# Validation Guide

# NYLON Microfiltration Membranes 0.2 µm

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# Section I
# INTRODUCTION

**Objective:**

This validation guide for Nylon 6,6 membrane aims to present an overview of the entire validation process, including its content and methodology. The objectives are to understand the necessity of the test parameters.

Enable customers and regulatory authorities to comprehend the validation approach. Ensure that once the filters are validated, they consistently meet performance and quality standards in both national and international markets. The guide provides comprehensive information on the validation strategy and the plans to be implemented during validation activities.

**Scope:**

This validation guide shall be used as an overall document for validation activities being performed. The validation guide is designed to include the complete validation plan, which shall include test parameters, method and results of filter paper.

**Abstract:**

This comprehensive report presents the validation data for Dorsan Nylon 6,6 membrane filter paper, a revolutionary product designed to revolutionize liquid filtration in the pharmaceutical industry. Developed Nylon 6,6 membrane filter paper demonstrates superior retention of fine particles. The report covers various validation tests, including Bacterial Challenge, Extractable, and Biological Safety these test showcasing the remarkable capabilities and potential applications of Dorsan Nylon 6,6 membrane Filter Paper.

**Methodology:**

The validation process involved a series of rigorous tests to evaluate the efficiency and safety of Dorsan Nylon 6,6 membrane filter paper. Key assessments included bacterial challenge test, where the membrane filter paper's ability to capture and retain bacteria was scrutinized. Additionally other test like Forward flow, Bubble point integrity, Extractable, Biological safety, Acute Systematic Injection, Intracutaneous, Implantation and Chemical compatibility test were performed to analyze the functionality of Dorsan Nylon 6,6 membrane filter paper.

**Bacteria Test Results:**

The data demonstrated that Dorsan Nylon membrane filter paper surpassed expectations in bacterial challenge test. Nylon 6,6 material effectively captured and retained bacterial contaminants, ensuring that the filtered liquid met stringent industry standards for purity and safety.

**Extractable Analysis:**

One of the essential aspects of filter paper validation is determining the level of extractable compounds that may leach into the filtered liquid. The results indicated that Dorsan Nylon 6,6 membrane filter paper exhibited remarkably low levels of extractable. Making it a reliable choice for critical pharmaceutical applications.

**Biological Safety Tests:**

Ensuring the biological safety of the filter paper is paramount in the pharmaceutical industry. The biological safety tests conducted on Dorsan Nylon 6,6 membrane filter paper confirmed its non-toxic nature, guaranteeing that the filtered liquid remains unadulterated and safe for medical use.

**Conclusion:**

The validation data presented in this report unequivocally establish that Dorsan Nylon 6,6 membrane filter paper as an exceptional solution for liquid filtration in the pharmaceutical industry. Nylon 6,6 material with better retention capabilities for fine particles, surpassing the performance of conventional filters. The membrane filter paper's high flow rate, extensive throughput, and minimal extractable properties make it an indispensable asset in diverse pharmaceutical applications. With the assurance of biological safety, Dorsan Nylon 6,6 Membrane Filter Paper emerges as the excellent choice for liquid filtration, elevating industry standards and redefining the landscape of pharmaceutical filtration technologies. This report is designed to assist the filter user in meeting the validation requirements of regulatory authorities within the pharmaceutical industry.

## 1.1 QUALITY ASSURANCE:

At Dorsan Filtration, quality assurance is paramount. All materials are meticulously selected in compliance with current regulations and our stringent in-house standards. Following our standard operating procedures (SOP), we ensure quality from material selection to dispatch, guaranteeing that all terms of delivery and acceptance are met.

Every finished Dorsan membrane filter paper undergoes rigorous final quality control. This includes 100% non-destructive testing of each lot of Nylon 6,6 membrane filter paper. No lot is released until all in-process and final quality control data are verified and meet our stringent standards.

## 1.2 TECHNICAL SPECIFICATION

**Product Information:**

The DORSAN 0.20 µm Nylon membrane filter paper is designed for effective particle removal and sterile filtration of aqueous solutions. Made from high-quality nylon, these filters deliver excellent flow rates, low extractable levels, and broad chemical compatibility.

Each lot of membrane filter paper undergoes rigorous 100% integrity testing during manufacturing to ensure reliable performance and compliance with sterilization standards. Ideal for pharmaceutical, biotechnology, and laboratory applications, the DORSAN Nylon 6,6 Membrane Filter ensures the purity and safety of critical processes.

**Material of Construction**

Filter Media: Nylon 6,6
Pore Size: 0.20 Micron

**Product Coding**

DORSAN Nylon 6,6 Membrane Filter product coding system for easy identification and traceability. Each code includes

| | Diameter | Nylon | Pore Size µm | Gridded | Sterile |
|---|---|---|---|---|---|
| M0 | 13 | NY | 0.20 | G | S |
| | 25 | | | | |
| | 47 | | | | |
| | 90 | | | | |
| | 142 | | | | |
| | 293 | | | | |

**Example: Dia.: 47mm, Pore Size: 0.20 Micron > M047NY020**

**Product Dimension**

All size are available as per customer requirement. Also we offer in roll form.

## SECTION – II:
## STUDIES ON BACTERIAL REMOVAL EFFICIENCY

### 2.1 Microbiological Validation using *Brevundimonas Diminuta* Liquid Challenge Test

#### 2.1.1 Introduction:

The guidelines set forth by the FDA regarding sterile products generated through aseptic processing declare, "A sterilizing filter refers to a filter that, when faced with the microorganism ***(Brevundimonas Diminuta*** ATCC No.19146), at a minimum density of $10^7$ organisms per $cm^2$ of filter surface, will yield a sterile outflow."

Establishing a correlation between microbial retention and a non-destructive integrity test forms a crucial facet in validating filters of sterilizing grade. The FDA guideline additionally asserts, "Upon successfully validating a filtration process for a specific product, process, and filter, it is imperative to ensure that the subsequent filter replacements (including the membrane and cartridge) employed in production runs, exhibit the same performance. One approach to achieving this entails establishing a link between filter performance data and filter integrity test results." The integrity tests utilized throughout this validation study encompassed the bubble point and forward flow tests.

#### 2.1.2 The Bubble Point Integrity Test:

During the Bubble Point Test, a filter is saturated with an appropriate test liquid. Following a period of stabilization, the gas pressure on the upstream side of the filter assembly is raised. This is done utilizing precise flow measurement apparatus, such as integrity test devices, to ascertain the pressure at which the gas flow rate undergoes a noticeable alteration.

#### 2.1.3 The Forward Flow Integrity Test:

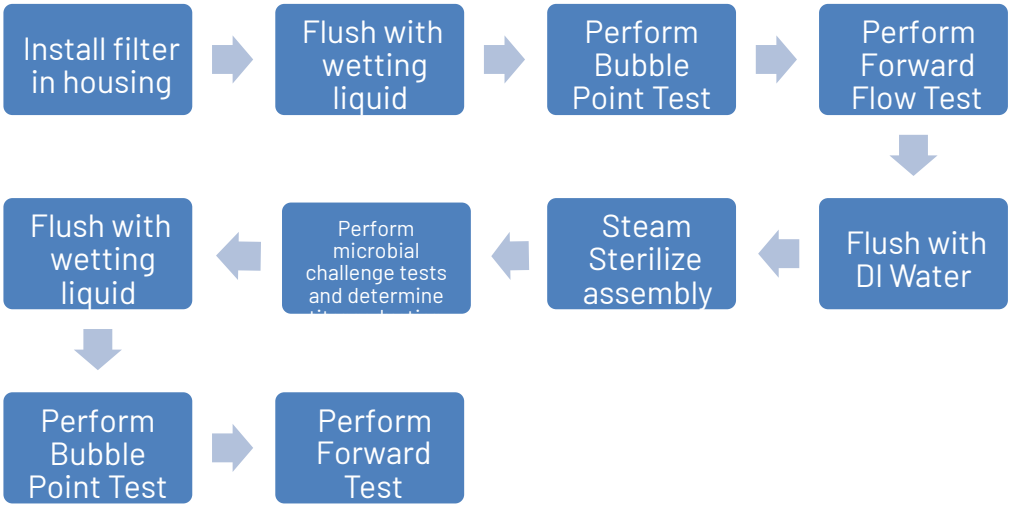
During the Forward Flow Test examination, a filter undergoes wetting with an appropriate test liquid, and a pre-established gas pressure is exerted on the upstream section of the filter arrangement. Following a period of stabilization, the flow of gas passing through the moistened membrane can be manually gauged on the upstream side.

**The aims of this series of tests were to:**

- Assess the effectiveness of Dorsan Nylon 6,6 Membrane Filter Paper in eliminating microorganisms through liquid challenge tests using ***Brevundimonas Diminuta***.

- Identify and define the parameters for conducting integrity tests.

## 2.2 Method of Analysis:




Dorsan Nylon 6,6 Membrane Filter Paper samples, exhibiting varying Bubble Point and Forward Flow characteristics. These samples were then subjected to rigorous microbial challenge tests, utilizing an aqueous suspension of ***Brevundimonas Diminuta.***

Before proceeding with the challenge tests, the filter sample was carefully installed within a housing and underwent integrity assessment employing both the Bubble Point and Forward Flow methodologies. Subsequently, the entire filter assembly was autoclaved at a temperature of 121˚C to 122˚C (250˚F) for a duration of 30 minutes. Following the autoclaving process, the filter assembly was meticulously connected to a pre-sterilized challenge apparatus in an aseptic manner.

To achieve a challenge level exceeding $1x10^8$ colony forming units (CFU) per $cm^2$, an aqueous suspension of ***Brevundimonas Diminuta*** was methodically passed through the filter. In all tests, a remarkable achievement of $1x10^8$ CFU was obtained. Upon the completion of the microbial challenge, a second round of Bubble Point and Forward Flow tests was conducted. Throughout the challenge test, the entire fluid containing the microorganism was subjected to filtration using a 0.20-micron filter. The resulting downstream liquid was collected and once again subjected to filtration through a 0.20-micron filter. The aforementioned filter membrane paper was subsequently incubated in both SCDM (Soybean Casein Digest Medium) and FTM (Fluid Thioglycolate Medium) media for further analysis.

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

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

**>Total number of organisms influent to the filter (e.g.>$1x10^8$)**

NOTE: Please contact Dorsan office if a more detailed description of the test methods is required.

## 2.3 Test Results:

### 2.3.1 Bubble Point Correlation:

The results for Bubble Point and retention of ***Brevundimonas Diminuta*** are displayed in Table 1 and graphically.

It was determined that all filters with Bubble Point values equal to or greater than 3400 mbar exhibited sterility in the effluent when exposed to a challenge of greater than >$1.0x10^8$ CFU (Colony Forming Units) of ***Brevundimonas Diminuta*** per filter.

| Filter Batch | Filter Identification | Bubble Point (mbar) | Sterile Effluent | Titer Reduction |
|---|---|---|---|---|
| Number | A | 3675 | Yes | >$1.0x10^8$ |
| | B | 3664 | Yes | >$1.0x10^8$ |
| | C | 3615 | Yes | >$1.0x10^8$ |
| 123045 | D | 3646 | Yes | >$1.0x10^8$ |
| | E | 3696 | Yes | >$1.0x10^8$ |
| | F | 3674 | Yes | >$1.0x10^8$ |
| | G | 3643 | Yes | >$1.0x10^8$ |
| | H | 3620 | Yes | >$1.0x10^8$ |

**Table 1:** Correlation of Bubble Point with ***Brevundimonas Diminuta*** Retention for Dorsan Filter Paper (47mm, 0.20 Micron)

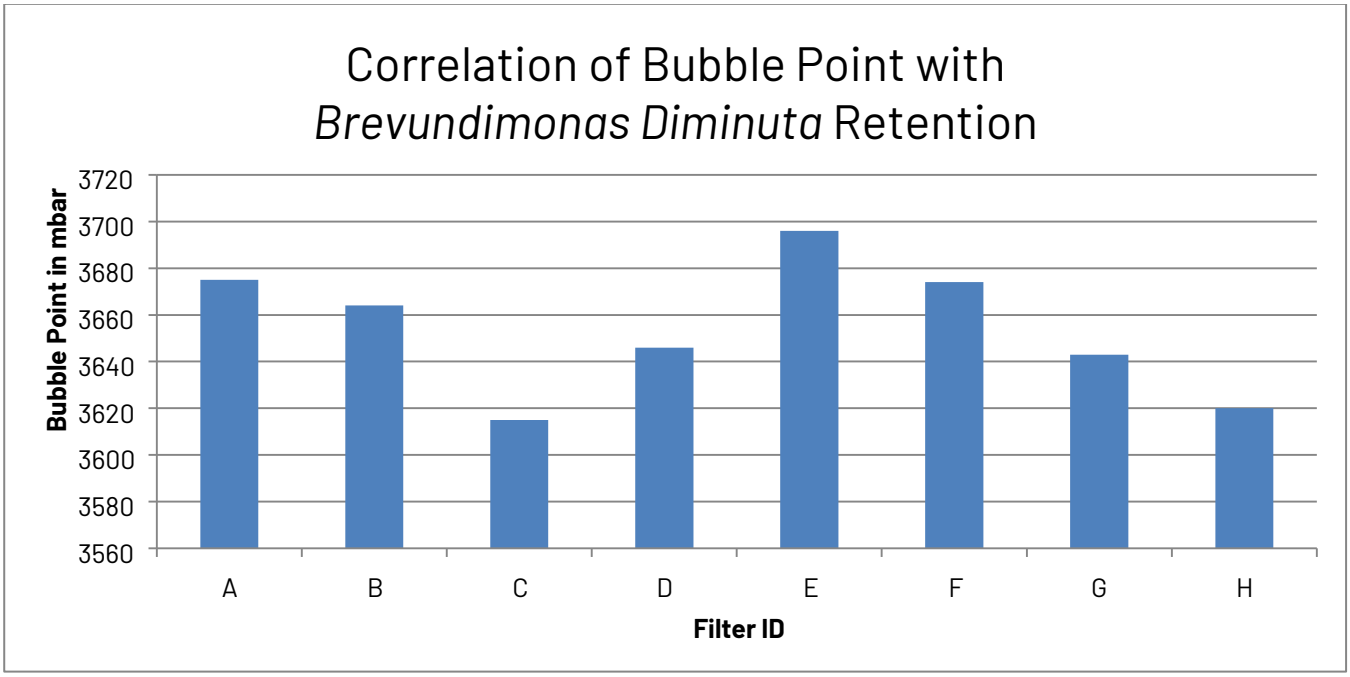



Figure 1: Correlation of Bubble Point with ***Brevundimonas Diminuta*** Retention for Dorsan Filter Paper (47mm, 0.20 Micron)

### 2.3.2 Forward Flow Correlation:

The results for Forward Flow and retention of ***Brevundimonas Diminuta*** are presented in the table 2. It was observed that filters with Forward Flow values nearby 12.5 ml/ min/$cm^2$ exhibited sterility in the effluent when subjected to a challenge of greater than $1.0x10^8$ CFU (Colony Forming Units) of ***Brevundimonas Diminuta*** per filter.

| Filter Batch Number | Filter Identification | Forward Flow (ml/min/$cm^2$ at Δ700 mbar @ 25°C) | Sterile Effluent | Titer Reduction |
|---|---|---|---|---|
| 123045 | A | 12.1 | Yes | $>1.0x10^8$ |
| | B | 12.3 | Yes | $>1.0x10^8$ |
| | C | 11.9 | Yes | $>1.0x10^8$ |
| | D | 12.4 | Yes | $>1.0x10^8$ |
| | E | 12.0 | Yes | $>1.0x10^8$ |
| | F | 12.0 | Yes | $>1.0x10^8$ |
| | G | 12.2 | Yes | $>1.0x10^8$ |
| | H | 12.3 | Yes | $>1.0x10^8$ |

**Table 2:** Correlation of Forward Flow with ***Brevundimonas Diminuta*** Retention for Dorsan Filter Paper (47mm, 0.20 Micron)

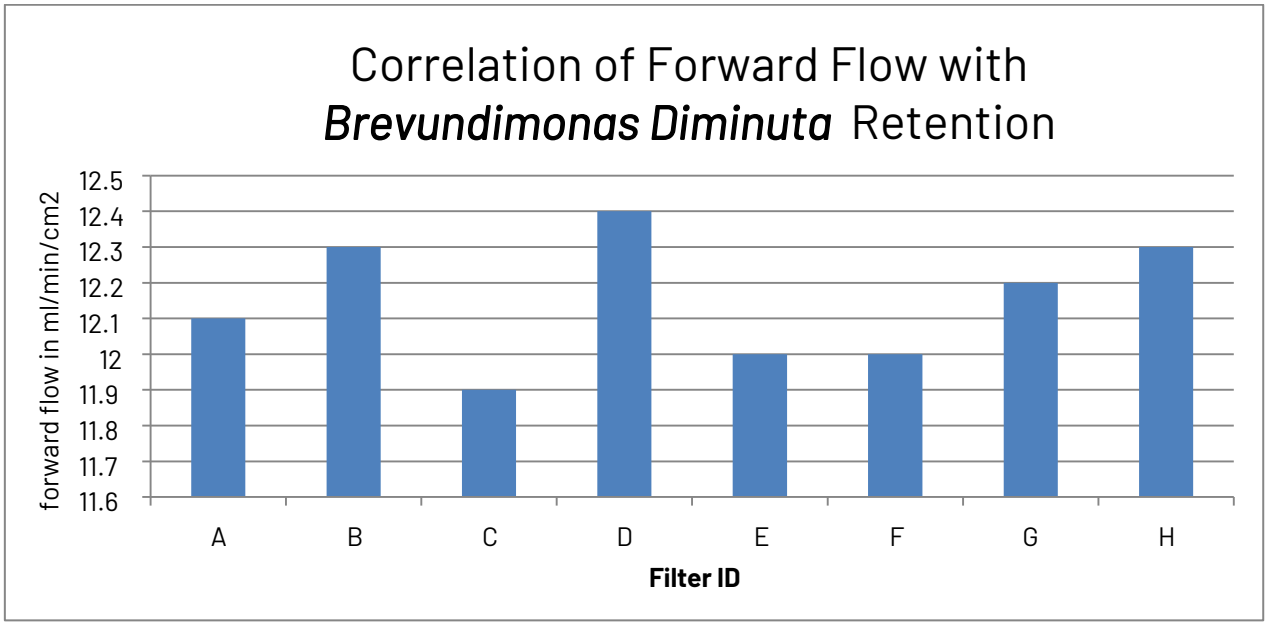



**Figure 2:** Correlation of Forward Flow with ***Brevundimonas Diminuta*** Retention for Dorsan Filter Paper (47mm, 0.20 Micron)

## 2.4 Observation

Based on the results of the validation study, both the Forward Flow and Bubble Point test methods were demonstrated to be suitable non-destructive integrity tests for Dorsan Nylon 6,6 Membrane Filter Paper. Integrity Test parameters for Dorsan Nylon 6,6 Membrane Filter Paper were set as follows:

**Bubble Point Test parameter**

| | |
|---|---|
| Wetting Liquid | DI Water |
| Test Gas | Air |
| Min. Pressure | 1000 mbar |
| Temperature | 25° C ± 2° C |
| **Minimum allowable Bubble Point Limit** | **≥ 3400 mbar** |

**Forward Flow Integrity Test Parameters**

| | |
|---|---|
| Wetting Liquid | DI Water |
| Test Gas | Air |
| Pressure | 1000 mbar |
| Temperature | 25° C ± 2° C |
| **Maximum allowable Forward Flow Limit** | **12.5 ml/min/cm$^2$ at Δ700 mbar @ 25°C** |

These Forward Flow and Bubble Point integrity test parameters:

Integrate a safety buffer to ensure an extra level of protection.

Deliver a robust guarantee of ***Brevundimonas Diminuta*** retention when exposed to a minimum of >1x10$^8$ CFU/cm$^2$ of effective filtration area.

Verify compliance of Dorsan Nylon 6,6 Membrane Filter Paper with the stringent criteria set forth for sterilizing grade filters.

Note: The integrity test values are regularly evaluated and monitored as part of routine production tests. These values are generated and overseen by the Quality Department at Dorsan. For additional information, please contact to Dorsan.

# SECTION – III
# VALIDATION OF PHYSICAL CHARACTERISTICS

## 3.1 Resistance to Autoclave Steam Cycle

### 3.1.1 Objective

The objective of this test is to validate the physical characteristics of the Dorsan Brand Nylon6,6 Membrane Filter (0.20µm) by assessing its structural integrity and performance after repeated autoclave cycles. This validation aims to evaluate the filter's strength and structural integrity after 30 autoclave cycles, perform a bubble point test after each cycle to ensure the filter's integrity and functionality remain intact, and visually inspect the filter after each cycle for any physical deformations such as bending, warping, or other signs of damage.

### 3.1.2

**Method of Analysis:**

The Dorsan Brand Nylon 6,6 Membrane Filter (0.20µm) was subjected to 30 autoclave cycles, with each cycle involving exposure to standard autoclave conditions, including a temperature of 121 to 122 degrees Celsius, and a duration of 30 minutes. After each autoclave cycle, a bubble point test was conducted to measure the pressure at which the first bubble passed through the wetted filter membrane, with the bubble point value being recorded for each cycle. Additionally, the filter was visually inspected after each autoclave cycle to check for any physical deformations, such as bending, warping, or other damage, and any observations were recorded.

### 3.1.3

**Test Results:**

The test results are summarized in the table below:

| Batch Number | Bubble Point Integrity Test (mbar) after following Number of 30 minutes Autoclave Cycles | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 4 | 8 | 16 | 24 | 30 |
| 452163 | 3675 | 3684 | 3648 | 3575 | 3567 | 3773 | 3762 | 3590 |
| | 3666 | 3658 | 3749 | 3654 | 3645 | 3733 | 3675 | 3567 |
| | 3568 | 3578 | 3698 | 3656 | 3600 | 3698 | 3690 | 3606 |
| | 3685 | 3743 | 3756 | 3754 | 3584 | 3678 | 3666 | 3615 |
| | 3684 | 3586 | 3749 | 3556 | 3685 | 3602 | 3783 | 3622 |

Wetting by purified water before autoclave cycle.

### 3.1.4 Observation:

The Dorsan Nylon 6,6 membrane filter paper demonstrates exceptional durability, capable of withstanding numerous autoclave cycles even in tough conditions. This durability is highlighted by its performance during high-pressure steam exposure at 121°C (255°F), which tests the filter's strength under extreme temperatures and conditions.

# SECTION – IV
# EXTRACTABLE TESTING

## 4.1 Introduction:

The evaluation of extractable substances is vital for confirming the suitability of Dorsan Nylon 6,6 membrane filter papers for applications requiring low levels of extractable. This procedure follows methodologies aligned with USP guidelines to ensure compliance with standards for minimal contamination.

## 4.2 Method of Analysis:

### 4.2.1 Preparation of Filter Samples:

To prepare the Dorsan Nylon 6,6 membrane filter papers for extractable substance testing, first, select representative samples of the filter paper, each with a diameter of 47 mm. Precondition these samples by drying them in an oven at 50°C for 1 hour to remove any pre-existing moisture that could interfere with the results. Ensure that the drying process is thorough, as residual moisture may affect the accuracy of the test. After drying, allow the filter papers to cool in a desiccator to prevent any reabsorption of moisture from the environment. Once cooled, handle the samples using clean, dry tools to avoid contamination and proceed with the extraction process. This preparation ensures that the filter papers are in a standardized condition for accurate assessment of extractable substances.

### 4.2.2

**Extraction Procedure:**

Flush each preconditioned Dorsan Nylon 6,6 membrane filter paper with 1 liter of purified water at room temperature, then collect the water in a clean container. Repeat the process with 1 liter of methanol. Allow the filter paper to remain submerged in each extraction medium for four hours. After collection, evaporate 50 mL aliquots of each solvent in acid-washed crucibles to dryness. Weigh the residues to determine the amount of non-volatile extractable substances in the filter paper.

### 4.2.3

**Analysis of Material Extracted:**

In the analysis, each solvent extract is processed by transferring 50 mL aliquots into acid-washed crucibles, which are then placed in an oven to evaporate the solvent completely. The remaining non-volatile residue is carefully weighed using an analytical balance. This weight measurement provides the amount of extractable substances present in the filter paper. The process is conducted separately for both water and methanol extracts to ensure comprehensive analysis.

The total mass of non-volatile residues from the solvent extracts is recorded and compared against established limits to assess compliance with regulatory standards. The results are then analyzed to determine whether the Dorsan Nylon 6,6 membrane filter papers meet the required specifications for extractable substances, ensuring their suitability for applications where low extractable levels are critical.

## 4.3 Test Results:

The non-volatile residues from the solvent extracts of the Dorsan Nylon 6,6 membrane filter papers were measured and found to be within the acceptable limits set by USP. This confirms that the filter papers meet the required standards for minimal extractable.

## 4.4 Observation:

The test results for the Dorsan Nylon 6,6 membrane filter papers were thoroughly documented, showing that the non-volatile extractable substances from both water and methanol extracts were below the USP limits. This confirms that the filter papers have low extractable levels and are suitable for sensitive applications. The process was conducted with meticulous care to ensure accuracy, and the results were consistent with the regulatory standards.

# SECTION – V
# BIOLOGICAL SAFETY TESTING

## 5.1 Introduction:

The purpose of these tests was to assess the biological compatibility and suitability of the materials used in the construction of Dorsan Nylon 6,6 Membrane Filter Paper. The following are the filter medium of construction employed in Dorsan Nylon 6,6 Membrane Filter Paper.

## 5.2 Method of analysis:

The tests conducted on the Dorsan Nylon 6,6 Membrane Filter Paper followed the guidelines outlined in the United States Pharmacopeia (USP) for Class VI Plastics, specifically pertaining to biological reactivity tests in vivo at a temperature of 121°C (250°F). These tests were performed at NABL approved testing lab.

The testing procedures specified in the USP involved the injection of extracts derived from the plastic materials as well as the implantation of solid materials into animal tissue. Here we have tested granules of Nylon 6,6.

To simulate parenteral solutions and body fluids, four extracting media were utilized, as prescribed by the USP. These included Sodium Chloride Injection, a 1 in 20 Solution of Alcohol in Sodium Chloride Injection, Polyethylene Glycol 400, and Vegetable Oil (specifically sesame or cottonseed oil).

According to the USP, extracts could be prepared under 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. Therefore, the filters underwent extraction at 121°C (250°F), ensuring the stringent

**5.2.1** conditions were met.

**Acute Systemic Injection Tests:**

To assess the potential of a single injection of an extract to induce systemic toxicity, an Acute Systemic Injection Test was conducted. Intravenous injections were administered using both Sodium Chloride Injection and 1 in 20 Solutions of Alcohol in Sodium Chloride Injection. On the other hand, intraperitoneal injections were employed for the Vegetable Oil extract and Polyethylene Glycol 400 extract. These

**5.2.2** injections were performed to evaluate the potential systemic effects resulting from the administration of the respective extracts.

**Intracutaneous Tests**

In order to assess the propensity of inducing tissue irritation through a single injection of an extract, an Intracutaneous Test was conducted. All four of the aforementioned extracts, namely Sodium Chloride Injection, 1 in 20 Solution of Alcohol in Sodium Chloride Injection, Vegetable Oil, and Polyethylene Glycol 400, were employed in these investigations. The objective was to evaluate the possible irritant effects on the skin tissue resulting from the injection of each respective extract.

### 5.2.3 Implantation Tests:

Additionally, implantation tests were conducted to subject the individual components of Dorsan Filter Paper to the most rigorous conditions outlined in the USP.

## 5.3 Observation:

Dorsan Nylon 6,6 Membrane Filter Paper has successfully met the stringent requirements set by the United States Pharmacopeia (USP) for Class VI (121°C) Plastics. The accompanying figure displays a copy of the test certificate, confirming its compliance. For access to detailed test reports, it is recommended to reach out to Dorsan.

## SECTION -VI

# 6.0 CHEMICAL COMPATIBILITY

## 6.1 Introduction:

Chemical compatibility of Dorsan Nylon 6,6 membrane filters refers to their resistance to chemical degradation or damage when exposed to various substances, making it crucial for reliable filtration in diverse applications. Understanding and assessing this compatibility is essential for ensuring the integrity and effectiveness of nylon membrane filters in specific chemical environments.

## 6.2 Method of Analysis:

Assessing chemical compatibility of Dorsan Nylon 6,6 membrane filters involves immersing them in representative chemicals for 48 hours and then conducting a bubble point integrity test. Analyzing the results helps confirm if the membrane is resistant or susceptible to the chemicals, guiding its suitability for specific applications.

## 6.3 Observation:

| Chemical | Observation |
|---|---|
| Acetic acid, glacial | Resistant |
| Hydrochloric acid, conc.35%) | Not Resistant |
| Sulfuric acid, conc. (96%) | Not Resistant |
| Methanol | Limited Resistance |
| Sodium hydroxide, 3N (11%) | Resistant |
| Butyl acetate | Limited Resistance |
| Ethyl acetate | Limited Resistance |
| Ethylene glycol | Resistant |
| Propylene glycol | Resistant |
| Toluene | Limited Resistance |
| Chloroform | Resistant |
| Acetone | Limited Resistance |
| Acetonitrile | Resistant |
| Formaldehyde, 37% | Resistant |
| Formaldehyde, 4% | Resistant |

**Note:** Compatibility can be affected by multiple factors, including temperature and concentration. It is advisable to test the compatibility with the specific solution you intend to filter prior to conducting the actual filtration process.

## 6.4 Conclusion:

The validation guide for Nylon 6,6 filter papers selected for validation having quality attributes and process parameter for said filters which are explained in the validation guide. The results of each testing parameters match with the required specific limit which also mentioned in the validation guide.

Above all data shows that Nylon 6,6 filter papers are complies all the tests which are required during validation and the same will be demonstrate during the intended use.