rates at set differential pressures across PolymidPure Nylon (0.2µm) filter cartridges.

2.2 Summary of Methods

The tests were performed on standard production filters (10inch, 0.62m^2). Test filters were installed in a stainless steel housing and flushed with DI water to pre-wet the filter membranes. Pre-filtered deionized water was then pumped through the assembly in the normal flow (out to in) direction. Pressure transducers on the upstream and downstream side of the test filter housing were monitored to calculate the differential pressure at different water flow rates.

Further measurements were taken with the housing only (no filter installed). The housing-only results were subtracted from the filter assembly results in order to provide flow/pressure characteristics for the filter only. All data were corrected to a standard temperature of 20°C (68°F).

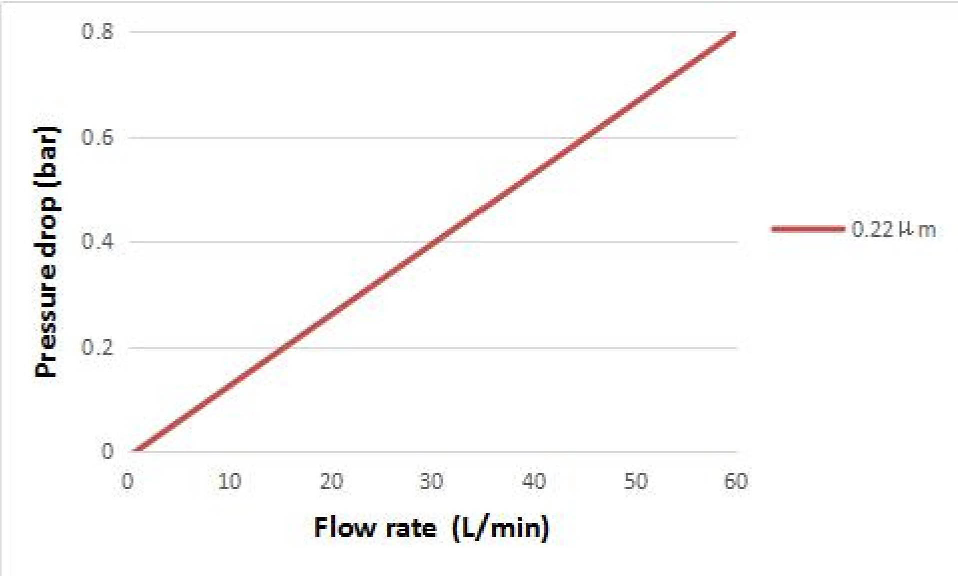
Please contact **PU** if further details about the test methods are required.

2.3 Test Results

The water flow measurements through typical filters (10inch, 0.62m^2) at differential pressure are shown in figure (3)，The water flow rates at 730 mbar (5 psig) differential pressure for PolymidPure Nylon (0.2µm) filter cartridges were found to range between 20~25L/min, These data can be used to form the basis of sizing filter systems using PolymidPure Nylon (0.2µm) filter cartridges.

Note: The differential pressures quoted are for liquids with a viscosity of 1cP. Differential pressures for liquids at other viscosities can be estimated by multiplying the differential pressure by the viscosity in cP. To obtain the total pressure drop of a complete filter assembly the housing pressure drop must also be added. Please contact **PU** for further details.

Figure (3)   Flow Rate/Differential Drop



Water Flow Rate (L/min), 20℃, 10inch

3. Resistance to Differential Pressure

3.1 Introduction

The aim of these tests was to determine the effects of Differential Pressure on the integrity of PolymidPure Nylon (0.2µm) filter cartridges.

3.2 Summary of Methods

The tests were performed on standard production filters (10inch, 0.62m^2). Test filters were installed in a stainless steel housing and flushed with DI water at differential temperature/Pressure for 2 hours, Test the integrity of the filters.

3.3 Test Results

The Results of integrity at differential Temperature/Pressure are shown in Table (4)

Table (4)   Pressure/Temperature

△P (psig) FF (ml/min) Temp	0 psig	10 psig	27 psig	45 psig	75 psig
Forward25℃	21.5 21.4	22.3 22.2	22.3 22.6	21.9 22.1	22.9 22.3
Reverse25℃	21.6 21.8	22.1 22.5	22.5 22.7	23.4 24.3	N/A N/A
Forward82℃	21.1 21.5	22.1 21.6	22.3 22.8	N/A N/A	N/A N/A

Wetting by DI water before steam sterilizing, Limit value: ≤23.0 ml/min, 29 psig (2000 mbar), 25 ℃,DI water.

The data presented in this section support the following conclusions:

Maximum Forward △P at 82℃ ≤27 psig (1900mbar)

Maximum Forward △P at 25℃ ≤75 psig (5200mbar)

Maximum Reverse △P at 25℃ ≤30 psig (2100mbar)

Please contact **PU** for further details.

Part Ⅳ. Extractables Testing

1. Introduction

The aim of this series of tests was to quantify the material, which can be extracted from PolymidPure Nylon (0.2μm) filter cartridges using water and ethanol.

2. Summary of Methods

Preparation of Filter Samples

Extractables tests were performed on typical production filter cartridges (10inch, 0.62m^2), which had been autoclaved in order to maximize the quantity of any extractable material present. The filters were wrapped in aluminium foil and autoclaved for half hour at 121°C (250°F), using a slow exhaust cycle. Visible droplets of water remaining on the filter elements were allowed to evaporate at room temperature before the extraction was performed.

Extraction Procedure

Dynamic extraction tests were performed. The test filters were immersed in 1800 mL of extraction fluid in a clean measuring cylinder， Use Ultrapure water flush the filters for 8 hours.

Analysis of Material Extracted

After the extraction, 1500mL of the extraction liquid was evaporated to dryness and the non-volatile extractables were determined gravimetrically.

The material extracted by ethanol were analyzed by Fourier Transform Infra Red spectroscopy.

Please contact **PU** if further details of the test methods are required.

3. Test Results

Table (5) shows the levels of extractables obtained using typical production PolymidPure Nylon (0.2µm) filter cartridges.

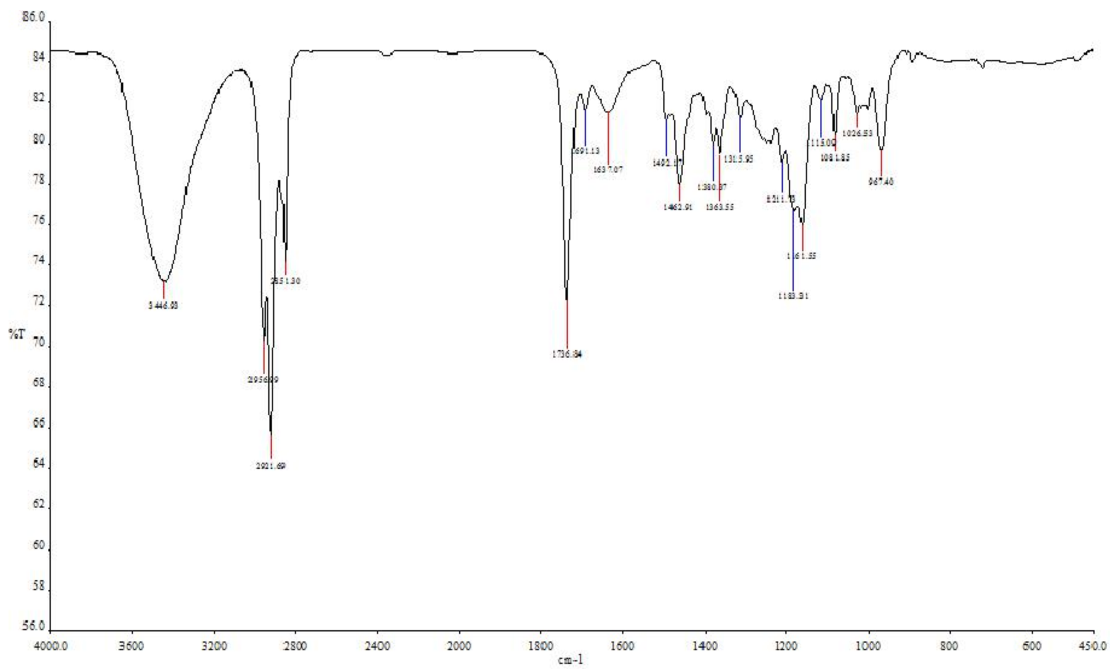
Infra red spectra of ethanol extracts from PolymidPure Nylon (0.2µm) filter cartridges (see Figure (4)) showed close similarities with those of polypropylene.

Table (5) Non-volatile Extractables using Typical (10") PolymidPure Nylon Nylon (0.2µm) cartridge filters

Extraction Fluid	Filter Serial Number	Residue

Ultrapure Water	16050704124	7.6mg
	16050702113	7.1mg
	16050701132	8.0mg
Ethanol	16050802521	24.8mg
	16050802117	25.5mg
	16050802141	24.3mg

Figure (4) Infra Red Spectrum of Ethanol Extracts from PolymidPure Nylon Nylon (0.2µm) cartridge filters



4. Conclusions

The levels of extractables determined for PolymidPure Nylon (0.2µm) filter cartridges were dependent on the solvent used. For most solvents tested, the gravimetric extractables were found to be extremely low. The results reported are typical for production elements.

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

Part Ⅴ. Biological Safety Testing

1. Introduction

The purpose of these tests was to evaluate the biological suitability of the materials of construction of the PolymidPure Nylon (0.2μm) filter cartridges. The materials of construction of PolymidPure Nylon (0.2μm) filter cartridges are as follows:

Filter Membrane:	Nylon 6,6
Support/Drainage layers:	Polypropylene
End cap/Adapter:	Polypropylene
Core/Cage:	Polypropylene/Polysulfone

2. Summary of Methods

The tests were performed in accordance with the Biological Reactivity Tests in vivo for Class VI Plastics (121°C) as described in the current United States Pharmacopeia. The tests were conducted NAMSA Laboratories.

The testing procedures described in the USP include:

- Injection of extracts of plastic materials
- Implantation of the solid material into animal tissu

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

- Sodium Chloride 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 (122°F) for 72 hours, 70°C (158°F) for 24 hours, or 121°C (250°F) for 1 hour. The most stringent condition not resulting in physical changes in the plastic is recommended, therefore the filters were extracted at 121°C (250°F).

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 materials of construction to the most stringent conditions included in the USP. Each of the components of the PolymidPure Nylon (0.2µm) filter cartridges was implanted separately.

3. Conclusions

PolymidPure Nylon (0.2µm) filter cartridges meet the requirements of the USP for Class VI (121°C) Plastics. Figure (5) show a copy of the test certificate. Please contact **PU** if copies of the test reports are required.

Figure (5) USP VI (121℃) Biological Safety Testing Report

11/08/2006 19:20 4543059 NAMSA PAGE 01/01

NAMSA

PEOPLE › SCIENCE › SOLUTIONS

Confidential
TCLAS-VI7

Kirit Patel
Donaldson Company, Inc.
85 Railroad Drive
Ivyland, PA 18974

Lab No. 06C_53079_10
P.O. No. Verbal
Test Facility: NAMSA
9 Morgan
Irvine, CA 92618

CERTIFICATE OF COMPLIANCE
USP BIOLOGICAL REACTIVITY TESTS, *IN VIVO*

USP PLASTIC CLASS VI

Test Article: Laminate 6502 Tetratex 0.2 Micron
ID No. Lot: 22072561-0301

USP Systemic Toxicity Study in the Mouse: The test article was prepared as indicated below and injected into mice. The saline, alcohol in saline, polyethylene glycol 400 and sesame oil extracts did not produce a significantly greater systemic reaction than the blank extractants.

USP Intracutaneous Toxicity Study in the Rabbit: The test article was prepared as indicated below and injected intracutaneously into rabbits. The saline, alcohol in saline, polyethylene glycol 400 and sesame oil extracts did not produce a significantly greater tissue reaction than the blank extractants.

USP Muscle Implantation Study in the Rabbit: The macroscopic reaction of the test article, implanted in rabbit muscle for one week, was not significant when compared to the USP negative control plastic.

The test article was prepared at a ratio of 120 cm^2:20 ml and extracted at 50°C for 72 hours. The test article extracts met the requirements of a USP Plastic Class VI.

nee Date Completed 11-8-06 Approved By R. Villani
Robert Villani, AALAS Certified
Tox Supervisor

Authorization for duplication of this report, except in whole, is reserved pending NAMSA's written approval. Page 1 of 1