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Preface

Filtration, whether pre- or final-, ultra- or diafiltration, is widely used within the biopharmaceutical industry. Especially sterilizing grade filtration, an essential part of aseptic processing, is increasing in importance due to the introduction of more and more biologically based drugs. The complexity of biopharmaceutical filtrations, the large variety of filter types that are available, and the many different purposes for which they may be employed make necessary the careful training of those who are to be engaged in filtration operations. Appropriate explanations of filter designs and properties, of causes and effects in their management, and instructions in their manipulation, all of which gained by experience, would be an ideal first step in such training. The regulatory authorities endorse training as being necessary for individuals working in biopharmaceutical processes one of these is filtration. Indeed, there is an obligation, stated by the FDA, to train those who are assigned such work. If not fulfilled, regulatory warnings or enforcement will be the consequence.

This work describes the individual filtration techniques available, the separation mechanisms at work, the production and design of different filters, the regulatory fulfillment of validation and integrity testing. Chapter 1 handles and explains the different filtration types and procedures available. It is not only important to realize the differences between specific filtration types, but also what the specific purpose of these types is. Prefiltration, for example, commonly does not receive the same attention as a sterilizing grade filter element. Nevertheless, the prefiltration step is essential for reducing the running costs within a production process, as it will protect the final filter or other process steps, such as reverse osmosis or chromatography systems. Testing of the different filter types during the investigative phase will help to find the optimal solution for the particular application. Testing of the filter with the product solution under process conditions will also verify the retentivity of the filter. Chapter 2 defines the different separation mechanisms which play a role in depth and membrane filtration. It explores the common belief that all filtration mechanisms are solely sieve retentive and set the record straight, that the most common case is otherwise. Due to the vast differences in separation mechanisms and the influence of these, appropriate validation with the product and process conditions has to be performed. Since sieve retention and/or adsorptive sequestration are dependent on the pore size and specifically the polymer. Chapter 3 describes the production processes and the different polymers in detail. Every membrane or depth filter polymer has

advantages in one application but disadvantages in another. Generally speaking, one can say that there is no overall best polymeric material for all applications. Similarly, the design of a filter requires appropriate evaluation to determine the performance with regard to its use. Chapter 4 describes the different designs and design criteria, which deserve attention when it comes to filter element development and filter choice in specific applications. As with polymers, the design and construction used, differs from application to application. Air filters are optimal for longevity and air flow rates, but not total throughput, as gases commonly have a low particulate load. Liquid filters, nevertheless, require a high dirt load capacity in some of the applications and therefore are optimal for this purpose. Every application will need testing to find the filtration system that fits optimally to the specifications defined by the user. Once the performance specifications are met, the filtration system requires validation. The performance has to be verified and documented to fulfill the user's specifications and regulatory requirements repeatedly and consistently. Chapter 5 describes the various guidelines available and the regulatory requirements defined in different regions by different authorities. These guidelines need to be met, whether they are regional, national or global since export to another region of the globe requires the fulfillment of the regional regulatory requirements. It also describes the individual tests, which are required to validate filters or filtration systems. The detailed description of such tests is helpful to anybody who requires to validate the filtration step into the process and this usually means everyone who utilizes a filtration step. Once a membrane filter is validated within the particular application, it requires to be tested as to whether it is integral or not, i.e. meets its performance criteria, especially retentivity. Chapter 6 describes the different integrity tests available and what needs to be observed when these tests are set-up and performed.

This volume creates an overview of the requirements of filtration within the biopharmaceutical industry. The choice, evaluation, optimization, validation and routine testing is not an easy task, indeed it is usually rather complex. The authors have tried to reduce the complexity and give practical guidance on what requires attention when choosing or utilizing a filtration system. We hope we have succeeded.

Manorville, November 2005 Maik W. Jornitz

Acknowledgement

We wish to acknowledge our colleagues and friends within the biopharmaceutical industry, and among the filter manufacturers and regulatory agencies for their consideration and collegiality in sharing their experiences and findings over the years. Their technical wisdom included in this book will now become even more widely available for the common good.

In particular, we would like to thank those individuals who supported this book with highly valued contributions, Dr. Richard V. Levy, Dr. Oscar W. Reif, Dr. Theodore H. Meltzer and Russel E. Madsen, Jr. We greatly appreciate the time they spent documenting their experiences and advice.

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Types of Filtration

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Abstract There are a multitude of filter designs and mechanisms utilized within the biopharmaceutical industry. Prefilters are commonly pleated or wound filter fleeces manufactured from melt-blown random fiber matrices. These filters are used to remove a high contaminant content within the fluid. Prefilters have a large band of retention ratings and can be optimized to all necessary applications. The most common application for prefilters is to protect membrane filters, which are tighter and more selective than prefilters. Membrane filters are used to polish or sterilize fluids. These filters need to be integrity testable to assess whether or not they meet the performance criteria. Cross-flow filtration can be utilized with micro- or ultrafiltration membranes. The fluid sweeps over the membrane layer and therefore keeps it unblocked. This mode of filtration also allows diafiltration or concentration of fluid streams. Nanofilters are commonly used as viral removal filters. The most common retention rating of these filters is 20 or 50 nm.

Keywords Prefiltration · Membrane filtration · Porosity · Retention rating · Pore size · Cross-flow · Microfiltration · Ultrafiltration · Viral filtration · Nanofilter

1
Types of Filtration

1.1
Prefiltration

Prefilters are most commonly depth filter types and are most often constructed of non-woven or melt-blown fiber materials such as polypropylene, polyamide, cellulosic mixed esters, glass fiber, mesh or sintered metals, and (before the interdiction of its use on account of its carcinogenicity) asbestos. These fiber materials are constructed into matrices by the random deposition of either individual or continuous fibers whose permanence of positioning is sought through pressing, heating, gluing, entanglements, or other forms of fixing. The actual pores of such filter constructions are formed from the interstices among the fibers. As shown in Fig. 1, the random deposition of the fibers during construction of the filter matrix results in a broad retentivity distribution, which causes a wide band of particle retention. The retentivity distribution can also be influenced by the thickness of the individual fiber or the degree of compression of the matrix.

Varying the different filter media will deliver a wide variety of properties; therefore prefilters can be manufactured for selected applications. For instance, polymeric properties can be chosen to ensure specific chemical, thermal, and mechanical stability, or to introduce adsorptive properties. Increasingly useful in today's biotechnological applications, charged matrices may be advantageous to remove haze, colloidal substances, or other oppositely charged particles.

Composed of discrete, non-attached fibers, these filters were regarded as being potentially fiber-releasing. Although support materials may help to eliminate shedding, this shedding is not necessarily eliminated by initial liquid flushing. Furthermore, shedding may be exacerbated by pressure fluctuations.

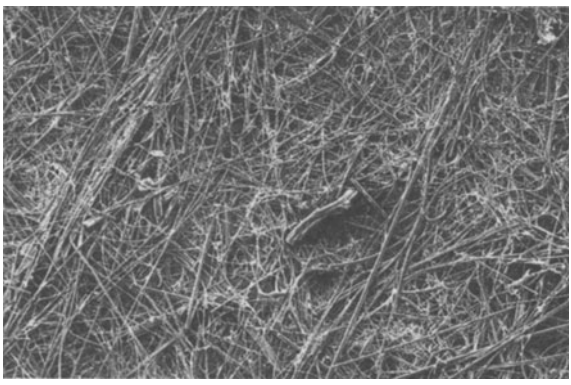

Fig. 1 Random depth filter matrix (courtesy of Sartorius Group)

Therefore, at least in the case of injectable drugs, their use must be followed by a final, membrane-based filter.

A major advance in depth filter design technology was made of melt-spun depth filter types and the introduction of heat stabilization of fiber fleeces. Depth filters of this classification commonly meet United States Pharmacopoeia (USP) or British Pharmacopoeia (BP) requirements. Additionally, these technologies allowed producing fleece construction of different fiber sizes within a filter matrix. Typically, the outer layers have a coarser retention rating than the inner layer of the filter. This allows for a prefiltrative effect, improving the total throughput. These filters are mainly used in applications with a wide spectrum of contaminant sizes, for example prefiltration of water.

A further advantage is gained with the introduction of longer melt-spun fibers, coupled with the thermal fusion that occurs in the manufacturing process. Unlike fiber migration which can occur with fiber yarn-wound filter designs, thermal fusion has the impact of reducing concerns about fibers coming loose and passing into the filtered effluent.

The melt-spun filter design offers additional advantages over traditional textile winding technology. First, the process produces a filter free of lubricants or finishing agents, eliminating the need for processing aids used to make yarn-wound filters. Second, the extrusion process produces a more controlled distribution of fiber diameter sizes. Although its distribution is relatively uncontrolled in this process, the mean fiber size can be smaller than the traditional staple fiber diameters. The smaller mean fiber diameter coupled with the graded density method can produce filters down to 0.5 µm nominal range, commonly testified as retentive at 99% of this particular particle size, measured by, for example, Arizona fine dust challenges.

Yarn-wound filter cartridges (Fig. 2) have commonly one retention rate throughout the filter depth, therefore the contaminants will either be retained on the surface of the filter or will penetrate through the filter. Therefore these filters are used to retain a specific particle size, commonly on the outside surface of the filter. These filters are generally very inexpensive, but also not very efficient compared to melt-blown depth or pleated filter types, as these filters do not have a high total throughput capacity. The surface area of the filters is relatively small and it would be advisable to utilized pleated devices with similar specific retentivity. The pleated device might be 8–10 times more expensive, but would have far more total throughput capacity. Additionally, yarn-wound filters are most often double open-ended filters, which presents the risk of fluid by-pass opportunities. In most pharmaceutical applications, a double o-ring filter cartridge adapter type is preferred.

Prefilter technology advanced with the advent of the first melt-blown type of cartridge that incorporated fiber of various diameters to achieve a graded pore design. In this process, the polymer is extruded through a multi-hole die and the polymer stream is stretched and attenuated by a high velocity heated air stream. The mean fiber diameter is changed as the filter is being made by adjusting the air velocity or one of the other variables that contribute to the for-

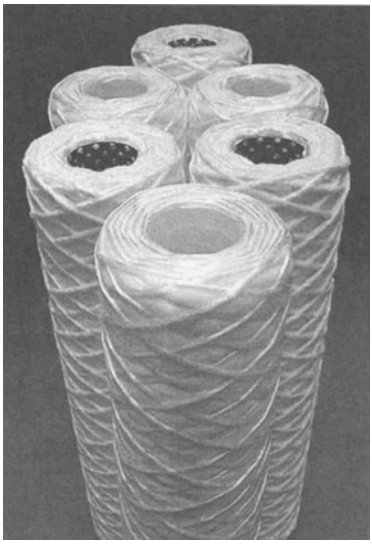

Fig. 2 Yarn wound filter cartridges

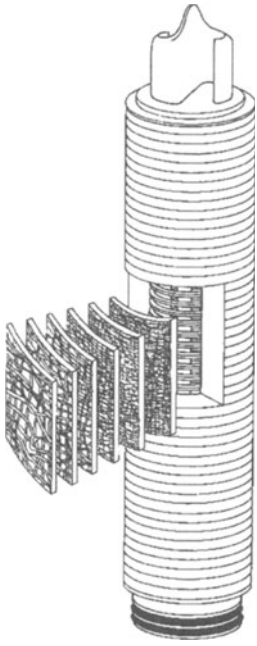

Fig. 3 Melt spun fiber depth filter cartridges with gradual tighter retention

mation of the fiber sizes, e.g., temperature or polymer pumping rate. So, rather than using density-based packing, this novel design is manufactured using a variation of standard melt-blowing equipment (Fig. 3).

The concept of using a graded or changing pore size to enhance filtration performance is a desirable one. This technique involves incorporating a series of prefilters into a single stage to maximize the use of the entire filter and extend filter life (dirt-holding capacity). This manner of filter construction results in prefilters with a wider particulate retention rate range. In fact, some filters have a nominal particle size removal from 0.5 μm up to 100 μm. Experimenting with variety of prefilters with varying retention ratings allows the user to find the optimal filter to protect the life and performance of more expensive membrane filters.

Prefilters can also contain supportive membranes, commonly composed of cellulose, mixed-esters of cellulose, or borosilicate. These prefilter types are utilized to remove a very fine band of particulate or contaminants from the fluid to specifically protect sterilizing grade membrane filters. Such filters may even require protective depth filter types in front of them, especially as their pore size rating decreases. The diversity and practical application of depth filters have been recently reviewed by Jornitz and Meltzer (2004).

1.2 Membrane Filtration

Membrane-based filters commonly contain a well-defined pore structure and consistent porosity range (Fig. 4). Although depth filters are produced under

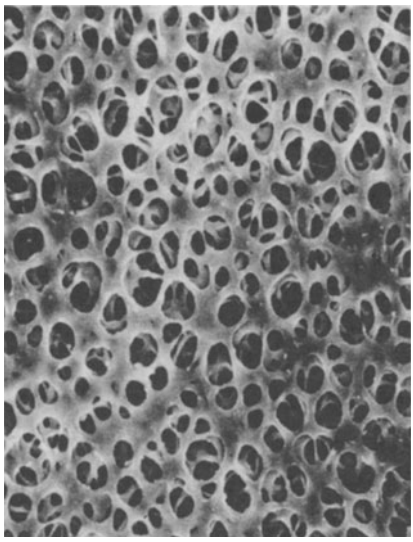

Fig. 4 SEM of the porous structure of a cellulose acetate membrane (courtesy of Sartorius Group)

controlled conditions, the randomness of the fibrous material does not result in a well-defined porous structure as can be seen in membrane filters. Often referred to as MF (microfiltration) microporous membrane filters, these filters offer a much more controlled degree of porosity than is available from the conventional depth filters. They are rated from 0.04 µm up to 8 µm, the most common being a 0.2 µm sterilizing rated filter. The complexity and practical application of membrane filters have been recently reviewed by Jornitz and Meltzer (2004).

1.2.1 Membrane Manufacturing Processes

Microporous membranes are manufactured by one of four methods: evaporation (air casting), quenching (immersion casting), stretching, or track-etched processes.

1.2.1.1 Phase Inversion Processes

Casting solutions intended for microporous membrane manufacture usually contain not only polymer in solution, but also a quantity of high-boiling (low-volatile) non-solvent. The resulting solution consists, then, of polymer molecules dispersed in a single, homogeneous liquid phase. As solvent (lower boiling) evaporates and the volume of solution diminishes, the polymer segments progressively come closer to one another. However, achievement of their potentially ultimate degree of propinquity is prevented by the action of the non-solvent. The point is reached where the composition of the remaining solution, modified from the original by loss of solvent, is too rich in non-solvent to support further the solubility of both the non-solvent and the polymer.

As described by Kesting (1971) at this point phase inversion occurs, with the appearance of two heterogeneous liquid phases – one rich in polymer and solvent, the other in non-solvent. With further evaporation of solvent, coalescence of the polymer-rich droplets into a wet gel distorts their spherical shapes into polyhedra (Maier and Scheuermann 1960). (A similar point, for particular casting solutions, may be reached by temperature manipulations, or be temperature triggered, rather than by the evaporation of solvent.)

In a somewhat over-simplified sequence, droplets of non-solvent separate within the solvent/polymer solution, and the polymer begins to condense out of solution. The polymer concentrates at the phase interfaces as it comes out of solution, thus leading to the formation of small droplets of non-solvent surrounded by a swollen polymer shell. As further solvent evaporation (or temperature lowering) takes place, more and more polymer comes out of solution and a thickening of the polymer shell occurs. The polymer-in-solution phase disappears and the polymer-surrounded droplets come into contact with each other, forming clusters that consolidate and distort into closed polyhedral cells

filled with residual non-solvent. Finally, the edges of the closed polyhedral cells accumulate polymer at the expense of the polyhedral cell walls, thereby leading to the thinning of such cell walls and their eventual rupture. An interconnecting, porous polymeric continuum is the result.

With the rupture and disappearance of the cell walls, the interconnecting pores are created, permitting the removal, by washing or evaporation, of the remaining solvent/non-solvent. The additional solvent removal, however, does not permit further significant spatial adjustments by the polymer segments. Such movements are inhibited by the high viscosity of the wet-gel state.

The attainment of phase inversion need not involve non-solvent pore former. Solution of the polymer can be managed by the use of cosolvent systems wherein two (or more) liquids, neither of them a solvent for the polymer, in combination do serve to dissolve it. Evaporative loss of one of the liquids upsets the system solvent properties and causes phase inversion.

Solution of polymer, as well as its precipitation from solution (the phase inversion), can also be managed by temperature manipulations rather than by solvent evaporation (Hiatt et al. 1985).

1.2.1.2 Air Casting, an Evaporative Process

In the air casting process the casting solution is applied onto a belt. In response to a specific defined temperature, belt speed, and atmospheric conditions (air flow and humidity), the solvent from the volatile casting solution begins to evaporate. This process leads eventually to phase inversion and the formation of the wet-gel form of the microporous membrane. The resultant changes in the casting conditions and to the casting solution itself lead to different pore structures and porosities. In evaporation of the solvent, two different diffusion mechanisms are involved: diffusion of the solvent in the liquid phase from the interior of the casting to its surface, and diffusion from the casting surface into the surrounding air. Hence, the dynamics of the evaporative process are affected by the temperature of the casting solution, by the temperature of the surrounding air, the ambient relative humidity, and the air velocity over the casting surface.

If the evaporation of solvent from the casting surface into the air is greater than the rate of solvent diffusion from the interior of the cast film to the surface, the result will be "skinning," the formation of a dense layer on the surface of the cast film. The evaporation of the solvent without adequate replacement by liquid diffusion from the film interior causes the surface of the liquid casting to represent a "bad" solvent condition; polymer precipitation results. The high rate of this process does not permit the formation of droplets of the non-solvent phase, or at best permits the formation only of very small droplets. The result is that the surface skin can be of a high degree of impermeability. However, this dense surface skin will moderate the solvent evaporation in the liquid layers below it. In these layers, therefore, coacervation will occur, and the bulk of the casting will be microporous.

1.2.2
Asymmetric or Anisotropic Membranes

The Loeb-Sourirajan RO cellulose diacetate membrane devised for water desalination is of such a morphology (Loeb and Sourirajan 1962). Asymmetric or anisotropic membranes have a pore disposition wherein the larger size pores are arranged at one surface and where the pore sizes become progressively smaller as they approach the opposite surface. The overall result, in effect, is an assembly of "V" shaped pores. The filter cartridges are so constructed that the more open ends of the V-shaped pores of the membrane are directed upstream. This enables them to accommodate larger deposits in their more open regions. The result is a possibility of larger dirt-holding capacity.

Kesting suggests, however, that the term "asymmetric" be reserved for skinned structures, and that the appellation "anisotropic" be employed for the non-skinned, pore-size gradient types. Less often, the term "anisomorphic" is used interchangeably with anisotropic, and even with asymmetric.

1.2.2.1
Stretched PTFE Membranes

Polytetrafluoroethylene (PTFE) polymer is best known by the DuPont trademark name Teflon. Aside from its carbon-carbon backbone linkages, it consists essentially solely of carbon-to-fluorine bonds. These are very stable chemically. The polymer is thus chemically inert to an exceptional degree. This suits the microporous membranes made of it for use with aggressive solvents. The polymer is hydrophobic and thus difficult to become wetted by water. This makes its microporous filters advantageous to use as air filters, given the relative ease with which water, accidentally condensed or intruded therein, can be expelled.

Microporous PTFE membranes can be manufactured from extruded films of PTFE by a stretching process. The resulting structure, as seen under a scanning electron microscope, consists of slits among separated strands of PTFE that are periodically bound together at nodules (Fig. 5).

The pore sizes of these microporous PTFE membranes become defined by the degree of stretch to which the PTFE film is subjected. It should be noted, however, that their pore shapes and ratings are different from those of conventional microporous filters, and that this may have unusual implications for particle and organism retentions. Melt extruded films are stretched under carefully defined process conditions to create a thin (commonly 60–100 µm) membrane.

PTFE filters are widely used in pharmaceuticals as sterilizing vent filters because of their inherent hydrophobicity. They are used to purify aggressive solvents, as in antibiotic manufacture, because of their chemical imperviousness. They are also used in the filtration of fermentation air because their unusual thinness minimizes their resistance to airflow and thus diminishes

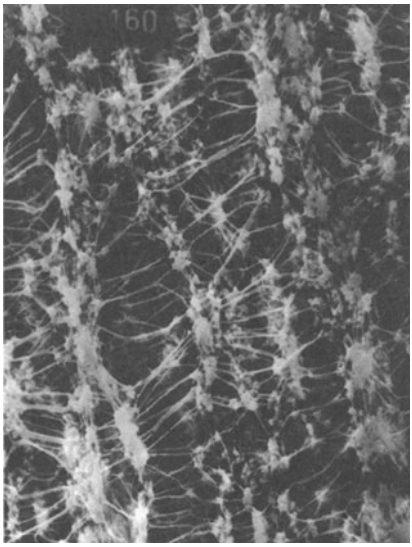

Fig. 5 SEM of the porous structure of a PTFE membrane

the kilowatt costs attendant upon pumping high quantities of air through them.

The PTFE filters are also used in filtering the off-gases of fermentations. These, usually at elevated temperatures, compromise the more common filters, usually of polypropylene fiber, by causing their oxidative decrepitude.

As described, membrane filters can be formed in a variety of structures for specific application purposes. An example is the formation of asymmetric membrane structures where the pore structure on the upstream side of the membrane filter is larger than the downstream side; this can enhance the dirt load capacity of such filter. Some applications require very distinct pore shapes to avoid premature blockage or, in case of the use of a membrane as microbiological test filter, the pore structure has to be very even to achieve appropriate nutrient distribution.

Membrane filters, as described above, are the most common filtration devices used in aseptic processing to remove organisms from liquids or gases. Due to the highly defined pore structure, these filters are extremely reliable with respect to the retention requirements and can be integrity tested, as will be described later in this chapter.

1.2.2.2 Track-Etch Membranes

The thinnest (10–20 μm) membrane films are created by the track-etch manufacturing process. Track-etch membranes are unique in their pore geometries. For their manufacture, thin polymeric films are bombarded by high-energy

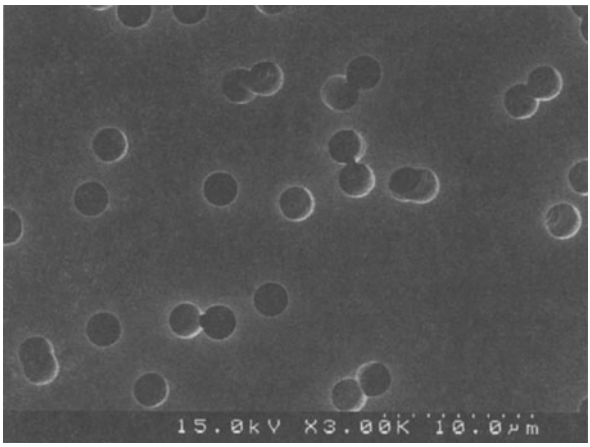

Fig. 6 Typical track-etch membrane pore structure

particles. The polymer is damaged along the bombardment track so that exposure to a caustic solution results in a pore being etched through the polymer film. The resulting pore is of a straight-through columnar shape whose diameter is a function of the etching line. This pore shape is distinct among filters and can be precisely measured under the scanning electron microscope. Although the manufacturers dispute this, it is generally held that in an effort to produce a high density of pores (i.e., a large total porosity or pore volume) an overlapping of pore paths is caused. Double or even multiple hits produce occasional larger pores. Of unpredictable occurrence, they compromise the dependability of retention of the track-etch membranes (see Fig. 6).

Several manufacturers supply filters of this type. Films of polycarbonate or of Mylar (a DuPont polyester) are one thousandth of an inch thick that have been bombarded by high-energy particles from a nuclear reactor. A process of French origin employs high-energy krypton ions to effect the same result (albeit, it is claimed, a somewhat higher total porosity). The total porosity of about 15% is generally not sufficient to give a filter of high flux. However, the thinness of the membrane enables filter cartridges to contain enough effective surface area to impart adequate flow rates to these filter devices.

Traditionally, track-etch filters have not been amenable to integrity testing, which has precluded their use in critical pharmaceutical processing. Recently, there have been promising improvements in this regard.

They may have the narrowest pore-size distribution of all membranes. This property is generally regarded as better ensuring the retention of particles larger than the filter's pore-size rating. However, by the same token, these filters do not measure up in the retention of smaller particles since they do not contain a measurable proportion of smaller pores.

Morphologically, the straight-through columnar pores of these filters offer less wall surface for adsorptive particle arrests than do the more conventional

microporous membranes with their particle intercepting tortuous passageway. However, the track-etch filters are used in electronic rinse water applications because they rinse up to acceptable resistivity levels using minimum flush volumes, due partly to the thin, straight columnar pores that predispose them to free drainage and their construction for thin polymeric films.

Furthermore, with its flat and clean surface, a track-etched membrane presents an ideal substrate for new rapid microbiological test methods, including those that use optical sensors to detect organisms grown or sitting on the membrane surface.

1.2.3 Pore-Size Distribution

All filters are characterized by a pore-size distribution function, membrane filters more narrowly so than others. However, this important filter parameter is not easy to measure. This may be the reason that pore-size distribution has been either neglected or misstated in the filter manufacturers' literature, although references exist in the technical literature (Badenhop et al. 1970; Badenhop 1983; Jacobs 1972; Pall 1975; Marshall and Meltzer 1976; Johnston 1983). A review of the subject was made by Richter and Voight (1974). In the usual characterization of microporous membrane filters, the "largest pore" and the mean-flow pore are more commonly specified. Yet knowledge of the "largest" and median pore sizes cannot predict the rate of flow characteristics, which are the product of the entire pore-size distribution, the total porosity. Similarly, predictions of filter blockage, and hence of the throughput volume of a suspension of a given particle-size distribution, necessitate elucidation of the true pore-size distribution present in the filter.

1.2.4 Fiber or Particle Sizes

Sand beds (multimedia beds), carbon beds, and even ion-exchange columns, all composed of layers or depths of discrete particles, are also depth-type filters, albeit of a non-structured variety. In the case of non-structured depth filters, the finer particles will yield greater retention efficiencies, along with lower flow rates, because of the closer packing and the smaller pores that result from the use of smaller particles. This is in accord with Kozeny's teaching that the volume average pore diameter, as distinct from the flow or number average pore diameter, is inversely related to the surface-to-volume ratio of the particles constituting the non-structural filter:

$$\bar{D}^2 = \frac{16\,E^2}{S^2(1-E)^2} \tag{1.1}$$

where E represents the void volume porosity.

The volume average pore diameter thus derived is smaller than that obtained from flow average calculations because the flow average number reflects the fourth-power flow relationship to the pore radius as in the Hagen Poiseuille equation. In any case, S, the ratio of surface to volume, becomes increasingly larger as the particle size declines; the smaller the filter aid particles, the smaller the volume average pore diameter. The Carman (1937) treatment of the Kozeny equation applies here as well. Where the surface-to-volume ratio of the individual fibers (or particles) is known, and the random packing of the fibers is carried out to a certain density, so that the porosity of the resulting depth medium is known, then the pore diameter can be calculated:

$$(\bar{d})^2 = \frac{16\,E^2}{(1-E)^2\,(S/V)^2} \tag{1.2}$$

Alternatively, where the depth filter is built of fibers:

$$(\bar{d})^2 = \frac{(\bar{d}_f)^2\,E^2}{(1-E)^2} \tag{1.3}$$

Where $\bar{d}$ is the average pore diameter (assuming a circle), E is the porosity of the medium, S/V is the surface-to-volume ratio and $\bar{d}_f$ is the diameter of the fiber.

From the inverse relationship of d and (S/V) in Eq. 2, and from the direct relationship of d to d_f in Eq. 3, it follows that the smaller the constituting particle or the thinner the fibers composing the depth filter the smaller the interstices or pore diameters. Therefore, the efficiencies of the resulting filters are increased (as measured by particle retention) but also, the consequent flow rates are lowered.

To summarize, depth-type filters are seen to have broader pore-size distributions because their technology of manufacture involves the laws of chance. This leads to the random placement of fibers (particles), with a resulting wider spread in the size of interstices of the resulting mat.

1.2.5 Polymer Chain Layering Effect

According to Piekaar and Clarenburg (1967) filters can usefully be thought of as consisting of superimposed planar layers, each with its own pore-size distribution. The total filter in its overall pore-size distribution is seen to reflect the averaging or narrowing effects occasioned by successive layering. The resulting filter structure, then, is not dissimilar to that formed in the case of the depth filter by the progressive laying down of fiber deposits, except that in the case of the membrane, the layers consist of overlapping deposits of polymer molecules. This conceivable view is given additional credence by the work of Pall and Kirnbauer (1978), who found that as one progressively stacks layers of

membrane filters, ultimately to a plateau value, the particle retention capabilities of the composite increases to a maximum level. The rationalization is that the successive layering, for the depth-filter construction, progressively narrows the pore-size distribution of the resulting filter to some constant level of uniformity.

Johnston (1998), however, explains the Pall and Kirnbauer findings differently. By way of graphical plots he demonstrates that while the subject data on a linear/linear plot do seem to lead to the conclusion that an increase in membrane thickness results in an increase in the bubble point (K_L in the Pall terminology) to some limiting plateau, plotting the same data on a log/log plot does not show this. This is in accordance with Johnston's theory that the bubble point, which is indicative of particle retention capabilities, does not increase with membrane thickness.

1.2.6 Solution Technology Effects

One explanation for the narrower pore-size distributions of microporous membranes relates to the solution properties of the casting formula. According to this hypothesis, the following details have significance: As stated, in the casting of inverse-phase membranes formulations are used that contain polymer in solution. Solvent is allowed to evaporate from thin films of these compositions until the polymer precipitates in the form of a wet get. (Alternatively, temperature changes may be used to induce the polymer precipitation.) At this point, the pore dimensions of the eventual dry membrane become prefigured by the size of the inter-segmented polymer spacings characteristic of the wet gel.

What is important in this process is that solution chemistry is involved. In any solution, the molecules of the dissolved solid or solute tend to disperse evenly through the entire volume of solvent, and therefore become spaced at similar distances from their neighbors; that is, they tend to become separated by spaces of equal dimensions. The rather equidistant separation of the solute or polymer molecules from one another is not an accident. It is an invariant consequence of the laws that govern solutions. This is significant because the pores of the microporous filters arise from these spaces, from the intersegmental spaces within the polymer solution.

Thus, solution technology assures that the spaces separating the polymer segments tend to be rather similar in dimension, and that the pores derived from these spaces are, therefore, also rather similar to one another in magnitude. Furthermore, the size of the one relates to the size of the other. To be sure, perfection does not prevail. The pores need not all be equal in size. Other influences may also be at work. Perhaps the result is some rather narrow Gaussian distribution, not necessarily symmetrical. In any case, there are some differences in the size of the pores, but the distribution about the mean is small because the pore size is being directed toward uniformity by the laws that gov-

ern solution. Thus, inverse-phase microporous membranes have narrower pore-size distributions.

1.2.7
Pore Distribution Analysis

When mercury is forced into a pore, the pressure required to fill that pore completely is in inverse proportion to its size. The relationship is, as for the capillary rise equation,

$$D = \frac{4\gamma \cos\theta}{P} \tag{1.4}$$

except that the minus sign is required by the non-wetting nature of mercury relative to membrane surfaces. Here P is the pressure, D the pore radius, γ the surface tension of mercury, and Θ the contact angle of mercury with the solid pore surfaces.

Assuming that Θ=130 °, γ has a value of 485 dynes/cm. Converting dynes per centimeter to psi yields D=181/P when the pore diameter in micrometers is inversely proportional to the mercury intrusion pressure in psi. In this procedure, the precise measurement of the mercury volume at any pressure, and hence a means of gauging the volumes intruded into the filter, is assessed dilatometrically, a method offering great accuracy.

Whatever its virtues, the method has serious shortcomings. Indeed, Badenhop (1983) concludes that mercury porosimetry is unsuited to the pore-size measurement of microporous membranes, and Williams (1984) states that, in principle, fewer than 20% of the largest apertures (pores) need be breached by the intrusion of mercury to fill the membrane entirely. Indeed, the very pressure it relies on may distort porous polymerics whose glass transition points do not render them immune from elongational effects, and its numerical conclusions involve the averaging of volume changes that may mask the true dimensions of internal metering orifices. The chief objection to mercury porosimetry arises from the artificialities its manipulations bear to the filtrative process, an operation that usually involves aqueous flow through a filter under rather moderate pressures, the very essence of the flow-pore regimen. In any case, using this procedure, measurements can be made of the cumulative volume of mercury introduced into a filter at different pressure levels. From this, the percentages of the various pore sizes become available, and also the pore-size distribution curve.

Early work was taken to suggest that membrane filters had a pore-size distribution of ±0.02 µm about their mean pore-size rating. This narrow distribution had significance, as it was suggested that these filters would be expected to exhibit "absolute retentions," and this was further supported by the successful use of such membranes in filter sterilizations. However, examination of four commercially available 0.45 µm-rated membranes, each from a different man-

ufacturer, by mercury porosimetry demonstrated that none of the tested filters had pore-size distributions as restrictive as ±0.02 μm. Therefore, it has been stated that the high reliability of their 0.2 μm-rated membranes for filtration sterilization applications must, therefore, be derived from one or more physical and/or physiochemical considerations (Marshall and Meltzer 1976).

1.2.8 Pore Lengths and Tortuosity

Except for the track-etch membranes, the pore passageways are not straight through and columnar. The pores of the phase-inversion membranes are tortuous and labyrinthine. Johnston (1998) describes the length of the pores as being the thickness of the filter medium multiplied by the tortuosity factor. He defines the latter mathematically for filter media composed of random units as being the reciprocal of the porosity.

What is of particular interest are the implications to particle retention of pore length and pathway tortuosity. A filter plate built up in thickness from contiguous thin-membrane layers all of the same pore-size distribution exhibits better retention than the same breadth of membrane disposed as separated layers. In the latter case, particles permeating a single thin membrane are carried into the inter-membrane liquid pool space. From there, the probability is high that the liquid flow will carry the suspended particles into to the larger (wider, less restrictive) pores of the next downstream membrane. Successive filters offer only the same pore-size distribution barrier the particles have already successfully permeated. Thus enhanced capture possibilities will derive mostly from the adsorptive effects of longer residence times within the pore system.

When the layered membranes are contiguous, however, a wider pore path of a thin membrane may become more restricted by being coupled with a narrower pore path of a second membrane, leading to better retention. To be sure, the restrictive pore paths of the first filter may also be extended by more open pathways in the second. This will, however, not diminish the overall retention of that pore path. Retention is defined by the narrowest dimension, by the smallest pore or aperture along the path. Where the wider pore path gains in restriction, however, added trapping efficiency is conferred on the filter. The overall effect of contiguous layering, then, is to increase the number of restricting pores along the pore pathway. Increased filter efficiency results. Increase in the number of restrictive pores engendered by contiguous layering may result in an increased opportunity for intra-membrane sieve retention as well as of enhanced adsorption, but flow rates may be somewhat reduced.

1.2.9 Implication of the Largest Pore

Where sieve retention of particles is the only consideration, the size of the largest pore (Pall 1975, recognizes more properly the assemblage of largest pores) pre-

sent in the filter is of overriding concern. In this oversimplified view of filtration, an organism small enough to fit geometrically through a membrane pore will not find within the filter impediments to its complete penetration.

Particularly in the filtrative sterilizations of pharmaceutical preparations, there is an emphasis on achievement of that particle/pore-size relationship that can produce organism removal solely by sieve retention. However, in theory, complete organism (particle) removal does not require the exercise of sieve retention. Adsorptive particle arrests can also be utilized. Indeed, sterilizing membranes, possessing narrower pore-size distributions, have sharper lower pore-size cutoffs than do the depth-type filters. Consequently, where small particles are concerned, it is possible for depth-type filters to be even more retentive in sieving than membranes simple because they do have many more smaller pores. Particles smaller than the mean pore size rating are likely retained by adsorptive sites within the membrane filters.

Microporous membranes are used in filtration sterilization because there is considerable surety of particle retention, which in most cases can be demonstrated to be independent of operating conditions. Sterilizing grade membranes are expected to have a pore-size distribution pattern wherein the largest pore is smaller than the smallest microbe whose retention is being sought. Sieve retention is thus assumed to be the sole particle-capture mechanism operative. This is the intended situation, for the "reliability" of sieve retention is seen in its freedom from the operational factors that influence the efficiencies of adsorptive removals, such as the organism challenge level, the magnitude of the applied differential pressure, and even such parameters as fluid temperature, viscosity, ionic strengths, the presence of wetting agents, etc., that constitute the contribution of the liquid vehicle (Levy et al. 1990, 1991; Mittelman et al. 1998). Actually, filter reliability, involving whatever mechanisms of particle removal, is demonstrated beyond doubt by the exercise of filter validation; and once established, it poses no continuing uncertainty, regardless of the particle-arresting mechanism.

Semantics enter the picture of the largest pore. As commonly considered, a penetrating particle encountering the filter enters by way of a large enough pore and completes its penetration unhindered. In this scenario, the large, inviting pore maintains its generous dimensions clear through the filter. In this sense, the bubble point assay measures the diameter of the entire pore passageway; for no distinction is made between the "largest pore" and any particle-restraining portion of the pore. Actually, the pore diameter not being uniform throughout, the bubble point measures the narrowest point of the overall widest pore.

Regrettably, the current use of the word "pore" is undifferentiated with regard to its meaning. Its use covers both the polyhedral chambers and their connecting, restrictive, smaller apertures. Williams intends the term "pore" to refer to the restrictive aperture, of significance to both flow and retention.

To empty a polyhedral chamber whose interconnecting apertures are the restrictive pores of interest in filtrations, one need only evacuate the two largest apertures of that multifaceted (multi-apertured) chamber. The smaller ones,

interconnecting with neighboring polyhedral chambers, may remain water filmed. The evacuation of water from these smaller apertures or pores is not necessary to the total evacuation of the water from the polyhedral chamber itself. Williams calculates that full evacuation of the liquid from a membrane, the full emptying of all the polyhedral chambers (the membrane pores in the conventional sense), can take place with only 20% of all the interconnecting apertures becoming blown free of their liquid films, depending on the liquid and the tendency of its films to burst or drain from the apertures under the differential pressure being applied.

In the Williams view, the pore passageways consist of an assemblage of larger and smaller apertures interconnecting the polyhedra. Overall, certain of these passageways are the largest in the sense that they are least restrictive. However large the passageways, it is their restrictive dimension that is measured by the bubble point. In this sense it is not the largest pore, the largest aperture leading from the polyhedron, but the narrowest of those comprising the largest pore path overall that comes to be measured. Strictly speaking, therefore, it is not the largest pores that are revealed in the bubble point measurement but the most restrictive ones associated with them in the overall largest pore path.

Therefore, Williams concludes that the ASTM method of determining flow-pore sizes (the bubble-point method) does not reveal a substantial portion of either the larger or smaller pores (interconnecting apertures) present in the filter. What the bubble point determination measures is the largest restrictive pores of the filter.

1.3 Prefilter and Membrane Filter Comparison

Depth type filters cannot dependably be used to produce sterile filtrates; membranes can. This dissimilarity is due to the difference in the pore-size distributions and the stability of the pore structure within both filter types. By whatever manufacturing technique filters are prepared, not all of the pores produced within a filter are of the same size. Given the relative homogeneous sizes of a suspension of particles (organisms) whose filtrative removal is being sought, the broader the pore size distribution, the more likely the encounter of a particle penetrating the filter.

Depth filters are manufactured by technologies involving the incorporation of discrete particles or fibers into some matrix or fixed form. These constitute the structured depth filters. The fabrication almost always requires the use of insoluble particles or fibers and a rather viscous dispersing medium. Uniform dispersal is a problem; the viscosity of the matrix, the preferred orientation of the fibers, insolubility of the fibers, insolubility of the heterogeneous phase, the usual mechanics of the mixing or lay-up, and the agglomeration of the primary particles all work against it. The tendency to diffusional equilibration that is the response to concentration gradients in the porous membrane-casting solutions is absent here. In principle, individual fibers, for example, are deposited on a sur-

face until the complete fiber mat becomes constructed. Each fiber falls largely in accordance with the laws of chance. The fiber mat irregularities reflect this random deposition. The spaces among the fibers constitute the filter pores. As indicated in Fig. 1, a modeled representation of the randomness of fiber deposition, the interstices vary greatly in size, reflecting localized low- or high-fiber population densities. Because the fiber, or other particle, deposition follows a random pattern, the consequent pore-size distribution is broad. The melt-spun and melt-blown processes randomly position the constituting fibers as well.

The breadth of the pore-size distribution of a depth-type filter will depend on the thickness of the fiber (particle) mat. Thicker mats can be considered as consisting of repetitive layers of a thin "unit mat." Each successive layer or increase in mat thickness will serve to diminish the pore-size distribution of the composite. The larger pores of one layer will come randomly to be coupled with the smaller pores of succeeding layers. The overall effect will be a progressive narrowing of the pore size. Eventually, some constant value of pore-size distribution will be approached, perhaps asymptotically, but it will never reach the stability and specification of a membrane structure.

Additionally, depth filter structures can be subject to process conditions. It is essential that the process conditions, especially pressure differential or pressure pulses fit the prefilter used. Such pressure conditions can either damage or loosen the filter structure and therefore have to be monitored accordingly. There have been examples of membrane filters being subjected to up to 72 psi (5 bar) of differential pressure and pulses. These membrane filters still passed the microbial retention and integrity test. A depth filter's fibrous structure could be damaged by such pressure conditions.

Depth filters, as is self-explanatory, remove any contaminants within the depth of the filter matrix, whereas membrane filters function mainly as surface retentive filters. This certainly depends on the contamination to be removed. The depth retention of prefilters make these the "work horse" of filtration processes due to the high dirt load capacity of such filters. Surface retentive filters' total throughput can only be enhanced by the porous structure (asymmetry), enlargement of the effective filtration area, or the use of depth filters as protection in front of the membrane filter. The aim is to find the best filter combination of pre- and final filters to achieve the desired retentivity, but also throughput need.

Membrane filters can be integrity tested, which is not possible with depth filters. To validate the membrane filter's performance and reach filtrative assurance integrity testing of these filters is a must. Depth filters commonly have the purpose to clarify and polish, but not to sterilize. For this reason an integrity test is unnecessary.

1.4 Cross-Flow Filtration

Cross-flow filtration differentiates itself from conventional "dead-end" filtration in that the fluid to be filtered flows parallel to the filtration surface rather than

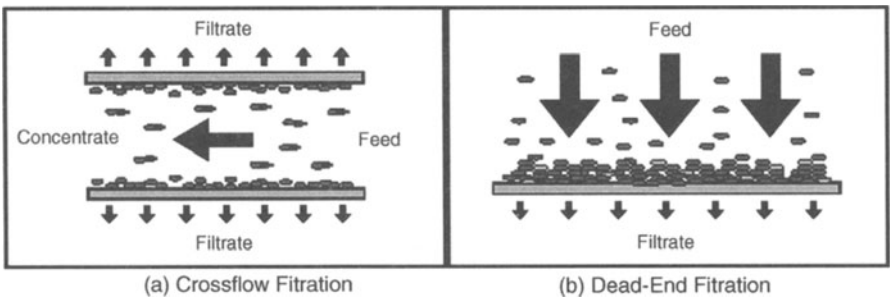

Fig. 7 Schematic of "dead-end" and cross-flow filtration (courtesy of Brose and Dosmar)

perpendicular to the filtration surface, the function shown in Fig. 7. The cross-flow generates shear that limits the build-up of a filter cake or gel layer. In conventional dead-end filtration the filter cake thickness increases with time, resulting in the eventual cessation of flow. In cross-flow filtration the feed stream flows parallel to the surface of the membrane, i.e., the feed flows tangential to the permeate or filtrate stream. A small fraction of the feed stream permeates the membrane (filtrate or permeate); the remaining fraction is retained by the membrane and exits as retentate or concentrate stream. The retentate or concentrate is recirculated over the membrane layers until the specified requirements are met.

Cross-flow filtration membranes have their origins in the 1950s when reverse-osmosis membranes were developed for water desalination. The Loeb and Sourirajan process of membrane formation by phase inversion created the first asymmetric membranes for reverse osmosis. The thickness of the asymmetric membrane's rejection layer was reduced 1000-fold over the thickness of symmetric membranes. The development of asymmetric membranes by Loeb and Sourirajan made it practical to use cross-flow filtration for industrial applications. It is this development, coupled with the development of new ultrafiltration membranes that gave birth to cross-flow filtration in the biotechnology and biopharmaceutical industries today.

In the biopharmaceutical industry, cross-flow filtration is used for both microfiltration (0.45, 0.2, and 0.1 μm) and ultrafiltration (1000–300,000 MWCO (molecular weight cut-off)). The microfiltration devices are mainly used for cell harvesting or cell debris removal, downstream of a fermentation process. In instances, cross-flow microfiltration devices are also used as a prefiltration step before conventional membrane filtration. Ultrafiltration systems are mainly used for fractionation, concentration, and diafiltration steps of proteins, peptides, or viral vectors. This technology enables the removal of undesired contaminants, buffer exchange, and concentration of a target protein without compromising or stressing (shear forces) the target.

Cross-flow filters have a variety of design, which range from plate and frame cassette systems to spiral wound and hollow fiber modules. The individual designs have to be properly evaluated when cross-flow is chosen, due to per-

formance differences in dead-volume, shear forces, cleanability, pressure conditions, energy inputs, and flow patterns. Cassettes or modules are placed into specific holding devices, which either can be manually driven or fully automatic systems. Plate and frame modules consist of flat-sheet membranes mounted into a framework, commonly silicone or polyurethane.

In the assembly of these systems each flow path is made up of two membranes that are facing each other. The upstream flow path must be sealed from the downstream permeate side of the membrane. Stacks of pairs of membranes are layered one on top of the other, and the permeate side of each membrane is supported by a rigid and porous spacer plate. The spacer plate may be smooth or have surface features that give the membrane an uneven surface for turbulence promotion. Flow paths are usually open and may be parallel and or in series. Spiral-wound modules utilize pairs of flat sheet membranes bound on the up and downstream sides by screens similar to those in cassette systems.

The membrane sandwich is sealed at three edges so that the feed is isolated from the permeate. The fourth side of the membrane sandwich is attached to a perforated permeate collection tube. The membrane pairs are then rolled around the perforated collection tube, thereby creating the spiral. Feed flow enters at one end of the spiral, flows tangentially along the axis of the cartridge, and discharges at the other end. Permeate flows at a right angle to the feed flow towards the center of the spiral and is collected in the core of the spiral.

Hollow fiber systems, as the name describes, are of a tubular, porous design, which is commonly bundled into a module. Liquid permeates the fiber wall, as with flat-sheet membrane, and permeate is collected on the opposite side of the fiber. Depending on the manufacturer, hollow fiber systems fed from the outside or from the inside (most commonly inside flow). In the case where the rejecting layer is on the inside (lumen) of the fiber, the feed solution enters the lumen of the fiber at one end, flows down the length of the fiber, and retentate exits at the other end. Permeate is collected on the outside (shell-side) of the fiber.

1.4.1 Design Considerations: Turbulence-Promoting Insertions

Insertion of static mixers into the flow path enhances the transition of the flow from laminar to turbulent. The use of screens or meshes as static mixers between membranes are found in variety of cross-flow devices. Screens are used in spiral-wound cartridges and in some plate-and-frame designs. These mesh-like spacers can cause considerable turbulence and have been shown to improve flux. There is some debate as to the nature of the flow through these systems. Belfort (1987) considers the flow to be laminar through systems with screened channels, whereas Cheryan (1986) reports the flow as turbulent, based on the pressure drop within the flow channel.

This matter can be resolved in a straight-forward manner by experimentally determining the slope of the log-log plot of flux as a function of velocity, as shown in Fig. 9.

There are potential disadvantages to the indiscriminate use of turbulence-promoting insertions for biopharmaceutical filtrations. Specifically, the mesh may cause product to hang up on the mesh, creating a cleaning problem as well as causing potential occlusion of the flow channel. If particulates tend to either hang up on the mesh or occlude the flow channel, then pre-treatment of the feed is required. Usually 50–200 μm prefiltration of the feed will alleviate this problem.

1.4.2 Flow-Path Length

The way to control gel polarization is to maintain high velocity and shear at the membrane surface. The length of the flow path has direct and indirect bearing on these hydraulic forces. First, as shown in Eq. 5, when flow is laminar the flux is proportional to the quantity $(1/L)^{0.33}$. Therefore, increasing the flow-path length has the effect of decreasing flux. In turbulent flow, the length of the flow path does not have a direct bearing on flux. Second, the flow-path length has an indirect bearing on flux for both laminar and turbulent flow because the pressure drop through the cross-flow device is proportional to the flow-path length because of frictional forces at the fluid-membrane interface. Therefore, the longer the flow path, the greater the pressure drop and the lower the flux. Third, because fluid is continually permeating the membrane, as the flow-path length increases the volumetric flow and velocity of the feed solution decreases. Based on Eq. 5 and Eq. 6, decreasing velocity causes a reduction in flux. Therefore, based on both direct and indirect reasoning, increasing the length of the flow path causes lower flux.

The dependence of mass-transfer coefficient on cross-flow velocity has been accurately correlated. For laminar flow the correlation is:

$$k = 0.816\left[\frac{\gamma}{L}D^2\right]^{0.33} \tag{1.5}$$

where γ is shear rate, and $\gamma=8v/d$ for flow through tubes, and $\gamma=6v/h$ for flow through rectangular channels; v is solution velocity; d is tube diameter; h is channel height; L is length of the membrane flow path; and D is solute diffusivity (Fig. 8).

When flow is turbulent, the mass-transfer coefficient is proportional to velocity raised to the 0.80 power instead of to the 0.33 power:

$$k = 0.023\left(\frac{1}{d_h}\right)^{0.20}\left(\frac{\rho}{\mu}\right)^{0.47}(D)^{0.67}(v)^{0.80} \tag{1.6}$$

where d_h is the hydraulic diameter and equals 4×(cross-sectional area)/(wetted perimeter), ρ is liquid density, and μ is liquid viscosity. Because of the greater dependence on velocity when flow is turbulent, improved benefits in flux can

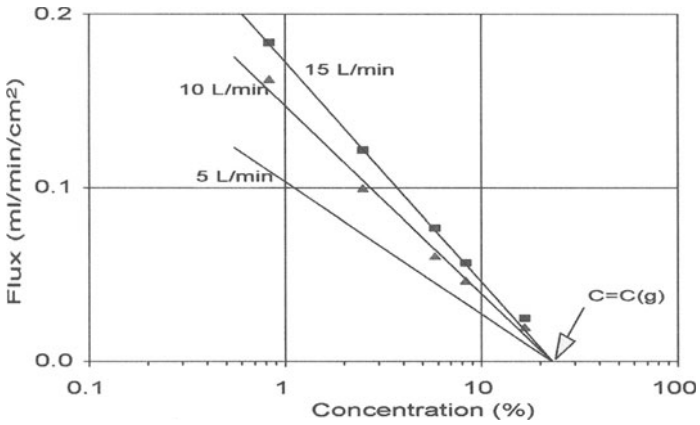

Fig. 8 Flux vs ln(C_f) for ultrafiltration of bovine serum using 100,000 MWCO polysulfone membrane at two different cross-flow rates (Sartorius Sartocon II, membrane area= 7000 cm^2)

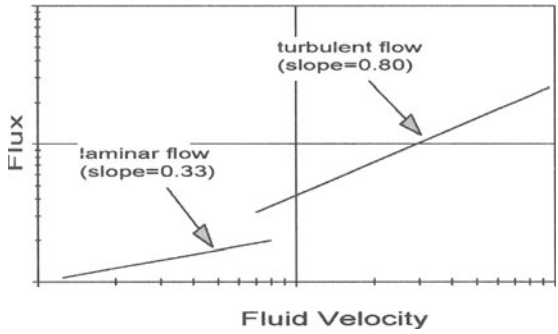

Fig. 9 Flux as a function of fluid velocity for laminar and turbulent flow in cross-flow ultrafiltration

be realized when flow is increased. The relationship between flux and velocity for both laminar and turbulent flow is shown in Fig. 9.

1.4.3 Flow-Channel Height

The flow-channel height also has direct and indirect bearing on flux in cross-flow ultrafiltration. As Eqs. 5 and 6 show, in laminar flow flux is proportional to the quantity $(1/d_h)^{0.33}$ and in turbulent flow flux is proportional to $(1/d_h)^{0.20}$. Therefore, as the channel height (or hydraulic diameter) increases the flux will decrease. The indirect consequence of changing channel height is to cause a change in the cross-flow velocity, assuming constant volumetric flow rate. That is, the cross-sectional area of the flow channel divided by the volumetric flow

rate gives the fluid velocity in the flow channel. By increasing the flow-path channel height the cross-sectional area is increased, resulting in a decrease in the velocity and the flux.

Increasing the volumetric flow rate in order to increase velocity may result in the need for very large pumps. Running pumps at high speeds results in high shear in the pump and the generation of excess heat by the pump. When possible the channel height should be as small as possible. However, care must be taken not to select a channel height that might trap recirculating particles or require a pump that might destroy existing particle aggregates in the attempt to achieve sufficient cross-flow rates.

1.5 Ultrafiltration Membranes

Ultrafilters are generally highly asymmetric (anisotropic), where the membrane's rejecting layer is thin (0.1–5 μm) with very small pores, and the underlying support structure is thick (~100 μm) with much larger pores (Fig. 10).

The materials used in UF membranes vary widely, and some of the common membrane polymers, in order from most hydrophilic to most hydrophobic, are: regenerated cellulose (RC), cellulose acetate (CA), modified polyvinylidene difluoride (PVDF), modified polysulfone (PS), polyethersulfone (PES), nylon and polypropylene (PP). Membranes made of these materials have been successfully used for isolation and purification of biopharmaceutical products by cross-flow filtration.

Ultrafiltration membranes are rated with a nominal "molecular-weight cut-off" (MWCO), however, there are no standardized guidelines for determining the MWCO of an ultrafilter. The rated MWCO and actual MWCO can vary by as much as 50–98%. Current convention for the rating of ultrafilters uses specific protein markers. Depending on the membrane manufacturer, a retention

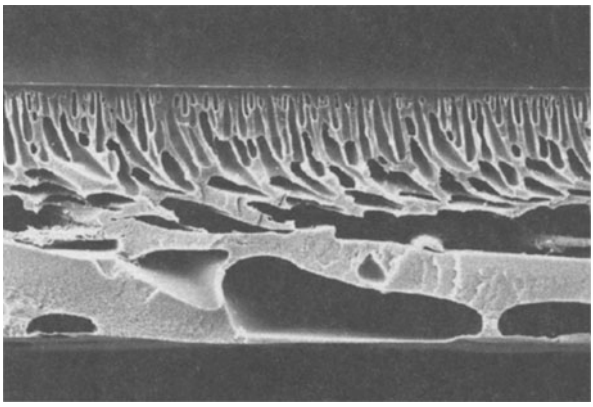

Fig. 10 Ultrafiltration membrane, with skin layer and finger support structure

of somewhere between 50% and 95% of the protein marker is the criterion for establishing a membrane's MWCO.

Typically a marker protein is filtered through a membrane and retention is determined. The use of single proteins as markers has many disadvantages. Proteins of the same molecular weight vary in size, shape and structure, isoelectric point, and lypophylicity. Consequently a membrane's retention of a single protein provides limited information with regards to the membrane's retention of other proteins.

For most applications, variation in the retention profile between vendors can be tolerated without any adverse impact on the process yield. However, if the process is sensitive to the membrane's retention profile, it may then be necessary to characterize the retention profile relative to the actual process. From a process and validation stand point, actual retention coefficients would be best determined by end users filtering actual process streams.

It has been suggested in the literature that rather than using protein markers, poly-dispersed dextran solution be used as the challenge solution. Using this approach a single challenge solution can be used to test membranes from 10,000–1,000,000 MWCO. The analysis of permeate and retentate samples via size-exclusion chromatography may be translated into a retention curve. However, when two or more solutes are being filtered, the retention of the smaller solute will be increased by the presence of the larger solute. Marshall et al. (1993) demonstrated that heterogeneous dextran mixtures and heterogeneous PEG mixtures, respectively, resulted in a membrane's retention shifting towards higher MWCO values.

The matter of retention rating, regardless of the method employed, should be viewed only as a guideline for the end user because retention will be affected by the nature of the feed stream.

1.6 Nanofilters (Viral Retentive Filters)

Nanofilters are specifically designed to separate viruses and other biomolecules using size exclusion as the predominate mechanism of removal. Viral retentive filters take the concept of ultrafilter to a new level of particle removal and reliability. Traditional ultrafiltration membranes exhibit defects that permit the passage of viruses at levels unacceptable for reliable removals. These new membranes do not. This is accomplished by taking patented membrane filter casting technology to a new level and, in some cases, by layering these nanofilter membranes to further increase the level of retention.

These nanofilters have water bubble points well in excess of practical testing capabilities. Therefore, integrity testing of these filters also called for the development of new test methodologies, such as liquid porosimetry (Phillips and Dileo 1996). This method uses two immiscible liquids, which are successively intruded by pressure into largest pores of the membrane. When properly designed and qualified, this method measures fluid flow through only

those pores that have been targeted, and is identical to methods that are universally accepted for conducting both bubble point and air diffusion tests. This statement is true with one exception: the liquid porosimetry test allows the use of lower differential pressures than would be expected from traditional air-water or air-water-alcohol integrity tests. Thus the practicality of the method in actual filter application is assured. These porosimetry measurements may be correlated to viral removal post-filtration, which allows the test to be used to validate viral removal in actual practice.

These filters, as well as ultrafilters, may be used in either normal (direct) flow or in tangential flow mode, depending on the nature of the filter itself and the application. Manufacturer's recommendations and published literature are critical sources of guidance in choosing a particular nanofilter and determining the optimal mode of operation. Experience has shown that successful application of these filters is dependent on knowing the boundaries of operation for each application.

In 1998, Levy et al. reviewed the use of nanofilters in the physical removal of viruses from biopharmaceuticals. The subject was further extensively reviewed by Aranha in 2001.

Nanofilters or viral retentive filters are an essential contaminant removal step within modern bioprocesses. A multitude of nanofilters are available for different applications and target contaminants. Most common retention ratings are 20 and 50 nm, also known to separate parvo- and retro-viruses. Since most of the biologic drug products are obtained by cell cultures, the possibility of viral contamination is increased and for this reason a downstream process has to have three robust viral inactivation and/or removal steps, of which one of them is commonly a filtration step.

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Modus of Filtration

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Abstract Experience teaches that particles larger than the pores of a filter cannot negotiate its passage. Other retention mechanisms are less obvious than sieve retention or size exclusion. They are electrical in nature, and find expression in the bonding alliances that mutually attract (or repel) filters and particles. The influence of hydrogen bonds, of van der Waals forces, of hydrophobic adsorptions, and of transient polarities on particle retentions are set forth in terms of the double electrical layer concept that also governs colloidal destabilizations. The origins of differences in membrane porosities is explained, as also the importance of the filtration conditions. The singularity of the particle-fluid-filter relationship on organism and/or pore size alteration is stressed.

Keywords Sieve retention · Adsorptive sequestration · Hydrophobic adsorption · Inertial impaction · Brownian motion · Partial charges · Electric double layer · Hydrogen bonds · Van der Waals forces · Zeta potential

1 Introduction

A major purpose of filtration is the separation of particulate matter from fluid streams. In pharmaceutical settings this finds its ultimate expression in the sterilizing filtration of drug preparations, especially those intended for parenteral application. At its most utilitarian level, it is enough to know that particle (organism) removals can be made using filters, and to understand how and when to manage the outcome; a detailed comprehension of the mechanisms is not necessary. An understanding of filtrative behavior is more likely, however, to lead to fuller and more successful applications. It is in pursuit of this goal that an inquiry into the mechanisms of particle removals is herewith undertaken.

Present understanding of the particle retention mechanisms is strongly based on the sieving effect that results from size exclusions wherein the particle's size makes impossible its passage through the pore. There is also the recognition that adsorptive influences, usually but perhaps not always electrical in nature, play a role in particle captures by filters. The several ways in which particles impact filter surfaces may also be considered retention mechanisms. The

logic of the size discrimination mechanism is self apparent. The forces involved in the adsorptive retentions require elucidation.

The chief protagonists in the filtration drama are the particles and the filter pores. However, other influences also bear on the outcome of a filtration. The nature of the fluid vehicle, the predilection of the membrane's polymeric composition for adsorptions, the compatibility of fluid and filter, and the particular filtration conditions employed, largely the differential pressure, and temperature, are all influencing factors. Nevertheless, since it is principally the size relationship of the pores and particles that will decide the outcome of the particle retention operation, some prior considerations should be paid them.

2
Particles and Pores

Although, as said, factors other than the pore/particle sizes are involved in the filtration process, in the first instance it is precisely this apposition that is of prime importance. It is from the appropriate pairing of pore and particle sizes (and shapes, albeit seldom known), that the direct interception of the particle by the pore results.

The particles whose filtrative removal may be sought are too varied to permit generalizations. They may come in any and all sizes and shapes. The usual tendency is to oversimplify by picturing the particle to be spherical. For this reason the spherical latex particles, discussed below, may be useful models of the generic particle.

2.1
Molecular Segmental Arrangements

Too little is known about the sizes and shapes of the membrane pores. An inquiry into membrane construction may be helpful. Consider a solution of polymer in solvent (plus non-solvent) from which the membrane filters are cast to a very precise thickness. The long polymer molecules are equally separated from one another, in accordance with the properties of solutions, to extents that reflect the degree of dilution and the ambient temperature. The molecular chains are flexible and convoluted, and their segments tend to coil and overlap, albeit they are more extended in diluter solutions. However, the segments that comprise the polymer chains, whether within or between molecules, are nonetheless separated from one another within the solution to extents that mirror its concentration.

The progressive removal of solvent, as by evaporation, reduces the volume of the solution, and results in the chain segments moving correspondingly closer to one another. Complete solvent removal results in the closest segmental convergence possible. A solid, unbroken polymer film is formed. However, within

the final solid matrix, intersegmental spaces still exist. They represent the equilibrium positions set by the opposing electrical attractive and repulsive forces, soon to be described, that characterize all solids. It is these intersegmental spaces that prefigure the "pores" in the finished polymeric membranes.

Differences in the intersegmental distances of the bulk state can be managed by manipulating the speed of solvent evaporation. Slow evaporation allows time for the chain segments to adjust their closer positions. This segmental movement becomes increasingly difficult as the viscosity of the casting increases with solvent loss, ultimately to yield the high viscosity of the solid state. Where this relaxation time is maximized, the smallest "pores" result from the closest positioning.

2.2 Reverse Osmosis Membranes

The ultimate segmental closeness, the sizes of the smallest "pore", reflect the molecular architecture of the particular polymer molecule. Thus, chain lengths, branching, substituent groups, etc. may so define the ultimate intersegmental distances of a given polymer as to create semipermeable membranes. The reverse osmosis membranes of cellulose acetate, and polyamides are examples. Water molecules are small enough to permeate them under pressure, ions enlarged by skirts of hydration are not. More rapid solvent removals, providing briefer relaxation times, further limit the shrinking of the intersegmental distances: progressively, the more open nanofilters, and the still more open ultrafilters result.

2.3 Nanofilters

The pore sizes of the nanofilters discriminate between permeation by the larger hydrated divalent ions, such as calcium and magnesium, and the smaller hydrated monovalent ions. This enables them to restrain the passage of the alkaline earth elements that cause water hardness. Accordingly, nanofilters are used in water softening. Their advantage over conventional softening by ion-exchange is that they remove the divalent ions while not adding sodium ions.

2.4 Ultrafilters

The intersegmental spaces of the ultrafilters are designed to separate larger molecular entities, such as proteins. Their pore sizing ratings are in terms of MWCOs (molecular weight cut-offs). They are also rated in daltons. The practical MWCO to use in an application is usually found by trial and error, since the rated cut-off is based on tests with pure standards such as serum albumin, Cytochrome C, bovine serum albumin, etc.

2.5
Microporous Membranes

The yet larger pore size ratings of the microporous membranes are attained by a transitioning of the dissolved polymer from its solution state to its solid state. This is managed by its premature precipitation as caused by non-solvent. Ongoing evaporation of the volatile solvent from the casting leaves it with a progressively increasing concentration of high-boiling non-solvent. At a point, the non-solvent portion of the casting formula is sufficiently ample to asserts its influence. The polymer comes out of solution as a wet gel whose intersegmental spaces prefigure the pores-to-be in the dry membrane. It is these filters that are relied upon to separate organisms from their suspensions.

2.6
Pore Paths

The microporous membrane analogy is that of a polymeric sponge. The oversimplified picture of the filter pores is that of irregular and tortuous capillaries composed of the interconnected spaces within the polymer matrix. As just explained, the structure derives from a polymer solution. The chain segments are separated from one another by distances that reflect the polymer dilution. It is the final interstitial distances that in their interconnections prefigure the pores of the finished membrane. Formulae of different polymer concentrations give rise to different intersegmental separations, ultimately to different porosities, when by proper manipulation the polymer is precipitated as a gel, to be washed and dried to its solid, microporous membrane state. There is inevitable a pore size distribution, and some anisotropic pore shape formation [1].

The casting solution consists of polymer dissolved in a mixture of solvent and high-boiling non-solvent. In terms meaningful to the polymer chemist, pore formation occurs as follows: As solvent progressively evaporates from the casting solution, the non-solvent increases in content to the point where phase separation takes place. Non-solvent droplets separate within the polymer/solvent phase, and polymer comes out of solution to concentrate at the phase interfaces. The swollen polymer shells surrounding the non-solvent droplets thicken as continuing solvent loss occasions more polymer deposition. The eventual disappearance of the polymer/solvent phase brings the polymer-surrounded droplets into mutual contact. They consolidate into clusters, and distort into polyhedral cells filled with non-solvent under the impetus of the area minimizing forces. Finally, the edges of the cells accumulate polymer at the expense of the cell walls. Thinning of the walls of the polyhedra leads to their rupture, and interconnection. The reticulation of the discrete cells of the polymeric matrix permits the removal of the non-solvent, as by washing. Not the polyhedral cells, but their interconnecting openings, thus formed, comprise the metering pores of the membrane [1].

3
Pore Size Distribution

The efficiency of particle removal varies inversely, in certain instances, with the challenge density. This can be explained on the basis of a pore size distribution wherein the number of smaller pores far outweighs the fewer large pores. Only when so great a number of organisms is present as to enable confrontations with the few larger pores do organisms escape capture.

The attention, especially in sterilizing filtrations, is so focused on restraining bacterial passage that only the largest pores, those which the organisms can negotiate on a size basis, are a matter of concern. Hence, the emphasis on the bubble point measurement of the set of largest pores. There is reason to believe that, despite their relative paucity, the larger pores are early on engaged by the hydrodynamic flow when diluter organism suspensions are fed to the filter [2–4]. If so, organism passage may occur. In any case, in this view the smaller pores, those adequate for the sieve retention of the organisms, can safely be ignored.

One factor that had delayed explanation of the dependence of organism retention on the challenge density was the de-emphasis of the pore size distribution. The pore size distribution of membranes had early on been explored by mercury porosimetry and had originally been reported to be a narrow 5%. The 0.45 μm-rated membrane was said to be ±0.02 μm in its distribution, "It reflects an extraordinary degree of uniformity." [5]. Subsequently, Marshall and Meltzer [6] demonstrated the actual value to be closer to ±100%. The exaggerated report of pore size uniformity confused the meaning of the pore size rating by trivializing its difference from the largest pores. Meant to signal the mean pore size, it also came to identify the set of largest pores, those that are the concern in particle retentions. Consequently, experts in the membrane field advised, "The membrane filter functions primarily as a screen filter. *It retains all particles larger than the pore size of the filter*" (emphasis added) [5]. This is now known not to be so, nor does the pore size rating value represent the pore dimensions. The erroneous concepts sustained belief in the exclusivity of sieve retention, and catered to the comforting reliance on absolute filters. The series of experimental findings in the 1970s that will subsequently be discussed corrected the record.

4
The Sieve Retention Mechanism

The sieving mechanism is perhaps the most common manifestation of filter action. Inevitably, the usual mental picture of a filtration is of particles that are retained by the filter because they are too large to enter or pass through the filter's pore. The logic is unquestioned, it is unfailingly understood.

The particle is retained because it is too large to fit through the filter mesh or pore. The principle of size exclusions is so obvious as to be an axiom of solid

geometry. Its effect is independent of the challenge density, or of the number of particles, or pores, or their ratio. It is independent of the filtration conditions. For example, the differential pressure motivating the fluid's flow, unless high enough to deform the suspended particles, does not affect the retention. The physicochemistry of neither the organism type nor the filter polymer type sways the results.

Even in situations where there are particle and pore size distributions, as long as the smallest particle is larger than the largest pore the filtration is absolute. However, only in that circumstance may the filter be characterized as being absolute. In different situations the removal of the same organism by the same filter might not take place, as when the organism decreases in size as a result of its suspension in a vehicle of high ionic strength. In any case, absoluteness is not a filter property. It is a description of the pore size/particle size relationship that may exists under particular circumstances.

The sieve retention mechanism is relied upon to effect the clarification of fluids. It is an ancient practice that removes visible particles, variously described as being from 20 or 40 μm in size, by filtration. On a less subtle scale, sieve action is equally exemplified by the netting of the birds of the air (Fig. 1), and the fish of the sea. The segregation of different size particles from their mixture can be managed using sieves of different mesh sizes (Fig. 2). The sieve retention mechanism is easily understood. The particles larger than the filter pores are restrained by size exclusion, the smaller particles and fluid are not so retained.

The serious use of polymeric membrane filters, chiefly of microporous ratings, followed acquisition of German technology [7]. Impressive successes, particularly in attaining sterilizing filtrations, led to the assumptions that the pores of the "sterilizing filter", then 0.45 μm-rated, were so small as to operate

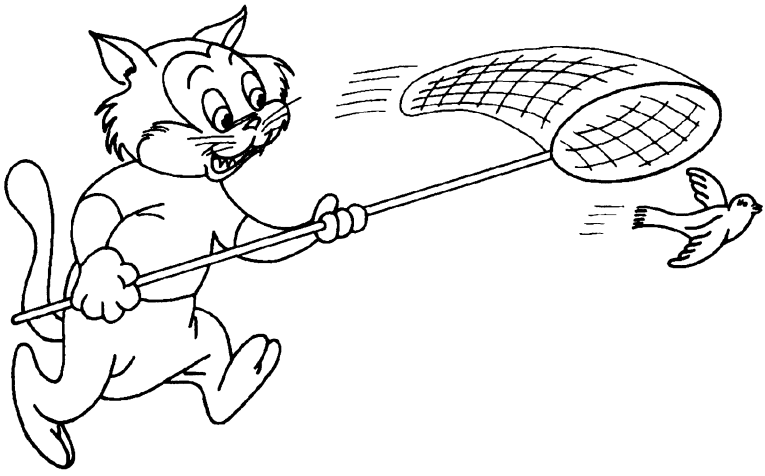

Fig. 1 Solid-gas separation by sieve-retention

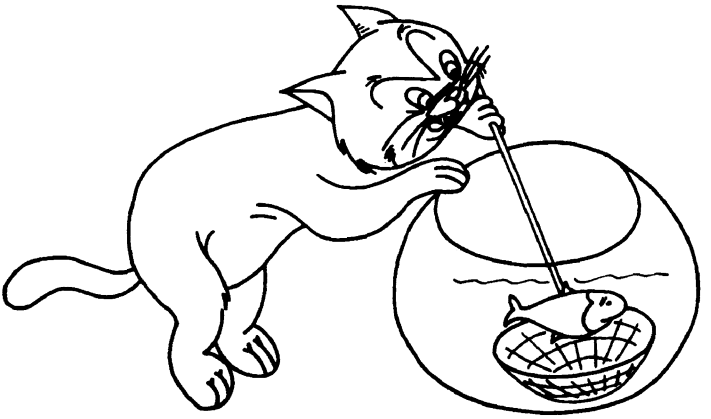

Fig. 2 Solid-liquid separation by sieve-retention

exclusively by the sieve retention mechanism, and that the filter action was absolute. Over the next several decades these assumptions would prove to be unfounded. Adsorptive influences, soon to be considered, would be seen to contribute and to reinforce the sieving action.

5 Inertial and Brownian Impactions[1]

The very term "Mechanism of Particle Removal" requires definition. Particle impactions on filters could also come to be considered mechanisms of particle removal. To some the term 'mechanism' denotes the several ways of a particle's coming together with the filter surface leading to its retention. Thus, the settling out of a particle from the air onto a filter surface due to the influence of gravity could be attributed to a mechanism formalized by the name of "gravitational impaction"

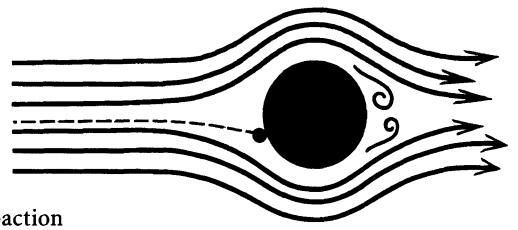

Fig. 3 Inertial impaction

[1] The impaction mechanisms are more important in gas filtrations. For pertinent details, and for other aspects of gas filtrations refer to [8]

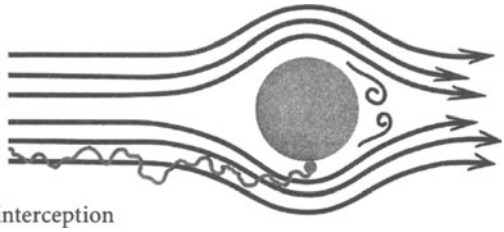

Fig. 4 Diffusional interception

In like manner, "impaction" or "inertial impaction" is often considered the mechanism involved in the encounter between a filter's surface and a particle suspended within a flowing air stream. Particles, conveyed by the moving fluid, possess the inertia that is the product of mass and velocity, the velocity being the more important of the two factors. Were the gas stream to veer suddenly from its path, the particle would continue on its trajectory, motivated by its inertia. In so doing, as shown in Fig. 3, it could impact the filter to be retained thereby. Inertial particle/surface contacts are more likely in the low viscosity of gas streams where their speed is less attenuated than in liquid, as by molecular collision.

Similarly, smaller particles, particularly those suspended within gas rather than liquid streams, are more apt to be vectored by Brownian motion into contacts with filter surfaces. This results from their longer mean free paths in the less dense medium, the distances they travel between the collisions that motivate them, and subsequent molecular collisions that interrupt their free movement. This situation is also one of impaction, albeit from a different cause.

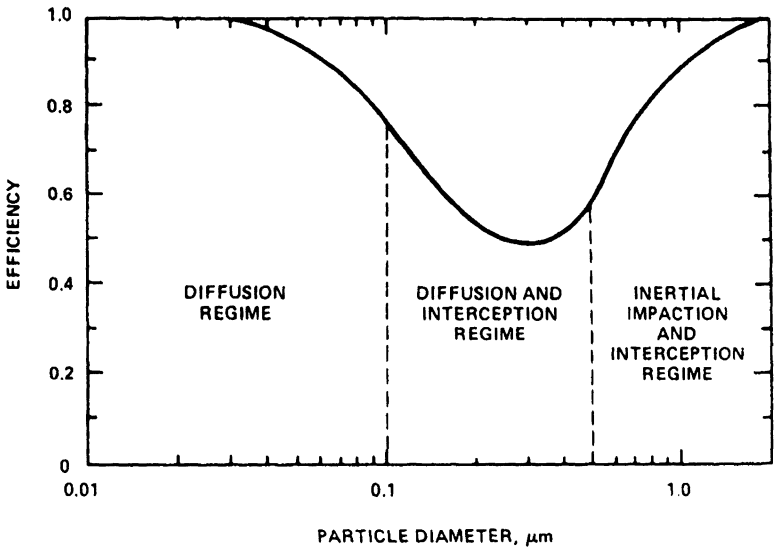

Fig. 5 Filter efficiency as a function of particle size (from [48])

Although the term in not often used, "Brownian impaction" could characterize the mechanism of such occurrences (Fig. 4).

5.1 Most Penetrating Particle Point

The response of a particle to the forces of inertia or of Brownian motion depends upon its size and mass. Heavier particles can acquire greater inertial energies from higher velocities, but are less motivated by Brownian collisions. However, their inertial energies are less affected as their size decreases. Conversely, it is the smaller particles that are most responsive to the molecular collisions that result in Brownian motion, and the least directed by inertial considerations. Also, their Brownian movement diminishes as the particle size increases. The likelihood of a particle's connecting with a membrane surface is largest when it is at an extreme in size, whether largest or smallest. Therefore, it is least likely to be vectored to an encounter with the surface of a filter when it is 'medium' in size, when it is least responsive to inertial and Brownian influences. That particle is described as "the most penetrating particle" (MPP) because in encountering a filter, particularly of the depth type fibrous construction often used in air filtrations, it is least amenable to restraints other than that of size exclusion. What the term 'most penetrating particle' implies is that on account of its size it is least likely to undergo impactive removals (Fig. 5).

The most penetrating particle is rated as being 0.3 μm in size for depth filters, like HEPA or ULPA filters utilized in clean room ceilings. DOP (dioctylphthalate) aerosol generators produce droplets that are essentially of

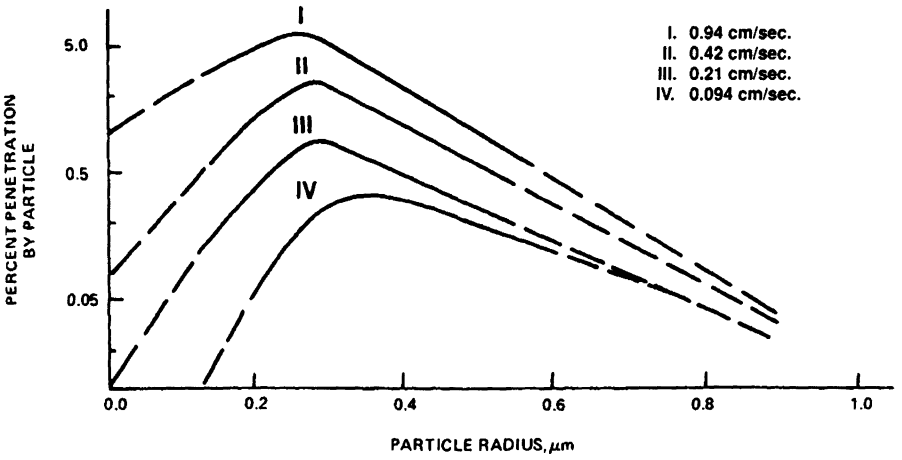

Fig. 6 Penetration of filters by DOP dioctylphthalate particles as a function of velocity and particle size (from [49])

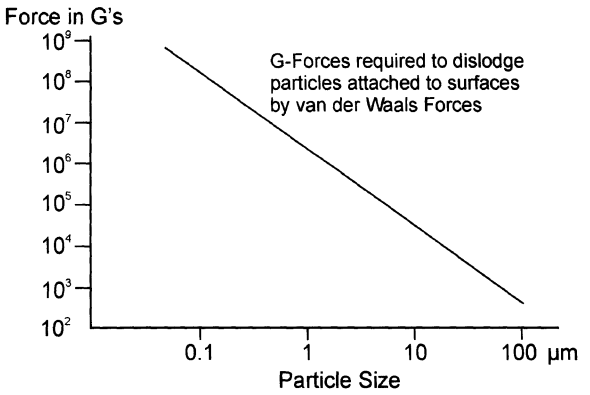

Fig. 7 Particle adhesion force. G-forces required to dislodge particles attached to surfaces by Van der Waals forces

that size. The efficiency of filters can, therefore, be measured using DOP aerosols [9].

As Fig. 6 indicates, smaller particles are more penetrating at higher velocities. This is in keeping with the reduced time available for Brownian motion to effect their capture at the higher speeds. As predicted, inertial impaction is similarly but less strongly affected.

6
Particle Binding Forces

The term "mechanism", then, can describe the several types of impaction that bring particles into intimate contact or collision with filter surfaces. However, it does not elucidate concerning the forces that manifest themselves between particle and surface once these come into close proximity, and which continue their mutual attractions, thereafter, until they are disrupted. That such forces exist and actively bind particle and surface is shown in Fig. 7 wherein are listed the forces in G strengths necessary to dislodge particles attached to surfaces. It is seen that the smaller the particle, the more difficult is its dislodgement. Depending upon the nature of the particle, the binding force may be thousands of times larger than the particle's mass.

These forces are understood to be electrical in nature. They are consequences of electrical-charges of various origins. Even the hydrophobic interactions that do not derive from obvious ion or dipole features that could initiate electrical interactions are hypothesized nevertheless to be electrical. It is the forces that stabilize the spatial relationship between particle and filter surface once their encounter has taken place. that are considered to be the "mechanisms" of particle capture in this writing. This usage of the term 'mechanism' explains why the particle/surface relationship continues and persists. It expresses the

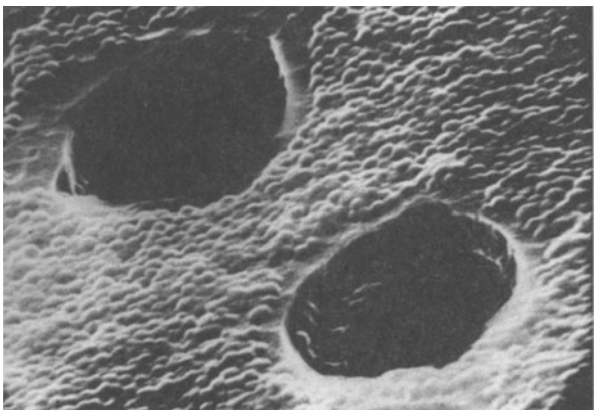

Fig. 8 S.E.M. of *Staphylococcus aureus* (0.8-μm) on polycarbonate track-etched membrane of pore size 12 μm

strength of the bonding between particle and filter. It is an explanation of these mechanisms that will be explored.

7
Adsorptions Onto Membranes

The adsorptive phenomenon is hardly a novelty in the physical or biological sciences. That polymeric membranes are capable of adsorbing various molecular entities is known. As far back as 1909, Zsigmondy pointed out that the filter surface has a certain adsorbing capacity that must be satisfied before unhindered passage of the dispersed phase through the filter occurs. Numerous investigators have since noted many specific adsorptions. Elford [10] reported that dyes could adsorptively be removed from true solutions by collodion membranes, cellulose nitrate being a most adsorptive material. The strong adsorption tendencies of the cellulose nitrate polymer had also been noted by Elford [11] in the case of viruses. The use of membrane filters to collect and isolate nucleic acids, enzymes, single-strand DNA, ribosomes, and proteinaceous materials adsorptively in scintillation counting operations is well established. Moreover, such adsorptive retentivity is utilized nowadays by introducing chromatography and membrane adsorber steps into the downstream purification process. Bovine serum, antigen/antibody, and antibody complex, and specific binding and receptor protein adsorption to cellulose nitrate has been shown to occur.

Berg et al. [12] investigated the adsorption of both inorganic and organic compounds upon polymeric such as cellulosic filter papers, nylon, polyethylene, and cellulose diacetate dialysis membranes. That water-soluble organics could adsorptively be removed from aqueous solutions by filters was observed by Chiou and Smith [13]. These investigators were thus led into a rather thor-

ough study of such adsorptions by filters. Udani [14] and Brose et al. [15] studied the adsorptive sequestration of such preservatives as benzalkonium chloride, chlorocresol, and chlorhexidine acetate from their solutions by membrane filters. The adsorptive removal of flu vaccine impurities and antibodies onto membrane filters has been reported [16]. Inorganic particulate matter can be removed filtratively through the adsorption mechanism. It is thus well documented that molecules and materials can be adsorbed onto filters, to become filtratively removed thereby.

7.1 Adsorptive Sequestration of Organisms

The adsorptive fixation of organisms to solid surfaces is reported in the literature. Pertsovskaya and Zvyagintsev [17] state that different groups of different bacteria are adsorbed by polymeric films composed of polyamides, polyacrylates, polyethylenes, or cellulose acetate. That various bacteria adsorb onto various surfaces was also disclosed by Gerson and Zajic [18]. Hjertin et al. [19] studied the adsorption of yeasts on nonionogenic, hydrophobic agarose, and the column adsorption of *S. typhimurium*. Zierdt and his associates in 1977 at the National Institutes of Health noted that both Gram-negative and Gram-positive organisms were retained on the surfaces of polycarbonate, and cellulose acetate membranes of pore sizes much larger than the bacteria. The organisms involved in the studies were *Escherichia coli* and *Staphylococcus aureus*. The adsorptive bonding of the bacteria to the polymeric filter surface withstood the mechanical and desorptive actions of washings with buffer solutions (Zierdt et al. 1977). SEM photographic evidence of 0.8 μm *S. aureus* organisms retained on the surfaces of (track-etched) polycarbonate membranes of 12 μm-rated pore size is shown in Fig. 8 [20]. The adsorptive removal of *B. diminuta* by a glass fiber prefilter in circumstances unattributable to sieve retention is shown in Fig. 9 [21].

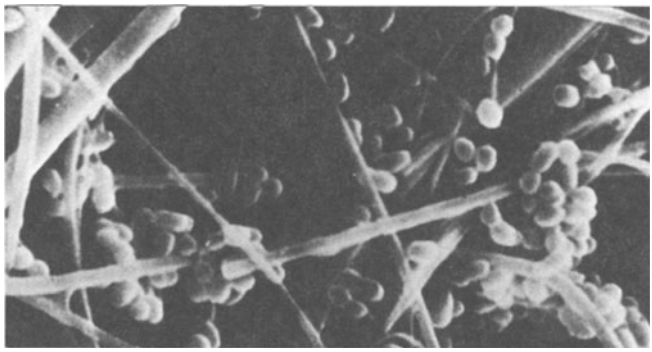

Fig. 9 SEM of fibrous depth filter (AP15) challenged with *P. diminuta* 19146. *Bar*=5 micrometers

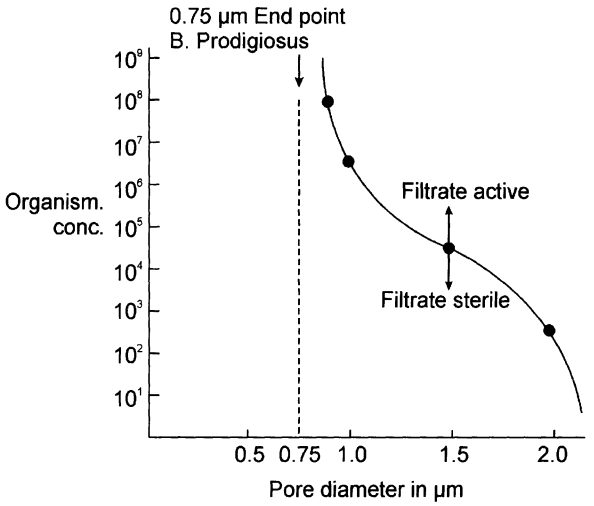

Fig. 10 Pore-diameter vs *Bacillus prodigiosus* concentration. Sterile or active effluent are a resultant of these parameters [10]

Tanny et al. [22] challenged the exclusivity of sieve retention as the mechanism of organism removal by membrane filters. It was postulated that the retention of *B. diminuta* by 0.45 µm-rated cellulose acetate membranes involved adsorptive sequestration. An initial reluctance by some to accept this view stemmed perhaps from the relative unfamiliarity with adsorption phenomena among filtration practitioners. A more substantial disinclination to abandon the straightforwardness of sieve retention, where it applies, in favor of the conditional actions of the adsorptive effect is evident even today. Hence, the drive to use membranes of lower pore size ratings, more assertive of size exclusions, where 0.2/0.22 µm-rated filters do not yield sterile effluent. Some 20 cases of such occurrences have been noted [23]. The 0.1 µm-ratings are championed as alternatives to the more conventional use of the 0.2/0.22 µm variety despite that the organisms escaping capture by the latter are not necessarily retained by the former, and also despite the penalty in flows where the advocated substitution is unnecessary [24].

7.2 Adsorptive Filtration Patterns

As stated, it was believed that sieving was the exclusive particle capture mechanism, and that sterilizing membranes were absolute in their retentions. Certain experimental findings required reconciliation. The term "absolute" posits, among other things, an outcome that is invariantly independent of the challenge level, and of filtration conditions, such as the differential pressure. Elford in 1933 reported that organism retention varied inversely with the challenge level (Fig. 10). Importantly, Wallhaeusser [25] confirmed this relationship, as

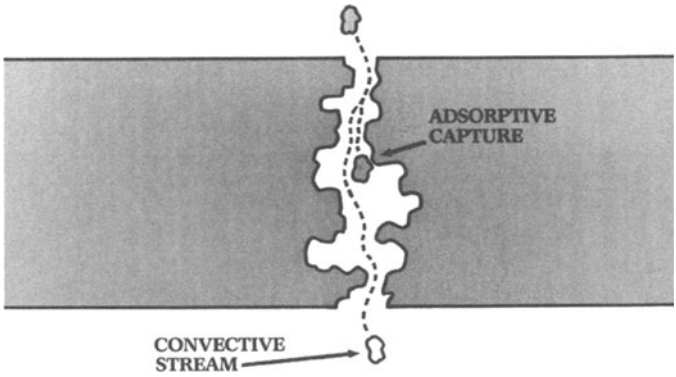

Fig. 11 Alternative paths for particles entering pores

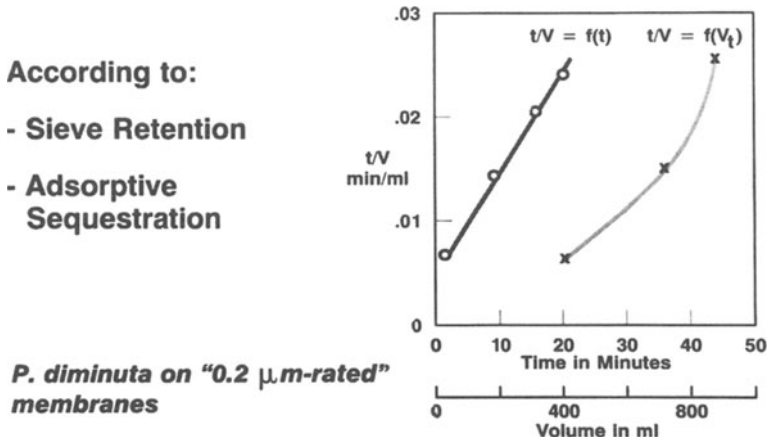

Fig. 12 Plotting of Wallhäusser data [25]

also did Zierdt et al. [20]. Rather than absoluteness, it bespoke a probability relationship. It could, however, be explain on the basis of pore size distribution, a little explored membrane feature even today. Conceivably, a challenge so large as to ensure organism encounters with the relatively few larger pores resulted in the organism passage. However, Leahy and Sullivan [21] found that organism retentions varied accord to the transmembrane pressure (Table 1). This dependence of sterility on filtration conditions relegated the belief in the absolute to a marketing ploy. Increasingly it became evident that capture mechanisms other than sieve retention were at work.

Tanny et al. [22] demonstrated that the ability of 0.45 μm-rated membranes to contain challenge densities of 2×10^7 CFU/cm^2 of filter area depended upon the pressure differential, and postulated organism retentions by adsorptive sequestration (Table 2). As mentioned above, SEM photographs of organisms

Table 1 Impact of pressure on passage (β ratio)

Filter type	Pore size (μm)	β Ratio		
		0.5 psid	5 psid	50 psid
GS	0.22	$>10^{10}$	$>10^{10}$	$>10^{10}$
HA	0.45	10^8	10^7	10^8
DA	0.65	10^4	10^4	10^3
AA	0.80	10^2	10^1	10^0

Table 2 Pressure dependent retention performance

Operating pressure (psi)	Total filtration time for 2000 mL min:s	500 mL	1000 mL	1500 mL	2000 mL	Avg. no. of org. in filtrate/mL
		(org 100/mL)				
5	189:30	0	0	0	0	0
5	75:00	4	12	7200	7200	
5	304:00	0	0	0	0	
15	108:27	0	13	19	39	10–20
15	69:30	3	2	0	7200	
15	43:58	6	15	12	11	
30	18:35	93	91	61	66	50–100
30	16:12	38	34	39	52	
30	50:02	7200	7200	7200	7200	

Cellulose triacetate 0.45 μm-rated membrane.
Challenged with *P. diminuta* suspension of 10^5 org/cm.
2000 mL over 9.6 cm^2 available surface (47 mm disc).
Total organism challenge level 2×10^7 org/cm^2.

retained by filters despite the absence of sieving conditions confirm that other capture mechanisms are operative. Leahy's SEM [50] shows adsorbed *Breundimonas diminuta* pendant from glass fibers (Fig. 9), the Zierdt et al. [20] SEM photograph illustrates *Staphylococcus aureus* adsorbed to the surfaces, both horizontal and vertical, of a polycarbonate membrane (Fig. 8). As stated, Zierdt et al. [20] found that a higher percentage of organism retentions occurred at challenges as low as 500 CFU–1000 CFU/mL rather than at 10^8–10^9 CFU/mL. At the higher densities increasing number of *Escherichia. coli* passed through the filter, although more were retained. This accords with adsorptive sequestration effects, not with sieving restraints.

Elford [10] wrote "The importance of adsorption in filtration has long been recognized". Nevertheless, the presumed certainties of "absolute" and of "sieve

retention" still retain their blandishments seven decades after. The contributions of adsorption sequestrations are not universally acknowledged. The operational advantages of sieving, where the choice exists, is its independence from the such filtration conditions as differential pressure, temperature, or viscosity. Absoluteness in the sense that employing a given filter will invariably yield sterile effluent is unattainable. The ultimate filtration results depend upon the specifics of the membrane, of the organism type, of the fluid's composition, and of their interactions, plus the choice of the filtration conditions. A greater likelihood of sieving would result from using tighter filters, especially where smaller organisms are involved, hence the suggestion that 0.1 μm-rated membranes rather than the 0.2/0.22 μm-rated be designated as the sterilizing filters. However, 0.1 μm-rated filters do not necessarily retain organisms that pass 0.2/0.22 μm-rated membranes [26]. Moreover, they will penalize fluid flows, and may unnecessarily foreshorten flow rates and throughputs, depending upon the extent of particle loading. The correct membrane to utilize is one that provides the proper degree of retention while permitting the most generous rates of flow.

7.3 Specific Experimental Findings

The dependence of adsorptive sequestration on the differential pressure is illustrated in Fig. 11. An organism entering the membrane pore can meet one of two fates, it can either emerge with the convective stream, or, because of Brownian motion, it can contact the pore wall to become adsorptively attached. The longer its residence time within the pore, the greater the probability of its pore wall encounter, and adsorption. The lower the stream velocity, as governed by the differential pressure, the longer the residence time. From this it becomes evident why a fluid's property, namely its viscosity, influences adsorptive captures. The higher the viscosity, the greater its capacity to abbreviate the mean free-path of the particle resulting from Brownian motion. Just as differential pressure is a process condition that influences a filter system's retention qualities, so too is temperature, for it is a moderator of viscosity in its inverse relationship. Particles of different sizes and shapes may be affected differently, and to different extents by the filtration's operational influences.

Sieving, the size-discriminating mechanism, is independent of the challenge level. Its only requirement is that the particles be larger than the largest pores. However, adsorptive sequestration depends upon an interaction of the several conditions that define a filtration, including the physicochemistry of the particle and of the membrane. Lacking singularity of cause introduces a probability factor into the adsorptive sequestration operation. It does depend upon the challenge density. Therefore, the larger the number of organisms that essay passage of the filter, the more are likely to emerge with the effluent.

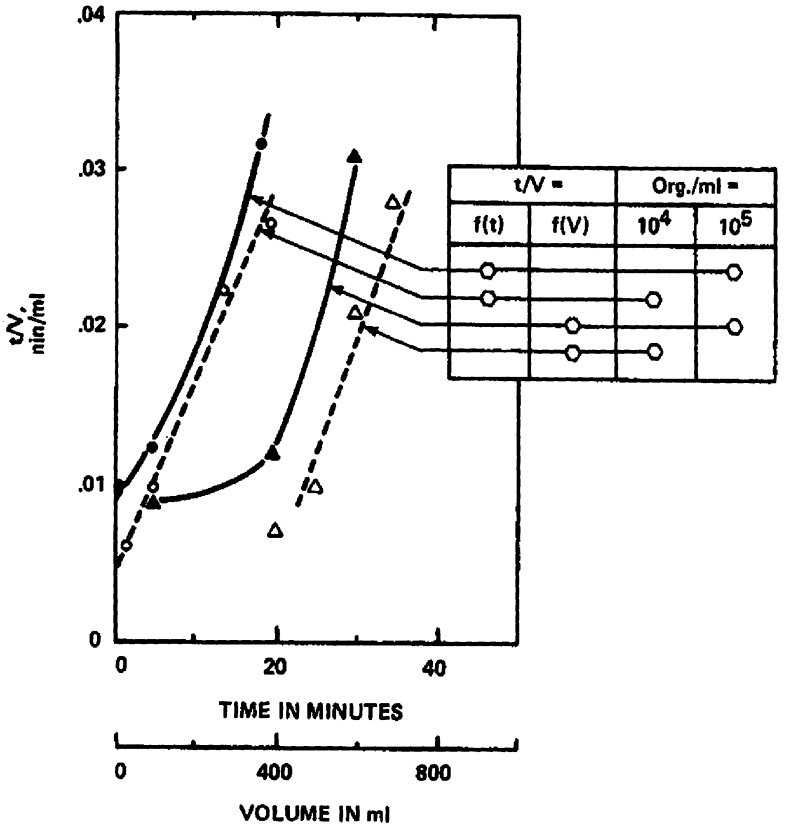

Fig. 13 Plot of Wallhäusser data showing adsorptive effects (from [22], courtesy of Journal of the Parenteral Drug Association)

7.3.1 Elford's Findings

Elford [10] confronted filters of different pore size ratings with organism challenges of different severities. He observed, as depicted in Fig. 10, that below a certain pore diameter the filter completely retain as many as 10^9 *Bacillus prodigiosus* (now called *Serratia marcescens).* Only at pore diameters larger that necessary for sieve retention is the efficiency of filtration independent of the organism challenge level. Above Elford's 'end-point' or critical pore size, adsorptive sequestration becomes a capture mechanism, reinforcing the effects of sieve retention, but subject to the influence of the particles that are present. Particle capture is now a matter of probability. The filter efficiency is greatest where the challenge level is lowest. For this reason, too, final filters ideally fill the roll of polishing filters, penultimately cleaning fluids already cleaned by prefilters.

Table 3 Dependence of organisms breakthrough on initial organism concentration

Initial *P. diminuta* concentration	10^3/ml		10^4/ml		10^5/ml	
Filtrate (ml)	0.2 µm rated	0.45 µm rated	0.2 µm rated	0.45 µm rated	0.2 µm rated	0.2 µm rated
100	0	0	0	1	1	1000
200	–	–	2	4	4	
1000	0	0	9	25	17	(10^4)
Filtration time for 1000 ml	6′52″	2′27″	2′12″	2′30″	3′15″	8′

Source: From [25].

7.3.2 Wallhaeusser's Findings

Shown in Table 3 are Wallhaeusser's findings [25] that confirm that organism retentions can reflect the inverse of their concentration. At the time of this experimental investigation the exclusivity of sieve retention and the absoluteness of membrane filtration were in vogue. Wallhaeusser's work proved the actual situation to be otherwise.

7.3.3 Leahy and Sullivan

The work of these investigators [21] provides a concise relationship among pore-size ratings, applied differential pressures, and organism challenge levels for mixed esters of cellulose membranes. As shown in Table 1, mixed esters of cellulose membranes of 0.22 µm-rating exhibit log reduction values of 10 against *B. diminuta* challenges whether at applied differential pressures of 0.5 or 50 psi (0.33 or 3.3 bar). The same type of filter in its 0.45 µm-rated manifestation shows a LRV of 8 at 0.5 psid, a LRV of 7 at 5 psid, and an LRV of 6 at 50 psid. The more open filters clearly show the influence of the applied differential pressure level on the adsorptive sequestration of the organisms. The capture mode for the 0.22 (0.2) µm-rated membrane is sieve retention, attested to by its freedom from the pressure differential influence. The 0.65 µm-rated membrane and its 0.8 µm-rated counterpart progressively show the increasing influence of the pressure differential as coupled with larger-diameter pores, as would be expected of adsorptive arrests. Interestingly, Aicholtz et al. [27] demonstrated the complete retention of *B. diminuta* ATCC-19146 by 0.22 (0.2) µm-rated membrane, even at 55 psid (3.7 bar).

Table 4 0.198-μm latex percent retention for various 0.2 μm-rated membranes as a function of pH

Filter type	Bubble point	pH 4	pH 6	pH 8	pH 9
Asymmetric polysulfone	51	100	100	100	100
Polycarbonate (track-etched)	63	100	100	100	100
Polyvinylidene difluoride	55	86.8	74.8	79.5	67.3
Cellulose esters	58	36.3	89.4	23.0	31.3
Nylon 66	45	99.9	82.1	23.7	28.4

Source: From [31].

7.3.4 Tanny et al.

Table 2 from Tanny et al. [22] demonstrate that lower differential pressures yield greater filter efficiencies, to the point where 2×10^7 CFU/cm^2 EFA are retained by a 0.45 μm-rated cellulose acetate membrane under a differential pressure of 0.5 psi (0.3 psi). Sterility did not result at higher differential pressure. This dependence of organism capture upon transmembrane pressure, among other factors, necessitates the validation of sterilizing filtrations.

7.3.5 Mathematical Modelling of Filter Blockage

The differentiation between particle retentions by sieving and by adsorptive sequestration may be sought through mathematical modeling. The mathematical treatment leading to a distinction between the two capture mechanisms assumes that bacterial retention is the controlling occurrence, the one

Table 5 Retention of various size latex particles for 0.2 μm-rated membranes

Latex particle size (μm)	0.091	0.198	0.305	0.460
Membrane type	Percent retention			
Asymmetric polysulfone	54.3	100	100	100
Charge-modified nylon	10.5	100	100	100
Polycarbonate (track-etched)	6.3	100	100	100
Polyvinylidene difluoride	23.4	19.2	84.5	100
Cellulose esters	17.7	25.1	48.6	100
Nylon 66	1.0	1.0	1.0	100

All solutions0.04% latex in 0.05% triton x-100.
Source: From [31].

leading to the eventual clogging of the filter. It distinguishes between the changes in flow that eventuate from sieving and adsorption. Sieve retention is assumed to block the surface of the membrane by an ever increasing filter cake formation. This introduces a growing hydrodynamic resistance to flow at constant pressure. The relevant factors are expressed mathematically by Ruth et al. [28]. Adsorptive sequestrations are presumed to take place within the pores, progressively reducing the total pore volume. This differs from the blockage by cake formation. The pertinent mathematics were elucidated by Hermans and Bredee [29].

The mathematical treatments embody certain assumptions. It is assumed that bacterial retention eventually leads to filter clogging. Also assumed is the non-compressibility of the filter cake, an assumption that is rather suited to more rigid particulates than bacteria. The assumption that sieve retention is a surface phenomenon can be challenged. Thin though the membrane is, its metering retention need not necessarily be a surface occurrence. For all these reasons the plotting of the flow decay data leads to non- rigorous results. They are, however, not without significant implications.

To perform this type study flow decay or flux decline studies are performed in constant-pressure filtrations. Periodic plotting is then made of the volume or throughput as a function of time. Flux decline during filtration will be a consequence of any retention mechanism, but will follow different time-volume relationships depending on the mechanism governing the filter's clogging.

7.3.5.1 For Sieve Retention

The most commonly used model [28] for bacterial filtration by sieve retention is that of a porous matrix whose pores are smaller than that of the organisms. In such a situation, the bacteria are retained on the surface of the membrane and create a filter cake that grows in thickness as the filtration progresses. Since this cake will add a resistance to the flow at constant pressure, the instantaneous rate of filtration at time t, $J_V(t)$, and the total volume of filtrate up to time t, V(t), will change in a disproportionate manner as a function of time. Assuming an incompressible cake and a constant pressure differential across the filter, the relation

$$\frac{t}{V(t)} = \frac{k}{2}[V(t) + 2V_f 1] \tag{2.1}$$

will be obeyed, where V_f is the volume of filtrate required to produce a change in total resistance equal to that of the filter, K is a 'filtration constant' that depends on pressure ΔP, viscosity η, filter area A, cake resistance R_c, and particle concentration C, in the following way:

$$K = \frac{2A^2 \Delta P}{\eta C R_c} \tag{2.2}$$

From these simple relations, it follows that a plot of t/V(t) vs V(t) should yield a straight line with a slope of K and an intercept of KV_f. Such a plot constitutes a first verification of the sieve retention, or more properly, the surface retention model.

7.3.5.2 For Adsorptive Sequestration

Adsorptive capture, whether of a particle or of a soluble or near-soluble entity from solution, involves the entry of that particle, viable or otherwise, into the pore channel. In these situations the body being adsorptively retained is smaller than the filter's pore. (Even though the pore entrance is larger than the organism, sieve statistics dictate that a substantial fraction of the bacteria will be excluded, approximately 99.9% for pores 10% larger in diameter than the particle.) The convective flow situation existing within the pores will tend to carry the particles that do enter through the membrane. However, the attractive forces, soon to be discussed, namely, the London-van der Waals interactions, the electrical double layer, and the hydrophobic attraction forces, act between the bacteria and the pore walls against the convective flow, and promote interception of the particles. In terms of the model, all these forces are combined and treated as a first-order reaction between the particle and the wall.

Each particle 'reacting' within the pore cavity, i.e., being adsorbed, reduces the total pore volume. (Intrapore sieve retention, as said, is also possible.) The equation expressing the adsorptive model of flux decrease is

$$\frac{t}{V(t)} = \frac{kt}{2} + \frac{1}{J_V(0)} \quad \text{or} \tag{2.3}$$

$$\frac{1}{J_V(t)} - \frac{1}{J_V(0)} = kt \tag{2.4}$$

where k is a filtration constant related to the internal pore area and the particle concentration.

A plot of t/V(t) vs t should yield a straight line with a slope of k/2, and such behavior constitutes a test of the model, wherein the particles are retained within the pores.

7.3.6 Analyses of the Blocking Mechanisms

The data reported by Wallhäusser, when plotted in accordance with the equations representing the two retention models, were seen to accord with that of adsorptive capture. This is indicated by the linearity of that line (Figs 12 and 13).

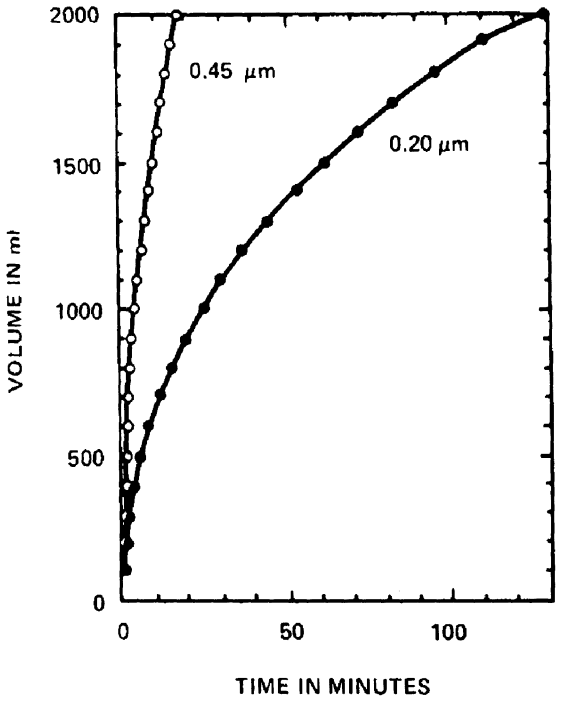

Fig. 14 Flow decline data compiled for 0.2 μm-rated and 0.45 μm-rated membranes. (from [22], courtesy of Journal of the Parenteral Drug Association)

By 0.45 μm – rated Cellulose Acetate Membranes

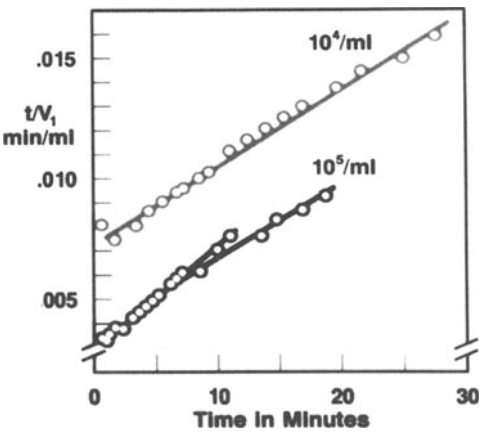

Fig. 15 *P. diminuta*, retention at two different concentrations

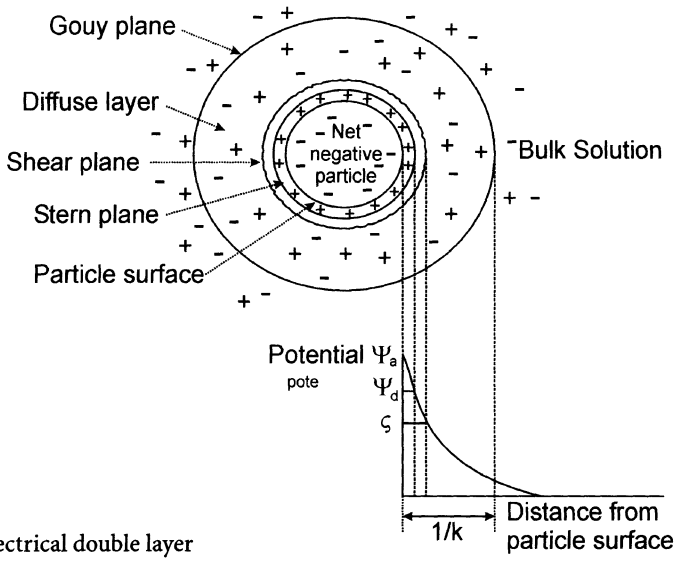

Fig. 16 Electrical double layer

Additionally, flow decay experiments were made [22] using *B. diminuta* concentrations similar to those employed by Wallhäusser. These yielded data showing that organism retention by 0.45 µm-rated cellulose triacetate membranes also depended on the adsorptive sequestration mechanism.

The flux decline exhibited in Fig. 14 plotted to the adsorption model for the 0.45-µm cellulose triacetate membrane shows an almost *straight-line* decrease, indicative of adsorptive retention, a consequence of its rather open porosity. In contrast, the flow decay of the corresponding 0.2 µm-rated filter, similarly plotted, traces a *curve*. The absence of linearity attests to the non-conformity to adsorption, sieving may be inferred. This seems reasonable, The 0.2 µm-rated filter shows a more precipitous decrease in flow, as cake formation on the more finely sized pores is more immediately manifested.

The plotting of the data of Fig. 15, in accordance with the adsorption mechanism equation (t/V)/t, yields a straight line for the bacterial feed concentration of 10^4/ml retained by the 0.45 µm-rated filter. This straight line indicates that the site of the bacterial arrest is within the pore path. It is, therefore, assumed to signal adsorptive sequestration. The line in Fig. 15 derived for the 0.45 µm-rated membrane using a bacterial feed challenge of 10^5/ml shows an initial straight line followed by the onset of a curve. This shape shows adsorptive retention, actually intrapore retention, leading to pore clogging (more rapidly realized as a result of the higher bacterial concentration), followed by sieve retention of the bacteria subsequently filtered out by the now clogged, and, hence, smaller-diameter pores.

7.4 Latex Bead Retentions

Polystyrene beads, rigid because they are crosslinked, and spherical because they are prepared by stirred emulsion polymerization, are available in rather precise, narrow size ranges. They have been usefully employed in retention studies because, like organisms they can be sieve retained, and are also susceptible to adsorptive influences. However, unlike organisms, surfactant present in their suspensions prevents their adsorption. They have been utilized, therefore, to differentiate between these two types of mechanisms.

Pall et al. [30] found that the efficiency of latex bead retention by membranes was affected by pH, more so at lower pHs. This is in line with the effect of high ionic strengths on the reduction of the zeta potentials. Pall et al. [30] also

Table 6 Retention as a function of pH

Asymmetric Polysulfone	51	100	100	100	100
Polycarbonate (track-etched)	63	100	100	100	100
Polyvinylidene fluoride	55	86.8	74.8	79.5	67.3
Cellulose esters	58	36.3	89.4	23.0	31.3
Nylon 66	45	99.9	82	23.7	28.4

Percent Retention of 0.198-μm Latex Particles for Various 0.2 μm-rated Membranes as a Function of pH [31].

Table 7 Retentions of latex spheres as influenced by surfactant

Latex particle size (μm) Membrane type	0.091	0.198	0.305	0.460
Percent retention All solutions 0.04% latex in 0.05% triton x-100				
Asymmetric polysulfone	54.3	100	100	100
Charge-modified nylon	10.5	100	100	100
Polycarbonate (track-etched)	6.3	100	100	100
Polyvinylidene fluoride	23.4	19.2	84.5	100
Cellulose esters	17.7	25.1	48.6	100
Nylon 66	1.0	1.0	1.0	100

Retention of Various Size Latex Particles for 0.2 μm-rated Membranes [31].

Table 8 Comparative retentions by various 0.2 μm-rated membranes

Filter type	In water	In 0.05% triton x-100
Polycarbonate	100.0	100.0
Asymmetric polysulfone	100.0	100.0
Polyvinylidene fluoride	74.8	19.2
Nylon 66	82.1	1.0
Cellulose esters	89.4	25.1

Percent retention of 0.198 μm-spheres by various 0.2 μm-rated membrane [33].

reported that the presence of surfactant diminished the latex bead retention, and that different surfactants did so to different extents. Confirmation of these findings were made by Wrasidlo et al. [31], in respect to both pH, and surfactant (Tables 4, 6 and 5, 7). Emory et al. [32] demonstrated that not all surfactants have the same effect with a given membrane.

Tolliver and Schroeder [33] compared the retention of 0.198-μm latex beads suspended in water, with those suspended in an aqueous solution of 0.05% Triton X-100. The comparisons were made using various commercially available 0.2 μm-rated membranes. Table 8 shows differences in results between the two vehicles. The dissimilarity is greatest for the nylon 66 membrane. The polyamide polymers are known to exhibit strong adsorptive interactions with non-specific proteins.

The action of surfactant in differentiating between the extents of latex particle retentions in otherwise similar situations is taken as a confirmation of the adsorptive sequestration mechanism.

7.5 Electrical Mediated Adsorptions

The adsorptive forces that in their interaction between the particle and the filter adhere the surface of the one to the surface of the other are electrical in nature. They may be operative as well in the sieving and impaction mechanisms once the mechanical manifestations of these retention mechanisms have brought the particle and filter together. It is generally comprehended that in electrical charge interactions opposite charges attract one another, while like charges mutually repel. Curiously, electrical attractive and repulsive forces are simultaneously present on both surfaces, as will be explained. The attractive charges of opposite sign situated on the one surface tend to attract those of the other, but being weaker than the repulsive forces do not prevail. The repulses arise from the like-charges sited on the two surfaces. Their powers of rejection are the stronger. Therefore, they can operate over larger distances. When, however, the distance separating the two surfaces are reduced, or when the repulsive force is sufficiently attenuated, the weaker attractive influences become

assertive, and their electrical interactions do bond the particle and filter. This is the nature of charge mediated adsorptive sequestration. Whether in the adsorption of a particle by a filter, or in the agglomeration of particles in colloidal destabilizations, the phenomenon is the same, namely, the coming together of two surfaces in an electrical bond. The terminology used in describing the situation differs, however, depending on the application.

7.5.1 Electrons and Electrical Charges

By convention, the electron is said to bear a negative charge. Therefore, atoms, or groups of atoms, such as radicals or molecules, that possess more electrons than they do in their neutral state, will be labeled as being negatively charged. If they contain fewer electrons than in their neutral state, they are designated as being positive or positively charged. Atomic and molecular entities whether solid, liquid, or gaseous react to one another in response to their plus or minus electrical states. They do so by acquiring or donating electrons in exchanges with other atoms. As a result of the electron sharing, a molecule is formed.

In an oversimplified view, an atom consist of a nucleus surrounded by shells or rings of electrons. Because it contains positrons, positive charged subatomic particles, the nucleus is plus (+) charged electrically. Electrons, each negatively (–) charged revolve around the nucleus. Each atom in its normal state possesses just enough electrons (negative charge) to neutralize the positive charges of the nucleus. Hence, atoms in their normal state are uncharged electrically. The electrons occupy a number of concentric shells or rings that surround the atom's nucleus. An element's atomic number is also the number of its planetary electrons. Each shell is limited to an occupancy by an exact number of electrons. An atom in forming a molecule with another atom will fill or empty its incomplete shell by accepting or donating electrons to another atom that is under the same compulsion. (The noble gases are inert because their outermost shells are completely filled. Therefore, they have no need to acquire or lose electrons by combining with other atoms.) The shared electrons form the chemical bond that ties the two atoms into a molecule.

7.5.2 Ionic Charges

Consider the union of a sodium atom and a chlorine atom. In the electron sharing just described, the electrically neutral sodium ion, now bereft of an electron (–), becomes changed into a positive charged sodium cation. The neutral chlorine atom, having acquired an electron (negative), is now negatively charged. It is now a chloride anion. They interact on the basis of their opposite charges to create a molecule that is a salt, sodium chloride. The molecular combination consists of an aggregate of positively charged sodium ions in lattice form jux-

taposed to an assembly of negatively charged chloride ions in lattice form, connected by the attractions of their opposite charges. The transfer of the electron that creates the ionic bond is total and complete. When the two oppositely charged ion lattices are separated, as by the salt being brought into aqueous solution, the electron whose transfer created the negative charge stays completely with the chloride ion. It is not shared whatsoever with the sodium ion. There are, however, other types of bonds that are characterized by a sharing of the bonding electron. The covalent bond consisting of two shared electron is a case in point [34].

7.5.3 Partial Charges

The cation/anion interaction is an example of the attractive forces that operate between the net or full opposing electrical charges of ions. It is possible, however, for two atoms to come together to share the two electrons of a covalent bond that connects them. Because of the sharing, the atoms have no individual primary charges as ions do. However, the electrons may be shared unequally. The consequence of this is a *partial* electrical charge on each of the two atoms, proportionate to the sharing of the bonding electrons. The atom next to which the electrons are closer is partially negatively charged, the other atom, somewhat deprived in the sharing, is partially positively charged. (The symbol for the partial charge is the lower case Greek letter delta, δ.) The partial charges are also weaker in their strengths. They can, however, undergo bonding interactions, albeit weaker, with other partially charged atoms of appropriate sign.

7.5.4 The Dipole Structure

Molecules may be electrically neutral overall but may be complex enough to contain sites that bear partial positive and negative charges. Neutral molecules with such unsymmetrically arranged electrical charges are called dipoles. The dipole, then, is constructed of one positive and one negatively charged particle, but the whole system acts as a single unit. There is a finite distance between the centers of positive and negative charge. The dipole moment is a measure of the polarity of the molecule. It is defined as the distance between the charges, multiplied by the magnitude of one of the charges.

The partial charges leading to interactions can arise from several sources. They are of different strengths depending upon the power of the electron-dislocation force. The unequal sharing of electrons may be induced in a neutral molecule by the proximity of a dipolar molecule. The molecule with the induced dipole will by its electronic imbalance be able to exercise its partial charge influences on other neutral molecules, etc. An even greater electron-pair dislocation, a greater partial charge, would be induced in an heretofore elec-

trically neutral neighbor by the full electrical charge of an ion. At the other extreme, as will be discussed, van der Waals forces are hypothesized to be induced dipoles, induced by induced dipoles. The VDWs are weak but significant electrical forces that are considered responsible for the charge interactions between molecular structures that possess no obvious polar features, e.g., hydrocarbons.

7.5.5 The Hydrogen Bond

A common interaction is one between two dipolar molecules, whether of structural origin, or induced. The hydrogen bond is an example. It arises from a dipole/dipole interaction. It manifests itself chiefly between the hydrogen atom and the atoms of the most electronegative elements, namely, fluorine, oxygen, and nitrogen in decreasing order. The bond is unique to the hydrogen atom whose small size enables a close approach to its bonding partner. This empowers the attraction forces that operate over a short-range.

The water molecule, H_2O, consists of two hydrogen atoms each bonded to the same oxygen atom. Because of its strong electronegativity, the nucleus of the oxygen atom pulls the bonding electrons more strongly to itself. The bonding is not disrupted, but the bonding elements become partially charged. The unequal sharing of the electrons makes the electron-richer oxygen partially negative, and the proportionately deprived hydrogen atoms partially positive.

The water molecule is tetrahedral in shape. The molecules of water in its solid (ice) state exist as tetrahedral hydrogen bonded structures. Much of this ordered form persists even in the mobile liquid. Each of the tetrahedral corners holds either a pair of electrons or an hydrogen atom. Each of the partly positive hydrogen atoms of one water molecule can form a hydrogen bond with a partly negative oxygen atom of each of two different water molecule. Thus, water molecules can hydrogen bond with each of four other water molecules. This process, repeated throughout the water volume, in effect creates an interconnected molecular network. Hydrogen bonding is responsible for many of the singular properties of water, such as its high boiling point, it high surface tension, its wetting and solution properties, its density/temperature relationship, etc.

The hydrogen bond is a weak bond, but it is an extremely important bond. It has a prominence in protein chemistry, for instance, and it plays a major role in maintaining the fine structural integrity of many biological macromolecules.

7.5.6 Solvating Effects

The solvating effect of charge interactions involving hydrogen bonds marks the hydration of ions brought into aqueous solution. This helps explain why high

ionic strengths attenuate the zeta potential. When placed in an electric field, dipoles, such as water molecules, will tend to orient themselves in head-to-tail chain-like fashion alternating their positive and negative partial-charge interactions. Such an array of dipoles intervening between the two charge-laden sites serves as a chain of subsidiary or partial-charges that attenuates the interaction, whether attractive or repulsive, between the charge sites. This will subsequently be detailed.

An example of a chain of electrical conduits interposing between electrical charges to cancel their interaction, in this case attractive, is given by the solubilization of an electrolytic salt by water. The water molecules' wetting and solvating capability follows: The electrolytic salt molecules considered above are ionic unions that exist as molecular entities as long as the electrical ionic bond created by the attractions of opposite charges persists. The ionic bond can, however, be weakened and disrupted by the insinuation of multi-electrical, albeit partial charges between the sodium and chlorine moieties, thus attenuating their strong mutual attraction. The addition of water to an electrolyte, such as salt, affects this ion/dipole interaction. Water, because of the potent electronegativity of its oxygen atom, is a dipolar molecule that has a high dielectric charge, its oxygen atom has a partial, hence weak, negative charge, and its two hydrogen atoms have each a partial positive charge as explained.

The water molecules, by way of their partial charges, also respond to the electrical charge forces of the ions, causing them to become hydrated. That is, the ions acquire skirts of water molecules attached by the electrical attractions of opposite charges. These new electrical alliances compete with and dilute and weaken the power of the primary ionic bonds forming the salt molecule. Heretofore, the electrical needs of the ionic charges had been exclusively satisfied by the counterions, but these interactions are now weakened by the competing dipolar influences of the many water molecules. The water's effect is to separate the ionic lattices by displacing the ionic bonds with its own dipolar allegiances. This brings the salt into solution. That is to say, each ion is now individual, released from its ionic lattice, and separated from the others by an envelope of water molecules that are attached to one another within their hydrogen bonded structures.

Unlike with water, sodium chloride will not be dissolved by liquid hydrocarbons because, not being polar, their molecules are not characterized by electrical charges Therefore, they do not detract from the ion/ion charge that defines the sodium chloride molecule.

7.5.7 van der Waals Forces

Discussed above were ion/ion, ion/dipole, and dipole/dipole charge interactions. There are also ion/induced dipoles, and dipole/induced dipoles. In all these cases some molecular polar entity, usually an oxygenated group, can be recognized as being the originating cause of the electron imbalance. In the absence of overt

causes it was concluded that non-electrical mechanisms of particle capture must also exist. Their influence, it was rationalized, arose from non-polar, hence hydrophobic origins. This conceivable explanation of hydrophobic adsorption will be discussed below.

It was theorized, however, that a similar imbalance of electrons could come about in molecules where no polar influence is evident. These are ascribed to induced dipole/induced dipole electrostatic forces. They give rise to a weak but very important attraction mechanism. This was deduced from experimental investigations of departures from the Perfect Gas Law. The noble gases are inert because their outer electron orbitals are completely filled. Therefore, they do not form covalent bonds. Neither are they in need of electron donating or borrowing. It was found, nevertheless, that they exhibit electronic attractions. These are named *van der Waals* forces (VDW) for their early investigator. VDW forces are not widely understood, and are difficult to explain because they require a quantum-mechanical treatment to be comprehended.

As a consequence of their obscurity to all but specialists, but with an appreciation of their reality, there are widespread erroneous references in the technical literature to the VDW forces that misidentify their genesis. All attractive forces of whatever partial charge origins are often referred to as VDW forces, or as "secondary valence" effects. Albeit incorrect, the end result suffices in that there is recognition that unsatisfied electronic expressions are at work. The VDW forces are universally operative, but are seen to be of prime importance among non-polar molecules, such as hydrocarbons, whose structures would seem not to hold possibilities for inducing dipole formation. The question is: What, if polar influences are absent, induces the dipole that next induces a dipole?

The VDW forces are fundamentally different from the classical models of the electrical interactions just considered. The VDW attractions are ascribed to *transient dipoles* that result from an *"instantaneous non-zero dipole moment"* that induces a momentary dipole in a neighboring molecule [34]. The electrons are in constant circulation around their nucleus. Therefore, the charge distribution, over a time period, is not in one fixed position. It is described in terms of a cloud to emphasize its ubiquitous positioning. However, although constantly changing, the molecule does at any instance have an immediate dipole moment. It is this that induces dipoles in adjacent molecules. The van der Waals forces operate as attractive influences, albeit weak and effective over only short distances. However, in their multitude they are of substantial import. A molecule is not limited to a single fluctuating dipole, but may have many transient dipoles, each capable of inducing a dipole in another molecule. The VDW, therefore, has a cumulative effect [34].

It may be of interest to know that in addition to being responsible for the adsorption of organisms and other particles to filter surfaces, and its similar action in destabilizing colloids, VDW forces govern the condensation of gases into liquids by their induced dipole/induced dipole interaction. For instance,

in the gaseous state, water molecules, like all vapors, are widely separated and remain so. When, however, they are squeezed together by pump action, the attractive forces acting among them become operative over the compressed intermolecular distances, and overcome the ever-present repulsive forces to create liquid water. (This is the operative principle of the vapor compression still.) This accords with the closer molecular propinquity of the liquid state and with its higher density. The dipole/dipole relationship resulting from the thermal condensation of steam has the same effect, but differs in the origin of the dipoles, the VDW being induced. VDW forces are involved in lipid-lipid interactions, in interactions among hydrocarbons, and even, as stated, among the noble gases.

The van der Waals forces are considered the electrical motivators of interactions involved where induced dipoles are induced by induced dipoles rather than by structural polar features.

7.6 Hydrophobic Adsorptions

Adamson [35] writes "The term 'hydrophobic bonding' is appropriate to conditions wherein there is an enhanced attraction between two surfaces (as of a particle and filter) exposed to a liquid, if the liquid-particle interaction is weaker than the liquid-liquid interaction." The term "hydrophobic" implies an antipathy for water. This derives from an absence of polar groups capable of hydrogen bonding to water. It is demonstrated by an immiscibility with water. Molecular structures, such as ester, and carboxylic groups, that contain oxygen atoms give rise to dipoles on account of the strong electronegativity of their oxygen atoms. The dipole/dipole and other electrical interactions account for the attractions between solid surface sites that result in adsorptive sequestrations, and also colloidal agglomerations. Such polar features are, however, absent from hydrocarbon molecules that, nevertheless, do interact in the manner that suggests adsorptive influences. The apparent contradiction requires clarification. It will be remembered that the van der Waals forces that exercise attracting interactions among hydrocarbons bereft of oxygen or other polarizing features were hypothesized as being due to induced-dipoles that resulted in attractions, albeit weak ones. This would explain the hydrocarbon interaction as also being charge related.

Another hypothesis that does not rely upon charge interactions between hydrocarbon molecules is possible. Hydrocarbon molecules, here taken as the archetypical non-polar substances, do connect with other hydrophobic molecules. This is in agreement with alchemists' observations, namely, "like prefers like". The implication is that the hydrocarbon molecules' van der Waals *attraction of the one for the other* is an important factor in hydrophobic adsorptions. However, Tanford [36] expostulates that it is the water molecules' alliances among themselves that rejects interactions with the hydrocarbon molecules, causing a concentration of the latter: "The free energy is representative of the at-

traction between the substances involved. The free energy of attraction between water and hexane or octane obtained at 25 °C is about –40 erg/cm^2 of contact area, the free energy of attraction of the hydrocarbons for themselves at the same temperature is also about –40 erg/cm^2, but the free energy of attraction of water for itself is –144 erg/cm^2. It is clearly the latter alone that leads to a thermodynamic preference for elimination of hydrocarbon-water contacts, the attraction of the hydrocarbon for itself is essentially the same as its attraction for water."

In the above discussion on the hydrogen bond it was stated "The water molecule is tetrahedral in shape. Each of its corners holds either a pair of electrons or an hydrogen atom. Each of the partly positive hydrogen atoms of one water molecule can form a hydrogen bond with a partly negative oxygen atom of each of two different water molecule, etc. This process, repeated throughout the water volume, in effect creates an interconnected gel. Thus, the molecules of water in its solid state (ice) exist as tetrahedral hydrogen bonded structures. Much of this ordered form persists even in the mobile liquid". The hydrocarbon molecules with little affinity for the water molecules intrude among these spatially, tetrahedrally-ordered arrangements. It is the network formed by the water molecules among themselves that in excluding the hydrocarbon molecules causes their segregation. In their coming together, the hydrocarbon molecules effect a reduction in the surface free energy, the driving force of hydrophobic adsorptions.

It is likely that micellar groupings are involved under the influence of area-minimizing forces.

It will be recalled, however, that comprehension of the van der Waals phenomenon was limited for many by the mysteries of quantum mechanics. In a similar vein it may be that the intricacies of thermodynamics may for some, including the authors, detract from an understanding of hydrophobic adsorptions. The different hypotheses may be only one made seemingly different by technical semantics.

In capsule form, the tendency is for hydrophobic entitles to consolidate into larger aggregates within an aqueous medium that is unsympathetic to their type of non-polar bonding. Hydrophobic molecules or particles in aqueous media tend to deposit onto hydrophobic areas of solid surfaces they encounter. A diminution of the surface free energy of the system is a result, caused by the joining of smaller, scattered hydrophobic areas or volumes into one large mass.

7.7
Attractive and Repulsive Forces

The opposing forces, both attractive and repulsive, are simultaneously latent in the affected molecules. Coulomb's law states that the electric force of interaction between two charged particles is directly proportional to the product of their charge and inversely to the square of the distance separating them. The forces of repulsion are the coulombic interactions between like-charged entities, and

the more general repulsions between any two atoms that are brought together close enough for their like-charged electron clouds to overlap. This latter repulsion is very short range. In effect it defines the atomic or molecular diameters. The attractive forces are also short-range and electrostatic. They are the various attractive dipole interactions, including the van der Waals forces just discussed. However, the repulsive strength manifests itself over greater distances than that of attraction. Until, therefore, the distances separating the two surfaces are sufficiently reduced for the attractive forces to assert themselves, the adsorption of particles to filters, or the settling out of colloids, both solid-surface to solid-surface adsorptions, will not occur. The extent of the distance necessary to thwart the attractive forces depends directly upon the strength of the repulsive forces. As will be seen, these strengths can be diminished, and the distances keeping the weaker attractive forces from being effective can be reduced by dipoles. Indeed, the dielectric constant of a material is a measure of how much its presence will reduce the magnitude of an electric field.

7.7.1 Attenuation of Repulsive Forces

It is possible to have two separated electrodes, one charged positively, the other negatively. Depending upon the magnitude of the charges, the distance of separation may be too large for the electrical charges to bridge, especially within

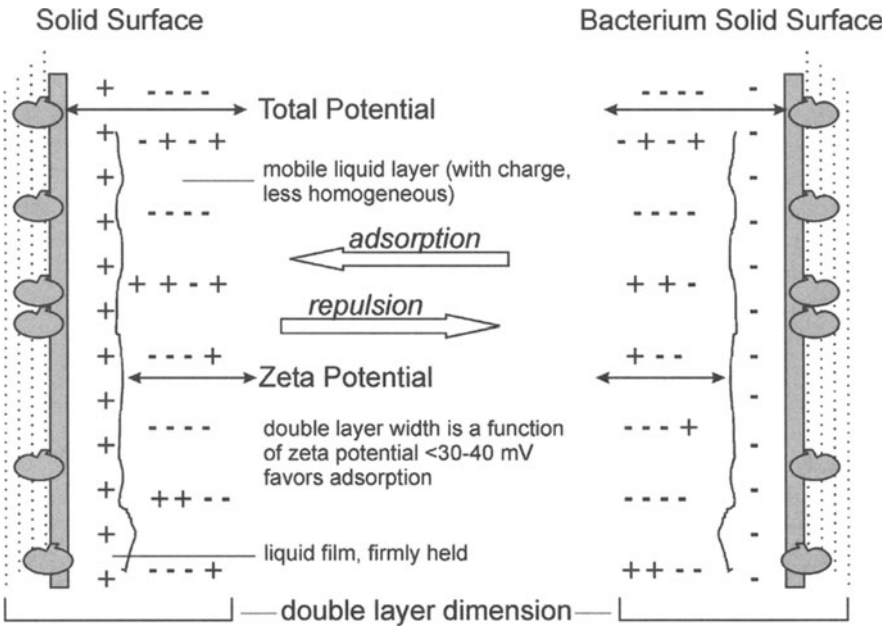

Fig. 17 Electric double layer

a vacuum. Indeed, this arrangement describes a capacitor. If, however, the space intervening between the electrodes were occupied by a medium that can carry an electrical current, an electrical discharge will take place over the closed circuit. The flow of electrons will have been enabled by the electrical conductivity of the intervening medium.

By contrast, dielectric materials cannot carry current, but they have the ability to reduce the strengths of the repulsive electrical interactions, and by so doing permit the attractive forces to prevail. This is due to the structure of the dipoles. As already stated, dipoles can set up an electric field and thereby influence other charged particles. They can also be influenced by external electric fields. Importantly, despite being electrically neutral, a particle's motion can be influenced if it has a dipole. Thus, an electrical field tends to align dipoles from their random positions. The stronger the field, the greater the alignment.

Dielectrics are electrically neutral materials that do not readily transmit charges. However, they consist of many dipoles, and their multiple dipoles do undergo alignment by electrical fields. The result is an enhanced array of aligned dipoles. The orientation of an aligned molecular dipole is always such that its electric field *opposes* the field orienting it. Therefore, the initial field strength is diminished by the orientation of dipoles and by the induction of temporary dipoles in the dielectric medium.

It is in this way that a medium consisting of multiple dipoles, namely, a dielectric interposed between charges, can reduce the repulsive strength to the point where the weaker oppositely charged attractive interactions, simultaneously present, come to predominate. It is this reduction of the zeta potential that governs the electric double layer arrangement that is central to particle/filter adsorptions.

7.8 Electrical Double Layer

The idea of the electric double layer was developed by four individuals, Derajaguin, Landau, Vervey, and Overbeek, and is, therefore, known as the DLVO theory. It is widely accepted to be correct in its teachings. The double layer hypothesis was early on developed to explain colloid destabilization which, like adsorption, involves the adsorptive bonding of solid surfaces to one another. It may be instructive to consider, therefore, the nature of colloids, and to comprehend their agglomeration into larger particles as practiced, for instance, in water clarification. As said, the same mechanisms are seen to be operative in the adsorption phenomena common to the filtrative retention of particles.

7.8.1 Colloidal Destabilization

Colloids consist of particles from 0.001 to 1 μm (10^{-7} to 10^{-4} cm) in size, too small to be visible under an optical microscope. In its simplest form the colloidal

state is a suspension of discrete particles that resist settling out for several reasons. Chiefly, each particle bears similar electrical surface charges that repel one another. The colloidal particle, of whatever composition, has a large surface area. This encourages the adsorption of ions and the concomitant acquisition of electrical charges. Colloidal charges can also result from the ionization of molecules on the surface of the particle, or from the dissolution of ions from the solid into the suspending liquid. Since like-charges repel, and since all the particles constituting a colloid bear the same charge, the discrete particles repel one another and do not agglomerate to form a sediment. The destabilization of colloids, the 'neutralization' of their charges, leads to the agglomerative interaction of the particles' surfaces with one another. Actually, the repelling charges are not neutralized in the sense of being eliminated. They are reduced by dipole alignment to the point where the attractive, electrical dipoles interactions avail. This is done through the agency of the dielectric orientation discussed above.

The joining of one colloidal particle to another involves the same forces of attraction that regulate the adsorption of molecules or organisms to filter surfaces. A most important consideration is the distance separating the molecules or particles being adsorbed and the adsorbing sites. It is over this distance that the attractive forces, whether they are of hydrophobic or more overt charge-related origins, must operate. Given their short-range effectiveness, the distance involved is very important. The process is one of overcoming opposing repulsive forces that are effective over greater distances.

It is believed that an electrical double layer surrounding the core particle interposes the distance-increasing charge dimensions that repulse. Solutions of high ionic strengths, including those of hydrogen (hydronium) ions as quantitated by pH, serve to shrink the double layer. This reduces the repelling charges and shortens the distance over which they are effective. This enables the attractive influences to result in adsorptive sequestrations, and in colloid destabilizations. The customary practice in water treatments is to utilize coagulation with alum, a double salt of aluminum and ammonium sulfates, to settle colloids. Alum adds the trivalent Al^{3+} cation to the solution, markedly increasing its ionic strength.

7.8.2 Double Layer Details

Consider a filter surface and that of a particle in contact with an aqueous electrolyte solution, each having a series of electrical charges, however acquired, firmly fixed to it. Each charged surface will attract a layer of oppositely charged ions from within the solution. These counterions, in hydrated form, will be very tightly bound to the charges on the filter surface. The cloud of remaining counterions in the solution will tend to form successive charge-alternating layers throughout the liquid, but with increasingly less charge-homogeneity as the ionic attractions attenuate with distance. At least three distinct layers form: the filter surface with its charges, the strongly bound ions of opposite charge within

the solution, and the less tightly held successive layers of diffuse ions within the solution.

The first part of the electrical double layer consists of the hydrated ions within the solution that are permanently bound to the charged boundary surface of the (filter) solid. It is also called the Stern layer or Helmholtz layer. The diffuse second layer, the less strongly bound layer of less homogeneous ions, is called the Gouy or Gouy-Chapman layer (Figs. 14 and 15). The impress of an electric current upon such an arrangement causes the movement towards the electrodes of the hydrated ions within the Gouy layer. A line of shear will form between the fixed counterions, and those present and migrating in the liquid bulk. An electrical potential can be measured from the permanently fixed charges and the interior of the liquid, i.e. from the solid (filter) surface to the plane of shear. It is usually characterized as the zeta potential, but is also referred to as the Stern potential.

Zeta potential is a measure of the charge density on the filter surface that is not satisfied by the permanently bound ions within the liquid. The charges required for electrical balance must come from the mobile, less tightly bound ions within the liquid phase. The higher the potential, the greater must be the distance over which its force extends into the interior of the liquid in order to involve, at a given concentration, enough ions to satisfy it. Therefore, the greater the zeta potential, the more extensive the double layer, or, as it is called, the Debye length. It measures the counterion 'cloud' within the liquid over which the zeta potential extends. The thickness of the double layer relative to the particle diameter is very small. In a 10^3 mol/l monovalent ion solution the thickness is approximately 100 Å (0.1 µm) [37]. Nevertheless, reductions in such dimensions promote adsorptions to filter surfaces, and to colloid destabilizations.

In summary, the net effect of the double layer is to inhibit the close approach of particle surfaces to one another, or to that of a filter. This preserves the stability of colloidal suspensions, and countervails particle adsorption to filters. However, as stated, the potential can be reduced by the addition of ions. Supplying the ions to a higher concentration reduces or eliminates the need for larger volumes of diluter solution as expressed by Debye lengths. The greater the ionic concentration, the smaller the Debye length. This serves to diminish the double layer distance. Moderating the long range repulsive forces enables the short range attractive van der Waals forces to dominate. The result is the adsorptive sequestration of particles to filter surfaces. The effect is manifest by solutions of high ionic strengths or of high osmolarities.

7.8.3 The Zeta Potential

The measure of the zeta potential represented by ζ is the difference in the electrokinetic potential that exists between the fixed boundary layer of the charges within the liquid and the mobile charges within the bulk of the solution. If the zeta potential of a particle in suspension in an electrolyte solution is of oppo-

site sign to the zeta potential of the pore walls of a filter similarly situated, the particles will become adsorbed to the wall. As stated, the core particle with its tightly adhering charges can be separated from the more diffusely held layers of ions by their movement toward an electrode in response to an imposed electric current. The separation of the colloid with its fixed surface-charges from its mobile charged layers reflects the potential at their plane of shear. To summarize, its magnitude is inversely related to the ease of destabilizing colloid particles, and/or encouraging adsorptions. Both are exercises that involve double layer shrinkage and charge neutralization. The lower the zeta potential, the smaller (thinner) the distance between the permanent charges of the first surface, and the opposite fixed charges of the second surface. Adsorption follows the interaction of the two (opposite) charges.

Jaisinghandi and Verdegan [38] provide a discussion of how to measure the zeta potential of a filter medium. However, the measurements of zeta potential, even by the zeta meters devised for that purpose, are time-consuming to a degree that reduces their practicality in assaying, for example, the quantity of alum needed for the clarification of a water to rid it, by agglomeration, of its colloidal content.

7.8.4 Streaming Current Potential

In the measurement of zeta potential, the core particle with its attached charges is caused to separate from its charge envelope by being moved electrically through the suspending (nonflowing) water toward an electrode. The same separation of the electrical double layers can be obtained by anchoring the particles, as by adsorption to surfaces, and causing the liquid to flow past them. This is called the streaming current potential technique. It is easier to perform than zeta potential measurements and also measures the voltage necessary to separate the double layers and hence helps determine the ease of colloid destabilization, and the likelihood of particle adsorption.

The streaming current potential is useful because it provides a measurement of the net surface charge of the colloidal particles. This correlates with how much coagulant must be added to the colloidal suspension to cause it to agglomerate. The coagulant, such as alum, supplies multivalent cations, Al^{+3}, to neutralize the negative charges of the first electrical layer. This charge neutralization destabilizes the colloidal suspension, *reducing the double layer dimension* and thus permitting the particles to agglomerate and to become large enough to be responsive to gravitational settling.

7.8.5 Adsorptive Particle Captures

An intriguing view of the effects of the zeta potential, the measure of the electrokinetic effect, vis a vis colloids is given by Pall et al. [30]. These investiga-

tors point out that colloidal suspensions are stabilized when their particles are endowed with net surface charges of similar sign in the magnitude of 30–40 mV or more. The mutually repulsive forces then suffice to repel the particles from one another. The double layer distance is then large enough to frustrate the shorter range attractive van der Waals forces. Therefore, no flocculation occurs, and the colloidal dispersion is stabilized. Below about 30 mV the double layer extent shortens, and the zeta potentials begin to reflect the growing involvement of the attractive secondary valence forces. Over and at the zero charge level attraction dominates. Flocculation occurs, adsorptions take place.

In the view of Pall et al. the same considerations hold when a suspension of particles in the approximate range of 0.1–0.6 µm is passed through the somewhat larger pores of a filter medium. If the zeta potentials of the pore walls and

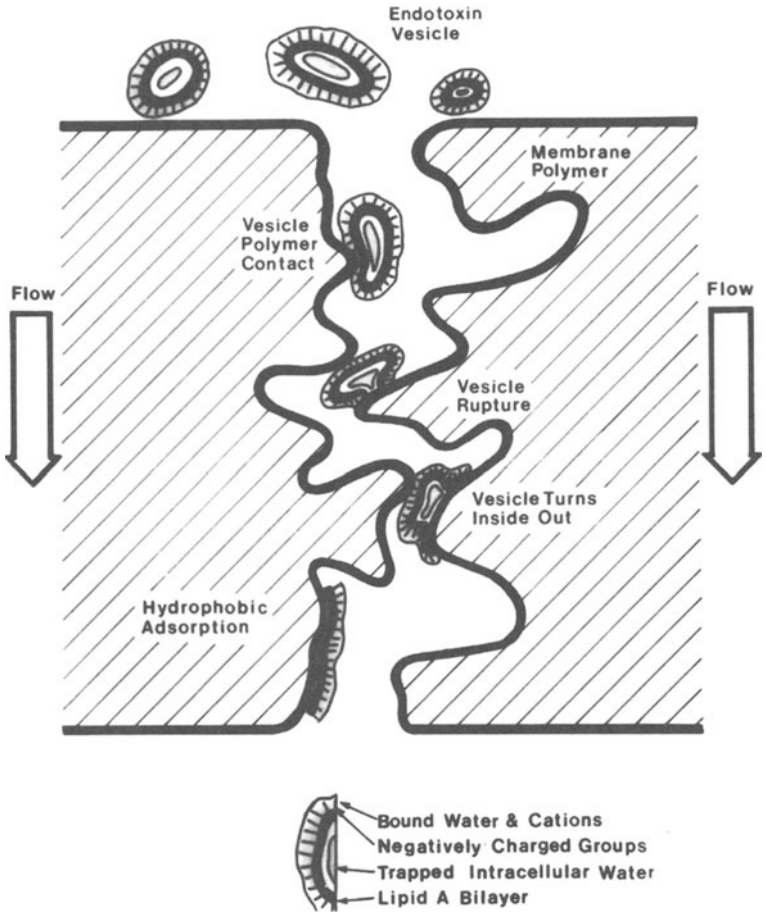

Fig. 18 Hydrophobic adsorption mechanism

the particles are of like charge, and if they are sufficiently strong, the particles will pass through the filter. If, however, particles and pore walls have opposite charges or are weak in their charge magnitudes, adsorption will occur, that is, the particles will adhere to the pore walls of the filter.

To be sure, the ionic strength of the suspending solution, like its pH, exerts an influence on the magnitude of the mutually repelling like charges. Low pH and/or high ion concentrations serve to attenuate the repulsive forces and so usually promote flocculation in colloids and may tend to influence adsorptive particle arrests. Nonionic surfactants also exert an influence. The potential energy barrier that prevents charged particles from approaching one another can be matched in its separating effect by the steric stabilization of the two surfaces caused by the hydrophobic adsorption of a surfactant layer that serves to envelop the particle. It has also been called entropic stabilization. This spatial barrier effect may be of a considerable magnitude, nonionic surfactants in particular can exert a significant influence on colloid stabilization and hence on the adsorptive sequestration of particles by filters.

7.9 Competition and Selectivity

Competition and selectivity may characterize the adsorptive sequestration event. Thus, latex particles may be adsorbed by filters from their suspensions in acetate buffer but not from potable liquids with their more complex constitutions [39], and yeast cells may be adsorptively removed by filters from buffered aqueous suspensions but not if competing sulfate ions are present. Carazzone et al. [40] show similar differences in pyrogen and organism retentions.

The Bowman et al. [41] early finding that occasioned the development of the 0.22 (0.2) μm-rated membrane – namely, that a mutant strain of *B. diminuta* present in certain protein solutions (penicillinase) could not be removed by 0.45 μm-rated membranes – is possibly a case in point. It is possible that modifications to adsorptive organism sequestration by competitive protein adsorption were involved. This demonstrates the influences of the liquid vehicle in which microbes are suspended.

Carazzone et al. [40] conclude, "Positively charged filter media are very interesting, but need careful preliminary studies in order to define their suitability and operational procedures."

7.9.1 Polymeric Influence on Adsorptions

Protein molecules in their complicated formats of polar and nonpolar regions undergo adsorption to appropriate surfaces by charge-related enticements as well as by hydrophobic interaction, chiefly the latter it is believed. It is not surprising, therefore, that proteins in their individual molecular makeup

adsorb dissimilarly onto diverse polymers whose molecules likewise reflect differences in polarity. This type of adsorptive complexity may also guide the response of different organisms adsorbing onto different filters whose molecular intricacies offer similar opportunities for adsorptive attraction.

From a study of organism attachment to and fouling of reverse osmosis membranes, Ridgway [42] reached the conclusion that different organisms adsorb variously to different membrane surfaces. Ridgway's investigations were in connection with the development of biofilms on surfaces in contact with water.

Using radioisotopically labeled Mycobacterium BT2-4 cells, Ridgway et al. [43–46] studied biofilm formation on cellulose acetate (CA) RO membranes. The adhesion of the organisms, without the impress of differential pressure, was surprisingly rapid and showed no log phase. The attachment phenomenon was biphasic: An initial rapid adhesion, straight line with respect to time, was followed by a much slower rate of attachment, also linear with time.

Ridgway et al. [43, 45] demonstrated that microbial adherence to RO membranes is by hydrophobic adsorption, as illustrated by the strong influence of low concentrations of nonionic surfactant. This is in contrast to the lack of effect by ionic strength or additions of charged polymer.

The nonionic surfactant adsorbs hydrophobically to the organisms and to the adsorptive sites and serves as a buffer between the two entities, preventing their adsorptive interaction. A direct correlation was shown to exist between the relative hydrophobicity of a microbial cell and its adherence to RO membrane surfaces. The ability of nonionic surfactants to disrupt mutual hydrophobic reactions, by masking the hydrophobic ligands and leaving the hydrophilic moiety of the surfactant to interact with the water, justifies the use of such surfactants in the removal of bacterial plaque. Such surfactants are used in many RO cleaning formulas [47].

That bacterial attachments may also involve electrostatic interactions is shown by the promotion of adhesion of mycobacteria to cellulose acetate RO membranes caused by certain quaternary ammonium surfactants [43]. This stimulation of adhesion is concentration-dependent and is presumably caused by a differential binding of the surfactant to the organism cell and to the membrane. Another effect of the quaternary compound was to inactivate the organisms. At low surfactant concentration the quaternary compound attaches to the organism, imparting a strong cationic charge to it. This enhanced charge interacts more strongly with the more electronegative RO membrane surface. The greatest degree of mycobacterial adhesion is to polyamide RO membranes. These contain anionic carboxylic acid and sulfonic acid groups. The less strongly charged CA membranes show adhesions reduced by five- or tenfold. Colonization of cellulose acetate RO surfaces by microbes is quite rapid, 3×10^5 cfu/cm^2 being evident after only three days [43, 44].

While holding that microbial attachment to RO membranes is by way of hydrophobic adsorption, Ridgway et al. [43, 45] stated that the five- to tenfold preference of mycobacteria for adsorption to polyamide filters over those

of cellulose acetate was due to the stronger electronegativity of the former polymer.

Interestingly, the continuous addition of 10 mg/L of monochloramine to the feedwater completely inactivated the fouling bacteria without interfering with their adhesion and subsequent attachment to the membrane surface. The implication is that such attachments are physicochemical, rather than being a result of such metabolic processes as exopolymer-mediated bridging of the electrical double layer or of chemotactic responses.

7.10 Management of Adsorption

Although colloidal destabilizations and adsorptive sequestrations are governed by the same principle, they are different in their manageability. As stated, the reduction in zeta potentials that lead to colloidal agglomerations can be manipulated by the addition of ions. In water treatment contexts the aqueous vehicle is converted to a medium of high ionic strength by the addition of ionic charge-bearing entities, the higher their valence, the more effective their influence. To know how much ion addition is necessary, zeta potential measurements are made. This is not the practice in filtrations. In that operation the filter selection having been made, the filtration is performed. Modifications of the preparations intended for filtration are not made in order to promote adsorptions or to deny them. That is seen to be the function of the filter as bestowed by its polymeric composition.

It is empirically known, for example, that non-specific protein adsorptions eventuate with polymers of more hydrophobic bend, such as the polyamides, and less so with hydrophilic membranes, such as the polyethersulfones, or cellulose acetates. Indeed, that is why insufficiently hydrophilic membranes are "hydrophilized" by chemical grafting with oxygenated substituents, to render them less likely to adsorb proteins when that is desired. Manipulations of aqueous preparations intended to promote the adsorptive sequestration of organisms are not yet a possibility.

The value of the adsorptive sequestration mechanisms is that they serve as means of reinforcing the retention of particles, organisms included, in filtrations. They do lack the straightforwardness of size exclusions. The maximization of adsorptive retentions is dependent upon attaining certain filtration conditions, upon the use of membranes of given polymeric compositions, upon the organism type, and upon the imperviousness of both organisms and pores to size alterations caused by the fluid. However, the certainty of the adsorptive sequestration mechanism is not compromised by the complexity of its background. Where particle retention by either mechanism takes place, it remains dependable as long as the conditions necessary to it remain in place. In any case, the attainment of sterility by any and all mechanisms requires validation, confirmation by documented experimental evidence. Uncertainty regarding the results is not an option. Filter dependability can be, and is required to be validated.

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Microfiltration Membranes: Characteristics and Manufacturing

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Abstract Membrane filtration is used within a multitude of processes ranging from dialysis to desalination processes to sterilizing filtration in the pharmaceutical industry. Membranes, nevertheless, have to have special characteristics and properties to serve such specific applications. Microfiltration membranes are utilized in a large range of membrane polymers and structures, which all have individual production process steps to achieve consistently the same membrane parameters. This chapter discusses membrane polymers and production processes in detail.

Keywords Microfiltration · Membrane polymer · Separation applications · membrane structure · Homogeneous membranes · Asymmetric membranes · Composite membranes · Membrane manufacturing

1 Microfiltration Membranes: Characteristics and Manufacturing

Membrane filtration is one of the key processing steps in numerous applications, providing a cost efficient and robust tool for the purification of various liquids and gases. The demand of different membranes is growing year by year by the development of new applications and new membrane technologies. The range of membranes vary in their basic material, the structure and function as well as their field of application, ranging from sea water desalination to haemodialysis or sterile filtration of pharmaceuticals. Within this field, microfiltration plays an important role in various applications in the biopharmaceutical, food and beverage and semiconductor industries. The following chapter will give a brief overview of the various membranes, the characteristics of the base polymers in general but will mainly focus on the microfiltration range.

2
Introduction to Membranes

2.1
History and Definition

Synthetic membranes have been used now for several decades. However, membranes have only become an important separation process in the last 50 years. Membranes started off as being relatively expensive and therefore were only applied in separation involving small, higher-value products. One of the first practical synthetic membranes was developed by Sartorius AG in the 1920s and 1930s [1, 2] using nitrocellulose as a membrane material. However its use was mainly for small scale laboratory separations. Membranes have subsequently developed into low-cost separators for a number of applications including sterile filtration, water preparation, haemodialysis [3], gas separation [4], and reverse osmosis [5]. One of the largest markets for membranes in the world is haemodialysis and haemofiltration with a volume of 2500 million USD in 2002. Microfiltration membranes especially with pore sizes between 0.1 and 20 µm are widely used for downstream processing in the pharmaceutical and biotechnological industry or the preparation of sterilized or purified water. Another larger application with comparable size is the semiconductor industry for the production of pure water, solvents and gas/air.

Membranes can be either natural (biological) or synthetic. Natural membranes – those derived form biological sources – are a broad subject and beyond the scope of this discussion. Synthetic membranes may be polymeric, metal, or ceramic. This discourse will focus on polymeric membranes as they are most often used for microfiltration and are dominated by some specific polymers. The main obstacle for inorganic membranes, e.g. ceramics, so far in microfil-

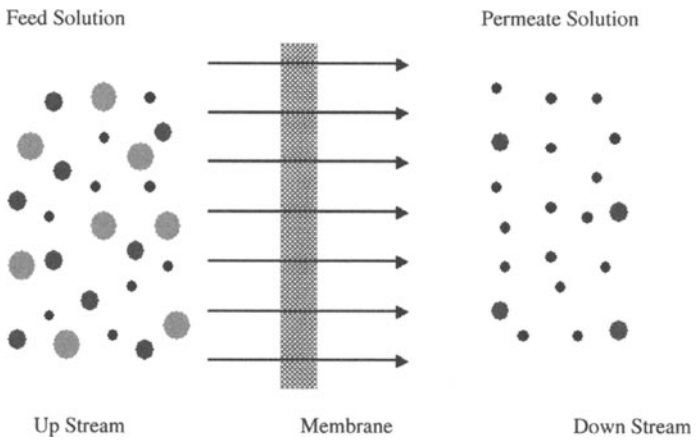


Fig. 1 Schematic of a membrane separation process

tration is their relative high cost, which can be higher by the factor of up to 5–20, depending on the application. Nevertheless, the market for ceramic membranes might grow with increasing demand for microfiltration membranes with a superior chemical and thermal robustness.

The very definition of a membrane is sometimes debated. For this review, a membrane shall be considered a barrier that selectively permits the passage of certain compounds as is illustrated in Fig. 1. General driving forces of the separation process are differences of solute concentrations, energy, temperature or pressure over the membrane barrier.

The feed is considered to be the upstream side while the permeate is considered to be the downstream side. The feed contains, at minimum, two compounds. The membrane preferentially allows the passage of Compound 1 (dark circles) compared to Compound 2 (light circles). The membrane does not need to allow *only* Compound 1 to pass. Compound 2 may pass through the membrane as well, albeit at a slower rate than Compound 1. The net result is that the feed side becomes concentrated in Compound 1, and the permeate side becomes concentrated in Compound 2. Either the feed side or the permeate side may be the "Product" to which value is attached.

2.2 Membrane Driving Forces

A driving force is always required for a separation to occur regardless of the technology involved. The driving force requires some form of energy. For a conventional process such as distillation, the driving force is created by the application of heat to take advantage of a difference in vapour pressures between two compounds. For membranes, the driving force can be one of several phenomena including, pressure, concentration, temperature, and electrical charge.

2.2.1 Pressure

A common driving force for membranes is pressure. Pressure is almost always used when separating gases by creating a higher partial pressure for the gases on the feed side compared to the permeate-side. The pressure gradient causes the gases to flow across the membrane. The membrane preferentially allows one or more gases to pass, thereby concentrating that gas on the permeate-side. Pressure is also the primary driving force for reverse osmosis, ultrafiltration, and microfiltration.

2.2.2 Concentration

Some membranes, such as those used for kidney dialysis, are operated without a substantial pressure gradient (a small pressure gradient may exist that allows

passage of fluid out of the patient). In dialysis, the higher concentration of the impurities in the blood, compared to the buffer creates the driving force. This driving force plays only a minor role in microfiltration applications.

2.2.3 Electricity

Electricity in the form of a voltage gradient can be use in a membrane-based electro-dialysis. Separation can be achieved by inducing a charged molecule to move across a membrane. Novel separation technologies combine pressure or concentration with charge/electricity driven process conditions. By using ultra- or microfiltration membranes with a limited pressure difference within an electrical field, molecules and larger particles can be separated not only by their size but by their charge. Such systems are still under development and their efficiency still has to be proven.

2.2.4 Temperature

The separation properties of membranes can be combined with intrinsic vapour pressure differences to enhance distillation. Membrane distillation, for instance, can break the azeotrope that is formed by water-alcohol. Most applications use nano- or ultrafiltration membranes in these applications.

2.3 Types of Membrane Separations

Separations addressed by membranes are defined by the size of the solutes that are retained. The solutes can range in size from atomic, e.g. nitrogen, (salt ions) to macro-molecules (e.g. proteins) and macro-particles (e.g. cells). Figure 2 shows the size of the solutes, their applications, and the membrane classification. The more important commercial applications include reverse osmosis, gas separations, dialysis, pervaporation, ultrafiltration and microfiltration.

Another way to differentiate between the membranes is to use the general applications. Membranes can have various tasks in the field of biomedical applications, ranging from haemodialysis, hemofiltration to artificial organs. Another field is the biopharmaceutical industry, where ultrafiltration and microfiltration membranes are widely used for concentration, purification and sterilization of the processed water, used gases or the pharmaceuticals. A comparable application is given in the food and beverage industry, for example during the processing of beer, wine or fruit juices. As mentioned before, another field is the preparation of pure water, solvents and gases in the semiconductor industry where particle removal is critical for the quality of the final electronic product. Other application fields for membranes are oil/water separation, waste and

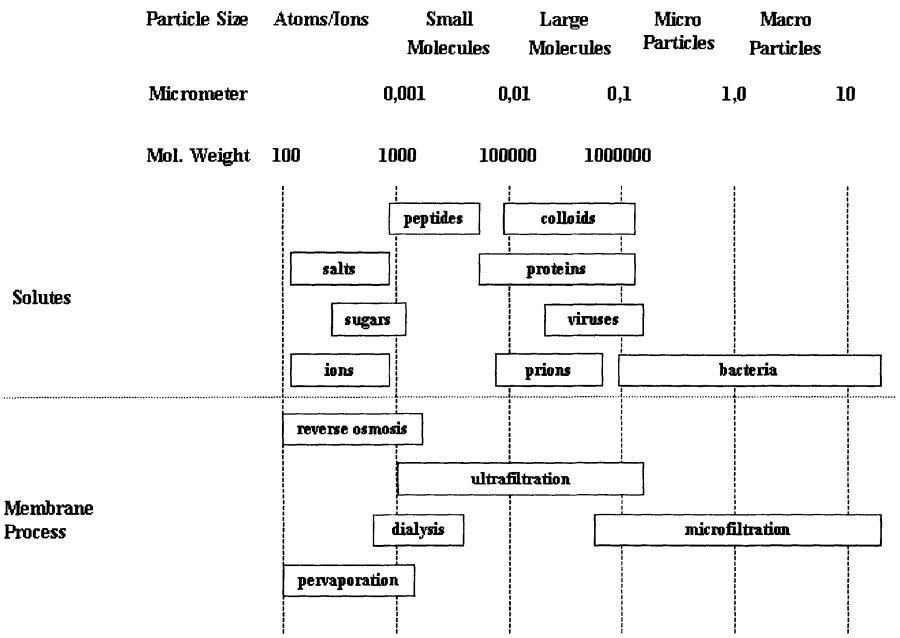


Fig. 2 Selected separation applications of membranes

drinking water preparation, gas and air purification, energy production (fuel cells), etc. where microfiltration plays no or a minor role than other membrane filtration technologies such as nano- and ultrafiltration.

2.3.1 Reverse Osmosis

Osmosis refers to the transfer of solvent but not of the solute through a membrane [6]. High pressure (50 bar) is used to create sufficient osmotic pressure of salt or brackish water so that water passes through a highly selective membrane to create potable water. Membranes in this field are commonly very dense and designed to withstand high pressures and physical stress.

2.3.2 Gas Separations

Pressure driven gas separations have been used to separate oxygen and nitrogen from air, carbon dioxide from natural gas, hydrogen from refinery synthesis gases, and to dehydrate compressed air. Polymeric membranes for gas separations are mainly dense membranes or films with a specific selectivity for the individual gases, thereby allowing controlled diffusion, permeation or adsorption of selected gas molecules.

2.3.3
Dialysis/Bioartificial Organs

Dialysis refers to the transfer of both the solvent and some of the solutes through a membrane. Most frequently considered is haemodialysis or kidney dialysis where a membrane allows the passage of low molecular weight impurities such as urea from the blood stream of a patient with end-stage renal disease [7]. Larger compounds such as proteins and blood cells cannot pass across the dialysis membrane and are retained by the patient. The dialysis membranes help manage the fluid balance in the body and can be used to supply nutrients to patients. Dialysis membranes are mainly hollow fibre membranes of cellulosic, polyethersulfone (PESU) or polysulfone (PSU) nano- and ultrafiltration membrane materials. A critical parameter of the suitability of these materials in the application is the biocompatibility and the robustness/reproducibility of the process due to the direct contact to the patient. This is even more important in the use of membranes as bioartificial organs such as liver. The biocompatibility, the possibility of providing a sufficient cell adhesion and growth combined with a good selectivity or separation results are essential for an efficient treatment of a living organism. PESU ultra and microfiltration membranes seem to offer a superior cell adhesion and growth rate than comparable cellulosic materials.

2.3.4
Pervaporation

In a pervaporation process, a liquid feed mixture contacts one side of a membrane while the permeate is removed as a vapour from the opposite side. The most important application is the dehydration of organic solvents, e.g. alcohols. For example the dehydration of liquid methanol can be accomplished by pulling a vacuum on the permeate side of a membrane that selectively passes water vapour. This is one of the few membrane processes that use both a liquid and vapour phase.

2.3.5
Ultrafiltration

Molecules such as peptides, proteins or other particles can be concentrated using ultrafiltration. With ultrafiltration, the separation is described by a molecular weight cut-off instead of a particle size. Membranes are optimised to allow molecules up to a certain molecular weight to pass. Ultrafiltration is generally performed with pressure of 1–6 bar and is used to concentrate molecules with a molecular weight of 1000 to 500 000. The same ultrafiltration membrane can be used to purify the proteins by dialyzing with a buffer (i.e. washing small molecular weight compounds through the membrane with buffer). Ultrafiltration processes often involve flowing the feed across the membrane at a high velocity to prevent the fouling of the membrane. Typically only 10% of the feed

is allowed to permeate per pass with the remaining feed (termed retentate) being recycled back to the feed tank. Membranes for ultrafiltration are dominated by cellulosic materials such as cellulose acetate, regenerated cellulose acetate or newer materials such as cross-linked regenerated cellulose acetate and polyethersulfone (PESU) or polysulfone (PSU). The membranes are typically asymmetric with a thin skin layer, where the separation is performed and a larger support layer with the only function to give the skin layer sufficient physical strength.

2.3.6 Microfiltration

Molecules and particles such as proteins with a molecular weight of 500 000 and higher, cells, and bacteria can be separated or concentrated with microfiltration. Microfiltration is often used to separate a produced protein from a fermentation broth. The cells that produced the protein are retained by a microfiltration membrane and the produced protein is allowed to permeate. The capability to efficiently remove bacteria and other microorganisms from a pharmaceutical drug without influencing or damaging the drug itself makes microfiltration membranes the method of choice of sterilization in the biopharmaceutical industry. The microfiltration membrane/device is sterilized prior use (steam, irradiation, ETO, etc.). Comparable to ultrafiltration membranes, fouling or blockage is the most serious problem in microfiltration, limiting the service life of the filter. To optimize the process, prefilters with larger pore sizes (depth filter, etc.) are often used to prevent an early blocking of a final microporous filter. As described before, another essential field of microfiltration membranes have the largest pore sizes (0.1–20 µm) of what are typically called membranes. These pore sizes overlap with the smaller pores of conventional dead-end filtration (sterile filters have a nominal pore sized of 0.2 µm). A broader range of polymers are utilized for microfiltration membranes such as polysulfone, cellulose acetates, polyamides, PVDF, PTFE, polycarbonates or olefins. Based on the individual characteristics of the polymers, the resulting membranes offer different advantages and disadvantages and have to be selected for the individual application or separation task.

3 Membrane Structure

3.1 Porous and Homogenous Membranes

A homogenous membrane is usually a dense film ranging from 10 to 200 µm (Fig. 3a). As an example, polyethylene can be formed into a film and used to separate air into an oxygen-rich permeate and a nitrogen-rich concentrate. A

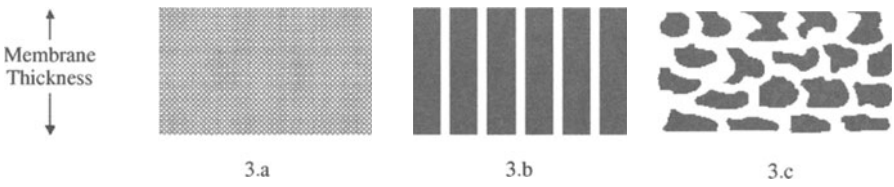


Fig. 3 **a** Homogenous membrane. **b** Porous membrane with cylindrical pore. **c** Porous membrane with tortuous pores

dense membrane relies on the intrinsic nature of the material for its separation properties. The polyethylene film will naturally permeate oxygen faster then nitrogen. With homogenous membranes there is a trade-off between the strength and productivity. While a homogenous membrane is made stronger with increasing thickness, the permeation rate across the membrane decreases. Homogenous membranes are generally used for the separation of materials on the molecular scale. The mechanism for selective separation for homogenous membranes involves the solubility of a compound in the material and rate at which that compound diffuses across the membrane. The equation for mass transfer across a dense membrane is

$$F = D \cdot S \cdot (C_{feed} - C_{permeate})/L$$

F = Flux
D = Diffusion coefficient
S = Solubility of solute in membrane
C_{feed} = concentration of solute in feed
$C_{permeate}$ = concentration of solute in permeate
L = membrane thickness

Thus, the solute molecules dissolve in homogenous, dense membranes and then move across the membrane via diffusion. Instead of concentration as shown in the equation, the driving force could be pressure, voltage or temperature.

The product of the diffusion coefficient and the solubility (DxS) is also called the permeability. When evaluating a homogenous membrane, the selectivity of one component compared to another component is equal to the ratios of their permeabilities.

A porous membrane understandably has a porous structure. The size and shapes of the pores largely determine the separation characteristics. As the pore size increases, the separation becomes more similar to that of a filter, where compounds are allowed to pass based on size. The intrinsic nature of the material can still have an effect on the separation by, for instance, slowing the passage of one compound due to molecular attractions. The pores in a porous membrane can be cylindrical (Fig. 3b). However, it is more common that the pores have a range of sizes and are tortuous path (Fig. 3c).

The separation mechanism for porous membranes is more similar to conventional filtration – larger particles or compounds cannot pass through the pores and are therefore retained.

3.2 Symmetric vs Asymmetric

In addition to porous and homogenous, membranes can be classified as symmetric and asymmetric. Symmetric membranes have a structure that is consistent throughout. Homogenous membranes are symmetric. Porous membranes can also be symmetric with pore sizes and pore shapes consistent throughout. Nevertheless, there is no general understanding, defined parameters or equation to classify a membrane as asymmetric or symmetric. Therefore, each membrane manufacturer and user has an own approach to the definition of this membrane parameter.

In general, an asymmetric membrane has a structure that is different on the surface compared to the interior. In one case, the surface, or skin, may be dense

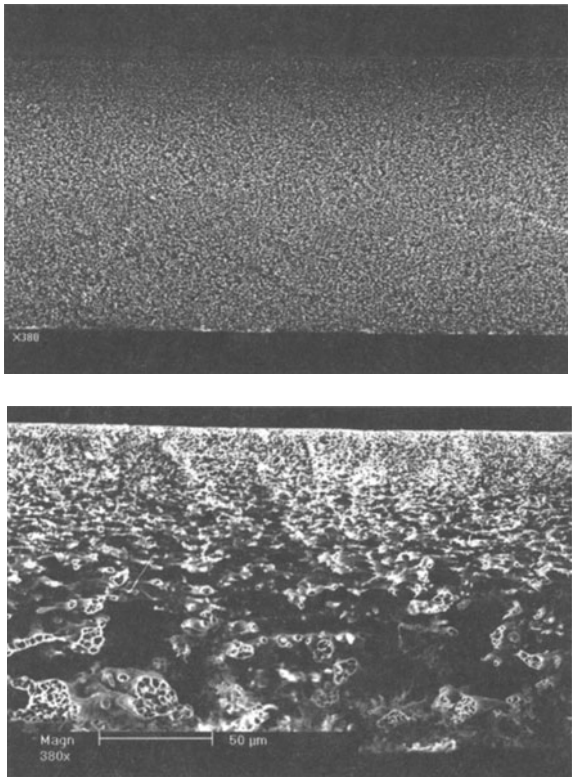
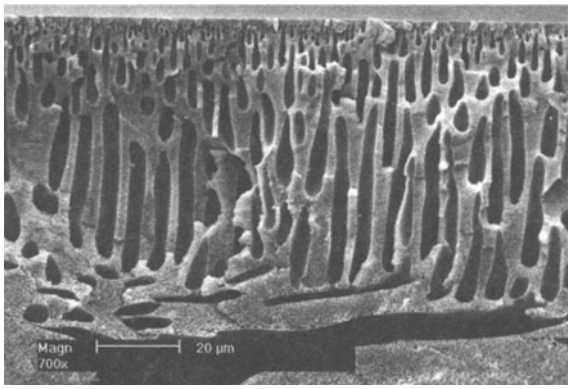


Fig. 4 **a** Symmetric porous membrane. **b** Asymmetric porous membrane. **c** Membrane with dense skin layer and porous support. **d** Membrane with skin layer and finger structure support



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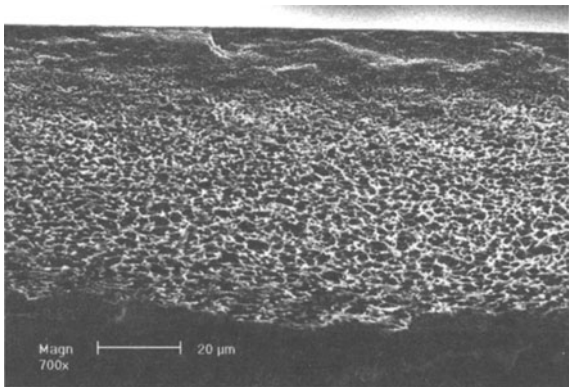


d

Fig. 4 (continued)

and the rest of the membrane is porous. Or the surface may have different sized pores compared to the membrane interior. Since most of the separation characteristics results from the surface, the surface can be tailored according to the application. For instance, a porous membrane could have an integral dense skin on the surface. Schematic examples are shown in Fig. 4. The dense skin is much thinner (0.1 to 1.5 µm) than a comparable homogenous membrane and therefore has higher permeability. This sort of membrane is usually more effective for gas separations and for reverse osmosis than a dense homogenous membrane made of the same material. The porous substructure of the membrane gives the membrane strength without adding to the resistance to mass transfer.

3.2.1 Composite Membranes

The process to create a dense membrane skin on a porous support from a single material is difficult. It is often simpler to deposit a coating on a porous membrane surface that acts as a dense, highly selective membrane. The coat-

ing can be a different polymer that is more selective for the application than the intrinsic properties of the polymer support layer. The polymer can be applied by many techniques, the most important of which are dip coating and interfacial polymerization.

4
Membrane Polymers and Selected Chemical Properties

4.1
Membrane Polymers

Most commercially viable synthetic membranes are polymeric and therefore will be the focus here. Polymers are high molecular weight molecules built from a basic group that usually repeats. Other microporous membranes can be made of inorganic materials such as metals, metal oxides, silicates, and other ceramic materials. As their application is still limited, these materials will not be discussed in detail here.

4.1.1
Hydrocarbon-Based Polymers

The most basic polymers are created from vinyl monomers (H_2C=CHR). The simplest, polyethylene, is made from the polymerisation of ethane to form a saturated carbon chain. In the case of polyethylene, the R-group is hydrogen. The position of the R-group after polymerisation has a significant effect on the properties of the polymer. Polymers with all of the R-groups on same side of the carbon chain (termed isotactic) are crystalline. Polymers with the R-groups randomly arranged on either side of the carbon chain (termed atactic) are amorphous. Polymers with R-groups regularly distributed on both sides of the carbon chain (termed syndiotactic) are partially crystalline. Polypropylenes strength and versatility result from a matrix of interlocking crystallites that allow the formation of rigid and tough polymer structures. Polypropylene membranes reach a limited porosity and are mainly of symmetric structure. The basic material is hydrophobic, limiting the material to organic solvents or requires a surfactant to reduce the hydrophobic influence on the membrane surface. Even through the final melting point of commercial PP lies in the range of 150–180 °C, the safer upper working temperature limit should be between 100–120 °C, depending on the stress. The material normally starts to soften at temperatures around 80 °C, so a sterilizing step with hot steam (121 to 134 °C) is limited. Irradiation results in a autocatalytic degradation of the polymer, which can only be inhibited by additives which reduce free radicals. PP is compatible with acidic and caustic solutions as well as with most solvents, offering a broad range of applications. Only powerful oxidizing agents and highly aromatic solvents are generally considered non-compatible. PP adsorbs some sol-

vents leading to a swelling of the PP matrix, thereby influencing the pore structure and size.

4.1.2 Cellulosic Polymers

Cellulose is a polysaccharide with a molecular weight up to 1,500,000 (Fig. 5). It can be formed into esters (cellulose acetate, cellulose nitrate) or into ethers (ethyl cellulose). The alcoholic hydroxyl groups of the cellulose are polar and can be substituted by nucleophilic groups under strong acidic conditions. The mechanism of esterification can be applied to various agents, but mainly nitric acid or organic acids (e.g. acetic acid) are used for generating cellulose ester polymers for microporous membranes. The regular repeating linear chain leads to a crystalline structure. It is extremely hydrophilic, making it useful for aqueous-based membrane processes such as kidney dialysis, microfiltration, and ultrafiltration. Cellulosic membranes have also been produced with dense, nonporous skins appropriate for gas separations. Cellulosic membranes have low adsorption characteristics making them useful for biopharmaceutical processes where proteins can cause rapid fouling. However, cellulose is unstable at high pH which limits the application. This problem has been overcome by chemical stabilization and can tolerate cleaning with 1.0 N NaOH for limited time periods [8]. The most common cellulosic material in microfiltration is cellulose acetate (Fig. 5) or mixtures of cellulose nitrate and cellulose acetate.

Cellulose acetate (CA) membranes are hydrophilic and stable against weak caustic and acidic solvents and stable against most mineral and fatty oils. The stability against high temperatures and physical stress combined with an extremely low unspecific adsorption of chemical entities or peptides and proteins make CA a membrane material of choice for the filtration of high value products. The CA membranes can be either symmetric or asymmetric and the physical strength can be even improved by the incorporation of support fleeces in the membrane matrix without influencing the pores structure or size.

The unique feature of cellulose nitrate (CN) is its extremely high unspecific adsorptive capabilities. Therefore, the use of cellulose nitrate in mixed ester membranes provide membranes for applications where an unspecific adsorption is desired (such as analytical, diagnostic or microbiological applications).

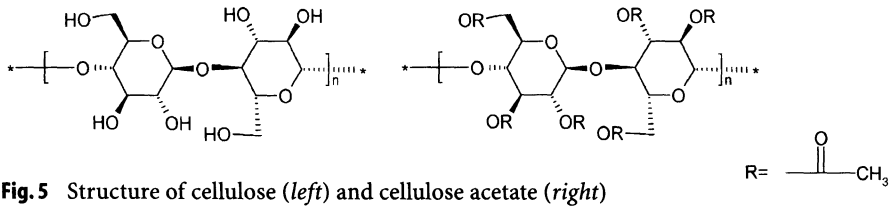


Fig. 5 Structure of cellulose (*left*) and cellulose acetate (*right*)

4.1.3 Polysulfone

Polysulfone is the generic term for all sulfone-containing polymers, which is one of the most important group of polymers in membrane science. All commercial polysulfones used as membrane polymers are essentially amorphous and are relative polar. They can adsorb only small amounts of water and therefore show nearly no swelling in aqueous solutions. The membrane polymer is extremely resistant to hydrolysis over the whole pH range, even in hot steam or water. Only organic solvents with a polarity similar to that of the polymer (for example: DMF, DMSO) or certain chlorinated hydrocarbons can show dissolving effects. Resistance against ionising irradiation and thermal stability up to >200 °C is excellent. The polar groups in the polysulfone chain result in a very flexible modulus and thereby robust membrane matrix.

Polysulfone and polyethersulfone (Fig. 6) are the mostly used commercial membrane polymers. They can be formed into homogenous membranes but are usually formed into porous membranes. The membranes can be either very symmetric or asymmetric or a combination of both and thereby offer the broadest range of membrane structures. The porosity of the membrane matrix is very high, resulting in excellent filtration rates. The flexibility and the thermo-physical toughness of the base polymer combined with the high chemical compatibility offer a broad range of applications. Therefore, they can be used for microfiltration, ultrafiltration, nanofiltration or as a base support for composite membranes. They have recently also been used for haemodialysis membranes with improved biocompatibility [9].

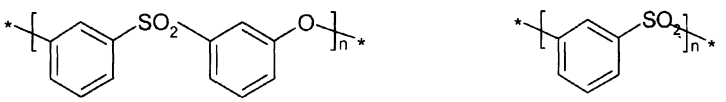


Fig. 6 Structure of polyethersulfone (*left*) and polysulfone (*right*)

4.1.4 Polyamides

Polyamides – generally characterized by the amide group as the recurring part of the chain and known as "nylons" – are widely used as base polymers for microfiltration membranes. Aliphatic polyamides (Fig. 7) are very common in a wide range of applications, but the aromatic polyamides are principally preferred as membrane materials due the their good chemical, thermal and physical compatibility. In particular, compatibility with most solvents makes it a membrane of choice for the filtration of those applications. The resistance to extreme high and low pH is limited, but the toughness, fatigue and abrasion resistance make it a very robust membrane polymer. Nevertheless, the compara-

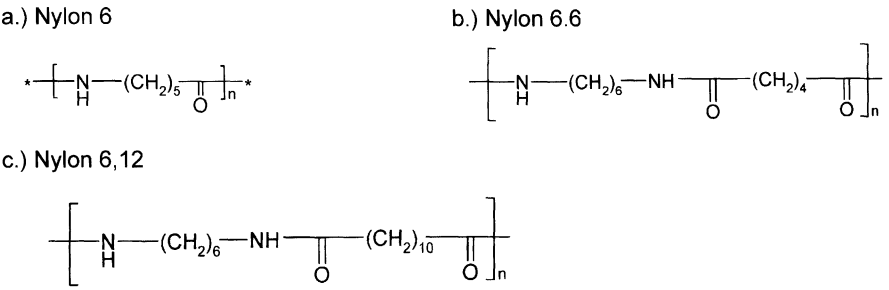


Fig. 7 Structures of a selection of polyamides used for membranes

ble low base polymer price and long time availability in the market make the aliphatic polymers very common in microfiltration. Due to the weak charges, the aliphatic polyamide membranes are hydrophilic and show very high adsorption capacities. This feature can be an advantage in processes where adsorption is essential as well as a disadvantage, when adsorption of a target molecule results in a loss of product. The adsorption of water into the matrix leads to a swelling effect, but does not influence the robustness of the membrane. The structure is limited to a more symmetric matrix and the porosity is not reaching levels of newer PESU membrane structures. But even with these limitations, PA-membranes are excellent filtration tools for solvents, where its chemical compatibility is of advantage.

4.1.5 Polycarbonates

The most typical – and economically successful – polycarbonate is the bisphenol A polycarbonate (Fig. 8). Due to its unique combination of extreme toughness, high heat resistance, low price and high transparency the PC is one of the most common polymer for construction and device design. The chemical compatibility is limited with strong acids and most halogenated and non-halo-

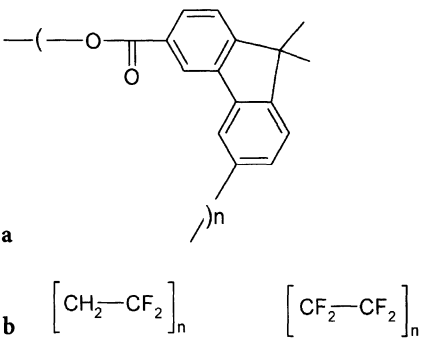


Fig. 8 **a** Structure of polycarbonate. **b** Structure of PVDF (*left*) and PTFE (*right*)

genated solvents, but good with water, alcohols and aliphatic solvents. Utilization as a membrane base polymer nevertheless has some limitations, due to very low porosities compared to other existing polymer matrices if the membrane is produced under standard procedures like evaporation of precipitation casting. But polycarbonates play a roll in the field of track etched membranes, where a membrane is produced by irradiation of a thin film followed by an etching with a strong acid. This procedure and the thereby generated symmetric pores and membranes matrices require a very physically robust and tough polymer. Therefore, most membranes of this type are based on polycarbonate.

4.1.6 Fluorpolymers

There are only a limited number of different polymerised fluorpolymers, of which poly(tetrafluorethylene) (PTFE) and poly(vinylidene fluoride) (PVDF) are the most common in general and especially in membrane science. All have in common a very high chemical and oxidative stability, but are not stable against irradiation. The compatibility with most solvents and the thermal resistance is outstanding.

For PTFE, the very high maximum use temperature of >260 °C and a resistivity against all known solvents make it a membrane polymer of choice for the filtration of chemicals or hot air. In particular, the extreme hydrophobicity of the polymer results in an excellent air filtration membrane with superior blow down properties after steam sterilization. Another consequent application is the classical utilization as a steam permeable but water repelling barrier. Due to its high resistance against solvents, a classical casting approach to manufacture a membrane from this material is not possible. The only membranes of PTFE are produced by stretching the still hot extruded PTFE film until a controlled and defined "micro-tearing" of the film results in a porous PTFE membrane structure. This process is rather unique for PTFE.

PVDF has comparable properties like PTFE with respect to the resistance against abrasion, hydrophobicity and physical robustness. It also shows high tolerances against elevated temperatures and is stable against most solvents. However, unlike PTFE, PVDF is not stable against most polar solvents. On the other hand, this fact offers the opportunity to produce cast membranes with higher porosities from this polymer material. As it is not as hydrophobic as PTFE, the applications in air filtration or as a water barrier are limited. The main utilization in filtration is sterile filtration of solvents and water based liquids. For this, the membrane has to be surface treated or grafted with a hydrophilizing agent, such as acrylic acid. This surface coating reduces the hydrophobic character of the membrane surface, but also reduces the chemical stability of the whole membrane. For example, the PVDF membrane is stable against extreme caustic conditions, while the acrylic coating starts to degrade under this conditions. The resulting membranes are mainly symmetric with high porosities, resulting in good flow rates but limited total throughput

values. Due to this parameters, the applications of PVDF are limited to certain ranges of microfiltration.

4.2 Selected Polymer Properties

4.2.1 Glass Transition Temperature

A key parameter for performance of a membrane polymer with respect to flexibility, physical and thermal robustness can be defined with the glass transition temperature. Polymers in the solid phase may be rubbery or glassy. At low temperatures little molecular motion occurs and a polymer molecule is stiff with a high modulus of elasticity. This is termed the glassy region. As a polymer is heated, the tensile strength will initially remain relatively constant as long as it remains in the glassy region. As the temperature continues to increase, the amplitude of atomic vibrations increase and groups of atoms begin to move. This causes a rapid drop in the modulus of elasticity (Fig. 9) and the temperature is termed the glass transition temperature (T_g). Above T_g, the polymer exhibits rubbery behaviour where the molecules coil and can achieve large stretching when tension is applied. The modulus of elasticity for rubbery compound is often two orders of magnitude lower than in the glassy region.

The structure of the polymer determines the glass transition temperature. Polymers that are flexible such as atactic (amorphous) polyethylene have low

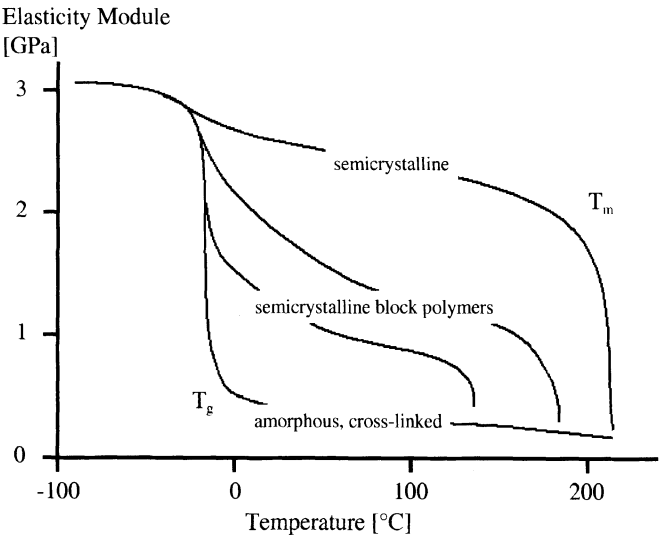


Fig. 9 Tensile strength as a function of temperature for a polymer for amorphous, semicrystalline, and crystalline polymers

glass transition temperatures and readily change to the rubbery state. Polymers that are crystalline (isotactic) and are less flexible, perhaps due to the presence of bulky side R-groups or unsaturated carbons in the carbon chain, may not have a T_g but instead have a temperature at which the polymer melts (T_m). Polymers that are partially crystalline may have a T_g that is not as sharp as that of an amorphous polymer.

Polymers that are rubbery at ambient temperatures are more commonly used as homogenous membranes where the intrinsic separation properties of the polymer are important. Polymers that are glassy at ambient temperatures are used to create porous membranes such as are used in ultrafiltration and microfiltration. A higher glass transition temperature leads to higher thermal stability and often higher chemical stability.

4.2.2 Chemical and Thermal Stability

Many membrane applications are operated under rather inert conditions – ambient temperature in water-based solutions. However, even in those situations, membranes are often cleaned with aggressive chemicals at elevated temperatures that can cause degradation of the polymer. Polymer degradation, for this discussion, can be a change in the polymer that is not reversed when the polymer is returned to its original conditions with respect to temperature and chemical environment. This membrane degradation implies an irreversible transformation of the membrane that may involve the cleavage of covalent bonds, or a change in the pore structure that permanently damages the membrane. Polymer degradation can also be the result of a reversible interaction with the environment. A polymer can swell in the presence of a solvent. While it is reversible once the solvent is removed, the mechanical properties change during contact and this is therefore a form of polymer incompatibility.

Table 1 Glass transition temperature for various polymers

Polymer	Tg [°C]
Polyethylene	−120
PVDF	−40
Polyvinyl acetate	29
Nylon-6	50
Cellulose Nitrate	53
Polyethylenetherephthalate	69
Cellulose Acetate	80
PTFE	126
Polycarbonate	150
Polysulfone	190
Polyethersulfon	230
Polyimide	300

The thermal stability of a polymer is indicated by the glass transition temperature (T_g) for amorphous and semi-crystalline polymers and the melting point (T_m) for crystalline polymers. As the temperature approaches T_g or T_m, the polymer chains begin to flow causing the membrane structure to be altered. Thermal stability is aided by crystalline structure. The crystalline structure typically is isotactic (side groups all on the same side of the polymer) and has limited flexibility. The flexibility is lowered by the presence of carbon double bonds or heterocyclic groups in the polymer chain. As an example, polysulfone, such as polyethersulfone, has a high T_g due to the inflexible and immobile phenyl sulfone groups (Fig. 6) and has a glass transition temperature of 230 °C. Table 1 shows the T_g for various polymers.

The chemical and thermal stability of a polymer are often, but not always, related. A PTFE membrane has excellent chemical stability but has a T_g of only 126 °C. The chemical stability of polymers is affected by the following general rules [11]:

1. Solubility is reduced and chemical resistance is enhanced by increasing the molecular weight.
2. Susceptibility to oxidation increases if the polymer contains unsaturated carbons.
3. Solubility is favoured and chemical resistance is reduced by chemical similarity between the polymer and the contacting solvent.
4. Chemical resistance is enhanced by chain branching and cross-linking.

5
Manufacture of Membranes

Membranes can be manufactured using one of several methods. Membrane manufacturing techniques include, but are not limited to, phase inversion, membrane stretching, and irradiation. Of these, phase inversion is the most common. Many of these methods can be applied to the two primary shapes of commercial membranes, flat sheets and hollow fibres. The combination of the choice of membrane material, membrane formation technique, and membrane configuration leads to numerous possibilities for membranes. Some of the more important possibilities are addressed in this section.

5.1
Phase Inversion

Phase inversion is probably the most important technique for commercial membrane production. The membrane is formed when two phases are formed. One phase has a high concentration of the chosen polymer and a low concentration of solvents and forms a solid. The other phase stays a liquid and has a lower concentration of polymer and a higher concentration of solvents and

forms the pores of the membrane. The polymer-rich phase can be precipitated using solvent evaporation, polymer cooling, and absorption of a non-solvent (e.g. water) from the vapour phase, and by precipitation in a non-solvent. Almost all reverse osmosis, ultrafiltration, microfiltration, and many gas separation membranes use phase inversion. Phase inversion techniques may be applied to a flat sheet or hollow-fibre membrane.

5.1.1 Solvent Evaporation

This is one of the earliest methods of membrane formation [2, 12]. A polymer is dissolved in a mixture consisting of a volatile solvent (i.e. acetone, hexane) and a non-solvent (i.e. water or an alcohol). The membrane is spread out on a solid surface such as glass. As the volatile solvent evaporates, the polymer precipitates as it reaches is solubility limit with the non-solvent. The non-solvent, which is not as volatile, remains in the polymer and forms pores. The pore structure and size can be controlled by the rate of evaporation and the endpoint of the evaporation – the formation of pores can be stopped by immersing the membrane in water or some other non-solvent.

5.1.2 Vapour-Phase Precipitation

Commonly used for microfiltration, a polymer mixture consisting of the polymer, a volatile solvent and sometime a non-volatile solvent is spread thinly or cast on a surface. The membrane is placed in an atmosphere saturated with the volatile solvent and containing a non-solvent (e.g. water vapour). The non-solvent penetrates the polymer mixture and causes the polymer to precipitate. The solvent is not able to evaporate into the solvent saturated atmosphere. This method may be performed on a continuous basis where the cast membrane is passed through a chamber with a controlled atmosphere. When the precipitation is complete, the remaining solvent can be evaporated and the membrane further processed (Fig. 10).

5.1.3 Polymer Cooling

A hot polymer solution is cast without a non-solvent. As the polymer cools, it phase-separates into a porous membrane with the pores formed by dispersed cells of the solvent. The rate of cooling determines the size of the pores with rapid cooling producing small pores. The total pore volume is determined by the amount of solvent in the polymer mixture. Polymer cooling can be used to make both flat sheet and hollow-fibres [13].

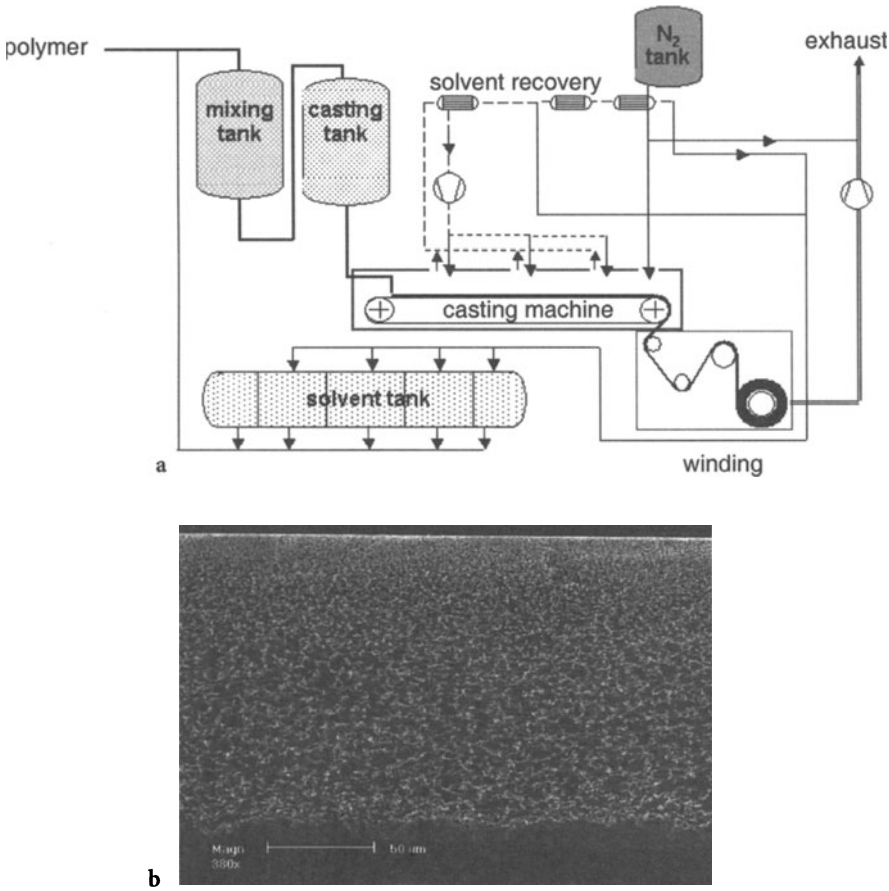


Fig. 10 **a** Casting machine for vapour-phase precipitation. **b** REM of a typical 0.2-µm PESU membrane

5.1.4 Precipitation in a Non-Solvent

The most common of the phase-inversion processes is the precipitation of the polymer mixture directly into a non-solvent – usually water. Membranes made by precipitation in a non-solvent are made as shown in Fig. 11. The polymer mixture, which may contain a non-solvent to enhance pore formation, is immediately precipitated upon contact with a bulk non-solvent phase containing one or more non-solvents. The membrane solution is cast onto a moving drum often along with a support layer. The membrane thickness is defined and controlled by the casting blade. The surface of the membrane precipitates quickly forming a relatively dense surface. The interior of the membrane precipitates more slowly allowing larger pores to form. The precipitated membrane is passed into a second tank where the remaining solvent is rinsed to stop the pore formation process.

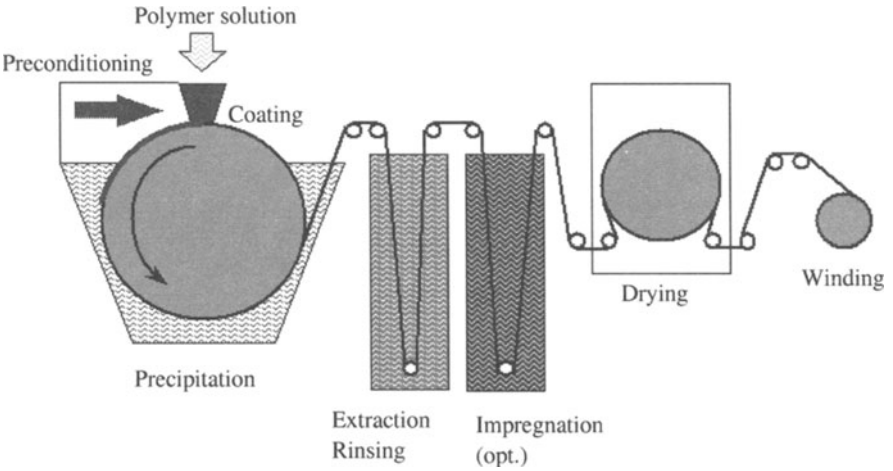


Fig. 11 Casting machine for precipitation with a non-solvent

The formation of a membrane using a three component mixture can be described using a ternary phase diagram (Fig. 12). The corners of the triangle are pure components – polymer, solvent, and non-solvent. There are two primary regions. The one-phase region, on the left side of the triangle, represents the polymer mixture prior to precipitation. The polymer and non-solvent can exist in a single phase that has a high concentration of solvent. The initial poly-

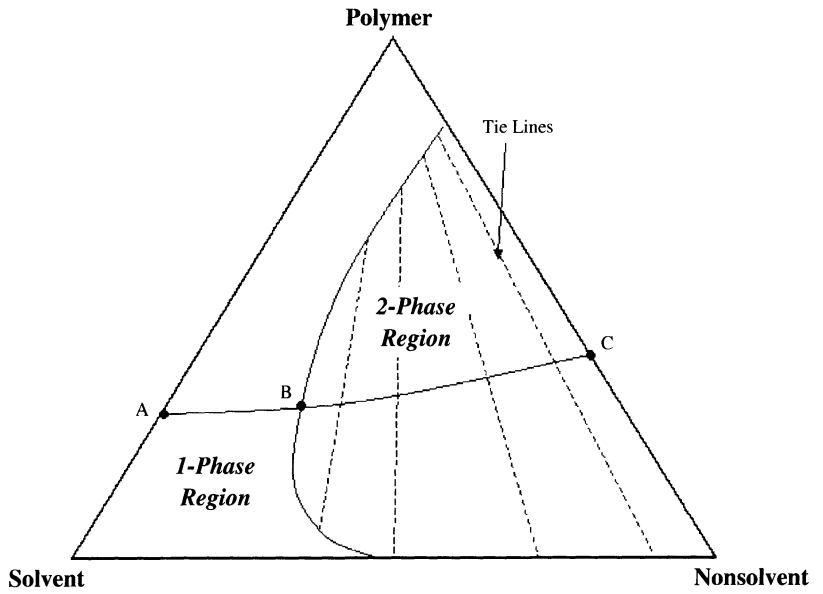


Fig. 12 Ternary phase diagram of membrane precipitation

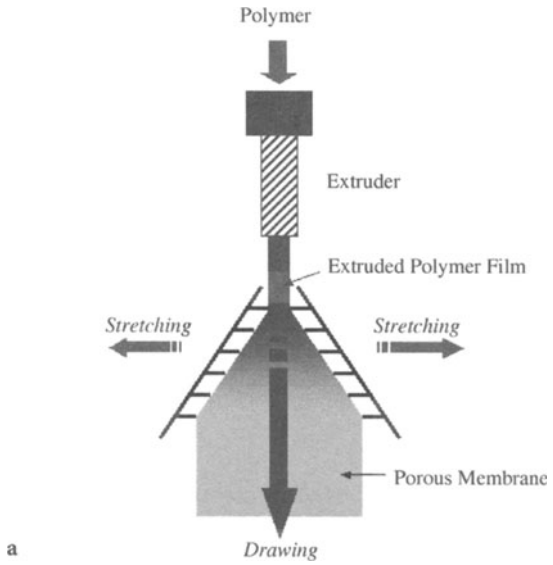
mer mixture is represented by Point A. The two-phase region on the right-hand side of the triangle is the region polymer precipitates into a solid, polymer-abundant phase and a liquid, solvent-rich phase. This represents the precipitated membrane with the liquid phase collecting in the pores. As the cast membrane (Point A) contacts the non-solvent, solvent passes out of the polymer and is replaced by non-solvent. This continues until the polymer reaches its precipitation point (Point B). While the polymer becomes a solid at Point B, it can still show substantial mobility and the pore structure is not set. As solvent continues to be replaced by non-solvent, the membrane solidifies to its final composition at Point C. At that point, the solvent as been completely removed and the membrane can be dried.

5.2 Membrane Stretching

Membrane stretching is commonly used to create porous, symmetric membranes from homopolymers. The most common polymers formed with membrane stretching are PTFE, polypropylene, and polyethylene. In this process, a crystalline or partially crystalline polymer is heated nearly to its melting point and extruded while being drawn down rapidly. This causes the polymer chains to become aligned or "oriented". The polymer is then stretched rapidly at a 90° angle to the original extrusion as shown in Fig. 13. This causes long, narrow slits to form which can be controlled to a specific nominal pore size. Membrane stretching is used to make porous membranes of which Gore-Tex, made from PTFE by W.L. Gore, is the most common [14].

5.3 Track Etching

All the membrane processes discussed thus far have been used to create porous membranes that do not have a single distinct pore size. Separation is achieved by a combination of the minimum pore diameter and the type of material chosen. Unlike membranes that have a range of sizes, track etching creates membranes that have uniform, cylindrical pores. Membranes created by this method have the advantage of having the most precise separation characteristics – a cylindrical pore of a given diameter that cannot pass a particle larger than the diameter. Membranes made by track etching have the disadvantage of having a relatively low overall porosity (about 15% maximum) limiting the throughput. The process for creating membranes using track etching was originally developed by Nucleopore Corp and is shown in Fig. 14. A film, usually polycarbonate or a cellulosic ester, is irradiated with charged particles that damage the polymer chains leaving behind weak spots. The polymer is then etched with an acid or alkaline solution. Pores form around the damaged spots. By control of the irradiation and the etching time, uniform pores can be created.



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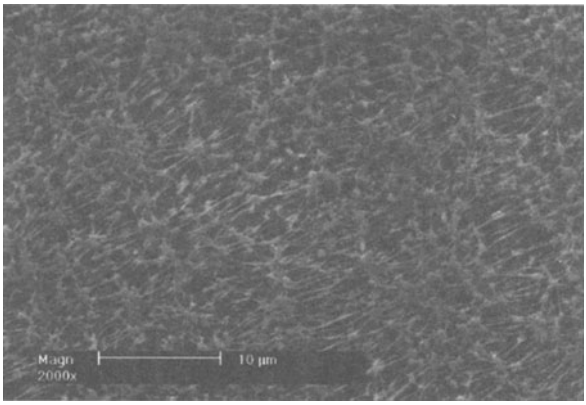
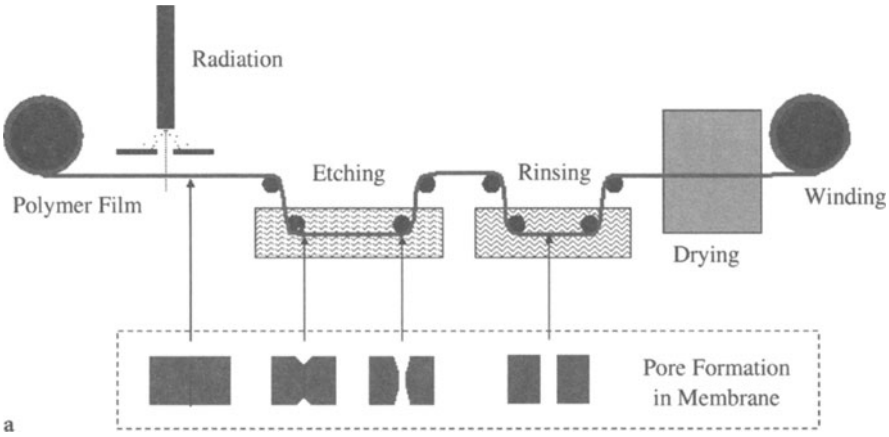


b

Fig. 13 **a** Schematic process of membrane stretching. **b** REM of a resulting 0.2-µm PTFE membrane

5.4 Extrusion Membranes

Another interesting technology is the production of polymeric membranes via an extrusion process. Basically, the process is identical to classical extrusion technologies used in the manufacturing of films, fibres and especially foams. For this purpose, blowing agents or gases are mixed with the membrane polymer in an extruder under high temperatures. At the die, the blowing agent expands in the polymer solution and forms voids and bubbles in the polymer solution. During the cooling phase, this structure is maintained. Most



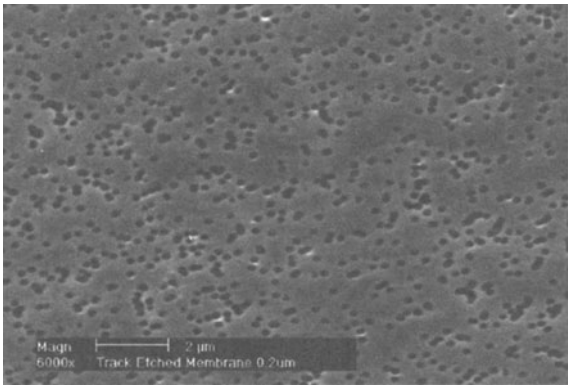
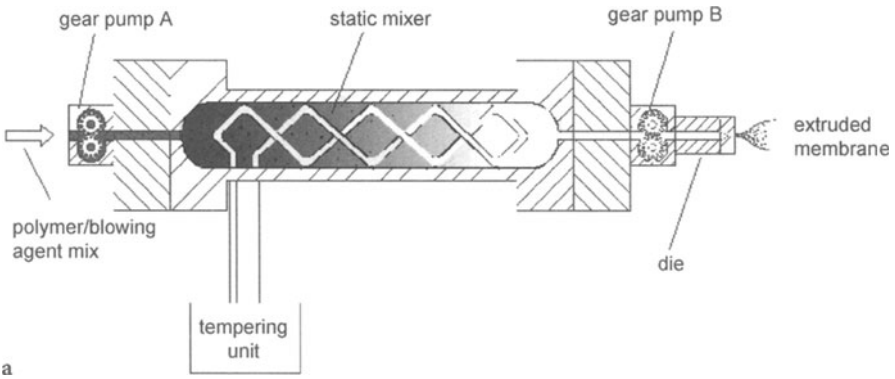


Fig. 14 **a** Schematic of membranes by track etching. **b** REM of a 0.2-µm track etched polycarbonate membrane

methods result in closed cell foams, and special mixtures and technologies have to be applied to generate open cell foams which allow a passage of liquids through the membrane. Resulting extruded membranes showed pore sizes of 30 µm and higher. For microfiltration membranes in the range of 0.2 µm only closed cell structures could be achieved, for a reduction in blowing agent resulted in smaller bubbles but did not break the cell walls to generate an open structure. To achieve open cells, the blowing agent has to be applied in higher concentrations, thereby increasing the bubble and resulting pore size.

Recently, new methods to overcome this problem were developed: To generate a microporous membrane with open cells in the sub-micron range, the polymer solution can be mixed with a blowing solvent or agent under high temperatures and high pressure until the critical point is reached. The supercritical solution expands at the die with high speed and the blowing solvent



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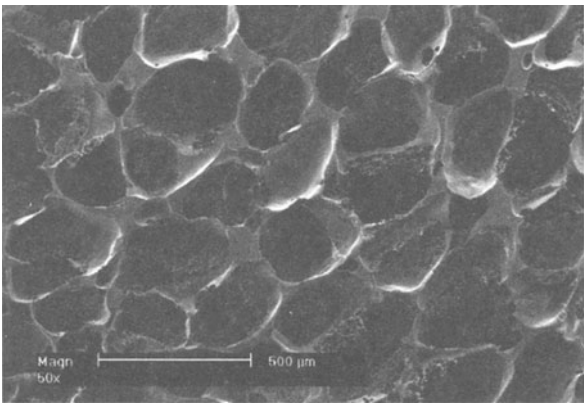

b

Fig. 15 **a** Schematic system for the production of extrusion membranes and **b** REM of an extruded membrane

forms small voids/bubbles in the extruded film. The force of the expansion of the dissolved blowing gas in the supercritical solution is strong enough to break the walls and to form a microporous membrane with sufficient permeability even in the sub-micron range. An example for such an extrusion system is shown in Fig. 15. The most critical point is to maintain precise temperature and pressure control, for the process conditions can reach more than 500 bar at temperature of several hundred degrees Celsius.

The resulting structures often show a combination of larger pores and smaller pores in a substructure, offering a combination of pore size ranges in a single matrix. The example in Fig. 15b shows a polypropylene foam membrane with a void fraction of at least 75% and 90% open cells at a membrane thickness of 500 µm.

5.5 Melt Spinning

Melt spinning is similar to the production of hollow-fibres or other fibres for the textile industry. A major advantage to melt spinning is that many fibres can be simultaneously spun resulting in a high production rate. A polymer solution consisting of a polymer and a solvent is extruded into a cooler atmosphere. The solvent is miscible with the polymer at the melt temperature. However, upon cooling, the solvent phase separates resulting in a porous hollow-fibre [15, 16].

5.6 Composite Membranes

As discussed in Sect. 3.2, composite membranes are created by coating a porous membrane. The coating is the primary separation layer while the porous base membrane acts as a support layer. A composite membrane has the advantage of achieving the selectivity of a homogenous polymer with a much higher flux due to the relative thin selective layer (less than 1 μm compared to 20–200 μm for a dense homogenous membrane). The coating can be specifically chosen for a particular separation. The support structure can be optimised for strength, porosity, chemical or thermal resistance, or created to be a flat sheet or hollow fibre. One support structure can be used for many applications by changing the selective composite coating.

The methods for the production of composite membrane include dip-coating, interfacial polymerisation, spray coating, in-situ polymerisation, plasma polymerisation, spin coating, and grafting. This discussion will focus on dip-coating and interfacial polymerisation because they are the most common. Dip coating conceptually represents spray coating and spin coating where a homogenous polymer is placed on the surface of the porous support. Interfacial polymerisation represents the other techniques where a new polymer is created on the surface of the support layer.

5.6.1 Dip Coating

Dip-coating is used to produce most reverse-osmosis [17] and some gas separation membranes [18]. The porous flat sheet or hollow fibre support structure is drawn into a bath containing a low concentration (~1%) of a polymer, prepolymer, or monomer. When the porous support is drawn from the bath, it is coated by a thin layer of the solution (Fig. 3.16). The membrane is then subjected to additional processing such as exposure to heat which causes the polymer to cross-link. The cross-linking is necessary for two primary reasons. First, it causes the polymer to form into the pores of the support thereby adhering the coating to support (there are no covalent bonds between

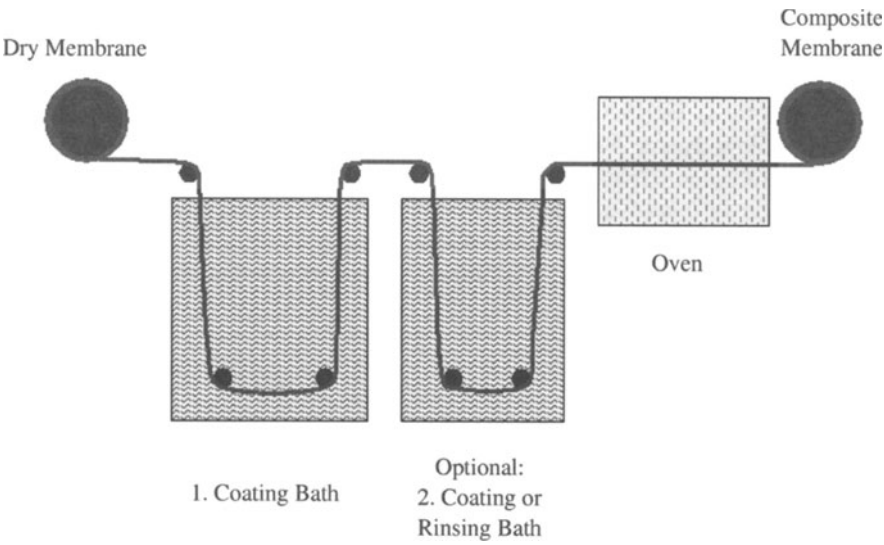

Fig. 16 Dip-coating of a porous support

the coating and the support, so the coating polymer needs to be thoroughly embedded in the support). Second, cross-linking is necessary to achieve chemical and thermal stability as well as to achieve the desired separation characteristics.

Membranes made by dip-coating can be optimised by the appropriate choice of the support structure and coating material. First, the support structure must be easy to coat and must have sufficient surface porosity to allow the coating to adhere. Yet, the surface pores must be small for a thinner coating. In addition, the porous structure must be defect-free as only a few, uncoatable defects can change the separation properties of an entire membrane module.

The polymer used for coating is a homogenous film are identical to those described in chapter three with regard to tensile strength, thermal stability, and chemical stability. The coating polymers tend to be rubbery polymers (e.g. silicone) that have limited tensile strength. Therefore, choosing polymers with a higher tensile strength allows the coating, and hence membrane flux to be higher. An example of this is the use of extremely high molecular weight silicone.

Several different technologies can be applied for the production of such a thin layer on a membrane or film support. Most commonly used processes are dip, spray and spin coating combined with an in-situ polymerisation or a grafting process. For hydrophilization, plasma polymerisation is increasingly used in production, since the first technologies for a continuous plasma treatment are now commercially available.

5.6.2 Interfacial Polymerisation

Coatings using interfacial polymerisation are used for many microfiltration, ultra filtration and reverse osmosis membranes [19]. Interfacial polymerisation involves polymerisation between reactants of an organic and aqueous phase that occurs on the surface of the support structure. The result is a highly cross-linked selective membrane layer. In interfacial polymerisation, an aqueous solution of a reactive prepolymer such as a polyamine is soaked into the pores of the porous support structure. The amine-loaded support is then immersed in a water-immiscible solvent (usually organic) containing a reactant such as an acid chloride. The polymerisation reaction is swift and the resulting coating is strong, chemically and thermally stable, and presumably tailored for specific separation properties (Fig. 17). There is little concern of the newly formed polymer plugging the pores of the support. First, the acid chloride prefers to stay in the organic solvent, and second, the coating forms a barrier to further diffusion of the reactants as soon as it is formed. Several different technologies can be applied to the production of such a thin layer on a membrane or film support. The most commonly used processes are dip, spray and spin coating combined with in-situ polymerisation. For hydrophilization, plasma polymerisation is increasingly applied.

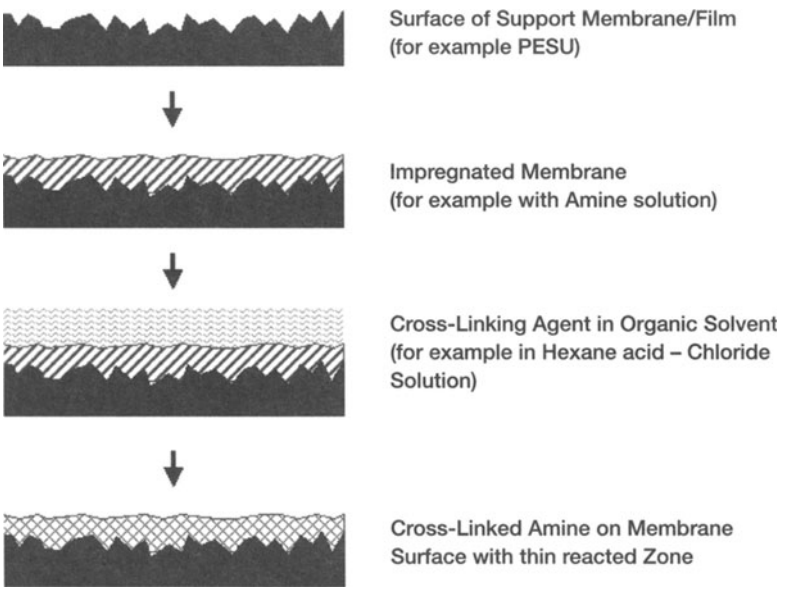

Fig. 17 Schematic of the interfacial polymerization process

6
Membrane Characterization

The characterisation of a membrane is an essential task, providing the correct tools for the selection of a porous matrix for a certain separation task or class of separations. Furthermore, the reproducibility of a membrane during manufacturing and the process control are critical, for even small changes in one of the key parameters can change the whole membrane matrix, and switch the structure from symmetric to asymmetric or from a porous to a non-porous matrix. A change of 2 °C in the evaporation conditions can lead to the formation of a skin layer on an otherwise non-skinned membrane.

Membrane characterization is therefore essential to relate structural and chemical membrane properties to such as pore size, porosity, pore size distribution, crystallinity and flexibility to the membrane separation performance properties. For example, the morphology of the polymer material used for the membrane directly affects its permeability. Other factors such as temperature and the solvent-polymer interaction have a strong influence on the segmental motions of the polymer matrix. Consequently, the material properties may change if the solvent, solvent composition or temperature are changed. To study and control these parameters and influences, the characterization of the membrane is essential.

Principally, in microfiltration two different characterization types for porous membranes can be defined. First, structure related parameters such as pore size, pores size distribution porosity, membrane thickness, skin layer thickness, skin layer porosity, flexibility and physical and thermal robustness. Second, permeation-related parameters, such as the flux of a solvent through the membrane and the selectivity of the membrane with respect to the size and nature of the applied solutes and particles. These parameter are generally application oriented.

In microfiltration, membrane and their structures are mainly characterized by bubble-point, diffusion and multi-point diffusion testing, scanning electron microscopy, intrusion porometry, elongation and burst pressure tests, adsorption isotherms and permeation and retention testing. No standards are defined, so each manufacturer and user has to apply its own standards in the procedures of these techniques. Consequently, the definitions and data of the individual membrane properties vary from source to source – even within the same field of applications and selected polymers. A robust and validated testing of the membrane prior to use is therefore essential for the successful utilization of micro- and ultrafiltration membranes in the designated applications.

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Filter Construction and Design

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Abstract Sterilizing and pre-filters are manufactured in different formats and designs. The criteria for the specific designs are set by the application and the specifications of the filter user. The optimal filter unit or even system requires evaluation, such as flow rate, throughput, unspecific adsorption, steam sterilizability and chemical compatibility. These parameters are commonly tested within a qualification phase, which ensures that an optimal filter design and combination finds its use. If such design investigations are neglected it could be costly in the process scale.

Keywords Sterilizing grade filter · Pre-filter · Capsule · Disc filter · Lenticular filter · Filter design · Cartridge filter · Scalability

1 Disc Filters

Disc or flat filters were the first filter configuration used within the biopharmaceutical industry, mainly as 293-mm discs within large stainless steel holding devices. Multiple membrane discs were assembled in a multi-stack filter housing. The assembly of such housing was/is difficult as one works with wetted flat filters and has to be extremely careful not to damage the filter membrane. Also wrinkles or bents during assembly might cause problems during the filtration process. These "process" filtration devices were replaced by pleated filter cartridge formats [1]. Disc filters are cut from the cast membrane sheet and are available in a large variety of size, either builds into a disposable plastic housing or placed into a filter holder. Common diameter sizes to be placed in filter holders are 4, 25, 47, 50, 90, 142, and 293 mm. Any of the different sizes are used for different types of applications. The most common 47

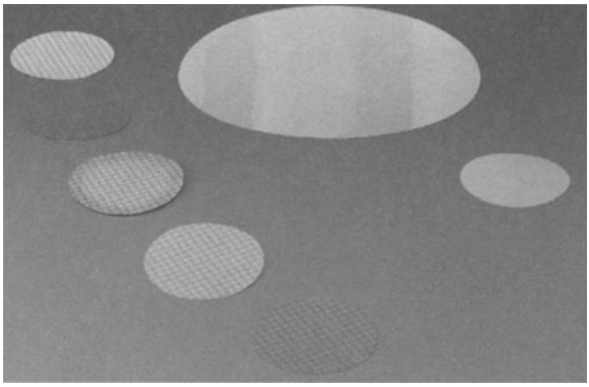

Fig. 1 Different flat filter types (courtesy of Sartorius Group)

and 50 mm are utilized as microbial (analytical) assessment filter (Fig. 1) and can have different colors or colored grids printed on the membrane. The grid structure on the membrane helps counting organisms per defined filtration area and therefore previous filtered volume. Such analytical filters commonly have a pore size of 0.45 µm and utilize adsorptive polymeric materials, for example Nylon or Cellulose Nitrate [2–4]. The reason for the material choice is the requirement of adsorptive capture of the organisms. The pore size is chosen to be 0.45 µm to assure the nutrient, on which the membrane is placed, penetrates through to the membrane surface to feed the captured organisms.

Since disc filters are restricted within its effective filtration area (EFA) pleated filter cartridge designs were developed to increase the filtration area without increasing the footprint of the filtration system or filter holder.

2 Cartridge Filters

The primary motivation to develop pleated membrane cartridges was the need of an increase in the filter area sufficient to secure the engineering advantages of lower applied differential pressures and larger volume flows (particularly advantageous with more viscous liquids). Achieving this goal in the pleated filter cartridge form meant, moreover, that less plant space needed to be allocated for filter installations. As described above, 293-mm discs utilized before pleated filter cartridges required large floor space due to the low effective filtration are of 0.5 ft^2 (0.05 m^2). To replace a common ten-inch filter cartridge and to achieve the same effective filtration area, 15 × 293-mm discs would be needed. Therefore the footprint of such system is by far larger than that of a ten-inch filter housing. Moreover, every disc filter required O-ring sealing, therefore the assembly was time-consuming and insecure.

The first pleated filter cartridge devices already contained approximately 4000 cm^2 of filtration area within the cylindrical pleat pack, which was resin bonded to the end caps (Fig. 2).

Polyester material was commonly used as pre- and support fleece. Both, the polyester and the resin used to bond the membrane to the end cap were reasons for the low chemical and thermal resistance of such filters, not to mention extractable levels, which would be unacceptable under today's standards [5–7]. The first membrane materials were cellulose acetate, cellulose nitrate, polyamide, and polyvinylidene fluoride. Often, these membrane materials were surface treated to achieve pleatability, wettability, and stability of the membrane, which required large water flush volumes before the filter could be used. Pleating polymeric membranes has been a major achievement due to the possibility of pleat breaks, which happens every so often if the right pleat parameters and chemical composition have not been found.

Nowadays available are pleated filters composed variously of cellulose acetates, Teflons®, polyvinylidene fluoride, polysulfone, polyethersulfon, Nylon, etc. The pleating arrangement, the back-and-forth folding of the flat membrane filter upon itself, permits the presentation of a large filter surface area within a small volume. A pleated membrane cartridge of some 2.75 inches (70 mm) plus in diameter and 10 inches (254 mm) in length can contain from 5 to 8 ft^2 (0.5 to 0.8 m^2) of filter surface, depending on the membrane thickness, pre-filtration layers, and construction detail. (Track-etched polycarbonate of 10 µm thickness has been offered in cartridges containing some 20 ft^2 (2 m^2) of membrane surface, required due to its low porosity). Pleated membrane cartridges are also offered in various lengths from 2 to 40 inches and effective filtration areas, from 0.015 m^2 to 36 m^2 (Fig. 3). This range of sizes and effective filtration areas are required for scale-up and down within the process and development steps. A pleated filter device should be able to scale-up linear from the pre-clinical volume size to process scale [1].

Moreover, pleated filter elements introduced the opportunity to combine various prefilter fleeces or membranes in front of the final filter membrane.

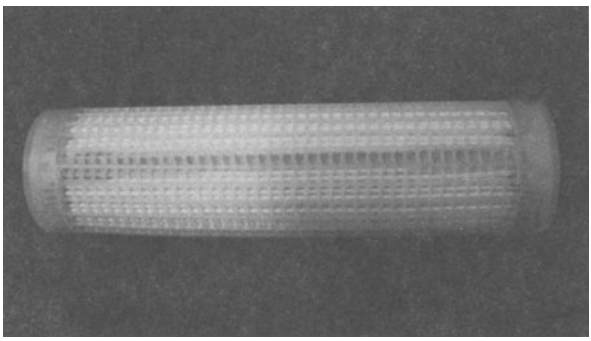

Fig. 2 Resin bonded pleated filter cartridge

Fig. 3 Different filter cartridge structures and types (courtesy of Sartorius Group)

Instead of stacking flat filter discs on top of each other with the risk of leaking due to insufficient sealing or unutilized effective filtration area due to air entrapment between the membranes, pleated filters already will have these prefilter combinations build into the element. The manufacturers gained the flexibility to combine filter combinations determined in filterability trials into a welded filter element. Therefore filtration applications could be optimized [8].

Typical construction components of the pleated filter cartridge are as follows.

End caps are the terminals for the cartridge and the pleat pack and are responsible for holding the cartridge contents together. The end caps are also responsible for providing the seal between the cartridge and the O-ring recess on the cartridge-housing outlet plate. Polypropylene end caps are frequently adhered to the membrane pleat pack, by the use of a polypropylene melt softened preferably by fusion welding. In the past the polypropylene was heated up to the melt point and the pleat pack dipped into it. This welding technique resulted often in excessive polypropylene melt running up the filter pleats, which caused either hydrophobic spots or weakened membrane areas. Fusion welding of the end-cap to the inner core, outer support area and the membrane pleat pack avoid such behavior. In instances, polypropylene end capping can cause hydrophobic areas on the pleat pack, for example with Nylon membranes. Therefore polyester end caps and melts were used, which is not completely unproblematic due to the lower chemical and thermal compatibility of the polyester. It has been reported that the polyester material became so brittle that one could rub it to dust. Such filter cartridge should be inspected on a regular

basis, if used in applications with multiple uses. In the past polyurethane adhesives are also used in end cap materials. In conjunction with polyurethane sealant, the use of polypropylene end caps has sometimes resulted in the falling off of end caps; therefore fusion welding is the most common bondage of end caps nowadays. Besides using similar components, means also a low extractable level. Polysulfone end caps are also used when required, as an inert polymeric material that can be adhered dependably to the pleat pack/outer support cage without creating hydrophobic spotting problems.

A stainless steel ring stabilizes the cartridge orifice against steam-induced dimensional changes and so preserves the integrity the O-ring seal against bypass. The use of such dimension-stabilizing rings is made in the construction of pharmaceutical-grade cartridges intended for sterilization(s), especially when polypropylene end caps are involved. Nevertheless it has been also found that such stainless steel ring, with different expansion rates during temperature changes can also cause problems in respect to hairline cracks and fissures within the adapter polymer or the welding sites. This could go so far that the adapter damage does not allow any longer proper O-ring sealing (Fig. 4). This effect often has been seen with adapter, which has not been molded from one piece. The welding starts cracking, liquid penetrates into the stainless steel ring cavity and expand during the next steaming [9]. To avoid the differences in expansion of the support ring and the adapter polymer, most of the adapters are constructed with a polymer support ring.

The outer support cage is responsible for forming the outer cylinder of the cartridge and for holding the pleated internal contents together. The outer support cage also provides for a backpressure guard in preventing loss of filter medium integrity as a result of fluid flowing in the opposite direction under excessive backpressure. Additionally, it eases the handling of the filter cartridge during installation. The user does not come in direct contact with the pleats and damage can be avoided.

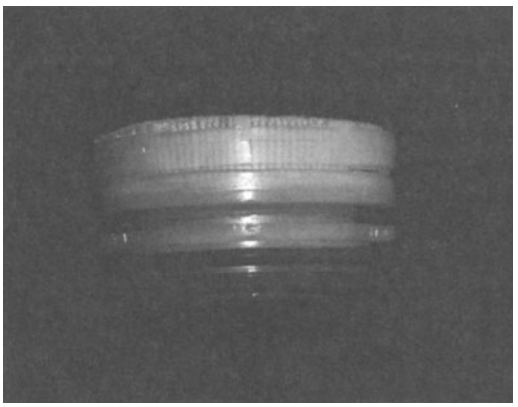

Fig. 4 Filter cartridge adapter damage (courtesy of Sartorius Group)

The outer filter pleated support layer serves as a multipurpose constituent. Pleating, and the assembly of the membrane into cartridge form, requires its inclusion in the cartridge. The supportive outer pleated layer aids in protecting the filter medium throughout the cartridge pleating and assembly operation. The material also serves as a pre-filter to extend the useful service life of the final membrane that lies beneath it. Lastly, the support maintains the structure throughout fluid processing. Without this layer, the pleats under pressure might be compressed, limiting the filter area available to the fluid processing.

The drainage or downstream screen, similar to the outer filter pleat support, stabilizes the pleating of the pleat pack. Additionally, it keeps the filter medium pleats separated during fluid processing to assure that maximum filtration area is open for optimum flow rates and drainage of remaining filtrate, i.e. reducing the dead volume or otherwise trapped fluids. The filter arrangement of the microporous membrane sandwiched between the support and drainage layers, all simultaneously pleated, is often called "the filter pack" or the "pleat pack".

As the sealing between the pleat pack, drainage fleeces, inner core and outer cage and the end caps, low-melting polypropylene sealants are widely used. Use of a low-melting sealant may involve some 1/2 in. of the pleat pack at each end of the filter assembly. A newer sealing technique utilizing polyolefin end caps relies on fusion welding of the cap to approximately 1/8 in. of each of the pleat pack. Valuable effective filtration area is retained thereby. The tendency in cartridge sealing is to utilize as few different materials as possible. Polytetrafluoroethylene, PTFE, or polyvinylidene fluoride, PVDF, microporous membranes are applied for their hydrophobicity (vent and air filters), or for their resistance to aggressive reagents such as certain solvents and oxidizers, or hot acids (semiconductor etchants). Thermoplastic fluorinated polymers, preferably as fluorinated as possible, are used for the cartridge components and in its sealed construction. The melts supported are then usually made of a porous Teflon® material or of PVDF, as is also the remainder of the cartridge hardware from the like polymer in its solid form.

The filter cartridge inner core serves as the inner hollow tube on which the pleated pack is supported. It confers strength upon the cartridge assembly. This component also determines the final assembly length of the cartridge. Lastly, the core is the outlet port of the cartridge. Through its perforations, the filtered fluid passes to be guided to the outlet plate of the filter housing. The cartridge core should not be flow limiting, but can be in high flow applications, i.e. air filtration or water filtration with pre-filter cartridges. It can be seen that the flow rate will not drastically increase by using a 30-inch filter size to a 20-inch filter (Fig. 5). The only benefit here is a higher service life, but not an increase in flow. For this reason air filtration systems are commonly sized with 20-inch filter cartridges.

The filter membrane is the heart of the filter cartridge, responsible for removal of the contaminants. Solutions permeate into and through the filter

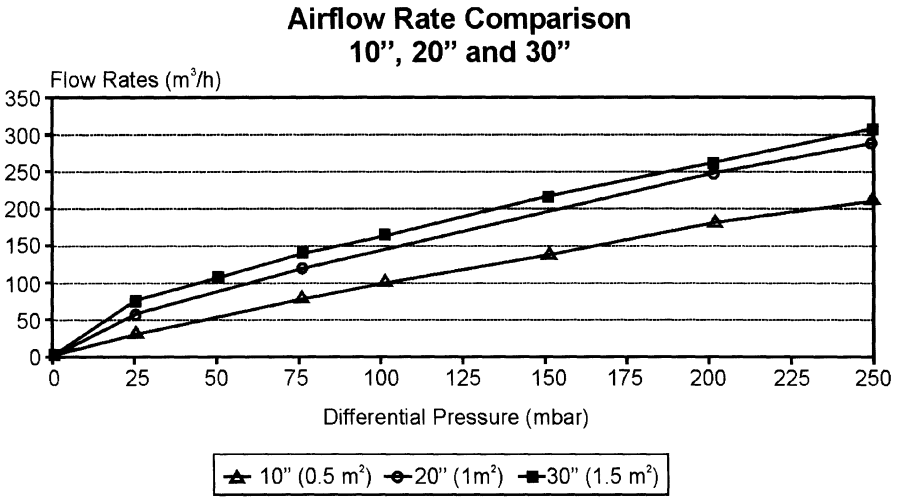

Fig. 5 Flow rate curves of 10-, 20-, and 30-inch filter cartridges sizes (courtesy of Sartorius Group)

medium and into the cartridge core, then proceed through the outlet assembly and filtrate piping. Once the filter medium has become fully wetted, processing can be continued until one of several flow decay indicators signals the need for cartridge replacement, as customer preference dictates.

Cartridge designs can be manifold and fit for the application. Not only size difference are applicable, but also cartridge adapters, i.e. plug-ins, which fit into filter housings sockets and recesses (Fig. 6). A single cartridge with an end plug is used as a ten-inch filter. Otherwise it can be joined by adapters to as many ten-inch double open-end cartridges as are necessary to form the ultimate unit length desired. The filter user needs stock only three items, namely, the double open-end cartridges, the adapters, and end plugs. Nevertheless, joining such ten-inch element together manually include also the risk of bypasses around the o-rings or gaskets used. Therefore these types of designs are undesirable in today's applications.

Single open-ended filter cartridges with bayonet locking are mainly used for sterilizing grade filter cartridges due to the reliability of the fit into the housing (Fig. 7). By-pass situations have to be avoided, which can only be accomplished, if the sealing between the filter cartridge and its holder is smug. In the case of the string-wound cartridges, no end caps are used, because the avoidance of product bypass is not as critical as in sterilizing grade filtration [1]; only the double open-end cartridges and the adapter pieces need be stocked.

In microporous membrane applications, frequent use is made of the single open-end ten-inch cartridge, usually in T-type housings. Therefore, such a unit is manufactured with an integral end cap. Such cartridges are also constructed

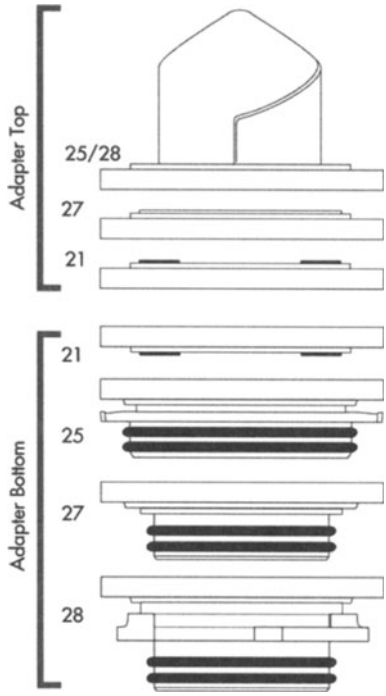

Fig. 6 Different filter cartridge adapter types and designs

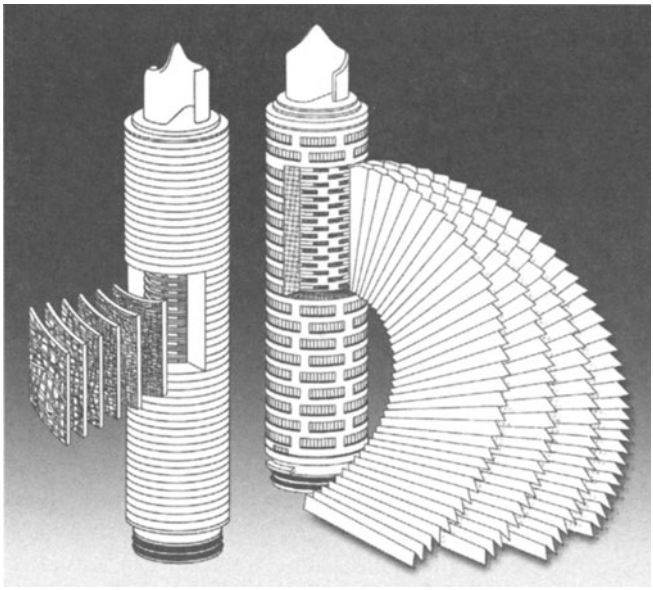

Fig. 7 Schematic of filter cartridge (courtesy of Sartorius Group)

in 20- and 30-inch lengths. Attempts have been made to offer pharmaceutical manufacturers the versatility of ten-inch single and double open-end units to be assembled via adapters with O-rings. As such an arrangement increases the critical sealing area, its acceptance has been limited. The more widespread use in critical pharmaceutical manufacture is of single open-end 10-, 20-, and 30-inch cartridges.

The O-ring materials used are also of critical importance, as the chemical compatibility of the O-ring material has to be determined towards the fluid to be filtered. The O-ring is the critical area of the separation between up- and down-stream side, therefore any incompatibility might be a hazard to the filtrate quality. Furthermore, in instances of multiple steam sterilization, the O-ring material has to be checked for so called heat-set. The O-ring experiences the pressure points from the housing wall and the cartridge adapter. When the temperature is elevated, as in the steaming process, the O-ring starts deforming at the pressure points. If the O-ring material is not flexible enough, the deformation (heat set) will be maintained. The O-ring will commonly show an oval shape. It is important that O-rings are visually inspected on a routine basis to see whether the O-ring is deformed. Any heat set might result into a by-pass situation. EPDM O-ring materials showed so far the highest heat set tendency, nevertheless are very compatible to chemicals. Silicone has commonly a high flexibility and low heat set [10].

In the past, the dimensions of the membrane cartridges are derived from those of the string-wound filters, roughly 10×2.5 inches. Increasing the diameters of these cartridges serves to increase their effective filtration area (per unit number of pleats). Most manufacturers supply cartridges with a 2.75-inch (70-mm) diameter. Diameters as well as adapters types are commonly standardized or similar, which creates the opportunity for the filter user to chose. Additional capital investments into different filter housings are not necessary due to the common adapter types utilized.

The resulting increase in the effective filtration area reflects two factors in addition to the cartridge diameter. The first consideration is the diameter of the center core of the cartridge. Each pleat consists of a membrane layer or of multiple membrane layers, sandwiched between two protective layers whose presence is necessary to avoid damage to the membrane in the pleating process, and which serve usefully in the finished cartridge as pleat separation and drainage layers. As a consequence of this sandwich construction, each pleat, naturally, has a certain thickness. Fewer of these thicknesses can be arranged around a center core of narrower diameter. Therefore, increasing the diameter of the center core increases the extent of its perimeter and the number of pleats that can surround it. This governs the number of pleats possible in the pleat pack that can comprise the membrane cartridge, thus increasing its effective filtration area.

One other consideration favors the use of center cores with larger diameters. Particularly in longer cartridges used under elevated applied differential pressures, the liquid flow through the microporous membrane may be so great

as to find restrictions to its passage through long center cores of 'narrower' diameters. Thus, in pleated cartridge constructions intended for the high water flows the outer cartridge diameter may be 12 inches to accommodate a maximum number of high pleats or greater arranged around a center core dimensioned at a 10-inch diameter. The concern, exclusive of pleat heights, is to increase the service life, the throughput of the filter, by increasing its effective filtration area. (In this application, high flow rates are accommodated within the ten-inch core diameter.)

Such restrictions to flow within cartridge center cores are generally not the concerns in critical pharmaceutical filtrations, where the applied pressure differentials are restrained in the interests of filter efficiency and longevity to yield.

To define a cartridge, therefore, designations must be made of such considerations as its pore-size designation [5], its diameter, its length, the type of outlet, e.g. the O-ring(s) sizes, the configuration of the outer end, e.g., open or closed, with or without fin, the type of O-ring or gasket seal, e.g., silicone rubber, EPDM rubber, and any nonstandard features. Manufacturer product numbers serve as shorthand substitutes for the detailed specifications.

The second factor governing the effective filtration area of a cartridge, in addition to its overall diameter and center core diameter, is the pleat height. Obviously, for any given pleat, the greater its height, the longer its surface area. Present pleating machines cannot fashion pleat heights beyond one inch or so. The designing of a cartridge usually begins with a defining of its overall outside diameter. Given a maximum pleat height of one inch, the maximum size of the center core becomes determined. However, if the pleat height is diminished in order for the center core diameter to be increased, the greater overall number of pleats that can be arranged around the wider core may more than compensate in effective filtration area for that lost through pleat height diminution.

The optimum number of pleats to be arranged about a center core of a filter cartridge may reflect the filtrative function for which it is intended [9, 11]. In the handling of rather clean, pre-filtered liquids, as in most pharmaceutical final filtrations, relatively few particles require removal. A crowding of as large a number of pleats as possible in order to enhance the filter area may be acceptable because the pleat separation layers will operate to make even the crowded surfaces individually available to the liquid being filtered. Where there are high solids loadings in the liquid, or a viscous fluid, a different situation may result. The particles being removed may be large enough to bridge across a pleat, to block the interval between two adjacent pleat peaks. Or, being small, they may, after their individual deposition on the filter, secrete and grow large enough to cause bridging. Whatever the mechanism, the bridging serves to deny the liquid being processed access to useful flow channels bordered by membrane.

In practice, pleated cartridges are built for general usage in what is still an artful construction [9, 10]. Nevertheless, there is said to be available an empirically

developed formula that relates the outer cartridge diameter to the maximum core diameter, and to the number of pleats of given height that should be used.

Care must be taken to protect the surface of the membrane during the pleating operation, and to avoid damage to the filter structure. Both these objectives are furthered by sandwiching the membrane between two support layers and feeding the combination to the pleater. The outlying support layers protect the membrane surfaces. Nevertheless the fleeces have to be chosen properly, for example a fleece too coarse could press too much on the membrane, at the pleating curvation and starts pressing into the membrane. In Fig. 8, one can see the result of coarse fleece compression on a PTFE membrane, which weakens the membrane and might be detrimental in long-term use of the filter. Especially air filters are used over a long period and experience multiple in-line steam sterilization. If the membrane shows impressions by the coarse filter fleece, this commonly means that the filter membrane in this area is thinning. Multiple steam sterilization could exaggerate this thinning and flaws can develop. On the other hand a fleece, which is too soft will not support the membrane sufficiently. Usually soft fleeces have a high fiber density and a small fiber diameter, which means liquid, would be bound within the fiber structure. Such phenomenon needs to be avoided, for example in air filtration, because it could cause water logging.

Additionally, the sandwich in its thickness minimizes opportunities for the membrane to be too strongly compressed at the pleat. What is required is a pleat having some radius of curvature rather than a sharp, acute angle of fold. This prevents the membrane from being subjected, at the pleat line, to forces in excess of its mechanical properties as expressed in the magnitude of its tensile and elongation values. Different polymeric materials will, of course, have different tensile and elongation qualities; various materials differ in their brittleness. Ad-

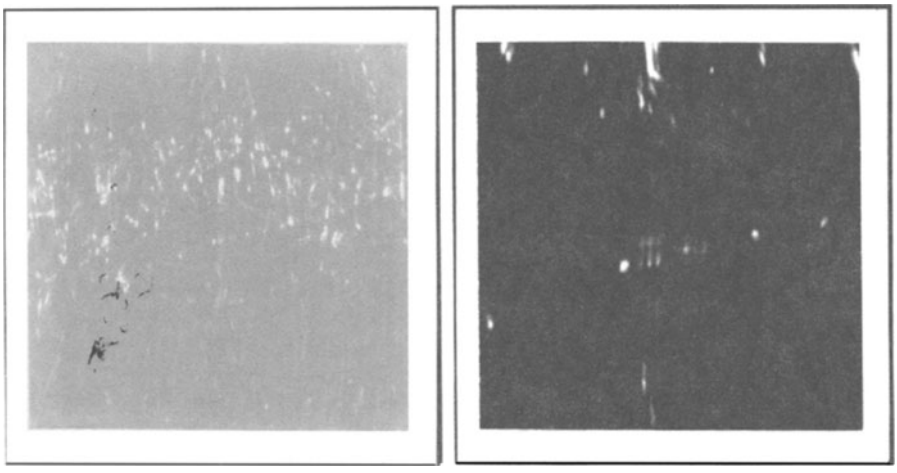

Fig. 8 Pre-filter fleece impression on a PTFE membrane (courtesy of Sartorius Group)

ditionally sharp pleat edges or pleatings with a high pleat density will have a gap in between the pleats, which would result into capillary activity, i.e. in air filtration condensate could potentially be trapped in between the pleats and the air filter might experience water blockage. Therefore, filter designs and construction require thorough investigation in development to achieve the best performance ratios. In instances the highest effective filtration are in the confined construction of a filter cartridge might not be the optimal solution, as the pleat density becomes too high. Nevertheless, effective filtration area should also not be too low as it will influence the flow rate and total throughput. Decreasing the diameter of the center core will serve to lessen the number of pleats, although in applications which require a high flow, for example air, the inner core becomes the flow restrictor. Therefore the inner core again needs to be optimized to the filter cartridge utilization. For example a 28-mm core diameter will require a 40–50% higher differential pressure than a 35-mm inner core to achieve an air flow rate of 100 scbm. This differential pressure increase might not seem to be high, but the costs involved running such pressure difference is substantial.

3 Capsule Filters

The disk and cartridge filters of commerce are usually disposables. It is their housings and holders, usually of metal, that are permanent. However, filters encapsulated into plastic housings have been devised wherein the entire unit is disposable (Fig. 9).

Fig. 9 Different types and styles of disposable Capsule filters (courtesy of Sartorius Group)

There are advantages to these devices. Among them is that many are available in presterilized conditions, by gamma radiation, steam or ethylene oxide. Another advantage, therefore, is their ready availability. They are in a standby condition on the shelf, available when needed. That they are disposables does not necessarily militate against the economics of their usage. Calculations show that where labor costs are reckoned, the installation of a single 293-mm filter disk in its housing is more costly than the equivalent filtration area in the form of a disposable filter device. The use of the disposables entails very little setup time, and no cleanup time. There is no need to sterilize the already presterilized units. Disposal after the single usage eliminates risks of cross-contamination.

One small volume parenteral (SVP) manufacturer adopted the use of disposable filter devices embodying flat disk filter design of essentially the same effective filtration area as a 293 mm disk to replace the latter. The cost savings, reckoned largely as labor, was considered significant. In making the substitution, there were such factors as flow rate vs differential pressure, throughput, rinse volume and time effect wetting and extractable removal, ability to be heat sterilized, confirmation by vendor of product non-toxicity, and freedom from pyrogenic substances [1, 5]. Another SVP manufacturer opted for the same type of replacements, selecting, however, the required effective filtration area in pleated filter capsule form. In both cases, the disposable device was equipped with sanitary connections, enabling a straightforward substitution. Pleated disposable device show commonly better performance due to the pre-filter fleeces and sometimes pre-filter membrane in front of the final filter membrane. Therefore, 293-mm disc filters could potentially also be replaced by 150- or 300-cm^2 disposable devices, even when such have a smaller effective filtration area.

In one application involving the filtration of serum through a 0.1 µm-rated membrane [7], a pleated filter capsule replaced a 293-mm disk because a steam-autoclaved disk holder assembly required a much longer period to cool down to use-temperature than did the plastic-housed disposable filter. The savings in time was judged substantial enough to merit being addressed.

The venting of disposable filter devices has been the recipient of good design considerations. One disposable-capsule manufacturer has taken care to so position the vents that they are on the highest point of the containing shell, exactly where they are most effective. Another design utilizes a self-venting device in the form of a hydrophobic membrane. This permits the self-venting of air while safeguarding against the passage of liquid or contaminants (in either direction). This is particularly useful in water installations, where intermittent use serves repeatedly to introduce air to the system. The self-venting feature reduces maintenance and increases the system efficiency.

There are often ancillary advantages to the use of disposable filter devices. Some manufacturers construct their shells of transparent polymers so that the filtration process is observable. The instruments are compact and relatively lightweight, hence, easy to handle. Nor does their construction lack the so-

phistication of their metal housing-contained counterparts. Thus, many of the disposable units are equipped with vent plugs and drain plugs. The identifying description they bear on their outer casings, make their traceability, in accordance with FDA record requirements rather certain [5]. Product and batch numbers become part of the permanent operational record. Above all, the use of these disposables obviates the need to expense or amortize stainless steel filter holders. No capital expenditures are involved.

Furthermore, the use of disposable filters can reduce costs in respect of cleaning, which would occur with stainless steel filter housings after every use. Cleaning validation, which needs to be performed with fixed equipment like filter housings, will be greatly reduced. The disposable filters do not go through such cleaning regime and therefore the validation of cleaning exercises is avoided. For this reason and the convenience of the use of disposable filters, the biopharmaceutical industry switches more and more to Capsule filters instead of filter housings. That use of disposable equipment becomes more common can also be seen in the fact that bags replace glass or stainless steel holding and storage vessel. Commonly a disposable Capsule filter is connected to such bag, both are available in different sizes for the individual purpose. Once the Capsule filter is connected the bag and filter are gamma irradiated to sterilize the entire set-up. Certainly the filter material and polymers need to be gamma stabile otherwise particle shedding or an excess amount of extractable can occur.

Another advantage is the fact that the user will not encounter the product filtered. This certainly could be the case when using cartridge filters within a housing. The cartridge has to be removed from the housing at the end of the filtration run, i.e. the user probably comes in contact with the filtered product remaining on the filter cartridge and housing, which may need to be avoided due to health hazards or biological activity. Disposable filters create the opportunity to replace a filter without being in contact with the product.

The disposable filter devices are available in a large variety of constructions, whether disk, multidisc, pleated cylinders of various lengths and of different effective filtration areas. Their expanse of filter surface runs from 4-mm discs suitable for affixing to hypodermic needles to 30-inch capsules of about 180 ft^2 (1.8 m^2) (Fig. 10). The filters are made of a variety of polymeric filter materials, both hydrophilic and hydrophobic, namely, cellulose esters, polyvinylidene fluoride, polysulfone polyethersulfone [11], nylon, polyethylene, Teflon, etc. Their shells are composed variously of polycarbonate, polyethylene, but most often polypropylene.

The versatility of these disposable filter instruments is increased by constructions involving integral pre-filters, as in one capsule unit having approximately the effective filtration area of a 293 mm disk. This is appropriate, as single disk filtrations most often involve applications that require the use of a pre-filter. Repetitive final filter constructions are also available in disposable unit form. These are used, for instance, in tissue culture medium filtrations where repetitive final filter arrangements are common.

Fig. 10 Large scale disposable Capsule filters (courtesy of Sartorius Group)

The increase in the tailoring of disposable filter device constructions to specific application needs helps explain the mounting popularity of their usage and heightens predictions of their continuing replacement of at least part of the more conventional filter/holder market.

The use of most cartridge filters accords with FDA emphasis on record keeping. Despite all the care with which filter manufacturers pack flat disk filters, the membranes themselves are unlabeled. Cartridge filters are, however, available with identifying data [1]. Most are identified with some code, if not on the cartridge itself then on its container. Some manufacturers stamp the cartridge end cap with the part number, its pore size identity, and its lot number as well. Indeed, some manufacturers even number each cartridge consecutively within each lot. Should the need ever arise to trace the components and history of these filters, and of their components, the ability to do so exists. Batch records in concert with the appropriate manufacturing QC records make this possible.

Because of the fragility of most membrane filters, appropriate and even extreme care is to be used in their handling. In the case of cartridge filters, this practice continues. However, the actual membrane surface of these instruments is out of reach ordinary handling. There is, therefore, far less possibility of damage to the filters. Overall, cartridges are used mostly for the more rapid flow rates and/or the large-volume filtration productions they enable, a consequence of their aggrandized effective filtration areas.

Cartridges are increasingly constructed so that their in-situ sterilization can be effected by the convenient use of the steam-in-place technique.

4 Lenticular Filters

Lenticular filter designs are mainly used as clarifying filters. Highly adsorptive cellulosic or kieselguhr containing depth filter pads are welded together in a plate format (Fig. 11). These plate formats commonly have a diameter of 12 or 16 inches and are welded together in stacks of 4 to 16 to create a depth filter unit.

The benefits of these depth filter materials are the tremendous dirt load capacity (total throughput). These filters are commonly used to prefilter solutions, which would blind membrane filters rapidly. The adsorptive depth filter material is ideal to separated colloidal substances and lipids, therefore these filters are very often found in plasma and serum applications. Recently these filters also find their use in the cell harvest step in downstream processing after the fermentation. Again the high dirt load capacity is appreciated within such application. When compared to the traditional technologies of centrifugation or cross-flow filtration, the combination of dirt hold capacity and reduction of the filtrates turbidity show better results than the quoted alternative technologies. Nevertheless, the selection of the separation technology of choice within the cell harvest application requires performance analysis, as the results can vary from application to application. It is detrimental to test the performance in small scale trials to utilize later in the process scale the optimal technology.

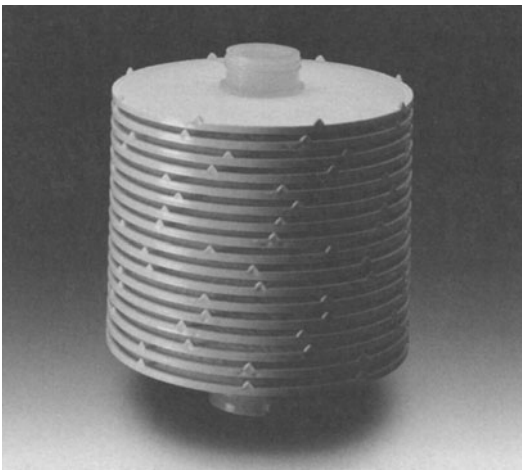

Fig. 11 Lenticular depth filter stack design (courtesy of Sartorius)

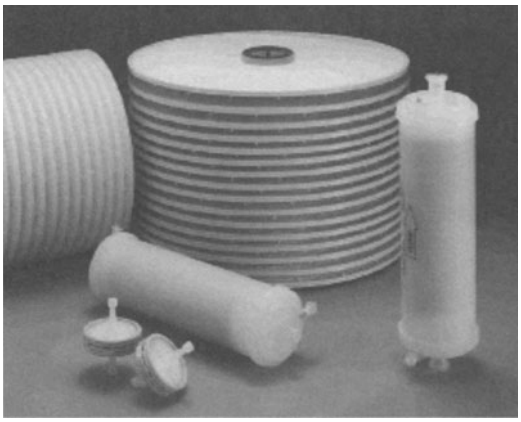

Fig. 12 Scalable lenticular depth filter range (courtesy of Millipore Corp.)

As with pleated membrane and prefilter cartridges, the possibility to scale the filter element is essential [12]. Large scale trials most often cannot be performed due to the lack of product and more so financial burden. The filter products require to be scaled-down to perform optimization and validation trials at the lowest possible burden on product volume requirements. The ability to scale-down the filter is one side of the story. More importantly, the results gained in small scale trials require to be linear scalable to process scale. Any trials performed with small scale filters, which have a different design in process scale, are of no value, as more tests are required in large scale due to the design change. For this reason, filter manufacturers designed specific small scale devices which mirror the larger scale process filter (Fig. 12).

Since such filters are utilized in biopharmaceutical processes, these filters required to be in-line steam sterilized and fully validated. Especially leachable levels of the filters need to be low or the flush volume required to achieve regulatory requirements need to be as low as possible. These critical parameters have been picked up by the filter manufacturers and current lenticular filters have a far higher mechanical and thermal stability than in the past. The construction and design of the support cages and fleeces, the welding and adapter technology evolved. The filters reached with these design changes a higher stability and safety level. Since most of the filter pads utilized in lenticular filters are resin bonded, the filters are pre-flushed within the manufacturers production process to achieve the low leachable level required. Nevertheless, as with pleated filter devices, the leachable level should be determined within the filter users´ production facility to evaluate any product or production process influences. Most of the filter manufacturers testing conditions are very specific and are commonly achieved utilizing water as a test fluid. As some products can have a different influence on the filters matrix and production parameters on the stability of the filter the filter requires to be validated into these conditions. Again small scale device might help in this exercise.

When lenticular filter combinations are tested, the tests do not only involve the total throughput of the filter element as it is commonly the case with pleated prefilter cartridges, but an important factor is the turbidity measurement of the filtrate. The turbidity measurement will create an indication of the protective properties of the lenticular filter retention rating used and how much of the contaminants are separated by the particular filter rating. Since the applications for lenticular filters vary, these filters have to undergo tests, which include the process conditions. The retentivity efficiency of these filters are very much dependent on the fluid contact time within the filter matrix. The longer the contact time the better the separation of contaminants, as the main separation force of these filters is adsorptive retention. Therefore the process conditions especially pressure and flow conditions require evaluation to find the optimal total throughput combination with the lowest turbidity level within the filtrate. At the beginning of a trial the lowest possible differential pressure is used, which fulfills the flow requirements. Samples are taken in specific time intervals and the turbidity measured. This gives and indication of which pressure conditions is the optimal for the filtration task, but also might show the exhaustion of the filter media, if after a certain filtered volume the turbidity of the filtrate starts rising. These tests will determine the process conditions required the filter needs to be used at. To determine which turbidity level is the optimal filtrates with specific turbidities are utilized with membrane filters, which commonly follow the lenticular prefilter. These trials will show, at which turbidity level the next membrane filter step will obtain the highest total throughput. Once the optimal process parameters are determined they are lock in the filtration protocol and the standard operating procedures.

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Filter Validation

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Abstract Validation of a sterilizing filtration process is critical since it is impossible with currently available technology to measure the sterility of each filled container; therefore, sterility assurance of the filtered product must be achieved through validation of the filtration process. Validating a pharmaceutical sterile filtration process involves three things: determining the effect of the liquid on the filter, determining the effect of the filter on the liquid, and demonstrating that the filter removes all microorganisms from the liquid under actual processing conditions.

Keywords Validation · Filter · Filtration · Integrity · Extractables · Sterilize · Challenge · Membrane

1
Filter Validation

Validation of a sterilizing filtration process is similar to validating any other process used in the production of pharmaceutical products, except perhaps for its criticality. Since it is impossible with currently available technology to measure the sterility of each filled container, sterility assurance of the filtered product can only be assured through validation of the filtration process. The FDA defines process validation as "Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes [1]."

Validating a sterile filtration process used for pharmaceutical liquids essentially involves three things: determining the effect of the liquid on the filter, determining the effect of the filter on the liquid, and demonstrating that the filter removes all microorganisms from the liquid under actual processing conditions, resulting in a sterile filtrate. These are discussed in detail in the following sections.

2 Guidelines and Documents

Sterile filtration for pharmaceutical products is the subject of many regulations, guidelines and standards. Regulatory agencies such as FDA, the EC Enterprise Directorate-General and EMEA have issued guidance documents addressing sterile filtration and associated validation practices and requirements. Compendial organizations such as the USP and the European Pharmacopoeia have addressed sterile filtration relative to extractables, particulate release and biocompatibility. ISO 13408-2:2003 specifies requirements for sterilizing filtration as part of aseptic processing of health care products. It also addresses set-up, validation and routine operation of sterilizing filtration processes. ASTM F 838-83 (withdrawn May 21, 2002, and not superseded as of May 2004) provides a standard test method for determining bacterial retention for membrane filters used for sterilizing filtration of liquids. The PDA Technical Report No. 26 contains a wealth of information on sterilizing filtration, including comprehensive treatment of microbial retention, extractables and process compatibility.

FDA's 2003 draft guidance document "Guidance for Industry, Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice" (draft AP guidance) addresses the use of sterilizing-grade filters for product filtration [2]. The document indicates that the "total time for product filtration should be limited to an established maximum to prevent microorganisms from penetrating the filter" and to "prevent a significant increase in upstream bioburden and endotoxin load." Additional provisions include replacing sterilizing-grade filters following each manufactured lot.

Validation of the sterile filtration process is covered in detail in the draft AP guidance document as follows:

Filtration is a common method of sterilizing drug product solutions. An appropriate sterilizing grade filter is one that reproducibly removes all microorganisms from the process stream, producing a sterile effluent. Such filters usually have a rated porosity of 0.2 micron or smaller. Whatever filter or combination of filters is used, validation should include microbiological challenges to simulate worst-case production conditions regarding the size of microorganisms in the material to be filtered and integrity test results of the filters used for the study. The microorganisms should be small enough to both challenge the nominal porosity of the filter and simulate the smallest microorganism that may occur in

production. The microorganism Brevundimonas diminuta (ATCC 19146) when properly grown, harvested and used, can be satisfactory in this regard because it is one of the smallest bacteria (0.3 micron mean diameter). Bioburden of unsterilized bulk solutions should be determined to trend the characteristics of potentially contaminating organisms. In certain cases, when justified as equivalent or better than use of Brevundimonas diminuta, it may be appropriate to conduct bacterial retention studies with a bioburden isolate. The number of microorganisms in the challenge is important because a filter can contain a number of pores larger than the nominal rating, which has the potential to allow passage of microorganisms. The probability of such passage is considered to increase as the number of organisms (bioburden) in the material to be filtered increases. A challenge concentration of at least 10^7 organisms per cm^2 of effective filtration area of B. diminuta should generally be used. A commercial lot's actual influent bioburden should not include microorganisms of a size and/or concentration that would present a challenge beyond that considered by the validation study.

Direct inoculation into the drug formulation provides an assessment of the effect of drug product on the filter matrix and on the challenge organism. However, directly inoculating B. diminuta into products with inherent bactericidal activity or into oil-based formulations can lead to erroneous conclusions. When sufficiently justified, the effects of the product formulation on the membrane's integrity can be assessed using an appropriate alternate method. For example, the drug product could be filtered in a manner in which the worst-case combination of process specifications and conditions are simulated. This step could be followed by filtration of the challenge organism for a significant period of time, under the same conditions, using an appropriately modified product (e.g., lacking an antimicrobial preservative or other antimicrobial component) as the vehicle. Any divergence from a simulation using the actual product and conditions of processing should be justified.

Factors that can affect filter performance normally include (1) viscosity of the material to be filtered, (2) pH, (3) compatibility of the material or formulation components with the filter itself, (4) pressures, (5) flow rates, (6) maximum use time, (7) temperature, (8) osmolality, (9) and the effects of hydraulic shock. When designing the validation protocol, it is important to address the effect of the extremes of processing factors on the filter capability to produce sterile effluent. Filter validation should be conducted using the worst-case conditions, such as maximum filter use time and pressure. Filter validation experiments, including microbial challenges, need not be conducted in the actual manufacturing areas. However, it is essential that laboratory experiments simulate actual production conditions. The specific type of filter used in commercial production should be evaluated in filter validation studies. When the more complex filter validation tests go beyond the capabilities of the filter user, tests are often conducted by outside laboratories or by filter manufacturers. However, it is the responsibility of the filter user to review the validation data on the efficacy of the filter in producing a sterile effluent. The data should be applicable to the user's products and

conditions of use because filter performance may differ significantly for various conditions and products.

After a filtration process is properly validated for a given product, process, and filter, it is important to ensure that identical filter replacements (membrane or cartridge) used in production runs will perform in the same manner. Sterilizing filters should be routinely discarded after processing of a single batch. Normally, integrity testing of the filter is performed prior to processing, after the filter apparatus has already been assembled and sterilized. It is important that integrity testing be conducted after filtration to detect any filter leaks or perforations that might have occurred during the filtration. Forward flow and bubble point tests, when appropriately employed, are two integrity tests that can be used. A production filter's integrity test specification should be consistent with data generated during filtration efficacy studies.

We recommend you consider use of sterilizing-grade filters in series; this is a common practice.

Another FDA guidance document, "Guidance for Industry – Changes to an Approved NDA or ANDA", lists the criteria that require various submissions to the agency detailing the changes [3]. The document defines minor, moderate, and major changes applicable to sterile filtration and sets forth the notification requirements for each.

Minor changes, which are to be reported to FDA in an Annual Report, are not applicable to sterile filtration because of the criticality of the process.

Moderate changes are broken down into two categories: changes being effected (CBE) and changes being effected in 30 days (CBE 30). In each case, the changes must be reported to FDA in a supplement to an NDA (or ANDA, etc.), notifying the agency that the change has been implemented (CBE) or will be effected in 30 days (CBE 30) unless the firm receives notification from FDA within 30 days that the change has not been approved.

Filtration changes in the CBE category include "elimination of in-process filtration performed as part of the manufacture of a terminally sterilized product."

Filtration changes in the CBE 30 category include "changes to filtration parameters for aseptic processing (including flow rate, pressure, time, or volume, but not filter materials or pore size rating) that require additional validation studies for the new parameters" and "filtration process changes that provide for a change from single to dual product sterilizing filters in series, or for repeated filtration of a bulk."

Major changes, which require a prior approval supplement, include "changes from sterile filtered or aseptic processing to terminal sterilization, or vice versa," and "changes in materials or pore size rating of filters used in aseptic processing."

The EC Guide to Good Manufacturing Practice, Revision to Annex 1, Manufacture of Sterile Medicinal Products, published by The European Commission Enterprise Directorate-General, discusses several factors to which attention

should be given when pharmaceutical products are sterilized by filtration [4]. The document recommends that "the time between the start of the preparation of a solution and its sterilisation or filtration through a micro-organism-retaining filter should be minimized" and "there should be a set maximum permissible time for each product that takes into account its composition and the prescribed method of storage." It also recommends that "all solutions, in particular large volume infusion fluids, should be passed through a micro-organism-retaining filter, if possible sited immediately before filling."

Annex 1 also contains a section specifically addressing "Filtration of medicinal products which cannot be sterilised in their final container." The contents of that section are as follows:

82. Filtration alone is not considered sufficient when sterilisation in the final container is possible. With regard to methods currently available, steam sterilisation is to be preferred. If the product cannot be sterilised in the final container, solutions or liquids can be filtered through a sterile filter of nominal pore size of 0.22 micron (or less), or with at least equivalent micro-organism retaining properties, into a previously sterilised container. Such filters can remove most bacteria and moulds, but not all viruses or mycoplasmas. Consideration should be given to complementing the filtration process with some degree of heat treatment.

83. Due to the potential additional risks of the filtration method as compared with other sterilisation processes, a second filtration via a further sterilised micro-organism retaining filter, immediately prior to filling, may be advisable. The final sterile filtration should be carried out as close as possible to the filling point.

84. Fiber shedding characteristics of filters should be minimal.

85. The integrity of the sterilised filter should be verified before use and should be confirmed immediately after use by an appropriate method such as a bubble point, diffusive flow or pressure hold test. The time taken to filter a known volume of bulk solution and the pressure difference to be used across the filter should be determined during validation and any significant differences from this during routine manufacturing, should be noted and investigated. Results of these checks should be included in the batch record. The integrity of critical gas and air vent filters should be confirmed after use. The integrity of other filters should be confirmed at appropriate intervals.

86. The same filter should not be used for more than one working day unless such use has been validated.

87. The filter should not affect the product by removal of ingredients from it or by release of substances into it.

In 1996 the EMEA Committee for Proprietary Medicinal Products published a note for guidance on manufacture of the finished dosage form [5]. This guidance document asserts that the maximum acceptable bioburden prior to filtration must be stated in the application. It says that a pre-sterilization bioburden not exceeding 10 CFU/100 ml is acceptable "depending on the volume to be filtered

in relation to the diameter of the filter." If this level is exceeded, a bioburden reducing filter must be used in front of the sterilizing-grade filter to reduce the bioburden to the acceptable level. While the guidance leaves room for interpretation in respect to what type of filter this could be it states that "pore sizes of 0.22 µm or less are acceptable without further justification, in accordance with Ph. Eur.," implying that additional validation is not required.

USP 27 specifies tests for biocompatibility, extractables, endotoxins (pyrogens) and particulate release that are applicable to the filter membranes and cartridges used to sterile filter pharmaceutical products [6]. The European Pharmacopoeia contains similar requirements [7].

Filter manufacturers test their pharmaceutical-grade filters for particulates to ensure the filtered product will meet USP and Ph. Eur. Requirements for visible and sub-visible particles. These tests typically are performed in the product qualification stage of the filter product validation, usually with high purity water. The polymers used in cartridge fabrication are subjected to biocompatibility testing to ensure they meet pharmacopeial requirements. In addition, assembled filters are extracted with high purity solvents such as water and isotonic saline to ensure freedom from objectionable levels of extractables. As with particulates and especially chemical compatibility testing, the extractable test provides important information about potential product/filter interactions or whether the filter releases substances that could degrade product quality or otherwise adversely affect the patient.

In 2003, ISO published standard 13408-2, Aseptic Processing of Healthcare Products-Part 2: Filtration [8]. The standard specifies sterilizing filtration requirements for aseptically produced health care products and contains guidance for validation as well as routine operation of the filtration process. It also provides a list of terms and definitions applicable to sterile filtration of pharmaceutical products.

In fact, the document is a comprehensive source of information about sterilizing filtration, including sections on:

- Selecting filtration equipment based on data supplied by the filter manufacturer
- The filtration process and process parameters
- Validation of microbial retention by means of bacterial challenge testing, including information on the challenge fluid, challenge microorganisms and the need for determining fluid-specific microbial retention
- Design of the filtration system
- Routine process monitoring and documentation
- Maintenance and change control
- Operator training

ISO 13408-2 also includes an informative (as opposed to normative) annex describing information that is usually available from filter manufacturers.

One of the first standard test methods for determining bacterial retention of membrane filters was ASTM F 838-83 [9]. The standard utilized the reten-

tion of *Pseudomonas diminuta* (currently known as *Brevundimonas diminuta*) to evaluate membrane filter systems used for liquid sterilization. The specified test procedure required the filter to be challenged with a suspension of *B. diminuta* (ATCC 19146) at a level of 10^7 organisms per cm^2 of effective filtration area at a maximum test filter differential pressure of 206 kPa and a flow rate of 2 to 4 L per min per cm^2. Subsequently, the filtrate is passed through an analytical membrane filter disc which is then incubated on a solidified growth medium, allowing organisms not retained by the test filter to form visible colonies on the analysis membrane. As stated previously, ASTM F 838-3 is currently obsolete (i.e., is no longer supported by ASTM) but it has not been superseded. It is still useful as a standard method for distinguishing between sterilizing-grade filters rated at 0.2 and 0.4 μm nominal pore size; *B. diminuta* will be retained by the former but will penetrate the latter.

PDA Technical Report No. 26 (TR 26) is an extremely comprehensive guidance document covering all aspects of sterilizing filtration [10]. TR 26 contains sections on how filters work, filter selection and characterization, physical and mechanical characteristics, validation and bacterial retention, integrity testing, filter sterilization, and several appendices, including one on toxicity and extractables testing.

The validation and bacterial retention section of TR 26 is especially detailed, providing practical information regarding integrity test protocol development, product and surrogate fluids, bacteriostatic and bactericidal challenge fluids and how to deal with them, the use of filter media in place of the filter device, pressure differential and flow rate, duration, sampling, selection of the analytical membrane and interpretation of results.

3 Bacteria Challenge Test

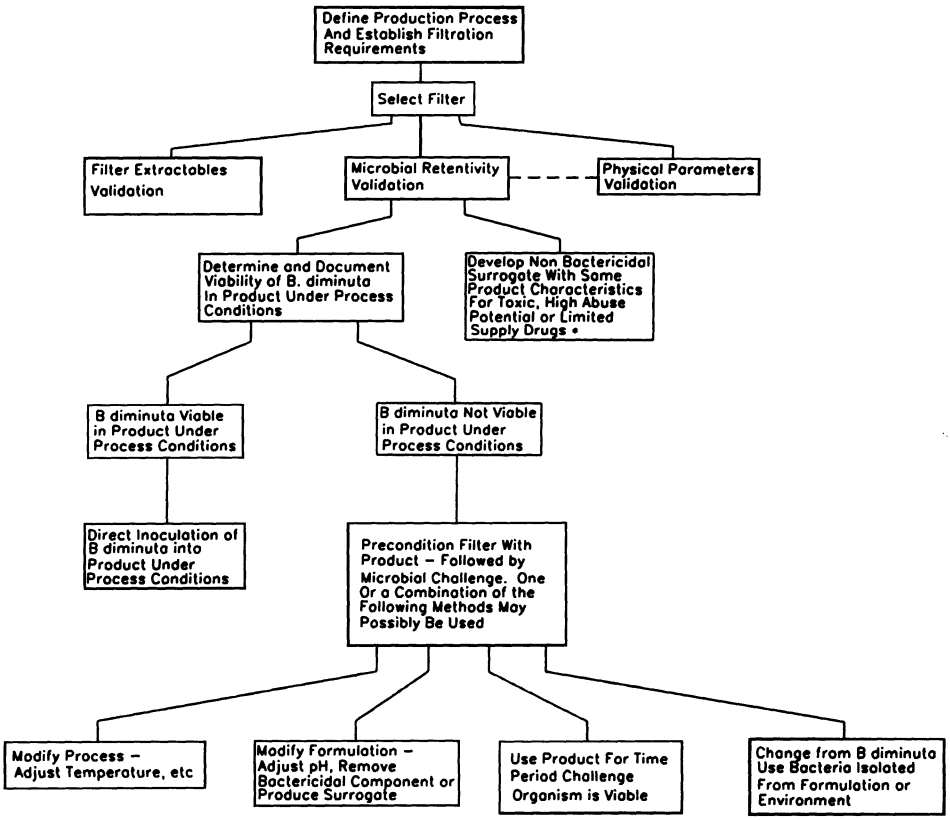
The purpose of a sterilizing-grade filter is to remove microorganisms that may be in the filtered solution from the filtrate. Successfully passing an integrity test demonstrates the filter's ability to remove (and to have removed) microorganisms from the filtered solution, but in the absence of data from a bacteria challenge test, the integrity test data are merely indirect indicators of the filter's ability to produce a sterile filtrate.

As previously mentioned, ASTM F 838-3 was developed as a standard bacterial challenge test, utilizing the retention of *Pseudomonas diminuta* at a minimum challenge level of 10^7 organisms per cm^2 of filter area to demonstrate effective bacterial retention of sterilizing-grade filters. The test utilizes an organism suspension of monodispersed cells in either saline lactose broth or normal saline. Specific methods for culturing the microorganism and preparation of the bacterial challenge stock and challenge suspensions are provided.

While the ASTM method provides a standardized means for evaluating the bacterial retention of sterilizing-grade filters, it fails to consider the potential

effects of the drug product solution on the filter medium or on the challenge microorganisms. The actual drug product may affect the pore structure of the filter, may have different electrostatic effects than the standard spore suspensions, and may change the size and shape of the challenge microorganisms [11]. FDA requires evidence that the sterilizing-grade filter will produce a sterile filtrate irrespective of the process parameters, solution properties or bioburden [10, 12].

In order to negate these potential effects, the microbial challenge is prepared using the actual drug product whenever possible. Before performing a bacteria challenge test with product, viability studies should be used to confirm that the drug product has no detrimental effects on the challenge organism. This can be accomplished by inoculating the challenge organism into the product to be filtered at a known level, then at intervals defined by the actual filtration process, the log value of the challenge organism concentration is determined.



*Concurrence of the appropriate regulatory agency should be sought prior to using this methodology.

Fig. 1 Decision tree for product Bacteria Challenge testing (Reprinted from [10] with permission)

If the challenge organism concentration is reduced due to the fluid properties, different bacteria challenge test methods may be used to overcome this incompatibility (Fig. 1).

While it is not a standard in the sense of ASTM F 838–83, TR 26 describes various bacteria challenge methodologies that can be used under various circumstances to evaluate the ability of the filter to retain organisms in the actual product to be filtered or a placebo product: nonbactericidal processes and fluids, placebo challenge, product recirculation with a challenge after recirculation, and use of resistant indigenous microorganisms in place of *B. diminuta.*

It is necessary to perform the bacterial challenge test in actual product under normal processing conditions for several reasons. The influence of the product and process parameters on the challenge microorganism has to be evaluated. The challenge organism could shrink due to high osmolarity of the product or prolonged processing times, or because of starvation due to the low nutrient content of the fluid. There also may be issues related to the compatibility of the filter with the product and the parameters of the process. The filter should not show any sign of degradation caused by exposure to the product. Also, the filter must not be adversely affected by the process parameters such as pressure, pressure pulses, flow rate, or time. Finally, there are two separation mechanisms involved in liquid filtration: sieve retention and retention by adsorptive sequestration [13–18]. In sieve retention the smallest particle or organism size is retained by the biggest pore within the membrane structure. The contaminant will be retained, no matter of the process parameters. This is the ideal. Retention by adsorptive sequestration depends on the filtration conditions. Contaminants smaller than the actual pore size penetrate such and may be captured by adsorptive attachment to the pore wall. This effect is enhanced using highly adsorptive filter materials, for example glass fiber as a pre-filter or polyamide as a membrane. Nevertheless certain liquid properties can minimize the adsorptive effect, which could mean penetration of organisms. When the fluid has such properties, the effect of adsorptive sequestration on retention will be reduced and may cause penetration. This has to be evaluated in specific product bacteria challenge tests.

If the product is nonbactericidal, the challenge test is performed by inoculating directly into the product a high level of the challenge organism, bearing in mind that the challenge level has to reach 10^7 per cm^2 at the end of the processing time.

If the mortality rate is too high, i.e., greater than one log, a different approach should be used. The product, and possibly the processing conditions, should be evaluated to determine why the challenge organism viability is being compromised. If the viability is affected by a toxic component in the product formulation the component might be removed or other product properties such as pH are modified as necessary to improve organism viability. This modified product is called a placebo. The placebo should match the product as closely as possible without adversely affecting the challenge organism. Critical variables are pH, ionic strength, osmolality, viscosity and surface tension.

If it is not possible to find a suitable placebo, the product itself can be circulated through the filter at the specific process parameters for the anticipated normal processing time, then flushing the filter extensively with water and then performing the challenge test as described in ASTM F838-38. Nevertheless such challenge test procedure would be more or less a filter compatibility test.

If the normal challenge organism, *B. diminuta,* is not viable in the product under normal processing conditions, other microorganisms which may be indigenous to the product may be suitable. These organisms may be isolated from the manufacturing environment or the product formulation and as such have the ability to survive within the product under actual production filtration conditions. Acceptable challenge bacteria should be capable of surviving or being propagated within the product to a concentration sufficient to deliver a minimum concentration of 10^7 per cm^2 of filter surface area, under actual processing conditions. The indigenous organisms should be able to be propagated in the actual product so their morphological and physiological characteristics are consistent with actual process isolates.

4 Extractable Test

Another important part of the validation process when applied to filtration in the pharmaceutical industry is to determine whether there are any substances related to the filter system that can be released into the process stream. Typically, filter cartridges are composed of various thermoplastic polymers used for the end caps and inner and outer cores, O-rings, gaskets and the membrane itself. Components of these materials include the monomers and polymers of which the materials are composed, degradation products of the thermoplastic compounds, plasticizers, anti-oxidants and various adhesives, which may be used in cartridge manufacture. All of these materials, their components and degradation products can potentially be extracted or leached into the drug product during the filtration process. This yields potentially a complex mixture of compounds with different functional groups, solubilities, and molecular weights at levels that challenge even the best analytical techniques. Nonetheless, tests for the presence of these compounds should be performed to ensure the purity of the drug product [10, 11, 19].

Filter manufacturers generally select the components of their pharmaceutical-grade filters based on meeting the requirements of the USP Biological Reactivity Tests, In Vivo, Class VI [20]. The physicochemical tests for plastics that are defined in USP 27 should be performed also [21]. The physicochemical tests require extracting a sample of the material to be tested with water at 70 °C for 24 h and evaluating the extract for nonvolatile residue, residue on ignition, heavy metals, and buffering capacity.

Advances in analytical technology have enabled investigators to determine with increased accuracy and higher sensitivity the substances that heretofore

were measured using nonvolatile residue (NVR) testing, prompting regulatory agencies in 1994 to dismiss NVR testing for this purpose [22]. Extractables testing is currently performed using a combination of methods such as gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared (FTIR), reverse phase high pressure liquid chromatography (RPHPLC), ultraviolet-visible spectrophotometry (UV-VIS), gel permeation chromatography with refractive index detection (GPC-RI), high pressure capillary electrophoresis (HPCE) and super-critical fluid extraction (SFE). Additional, "classical" analytical methods that can be used to evaluate extractables include pH, oxidizable substances, conductivity and heavy metals.

It is sometimes difficult, but not impossible, to measure extractables in the drug product because of interferences. Extractables from pharmaceutical-grade filter cartridges are normally in the microgram level and even the best analytical methods sometimes do not allow their detection in the presence of the drug product [23–25]. For this reason, water and other pure solvents are often used for this testing. Reif et al. used water and ethanol since much of the time these solvents are used for pharmaceutical production and purification processes, allowing their study to support the extractables analysis of most drug product solutions [19]. They used the extraction and analysis scheme shown in Fig. 2 to measure extractables from a variety of pharmaceutical-grade filter cartridges as shown in Fig. 3.

While this study was performed with water and ethanol, such conditions do not represent true process realities and it may be advisable, depending on

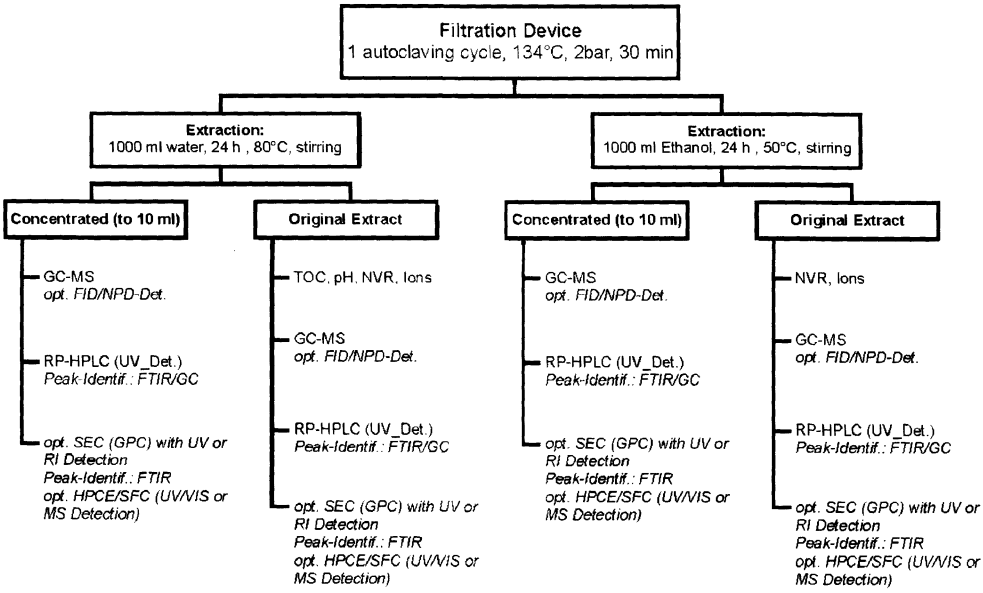


Fig. 2 Extractable test schematic for water and ethanol, 24 h at 80 and 50 °C, respectively (from [19] courtesy of Reif)

Cartridge G (PP fleece prefilter)	Cartridge B (GF fleece prefilter)	Cartridge C (Membrane prefilter)	Cartridge D[a] (PP fleece prefilter)	Cartridge E[a] (GF fleece prefilter)	Cartridge F[a] (PP fleece prefilter)
Cyclohexane	Succinic acid	Methyl-2,4-pentadiol	Caprolactam	Caprolactam	Propionic acid
2,6-Di-*tert*-butylphenol	8-Oligoaliphates	Glycerol	Propionic acid	Dodecanol	Butyl-1-methoxybenzene
Hydroxybenzoic acid	2,4-Bis(1,1-dimethylethylphenol	2,4-Bis(1,1-dimethylethyl)phenol	4-(1-Methyl-1-phenylethyl)phenol	High MW N-containing compound	Naphthalenic compound
2,6-Di-*tert*-butylcresol	Ethoxyethyl benzoate	Butylphenoxyacetic acid	2,6-Di-*tert*-butylcresol	2,6-Di-*tert*-butylcresol	Polyether
Stearic acid	Diethyl terephthalate	Ethoxybenzoic acid	Benzoic acid	High MW aromatic	2,6-Di-*tert*-butylcresol
8-Oligoaliphates	Myristic acid	4-Oligosiloxanes	Dibutylphthalate	High MW aromatic	Dibutylphthalate
4-Oligosiloxanes	Palmitic acid	6-Oligoaliphates	2,2-Dimethoxy-1,2-diphenylethanone	Cyclododecane	High MW N-containing compound
Polyalkylic ether	Octadecene	Diethylphthalate	Butylmethoxybenzene	Butyl-4-methoxyphenol	Hexadecene
3-Oligosiloxanes	Stearic acid	Polyalkylic ether	5-Oligosiloxanes	3,3-Thiobispropionic acid	
	Polyalkylic ether		7-Oligoaliphates	7-Oligoaliphates	
	2-Oligosiloxanes		Caprolactam derivative	6-Oligosiloxanes	
	Glass fibers		Triphenylphosphine oxide	Polyether	
				Glass fibers	

[a]Identification of the RP-HPLC peaks by FTIR is still in progress; extractables list may be incomplete.
Source: Reif et al. (1995b).

Fig. 3 Extractable listing of different sterilizing grade filters (from [19] courtesy of Reif)

the process conditions and the solvents used, to perform extractable tests with the drug product itself. Formerly, these tests were performed only with the drug product solvent, but not with the actual drug product because the drug product interfered with the analysis. However, using modern analytical techniques, it may be possible to evaluate the extractables using the actual drug product.

The data generated from water and ethanol extractions by various analytical methods provide a comprehensive picture of the type and amount of material that might find its way into the filtered product. Figure 3 shows the compounds extracted from various pharmaceutical-grade filters produced by various manufacturers. It is important to note that this testing represents "worst case" conditions, concentrating the compounds into a relatively small volume of extract over 24 h at 80 °C in water and 50 °C in ethanol. Even under those conditions, the extractables from pharmaceutical-grade filter cartridges produced by various manufacturers were determined to be less than 1 ppm in an extraction volume of 1300 mL [19]. In actual production filtration, the filtered volumes are often thousands of liters and the temperature rarely varies much from room temperature, allowing one to conclude that extractables should not present a problem under actual conditions of use for the majority of pharmaceutical products.

Most pharmaceutical manufacturers have in their quality control and research laboratories the sophisticated analytical instruments necessary to perform extractables testing in-house. In the event such equipment is unavailable, the validation services units of most filter manufacturers are capable of performing extractables testing using sophisticated separation and detection methodologies such as GC-MS, FTIR, RP-HPLC, UV-VIS, GPC-RI, HPCE and SFC.

5 Chemical Compatibility Test

Chemical compatibility testing is used to determine the effect of the liquid on the filter and the effect of the filter on the liquid. Most filter manufacturers perform chemical compatibility testing on their filters over a period of seven days, after which the filter elements are checked for performance and integrity [11]. Despite the tests performed by the filter manufacturer, additional, specific chemical compatibility testing should be performed to ensure the filter is compatible with the solution to be filtered. All of the filter system components under investigation should be included in the chemical compatibility test. In addition to the membrane, these include membrane support materials, cartridge shell and housing material, and o-rings used to seal the cartridge and the housing.

For microbial challenge studies, the FDA recommends use of the drug formulation to provide an assessment of the effect of drug product on the filter matrix and includes "compatibility of the material or formulation components with the filter itself" in a list of factors that can affect filter performance [2].

Loss of yield or product ingredients due to adsorption can be a problem for certain products and the product and filter should be evaluated to ensure these effects do not occur, or if they do, they do not adversely affect the filter or the solution to be filtered [26]. For example, certain filter membranes can adsorb preservatives such as benzalkonium chloride and chlorhexadine. These membranes may to be saturated by the preservative to avoid product preservative loss, which can be detrimental to its microbiological quality and stability.

Similarly, adsorption of proteins from a biological product is an unwanted condition. To optimize the yield of such proteins within an application, adsorption trials have to be performed to find the optimal membrane material and filter construction. Flow conditions and pre-rinsing procedures can then be developed on the basis of these tests.

Chemical incompatibilities can be subtle and often are influenced by a combination of factors related to the composition of the product formulation. The aim of chemical compatibility testing has to be to find subtle incompatibilities, which may occur due to a mix of chemical components and entities or specific process conditions. Testing with individual components of the product formulation may not reveal these incompatibilities.

Elevated temperatures or prolonged filtration times also may result in filter incompatibility (Fig. 4).

Chemical incompatibility can affect filter extractables and leachables even if the microbial retention capability of the filter membrane is not compromised. Because of this, appropriate compatibility tests have to be performed with the actual drug product and process conditions. In many instances

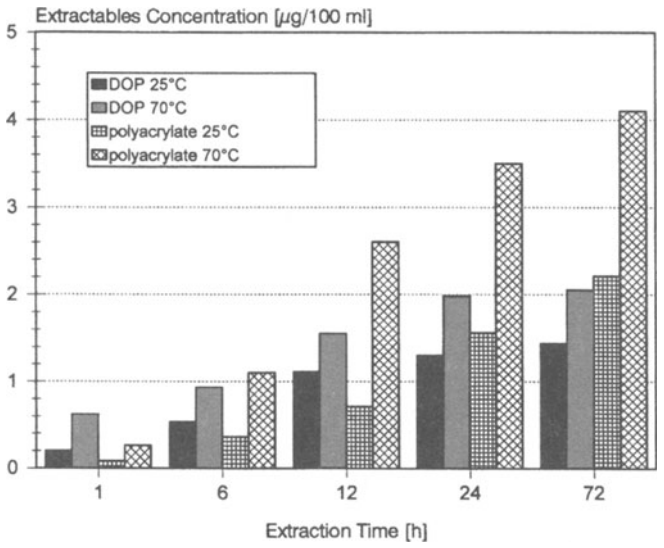


Fig. 4 Example of leachables increases due to temperature increases (courtesy of Sartorius Group)

	Bubble point [bar]	Burst Pressure [bar]	NVR [mg/l]	BC-Test Brev. Dim.
After extraction with RO-Water	3.6	0.42	11	sterile
After extraction with 0,1 M HCl	3.5	0.14	156	non sterile

Fig. 5 Example of subtle incompatibilities of a filter membrane (from [19] courtesy of Reif)

integrity tests before and after the submersion of the filter in the solution to be filtered product will reveal incompatibilities. Incompatibilities often will be revealed, however, only by subjecting the filter to process conditions with the product. In any case, sole reliance should not be on integrity testing. Non-volatile residue testing along with integrity testing can be useful where the filter is integral but shows elevated extractable levels (Fig. 5). Scanning electron microscopy can be utilized in these situations to detect any chemical attacks on the membrane surface.

6 Other Tests

USP 27 includes a general chapter, Biological Reactivity Tests, In Vitro, designed to evaluate the effect of elastomeric and polymeric materials on mammalian cell cultures [27]. Three tests are described: the Agar Diffusion Test, the Direct Contact Test, and the Elution Test. The chapter requires a decision regarding which type and number of tests are required based on the material, the product and its intended use. It also cautions "Other factors that may also affect the suitability of sample for a specific use are the polymeric composition; processing and cleaning procedures; contacting media; inks; adhesives; absorption, adsorption, and permeability of preservatives; and conditions of storage. Evaluation of such factors should be made by appropriate additional specific tests before determining that a product made from a specific material is suitable for its intended use." The extraction is typically performed using sodium chloride injection (0.9% NaCl), serum-free mammalian cell culture media or serum-supplemented mammalian cell culture media.

Particulates are critical in sterile filtration, specifically for injectable products. The USP provides tests and acceptance criteria for particulates in injectable solutions. Sterilizing-grade filters that are incompatible with the filtered solution or which release particulates because of inadequate flushing can result in failure of the filtered solution to meet pharmacopeial particulate requirements. Filters should be tested for particulate release, evaluating the filtrate with particle counters. Such tests also should be performed with the actual product under process conditions to ensure that the product and the process conditions

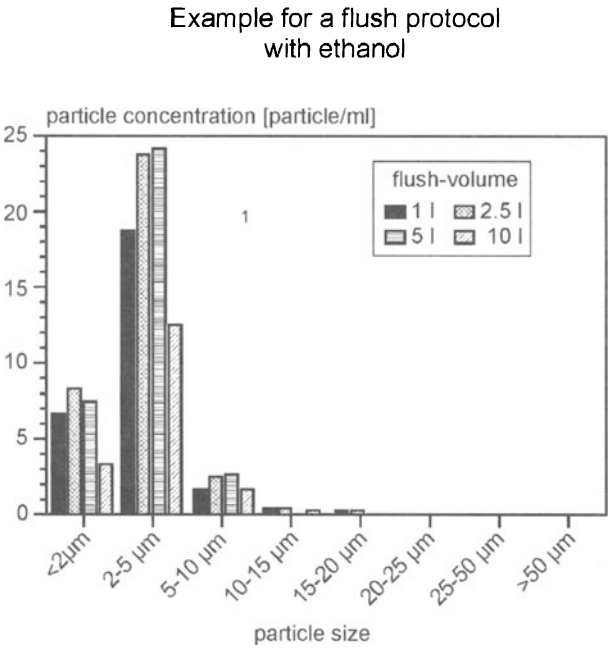


Fig. 6 Example of a flush protocol of a filter cartridge (courtesy of Sartorius Group)

do not result in an increased level of particulates within the filtrate. Specific flushing protocols can be established (Fig. 6). Additionally, these tests are useful for pre-filters to lower the risk of a particulate contamination in the filtration process.

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Integrity Testing

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Abstract To ensure that sterilizing grade filters in aseptic processing worked as required, such filters are integrity tested. Integrity tests, like the bubble point, diffusive flow or pressure hold test, are non-destructive tests, which are correlated to a destructive bacteria challenge test. This correlation verifies the integrity test limits the filters have to pass. Integrity tests are required by regulatory authorities. The post filtration integrity test is a must, pre filtration integrity testing is recommended. The different tests have specific limitations therefore there is no overall, best integrity test, which can be utilized for every filtration system.

Keywords Membrane filter · FDA · EMEA · Bacteria challenge test · Bubble point test · Diffusion test · Pressure drop test · Water intrusion test · Multipoint diffusion test · Automatic integrity tester

1 Guidelines and Documents

Sterilizing grade filters require to be tested to assure the filters are integral and fulfill its purpose. Such filter tests are called integrity test and can be performed before and after the filtration process. Sterilizing grade filtration would not be admitted to a process, if the filter would not be integrity tested in the course of the process. The integrity testing of filters is central to the practice of aseptic processing. The exercise is seen to stand between certainty and potential failure. At the moment when a filter is removed from its shipping container preparatory to use, only the proper performance of an integrity test attests to its appropriateness. Even its identifying label is no guarantee, mistakes do occur. This fact is also established in several guidelines, recommending the use of integrity testing, pre- and post filtration. This is not only valid for liquid, but also air filters.

The FDA, Draft Guidance "Sterile Drug Products Produced by Aseptic Processing" (2003) quotes "Normally, integrity testing of the filter is performed prior to processing, after the filter apparatus has already been assembled and sterilized. It is important that integrity testing be conducted after filtration to detect any filter leaks or perforations that might have occurred during the filtration. Forward flow and bubble point tests, when appropriately employed, are two integrity tests that can be used. A production filter's integrity test specification should be consistent with data generated during filtration efficacy studies." There is a major difference in this draft guidance, as it describes the pre-filtration, post sterilization integrity test as a requirement. This factor can be of disadvantage to the filter users, as the pre-filtration post sterilization integrity test is in instances difficult to perform. Furthermore, the major value of such integrity test is mainly economical, as if the filter fails post filtration testing, the filtered batch needs to be reprocessed or discarded. The choice, whether the pre-filtration test is of value should stay with the filter user and not necessarily be dictated by regulations. A similarity to the FDA Draft can be seen in the following guideline.

EC Guide to GMP, Revision to Annex 1 (May 2003), "Manufacturing of Sterile Medicinal Products" quotes "The integrity of the sterilised filter should be verified before use and should be confirmed immediately after use by an appropriate method such as a bubble point, diffusive flow or pressure hold test. The integrity of critical gas and air vent filters should be confirmed after use. The integrity of other filters should be confirmed at appropriate intervals." Again, the pre-filtration integrity test is recommended.

Other guidelines of interest are as follows.

"Guide to Inspections of High Purity Water Systems", "Guide to Inspections of Lyophilization of Parenterals" and also in the *CGMP document 212.721 Filters*

1. The integrity of all air filters shall be verified upon installation and maintained throughout use. A written testing program adequate to monitor integrity of filters shall be established and followed. Results shall be recorded and maintained as specified in 212.83.
2. Solution filters shall be sterilized and installed aseptically. The integrity of solution filters shall be verified by an appropriate test, both prior to any large-volume parenteral solution filtering operation and at the conclusion of such operation before the filters are discarded. If the filter assembly fails the test at the conclusion of the filtering operation, all materials filtered through it during that filtering operation shall be rejected. Rejected materials may be refiltered using filters whose integrity has been verified provided that the additional time required for refiltration does not result in a total process time that exceeds the limitations specified in 212.111. Results of each test shall be recorded and maintained as required in 212.188(a).

ISO 13408 "Aseptic processing of health care products", (2003) [15]

Section 7: Filtration process

7.1.2 Written integrity test procedures shall be established including acceptance criteria and methods of failure investigation and conditions under which the filter integrity test can be repeated.

Notes

1. Information from the filter manufacturer can be useful in designing and validating integrity test procedure(s) based on gas flow through wetted filter.
2. It should be demonstrated that the integrity test conditions can be supported by standardized bacterial retention testing. The standardized bacterial retention tests should use a challenge level of at least 10^7 colony forming units per square centimeter, with filters representative of standard production filters approaching the acceptance test limit.

7.1.3 One or more appropriate wetting fluids shall be selected. These shall be the filter manufacturer's recommended reference wetting fluid or the actual fluid to be filtered. In the latter case, the appropriate integrity test value specification shall be established and validated.

7.1.4 For air and gas filters, appropriate frequency for physical integrity testing shall be established.

Section 8: Filtration system design

8.10 The filtration system should be designed to permit in-place integrity testing as closed system prior filtration.

Section 9: Routine process

9.1 The routine process for filtration shall be documented in a written procedure.
9.2 Due consideration shall be given to:
f) Control testing including integrity test procedures and bioburden monitoring.
9.3 The validated physical integrity test of a sterilizing filter shall be conducted after use without disturbing the filter in its housing. Physical integrity testing of a sterilizing filter shall be conducted before use where the design of the filtration system permits.

Section 10: Process documentation

10.1.2 Batch manufacturing records shall include, where appropriate:
h) Filter integrity test result and assessment

Section 11: Maintenance and change control

11.1 The filter user shall establish, document and execute calibration and maintenance procedures for the filter and filtration system and test instrument. A change control procedure shall be defined and documented for any change of the defined process parameters.

Section 12: Operator training

Filtration-specific operator training shall be implemented, for:
a) Integrity test theory
b) Failure investigation procedures and measures taken in case of integrity test deviations

USP (United States Pharmacopeia) 27 (2004)
Guide to Good Pharmaceutical Manufacturing Practice (Orange Guide, U.K., 1983)
PIC/S, July 31, 2001 – Recommendation on the Validation of Aseptic Processes
PDA (Parenteral Drug Association), Technical Report No. 26, "Sterilizing Filtration of Liquids" (March 1998) [13] it quotes in Section 4: Physical Integrity testing "Integrity testing is required for all sterilizing filtration applications. Physical integrity tests are based upon the gas flow rate through a filter wetted with a suitable liquid, as a function of applied test pressure. Hydrophobic filters also can be tested by measuring the membrane's resistance to water flow as a function of applied pressure.

Manual and automated test methods are available. The chosen integrity test method and acceptance criteria must be validated and should correlate to bacterial retention."

The PDA Technical Report 26 [13] is probably the most comprehensive document, which not only describes the integrity test methodologies, criteria and

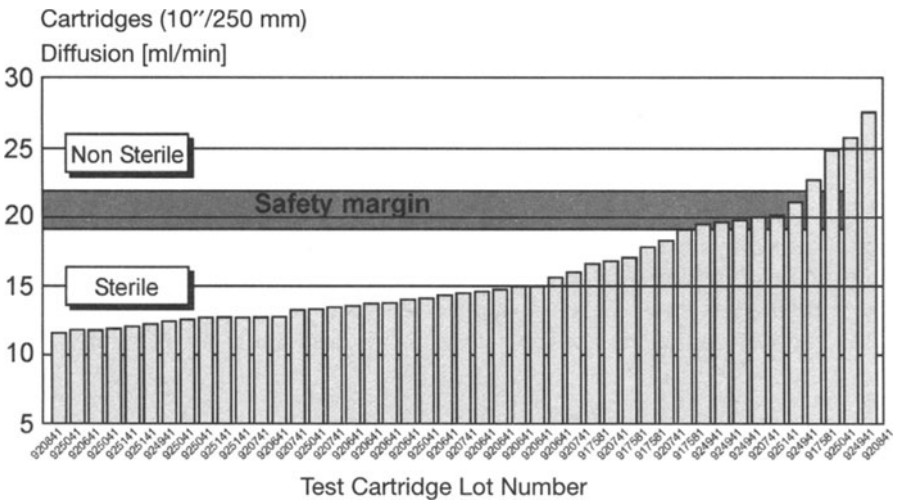

Fig. 1 Integrity test/bacteria challenge correlation chart (courtesy Sartorius AG)

maximum allowable repeats of an integrity test, but also liquid filter validation in details. This document also describes the advantages and disadvantages of the individual test methodologies, which can be of help, when one requires a trouble shooting guide.

Integrity tests, such as the diffusive flow, pressure hold, bubble point or water intrusion test, are non-destructive tests, which are correlated to the destructive bacteria challenge test with 10^7 per square centimeter *Brevundimonas diminuta*. Derived from these challenge tests specific integrity test lim-

Table 1 Bubble point values for different wetting agents using cellulose acetate 0.2 µm (courtesy Sartorius AG)

Product	Bubble point value
Water	3.20 bar
Mineral oil	1.24 bar
White petrolatum	1.45 bar
Vitamin B complex in oil	2.48 bar
Procainamide HCl	2.76 bar
Oxytetracyline in PEG base	1.72 bar
Vitamin in aqueous vehicle	2.07 bar
Vitamin in aqueous vehicle	2.69 bar
Iron dextran	2.83 bar
Vitamin E in oil base	1.66 bar
Solution preserved with benzyl alcohol	2.14 bar
Diazepam in glycol base	1.93 bar
Digoxin in glycol base	2.14 bar

its are established, which are described and documented within the filter manufacturer's literature (Fig. 1). The limits are water based, i.e. the integrity test correlations are performed using water as a wetting medium.

If a different wetting fluid, respectively filter or membrane configuration, is used, the integrity test limits may vary. Integrity test measurements depend on the surface area of the filter, the polymer of the membrane, the wetting fluid, the pore size of the membrane and the gas used to perform the test. Wetting fluids may have different surface tensions, which can depress or elevate the bubble point pressure (Table 1). The use of different test gases may elevate the diffusive gas flow. Therefore appropriate filter validation has to be established to determine the appropriate integrity test limits for the individual process.

2
Pre-Requisites for Integrity Testing

2.1
In General

There are a number of possible integrity tests, for example bubble point, diffusive airflow measurements, both single-point and multipoint, pressure hold or decay evaluations, and water intrusion assessments, whose endpoints are correlative with the organism retention stipulated for "sterilizing" filters [14]. These tests can be performed manually, or by use of automated testing machine. The automatic test instruments are to be preferred as they eliminate the subjectivity of the manually performed analyses.

With the exception of the water intrusion test, designed expressly for assaying hydrophobic filters, the other integrity tests are based on measurable airflow phenomena that result when wetted membranes are exposed to air pressures. The two principal integrity tests are the bubble point and diffusive airflow measurements, whether performed manually or by automated test machine. The pressure hold/decay test is derivative of diffusional airflow testing.

2.2
The Wetted Membrane

The integrity testing of hydrophilic membranes is based on air passage through wetted filters. The wetting fluid, commonly water, is retained within the pore structure by capillary forces. The smaller the pore the higher the pressures are required to free the pore of the wetting agent. On the other hand smaller pores have also been found to be difficult to wet. This probably is due to the fact that the wetting agent flows through the larger pore sections without even entering the smaller pore sizes. For this reason specific techniques are utilized to achieve reliably complete wetting of the entire pore structure. Reliable results require

that the filter pores be thoroughly filled with wetting solution. Many filter polymers not being completely hydrophilic, care must be taken to ensure complete wetting.

2.3 Imperfect Wetting Effects

Meltzer and Meyers [2] explored the effect of wetting agent on the bubble point of hydrophobic microporous membrane using different liquids, both aqueous and non-aqueous. Bubble point measurements using water as a test fluid with materials having any degree of hydrophobicity are rather unreliable. Less-polar solvents are more effective, hence, the use of alcohols and of aqueous alcoholic solutions. For the liquid-filter couple to involve a complete wetting action, both components must have matching polarities, the result of similar cohesive densities.

The greater the reluctance of the polymeric filter to become wetted by water, the greater the possibilities of imperfect wetting. Thus, there is a greater variability in the bubble points of microporous fluorocarbon membranes like PTFE, even when wetting agent is used, than there is for more wettable polymeric membranes.

In imperfect wetting, a pore channel may be so partially filled with the liquid as to give any "bubble point" value between zero and the true bubble point magnitude (Fig. 2). If prewet with isopropanol, for example, a hydrophobic 0.2 µm-rated membrane tested with water can exhibit a bubble point of anywhere between about 20 and 60 psig (1.38–4.13 bar), corresponding to the respective surface tension of isopropanol (20–22), and of water (68–74 dynes/cm) – about a threefold difference [3].

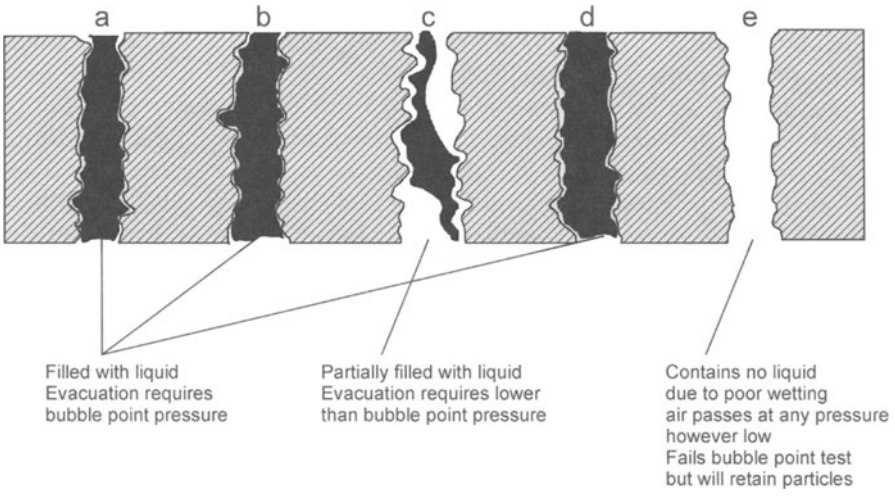

Fig. 2a–e Bubble point failure due to imperfect wetting (courtesy of [12])

2.4 Wetting Procedure for Membrane Filters

Membrane filters must be fully wetted preparatory to performing an integrity test, whether of the bubble point or diffusive airflow type. There is no uniform standard procedure. It seems reasonable that the filter manufacturer's protocol should be followed. Many filter manufacturers recommend specific flush protocols for their individual filter configurations and membrane polymers. Some filter manufacturers provide troubleshooting leaflets for use if wetting problems occur. Such troubleshooting guides lead the user through a flowchart that gives practical advice and recommendations if wetting the filter membrane is a problem. This is especially important when wetting problems of the filter result in a false negative integrity test result.

Usually, soaking the filter by placing cartridges into a container of water will not suffice, depending on the membrane material and pore size of the filter element. A dynamic water flow is required. One manufacturer stipulates that the 10-in. filter cartridge should be soaked in the housing for 5 min under enough water pressure to expel all the air and fill the housing, as evidenced by water exiting from the vent valve. Another filter supplier recommends the use of a differential pressure of 7 psi (0.5 bar), the inlet pressure exceeding 14.5 psi (1 bar), and the outlet side pressure (so-called backpressure) being maintained at 7 psi (0.5 bar). The flushing period under these conditions should last for 5 min with an appropriately vented filter system. One procedure calls for water to be rinsed through the housing for 2–3 min at 15–30 psi (1–2 bar) with the downstream valve so regulated as to permit a flow of 2–3 gal/min (8–12 L/min). An extensive study involving 10-inch cartridges of polyvinylidene fluoride (PVDF), hydrophilic PVDF, polyethersulfone, each from more than one manufacturer and of Nylon 66 is reported. Wetting conditions such as those just described were used effectually [4]. This technique, having been applied successfully to several cartridge types secured from different filter suppliers, would seems to be of proven general effectiveness.

Recalcitrant cases may benefit from the use of hot water (100–200 °F, 38–84 °C). Nevertheless temperature influences have to be avoided by flushing the filter system with cold water afterwards or to let the system cool down before the integrity test is performed. The use of an aqueous alcoholic solution such as 50–70% isopropanol or ethanol, followed by a water flush (promptly, to prevent evaporation of the alcohol) is a very effective means of achieving wetting. Such fluids usually wet the filter more readily than water or product. This procedure is usually used as a drastic measure to achieve a properly wetted membrane.

An aqueous alcoholic bubble point determination serves as a referee test in cases where incomplete water wetting may be a problem. For example, such test is recommended within PDA Technical Report No 26 [13] to assure a failed integrity test measurement is not a false negative test result due to wetting problems.

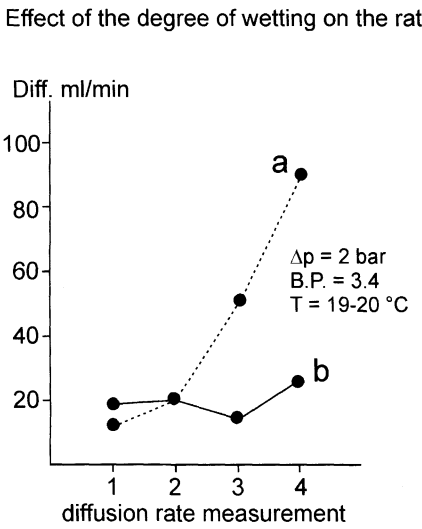

Fig. 3 Diffusive flow measurements at different stages of wetting (from [18])

When utilizing the diffusive flow test, as in bubble point testing, wetting of the membrane to be tested is essential, perhaps even more so than in bubble point testing, because even non-wetted smaller pores will be detected by the diffusive flow test. In Fig. 3 the two curves show the measurement of diffusive flows at two different wetting stages. Curve "a" shows four repeated diffusive flow measurements made without rewetting the membrane tested. There are two mechanisms that increase the diffusive level over the repeated tests: thinning of the water layer within the membrane matrix and drying out of some of the pores. In test "b" the membrane was wetted out after each individual diffusion test. As is apparent, if the entire thickness of the membrane is not wetted properly, the diffusive flow test will give a false negative result. It is of absolute importance that the entire membrane be flushed and completely wetted.

2.5 Product as the Wetting Liquid

More often, post-filtration integrity testing is performed using the product as the final wetting agent simply because removing it by water flush may require too large an amount of water, which would elevate the process costs [5]. This wetting procedure also finds its use during the pre-filtration integrity test to avoid any dilution of the actual product with a foreign wetting agent, for example water. Contact between certain membranes and various pharmaceutical preparations can produce depressed bubble points, lower than the values for water (Table 1). Depending on the filter material and/or product ingredients used, the depressed bubble point can be restored, more or less, but mostly less,

by copious washing with water. Subtle wetting effects may be at work here whose surface physics is not comprehended. The surface tension differences between the product and water contribute to the anomaly.

Efforts to flush with water before the final integrity test is performed in order to obtain pre- and post-filtration bubble points for water may not avail. For example, it was reported that Nylon membranes became so fouled by proteins in an albumin filtration process that often the filters could not be wetted by water and false negative results were obtained. This was also found to occur with products containing Tween, a synthetic detergent. Even after flushing with large volumes of water, surface tension-reducing properties were seen. In such cases, pre- and post-filtration comparisons may usefully be performed using product as the wetting liquid. The displacements in bubble point values are ascribed to unknown wetting effects, largely to the influence of the surface tension values of the product. They are assumed not to reflect on the organism removal capabilities of the membranes.

2.6 Temperature Stability

It is important that during integrity testing the temperature should be kept constant and within a defined range as recommended by the filter manufacturer. The temperatures of the test liquid, filter system, and test gas should be the same, otherwise irregularities will result. For example, it has been evaluated that a 10 °C temperature increase was found to translate into a 2% decrease in the bubble point value owing to lower surface tension of the test liquid, depending on the test liquid. The bubble point phenomenon is sensitive to the influences of temperature through its effect on the liquid's surface tension λ, which is one of the parameters defining it. The influence of temperature on surface tension is shown in Fig. 4. The higher the temperature, the lower the surface tension. This causes diffusive flow increases to an extent dependent on

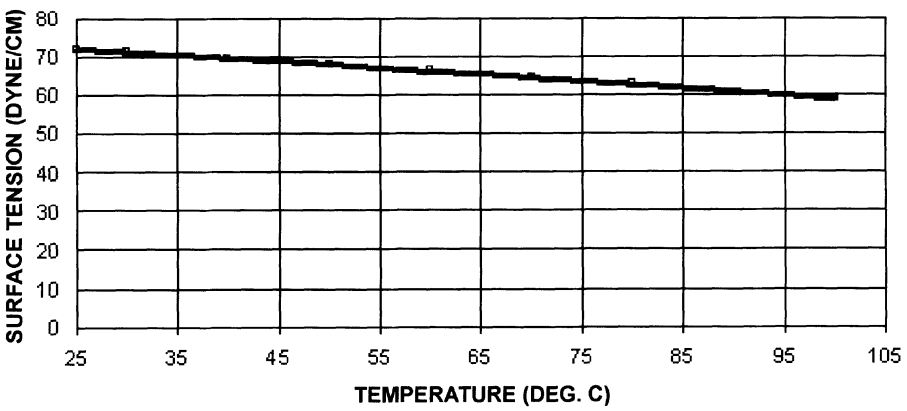

Fig. 4 Decline in surface tension of water due to temperature increase (data from literature)

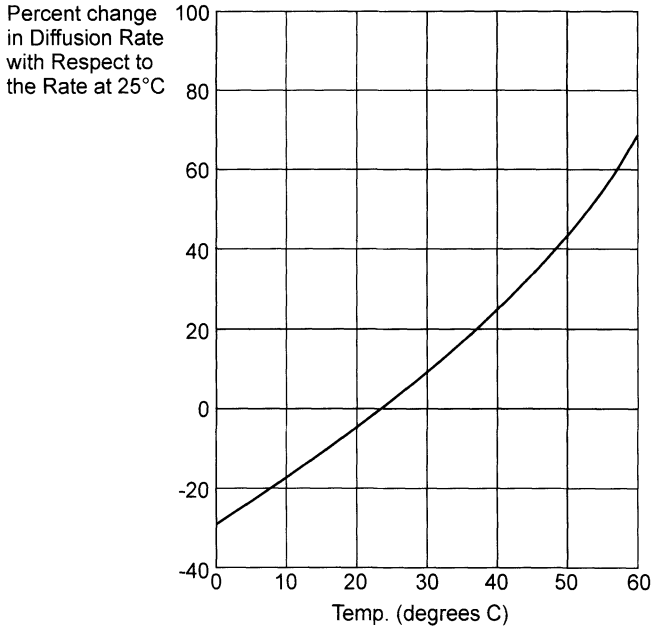

Fig. 5 Diffusive flow change due to temperature increase (data from literature)

the wetting liquid. Moreover, if the surface tension were to decreases enough the diffusive flow pressure measurement would become the bubble point.

Diffusive airflow values reflect the workings of Henry's Law governing the solubility of gases in liquids. Unlike bubble point, the polymeric nature of the filter, as also the chemical and physical character of the wetting liquid have no influence on the integrity test values other than those that impact the gas-in-liquid solubility and the thinning of the wetting liquid layer. Temperature does, however, affect the solubility of gas in liquid (Fig. 5).

The effect of temperature on nitrogen gas diffusion was found to depend on two factors, the solubility of the gas in the liquid and the diffusion coefficient of the gas. The solubility of gas in liquid decreases with increases in temperature. There is therefore less gas to diffuse, diffusion is minimized. The reverse is true for the diffusion coefficient, a quantity that reflects the viscosity of the liquid, which decreases with temperature and promotes the rate of diffusion. For example, up to 60 °C, nitrogen gas diffusion increases with decreasing liquid viscosity at a faster rate than the solubility of the nitrogen decreases. The result is an overall increase in diffusion as temperature increases, until the 60 °C level is attained.

More important are temperature changes during the integrity test. Most automated integrity test systems measure the pressure drop within the upstream volume of the housing and convert this pressure drop to a diffusive flow level. If the temperature increases, the pressure drop will be masked by an increase in the upstream pressure due to the temperature. This would create a false pos-

itive integrity test result. An upstream pressure change caused by temperature changes can be calculated:

$$\Delta P = \frac{P_{test}\,(T_1 - T_o)}{T_o} \qquad \text{(eq. 1)}$$

where ΔP=upstream pressure change (mbar), P_{test}=diffusion test pressure (mbar), T_o=absolute temperature (K) at time 0, and T_1=absolute temperature (K) at time *t*.

A temperature change in the system from 20 to 21 °C (68 to 70 °F) at a test pressure of 40 psi (2.7 bar) results in a pressure change of 9.2 mbar, a significant quantity.

Hofmann [6] illustrated the temperature effect on pressure change that was caused simply by hand-touching a 10-inch (25.4 cm) sanitary test housing for about 5 s. Filter housings and integrity test connections such as tubing should not be touched, because this will have an immediate effect on the pressure. Air conditioning units that start up during the test and are close by may affect the temperature and with it the test results.

Wallhäusser [7] holds that because of the sensitivity of diffusive airflow to temperature, it may not be suited to initial integrity testing. Initial integrity testing might be performed after the steam sterilization of the filter, to detect pore-size changes, if any, that might be caused by heat-induced stress releases. Wallhäusser points out that the cooling phase following steam sterilization of the filter and housing assembly would necessarily be prolonged. Diffusive air flow testing could be impractical for reasons of the time required. Figure 6 shows an example of test failures due precisely to such temperature influences. In this case filter capsules (disposable filter units) were tested directly after autoclaving. The filters in tests 1 and 2 (left and middle) were still warm when the integrity test was performed. The test gas cooled down during the test period, and therefore an excessive pressure drop was measured. These tests showed a false negative result.

Scheer et al. [8] elaborate on the situation, pointing out that passive cooling following steam treatment produces temperature imbalances due to vastly different heat transfer rates between metallic and polymeric components of the system, while active cooling with either a gas or liquid can easily be taken past ambient. The test equipment should be allowed to equilibrate at room temperature before being utilized.

Erdem [9] observes that among the temperature deviations that can affect the integrity test is caused by the temperature difference between the air surrounding the filter housing and the product to be filtered, which will also be used as the wetting agent. For example, in large-volume parenteral (LVP) production the water is usually at a higher temperature when filled into the mixing vessels. The mixture is then used to wet the filter to perform the prefiltration integrity test. If the vessel is not cooled under controlled conditions, the temperature of the wetting liquid can vary. This will affect the integrity test

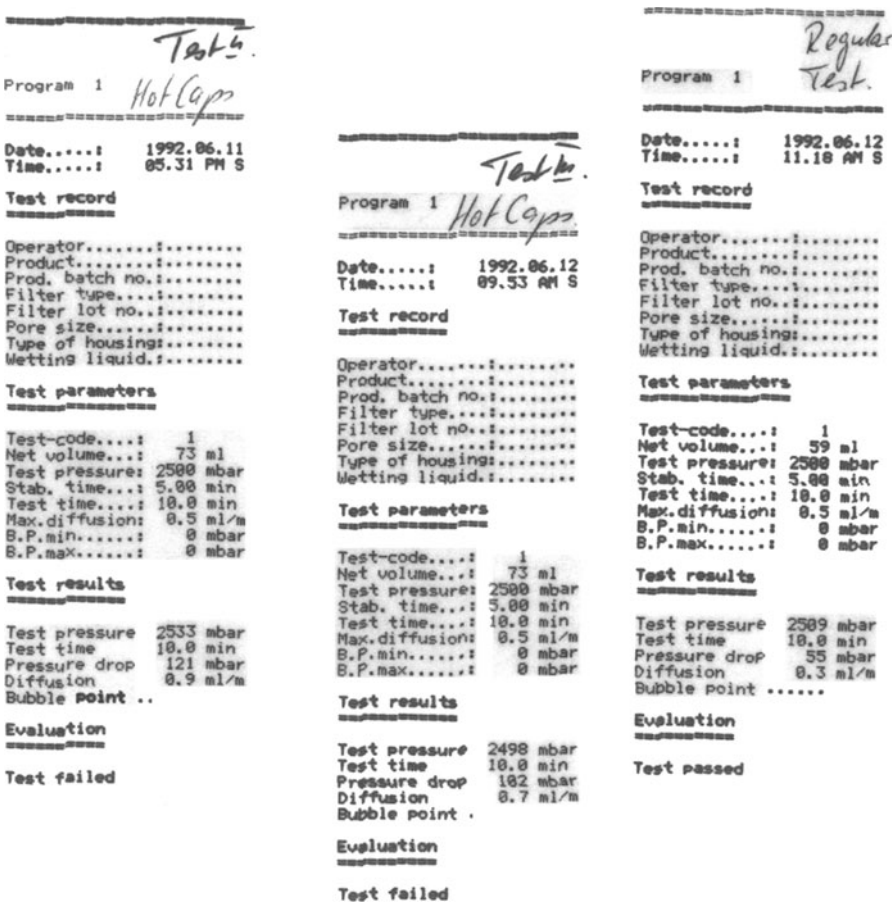

Fig. 6 Examples of test failures due to temperature differences

results. Temperature deviations are possible due to air movement, personnel, or even other equipment that is close by.

Temperature aberrations will cause errors in the results. For this reason, commonly used integrity testers print out a graph of the integrity test conditions during the test interval. It is then easy for the user to see when a temperature increase, and with it a pressure rise, occurred. Moreover, the test unit's software should be able to compare the measured values between tests. If a measured value drifts out of range, the test machine should abandon the test and display an alarm.

Scheer et al. [8] studied the effects of temperature on the diffusion test "because it is the least subjective of the integrity measures". These investigators confirm that serious errors in test results are possible unless temperature and volume factors are recognized and accommodated. They observe that "the exigencies of field filter testing may only rarely allow the needed degree of con-

trol". Scheer et al. conclude, however, that it should be possible to establish an offset or bias as a correction factor when comparing the test results in the field with those that the filter manufacturer presents.

2.7 Extent of Wetting

The bubble point equation is

$$P = 4\lambda \cos\theta/d$$

where λ=surface tension of the liquid, cosine θ=angle of wetting, d=pore diameter.

Perfect wetting has an angle of zero, the cosine of which has the value of 1.

However, the surface tension of the wetting liquid, as also its viscosity, diminishes with mounting temperature, while the angle of wetting increases, and its cosine decreases with the hydrophobicity of the filter polymer. In other words, the less hydrophilic the polymer, the less perfectly does it wet, particularly with aqueous liquids. Therefore, the bubble point is a specific product of the each particular filter/liquid couple. That the bubble point of a filter differs for different wetting liquids is commonly known. That it differs also for polymeric materials is less appreciated. Table 2 shows the surface tensions corresponding to various liquids. Emory et al. [10] report on the effects of surfactants of different types in variously lowering the surface tension of aqueous solutions. Table 3 illustrates how the cos θ values differ for various wetting angles, reflective of different polymeric polarities.

It has usually been assumed in the ordinary bubble point measurement that the liquid wets perfectly, regardless of the polymeric makeup of the membrane, and that cos θ equals one, if not exactly, then at least sufficiently to make the equation applicable. Quite aside from the improbability of identical pore-size dis-

Table 2 Characteristics of liquids commonly used in bubble point measurements

Description	Surface tension (dynes/cm)	Conversion factor[a]
Water	72	41.2
Kerosene	30	12.5
Isopropanol	21.3	8.9
Freon TF	19	7.9
Silicone fluid[b]	18.7	7.8
Fluorocarbon fluid[c]	16	6.7
0.5% Triton X-100	30	12.5
0.1% Triton X-100	30	12.5

[a] Conversion factor divided by pressure in psi equals maximum pore in micrometers.
[b] Dow Corning 200 fluid, 2 centistoke.
[c] 3M Company, Fluorochemical FC-43.

Table 3 Magnitude of cos θ variations

$$D(\mu m) = \frac{4\gamma \cos \theta}{P\ (psi)}$$

cos 0°=1	cos 50°=0.64
cos 10°=0.98	cos 60°=0.5
cos 20°=0.94	cos 70°=0.34
cos 30°=0.87	cos 80°=0.17
cos 40°=0.75	cos 90°=0.00

tributions, wherein the pore-size rating (presumably mean-flow pore) and the largest pore will be in an invariant relationship for all membranes, the above reasoning ignores the fact that membranes with similar pore-size ratings do exhibit bubble point values that reflect differences in their polymeric composition [11].

In this context, it may be instructive to examine the theoretical basis for bubble-point value differences occasioned by the solid/liquid wetting interaction.

2.8 Cohesive and Adhesive Forces in Wetting

Within a mass of material, it is the intermolecular forces of attraction that serve to bind the numerous molecules into a coherent whole. Depending on the relative strengths of these forces, as well as on geometric molecular factors, a crystalline, a solid-state morphology or the liquid state, ranging from a mobile fluid to a glass, may result. The crystalline and glass manifestations can also be influenced by fabrication and post-fabrication variables. In all these cases, the intermolecular forces that operate within one state of matter are defined as cohesive.

Attractions operating between two states of matter, such as between a solid and a contacting liquid, are called adhesive forces. When two bodies come into contact, there are always interacting forces across the contact boundary. In the case of contact between a liquid and solid, these forces are higher where complete liquid spreading occurs and, indeed, account for the greater degree of spreading, otherwise the cohesive forces within the liquid could not be overcome. By the same token, partial spreading denotes weaker adhesive forces (Fig. 7).

When water is placed on a clean glass surface, the intermolecular adhesive forces are so strong that they overcome the liquid's substantial cohesive forces and cause it to spread over the glass. In the case of a drop of water placed on a hydrophobic polymeric surface, the cohesion of water is sufficiently strong not to be disrupted by the weaker attractive forces operating across the solid/liquid boundary. Spreading of the water does not normally occur, therefore, on hydrophobic polymeric surfaces, and a finite, measurable contact angle results whose value depends on the chemical structure of the polymer. In any instance,

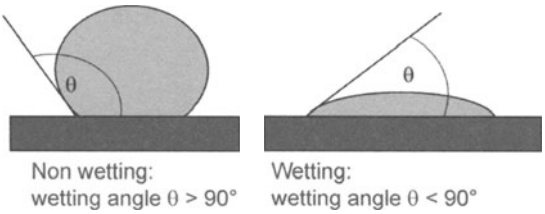

Fig. 7 Liquid surface interaction at different wetting properties

as already stated, the degree of wetting of the solid by the liquid is understood to reflect the degree of 'sameness' in the bonding constituting the solid phase and the liquid phase. It is an expression of the cohesive energy densities of the molecular species involved.

Since wetting is a result of the solid/liquid interaction, one can speak of a work of adhesion. This may be defined as a function of the intermolecular forces operating across the solid liquid interface. More quantitatively, the solid/liquid interface can be characterized thermodynamically in terms of the work necessary to overcome the wetting of the one phase by the other, that is, to separate the liquid and solid. Angle θ between the solid and liquid may be higher than or equal to zero, or may be smaller than or equal to 180°, depending on the nature of both the liquid and the solid. From this it is derived that if, in a given instance, the solid is the same but the liquid is changed, a different value of θ will result. Similarly, if the liquid remains unchanged but a different solid is involved, the value of θ will also change.

2.9 Polymer Contribution

It is evident that the nature of the solid contributes to the wetting interaction of the solid/liquid interface, and that as a result bubble point test measurement values will reflect the influence not only of the selected test liquid but also of the specific solid membrane component being tested (Fig. 8).

Since the bubble point test values reflect the indivisible combination of both solid and wetting liquid, one can neither arbitrarily set a standard bubble point test liquid to characterize all membranes, nor regard the resulting bubble point test value as an independent parameter for the purpose of characterizing the filter performance of all membranes.

A contrary view is advanced on occasion, citing as its support the fact that the bubble point values of many, for instance, 0.2 µm-rated membranes composed of a number of different polymers, as listed in various manufacturers' catalogs, are all of rather the same psi bubble point level. The reason for this is not that all the so-called 0.2 µm-rated membranes exhibit a 50 psi (3.49 bar) bubble point, but rather that manufacturers label as 0.2 µm filters those that do show a 50 psi (3.49 bar) bubble point.

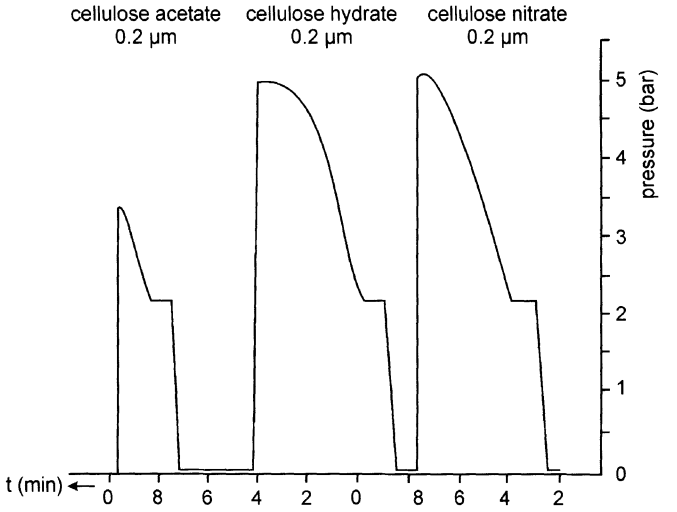

Fig. 8 Bubble point values of different polymers (from Karbachsch 1982)

It bears repeating, however, that the practice of rating membrane filters by the similarity of their bubble points is a technical absurdity when extended to prognostications of similarities in terms of flow characteristics or particle retentions. These latter performance characteristics reflect the pore shapes and sizes, about which little is known, as well as and the nature of the filter's polymeric identity.

3
Bubble Point Test

Microporous membranes pores, when wetted out properly, fill the pores with wetting fluids by imbibing that fluid in accordance with the laws of capillary rise. The retained fluid can be forced from the filter pores by air pressure applied from the upstream side to the degree that the capillary action of that particular pore is overcome. During the bubble point test, the pressure is increased in gradually in increments. At a certain pressure level, liquid will be forced first from the set of largest pores, in keeping with the inverse relationship of the applied air pressure P and the diameter of the pore, d, described in the bubble point equation:

$$P = \frac{4\gamma \cos \theta}{d}$$

where γ is the surface tension of the fluid and θ is the wetting angle, P is the upstream pressure at which the largest pore will be freed of liquid, d is the diameter of the largest pore.

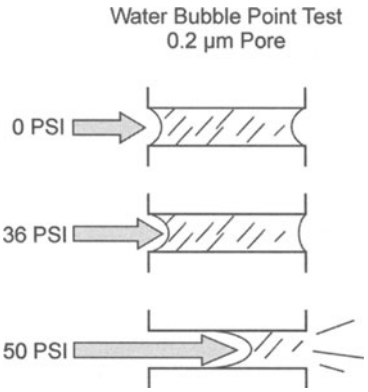

Fig. 9 Schematic of the bubble point and pressure at which the wetting fluid will be expelled (courtesy Sartorius AG)

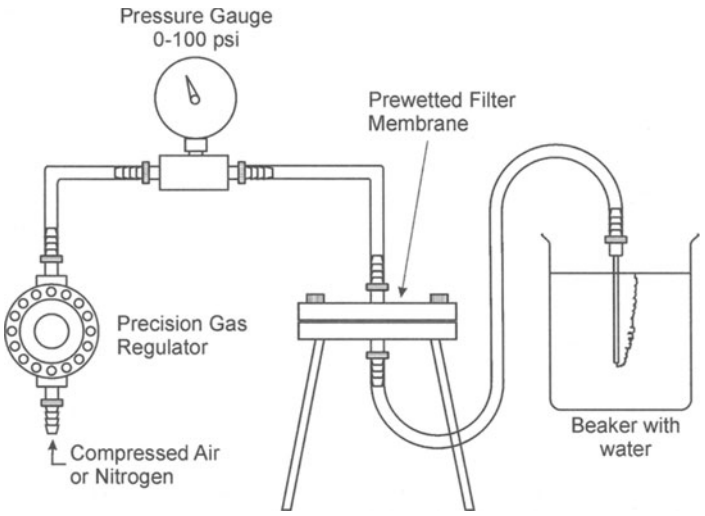

Fig. 10 Manual bubble point test set-up (reprinted, with permission, from [13])

When the wetting fluid is expelled from the largest pore, a bulk gas flow will be evaluated on the downstream side of the filter system (Figs. 9 and 10). The bubble point measurement determines (to a certain degree) the pore size of the filter membrane, i.e. the larger the pore the lower the bubble point pressure. Therefore filter manufacturers specify the bubble point limits as the minimum allowable bubble point and correlate the bubble point test procedure to the bacteria challenge test. During an integrity test the bubble point test has to exceed the set minimum bubble point.

Key for a successful bubble point test is the qualified wetting fluid and its surface tension. The bubble point will be highly influenced by surface tension

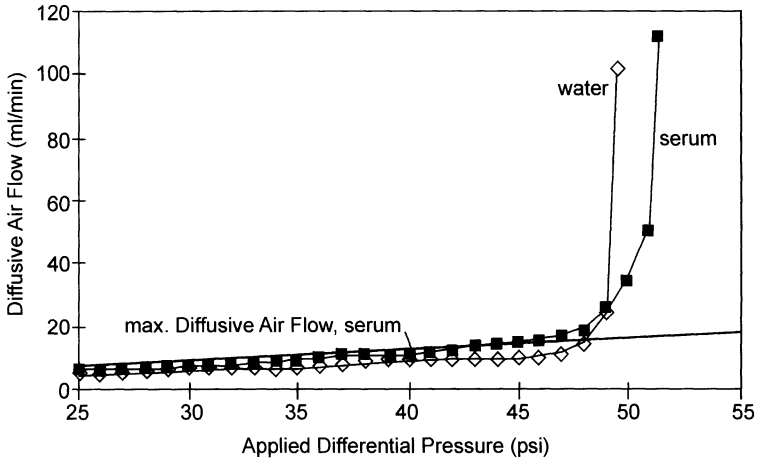

Fig. 11 Bubble point shift due to different wetting fluids

changes within the wetting fluid. Figure 11 shows two different possible wetting fluids and the bubble point changes of such, utilizing the same membrane. Wetted with serum the bubble point is actually higher than with water.

However, the surface tension of the wetting liquid, as also its viscosity, diminishes with mounting temperature, while the angle of wetting increases, and its cosine decreases with the hydrophobicity of the filter polymer. In other words, the less hydrophilic the polymer, the less perfectly does it wet, particularly with aqueous liquids. Therefore, the bubble point is a specific product of the each particular filter/liquid couple. It varies from one polymer to the other and therefore bubble point values given and obtained are not equal, even for the same pore size rating. That the bubble point of a filter differs for different wetting liquids is commonly known. That it differs also for polymeric materials is less appreciated (Table 4).

The bubble point test can only be used to a certain filter size. The larger the filter surface, the larger the influence of the diffusive flow through the membrane. The diffusive flow would cover the actual bubble point due to the extensive air flow due to the greater number of largest pores present in the more extensive area. Filter area affects the *perceived* bubble point, the instance when enough bulk air has passed through the filter to coalesce into visible bubbles.

Table 4 Bubble point values for different polymeres using water

Pore size (µm)	Cellulose acetate	Cellulose nitrate	Poly-carbonate	Nylon	Poly-sulfone
0.1	~4.2	~9.0	>7.0	~5.5	~4.5
0.2	~3.4	~4.8	~4.2	~3.3	~3.1
0.45	>2.0	~3.1	~2.3	~2.4	~1.7

Table 5 Trouble shooting – manual bubble point test

Symptom	Possible cause	Required actions
No test pressure buildup	1. Filter system leakage, i.e. damaged seal, valve open, clamp improperly closed, damaged filter	1. Check and tightening of all clamps, valves, connections – repeat test
	2. Improperly wetted filter	2. Reflush filter with appropriate pressure conditions or use solvent as wetting agent
	3. Hydrophobic spots or surface tension lowering contaminant or remaining product	3. Either force flush at high pressures or temperatures or solvent wetting
	4. Inappropriate wetting medium, e.g., solvent instead of water	4. Recheck wetting agent, either change to proper agents or change test parameters
	5. Wrong filter pore size or pre-filter assembled	5. Check package label, reassemble with correct filter
	6. Improper inlet valve setting and too fast a pressure increase	6. Rewet the filter and repeat test with appropriate pressure steps
	7. Malfunctioning pressure gauge	7. Check pressure gauge with reference gauge, possibly change gauge
No bubble point observed	1. Damaged filter	1. Reassure filter is damaged by steps 1, 2 and 3 above
	2. Improperly wetted filter	2. Rewet under higher pressure conditions or use solvent
	3. Inappropriate wetting medium, e.g., solvent instead of water	3. Recheck wetting agent, either change to proper agents or change test parameters
	4. Wrong filter pore size	4. Check package label
	5. Too speedy pressure increase	5. Rewet and increase with appropriate pressure steps
	6. Malfunctioning pressure gauge	6. Check gauge and change
	7. User subjectivity	7. Train filter user appr. or use automatic test machine
	8. Wrong test gas (e.g. CO_2, which causes higher diffusive flow)	8. Check connected gas line or bottles connected to the line

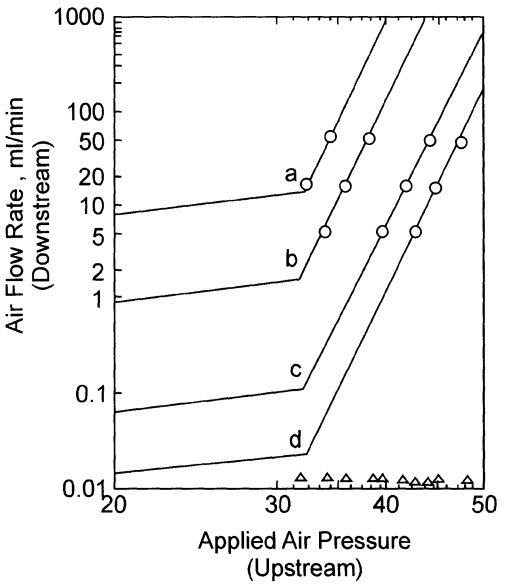

Fig. 12a–d Shift in bubble points as a function of filter area for different areas of a 130 µm thick, 0.2 µm rated membrane: **a** 4545 cm^2 in a pleated cartridge filter; **b** 589 cm^2 in a 293-mm diameter disc; **c** 44 cm^2 in a 99-mm disc; **d** 9.6 cm^2 in a 47-mm disc (from Johnston et al. 1981) [16]

A larger filter simply permits the passage of more air per unit time, because of its more numerous pores at every pore size (Fig. 12).

Therefore the bubble point finds its ideal use with very small system to medium size systems (some mentioned the critical borderline to use the bubble point is a 3×20-inch multiround filter housing, depending on the pore size). In some instances of multiround filter housing testing, the multipoint diffusion test is utilized, as bubble point and single point diffusion tests have their disabilities and cannot be utilized completely reliable (Table 5).

4
Diffusive Flow Test

A completely wetted filter membrane provides a liquid layer across which, when a differential pressure is applied, the diffusive airflow occurs in accordance with Fick's law of diffusion (Fig. 13).

This pressure is called test pressure and commonly specified at 80% of the bubble point pressure. In an experimental elucidation of the factors involved in the process, Reti simplified the integrated form of Fick's law to read

$$N = \frac{DH\,(p1 - p2)}{L} \cdot \rho$$

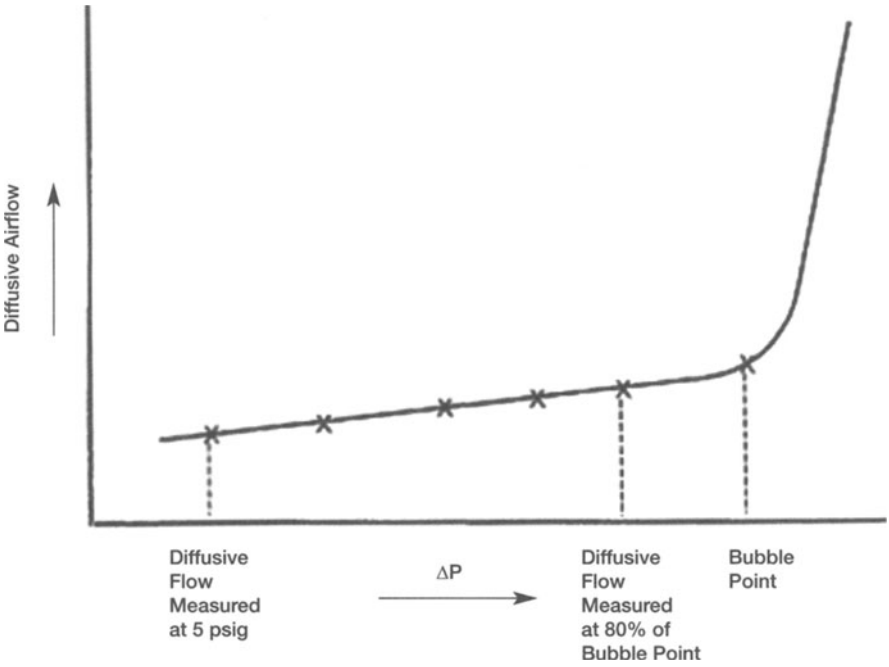

Fig. 13 Diffusive air flow at different pressure settings (courtesy [12])

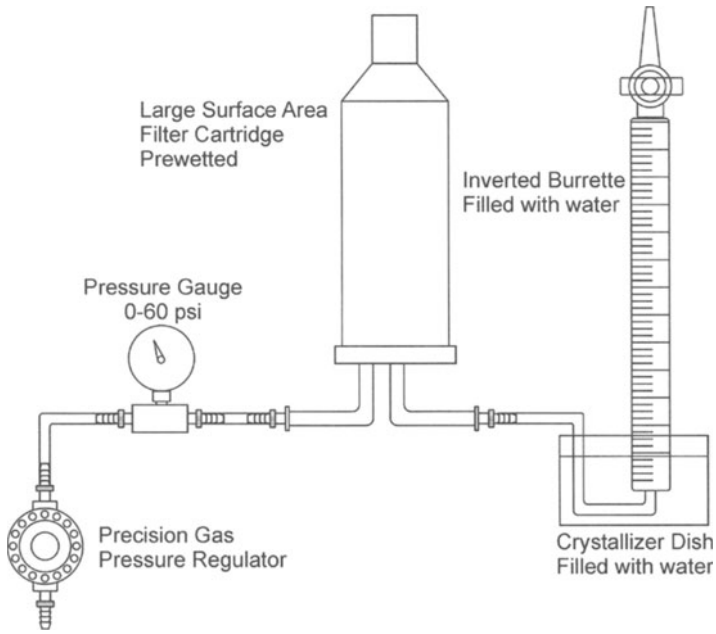

Fig. 14 Manual diffusive flow test set-up (reprinted, with permission, from [13])

where N is the permeation rate (moles of gas per unit time), D is the diffusivity of the gas in the liquid, H is the solubility coefficient of the gas, L is the thickness of liquid in the membrane (equal to the membrane thickness if the membrane pores are completely filled with liquid), P (p1–p2) is the differential pressure, and ρ is the void volume of the membrane, its membrane porosity, commonly around 80%.

The size of pores only enters indirectly into the equation; in their combination they comprise L, the thickness of the liquid layer, the membrane being some 80% porous. The critical measurement of a flaw is the thickness of the liquid layer. Therefore a flaw or an oversized pore would be measured by the thinning of the liquid layer due to the elevated test pressure on the upstream side. The pore or defect may not be large enough that the bubble point comes into effect, but the test pressure thins the liquid layer enough to result into an elevated gas flow. Therefore filter manufacturer specify the diffusive flow integrity test limits as maximum allowable diffusion value. The larger the flaw or a combination of flaw, the higher the diffusive flow.

Table 6 Trouble shooting – manual diffusion test

Symptom	Possible cause	Required actions
Failure of diffusive flow	1. Damaged filter	1. Rewet, repeat test and/or replace filter
	2. Improperly wetted filter	2. Reflush filter with appropriate pressure conditions or use solvent as wetting agent
	3. Inappropriate wetting medium, e.g., solvent instead of water	3. Recheck wetting agent, either change to proper agents or change test parameters
	4. Wrong filter pore size	4. Check package label, reassemble with correct filter
	5. Too speedy a pressure increase	5. Rewet and increase with appropriate pressure steps
	6. User subjectivity	6. Train filter user appr. or use automatic test machine
	7. Wrong test gas	7. Check connected gas line or bottles connected to the line
	8. Temperature shifts during the test	8. Repeat test avoiding any possible temperature shift by the user, test gas/liquid temperature, room conditions or other equipment close by
	9. Improper test pressure setting	9. Rewet filter and retest at appropriate test pressure
	10. Insufficient stabilization time	10. Rewet and repeat test to manufacturers guidelines
	11. Inappropriate downstream test tubing	11. Replace tubing to thin tubing and rewet/repeat test

The diffusive flow cannot be used for small filter surface, due to the low diffusive flow with such surfaces (Fig. 14). The test time would be far too extensive and the measured test value to unreliable to be utilized. Nevertheless, the diffusive flow, as well as the pressure drop test are best used for larger filtration surfaces, where the bubble point test finds its limitations (Table 6).

5 Pressure Hold Test

The pressure hold test is a variant of the diffusive airflow test. The test set-up is arranged as in the diffusion test except that when the stipulated applied pressure is reached, the pressure source is valved off (Fig. 15). The decay of pressure within the holder is then observed as a function of time, by using a precision pressure gauge or pressure transducer.

The decrease in pressure can come from two sources: a) the diffusive loss across the wetted filter – since the upstream side pressure in the holder is constant, it decreases progressively all the while diffusion takes place through the wetted membrane; b) source of pressure decay could be a leak of the filter system setup.

An important influence on the measurement of the pressure hold test is the upstream air volume within the filter system (Fig. 16). This volume has to be determined first to specify the maximum allowable pressure drop value. The larger the upstream volume, the lower will the pressure drop be. The smaller the upstream volume, the larger the pressure drop. This means also an increase in sensitivity of the test, but also an increase of temperature influences, if changes

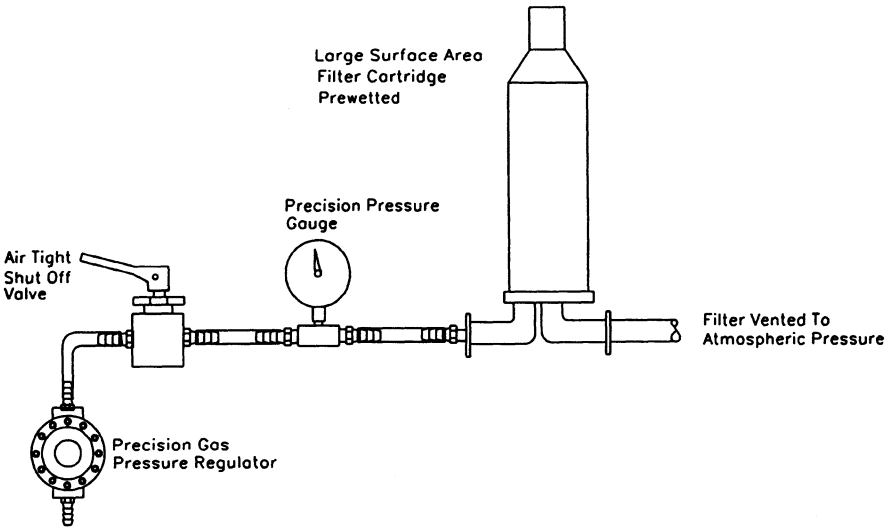

Fig. 15 Manual pressure-hold test set-up (reprinted, with permission, from [13])

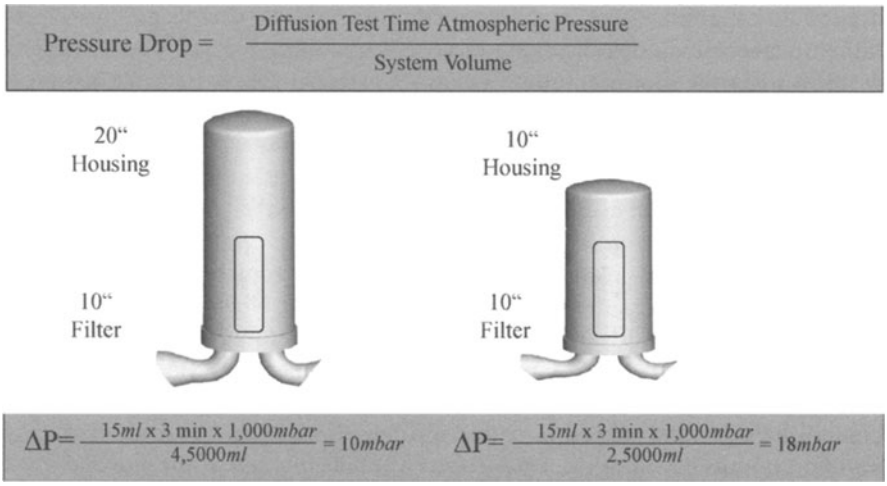

Fig. 16 Pressure-hold test volume influence (courtesy of [19])

Table 7 Trouble shooting – manual pressure hold test

Symptom	Possible cause	Required actions
Failure of pressure decay/drop	1. Damaged filter	1. Rewet, repeat test and/or replace filter
	2. Improperly wetted filter	2. Reflush filter with appropriate pressure conditions or use solvent as wetting agent
	3. Inappropriate wetting medium, e.g., solvent instead of water	3. Recheck wetting agent, either change to proper agents or change test parameters
	4. Wrong filter pore size	4. Check package label, reassemble with correct filter
	5. Too speedy a pressure increase	5. Rewet and increase with appropriate pressure steps
	6. User subjectivity	6. Train filter user appr. or use automatic test machine
	7. Wrong test gas	7. Check connected gas line or bottles connected to the line
	8. Temperature shifts during the test	8. Repeat test avoiding any possible temperature shift by the user, test gas/liquid temperature, room conditions or other equipment close by
	9. Improper test pressure setting	9. Rewet filter and retest at appropriate test pressure
	10. Insufficient stabilization time	10. Rewet and repeat test to manufacturers guidelines
	11. Inappropriate gauge sensitivity	11. Replace gauge for high sensitivity gauge

occur. Filter manufacturers specify maximum allowable pressure drop values, utilizing their maximum allowable and correlated diffusive flow value and convert this diffusive flow maximum with the upstream volume into a maximum allowable pressure drop.

Another major influence, as mentioned, has the temperature. Any temperature change during the test will distort the true result, as an increase in the temperature will lower the pressure drop and a decrease will artificially elevate the pressure drop. Therefore the temperature conditions during the test should only vary slightly. This also means that the wetting agents used should have a similar temperature as the environmental temperature surrounding the test set-up. Temperature differences between the wetting solution and the test gas and the temperature of the environment will influence the true test result.

The pressure hold test (Table 7) is an upstream test, even when performed manually. Both tests, bubble point and diffusive flow, require downstream manipulation and therefore cannot be used after steam sterilization of the filter system. The pressure hold, as it measures the pressure drop on the upstream side, can be used without downstream evaluation.

6 Water Intrusion Test

The water intrusion (also called water pressure hold) test is used for hydrophobic vent and air membrane filters only. The upstream side of the hydrophobic filter cartridge housing is flooded with water. The water will not flow through the hydrophobic membrane (Fig. 17). Air or nitrogen gas pressure is then applied to the upstream side of the filter housing above the water level to a defined test pressure.

This is done by way of an automatic integrity tester. A period of pressure stabilization takes place over, by the filter manufacturer recommended, timeframe,

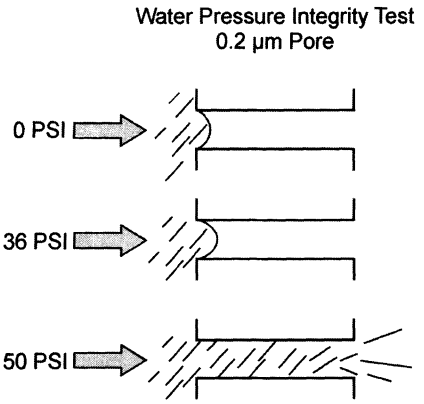

Fig. 17 Water intrusion schematic (courtesy [20])

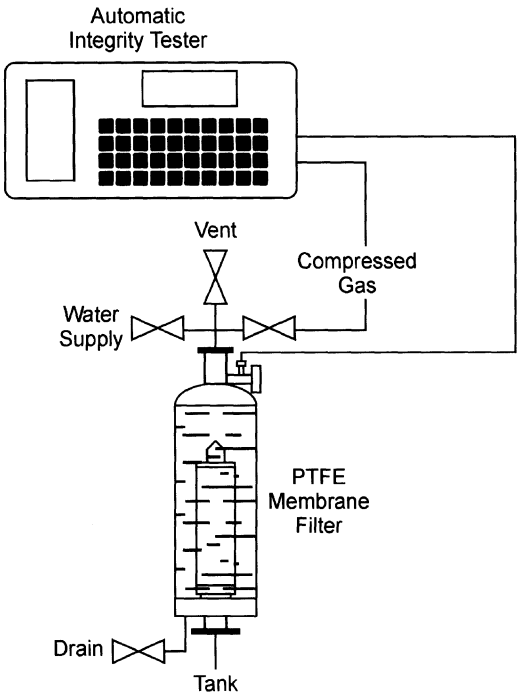

Fig. 18 Water Intrusion test schematic of different pressure conditions (courtesy of Tarry 1993)

during which the cartridge pleats adjust their positions under imposed pressures. After the pressure drop thus occasioned stabilizes, the test time starts and any further pressure drop in the upstream pressurized gas volume, as measured by the automatic tester, signifies a beginning of water intrusion into the largest (hydrophobic) pores, water being incompressible and water vapor flow through the membrane (Fig. 18). The automated integrity tester is sensitive enough to detect the pressure drop. This measured pressure drop is converted into a measured intrusion value, which is compared to a set intrusion limit, which has been correlated to the bacteria challenge test. As with the diffusive flow test, filter manufacturers specify a maximum allowable water intrusion value. Above this value a hydrophobic membrane filter is classified as non-integral.

The water intrusion test offers several advantages. For example:

1. The test is highly sensitive because its test pressures are in the range of the water penetration pressure of 0.45 µm-rated filters (Fig. 19).
2. Contaminants such as solvent mixtures are avoided.
3. In addition to the integrity, the validated hydrophobicity is tested, i.e., the presence of contaminants on the membrane can be discovered.
4. The test can be performed in place, after steam sterilization.
5. Test times are greatly reduced, because contaminants do not have to be flushed off, and in-place testing is not necessary.

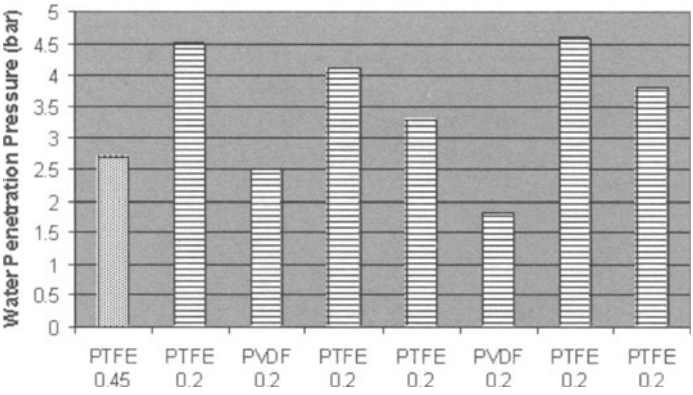

Fig. 19 Water penetration pressure of different hydrophobic membrane filter materials, one having a pore size of 0.45 µm

The specifications defined by the filter manufacturers have to be observed to achieve reliability. In most cases the test is performed with automated test machines. This may be considered a disadvantage because of capital costs incurred. Nevertheless, automated test machines are usefully versatile and are also commonly used to perform other integrity tests, such as the diffusive airflow and bubble point tests.

After the water intrusion test was introduced in the early 1990s, it became the standard test for hydrophobic vent filters. It replaced the commonly used bubble point or diffusive flow test, after the hydrophobic filter has been wetted with a water/solvent mixture. The water/solvent test did not allow the vent filter user to test the filter within the system, but only off-line. Therefore the frequency of testing was limited and could not be performed after in-line steam sterilization. Nevertheless, since vent or compressed gas filters are used multiple times, after multiple steaming, a routine test has been desirable. The in-situ water intrusion test does not only find its use within vent filter applications on tank vents or compressed gas housings for the fermentation process, but is now also established as a fully automated test in equipment like autoclaves and freeze dryers. Such equipment implemented tests utilize the software and control functions of the machine and test the vent filter automatically without user intervention. Automated test configurations also avoid the need for a filter user to test a filter in difficult to access areas or locations, as the machine would do so.

A very common concern in respect to the water intrusion test is the fact that the filter up-stream side is filled with water and whether or not the filter will be water blocked. To find out whether the filter still performs as required so called blow-down tests are performed (Fig. 20). Within these test procedures the filter's initial, dry air flow rate is measured, afterwards the water intrusion test performed and immediately after the integrity test, the air flow is tested once again. The second air flow rate is compared to the initial air flow rate. Depending on the filter´s configuration and the membrane polymer, the outcome

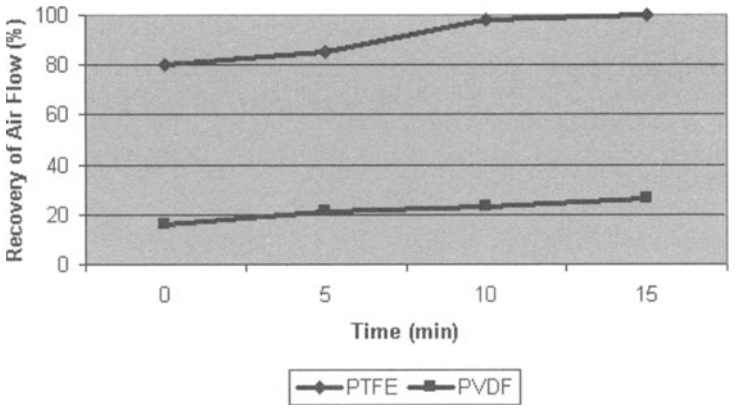

Fig. 20 Blow-down time or air recovery of different vent filter polymers

or deviation from the initial air flow might be considerable. Therefore it is of use to investigate the blow-down time performance of different vent filters at the performance qualification phase. Commonly the blow-down time is low, as the highly hydrophobic filter material is repelling the water on its up-stream side. After the test pressure is released the water repels from the membrane surface and builds droplets, instead of a continuous film of water. The air flow is therefore not prevented, but a free air flow is experienced.

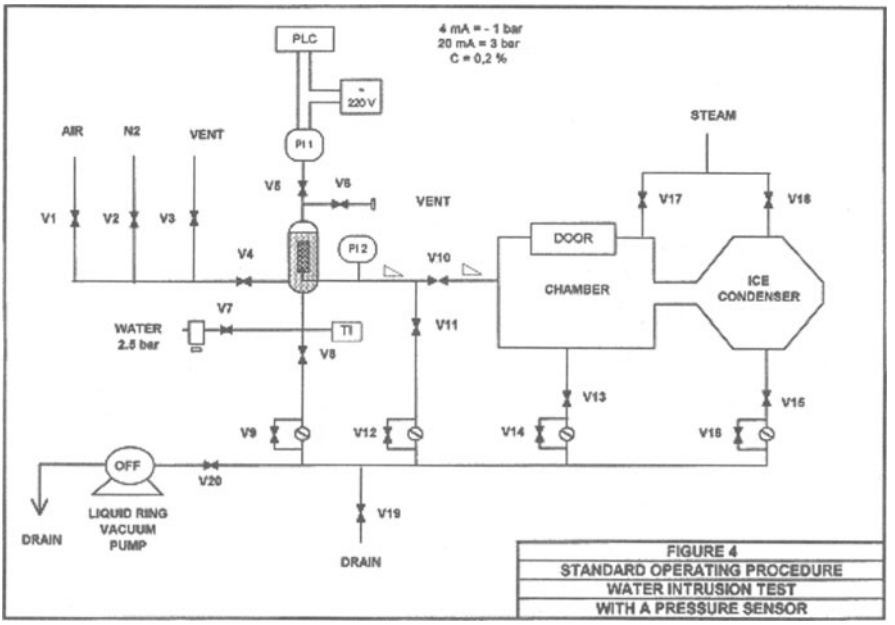

Fig. 21 Freeze dryer vent filter set-up (courtesy of Sartorius)

In certain vent filter applications, the filter needs to be dry to prevent any moisture flow into the product or equipment. For example vent filters on freeze dryers need to be dried after the integrity test, as the residual moisture within the filter fleece and pleating structure would influence the drying process. To dry the filter, it has been found that a vacuum pulse through the filter will dry a 0.2 m^2 filter within 30 min (Fig. 21).

7 Multipoint Diffusion Test

In single-point diffusive flow testing, the test is performed at a defined test pressure, which is commonly around 80% of the bubble point value. Therefore the area between the diffusive flow test pressure and the bubble point value is not tested and stays undefined. In comparison, the multipoint diffusive airflow test is performed at a multitude of test pressures. Usually this test is performed with an automated test machine, which allows defining the individual test pressure points. In any case, the multipoint diffusion test should be performed right to the bubble point. Therefore the entire graph with its linear and exponential section is plotted (Fig. 22).

The additional benefit of an automated test machine is the accuracy of its measurement. Moreover once the pressure points are defined the machine performs the test without the need of supervision. Therefore valuable time and resources are not bound. To the benefit of data storage, the test machines also print an exact graph of the test performed, therefore any irregularities will be detected.

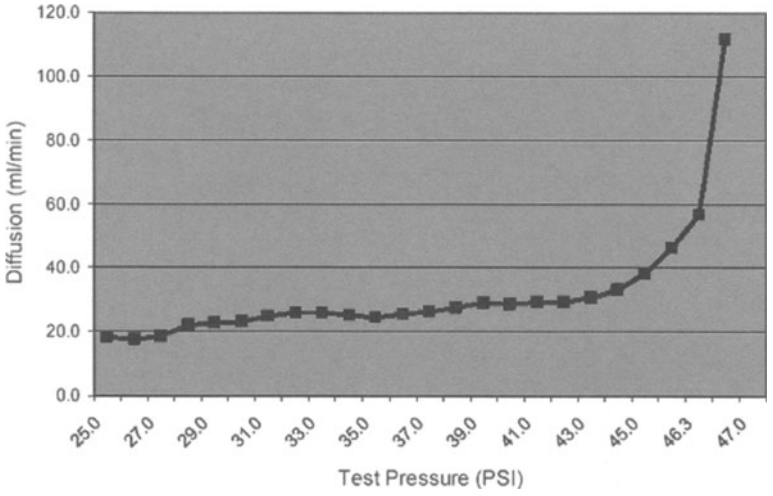

Fig. 22 Multipoint diffusive flow graph at different test pressures (courtesy of Sartorius Group)

Multipoint diffusive testing has advantages over single-point diffusive testing, because it can more rapidly detect a pending product failure due to gradual filter degradation. A multipoint integrity test could indicate a trend of increasing diffusion over time that might be overlooked with single-point diffusion testing and even through bubble point testing (Fig. 23). Take as an example the case of a hydrophobic vent filter cartridge on a water-for-injection tank. If the system is in-line steam-sterilized daily, potentially stressing the filter membranes with each cycle, the filter may eventually lose its integrity and fail both a single-point diffusive airflow test and a bubble point test. The bubble point value in this example may also never quite decrease to the point at which the filter actually loses its integrity. The same may be true for the case of single-point diffusion testing. However, a trend may be elucidated if a reduction in membrane integrity is demonstrated as a function of time and not as a single stressful incident. Better estimates of the service life of these vent filters may be made available through such validation of the filters over their operating service life. Such a test could be performed within the performance qualification (PQ) stage, where the vent filter would be subjected to multiple steam sterilization cycles to evaluate the resistance of such filter to the individual steaming cycle used in the process. The lifespan of the vent filter could be evaluated during such test series, using the multipoint diffusion test.

These tests were performed at steaming cycle temperature of 134 °C. The results of these tests showed that initially these filters fell within the acceptable air diffusion range suggested in the literature. CA filter #1 showed an initial increase after the first sterilization then remained lower until the seventh

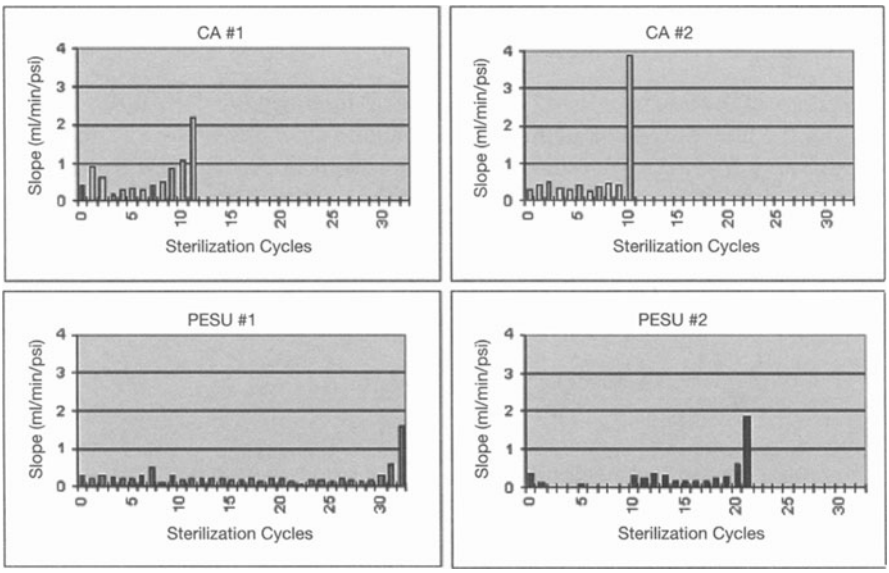

Fig. 23 Multipoint diffusion test slope at multiple steaming cycles (courtesy of [17])

cycle. At this point the air diffusion rate slowly increased to the tenth cycle. At which point the rate exceeded 15 ml/min at 36.8 psi. In this case eventual filter failure was forewarned by the increase in slope. The second CA filter (CA #2) was within acceptable limits until the tenth cycle in which failure was abrupt and not preceded by an increase in slope. PES filter #1 had an initial decrease in air diffusion after sterilization, then began to show an increase in slope between the seventeenth and twentieth cycle. This slope increase indicated a pending filter failure. PES filter #2 did not show a marked change in air diffusion after sterilization but eventually did show an increase in slope prior to filter failure. It shows that the multipoint diffusion test creates a possibility to predict filter failure at certain steaming conditions. The steaming cycle performance given by the filter manufacturer can only be an implication. Due to the individual steaming procedures within the users facility, one should perform a filter steaming qualification. When multiple steaming cycles are used the multipoint diffusion test can be a useful tool to support such qualification efforts.

Additionally, multipoint diffusive testing is invaluable in the characterization of a filter's diffusive flow when wetted with a drug product. Instead of using a single-point determination, which can cause inaccuracies, one measures the diffusive flow graph for water and for the product to be used. The measurement especially evaluates the slope of the linear section of the diffusive flow measurement and the shift of the bubble point. The slope will arise from the differences in diffusivity and solubility of the test gas in the different wetting media. The linear section of the diffusive flow will follow the described equation:

$$N = \frac{D \cdot H \cdot \rho}{L} \cdot P_1 + \frac{-D \cdot H \cdot \rho \cdot P_2}{L}$$

$$N = \text{slope} \cdot P_1 + (y - \text{intercept}) \qquad \text{(eq. 2)}$$

The slope of the line is ($DH\rho/L$) and the line's y-intercept is ($-DH\rho P_2/L$). The values for the filter porosity and thickness are identical for any of the wetting agents (water and product). Therefore, the differences in slope will arise from differences in diffusivity and solubility of air in the wetting liquid, and these differences should be constant over a pressure range if D and H are constant over this pressure range. Indeed, if D or H changes with pressure, then we would not observe a line at low pressure, but a curve. Therefore, to predict a value for N (diffusion rate) with a product as the wetting agent, one would use this equation:

$$\frac{N_{product}}{N_{water}} = \frac{(D \cdot H)_{product} \cdot P_1 + (-D \cdot H)_{product} \cdot P_2}{(D \cdot H)_{water} \cdot P_1 + (-D \cdot H)_{water} \cdot P_2} \qquad \text{(eq. 3)}$$

This equation reduces itself to the ratio of the slopes, which is required to evaluate the correction factor for the maximum allowable product wet diffusion:

$$\frac{N_{product}}{N_{water}} = \frac{(D \cdot H)_{product}}{(D \cdot H)_{water}} \qquad \text{(eq. 4)}$$

For example:

Slope value for serum with CA = 0.264 ml/min/psi
Slope value for water with CA = 0.343 ml/min/psi
Serum/water duffusion ratio of slope = 0.264 ÷ 0.343 = 0.769

This ratio is then multiplied by the maximum allowable diffusion limit set by the filter manufacturers at a certain test pressure, which is correlated to the bacteria challenge test. Once the proper diffusion curve limit is defined by multipoint diffusive testing, done during the performance qualification (PQ) phase, the reliability of the single-point diffusive airflow test becomes established.

For example the maximum allowable air diffusion through water at 36.8 psi described in the validation guide of the filter vendor and correlated to the BC test, to determine the maximum acceptable air diffusion through serum at 36.8 psi:

Maximum allowable air diffusion through serum in CA
at 38.8 psi = 0.769 × 15 ml/min = 11.5 ml/min

The value of 11.5 ml/min would be the maximum allowable product diffusion value used in production for a single point diffusion test at a test pressure of 36.8 psi (2.5 bar). The same can be done with any other filter material, wetting agent, and test pressure. Nevertheless the foundation for this maximum product diffusion value is the bacteria challenge test correlated maximum allowable water diffusion value, which can be obtained from the individual filter manufacturers. In any case the determination of the maximum allowable diffusion value using the multipoint diffusion test instead of a single point determination has a by far higher accuracy, due to the multitude of test points. The ratio of slopes is measured at several test pressure points, within a fixed frame, set by the user and the linearity of the graph. These data create a statistical firm basis, contrary to the product wet single point test.

Furthermore the multipoint diffusion test has seem to have the ability to test multiround housings reliably. As described in the bubble point and diffusive flow test section, both tests have their limitation integrity testing multiround filter housings. A single-point diffusive flow test may not be able to find a flawed filter within the multitude of filters. The bubble point may be covered by an excessive diffusive flow.

In any case the multipoint diffusive flow test seem to be able to find a flawed filter due to the change of the slope of the linear section of the diffusive flow. As seen in Fig. 24, a single flawed filter cartridge can be detected within a three round filter housing, where a single-point test would not have determined the defect. Such a test may well take longer in its test time, but will add to the overall accuracy of integrity testing multiround housings. Certainly, as with the other tests, the multipoint diffusion test will find its limits, with increasing size of the filter system. At one point the automatic integrity test machine will not

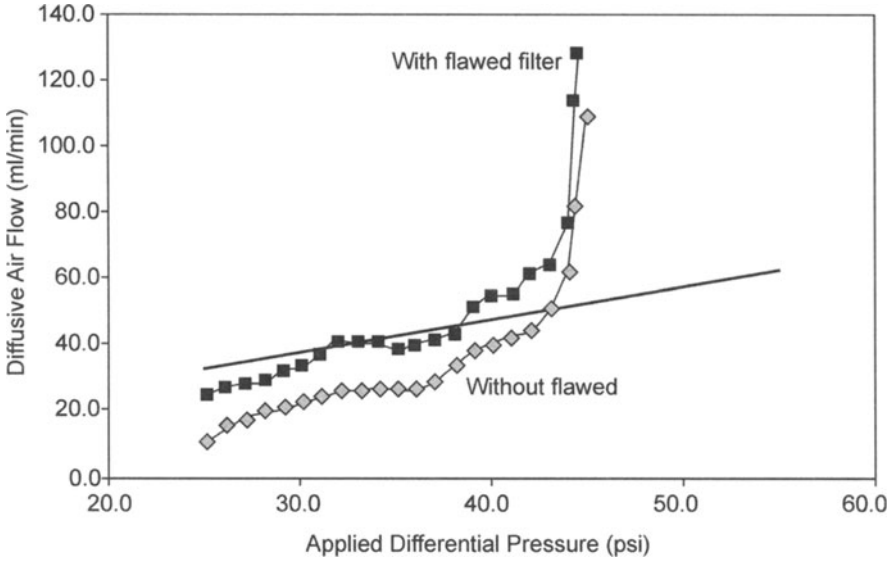

Fig. 24 Multipoint diffusion test with multiround housings (courtesy of [17])

be able to test the size of housing or the amount of filters used. As far as one filter manufacturer claims, one can perform such a test to the multiround filter housing size of 5 round 30 in.

In some instances the multipoint diffusion test is also useful in the analysis of failed filter integrity tests. For instance, when a filter failed the single point diffusive flow test or bubble point test, one should aim for testing the filter with a multipoint diffusion test to see the entire graphic. This result could be compared to the graphs established during the performance qualification phase. Commonly there are distinct test graphics, which show whether the filter has a flaw or not and if so what the cause of the flaw could be. Often enough, failed filter integrity tests are caused by wetting problems or product residues within the filter membrane matrix or contaminants in the steam. Such problems can be evaluated by using the multipoint diffusion test and run the graphic of the failed filter in comparison to a passed filter. The user has now the opportunity to either discovers the reason for failure by himself or is able to send such graphs to the filter manufacturer for evaluation and answers. Single point diffusion testing and bubble point testing are not able to show the reason of a failure in the same scale as the multipoint diffusion test.

8 Automated Filter Integrity Testing

Pharmaceutical and healthcare-related industries are accelerating the incorporation of automated equipment into their manufacturing processes. In the

aseptic processing of pharmaceuticals, verification of the integrity of sterilizing grade filters is a necessity and by automated integrity test instruments is a highly developed technology used to provide accurate and reproducible filter integrity test results.

Manual integrity tests, still performed by a majority of filter users (PDA Tech. Report No. 23), always involve human subjectivity and error, which cannot be allowed in such critical processes as aseptic processing in the pharmaceutical industry. Integrity tests are by far the most reliable and least ambiguous when performed using an automated test device. These instruments have the additional considerable advantage, besides that of not requiring the invasion of the system downstream of the filter, in that most automated integrity test units are connected to the upstream side of the filter system to perform the test:

1. The integrity testing of a given filter should be carried out in conformity with its manufacturer's protocol. The test limits and parameters are programmed into the automated device.
2. Single-point diffusive airflow testing relies on empirically established correlations to organism retentions. Multipoint testing through the bubble point creates an additional accuracy. Such test can only be performed with an automated integrity test machine.
3. As commonly performed without benefit of automated instruments, both the bubble point and diffusive airflow measurements necessitate invasion of the system downstream of the filter, risking asepsis.
4. The pressure hold test does not violate downstream system integrity. It can be a useful indicator of system leaks, whether of seals or filters or both. Its readings must be correlated with retention levels. Its performance needs the sensitivity of automated testing devices.
5. The water intrusion test is suitable for the determination of hydrophobic membrane integrity. Again a high sensitive device is required to perform such test, i.e., the test should be performed with an automated device.
6. Initial integrity tests should be performed, best post-steam sterilization. As a matter of practicality and contamination control, automated devices should be used, which will not compromise the downstream side.
7. Most of the integrity test units provide a hard copy print-out, which can be used for the batch record, but also as an investigative tool, due to the plot of the test graph.
8. Some of the units provide data storage via memory cards or direct connection to the process database system (electronic batch records).

The computerized systems used to control pharmaceutical manufacturing processes, although not fundamentally different from those used in automated integrity test instruments, are required to undergo detailed software and hardware validation. Such systems can be very complex and influence the production process, therefore such system needs to go through qualification stages to verify that the unit performs to user specifications and also to evaluate any

risks involved. An automated integrity test unit has an indirect, auxiliary role and does not control the actual manufacturing process and monitor its performance. Nevertheless, the automated integrity test unit has its important role to verify whether the sterilizing step, done by filtration worked appropriately or not. Due to this the critical nature of the testing provided by these automated integrity test instruments, most regulatory and industry validation groups require these units to meet minimum qualification requirements. These standards and specifications, as detailed below, have to be provided by the integrity test manufacturer in its support documentation and service.

Filters perform a critically important role in pharmaceutical production. The removal and retention of particulates and microorganisms must be ensured to provide safe and effective high quality products. Therefore integrity testing of sterilizing grade filters has to be a part of the filter validation process. This validation process sets the integrity test limits for specific filter types, retention ratings and wetting fluid in conjunction with the test gas. Once the limits are set, one has to revalidate the filter system, if one changes any of such integrity test limits. Any change will have an influence to the process. Integrity testing is to critical to underestimate such changes. Validation is defined as "Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes."

Relating this statement to the validation of a filter integrity test instrument, this would mean documenting that when one instrument performs the test it follows a specified operating procedure, uses the correct test parameters, measures within set sensitivity specifications and tolerances, provides accurate and documented results. Computer-related system validation (CRSV) is defined as (PDA Technical Report No. 18) "The procedure to establish documented evidence that provides a high degree of assurance that a specific computer-related system will consistently operate in accordance with predetermined specifications."

Automated integrity test unit manufacturers have to provide specific documentation for such units to fulfill the above requirement of systems validation. The user has to specify all components used in such machine, furthermore needs to test all the components to establish the set specifications by the component manufacturer. The software involved in such a unit needs to be well documented, change procedure protocols and software verification has to be established. These validation procedures and protocols are well documented by filter integrity test instrument manufacturers. The following sections describe these processes in detail and outline the testing and documentation needed to fulfill the instrument qualification in the current regulatory and industrial environment.

Integrity test instruments are electropneumatic devices that use mass flow meters or pressure transducers as their primary measurement transducers. Test methods and testing protocols vary with the type of electropneumatic measurement device used, pressure decay or mass flow transducers, and the device

manufacturer's specific testing sequence, measurement, and test parameters. The integrity test instrument, regardless of manufacturing source, is connected to the upstream side of the filter/housing system to prevent any potential downstream contamination.

Automated integrity testing, like manual tests, determines the construction integrity and proper micrometer rating and installation of filters in a filter system as well as the leak-tightness of the piping and connections.

All instruments perform integrity tests that are widely accepted and recognized by regulatory and industrial agencies and advisory bodies, diffusive airflow, bubble point, and water intrusion tests. Some of the instruments are also able to perform a multipoint diffusion test.

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