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THE STERILIZING FILTER

HISTORY

The first microporous membranes manufactured in the United States and intended for sterile filtrations were rated as being 0.45 μm in pore size. They met with impressive successes. The common view concerning filters at that time was, indeed still is, that they retain particles, whether viable or not, solely by sieving; the size discrimination arising because the pores are smaller than the particles, thus interdicting their passage. Adsorptive effects were recognized as being capable of removing certain dissolved molecules from solution. However, despite an ample published literature (Kramer, 1927; Nash et al., 1967; Pertsovskaya and Zvyagintsev, 1971; Gerson and Zajic, 1978; Hjertin et al., 1974; Zierdt, 1978; Tanny et al., 1979) the adsorptive removal of organisms by filters was then, as even now, not too widely recognized. The exclusivity of the sieve retention mechanism was assumed. It promised reassuring absoluteness independent of challenge density, of the filtration conditions, and of the influence of the suspending fluid.

The many successful trials with these membranes encouraged a belief that the organism types typically present in pharmaceutical preparations had most likely been encountered in the several applications, and that they had all been filtratively retained. From this it was concluded that the pore sizes of the membranes were small enough to make them rather universally applicable to the sterilization of pharmaceutical preparations. As stated, these membranes were rated at a **pore size of 0.45 μm**.

THE 0.2/0.22 μM RATED MEMBRANE

When subsequently, Bowman et al. (1967) discovered a smaller organism, *Pseudomonas diminuta*, (reclassified as *Brevundimonas diminuta* by Segers et al., 1994) that was not sieve retained by these 0.45 μm rated filters, the pore size was reduced by half to a 0.22 μm rating. (Some filter purveyors assigned the rating of 0.2 μm. Both size designations are arbitrary and essentially undistinguishable.)

This became the 0.2/0.22 'sterilizing filter', so defined in 1987 by the Food and Drug Administration (FDA) on the basis of its retaining a minimum of 1×10^7 colony forming units (cfu) of *B. diminuta* per cm^2 of effective filtration area (EFA). The Health Industry Manufacturers Association (HIMA) in 1982 recommended the same retention but had stipulated that this apply to differential pressures of up to 30 psi (2 bar) for flat stock, and in the case of cartridges at a flow rate of 3.86 liters/min/0.1 m^2 of EFA. The flow restriction was designed to render practical the volume of culture medium that would be required. The *B. diminuta* organism was not selected as being the smallest sized organism, but rather as a suitable model for the small bacteria thought likely to be encountered in a pharmaceutical setting. (See *The 0.1 μm Rated Membranes* in this Chapter.)

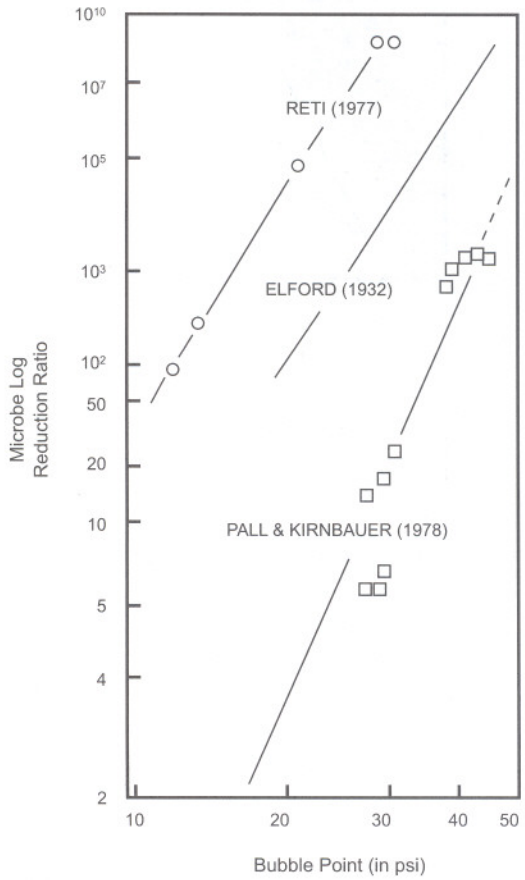
It was determined by several investigators that for the several types of microporous membranes a straight line relationship existed between the bubble point integrity test values of the different pore size ratings and their *B. diminuta* retentions (Fig. 1.1). This enabled the affirmation of the 'sterilizing' filter to be made by way of integrity testing. One need only read from a graph the bubble point value corresponding to the necessary level of retention forthcoming from the selected 0.2/0.22 μm rated filter. The matching of the integrity test value with the desired retention level constituted validation of the filter (Fig. 1.2).

By the same token, it was believed that the 0.45 μm rated membranes, on the basis of the size exclusion or sieve retention mechanism could not yield sterile effluents, as witnessed by the Bowman et al. (1967) experience. Investigations by Tanny et al. (1979), and by Trotter et al. (2002) illustrated, however, that 0.45 μm rated membranes can be depended upon to yield sterile effluent. It all depends upon the conditions under which the filtration is conducted. This in itself points to a mechanism, adsorptive sequestration, that under the proper circumstances operates to reinforcing sieving. Indeed, the very experience of Bowman et al. (1967) itself indicates the same. The 0.45 μm rated membrane they used did provide sterile effluent under the condition that protein (penicillinase) did not preempt the adsorption sites.

All the 0.2/0.22 μm rated membranes of commerce do meet the FDA definition of 'sterilizing' membrane, but all do not retain *B. diminuta* organisms to the same extent. The necessary bacterial challenges of filters to determine their suitability for the 'sterilizing' grade identification are performed by each of the individual filter

manufacturers using a procedure based on an American Society for Testing and Materials (ASTM) test in 1988. However, there is not an industry-wide standard for the specifics of the organism challenge test protocol. The relative influences of such parameters as bacterial density, volume of test solution, test duration, applied pressure differential, etc., have not been investigated. Therefore, the distinctions found among different membranes are of an unknown significance. They may reflect the variations in the non-standard testing.[1]

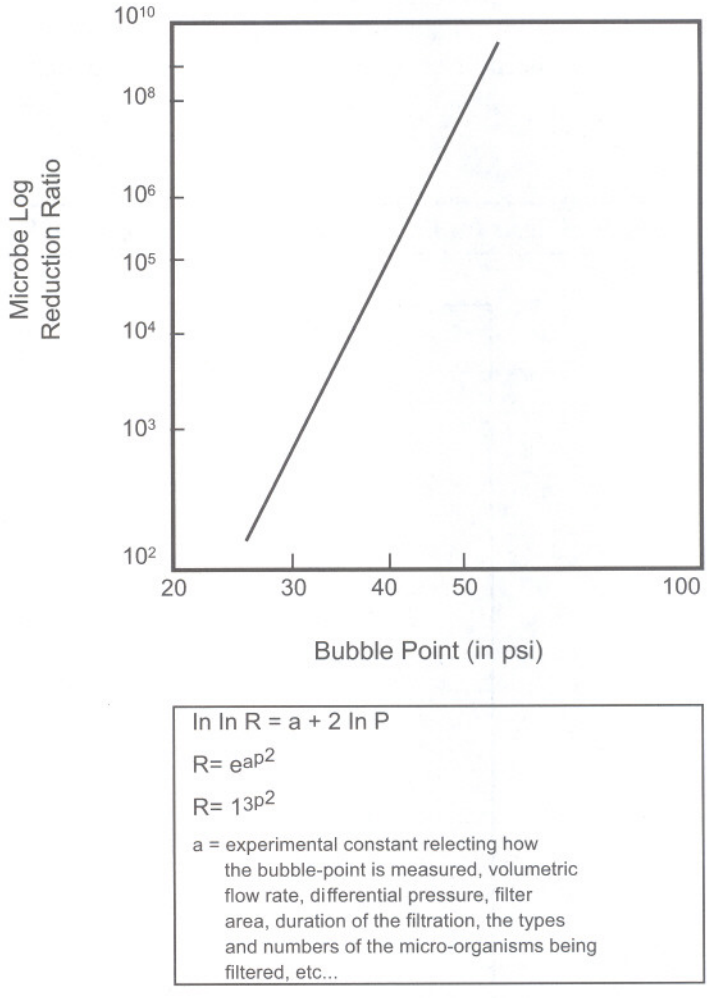
Figure 1.1 Experimental Basis for Correlation of Bubble Point and Retention



Johnston and Meltzer, 1979; Courtesy of *Pharmaceutical Technology*

[1] This does not inhibit certain filter purveyors who choose to test with higher organism concentrations from making marketing claims of having a more retentive filter.

Figure 1.2 General Bubble Point Retention Correlation



Johnston and Meltzer, 1979; Courtesy of *Pharmaceutical Technology*

Until rather recently, then, it was believed that the sterilization of fluids could be achieved by their filtration through a 'sterilizing' membrane whose proper and pertinent identity was confirmed by its pore size rating, which was itself determined by integrity testing. Developments in filtration practices showed this belief to be too generally founded. What had once seemed simple is now recognized as being quite complex. It was discovered that the positive conclusions based on pore size ratings were subject to modification by the physicochemical specificity of the organism-suspending fluid; by the individuality of the organism type in its size-changing response to the fluid; in the possible change in pore size induced by the fluid; and by

the adsorptive qualities of the filter resulting from its particular polymeric composition; all influenced by the filtration conditions in their numerous varieties, but especially by the transmembrane pressure. A filter may not sterilize the same preparation under different filtration conditions, especially under dissimilar differential pressures (Leahy and Sullivan, 1978). A given membrane may or may not retain a particular organism type suspended in a different drug vehicle (Bowman et al., 1967). The organism type need not remain constant in size, but may alter in response to its suspending fluid (Gould et al., 1993; Leo et al., 1997; Meltzer et al., 1998). The effect of the vehicle upon the polymeric membrane may cause a change in its pore sizes (Lukaszewicz and Meltzer, 1980).

Conclusions cannot be made regarding the sterile filtration of microorganisms unless methods of quantifying them by culturing and counting are available. Organisms such as the L-forms, nanobacteria, and 'viable but non-culturable' entities may not be amenable to such analyses. Concerns about their presence may be justified, but without the means to cultivate and count them, it is impossible to attest to their complete absence. It follows that a sterilizing filter can be judged only by its performance in the removal of identifiable and culturable organisms known to be present in the drug preparation (Agalloco, 1998).

The certainty of obtaining sterile effluent requires far more than the identification of a 'sterilizing filter' by a pore size rating. The complex of influences governing the outcome of an intended sterilizing filtration necessitates a careful validation of the process, including that of the filter (PDA Technical Report # 26). The very drug preparation of interest, the exact membrane type, the precise filtration conditions, and the specific organism type(s) of concern must be employed in the necessary validation.

Given the complexity of the organism removal operation, it is doubtful whether a universal sterilizing filter can be devised. Certainly, there is no known absolute filter, one that will retain all organisms under all conditions, especially if viruses are included (Aranha, 2004). Therefore, the successful attainment of a sterile filtration with regard to specified organisms of interest must in every individual case be attested to by the documented experimental evidence that constitutes validation.

ADVOCACY FOR THE USE OF 0.1 µM RATED MEMBRANES

The FDA (1987) defined a sterilizing filter as one that retains a minimum of 1×10^7 cfu of *Brevundimonas diminuta* ATCC 19146/per cm^2 of effective filtration area (EFA). The FDA, although cognizant of smaller organisms that penetrated 0.2/0.22 µm rated membranes (Howard and Duberstein, 1980), selected *B. diminuta* as the model organism for sterile filtrations. Although not the smallest organism known, *B. diminuta* was considered diminutive enough to represent whatever smaller size organisms were

likely to be present in pharmaceutical preparations. Since then, some 25 cases have been noted wherein the 0.2/0.22 µm rated membranes did not yield sterile effluent (Sundaram et al., 2001, Part 1). Obviously, *B. diminuta* is not a universal model for all organisms. More especially, it was not, nor is it as yet, sufficiently recognized that organisms penetrating the 0.2/0.22 µm rated membranes were likely to have undergone size reductions occasioned by exposures to particular drug preparations.

Realizing that 0.2/0.22 µm rated membranes do not always produce sterile effluents, it has been argued that the tighter 0.1 µm rated filter should be designated as the 'sterilizing' filter. It is, indeed, reasonable to expect that the tighter filters will more likely restrain the passage of smaller organisms, both by sieve retention and by adsorption. Thus, the use of tighter filters may well be appropriate where smaller size organisms are the concern as indicated by experimental investigations (Jornitz and Meltzer, 2001; p. 543).

This is of interest where the phenomenon of grow-through is of concern. The regulatory authorities have long been anxious about grow-through, a condition whereby organisms retained on one side of a membrane are found to penetrate it over time to appear on the other side. It has been raised as a hypothesis that grow-through occurs when the organism undergoes binary fission in its reproductive cycle. True or not, this explanation recognizes the obvious, namely, that the organism has had to undergo a shrinkage in size as a function of time for it to pass through the filter. In its proposed Good Manufacturing Practices (GMPs) for large volume parenterals (LVPs), the FDA in 1976 addressed the problem by requiring that the mixing and filtration of a batch be completed within the 8 hour period of a single shift, an interval too brief for grow-through to occur. Although this proposal was never finalized, in the actual event it became the rule. Its application discouraged the long-term use of membrane filters. Similarly, the *FDA Aseptic Guideline*, Draft 2003 and Section 211,111 specifies processing time as a critical factor that requires validation. This may be a reason that the European Agency for the Evaluation of Medicinal Products (EMEA) and Committee of Proprietary Medicinal Products (CPMP) *EMEA/CPMP Guide* QWP 486-95 states that the bioburden level in front of a 0.2 µm filter should not exceed 10 cfu/100 mL, and if necessary a prefilter is needed to reduce the level to this limit. (See *Chapter 13,* Section: *Grow-Through*.)

Another cause of organism size diminishment has been highlighted by the impressive work of Dr. Sundaram and his associates. Sundaram et al. (1999) investigated the size reduction that certain microbes, notably *Ralstonia pickettii*, undergo over time as a result of their exposure to certain drug preparations. Such morphological happenings had previously been observed (Geesey, 1987; Gould et al., 1993; Givskov et al., 1994). It occurs when, among other circumstances, organisms are suspended in high purity compendial waters that are nutritionally poor for them. As a survival mechanism against starvation, organisms minimize their cell size, and increase their surface to volume ratios (Gould et al., 1993; Mittelman, 1998). A thinner

or small form may more easily pass through a filter pore. This event may be distinguished from grow-through, although the consequences are the same, by being called 'penetration'. Organism penetrations also take place with preparations of high osmolarities in response to the Donnan equilibrium, and with lipid-containing solutions. The two apparent factors governing grow-through are the nature of the drug formulation, and the length of the exposure. Therefore, processes involving long-term filtrations all provide opportunities for organism shrinkage. For example, blow/fill/seal processes are known to be prolonged filling operations wherein filtered air is utilized to blow open the parison to form the molded container [2] (Wilson, 1994; Leo et al., 2004; Kawamura et al., 2000). It is in such operations where prolonged liquid filtration can result in microbial penetration, or grow-through that 0.1 µm rated membranes should be considered. Such occurrences do argue for the use of tighter membranes. To investigate the actual situation with regard to organism size reductions the following are required:

- Routine bioburden analyses of the organisms' species and levels.

- Perform product and process related filter validations for each of the filters, using the actual drug product, following a time interval, after its having been compounded, equal to that required for its production filtration and filling.

- If possible, the actual bioburden species recovered from the drug preparation should be added to a newly formulated preparation to fix the initial time point. These investigations can determine whether the retention rating should be 0.2/0.22 µm or 0.1 µm; and can help in devising advantageous membrane combinations.

[2] In the process there are pressure cycles at the rate of about one per second. The pressure spikes exert high stresses upon the filter cartridges and may well strain the membrane matrix. Thus, the filters used, whether for air or liquid, require being tested under the process conditions (performance qualification [PQ] and process validation). Only such tests can verify that the filters will withstand the severe process conditions. Some filter manufacturers apply pulsation testing to the filter cartridges. The data show that such filters can withstand up to 20,000 pulsations at a differential pressure of 5 bar. However, these pulsation trials are performed under the manufacturer's process conditions. They, therefore, still require verification under the actual pharmaceutical filling process.

CONSIDERATIONS REGARDING THE 0.1 µM RATED FILTERS

A serious objection to the 0.1 µm rated membrane is that its filter classification is not defined by any industry or regulatory standard. Sundaram et al., Part III (2001) report that three out of seven types of 0.1 µm rated filters failed to retain "*naturally occurring waterborne bacteria*". This is hardly a basis for defining a class of membranes. These experimenters agree that the "*0.1 µm rating associated with a filter is meaningless, unless there is a functional microbial removal claim that serves as a basis for such a rating.*" There are no industry standards for identifying 0.1 µm rated filters. As regards the use as a 'sterilizing' filter, neither of the two filter ratings, 0.1 or 0.2/0.22, is capable of retaining all possible microorganisms, and certainly not under all conditions.

There are certain operational disadvantages that accrue to the use of the 0.1 µm rated membranes. For example, it would not be practical to routinely apply the bubble point integrity test to the 0.1µm rated membrane. The test pressures needed for the bubble point testing would be too high. For 0.1 µm rated membranes it is typically about 73 psi (> 5 bar). The 0.2/0.22 µm rated membrane typically requires 58–73 psi (4–5 bar). Filter integrity testing could still be performed using diffusive airflow testing. However, integrity testing to more precisely determine the bubble point requires that both tests be conducted (Jornitz and Meltzer, 2003, p. 61).

The very feature that commends the use of the 0.1 µm rated membrane for more reliable retentions, namely, its tighter pore passageways, creates certain operational shortcomings. The tighter membrane would gratuitously remove particles whose presence in the drug is not objectionable. Depending upon the degree of loading, reductions in throughput may be promoted by the tighter filter. Longer processing times, finer prefiltrations, higher differential pressures, or larger filter area would be required to achieve the same rate of effluent production forthcoming from 0.2/0.22 µm rated membranes. An unnecessary replacement of the more open filter by the tighter 0.1 µm rated membrane would seem to be unwise unless definitely required, as indicated by a validation study. A blanket substitution of the 0.1 for the 0.2/0.22 will, therefore, not suffice, nor is it advocated (Sundaram et al., 2002).

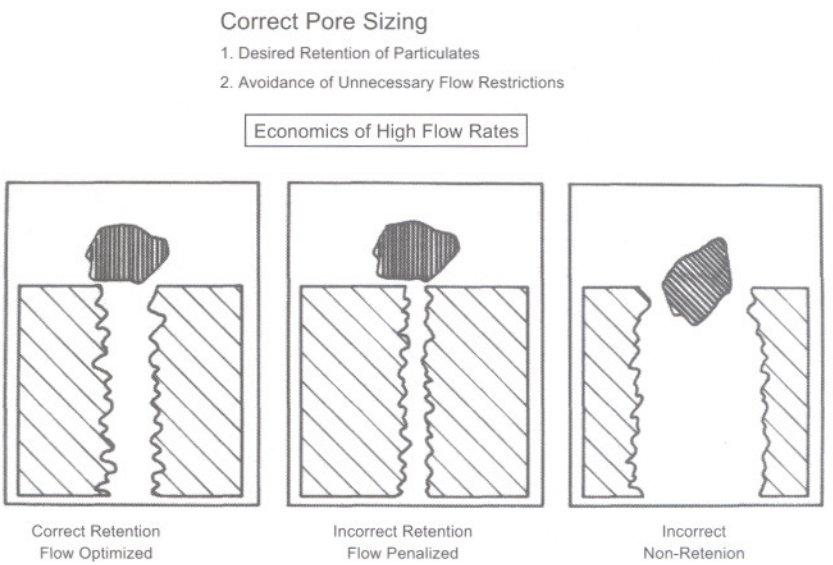
FLOW RATE COMPARISONS: 0.1 µM VERSUS 0.2/0.22 µM

Central to the practical disadvantages of the generic 0.1 µm rated membrane is its lower flow rate (actually flux) as compared to that of its generic 0.2/0.22 counterparts. Experience has led to the general belief that the relationship among pore size, flow, and retention is that smaller size pores retain finer, and flow slower. The 0.1 membrane has the advantage of a tighter pore diameter. This gives greater assurances of organism retention, whether by sieving or by adsorptive sequestration. This expedient property

has value in its proper context. Yet, the use of the 0.1 μm rated membrane would ordinarily be expected to yield a significantly reduced rate of flow, even for clean fluids, it being a tighter filter. The allegation that there are 0.1 μm rated membranes that flow as fast or faster than 0.2/0.22 μm rated membranes seems profoundly contrary to widespread experience. How is the seeming contradiction to be reconciled?

As mentioned, there are two components to the practical application of the filtration technique. Each depends upon the size of the filter pore. It is the size of the pore that restrains the passage of particles such as organisms, and through which the fluid flows. In choosing a filter, one seeks to ensure the desired organism retention while maximizing the fluid velocity (Fig. 1.3). This choice, as expressed by filter users in the marketplace, can be the object of commercial rivalries. The technical clarification of such situations can be of benefit to filter users.

Figure 1.3 Correct Pore Sizing



Courtesy of Capitola Presentations

Perhaps the terms 'filter' and 'membrane' as used in the present context should be differentiated. By 'membrane' is meant the component of the 'filter' device that by way of its pores effects particle separations from the liquid. The 'filter' is the complete device in its entirety including the membrane, the cartridge size, the center core diameter, etc. It is legitimate to compare 'filters' even if they contain different effective filtration areas or even if they differ in their pore size ratings. Different instruments or devices may be compared for whatever reason.

As regards membranes, superior flows may result from filters having larger membrane areas, and greater porosities, as well as thinner membranes, and less tortuous pore passageways. However, comparisons among membranes, presumably of the same pore size rating, necessitates a measurement of flux. Flux is defined in terms of volume per EFA per unit time. What is needed for the comparisons is the quantitation of the flow that is inherent in each membrane structure as a consequence of its porosity and thickness, but also filter design. Equal EFAs require being compared using identical challenges under identical test conditions. Comparing the flow properties of two membranes compels the use of identical areas of each. However such comparison does not reflect process scale reality, as the filtration areas of process scale filter cartridges differs with regard to flow. An optimal comparison condition would therefore be the use of 10" filter cartridges in a parallel setting at the same process parameters using the actual fluid stream or water.

Average Pore Size

According to Johnston (2003, p. 5), the complicated geometry of the sponge-like membrane results in the pores' ratios of cross-sectional areas to perimeters, called the hydraulic parameters, varying over its entire thickness. Moreover, filtration is not a simple sieving process, except perhaps in the case where the filter is a track-etched membrane with pores passing straight through a much thinner film (approximately 1 μm). However, for the membranes prepared by the casting process (thinness approximately 150 μm) a fluid on passing through encounters pores of different diameters and surface areas. Particles are separated from the fluid by attachment to the pore surfaces, as well as by size exclusions of the particles. Thus, the meaning of the average pore size is necessarily an oversimplification.

One may speak of comparing the flux of like-rated filters, but legitimately only by assuming a common pore size rating system. However, this is hard to come by. Actually, there is no industry-wide pore size rating standard. The pore size rating systems employed by each of the several filter manufacturers are not the same.

Pore size designations can be assigned by measuring the largest size particle that can just permeate the membrane, or they can be derived from the rate of liquid flow through the filter. The particle retention method is detailed in *Chapter 3,* Section: *Pore Size Rating*. It suffers from the need to assume a cylindrical pore and a spherically shaped particle free of adsorptive influences. The pore size classification based on liquid flows is likewise flawed by requiring simplifying assumptions. Indeed, the very concept of 'pores' when applied to microporous membranes other than the straight-through columnar pores that characterize track-etched membranes, is an artificiality (Johnston, 2003; Chapter 3). Nevertheless, we will assume that the assigned pore size ratings do represent the average pore size dimensions, and that they were defined by a standard rating system.

Flow Rates versus Pore Size Ratings

The flow characteristics of single pores are described by the *Hagen-Poiseuille Law* wherein a fluid of viscosity, η, with an average velocity, u, of the fluid is related to the tube diameter, d, and pressure drop, ΔP along a length.

Equation: $d^2/32 = u \times \eta \times z/\Delta P$ (Johnston, 2004)

Consider a fluid of a given viscosity flowing at the same average velocity through two pores of similar length, the same porosity and under the impetus of an identical pressure differential. All the factors being equal except for the pore diameters, the rate of flow for the pores of the two membranes will differ as the squares of their diameters, or as the fourth power of their radii. If, however, the diameters of the pores are the same, differences in the flow velocities must derive from differences in porosity. It is this very situation that is claimed by the proponents of faster flowing 0.1 µm rated membranes.

In the case of individual pores, according to Hagen-Poiseuille, the flow rate would be greater for the pore with the larger diameter. However, in comparing membranes we are not dealing with single pores, but with membrane areas. These each contain large numbers of pores. Nevertheless, as a first approximation, it would seem from the results forthcoming from single pores that the flows from identical filter areas should favor membranes with greater porosities; i.e., with more pores and/or larger pores.

Therefore, to state that a particular 0.1 µm membrane has as large a flux as the 0.2/0.22 µm membranes is also to assert that it has the porosity of a 0.2/0.22 µm rated membrane. It differs from the conventional 0.1 µm rated membranes by having a greater porosity and/or shorter pores (thinness). In essence its pore size rating is arrived at from its flow characteristics, its retentivity is not impugned.

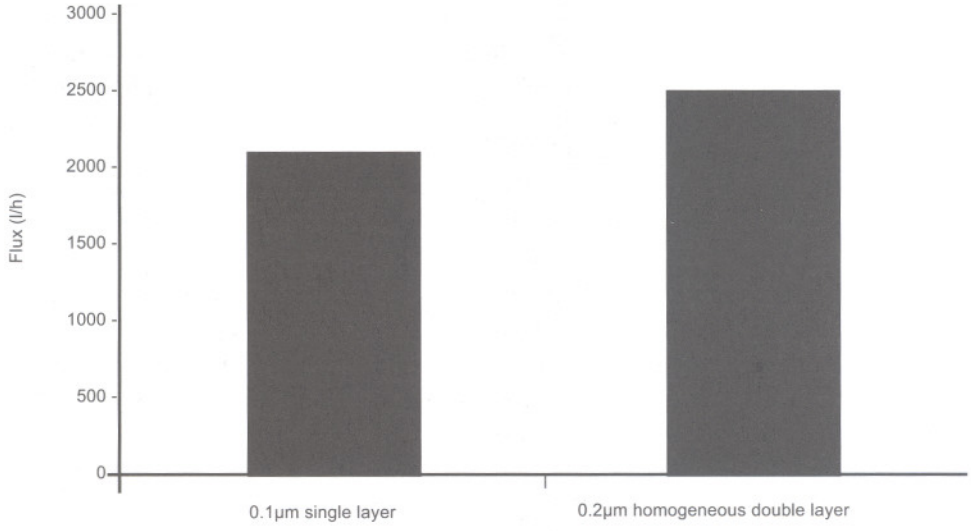
The rule of thumb has a halving in membrane thickness, as permitting the doubling of the number of permeating particles. Since the purpose of the membrane's selected pore size is the complete retention of the particular organisms of interest, it will be assumed that thinness was not varied in order not to jeopardize complete retention. This leaves the increase in porosity as the sole cause of greater flows. Indeed, this is the claim of one manufacturer of 0.1 µm rated membrane.

It is also possible that the 0.1 and the 0.2/0.22 µm rated membranes being compared bear different pore size designations because they were rated to different standards. They do have rather similar fluxes. They may each merit the same pore size label, and perhaps would be so classified were a standardized rating system in place. This possibility should be explored. (See *Chapter 3,* Section: *Assigning Pore Size Ratings*.)

RATING AMBIGUITIES

To repeat, it is possible that because of non-standard methods of pore size rating, the two membranes being compared are really of the same rating. It could be that their different pore size ratings are the product of different rating standards. The correlation of a membrane's flow with its pore size rating is made tenuous by the non-standard pore-size labeling techniques. Within any given pore size designation available in the market, there exists some range of measurements. In its manufacture, a membrane lot may, and indeed will, incur some variation in its pore size (Fig. 1.4).

Figure 1.4 Flow Rate: Single-Layer 0.1 µm and Double-Layer 0.2 µm rated Membrane



Meltzer and Lindenblatt; Courtesy of *European Journal of Parenteral Science*

As Schroeder (2001) points out, the pore size/retention correlation is not a step function. Hence, the possibility exists for filter manufacturers to assign pore size labels somewhat arbitrarily. One fabricator may, on the basis of flux, consider a membrane to be an 'open' 0.1. Another filter producer, using a different rating system might classify it as being a 'tight' 0.2. This could give rise to a labeled 0.1, not so handicapped by reduced flows, being compared with a labeled 0.2, not so advantaged by enhanced flows. The more 'open 0.1' may not flow faster than an average 0.2, but may do so against a 'tight 0.2'.

There are also possibilities for comparisons to be based on misconceptions. Double membrane constructions are often classified by the rating of the downstream membrane alone. Thus, a 0.2 μm over a 0.2 μm construction, may simply be labeled as being 0.2 μm rated. This so-called 0.2 filter, actually a double layered 0.2 filter, its flow disadvantaged by the extra 0.2 membrane layer, may undergo comparison with a single, more open 0.1 filter, to the advantage of the latter (Fig. 1.4).

The prevailing situation is that the several filter makers do use different standards. Therefore, the claim that a particular 0.1 μm rated filter has a higher flux than some 0.2/0.22 μm rated filter is of a logic commonly found in comparisons of apples with pears. The only valid comparison can be that of 0.1 and 0.2 μm rated filters of the same filter manufacturer, i.e., the same branded type, the same standard for pore size rating, the same polymer, same manufacturer, same design, etc. This would eliminate non-standard rating as a cause of apparent but erroneous differences in flux. Differences in flux would then be understood to derive from differences in porosity (and/or thickness).

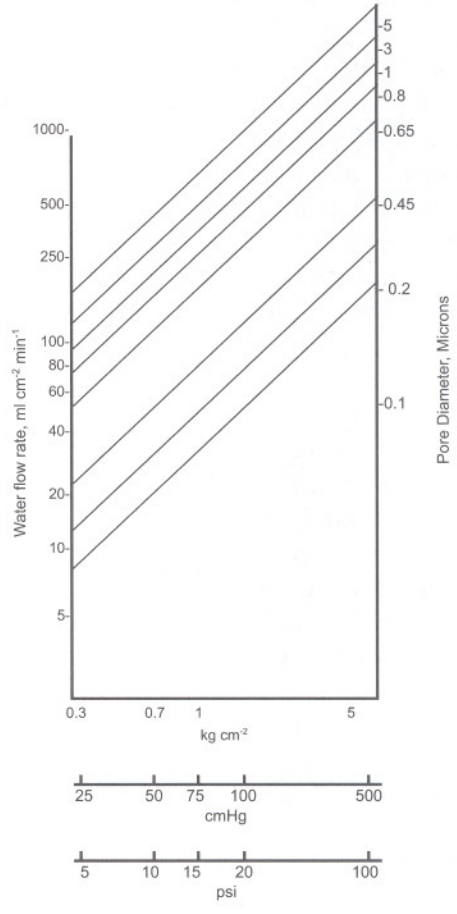
Relevant data exist that enable such a comparison. Brock (1983) lists the flux of a series of membranes of different pore size ratings prepared by one filter manufacturer, and pore-size rated by the fabricator's single standard. The several pore-sized membranes exhibited substantial differences in flux, especially between those of the 0.2/0.22 μm rated and 0.1 μm rated membranes. On the basis of these data is seems improbable, if not impossible, for the 0.1s to have rates of flow (flux) approaching those of the 0.2/0.22s. Figure 1.5 illustrates that the flux of a mixed cellulose ester membrane of 0.45 μm-rating is 22 mL per second per unit area; for a 0.22 μm rated filter of identical type it is 8 mL; for a 0.1 μm-rating it is 2 mL. The differences in flows among the various pore sizes are hardly subtle; only 1/3 the flow through a 0.45 pore diameter (22 mL/min/cm^2) was given by the 0.2 pore diameter (8 mL/min/cm^2); and of that flow only 1/8 was exhibited by the 0.1 pore diameter (1 mL/min/cm^2). Roughly 65% of the 0.45's flux is lost in changing to the 0.2. The shift to the 0.1 pore diameter from the 0.2 lost about 87% of that flow (Brock, 1983). The substantial loss in flow rates may be more evident in the form of a simple line graph as presented in Figure 1.6. It illustrates the same relationship between pore size (diameter) and flux as does Figure 1.5.

Cartridge Construction Influences

A confirming judgment regarding the competitive flows of 0.1 and 0.2/0.22 membranes is forthcoming from their flow performances in cartridge form. The more complex constructions afford opportunities for influencing the optimum flows of their membrane components. Unless cartridges of 0.2/0.22 μm rated membrane are so poorly designed as to be handicapped by inappropriate flow-impeding support and

drainage layers, or by poor pleat constructions, etc., they will produce faster flows than shown by their 0.1 μm rated counterparts.

Figure 1.5 Flow Rate as Function of Pore Size Rating

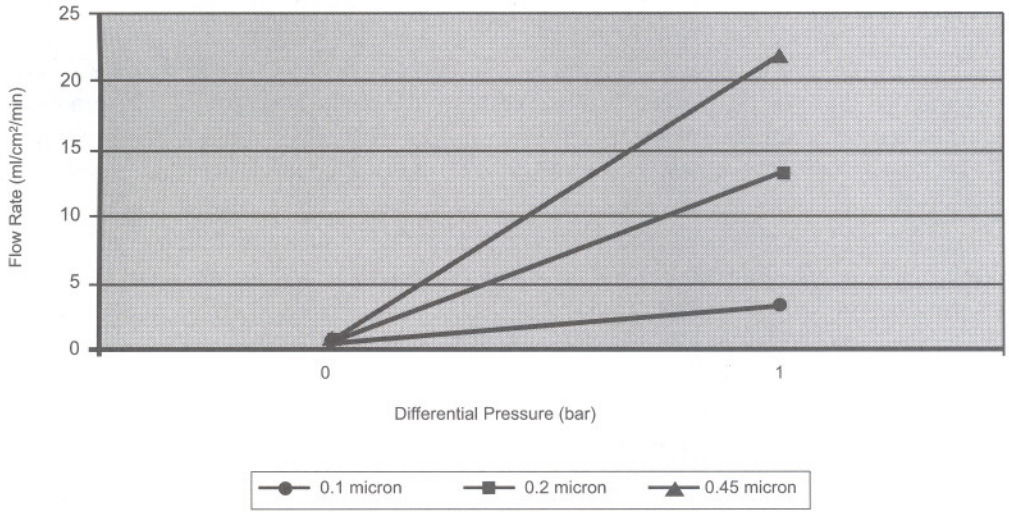


Brock, 1978; Courtesy of Science Tech, Inc.

IN SUMMARY

Above all, flow comparisons may be made only between membranes classified by the same pore size rating system. Only by assuming a common rating system can one attribute dissimilarities in flows to differences in porosities or thickness. Measurements of porosities or thicknesses for commercially available membranes are seldom, if ever, mentioned or referred to by filter manufacturers, as they would be if their actual variations were the factors governing membrane flux.

Figure 1.6 Flow Rates of Different Pore Sizes



Jornitz, 2006; Courtesy of Sartorius

Significantly, there are no published data substantiating the assertion that there is a 0.1 µm rated membrane with a flux similar to that of 0.2/0.22 µm rated membranes. In one published paper it is stated: "*Figure 1 shows the typical flow rates for two kinds of traditional 0.1 µm rated filters ... Also shown are typical flow rates for commercially available 0.2 µm filters. The data indicates (sic) that in addition to offering a substantially enhanced flow rate, compared with other 0.1 µm rated filters, the new filters actually outperform many 0.2 µm rated filters.*" Incredibly, the referenced 'data' include no numbers. The 'data' in the referenced Figure 1 are cartoon-like images of water flowing from faucets. The Figure is completely devoid of any numerical quantification whatsoever. Comparisons of flows on the basis of measured values is, therefore, impossible.

One is reminded of Lord William Thompson Kelvin's famous dictum, long a guiding principle of the scientific approach:

> "*When you can measure what you are speaking about and express it in numbers, you know something about it.*"

The conditions necessary to this discussion remain unfulfilled. Measured numbers have yet to be disclosed.

THE 0.2/0.22 μM VERSUS 0.1 μM RATED MEMBRANE

An advocacy for utilization of tighter filters stems from concerns about the possible presence of organisms small enough not to be retained by 0.2/0.22 μm rated membranes. Hence, the drive to use membranes of lower pore size ratings, more assertive of the size exclusion mechanism. Certain organisms exposed to certain drug preparations undergo size reductions that result in the penetration of 0.2/0.22 μm rated membranes. Sundaram et al. (1999) describe such size reductions involving *Berkholderia cepacia* and *Ralstonia pickettii*. In the case of the latter, the size of 1.35 μm × 0.37 μm in the culture reduced by 40% in the product. Also, certain bacteria when exposed to given enzymes or antibiotics lose their cell walls. The morphological alteration detracts from their rigidity. These 'L-forms', as they are labeled, being deformable, are liable to penetration 0.2/0.22s under the imposed differential pressure. One is reminded of Bergey's (1986) statement that organism sizes in cultures are not the same as they are in pharmaceutical processing contexts.

Sundaram et al. found that *R. picketii* and *B. cepacia* in their reduced sizes penetrated 0.2/0.22 μm rated filters, but were retained by a particular 0.1 μm rated membrane. This should not be taken to mean that other commercially available 0.1 type membranes would perform similarly. It should also be remembered that organisms not retained by 0.2/0.22 μm rated filters are not necessarily retained by those of a 0.1 μm-rating.

The question is: *Which filter should be relied upon*? Obviously, where applications of the 0.2/0.22 μm rated filters have been successfully validated in respect to microbial retentivity, there is no reason to discontinue their use. This assumes, however, that organism shrinkage may have been encountered, perhaps unknowingly, and did not pose problems. The same applies, of course, to the 0.1 μm rated membranes. Uncertainty of choice enters the picture when new applications, not guided by previous experience, are being addressed. The aim is to achieve a sterile filtration without the needless expenditure of time, or the cost of additional filters. Validation is the means to this achievement.

The FDA is said anecdotally to require that a drug be formulated and filtered within an 8 hour period, an interval within which organism shrinkages have not been seen to occur. This had been proposed by FDA in the current Good Manufacturing Practice (CGMP) for LVPs (1976) that were never finalized. It could be advantageous to learn in advance how long it would take for shrinkage, if any, to occur. If this is experimentally determined and documented, the FDA would, due to this validation, have no reason to prohibit processing times longer than those validated.

However approached, validation must be depended upon to demonstrate which, if either filter type, will suffice to produce the required retention within the time interval devoted to the filtration and processing. The pore size classification systems of the

different filter manufacturers not being standardized, the flow properties of filters cannot be judged from their designated pore sizes. The flow qualities must also be determined experimentally. The validation will deal with the selected filter's porosity and area, in conjunction with such specific filtration conditions as differential pressure, temperature, etc. Therefore, the conclusions derived from this validation exercise will be valid only for the membrane types examined. If for the filter types selected, both pore size ratings qualify regarding retentions, the one yielding better flow rates is to be utilized.

The avoidance of the effort and costs of two validations is obviously preferred where one can serve. The question then becomes: *For which membrane should validation be sought first*? If the selected 0.2/0.22 type is tried first and is validated as ensuring the requisite results, it should be used. There is no need for a tighter filter. The necessary retention has been achieved without an unnecessary diminution of flow. If the 0.1 is validated first and succeeds, it will likely be used, perhaps to avoid the second validation effort. This decision, in the absence of an effort to validate the 0.2/0.22 membranes, could entail a loss in flow rate, and possibly the other consequences previously mentioned.

The choice, then, comes down to whether to overdesign, in the classic response to uncertainty, or to seek the guidance of the added validation exercise. The choice of the former may involve unnecessary filtration penalties. The latter selection, less emotionally based, is in the direction of good science. Validation is to be preferred.

If the validation exercise does not totally resolve the question of which selected pore size designation is the better of the two in terms of a potential penetration due to the filling speed, process or organism species, the 0.1 μm rated membrane should be selected. Full retention is the primary objective. This filter can then be sized to the requirements of the process. This sizing will determine the costs involved. Once this is done, it will be possible to compare an existing process with a conceivable future process to determine the potential improvements and savings.

Filtration, including filter selection, should be a disciplined practice confident in its dependence upon good science, unalloyed by the desire, however understandable, of gaining feelings of security from wasteful overdesign. The required validations need to be performed.

THE AUTHORS' INTENTIONS

This writing is designed to persuade that there are particle retention mechanisms other than size exclusion. The adsorptive effects are especially emphasized. Focusing on the less recognized retention mechanism is not intended to promote a reliance upon

adsorptive organism-captures, even though they reinforce the sieving action. It is to negate the belief that, practically speaking, sieving is essentially the means of organism removal. Sieve retention usually is the dominant mechanism; but ignoring the adsorptive contribution inevitably, and periodically leads to the call for tighter membranes to achieve sterility. Advocating the replacement of the 0.2/0.22 μm rated membranes by those that are rated 0.1 μm is a repeat of the replacement in the 1960s of the 0.45 μm rated membranes by the 0.2/0.22 μm ratings. At that time it was also believed that sieve retention was essentially the only retention mechanism that merited consideration, and that filter validation required only the confirmation of an integrity test. With a repetition of this reasoning, perhaps the next progression will be to yet tighter filters to retain viable but non-culturable organisms!

The emphasis on adsorption sequestration is not an expression of preference for one mechanism or another. In fact, rarely is a means of filtration selected on the basis of the mechanism of retention it entails, although the opportunity to do so sometimes presents itself. The use of 'hydrophylized' hydrophobic membranes to avert the attachment of proteins to filters by the mechanism of hydrophobic adsorption is a case in point. From a strictly utilitarian point of view, it is not necessary to understand how (by what mechanism) an organism is retained, only that it be retained. However, better understanding the causality may better enable its governance.

Where the mechanism of particle retention can be selected, sieve retention or size exclusion should be the choice. Its certainty is less conditional. Its restraint on the passage of an organism through a pore depends essentially only on their size relationship. Sieving is free of the many influences that govern adsorptive sequestrations, such as the number of pores, the challenge density, the adsorptive propensity of the polymeric filter, the differential pressure, temperature, viscosity, ionic strength of the solution, etc. The axiomatic nature of the size exclusion mechanism is assuring in its simplicity. It should be understood, however, that the complexity of adsorptive sequestrations does not reflect on its reliability as a mechanism of retention. In any filtration, the retention mechanisms that become engaged are determined by the selection of the filter and of its conditions of operation. Adsorptive retentions are conditional, but they are viable and dependable given the fulfillment of the operational conditions.

The understanding of adsorption effects is not necessary, provided that the omission does not equate with an ignorance of their influences. If one understood only the operation of sieve retention, then it would be possible to conclude that the challenge density is not an important factor in filtrations (especially when pore size distribution is seldom of concern). It may even be concluded that differential pressure is not a prime determinant of retention, unless its level is high enough to distort the particle, allowing for its permeation of the filter. The efficacy of low differential pressure applications in achieving desired retentions is well understood by filter practitioners, but perhaps largely as a rule of thumb. As such it has value. However,

comprehending that the differential pressure governs the residence time of the microbe within the pore pathway, that this in turn reflects on the probability of pore wall encounters, and that this can influence the likelihood of adsorptive captures, offers the advantages that derive from understanding the phenomena involved.

It might not have mattered if Bowman and her associates could have filtered *B. diminuta* from a penicillinase solution without understanding the retention mechanism. It turned out to be important, however, to learn that proteinaceous materials, by way of a different mechanism, could interfere with the retention of *B. diminuta*, and to understand how by use of a second mechanism this interference could be avoided. Similarly, it is worthwhile to understand how endotoxin can be removed by filters in different ways, by electrostatics and by adsorption, and to understand why there is a difference; and a choice. Knowledge empirically acquired is available to utilitarian application. It is, however, the comprehension of the relevant phenomena that may permit a more fruitful extrapolation.