Validation and Qualification

for Durapore® Sterilizing-Grade (0.22 µm) Membrane VMF4

Introduction – A Technical and Historical Perspective

This document contains Millipore's most current Validation and Qualification information for Durapore, Sterilizing-Grade (0.22 µm) Membrane VMF4. This membrane is a hydrophobic polyvinylidene fluoride (PVDF) backbone that is rendered hydrophilic by applying a surface modification of cross-linked hydrophilic polymer.

In 1995, consultants and regulators began to discuss the need to use worst case or minimum bubble point membranes to validate the capability of a membrane to sterilize a drug solution as part of an aseptic process. As a result of this discussion, PDA Technical Report 26, "Sterilizing Filtration of Liquids," was published. The Technical Report stated that "a physical integrity test is meaningful only when it can be related to specific filter-retention characteristics." This statement focuses on the need to support any membrane designated as sterilizing-grade with a documented correlation between bacterial retention and an integrity test value.

Millipore's approach to providing this correlation between bacterial retention performance and integrity test value is the hydrophobic membrane structure of Durapore.

A hydrophilic bubble point LRV correlation was not developed because hydrophilization does not impact retention. The hydrophobic structure of the Durapore membrane is the primary determinant of this membrane's retention characteristics. Hydrophilization, which is the second step in manufacturing, simply makes the initially cast membrane hydrophilic. It does not change the pore size, membrane structure, methanol bubble point or microbial retention characteristic for sterilizing-grade Durapore.

The Durapore membrane structure can be changed to yield a series of membranes with different pore sizes and corresponding bubble points. By testing this series of membranes using the standard ASTM test, one can demonstrate that through a range of bubble points, membrane retention performance undergoes a transition from incomplete to complete bacterial retention. This demonstration fulfills the regulatory requirement for a demonstrable correlation between integrity test value and bacterial retention performance.

Although the hydrophobic structure provides the bulk of particle retention, most filtration applications require a hydrophilic membrane. Because the hydrophobic base membrane cannot be easily water wetted, this membrane is surface-modified with a hydrophilic cross-linked polymer. Although this hydrophilic membrane has a water-wet bubble point value, the hydrophobic bubble point is the integrity test value most closely linked to intrinsic particle-retention performance. This rational is explained in further detail below, along with the data that support this correlation.

Abstract

A correlation between bubble point and LRV was established experimentally on hydrophobic PVDF membrane. From this correlation, the bubble point of sterilizing-grade hydrophobic membrane can be predicted using statistical methods of analysis. Several statistical analysis methods were used in this study. The method chosen as most appropriate for the data was a method that combined Poisson regression and the negative binomial. This statistical method shows that the rapid transition from non-retentive to retentive membrane occurs over the range of membranes spanning 14 to 16 psi methanol bubble point. Additionally, membranes with the manufacturing release specification of 18 psi methanol bubble point are shown to be outside of this transition region, and well above the bubble point range in which the statistical method provides a meaningful estimate of the probability that a disk with a given bubble point value will yield a sterile filtrate.

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Statistical Analyses

The bubble point and LRV data for hydrophobic membrane were analyzed using linear regression, survival analysis and Poisson regression.

Linear regression (Weisberg) is a statistical tool in which data are analyzed to determine the equation for a line that best represents the relationship between LRV and bubble point. Using this technique, only data from non-fully-retentive disks can be analyzed, because fully-retentive disks do not have a real number LRV associated with them. Typically, this technique has been used to derive a linear relationship between LRV and bubble point over the range of several bubble point units. Importantly, since this method cannot include data of fully retentive disks, extrapolation of the linear relationship is required to arrive at a value for bubble point that represents a "bubble point cutoff" for retentive membrane.

Results

Four trials were executed in this study. Each trial was done on a different day.

Microbial Challenge Suspensions

In this study, a sterilizing-grade filter is defined as one that yields a sterile filtrate when challenged with at least 10^7 organisms per square centimeter, which is 1.38×10^8 CFU/disk when using 47 mm disks. A filter that retains all organisms at this challenge level is reported to have an LRV of greater than 8.14. In the trials presented, every effort was made to keep the challenge level the same so that this variable could be considered a constant in the analysis of the data. Subsequently, all challenge suspensions were between 1.67×10^8 and 2.30×10^8 CFU/disk and retentive filters had LRVs reported as ">8.22" to ">8.36". The data from these experiments are found in Table 1.

Table 1. Summary of Experimental Data

Trial Number	Initial Bubble Point (MeOH)	LRV	Measured Thickness (µm)	Trial Number	Initial Bubble Point (MeOH)	LRV	Measured Thickness (µm)
47	10.1	3.72	115	55	13.6	7.45	108
46	10.3	3.77	116	43	13.6	7.55	104
38	9.7	3.85	117	8	13.4	7.60	105
37	10.0	3.94	116	45	13.4	7.64	101
39	10.8	3.95	115	26	13.4	7.66	106
19	10.2	4.01	114	58	14.1	7.75	91
51	11.0	4.11	90	27	13.6	7.76	109
50	11.0	4.13	89	9	13.1	7.99	102
2	10.2	4.17	118	59	14.0	>8.22	87
49	10.9	4.17	94	60	14.9	>8.22	115
48	11.0	4.21	95	61	15.0	>8.22	114
3	10.0	4.22	116	62	16.2	>8.22	94
1	10.2	4.29	117	63	16.0	8.22	96
21	10.2	4.30	118	7	13.6	>8.29	106
20	10.3	4.35	113	10	14.1	>8.29	99
54	12.6	6.22	108	11	13.8	>8.29	93
22	12.4	6.25	104	12	13.6	>8.29	89
40	12.6	6.33	106	13	14.9	>8.29	114
53	12.8	6.35	106	14	15.0	>8.29	116
42	12.8	6.38	104	15	14.5	>8.29	115
6	12.8	6.50	104	16	16.0	>8.29	98
41	12.8	6.50	104	17	15.9	>8.29	98
52	12.6	6.50	108	18	15.6	>8.29	92
23	12.6	6.53	106	28	14.1	>8.36	85
4	12.4	6.59	108	29	14.0	>8.36	88
5	12.5	6.75	105	30	14.0	>8.36	90
24	12.9	6.88	110	31	14.8	>8.36	113
56	13.4	6.98	114	32	15.2	>8.36	112
57	13.5	7.08	105	33	14.8	>8.36	116
44	14.2	7.29	104	34	15.6	>8.36	92
25	13.4	7.41	103	35	15.8	>8.36	95
				36	16.0	>8.36	102

Retention Correlation

The data in Table 1 were analyzed by linear regression, survival analysis and Poisson regression.

The data fit the following linear regression equation:

LRV = 1.02811*bubble point - 6.5255 + error

The coefficient of regression, R^2, is 0.932. This curve intersects an LRV of 8.14 (the value representing sterilizing membranes) at a bubble point of 14.3 psi.

Using the mean square error (sigma), for the "error" value, the following equation is derived:

bubble point=(LRV-b-sigma)/m

The bubble point of a membrane predicted to yield a sterile effluent can be calculated for any confidence level by multiplying sigma by a z factor. The practical interpretation is that if the entire experiment were repeated 100 times, 99% of the time the prediction line (regression line) would intersect LRV=8.14 at a bubble point of 14.9 or lower. The same data can be used to calculate predicted "sterilizing" bubble points for any confidence level, as is shown in Table 2.

The same data were analyzed by Poisson regression. A summary of this analysis is provided in Table 5. Poisson regression gives statistically derived probabilities that an individual disk with a given bubble point will yield a sterile effluent. For example, according to the data in Table 3, a disk with a 17.0 psi bubble point will have a 99.96% chance of yielding a sterile effluent when tested according to the methods used in this report.

Table 2. Bubble Points of Sterilizing-Grade Hydrophobic PVDF Membrane as Calculated by Two Statistical Methods

Statistical Method	BubblePoint[1] LRV=8.14	Confidence Interval[2]			
		99.5%	99%	97.5%	95%
Linear Regression Analysis (retentive data excluded)					
Linear regression	14.3	15.3	15.2	15.0	14.9
Log linear regression	14.0	15.0	14.9	14.7	14.6
Survival Analysis (retentive data included)					
Linear analysis	14.0	15.0	14.9	14.7	14.6
Log linear analysis	13. 8	14.8	14.7	14.5	14.4

[1] Bubble point at which the curve predicted from a given statistical model intersects an LRV of 8.14

[2] Bubble points that give the indicated level of confidence that a filter disk with this bubble point will yield a sterile filtrate

Table 3. Poisson Regression Analysis: Prediction of the Ability of Hydrophobic PVDF Membrane to Yield a Sterile Filtrate

Visual Methanol Bubblepoint	$P_{(0cfu)}$*
15.0	95.48
16.0	99.59
17.0	99.96
18.0	>99.99
19.0	>99.99
20.0	>99.99
21.0	>99.99

* Probability that an individual disk with the designated visual methanol bubble point will yield a sterile effluent in the standard microbial retention test

Conclusion and Discussion

In this study, microbial LRV was compared to membrane bubble point, and a correlation between the two variables was established. The data were analyzed by five different statistical methods.

The Poisson regression was chosen as the primary statistical method. The primary value of the Poisson regression method is in describing the relationship between bubble point and microbial retention of filters that are in the narrow region of transition from not-fully-retentive to fully-retentive filters. Therefore, the transition region can be very well characterized.

From a practical perspective, the most relevant use of the data and the statistical modeling of the data is to compare them to the current manufacturing release specification for hydrophobic Durapore, which is 18 psi. The statistical model derived from the data verifies that the 18 psi specification is well beyond the region of transition to fully-retentive filters, and is well into the region of fully-retentive filters. Therefore, the 18 psi specification remains solid, and this work has **requalified hydrophobic Durapore with a bubble point of 18 psi or greater as a sterilizing-grade membrane.**

Correlation of the bubble point of Durapore to microbial retention fulfills a regulatory and industry requirement of sterilizing-grade filters. This report will assist in validating the use of Durapore filters in manufacturing processes.

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