Life Sciences

Validation Guide

USTR 2303

Validation Guide for Pall® Supor® UEAV Filter Cartridges

*Filtration. Separation. Solution.*SM

CONTENTS

1. Overview

1.1 Introduction

Pall Supor UEAV bioburden reduction filters with hydrophilic asymmetric polyethersulfone membrane are constructed with patented **Pall** Ultipleat® technology. This crescent-shaped pleat configuration maximizes membrane area in order to increase flow rates and maximize filter life. The filters are suitable for the filtration of a wide range of fluids including buffers, biological fluids, tissue culture media and ophthalmic products. **Supor** UEAV bioburden reduction filters can also be used to protect downstream process equipment such as storage or transfer tanks, tangential flow filter systems and chromatography columns.

The purpose of this report is to summarize the tests that were performed to qualify the performance of **Supor** UEAV filters under standard test conditions. The qualification program included:

- **Microbial Challenge Tests**
- **Endurance to Steam Sterilization**
- **Determination of Water Flow Characteristics**
- **Extractables Testing using Water**
- **Protein Transmission Studies**
- **Biological Reactivity Tests**

Note: The units of pressure quoted in this document are bar and pound/inch^2 (psi). The following figures can be used to convert these units of pressure to Pascal (Pa):

- **1 bar = 1 x 10^5 Pa**
- **1 psi = 6.894757 x 10^3 Pa**

1.2 Summary of Conclusions

Microbial Challenge Tests

Typical **Supor** UEAV filters from production, part number AB1UEAV7PH4, were found to provide sterile effluent when subjected to aqueous microbial challenge tests using *Brevundimonas diminuta (B. diminuta)* at a challenge level of > 1 x 10^7 CFU. The filters that were tested had Forward Flow values ranging from 16.1 to > 60 mL/min when tested water wet at 2060 mbar (30 psi) air test pressure.

These data support the use of the following Forward Flow integrity test limits.

Forward Flow Integrity Test Parameters*

Test pressure	2060 mbar (30 psi)
Wetting liquid	Water
Temperature	20°C ± 5°C (68°F ± 9°F)
Test gas	Air
Maximum allowable Forward Flow limit**	50 mL/min

* See Section 2.2 for test procedure.
** During the test period the temperature of the filter assembly should not vary by more than ± 1°C (1.8°F).

Endurance to Steam Sterilization

Supor UEAV filters (part number AB1UEAV7PH4) have been demonstrated as being capable of withstanding multiple in-line steam sterilization cycles. Five filters were tested and all were found to retain integrity following exposure to 19 one-hour steam cycles at 125°C (257°F), which is an adequate safety margin for the 10 cycle claim.

Determination of Water Flow Characteristics

Water flow rates at set differential pressures have been determined. The average pressure drops for AB1UEAV7PH4 filters have been presented at a number of applied clean water flow rates. These data can be used to assist users in sizing filter systems employing **Supor** UEAV filters.

Extractables Testing using Water

The typical amount of non-volatile residue (NVR) extracted from **Supor** UEAV filters has been determined using water as the extraction fluid. For the 254 mm (10 inch) filter elements that were tested (part number AB1UEAV7PH4), the non-volatile residue measured was ≤ 50 mg per 254 mm (10 inch) filter.

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

Transmission of Proteins

Protein adsorption studies have been performed using **Pall Supor** UEAV filter membrane with Bovine Serum Albumin (BSA) and IgG. The results demonstrate that > 98% protein transmission can be obtained when filtering dilute solutions.

Biological Reactivity Tests

All of the materials used in **Supor** UEAV filters meet the requirements of the USP Biological Reactivity Tests *(in vivo)* for Class VI (121°C) plastics. The tests included the systemic injection test, the intracutaneous test and the implantation test.

2. Microbial Challenge Tests

2.1 Introduction

The aim of this study was to determine the microbial removal efficiency of typical **Supor** UEAV filters from production in liquid challenge tests using *Brevundimonas diminuta* (ATCC 19146).

2.2 Summary of Methods

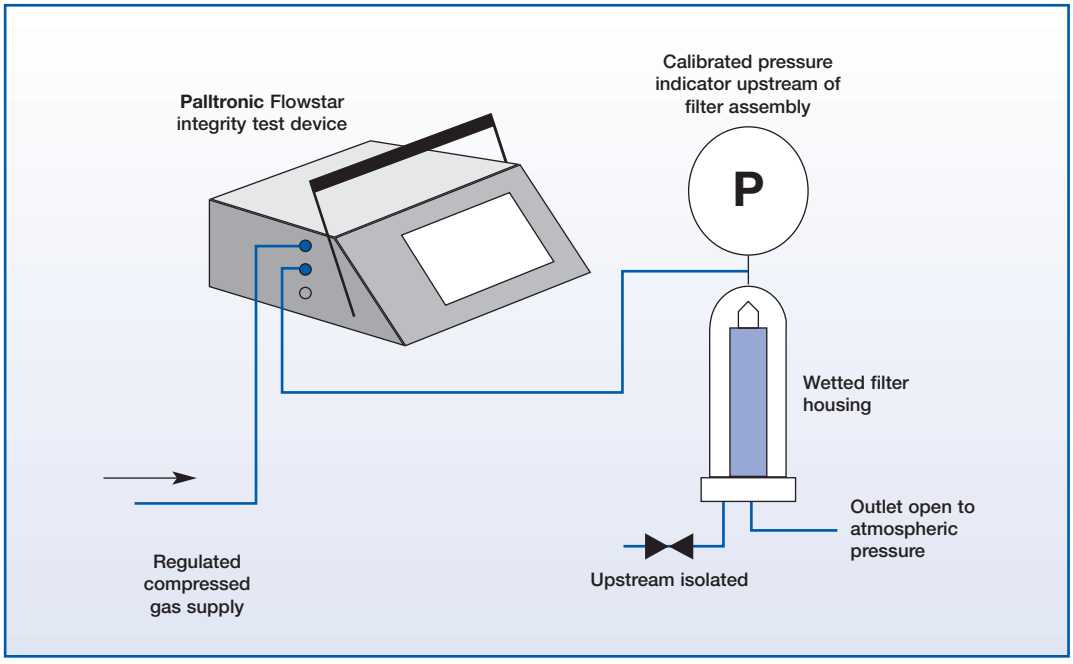
Typical **Supor** UEAV filters were selected randomly from three separate manufacturing batches (part number AB1UEAV7PH4, lot numbers: IG6364, IG6729 and IG6730). In addition, filters from a further batch (lot number IG5488) were included. These filters were specifically chosen as they had high integrity test values when they were tested in production.

During this test procedure the filters were integrity tested using the Forward Flow test method, subjected to a microbial challenge test, and then Forward Flow integrity tested again.

The Forward Flow Integrity Test

In the Forward Flow test, a filter is wetted with an appropriate 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 on the upstream side, using sensitive flow measurement equipment such as the Palltronic® Flowstar filter integrity test instrument, see Figure 2-1.

Figure 2-1 The Automated Forward Flow Integrity Test

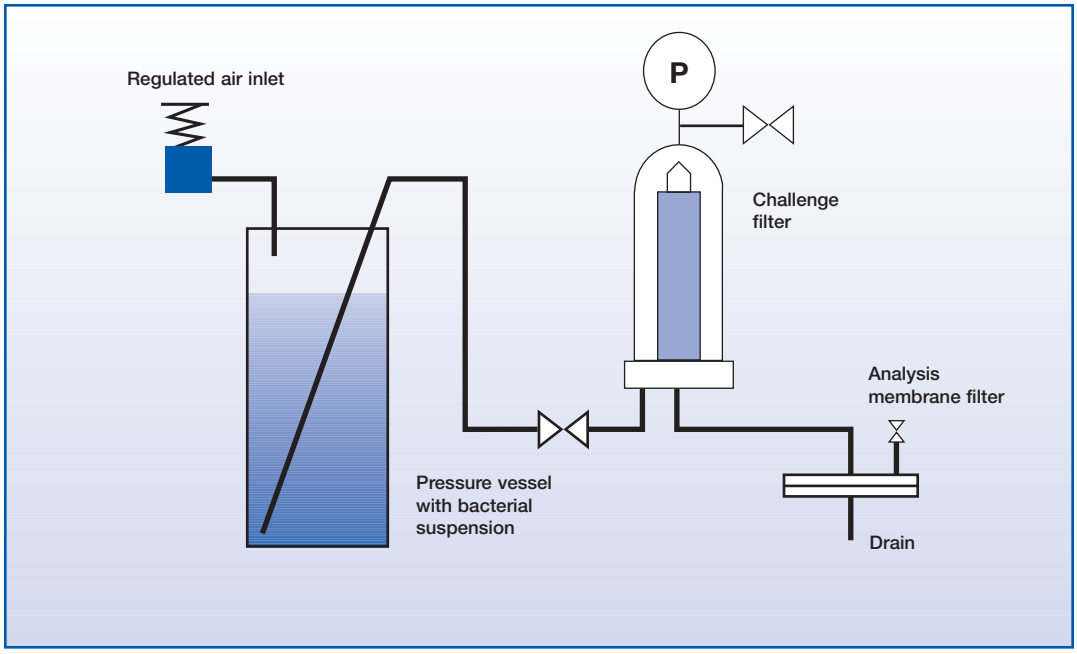


During this study **Supor** UEAV filters were wetted with deionized (DI) water at a flow rate of 10 L/min for 10 minutes and the Forward Flow values were determined using a **Palltronic** Flowstar integrity test instrument. A test pressure of 2060 mbar (30 psi) was used and the test gas was compressed air.

The Microbial Challenge Test

Prior to the challenge tests the filters were installed in an appropriate housing and Forward Flow integrity tested as described above. The filter assemblies were then sterilized in an autoclave at 121°C (250°F) for 60 minutes and then aseptically connected to the pre-sterilized challenge apparatus, as shown in Figure 2-2.

Figure 2-2 Microbial Challenge Apparatus



An aqueous suspension of *B. diminuta* was passed through the filter to achieve a total challenge level of ≥ 1 x 10^7 colony forming units (CFU).

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 agar and following incubation, the disc was examined to determine if any colonies had grown, indicating whether or not 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. > 1 x 10^7)

On completion of the challenge test the filter assemblies were autoclaved, flushed and Forward Flow integrity tested as described previously.

2.3 Results

The Forward Flow and *B. diminuta* retention results are shown in Table 2-1. The higher of the pre and post challenge Forward Flow values are presented and the data are arranged in order of increasing Forward Flow value.

All of the filters that were tested gave sterile effluent when challenged with ≥ 4.49 x 10^7 colony forming units. These results included filters that had Forward Flow values in excess of 60 mL/min.

Table 2-1 Results of Forward Flow and *B. diminuta* Retention for Typical Supor UEAV Filters (Part Number AB1UEAV7PH4)

Pall Filter Serial Number	Forward Flow (mL/min)	Effluent	Titer Reduction
IG6730028	16.1	Sterile	> 4.54 x 10^8
IG6729032	22.7	Sterile	> 6.28 x 10^7
IG6364039	22.9	Sterile	> 4.42 x 10^8
IG6364011	23.1	Sterile	> 3.52 x 10^8
IG6364068	24.9	Sterile	> 3.42 x 10^8
IG6364060	25.6	Sterile	> 4.09 x 10^8
IG6729057	26.3	Sterile	> 4.49 x 10^7
IG6729020	27.1	Sterile	> 5.19 x 10^7
IG5488012	27.4	Sterile	> 6.20 x 10^8
IG6730057	28.0	Sterile	> 3.80 x 10^8
IG6730010	28.5	Sterile	> 1.53 x 10^8
IG5488037	34.1	Sterile	> 7.80 x 10^8
IG5488039	37.5	Sterile	> 8.60 x 10^8
IG5488051	39.1	Sterile	> 8.40 x 10^8
IG5488040	> 60	Sterile	> 9.00 x 10^8
IG5488054	> 60	Sterile	> 7.95 x 10^8

* Forward Flow values at 2060 mbar (30 psi) air test pressure, wet with water, temperature 20°C ± 5°C (68°F ± 9°F), maximum allowable limit value 50 mL/min.

2.4 Conclusions

Typical **Supor** UEAV filters from production, part number AB1UEAV7PH4, were found to provide sterile effluent when subjected to aqueous microbial challenge tests using *B. diminuta* at a challenge level of > 1 x 10^7 CFU. The filters that were tested had Forward Flow values ranging from 16.1 to > 60 mL/min when tested water wet at 2060 mbar (30 psi) air test pressure.

These data support the use of the following Forward Flow integrity test limits.

Forward Flow Integrity Test Parameters*

Test pressure	2060 mbar (30 psi)
Wetting liquid	Water
Temperature	20°C ± 5°C (68°F ± 9°F)
Test gas	Air
Maximum allowable Forward Flow limit**	50 mL/min

* See Section 2.2 for test procedure.
** During the test period the temperature of the filter assembly should not vary by more than ± 1°C (1.8°F).

Users of **Supor** UEAV filters can be assured that filters that pass the Forward Flow test will provide typical titer reductions in excess of 1 x 10^7 as demonstrated using aqueous challenge tests with *B. diminuta.*

3. Endurance to Steam Sterilization

3.1 Introduction

The purpose of these tests was to determine the effects of repeated in-line steam sterilization cycles at 125°C (257°F) on filter integrity using standard **Supor** UEAV filters from production.

3.2 Summary of Methods

Typical **Supor** UEAV filters from three production batches (part number AB1UEAV7PH4) were used for the tests. The filters were flushed with DI water at a flow rate of 10 L/min for 10 minutes and then Forward Flow integrity tested using an air test pressure of 2060 mbar (30 psi).

The filters were subjected to a one-hour in-line steam cycle at 125°C (257°F). As the steam was initially introduced to the filter assembly, the vent and drain valves of the housing were slightly open and the steam flow was controlled so that the differential pressure across the filter assembly did not exceed 300 mbar (4.35 psi) in the forward direction. On completion of the steam cycle, dry compressed air was flushed across the upstream side of the filter for 30 minutes in order to replace the steam and cool the filter assembly. The filters were then flushed with water again prior to starting the next steam cycle.

The above sequence was repeated until each filter had been exposed to 19 steam cycles. At intervals during the steam cycles the filters were cooled to room temperature and integrity tested using the Forward Flow test method.

For further details about steaming Pall filter cartridges, please refer to 'Steam Sterilization With Replaceable Filter Cartridges', **Pall** publication USTR805.

3.3 Results

The Forward Flow integrity test results for the **Supor** UEAV filters (part number AB1UEAV7PH4) measured at intervals during exposure to one-hour steam cycles at 125°C (257°F) are shown in Table 3-1. Filters from three separate manufacturing batches (lot numbers: IG6364, IG6729 and IG6730) were included in the tests and all of the filters retained integrity following exposure to 19 one-hour cycles at 125°C (257°F).

Table 3-1 Effects of Exposure to In-Line Steam at 125°C (257°F) on Filter Integrity for Supor UEAV Filters (Part Number AB1UEAV7PH4)

Pall Filter Serial Number	Forward Flow* (mL/min) Measured At				
	Start of Test	After 3 cycles	After 6 cycles	After 9 cycles	After 19 cycles
IG6364074	20.9	22.9	20.4	22.1	21.4
IG6364016	25.2	25.4	25.1	21.6	22.5
IG6729053	22.7	23.4	21.5	21.7	22.6
IG6730051	24.5	27.2	24.6	25.3	24.8
IG6730004	27.4	30.2	26.3	27.7	26.1

* Forward Flow values at 2060 mbar (30 psi) air test pressure, wet with water, temperature 20°C ± 5°C (68°F ± 9°F), maximum allowable limit value 50 mL/min.

3.4 Conclusions

Supor UEAV filters (part number AB1UEAV7PH4) have been demonstrated to be capable of withstanding multiple in-line steam sterilization cycles. Five filters were tested and all were found to retain integrity following exposure to 19 one-hour steam cycles at 125°C (257°F), which provides an adequate safety margin for the 10 cycle claim.

4. Determination of Water Flow Characteristics

4.1 Introduction

The aim of these tests was to determine the typical differential pressure measurements across **Supor** UEAV filters, part number AB1UEAV7PH4, at set water flow rates.

4.2 Summary of Methods

The tests were performed on standard production filters (part number AB1UEAV7PH4). Four filters were tested and these included filters sampled from three different manufacturing batches (lot numbers: IG6364, IG6729 and IG6730). The test filters were installed in an appropriate housing and pre-filtered DI water was pumped through the filters in the normal flow ('out to in') direction. Pressure transducers positioned on the upstream and downstream sides of the filter element were monitored to calculate the differential pressure at set water flow rates. The pressure transducers were positioned in such a way that the pressure losses from the housing and associated pipes were eliminated. All data were corrected for a standard temperature of 20°C (68°F).

4.3 Results

The water flow/differential pressure measurements obtained using **Supor** UEAV filters (part number AB1UEAV7PH4) are shown in Figure 4-1 and Table 4-1. The data in the graph represent the average values from measurements taken using four different filters.

Figure 4-1 Water Flow/Differential Pressure Characteristics of Supor UEAV Filters, Part Number AB1UEAV7PH4

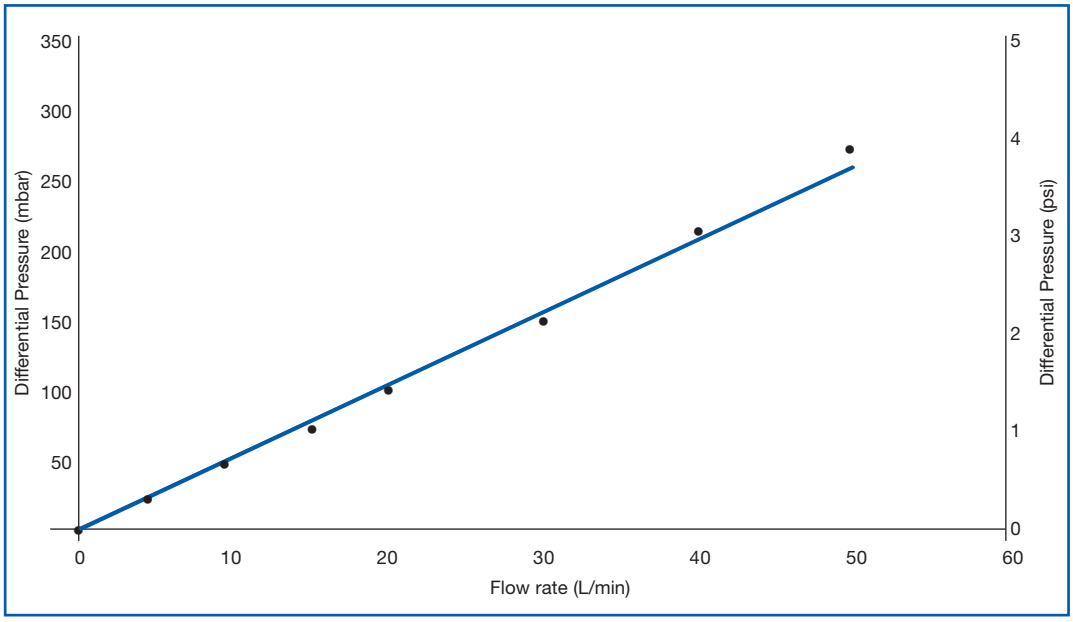


Table 4-1 Water Flow/Differential Pressure Characteristics of Supor UEAV Filters, Part Number AB1UEAV7PH4

Pall Filter Serial Number	Differential pressure at 20°C (68°F) at following water flow rates				
	10 L/min	20 L/min	30 L/min	40 L/min	50 L/min
IG6729038	46 mbar (0.67 psi)	97 mbar (1.41 psi)	150 mbar (2.18 psi)	213 mbar (3.09 psi)	271 mbar (3.93 psi)
IG6730002	47 mbar (0.68 psi)	102 mbar (1.48 psi)	148 mbar (2.15 psi)	218 mbar (3.16 psi)	269 mbar (3.90 psi)
IG6364028	46 mbar (0.67 psi)	94 mbar (1.36 psi)	147 mbar (2.13 psi)	209 mbar (3.03 psi)	267 mbar (3.87 psi)
IG6364058	48 mbar (0.70 psi)	98 mbar (1.42 psi)	153 mbar (2.22 psi)	209 mbar (3.03 psi)	275 mbar (3.99 psi)
Average Values	47 mbar (0.68 psi)	98 mbar (1.42 psi)	150 mbar (2.17 psi)	212 mbar (3.08 psi)	270 mbar (3.92 psi)
Standard Deviation	0.96 mbar (0.01 psi)	3.30 mbar (0.05 psi)	2.64 mbar (0.04 psi)	4.27 mbar (0.06 psi)	3.42 mbar (0.05 psi)

4.4 Conclusions

Water flow rates at set differential pressures have been determined. The average pressure drops for AB1UEAV7PH4 filters have been presented at a number of applied clean water flow rates and these data can be used to assist users in sizing filter systems employing **Supor** UEAV filters.

5. Extractables Testing using Water

5.1 Introduction

The aim of this series of tests was to quantify and characterize the material that can be extracted from **Supor** UEAV filters (part number AB1UEAV7PH4) using water.

5.2 Summary of Methods

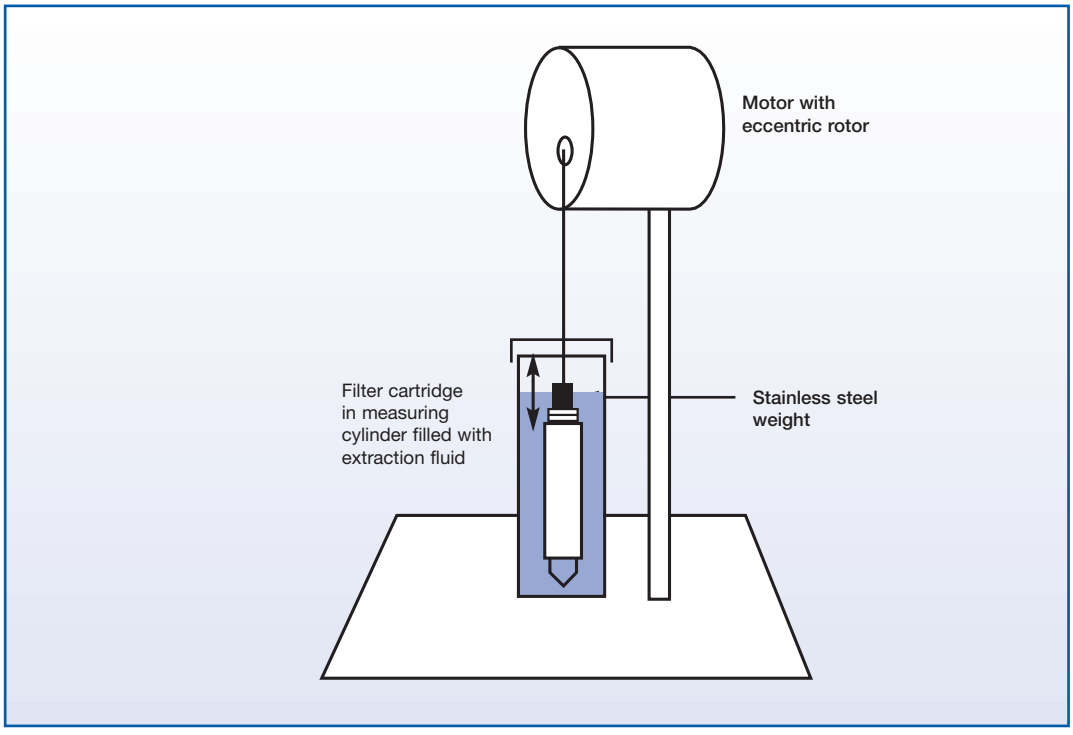
Preparation of Filter Samples

Tests for extractables were performed on typical filter cartridges that had been autoclaved in order to maximize the quantity of any extractable material present. The filters were wrapped in aluminum foil and autoclaved for one hour at 121°C (250°F). Visible droplets of water remaining on the filter elements were allowed to evaporate before the extraction was performed.

Extraction Procedure

Dynamic extraction tests were performed in water at ambient temperature 20°C (68°F) ± 3°C. The test filters were immersed in 1500 mL of water in a clean graduated measuring cylinder, as shown in Figure 5-1. For four hours the filter was gently moved up and down. This movement of the filter created flow through the membrane as a result of the pressure head that was created each time that the filter was partially lifted out of the liquid.

Figure 5-1 Filter Extraction Apparatus



Analysis of Material Extracted

After the extraction, a known volume of the extraction fluid was evaporated to dryness and the non-volatile extractable material was determined gravimetrically. A correction to the measured residue was made to take account of the material extracted in the full 1500 mL volume. A sample of the material extracted was analyzed by Fourier Transform Infra Red Spectroscopy (FTIR).

5.3 Results

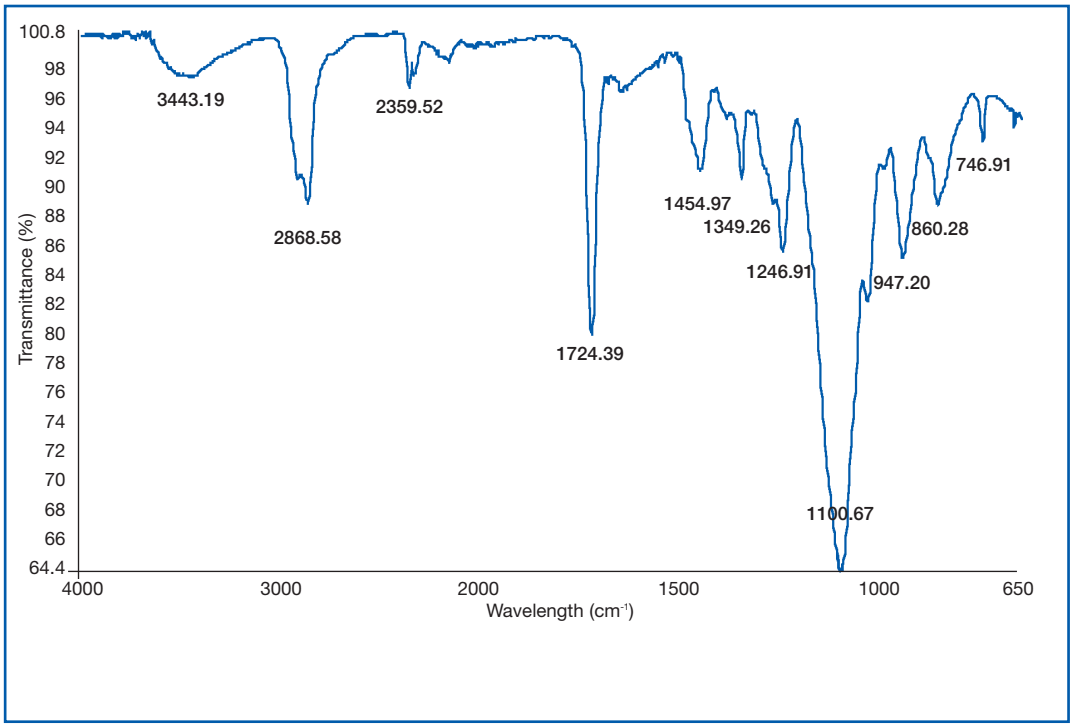
Table 5-1 shows the typical level of aqueous extractables obtained from filters taken from three separate production batches (lot numbers IG6364, IG6729 and IG6730) of **Supor** UEAV filters (part number AB1UEAV7PH4).

Table 5-1 Non-volatile Aqueous Extractables obtained using Supor UEAV Filters, Part Number AB1UEAV7PH4

Pall Filter Serial Number	Non-volatile Residue (mg)
IG6364004	16
IG6364006	20
IG6364070	23
IG6729019	24
IG6729026	44
IG6729047	50
IG6730021	21
IG6730042	50
IG6730050	43

A typical infra red spectrum (Figure 5-2) of one of the aqueous extracts obtained from a **Supor** UEAV filter indicates the presence of extractables typical of polyethersulfone resins and the methacrylate copolymer used to render the membrane hydrophilic.

Figure 5-2 Infra Red Spectrum of the Aqueous Extractables from Supor UEAV Filters



5.4 Conclusions

The typical amount of non-volatile residue extracted from **Supor** UEAV filters has been determined using water as the extraction fluid. For the 254 mm (10 inch) filter elements that were tested (part number AB1UEAV7PH4), the non-volatile residue measured was ≤ 50 mg per 254 mm (10 inch) filter.

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

6. Transmission of Proteins

6.1 Introduction

The aim of this study was to determine the transmission of protein solutions through **Pall Supor** UEAV filter membrane using Bovine Serum Albumin (BSA) and IgG in a perfusion challenge technique.

6.2 Summary of Methods

Solutions of 125I-labelled BSA and IgG were prepared in phosphate buffered saline (PBS). The protein concentration in the challenge solutions was 0.1 mg/mL.

Prior to the tests, the filter disc holders and associated tubing were exposed to the challenge protein solution in order to minimize any subsequent protein adsorption by the test assembly.

Discs of **Supor** UEAV filter membrane (13 mm diameter) were then installed in the disc holders and the challenge solutions were pumped through the discs at a flow rate of 0.5 mL/min until a total volume of 2.5 mL had been passed through each disc. Following filtration excess liquid was carefully blotted from the surface of the discs. The amount of 125I-labelled protein retained by each disc was determined using a gamma counter.

Protein transmission was calculated by subtracting the amount of protein on the filter disc from the total in the original challenge solution. Each test was performed in triplicate.

6.3 Results

The transmission of BSA and IgG through **Supor** UEAV membrane is shown in Tables 6-1 and 6-2. In all cases the measured protein transmission was in excess of 98 %. The volume filtered through the discs in this study represents less than 50 L being passed through a full-size 25 cm (10 in.) filter cartridge.

Table 6-1 Transmission of BSA through Supor UEAV Filter Membrane

Filter	Transmission of BSA
Sample 1	99.0 %
Sample 2	98.9 %
Sample 3	99.2 %

Table 6-2 Transmission of IgG through Supor UEAV Filter Membrane

Filter	Transmission of BSA
Sample 1	98.9 %
Sample 2	98.9 %
Sample 3	98.9 %

6.4 Conclusions

This study demonstrates that very high protein transmission can be obtained when filtering dilute solutions through **Supor** UEAV filter membrane.

7. Biological Reactivity Tests on the Materials of Construction

7.1 Introduction

The aim of this study was to evaluate the biological suitability of the materials of construction of **Supor** UEAV cartridges. The materials of construction of the filters are as follows:

Membrane	**Pall** hydrophilic asymmetric polyethersulfone membrane
Membrane support and drainage layers	Polypropylene
Core and endcaps	Polypropylene
Filter cage	Polypropylene
O-rings	Silicone elastomer for 'H4' option

7.2 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 Pharmacopoeia (USP).*

The testing procedures described in the USP include:

- **Injection of extracts of plastic materials**
- **Implantation of the solid material into animal tissue.**

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 filter materials 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 materials of the **Supor** UEAV were implanted separately.

7.3 Results

No biological response was observed in any of the tests performed and therefore the materials used in **Supor** UEAV filters passed all of the tests specified.

7.4 Conclusions

The materials used in **Supor** UEAV filters met the requirements of the USP Biological Reactivity Tests *(in vivo)* for Class VI-121°C plastics. The tests included the systemic injection test, the intracutaneous test and the implantation test.

Life Sciences

2200 Northern Boulevard
East Hills, New York 11548-1289

+1 516 484 5400 phone
+1 516 801 9548 fax
pharmafilter@pall.com e-mail

Europa House, Havant Street
Portsmouth PO1 3PD, United Kingdom
+44 (0)23 9230 3303 phone
+44 (0)23 9230 2506 fax
BioPharmUK@europe.pall.com e-mail

Visit us on the web at www.pall.com

Pall Corporation has offices and plants throughout the world in locations including: Argentina, Australia, Austria, Belgium, Brazil, Canada, China, France, Germany, India, Indonesia, Ireland, Italy, Japan, Korea, Malaysia, Mexico, the Netherlands, New Zealand, Norway, Poland, Puerto Rico, Russia, Singapore, Spain, South Africa, Sweden, Switzerland, Taiwan, Thailand, United Kingdom, the United States and Venezuela. Distributors are located in all major industrial areas of the world.

10.2004

Filtration. Separation. Solution.SM