Chapter 12

Sterilization

12.4 Filtration sterilization

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1 Introduction

Early attempts to purify water were made by allowing it to percolate through beds of sand, gravel or cinders, and a complex ecosystem thus developed on these filters. An increasing knowledge of bacteriology and an awareness of the involvement of water-borne bacteria, pathogenic protozoa and worms in disease and epidemics, eventually led to a more thorough study of filtration devices.

Chamberland, a colleague of Louis Pasteur, invented a thimble-like vessel, made by sintering a moulded kaolin and sand mix. These so-called Chamberland candles were the first fabricated filters and represent another example of the inventive output from the Pasteur school (Chamberland, 1884). Later to be made by the English firm of Doulton and other ceramic manufacturers, they were essentially of unglazed porcelain. These filters enjoyed a great vogue in the pharmaceutical industry until the advent of membrane filters (section 2.4) rendered them practically obsolete in this area.

2 Filtration media

The ideal filter medium to remove microorganisms from solutions destined for parenteral administration should offer the following characteristics: efficient removal of particles above a stated size; acceptably high flow rate; resistance to clogging; steam-sterilizable; flexibility and mechanical strength; low potential to release fibres or chemicals into the filtrate; low potential to sorb materials from liquids being sterilized; non-pyrogenic and biologically inert.

Additionally, when such a medium is mounted in a holder or support, it must be amenable to *in situ* sterilization and integrity testing. The medium most frequently employed, and which most nearly

approaches the ideal, is the polymeric membrane, usually in the format of a flat disc or a pleated cartridge (section 2.4). As a consequence, this medium is by far the most important in current use, but several other filter media have been used in the past, which are deficient in one or more of the above and yet retain limited and specialized applications (sections 2.2 and 2.3).

2.1 Filters of diatomaceous earth

Diatomaceous earth, added to liquid products to form a suspended slurry, has been widely used as a filter aid in the pharmaceutical industry. The slurry is deposited on porous supports and the liquid then passes through, leaving coarse particulate matter entrained within the retained filter cake. Such an approach has been employed in rotary-drum vacuum filters (Dahlstrom & Silverblatt, 1986), as used in antibiotic manufacture for instance, where the drum rotates within the slurry, pulling filtered liquid through the retained cake under vacuum and leaving the cell debris behind.

2.2 Fibrous-pad filters

Originally constructed of asbestos fibres, until the toxicity of asbestos was recognized, microfibres of borosilicate glass are now employed to create these filters. They have found widespread application in filter presses and as prefilters for clarification of pharmaceutical solutions. It is usual to employ such filters with a membrane filter (section 2.4) downstream to collect any shed fibres.

Other materials used in the construction of this type of filter include paper, nylon, polyester and cellulose-acetate fibres.

2.3 Sintered or fritted ware

This type of filter was made by taking particles of glass or metal (stainless steel or silver), assembling them in suitable holders and subjecting them to a heat process, so that the particles melted or softened on their surfaces and, on cooling, fused together. It is clear that a complete melting would defeat the object of the technology and this partial melting, followed by surface fusion, was called sintering or frittering. Such a process will give rise to a porous sheet of material, which can then act as a filter (Smith, 1944). This process differs from the sintering process used in the manufacture of unglazed porcelain, in that the latter contains several components and the process is accompanied by chemical changes in the constituents.

2.4 Membrane filters

Membrane filter technology has had over 80 years in which to develop, since the first description, by Zsigmondy and Bachmann in 1918, of a method suitable for producing cellulose membrane filters on a commercial scale. The full potential of membrane filters was not recognized until their successful application in the detection of contaminated water-supplies in Germany during World War II (Gelman, 1965). Following their commercial exploitation in the 1950s and 1960s, a number of large international companies evolved which now offer a wide array of filters and associated equipment from which to choose. Undoubtedly, the role played by membrane filters continues to expand, both in the laboratory and in industry, and they are now routinely used in water analysis and purification, sterility testing and sterilization. Their future is assured, at least in the pharmaceutical industry, unless other, as yet undiscovered, techniques emerge, since they represent the most suitable filtration medium currently available for the preparation of sterile, filtered parenteral products to a standard accepted by all the various regulatory authorities.

2.4.1 Methods of manufacture

There are four major methods of membrane-filter manufacture currently employed on an industrial scale. These involve either a gelling and casting process, an irradiation-etch process, an expansion process or a procedure involving the anodic oxidation of aluminium. Each method produces membranes with their own particular characteristics.

Gelling and casting process

This is perhaps the most widely used process, and

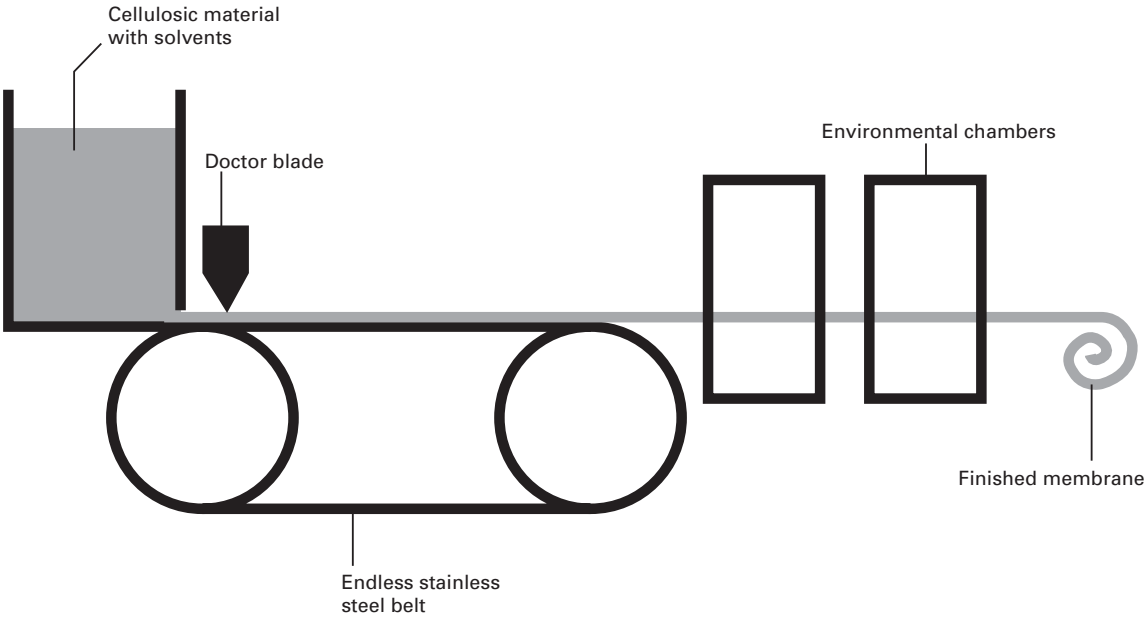

Figure 12.4.1 Membrane manufacture – the casting process.

all the major filter manufacturers offer filters prepared by this method. Cast polymeric membranes, as they are known, are principally derived from pure cellulose nitrate, mixed esters of acetate and nitrate or other materials offering greater chemical resistance, such as nylon 66 (Kesting *et al.*, 1983), polyvinylidine fluoride (PVDF) or polytetrafluoroethylene (PTFE) (Gelman, 1965). The properties of these, and other polymers, appear in Table 12.4.3.

In essence, the process still utilizes the principles outlined by Zsigmondy and Bachmann in 1918, where the polymer is mixed with a suitable organic solvent or combination of solvents and allowed to gel (Ehrlich, 1960). In the modern process, a minute quantity of hydrophilic polymer may be present as a wetting agent, ethylene glycol may be added as a 'pore-former' and glycerol is often included to afford flexibility to the finished membrane. The mixture is then cast on to a moving, perfectly smooth, stainless-steel belt, to give a film 90–170 µm thick (Fig. 12.4.1). By carefully controlling the temperature and relative humidity, the solvents are slowly evaporated off, leaving a wet gel of highly porous, three-dimensional structure, which dries to give a membrane of considerable mechanical strength (Fig. 12.4.2). Pore size and other membrane characteristics are determined by the initial concentration of the polymer, the mixing process, including the

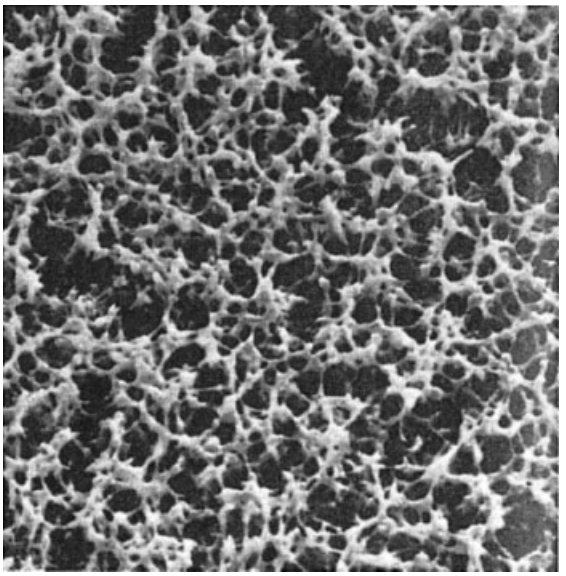

Figure 12.4.2 Scanning electron micrograph (4000×) of the surface of a 0.22-µm pore-size cast cellulose membrane filter.

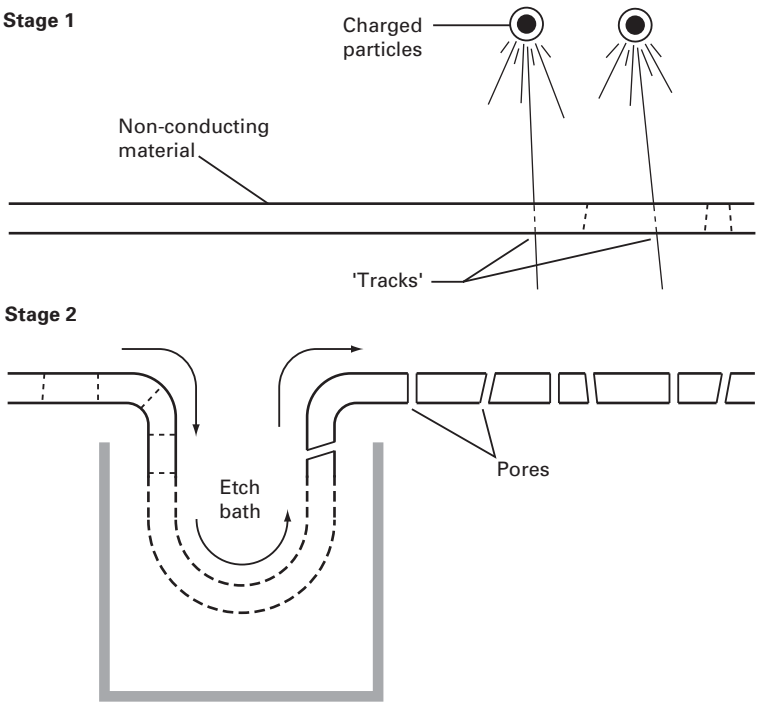

Figure 12.4.3 Membrane manufacture – the irradiation-etch process (see text for details of stages 1 and 2).

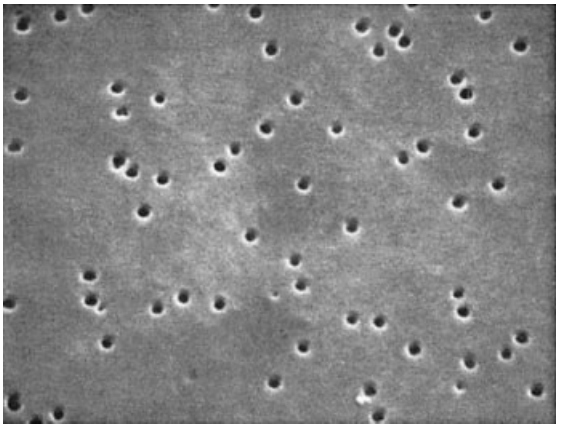

Figure 12.4.4 Scanning electron micrograph (10 000×) of the surface of a 0.2-μm pore-size polycarbonate track-etch membrane filter.

solvents added, and the environmental drying conditions.

Track-etch (irradiation-etch) process

Developed from the method of Fleischer *et al.* (1964) and originally patented with the Nuclepore Corporation, this process is operated in two stages. First, a thin film (5–10 μm thick) of polycarbonate or polyester material is exposed to a stream of charged particles in a nuclear reactor; this is followed by a second stage, where the fission tracks made through the film are etched out into round, randomly dispersed cylindrical pores (Fig. 12.4.3). Pore density and pore size are controlled by the duration of exposure of the film within the reactor and by the etching process respectively. The finished track-etched membranes are thin, transparent, strong and flexible (Fig. 12.4.4).

Expansion process

Stretching and expanding of fluorocarbon sheets (e.g. PTFE), along both axes is sometimes undertaken to provide porous, chemically inert membranes. A support of polyethylene or polypropylene is usually bonded to one side of the membrane to improve handling characteristics. Their hydrophobic nature ensures that these filters are widely employed in the filtration of air and non-aqueous liquids.

An alternative method of production for PTFE

filters is by a process that forms a continuous mat of microfibres, fused together at each intersection to prevent shedding into the filtrate. These filters usually have no supporting layer to reduce their chemical resistance.

Anodic oxidation of aluminium
This procedure is employed to produce ultrathin membranes, with a honeycomb-pore structure, in which the pores have a narrow size distribution (Jones, 1990). These membranes are hydrophilic and offer several advantages over polymeric membranes, including very high temperature stability (up to 400 °C) and minimal levels of extractable materials, because monomers, plasticizers and surfactants are not used in the production process.

Other methods of filter construction
Other methods of manufacture include solvent leaching of one material from a cast mixture leaving pores, the production of bundles of hollow fibres and deposition and etching of sacrificial layers of silicon (Desai *et al.*, 1999).

2.4.2 The mechanisms of membrane filtration

Membrane filters are often described as 'screen' filters and are thereby contrasted directly with filter media that are believed to retain particles and organisms by a 'depth' filtration process. By this simple definition, filters made from sintered glass, compressed fibre or ceramic materials are classified as depth filters, while membranes derived from cast materials, stretched polymers and irradiated plastics are classified as screen filters. In essence, during depth filtration, particles are trapped or adsorbed within the interstices of the filter matrix, while screen filtration involves the exclusion (sieving out) of all particles larger than the rated pore size.

Unfortunately, classification of membrane filters is not nearly as simple as this scheme might suggest. For example, some manufacturers use the terms 'screen' filter and 'depth' filter respectively to describe membranes with capillary-type pores, i.e. track-etch membranes, and those possessing tortuous inter-linked pores made by gel casting. It is now recognized that the filtration characteristics of many membrane filters cannot be accounted for in terms of the sieve-retention theory alone. In 1963, Megaw and Wiffen pointed out that, although membrane filters would be expected to act primarily by sieve retention, they did possess the property of retaining particles that were much smaller than the membrane pore size, larger particles being trapped by impaction in the filter pores. This aspect is discussed in more detail below. A more precise classification might be expected to take into consideration the considerable variation in membrane filter structure (see section 2.4.1) and the subsequent influence that this may have on the mechanism of filtration.

The influence of membrane-filter structure on the filtration process
Several studies have reported a marked difference between the pore structure of the upper and lower surfaces of polymeric membrane filters. Of particular note are the works of Preusser (1967), Denee & Stein (1971) and Marshall & Meltzer (1976). These workers have all shown one surface to have a greater porosity than the other. This phenomenon can be used to advantage in filtrations, since it confers a depth-like filtration characteristic on the membranes when used with the more open side upstream. Particles can now enter the interstices of the filter, increasing the time to clogging. The variation in flow rate and total throughput resulting from the different directions of flow can exceed 50%. Most filter manufacturers recognize the asymmetry of their membranes; indeed, several emphasize it in their technical literature and ensure that all disc filters are packed in the preferred flow direction (top to bottom). Highly anisotropic membranes, with superior filtration characteristics to those of conventional mixed-ester membranes, have been described (Kesting *et al.*, 1981; Wrasidlo & Mysels, 1984). Exactly the same principle is applied in the manufacture of depth filters where increased filter life and dirt holding capacity are achieved when the density of the filter medium increases from the upstream direction. The improved dirt retention is particularly useful when depth filters are used as a prefilter for a sterilizing-grade screen membrane.

A membrane filter can be further characterized by its pore-size distribution and pore numbers. Manufacturers have traditionally given their mem-

branes either an 'absolute' or 'nominal' pore-size rating, usually qualified by certain tolerance limits. There has been increasing recognition, however, that the designation 'absolute' is misleading. Complete removal of all suspended material can only be assured when a sieving mechanism is operative and all the particles are larger than the largest pore in the membrane, but the situation rarely prevails in which the diameters of the smallest particle and the largest pore are known with certainty. Even if this situation were known to exist, an 'absolute' filter could only be expected to remove all suspended material for a limited time, because on prolonged use there is the possibility of microorganisms growing through the membrane. 'Nominal' pore size implies that a certain percentage of contamination above that size is retained. Graphs depicting pore-size distribution have been offered by several filter manufacturers (Fig. 12.4.5). It must be remembered that the techniques used to establish pore size vary from manufacturer to manufacturer, and the values obtained are not necessarily comparable. Indeed, not only are manufacturers not obliged to measure pore size by a standardized method, but they are also under no obligation to give any details of the particle size distribution (although this may be available on request). If these facts are considered together with the observation that pore size measurements based upon bubble point determinations (section 4) may be influenced by membrane thickness and the nature of the membrane polymer (Waterhouse & Hall, 1995), it is not surprising that membranes having the same labelled pore size display substantial differences. Table 12.4.1 (adapted from Kawamura *et al.*, 2000) shows that the largest pores measured in the 0.2 µm membrane of one manufacturer were, in fact, almost twice that dimension, and the average pore size in another membrane was 35% greater than the labelled value.

It is apparent from these data that the designated pore size should not be regarded as absolute, but would be better interpreted as a label indicating the likely suitability for a particular purpose.

Jacobs (1972) first described the distribution of pore diameters in graded ultrafilter membranes and discussed the maximum pore diameters and average pore diameters of various commercially avail-

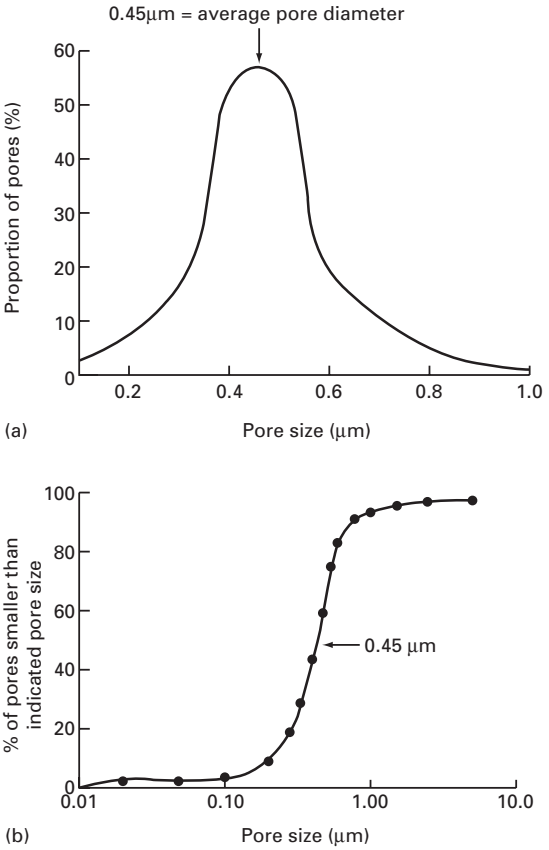

Figure 12.4.5 Typical pore-size distribution curves for 0.45 µm rated cellulose membranes obtained from mercury intrusion tests.

Table 12.4.1 Pore size characteristics of three nominal 0.2 µm membranes from different manufacturers.

Filter supplier	*Minimum µm*	*Average µm*	*Maximum µm*
1	0.16	0.225	0.33
2	0.195	0.264	0.388
3	0.203	0.269	0.367

able membranes. Subsequently, other workers were unable to confirm a pore size distribution of ±0.03 µm about a mean value, as is claimed for certain 0.45-µm filters (Pall, 1975; Marshall & Meltzer, 1976). While it has long been established that track-etched filters normally possess a greater uniformity than cast polymeric membranes, it is, nevertheless, clear that track-etched filters may not be entirely free from irregularities in pore size and shape (Pall, 1975; Alkan & Groves, 1978). A broader pore-size distribution within a membrane filter is not necessarily considered a failing, since it offers resistance to early clogging occasioned by too close a match between the dominant pore size and the prevailing particle size.

Cellulose filters (available in a range of pore sizes from around 12 µm down to 0.025 µm) possess between 10^7 and 10^{11} pores/cm^2, the number increasing as the pore size decreases. This contrasts with the 10^5 to 6 x 10^8 pores/cm^2 offered by a similar size range of track-etched filters. The number of pores and their size distribution will contribute to the overall porosity (void volume) of the filter system, which is considered to be approximately 65–85% for cellulose filters (decreasing with decreasing pore size) and only 5–10% for the track-etched product. Overall fluid-flow characteristics are similar for both types of filter (Ballew *et al.*, 1978), since the greater thickness of cellulose filters (≈150 µm) and their tortuous pore system afford approximately 15 times more resistance to flow than the 10-µm-thick track-etched filter.

There appears little justification for assuming a uniform pore structure, at least within the cast polymeric membranes, and the simple capillary pore model does not describe correctly the typical membrane filter. Duberstein (1979) states that the bacterial-removal efficiency of membrane filters depends on the membrane pore size distribution and on the thickness of the membrane; the latter is in disagreement with the sieve theory (see below), which relies solely on retention associated with the pore size of the surface pores. Furthermore, these two factors are not the only ones that have a bearing on the bacterial-removal efficacy; both the tortuosity of the pores through the membrane and its chemical composition (and hence its surface charge) will influence the extent of removal. The characteristics of the fluid being filtered (pH, ionic strength, presence of surfactants etc.), the character of the suspended organism or particle and the differential pressure across the membrane (Lee *et al.,* 1998) are additional factors that all have a bearing on the efficiency of particle retention. Indeed, the extent to which particle retention efficiency is dependent upon such physicochemical parameters gives an indication of the relative contributions of sieving and adsorption to the particle removal process.

For the thin track-etched membrane, the contribution made by the thickness of the filter towards the retention process may be considered small, especially in the light of their relatively uncomplicated pore structure, and the term 'screen' filter may adequately described this type of membrane (Heidam, 1981). The thicker cast polymeric membranes, as exemplified by the cellulose filters, however, offer characteristics between those of a true depth filter and those of a true screen filter and may best be described as membrane 'depth' filters. With these filters, very small particles will be retained by adsorption, but a point must be reached beyond which the smallest particle confronting any filter is larger than that filter's largest pore, in which case the sieve mechanism can adequately describe the filtration phenomenon.

The removal of microorganisms from liquids by filtration

Sterile filtration is considered to be the absolute removal of bacteria, yeasts and moulds but not viruses (PDA, 1998). It should by definition be able to deliver a sterile effluent independently of the challenge conditions, even when these are severe (Reti, 1977). In practice, this can be achieved by means of a 0.22- (or 0.2)-µm filter, although various authors have, in fact, shown that this filter is not absolute. Bowman *et al.* (1967) described the isolation of an obligate aerobe (cell diameter <0.33 µm), then termed a *Pseudomonas* sp. ATCC 19146 (later called *Pseudomonas diminuta*, now *Brevundimonas diminuta*), which could pass through a 0.45-µm membrane filter (see below); this poses a severe challenge to sterilization by filtration. The idea that sterile filtration is independent of the challenge condition is untenable. One of the prerequisites for successful filtration is an initial low number of

organisms; as the number of *B. diminuta* in the test challenge increases, the probability of bacteria in the filtrate increases (Wallhausser, 1976). An early report (Elford, 1933) had likewise shown that a filter's ability to retain organisms decreased as the number of test organisms (in this case, *Serratia marcescens*) increased and as the filter's pore-size rating increased. Approximately 0–20 *Pseudomonas* organisms per litre can pass through even so-called absolute filters (Wallhausser, 1979); the extent of the passage of *B. diminuta* through membrane filters is encouraged by increasing pressures (Reti & Leahy, 1979).

Leptospira species, together with other water-borne bacteria, have also been reported in the filtrate of well water that had passed through a 0.2-μm-rated membrane (Howard & Duberstein, 1980), and even the larger cells of *S. marcescens* can also pass through a 0.2-μm filter, although to a much smaller extent than *B. diminuta* (Wallhauser, 1979). Mycoplasmas, which lack rigid cell walls and consequently have a more plastic structure than bacteria, can pass through 0.22-μm filters (Lukaszewicz & Meltzer, 1979b), and such an organism, *Acholeplasma laidlawii*, has been used to validate 0.1-μm-rated sterilizing filters (Bower & Fox, 1985). The variety of organisms that have now been reported as capable of penetrating 0.2 (0.22)-μm membranes is substantial. In addition to those mentioned above, Sundaram *et al.* (1999) have identified reports of filter transmission of bacterial L forms, several genera of water-borne bacteria, spirochaetes, Gram-negative opportunist pathogens such as *Ralstonia pickettii*, corynebacteria and streptomycetes. Whilst the early reports were confined to specific membrane types, high bacterial challenge levels and non-pathogens that were unlikely to arise in pharmaceutical materials, the more recent ones demonstrated that this was not invariably the case. This has led to the same authors strongly supporting the proposal first put forward by Robinson (1984) that the 0.2- (0.22)-μm membranes should no longer be regarded as the routine sterilizing grade, but replaced with 0.1 μm membranes for this purpose (Sundaram *et al.*, 2001a–c). This is by no means the consensus view, however, and several observers consider there to be no need to consider an industry-wide change in this respect. Rather, they contend that thorough validation studies (section 4) using realistic bioburden isolates are likely to ensure a satisfactory level of sterility assurance (Waterhouse & Hall, 1995; Kawamura *et al.*, 2000; Bardo *et al.*, 2001; Levy 2001a). There is general agreement, however, that the circumstances in which 0.1-μm membranes are appropriate for sterilization include the following: (1) when there is evidence of mycoplasmas present in the normal bioburden; (2) when the product is, or contains, serum; (3) when manufacturing water for injection or pharmacopoeial purified water from a source likely to contain small bacteria (since many organisms are known to minimize their surface to volume ratio and become smaller in conditions of nutrient deprivation).

Wallhausser (1979) emphasizes the pore-size distribution of filter materials, which may be heterogeneous in form and composition, and the fact that pore size itself cannot be taken as an absolute yardstick for sterile filtration. It is to be expected, therefore, that two filters with the same nominal pore size can have markedly different filtration efficiencies, not only because the number, tortuosity and sorption characteristics of the channels within them may vary, but also because they have been characterized using different methods. Clearly therefore, care must be exercised in selecting a filter, particularly in an industrial setting, when there are several alternatives of the same nominal grade to choose from. There are dangers in attempting to select on the basis of price alone.

The reduction in bacterial concentration used as a parameter of filter efficiency is normally termed the titre reduction value (Tr). Because it is the ratio of the number of organisms challenging the filter to the number of organisms that pass through, the production of a sterile filtrate will, axiomatically, give a Tr of infinity. Under these circumstances, convention places '>' in front of the challenge number, so that a sterile filtrate resulting from a challenge of 10^7 is represented as a Tr of $> 10^7$. The Tr may also be represented as its logarithmic value, i.e. 7, when it is called a log removal factor or log reduction value (LRV).

The foregoing thus suggests that sieve retention is only one mechanism responsible for sterile filtration. Other contributing factors include van der

Waals forces and electrostatic interactions (Lukaszewicz & Meltzer, 1979b). Tanny *et al.* (1979) showed that many *B. diminuta* cells could be removed from suspension by adsorptive sequestration, using a 0.45-μm membrane filter, and postulated that an organism could actually enter the pore but be retained there by this mechanism.

The retention mechanisms operating during membrane filtration are elegantly illustrated in the scanning electron micrographs of Todd & Kerr (1972), where the screen-filter action of a track-etched filter is clearly contrasted with the depth-filter characteristics of a cellulose membrane filter. Similarly, Osumi *et al.* (1991) published scanning electron micrographs clearly showing that many of the pores in a 0.2-μm-rated membrane were much larger than the *B. diminuta* cells that were entrapped within them, and that the bacteria were usually retained by the membrane within the first 30 μm of the filter depth. The dominance of adsorptive effects during the filtration of plasma proteins and influenza vaccine through 0.22-μm and 0.45-μm membrane filters respectively, has been recognized (Hawker & Hawker, 1975; Tanny & Meltzer, 1978). Track-etched filters show few adsorptive properties and this can be attributed to their thinness, lack of tortuous channels and hence purely sieve-like properties. Adsorptive sequestration is not an inherent quality of a filter, but rather describes the ability of that filter to capture organisms of a given size (Lukaszewicz *et al.*, 1978; Lukaszewicz & Meltzer, 1979a). Depth-type filters, with a broad distribution of pore sizes, are believed to retain organisms largely by adsorption (Lukaszewicz & Meltzer, 1979a). Bobbit & Betts (1992) compared bacterial retention at a range of pore sizes on both screen-type polycarbonate membranes and cellulose-ester membranes. They observed that the former exhibited a much more distinct size threshold at which no further cells would pass through the membrane, and so had greater potential for the selective removal of bacteria from suspension according to size.

Thus, sieve retention may yet be the most important mechanism whereby sterile filtration is achieved, but it is unlikely to be the sole contributory factor. Although many membrane filters can no longer be considered to act simply as sieves, their thinness and greater uniformity of pore size give them several advantages over conventional depth filters (section 2.4.5), a fact that is widely exploited in filtration technology.

2.4.3 Crossflow filtration

The traditional mode of filtration (sometimes termed normal filtration) is that in which the liquid approaches the filter perpendicularly, and all of it passes through as a result of either upstream pressure or, less commonly, downstream vacuum. A problem that often arises using this form of filtration is that the filter membrane becomes blocked with suspended solids. Such blockage is minimized in crossflow filtration where the liquid to be filtered is pumped in a direction parallel with the membrane surface and the filtrate (also called permeate) passes through the membrane as a result of a pressure differential – the trans-membrane pressure (Fig. 12.4.6). The principle of crossflow membrane filtration can be applied not only to suspended solids of microscopic dimensions – where it is termed microfiltration, but also to the separation of dissolved molecules from the solvent. Ultrafiltration is the term applied to the separation of solutes having molecular weights of the order of 10 000–100 000 Da whilst nanofiltration and reverse osmosis describe similar separations of progressively smaller molecules. The term tangential flow is also used to describe crossflow filtration, although, in reality, it is a misnomer because the liquid does not approach the membrane at a tangent.

In crossflow microfiltration, the speed at which the fluid passes over the surface is critical; it is usually 1–6 m/s, and if reduced below this range the tendency to blockage of the membrane pores is much increased. Just as in any other form of filtration, the rate at which the suspending medium is separated from the solids is increased as the surface area of the membrane increases. Although the membrane can be flat, a tubular form is also common, and it is beneficial to design a system with maximum tube circumference and minimum cross sectional area. This means that it is more efficient to have tubes that have infoldings so they are star-shaped in cross-section, or multiple small-bore

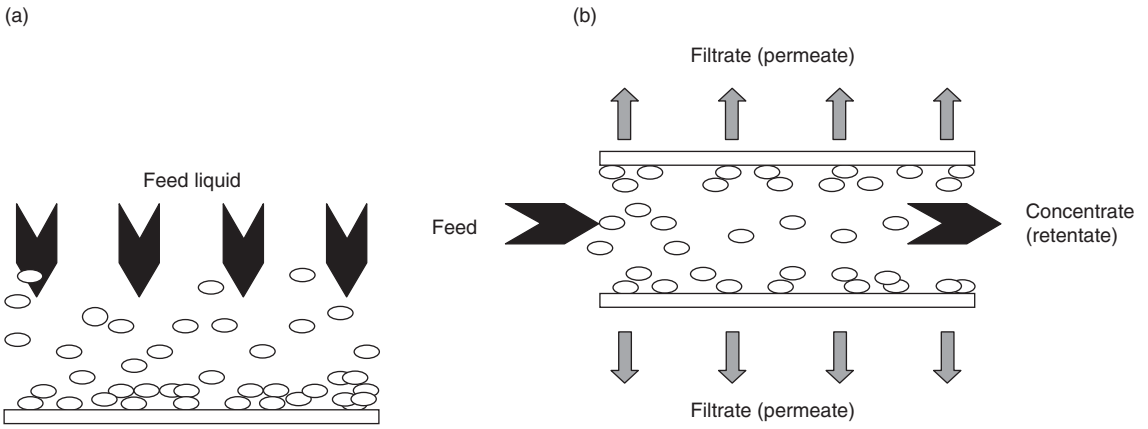

Figure 12.4.6 Comparison between (a) normal and (b) crossflow filtration.

tubes rather than a single tube of large diameter. Small lumens result in high pressures within the tube (typically 10–35 p.s.i., equivalent to approximately 70–240 kPa) and require powerful pumps to achieve the required velocity; on an industrial scale this may mean significant energy costs. The high costs, however, are offset by the fact that the membrane is less inclined to block than a traditional screen or depth filter, and in situations where the filter may be reused, it is easier to clean by back-washing.

Not all of the suspending medium or solvent in the feed liquid passes through the membrane, and that which is retained, the retentate (concentrate), has an increased concentration of suspended solid or solute; it may be recirculated via a holding tank. The ratio of permeate to feed volume is termed the recovery, and recovery values up to 80% or more may be achieved under optimized conditions. The membranes used in crossflow systems are highly asymmetric with the surface facing the feed liquid having a fine and carefully controlled pore size; this surface may be formed as a film approximately 0.1–5.0 μm deep on a much more porous support up to 50 μm or more thick. Multiple pairs of flat membranes can be mounted into cassettes with porous separators between each pair; alternatively, crossflow membranes may be coated onto the inside wall of a tubular element having a typical diameter of approximately 0.6–2.5 cm. Several such elements can be mounted together in the filter device to produce modules having from three, to more than 100 elements. As part of an extensive review of the process, Dosmar & Brose (1998) give a well-illustrated account of the various designs of crossflow filtration devices that are currently available.

The filter membrane itself can be manufactured from the various polymers that are used for traditional perpendicular (normal) flow filtration systems, but ceramic or metallic membranes are also available, and all can achieve the 0.1–10 μm pore size range typical of clarifying and sterilizing membranes used in traditional filtration systems. Ceramic membranes are usually made from alumina, and although more expensive, afford better heat resistance than polysulphone membranes (a common alternative) without any loss of chemical resistance. Crossflow filtration systems employing flexible textile supports are used for water purification; here the filter membrane is formed by deposition of the suspended material as a thin film on the inner surface of the tube. If the quantity of suspended material in the feed liquid is insufficient to form the membrane, a filter aid may be added initially to the feed water.

2.4.4 Membrane filters used for sterilization

The most suitable pore size for a sterilizing-grade filter is chosen, in part, by considering the minimum dimension (frequently less than 1 μm) of the microorganism to be retained. The efficient removal of all bacteria from contaminated solutions may sometimes require a 0.1-μm-rated membrane filter

(see section 2.4.2). Experience has shown, however, that, under normal pharmaceutical good manufacturing practice (GMP) conditions (Medicines Control Agency, 2002), the sterilization of pharmaceutical and blood products can be assured by their passage through a 0.20–22-μm membrane filter, but part of the process validation must include regular sterility tests.

In other areas, where the likely contaminants are known or additional filtrative mechanisms are at play, a membrane filter of larger pore size may be considered sufficient to ensure sterility. For instance, the sterilization of air and gases during venting or pressurizing procedures can often be assured by passage through filters of 0.45–0.8-μm-rated pore size. The removal of yeast during the stabilization of beers and wines can be effected by a 0.6-μm membrane filter. In general, however, such filters are only employed in systems where a reduction in bacterial numbers and not complete sterilization is demanded. An ideal example of this is the routine filtration through a 0.45-μm-rated filter of parenteral solutions that are later to be terminally sterilized. This reduces the likelihood of bacterial growth and pyrogen production prior to autoclaving.

Sterilizing membrane filters are available in discs ranging from 13 to 293 mm in diameter and their filtrative capacities make them the ideal choice for the small- and medium-scale processes normally encountered in the laboratory or hospital pharmacy (Table 12.4.2).

The flow rate of a clean liquid through a membrane filter (volume passed per unit time) is a function of that liquid's viscosity, the pressure differential across the filter and the filtration area and is given by:

$$Q = C(AP/V)$$

where Q = volumetric flow rate, A = filtration area, V = viscosity of the liquid, P = pressure differential across the membrane and C = resistance to fluid flow offered by the filter medium, governed in part by the size, tortuosity and number of pores.

The industrial manufacturer of sterile fluids needs to filter very large volumes and, as a consequence, demands a flow rate far beyond the capabilities of the largest available membrane disc. To provide the filtration area needed, multiple-plate filtration systems have been employed, where up to 60 flat filter discs of 293 mm diameter, separated by screens and acting in parallel, can be used to provide a total surface area of 3.0 m^2. A typical multiple-plate filtration system is illustrated in Fig. 12.4.7.

A second approach can be to use cartridge filters (Cole *et al.*, 1979). These are essentially hollow cylinders formed from a rigid perforated plastic core, around which the membrane filter, supported by a suitable mesh and sometimes protected by a prefilter, is wound. An outer perforated plastic sleeve provides protection against back-pressure and is held in place by bonded end-caps (Fig. 12.4.8). The cartridge filter combines the advantages of increased filtration area with ease of handling. Since the filter is no longer in the form of a fragile disc, it can be easily installed in special holders. Multiple cartridge units are available, which may contain, for example, up to twenty 79-cm filter

Table 12.4.2 Effect of filter diameter on filtration volumes.

Filter diameter (mm)	*Effective filtration area*[a] *(cm^2)*	*Typical batch volume*[b] *(L)*
13	0.8	0.01
25	3.9	0.05–0.1
47	11.3	0.1–0.3
90	45	0.3–5
142	97	5–20
293	530	20

[a]Taken from one manufacturer's data and to some extent dependent on the type of filter holder used. Values may well vary from manufacturer to manufacturer.

[b]For a low-viscosity liquid.

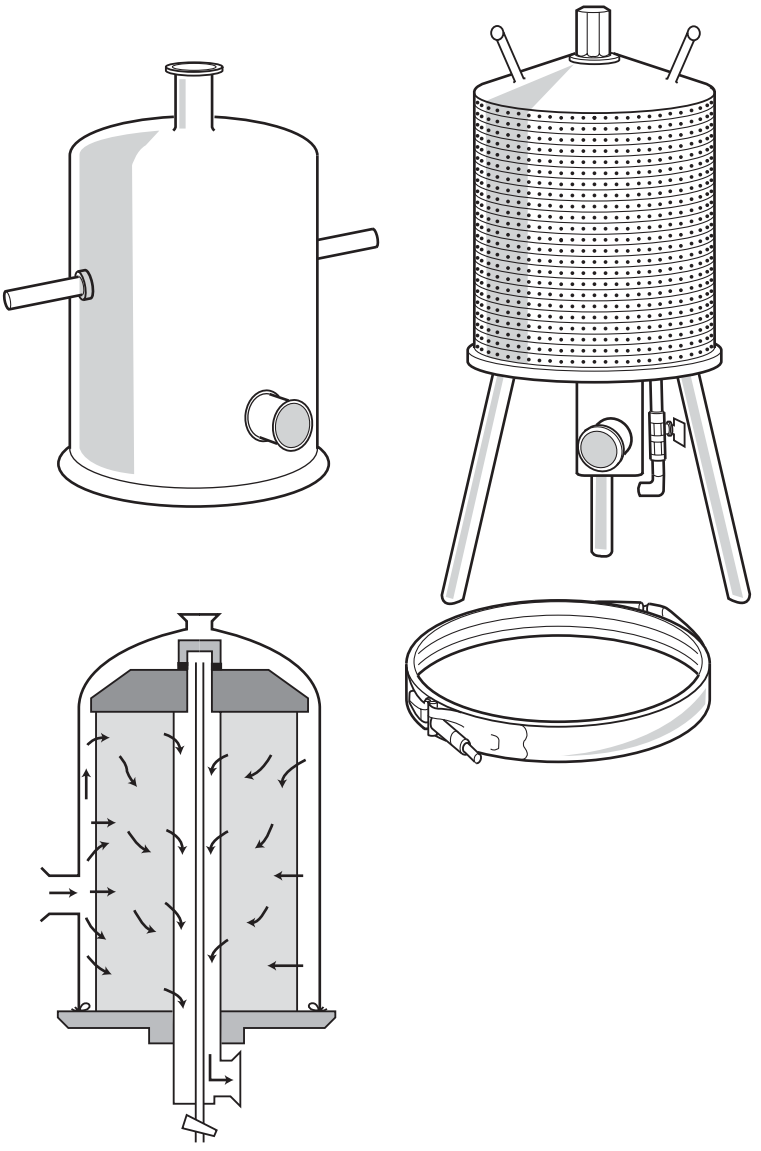

Figure 12.4.7 A typical multiple-plate filtration system, with inset showing the fluid-flow path during filtration.

tubes (of 5.7 cm diameter), giving a maximum filtration area of approximately 2.4 m^2.

The most common filter format for use in large-scale filtration systems is the pleated-membrane cartridge. Early devices were manufactured from a flexible acrylic polyvinylchloride copolymer membrane, incorporating a nylon web support (Conacher, 1976); other membranes have now evolved, which can also be pleated without damage (Meltzer & Lukaszewicz, 1979), and the range of materials includes cellulose esters, polyvinylidene fluoride (PVDF), PTFE, nylon, acrylic and polysulphone. The pleated configuration of the membrane ensures a far greater surface area for filtration than a normal cartridge filter of similar dimensions. For comparison, a single standard pleated-polycarbonate membrane cartridge of 24.8 cm length and 6.4 cm diameter, such as that illustrated in Fig. 12.4.8,

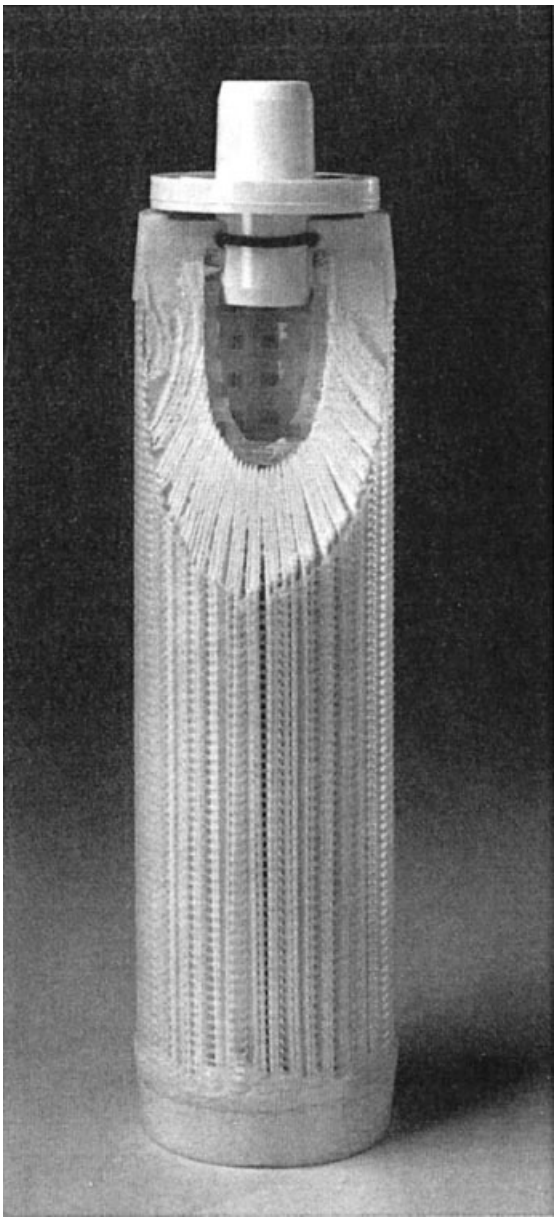

Figure 12.4.8 Cutaway showing the construction of a pleated polycarbonate membrane cartridge filter.

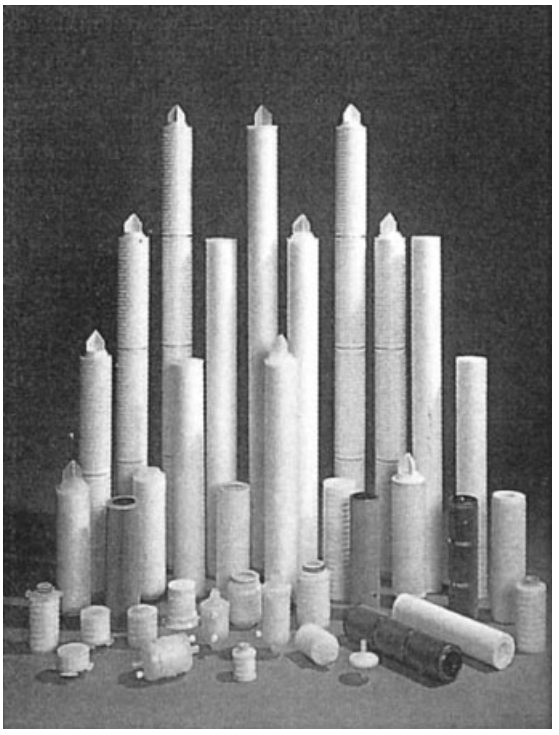

Figure 12.4.9 A selection of cartridge and capsule filters, which illustrates the variety available from a major manufacturer.

can offer a filtration area approaching 1.7m^2, approximately 30 times that afforded by a typical 293-mm membrane disc; the effective area can be increased even further by connecting these cartridges in series. Pleated cartridges are also manufactured as units in sealed plastic capsules, which are disposable and convenient to use, but relatively expensive. Figure 12.4.9 shows the diverse range of cartridges and capsules currently available.

To ensure the widest application for their filters, manufacturers offer their membranes in a wide variety of constituent materials and formats (Fig. 12.4.9). This permits the selection of a suitable filter type for use with most of the commonly encountered solvent systems (Gelman, 1965; Brock, 1983). Extensive chemical-compatibility lists are included in the catalogues of most manufacturers and further guidance can often be obtained through their technical-support services. Subtle changes in filter structure do occur, however, when processing mixtures of liquids, the complex fluid presenting entirely different solvent properties to the membrane from what could be predicted from compatibility studies involving the individual liquid components. In a number of instances, these changes have resulted in filter failure, and com-

patibility tests should always be undertaken when mixed-solvent systems are to be processed (Lukaszewicz & Meltzer, 1980). It is as well to remember, also, that any system is only as compatible as its least resistant component, and attention must be paid to the construction materials of the filter holder, seals, tubing and valves. Table 12.4.3 describes the properties of polymers commonly used in membrane construction.

Hydrophobic filters (e.g. PTFE) are available for the sterile aeration of holding tanks and fermentation vessels in the beverage and biotechnology industries, for the supply of fermentation tanks with sterilized gas, for the filtration of steam and for the removal of water droplets from an oily product. They can be used to filter aqueous solutions by first wetting the membrane with a low-molecular-weight alcohol, such as ethanol. Hydrophobic-edged filters, derived from cellulose nitrate or acetate, whose rims have been impregnated to a width of 3–6 mm with a hydrophobic agent can also be obtained. These find wide application in filtrations requiring that no residual solution remains trapped under the sealing ring of the filter holder, such as during the sterility testing of antibiotics. They also have the advantage that air or gas trapped behind a filter can escape through the rim and thus prevent airlocks or dripping during a filtration process.

To ensure the production of a sterile filtrate, the final filter and its holder, together with any downstream distribution equipment, must be sterilized. To minimize aseptic manipulations, it is customary to sterilize the membrane filter after mounting it in the filter holder. The sterilization method is usually selected from among the following: autoclaving, in-line steaming, dry heat, ethylene oxide and γ-irradiation. The choice depends largely upon the heat resistance of the filter and its ancillary equipment, and, before embarking upon any sterilizing procedure, it is first necessary to confirm their thermal stability. In extreme cases, chemical sterilization (for example, by immersion in a 2–3% formaldehyde solution for 24 h) may be the only satisfactory method.

Most filter types will withstand autoclaving conditions of 121 °C for 20–30 min and, as a result, the routine autoclaving of assembled small-scale filtration equipment is common practice. Similarly, in-line steaming is a widely used process, in which moist steam is forced through the assembled filter unit (and often the entire filtration system) under conditions sufficient to ensure an adequate period of exposure at 121 °C or other appropriate temperature (Kovary *et al.*, 1983; Chrai, 1989). This method is of particular value in large systems employing cartridge filters. It has the added advantage that the complete system can be sterilized, thereby lowering the bacterial contamination upstream from the final bacteria-proof filter. Voorspoels *et al.* (1996) undertook temperature mapping and process-lethality determinations at different locations within assembled cartridge filters, and their findings are particularly pertinent to the design of *in situ* sterilization-validation protocols. If the sterilization temperature or time exceeds the limits which are imposed by the manufacturer, 'pore collapse' may occur, with a subsequent reduction in membrane porosity. Frequently, cartridge filters are validated for a fixed number of resterilizations (e.g. four exposures, each of 15 min at 121 °C). For this reason, dry heat sterilization is rarely used, since the conditions employed are often too severe. For convenience, certain membrane filters may be obtained in a presterilized form, either individually packed or ready-assembled into filter holders, as single-use devices. Sterility is, in this case, usually achieved by ethylene oxide treatment or γ-irradiation.

2.4.5 Advantages and disadvantages of membrane filters

Membrane filters have several advantages over conventional depth-filtration systems, a conclusion emphasized by the technical literature supplied by the major membrane-filter companies. Table 12.4.4 summarizes the more important characteristics of membrane filters and compares them with conventional depth filters. Several features require further discussion, since they have considerable bearing on the quality of the final filtered product.

A problem usually associated only with conventional depth filters is that of 'organism growth-through'. If a bacterial filter is used over an extended period of time, bacteria lodged within the matrix can reproduce and successive generations

Table 12.4.3 Properties of polymers used in filter membrane construction.

Material	*Cellulose esters*	*PVDF*	*Polypropylene*	*Nylon 66*	*PTFE*	*Polysulphone/ polyether-sulphone*	*Polycarbonate*	*Polyamide*
Typical minimum pore size (μm)	0.025	0.1	0.2	0.04	0.1	0.04	0.05	0.2
Autoclavable at 121 °C?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Solvent resistance	Poor to moderate	Limited	Good	Good	Good	Limited	Good	Good
Extractables	Varies with grade	Low	Low	Very low	Low	Low	Very low	Low
Wettability	Hydrophilic	Naturally hydrophobic but available as hydrophilic	Hydrophobic	Hydrophilic	Normally hydrophobic, but hydrophilic available	Hydrophilic	Hydrophilic but available as hydrophobic	Hydrophobic
Protein binding	Acetate low, but nitrate and mixed esters high	Hydrophobic high; hydrophilic very low	Low	Very low binding grades available	Hydrophobic high; hydrophilic low	Low	Low	High
Special properties	Good strength and heat resistance but may be brittle	Not brittle. Grades having good virus removal available	Flexible and strong	Positively charged grades enhance endotoxin removal	Tolerance of pH extremes, solvents and high temps	Polyether-sulphone membranes having high flow rates are available	Usually track-etched membranes of high tensile strength	Tolerant of solvents and bases
Uses	Sterilization of aqueous solutions	Sterilization of aqueous solutions; hydrophobic membranes for gases	As an alternative to PTFE in many applications	Sterilization of aqueous solutions	Filtration of acids, bases, gases and solvents	Sterilization of tissue culture media and protein solutions	Microscopical observation of particles on filter surface	Highly alkaline solutions

PVDF, polyvinylidene fluoride; PTFE, polytetrafluoroethylene.

Table 12.4.4 Characteristics of membrane and depth filters.

Characteristic	*Membrane*	*Depth*
1 Filtration (retention efficiency for particles > rated pore size)	100%	<100%
2 Speed of filtration	Fast	Slow
3 Dirt-handling capacity	Low	High
4 Duration of service (time to clogging)	Short	Long
5 Shedding of filter components (media migration)	No	Yes
6 Grow-through of microorganisms	Rare (see text)	Yes
7 Fluid retention	Low	High
8 Solute adsorption	Low	High
9 Chemical stability	Variable (depends on membrane)	Good
10 Mechanical strength	Considerable (if supported)	Good
11 Sterilization characteristics	Good	Good
12 Ease of handling	Generally poor	Good
13 Disposability	Yes	Not all types
14 Leaching of extractables	Variable (depends on membrane)	Unlikely

will penetrate further into the filter, eventually emerging to contaminate the filtrate. The extent of this phenomenon will be a function of, at least in part, the nutritional status of the medium being filtered and the nutritional requirements of the contaminant. This problem is no longer considered to be exclusive to conventional depth filters and has been recognized to occur with some 0.45-μm membrane 'depth' filters (section 2.4.2) (Rusmin *et al.*, 1975). For this reason, it is recommended that the duration of filtration be as short as possible (Lukaszewicz & Meltzer, 1979a; *United States Pharmacopeia*, 2003).

Solute adsorption by filter is rarely a major problem in large-scale industrial processes, but it can be of greater consequence in the filtration of small volumes containing medicaments at high dilution. Conventional depth-filtration media have been implicated in the adsorption of antibiotics from solution (Wagman *et al.*, 1975), while the thinner membrane filters appear to suffer less from this disadvantage (Rusmin & DeLuca, 1976). Bin *et al.* (1999) observed between 116 and 429 μg benzalkonium chloride adsorption per 47-mm diameter disc, with the higher values arising on hydrophobic or anionic membranes. S.P. Denyer (unpublished results) has observed a similar loss (38%) of tetradecyltrimethylammonium bromide after filtration of 10 mL of a 0.001% w/v solution through a 0.22-μm cellulose membrane filter. Drug adsorption has been reported by De Muynck *et al.* (1988), and a method for its control suggested by Kanke *et al.* (1983). Presumably, adsorption sites are rapidly saturated in these thin membranes, and the passage of additional solution would probably occur without further loss. Nevertheless, it emphasizes the need to select the most compatible filter material and to discard, if at all possible, the first few millilitres of solution run through any filtration system. Flushing through to remove downstream particles is often an integral part of the filtration process anyway.

Care should be taken in the choice of filter in special operations, particularly where the loss of high-value material could be of significant economic importance. For instance, proteins (in particular those of high molecular weight) are readily removed from solution on passage through cellulose-nitrate and mixed-ester filters, and nylon (Hawker & Hawker, 1975; Olson *et al.*, 1977; Akers *et al.*, 1993). This is not so evident for fluorocarbon and cellulose-acetate filters, which would therefore be more suitable for filtration of pharmaceutical protein preparations (Pitt, 1987). The conformational changes elicited in proteins by filtration through filter media have been highlighted by Truskey *et al.* (1987).

A further problem associated with some mem-

brane filters is the leaching of extractives, some of which may be potentially toxic (Brock, 1983; Kristensen *et al.*, 1985). Surfactants, glycerol and other extractable materials added during the manufacturing process may leach from these filters during use, and limited flushing beforehand has been recommended (Olson *et al.*, 1980). As an alternative to flushing, a leaching process has been suggested, which requires boiling the new filter for 5–10 min in two changes of apyrogenic water. The level of extractable material ranges from 0% to 15% of the filter weight and varies according to filter type and filter manufacturer, and Kao *et al.* (2001) have recently shown proton nuclear magnetic resonance spectroscopy to be a convenient means of characterizing extractables. Special low-water-extractability filters, e.g. those constructed of anodized aluminium (Jones, 1990), are available for use in highly critical applications involving sensitive biological systems, e.g. tissue-culture work, or very small volumes of filtrate. Track-etched membranes yield no leachable material and need not be treated before use.

One problem associated with membrane filters of all types, and of considerable economic importance, is the rapidity with which they clog when a large volume of solution or highly contaminated fluid is processed. To overcome this, it is possible to introduce a depth filter, as a prefilter, into the system, the high 'dirt'-handling capacity of which will remove much of the initial solids and complement the filtering efficiency of the final (sterilizing) membrane filter (Lukaszewicz *et al.*, 1981a). Such a prefilter is generally constructed of bonded borosilicate glass fibre and is available from most manufacturers in sizes and grades compatible with their membrane filters. For use on a large scale, prefilters are often supplied as cartridges. In the critical area of parenteral-product filtration, cellulose-webbing prefilters that do not shed particles are available. By selecting the correct grade of prefilter, the throughput characteristics for any membrane-filtration assembly can be improved significantly (Fig. 12.4.10).

The correct matching of prefilter grade with membrane pore-size rating does not, on its own, provide the most economical and efficient system. Consideration must also be given to the prefilter membrane surface-area ratio, since too small a prefilter area will result in premature plugging with usable life still remaining in the membrane. Conversely, if the area of the prefilter is too large, it will be left only partly used when the membrane becomes blocked. The ideal ratio will make for the

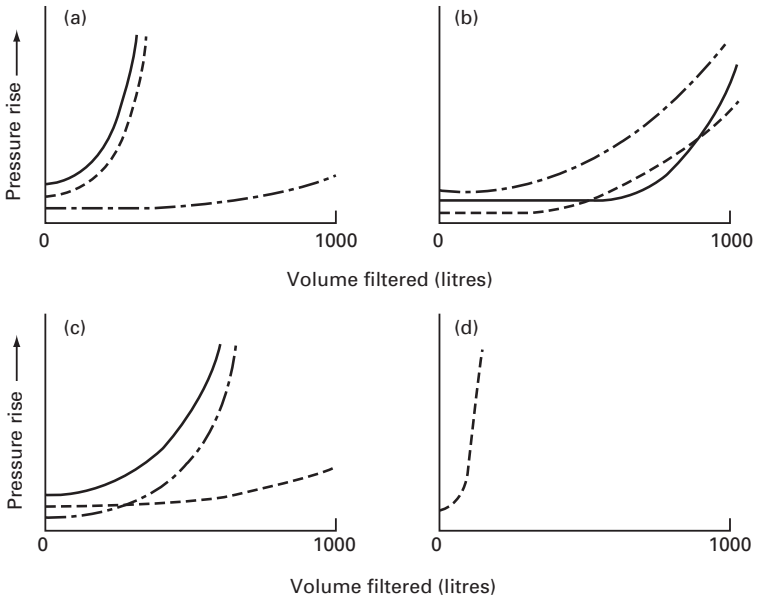

Figure 12.4.10 Effect of prefilter characteristics on the volume filtered and filtration pressure. – – –, Combination of membrane filter prefilter; ——, prefilter alone; –·–·–, membrane filter alone. (a) Prefilter too coarse; insufficient preseparation, membrane filter clogs rapidly, pressure rises rapidly. (b) Prefilter too fine: prefilter clogs faster than membrane filter, poor effective filter life. (c) Correct prefilter: prefilter and membrane filter exhaust themselves approx. simultaneously, optimum effective filter life. (d) Membrane filter without prefilter: rapid rise in pressure, short effective filter life.

most economic filtration and must be determined for each new system.

2.4.6 Removal of viruses, prions and endotoxins by filtration

Despite sterilizing filtration still being defined as the removal of all microorganisms *except viruses* from a fluid (PDA Tech Rep 26, 1998) there has, nevertheless, been an increased interest in recent years in the subject of virus removal or reduction by filtration. This has stemmed in part from the rise in numbers of biotechnology products and from a greater awareness of the potential of plasma products to act as vectors of viral transmission (Aranah, 2001). These developments, combined with the improved characterization of bacteriophages and mammalian viruses to act as size markers, have led to more detailed guidelines for validation of virus removal from biotechnology and other vulnerable products (Anon, 1998).

Mammalian viruses vary in size from about 300 nm (e.g. vaccinia) down to about 20–25 nm (e.g. polio and parvoviruses), so those at the top of this range approximate in size to small bacteria (*B. diminuta* is 300 × 700 nm and *Acholeplasma* species about 300 nm diameter). It is to be expected, therefore, that sterilizing filters rated at 0.22 μm are likely to effect a reduction in concentration of some viral species by size exclusion, but the majority of viruses could only be removed by adsorptive mechanisms operating within such membranes. Because the efficiency of adsorption as a removal mechanism is much influenced by process-related factors like flow rate, pH and ionic strength of the fluid, pressure differential and temperature of the filter itself, it is considered less desirable to rely on adsorption-based filtration systems than size exclusion (sieving) ones. For that reason, several of the major manufacturers have produced virus-retentive membranes having pore sizes in the ultrafiltration range, but these provide a confusing array of nominal ratings. Some are, or were originally, rated on the basis of exclusion of polymers, usually dextrans or proteins of known hydrodynamic diameters, others are rated on average pore size, and yet others on the basis of the log reduction values that result from challenges with viruses of known dimensions. Despite the intention that virus-retentive membranes should operate on the basis of size exclusion rather than adsorption, Bechtel *et al.* (1988) reported that a membrane having virus-sized pores was unable to discriminate between three viral species of significantly different dimensions, producing approximately the same log reduction for each species; such a finding would not be expected if a sieving mechanism were operative. This failure was attributed to mechanical imperfections inherent in ultrafiltration membranes that result in the creation of a small fraction of exceptionally large pores that may have a disproportionately large impact on virus retention.

The virus-retentive membranes available are commonly manufactured from PVDF or PES although regenerated cellulose is also used, and they are available for direct flow and crossflow filtration systems in the usual range of flat discs, capsules and pleated cartridges. Because pores of a size sufficient to retain small viruses also retard the passage of large polymer molecules, several of the virus filters are rated according to their molecular weight cut-off. In selecting a filter, therefore, it is particularly important to know the size of the viruses to be removed and the molecular weights of the protein(s) that need to pass into the filtrate in order to achieve optimal filter performance. A typical specification for a virus-retentive membrane is currently a log reduction value of approximately 6 for larger viruses (human immunodeficiency virus, influenza and human T-cell leukaemia viruses all at approximately 100 nm) with an LRV of 3 for parvoviruses whilst permitting >95% recovery of proteins up to 150 kDa; use of filters in series permits LRVs of approximately 6 even for small viruses (Abe *et al.*, 2000). Levy *et al.* (1998) and Aranah (2001) have compiled comprehensive tabulations of LRVs published for a wide range of viruses and the filters of the major manufacturers.

The concerns about serum-derived products acting as vectors for the spread of viral infection are equally valid in respect of prions. These agents of transmissible spongiform encephalopathies are resistant to heat, radiation and chemical methods of sterilization yet capable of transmission via residues on surgical instruments and in therapeutic products manufactured from human tissues (Levy

et al., 1998; Taylor 1999). Virus removal filters having a pore size of 9 nm were shown to achieve over 5 log reductions of scrapie agent ME7 in artificially contaminated albumin solutions, but the performance was much reduced by the presence of detergents and by increase in pore size to 35 nm (Tateishi *et al.*, 2001).

Many sterile medicinal products must also satisfy pharmacopoeial limit tests for endotoxins (bacterial lipopolysaccharide), so the ability to remove or retain such material is a desirable property in a filter membrane. Just as membranes designed specifically to effect viral removal have been introduced in recent years, so, too, have membranes intended for endotoxin removal. Because lipopolysaccharides are negatively charged, the filters by which they are most effectively removed are treated to exhibit a positive charge so that removal is achieved by adsorption; size exclusion pays no part in the removal process since the endotoxin molecules are much smaller than the membrane pores. Hydrophilic PVDF is the material most commonly used in membrane manufacture, although positively charged nylon filters can also be effective (Vanhaecke *et al.*, 1989). On a research rather than production basis, it has been shown that immobilization of polymyxin B, deoxycholate and other materials onto polymer membranes can achieve such high affinity that endotoxin levels may be reduced below those required for parenteral products with residence times of only 6 s (Anspach & Petsch, 2000). Although non-polymer membranes have been introduced successfully in other filtration applications, membranes made of either ceramics or aluminium were shown by Bender *et al.* (2000) to be unsuitable for endotoxin removal.

Because the removal mechanism is adsorption there is a finite amount of endotoxin that can be retained on each membrane, and the maximum weight of pure endotoxin that can be adsorbed per unit area of membrane (ng/cm^2) should be the least ambiguous way of expressing filter performance. Despite this, some manufacturers make claims for endotoxin removal in terms of log reduction values without specifying both the volume and concentration of endotoxin in the solution with which the filter is challenged.

The performance of a filter membrane is influenced by the physical conditions under which the filtration occurs and the nature of the fluid being filtered. Vanhaecke *et al.* (1989) found endotoxin retention by nylon filters was much reduced when sodium chloride was added to 5% glucose solution compared with the value for glucose alone, although Brown & Fuller (1993) noted that retention improved with increasing molarity and decreasing pH. Because the extent of endotoxin removal is markedly affected by the nature of the fluid passing through the membrane and the possibility of competitive adsorption of other negatively charged molecules, thorough validation of the process is necessary before filtration can be relied upon as a means of endotoxin removal. Furthermore, the efficiency of adsorption might be reduced at high flow rates, so it is necessary to specify maximum pressure differentials in order to achieve satisfactory removal. Because of these constraints, a strategy of avoiding endotoxin accumulation in the process fluid in the first place is generally preferred to one of attempting to remove it at the end.

3 Applications and limitations of filtration

3.1 Filtration sterilization

Sterilization by filtration is widely used industrially and in hospitals. In brief, it may be employed for the sterilization of thermolabile solutions and soluble solids, as well as in the sterilization of air and other gases. Air sterilization is of particular importance in areas involving the aseptic production of many pharmaceutical products (Hargreaves, 1990; Denyer, 1998; Medicines Control Agency, 2002), in surgical theatres and in hospital wards specially designed for patients with a low resistance to infection. It would, however, be erroneous to imply that filtration sterilization has no disadvantages or limitations, and these will also be considered where appropriate.

3.1.1 Sterilization of liquids

Wherever possible, solutions should be sterilized by heating in an autoclave, because this eliminates the contamination risks associated with the transfer of

filtered liquid to sterilized containers. Some solutions are unstable when heated and consequently an alternative sterilizing procedure has to be sought. Ionizing radiation has been studied extensively, but, unfortunately, many substances that can be sterilized by this process in the solid state are unstable when irradiated in solution. Filtration is an obvious choice, although it must be added that another alternative for substances thermostable in the solid form but unstable in solution (even at ambient temperatures) is to sterilize the solid by dry heat and prepare the solution aseptically immediately before use.

Filtration cannot, in fact, be regarded as a true sterilization process. Admittedly, it will remove microorganisms (see section 2.4.2 for a discussion of the possible mechanisms of filtration), but the filtration process must then be followed by an aseptic transference of the sterilized solution to the final containers, which are then sealed, and recontamination at this stage remains a possibility.

Sterility assurance levels for products that have been filter-sterilized and aseptically filled are typically of the order of 10^{-3} (Gilbert & Allison, 1996), and it is for this reason that such products are much more heavily reliant on tests for sterility than heat-processed ones, which have sterility assurance levels of at least 10^{-6} and usually much better than this. Persuasive arguments, based on a statistical appraisal of the information conventional sterility tests can supply, have been put forward for their abandonment as a means of monitoring thermal-sterilization processes, the tendency now being to validate these processes by biological indicators (see Chapter 16; Brown & Gilbert, 1977). Nevertheless, although there might be much scientific merit in their abandonment, they do form an additional defence in the case of litigation following trauma from a suspected contaminated product, and sterility testing should always be carried out on samples of any batch prepared by an aseptic method. This would mean, in essence, that a solution which can be sterilized rapidly by filtration should ideally not be used until the test sample has passed the sterility test, which may take several days. In an emergency, however, it may well be that clinical judgement has to come down in favour of a hospital-prepared product which has not yet passed a test for sterility, if failure to use it poses a greater risk to the patient.

Despite these criticisms, filtration sterilization is performed on a wide range of liquid preparations (McKinnon & Avis, 1993; Avis, 1997) and routinely on liquid parenteral products (including sera) and on ophthalmic solutions. It is often the only method available to manufacturers of products that cannot be sterilized by thermal processes. Information as to the actual procedures may be found in the *United States Pharmacopeia* (2003), *British Pharmacopoeia* (2002) and other national and international pharmacopoeias. It must be emphasized that membrane filters are almost exclusively used in this context and that filtration with a filter of 0.22 (or 0.2)-μm pore size, rather than one of 0.45 μm, is recommended for this purpose.

Membrane filters find an equally important application in the small-scale intermittent preparation of sterile radiopharmaceuticals and intravenous additives. As a result of the special circumstances surrounding the preparation and use of such products, disposable, sterile filters attached to a syringe are generally used. Preparation of these products is best performed under laminar air flow (LAF) conditions (section 3.1.3).

The use of sterilizing-grade filters in parenteral therapy is not confined to the production stage alone. In-line terminal membrane filtration has been widely advocated as a final safeguard against the hazards associated with the accidental administration of infusion fluids contaminated with either particles or bacteria (Maki, 1976; Lowe, 1981; McKinnon & Avis, 1993; Voorspoels *et al.*, 1996). These filtration units, generally of 0.22-μm rating, may comprise an integral part of the administration set or form a separate device for introduction proximal to the cannula. In addition to affording some protection against particles and microorganisms introduced during the setting up of the infusion or while making intravenous additions (Holmes & Allwood, 1979), terminal filters also reduce the risk of an air embolism from air bubbles or when an intravenous infusion runs out (a wetted 0.22-μm membrane filter will not pass air at a pressure below 379 kPa (55 p.s.i.)). The properties of a wetted membrane filter have been further exploited in infusion-burette devices, where they act as an air

shut-off 'valve', designed to operate following administration of the required volume.

Although conventional wisdom formerly suggested that membrane filtration cannot be employed successfully in the sterilization of emulsions (McKinnon & Avis, 1993), recent reports have shown this not to be so, and parenteral emulsions (Hosokawa *et al.*, 2002), liposome suspensions (Goldbach *et al.*, 1995) and nanoparticle suspensions (Konan *et al.*, 2002) have all been sterilized by this method.

3.1.2 Sterilization of solid products

The *British Pharmacopoeia* (2002) lists four methods that may be used to sterilize powders: ionizing radiation, dry heat, ethylene oxide and filtration. The principle of the filtration process is that the substance to be sterilized is dissolved in an appropriate solvent, the solution filtered through a membrane filter and the sterile filtrate collected. The solvent is removed aseptically by an appropriate method (evaporation, vacuum evaporation, freeze-drying) and the sterile solid transferred into sterile containers, which are then sealed. Such a method was originally used in the manufacture of sterile penicillin powder.

It appears likely that the probability of contamination occurring during the postfiltration (solid-recovery) stage is higher than that described above for sterilizing solutions.

3.1.3 Sterilization of air and other gases

Air is, of course, the most common gas which is required in a sterile condition, although there is a less frequent, but nevertheless significant, requirement for other sterile gases (e.g. nitrogen for sparging the head-space above oxidation-prone liquids and oxygen administered to patients with breathing difficulties). Filters intended to sterilize air are employed in a variety of industrial applications, often as part of a venting system on fermenters, centrifuges, autoclaves and lyophilizers (Ljungquist & Reinmuller, 1998), or in hospitals to supply sterile air in operating theatres or through respirators to patients vulnerable to infection. In both the industrial and hospital settings, sterile air is also required for 'clean rooms' used for aseptic manufacturing or testing.

Many aspects of liquid filtration have direct parallels in the filtration of gases, although there are certain features specific to the latter. Prominent among these is the fact that particles suspended in a gas are exposed to Brownian motion, as a result of bombardment by the gas molecules. This phenomenon, which operates to an insignificant degree in liquids, means that particles suspended in the gas occupy an effective volume greater than that which would be expected from their real size, and so a filter with a given pore structure will remove much smaller particles from a dry, unwetted gas than it will from a liquid (provided that it is not wetted during use). Filters of up to 1.2-μm pore size have been found suitable for the provision of sterile air. Nevertheless, at these larger pore sizes occasional problems with moisture condensation and subsequent grow-through of bacteria can occur, and GMP regulations generally require a 0.2–0.22-μm filter for air sterilization.

Air filters may be made of cellulose, glass wool or glass-fibre mixtures, or of PTFE with resin or acrylic binders (Underwood, 1998). Depth filters, such as those made from fibreglass, are believed to achieve air sterilization because of the tortuous passage through which the air passes, ensuring that any microorganisms present are trapped not only on the filter surface, but also within the interior. The removal of microorganisms from air occurs as a result of interception, sedimentation, impaction, diffusion and electrostatic attraction (White, 1990). However, reproduction of microorganisms on the filter and their subsequent release into the atmosphere is one cause of 'sick building syndrome' (Kelly-Wintenberg *et al.*, 2000)

The quality of moving air is described by the maximum level of contamination permitted. In the USA Federal Standard 209 recognized six classes, namely Class 1, Class 10, Class 100, Class 1000, Class 10 000 and Class 100 000, where the maximum numbers of particles 0.5 μm or larger were respectively, $1/ft^3$ (0.035/L), $10/ft^3$ (0.35/L), $100/ft^3$ (3.5/L), $1000/ft^3$ (35/L), $10\,000/ft^3$ (350/L) and $100\,000/ft^3$ (3500/L). In the UK, environmental cleanliness is stated in terms of size and maximum permitted number of airborne particles, and four

grades designated A–D now exist (Medicines Control Agency, 2002). Grade A is the equivalent of Class 100 of the Federal Standard, with a particle count not exceeding 3500/m^3 for 0.5 μm size or greater. ISO 14644-1 was published in 1999, and it should be the classification described in this standard that should prevail in future. The relationship between the three classification schemes is described in Table 12.4.5.

Only Federal Standard 209 Class 100 air or better is acceptable for aseptic (sterile-area) purposes and the viable particle count is 0.1/ft^3 (0.0035/L) (Avis, 1997; Neiger, 1997). High-efficiency particulate air (HEPA) filters are available that remove particles of 0.3 μm or larger (Wayne, 1975) and, indeed, for strict aseptic conditions, Phillips & Runkle (1972) state that they will remove particles much smaller than this. Passage of phage particles (0.1 μm diameter) through ultrahigh-efficiency filters is remarkably low and it is considered that these filters provide excellent protection against virus aerosols (Harstad *et al.*, 1967).

An important type of air filtration incorporates the principle of laminar air flow (LAF). This was introduced by Whitfield in 1961 (Whitfield, 1967; Soltis, 1967; Whitfield & Lindell, 1969), and is defined as unidirectional air flow within a confined area moving with uniform velocity and minimum turbulence. Close control of airborne contamination may be a difficult problem, partly because of the non-uniform nature of the air-flow patterns in a conventional clean room, partly because they do not carry particulate matter away from critical work areas and partly because airborne contamination is not removed as quickly from the room as it is brought in (Whitfield, 1967; Avis, 1997; Neiger, 1997). Whitfield (1967) concluded that a uniform airflow pattern was needed to carry airborne contamination away from the work area. Laminar air flow was designed originally to remove dust particles from air by filtration, but it will also remove bacteria (Coriell & McGarrity, 1967). It was employed initially in the electronics and aerospace industries for the purpose of producing air with low particulate levels, necessary to prevent instrument and circuitry malfunction, but is now widely used by the pharmaceutical, cosmetic and other industries.

Laminar air flow can be used in the form of:

1 LAF rooms with wall or ceiling units, the air flow originating through one wall or ceiling and exiting at the opposite end, to produce a displacement of air;

2 LAF units (see below) suitable for small-scale operations, such as the LAF bench used for aseptic processing and sterility testing (White, 1990; Avis, 1997).

Thus, airborne contamination is not added to the work space, and any generated by manipulations within that area is swept away by the laminar air currents (Coriell, 1975). Nevertheless, there are limitations to the use of LAF, namely it will not sterilize a contaminated product or area (Wayne, 1975). Laminar air flow controls only airborne

Table 12.4.5 Clean room classifications based on airborne particulates.

Classification system	*Federal Standard 209 Class*	*1*	*10*	*100*	1000	*10 000*	*100 000*
	EU GMP Class	–	–	*A/B*	–	*C*	*D*
	ISO 14644-1 Class	*3*	*4*	*5*	*6*	*7*	*8*
Performance specifications[a]	0.1 μm	35	350	–	–	–	–
	0.2 μm	7.5	75	750	–	–	–
	0.3 μm	3	30	300	–	–	–
	0.5 μm	1	10	100	1000	10 000	100 000
	5.0 μm	–	–	–	7	70	700

[a]Performance specifications for the control of airborne particulates defined as limits on the number of particles of given size (μm) that may be present in a cubic foot of air.

particulate contamination and does not remove surface contamination (Phillips & Brewer, 1968; Brewer & Phillips, 1971). Correct techniques must be used, since poor aseptic technique can nullify LAF, and holes in the HEPA filter or air leaks in the system may allow contaminated air to enter the aseptic area (Stockdale, 1987; Neiger, 1997).

Filters that are used in LAF devices are HEPA filters, mentioned above. These have been designed with a bacterial removal efficiency of greater than 99.99% (White, 1990; Avis, 1997; Neiger, 1997) and often possess particle-removal efficiencies in the order of 99.9997% against 0.3 μm particles, a standard sufficient for even the most exacting pharmaceutical purposes. Their life can be prolonged by employing low-efficiency filters upstream to intercept most of the larger particles and some smaller ones before they reach the expensive HEPA filters. High-efficiency particulate air filters are most efficient when air passes through them at an average velocity of 30 m/min (100 ft/min; Coriell, 1975).

Laminar-air-flow units providing Class 100 (grade A) clean air are of two types, horizontal and vertical, depending upon the direction of the air flow. In vertical LAF (Fig. 12.4.11), a supply fan passes air down through an ultrahigh-efficiency filter into the work area, and the air exhausts through a grated work surface, often with the aid of a second fan. A slight negative pressure is maintained by adjusting the fans to exhaust more air than is supplied; this causes ambient air to move from the operator towards the external periphery of the work area, so that a protective curtain of air is created (Favero & Berquist, 1968). A vertical LAF of 30 m/min (100 ft/min) maintains a Class 100 condition, whereas 18 m/min (60 ft/min) does not (Loughhead & Vellutato, 1969). In horizontal LAF (Fig. 12.4.12), air passes from back to front through an HEPA filter at an average velocity of 30 m/min (100 ft/min), travels horizontally with minimum turbulence and exits at the front of the unit (Coriell & McGarrity, 1968, 1970).

Laminar-air-flow units have three general areas of usefulness: (1) for product protection, e.g. in sterility testing or aseptic filling; for these purposes, a standard horizontal LAF is suitable; (2) for personal protection, i.e. protection of personnel processing infectious material, where a horizontal LAF is obviously unsuitable; here, a vertical LAF is essential; and (3) for product and personnel protection, in which case a vertical LAF must be used.

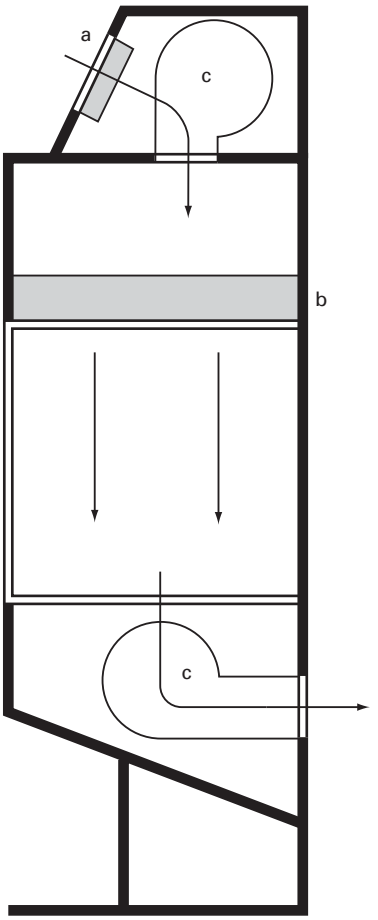

Figure 12.4.11 Vertical laminar-air-flow unit. (a) Prefilter; (b) HEPA filter; (c) fan.

LAF rooms have found additional uses, for example:

1 In conferring protection to patients undergoing bone-marrow transplants. In this procedure, LAF, in conjunction with a strict aseptic technique, produces maximum protection against microbial contamination from the environment (Solberg *et al.*, 1971).

2 In conferring protection from the environment upon leukaemic patients undergoing immunosuppressive (radiomimetic) and anticancer drug therapy. Results suggest that the incidence of infec-

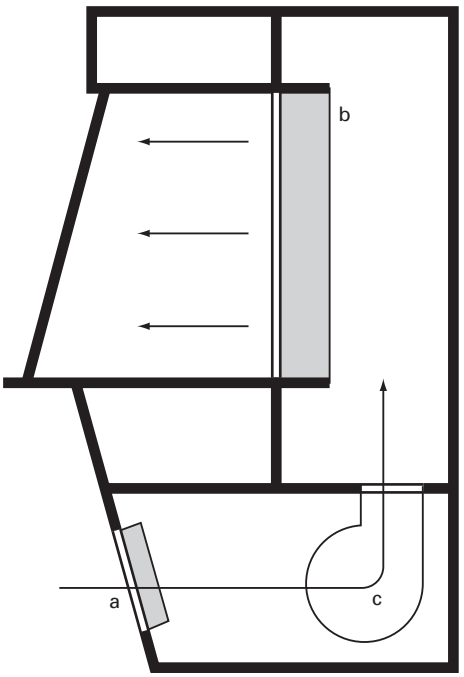

Figure 12.4.12 Horizontal laminar-air-flow unit. (a) Prefilter; (b) HEPA filter; (c) fan.

tion of leukaemic patients in LAF rooms is substantially less than for those treated elsewhere (Bodey *et al.*, 1969).

3 For preventing cross-contamination in germ-free mice (van den Waaij & Andres, 1971).

4 For aiding in the treatment of burns (Anon., 1975).

On a smaller scale, the sterile filtration of air (and other gases) for venting, aeration or pressuring purposes can often be accomplished through membrane filters. In line, these filters can also ensure the clarification and sterilization of medical gases. Mechanical patient ventilators may incorporate bacteria-proof filters commonly constructed from hydrophobic glass fibres (Nielsen *et al.*, 1996), although Das & Fraise (1998) have questioned their value in reducing cross-infection. Most membranes used are deliberately of the hydrophobic type, so that they will resist wetting by entrained water droplets, which might otherwise cause an airlock. Hydrophobic filters of 0.2 µm have been used to replace the conventional airways needed with rigid infusion and irrigation containers. The hydrophobic material will support the solution but allow filtered sterile air to enter as the fluid is used.

3.1.4 Microbiological safety cabinets

Microbiological safety cabinets are of three types: class III, which provides the highest degree of containment for handling category 4 pathogens; class II (laminar-flow recirculating cabinet), which protects both the work and the operator from contamination; and class I (exhaust protective cabinet), which protects the worker against bacterial aerosols possibly generated when handling pathogenic material (Clark, 1980). The cabinets employ HEPA and prefilters, and further information can be obtained by consulting BS 5726 (1992) and Newsom (1979a,b).

3.2 Non-sterilizing uses of membrane filtration

Apart from their use, described above, as a method of sterilization, filters (and especially membrane filters) have wide applications in other microbiological areas.

Membrane filtration in the sterility testing of antibiotics was first described by Holdowsky (1957) and this method is now commonly employed in sterility testing generally (Russell *et al.*, 1979; Akers *et al.*, 1993; Opalchenova & Keuleyan, 1993; *United States Pharmacopeia*, 2003; *European Pharmacopoeia*, 2002; *British Pharmacopoeia*, 2002).

One method of determining the numbers of colony-forming units (cfu) in bacterial suspensions or in fluids that may be contaminated by microorganisms is by means of membrane filtration. Basically, this procedure consists of filtering a suitable dilution of the suspension through a membrane filter, which retains the organisms and which is then transferred to the surface of an appropriate solid medium. This method has been used for the bacterial examination of water (Windle-Taylor & Burman, 1964) and for the determination of bioburdens in parenteral solutions prior to heat sterilization (Boom *et al.*, 1991), and is routinely employed for evaluating bacterial retention of other sterilizing-grade filters (Carter, 1996).

Suitable adaptations have been made to this procedure for determining the numbers of cells surviving treatment with antibiotics (Meers & Churcher, 1974) or disinfectants (Prince *et al.*, 1975). The amounts of disinfectants, for example benzalkonium chloride, phenylmercuric borate or chlorhexidine gluconate, adsorbed on to most types of membrane filters are apparently small (Van Ooteghem & Herbots, 1969; but see above and Naido *et al.*, 1972). Russell (1981) has described a method employing membrane filtration for demonstrating the inactivation of disinfectants by neutralizing agents. The *British Pharmacopoeia* (2002) recommends the use of membrane filtration in preservative-efficacy tests when the preservative cannot be readily inactivated by dilution or specific neutralizing agents. Membrane filtration combined with epifluorescent microscopy [known as the direct epifluorescent filtration technique (DEFT)] has been employed for the rapid enumeration of contaminating microorganisms in the water industry (Hobbie *et al.*, 1977), dairy and food products (Pettipher, 1983), ultrapure water (Mittelman *et al.*, 1983, 1985) and parenteral pharmaceutical products (Denyer & Lynn, 1987; Newby, 1991), and as a rapid method in preservative evaluation (Connolly *et al.*, 1993).

A further analytical application for membrane filters is in the bacteriological sampling of moist surfaces, using a simple contact technique (Craythorn *et al.*, 1980). In this method, the sterile membrane (3–5-μm pore size) is placed in direct contact with a contaminated surface for 5 s and then removed, incubated in the conventional manner on the surface of a solid nutrient medium and the resultant colonies counted. A comparison with traditional contact-sampling techniques indicates that the membrane-filter method can be successfully employed for the quantitative bacteriological examination of contaminated clinical surfaces (Craythorn *et al.*, 1980).

Membrane filtration has been adapted, by means of tangential-flow filter systems, to provide an alternative to centrifugation for the small-scale harvesting of cultures (Tanny *et al.*, 1980; Brock, 1983; Kemken *et al.*, 1996). These filtration devices combine normal fluid flow through the membrane with a washing action, and as a result manage to keep the majority of filtered material in suspension, thereby preventing rapid clogging of the filter (Lukaszewicz *et al.*, 1981b). The technique is reported to have little effect on cell viability and offers a recovery efficiency of up to 75% (Tanny *et al.*, 1980). For the concentration of particularly delicate organisms, a 'reverse-flow' filtration system has been developed (Brock, 1983; Kempken *et al.*, 1996). Other applications of tangential filtration have been described by Genovesi (1983).

Ultrafilter membranes have been used in the purification of water by reverse osmosis (Pohland, 1980). This process may be defined as a reversal of the natural phenomenon of osmosis. If a solution of dissolved salts and pure water is separated by a semipermeable membrane, water will pass through the membrane into the salt solution. This is osmosis itself. Solutes dissolved in the water diffuse less easily, and if their molecular weight is greater than 250 they do not diffuse at all. To reverse the process of osmosis, a pressure in excess of the osmotic pressure of the salt solution is applied and water is thereby forced out of this solution through the membrane in the reverse direction. Since the typical reverse osmosis membrane has pores approaching 2 nm in diameter, this process will remove bacteria, viruses and pyrogens and the purified water produced will be sterile and apyrogenic; it must, however, be added that contamination could occur after production. Ultrafiltration membranes are also exploited in haemodialysis.

4 The testing of filters

Confidence in the integrity and suitability of a filter for its intended task is of paramount importance in filtration sterilization, and this must ultimately rely on stringent testing.

The list of desirable properties which a filter medium should possess (section 2) gives a guide to the parameters that are controlled during manufacture and the specifications of the finished product. Each manufactured batch of filters should conform to specifications regarding their release of particulate materials, mechanical strength, chemical characteristics, including for example, oxidizable materials and the leaching of materials which may

cause a pH shift when flushed with water, and their pyrogenicity. However, filtration performance is of paramount interest, and this, basically, can be tested in two ways. A challenge test is the only true measure of what a filter is capable of removing from suspension, but this is a destructive test and so it cannot be applied to each individual unit in the manufactured batch. What can be, and is, normally applied to each cartridge filter is an integrity test, and the data it provides can be correlated with those from a challenge test in order to assess the validity of the non-destructive procedure as a substitute. The tests described below are most frequently applied to membrane filters but the underlying principles will apply equally well in the validation of most other filtration media (section 2).

4.1 Filters used in liquid sterilization

The challenge test, which is the most severe and direct test to which a bacteria-proof filter can be subjected, involves filtration of a bacterial suspension through a sterile filter assembly, with subsequent collection into a nutrient medium and incubation of the filtrate (Bowman *et al.*, 1967). In the absence of passage of organisms, no growth should be visible.

In the filter industry, such tests are employed for validation purposes (Wallhausser, 1982). They generally use *S. marcescens* (minimum dimensions approximately 0.5 μm) and *Saccharomyces cerevisiae* to challenge 0.45 μm and 0.8 μm pore-size filters respectively, while a more rigorous challenge is applied to the 0.2–0.22-μm-rated and 0.1-μm-rated sterilizing filters. Such filters are defined as being capable of removing completely from suspension *B. diminuta* ATCC 19146 (minimum dimension approximately 0.3 μm) or *A. laidlawii* respectively. Guidelines on testing procedures have been produced by a number of regulatory and professional bodies including the American Society for Testing and Materials (ASTM), the Health Industry Manufacturers Association (HIMA), the United States Food and Drug Administration (FDA, 1987), the Parenteral Drug Association (PDA, 1998) and the European and United States Pharmacopoeias. Both Waterhouse & Hall (1995) and Carter & Levy (2001) have reviewed these methods and the latter authors have tabulated comparisons between them. Technical report 26 (PDA, 1998) provides much practical guidance on the conduct of filter validation tests in general.

Bacterial retention determinations are undertaken as part of the validation process of filter sterilizing a product, but they cannot be conducted using the normal manufacturing facilities because they would entail introducing microorganisms into an area from which they should be excluded. If the manufacturer conducts the tests at all, they would be undertaken using a scaled down testing plant, but more commonly they are undertaken in the testing laboratories of filter manufacturers. A typical protocol would involve exposure of a sterile filter at a pressure of 206 kPa (30 p.s.i.) to a volume of culture medium containing 10^7/mL *B. diminuta* cells to result in a total challenge of approximately 10^9 organisms. Either the filtrate is passed through a second 0.22 μm membrane disc, which is then placed on an agar plate and incubated for 2 days, or the effluent itself is collected in a sterile flask and incubated for up to 5 days. Any sign of growth would result in failure of the filter. Griffiths *et al.* (2000) have recently shown that the 48-h incubation period can be halved by use of bioluminescent and fluorescent recombinant strains of *B. diminuta*. A satisfactory filter would be expected to have a Tr of 10^7 (Osumi *et al.*, 1991). In addition to more obvious factors like membrane thickness, transmembrane pressure and filtration rate, the ability of a filter to retain *B. diminuta* will be influenced by the physical characteristics of the liquid in which the bacteria are suspended, and the size, charge and aggregation potential of the bacterial cells as determined by their growth conditions (Mittelman *et al.*, 1998; Levy, 2001b; Lee *et al.*, 2001). Consequently, the bacteria are preferably grown in a lactose broth rather than tryptone soya broth in order to achieve individual cells of consistently small size, and they should be suspended in the process liquid that is to be sterilized; if this liquid proves inimical to the bacteria a surrogate (placebo) fluid may be used. Ensuring that the substitute is as realistic as possible is particularly important since it has been shown that the fluid composition may influence not just the size of the bacteria but also the size of the pores in the membrane (Jornitz *et al.*, 2002). Regulatory guide-

lines for aseptic manufacture of pharmaceuticals recommend validation of sterilizing filters by bacterial challenge under 'worst-case' conditions; a validation protocol applicable to the filter sterilization of high-viscosity fluids at high differential pressures has been described by Aranah & Meeker (1995). Meltzer (1995) has pointed out, however, that the need to test under worst case conditions only applies for filters that act by adsorptive retention rather than by sieving. It is possible to determine the extent to which these two mechanisms contribute to particle retention by flow decay studies (plotting flow rates as a function of time), and if it were shown that a filter acts solely by sieve retention, that would suffice to validate the filter for all pharmaceutical filtrations and eliminate the need for individual validation for each product and operating condition.

The bacterial retention tests described above are destructive tests and could not be used by the manufacturers of parenteral products to substantiate the efficacy and integrity of the membrane before and after use, as required by a number of regulatory authorities (Olson, 1980). Similarly, the physical method of mercury intrusion, frequently used to determine pore-size distribution (Marshall & Metzer, 1976), does not offer a satisfactory in-process test. What is required is a simple, rapid, non-destructive test that can be performed under aseptic conditions on sterile membranes to ensure the integrity of the membrane and the use of the correct pore size (Springett, 1981). With this aim in mind, a considerable proportion of the industry's research effort has been directed towards validating existing indirect tests and establishing new ones.

The oldest and perhaps most widely used non-destructive test is the bubble-point test (Bechold, 1908), which is the subject of BS 1752 (1983). To understand the principles behind this test, it is necessary to visualize the filter as a series of discrete, uniform capillaries, passing from one side to the other. When wetted, the membrane, will retain liquid in these capillaries by surface tension, and the minimum gas pressure required to force liquid from the largest of these pores is a measure of the maximum pore diameter (d) given by:

$$d = (K\sigma\cos\theta)/P$$

where P = bubble-point pressure, σ = surface tension of the liquid, θ = liquid to capillary-wall contact angle and K = experimental constant.

The pressure (P) will depend in part upon the characteristics of the wetting fluid, which for hydrophilic filters would be water, but for hydrophobic filters may be a variety of solvents (e.g. methanol, isopropanol).

To perform the test, the pressure of gas upstream from the wetted filter is slowly increased and the pressure at which the largest pore begins to pass gas is the first bubble point (Fig. 12.4.13). In practice,

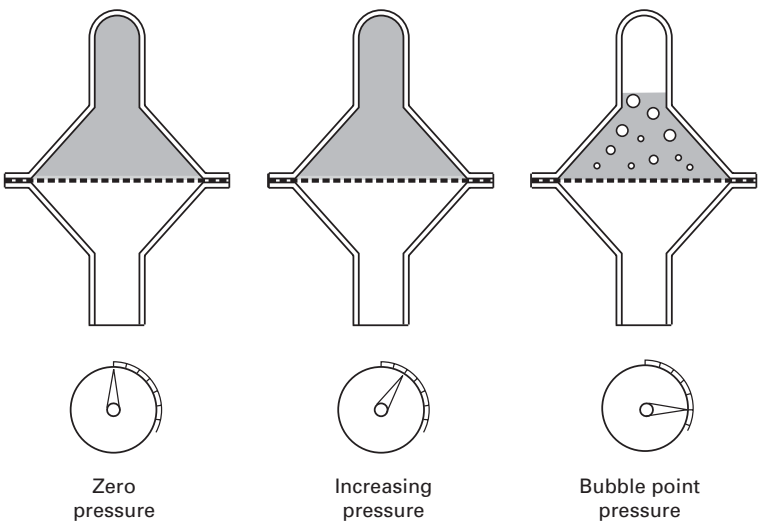

Figure 12.4.13 Stages in the bubble-point test.

this value is frequently taken as the lowest pressure required to produce a steady stream of bubbles from an immersed tube on the downstream side. The bubble point for a water-wet 0.22-μm-rated filter is 379 kPa (55 p.s.i.). An automated method for bubble-point testing has been developed (Sechovec, 1989).

The inadequacies of the capillary-pore model for describing the membrane structure have already been discussed (section 2.4.2). The bubble-point test is unlikely, therefore, to provide an exact indication of pore dimensions (Lukaszewicz *et al.*, 1978; Meltzer & Lukaszewicz, 1979) and it does not, in itself, indicate how efficient the filter is. Instead, its value lies in the knowledge that experimental evidence has allowed the filter manufacturer to correlate bacterial retentivity with a particular bubble point. Thus, any sterilizing-grade filter having a bubble point within the range prescribed by the manufacturer has the support of a rigorous bacterial challenge test regimen to ensure confidence in its suitability. In the words of one manufacturer, 'An observed bubble point which is significantly lower than the bubble point specification for that particular filter indicates a damaged membrane, ineffective seals, or a system leak. A bubble point that meets specifications ensures that the system is integral.'

Small volumes of fluid are often sterilized by passage through a filter unit attached to a hypodermic syringe. The following approximation to the bubble-point test can be applied to such a system to confirm its integrity after use. If the syringe is part-filled with air, then any attempt to force this air through the wet filter should meet appreciable resistance (the bubble-point pressure). Any damage to the membrane would be immediately indicated by the unhindered passage of air.

The bubble-point test has been criticized because it involves a certain amount of operator judgement and is less precise when applied to filters of large surface area (Trasen, 1979; Johnston *et al.*, 1981; Springett, 1981). Johnston & Meltzer (1980) recognized an additional limitation to the accuracy of this test; commercial membranes often include a wetting agent (see section 2.4.1, 'Methods of manufacture'), which may well alter the surface-tension characteristics of water held within the filter pores and hence the pressure at which bubbles first appear. This wetting agent is frequently extracted from the membrane during aqueous filtrations, rendering invalid any attempt to make an accurate comparison between before and after bubble-point values (Johnston & Meltzer, 1980). These authors have proposed an additional test based on the flow of air through a filter at pressures above the bubble point. The robust air-flow test examines the applied-pressure/air-flow rate relationship and is amenable to both single-point and multiple-point determinations. This test is described as convenient to use and would, if several readings were taken at different applied air pressures, be more accurate than the single-point bubble-point determination.

The passage of a gas through a wetted filter is not confined solely to bulk flow at applied pressures in excess of the bubble point; it can also occur at lower pressure values by a molecular-diffusion process. With filters of small surface area, this flow is extremely slow, but it increases to significant levels in large-area filters and provides the basis for a sensitive integrity test (Reti, 1977). This test finds its widest application in large-volume systems, where the need to displace a large quantity of downstream water before the detection of bubbles makes the standard bubble-point test impracticable. To perform this diffusion test, gas under pressure is applied at 80% of the bubble-point pressure (Reti, 1977; Olson *et al.*, 1981) for that particular wetted filter and the volumetric gas-flow rate determined by measuring either the rate of flow of displaced water or the volume of gas passed in a specified time (Trasen, 1979). A marked increase in gas flow seen at lower pressures than would normally be expected for that filter type indicates a breakdown in the integrity of the system.

Jornitz *et al.* (1998, 2002) have advocated the use of multipoint diffusive testing rather than testing at a single pressure because it can more rapidly detect a pending product failure due to gradual filter deterioration. In such a multipoint test, the gas flow rate is related to pressure in a manner similar to that shown in Fig. 12.4.14. Impending filter failure may be indicated by an increase in slope of the early portion of the plot corresponding to diffusional flow. This approach can be used to assess pore-size distribution; a narrow distribution would be indicated by a significant rise in gas flow at applied pressures

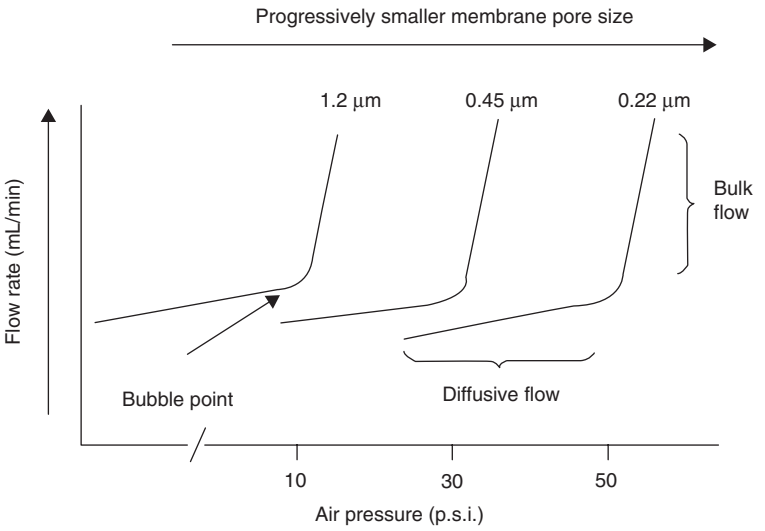

Figure 12.4.14 The relationship between air pressure and flow rate in diffusive flow filter testing.

only marginally above the bubble-point, while a wide distribution would cause a more gradual increase in gas flow.

Virus-retentive filters pose particular problems with respect to validation because their pore sizes correspond to wet bubble point pressures of the order of 2060 kPa (300 p.s.i.). These cannot easily be achieved in conventional testing equipment, so diffusive flow integrity testing (section 4) is the preferred procedure. There is the possibility of destructive filter testing using a viral challenge analogous to the testing of bacterial filters with *B. diminuta*. The problems that exist with bacterial integrity testing with respect to ensuring uniformity of cell size and non-aggregation of the test organism are also seen with viral testing. Whilst the sizes of many common pathogenic viruses are now well-defined, using these as challenge organisms may pose unacceptable infection risks to the operators and problems of enumeration, since plaque counts in cell monolayers are not particularly easy or precise. For these reasons Aranah-Creado & Brandwein (1999) have advocated the use of appropriately sized bacteriophages as a safe, economical and effective method of filter testing. However, even bacteriophages do not present problem-free alternatives to human viruses because they, like bacteria, display the potential to aggregate and so form unrealistically large particles of variable and unknown dimensions. Parks *et al.* (1996) quoted LRVs of the order of 9 for MS-2 coliphage during the operating of air filters, but the phage were generated as aerosols, and the authors acknowledged that the droplets were likely to be much greater than the dimensions of the individual phage particles (23 nm).

All the major suppliers of cartridge filters have developed and supply to their customers integrity-testing instrumentation, which can evaluate the diffusive flow characteristics of the filters at any time during their working life. The testing procedures tend to be named differently by the various manufacturers, e.g. 'pressure decay test', 'pressure hold test', 'forward flow test' or 'diffusive flow test', but they all operate on similar principles. A recent review of integrity tests performed by United States' pharmaceutical manufacturers showed that diffusive flow and bubble-point tests were undertaken by 63% and 87% respectively of the companies responding (Madsen & Meltzer, 1998).

4.2 Filters used in gas sterilization

The bubble-point and diffusive-flow tests described in the previous section are also applicable, with modifications, to membrane filters used for gas sterilization in venting systems. The major difference is that air filters are hydrophobic, so it is necessary to use a liquid with a lower surface tension than water

in order to achieve adequate wetting; isopropyl alcohol mixed with water is most commonly used for this purpose. Water-based testing procedures have been developed for use in situations where alcohol is undesirable, and these are similar in principle to the bubble-point procedure (Dosmar *et al.*, 1992). In these so-called water-intrusion tests, the parameter measured is the pressure required to cause water to be forced through a hydrophobic filter, rather than air to be passed through a wetted filter.

The continuous production of high-quality filtered air by any HEPA filtration system (section 3.1.3) can be assured by the application of rigorous efficiency tests to the filter, both at the time of installation and at intervals throughout its service life. One of the most exacting test methods available is the diocytylphthalate (DOP) smoke test (Gross, 1976, 1978). In this test, DOP is vaporized upstream of the filter to produce an aerosol of particles which can be detected in the filtered air using a suitable photoelectric device. For efficiency testing by the filter manufacturer, DOP smoke should be thermally generated, to give monosized particles of approximately 0.3 µm diameter, but cold DOP aerosols of larger polydisperse droplet size (Caldwell, 1978) have been recommended for detecting small flaws and leaks that may develop in a filter during use (Gross, 1978). The passage of DOP particles is best examined in a LAF unit by using a small probe to scan the filter-surface closely in an overlapping pattern (Gross, 1976). This will detect any areas of particular weakness, such as pinholes or poor seals (McDade *et al.*, 1969). A HEPA filter is expected to have an overall minimum retention efficiency of 99.97% to hot DOP (Gross, 1978), this value being increased to 99.999% for ultra-HEPA filters (Groves, 1973). Mika (1971) has suggested that filtration efficiency is at a minimum for airborne particles of 0.2–0.3 µm diameter, and the bacterial-retention properties of a HEPA filter (section 3.1.3) may well be underrated by this test.

Alternatively, filters can be examined using the sodium-flame test (BS 3928, 1969), in which a minimum retention efficiency of 99.995% is expected of all HEPA filters used to prepare air to grade A standard (BS 5295, 1989). An aerosol is produced from a sodium chloride solution, upstream of the filter, and rapid evaporation of these droplets then ensures that the air arriving at the filter contains minute particles of sodium chloride. Retention efficiency is evaluated by downstream sodium-flame photometry. Other testing methods involve discoloration by atmospheric dust or weight gain during filtration, and are generally confined to filters of a coarser grade.

A bacterial aerosol challenge test has been developed to study the filtration efficiency of air and gas filters (Duberstein & Howard, 1978). Other workers (Harstad & Filler, 1969; Mika, 1971; Regamey, 1974) have suggested using phage particles, vegetative organisms and spores as a suitable challenge for HEPA filters.

5 Designing a filtration system for the preparation of medicinal products

Sterilization and clarification by filtration are routinely applied to a variety of liquids, which often differ markedly in their filtration characteristics. The first stage in designing any filtration system, therefore, is to classify the fluid to be processed according to the ease with which it can be filtered. The majority of aqueous solutions for intravenous, ophthalmic and irrigation purposes pass easily through a sterilizing-grade membrane filter, while, at the other end of the spectrum, oils and fluids with a high particulate or protein content (e.g. vaccine, serum, plasma and tissue culture media) will, without exception, require some form of pretreatment before final processing. The early methods of pretreatment, which included centrifugation and settling, have largely been replaced by extensive prefiltration (see section 2.4.5 and Fig. 12.4.10) or by sequential filtration through a series of membrane filters of progressively smaller pore size. Often, this series consists of a stack of membrane discs, separated by a support mesh, assembled together in a single filter holder. For ease of handling, it is advisable to arrange the stack of filters in a separate holder from the final sterile, 0.22 µm, sterilizing filter. The serial filters can then be replaced when they become clogged, without jeopardizing the sterility of the final filter. The successive filtration of serum through various grades of prefilter, followed by passage through 1.2, 0.8, 0.45 and 0.22 µm mem-

branes, provides a typical example of serial filtration. The pore size of the final filter is dictated by the need to provide a sterile product.

Small-volume parenteral, ophthalmic and other hospital-produced products are routinely passed through single-disc filter systems capable of processing batches in the region of 500 L. Bulk industrial production, however, with its larger volumes and attendant high capital investment, requires a more sophisticated approach to system design. Invariably, this will demand a pilot study, where results obtained from flow-decay tests performed on approximately 0.1% of the batch volume or with small-capacity filters, can be used to provide sufficient information for the scaling-up operation (Meltzer & Lukaszewicz, 1979). Major filter firms may offer an on-site analysis programme, culminating in a computer-assisted appraisal of the filtration process. Any system finally chosen must attempt to optimize total fluid throughput, flow rate and filter and prefilter life.

The ancillary equipment required for an evolving filtration system is determined, at least in part, by the scale of the process. Large industrial systems will make many individual demands for specialized equipment, which may include pumps, holding tanks, cartridge-filter holders and extensive stainless-steel plumbing. This combination of components is rarely found in small-scale hospital units.

Accumulated expertise has now clearly demonstrated that, when selecting equipment for assembly into any filtration system, no matter what its size, the following important points must be taken into consideration.

1 Is filtration to be performed under positive or negative pressure? Vacuum filtration is well suited for small-scale analytical processes, such as sterility testing, but should not be used for production purposes. Positive pressure, provided by syringe, pump or nitrogen gas under pressure, offers the important advantages of high flow rates and easier bubble-point testing, and also protects against the ingress of unsterile air and solvent evaporation. Equipment should be designed, therefore, to withstand the pressures employed during the filtration process.

2 Is filtration to be a batch or continuous process? In a continuously operating large-scale system, provision must be made to allow filter changes without interrupting the process. To do this, a valve must be included to switch flow over to another unit fitted with a fresh filter.

3 The system must be amenable to regular maintenance and cleaning. If not, the filters may well be exposed to challenge levels in excess of their capabilities.

4 The amount of particulate contamination within a system is directly proportional to the number of valves, joints and junctions. It is considered advisable, therefore, to keep any system as simple as possible.

5 All valves shed particles during use and must be placed upstream of the final filter.

6 It is axiomatic that the final membrane filter must be placed at the last possible point in the system.

A system that pays attention to all these points should be capable of providing parenteral products of a standard acceptable to all the regulatory authorities. As a final cautionary word, however, the quality of the finished product does not depend solely upon the design and efficiency of the filtration system; it will also owe a great deal to the standard of the production environment, containers used and personnel employed and must, therefore, depend ultimately upon the continued observance of all pharmaceutical GMP requirements (BS 5295, 1989; Medicines Control Agency, 2002; see also Chapter 21).

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