



Life Sciences

Validation Guide

USTR 2376 Rev A

Pall Supor[®] TFF Membrane Cassettes

Centramate[™], Centrasette[™], Maximate[™], Maxisette[™]



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1 Purpose of this Document

This document provides validation support information for Pall Supor® TFF membrane cassettes, and includes summary data to support testing conducted for biological safety, extractables, chemical compatibility, physical and performance attributes, as well as usage conditions (temperature limits, chemical limits, cleaning, flushing, integrity testing, operating methods).

The data contained in this Guide is generated under standard conditions as specified. The methods and information contained in this Guide are designed to provide the user with an acceptable approach for validation of Supor TFF membrane cassettes under actual conditions of use.

Pall Life Sciences offers technical support to customers to develop, troubleshoot, and validate tangential flow filtration procedures.

1.1 Validating Filtration Processes-General Concepts

Tangential flow membrane cassettes play an important role in purifying, concentrating, and separating biopharmaceutical solutions and products. Typical applications include concentrating human plasma fractions, downstream processing of enzyme and protein solutions, and harvesting mammalian or bacterial cells. Hence, the validation of tangential flow filtration processes utilizing membrane cassettes is an essential part of ensuring the manufacture of safe and efficacious products.

The U.S. Food and Drug Administration defines validation as "establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specification and quality attributes" (Current Good Manufacturing Practice (cGMP) for Finished Pharmaceuticals, 21 CFR 210.3). With respect to a tangential flow filtration process, validation involves providing assurance that the filtration process operates reproducibly and consistently.

For any given process, a Functional Design Specification must be written based on the requirements of the process and data generated at the pilot scale. For a tangential flow filtration process utilizing the Supor TFF membrane cassette, this will include developing operational protocols within performance limits outlined in this validation guide and based on the individual cassette operating instructions and care and use manuals supplied with cassettes.

A process system can then be designed and built to allow direct scale-up to meet specifications established at pilot or bench scale. Since tangential flow membrane cassettes are incorporated into complex systems, three stages of system validation are followed: Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ).

1.2 Installation Qualification (IQ)

This checks that the cassettes selected for the process are the ones supplied and installed in the system, and that specified installation procedures such as torque settings have been adhered to. Additionally, it is confirmed that all required documentation has been received (operating instructions, and certificates of conformance) with the cassettes.

1.3 Operational Qualification (OQ)

Engineers test and document the range and operational limits of the filtration process with cassettes in place. Operational qualification (OQ) does not have to be conducted in the customer process manufacturing area. Engineers normally simulate worst-case production conditions for these studies, using water or another surrogate process fluid, to deliberately trigger alarm conditions. As part of the operational qualification, engineers also verify and document procedures such as flushing and sanitizing that are associated with the operation of the membrane cassettes.

1.4 Performance Qualification (PQ)

Performance qualification (PQ) involves testing the cassette filtration process during production of the final product under actual operating conditions, including installation, sanitizing, conditioning, concentration, diafiltration,

product recovery, cleaning, etc. Critical elements of performance qualification include verification of chemical compatibility and retention characteristics. Since validating a process ensures the process accomplishes what is intended, performance qualification provides the most meaningful process validation data (which will be confirmed by ongoing performance data collected during system operation) because the data is derived from the process itself, utilizing the intended operating conditions. PQ may not necessarily provide data on the operation of the system at the design limits (alarm conditions), as the process may never reach these.

Manufacturers of regulated products must develop and submit protocols, qualification documents, and validation documents for their specific product to be granted approval to manufacture and market their product.

2 Product Specifications — Supor TFF Membrane Cassettes

To help you prepare IQ documentation, this section provides you with information on the materials of construction, physical characteristics, and basic performance of Supor TFF membrane cassettes.

Membrane cassettes are individually packaged in heat-sealed plastic bags with the following information printed on the cassette edge (Figure 1):

- Company name
- Membrane type
- Molecular weight cut-off or pore size
- Cassette format
- Membrane area
- Feed channel format
- Part number
- Serial number (formerly called a lot number)

are shipped in a box containing two silicone gaskets in a plastic bag, Certificate of Test, *Membrane Cassettes Care and Use Procedures* (R00640 Rev A), and MSDS documents (where appropriate).

2.1 Part Numbers

Part numbers give specific information about the cassette. For example, the part number PSM20F07 represents a Supor 0.2 µm membrane, 0.5 m² (5.4 ft²) Centrasette™ II suspended screen channel cassette.

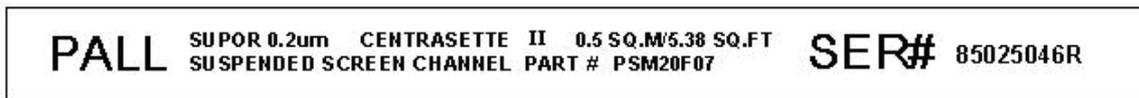


Figure 1: Example of Information Printed on the Side of Cassettes

The Part Number for a cassette can be interpreted to identify specific information about the cassette characteristics.

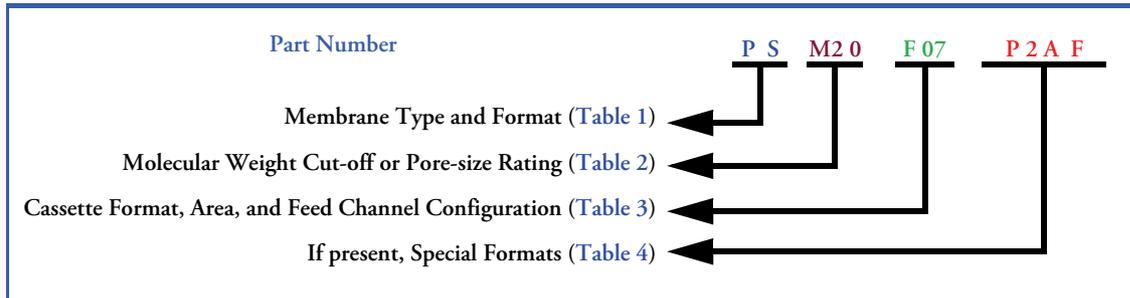


Figure 2: Part Number Code

Table 1: Identification Code for Membrane Type

| Part Number (Digits 1 – 2) | Membrane Format |
|----------------------------|--------------------|
| PS | Supor TFF Cassette |

Table 2: Identification Codes for Pore Size Rating

| Part Number (Digits 3 – 5) | Pore Size |
|----------------------------|-----------|
| M10 | 0.1 µm |
| M20 | 0.2 µm |
| M45 | 0.45 µm |
| M65 | 0.65 µm |

Table 3: Identification Codes for Cassette Format and Feed Channel Configuration

| Part Number (Digits 6 – 8) | Cassette Format, Feed Channel Configuration | Membrane Area (Nominal) | |
|----------------------------|---|-------------------------|----------------|
| | | ft ² | m ² |
| C11 | Centramate™ Suspended Screen ⁽¹⁾ | 1 | 0.093 |
| F07 | Centrasette™ II Suspended Screen | 5.4 | 0.5 |
| F22 | Centrasette™ II Suspended Screen | 21.5 | 2.0 |
| F27 | Centrasette™ II Suspended Screen | 26.9 | 2.5 |
| G02 | Maximate™ Suspended Screen | 2 | 0.19 |
| C52 | Maxisette™ Suspended Screen | 30 | 2.8 |

(1) Suspended screen cassettes have fine screens in the filtrate channels and fine screens suspended between spacers in the feed channel.

Table 4: Identification Codes (if present) for Special Formats

| Part Number (Digits 9–12) | Membrane Area, Grade or Special Designation |
|---------------------------|---|
| P2 | 0.2 ft ² (Centramate) |
| AF | 0.2 – 0.3 N NaOH storage agent |
| SXXX | Special Designations |

2.2 Serial Number

Unique serial numbers enable the tracing of the following cassette information:

- Date of manufacture
- Components used in manufacture
- Water permeability of membrane lot used in construction
- Membrane integrity test results
- K_L test values for membrane lot
- Manufacturing plant location
- Name of the technician who manufactured the cassette

From the serial number and production records, components can be traced back to their source.

2.3 Materials of Construction

2.3.1 Membrane

Supor membranes are cast unsupported from polyethersulfone resins. Released lots of membrane are laminated on a highly porous polyolefin backing (substrate) that imparts strength and rigidity to the finished membrane.

2.3.2 Screens

Screens are constructed of polyester.

2.3.3 Spacers

Supor TFF suspended screen channel cassettes incorporate a screen suspended between spacers in the feed/retentate channel. The spacers are constructed of natural HDPE with a nominal thickness of .18 mm (.007 in.)

2.3.4 Encapsulant

The encapsulant is polyurethane.

2.3.5 Gaskets

Gaskets are constructed from USP Class VI medical grade, platinum-cured silicone.

All Supor cassettes except F22 and F27 cassettes use silicone gaskets with a nominal thickness of 0.79 mm (1/32 in.). Supor F22 and F27 cassettes use thicker silicone gaskets with a nominal thickness of 1.6 mm (1/16 in.).

2.4 Dimensions

Pall membrane cassettes are manufactured in a range of formats, and membrane areas (Table 5). This allows the ability to directly scale up or down depending on requirements.

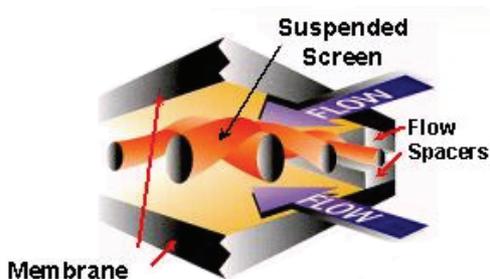


Figure 3: Cassette Suspended Screen Channel Configurations

Table 5: Physical Dimensions of Supor TFF Suspended Screen Channel Membrane Cassettes (nominal)

| Characteristic | | Centramate | | Centrasette II | | | Maximate | Maxiset |
|---|--------------------|------------|-------|----------------|------|-----|----------|---------|
| | | | | | | | | |
| Membrane Area | (m ²) | 0.019 | 0.093 | 0.5 | 2.0 | 2.5 | 0.19 | 2.8 |
| | (ft ²) | 0.2 | 1 | 5.4 | 22 | 27 | 2 | 30 |
| Weight (kg) Suspended ⁽²⁾ | | .04 | 0.17 | 0.45 | 2.4 | 3.0 | 0.36 | 3.2 |
| Thickness (cm) suspended | | 0.4 | 1.8 | 1.8 | 8.2 | 10 | 2.1 | 2.1 |
| Length (cm) | | 20 | 20 | 20 | 20.8 | | 33 | 58 |
| Width (cm) | | 5.4 | 5.4 | 17 | 17.8 | | 6.5 | 33 |
| Flow path length (cm) (port center to center) | | 17 | 17 | 17 | | | 28 | 28 |
| Flow path width (cm) | | 2.8 | 2.8 | 14 | | | 4.0 | 54 |
| Port diameter feed (cm) | | 0.9 | 0.9 | 1.0 | | | 1.2 | 1.2 |
| Number of feed ports | | 1 | 1 | 5 | | | 1 | 15 |
| Port diameter retentate (cm) | | 0.9 | 0.9 | 1.0 | | | 1.2 | 1.2 |
| Number of retentate ports | | 1 | 1 | 5 | | | 1 | 15 |
| Port diameter permeate (cm) | | 0.7 | 0.7 | 0.6 | | | 0.9 | 1.2 |
| Number of permeate ports | | 2 | 2 | 8 | | | 2 | 14 |

(2) Approximate weight of a cassette as shipped in plastic bag (no outer packaging). The cassette is wetted with a storage solution and drained. Weights may vary due to different amounts of storage solution remaining in the cassette.

2.5 Operating Pressures and Temperatures

Membrane cassettes have operating limits for pressure, temperature, and pH (Table 6).

Table 6: Cassette Operating Limits of Pressure, Temperature, and pH

| Membrane | Temperature | Maximum Recommended Operating Pressure | | pH Range | |
|-------------------------|-------------|--|--------------------------|---------------------|-------------------|
| | | | | Continuous at 25 °C | Cleaning at 25 °C |
| Supor TFF (all formats) | 4 – 25 °C | 3 barg ⁽³⁾ | (45 psig) ⁽³⁾ | 1 – 14 | 1 – 13 |
| | 25 – 50 °C | 2 barg | (30 psig) | 1 – 14 | 1 – 13 |

(3) Clamping pressure must be set to the recommended level to avoid leaks.

Two Supor TFF cassettes (PSM65F22) were tested at 4 bar (60 psi) pressure at 25 °C in 0.5 N NaOH for 40 hours (10 cycles of 4 hours). Cassettes met release specifications following the test (Section 3.4 "Evaluation of Supor TFF Cassettes" on page 12). When operating at maximum pressure, cassette holders may need to be set near the upper end of the torque/hydraulic pressure range (Table 13: Recommended Torque Values for Manual-torque Cassette Holders on page 18 and Table 14: Recommended Hydraulic Pressure Values for Supor TFF Membrane Cassettes on page 19).

2.6 Water Flux Ranges

Membrane water flux is a measure of the membrane permeability. Water quality, temperature, and pressure affect the water flux rate. At a minimum, the water used to measure flux should be distilled, deionized (DI), 0.2 µm filtered, or preferably, pharmaceutical grade (USP Water for Injection, hereafter called WFI). The presence of biological organisms, organic materials, or minerals in the water may affect water flux results.

2.6.1 Water Flux Specifications for Supor TFF Membranes

Water flux is measured in stirred cells on 47 mm disc membrane samples stamped out from the beginning, and end of each production lot of membrane (Table 7).

Water flow is determined using a 47 mm disc filter holder. Membrane is wetted out in water and inserted in the disc holder. A measured volume of water at a temperature between 29 – 32 °C is added to the filter holder.

Vacuum is applied (24 inches Hg) and the time is measured to draw the water through the membrane. The water flow is calculated and reported in mL/min/cm². Membranes used in cassette construction must meet the water flux specifications.

Table 7: Water Flux Specifications For Supor TFF Membranes

| Membrane | Water Flux on Disc Membrane | | |
|----------|---|-----|---------------------------------------|
| | mL/min/cm ² @ 24 inches Hg, 30 °C | | Calculated Average LMH/psi @ 20 °C |
| 0.1 | 2.8 | 7.4 | 204 |
| 0.2 | 19 | 35 | 960 |
| 0.45 | 34 | 100 | 2124 |
| 0.65 | 65 | 170 | 4280 |

2.7 Membrane Characterization — K_L Test

Supor TFF membrane is characterized by performing a K_L (quantitative bubble point) test on samples taken from each roll of membrane. Membrane samples are wetted out in water or water/isopropyl alcohol (IPA). Measurements are performed on a K_L Test Stand which records air flow as a function of applied pressure and determines the K_L point (knee location) from programmed algorithms.

2.8 Membrane Integrity — Forward Flow Test Values

The membrane integrity test measures air forward flow rates at specified pressures to determine the integrity of membranes. The air forward flow is a measure of air diffusion through the liquid in the membrane pores plus air leakage around seals. The test identifies gross defects in the cassette membrane or membrane seals. Membrane integrity forward flow test values are given in Table 8 for the different cassette formats. The same integrity test limit values apply for cassettes flushed out with water or preservative solution (20% glycerin, 0.1% NaN₃, or 0.3 N NaOH). The test procedure is described in Section 6.8: Integrity Testing on page 20.

Use only dry filtered air or nitrogen from cylinders (instrument-quality) when using integrity analyzers incorporating mass flow meters. Fluctuations in house air and nitrogen supplies as well as changes in temperature can cause inconsistent results. Fully wet-out the membrane in a cassette prior to performing the membrane integrity test or high forward flow values may be obtained. The procedures for wetting out cassettes and measuring forward flow are described in *Membrane Cassettes Care and Use Procedures*.

Table 8: Membrane Integrity Test — Forward Flow Limits

| Supor TFF Membrane | | | Maximum Air Forward Flow ⁽⁴⁾ | |
|--------------------|-------------|-----------------------------|---|------------------------|
| Cassette Format | Rating (µm) | Test Pressure bar (psig) | (sccm/ft ²) | (sccm/m ²) |
| Centramate | 0.1 – 0.65 | 0.7 (10) | 25 | 270 |
| Centrasette | | | | |
| Maximate | | | | |
| Maxisette | | | | |

(4) Nitrogen can be used in place of air. However, a correction factor on the maximum diffusive flow of 0.84 must be applied if Nitrogen is used as the test gas.

2.9 Shelf Life of New and Used Cassettes

The recommended shelf life of cassettes packed in glycerin and sodium azide is three years from date of manufacture. To achieve satisfactory performance, it is recommended that the cassettes be stored unopened in the original packaging at 4 – 25 °C and protected from direct light. For cassettes packed in 0.3 N sodium hydroxide, shelf life is one year with expiration date shown. Real-time storage studies are on-going. Users should test the membrane integrity prior to use. Contact Pall for shelf life of cassettes packed in alternate solutions.

The useful life of cassettes that are properly conditioned, used, cleaned, stored, and maintained is often more than one year. However, it is not possible to specify a shelf life or useful life of a cassette that has been used or removed from the original packaging. The actual useful life for a cassette will depend on the character and complexity of the product to which it is exposed, composition of process fluids, process temperatures, operating pressures, and cleaning regime. Therefore, customers should validate reuse and the useful life of a cassette in their process. Pall Life Sciences makes no claims of warranty or guarantee of performance related to reuse of cassettes. Consult Pall Life Sciences' *Membrane Cassettes Care and Use Procedures* for recommended storage conditions.

2.10 Chemical Compatibility—Sodium Hydroxide, 0.5 N, 45 – 50 °C

Sodium hydroxide is the most effective and commonly used agent in biological applications for cleaning, sanitizing, and depyrogenating membrane cassettes. To evaluate the chemical resistance of Supor TFF membrane cassettes TFF membrane cassettes, a study was performed in which cassettes were first put through a standard pre-conditioning, characterized and then cycled 20 times (1-hour cycle time) in 0.5 N NaOH at 45 – 50 °C. The following function tests were performed on the cassettes: membrane integrity, water flux, and pressure drop.

Results

After twenty cycles of 1 hr at 45 – 50 °C, the samples were within specification for membrane integrity, and water flux. Details of the procedure are given in [Section 3.3 on page 11](#). Results from testing are given in [Table 11](#) and [Figures 4 and 5](#).

2.11 Chemical Compatibility — General

Chemical compatibility of membrane cassettes can be described in terms of changes in physical characteristics as a result of continuous contact with a chemical solution for several hours. Changes can affect dimensions, hardness, swelling, integrity of internal seals, and membrane integrity. Changes can also be described in terms of functional characteristics of the membrane (such as water permeability, retention characteristics).

[Table 9](#) illustrates the compatibility of Supor TFF membrane cassettes at 20 °C (unless otherwise noted) with respect to physical characteristics. [Table 9](#) should be used only as a guide. Cassettes should be tested in the appropriate solvent and product under actual operating conditions and for an appropriate time to determine compatibility for the specific application. Membrane porosity— and consequently both water permeability and retention characteristics — may be affected. Physical changes to the cassette may be permanent or reversible. To determine if changes are permanent, flush and then soak the cassette in water for one to two days and then test the sample again. Changes in water permeability and solute retention may be due to physical changes in the membrane. Changes in solute retention may be a result of conformational changes in the molecules used to measure retention, or a combination of factors.

Table 9: Membrane Chemical Compatibility Chart ⁽⁵⁾

| | Compatible ✓ |
|----------------------------------|------------------|
| Reagent | Not Compatible ✗ |
| pH Range | 1 – 14 |
| Acetic Acid (5%) | ✓ |
| Alconox* (1%) | ✓ |
| Ammonium hydroxide (5%) | ✗ |
| Citric acid (1%) | ✓ |
| Ethanol (70%) | ✓ |
| Formaldehyde (1%) | ✓ |
| Glycerine (50%) | ✓ |
| Guanidine HCl (6 M) | ✓ |
| Hydrochloric acid (0.1 N) | ✓ |
| Hydrogen peroxide (1%) | ✓ |
| Phosphoric acid (0.1 N) | ✓ |
| Sodium dodecyl sulfate (0.01M) | ✓ |
| Sodium hydroxide (0.5 N @ 50 °C) | ✓ |
| Sodium hypochlorite (0.05%) | ✓ |
| Terg-a-zyme* (1%) | ✓ |
| Triton** X-100 (0.002 M) | ✓ |
| Urea (25%) | ✓ |

(5) Data for cassette membrane and components at 20 °C, 24-hour exposure, unless otherwise noted. There may be changes in porosity and/or selectivity of membrane.

* Alconox and Terg-a-zyme are trademarks of Alconox, Inc.

** Triton is a trademark of Dow Chemical Company.

2.12 Protein Binding Characteristics

Supor TFF membranes have low nonspecific adsorption characteristics. The actual amount of protein or other substances that will adsorb (nonspecifically bind) to the membrane is dependent on the specific characteristics of that substance. Adsorption of a molecule to a membrane can vary to a great extent depending on its environment (i.e., the chemical composition of the solution it is in, as well as the number and concentration of other solutes present). Changes in pH, ionic strength, temperature and concentration have a significant effect on binding properties. Buffer salts, detergents, and organic solvents also influence binding. If adsorption is a concern, then a study can be performed on a disc membrane using the actual sample and buffer to determine the level of nonspecific adsorption.

3 Validation Procedures

This section describes the procedures Pall used to validate specific chemical and physical characteristics of the Supor TFF membrane cassettes.

3.1 Extractables Test

3.1.1 Introduction

The purpose of this test is to quantify and characterize the nonvolatile materials that may be extracted from Supor TFF membrane cassettes into aqueous products. The extracted material was analyzed by infrared (FTIR) and ultraviolet spectrophotometry. The cassettes tested contained approximately 0.5 m² (5.3 ft²) of a polyethersulfone filter membrane interleaved between polyester screens. Polyolefin spacers suspend the screens in the feed/retentate channels. The repeating layers of membrane, screen, and spacer are encapsulated with a polyurethane sealant. Centramate™, Centrasette™, Maximate™ and Maxisette™ cassettes are constructed from the same raw materials and by the same procedure. Therefore the type of extractables determined for one format should be the same for all formats.

3.1.2 Summary of Method

Supor TFF suspended screen channel membrane cassettes from inventory, catalog number PSM65F07, were tested for extractable material. The cassettes were flushed and sanitized following protocols generally outlined in *Membrane Cassettes Care and Use Procedures*. The test cassettes were then subjected to a rigorous extraction procedure by recirculating either water at 46 – 50 °C or 25% ethanol at 20 – 25 °C for 16 hours according to the extraction procedure described in [Section 7.1: Characterization of Extractables on page 24](#). After this extraction period, the fluid was drained and collected from the system for analysis. A second 16-hour extraction of each cassette (with the same solvent and conditions) followed by recovery of the extraction fluid was then performed to demonstrate presence of additional extractables. These test conditions were chosen to maximize the amount of extracted matter. A negative control (holder and cut-out gasket only, no cassette) and positive control (known amount of a known substance) were performed for each test solvent. Collected samples and controls were analyzed by ultraviolet/visible absorption spectroscopy over the range 200 – 800 nm. A 1,000 mL aliquot of each extract was evaporated to dryness and the amount of nonvolatile residue was weighed and characterized by infrared spectroscopy. Details of the test methodology are provided in [Section 7.1](#).

3.1.3 Results

Two successive 16-hour extractions of three Pall Supor TFF suspended screen channel membrane cassettes (P/N PSM65F07) in Water and an Aqueous 25% Ethanol Solution produced a total nonvolatile residue (NVR) of under 50 and 73 mg per cassette, respectively. Infrared spectroscopy of the NVR showed that the materials extracted were composed primarily of glycerin as well as trace amount of oligomers from polyurethane. Successive extractions of each filter showed a significant decrease in nonvolatile residue, demonstrating depletion of extractables available to solvent. Therefore, these results will be valid regardless of volume of solution processed, and for contact times up to, and probably beyond 32 hours. We conclude that extractables from a PSM65F07 cassette [membrane area 0.5m² (5.3 ft²)] into dilute aqueous pharmaceutical products containing less than 25% compatible organic constituents that can be modeled with ethanol are unlikely to significantly exceed 73 mg nonvolatile residue, and will be composed primarily of the materials described above.

A sample representative of this filter cassette type (or of its component parts) met the requirements of the *United States Pharmacopeia* (USP) Class VI-70 °C USP Class VI, 70 °C Biological Reactivity Tests for Plastics, demonstrating that extractable materials did not produce any detectable toxic effects under the test conditions.

The results listed above are based on cassettes that have been pre-conditioned following a relatively short flushing and sanitization protocol. Using a more extensive flushing and sanitization protocol will significantly reduce extractables that could potentially get into pharmaceutical products.

3.2 Cassette Flushing Procedures to Remove Storage Agents

Supor TFF membrane cassettes are shipped wet, in liquid containing a humectant and bactericidal storage solution. This solution consists of DI water containing approximately 15 – 20% glycerin, which serves to prevent the

membranes from drying out, and 0.05 – 0.1% sodium azide, a bactericidal agent. The storage solution must be removed and the cassette flushed well with water prior to use to prevent product contamination. The purpose of this study was to determine the effectiveness of recommended flushing procedures for removal of these agents.

3.2.1 Sanitization and Flush Procedure for Supor TFF Membrane Cassettes

The procedure recommended for flushing these agents out of the cassette prior to use is described in *Membrane Cassettes Care and Use Procedures*.

3.2.2 Summary of Method

To evaluate the effectiveness of this procedure for removal of the storage solution from Supor TFF, suspended screen channel membrane cassettes, a test was conducted using the following procedure:

1. A Centrasette II cassette with 0.2 μm Supor TFF membrane (PSM20F07) was installed in a cassette holder that had been previously flushed. The system was flushed with water, approximately 4 L/ft² through the retentate followed by 4 L/ft² through the permeate.
2. Cassettes were sanitized using 0.1 N NaOH (1 L/ft²) at room temperature by recirculating the solution through both the retentate and permeate for 1 hr.
3. The sanitizing solution was drained from the system.
4. Distilled Water was flushed through the retentate for two minute at 1 L/min/sq. ft.
5. The permeate valve was opened and retentate valve adjusted until the retentate flow rate was approximately 10% of the feed flow rate (1 L/min/ft²).
6. Timer started with samples taken at start and then at periodic intervals until TOC was reduced to below 500 ppb.

In addition to the flushing study described above, a study on the flushed cassettes was performed to evaluate any residual material in the cassettes that could be extracted.

3.2.3 Summary of Extraction Method

1. Drain and add 5 L of water to system
2. Adjust feed flow rate to 5 L/min.
3. Open permeate valve and then close retentate valve until the retentate flow rate is approximately 10% of the feed flow rate.
4. Circulate for 1 hour.
5. Determine TOC of solution in reservoir.

3.2.4 Results

The flushing procedure was performed in triplicate. The TOC level of the flushing solution was less than 500 ppb at the initial sampling after completion of the sanitization step. Results show that using an initial water flush (4 L/sq. ft. through retentate followed by 4 L/sq. ft. through permeate) followed by a sanitization step (0.1 N NaOH, 5L/sq. ft., 1 hr recirculation) effectively removed the storage agent in the cassette. Additional flushing is still necessary to completely remove the sanitizing solution. Specific volume and additional flushing required may need to be evaluated for particular applications.

An extraction study showed that there was still a trace level of material diffusing out from the cassette after flushing. Approximately 6 ppm in 5 L of water (1 L/sq. ft.) after 1-hour recirculating at ambient temperature (for an average total residual of 30 mg) was measured. From previous work, the TOC is expected to be residual glycerin.

Table 10: Results of Supor TFF Cassette Flushing Study

| Supor TFF 0.2 micron suspended screen cassette - PSM20F07 | | | |
|---|-----------------------------------|--------------|---------------------|
| Cassette Serial Number | Rinse down time* (min) to 500 ppb | Residual TOC | Total Residual (mg) |
| 82260011R | Immediate | 4.8 ppm | 24 mg |
| 82260012R | Immediate | 5.9 ppm | 30 mg |
| 82308036R | Immediate | 7.2 ppm | 36 mg |

3.2.5 Conclusions

The concentration levels of glycerin and sodium azide decreased rapidly from both the retentate and filtrate channels of 0.2 µm Supor TFF Centrasette membrane cassettes with DI water flushing.

3.3 Chemical Compatibility — Sodium Hydroxide, 0.5 N, 45 – 50 °C

To evaluate the chemical resistance of Supor TFF membrane cassettes TFF membrane cassettes, a study was performed in which cassettes were first put through a standard pre-conditioning, characterized and then cycled 20 times (1 hr cycle time) in 0.5 N NaOH at 45 – 50 °C. Conditions were chosen to simulate *worst case*. For this study 0.2 µm Supor TFF membrane cassettes (PSM20F22) were used. The following function tests were performed on the cassettes: membrane integrity, water flux, and pressure drop.

Summary of Method

1. Install cassettes in a Centrasette AT Cassette Holder using 1/16 in. gaskets between cassettes. Set clamping force to 1100 psig.
2. Flush with water (DI / 0.2 µm filtered).
3. Characterize cassette as a stack for NWP, Air Integrity and Pressure drop
4. Perform each cleaning cycle using 0.5 N NaOH, at the following conditions:
 - (i) Time = 1 hour
 - (ii) Feed Pressure 2 bar
 - (iii) CFF = 1.5 - 2.0 L/min/ft²
 - (iv) Temp. = 50 °C
5. Cool down
6. Flush with water (ambient temperature).
7. Characterize cassettes as stack for NWP, Air Integrity and Pressure drop at required intervals
8. Repeat cleaning cycle and flush cycles for a total of 20 cycles.
9. Characterize cassettes as stack for NWP, Air Integrity and Pressure drop
10. Compare initial and final results for individual and stack cassette conditions.

Results

After twenty cycles of 1 hr at 45 – 50 °C, the samples were within specification for membrane integrity, and water flux.

Table 11: Results from Compatibility Study of Supor TFF Cassettes with 0.5N NaOH

| Cassette & Serial No. | Initial Characterization (average values) | | | Final Characterization (20 cycles) (average values) | | |
|-----------------------|--|-----------------------------------|---|--|-----------------------------------|---|
| | NWP (LMH/psi) | ΔP @ 1.0 L/min/ft ² | Integrity @ 5 psi sccm/ft ² | NWP (LMH/psi) | ΔP @ 1.0 L/min/ft ² | Integrity @ 5 psi sccm/ft ² |
| PSM20F22 | | | | | | |
| 84180014R | 60 | 9.5 | < 1 | 50 | 9.0 | <1 |
| 84175037R | | | | | | |
| 84175038R | | | | | | |
| PSM65F22 | | | | | | |
| 84014018R | 230 | 2 | 3 | 190 | 3 | 4 |
| 84014019R | | | | | | |
| 84014020R | | | | | | |

The graphs below show the Water Permeability vs. TMP and DP vs. CFF data generated from cycle testing of the PSM65F22 cassettes.

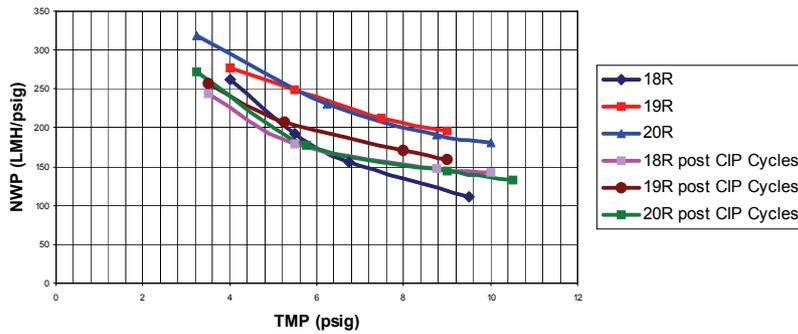


Figure 4: Water Permeability vs. Transmembrane Pressure

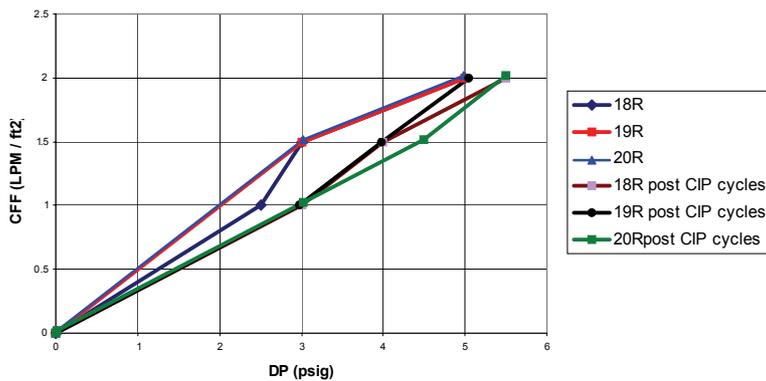


Figure 5: Cross Flow Flux Rate vs. Delta P

3.4 Evaluation of Supor TFF Cassettes at an Operating Pressure of 4 bar (60 psi) @ 25 °C in 0.5 N NaOH

3.4.1 Summary

Two Pall Supor TFF Cassettes, PSM65F22, were installed in a holder, preconditioned and characterized for water permeability, air integrity, pressure drop (feed/retentate) and edge thickness. A solution of 0.5 N NaOH @ 25 °C was first flushed through the cassettes then circulated through both the retentate and permeate at a cross flow rate between 0.5 – 1.0 LPM/ ft² and a feed pressure of 4bar (60 psi) for 4 hrs. Cassettes were then flushed with water and characterized as before the test. The characterization (in water) and circulation procedure in 0.5 N NaOH @ 25 °C and 4 bar (60 psi) feed pressure, was repeated for a total of 10 cycles (40 hours).

3.4.2 Test Results:

No significant changes were noted to either test cassette with respect to water permeability, air integrity, pressure drop (feed/retentate) or dimensions (thickness). Both cassettes were within release specifications following the test. Supporting data is presented in Table 12 and Figures 6, 7, and 8.

3.4.3 Equipment:

Two 0.65 µm Supor TFF suspended screen channel cassettes, 2 m² (PSM65F22) Serial Numbers 85186095R and 85183008R

Centrasette-10 holder with 3 gauge fitting kit

Stainless steel tank with conical bottom, 5 gal capacity (approximate)

Quattroflow* 4000 Pump

*Quattroflow is a trademark of Quattroflow Fluid Systems.

3.4.4 Procedure:

1. Measure dimension (thickness) of the cassettes at urethane edges
2. Perform characterization on two cassette samples for NWP, Delta P, and Integrity (Test air integrity (IT) at 3 and 10 psi).
3. Place both cassettes in C-10 AT holder and apply 1100-psi hydraulic clamp pressure.
4. Flush retentate to drain with 15 gallons of deionized water with filtrate slightly open (do not exceed 30 psi feed).
5. Flush filtrate to drain with 50 gallons of deionized water with retentate closed or slightly open (do not exceed 30-psi feed)
6. Prepare 0.5 N NaOH @ 25 °C (approximately 20 L/cycle)
7. Recirculate caustic and slowly increase flow rate
8. Achieve 60 psi feed pressure and if possible maintain the cross flow rate between 0.5 – 1.0 LPM/ft²
9. Run 4-hour recirculation
10. Flush the cassette (repeat steps 4 and 5), check pH.
11. Repeat steps 1 and 2, (check the dimensions immediately after removing cassettes from the holder, and characterize cassettes within 24 hours).
12. Repeat steps 1 through 11 for additional nine cycles, for a total of 10 cycles.

Note: Information contained in this report is for reference only, and is not intended as recommendations for normal operation conditions for Supor TFF Cassettes.

Table 12: Measurement of cassette thickness before and after cycle test

| Cassette Thickness Urethane Edge | | Initial Thickness of Urethane edge (inches) | Thickness after 5 th Cycle (inches) | Thickness after 10 th Cycle (inches) |
|----------------------------------|---------------|---|--|---|
| Cassette #85186095R | Minimum (in.) | 3.042 | 3.033 | 3.049 |
| | Maximum (in.) | 3.110 | 3.100 | 3.096 |
| | Average (in.) | 3.089 | 3.078 | 3.080 |
| Cassette #85183008R | Minimum (in.) | 3.038 | 3.038 | 3.045 |
| | Maximum (in.) | 3.124 | 3.086 | 3.083 |
| | Average (in.) | 3.092 | 3.067 | 3.065 |

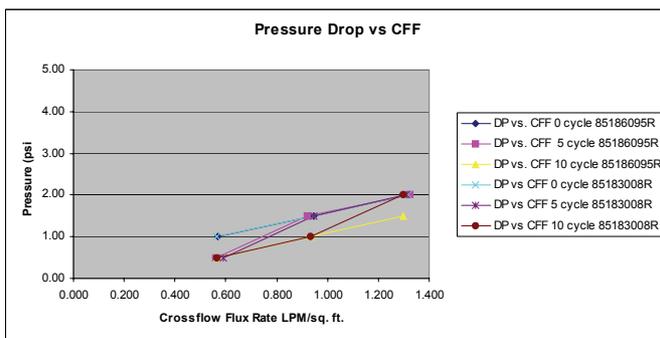


Figure 6: Comparison of Pressure Drop vs. Cross Flow Flux

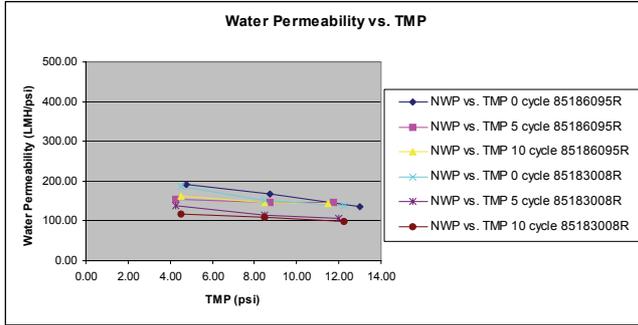


Figure 7: Water Permeability vs. TMP

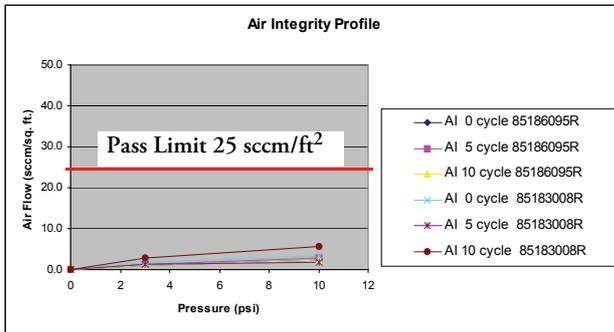


Figure 8: Air Integrity Profile

Reference: SLS Lab Report LLK 1022

4 Quality Assurance

Membranes and cassettes are produced in conformance with the Pall Corporation manufacturing documentation. Cassette components meet current standards for USP Class VI, 70 °C Biological Reactivity Tests for Plastics.

4.1 Quality Control Measures

Raw materials used in production are logged in for traceability and quarantined upon receipt. After inspection by the Quality Control Department, approved raw materials are issued to the warehouse for storage.

During manufacturing, multiple samples from the beginning, and end of each lot of membrane are tested for quality. Tests include water permeability, KL (bubble point), and thickness measurement.

Quality control inspects each cassette and lot card for completeness. The cassettes are then flushed with a glycerin/sodium azide solution and membrane integrity is tested.

4.1.1 Quality Assurance Certificates

A quality assurance certificate is packaged with each tangential flow cassette.

4.1.2 Labels

Each cassette, sealed in a plastic bag, is inserted into a cardboard cradle, and then packaged in a box. Labels affixed to the cradle, box and bag describe the contents. The label identifies the cassette format and contains the part number, and the serial number. This information should match the information printed on the side of the cassette.

4.1.3 Cassette

The following information is printed on the side of each cassette (Figure 9):

1. Company name
2. Membrane type
3. Molecular weight cut-off or pore size
4. Cassette format
5. Membrane area
6. Feed channel format
7. Part number
8. Serial number (formerly lot number)

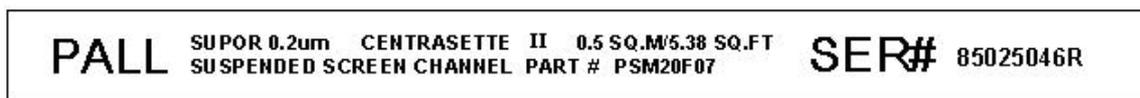


Figure 9: Example of the Information Printed on Supor TFF Cassettes

5 Biological Evaluation and Test Procedures

5.1 Introduction

The purpose of the biological evaluations and tests was to evaluate the biological suitability of the materials of construction of the Supor TFF membrane cassettes. These tests were performed by an outside contract laboratory. Tests performed included the *Biological Reactivity Tests, In Vivo, for Plastics* (hereafter called the *biological reactivity tests*), as described in the *United States Pharmacopeia*, Chapter <88>; as well as the *Hemolysis Test*, and the L929MEM—*Cytotoxicity Test* (hereafter called the *cytotoxicity test*).

5.2 Summary of Test Procedures

The *biological reactivity tests* described in the *United States Pharmacopeia* include injection of extracts of plastic materials, as well as implantation of the material itself into animal tissue. Four extracting media are listed which simulate parenteral solutions and body fluids. These include: (1) sodium chloride injection, (2) 1-in-20 solution of alcohol in sodium chloride injection, (3) polyethylene glycol 400, and (4) vegetable oil (sesame or cottonseed oil). Extracts are prepared at one of three standard conditions: 50 °C for 72 hours, 70 °C for 24 hours, or 121 °C for one hour. Since Supor TFF membrane cassettes have a recommended operating temperature limit of 50 °C, cassette components were extracted at 70 °C to provide for the most stringent test condition not resulting in physical changes in the plastic itself.

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 extracts were injected intravenously. Vegetable oil extract and polyethylene glycol 400 extract were injected intraperitoneally.

An intracutaneous test was performed to evaluate the potential of a single injection of an extract to produce tissue irritation. The four specified extracts were used.

Implantation was also performed in order to subject the materials of construction to the most stringent conditions included in the *United States Pharmacopeia*. Each of the components of the filter cassette was implanted separately.

The *hemolysis test* and *cytotoxicity test* were conducted to determine the potential toxicity resulting from direct contact of the materials of construction with blood or tissue. The *hemolysis test* determines the degree of red blood cell lysis caused by contact of the test material. Using cell culture techniques, the *cytotoxicity test* determines the lysis of cells and the inhibition of cell growth caused by extracts of the test materials.

5.2.1 Results

All Supor TFF membrane cassette components were found to meet the requirements of the USP, In Vivo Class VI-70 °C Plastics. Additionally, test samples meet the requirements of the *hemolysis test* and *cytotoxicity test*.

Tests were conducted by STS *division of Ethox Corp*, 7500 W. Henrietta Road, Rush, NY 14543 and by Toxicon Laboratories, 225 Wildwood Avenue, Woburn Massachusetts 01801.

Details of test reports are available by contacting Pall Life Sciences, Quality Assurance department.

5.3 Summary of Materials of Construction

- Polyethersulfone—Meets FDA 21 CFR, part 177.2440, USP Class VI plastics at 70 °C.
- Polyolefin membrane support—Meets 21 CFR, part 176.170, 177.1520, 177.2800, USP Class VI plastics at 70 °C.
- Polyester screen—Meets 21 CFR, part 177.1630, USP Class VI plastics at 70 °C.
- Polyolefin spacers—Meets 21 CFR, part 177.1520, USP Class VI plastics at 70 °C.
- Polyurethane encapsulant—Meets 21 CFR, part 175.103, 175.300, 177.2600, USP Class VI plastics at 70 °C.
- Silicone gaskets—Platinum cured, medical grade, Meets 21 CFR, part 177.2600, USP Class VI plastics at 70 °C.
- Glycerin—CP/USP grade, plant origin, (added as humectant; removed with flushing).
- Sodium azide — 0.05 – 0.1%, (added as bacteriostat, removed with flushing).
- Spacer in suspended screens-Natural HDPE sheet, USP Class VI.

6 Operational Procedures

6.1 Installing Cassettes in Holders

Follow these steps to install Supor TFF membrane cassettes in holders. The exact procedure may vary with different holders:

1. Remove the top plate of the cassette holder and ensure the sealing surfaces are clean and undamaged.
2. Wet the silicone gaskets with deionized or pharmaceutical grade water. Place one gasket against the manifold plate of the holder and align the holes in the gasket with the holes in the manifold.
3. Place the cassette into the holder on top of the installed gasket, and place the second gasket on top of the cassette. Align the holes in the gasket with the holes in the cassette.
4. If your application requires multiple cassettes, place the next cassette on top of the gasket on the first cassette. Place another gasket on top of the cassette. Repeat this process for each additional cassette.
5. Check the alignment and orientation of the cassettes and gaskets to the holder. Place the end plate on the holder.
6. Add tie-rod spacers if required, and hand-tighten the hex nuts on the tie rods. Manually or hydraulically compress the holder closed as described in the following sections.

Pall supplies two gaskets with each cassette. Installing the first cassette requires two gaskets. Installing each additional cassette requires only one gasket. Save extra gaskets to replace worn or damaged gaskets. Pall recommends replacing gaskets after six months of use.

6.2 Cassette Holder Torque Specifications

Membrane cassettes and gaskets are installed in a holder and the holder must be compressed to exert sufficient force on the cassettes to prevent leaking when the cassette is operated under pressure. If insufficient compression is applied, air or liquid leaks from the holder. If excessive compression is applied, cassette damage may occur. The appropriate torque value depends on several factors including the operating pressure, the number of cassettes installed and the condition of gaskets and holder surfaces. Using the lowest acceptable torque value helps to prolong the useful life of a cassette.

6.3 Tightening Manual Torque Cassette Holders

Torque Value Selection and Tightening Sequences

Manual-torque cassette holders require proper torquing to prevent leaks and ensure consistent performance. Over-tightening a cassette can result in permanent damage. Therefore, you should normally use the lowest torque value that will assure system integrity. Select the recommended torque range from [Table 13: Recommended Torque Values for Manual-torque Cassette Holders on page 18](#).

Never exceed the torque limits shown—excessive torque shortens the operating life and degrades performance of membrane cassettes. Cassette holders require adjustment periodically as torque decreases over time due to cassette and gasket compression or changes in temperature. Changes tend to be greatest for new cassettes.

After you hand-tighten the assembly nuts, use a calibrated torque wrench to tighten each nut according to the specific pattern recommended for your holder ([Figure 10: Pall Manual-Torque Cassette Holders and Torquing Sequence on page 18](#)). As you tighten the nuts with the torque wrench, do not turn the nuts more than ¼ turn at a time. After reaching a ¼ turn, move and begin tightening the next nut in the specified sequence. Tighten the nuts in this fashion until the specified torque value is reached.

You must recheck the torque prior to using a cassette/holder assembly. For manual assemblies, checking torque prior to use is essential. Processes where temperature of the process fluid varies may require periodic adjustment of torque due to expansion and contraction of cassettes and gaskets.

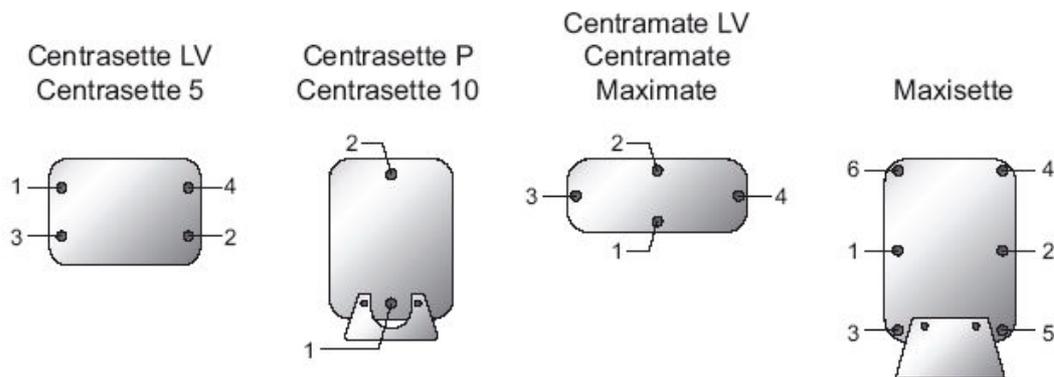


Figure 10: Pall Manual-Torque Cassette Holders and Torquing Sequence

Table 13: Recommended Torque Values for Manual-torque Cassette Holders

| Holder Type | No. Bolts on Holder | Recommended Torque Range for Manual -Torque Cassette Holders | |
|---|---------------------|--|----------|
| | | (in-lb.) | (Nm) |
| Supor TFF Membrane Cassettes | | | |
| Centramate LV | 4 | 50 – 60 | 6 – 7 |
| Centramate | 4 | 60 – 90 | 7 – 10 |
| Centrasette 5, Centrasette LV | 4 | 300 – 450 | 35 – 50 |
| Centrasette 10, Centrasette P | 2 | 600 – 900 | 70 – 100 |
| Centrasette 10 | 4 | 300 – 450 | 35 – 50 |
| Maximate | 4 | 100 – 160 | 11 – 18 |
| Maxisette 25 Maxisette 50 Maxisette 100 | 6 | 700 – 1050 | 80 – 120 |

6.4 Tightening Auto-Torque (AT) Holders

To tighten a Pall auto-torque holder, select and apply the required hydraulic pressure to the hydraulic cylinders on the holder (Table 14: Recommended Hydraulic Pressure Values for Supor TFF Membrane Cassettes on page 19). Refer to the AT-holder operating instruction manual for the detailed operating procedure. If you are using the Pall Hydraulic Pump, refer to the owner's manual for operating procedures. Auto-torque assemblies do not need periodic pressure adjustment. The AT system eliminates possible operator error that can occur with a manual torque cassette holder. The AT system maintains the specified clamping force under conditions that would cause a manually-torqued cassette to require retorquing (i.e., temperature reduction or cassette-gasket compression).

Table 14: Recommended Hydraulic Pressure Values for Supor TFF Membrane Cassettes

| Holder Type | No. Hydraulic Pistons On Holder | Recommended Hydraulic Pressure Range for AT Holders ³ | |
|--|---------------------------------|--|----------|
| | | (psig) | (bar) |
| Supor TFF Membrane Cassettes | | | |
| Centrasette 5 AT | 4 | 500 – 800 | 34 – 54 |
| Centrasette 10 AT (F22, F27 Cassettes) | 2 | 1100 – 1600 | 75 – 110 |
| Centrastak AT (F22, F27 Cassettes) | 2/level | 1100 – 1600 | 75 – 110 |
| Maxiset 25 AT | 6 | 1400 – 1600 | 95 – 122 |
| Maxiset 50 AT | 6 | 1400 – 1600 | 95 – 122 |
| Maxiset 100 AT | 6 | 1400 – 1800 | 95 – 122 |

6.4.1 Effect of Temperature on Set Torque Value

At a given torque setting, changes in temperature will change the clamping force exerted by the holder on the gaskets and cassettes. If the temperature decreases more than 5 °C from the temperature at which cassette was last torqued, retorque the holder. If the temperature will increase more than 20 °C, use lowest possible starting torque, which maintains system integrity.

6.5 System Hold-up Volume

The system hold-up volume equals the total volume of liquid contained in the feed/retentate flow path (Figure 11). The feed/retentate flow path includes the cassette feed/retentate channels, holder ports, pump, valves, gauges, connectors, and tubing. You must determine the system hold-up volume of your system because each installed system varies due to specific applications.

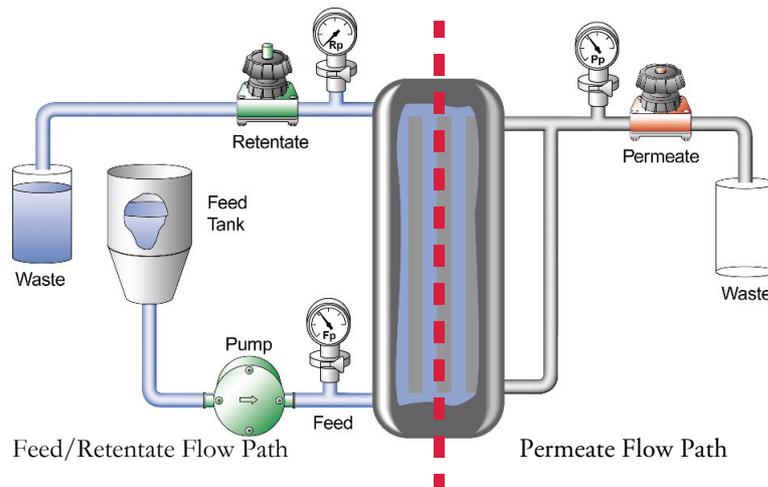


Figure 11: TFF System Schematic with Feed/Retentate Flow Path

6.6 Sanitization

Cassettes are not supplied sterile nor does Pall claim they are pyrogen (endotoxin) free. New cassettes contain 0.05 – 0.1% sodium azide as a bacteriostatic agent to prevent bacterial growth. Cassettes should be washed free of

storage agents, sanitized, and washed free of any trace of pyrogens prior to use. After use, cassettes are normally stored in an aqueous solution of 0.1 N sodium hydroxide to prevent bacterial growth.

Method for Sanitization

The recommended procedure for sanitizing and depyrogenating cassettes is described in *Membrane Cassettes Care and Use Procedures* (R00640 Rev 01).

6.7 Flushing

Flushing removes storage agents or cleaning agents from the cassette prior to use in order to prevent contamination of the product.

Flushing Method

The Pall BioPharmaceutical *Membrane Cassettes Care and Use Procedures* describes the recommended procedure for flushing.

6.8 Integrity Testing

Integrity testing enables you to determine that the filtration system components and membranes are not leaking or damaged. You can perform two types of integrity test:

1. A system integrity test indicates the presence of air leaks in the system, such as in the holder, fittings, and gaskets.
2. When performed after a system integrity test, the membrane integrity test indicates the absence of holes, tears, seal failures, or defects in the membrane cassette.

The membrane integrity value is a measurement of air that either diffuses through the liquid that fills the pores in the membrane or passes through pinholes or defects in the membrane or around the seals. Membrane integrity measurements are made at a specified air (or nitrogen) pressure. The diffusive membrane integrity value is related to pore size, pore density, membrane density (surface skin thickness), temperature, and pressure. However, differences in membrane integrity values due to these factors are small compared to the large increases that result from the presence of even a tiny pinhole.

Prior to performing the membrane integrity test, you must flush and condition the tangential flow filtration system with the cassettes installed. The method and equipment you use can also affect results. Pall recommends using mass flow meters connected to the upstream side of the cassette to yield accurate and consistent results.

The schematic in [Figure 12: Membrane Integrity Test Setup Using a Pall Integrity Analyzer on page 21](#) shows an integrity test setup using the Pall Integrity Analyzer. This instrument regulates the air flow rates and pressures to perform the integrity tests. A sensitive mass flow meter measures and displays the rate of air that is diffusing through the membrane. The following sections describe how to perform the system integrity test and the membrane integrity test.

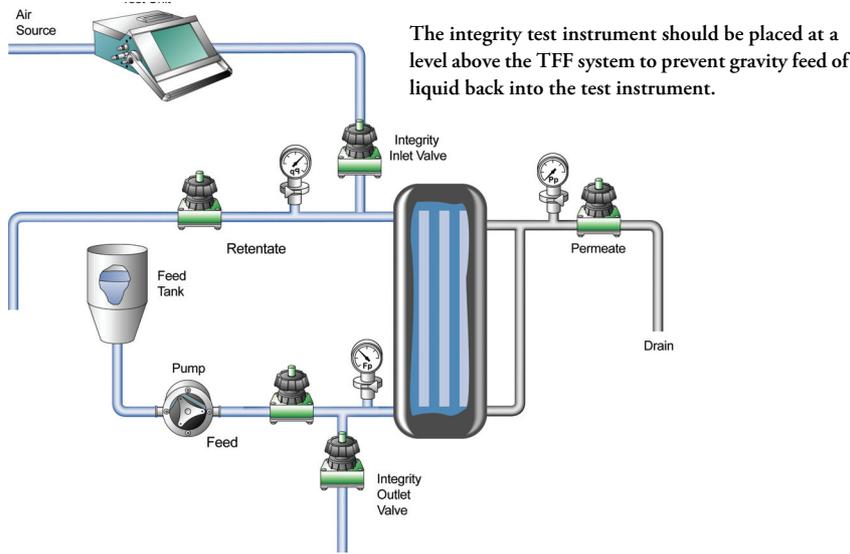


Figure 12: Membrane Integrity Test Setup Using a Pall Integrity Analyzer

6.8.1 System Integrity Test

During the integrity test, you pressurize the system with compressed air, and monitor the airflow. The airflow should gradually drop to zero. Test the integrity of the system before testing the integrity of the membrane. There should be little to no air flow if the system is sealed.

Integrity Test Method

The system integrity test procedure is described in *Membrane Cassettes Care and Use Procedures*.

6.8.2 Membrane Integrity Test

The membrane integrity test checks the membrane and internal seal assemblies of the cassette. The cassette must be fully wetted-out prior to making a measurement. The pressure on the feed/retentate side of the membrane should be set to the appropriate value prior to opening the filtrate valve. The membrane integrity measurement should be made within fifteen minutes of having the water expelled from the feed/retentate channel to prevent the pores from drying out. You should perform the system integrity test before performing a membrane integrity test.

The membrane integrity test should be performed separately on each cassette. When multiple cassettes are used in one system, the cassettes can then be installed and both a system and membrane integrity test performed. The air Forward Flow values obtained for the combined stacks of cassettes should be approximately equal to the sum of the individual air Forward Flow values. If after use, the air Forward Flow values obtained for the stacks increases by more than the limit value for one cassette, the stacks should be broken down and each cassette tested separately. This is done to eliminate the possibility that there is a single defect in one cassette that accounts for the total increase in Forward Flow.

Method

The membrane integrity test procedure is described in *Membrane Cassettes Care and Use Procedures*.

6.9 Method for Determining Water Flux and Normalized Water Permeability

Membrane water flux is a measure of the permeability of the membrane to clean water at a specified transmembrane pressure and temperature.

Method

Procedure to determine water flux and normalized water permeability is described in *Membrane Cassettes Care and Use Procedures*.

6.10 Cleaning the System and Cassettes

Proper cleaning of cassettes after use is critical to their reuse. You must establish a cleaning protocol that removes foulants and recovers the initial water permeability. The key cleaning parameters include:

- Cross flow rate
- Cleaning agent and concentration
- Temperature
- Time

6.10.1 Cross Flow (Retentate Flow Rate)

Increasing cross flow rates improves cleaning effectiveness. Pall recommends a cleaning cross flow rate of 1.5 – 2 times the normal operating cross flow rate. The retentate valve should be fully open. For suspended screen cassettes, the filtrate valve should not be completely closed. Since most foulant is lodged at the surface of a skinned, anisotropic membrane, the objective is not to drive it into the membrane but to hydrolyze, oxidize, or resolubilize the material and remove it.

6.10.2 Cleaning Agent and Concentration

The choice of cleaning agent depends on the possible foulant. Sodium hydroxide at concentrations up to 0.5 N usually is sufficient to remove most biological foulant. The addition of 400 ppm of sodium hypochlorite may help remove additional materials not removed with NaOH alone. This mixture is particularly effective for removing cells and cellular debris from the cassette. A list of recommended cleaning agents and the type of foulants they may remove is presented in *Membrane Cassettes Care and Use Procedures*. In general, higher concentrations will improve cleaning and/or reduce time. However, cassette life may be compromised, especially when using concentrated solutions at elevated temperatures.

6.10.3 Temperature

Increasing temperature normally improves cleaning significantly. If possible, heating and maintaining the temperature of cleaning solutions between 35 – 45 °C is strongly recommended. If the foulant is very hydrophobic, reducing the temperature may sometimes help.

6.10.4 Time

Cleaning should take from 30 – 120 minutes. If a membrane recovery of greater than 80% of initial water permeability cannot be established within two hours, it may indicate that a different cleaning agent is required. The time required to achieve the required level of cleaning should be established as part of a carefully performed cleaning study.

6.11 Recommended Cleaning Procedures

Procedures for flushing and cleaning cassettes after use are described in the *Membrane Cassettes Care and Use Procedures*.

6.12 Cleaning Study Protocol

You should perform a cleaning study to identify the cleaning time, temperature, and concentration that provide the optimal recovery of the initial water permeability. The following steps describe, in general, how to perform a cleaning study. Actual times and volumes can be changed:

1. After recovering concentrated product, open retentate valve, and close filtrate valve (screen channel cassettes only). Flush the cassette to waste with 10 – 20 L/m² of spent filtrate; or if product was in filtrate, with fresh buffer.
2. Add additional spent filtrate or buffer to the feed reservoir (5–10 L/m²). Circulate it for 15 minutes at twice the cross flow rate used for processing. Spent filtrate makes a good flushing agent because it was used to solubilize the product initially and saves the cost and time of making up fresh buffer. Furthermore, the spent fil-

trate was already ultrafiltered and may be cleaner than fresh buffer. Direct the retentate and permeate to drain. Pump the system dry.

3. Select a cleaning agent, concentration, and temperature. The recommended starting solution is 0.5N NaOH, at 35 °C. Add 20 – 30 L/m² of the cleaning solution to the feed reservoir. Open retentate and filtrate valves, and flush approximately 5 L/m² of the cleaning solution to waste.
4. Set the system for recirculation and recirculate for 30 minutes. At 30 minutes, pump out the cleaning solution to waste.
5. Add 30-40 L/m² of water to the feed reservoir. Flush the cassette with water (10 L/m² through retentate, then 10 L/m² through filtrate). Recirculate through both retentate and filtrate for 10 minutes. Increase cross flow to increase feed pressure by about 0.2 – 0.5 barg (3 – 5 psig) to remove any air trapped in the cassette. Reduce the flow after a few seconds. Repeat several times until no air is observed exiting from the retentate or permeate lines.
6. Measure the water permeability.

If you have recovered more than 90% of the membrane's initial water permeability, you can accept the process *as is* or you may try a lower concentration or temperature after the next process is run on the membrane. Less stringent conditions may prolong membrane life. If after the cleaning, you recover less than 90% of the membrane's initial water permeability, repeat steps 3 through 6. If the second cleaning improves membrane recovery, repeat steps 3 through 6 a third time. If the second cleaning does not improve recovery to at least 80%, you should consider changing cleaning solution; for example, adding 400 ppm NaOCl to the NaOH, and repeating the process. The inability to recover at least 80% of the initial water permeability does not necessarily mean that the cassette has not been cleaned. Cassette compression can cause a reduction in the calculated value for water permeability. It is important not to over-torque cassettes. Increasing the temperature of the cassettes after they have been properly torqued, will cause an increase in compression.

7 Method Details

7.1 Characterization of Extractables

Characterization of Extractables from Pall Supor TFF Suspended Screen Channel Membrane Cassettes (Part No. PSM65F07) into Water and an Aqueous 25% Ethanol Solution

7.1.1 Summary

Two successive 16-hour extractions of three Pall Supor TFF suspended screen channel membrane cassettes (Part No. PSM65F07) in Water and an Aqueous 25% Ethanol Solution produced a total nonvolatile residue (NVR) of under 50 and 73 mg per cassette, respectively. Infrared spectroscopy showed that the materials extracted were composed primarily of glycerin as well as trace amount of oligomers from polyurethane. Successive extractions of each filter showed a significant decrease in nonvolatile residue, demonstrating depletion of extractables available to solvent. Therefore, these results will be valid regardless of volume of solution processed, and for contact times up to, and probably beyond 32 hours. We conclude that extractables from a PSM65F07 cassette into dilute aqueous pharmaceutical products containing less than 25% compatible organic constituents that can be modeled with ethanol are unlikely to significantly exceed 73 mg nonvolatile residue, and will be composed primarily of the materials described above.

7.1.2 Test Methods

Test Parameters

| | |
|-------------------------|--|
| Test Filter | PSM65F07 (three cassettes for water extractions; three cassettes for aqueous 25% ethanol solution extractions) |
| Test Time | 16 hours per extraction |
| Test Temperature | 46 – 50 °C Water extraction and 20 – 25 °C for Aqueous 25% Ethanol Solution |
| Agitation method | Recirculation |
| Test Solvents | Water and Aqueous 25% Ethanol Solution |

Extraction and Analysis

1. Samples, solvents, conditions
 - (i) Deionized Water (18 MΩ-cm) from Pall DI water Plant and Ethanol (200 proof) from EM Science (L/N 05K03) were used as extracting solvents.
 - (ii) Pall Supor TFF suspended screen channel membrane cassettes (P/N PSM65F07) were tested. Test was conducted in triplicate.
 - (iii) Extractions were conducted at 46 – 50 °C for Aqueous 25% Ethanol Solution and at 46 – 50 °C for Water extraction.
 - (iv) Potassium hydrogen phthalate (KHP) was purchased from J. T. Baker (L/N A46634).
2. Preparation
 - (i) Equipment: 2-liter glass beakers and graduated cylinders, PTFE diaphragm pump and Tygon tubing lined with FEP, stainless steel housing, rotary flash-evaporator, PTFE diaphragm vacuum pump, hot water bath, covered crucibles, desiccator, and drying oven.
 - (ii) Equipment was acid-cleaned and rinsed with 18 MΩ DI water (crucibles were ignited in a muffle furnace).

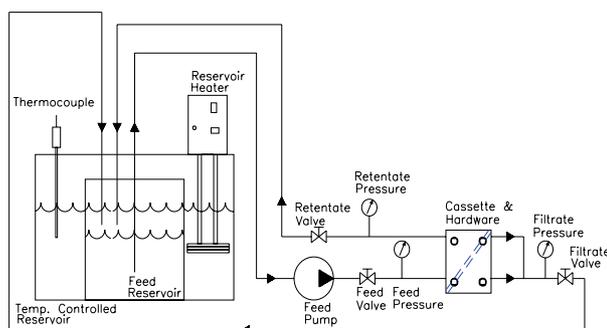


Figure 13: Diagram of Extractables Testing Setup

3. Extraction:
 - (i) Two recirculation setups were used for Water extraction and Aqueous 25% Ethanol Solution extraction, respectively. The diagram of the setup is shown in Figure 13.
 - (ii) A Water sample or an Aqueous 25% Ethanol Solution circulated through the system (without cassette) was used as negative control.
 - (iii) A positive control was prepared by dissolving 51.2 mg of potassium hydrogen phthalate in 2.0 liters of Water or 2.0 liters of Aqueous 25% Ethanol Solution.
 - (iv) Lubricate a set of cut-out gaskets only with DI water and install them in the cassettes holder. Connect the holder to the in-house DI water supply. Adjust the flow to achieve 2 L/min. Adjust the retentate valve to obtain 90% filtrate /10% retentate and flush 25 liters to a drain.
 - (v) Place pump inlet, filtrate line, and retentate line into a vessel charged with 4 liters of sanitizing solution (0.5 N NaOH) at 40 °C and recirculate for 40 minutes. Open the filtrate valve completely and then close retentate valve until the retentate flow rate is approximately 10% of the feed flow rate. Stop the pump, open retentate valve, and drain the system.
 - (vi) Connect the inlet to in-house DI water source. Adjust the retentate valve to obtain 90% filtrate/ 10% retentate, and flush to a drain at 2 L/min for 10 minutes. Take a water sample at the permeate port and measure TOC and pH. If the TOC is over 1 ppm or if the pH is above 8, continue flushing for an additional 10 minutes and check effluent TOC and pH again. Repeat the flush until both TOC and pH meet the requirements. Drain the system.
 - (vii) Fill the reservoir with 2 liters of DI water and bring to 46 – 50 °C or 2 liters of Aqueous 25% Ethanol Solution at 20 – 25 °C. Place the pump inlet tubing, filtrate line and retentate line into the reservoir. Pump the extraction solution through the system at 2 L/min, adjusting the filtrate valve to obtain 10% of total flow through the filtrate line.
 - (viii) At the end of 16 hours, stop the pump, transfer the extraction solvent into a critically pre-cleaned vessel (2-L graduated glass cylinder) labeled with the solvent name and *negative control*. Pump again to get the remaining solvent in the system. Add the remaining solvent to the cylinder. Record the volume.
 - (ix) Repeat steps (iv) to (vii) but using a cassette instead of cutout gasket.
 - (x) At the end of 16-h time, pump the extraction solvent into a clean vessel labeled with the solvent name, cassette serial number, and *extract 1*, and place 2 liters fresh solvent into the reservoir. Allow the solvent to reach the test temperature, and then recirculate the fresh solvent for another 16 hours. Pump the second extract into a clean vessel labeled with the solvent name, cassette serial number, and *extract 2*.
 - (xi) This *rinse, sanitization, and extraction* procedure was carried out for one negative control and three cassettes using DI water. For Aqueous 25% Ethanol Solution, one negative control was carried out for the three cassettes. However, since there was a pump failure during the first extraction for the third cassette, a replacement cassette (S/N 86035002R) was extracted. And prior to the extraction of the replacement cassette, another negative control was performed to make sure the cleanliness of the system.

4. Preparation of NVR:
 - (i) 1.0 liter each of negative controls, positive controls, and extracts was evaporated, reserving the remainder for other analyses. Evaporation of each sample was conducted in a separate round-bottom flask, using a rotary evaporator under vacuum and a water bath set at 60 – 80 °C. Volume of each sample was reduced to below 10 mL.
 - (ii) Each sample was transferred to a clean pre-weighed crucible, with solvent rinses, covered, and dried to constant weight in a drying oven at 60 °C over night. The obtained non-volatile part was designated as Non Volatile Residue (NVR).
 - (iii) Calculations: net weight of NVR for 1.0 liter of filter extracts, negative and positive controls; net weight of NVR for 2.0 liters of each. Subtract negative control values; calculate percent recovery of positive control.
 - (iv) Criteria for quantitative results: negative control less than 5 mg or 20% of sample (first extract); positive control between 80-120% recovery.

5. Infrared spectroscopy:

- (i) NVR from filter extracts, negative control, and positive control were analyzed by FTIR (Fourier Transfer Infrared Spectrometry). Spectral libraries supplied with the instrument (Thermo-Nicolet FTIR Spectrometer, Model 6700) were used to obtain reference spectra for comparison, and spectral matches.
 - (ii) Criteria for successful characterization: A recognizable potassium hydrogen phthalate spectrum from the positive control, and assignment of significant spectral features for filter extracts to spectra of materials known or expected to be extracted from polymers in the cassette.
6. Additional analyses of liquid extracts.
- (i) UV-Vis (Ultraviolet-Visible) spectra of each sample and control were recorded between 200 and 800 nm using instrument subtraction of a solvent blank. The UV instrument used was Agilent Model 8452A.
 - (ii) TOC (total organic carbon) analysis was performed on Water extracts only. The instrument used was Tekmar-Dohrmann Phoenix 8000, which was based on persulfate oxidation under UV-light catalysis. The calibration was performed prior to sample analysis using the TOC Standard from Alfa Aesar (L/N 009319H).

7.1.3 Test Results And Discussion

Part One: Water Extractions

NVR Results

Total extractables as non-volatile residue (NVR) from the two extractions of each cassette (identified by serial number), the solvent and the system blank, and the positive control, are shown in [Table 15](#). The extracting solvent alone (no cassette) produced a blank of 3 mg per 2.0 liters. The positive control yielded 48.6 mg per 2.0 liters (95%). Both controls met predetermined acceptance criteria for quantitative results.

The NVR dropped significantly with a second extraction. The totals for 32 hours of extraction are less than approximately 50 mg.

Table 15: Cassette NVR (mg) in Two Successive 16-Hour Extracts

| Sample | NVR1 | NVR2 |
|------------------------------|---------------------|--------------------|
| Cassette S/N 85285068R | 42.2 ⁽⁶⁾ | 7.4 ⁽⁶⁾ |
| Cassette S/N 85285071R | 12.0 ⁽⁶⁾ | 4.2 ⁽⁶⁾ |
| Cassette S/N 85278020R | 35.4 ⁽⁶⁾ | 9.2 ⁽⁶⁾ |
| Blank for Cassettes | 3.0 | N/A ⁽⁷⁾ |
| 51.2 mg KHP Positive Control | 48.6 ⁽⁶⁾ | N/A ⁽⁷⁾ |
| Blank for Positive Control | 0.4 | N/A ⁽⁷⁾ |

(6) Net NVR as NVR less blank.

(7) N/A, measurement not applicable.

The results of the second extractions show the amount of additional extractable material that becomes available due to diffusion from within the polymers in cassettes during a 16-hour period. Availability of extractables at cassettes' fluid-contacting surfaces decreased during the test time.

UV-Vis Spectroscopy of Extracts

UV-Vis (Ultraviolet-Visible) spectra of the water extracts after subtracting that of the negative control exhibited no maximum in the region of 200 – 800 nm ([Figure 14](#)). In fact, the absorbances of all the extracts were lower than 0.1 at wavelength longer than 220 nm.

Infrared Spectroscopy of NVR

The spectra of the first extracts of the filters are shown in [Figure 15](#), and the second-extract spectra in [Figure 16](#). The positive control produced a spectrum characteristic of Potassium hydrogen phthalate ([Figure 17](#)).

The infrared results are consistent with a NVR composed primarily of glycerin. A reference spectrum of glycerin appears in [Figure 18](#). Glycerin is used as part of the cassette preservative solution. An additional signal at 2030 – 2044 cm^{-1} may be attributable to trace amounts of decomposition and/or polymerization byproducts from the polyurethane. Minor signals at 1550 – 1780 cm^{-1} , typical of carbonyl moieties, may represent traces of oligomers

from the polyurethane sealant used to encapsulate the membrane. Signals at about 2500 and 880 cm^{-1} were also observed in the spectrum of the negative control. These results are as expected from the materials of construction of this cassette, and are typical of this cassette type.

Total Organic Carbon (TOC) Analyses of Extracts

The TOC results are listed in Table 16. The carbon content in glycerin is 39.13%. The mass balance can be elucidated using an example from the first extract from cassette 85285068R. Assuming all the NVR (42.2 mg/2 L as shown in the Table 15) is from glycerin, that will add 8.26 $\mu\text{g}/\text{mL}$ organic carbon, resulting in a total of 88.46 $\mu\text{g}/\text{mL}$. The test value of the TOC is $86.47 \pm 0.98 \mu\text{g}/\text{mL}$ (as shown in the Table 16), which is well balanced with 88.46 $\mu\text{g}/\text{mL}$.

Table 16: TOC ($\mu\text{g}/\text{mL}$) in Two Successive 16-Hour Water Extracts

| Sample | First Extract | Second Extract |
|------------------------|------------------------|------------------------|
| Cassette S/N 85285068R | $86.47 \pm 0.98^{(8)}$ | $38.56 \pm 0.57^{(8)}$ |
| Cassette S/N 85285071R | $99.51 \pm 0.55^{(8)}$ | $45.42 \pm 0.03^{(8)}$ |
| Cassette S/N 85278020R | $87.99 \pm 0.87^{(8)}$ | $34.54 \pm 0.42^{(8)}$ |
| Blank for Cassettes | 0.79 ± 0.01 | N/A ⁽⁹⁾ |

(8) TOC value was not subtracted from that of the blank.

(9) N/A, measurement not applicable.

Part Two: Aqueous 25% Ethanol Solution Extractions

NVR Results

Total extractables as non-volatile residue (NVR) from the two extractions of each cassette (identified by serial number), the solvent and system blanks, and the positive control, are shown in Table 17. The extracting solvent alone (no cassette) produced blanks of 3 mg and 2 mg per 2.0 liters, respectively. The positive control yielded 47.4 mg per 2.0 liters (93%). Both negative and positive controls met predetermined acceptance criteria for quantitative results.

The NVR dropped significantly with a second extraction. The totals for 32 hours of extraction are less than approximately 73 mg.

Table 17: Cassette NVR (mg) in Two Successive 16-Hour Extracts

| Sample | NVR1 | NVR2 |
|-------------------------------|----------------------|----------------------|
| Cassette S/N 85285069R | 36.0 ⁽¹⁰⁾ | 21.0 ⁽¹⁰⁾ |
| Cassette S/N 85285072R | 39.8 ⁽¹⁰⁾ | 18.2 ⁽¹⁰⁾ |
| Cassette S/N 86035002R | 48.8 ⁽¹¹⁾ | 23.8 ⁽¹¹⁾ |
| Blank 1 | 3.0 | N/A ⁽¹²⁾ |
| Blank 2 | 2.0 | N/A ⁽¹²⁾ |
| 51.2 mg KHP, Positive Control | 47.4 ⁽¹³⁾ | N/A ⁽¹²⁾ |
| Blank for Positive Control | 0.6 | N/A ⁽¹²⁾ |

(10) Net NVR as NVR less blank 1.

(11) Net NVR as NVR less blank 2.

(12) N/A, measurement not applicable.

(13) Net NVR as NVR less blank for positive control.

The results of the second extractions show the amount of additional extractable material that becomes available due to diffusion from within the polymers in cassettes during a 16-hour period. Availability of extractables at cassettes' fluid-contacting surfaces decreased during the test time.

UV-Vis Spectroscopy of Extracts

UV-Vis (Ultraviolet-Visible) spectra of the aqueous 25% ethanol solution extracts after subtracting that of the corresponding negative control exhibited no maximum in the region of 200 – 800 nm (Figure 19). In fact, the absorbances of all the extracts were lower than 0.13 at wavelength longer than 220 nm.

Infrared Spectroscopy of NVR

The spectra of the first extracts of the filters are shown in Figure 20 and the second-extract spectra in Figure 21. The positive control produced a spectrum characteristic of Potassium hydrogen phthalate (Figure 22).

The infrared results are consistent with a NVR composed primarily of glycerin. A reference spectrum of glycerol appears in Figure 18. Glycerin is used as part of the cassette preservative solution. An additional signal at 2030 – 2044 cm^{-1} may be attributable to trace amounts of decomposition and/or polymerization byproducts from the polyurethane. Minor signals at 1550 – 1780 cm^{-1} , typical of carbonyl moieties, may represent traces of oligomers from the polyurethane sealant used to encapsulate the membrane. These results are as expected from the materials of construction of this cassette, and are typical of this cassette type.

7.1.4 Conclusions

NVR from successive extractions of Pall Supor TFF suspended screen channel membrane cassettes (P/N PSM65F07) in Water and Aqueous 25% Ethanol Solution, produced a total of less than 50 mg and 73 mg per cassette (5.38 ft^2 effective filtration area), respectively. Infrared spectroscopy showed that the materials extracted were composed primarily of glycerin as well as trace amount of oligomers from polyurethane.

Two successive extractions of each filter cassette showed a significant decrease in non-volatile residue, demonstrating depletion of extractables available to solvent. This depletion test shows that neither larger volumes of extracting solvent, nor longer extraction times, will produce any significant increase in the amounts or change in the composition, of extractable materials. Therefore these results will be valid regardless of volume of solution processed, and for contact times up to, and probably substantially exceeding 32 hours.

We conclude that nonvolatile extractables from Pall Supor TFF suspended screen channel membrane cassette (P/N PSM65F07) into water at 46 – 50 °C and aqueous 25% ethanol solution at 20 – 25 °C, are unlikely to significantly exceed 50 mg and 73 mg, respectively, and will be composed primarily of the materials described above.

The following figures give the actual spectral outputs for the UV/Visible and FTIR analytical results. Examples are given for one cassette from each extraction (water @ 46 – 50 °C and 25% ethanol @ 20 – 25 °C). Spectral outputs are available for all of the extractions as well as blanks.

Reference: SLS Report 13354WD

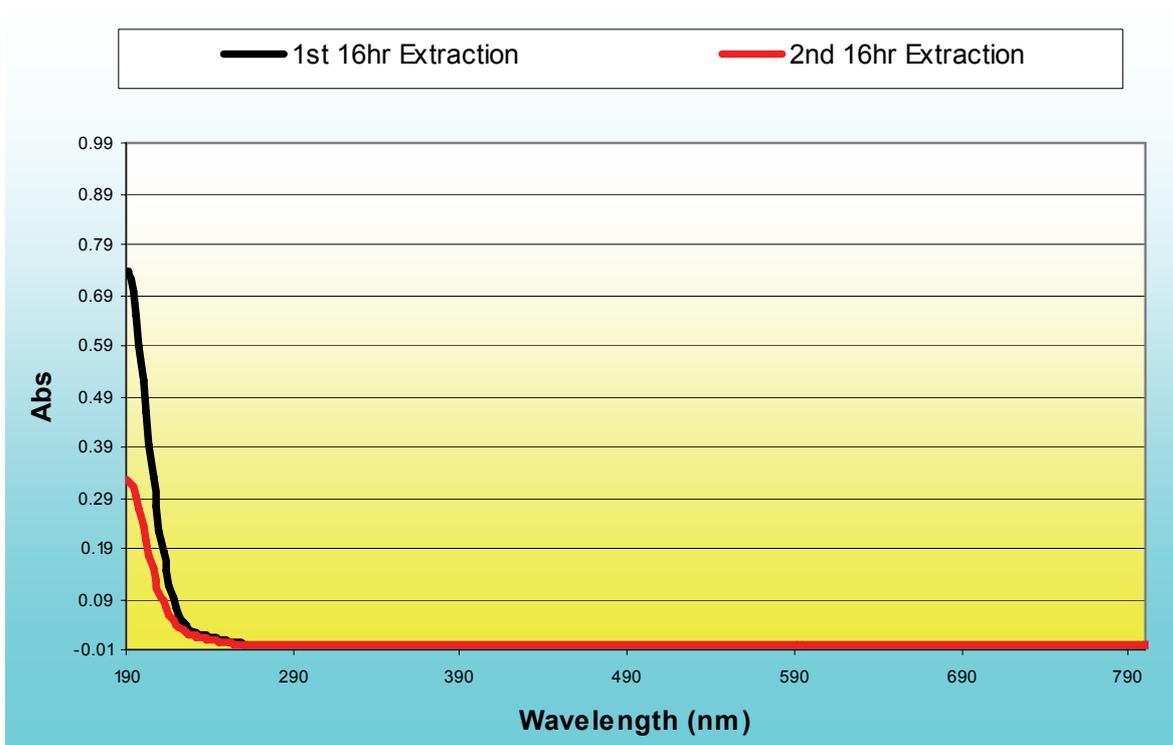


Figure 14: UV Spectra of Extracts from Cassette 85285068R in Water

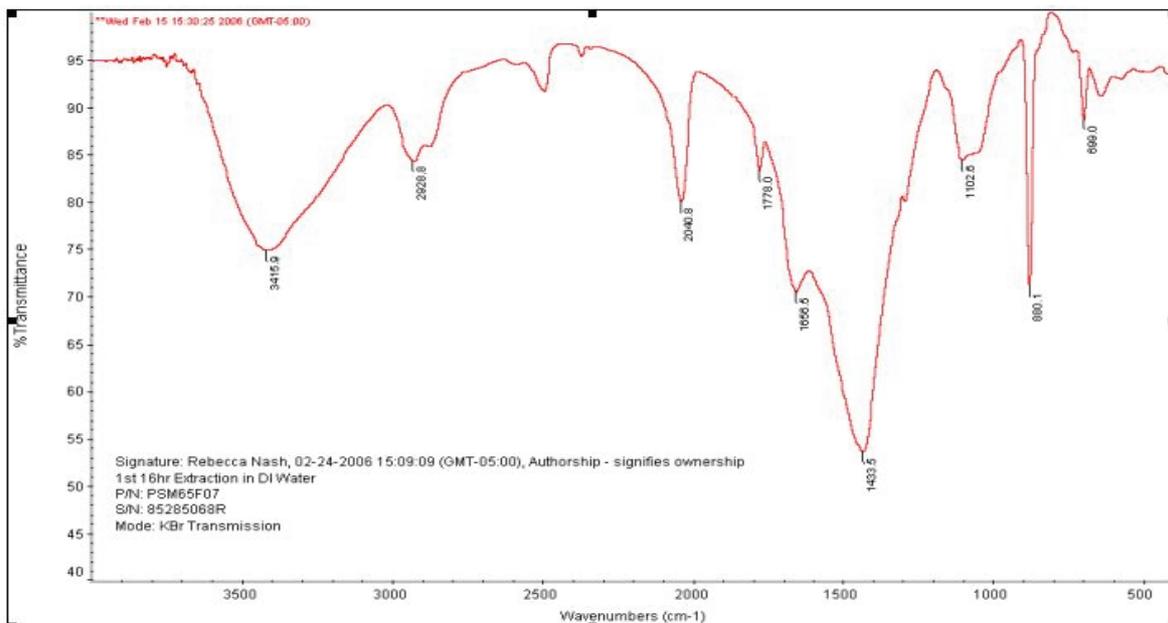


Figure 15: Infrared Spectrum of Filter 85285068R NVR1

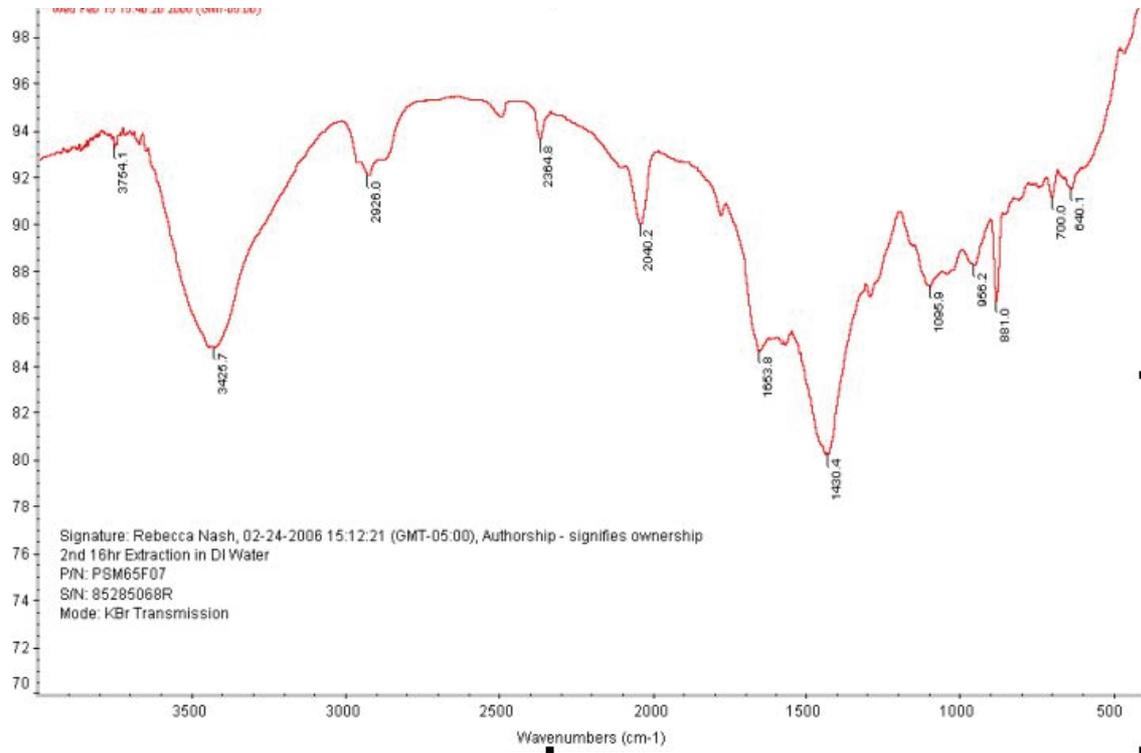


Figure 16: Infrared Spectrum of Filter 85285068R NVR

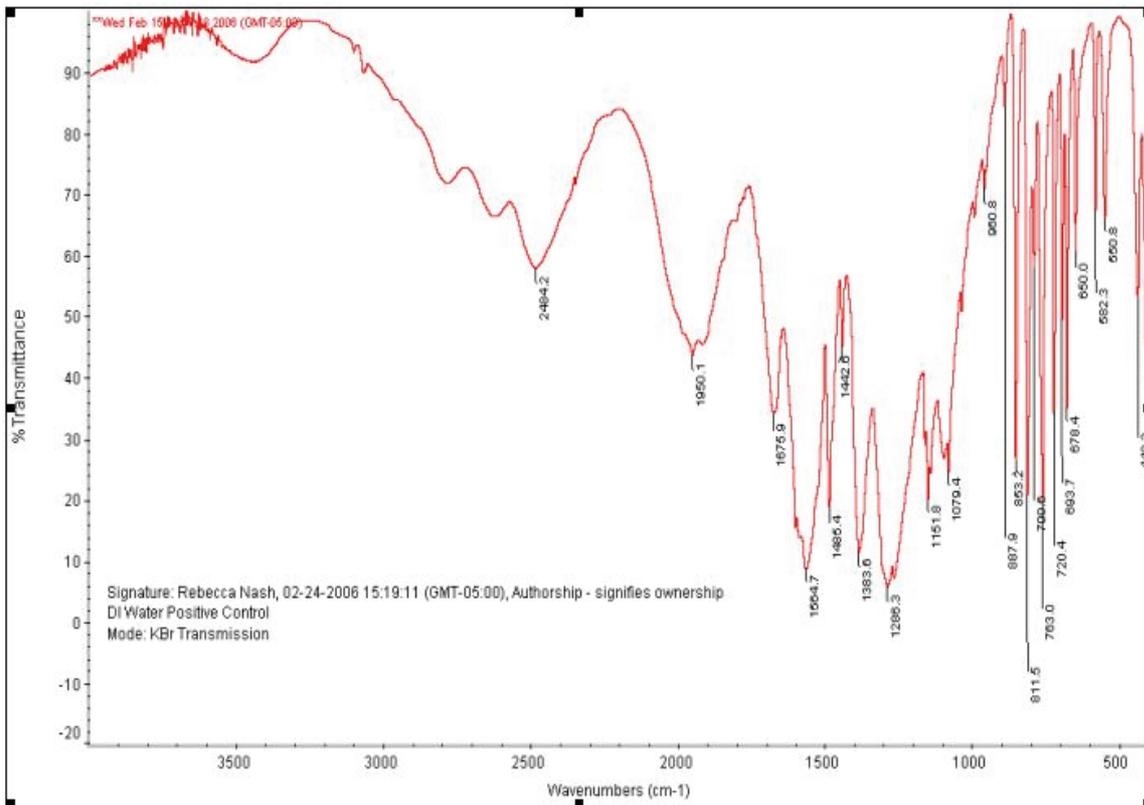


Figure 17: Infrared Spectrum of Positive Control (KHP) in Water

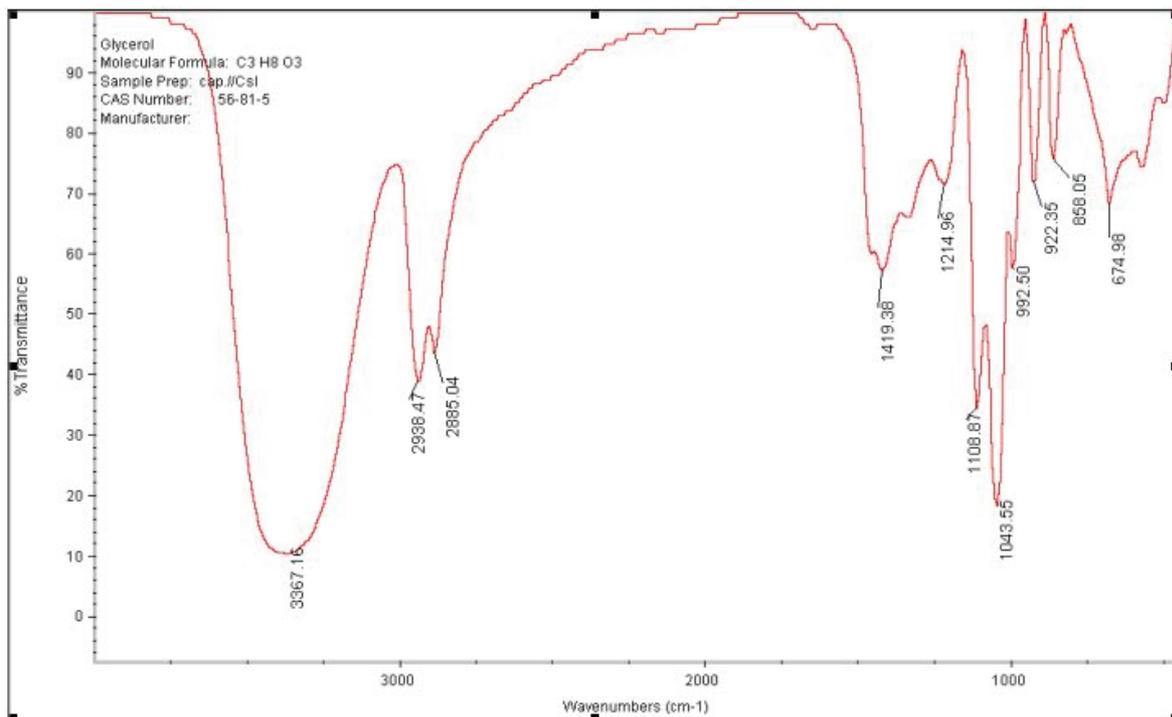


Figure 18: Infrared Spectrum of Glycerin

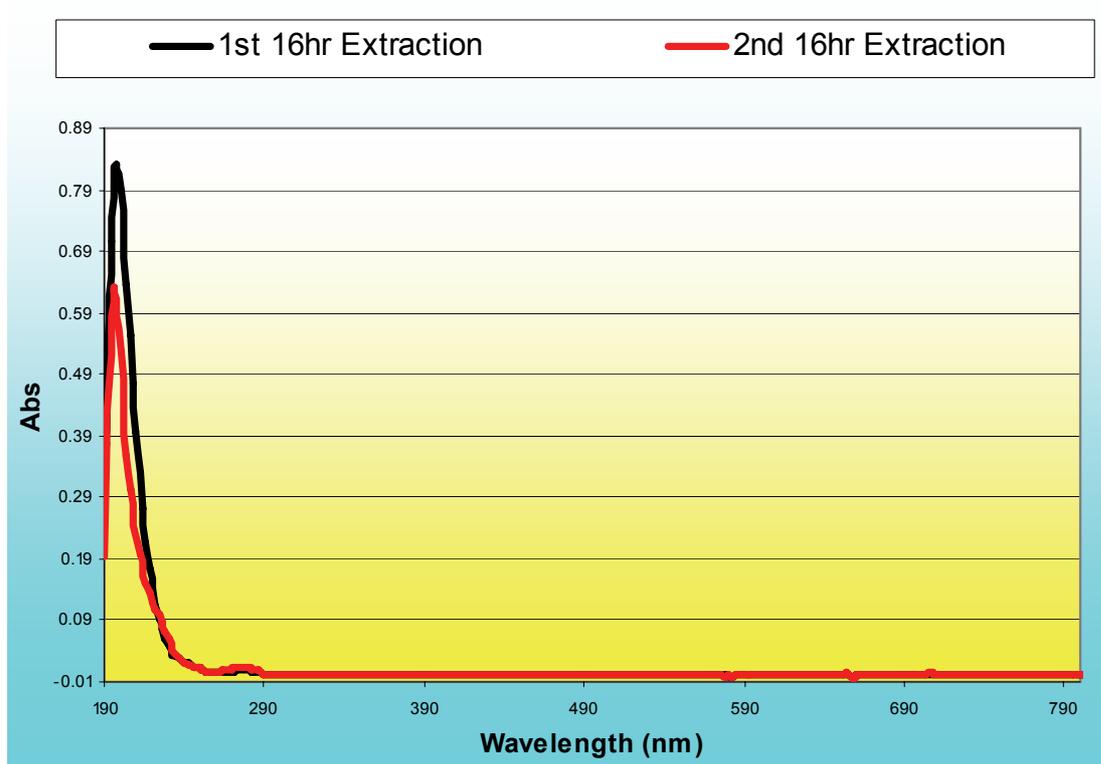


Figure 19: UV Spectra of Extracts from Cassette 85285069R in Aqueous 25% Ethanol Solution

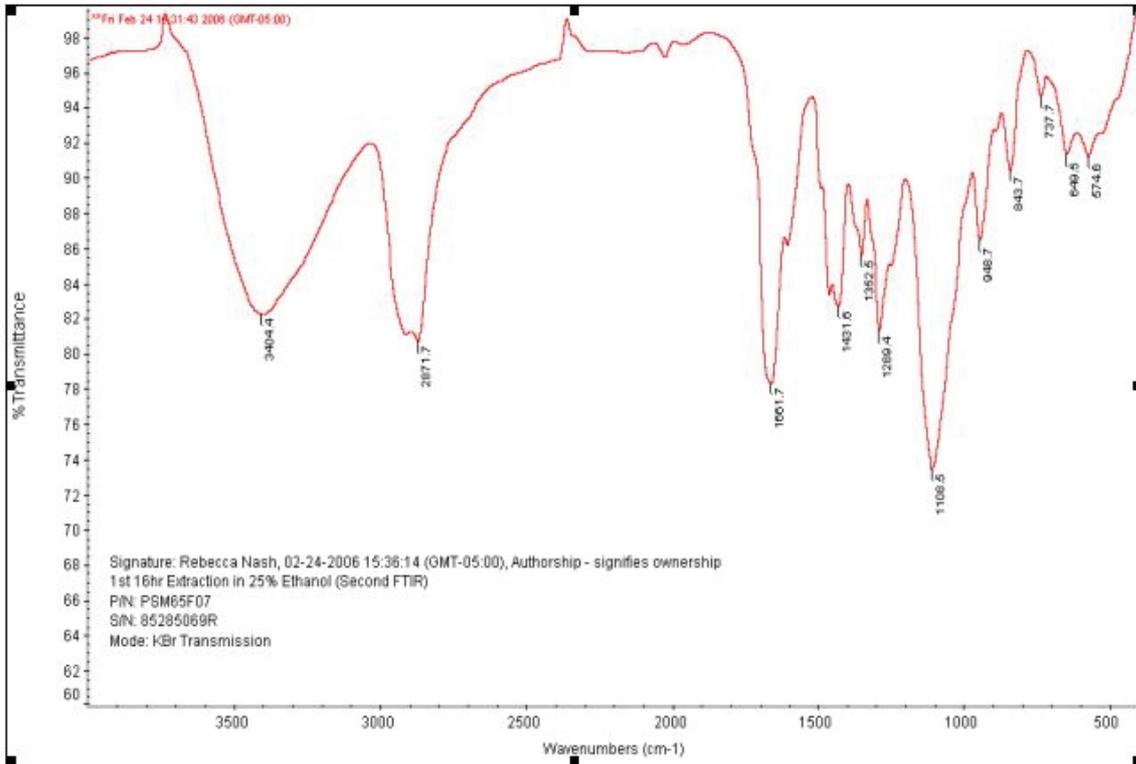


Figure 20: Infrared Spectrum of Filter 85285069R NVR1

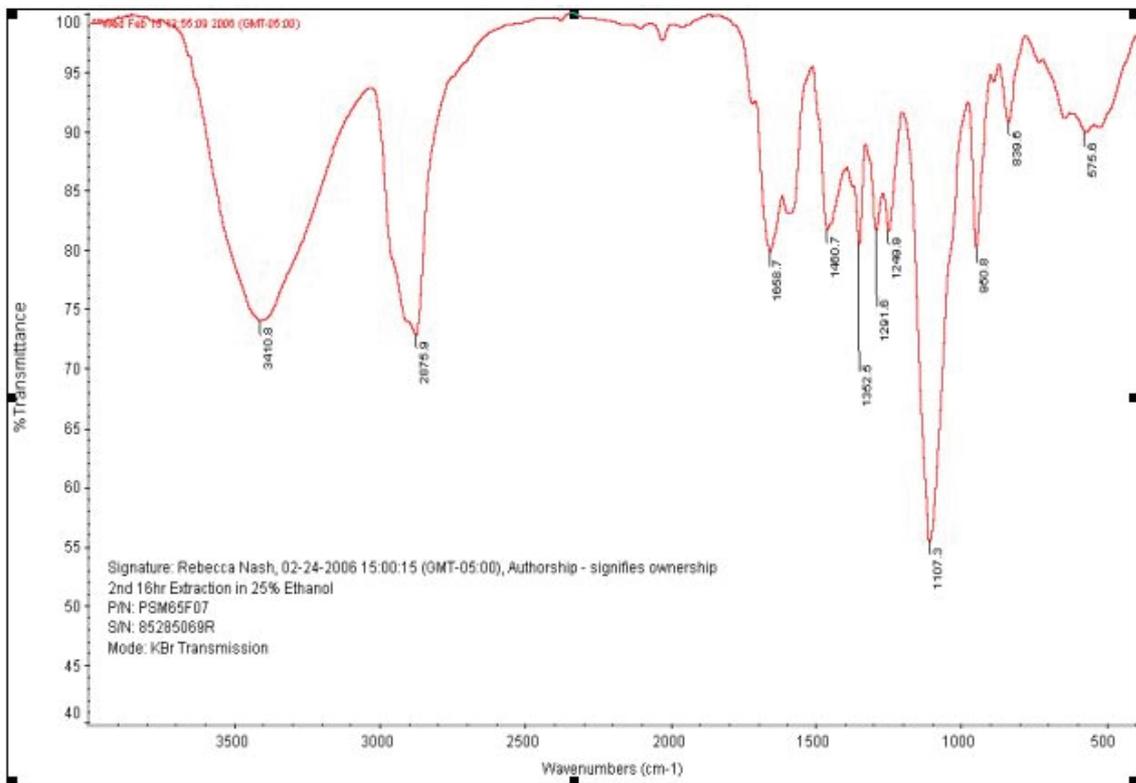


Figure 21: Infrared Spectrum of Filter 85285069R NVR2

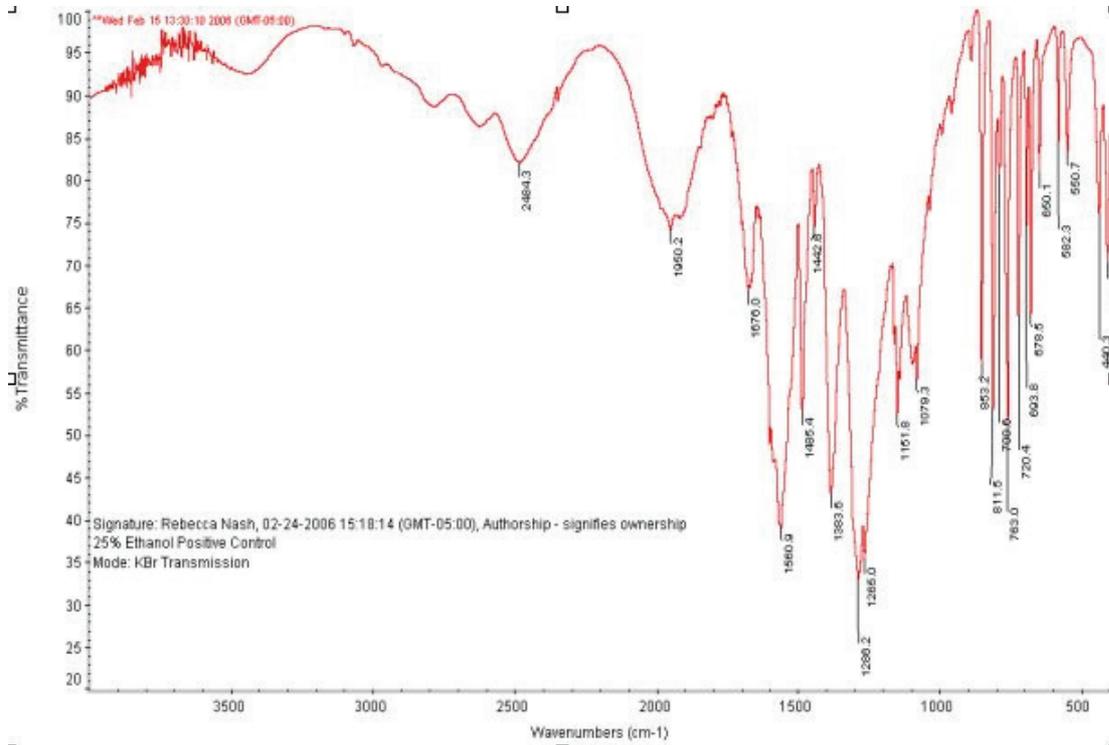


Figure 22: Infrared Spectrum of Positive Control (KHP) Aqueous 25% Ethanol Solution



Life Sciences

2200 Northern Boulevard
East Hills, New York 11548-1289

| | |
|--|-----------|
| 1.800.717.7255 | toll free |
| 1.516.484.5400 | telephone |
| 1.516.801.9548 | fax |
| biotech@pall.com | email |

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