

# The Usefulness of 0.45 $\mu\text{m}$ -Rated Filter Membranes

A. Mark Trotter,\* Patrick J. Rodrigues, and Laura A. Thoma



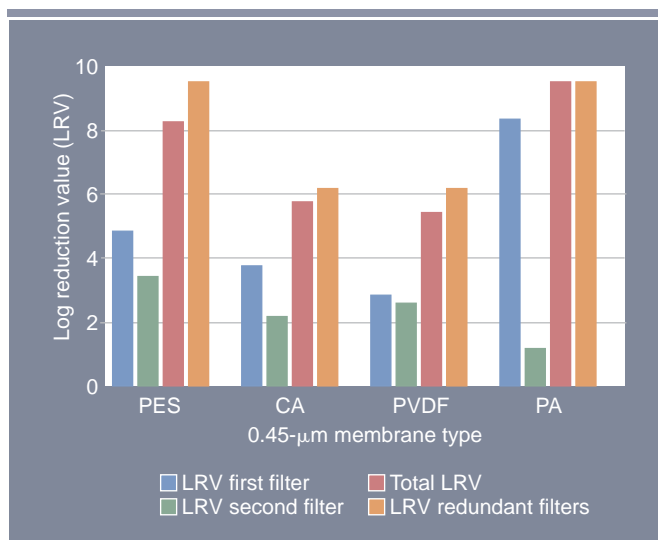
This study examines the retention of *Brevundimonas diminuta* (*B. diminuta*, ATCC 19146) by four different types of 0.45  $\mu\text{m}$ -rated membranes at two differential test pressures, 5 and 30 psi. The results demonstrated the ability of 0.45  $\mu\text{m}$ -rated filter membranes to retain at least  $1 \times 10^3$  organisms at 5 psi and  $>1 \times 10^8$  organisms at differential pressures of 30 psi. These findings, in addition to those referenced here, indicate that **the 0.45  $\mu\text{m}$ -rated filters can serve as sterilizing and bioburden reduction filters when challenged with significant numbers,  $1 \times 10^8$  cfu/cm<sup>2</sup> of *B. diminuta* in a wide range of differential pressures.**

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Filtration is an important practice in aseptic processing. Microporous membranes are relied upon because of their relatively narrow pore-size distributions. Traditionally, filters with pore-size ratings of 0.2/0.22  $\mu\text{m}$  have been considered “sterilizing grade,” meaning that they are expected to produce sterile effluents. Most drug preparations do result in sterility but not all. Therefore, it has been suggested that 0.1  $\mu\text{m}$ -rated membranes should be used to remove smaller organisms more thoroughly by sieve retention. A universal substitution of 0.2/0.22  $\mu\text{m}$ -rated filters by 0.1  $\mu\text{m}$ -rated filters is considered unnecessary, although contexts exist in which filters tighter than a 0.2/0.22  $\mu\text{m}$  can be used advantageously. However, one should consider that the smaller the pore-size designation of the filter, the more restricted its flow rate is, and depending upon the degree of particle loading in the fluid, the smaller the throughput that may be obtained.

These practical considerations should give technicians pause before using tighter filters than are necessary. Ratings tighter than 0.2  $\mu\text{m}$  do not necessarily mean better or more-secure retention. Adsorptive capture effects also are operative, and the attainment of a sterile filtration involves more than a sterilizing-grade membrane. However, the point here is that when use of



**Figure 1:** Log reduction value at 5 psi.

a 0.2/0.22  $\mu\text{m}$ -rated filter fails to yield sterile effluent, the tendency almost invariably is to substitute it with a tighter filter. A substantial history exists that relates to the success of a more open filter, namely the 0.45  $\mu\text{m}$ -rated membrane, for this very purpose.

### The sterilizing filter

The 0.2/0.22  $\mu\text{m}$ -rated membrane was selected to retain the *Brevundimonas diminuta* (*B. diminuta*, ATCC 19146) organism. Bowman and Holdowsky used such a filter for that very purpose and found that its 0.45- $\mu\text{m}$  counterpart removed as many as  $1 \times 10^5$  such organisms from aqueous suspensions (1). The 0.45- $\mu\text{m}$  filter failed in this retention only when penicillinase was present in the solution. Presumably, the presence of the protein competitively preempted the adsorptive sites. This circumstance prevented the adsorptive removal of the or-

ganisms and necessitated their sieve removal by the use of 0.2/0.22  $\mu\text{m}$ -rated membranes. In the absence of protein and other interferences, solutions containing *B. diminuta* can be sterilized using 0.45  $\mu\text{m}$ -rated filters. Leahy and Sullivan found that 0.22  $\mu\text{m}$ -rated membranes from mixed esters of cellulose yielded sterile filtrate at 5 psid and at 50 psid with a beta ratio of  $>10^{10}$  when confronted with *B. diminuta* challenges (2). The corresponding 0.45  $\mu\text{m}$ -rated filters gave beta ratios of  $1 \times 10^8$  cfu/cm<sup>2</sup> *B. diminuta* at 50 psi differential pressure and  $\sim 10^6$ /cm<sup>2</sup> at 5 psid. Tanny et al. showed that sterile effluent could be secured with 0.45  $\mu\text{m}$ -rated membranes when filtering  $2 \times 10^7$  cfu/cm<sup>2</sup> *B. diminuta* at 0.5 psid (3). Carter and Levy discuss the appropriateness of using 0.45  $\mu\text{m}$ -rated filters to recover organisms that already have passed through a sterilizing-grade filter (4).

The present study examined retentions of *B. diminuta* with four different types of membranes at two differential pressures, 5 and 30 psi. The results show that the first test filters retained at least  $1 \times 10^3$  organisms at 5 psi and  $>10^8$  organisms at 30 psi (see Figures 1 and 2). The four types of membranes investigated were composed either of poly(ether sulfone) (PES), cellulose acetate (CA), poly(vinylidene difluoride) (PVDF), or polyamide (PA).

The organism-retention action of the filters is not absolute given the varying contributions of adsorptive effects, but neither is the efficacy of the 0.2/0.22- $\mu\text{m}$  or the 0.1- $\mu\text{m}$  membranes as sterilizing filters (5). However, these findings and those referenced in the cited literature indicate that the 0.45  $\mu\text{m}$ -rated filters can serve as sterilizing and bioburden-reduction filters against significant populations such as  $1 \times 10^8$  cfu/cm<sup>2</sup> of *B. diminuta* in a wide range of differential pressures.

Relying on 0.45  $\mu\text{m}$ -rated membranes for sterile applications can present some advantages. FDA has advised that when viscous media are involved, two 0.45  $\mu\text{m}$ -rated filters should be used in series for sterility (6). Flow rates for filters with larger pores are higher, and throughputs are less likely to be abbreviated by higher particle loadings, which may be expressed in shorter processing times.

Therefore, the tendency to seek greater security for the attainment of sterile filtrations is not necessarily dependent on the use of tighter filters. Before committing to tighter membranes and their restrictive flows, filter trials and bioburden studies should be conducted to see whether the use of 0.45  $\mu\text{m}$ -rated membranes is advantageous for the purpose of securing sterility.

### Bubble points and retentions

Bubble-point values are taken to imply the size of the largest set of pores through which organisms are most likely to penetrate the filter. The higher the bubble point is, the smaller the pores are, which results in a greater degree of retention. The fact that the experimental data are in agreement with this known relationship is not surprising. However, comparing membranes of different polymeric compositions, each with a pore-size rating assigned

**Table I:** Three separate filters at 5 psi.

Membrane	LRV*		LRV		Total LRV
	First Filter	BP**	Second Filter	BP	
PES	4.83	40	3.35	41	8.18
CA	3.73	29.5	2.07	30	5.80
PVDF	2.78	27	2.61	28	5.39
PA	8.30	37.5	1.20	37.5	9.50

\* Log reduction value

\*\* Bubble point

**Table II:** Three separate filters at 30 psi.

Membrane	LRV*		LRV		Total LRV
	First Filter	BP**	Second Filter	BP	
PES	4.63	40.5	3.00	40	7.63
CA	3.58	30	1.80	30	5.38
PVDF	2.00	26	0.85	27	2.85
PA	7.87	37	1.60	37	9.47

\* Log reduction value

\*\* Bubble point

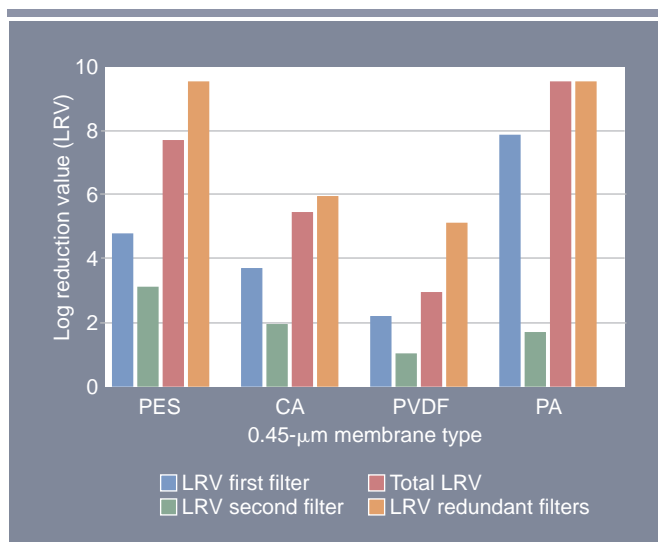


Figure 2: Log reduction value at 30 psi.

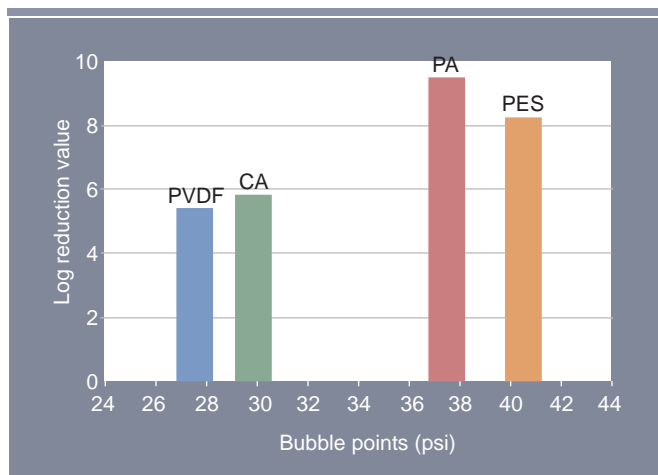


Figure 3: Total LRV versus bubble points at 5 psi.

by a different filter manufacturer, is an uncertain exercise. As will be discussed, membranes of the same pore-size rating but made from different polymers should exhibit different bubble points, although the differences may not be large.

Figures 3 and 4 show that the PVDF filters consistently maintained the lowest bubble points of the tested membranes. Also, they almost always yielded the least amount of organism retention, as would be expected. On the other hand, the PA mem-

branes, when compared with 0.45  $\mu\text{m}$ -rated membranes with similar bubble-point values (e.g., PES), showed higher retentions. This may be ascribed to the strong adsorptive tendency of this polymer noted in other contexts (7,8). A similar observation can be made for the CA membrane when compared with the PVDF membrane.

This conclusion is tentative. The contributions of the adsorptive propensities of the polymers composing these filters have not been quantified. Bubble-point pressures reflect, in addition to pore sizes, the additive effect of the attractive force involved in the wetting of the solid filter surface with the wetting liquid. The polymers, which have different structures, manifest different binding strengths with the same liquid. Therefore, the bubble points, the pressures that are needed to separate the various solid-liquid interfaces, are different for different materials. It is difficult to estimate how much of the difference derives from the individual polymers and how much is from the rating values that were conferred using individual (dissimilar) standards. Nevertheless, the same pore size-rated filters should not be expected to show the same bubble-point values if they are composed of different polymeric materials (9).

## Materials and methods

**B. diminuta preparation.** The challenge solution was prepared using *B. diminuta* freeze-dried cultures (No. 19146, American Type Culture Collection, Manassas, VA). The cultures were rehydrated aseptically with 0.5 mL of trypticase soy broth (TSB, Becton Dickinson, Sparks, MD). The entire contents were transferred to a culture tube containing 5 mL of TSB and incubated at  $30 \pm 2^\circ\text{C}$  for 24 h.

After incubation was complete, 20 trypticase soy agar (TSA) slants were inoculated with 75  $\mu\text{L}$  of the 5-mL TSB culture. The TSA slants were incubated at  $30 \pm 2^\circ\text{C}$  for 48 h and then stored at  $4^\circ\text{C}$ . At this point, the purity of the cultures was verified by a gram-stain and chrome oxidase test, and the cultures were checked for uniform colonial morphology. The slants could be stored for as long as one month.

The challenge inoculum was prepared 54 h before performing a test. A culture tube containing 30 mL of TSB was aseptically inoculated with a loop full of cells from a TSA slant and incubated at  $30 \pm 2^\circ\text{C}$  for 24 h. The seed culture broth was checked again with a gram-stain test. Seed culture (4 mL) then was used to inoculate 1 L of saline lactose broth (SLB, lactose broth, Difco, Detroit, MI) and incubated at  $30 \pm 2^\circ\text{C}$  for 30 h. At this time, the purity of the culture was checked again, and a serial dilution count was taken to determine the average cell density per milliliter. The total cell number was determined by a direct microscopic count of the dilution sample of the *B. diminuta* culture. The viable cell number was determined by a bacterial colonial count on a TSA plate. The challenge solution was prepared by diluting an appropriate volume of *B. diminuta* SLB culture (the bacterial challenge number determined by the viable count) with SLB broth (10).

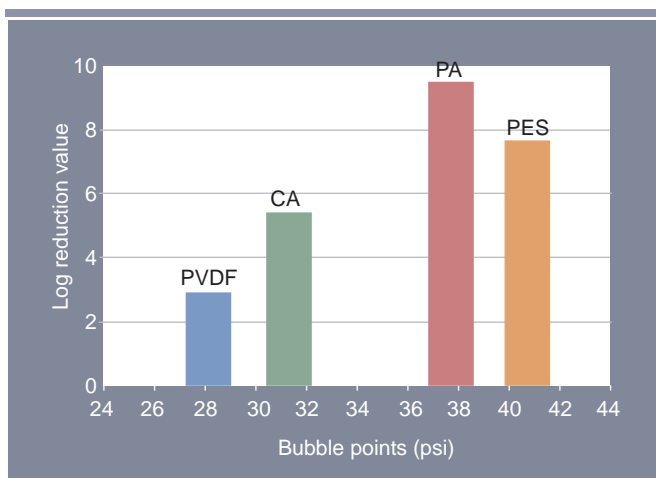
**Experiments.** Two types of experiments were conducted. In the Type I experiments, three filters of the same type (same material and  $\mu\text{m}$  rating) were loaded individually into separate filter housings. Each filter was wetted with sterile water using a

Table III: Redundant filters at 5 and 30 psi.

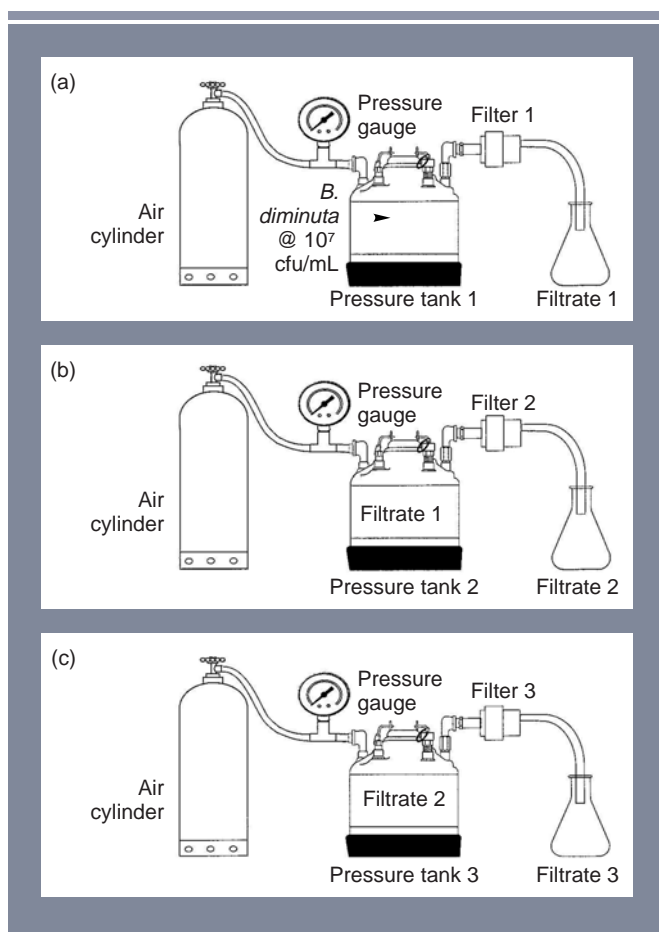
Membrane	5 psi		30 psi	
	LRV*	BP**	LRV	BP
PES	9.47	41	9.47	40
CA	6.14	29	5.93	29
PVDF	6.13	30	5.00	30
PA	9.47	39	9.47	39

\* Log reduction value

\*\* Bubble point



**Figure 4:** Total LRV versus bubble points at 30 psi.



**Figure 5:** Three separate filters.

peristaltic pump (model 501Z, Watson Marlow, Ltd., Cornwall, UK). After wetting, the bubble point of each filter was measured in sterile water using a compressed-air cylinder to ensure filter integrity. Each filter then was sterilized with the housing in an autoclave at 121 °C for 15 min. When cool, each filter was presented with 100 mL of sterile SLB solution.

A stainless steel pressure tank received 300 mL of SLB with a *B. diminuta* concentration of  $1 \times 10^7$  cfu/mL. Using a compressed air cylinder, this bacterial suspension was pumped

through the first filter at 5 psi. The filtrate was collected, the volume was measured, and it was transferred to a second pressure tank. This filtrate then was pumped through the second filter at 5 psi. Once again the filtrate was collected, the volume was measured, and it was transferred to a third pressure tank where it was pumped through the third filter at 5 psi. The resulting filtrate was collected in a sterile Erlenmeyer flask and incubated at  $30 \pm 2$  °C for 48 h (see Figure 5).

A 1-mL sample from each filtrate was removed and was used for plating on TSA plates by removing 250  $\mu$ L of the filtrate and spreading it onto a TSA plate. A 10-fold dilution of the filtrate also took place, which also was plated on TSA plates. Each filter was carefully removed from its housing and placed on a TSA plate. The experiment was conducted in triplicate. All plates were incubated at  $30 \pm 2$  °C for 48 h, at which time the plates were checked for bacterial colonies. The counts on the plates were used to determine the log reduction value (LRV).

The same experiment was repeated with each filter type. The results are presented in Table I. The entire experiment was repeated with all four filter materials at 30 psi, and the results are presented in Table II.

In the Type II experiments, three filters of the same type were tested. However, two filters were loaded into a single housing in direct contact with each other. The third filter, serving as an analytical membrane, was loaded into a separate housing. All three filters were wetted with sterile water using a peristaltic pump (model 501Z, Watson Marlow, Ltd.). After wetting, the bubble points of the two-filter set and the analytical membrane filter were measured in sterile water using a compressed-air cylinder to ensure filter integrity. The two-filter set and the analytical membrane filter then were sterilized along with the housings in an autoclave at 121 °C for 15 min. When cool, 100 mL of sterile SLB solution was added to the filters.

A stainless steel pressure tank received 300 mL of SLB having a *B. diminuta* concentration of  $1 \times 10^7$  cfu/mL. Using a compressed-air cylinder, this bacterial suspension was pumped through the two-filter set at 5 psi. The filtrate was collected, the volume was measured, and it was transferred to a second pressure tank. This filtrate then was pumped through the analytical membrane filter at 5 psi. The resulting filtrate was collected in a sterile Erlenmeyer flask and incubated at  $30 \pm 2$  °C for 48 h (see Figure 6).

A 1-mL sample from the filtrate was removed and was used for plating on TSA plates by removing 250  $\mu$ L of the filtrate and spreading it onto a TSA plate. A 10-fold dilution of the filtrate also took place, which also was plated on TSA plates. The two-filter set was carefully removed from its housing and placed on a TSA plate. The analytical membrane filter also was removed from its housing and placed on a TSA plate.

The experiment was conducted in triplicate. All plates were incubated at  $30 \pm 2$  °C for 48 h, at which time the plates were checked for bacterial colonies. The counts on the plates were used to determine the LRV.

The same experiment was repeated with each filter type. The results are presented in Table III. The entire experiment then was repeated with all four filter materials at 30 psi, the results of which also are presented in Table III.

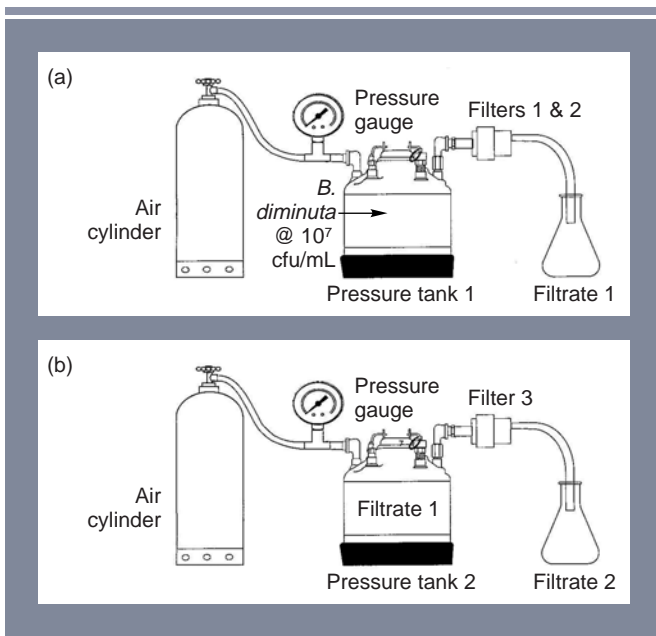


Figure 6: Redundant filters.

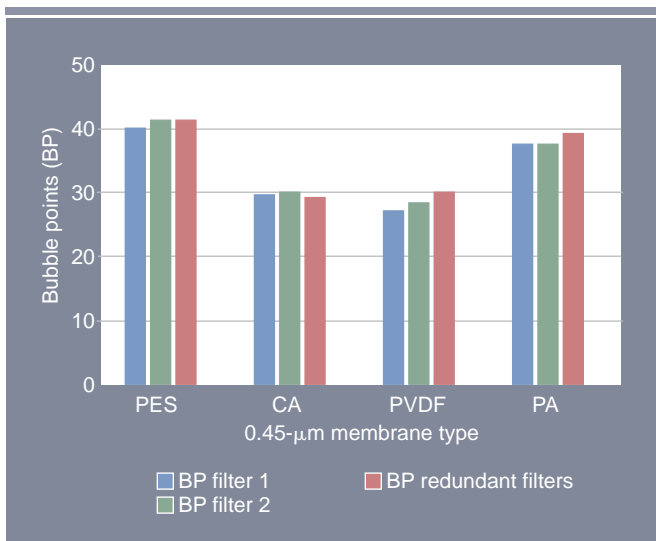


Figure 7: Bubble points of membranes used at 5 psi.

**Calculations.** LRV is the logarithm to the base 10 of the ratio of the number of microorganisms in the challenge to the number of organisms in the filtrate. Our challenge suspension had a concentration of  $1 \times 10^7$  cfu/mL. Because we used 300 mL of the challenge solution, the total challenge was 300 times  $1 \times 10^7$  cfu/mL, which equals  $3 \times 10^9$  cfu/300 mL or  $2.4 \times 10^8$  cfu/cm<sup>2</sup>. This number was taken as the starting challenge number. The number of organisms in the filtrate was calculated by reading the TSA plate counts for the filtrate and then multiplying by the volume collected.

### Separated or congruent double filters

The 0.45  $\mu\text{m}$ -rated membranes investigated in this study can serve very adequately as filters for the retention of *B. diminuta*. Tables I and II show the reductions in organism concentrations that are performed by each of the four membrane material types

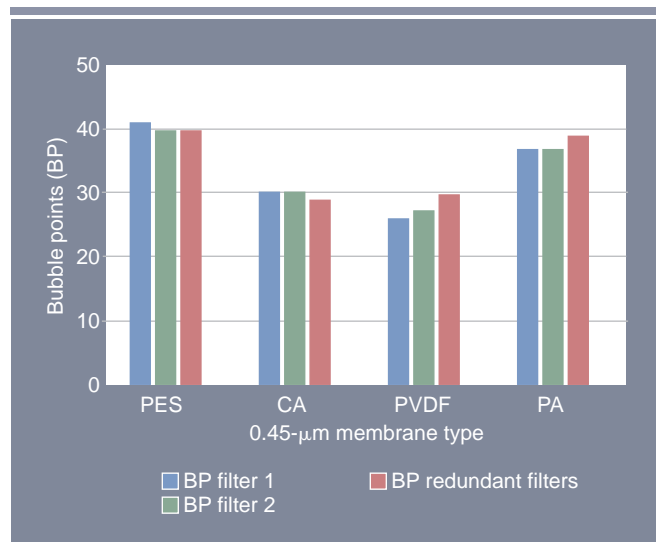


Figure 8: Bubble points of membranes used at 30 psi.

at differential pressures of 5 and 30 psi. The bacterial reduction achieved by the use of a separate second membrane also is shown. Table III reports the enhanced bacterial reduction that results when the two membranes are used in close proximity to each other. The explanation for the superior organism removal performance of the two congruent membranes is that an overlay of the larger pores of one filter by smaller pores of the second creates a longer pore path that is characterized by the retentive constriction of the smaller pore.

Where the filters are separated, particles escaping the first filter enter a liquid pool between the filters from which they may be directed toward the larger pores of the second filter guided by the hydrodynamic flow. The higher differentials and resultant higher flows diminished the time for particle alignment within the interfilter pool. Time allows for the flow to favor the larger pores and less time is required with the higher-pressure retentive constriction of the smaller pore (i.e., flow paths favor the more-open pore structures).

### Repetitive or double filters

**Nonadditivities of LRVs.** Double filters, or two final filters in series, are used widely in the industry. At least two intentions for this practice exist. The first is an exercise in prudence, to rely on safeguards in a contingency. It can be argued that upsets and deviations do occur and should be guarded against. The possibility of smaller, not-yet-discovered organisms is one example. This lends encouragement, however, to an endless, unassessable fear of the unknown and to an increasingly perceived need for dependence on tighter filters. Using double filters from this perspective is motivated by the need for emotional security, which will not be addressed in this article.

A second reason for the use of double filters is to compound the LRV of the filter type. It has been reasoned that whatever removal effect one filter may have, a second filter, with the same properties, should double that effect. The cited literature contains examples of the additivity of filter LRVs being assumed (11).

At least two reasons for log reduction values not to be ad-



ditive exist. The pores of any microporous membrane are represented by a distribution. The particles also are characterized by a size distribution. A given particle (organism) may encounter a pore small enough to arrest it, or it may find a larger pore through which it can escape capture. Analysts have reason to believe that what occurs is not strictly a matter of chance. The hydrodynamic flow exerts an influence toward the larger pores. The comparative number of more smaller pores than larger has the opposite effect. Even the concentration of particles (organisms) in the fluid may govern a particular pore-organism encounter (12,13).

Some particles may escape the first filter to be arrested by the second. Typically, only the organisms that, in their size distribution, are too small to be retained by the first filter will impinge upon the second. (For the same reason, they may not be stopped by the second.) The second filter, then, will not be confronted by the same size or number of organisms. Therefore, the two filters will exhibit different LRVs. Figures 1 and 2 show that the LRVs for successive same-type filters were not additive for each of the filter types examined.

However, legitimate reasons for the use of double filters do exist. These reasons arise from the limitations and the sensitivity of filter integrity testing. Hofmann, in discussing the integrity test, quantifies certain shortcomings that may derive from flaws too small to be discerned by the testing (11). If double filters are used, the flawed areas of one membrane likely will be overlaid and canceled by the integral portions of the other. The two membranes in close-contact arrangement serve as insurance against the possible but unlikely existence of such pores, which are undetectable by integrity testing (see Figures 7 and 8).

This fact pertains to a forthcoming statement from the Committee for Proprietary Medical Products (CPMP) to the effect that, in most cases, 10 cfu/100 mL will be the maximum acceptable bioburden before sterilization by filtration (14). If this requirement is not met, prefiltration with a bacterial-retentive filter will be required. Filters with pore sizes of 0.22  $\mu\text{m}$  or less are acceptable without further justification as bacteria-retaining filters. This standard will result in a series arrangement of two same-type membranes that show that they do not have additive LRVs. A better filter construction might be the classical prefiltration format wherein a membrane of larger pore-size rating precedes the final filter of a lower rating. Most of the larger organisms will be removed by the prefilter and therefore will not compete with the smaller organisms for the more-constricted pores. This mechanism should result in a larger proportion of the smaller organisms engaging the smaller pores of the final filter and being retained. The 0.45  $\mu\text{m}$ -rated membrane could do service as the prefilter.

One may question whether the CPMP's repetitive 0.2/0.22 filter arrangement is required to achieve  $<10$  cfu/100 mL for solutions being readied for sterile filtration, even if they contain the unlikely load of  $1 \times 10^7$  cfu/mL. Figures 1 and 2 show that the use of the redundant 0.45  $\mu\text{m}$ -rated membranes tested in this study would reduce *B. diminuta* concentrations ranging from  $1 \times 10^7$  to  $1 \times 10^9$  cfu/mL by 4 to 9 log even at applied differential pressures of 30 psid.

One can conclude on the basis of this study that, as modeled by the *B. diminuta* retention, 0.45  $\mu\text{m}$ -rated filters are sufficiently proficient at bacterial removal. As a result, they can be used as prefilters in bioburden reduction activities and, absent certain adsorptive interferences, as final filters in low bacterial contamination applications.

## References

1. F.W. Bowman and S. Holdowsky, "Production and Control of a Stable Penicillinase," *Antibiotics and Chemotherapy* **10** (8), 508 (1960).
2. T.J. Leahy and M.J. Sullivan, "Validation of Bacterial Retention Capabilities of Membrane Filters," *Pharm. Technol.* **2** (11), 64-75 (1978).
3. G.B. Tanny et al., "The Adsorptive Retention of *Pseudomonas diminuta* by Membrane Filters," *J. Parenteral Drug Assoc.* **33** (1), 43-51 (1979).
4. J.R. Carter and R.V. Levy, "Microbial Retention Testing in the Validation of Sterilizing Filtration," in *Filtration in the Biopharmaceutical Industry*, T.H. Meltzer and M.W. Jornitz, Eds. (Marcel Dekker, New York, NY, 1998), p. 584.
5. K. Kawamura, J.W. Jornitz, and T.H. Meltzer, "Absolute or Sterilizing Grade Filtration — What is Required?" *PDA J. Pharm. Sci. Technol.* **54** (6), 485-492 (2000).
6. FDA, "CDER Guideline on Sterile Drug Products Produced by Aseptic Processing" (June 1987), 21 CFR Part 10.90, p. 28.
7. D.B. Pall, E.A. Kirnbauer, and B.T. Allen, "Particulate Retention by Bacterial Retention Membrane Filters," *Colloids and Surfaces* **1**, 235-256 (1980).
8. T.H. Meltzer, *Filtration in the Pharmaceutical Industry*, (Marcel Dekker, New York, NY, 1987), p. 270.
9. A. Baszkin, D.J. Lyman, and T.H. Meltzer, "Theoretical Consideration of Bubble-Point Measurements," *Pharm. Tech. Internat.* **2** (1), 22-31 (1978).
10. A.M. Trotter et al., "The Effects of Bacterial Cell Loading," *Pharm. Technol.* **24** (3), 72-80 (2000).
11. F. Hofmann, "Integrity Testing of Microfiltration Membranes," *J. Parenteral Sci. Technol.* **38** (4), 148-159 (1984).
12. D.C. Grant and J.G. Zahka, "Sieving Capture of Particles by Microporous Membrane Filters from Clean Liquids," *Swiss Contamination Control* **4a**, 160-164 (1990).
13. A.M. Trotter et al., "Investigation of Filter Structure by Microbial Retention Studies: A Synthesis and Elaboration of Prior Findings," *PDA J. Pharm. Sci. Technol.* **55** (2), 127-133 (2001).
14. CPMP-EMEA, "Note for Guidance on Manufacture of the Finished Dosage Form," CPMP/QWP/486/95, pp. 5-6 (April 1996). **PT**

## FYI

### International student poster competition

The International Society for Pharmaceutical Engineering (ISPE) is holding its third annual international poster competition for graduate and undergraduate student members.

Participants may submit their posters to their local ISPE chapters; selected local winners will advance to a national competition at the annual meeting 3-7 November 2002 in Orlando, Florida.

Winners in both graduate and undergraduate categories will receive cash awards and the opportunity to have their findings published in ISPE's journal *Pharmaceutical Engineering*.

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