THE **ABCs** OF **FILTRATION** AND **BIOPROCESSING** FOR THE **THIRD MILLENNIUM**

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about this book...

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Spectrum has published *The ABCs of Filtration and Bioprocessing for the Third Millennium* to assist in the understanding and development of the most efficient methods for separating and bioprocessing liquids and gases using hollow fiber membrane technology.

Founded in 1970, Spectrum is a global leader in selected markets for hollow fiber and tubular membrane separation products that are used by research laboratories and biotechnology and pharmaceutical companies worldwide.

Hollow fiber separation is a dominant technology in various applications as demonstrated by its success in hemodialysis, blood gas exchange and pharmaceutical filtration. That hollow fiber filtration and separation is the modality of choice is also subscribed to by such well known institutions as SRI International, National Institute of Standards and Technology (NIST), large pharmaceutical and biotechnology companies and millions of hemodialysis patients around the globe. Spectrum is forecasting that many applications where membranes are used to separate, isolate and purify liquids and gases will upgrade to the performance and economy of scale of hollow fiber technology.

FILTRATION AND BIOPROCESSING

Spectrum has developed and patented a new generation of hollow fiber configurations that greatly minimize membrane fouling and improves the filtration efficiencies of hollow fiber modules by at least an order of magnitude. These products and technology will lower the cost of membrane bioprocessing of aqueous streams by fifty percent when compared to existing products.

SELECTIVE MOLECULAR EXTRACTION

Spectrum has developed and acquired a novel class of membranes for the selective extraction, concentration and recovery of organic compounds from dilute aqueous solutions. This novel class of polymeric liquid membrane technology will have significant application to improving the taste and flavor of beer and wine, water remediation by the extraction of VOCs from aqueous streams and the extraction and separation of industrial compounds such as butanol, ethanol, acetic acid and others. Research indicates that this technology reduces the requirement for processing energy by a significant degree when compared to existing competitive technologies.

MODULAR, MULTI-PHASE HOLLOW FIBER TECHNOLOGY

Spectrum is a pioneer in the development of modular, multi-phase hollow fiber technology where three independent membrane compartments, within the same modular structure, provide unique means of efficient triple streams processing of aqueous or non-aqueous solutions. This unique multi-compartment module can be used for: mammalian cell expansion in bioreactors; to separate blood formed elements simultaneously in parallel streams and in foods and beverages.

We welcome your inquiry.

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The ABCs of Filtration

Introduction

Filtration is the process of separating, fractionating or concentrating particles, molecules or ions within or from a fluid by forcing the material through a porous or semi-porous barrier. The force can be pressure, vacuum, concentration gradient, valence or electrochemical affinity. The fluid can be either a liquid or a gas.

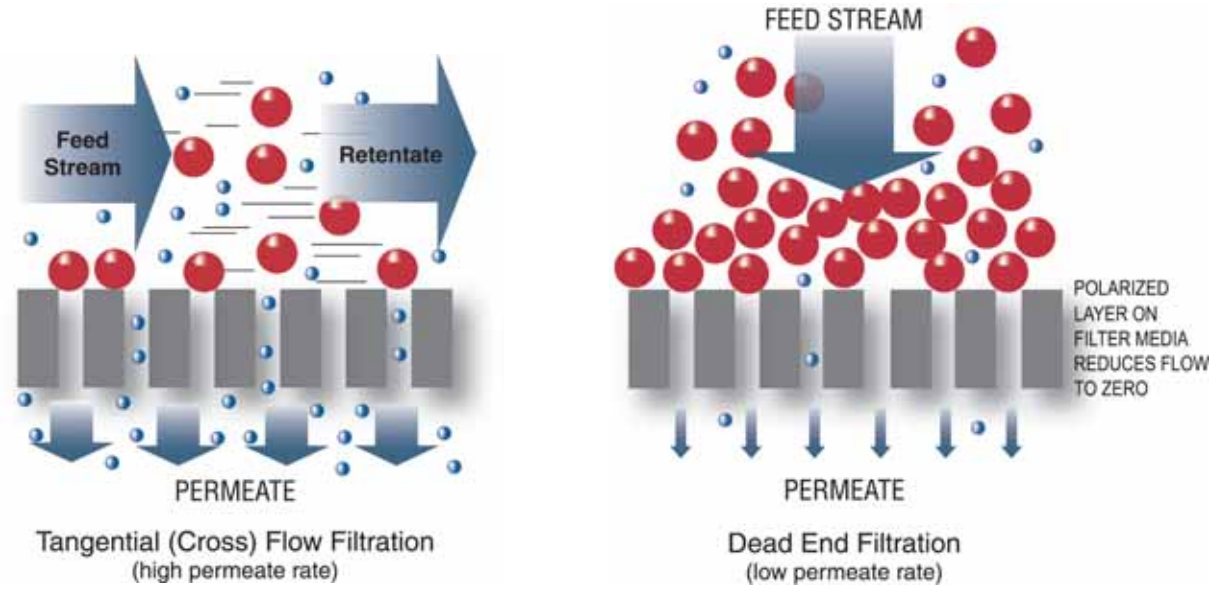


Figure A-1 The limitations of dead end filtration are overcome by tangential (cross flow) filtration.

Filtration is a cost-effective technology, generally requiring only the application of a force. There is no need to heat the process material as in the case of evaporation or high temperature sterilization. The latter are energy expensive and can affect the quality of the filtrate.

The main objective of filtration is to either achieve a clean liquid or gas, or to extract, concentrate, blend or analyze matter separated from liquids or gases by the filter. In most cases, the filtration efficiency depends primarily on the characteristics of the filter media, the properties of the fluid and the operating conditions.

Distinctive differences exist in the types of materials used to construct filter media. Most modern filtration media are man-made from either natural or synthetic polymeric materials. These materials are fabricated into various filter materials with a specifically designed structure, porosity, and performance characteristics. Filtration media are often classified as depth filters, screen filters (mesh) or membranes.

Depth Filters

Depth media are mostly used for coarse filtration and fluid clarification. They are also used to protect microfiltration, ultrafiltration, and reverse osmosis membranes. Depth filters are most often manufactured from fibrous materials, woven or non-woven polymeric material or inorganic materials.

Depth filters are not absolute and therefore do not have a precisely defined pore size or structure. Particles that are larger than the "aperture" or "pore size" of the filter will be trapped more or less on the surface of the filter. In the case of smaller particles, depth filters rely on the random entrapment and adsorption of matter within the structure, or interstices, of the media. In addition, the depth filter may have an electric charge (zeta potential, described later), that will attract and trap small particles. The result is efficient retention of a wide range of particle sizes by depth filters.

Being of thicker construction and higher porosity, depth filters exhibit the advantage of higher flow rate and dirt loading capacity when compared to screens and membrane filters. Depth filters are also less expensive than many screens and all membrane filters.

Screen Filters (Mesh)

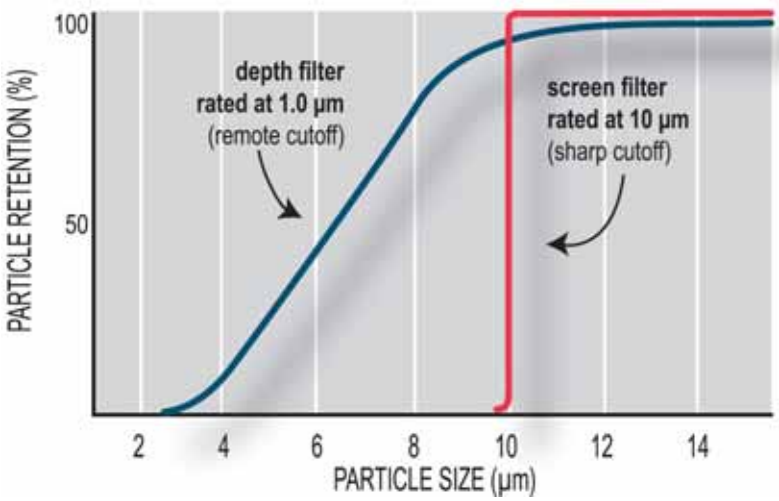


Figure A-2 Retention characteristics of screens and depth filters

Particles are captured directly on the surface of screen filters. Therefore, screens retain with certainty only those particles the same size or larger than the aperture size of the screen. Neglecting diffusion and inertial impaction, most particles smaller than the aperture size pass unimpeded through the screen.

For that reason, screens are preferred if the user needs low non-specific binding or low adsorption or absorption of the filtrate.

Should the user need maximum removal of all particles and/or a high binding capacity, the depth filter should be selected. Since the depth filter has a much larger available surface area than the screen, it has a much larger particle loading capacity and many more sites where proteins, viruses and other small particles can bind.

Figure A-2 shows the "remote cutoff" that is characteristic of virtually all depth filters compared to the "sharp cutoff" characteristic of screen filters.

Figure A-3 Spectra/Mesh® screens are available from 5 µm through 1,000 µm

Membrane Filters

Membrane filters are available in a variety of materials and configurations for a variety of applications. They are used routinely in divergent applications such as purification, diafiltration, desalination, particle fractionation and removal, molecular concentration and separation, fluid sterilization, fluid clarification and many other applications.

Membrane filters are manufactured from polymeric as well as other materials by processes such as solvent casting, sintering, stretching or nuclear particle track etching. The operating characteristics of membrane filters depend largely upon the manufacturing process and the polymeric materials used. Each of these processes will produce a thin membrane with a relatively well defined flow rate, pore size, pore structure, pore density, bubble point and tensile strength.

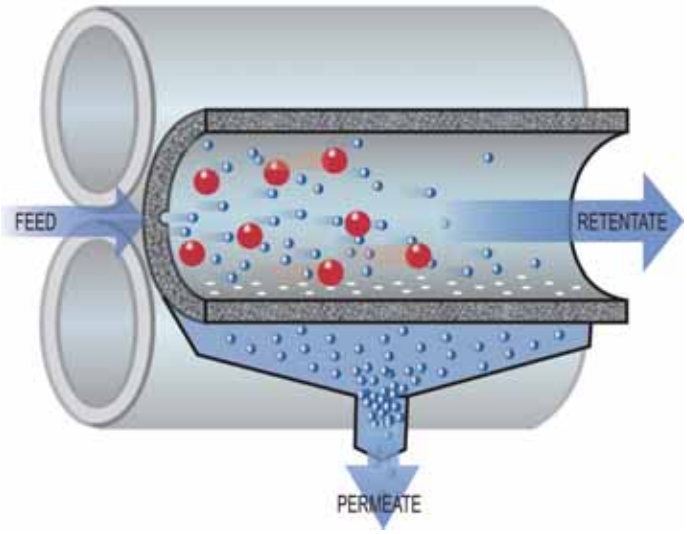


Figure A-4 Schematic of hollow fiber membrane showing tangential (cross flow) filtration

Due to these predefined characteristics, the filtration efficiency of membrane filters can be readily predetermined with a high degree of accuracy for a wide range of applications and process conditions.

Microporous membrane filters function primarily by surface capture or rejection of matter larger than the rated pore size of the membrane. However, random interstitial entrapment of particles smaller than the rated pore size is also an important property of some membrane filters.

Microporous membrane filters exhibit lower flow rates and dirt loading capacity when compared to depth filters. However, membrane filters offset this disadvantage with well defined filtration characteristics and an ability to fractionate suspended matter of a defined size from a fluid. If cross flow filtration is used, the feed solution that sweeps the filter surface continuously can provide throughput comparable to some depth filters.

Ultrafiltration and dialysis membranes that provide concentration, fractionation and other molecular separations represent significant improvements over previously used methods such as centrifugation, evaporation and heat treatment. The latter are energy expensive and may have deleterious effects on both the concentrate/retentate and the permeate/filtrate.

Newer applications for microporous and ultrafiltration membrane filters include: protein purification, cell harvesting, washing, clarification and concentration; cell perfusion; virus clarification and concentration; cleaning latex particles and many others. Representative laboratory and process applications are described in more detail in later chapters.

Membrane filters are primarily available in hollow fibers and flat sheets (pleated, stacked, or spiral wound configurations). Hollow fiber modules are preferred in many applications because of their superior flow dynamics, packing density (large membrane filtration surface area per unit of volume), continuous “sweep” cleaning of the membrane surface by cross flow, ease of handling, lower cost and disposability.

The Nature of Particles

Membrane filtration is used for a wide range of particle and molecular sizes as shown

in figure A-5 (inside back cover). Considering physical sieving only (excluding ions), this range spans from molecules with a molecular weight of 100 (very roughly 10 Å in size) to particles upwardly of 20 µm in size, (200,000 Å), a 2,000 order of magnitude.

Particle shape is also a factor. Molecules can range in shape from essentially spherical or globular (such as gamma globulin) to linear (such as tropocollagen). Particles can be long and slim (such as glass and polymer fibers) or somewhat spherical. They can be primarily rigid or deformable; and if rigid, they can be crystalline or non-crystalline. Furthermore, they can have an electric charge.

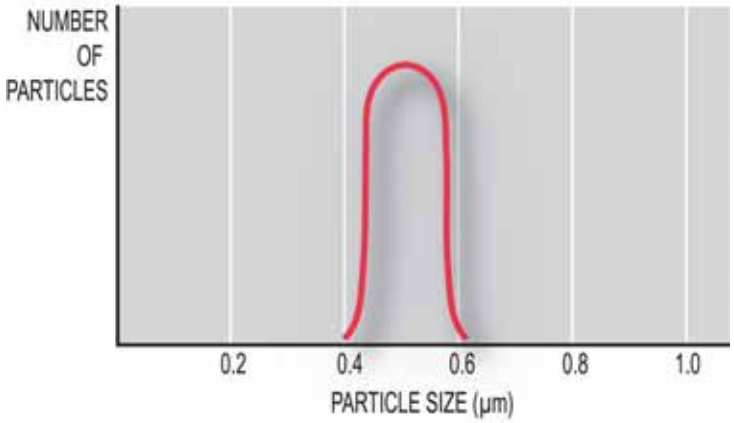


Figure A-6A Schematic showing narrow particle size distribution

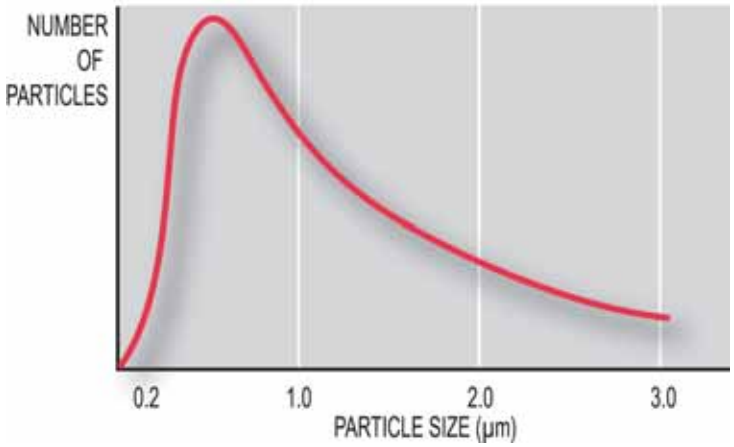


Figure A-6B Schematic showing broad particle size distribution

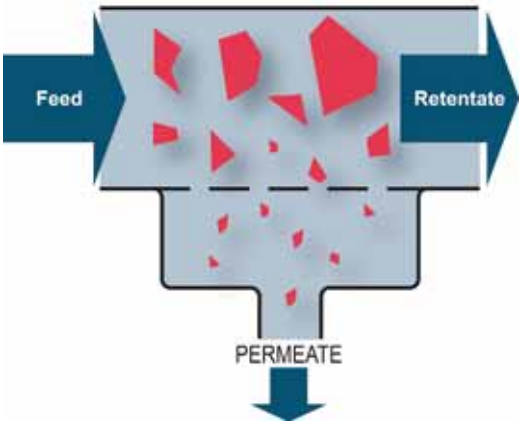


Figure A-7A Rigid particles smaller than the pore size will go through the filter.

The electric charge possessed by some filter media is called its "zeta potential". Zeta potential can be either a positive or a negative charge. When it is positive, it will electrokinetically attract particles in the feed stream that have a negative charge and vise versa. Zeta potential allows some filters to remove particles that are much smaller than those ordinarily trapped by simple sieving.

Since the nature of the particles to be removed or retained are often known, a highly selective separation system can be designed for the filtration objectives. However, in most fluids, the particle load is randomly dispersed and the filtration objective is to remove or retain 90% or more of everything above a given particle size.

As an example of the former case, the particle distribution and filtration objective may be to remove or retain bacteria of a given type from a culture consisting entirely of the bacterial cells. In such a case, the particulate distribution may be as shown in figure A-6A.

Far more common is the latter case. Figure A-6B illustrates the typical distribution of particles in a fluid. Note that the range of particle sizes is very broad with a higher percentrage in the submicron range.

Of equal importance to particle size is the nature of the particles themselves (refer to figures A-7A and A-7B). Rigid particles are generally much easier to filter than compressible particles. Since the latter can deform, they are much more likely to pass through the filter. Thus, deformable particles may behave similar to a smaller particle in their separation characteristics. In addition, compressible particles tend to compact on

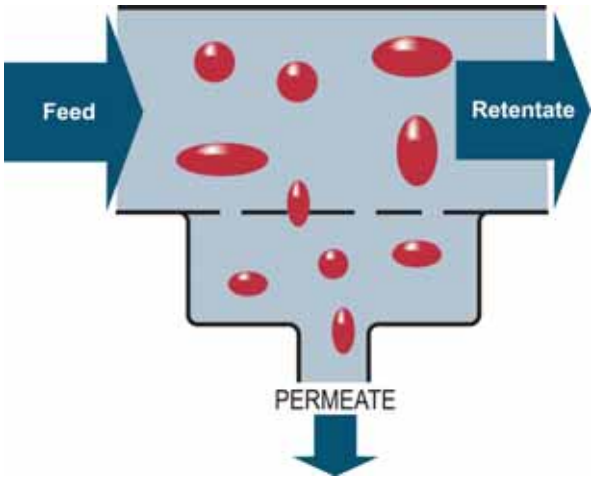


Figure A-7B Compressible particles will often deform and go through the filter.

the surface of dead end filters, effectively cutting off flow much sooner than would be the case with rigid particles.

Some particles tend to agglomerate, i .e. adhere to each other if they come in contact. The result is a much larger particle that, if stable, is easier to filter than smaller particles. In some cases, chemical agents can be added to a solution to encourage (or discourage) agglomeration.

Retention Efficiency

Referring to figures A-8A, B and C the upstream particle distribution previously described is shown as a solid line. The dashed lines show typical particle distributions downstream from a depth filter, a microporous membrane and a screen filter, all rated at 1 µm.

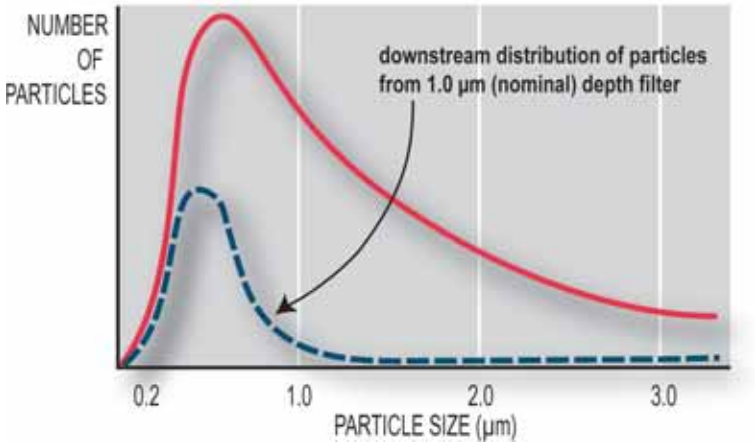


Figure A-8A Downstream particle distribution for a depth filter

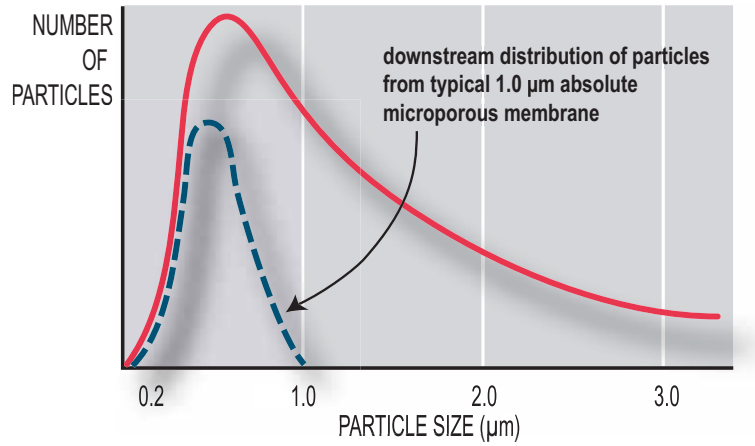


Figure A-8B Downstream particle distribution for a microporous membrane

The most efficient filter for removing the maximum number of particles, or reducing the particle load is the depth filter as shown in figure A-8A. However, depth filters have the disadvantage that they are not absolute. Microporous membranes are absolute and are also quite efficient in reducing the number of particles as shown in figure A-8B. Note that only those particles smaller than 1 µm pass through the filter.

Least efficient in terms of total number of particles removed is the screen filter. Figure A-8C illustrates the retention characteristic of screens. All particles greater than the screen pore or aperture size are trapped on the filter surface, while virtually all particles smaller than the screen pore or aperture size pass through the filter.

If the filter must be absolute and still retain the maximum number of particles, the filtration combination of choice would be the microporous membrane alone or in combination with a depth filter. The screen can also be used in combination with a depth filter.

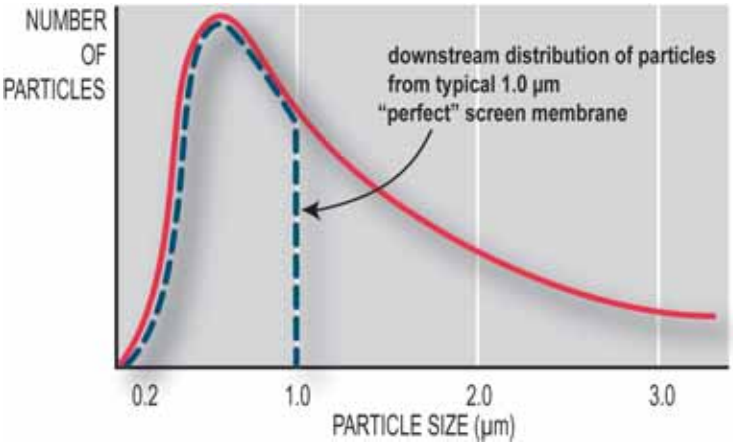


Figure A-8C Downstream particle distribution for a screen filter.

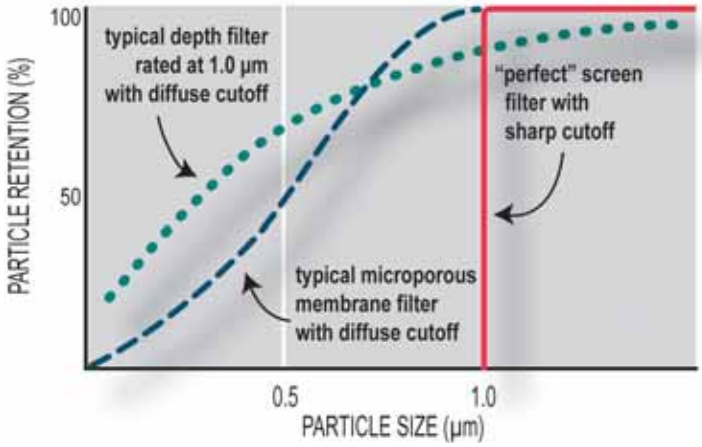


Figure A-9 Retention characteristics of depth filters, microporous membranes and screen filters.

The data from these three idealized curves have been replotted in figure A-9 to illustrate the particle size cut off rating of the three types of filters. Characteristic of depth filters and microporous membranes is a gradual reduction in retention efficiency for particles below their rating. This is called "diffuse or remote cutoff". Since screens trap only those particles at or larger than its pore size rating, they are said to have a "sharp cutoff".

For removal of particles from a gas stream, the retention characteristics for the three types of media above are often dramatically different than is the case for liquid filtration.

Plugging Characteristics

Throughput as a measure of filter life has been defined as that amount of fluid that can be filtered before an unacceptable pressure drop is reached across the filter. It is not only affected by how the filtration process is managed, but also by the interaction that takes place between the filter media and the particles to be separated.

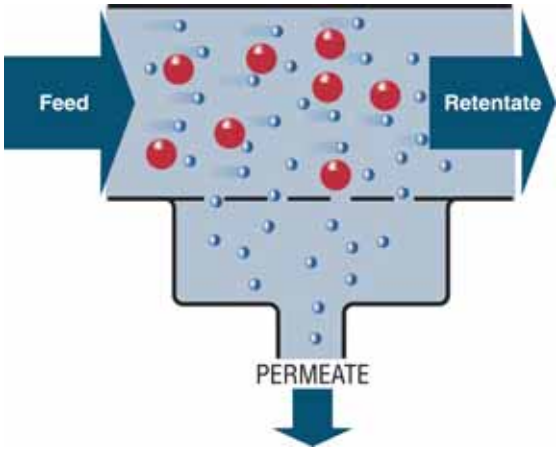


Figure A-10A "Sieving" mode of plugging

In the simple "sieving" mode of plugging, (Figure A-10A & B) particles smaller than the pores readily pass through the filter and particles larger than the pores are trapped on the surface of the filter.

Figure A-11 shows a phenomenon that occurs in filtration if the particles in the fluid are considerably smaller than the pores of the filter but are rigid. They may collect on the surface of the filter and begin to bridge over the pore openings.

In time, these particles will completely

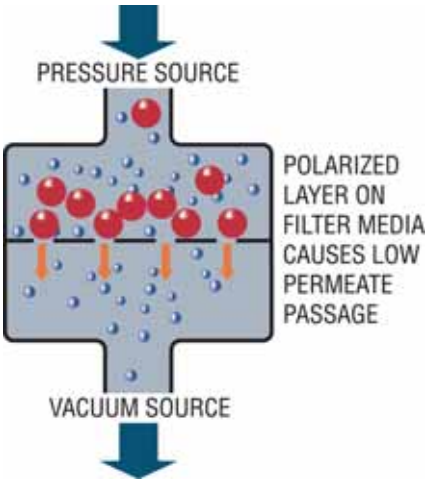


Figure A-10B "Sieving" mode of plugging

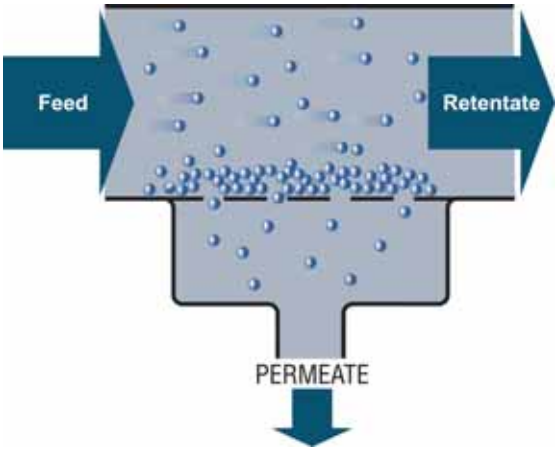


Figure A-11 Plugging by pore bridging

bridge the pores. These rigid particles do not deform and the result is that a filter cake is formed on the surface of the filter that may be many times more efficient as a filter than that of the filter matrix itself. In dead end filters, these filter cakes can build up to a considerable depth. As they become thicker they offer more and more resistance to flow. Therefore, there is a practical limit where the added filtration efficiency of the cake is more than offset by the increase in flow resistance. An effective filter cake must be formed of rigid particles since deformable particles will nearly always squeeze into a compacted mass that will effectively reduce flow to an unacceptable level.

Throughput can often be dramatically increased by analysis of the fluid to be filtered. In the case of a very dirty liquid, for example, the liquid can be allowed to remain at rest for an extended period during which time gravity will settle some of the particles, particularly those of large size. In other cases, smaller particles can be made to agglomerate into larger particles that are much easier to filter. However, even with these pre-treatments, it is nearly always necessary to additionally turn to other techniques to increase throughput. These can include use of a suitable pre-filter in combination with the final filter, agitation, cross-flow, back washing, serial filtration with two final filters, and careful consideration of the proper installation of the final filter media in its holder.

Fundamentals of Flow

Flow rate is defined as the volume of fluid that flows past a given point per unit of time. It is directly or inversely proportional to several parameters as shown below:

$$Q \text{ is proportional to } \frac{A\,\Delta P}{R\,Ó}$$

Where:

Q is the flow rate
R is the resistance to flow
A is the surface area of the filter
ΔP is the pressure differential
Ó is the viscosity of the fluid

The terms "flow rate" and "flux" are often used interchangeably. However, flux is generally considered to be the flow rate through the filter per unit of filter surface area.

In dead end filtration, flow rate and flux, or flux rate, have essentially the same meaning. The term flow rate is most commonly used however.

In common use, for tangential (cross) flow the rate of flow through the filter is often called flux or flux rate, while the term flow rate designates the rate of flow across the face of the filter.

Shown in figures A-12, A-13 and A-14 are the concepts of pressure drop and flux rate as they relate to simple and compound systems. In a simple system (figure A-12), the pressure differential is simply the upstream pressure minus the downstream pressure. When the filters are in series (figure A-13), the total pressure differential is the sum of the pressure drops across each of the three filters (or the pressure drop

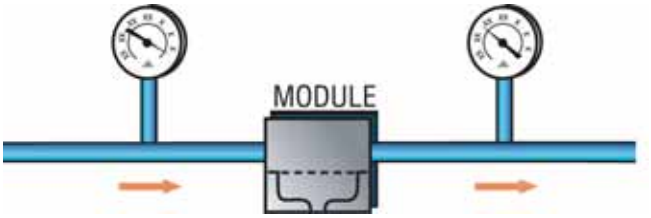


Figure A-12 Simple flow across a module

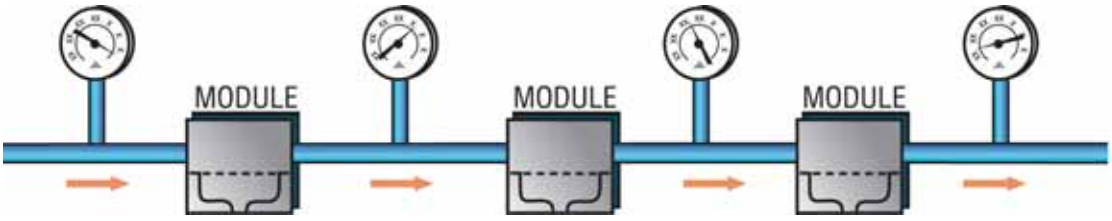


Figure A-13 Series flow

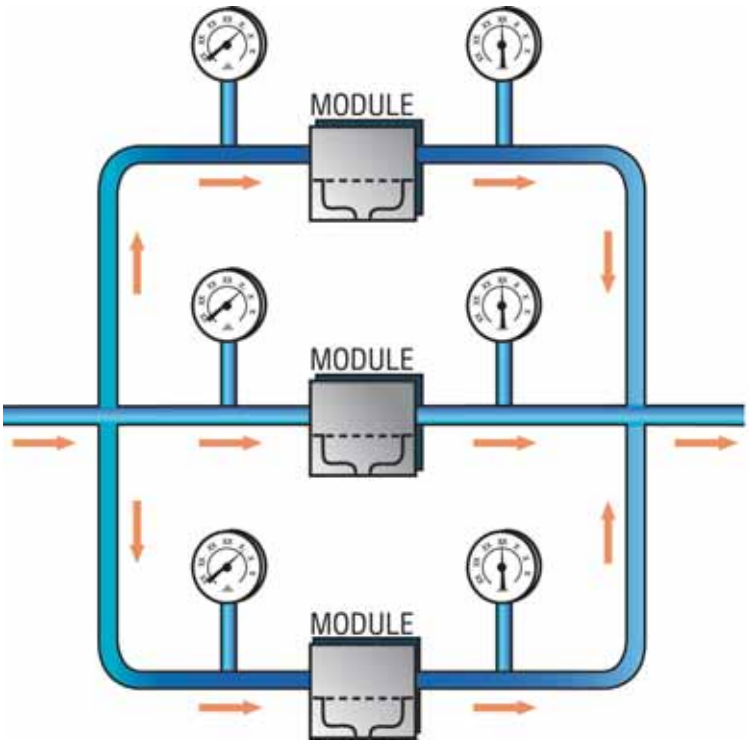


Figure A-14 Parallel flow

between the first and last filter). When the filters are in parallel (figure A-14), the pressure drop across the three filters is equal, but the total flux rate is the sum of the flux rates through each of the three filters.

Other general considerations include the following:

1. Increasing the pressure differential increases the flux rate in direct proportion. However, if the increase in pressure drop causes compaction of the filter or its cake, if the particles are compressible, or if flow becomes limited by the filter holder, flux rate may decrease.

2. Increasing the cross sectional area of the filter increases the flux rate in direct proportion.

3. Decreasing the viscosity of the fluid by increasing its temperature will increase flux rate in inverse proportion.

In many cases, it is necessary to

calculate the size of the filter module required for a specific filtration application. This can be done as follows:

$$A = \frac{V}{(t) \times (F)}$$

Where:

A = required filter surface area in square meters (1 m^2 = 10,000 cm^2)
V = filtrate volume desired in liters (L)
t = processing time in hours
F = steady state flux in liters per square meter hour (L/m^2hr)

Example:

900 liters of 0.2 µm clarified *E. coli* lysate is desired
The steady state flux is 50 L/m^2hr
The desired processing time is 6 hours

$$A = \frac{900 \text{ liters}}{6 \text{ hours} \times 50 \text{ L/m}^2\text{hr}} = 3.00 \text{ m}^2$$

.

Tangential (Cross Flow) Filtration vs. Dead-End Filtration

Throughput can often be significantly improved by using a cross flow system rather than a dead-end flow system. (The terms tangential flow and cross flow generally have the same meaning; although tangential flow is sometimes used to designate primarily laminar flow along the membrane surface and cross flow can designate either laminar or turbulent flow along the membrane surface.)

As shown in figure A-15, in dead-end flow the flow rate gradually decreases as a polarized layer builds up on the surface of the filter. Eventually, an unacceptable pressure differential is reached.

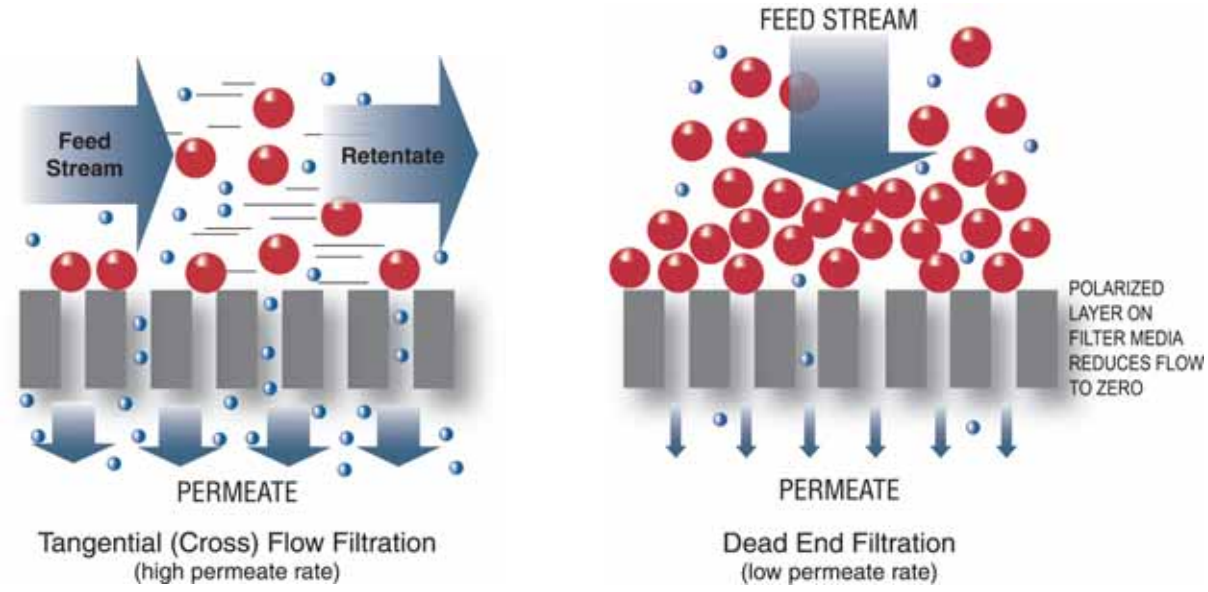


Figure A-15 Tangential vs. dead-end flow

In the case of the tangential or cross flow system configuration the particles or molecules are continuously swept away from the surface of the membrane by the flow stream across the surface. (More details about dead-end and cross flow configurations are included in a later section.)

Membrane Filtration

Membrane filtration has been known almost 100 years, yet it has only been since the mid-twentieth century that the membrane filter became a readily available commercial product. Originally marketed for water bacteriology, it soon became clear that membranes had a wide range of other applications.

Application of membrane filters to biochemistry was a major advance in the 1950's. In the 1960's membrane filters were used for the first time in nucleic acid hybridization. And in the 1970's, development of recombinant DNA technology led to the use of membranes in gene cloning.

In the pharmaceutical industry membrane filters are widely used for the cold sterilization of drugs, serum and large-volume parenterals. Membrane cartridges have provided particle and pyrogen free water for many industries, especially in microelectronics and pharmaceutical products. Membrane filtration is also used in air filtration for contamination control, air pollution analysis, and gas sterilization.

Continuing development of membrane technology has led to membranes being routinely used in a wide spectrum of research, laboratory and process applications. Descriptions of many of these applications follow in later chapters.

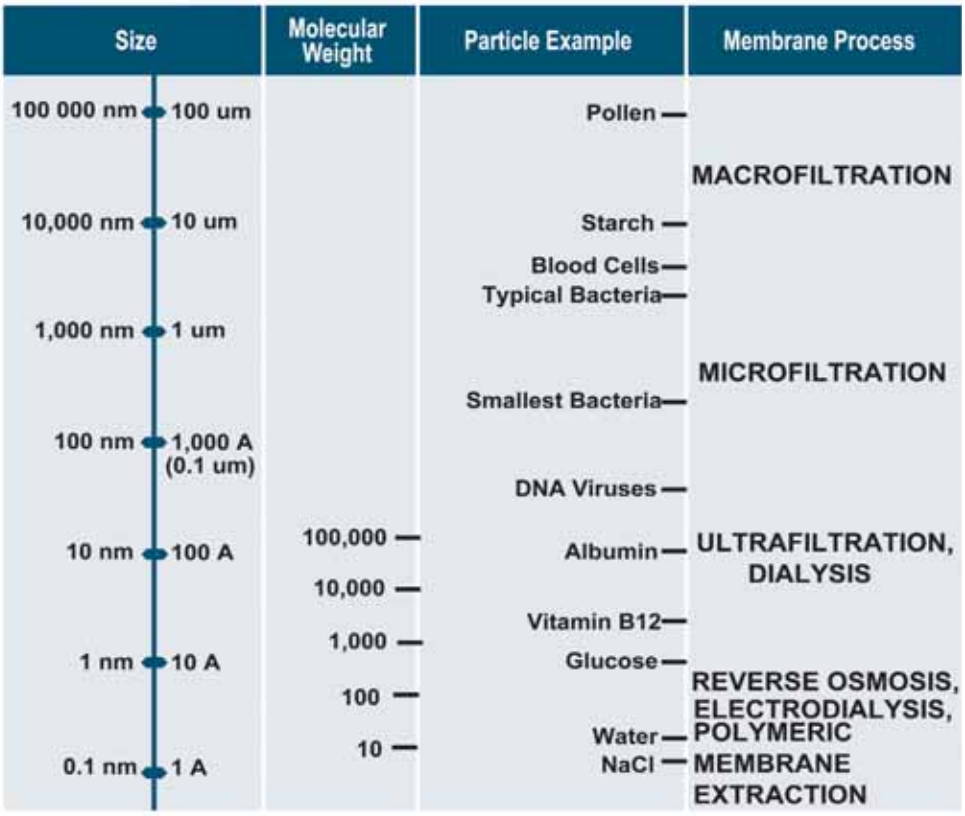


Figure A-16 Comparison of various membrane and filtration processes

Disposable membrane modules are now used routinely for manufacturing biopharmaceutical products. This eliminates the need to requalify filtration systems that utilize reusable modules and membranes.

Filtration membranes are often classified according to the size of the particle to be retained with or separated from the fluid. This is shown in figure A-16.

More complex separations might involve other membrane and feed fluid/particle characteristics such as electric charge, valence and concentration gradient, for example. In this case, in conjunction with the particle size, these additional characteristics might be the separation mechanism.

MACROFILTRATION is the separation of particles of one size from particles of another size where at least one of the particles is larger than 5 micrometers (µm).

MICROFILTRATION is the separation of particles of one size from particles of another size in the range of approximately 0.05-5.0 µm. The fluid may be either a liquid or a gas.

ULTRAFILTRATION is the separation of molecules of one size from particles and/or molecules of another size. The size of the molecules may range from 10 to 500 angstroms (Å) (0.001 to 0.05 µm).

DIAFILTRATION is the removal or washing of molecules by the addition of solvent directly to the solute being purified or retained. Constant volume and particle concentration is thus maintained. It is also used for desalting or buffer exchange.

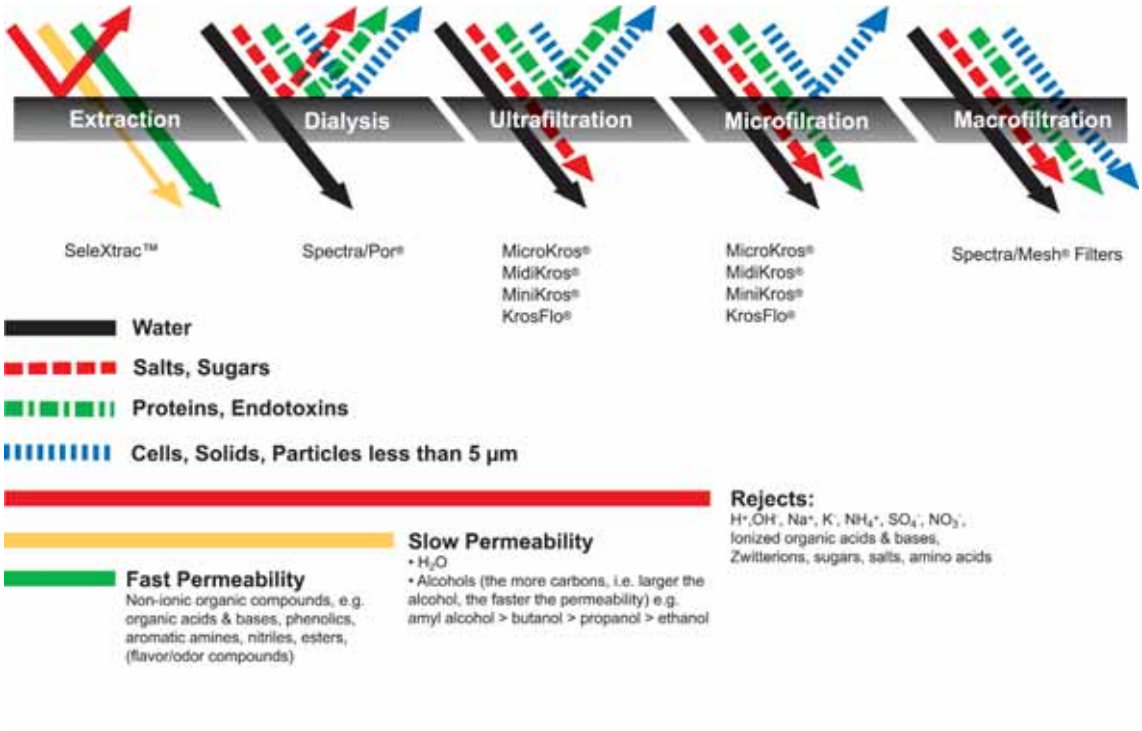
DIALYSIS is the separation of solutes in a solution based on size exclusion across a semipermeable membrane where the driving force is the concentration gradient, with particles moving from an area of higher concentration to an area of lower concentration. Additional information about dialysis is contained in a later chapter of this book.

REVERSE OSMOSIS is a process that separates small molecules and ions, less than 10 Å in size, from a liquid such as water.

ELECTRODIALYSIS is the transport of ions through a semipermeable membrane as the result of an electrical driving force.

POLYMERIC MEMBRANE EXTRACTION is a new technology for the selective extraction and concentration of organic compounds from dilute aqueous solutions and complex processing mixtures based on molecular properties.

Guide to Filtration, Bioprocessing and Selection of Spectrum Membranes



Volume of Liquid Processed Based on Module Type

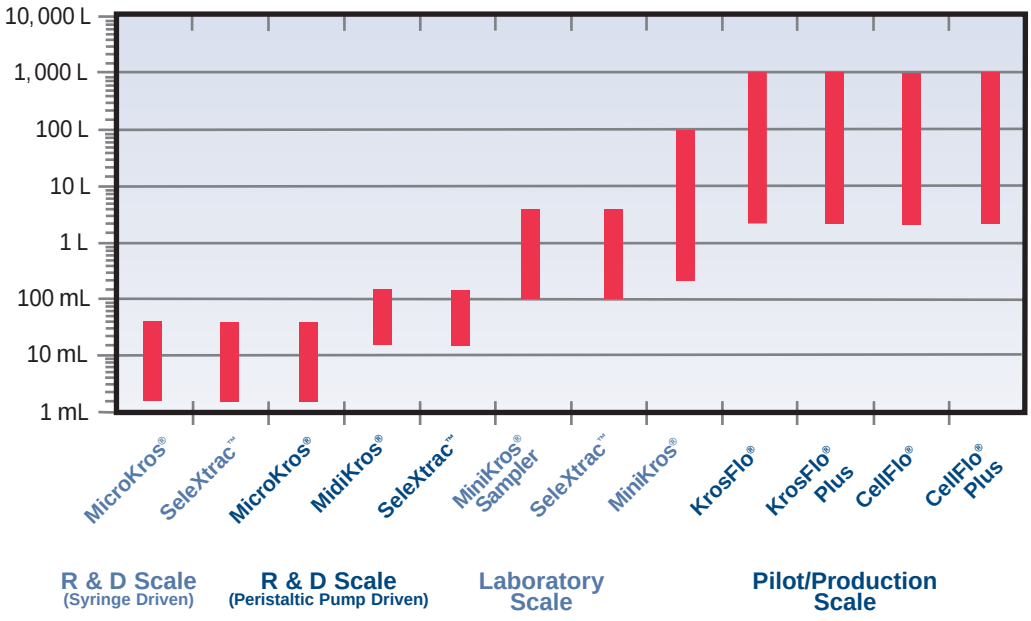


Figure A-17 Filtration characteristics of Spectrum products

Dialysis

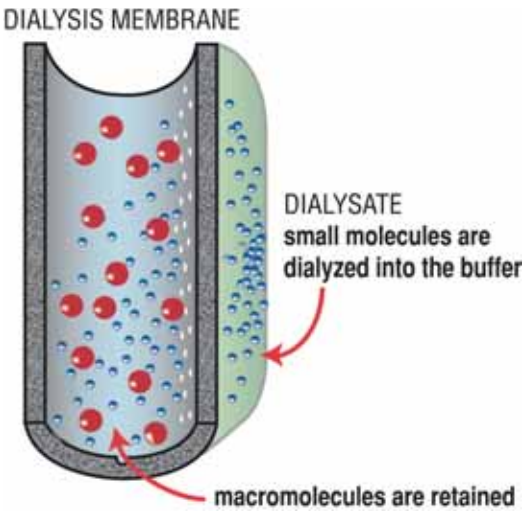


Figure A-18 Operation of hollow fiber dialysis membrane

Primarily used in laboratories and process applications for concentrating, desalting and purifying proteins and enzymes, dialysis is a means of separating molecules of different sizes. As shown in figure A-18, it involves placing a solution to be dialyzed on one side of a semipermeable membrane that has a higher degree of selectivity for small molecules and solvents than for larger molecules (macromolecules).

While the retentate solution is on one side or in the lumen of a tube or hollow fiber module, a buffer solution is on the other side. Small molecules will passively diffuse through the membrane in both directions, driven by the concentration gradient until equilibrium is reached. The membrane retains macromolecules. Generally little or no pressure is used to assist in the transport of the smaller molecules into the dialysate buffer.

Dialysis membranes and hollow fiber modules are available for separating molecules with a range of molecular weights from 100 to 1,000,000 Daltons.

Dialysis separation is presented in much greater detail in a later chapter of this book.

Electrodialysis (ED)

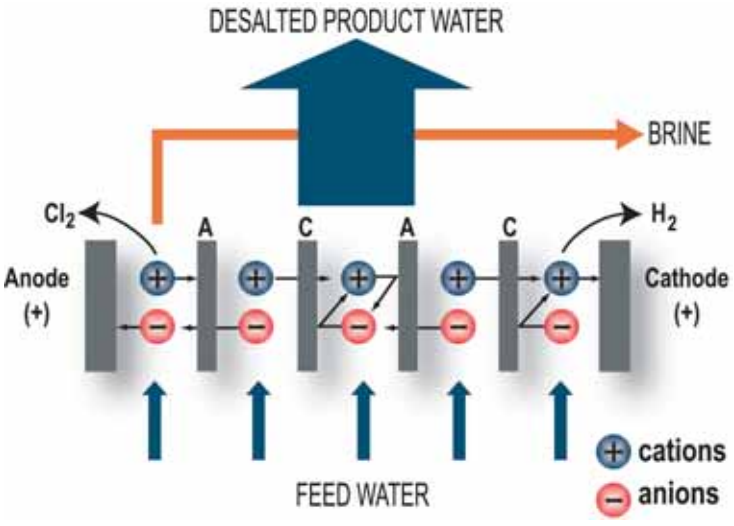


Figure A-19 Principle of operation of electrodialysis

Electrodialysis is defined as the transport of ions through a semipermeable membrane as the result of an electrical driving force.

Although the most frequent use of electrodialysis is in removing salt from water, with the proper membrane it can be used for separating electrolytes from nonelectrolytes, cations from anions, univalent ions from multivalent ions, etc. Secondary only to the desalting of water is the use of ED in the production of chlorine and caustic soda.

Figure A-19 illustrates the basic process involved in the separation of salt from water, which has two cations, H+ and Na+, and two anions, OH- and Cl-.

A voltage is established across a feed-

water solution separated by anion-permeable (but cation-impermeable) membranes, marked A, and cation-permeable (but anion-impermeable) membranes marked C. The positive sodium and hydrogen cations are attracted toward the negative cathode. They readily pass through the cation permeable membranes but are rejected by the anion permeable membranes. If there is no membrane in the path of the hydrogen cation, it is released at the cathode in the form of gas (H^2). In reverse, the same thing happens to the oxygen and chloride anions. As a result, in those sections of the electrolytic cell where there is a rejection of the hydrogen and sodium ions and transport of the oxygen and chloride ions, the ions partially recombine to form saltier water. In those sections of the cell where there is transport of both the hydrogen and chloride ions, only pure water will remain.

Reverse Osmosis (RO)

Osmosis is a natural phenomenon that takes place when water passes from a less concentrated solution through a semipermeable barrier to a more concentrated solution.

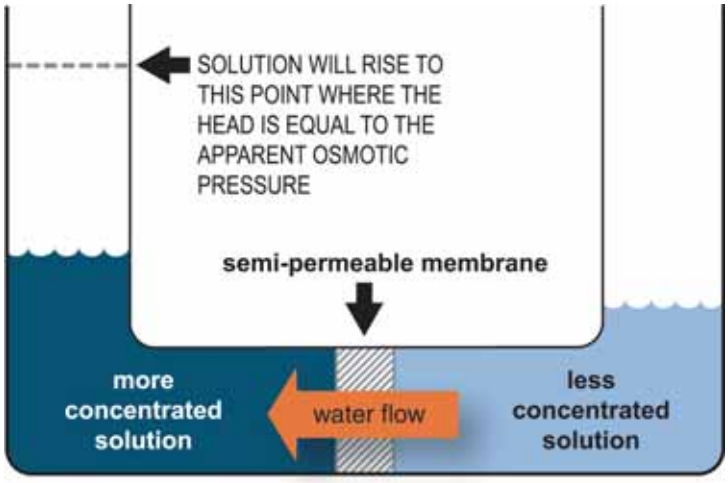


Figure A-20 Natural osmosis

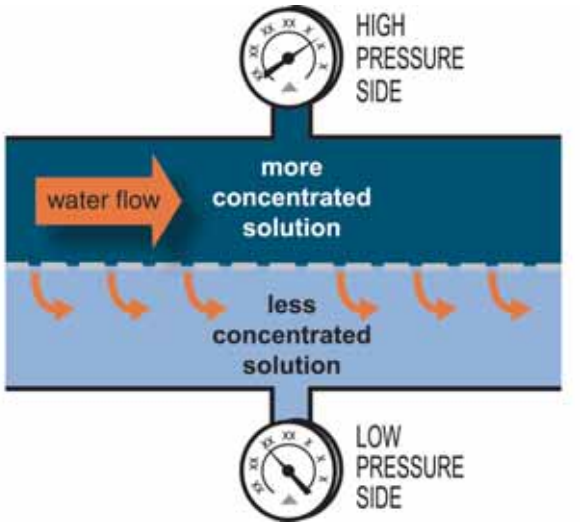


Figure A-21 Schematic of operation of reverse osmosis (RO)

Figure A-20 illustrates simple osmosis. A semipermeable barrier separates a dilute and a concentrated solution. Flow occurs from the dilute to the more concentrated solution until the two solutions are equalized in concentration. At equilibrium the head that develops is called the "osmotic pressure". For water this is equal to approximately 1 psi for each 100 parts per million (ppm) total dissolved solids.

Reverse osmosis occurs when a pressure is applied to the side containing the more concentrated solution as shown in figure A-21. At a pressure equal to the osmotic pressure, flow will cease. At a pressure greater than the osmotic pressure, flow is reversed.

In most reverse osmosis applications, tap water is forced through a membrane under pressure, generally in a cross flow configuration where the concentrate of tap water not transported through the membrane goes to drain.

Reverse osmosis rejects not only a high percentage (90-97%) of salts but also a very high percentage of organic matter and other particulates.

Figure A-22 shows the mechanism for salt rejection in which a pure water layer of about 10 Å (0.00l µm) thickness develops on the surface of the membrane due to the chemical nature of the

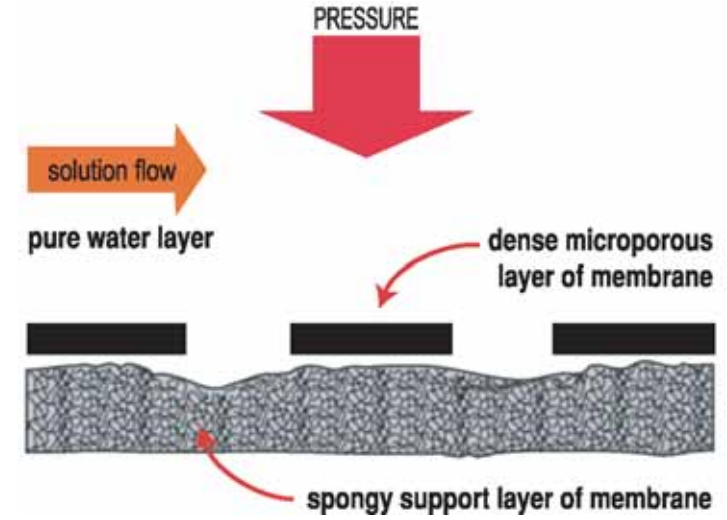


Figure A-22 Salts are repelled from the surface of RO membranes according to molecular weight

membrane. Salts are repelled from the surface of the membrane, with higher valence ions being repelled to a greater distance from the membrane than lower valence ions.

Organic and other particulate matter is rejected entirely on the basis of the size and configuration of the particulate or organic molecule as shown in figure A-23. This is the familiar filtration mechanism except that the "pores" of the RO membrane will filter down to very small molecules. Since the typical RO membrane has 20 Å pores, nearly all molecules above a molecular weight (MW) of 200 are rejected and depending on molecular shape, a substantial percentage of molecules of MW 100-200 are rejected. Thus, sugar, with a MW 342 is rejected and formaldehyde with MW 30 is passed. This latter feature is important since it allows the RO equipment to be sterilized by a simple formaldehyde flush.

Upwards of 90-95% of all pyrogens, viruses and bacteria are rejected by the RO membrane.

The most successful RO membranes for commercial use are spiral wound polymeric membranes with a very thin skin supported by a porous substructure. In these membranes, the substructure acts only as support for the active layer and generally has no effects on the filtration characteristics of the membrane.

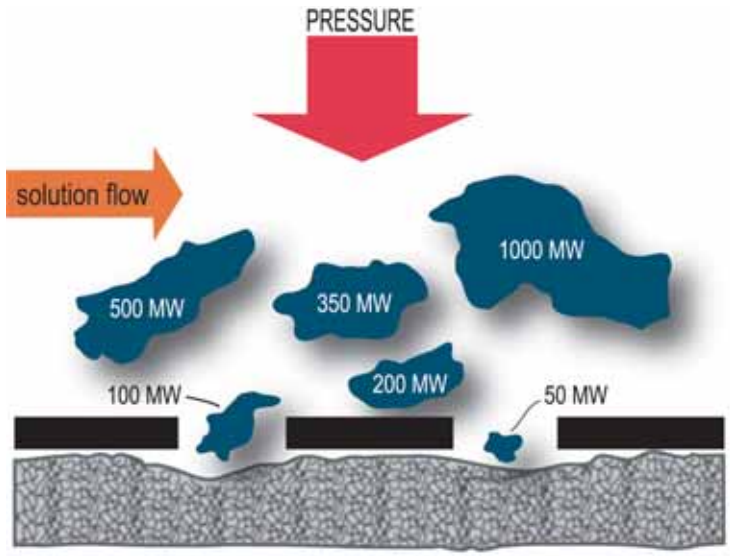


Figure A-23 RO membranes reject most molecules with MW > 200

Since the actual discriminating barrier is extremely thin, very high flow rates can easily be achieved. Due to the highly asymmetric structure, all rejected materials are retained on the membrane surface and internal pore fouling is virtually eliminated.

The largest application for reverse osmosis has been for the production of ultrapure water for pharmaceutical, medical, electronic and other industrial applications. There is also a growing market for household RO systems.

Selective Gas Permeable (Permselective) Membranes

Although the first reported observation that different gases permeate through media at unequal rates occurred in 1831, significant work on permselective membranes

did not begin until the mid 1940's with the availability of new polymeric materials. Basically, permeation of a gas through a nonporous membrane involves adsorption of the gas on one side of the membrane; diffusion of the gas through the membrane; and desorption of the gas from the opposite side of the membrane.

Gas separation now ranks as one of the most important membrane applications. Major applications include separation of helium from natural gas, oxygen enrichment from air, recovery of hydrogen from ammonia synthesis, enhanced oil recovery, separation of hydrogen and carbon dioxide from ammonia and methanol purge gases and removal of sulfur dioxide from processing high sulfur coal.

Polymeric Membrane Extractions

A novel class of membranes has been recently introduced for the selective extraction and concentration of organic compounds from dilute aqueous solutions. The pores of these advanced hollow fiber membranes are impregnated with a polymeric liquid having an affinity or attraction for the organic compound of interest. This technology of separating compounds from a liquid or gas stream depends primarily on the chemical properties of the liquid polymer used and not on the conventional sieving or rejection of molecules or particles through the membrane pores.

These liquid membranes selectively transport and extract specific compounds by utilizing various molecular properties such as hydrophobicity, hydrogen bonding capability, etc.

Figure A-24 illustrates a laboratory scale setup using syringes as pumps. The SeleXtrac™ membrane module is designed with two sets of inlet and outlet ports for circulating two different mixtures of a liquid or a gas or both. One set of syringes is used to circulate the "Feed" solution" (sample mixture). The other set of syringes is used to circulate the "Strip" solution" in a countercurrent flow direction.

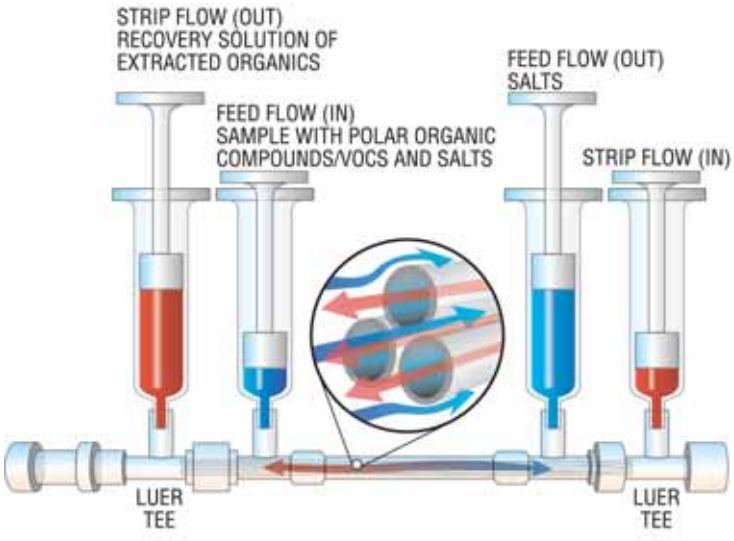


Figure A-24 Schematic of syringe pumped SeleXtrac™ module

By chemical interaction, targeted organic molecules are transported from the Feed solution through the liquid polymer contained in the pores of the hollow fiber membranes and into the Strip solution that is being recirculated through the fiber lumen. Either the Feed solution or the Strip solution may be a liquid or a gas mixture.

Larger scale extractions use either peristaltic or large volume pumps to circulate the two solutions.

A complete description of polymeric membrane extraction is included in a later chapter of this book.

Integrity Testing Membrane Filters

It is often important to check for complete integrity of the filter mounted in its holder. The most widely accepted test over the years has been the "bubble point" test.

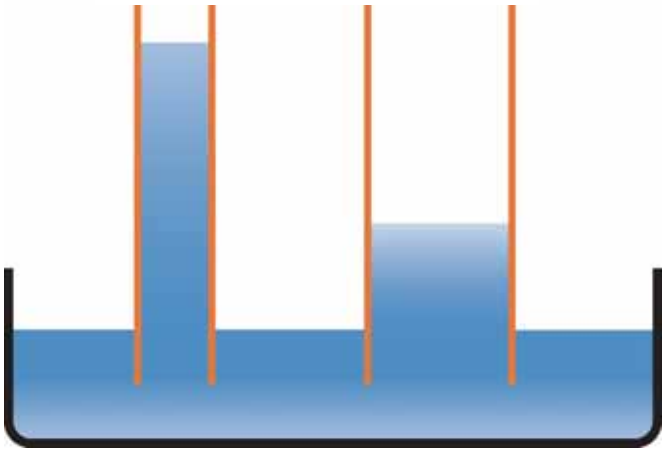


Figure A-25 Illustration of capillary pressure

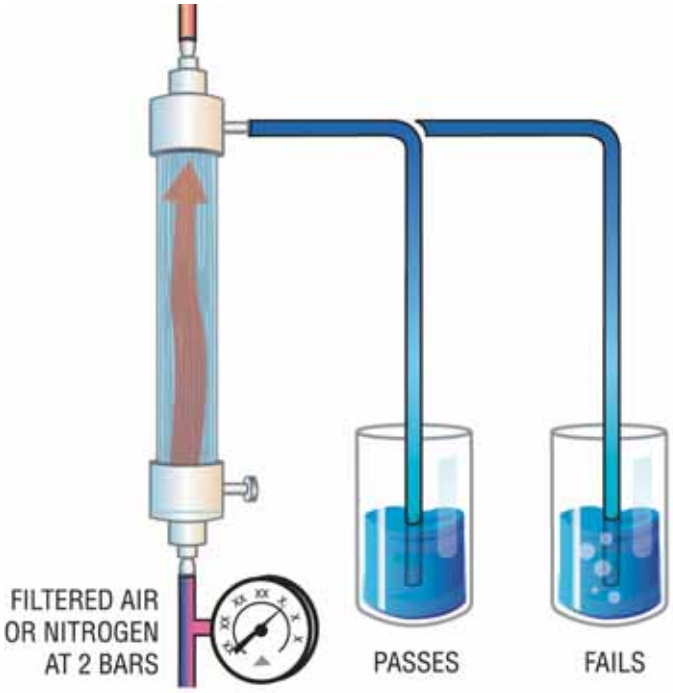


Figure A-26 Filter integrity test

Referring to figure A-25, when capillaries are full of liquid, the pressure required to force the liquid out of the capillary must exceed the surface tension of the liquid. The capillary pressure is higher in the case of a small capillary than for a large capillary, i.e., a higher air pressure is required to force water from the smaller diameter. The same is true of the pores in a membrane.

Figure A-26 illustrates the operation of the bubble point test. When the bubble point pressure is reached bubbles will form downstream of the filter. This is called the bubble point. As pressure is further increased, rapid bubbling will begin to occur. If bubbling occurs before reaching the bubble point pressure, the test has failed.

In performing a bubble point test, it must be considered that air has a finite diffusion rate through a liquid and over a period of time small bubbles may form on the downstream side of the membrane at pressures well below the bubble point. For example, a membrane rated at 0.22 micrometers might have a typical diffusion rate for air through water of approximately 0.04 ml/min/psi/sq. ft.

As a variation of the bubble point test, Spectrum has developed the pressure decay test. In this test, the module is fully primed to ensure complete wetting and a pressure of 5 psi of filtered air or nitrogen is connected to the system feed inlet. An integral system and membrane will maintain 5 psi of pressure (less gaseous diffusion) after shutting off the gas supply. To compensate for gaseous diffusion through the membrane, a pressure decay less than 0.1 psi/min indicates an integral membrane and system.

If pressure decays more rapidly than 0.1 psi/min, the filtrate tubing is then submerged in water. If bubbles are seen, the membrane either has not been completely wetted or it has a defect and must be changed. If bubbles are not seen, then air is leaking from the system upstream of the membrane.

The equation for determining bubble point pressure is:

$$P = k \frac{4 \acute{O} \cos \Omega}{d}$$

Where:

P = bubble point pressure
d = pore diameter
Ó = surface tension of the liquid
Ω = liquid to membrane contact angle
k = pore shape correction factor (the value of k is 1 for a perfect screen)

In the case of a hydrophobic membrane, a test similar to the bubble point test is often used. Water is placed on one side of the hydrophobic filter and the water pressure is increased until the filter wets on the dry side of the filter. This pressure is called the water intrusion pressure and is a good measurement of the hydrophobicity of a membrane. In general, the water intrusion pressure for a hydrophobic 0.2-micrometer membrane will be on the order of 25 to 30 psi versus the bubble point pressure of 70 to 90 psi for a hydrophilic membrane.

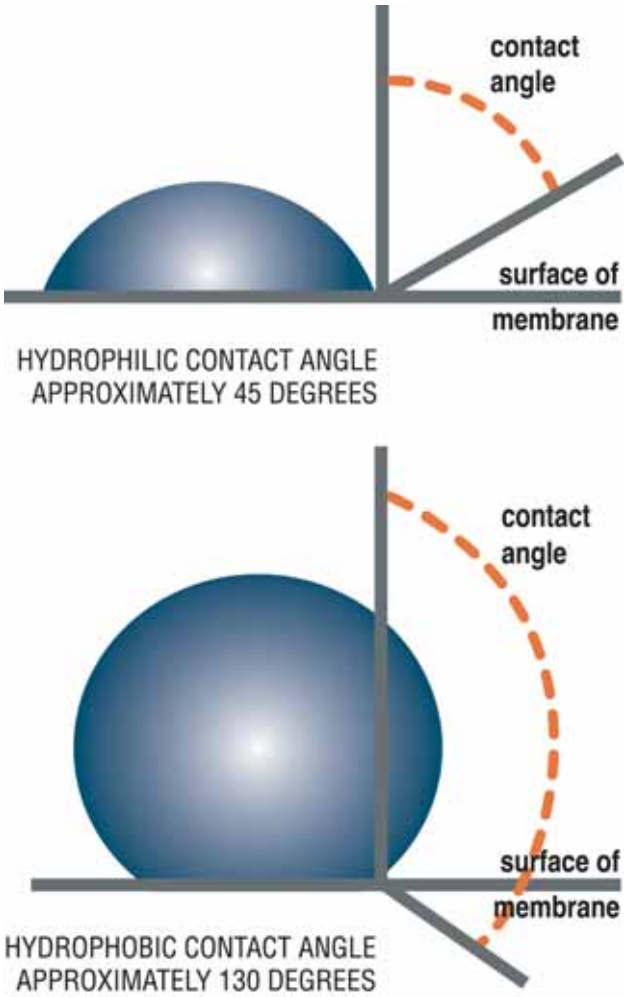


Figure A-27 Contact angles for hydrophilic and hydrophobic membranes

Hydrophobic membranes are most often used when filtering moist air since the pores of a hydrophilic membrane would soon plug from condensation of the water in its pores. A hydrophobic membrane, on the other hand, will condense water on its surface but will not condense water within its pores.

As shown in figure A-27, the contact angle of a liquid with a membrane is defined as the angle between a line that is perpendicular to the exact point at which the liquid contacts the filter and a line that is perpendicular to the surface of the filter.

Filters with water contact angles of 0 to 40° are considered hydrophilic. Contact angles of 90° or more are defined as hydrophobic. The contact angles of membranes vary from nylon with a contact angle near zero (very hydrophilic), through most hydrophilic membranes with typical contact angles of 25°, to PTFE with a contact angle of approximately 130° (very hydrophobic).

Tangential (Cross Flow) Hollow Fiber Module Ultrafiltration

Ultrafiltration (UF) separates dissolved particles and molecules according to size and configuration by flowing a solution that contains these molecules through a membrane. The membrane will retain most particles and molecules above its retention rating and will allow most smaller molecules, along with the solvent, to pass through the membrane.

Ultrafiltration is similar to reverse osmosis in that it filters on the basis of molecular size, but it differs in that it does not separate on the basis of ionic rejection. Another distinction is that UF operates at a moderate pressure, (on the order of 10 to 50 psi), while RO, depending on the concentration of the dissolved solids, may require pressures upward of 1200 psi.

The main difference between ultrafiltration and microfiltration (MF) is the pore size. MF separates micron and submicron sized particles. UF separates particles and molecules from molecules of significantly lesser molecular weight. The principal difference between UF and dialysis is that UF involves the application of pressure. Dialysis does not. Dialysis depends entirely on the concentration gradient as its driving force.

Although the UF membrane is not absolute, it does retain most macromolecules above its molecular weight cutoff while allowing smaller molecules and the solvent to pass through the membrane. As in the case of RO it does not involve phase changes nor temperature extremes.

The selection of an ultrafiltration membrane requires the consideration of a number of important performance parameters. These include retentivity, selectivity, molecular weight cutoff, filtration (flux) rate, adsorption, and temperature and chemical resistance.

Retentivity

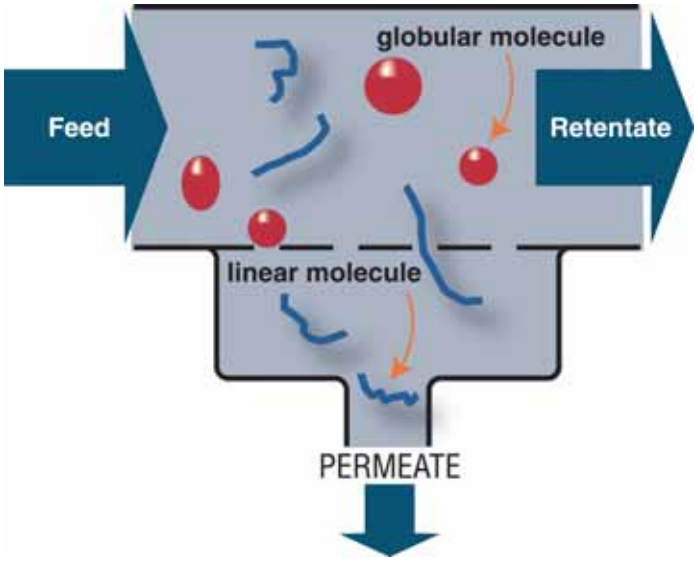


Figure A-28 Retention of globular and linear molecules by UF membrane

Ultrafiltration membranes are rated according to the retention of a substantial percentage of molecules at or larger than its pore size rating. This is defined as its nominal molecular weight cutoff (MWCO). Since UF membranes are not absolute, the shape of the molecule to be retained (or passed) has a major effect on retentivity. It can be seen in figure A-28 that a linear molecule may pass through a UF membrane while a globular molecule of the same molecular weight may be retained.

In addition to the physical configuration of the molecule, the electrical charge of a molecule will affect the separation characteristics of the membrane. Furthermore, retention varies because of the pore size distribution that is characteristic of all membranes. Therefore, it is

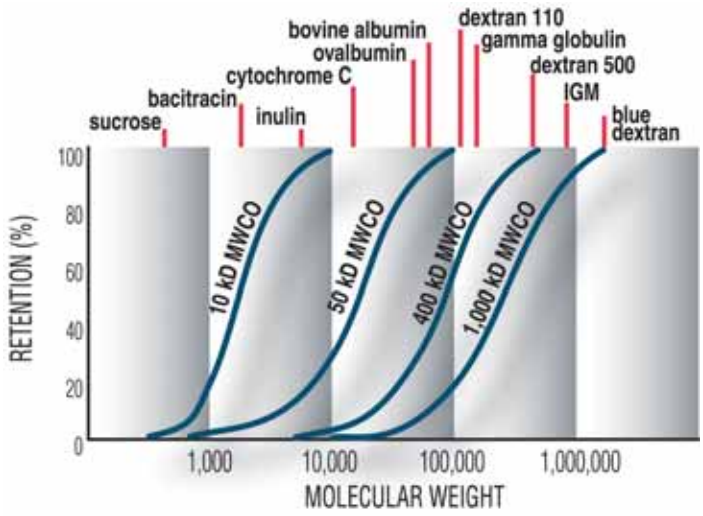


Figure A-29 Solute retention of Spectrum UF membranes

difficult to precisely characterize the molecular weight rating for ultrafiltration membranes.

In many cases, UF membranes are rated at that MWCO where at least 90% of spherical (globular) uncharged molecules of that same molecular weight will be retained. However, as previously noted, a substantial number of linear molecules with a molecular weight equal to or greater than this MWCO rating may pass through the membrane. Figure A-29 shows solute retention efficiency versus molecular weight for Spectrum Laboratories' ultrafiltration membranes.

Specific retention characteristics of Spectrum hollow fiber UF membranes for various molecular weights is shown in table A-1. The bold retention percentage indicates the specified MWCO for that particular membrane.

Retention of Spectrum Membranes (percent)				
Solute Size	**SP-10 kD**	**SP-50 kD**	**SP-400 kD**	**SP-50nm**
1 kd	20%	0%	0%	0%
3 kD	70%	5%	0%	0%
5 kD	90%	15%	0%	0%
10 kD	**99%**	30%	5%	0%
20 kD	100%	57%	16%	0%
30 kD	100%	75%	25%	2%
40 kD	100%	85%	33%	3%
50 kD	100%	**90%**	41%	5%
60 kD	100%	94%	48%	8%
100 kD	100%	99%	67%	18%
150 kD	100%	100%	80%	27%
200 kD	100%	100%	90%	39%
300 kD	100%	100%	97%	55%
400 kD	100%	100%	**99%**	66%
500 kD	100%	100%	100%	75%
630 kD	100%	100%	100%	83%
750 kD	100%	100%	100%	88%
800 kD	100%	100%	100%	90%
900 kD	100%	100%	100%	92%
1,000 kD	100%	100%	100%	**94%**
2,000 kD	100%	100%	100%	100%

Table A-1 Molecular retention by Spectrum UF membranes

MWCO Range

The retention efficiency, high selectivity and complete range of MWCO ratings available with Spectrum Laboratories UF membranes give the user a precision of separation previously unavailable.

Selectivity

Also of importance in evaluating membranes is the degree of retention of molecules with molecular weights somewhat below the MWCO rating of the membrane. If a membrane retains few of these molecules it has high selectivity (or a sharp cutoff), a very desirable characteristic. If a membrane retains substantial numbers of molecules with a molecular weight somewhat below the membrane MWCO rating, it is said to have low selectivity (or a diffuse cutoff). Spectrum UF membranes show a narrow pore size distribution which guarantees a relatively high selectivity and sharp MWCO.

Adsorption

All UF membranes will adsorb nonspecifically some percentage of rejected substances. Such adsorption can cause erroneous and misleading results in critical applications, such as in clinical laboratories or in sensitive research areas. Adsorption losses increase with membrane surface area, especially in the case of proteins. In general, protein adsorption can be expected to be between 5 and 20 $\mu g/cm^2$.

Temperature and Chemical Resistance

Temperature and chemical resistance characteristics are important for non-aqueous solutions and for operating conditions higher than room temperatures. Different membrane materials offer different resistance characteristics. UF membranes are typically made from polypropylene, polysulfone or cellulosic polymers. Refer to Chemical Resistance Table on page F-150

In addition, sterilization can subject the membrane or system to high temperatures or chemicals.

Filtration (flux) Rate

The membrane filtration rate will increase with increasing MWCO rating of the membrane and with increasing temperature. In some cases membrane flux will also increase with increasing pressure, depending upon the molecular species retained, the concentration of the solute and the hydrodynamic conditions at the membrane surface. In addition, the achievable flux rate depends on the concentration and heterogeneity of substances in solution. While membrane flux rates are normally stated for ultraclean water, in practice the flux will be lower when processing biological solutions due to the phenomenon of concentration polarization.

Concentration Polarization

In addition to flow reduction caused by physical plugging of the membrane pores, membranes can also experience filtration reduction, at least temporarily, by the formation at the membrane surface of a layer of concentrated macromolecules or particles. Such a layer is called “concentration polarization” in the case of macromolecules. This layer will form to reduce flux even in the absence of physical plugging.

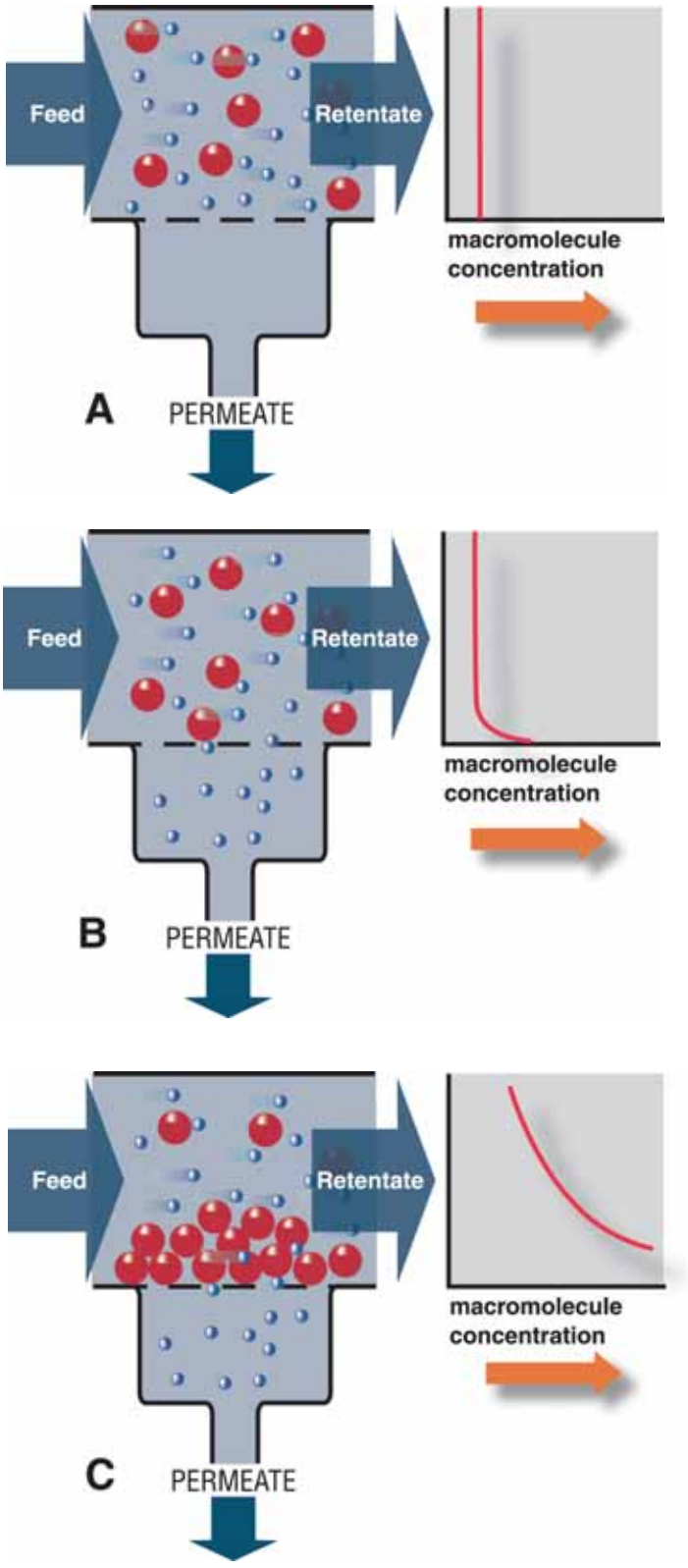


Figure A-30 Concentration polarization on surface of UF membrane

Figure A-30 illustrates the principle of concentration polarization. Initially all solute species are uniformly distributed throughout the solution, as shown in figure A-30A. In figure A-30B, as pressure is applied, the solution and small molecules pass through the membrane. Macromolecules are retained at the surface of the membrane. Since the back diffusion rate of these large molecules is slow due to their size, they accumulate in a layer above the membrane. At this point, filtration is still limited only by the characteristics of the membrane and the pressure. This is known as "membrane controlled filtration".

As shown in figure A-30C, as pressure is increased the number of these molecules in the boundary layer may increase until a limiting concentration is reached. At this time further increases of pressure may become ineffective, and the concentration polarization itself may control the filtration. This is often referred to as "gel controlled filtration".

The highest concentration occurs at the membrane surface and decreases exponentially toward the solution. In the case of higher molecular weight substances, the solubility limit is often reached at the membrane surface. The precipitated layer acts as a secondary membrane (the so-called "gel layer"), which means that the flux rate may decrease considerably. In addition, this "gel layer" may have a higher retention rating than the membrane itself.

Several factors may act to control formation of the gel layer. Most significant is molecular diffusivity that is, among other things, a function of molecular weight and configuration. Linear molecules tend to diffuse more slowly and thus form a gel sooner than is the case with globular molecules as shown in figure A-31.

Agitation to increase the diffusion of the macromolecules back into the solution partially or totally eliminates concentration polarization or gel limited filtration. Figure A-32 illustrates the concept of

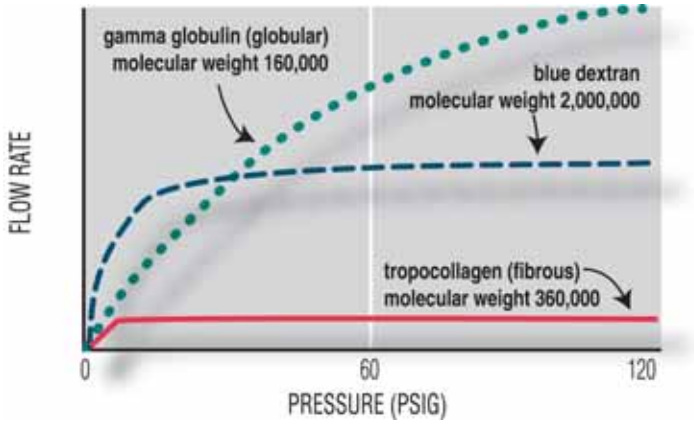


Figure A-31 Relative diffusivity of linear and globular membranes

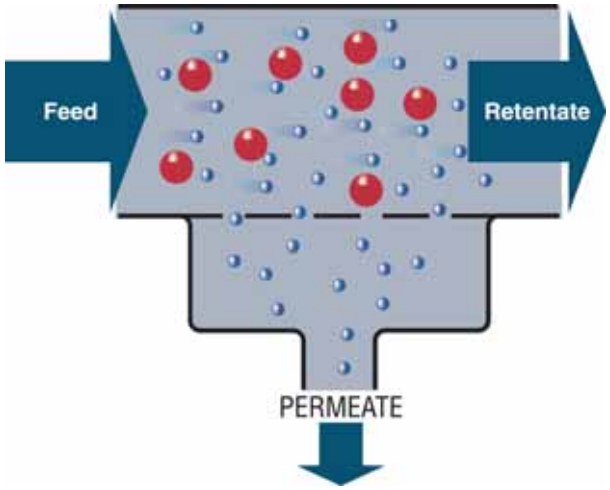


Figure A-32 Agitation of the solution will often assist diffusivity of the molecules for higher flux

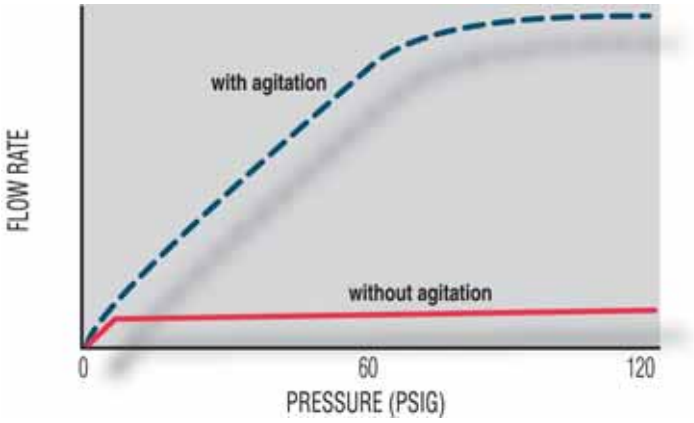


Figure A-33 Agitation of the solution will often dramatically increase the flow rate

using agitation to assist diffusivity and thus filtration.

Sometimes flux rate increases quite dramatically with agitation as shown in figure A-33.

Agitation includes not only physical movement of the solution such as stirring, shaking, etc., but also tangential or cross flow which is very effective in clearing the gel layer. However, the method of agitation must take into consideration the effects on the solutes such as denaturing, shearing, etc.

Feed flow in Spectrum hollow fiber membranes is always through the center, or lumen, of the membrane. This assures laminar rather than turbulent flow along the interior surface (or skin) of the membrane. Although a gel layer may form on the membrane surface, a backflush will generally break up and clear the gel layer, allowing for extended throughput.

Tangential (cross flow) filtration shows particularly dramatic results when filtering a fluid containing deformable particles. In normal (dead-end) flow these particles tend to compact on the filter surface and plug the filter very rapidly. In cross flow fluid systems, these particles are continuously swept away by the feed stream, greatly inhibiting plugging of the filter. Figure A-34 illustrates a typical microfiltration application, the concentration of deformable particles such as yeast, that reflects the same phenomenon that occurs in ultrafiltration. The cross flow fluid management technique clearly reduces the plugging of the filter when compared to the same solution in dead-end flow.

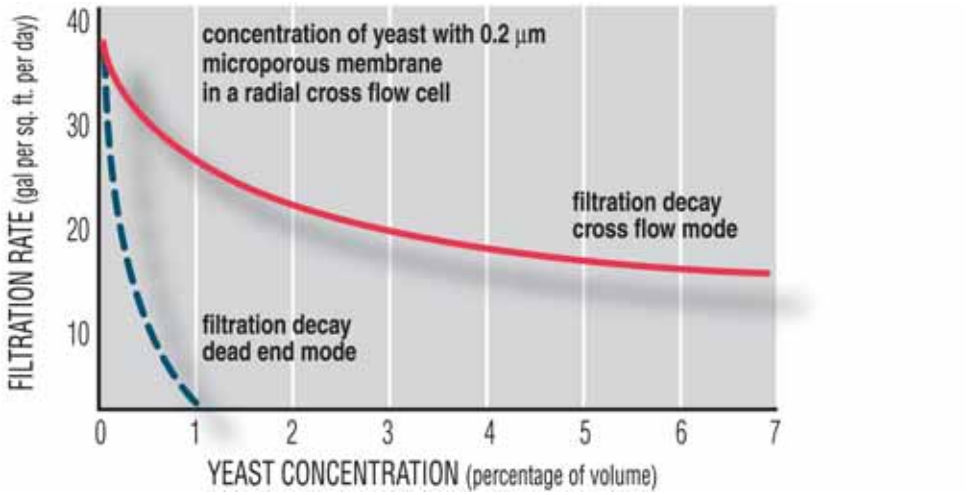


Figure A-34 Decay of flow rates for concentration of yeast in cross flow and dead end filtration modes

Other Factors Affecting Membrane Flux Rates

TEMPERATURE - Water flux and process fluid flux will increase with increasing temperature. Clean water flux varies linearly with changes of water viscosity. Over a range of 57° to 97° F changes in water viscosity varies with the ratio of the temperature change in degrees Fahrenheit.

Temperature Corrected Flux = (Flux At Standard Temperature) $\frac{(T_a)}{(T_s)}$

Where:

T_s = Standard temperature (e.g. 77°F)

T_a = Actual temperature (°F)

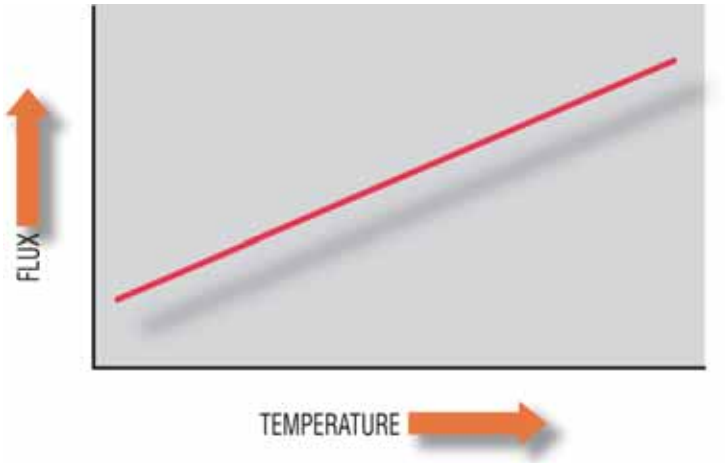


Figure A-35 Flux increases with increasing temperature

Process flux will also increase with temperature as illustrated in figure A-35. However, the degree of process flux improvement is less predictable than with clean water since both a “gel” layer and a “fouling” layer on the membrane surface contribute to flux resistance.

As a general rule, operation should be at the highest acceptable temperature, taking into consideration temperature limitations of the feed stream and the membrane.

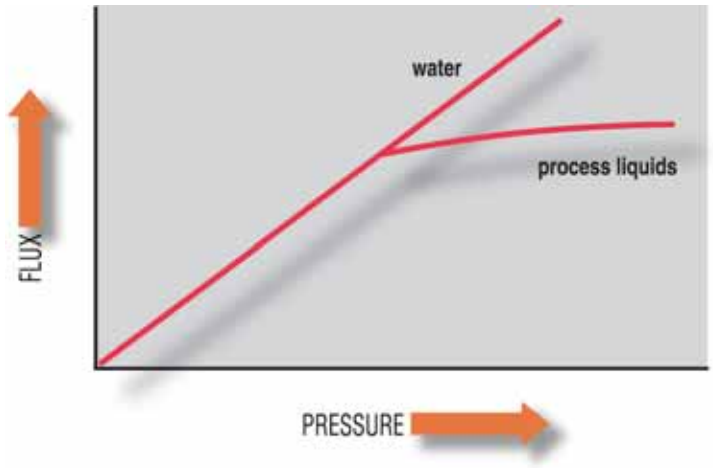


Figure A-36 Flux will increase with increased transmembrane pressure until gel limited flow is reached

TRANSMEMBRANE PRESSURE (TMP) Clean water flux will increase linearly with increasing transmembrane pressure, as shown in figure A-36. Process liquid flux will typically increase as a function of transmembrane pressure. However, depending on the circulation rate, the improvement in flux may become asymptotic since the "gel" layer's resistance to flux will increase from compaction of the macromolecules from the TMP.

Controlling the permeate back pressure (or permeate flux rate) may reduce the tendency of the membrane to foul in the initial stages of a concentration, providing an overall higher average flux rate.

The transmembrane pressure may be calculated as shown below:

$$\textbf{Transmembrane Pressure (TMP)} = \frac{P_{inlet} + P_{outlet}}{2} - P_{permeate}$$

CIRCULATION RATE - The circulation rate for clean water will have little or no effect on flux since there is no gel layer nor fouling layer to restrict permeation.

In the case of process liquids, cross flow filtration will reduce gel layer formation. Cross flow management will lower the resistance to filtration and will improve flux.

Thin feed flow channel devices (hollow fibers, spiral-wound cartridges, plate-and-frame devices, etc.) all operate in laminar flow. Increasing the circulation rate will increase the shear force and typically enhance the rate of filtration.

However, the pressure drop across thin channel devices will increase with increased circulation rate. This limits the degree to which feed velocity can be raised. Generally, to achieve high velocities with thin channel devices, the feed flow path should be as short as practical.

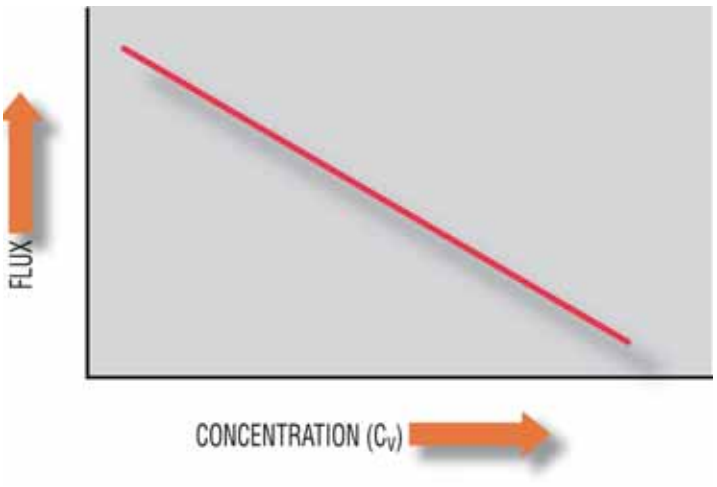


Figure A-37 Flux generally declines with increased solution concentration

CONCENTRATION - Process liquid filtration is highly dependent on feed components and the overall solute concentration. As would be expected, flux declines with concentration. The rate of decline generally decreases as shown in figure A-37.The volume concentration factor (C_v) may be calculated as shown on the next page:

$$C_v = \frac{V_o}{V_o - V_p}$$

Where:

V_o = Original Feed Volume
V_p = Volume of Collected Permeate

The system conversion percentage (S_p) may now be calculated as follows:

$$S_p = \left(1 - \frac{1}{C_v}\right) 100$$

TIME - Filtration declines with time, even with "clean" water. The influence of time on the rate of flux decline may, however, be insignificant compared to the effect of concentration. A rapid flux decline, while concentrating a solution indicates either the circulation rate is too low or the stream contains bad foulants. Flux might also decline over time due to gel layer compaction.

PARTICLE LOAD IN FEED STREAM – As previously shown, the feed stream flow rate has a major effect on permeate flux. However, consideration must also be given to the feed stream particle load in determining the optimum flow rate.

Low fouling streams exhibit stable flux rates over time with low circulation rates. The flux of low-fouling streams is basically concentration dependent. Please refer to figure A-38.

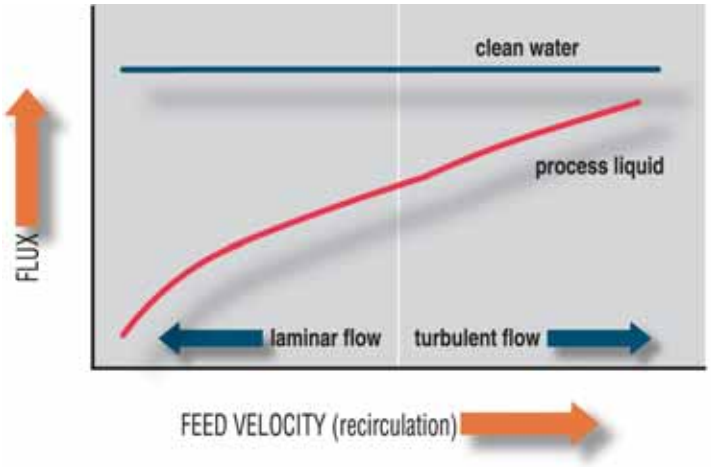


Figure A-38 Variation of flux with tangential shear force for pure water and process liquids

A circulation rate that provides an intermediate shear force, between 4,000 to 8,000 sec^{-1}, is a good starting point for processing low fouling streams. However, for feed streams containing fragile components that may be damaged by high circulation rates or high temperatures, shear forces on the order of 2,000 to 4,000 sec^{-1} are recommended.

If the stream is high fouling and there are no fragile components in the stream, increasing the feed stream circulation rate to at least 8000 sec^{-1} will generally reduce gel layer thickness and increase flux.

Diafiltration

When using ultrafiltration for purification, desalting and/or buffer exchange, small molecules and solvent pass through the membrane while macromolecules are retained. The simplest method for this therefore is to replace the solvent that had passed through the membrane with contaminant free solvent. This can be done on an intermittent or continuous basis.

To illustrate the intermittent method, imagine that 90% of a solvent containing 1% salt passes through a membrane. The feed solution is then brought back to its

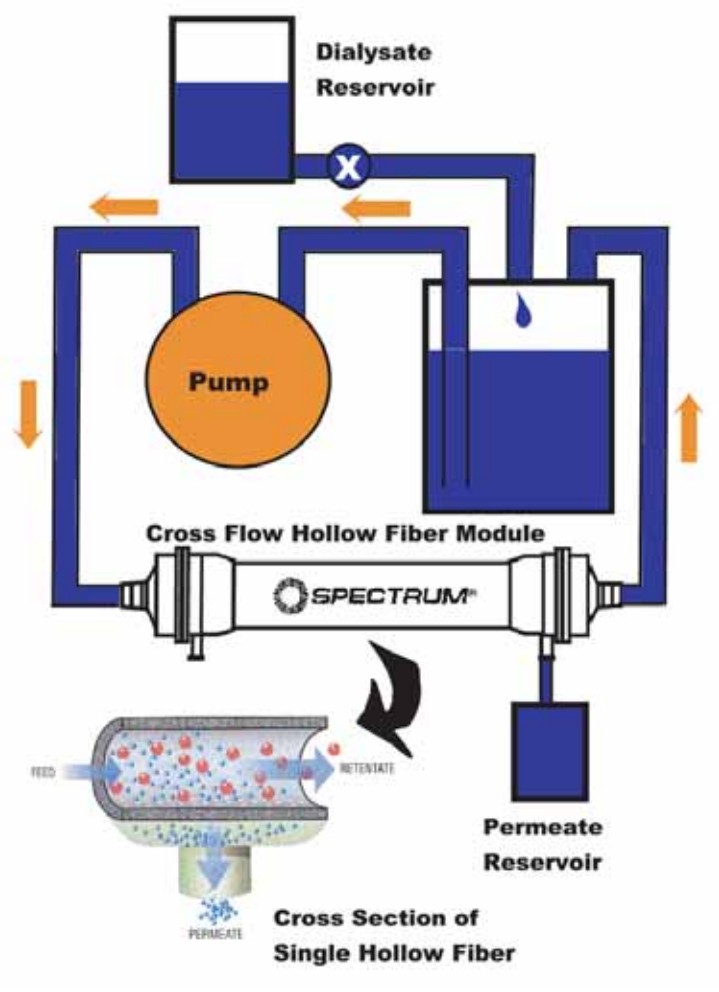


Figure A-39 Schematic of continuous diafiltration system

original volume by the addition of salt free solvent. The feed solution would now contain only 0.1% salt and the same original number of macromolecules. Repeating this process again, the resulting feed solution would then contain only 0.01% salt. Since the rate of removal of the salt is proportional only to the pressure driven flux through the membrane, and not to the impurity concentration, low concentrations can be reached very quickly.

Continuous diafiltration is a much more efficient procedure in using ultrafiltration for desalting and/or buffer exchange than the simple one described above. In this process, the solvent that is removed is continuously replaced at the same rate by contaminant-free solvent. This allows virtually complete purification. As shown in figure A-39, the wash fluid is pressurized by an external gas source or pulled by vacuum, forcing this fluid into the cell to replace the solvent volume that filters through the membrane. The volume of solution and the number of macromolecules remain unchanged while the micromolecules are removed through the membrane.

In practice, a pressure balance or a liquid level controller may be used on the reservoir to insure dialysate addition at the same rate as filtrate removal.

For constant volume diafiltration, each wash volume of filtrate removed reduces the solute concentration by factor of e (2.718..). For example, a four fold constant volume diafiltration will reduce the concentration of solute by a factor of e^4, 50 fold or over 98%. Using this technique, the concentration of solute can be monitored in the filtrate until the desired level of purification or product recovery is achieved. The system set up is the same as for topped-off batch concentrations or clarifications except that the wash buffer enters the vessel instead of feed.

Figure A-40 shows a schematic of a Spectrum hollow fiber system operating in the diafiltration mode.

As permeate passes through the hollow fiber module from the processing reservoir, liquid flows from the feed or buffer reservoir to compensate for the removed permeate.

Tangential (Cross Flow)

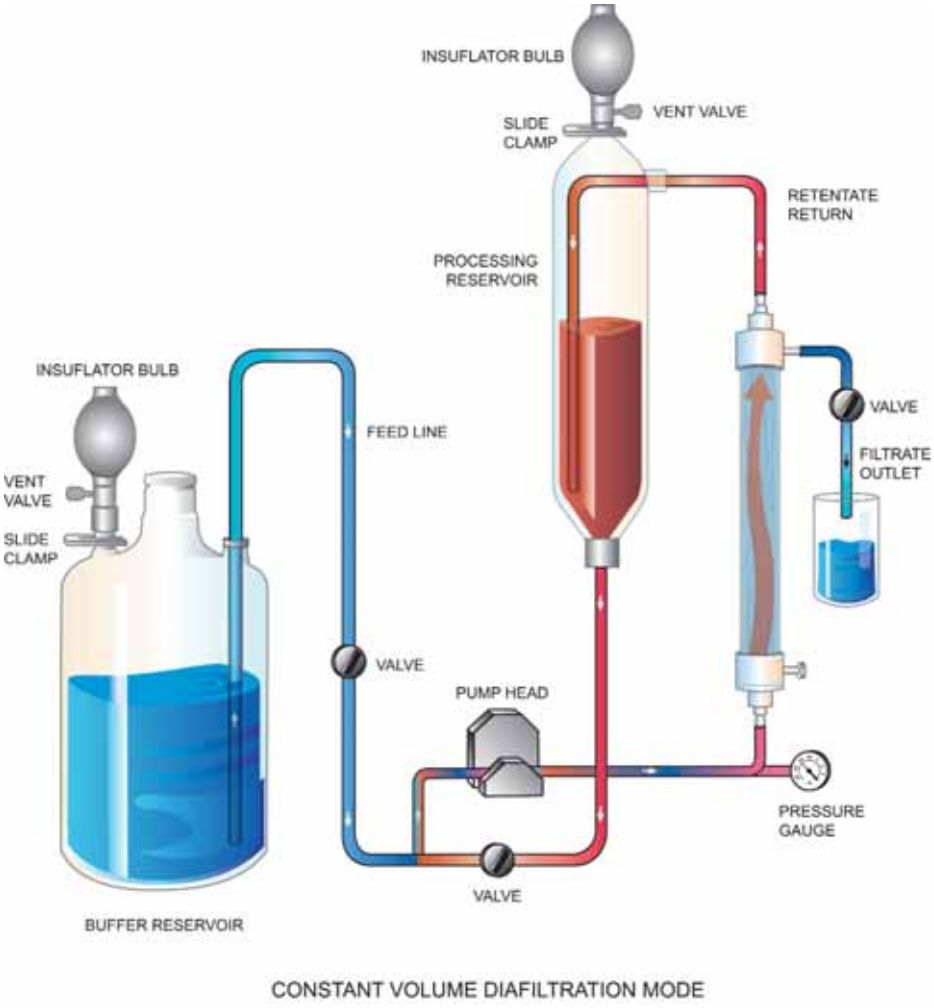


Figure A-40 Schematic of Spectrum diafiltration system using MiniKros® module with reservoir in a constant volume diafiltration mode.

Hollow Fiber Module Microfiltration

Microfiltration is the separation or removal of particles of more than 0.05 µm and less than 5 µm in size from a fluid or from particles of other sizes. The fluid may be either a liquid or a gas. Particles include both rigid and deformable types.

Microfiltration media are available in a wide variety of materials and methods of manufacture, and are rated "absolute" or "nominal" depending upon the percentage of capture of particles of equal size or larger than the retention rating of the media.

Nominal microfiltration media include fibrous mats and similar materials having a retention efficiency less than 100%. The performance rating for this media is often specified as capture of 95% or 98% of all particles equal to or larger than the stated pore size of the filter.

Figure A-41 shows retention characteristics for a typical depth filter rated to capture 98% of all particles equal to or greater than 1 µm. The retention curve approaches the 100% mark on an asymptote of performance eventually reaching 100% retention for very large particles. Although this curve shows no particle capture below 0.2 µm, in practice nearly all fibrous media have an electric charge that is effective in the capture of very small particles. This charge is called zeta potential.

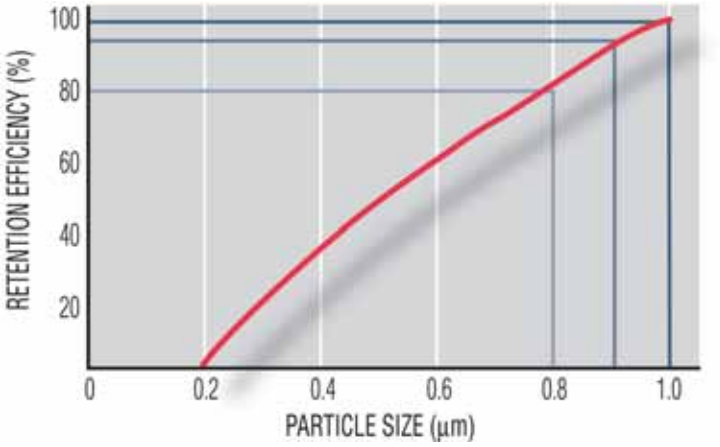


Figure A-41 Retention characteristic for a typical depth filter rated at 98% capture of 1 µm particles

Only media that retains 100% of particles at its pore size rating (within a finite time span) are considered absolute. In general, only macrofiltration screens and microporous membrane filters fall within this definition of absolute.

Microporous membrane filters have sometimes been classified by their pore structure, either capillary pore or tortuous pore.

Capillary pore membrane filters are true screens, similar to macroporous screens. They have round cylindrical pores, more or less normal to the surface of the membrane, with even random pore dispersion over the surface of the membrane. Capillary pore screen membrane filters are commercially available in thin films of polycarbonate or polyester, and are manufactured in a two step nuclear track and etch process.

Tortuous pore microporous membrane filters have a relatively rough surface where there appears to be many openings considerably larger than the rated pore size. These membranes are nevertheless absolute since they depend upon the random tortuosity of the numerous flow paths to achieve their pore size rating. These membranes are commercially available in various cellulosic compounds, nylon, polysulfone, polyethersulfone, polyvinyl chloride, pure silver, polytetrafluoroethylene (PTFE), polyvinylidine difluoride (PVDF) and many other materials.

Filtration Modes (Configurations)

Membrane and Module Configurations

Microfiltration membranes are primarily available in flat sheets, pleated cartridges, and spiral wound, plate and frame or hollow fiber modules. Sheet membranes and pleated cartridges are mostly used in dead end flow configurations.

Spiral wound modules and hollow fiber modules are generally in a tangential (cross flow) flow configuration.

Although dead end flow is much more widely used in filtration applications, plugging of the filter frequently becomes a major problem. Often throughput is significantly improved by using tangential flow instead of dead end flow.

Ultrafiltration membranes are primarily available in sheets, spiral wound modules and hollow fiber modules. Because of the rapid plugging that occurs when using UF membranes in dead end flow, tangential (cross) flow is used almost universally in UF applications.

Figures A-42 and A-43 illustrate the flow paths for the two types of systems. The dead-end flow system experiences molecular or particle concentration on the surface of the filter, reducing the permeation rate and eventually causing an unacceptable flow rate or pressure differential.

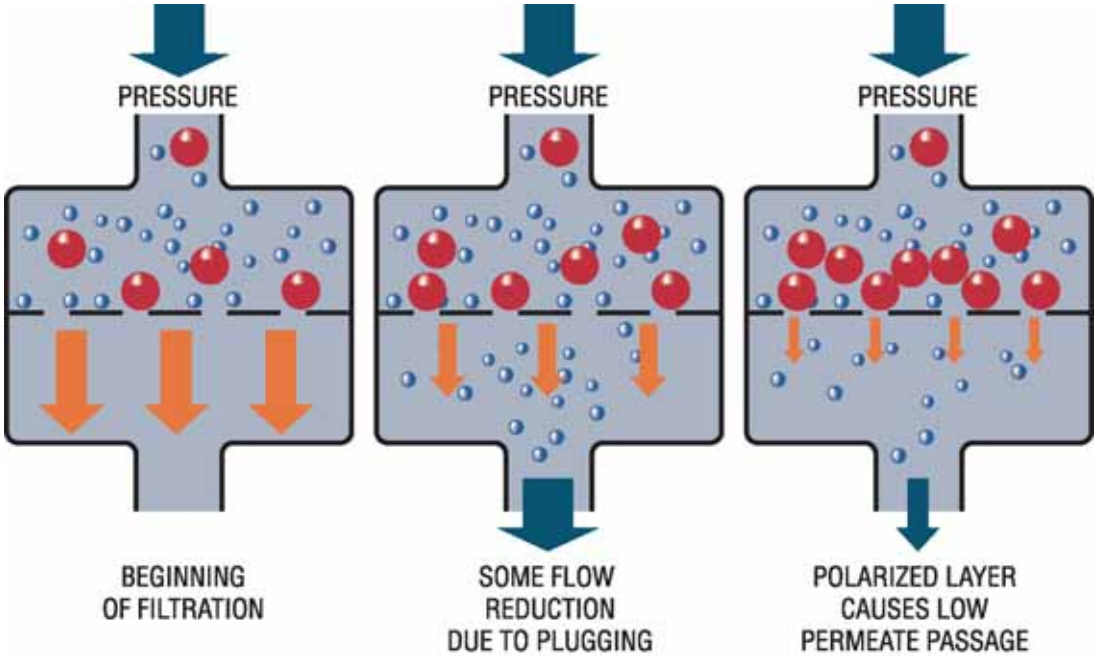


Figure A-42 Build up of polarized layer in dead end filtration mode

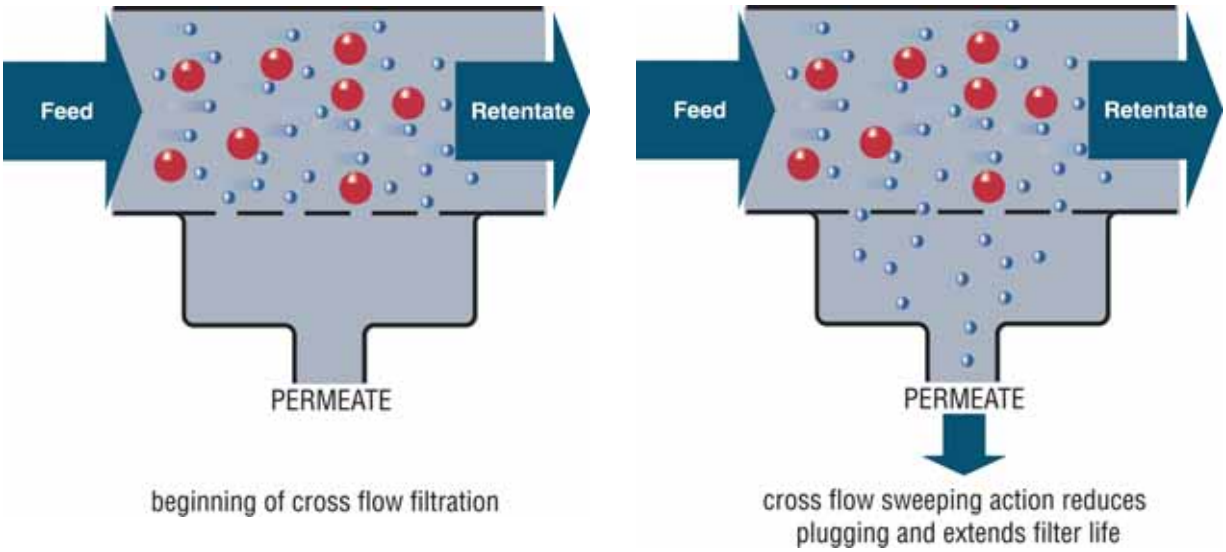


Figure A-43 Cross flow mode reduces concentration polarization and extends filter life

For dead end flow, the Transmembrane Pressure (TMP) may be calculated as follows:

$$TMP = P_{feed} - P_{filtrate}$$

Tangential flow (or cross flow) separations are an efficient way to separate streams that become quickly plugged using dead end techniques. When using tangential flow techniques, most of the process fluid flows along the membrane surface rather than passing through the membrane structure. Fluid is pumped at relatively high velocity parallel to the membrane surface.

In tangential flow, except for water treatment applications, the permeate is only a small percentage of the tangential flow along the membrane surface. In most cell and particle separations, for example, only 1% to 5% of the feed flow becomes permeate. The remaining 95% to 99% exits the membrane device as retentate. Retentate is recirculated back to a feed reservoir or to the module inlet.

Various tangential flow membrane geometries include: stacked plate and spiral devices that utilize flat sheet membranes; tubular devices; and shell and tube devices that use hollow fiber membranes.

In the case of tangential flow separations, the driving force (transmembrane pressure, TMP) is the difference between the average of the membrane feed and retentate pressures, and the permeate pressure as shown below:

$$TMP = \frac{P_{feed} + P_{retentate}}{2} - P_{permeate}$$

Retentate flow results in a build up of components on the membrane inside surface. Generally these components are carried down the length of the membranes and out the end of the module by the sweeping action of the recirculating fluid. However, under certain conditions a cake layer accumulates on the surface of the membrane. This boundary layer is composed of solids and/or solute macromolecules which are retained by the membrane during the course of filtration. This phenomenon, concentration polarization, can affect module performance by reducing the apparent size of the membrane pore. In other words, the cake layer becomes the membrane barrier, a "dynamic membrane".

The extent of caking is influenced by such fluid variables as the degree of solvation, concentration and nature of the solids and solutes, fluid temperature and operating variables such as solution velocity along the membrane and transmembrane pressure. Controlling this phenomenon is key to optimizing flux and solute passage.

Caking can usually be controlled by ensuring adequate solution velocity at the liquid-membrane wall. Solution velocity, controlled by the pumping rate, varies according to application. Generally it is necessary to test in laboratory scale modules before scaling up to large volumes.

When protein passage through the membrane structure is important, particular attention should be paid to feed (or recirculation rate). In general, high feed rates allow more efficient protein passage. Depending on the characteristics of the retained components (cells, particles, cell debris), a caking layer can form on the membrane wall that is actually tighter than the membrane pores. In these instances, high recirculation rates and low transmembrane pressures often help.

Other variables such as viscosity or shear sensitivity of the solution are also important considerations.

Tangential (Cross Flow) Configurations

Several physical configurations are used for cross flow fluid management, including flat media in an appropriate holder, plate and frame (also called "stacked or stacked plate"), tubular modules, spiral wound modules and hollow fiber modules. Spiral wound modules are the most widely used configuration because of their widespread use in reverse osmosis, electronic and pharmaceutical water applications.

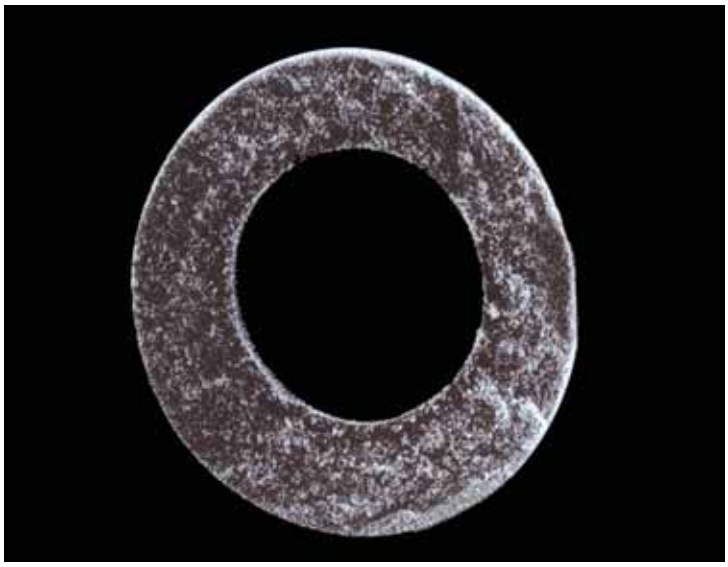


Figure A-44 Micrograph of Spectrum mixed ester hollow fiber membrane

However, the packing density, efficiency, easy scalability, disposability and cost savings available with hollow fiber modules are gradually making them the preferred configuration.

Hollow fibers are manufactured by spinning or casting membrane material into fine fibers, having diameters of 80-1000 µm with a wall thickness of approximately 30-200 µm (see figures A-44 and A-45.

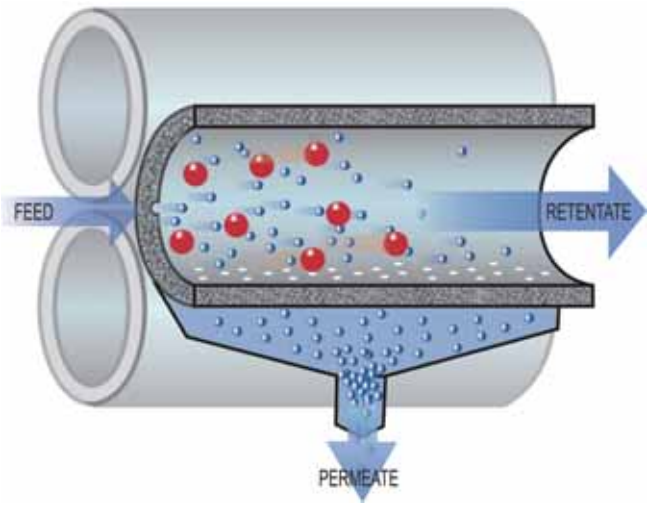


Figure A-45 Schematic of hollow fiber membrane

Figure A-46 Schematic of a typical tangential (cross flow) hollow fiber module

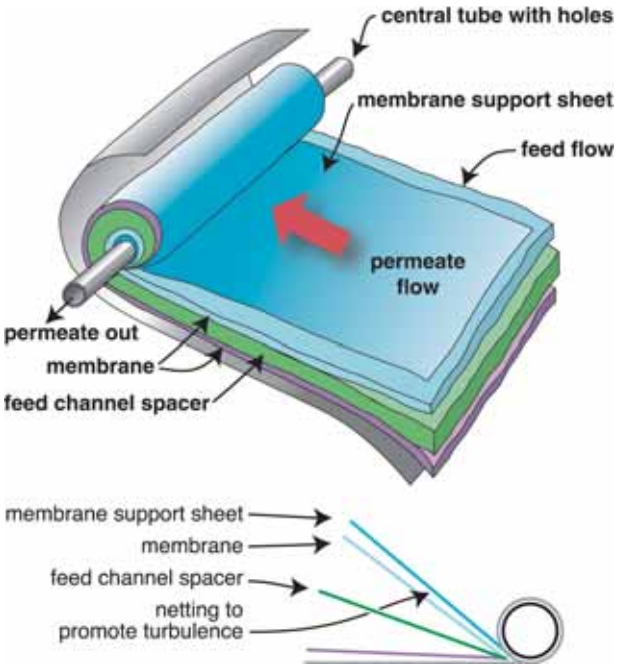


Figure A-47 Schematic of spiral wound cross flow module

Most hollow fibers are asymmetric in structure. The active surface (skin layer) of the membrane in contact with the feed stream is a high density skin cast onto a highly porous substructure. The substructure acts only as support for the thin active layer and generally has no effect on the filtration characteristics of the membrane. Since the actual discriminating barrier is extremely thin, very high flux rates can easily be achieved. Due to the highly asymmetric structure, all rejected materials are retained on the membrane surface and internal pore fouling is virtually eliminated.

As shown in figures A-45 and A-46, pressurized fluid flows into the centers (or lumen) of the hollow fibers, then the permeate flows through the walls of the hollow fibers and is collected in the housing. The permeate then flows out of the housing.

In the manufacture of hollow fiber modules, the two ends of a large bundle of fibers are potted into headers. The headers are sawed off to expose the centers of the fibers and the bundle is cast into an outer housing forming a module.

The hollow fiber module configuration permits a very large amount of membrane surface area to be packed into a small device.

In addition, hollow fiber modules have the advantages of ease of handling, direct scalability from lab to process applications, and easy cleaning. Spectrum hollow fiber modules are unique in that they combine disposability with high quality and exceptional performance. This eliminates the need for cleaning, retesting and revalidation of the hollow fiber modules.

A schematic of a typical spiral wound module is shown in figure A-47.

In this configuration alternate layers of filter media and separator material are rolled into a spiral configuration and the

ends of the spiral “pack” are potted to form the proper flow channels.

Parameters	Hollow Fiber	Flat Sheets
Geometry	narrow tubes	broad sheets
Cleaning	disposable	difficult
Module Configuration	bundled	stacked or spiral wound
Relative Membrane Surface area	large	small
Relative Module Size	small	large
Flow Path	uniform	non-uniform
Scalability	direct	difficult
Relative Re-usability	not necessary	low
Relative Material Cost	low	high

Table A-2 Comparison of tangential (cross flow) configurations, hollow fiber vs. flat sheets

Tangential Flow Membrane Technology using Hollow Fibers

Spectrum hollow fiber tangential flow membrane technology spans both microfiltration (MF) and ultrafiltration (UF) separations. Membrane technology for MF separations include retention ratings of 0.5 µm, 0.2 µm, 0.1 µm and 0.05 µm. UF technology includes membranes with MWCO ratings of 1,000 kD, 400 kD, 50 kD and 10 kD.

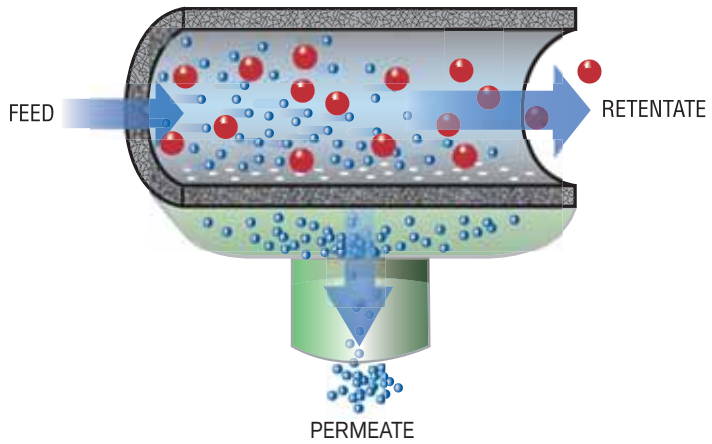


Figure A-48 Schematic of operation of a single hollow fiber

The hollow fibers are cast from either polysulfone (PS), mixed cellulose ester (ME), polyethersulfone (PES) or polyvinyl difluoride (PVDF) with fiber diameters of 0.5 mm and 1 mm. From these uniform hollow fibers, as shown in figure A-48, filtration modules are constructed into several configurations with effective membrane surface areas ranging from 5 cm^2 up to 3.3 m^2.

Spectrum Hollow Fiber Membrane Modules

Spectrum membrane modules utilize advanced hollow fiber flow geometry in which every fluid path is identical in length and dimension. Uniform flow distribution makes it practical to process difficult streams such as those containing suspended solids, fibers, particles and cells.

Process liquid flows inside the fibers under pressure and is continuously recirculated to take full advantage of the benefits of tangential flow. The retained solutes (retentate) are progressively concentrated unless additional liquid is added as in the case of diafiltration. Solvent and small solutes (permeate) flow through the hollow fiber pores and exit to the filtrate container.

All Spectrum membrane modules are assembled in a certified clean room environment under Good Manufacturing Practices and ISO 9001 standards. They feature low bioburden, meet all USP Class VI standards for biocompatibility and are non-pyrogenic by LAL test.

Spectrum tangential (cross flow) membrane modules differ from traditional tangential flow membrane devices in that they are supplied non-pyrogenic, ready for use and are priced to be disposable. The initial investment is low compared to competitive devices that must be repeatedly cleaned, retested and reused to be cost effective.

This new approach closely fits industry standards. Single use disposable pleated cartridges have been the industry standard for dead end membrane devices for more than thirty years.

Advantages of Single Use Disposable Membrane Modules:

1. Modules do not need to be cleaned subsequent to use.

2. Cleaning chemicals do not have to be rinsed from the modules after cleaning. For parenterals, expensive pyrogen free water for injection must be supplied for rinsing non-disposable modules.

3. Module cleaning does not need to be documented.

4. Tests for effective removal (rinsing) of cleaning chemicals are not required.

5. Process development is simplified. Repeated and exhaustive cleaning and rinsing studies are not required to guarantee a successful scale up.

6. It is not necessary to preserve or store modules between processed batches.

7. Consistent initial performance and no loss of performance with repeated use.

Scalability of Hollow Fiber Filtration

Spectrum Laboratories hollow fiber filtration systems cover a complete range of processed liquid volumes from hand pumped syringe disposable sampler products with liquid volumes as low as 2 mL to pilot and production scale systems to handle liquid volumes greater than 10,000 liters (2,580 gallons). This range allows the user complete scalability since the data that are taken with research and development systems testing is directly applicable to production volume levels.

Consideration of the expense and time span involved in bringing new biopharmaceutical products to market emphasizes the need to readily scale up from research liquid volumes, through prototype liquid volumes to process liquid volumes. Further, it is important to have as much stability as is possible at all stages of new product introduction. An important part of this stability is in the filtration steps involved.

One of the greatest advantages of hollow fiber filtration over other filtration configurations is that hollow fiber filtration is directly scalable as long as the fiber path length is held constant. A steady state flux rate (mL/min/cm^2) is first determined on a smaller, more manageable and less expensive scale. Then, by holding these same operating parameters constant and scaling up only the membrane surface area and recirculation rate, larger scale volumes can be processed in the same

amount of time as the small scale operation.

There are three ways to increase surface area for hollow fiber filtration.

1) Increase the length of the fibers

2) Increase the number of fibers

3) Increase both the length and the number of fibers

For a direct scale-up, the number of fibers are increased. If the user is scaling up by increasing the fiber length, then the same recirculation rate must be used which will result in different flux rates.

Figure A-49 illustrates the scalability of the range of Spectrum systems and their corresponding range of liquid volumes.

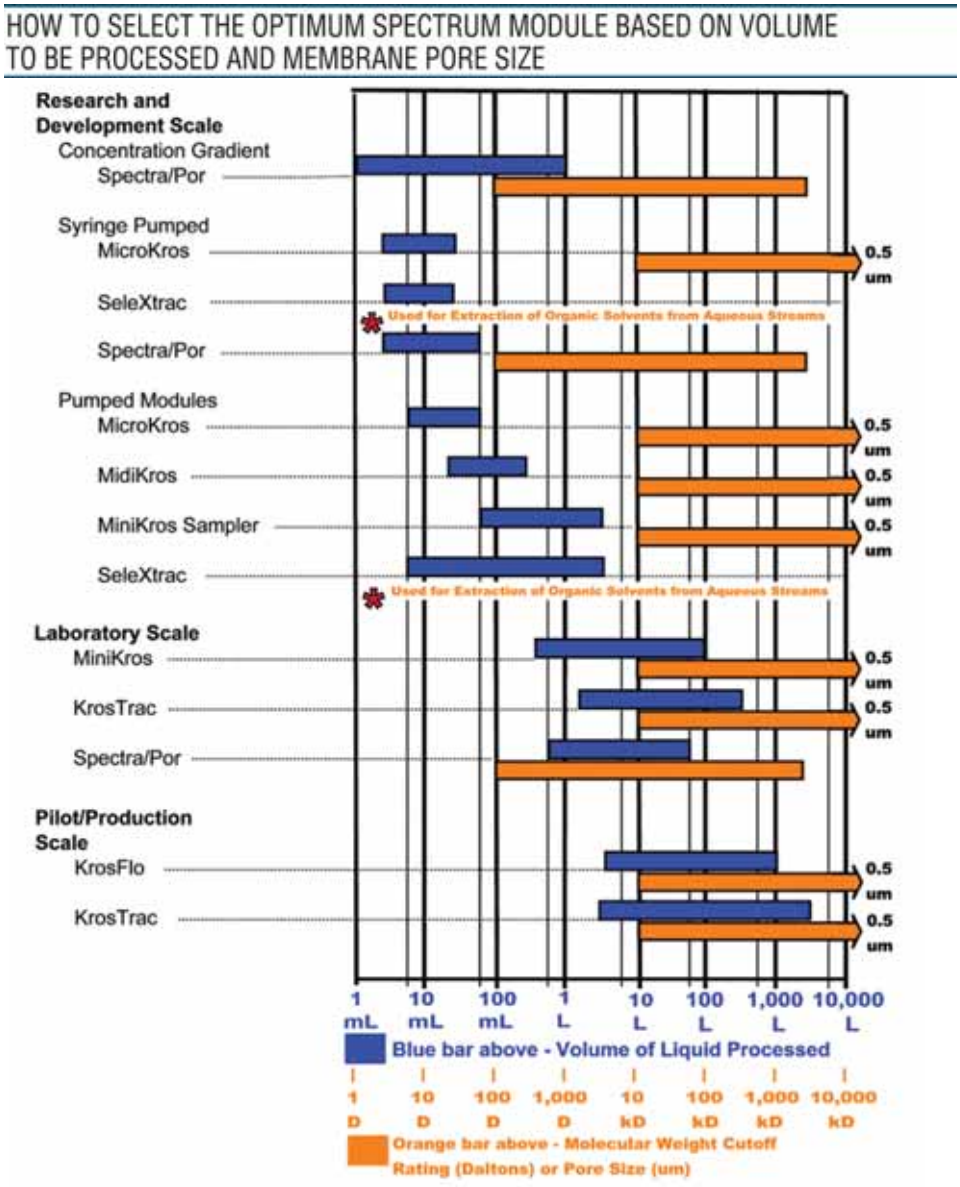


Figure A-49 Scalability of Spectrum hollow fiber membrane filtration systems

MicroKros® Hollow Fiber Membrane Modules

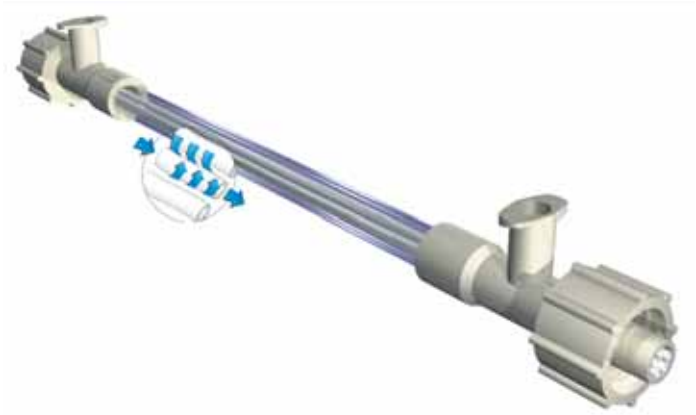


Figure A-50 MicroKros® Modules with process volumes from 2 to 50 ml

MicroKros® Modules
Process Volumes: 2 - 50 ml
Diameter: 3.5 mm
Total Length: 102 mm to 232 mm

Figure A-51 Schematic of MicroKros® module

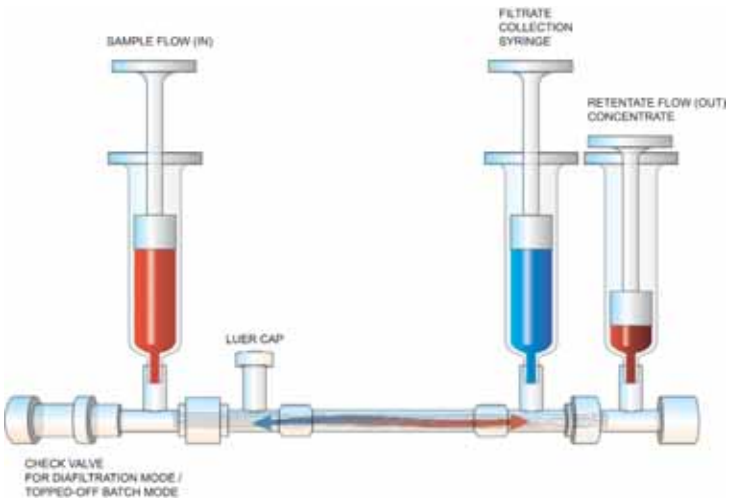


Figure A-52 Schematic of MicroKros® syringe system

MODULES: Mixed cellulose ester (0.1 µm, 0.2 µm); polyethersulfone (0.1 um, 0.2 µm, 0.5 µm) and low protein binding polysulfone (10 kD, 50 kD, 400 kD, 0.05 µm) all in polysulfone housings

LIQUID VOLUMES: 2 mL - 50 mL (syringe); 20 mL – 200 mL (pumped)

Designed for separations of extremely small volumes, MicroKros® disposable modules are the first practical tangential flow devices suitable for processing volumes as small as 2 mL. (Figures A-50, A-51 and A-52).

They are an ideal alternative to centrifugation for applications where pellet formation is undesirable. Flow can be supplied using either syringes or a peristaltic pump.

MicroKros® modules provide highly advanced hollow fiber membranes in a polysulfone housing. They are autoclavable and, depending on model, have a total membrane surface area ranging from 5 to 20 cm^2.

Due to their low cost and reproducibility they are directly scalable to pilot plant or production scale liquid volumes.

Applications of the MicroKros® membrane system include the following:

1. Diafiltering (washing) uniform latex particles and other diagnostic particles

2. Clarifying lysates to remove cell debris from soluble proteins

3. Washing and concentrating protein precipitates

4. Recovering serum from whole blood

5. Media studies for hollow fiber bioreactors

6. Protein concentration and washing

MidiKros® Hollow Fiber Membrane Modules

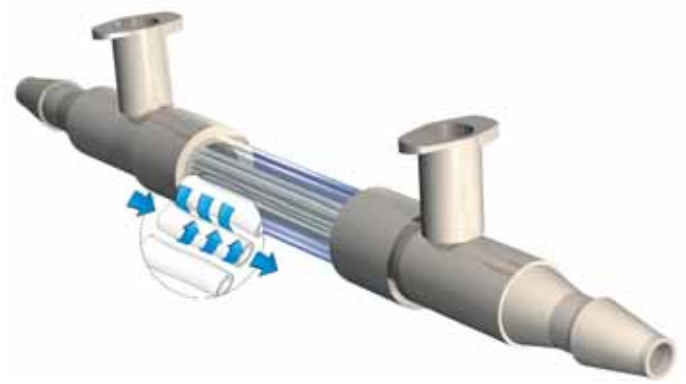


Figure A-53 MidiKros® module

MidiKros® Modules
Process Volumes: 20 to 200 ml
Diameter: 7.8 mm Total Length: 132 mm to 258 mm

Figure A-53A Schematic of MidiKros® module

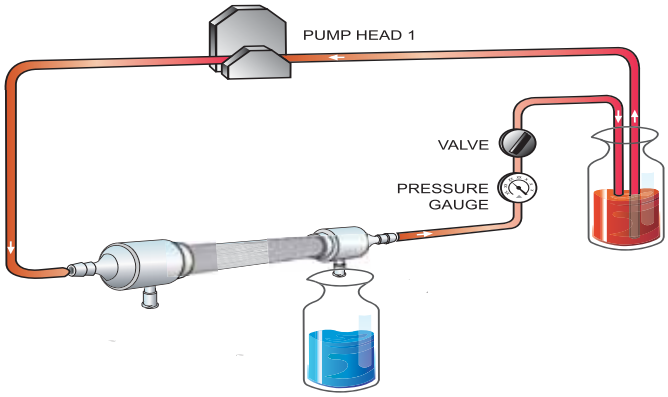


Figure A-54 Schematic of MidiKros® system set up

MODULES: Mixed cellulose ester (0.1 µm, 0.2 µm); polyethersulfone (0.1 um, 0.2 µm, 0.5 µm) and low protein binding polysulfone (10 kD, 50 kD, 400 kD, 0.05 µm) all in polysulfone housings

LIQUID VOLUMES: 20 mL – 200 mL

Designed for separations of small volumes, MidiKros® disposable modules are practical tangential flow devices suitable for processing volumes ranging from 20 mL to 200 mL. (See figures A-53 and A-54).

These modules are an ideal alternative to centrifugation for applications where pellet formation is undesirable. Flow is supplied using a peristaltic pump.

MidiKros® modules are hollow fiber membranes encased in a polysulfone housing. They are autoclavable with a total membrane surface area of 20 to 145 cm^2, depending on model.

Due to their low cost and reproducibility they are directly scalable to pilot plant or production scale liquid volumes.

Applications for the MidiKros® module are similar to those listed for the MicroKros® module above.

Volume Scalability from MicroKros® and MidiKros® Modules to MiniKros® Sampler Module, MiniKros® Module and KrosFlo® Module

A direct scale up of MicroKros® and MidiKros® systems is achieved with the MiniKros® and KrosFlo® modules and systems on pages 40 and 41.

Spectrum KrosFlo® Steam-In-Place (SIP) hollow fiber modules are a feasible alternative to expensive, reusable SIP filters. They can be sterilized by steam (maximum 142°C) or they can be autoclaved. Since their low cost allows modules to be discarded after use, they have numerous advantages when compared to reusable modules.

Reusable membrane modules require difficult and expensive chemical cleaning, storage and revalidation after each use.

MiniKros® Sampler Module
Process Volume: 50 ml to 4 liters
Diameter: 18.8 mm
Total Length: 185 mm

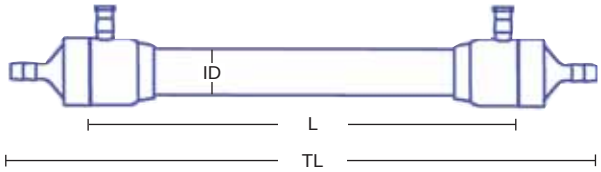


Figure A-55 MiniKros® Sampler Module

MiniKros® Module
Process Volume: 300 ml to 100 liters
Diameter: 18.8 to 31.2 mm
Total Length: 276 mm to 510 mm

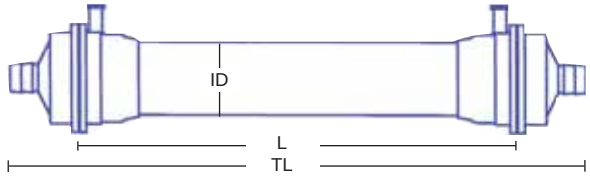


Figure A-56 MiniKros® Module

KrosFlo® Module
Process Volume: 3 to 1000 liters
Diameter: 70 mm
Total Length: 229 to 691 mm

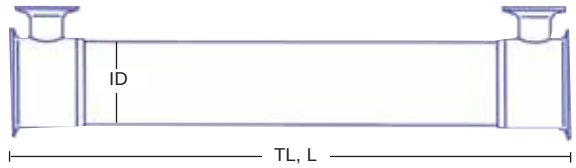


Figure A-57A KrosFlo® Module

KrosFlo® Plus Module
Process Volume: 3 to 1000 liters
Diameter: 70 mm
Total Length: 298 to 760 mm

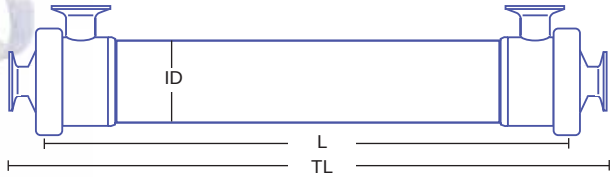


Figure A-57B KrosFlo® Plus Module

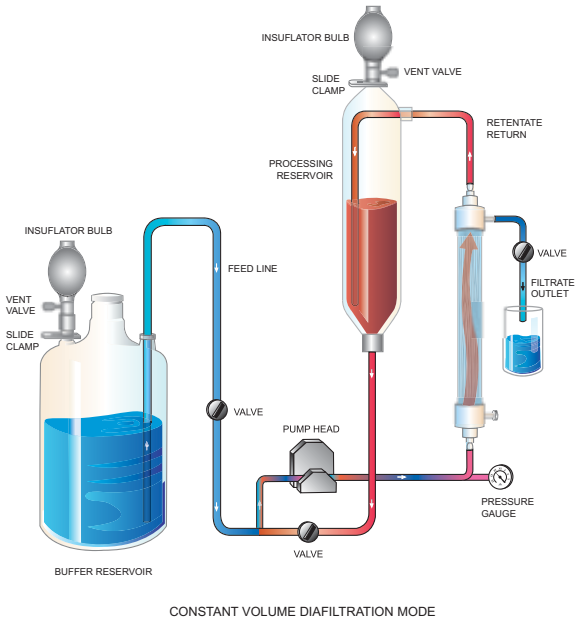


Figure A-58 MiniKros® Lab System with reservoir used in a constant volume diafiltration mode

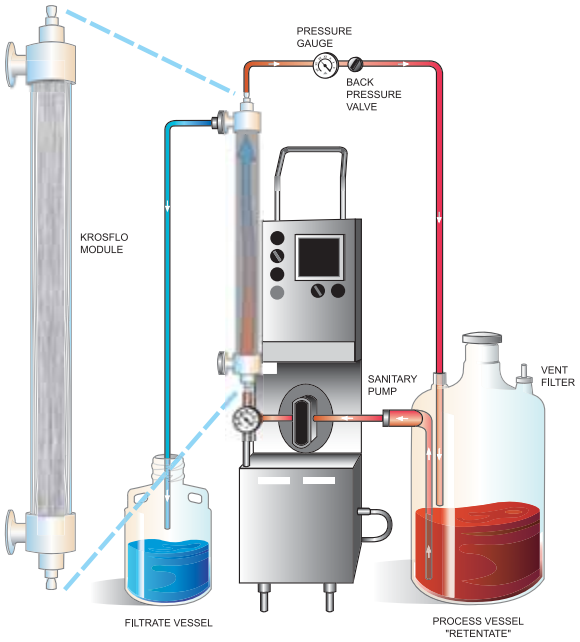


Figure A-59 KrosFlo® large volume pilot system in a batch concentration mode

Figure A-60 KrosFlo® SIP Module

Disposable SIP modules make validation easier, require no cleaning or validation of cleaning procedures, do not require special storage or storage and retesting of reprocessed modules, eliminate potential product contamination associated with module reuse and are not affected by the diminishing filtration performance that is characteristic of reusable membrane modules.

KrosFlo® SIP hollow fiber modules fit into most standard new or installed stainless steel pressure housings. They are manufactured with hydrophilic, 0.2 and 0.5 µm polyethersulfone (PES) hollow fibers potted in biocompatible epoxy resin. The modules are available with 0.8 and 1.8 m^2 total membrane surface areas. O-rings assure leak-free inlet and outlet seals.

Sterilization of Spectrum Membrane Modules

For aseptic operation, Spectrum membrane modules may be sterilized as follows:

AUTOCLAVING - Autoclave at 121° C for 30 minutes. Do not expose to temperatures above 124°C.

IRRADIATION - Dry modules may be exposed to 2.5 megarads of gamma radiation or electron beam radiation.

ETHYLENE OXIDE - Dry modules may be sterilized by ethylene oxide.

CHEMICAL - Modules may be chemically treated using 1500 ppm of peracetic

acid (Minntech Corporation), 1500 ppm of sodium hypochloride (bleach) or glutaraldehyde solution. Follow supplier recommendation for each of these chemical sterilants.

Modes of Operation of Hollow Fiber Filtration Systems

Most applications of crossflow microfiltration and ultrafiltration can be grouped into five major operational modes:

BATCH CONCENTRATION of cells, cell debris, particles, precipitates or proteins.

BATCH CLARIFICATION of solutions containing cells, cell debris, particles, precipitates or proteins.

TOPPED OFF BATCH for reducing larger process volumes to smaller manageable volumes for concentration or further process work.

CONSTANT VOLUME DIAFILTRATION (washing) of cells, cell debris, particles, precipitates or proteins.

DEAD END FILTRATION to recover extra product following clarification.

Batch Concentration

Generally, the term "concentrate" is applied to applications where materials being retained by the filter are (or contains) the desired product. As the process fluid circulates through the filter, there is a loss of volume in the process vessel due to the removal of filtrate. As a result, product is concentrated in the process vessel. This mode of operation is used, for example, in fermentation recoveries where the desired product is the cell itself or as an initial process step where the product is intracellular.

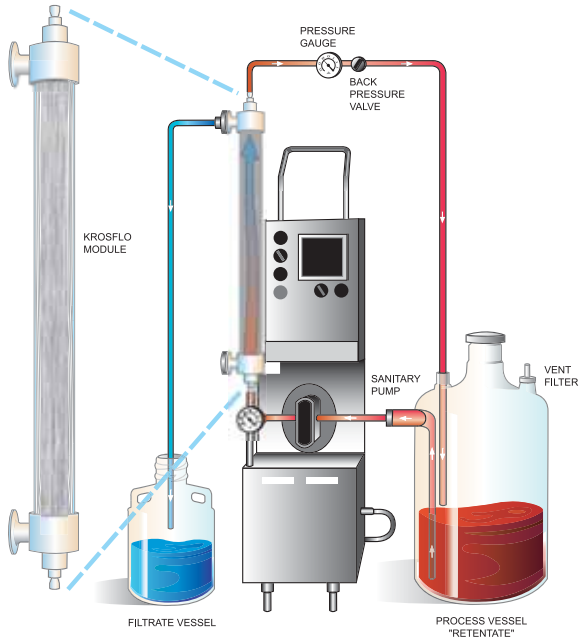


Figure A-61 KrosFlo® Pilot System used in a batch concentration mode

Batch Clarification

The term clarification is generally used for applications where the desired product is in the filtrate such as soluble proteins. This mode of operation is used, for example, to harvest animal or bacterial cell cultures where the desired product is secreted by the cells or microbial fermentation where the desired product has been released into solution by cell lysis.

When operated in concentration or clarification modes, the membrane quantitatively removes materials larger than the pores of the filter and allows the passage of soluble materials that are smaller than the membrane pores.

The system set-up is the same for both batch concentration and batch clarification filtrations.

As filtrate is removed, the solution in the process vessel becomes more concentrated in the components that the membrane retains. In addition, the volume is reduced. The degree of concentration, called the concentration factor (CF) or volume reduction factor (VRF), is given by the following equation where V_i is the initial volume and V_f is the final volume:

$$\textbf{Concentration Factor = Volume Reduction Factor =} \quad \frac{V_i}{V_f}$$

Topped off Batch Operation

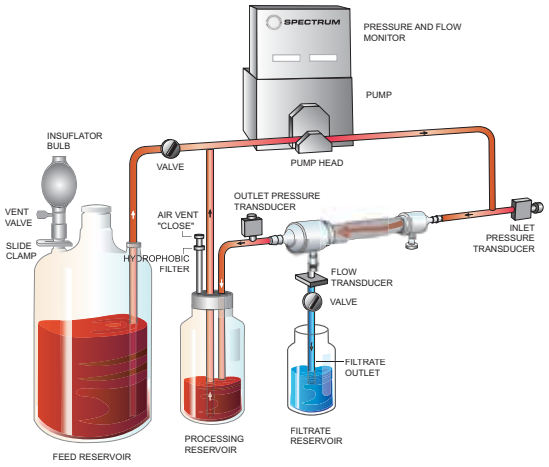


Figure A-62 MiniKros® Sampler System in Topped Off Batch Mode

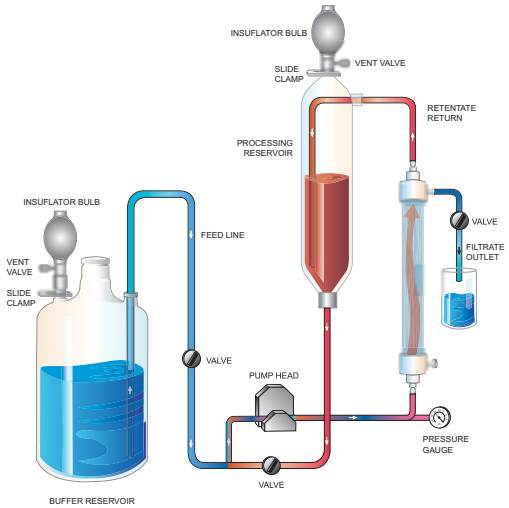


Figure A-63 Constant volume diafiltration mode using MiniKros® Lab System with Reservoir

A disadvantage of batch operations is the relative high flow rate needed (20 to 100 times the filtrate rate) for efficient tangential flow. As a result, it is difficult to achieve high concentration factors without foaming or vortexing in the processing vessel. A way to avoid this problem is to operate the membrane in a "topped-off-batch" mode, where a smaller processing reservoir is used.

Referring to figure A-62, the small processing reservoir is kept full by continually adding feed material at the same rate that filtrate is removed.

Constant Volume Diafiltration

Materials that pass through the membrane can be washed away from materials that are retained by the membrane (cells, particles, etc.). The technique is used to recover additional product in clarification applications, and to achieve better product purity in concentration applications. For best efficiency, the wash buffer should be free of the solute that is being recovered or removed.

Diafiltration may be accomplished either by adding buffer at the same rate as the filtration rate (constant volume diafiltration), or by reducing the volume in the reservoir and re-adding buffer to regain the original volume (intermittent diafiltration). The amount of diafiltration performed can be expressed by the amount of wash buffer added divided by the batch volume, i.e. the number of "wash volumes".

During a constant volume diafiltration where soluble components pass freely through the membrane, each wash volume of filtrate removed reduces the solute concentration by a factor of e (2.718…). For example, a four-fold constant volume diafiltration will reduce the concentration of solute by a factor of e^4,

50 fold or over 98%. Using this technique, the concentration of solute can be monitored in the filtrate until the desired level of purification or product recovery is achieved. The equipment set up is the same as for topped-off batch concentrations or clarifications except that the wash buffer enters the vessel instead of the feed solution.

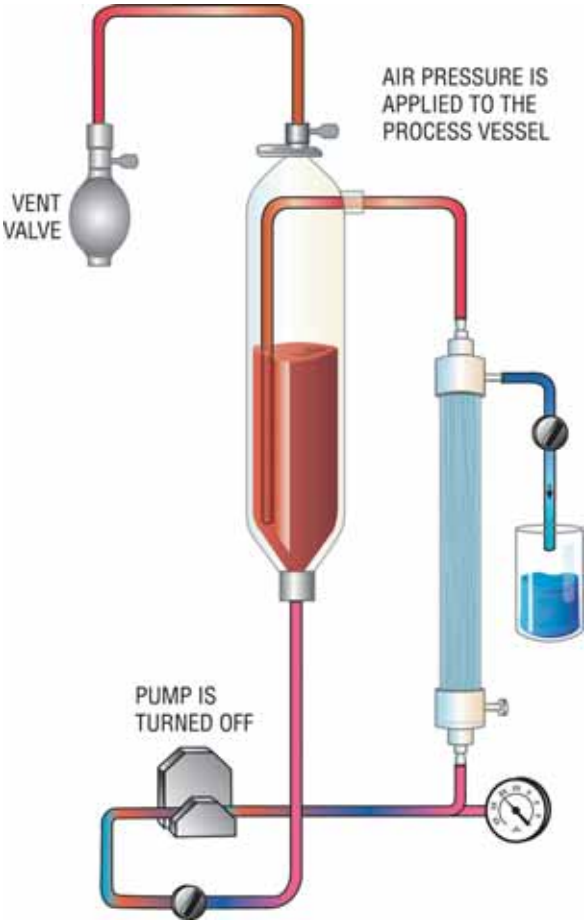


Figure A-64 Dead end operation

Dead End Operation

While the standard mode of operation involves tangential flow, Spectrum cross flow membrane modules can also be operated in the normal (dead end) mode. In this mode, shown in figure A-64, the retentate line is capped or blocked by a valve so that all of the solution being filtered passes through the membrane wall. While the efficiencies of tangential flow filtration are lost, in certain circumstances, such as at the end of a run where maximum filtrate recovery is desired, running dead end is advantageous.

Conventional tangential flow membranes cannot economically be used in dead end flow. Their high membrane costs require that they be cleaned and re-used. Dead end techniques are avoided because they interfere with membrane cleaning.

Spectrum disposable membranes, however, lend themselves to this mode of product recovery. Since the membrane investment is substantially lower than for reusable modules, cleaning a Spectrum module subsequent to use can be avoided and dead end techniques are economically feasible.

Extending Module Throughput by Forward, Reverse and BackFlushing

Forward Flushing

Shutting the permeate valve/clamp while recirculating sets up a backflushing condition in the downstream half of the module as shown in figure A-65.

The permeate pressure will rise and exceed the retentate pressure in the downstream half of the module. In the downstream region the permeate will flow from

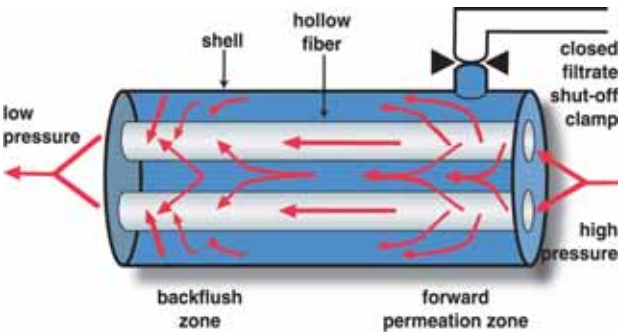


Figure A-65 Forward Flushing of Module

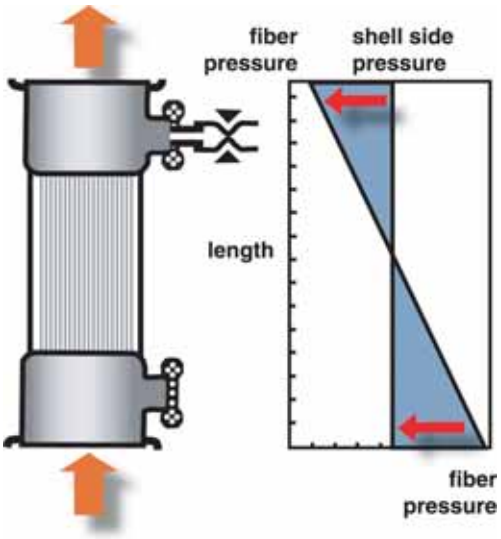


Figure A-66 Forward flushing of module

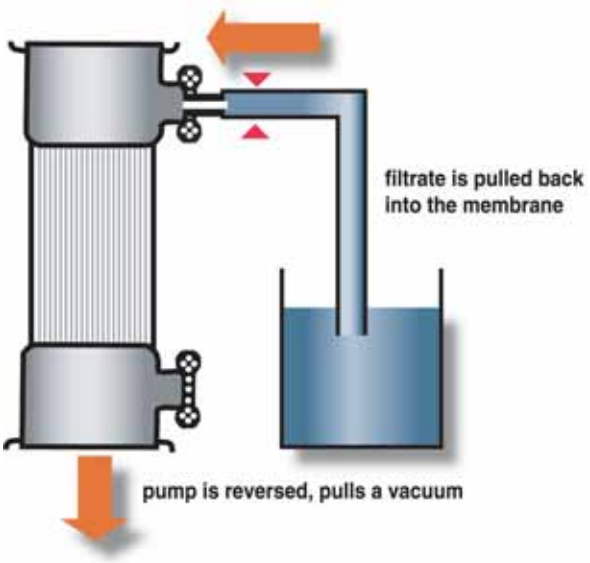


Figure A-67 Back flushing of module

the outside of the membrane to inside. This will loosen and carry away cake material.

The principle of forward flushing is further illustrated in figure A-66. When the permeate is shut-off, the net permeation rate in the module is zero. But permeation still occurs internally. The inlet half of the membrane module (the high pressure end) generates permeate that backflushes the downstream half of the membrane module (the low pressure end).

Normally forward flushing for 30 seconds is sufficient to clean the downstream half of the membranes.

Reverse Flushing

Reversing the pump with the permeate valve/clamp open will serve to backflush the other half of the module. In this case, the hollow fibers are flushed from outside in.

Back Flushing

Back flushing the entire filter (both halves) simultaneously can be achieved by reversing the pump while the permeate valve/clamp is open, as shown in figure A-67. Reversing the pump will create a negative pressure in the lumen side of the fibers drawing in filtrate or rinse buffer from the shell side. This will act to clean the entire length of the fiber internal surface. Note that the volume of the retentate solution will increase. An air vent will be required to allow for this volume increase.

Microfiltration Applications

Most applications of microporous membranes can be grouped into the following broad categories: cold sterilization of fluids; testing for microorganisms; clinical and general laboratory applications; harvesting of animal or bacterial cell cultures, cleaning of particles; clarification of fluids and processing of cells, bacteria and viruses.

Cold Sterilization of Fluids

Membranes with pore sizes of 0.2 µm to 0.45 µm are used to remove microorganisms from pharmaceutical liquids and air, beverages, ultrapure water, air, etc. This type of sterilization requires normal (dead end) flow in most applications since 100% of the organisms must be removed for complete sterility.

Testing for Microorganisms

Membranes with a pore size of 0.45 µm are used to test liquids and gases for the presence and type of bacteria. This was the first application of microporous membranes used widely in Germany during World War II to test drinking water supplies. Membranes with a pore size of 0.65 and 1.2 µm are used to test for yeasts.

Clinical and General Laboratory Applications

Microporous membranes are used in numerous clinical and laboratory applications, such as cytology, cell culture, analysis, chemotaxis, blood studies, virus and particle fractionations, and for general cold sterilization of fluids.

Cleaning of Particles and Clarification of Fluids

Cleaning Uniform Latex Particles to Remove Excess Free Protein

in vitro diagnostic tests using latex microparticles often require that the particles be cleaned before and after an appropriate protein (e.g. albumin, antibodies, antigens or other ligands) is attached to the particle surface. After passive adsorption or covalent binding, excess free protein must be quantitatively removed to ensure reliable test results and optimum sensitivity.

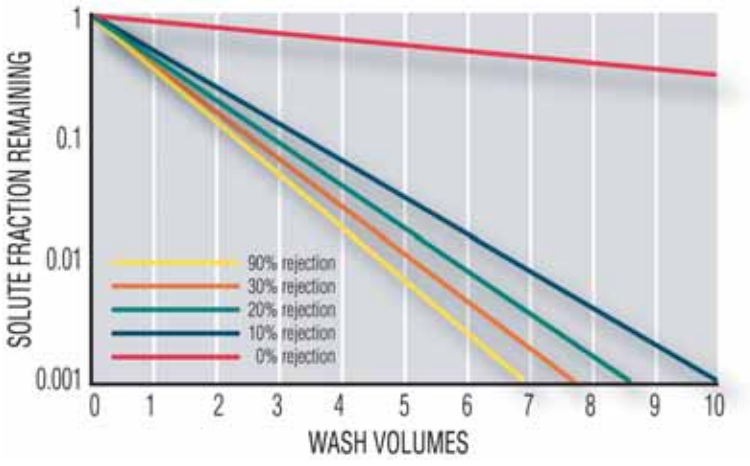


Figure A-68 Effect of Solute Rejection on Constant Volume Diafiltration

Spectrum cross flow membranes are a fast, effective and convenient alternative to ultra-centrifugation for cleaning uniform latex particles. Using tangential flow, latex particle preparations are pumped along the membrane surface as shown in figure A-69. Fluid pressure causes the particle solution to flow through the membrane device tangential to the membrane surface. The membrane retains particles while allowing solvents, unbound proteins and other solutes to permeate the membrane. Concentrated particles that are retained by the membrane flow out of the device

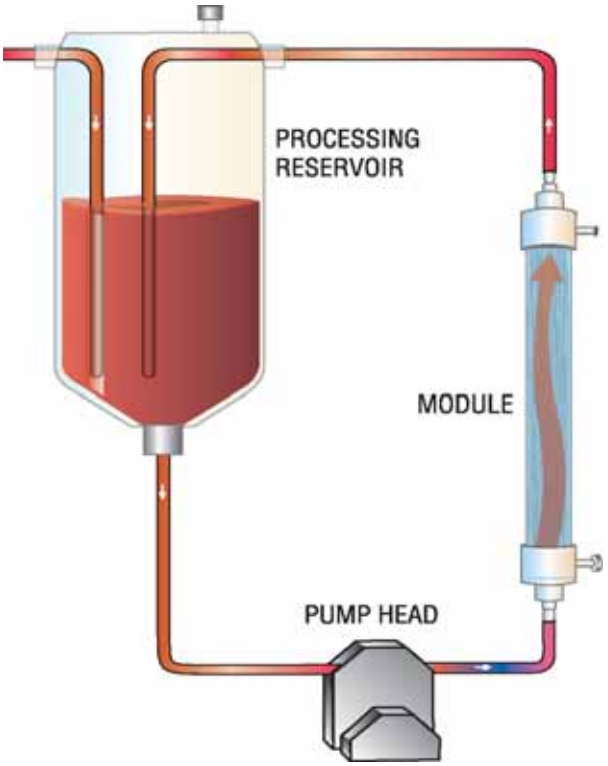


Figure A-69 Typical Module Setup

while particle free filtrate is removed separately. Depending on the specific application, membranes with pore sizes of 0.1 µm, 0.2 µm and 0.5 µm are used.

BATCH CONCENTRATIONS As particle free filtrate is removed, the concentration of latex particles is increased by a factor equal to the starting volume divided by the ending volume.

DIAFILTRATION The processing volume can be maintained by continuously adding buffer solution. Called constant volume diafiltration, this technique is used for buffer exchange of the latex particles and for removal of surfactants or other unwanted soluble components. Using this technique, the concentration of solutes that pass freely through the membrane are reduced by a factor of e (2.718...) for each volume exchanged. The influence of the membrane retention on the solute washing is shown in figure A-68.

Constant volume diafiltration using Spectrum MiniKros® and larger KrosFlo® disposable modules is a fast and effective alternative to multiple centrifugation/resuspension steps. They offer several advantages over filtration devices that must be cleaned repeatedly. These advantages include higher energy efficiency, improved quality and yield of product and lower cost.

Cell, Bacteria and Virus Processing

Recombinant Chinese Hamster Ovary Cell Separation

Many new biopharmaceuticals are being produced by mammalian cells. Hollow fiber microporous filtration is a valuable technology for separation of a protein secreted by mammalian cells.

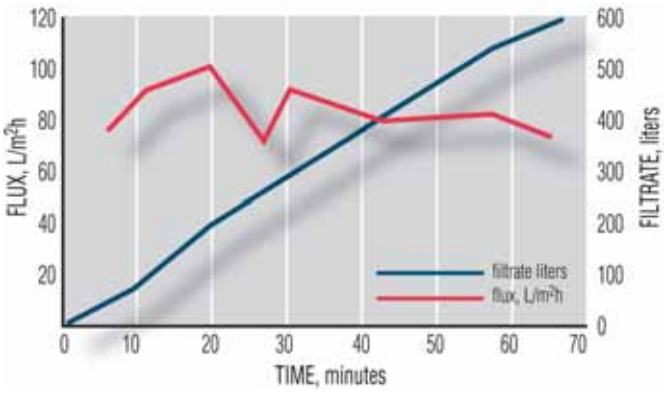


Figure A-70 FIux and Throughput vs. Time for Recombinant Chinese Hamster Ovary Cell Separation

In this example shown in figure A-70, the harvest has an initial concentration of about 2 x 10^5 cells/ml in cell culture media. The extracellular protein product is a 10 kD lymphokine similar to Interleukin-2 and is a potential cancer therapeutic. The result desired is removal of cells and particulates without lysing the remaining cells, while achieving high protein recoveries. Processing time is critical because the product is thermal labile and must be kept below 10°C.

Two parallel Spectrum KrosFlo® modules with a pore size of 0.2 µm and 3.3 m^2 of membrane surface are used. As shown in figure A-71, this allowed for separation of extracellular proteins from 600 liters of recombinant mammalian cells in 65 minutes with 94% product recovery and

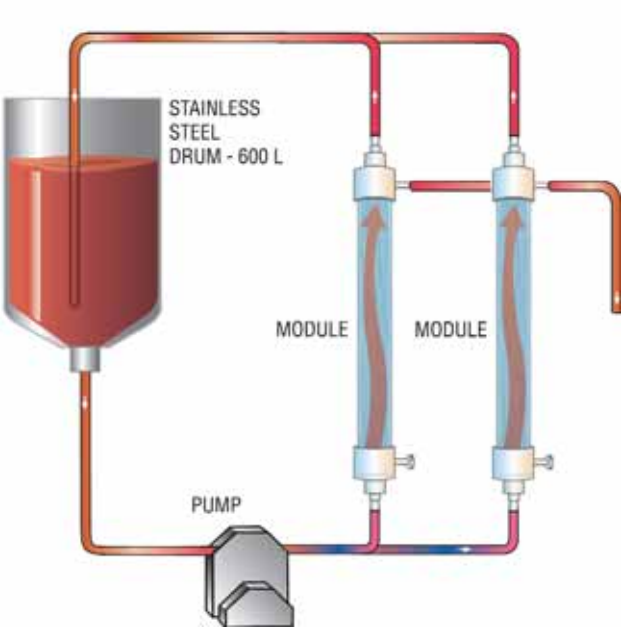


Figure A-71 System setup for Recombinant Chinese hamster ovary Cell Separation

insignificant flux decay.

KrosFlo® cellulosic membranes were used because of their low protein binding capacity. As single use modules, they allow consistent membrane characteristics and eliminate the risk, cost and time associated with cleaning procedures.

Escherichia coli Vaccine Cell Concentration

Fatal infant bovine diarrhea (calf scours) is prevented by an *E. coli* vaccine grown in batch fermentation. Once the culture reaches the desired density, cells are concentrated as the first step toward recovering cell wall-associated antigens.

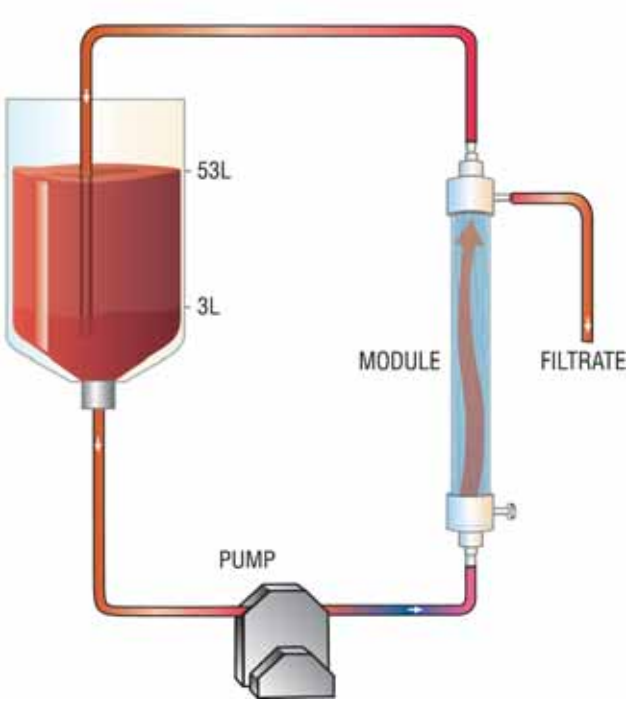


Figure A-72 System Setup for *E. coli* Vaccine Cell Concentration

Selection of the proper filtration module and process conditions are essential for cost effective cell concentration. A fast processing time with minimal membrane fouling is the key consideration.

This concentration process is often done by continuous centrifugation. Centrifuges have certain disadvantages such as lengthy cleaning cycles, mechanical complexity and significant product losses in the supernatant. In addition, unless special containment features are incorporated, aerosol creation can be hazardous to operators (especially when processing pathogens).

As shown in figures A-72 and A-73, a 0.2 µm rated KrosFlo module with 2.1 m^2 of membrane surface area was used. This setup concentrated 53 liters (with initially 1.8 x 10^9 cells/mL) to just over 3 liters in only 24 minutes. This represented a 17-fold volume reduction at a steady state flux of 58 L/m^2/hr.

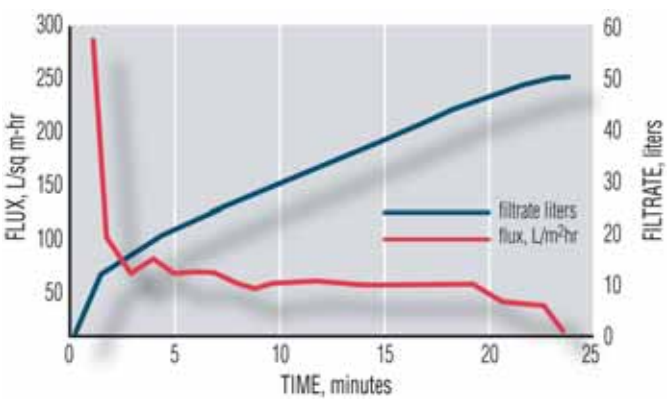


Figure A-73 Flux and Throughput vs. Time for *E. coli* Vaccine Cell Concentration

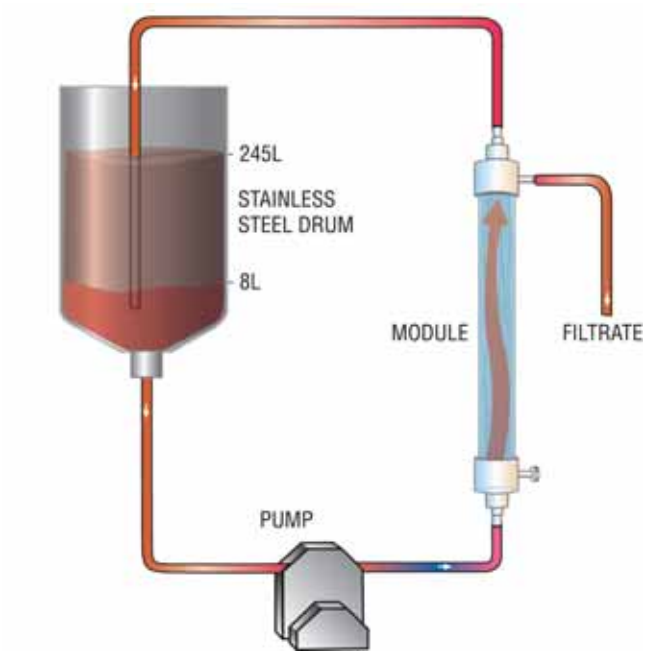


Figure A-74 System Setup for *E. coli* Lysate Cell Debris Removal

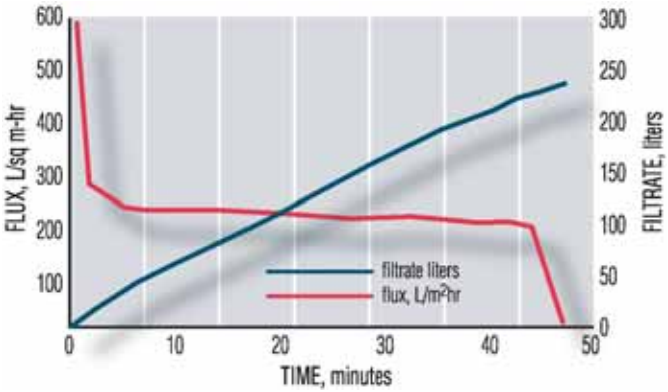


Figure A-75 Flux and Filtrate Flow vs. Time for *E. coli* Lysate Cell Debris Removal

Single-use Spectrum KrosFlo® modules provide consistent and economical run-to-run performance without risk, time loss and expensive cleaning procedures.

Escherichia coli Lysate, Cell Debris Removal

A genetically engineered *Escherichia coli* was fermented, mechanically lysed and the lysate was processed to recover an intracellular protein, an interferon with cancer treatment potential. Choice of the proper filtration module and process conditions are essential for cost-effective separation of the component of interest from cell debris.

As shown in figure A-74, a single 0.2 µm Spectrum KrosFlo module containing 1.0 m^2 of membrane surface was used. With an initial batch of 245 liters of lysate, the system purified 237 liters of filtrate in 65 minutes, figure A-75. The result was a 30-fold volume reduction and an equilibrium flux of 200 L/m^2/hr. Passage of the desired protein was 99%.

Spectrum KrosFlo® filtration modules are an effective and economical means of separating soluble proteins from cell debris subsequent to lysis.

Single use modules offer several advantages over multiple use filters. Starting each batch with a disposable non-pyrogenic filter eliminates concerns about inadequate cleaning and rinsing of re-used filters. The filter is no longer a possible source of batch-to-batch contamination. Finally, consistent high performance is assured during each run.

Cell Recycle Perfusion of Animal Cell Bioreactors with CellFlo® Technology

Figure A-76 illustrates the CellFlo® technology using hollow fiber membranes arranged in a shell and tube configuration for increased bioreactor output. In this application, designed to separate animal cells from spent media, cells are gently pumped through the lumen of the hollow fiber membrane tubes. Secreted proteins permeate the membrane structure and enter the shell side of the module. Using this technique, spent media is removed (along with secreted proteins and unwanted metabolites) while fresh media is continuously added to maintain bioreactor volume. CellFlo® technology allows control of the extracellular environment to achieve optimal conditions for product secretion and culture viability.

Using CellFlo® perfusion, ten-fold daily production increases have been achieved. Other major benefits of perfusion culture include:

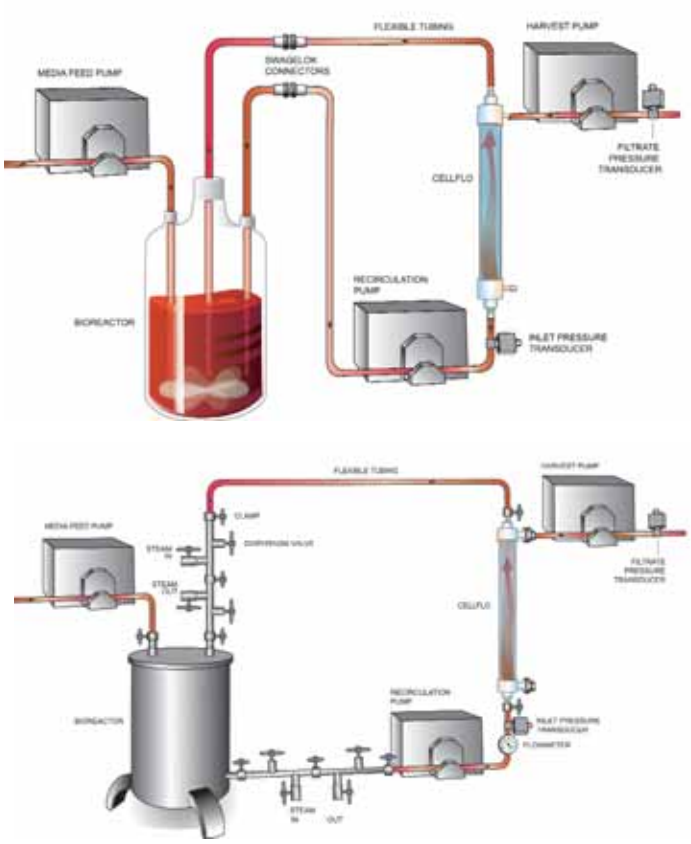


Figure A-76 Typical Perfusion Setup. Arrows indicate the direction of solution flow

1. Increased total output
2. Less media usage per gram of product recovered
3. Greater cell density
4. Simplified downstream purification
5. Longer useful culture life
6. Reduced in-reactor product degradation

Spectrum CellFlo® modules are manufactured from non-cytoxic USP XXI class VI materials. Large diameter hollow fiber flow channels minimize plugging due to cell agglomeration.

They are non-pyrogenic by LAL testing.

CellFlo® modules can be sterilized by autoclaving and are priced to be disposable. Single use disposability eliminates the costs and risks associated with cleaning and rinsing, simplifies validation and ensures consistent optimum performance and protein passage.

CellFlo® technology is included in greater detail in a later chapter of this book.

Ultrafiltration Applications

Most applications for UF may be grouped under three principal categories:

Concentration of a retained molecular species by the removal of a solvent through the membrane.

Desalting or buffer exchange by passing microsolutes through the membrane.

Fractionation or clarification by retaining larger species and passing smaller species through the membrane.

Concentration

Concentration will reduce large solvent volumes during isolation and purification procedures, and/or will improve the ability to detect or analyze small quantities of dissolved molecules. Ultrafiltration is often used for this purpose, replacing older techniques such as precipitation, evaporation, pervaporation, dialysis and gel filtration.

Figure A-77 shows a schematic diagram of a typical laboratory configuration for concentration.

UF offers these advantages:

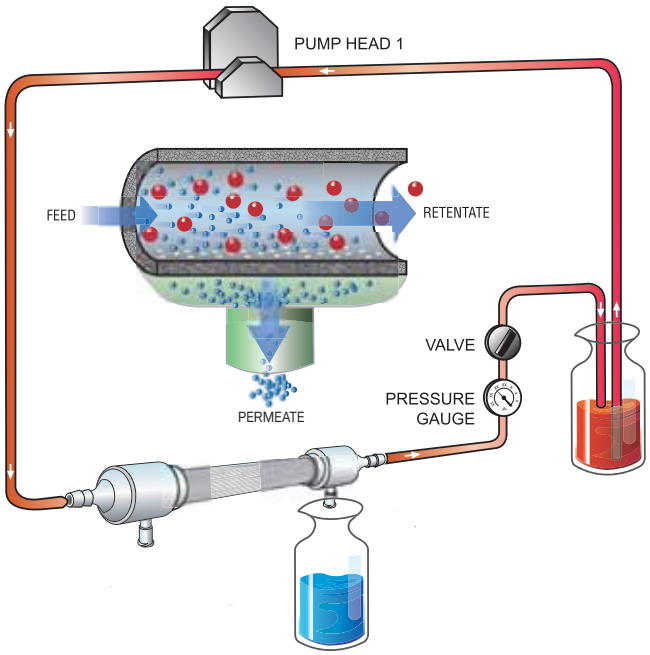


Figure A-77 Schematic diagram of typical laboratory configuration for concentration

Faster than dialysis.

Faster and less expensive than freeze-drying.

Minimizes denaturation of molecules compared to precipitation.

Produces higher yields than concentration by dialysis.

Faster than pervaporation and does not require prior dialysis.

Does not cause hyperconcentration of salts (maintains ionic strength).

For larger scale process applications, UF is rapid, simple, and relatively inexpensive as compared to the older techniques listed above.

Examples of the use of UF for concentration include:

Concentrate and/or desalt peptides and small molecules (MWCO 10kD)

Concentrate and/or desalt proteins, enzymes and viruses (MWCO 50kD)

Concentrate and diafilter albumin (MW~67,000) (MWCO 50kD)

Concentrate monoclonal antibodies and other macromolecules (MWCO 50kD or 400kD)

Concentrations in the Clinical Laboratory

CEREBROSPINAL FLUID. The concentration of CSF by UF makes possible definitive electrophoresis patterns for diagnosis of proteins in the concentrate.

URINE. High protein levels in urine indicate an abnormality that can often be detected by the analysis of the proteins. In most cases, protein concentrations in urine are too low for either electrophoresis or immunoelectrophoresis. Therefore, prior concentration of these proteins is essential.

SERUM. A membrane with 100,000 MWCO retains the antigen associated with serum hepatitis (HAA). Concentration of the HAA by the membrane will greatly increase the test sensitivity, but will not interfere with the specificity of the test.

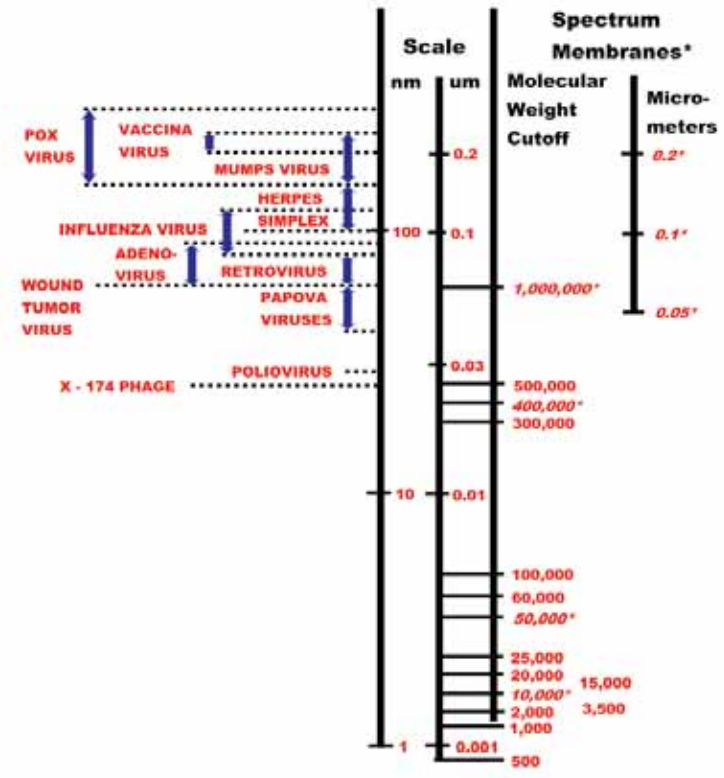


Figure A-78 Comparison of typical virus sizes with Spectrum membranes retention ratings

Virus Concentration and Purification

In medical research and in the manufacture of vaccines, UF has been used to remove viruses from process streams and to concentrate viruses and viral fragments from crude cell lysates as well as from partly purified solutions. In addition UF has been used to remove salts and other low molecular weight contaminants from viral preparations which have previously been purified by other methods. UF has found this broad application in the concentration and purification of viruses because of its ease of use, its rapidity, and its gentle treatment of viral particles.

Concentration, Purification and Fractionation of Blood

Bovine and fetal bovine serum are widely used in research and in the manufacture of pharmaceuticals. The first step is plasmaphoresis, or the removal of cells from the blood by MF membrane separation. Then, it is desirable to concentrate the serum albumin by "dewatering" the

serum (generally with a membrane with MWCO of 30,000-50,000) and then purifying by diafiltration or other UF desalting procedures in the same UF cell. UF is ideal for accomplishing any of these procedures since UF does not denature the proteins, and it is also possible to purify each serum fraction by diafiltration.

Desalting and Buffer Exchange

There is often a need to purify solutions containing macromolecules either as an additional step to concentration, or as an entirely independent procedure. This purification generally involves the removal of unwanted low molecular weight contaminants, such as salts or solvents, from the solution without reducing significantly the total number of macromolecules in the solution. Or in some cases, it is desired that an entirely different buffer replace the solvent or buffer now present in the solution.

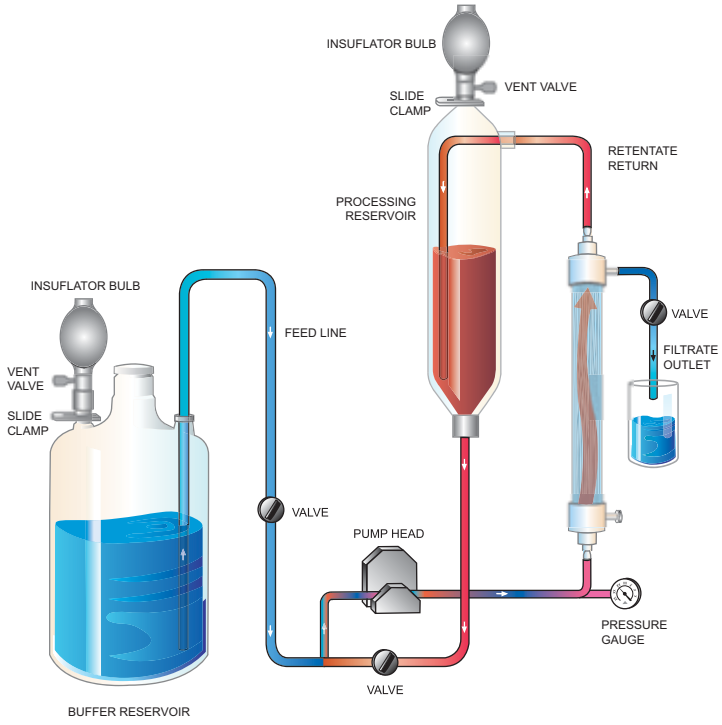


Figure A-79 Schematic of System Setup for Desalting and Buffer Exchange

The standard technique for most of these applications is dialysis. However, dialysis is slow and requires large volumes of dialyzing buffer to accomplish purification. Additionally, since the rate of diffusion across a dialysis membrane is proportional to the difference in concentration on either side of the membrane, dialysis is particularly slow at low concentrations of microsolutes, the case in many solutions.

Ultrafiltration rapidly removes all these contaminants (under hydraulic pressure) while using less buffer than dialysis. In addition, once these contaminants have been removed, concentration of the macromolecules can be accomplished in the same filtration apparatus.

In using UF for desalting and/or buffer exchange, small molecules quantitatively pass through the membrane along with the solvent, while macromolecules are retained by the membrane. The simplest method of desalting therefore would be to replace the solvent that had passed through the membrane with contaminant free solvent. For example, suppose one began with a solvent containing 1% salt, passed 90% of the solvent through the membrane, and then brought the solution to its original volume by the addition of salt free solvent. The solution would now contain only 0.1% salt and the same original number of macromolecules. Since the rate of removal of the salt is proportional only to the pressure driven flow rate through the membrane, and not to the impurity concentration, low concentrations can be reached much more quickly with UF than with dialysis. Additionally, much lower concentrations can be reached with significantly less solvent. And since the surface area of a UF membrane is less than that for a dialysis bag, less surface denaturation of macromolecules occurs with UF than with dialysis.

Diafiltration is a much more efficient procedure in using UF for desalting and/or

buffer exchange than the simple one described above. In this process shown in figure A-79, the solvent that is removed is continuously replaced by contaminant-free solvent. In this method virtually complete purification can be achieved. The dialysate fluid may be pressurized by means of an external gas source, forcing this fluid into the cell to replace the solvent volume that flows through the membrane. The volume of solution and the number of macromolecules would remain unchanged while the micromolecules are removed through the membrane.

In diafiltration, 99% of the micromolecules are removed when the solvent volume added is five times the original volume.

Fractionation

Fractionation is simply the separation of different size molecules. However, fractionation is normally performed by diafiltration of dilute solutions. (Gel polarization interferes with fractionation of more concentrated solutions). The dilute mixture of components with different molecular weights is filtered through a membrane rejecting the component with the higher molecular weight. Smaller molecules pass through the membrane enriching the higher molecular weight component in the retentate.

If desired, the low molecular weight component can be recovered from the filtrate by ultrafiltration with a second membrane that rejects this component.

Applications for the use of UF in fractionation of molecular species include:

Deproteinization of Blood and Urine Samples

To analyze for creatinine, glucose, urea, nitrogen, or other diagnostically important metabolites, proteins must be removed, otherwise turbidity, precipitates and other reactions with test reagents will result.

The standard technique in removing proteins from blood and urine samples is to precipitate these proteins with various reagents. The disadvantages of this technique include: ions may be added which become a potential source of interference and error; precipitation can change the concentration of solutes; some low molecular weight serum constituents are co-precipitated; and different precipitation methods are required for analysis of creatinine, glucose, urea, nitrogen and uric acid.

UF adds no ions, does not change the concentration of solutes, does not precipitate low molecular weight molecules and can be used for all analyses. Additionally, when working with urine or cerebrospinal fluid, the protein-enriched concentrate from the same sample can be used for electrophoresis.

Binding Studies in Pharmacology

The therapeutic and toxic effects of drugs are directly related to the level of free (or unbound) drug in the blood. Blood plasma proteins bind significant fractions of many drugs. With the bound fraction pharmacologically inactive, it is important that the level of unbound drug be determined.

The extent of binding varies from patient to patient. Recent studies have recommended routine evaluation of plasma binding of certain drugs, particularly anti-convulsants and heart-treatment drugs to determine the proper dosage for each patient.

Low membrane adsorption is an absolute necessity in binding studies since "unbound species" must be freely permeable to the membrane. High adsorption will give erroneous results with the membrane acting as a plasma protein and adsorbing "unbound species".

UF is faster than equilibrium dialysis and gel filtration. The latter are slow, requiring 24 to 48 hours, during which time the drug binding components of plasma may be altered. The bound complex may dissociate during this period as unbound species diffuse away, thus a rapid separation method is desirable. UF offers the advantage that weak binding interactions can also be assessed because the fractionation occurs rapidly.

System And Membrane Selection Guides

Membrane Application Guide

Figure A-80 illustrates representative separation applications and the optimum membrane module for each application.

The module should be matched to a system that will allow the maximum operational efficiency considering the nature of the feed stream, the objective of the separation and other important operating parameters.

APPLICATION GUIDE BASED ON MEMBRANE TYPE

	Application	Membrane Selection	Membrane Material
UF Applications	Concentrate and/or desalt peptides and small molecules	10 kD	(PS) Polysulfone
	Depyrogenation	10 kD, 50 kD	(PS) Polysulfone
	Concentrate and/or desalt proteins, enzymes, viruses	50 kD	(PS) Polysulfone
	Concentrate and diafilter albumin, (~MW 67,000)	50 kD	(PS) Polysulfone
	Concentration of monoclonal antibodies and other macromolecules	50 kD, 400 kD	(PS) Polysulfone
MF Applications	Bacculovirus and retrovirus concentration and washing	400 kD, 0.05 µm	(PS) Polysulfone
	Clarification of cell lysates to recover proteins	0.1, 0.2, 0.5 µm	(ME) Mixed Ester (PES) Polyethersulfone
	Concentration and washing of bacterial and/or mammalian cells	0.1, 0.2, 0.5 µm	(ME) Mixed Ester (PES) Polyethersulfone
	Continuous perfusion of cell culture	0.1, 0.2, 0.5 µm	(ME) Mixed Ester (PES) Polyethersulfone
	Clarification of viral cultures	0.1, 0.2, 0.5 µm	(ME) Mixed Ester (PES) Polyethersulfone
	Washing uniform latex diagnostic particles	0.1, 0.2, 0.5 µm	(ME) Mixed Ester (PES) Polyethersulfone
	Washing and concentration of protein particulates	0.1, 0.2, 0.5 µm	(ME) Mixed Ester (PES) Polyethersulfone

Figure A-80 Membrane Application Guide

INDUSTRIAL APPLICATIONS FOR HOLLOW FIBER MEMBRANE SYSTEMS

Industry	Application Examples
Pharmaceutical/ Biotech	Latex particle filtration Protein purification, desalting and concentration Enzyme isolation Blood fractionation Fermentation broth Pyrogen removal Monoclonal antibody production Cell expansion for cell therapy
Chemical	MTBE removal from aqueous streams Waste water processing Dyestuff desalting Latex concentration Catalyst recycling
Environmental	Surface water purification Filtration of waste water from car wash facilities Treatment of waste water from oil mills Printing ink removal COD reduction (removal of organic matter) Sludge concentration Filtration and concentration of landfill leachates Concentration and recovery of photo emulsions
Metals	Separation of oil/water emulsions Extension of life of degreasing baths
Paint	Filtration of electrocoat paint Recovery of water-based spray paint
Textiles	Removal and recovery of dissolved and suspended dyes Recovery of sizing agent
Paper	Recycling of coating color COD reduction
Food and Beverages	Concentration of milk and whey proteins Cheese production Wine and vinegar filtration Fruit juice clarification Starch and sugar concentration
Electronic	Prefiltration of ultrapure water

Table A-3 Industrial Applications for Hollow Fiber Membrane Systems

Sizing Hollow Fiber Systems

To properly size a system, use the following equation:

$$\mathbf{A} = \frac{V}{T \cdot f}$$

Where:

A is the required membrane surface area in square meters
V is the filtrate volume desired in liters
T is the desired processing time in hours
f is the steady state flux in liters per square meter hour (L/m^2hr)

Example:

900 liters of 0.2 µm clarified *E. coli* lysate is desired
The steady state flux is 50 L/m^2hr)
The desired processing time is 6 hours

$$\mathbf{A} = \frac{900}{6 \cdot 50}$$

$$\mathbf{A} = 3 m^2$$

Representative Spectrum Hollow Fiber Systems

MicroKros® System For Low Liquid Volume Laboratory Separations Concentration Operation Using Syringes

MicroKros® filters are designed for cross flow membrane separation of extremely small volumes. These disposable filters are the first practical tangential flow devices for processing volumes as small as 2 mL using a syringe as a pressure source. MicroKros® modules are also an ideal alternative to centrifugation for applications where pellet formation is undesirable. Flow can also be supplied with a peristaltic pump.

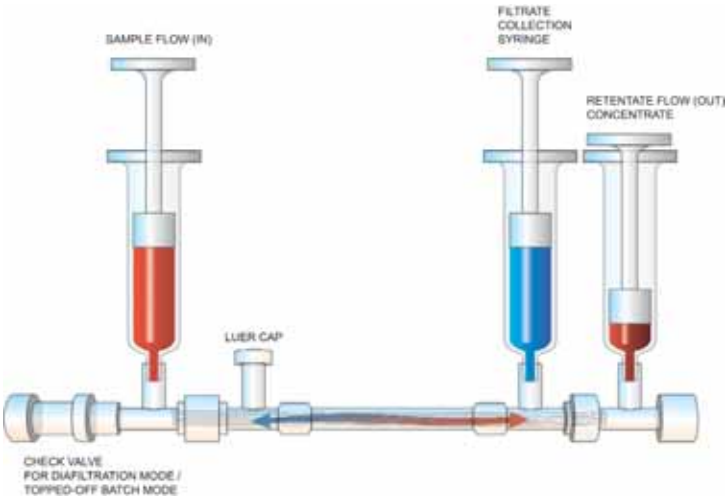


Figure A-81 MicroKros® Module Setup with syringes for low liquid volume in laboratory separations

In figure A-81, tangential flow filtration is initiated by applying pressure on the inlet syringe containing the fluid to be processed, while allowing the other two syringes to move freely. When the inlet syringe is empty, pressure is applied to the outlet syringe until it is empty. The process is continued by applying pressure first to one syringe then to the other until the desired concentration volume is obtained.

The permeate is collected by the middle syringe.

Applications of the syringe pumped MicroKros® system include:

Concentration of proteins, antibodies and microbial cells

Diafiltering (washing) proteins or uniform latex particles and other diagnostic particles

Clarifying lysates to remove cell debris from soluble proteins

Recovering serum from whole blood

Media clarification for hollow fiber bioreactors

Virus separation

MiniKros® Sampler System (Process Volumes 50 mL-3 L)

The MiniKros® Sampler System is designed for rapid cell separation, concentration, diafiltration or diagnostic particle washing with aqueous based solutions at volumes from 50 mL to 3 liters.

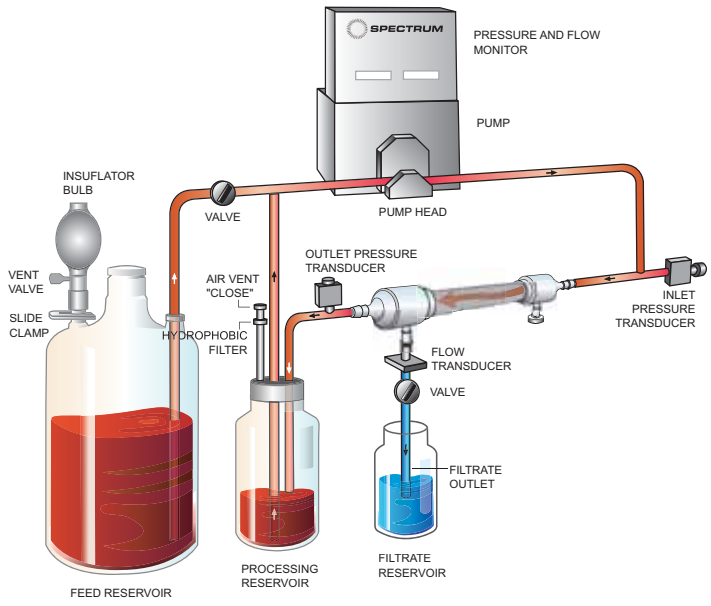


Figure A-82 MiniKros® Sampler System in topped off batch mode

Referring to figure A-82, the pump circulates the fluid to be processed in a closed loop with a pressure meter monitoring the inlet pressure. As filtrate is generated, more feed or wash buffer is pulled into the loop automatically. The system is particularly well-suited for processing shear sensitive solutions such as mammalian cell cultures.

The MiniKros® Sampler System provides an economical means of evaluating separation procedures that can be scaled up to larger MiniKros® and KrosFlo® modules.

Membrane modules for the MiniKros® Sampler System are available in retention ratings of 0.5 µm, 0.2 µm, 0.1 µm, 0.05 µm, 400 kD, 50 kD, and 10 kD and with surface areas from 120 cm^2 to 615 cm^2.

Typical applications for the MiniKros® Sampler System include:

1. Latex washing
2. Single stage prefiltration of biologicals
3. Filtration of fluids with high suspended solids
4. Concentration of cellular suspensions
5. Cell washing and/or nutrient feeding
6. Sterilizing filtration
7. Mass culture of mammalian cells/cell perfusion
8. Protein isolation/purification
9. Virus concentration/removal
10. Desalting/buffer exchange

MiniKros® Lab System (Process Volumes 300 mL - 100 L)

MiniKros® Lab Systems are designed for laboratory scale tangential flow separation volumes of 300 mL to 100 liters. Applications include concentration of cells, separation of insoluble components from suspensions, small batch filtration, protein concentration, diafiltration and scale-up studies for larger volume applications.

MiniKros® Lab Systems operate a single MiniKros® hollow fiber membrane module with lumen diameters of 0.6 mm and 1.0 mm, and surface areas from 170 cm^2 (0.16 ft^2) to 6600 cm^2 (7.1 ft^2). The system is autoclavable with typical permeate rates of 50 ml to 7.5 liters per hour.

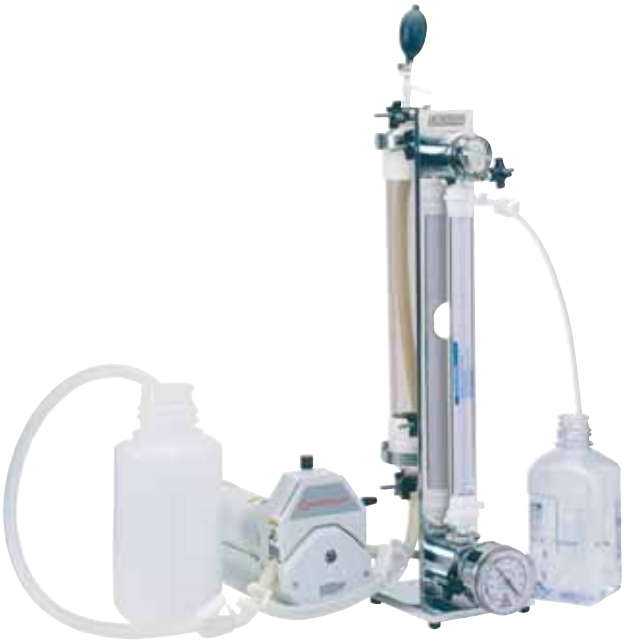


Figure A-83 The MiniKros® Lab System

The use of disposable MiniKros® modules eliminates the uncertainties and costs associated with cleaning and rinsing and the need for expensive and time-consuming validation of the membrane cleaning and rinse cycles.

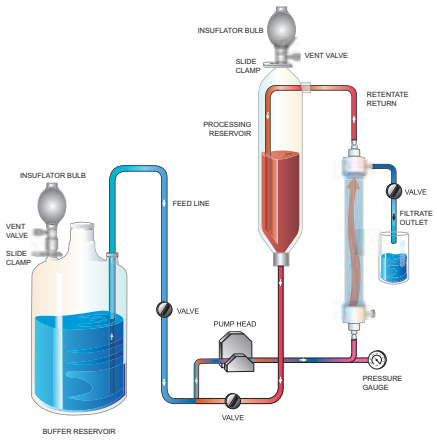


Figure A-84 Schematic of MiniKros® Lab System with Reservoir

Experimental laboratory results with the MiniKros® Lab System indicate feasibility of a ready scale-up to production level membrane modules and systems.

KrosFlo® Pilot System (Process Volumes 3 L-1000 L)

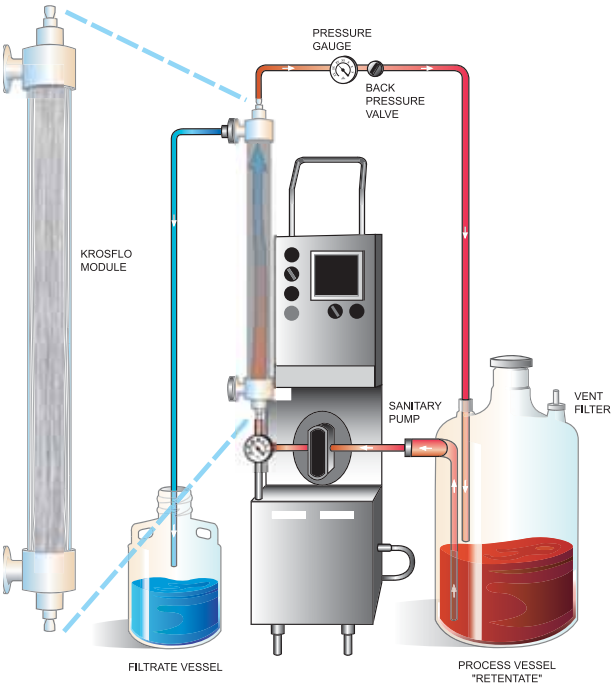
The CE certified KrosFlo® Pilot System is designed for production scale processing of pharmaceutical and other aqueous solutions. The system can process from 3 liter to 1,000 liters of solution, with permeate rates ranging from 20 to 1000 L/hour.

A wide range of membrane module configurations are available with the KrosFlo® system. These include retention ratings of 0.5 µm, 0.2 µm, 0.1 µm, 0.05 µm, 400 kD, 50 kD and 10 kD and with membrane areas from 0.32 m^2 to 5.1 m^2.

The system is mobile with a carting design that allows the system to be wheeled in and out of storage or from one location to another.

The Pilot System is also Steam-In-Place (SIP) compatible.

Using disposable and autoclavable KrosFlo® modules, the KrosFlo® Pilot System is easily converted to a SIP operating mode. The disposable KrosFlo® modules are price competitive making them economically disposable after each use when compared to reusable filters.



Figures A-85 Schematic of KrosFlo® Pilot System in batch concentration mode.

Figure A-86 Photograph of KrosFlo® Pilot System

Applications of the KrosFlo® Pilot System include:

1. Diafiltering (washing) uniform latex particles and other diagnostic particles
2. Clarifying lysates to remove cell debris from soluble proteins
3. Washing and concentrating protein precipitates
4. Recovering serum from whole blood
5. Media studies for hollow fiber bioreactors
6. Protein concentration and washing

The CE Certified KrosFlo® Pro Pressure and Flow Monitor

The KrosFlo® Pro Pressure and Flow Monitor shown in figure A-87, is a compact, book-size monitor equipped with an adjustable stand. The monitor can read and display inlet, outlet and permeate pressures simultaneously. Additionally, it can calculate and display the transmembrane (TMP) pressure.

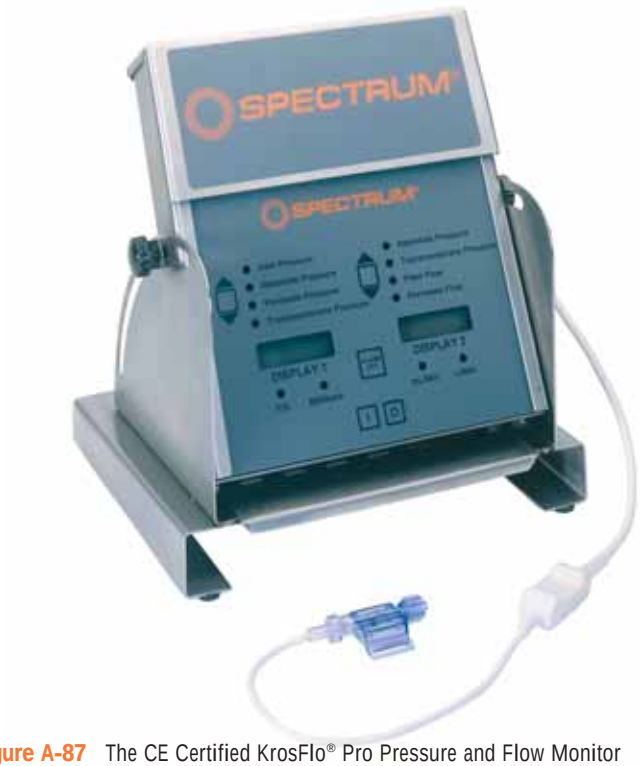


Figure A-87 The CE Certified KrosFlo® Pro Pressure and Flow Monitor

The monitor is equipped with an audible and visual pressure alarm. Should the alarm mode be triggered, the monitor sends a de-energized signal that can be detected by the on-off LiquiSense® electronic cell. The pump then shuts off.

SYSTEM COMPATIBILITY. The KrosFlo® Pro monitor is used with Spectrum tangential flow filtration systems such as the MiniKros® Lab and KrosFlo® Pilot systems or as a stand alone unit.

The KrosFlo® Pro interfaces with Windows 95/98. Through an RS-232 cable, the monitor can send pressure and flow readings to a computer that displays and records the process data.

Selecting the Optimum Membrane System

Many factors must be carefully considered and technical and economic tradeoffs made before one can select the optimum membrane system for their own application. Some of these factors and tradeoffs are shown in figure A-88, greatly simplified for illustration. Spectrum Laboratories maintains a staff of technically qualified scientists and engineers to assist customers in examining their filtration and/or bioprocessing requirements and arriving at the optimum selection. This service includes recommendations on other resources when Spectrum is unable to fill customers needs.

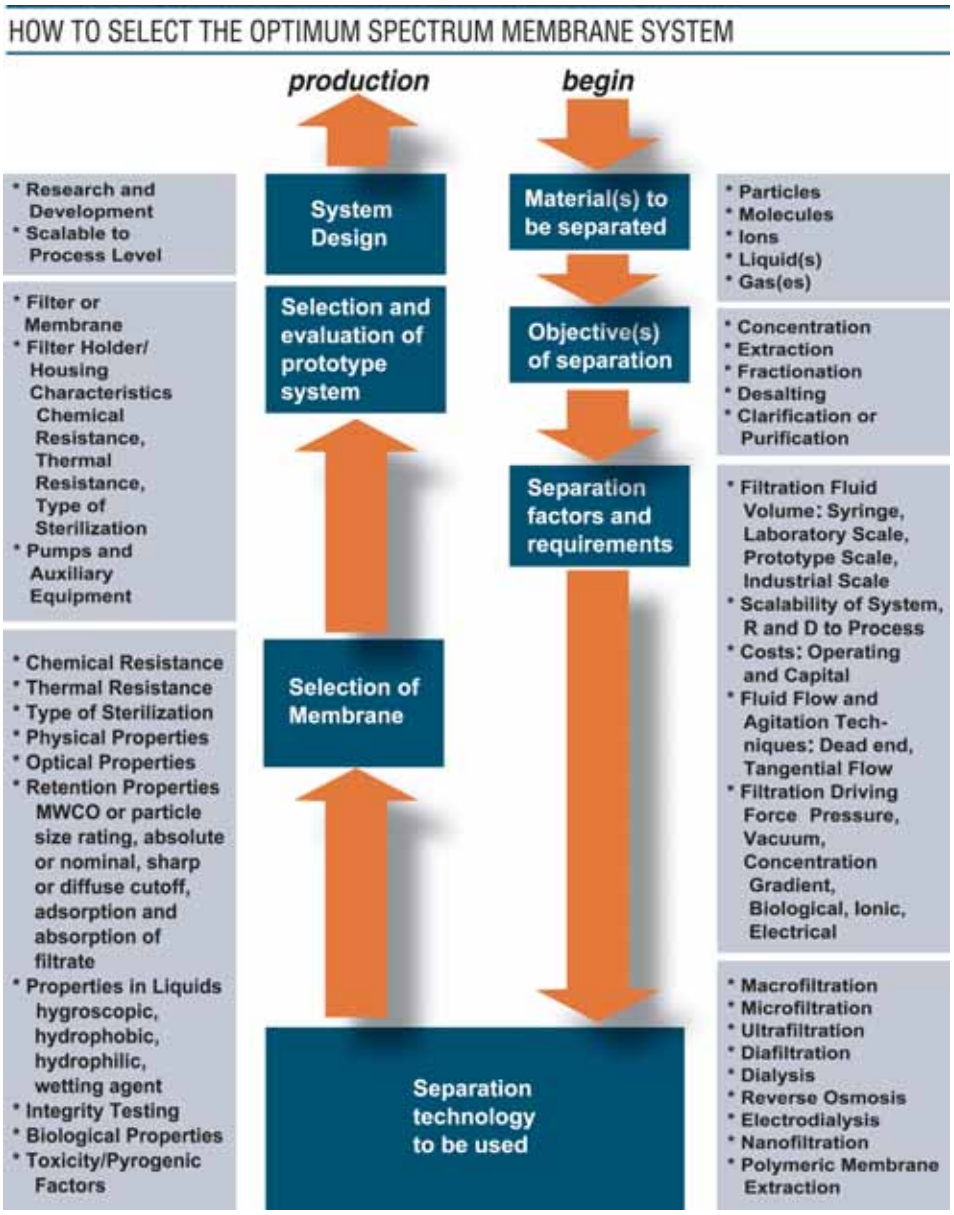


Figure A-88 Selecting the Optimum Spectrum Membrane and System

Macrofiltration

Figure A-89 Nylon screen filter (5 µm)

Figure A-90 Fleaker® with macroporous screen filter

Macrofiltration has been defined as the separation of particles of one size from particles of another size where at least one of the particles is larger than 10 micrometers. It is generally achieved by the use of screens or depth media. Screens have the major advantage that they have very low adsorption and absorption of the filtrate. This feature is essential when maximum recovery of particles in the filtrate is required such as in fractionation applications.

Macrofiltration screens are primarily available in electroetched or sintered metals, woven stainless steel wire and woven polymeric materials. Woven polymeric screens are principally available in fluorocarbon, nylon, polyester, polypropylene and polyethylene.

Woven stainless steel screens are available in mesh openings from 30 to 1000 µm. Woven polymeric screens are available in mesh openings from 5 to 1000 µm, depending on the polymer.

The selection of the optimum macrofiltration filter is based on conditions of the intended application, including flow rate, particle size and the operating environment (pressure, temperature, abrasion resistance, resistance to corrosive chemicals, etc.)

The characteristics of Spectra/Mesh® macrofiltration screens allow them to be used successfully in a wide variety of environmental, pharmaceutical, chemical and other industrial applications as well as in research.

Figure A-91 Spectra/Mesh® macroporous filters range in pore size from 5 µm to 1,000 µm

These characteristics include:

1. Precise mesh openings for accurate particle separations.

2. Most Spectra/Mesh® filters have negligible adsorption/absorption for precision fractionations of particles and similar applications. Nylon Spectra/Mesh® filters feature non-specific high binding capacity.

3. Essentially zero leaching eliminates contamination of the filtrate.

4. Excellent thermal and chemical resistance for filtration of aggressive solutions.

5. Can be backwashed to significantly extend filter life.

6. High flow rates permit lower initial capital investment.

7. Excellent physical strength for high-pressure differentials.

U.S. STANDARD SIEVE SIZES

Bureau of Standards Sieve Mesh Size*	Mesh Opening Microns (µm)	Bureau of Standards Sieve Mesh Size*	Mesh Opening Microns (µm)
4	4760	45	354
5	4000	50	297
6	3360	60	250
7	2830	70	210
8	2380	80	177
10	2000	100	149
12	1680	120	125
14	1410	140	105
16	1190	170	88
18	1000	200	74
20	841	230	63
25	707	270	53
30	545	325	44
35	500	400	37
40	420		

* Specifications adopted by U.S. Bureau of Standards and American Society of Testing Materials. Sieve Numbers are arbitrary designations and do not refer to the mesh count per inch.

Figure A-92 U.S. Standard Sieve Sizes – Specifications adopted by U.S. Bureau of Standards and ASTM. Sieve Numbers are arbitrary designations and do not refer to the mesh count per inch.

The ABCs of Laboratory Dialysis

Introduction

Dialysis is the process of diffusion of solutes through a semipermeable membrane from a liquid with higher solute concentration on one side of the membrane to a liquid with a lower concentration on the other side. See figure B-1. The membranes are semipermeable because they allow some molecules to pass while preventing others from passing. The process has long been used for the molecular separation of small molecules from macromolecules.

This relatively simple process is desirable and is performance and cost effective for several reasons:

1) Wide Range of Sample Volumes

2) Gentle Conditions

3) Inexpensive Membranes and Equipment

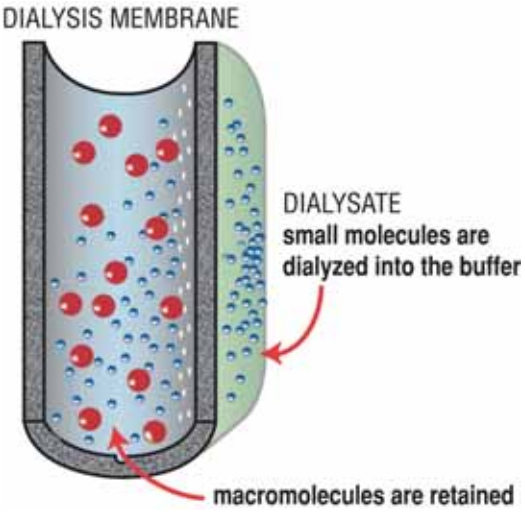


Figure B-1 Schematic of hollow fiber dialysis membrane

Common dialysis applications utilize tubular forms of membranes and involve placing a "sample" inside the membrane and a "buffer" outside the membrane. The process is run until the desired degree of separation is attained. Molecules smaller than the pores will eventually be equally distributed between the two solutions. Usually, a very large volume of buffer is chosen so that the permeable species are greatly diluted and therefore reduced-to very small concentrations in the remaining sample solution. Commonly, dialysis processes require several hours to complete.

Dialysis Membranes

The original type of dialysis membranes utilized for diffusion studies were cellulose nitrate. These membranes were not ideal because they tended to have high protein adsorption, thus limiting their use in the purification and separation of biological solutions. Regenerated cellulose membranes were then used because they alleviated the protein adsorption problems. Regenerated cellulose also exhibited good chemical and temperature resistance. The membranes, however, were limited in Molecular Weight Cut Off (MWCO).

Further advances in dialysis membrane development were made as a result of research to provide relief from renal disease by means of hemodialysis, a pressure driven rather than concentration gradient driven process.

Greater membrane permeability was achieved through the use of cellulose ester.

These solutions could be formulated to yield a wider range of pore sizes. Cellulose ester membranes are now widely used for clinical and laboratory dialysis. Membranes used for dialysis have pore sizes ranging from 100 to 300,000 Daltons. Sample volumes have also been greatly reduced to allow dialysis of small quantities of precious samples, particularly where maintaining enzyme activity is desired.

Dialysis Applications

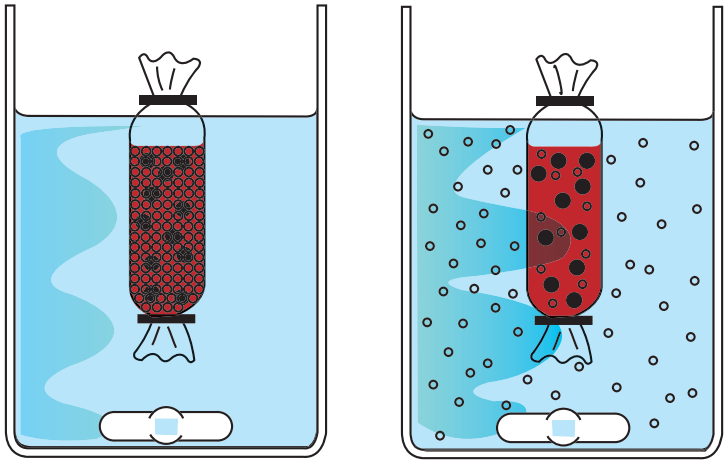


Figure B-2 Dialysis using conventional tubular membranes

For the simple dialysis applications of removing salts or small molecules, the use of membrane tubing is convenient, efficient and inexpensive. This can be accomplished with a length of membrane tubing in a container of buffer as shown in figure B-2. Stirring the buffer is recommended to increase the dialysis rate. Dialysis closures are used to provide an easy method for sealing the ends of the dialysis tubing. In addition, a range of new products allow dialysis of much smaller sample volumes.

Typical Dialysis Applications

Desalting

Concentrating Plasma or Serum

Buffer and pH Change of Sample Solution

Preparation of Diluted Proteins prior to Electrophoresis

Concentration of Antibodies

Contamination Removal

Binding Studies

Flow Dialysis/Batch Analysis

Temperature Regulated Dialysis

Tissue Culture Extract Purification

Protein Removal from Gels after Electrophoresis

Removal of Oligosaccharides from Protein Solutions

Dialysis is Commonly Used in the Following Fields of Research

- Biochemistry
- Molecular Biology
- Biomedical Research
- Hematology
- Oncology
- Microbiology
- Pharmacology
- Virology
- Renal Biology
- Neurology
- Physiology
- Genetics
- Rheumatology

Factors that affect the Rate of Dialysis

Molecular Weight Cut Off & Selectivity

Dialysis membranes are characterized by molecular weight cut off (MWCO). MWCO is determined by testing the degree of permeability for several solutes of different molecular weights. The MWCO rating for the membrane is the molecular weight of the smallest solute that is 90% retained in a 17-hour dialysis test. Molecular weight cut off ratings are used as a guide and not an absolute prediction of performance with every type of solute. A membrane MWCO size rating should be chosen as high as possible in order to achieve the maximum dialysis rate while still preventing the loss of the desired solute.

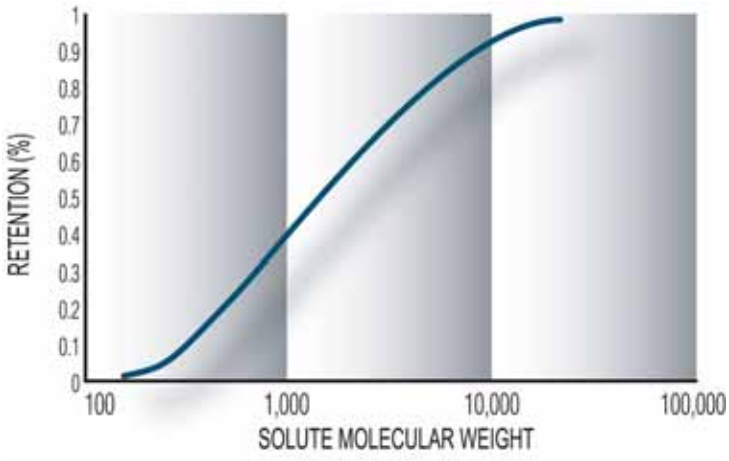


Figure B-3 Retention of idealized dialysis membrane vs. solute molecular weight using a 10,000 MWCO membrane

Plotting the results of a MWCO test in the form of retention versus the solute molecular weight would ideally produce a sigmoid curve. The steepness of the curve is a measure of the selectivity of the membrane. Figure B-3 illustrates an ideal curve for a cellulose ester membrane with 10,000 MWCO. It is shown on this curve that the central portion of the curve is approximately a linear relationship that crosses the 0.9 retention axis at 10,000 molecular weight.

Flux and Permeation Rate

The driving force for laboratory dialysis is the concentration difference across the membrane. The flux (or permeation rate) is directly proportional to the concentration difference, i.e. the greater the difference, the greater the rate. However, the dialysis rate is also influenced by other variables such as:

DIFFUSION COEFFICIENT. Different size molecules pass through a membrane at different rates. Larger molecules have a smaller coefficient and a lower rate of diffusion across the membrane.

MOLECULAR SHAPE AND CHARGE. Linear molecules permeate faster than globular molecules. The pH and ionic strength also affect the rate of dialysis.

CONCENTRATION POLARIZATION. As molecules diffuse across a membrane, they first move through the bulk of the sample solution to the surface of the membrane. The thin region next to the membrane has a higher concentration of solutes than the bulk solution. This build up is termed “concentration polarization” and is caused by depletion of small molecules at the surface of the membrane. This polarized layer causes resistance to the movement of molecules across the membrane. Finally, after passing through the membrane, the molecule often meets a thin layer of concentration higher than the bulk solution, further slowing the passage. These layers which form on either side of the membrane are called “fluid boundary layers” or “gel layers”.

FLOW DIRECTION AND AGITATION OF THE SOLUTION. Sample and buffer that flow perpendicular (or normal) might cause the membrane to plug. Sample and buffer mixing during dialysis can reduce this phenomenon. Mixing can be achieved by either stirring or by passing the sample parallel (or tangential) to the membrane. Parallel flow promotes higher permeation rates. The higher the stirring rate, the higher the dialysis rates (Concentration polarization is reduced by increased stirring rates).

TEMPERATURE. Higher temperatures promote more rapid molecular movement and therefore increase diffusion rate.

MEMBRANE THICKNESS. Membrane properties effect the dialysis rate. Thicker membranes will require a longer time for molecules to pass through.

MEMBRANE SURFACE AREA. The larger the membrane area, the faster the dialysis rate.

HYDRODYNAMIC PROPERTIES. Viscosity of the fluid and the membrane porosity affect the permeation rate. Low viscosity and high porosity are ideal for higher rates.

Dialysis Membrane Selection Guide

Spectra/Por® Dialysis Membranes

Spectrum offers three types of dialysis membranes:

SPECTRA/POR® REGENERATED CELLULOSE (RC) - good chemical compatibility for selective organic solvents, pH range of 2 to 12 and temperature stability of up to 60°C. There are two types of RC membranes:

Spectra/Por® Biotech Regenerated Cellulose - ultra pure hydrophilic membranes ready for use with precise MWCO selection of 3.5K to 60K.

Spectra/Por® Standard Regenerated Cellulose (Series 1 through 7) - hydrophilic standard membranes widely used within the biotech community. However, there are trace levels of heavy metals and sulfides contained in this series that are commonly treated by "boiling" the membranes in chemical solutions. Spectra/Por® 7, however, is chemically precleaned and ready to use.

SPECTRA/POR® BIOTECH CELLULOSE ESTER (CE) - ready to use membranes with a wide selection of MWCO ratings ranging from 100 to 300,000 Daltons. Biotech CE membranes are stable at pH 2 to 9 and temperature not exceeding 37°C. CE membranes, however, are not compatible with organic and strong polar solvents.

SPECTRA/POR® POLYVINYLIDENE DIFLUORIDE (PVDF) - hydrophobic, ready to use, with excellent chemical resistance, three MWCOs (250,000; 500,000 and 1 million Daltons).

SPECIFICATIONS OF SPECTRUM REGENERATED CELLULOSE DIALYSIS MEMBRANES

Specifications	*Spectra/Por® 1*	*Spectra/Por® 2*	*Spectra/Por® 3*	*Spectra/Por® 4*	*Spectra/Por® 5*	*Spectra/Por® 6*	*Spectra/Por® 7*
MWCO	6K-8K	12K-14K	3.5K	12K-14K	12K-14K	1K, 2K, 3.5K 8K, 10K, 15K, 25K, 50K	1K, 2K, 3.5K 8K, 10K, 15K, 25K, 50K
Physical properties	Transparent, Flexible	Transparent, Flexible	Transparent, Flexible	Transparent, Flexible	Thick, Rigid	Transparent, Flexible	Transparent, Flexible
Packaging:	Dry	Dry	Dry	Dry	Dry	Wet	Wet
Preservative	Glycerin	Glycerin	Glycerin	Glycerin	Glycerin	1% hydrogen peroxide	1% hydrogen peroxide
Trace Elements Contents:	Heavy Metals, Sulfides	Heavy Metals, Sulfides	Heavy Metals, Sulfides	Heavy Metals, Sulfides	Heavy Metals, Sulfides	Heavy Metals, Sulfides	Minimal
Flat width range (mm)	10 to 120	8 to 120	18 to 54	10 to 75	65 to 140	6 to 54	6 to 54
Sample Volume Range (ml):	1 to 1000	1 to 1000	2 to 200	1 to 300	25 to 1200	1 to 150	1 to 150
Disc/Flat Sheet:	Yes	Yes	Yes	Yes	Yes	No	No

pH Limits: 2 to 12 • **Suggested Temperature Limit:** 60°C • **Organic Solvent Tolerance:** good

Table B-1 Specifications of Spectrum regenerated cellulose dialysis membranes

Standard Regenerated Cellulose (RC) Dialysis Membranes

This series of membranes is composed of cellulose reconstituted from cotton linters. Spectra/Por® RC membranes carry no fixed charge and do not absorb most solutes. Standard RC dialysis membranes are used for general laboratory dialysis functions, such as desalting, buffer exchange, or molecular separation. Spectra/Por® RC Series 1 through 6 membranes contains small amounts of impurities that are easily removed by washing. Table B-1 shows the major characteristics of standard RC dialysis membranes.

Spectra/Por® Biotech Membranes

Spectrum's Biotech membranes are manufactured in a process that eliminates the use of metal salts in the manufacturing process. Thus, no boiling or soaking the membrane is required. Other advantages include precisely controlled Molecular Weight Cut Offs (MWCO) for general dialysis applications and small tubing diameters for very small samples.

Biotech Regenerated Cellulose (RC) membranes are recommended when resistance to various organic solvents (such as DMSO, DMF or alcohols) is required.

Biotech Cellulose Ester (CE) membranes provide a wide selection of rigidly controlled MWCOs ratings ranging from 100 to 300,000 Daltons.

Biotech Polyvinylidene Fluoride (PVDF) membranes provide not only excellent chemical resistance, but also the ability to heat-seal the membrane for encapsulation and implantation research.

SPECIFICATIONS FOR SPECTRUM BIOTECH DIALYSIS MEMBRANES

Membrane Type:	*Symmetric Regenerated Cellulose*	*Symmetric Cellulose Ester*	*Symmetric PVDF*
MWCO:	3.5k, 8k, 10k, 15k, 25k and 60k Daltons	100, 500, 1k, 2k, 3.5k, 8k, 10k, 15k, 25k, 50k, 100k, and 300k	250k, 500k and 1 million Daltons
Physical Appearance:	Opaque, Flexible	Opaque, Rigid	Opaque, Rigid
Organic Solvent Tolerance:	Good	Fair	Very Good
Packaging:	Dry with glycerin as a humectant (available irradiated wet in deionized water)	Wet with 1% hydrogen peroxide (available irradiated wet in deionized water)	Wet with 1% hydrogen peroxide
Flat width (mm):	4, 8, 10 and 16	8, 10, 12, 16, 24, and 31	8, 12, 16, and 24
Disc/Flat Sheet:	No	Yes, Asymmetric	No
Sample volume range:	0.5 ml to 30 ml	0.5 ml to 60 ml	0.5 ml to 30 ml
pH limits:	2 to 12	2 to 9	1 to 14
Suggested Temperature Limit:	60 °C	37 °C	130 °C

Table B-2 Specifications for Spectrum Biotech dialysis membranes

Applications for Biotech dialysis membranes include:

Removal of salts, surfactants, detergents and solvents

Buffer and pH adjustment of sample solutions

Concentration of proteins, peptides or antibodies

DNA electroelution

Preparation of proteins prior to electrophoresis, HPLC

Removal of contaminating micromolecules

Binding studies

Tissue culture extract purification

Chemical Compatibility

Refer to the chemical compatibility table as the first step in selecting the proper membrane. Variables in temperature, concentrations, duration of exposure and other factors may also affect the performance of the membranes. PVDF membranes have the best chemical resistance. Generally, Regenerated Cellulose (RC) membranes have a better resistance to most chemical solutions than Cellulose Ester (CE) membranes.

MWCO Selection

Selecting of the correct molecular weight cut off (MWCO) of the membrane is based on the size of the molecular weight of the macromolecules to be retained inside the membrane and the molecular weight of the molecules to be removed. The ratio of the two molecular weights should be a minimum 25 to 1 to achieve the maximum 90% retention.

Tubular Membrane “flat width” Selection

Smaller tubing will dialyze more quickly than larger tubing. The latter will dialyze more slowly due to the longer diffusion distances involved.

Closure Selection

A closure 4 to 10 mm wider than the flat width of the membrane tubing should be selected. This will allow the closure to securely seal the tubing.

Spectrum recommends two types of closures: polypropylene and nylon

POLYPROPYLENE CLOSURES (SPECTRA/POR®): are autoclavable and are commonly used for RC membranes. There are 3 types of polypropylene closures:

• Standard closures that seal at the top of the membrane tubing.

• Weighted closures that contain a stainless steel bar embedded in the standard closures. The weighted closures are applied at the bottom of the membrane tubing to keep it in a vertical floating position.

• Magnetic weighted closures that contain a magnetic stir bar. There is no need for an additional magnetic stir bar when the magnetic weighted closure is used at the bottom of the membrane tubing.

NYLON CLOSURES (UNIVERSAL) are specially designed for rigid membranes such as CE and PVDF, although they can be used for all types of tubing membranes.

Representative Spectrum Dialysis Products

Spectra/Por® Float-A-Lyzer™

Dialysis for Sample Volumes from 300 uL to 10 mL

These floatable, presealed and ready-to-use tubes are available for dialysis of six sample volume sizes: 300 uL, 500 uL, I mL, 3 mL, 5 mL and 10 mL. The Float-A-Lyzer™ contains Spectra/Por® Biotech Cellulose Ester or Regenerated Cellulose dialysis membranes with 13 choices of Molecular Weight Cut Off ratings ranging from 100 to 300,000 Daltons

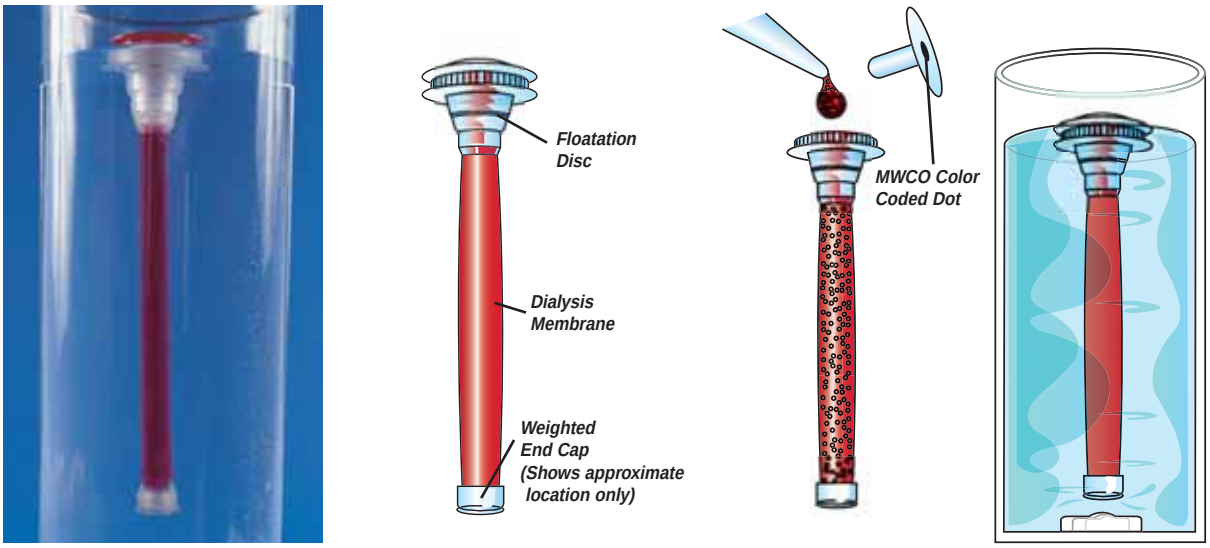


Figure B-4 Spectra/Por® Float-A-Lyzer™

The Float-A-Lyzer™ has an easy open cap for loading and recovering samples with the provided pipette. Multiple samples can be processed in one reservoir

The high purity membranes yield excellent sample recovery for biological samples such as proteins, peptides, antibodies, DNA, etc. In addition, removing small molecular weight contaminants, desalting, buffer exchange and concentration are quick and easy to handle with the Float-A-Lyzer™.

There is no danger of puncturing the membrane when loading or recovering the sample from using hypodermic needles and syringes. The floatable cap makes it easy to load and recover the sample with the provided pipette. It also allows for samples analysis during dialysis.

Spectra/Por® Micro DispoDialyzer®

The Micro DispoDialyzer® is a dialysis device for processing ultra small sample volumes from 10 uL to 100 uL. It features easy handling using micropipettes for small samples and set up, and Spectra/Por® Biotech Regenerated Cellulose dialysis membranes

The Micro DispoDialyzer® is available in 3 colored sample volume sizes: 10 uL, 50 uL and 100 uL, and Molecular Weight Cut Offs (MWCO): 3.5 kD, 8 kD, 15 kD, 25 kD, and 60 kD with a color-coded cap for easy sample identification.

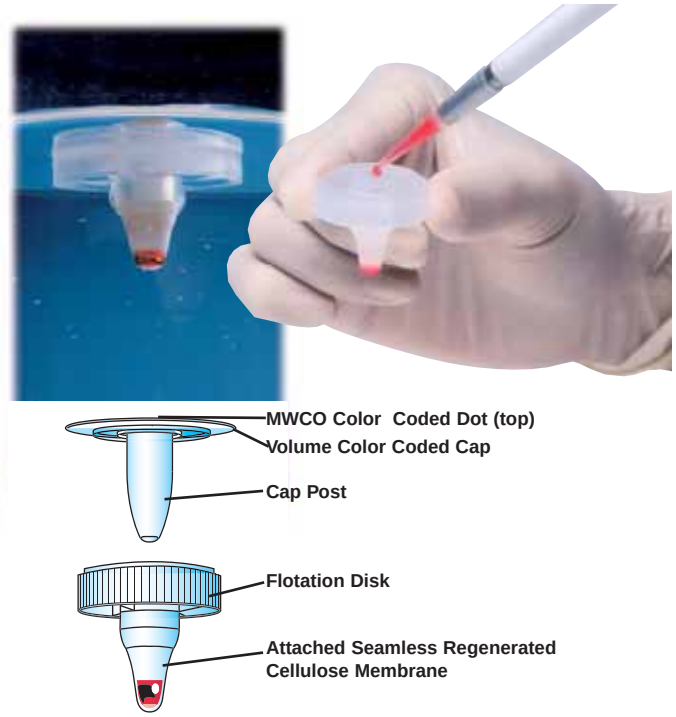


Figure B-5 Spectra/Por® Micro DispoDialyzer®

It incorporates a high membrane surface area to volume ratio for a faster dialysis rate and features maximum macro solute recovery for both liquid and solid phase samples

Multiple Micro DispoDialyzer® units can be dialyzed in one dialysis reservoir

No flotation device, syringe or needle accessories are required.

Applications include:

Removal of salts, surfactants, detergents and solvents

Adjustment of sample buffer and pH

Purification of proteins, DNA, oligonucleotides, peptides, polymers, antibodies and other biomolecules

Spectra/Por® DispoDialyzers®

Figure B-6 Spectra/Por® DispoDialyzer®

The Spectra/Por® DispoDialyzer® is a ready-to-use dialyzer for small sample volumes from 500 uL to 5 mL. It is available with both regenerated cellulose and cellulose ester membranes having a wide selection of MWCOs ranging from 100 to 300,000. The DispoDialyzer® is color coded by molecular weight cut off.

By allowing a small headspace during filling, the DispoDialyzer® will float vertically and remains fully immersed during dialysis.

Multiple DispoDialyzers® can be simultaneously used in the same buffer solution.

The ABCs Of Polymeric Membrane Extraction

Selextrac™ Technology Summary

Spectrum Laboratories, Inc. has introduced SeleXtrac™, a novel class of membranes for the selective extraction and concentration of organic compounds from dilute aqueous solutions.

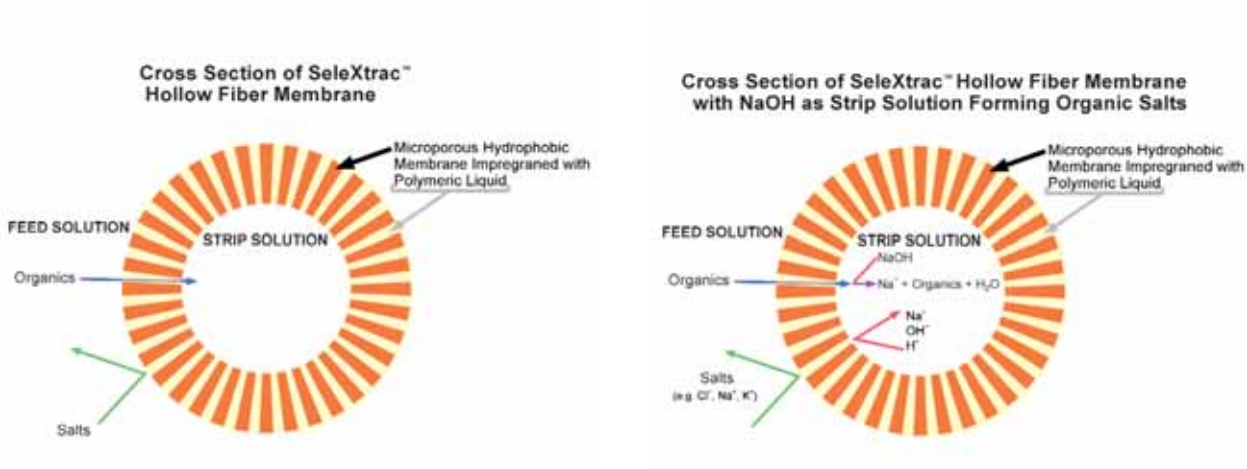


Figure C-1 SeleXtrac™ membrane modules are designed with advanced hollow fiber membranes that have the pores impregnated with a polymeric liquid that has an affinity or attraction for the organic compound of interest.

SeleXtrac™ membrane modules are designed with advanced hollow fiber membranes that have the pores impregnated with a polymeric liquid that has an affinity or attraction for the organic compound of interest. When membranes are prepared in this fashion, the ability of the membrane to separate compounds from a liquid or gas stream depends primarily on the chemical properties of the liquid polymer used and not on the conventional sieving or rejection of molecules through the membrane pores.

SeleXtrac™ liquid membranes selectively transport and extract specific compounds by utilizing various molecular properties, such as hydrophobicity, hydrogen bonding capability and the degree of dissociation as indicated by the pKa.

The housing of the SeleXtrac™ membrane module is designed with inlet and outlet ports for recirculating two different mixtures of a liquid or a gas or both. Driven by selective extraction, the SeleXtrac™ System operates with a dual head peristaltic pump or syringes. One pump head recirculates the Feed Solution (sample mixture) through the inlet and outlet sideports located on the side of the module. The other pump head recirculates the “Strip Solution” in a countercurrent flow direction through an inlet and outlet port at each end of the module that provides access to the lumens of the hollow fibers.

The Feed Solution, consisting of a liquid or a gas mixture, is recirculated on the outside of the SeleXtrac™ hollow fiber membrane. By chemical interaction, targeted organic molecules are transported from the Feed Solution through the liquid polymer contained in the pores of the hollow fibers and into the Strip Solution that is being recirculated through the fiber lumen. The Strip Solution may be a liquid or a gas mixture. Its selection depends on the nature of the organic compounds to

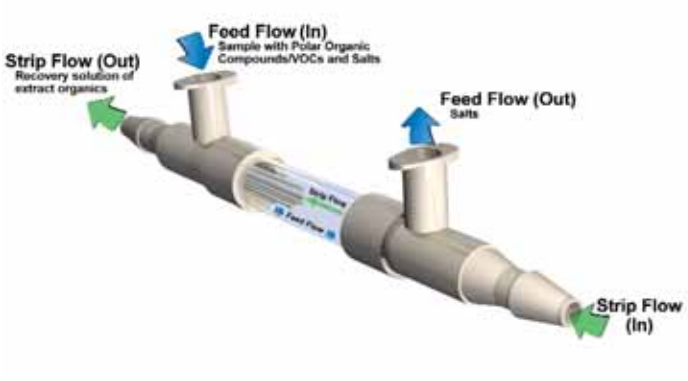


Figure C-2 Schematic of flow for SeleXtrac™ Hollow Fiber Membrane Module

be extracted. For example, water as the Strip Solution for soluble, neutral or non-volatile organic compounds; acidic Strip Solution (e.g. HCl) for basic organic compounds and basic Strip Solution (e.g. NaOH) for acidic organic compounds; and gaseous strip for volatile organics. The rejected ionized organic molecules remain in the Feed Solution sample throughout the extraction process. The Feed and Strip Solutions are recirculated through the SeleXtrac™ System until the desired concentration of the selected compound is achieved. The Strip Solution containing the extracted compound is collected as the "Extract".

SeleXtrac™ Features

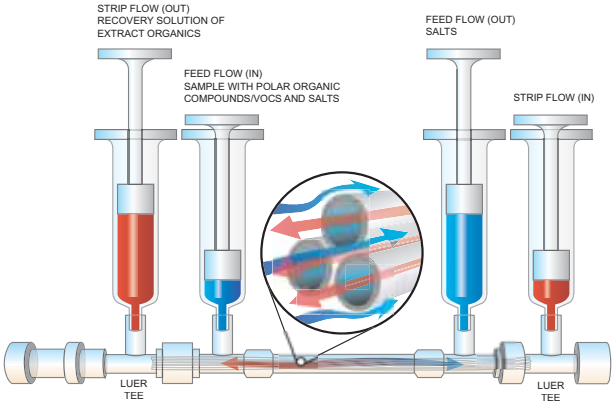


Figure C-3 The SeleXtrac™ Module can be used with syringes for small volume applications and for feasibility studies prior to scale-up

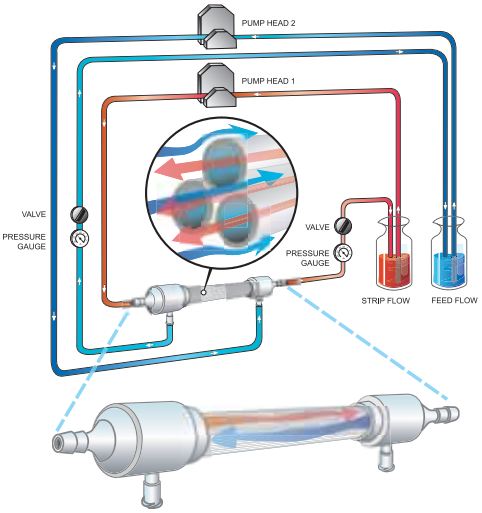


Figure C-4 SeleXtrac™ Module in Pumped Configuration for prototype and process scale quantities

Fast extraction procedure with no toxic solvents or phase separation.

Versatile extraction of liquid-liquid, liquid-gas and gas-gas mixtures.

Available in two types of easy-to-use hollow fiber modules:

Type S for extraction of hydrophobic organic compounds - more solvent resistant.

Type P is recommended for polar compounds.

Hollow fibers provide a large surface area in a compact module.

Three module sizes are available for processing volumes from 5 ml to several liters depending on sample size and type.

Higher yields due to smaller sample volumes.

Stable condition for biological samples.

Laboratory and prototype testing results can generally be scaled up to production levels.

Transport Rates of Organic Compounds through SeleXtrac™ Membranes

Transport rates for selected organic compounds across SeleXtrac™ membranes organic compounds are shown in figure C-5.

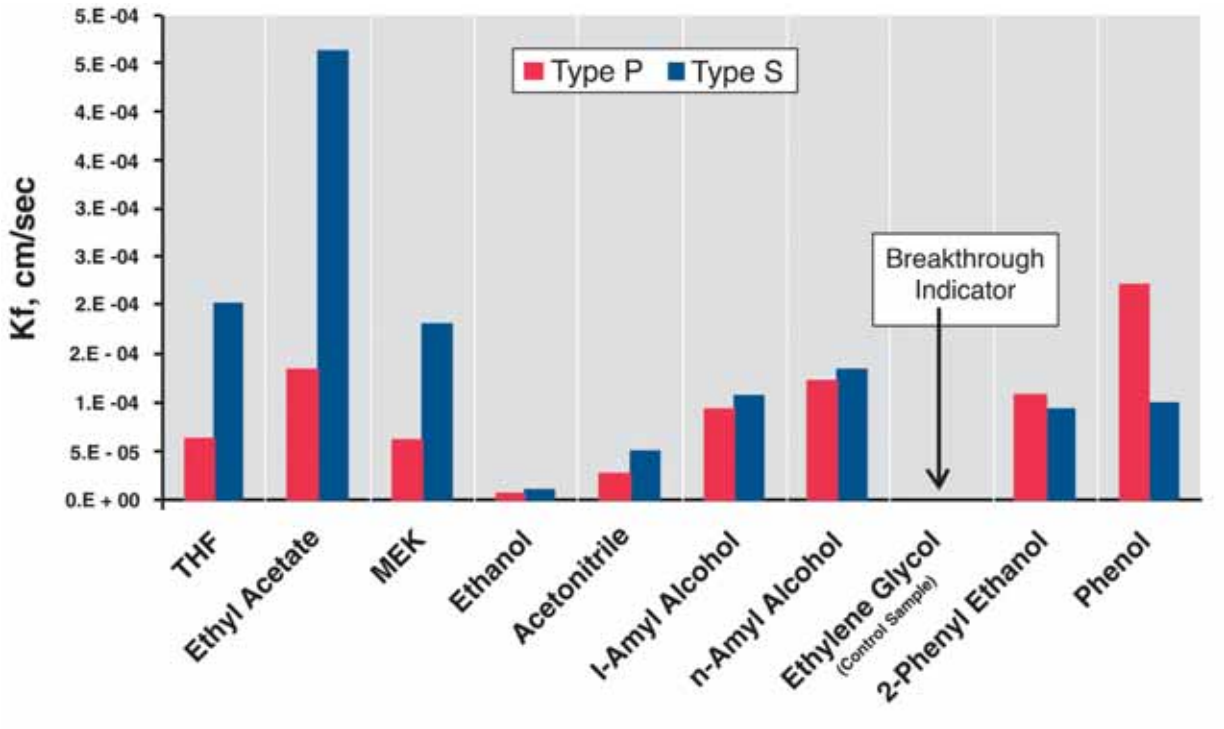


Figure C-5 Transport of selected organic compounds across Type S and Type P SeleXtrac™ Membranes

SeleXtrac™ Applications

Simultaneous extraction of hydrophobic organic compounds from biological samples, cell culture media, reaction solutions, fermentation broth, flavor and waste water processing in Life Sciences and in bulk industrial applications.

Extraction of non-ionic acids, basic organic compounds such as phenol and phenolics, nitriles, esters, carboxylic acids, aromatic amines, chlorinated hydrocarbons and other related compounds.

Rejections of ionized organic acids and bases, Zwitterions, sugars, salts, proteins and amino acids from aqueous solutions.

Extraction of volatile organic compounds such as alcohols, esters and chlorinated solvents using the SeleXtrac™ System in the pervaporation mode.

Example - Extraction of Volatile Organic Compounds (VOCs)

Removal of MTBE from Contaminated Water

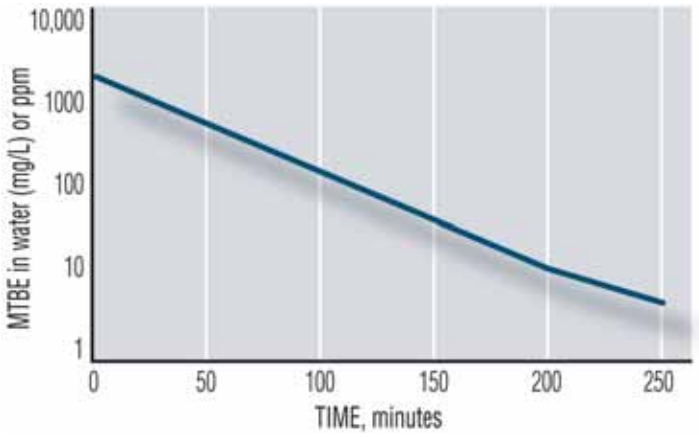


Figure C-6 The SeleXtrac Type S Membrane System is highly effective in the removal of MTBE from contaminated water

Methyl Tertiary Butyl Ether (MTBE), a gasoline oxygenate additive, is also a groundwater contaminant present in many states, especially in California. Due to its hydrophilic properties, MTBE is highly soluble in water and has a low affinity for common absorbents. The SeleXtrac™ Type S hydrophobic hollow fiber membrane module has proven to be an effective method to extract MTBE from contaminated water in a pervaporation mode. Once permeation through the liquid polymer in the membrane takes place, MTBE can be effectively removed by a "strip" gas such as air or nitrogen or removed by applying a vacuum. Water exhibits a very low permeation rate due to the membrane's hydrophobic nature.

In the experiments described in the discussion section below, the MTBE water mixtures used as the Feed Solution were recirculated through the lumen of the hollow fibers.

The water flow rates were varied to determine the significance of liquid film mass transfer resistance versus the liquid membrane resistance. Nitrogen was used as the ventilating gas on the outside surface of the membrane. The high nitrogen flow rate was maintained constant to minimize gas film resistance and to achieve the highest concentration gradient for MTBE.

Example - Extraction of Water Soluble Polar Organic Compounds

Removal of Para-Nitrophenol (PNP) From Wastewater

Industrial wastewater often contains phenolic compounds such as PNP and high concentration of inorganic salts that can be removed and concentrated for recycling. Due to its extraction affinity for polar compounds, the SeleXtrac™ Type P hollow fiber module can be used for removing PNP while rejecting the salts.

In the extraction process experiment described in the discussion below, 0.1 N NaOH solution (pH 13) is used as an alkaline Strip Solution and recirculated through the lumen of hollow fiber membranes. The PNP-containing wastewater at pH 3.8 is used as the Feed Solution and is recirculated on the outside of the membrane surface. During the experiment, pH values in both solutions remained fairly constant indicating no significant transport of H+ and OH- ions across the polymeric liquid membrane.

Discussion and Results: PNP removal was determined by measuring its concentrations in the Feed and Strip Solutions as a function of time. Figure C-7 shows that PNP concentration on the Feed side steadily declined. The SeleXtrac™ mem-

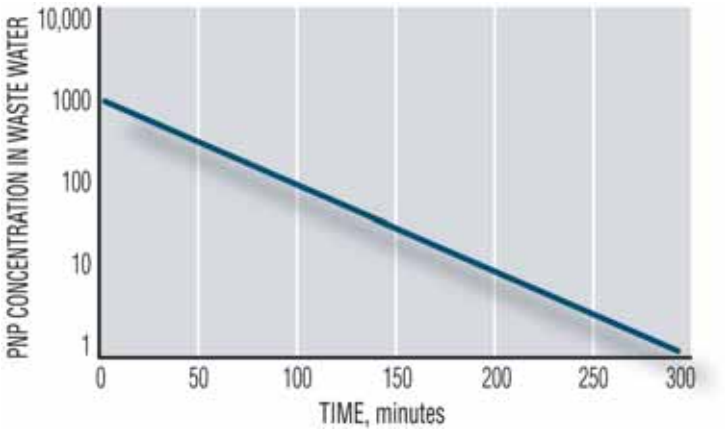


Figure C-7 The SeleXtrac™ Type P Membrane System is highly effective in the removal of water soluble polar organic compounds from contaminated water

brane module removed PNP in the Feed Solution from an initial value of 1000 mg/L to less than 1 mg/L. NaOH in the Strip side reacted with the permeating PNP to form sodium p-nitrophenate (Na+PNP-), which in this ionic state will not permeate back into the Feed side.

Thus simultaneous removal of PNP from the Feed and its concentration in the form of sodium PNP on the Strip side can easily be achieved.

Discussion

SeleXtrac™ Membranes for Polymeric Extraction of Organics from Aqueous Streams

Introduction

The majority of industrial membrane applications involving liquid solutions are based on size difference for the desired separation (1-4). Microfiltration membranes are widely used for removing particulates for solution clarification. Ultrafiltration membranes with a range of molecular weight cutoffs (MWCO) are typically used to retain dissolved compounds ranging from protein size (~100,000 MWCO) to smaller molecules (~1000 MWCO). Nanofiltration membranes get into the range of small molecules (MWCO of a few hundred) and ions. Reverse osmosis membranes, while not strictly functioning based on size, complete the range by allowing primarily only water to pass through and are thus widely used for recovering fresh water from seawater and brackish solutions. Another type of membrane applications is based on charge difference of the dissolved molecules or ions, such as electrodialysis.

There is a clear need for membrane separation based on the molecular properties of the dissolved compounds in addition to just size or charge, such as the wide range of industrial separations accomplished with solvent extraction. This concept has been cleverly employed in an approach called supported liquid membranes in which the extracting solvent resides ("supported") inside the pores of a microfiltration or ultrafiltration membrane, allowing membrane contact with the feed solution on one side and the strip solution on the other side. Consequently, all the separation capabilities of solvent extraction, including the use of selective carriers for specific separations, are directly translated into a membrane configuration in which very little solvent is needed and two separate unit operation steps are combined into one.

Conventional supported liquid membranes typically employ common organic solvents like kerosene and alcohols as the liquid membranes along with some specific carriers for enhanced transport rate and selectivity. These membranes have been extensively studied for transport of ionic compounds such as salts and metal ions (7,8) and for transport of organics (9-11). Despite their powerful and diverse separation capabilities, these supported liquid membranes turn out to be not very practical for industrial applications, primarily because of the instability of the membranes. This problem has been attributed to partial dissolution of the solvents, leaching of the carriers, and displacement of the solvents from the pores of the support matrix (12,13).

Spectrum has acquired from Monsanto Co. an interesting class of membrane called SeleXtrac™, a supported polymeric liquid membranes in which the liquid is a functional liquid polymer rather than an organic solvent. SeleXtrac™ membranes have been found to be much more stable, especially under very high osmotic pressure differences (5,6,14), probably because of the typical physical properties associated with the polymeric liquids used, such as high viscosity, extremely low volatility and insolubility in water. One unique application of this capability is for treating aqueous wastes discharged from chemical plants that typically contain low levels (a few percent or less) of small organic compounds (molecular weight <200) but also salts with concentrations reaching 30 wt% or

higher. The small size of the organics in these streams excludes the use of ultrafiltration and microfiltration, and the osmotic pressure limitation from the high salt content makes reverse osmosis impractical. The non-volatile nature of polymeric liquids also allow SeleXtrac™ membranes to be operated in the pervaporation mode, thus having similar capabilities as hydrophobic (solid) membranes that have been shown to work well for recovering relatively hydrophobic "volatile" organics (VOCs) from dilute aqueous wastes (4).

SeleXtrac™ membranes using certain liquid polymers have also been found to exhibit high permeability for flavors and aroma compounds, which could result in important applications in the beverage and food processing areas. Other potential applications include analytical and biomedical. This chapter will cover the concept and characteristics of SeleXtrac™ membranes, and the various key applications that have been identified.

Basic Theory

SeleXtrac™ Supported Polymeric Liquid Membranes

Concept

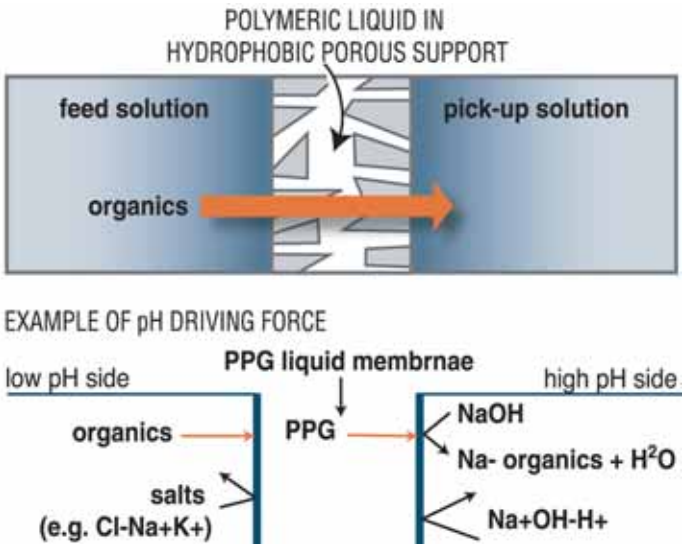


Figure C-8 Schematic diagram of SeleXtrac™ membranes

SeleXtrac™ membranes are prepared by filling the pores of microfiltration or ultrafiltration membranes with polymeric (oligomeric) liquids having affinity for the organic compounds of interest. These polymeric liquids are polyamphiphilic, i.e., they consist of repeating monomeric units that contain both hydrophobic and polar groups. The chemical nature of these groups and the number of monomeric units in each polymer chain can be manipulated to vary the chemical and physical properties of the polymeric liquids, thus conveniently creating membranes of diverse separation capabilities. Figure C-8 shows a schematic diagram for SeleXtrac™ membranes, with the example showing how ionizable organic compounds can be extracted from a high-salt aqueous solution and concentrated in a caustic solution.

The use of functional polymers in SeleXtrac™ membranes provides the flexibility of altering the liquid physical properties (solubility, viscosity) by varying the polymer size (average molecular weight) without compromising the chemical functionality of the polymers. For example, polyglycols are available as polyethylene glycols (PEG), PPG, PBG and so on. The PEG series are not suitable as liquid membranes for treating aqueous solution since they are fairly water soluble up to MW of several million. In contrast, PPG-4000 in the polypropylene glycol series has been found to remain stable as SeleXtrac™ membranes between 20% KCl and 0.1N NaOH aqueous solutions, thus withstanding the tremendous osmotic pressure difference (data presented below). Supported PPG-1200, however, lost its

membrane integrity within a few hours between such solutions, i.e., allowing equalization of pH and salt concentrations between the two sides. PBGs being more hydrophobic and more viscous than PPG at the same MW were relatively stable as SeleXtrac™ membranes at MW down to 1000.

The SeleXtrac™ membrane concept is equally applicable to other hydrophobic liquid polymers possessing different separating capabilities [6]. Other polymeric liquids that have functioned well as SeleXtrac™ membranes include various grades of liquid silicone and its derivatives, liquid hydrocarbons, and liquid polybutadienes. Obviously, known liquid polymers can be chemically modified to alter the functionality, or new ones can be synthesized for a particular application.

Characteristics of Polyglycol SeleXtrac™ Membranes

Compound	Partition Coefficient (PPG/Aqueous)	Membrane Permeability, (10^{-4} cm/s)
Ethanol	0.2	0.07
Butanol	4.8	ND
Benzyl Alcohol	ND	13
Acrylic Acid	4.6	0.5
Butyric Acid	7.2	0.7
Valeric Acid	24.6	1.6
Caproic Acid	83	4.7
Benzoic Acid	110	4.0
Cinnamic Acid	178	6.0
Hydrocinnamic Acid	98	5.4
Ethyl Acetate	440	7.9
Ethyl Benzoate	511	8.7
Fumaronitrile	25	2.8
Benzonitrile	240	9.0
Cyclohexanone	4.3	0.6
Hydroquinone	34	0.9
Maleimide	6.5	0.5
p-Nitrophenol	200-450	3.4 to 8.1
Phenol	174	6.0
Chloro nitrophenol	230	6.0 to 14
Recorcinol	67	1.7
p-Nitroaniline	318	6.5
Toluene	525	ND
(Benzene, Xylene)		
Dichloroethane	43	4.4
Trichloroethylene	245	15.5
Tetrachloroethylene	549	>20

The concept of SeleXtrac™ membrane was first developed using polyglycols as the polymeric liquids. The polyglycol that has been tested extensively is polypropylene glycol with an average molecular weight of 4000 (PPG-4000), and to a lesser extent, polybutylene glycol PBG-4800) manufactured by Dow Chemical. Therefore, it is worthwhile to review the general properties of these SeleXtrac™ membranes. Each monomeric unit in PPG has the formula -CH2-CH(CH3)-O- in which -CH2-CH(CH3)- represents the hydrophobic group and the ether oxygen has the role of the polar group that can form hydrogen bonds (5). Due to its highly hydrophobic nature, PPG appears to form an effective barrier to charged species in solution. This has been shown to include both small ions from dissociation of inorganic salts, H+ and OH-, and larger charged molecules. Only neutral compounds can pass through. This characteristic forms the basis for the use of PPG SeleXtrac™ membranes to recover organics from an aqueous saline waste illustrated in Figure C-8. For effective separation across such a membrane, the strip solution should provide an environment (a “sink”) which limits the back diffusion of the organic across the membrane into the

Table C-1 Partition and transport of organic compounds with impregnated PPG SeleXtrac™ membrane.

feed solution. This could be accomplished in several ways including pH change, reaction, chemical complexation, biodegradation, pervaporation, etc.

Ions which have been shown experimentally to be effectively blocked by the PPG membrane include H+, OH-, Na+, K+, Cl-, NH_4+, NO_3-, and SO_4. Charged organic molecules are also effectively rejected by the PPG SeleXtrac™ membrane. Zwitterions, which by definition are ionized at any pH, such as amino acids, could not pass through the PPG membrane at all.

For neutral compounds, the rate of transport through the PPG SeleXtrac™ membrane is sensitive to their chemical structures. The rate of transport for a compound appears related to its ability to form hydrogen bond with the ether oxygens of PPG, and to its hydrophobicity. Table C-1 shows the partition coefficients and the transport rates for various compounds such as phenol, amines and carboxylic acids. The partition coefficient is defined as the ratio of the concentration of the organic compound in the polymeric liquid over that in water, thus an indication of the interaction between the polymeric liquid and the compound. Rates are reported as the overall mass transfer coefficient (cm/s), which lumps the mass transfer resistances of the two aqueous boundary layers on the sides of the membrane with the resistance due to permeation through the SeleXtrac™ membrane itself. In these experiments, the pHs of the feed solutions were such that the organic compounds were un-ionized (pH about 2 units below pKa) and NaOH was used on the recovery side for weak acids, and HCl or HNO_3 for weak bases. Note that all these compounds have quite high partition coefficients, indicating a high degree of affinity for PPG.

Table C-1 shows the partition coefficients and the corresponding mass transfer coefficients for various organics with a polypropylene glycol supported SeleXtrac™ membrane. As can be seen, ester compounds and higher alcohols both partition very favorably into PPG from water and have very high mass transport rates compared to ethanol. It is this observation that let to the exploration of the potential application of this membrane technology for enhancing the flavor of consumable liquids such as beers, wines, fruit juices and soft drinks.

Advantages of SeleXtrac™ Membrane Systems

SeleXtrac™ membranes with polyglycols appear to exhibit relatively rapid transport rates for many organics including phenolics, aromatic acids and amines, carboxylic acids, alcohols and esters. Attractive features of this membrane process include the ability to recover the contaminants in concentrated form for either recycle or more economical disposal, low pressure (ambient) operation, ease of regeneration, and simple scale-up using commercial hollow fiber modules.

Polymer	Film thickness (μm)	Effective diffusion coefficient, cm^2/sec
Polyethylene	14.1	0.11×10^{-7}
Polyvinylidene chloride	10.0	0.06×10^{-7}
Styrene-butadiene copolymer	41.0	0.71×10^{-7}
XD-7 (Silane)	68.6	2.06×10^{-7}
Ethyl cellulose	35.2	0.96×10^{-7}
PPG- Celgard 2400(b)	25.0	15.0×10^{-7}

Table C-2 Phenol permeability through various polymeric membranes

The magnitude and practical significance of the overall mass transfer coefficients obtained with the PPG SeleXtrac™ membrane shown in Table C-2 can be appreciated by comparison with other membrane approaches for phenol transport.

Klein et al [14] reported effective diffusion coefficient (D in cm/s = permeability x membrane thickness) for phenol extraction using various polymeric films as membranes. With the PPG-polypropylene membrane (25µm thick) the permeability of phenol is 6.0 x 10^{-4} cm/s (Table C-1), which gives an effective diffusion coefficient of 15 x 10^{-7} cm^2/s. This is almost an order of magnitude higher than the best rate achieved by these workers (D = 2.06 x 10^{-7} cm/s) with the membrane made from a polymethylsilane-polycarbonate copolymer.

Another interesting comparison is with the hollow fiber contained liquid membrane approach by Sengupta et al. [15]. In this approach, the shell side of a membrane module that contained two sets of intermingled hollow fiber bundles was filled with an organic solvent that functioned as the liquid membrane. The feed solution flowed through the lumen of one set of fibers, and the strip solution through the other. With methyl isobutyl ketone as the contained liquid membrane, they reported an overall mass transfer coefficient for phenol of about 3 x 10^{-4} cm/s. This is about half of the rate obtained with the PPG-SeleXtrac™ membrane, which, incidentally, would utilize all of the hollow fiber membrane area available for transport rather than only half of it as in the contained liquid membrane approach.

The PNP transport rates obtained with the polypropylene PPG SeleXtrac™ membranes also compare well with the transport of PNP through octanol-filled SeleXtrac™ hollow fiber membrane [13]. The referenced work, which used the membrane-supported solvent extraction approach with octanol as the extraction solvent, reported a mass transfer coefficient of 6.5 x 10^{-4} cm/s. This is within the range of 3.4 to 8.1 x 10^{-4} cm/s obtained with the PPG SeleXtrac™ membrane (Table C-1) The obvious advantage of the PPG SeleXtrac™ membrane is that the separation can be accomplished in one step combining extraction and stripping, thus avoiding the additional step of stripping PNP from the solvent such as the case with octanol.

Mass Transfer Theory and Rate Measurements

Mass Transfer Theory

In an extraction process using membranes, the three resistances to consider are the membrane resistance and the two boundary layer resistances. The mass transfer theory for extraction processes using SeleXtrac™ membranes is briefly summarized below.

The overall mass transfer coefficient, K_f, for steady-state operation is:

$$\frac{1}{K_f} = \frac{1}{k_f} + \frac{1}{k_m K_p} + \frac{1}{k_s K_o} \qquad (1)$$

Where:

K_f : overall mass transfer coefficient in cm/s
k_f : feed-side mass transfer coefficient
k_m: membrane mass transfer coefficient
K_p: partition coefficient of organic compounds between liquid polymer and feed
k_s: strip-side mass transfer coefficient
K_o: partition coefficient of organic compounds between strip and feed

For reactions on the strip side such as acid-base reactions), the permeating compounds undergo rapid irreversible reactions as they enter the strip solution. Thus Ko is very large and the resistance due to the strip boundary layer could be ignored. For flat sheet, the overall membrane coefficient is:

$$k_m = \frac{D_m \varepsilon}{\tau Z} \qquad (2)$$

Where:

D_m: diffusivity of the organics in the polymeric liquid, cm^2/s
ε: membrane porosity
τ: membrane tortuosity
Z: membrane thickness, cm

For hollow fiber membranes, an area correction is added since the membrane area and K_f are based on the outer diameter of the fibers

$$k_m = \frac{D_m \varepsilon}{\tau Z}\left(\frac{d_{lm}}{d_o}\right) \qquad (3)$$

The two parameters, K_p ad K_f, sufficiently describe each organic/liquid polymer membrane system. K_p values indicate the relative solubility of the organic compound in the liquid polymer versus the aqueous solution. Higher Kp values would favor the extraction of the organics from the aqueous solution and typically result in higher transport rates through the membrane.

As indicated above, the definitions for K_p and K_f are:

$$K_p = \frac{\text{Concentration of the organic compound in the liquid polymer}}{\text{Concentration of the organic compound in the aqueous solution}}$$

K_f is the overall mass transfer coefficient as defined in the following equation:

$$\text{Membrane Flux} = K_f (C_f - C_s)$$

Where:

Flux is in units of g compound per second per cm^2 membrane area,
K_f : cm/s
C_f: concentration of the organic compound in the feed solution, in g/cm^3
C_s: concentration of the organic compound in the strip solution, in g/cm^3

K_f shows how fast the compound permeates through the SeleXtrac™ membrane, which is a function of many variables such as temperature, membrane support thickness, pore size and porosity, the partition coefficient Kf and possibly the liquid boundary layers on either membrane surface. The values for K_f were determined using a small stirred cell under conditions that minimize the resistance due to the boundary layers. However, for fast transport rates ($K_f > 10^{-3}$ cm/s), the contributions of the liquid boundary layers may be significant. The significance of determining the K_f value for each system is that it can be used to estimate the total membrane area required for applications at any scale.

Transport Rate Measurements

STIRRED CELLS WITH FLAT SHEET MEMBRANES Transport measurements with flat sheet membranes were conducted using stirred cells. These cells are convenient for rapid membrane screening as well as for determining the effects of various operational parameters on transport kinetics. Stirred cells were made up of two glass compartments separated by a sheet of membrane and held together by a clamp. Liquids in the compartments were magnetically stirred. The cell can contain approximately 30 ml of fluid in each cell compartment. The surface area of the membrane in contact with the fluids is 8 cm^2 for the normal size cell. Figure C-9 shows the picture of the small stirred cell.

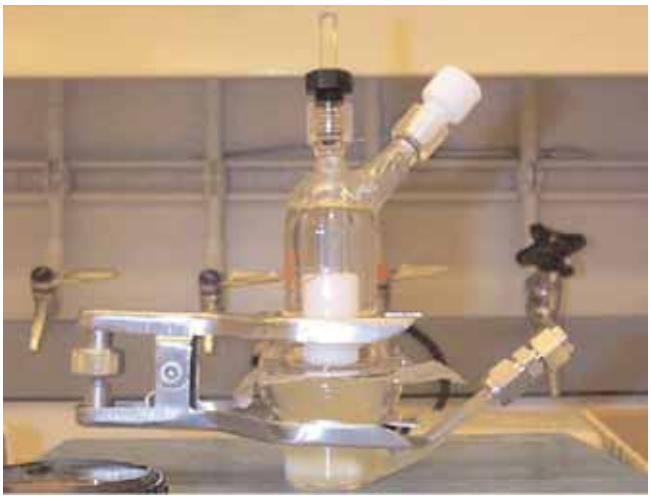


Figure C-9 Small stirred cell for transport measurements with flat sheet membranes

For stirred cells the following equation can be used for analyzing the transport data, allowing for back permeation of the solute from the pick-up side to the feed side:

$$\mathrm{Ln}\left[C_f\left(1+\frac{V_s}{V_f}\right)-C_{fo}\right] = -\frac{K_f A_m\,(1+V_s/V_f)}{V_s}\,t + \mathrm{Ln}\left[C_{fo}\,\frac{V_s}{V_f}\right] \qquad (4)$$

Where:

A_m: membrane area, cm^2
K_f: overall mass transfer coefficient in cm/s
C_f: solute concentration in the feed solution, mg/L, at time t
C_{fo}: initial solute concentration in feed solution, mg/L
V_f: liquid volume of the feed solution, mL
V_s: liquid volume of strip solution, mL
t : operating time, s

The plot of $\mathrm{Ln}\left[C_f\left(1+\frac{V_s}{V_f}\right)-C_{fo}\right]$ vs. Time can be fitted to a straight line whose slope is equal to and can be used to calculate the overall mass transfer coefficient K_f.

HOLLOW FIBER MEMBRANE SYSTEMS With hollow fiber membranes, fluids are circulated through the lumen side and the shell side. Organic concentrations of the feed and strip solutions are measured as a function of run time, from which the overall mass transfer coefficient can be calculated using the following analysis.

For hollow fiber membrane systems operating in a batch mode, the same equation as the stirred cell (equation 1) can be used provided that:

$$A_m K_f / Q_f < 0.1$$

Where:

Q_m: feed flow rate, cm^3/s

The term $A_m K_f/Q_f$ represents the ratio of the mass of material transported through the membrane to that passing through the module per single pass. For batch operation in small modules, whose membrane areas are low relative to the circulation rate, the change in feed concentration per pass through the module is very low and the whole system (feed reservoir and membrane) can be accurately treated as a perfectly mixed unit, therefore the applicability of equation 1. For large modules and with relatively low circulation rate, the removal per pass through the membrane unit can be significant and more complex mathematical model is needed for the analysis:

Key Variables

Effects of Polymeric Liquids and Permeating Molecules

Transport characteristics of the polyglycols SeleXtrac™ membrane regarding types of organics, effects of solid support, and type/molecular weight of the liquid polymer have been described elsewhere (17,18). A brief summary is given below.

Due to its highly hydrophobic nature, PPG forms an almost impenetrable barrier to charged species in solution. These include small ions (ions generated by salts, H+ and OH-) and larger charged molecules. Only neutral compounds can pass through. The driving force for transport includes concentration gradient, pH, chemical reactions, chemical complexation, biodegradation, etc.

Polar organics of intermediate solubility in water such as phenols, nitrophenols, nitroanilines, and benzoic acid exhibit quite high partition coefficients (>100) and relatively rapid permeation rates through the PPG SeleXtrac™ membranes (~ 4.0 x 10^{-4} cm/s or higher at room temperature). Partitioning and transport of several monobasic carboxylic acids (C2 to C6) through the PPG liquid membrane show that both the partition coefficients and the transport rates increase drastically with the number of carbons. This behavior is probably a consequence of the different solubility of these carboxylic acids in the PPG phase (hydrophobic interaction).

Effects of the number of hydroxyl groups on phenolic compounds were explored with resorcinol, hydroquinone, pyrocatechol, and phloroglucinol. More hydroxyl groups attached to the ring make the compound more hydrophilic, which results in lower transport rates. Also very interesting is the behavior of amino acids and its analogs. Being zwitterions, which are always ionized, amino acids, even the most hydrophobic ones such as tryptophan, phenylalanine and tyrosine, are totally rejected by the PPG membrane. Yet hydrocinnamic acid, which is phenylalanine without the amino group, permeates very fast (comparable to p-nitrophenol) through the PPG liquid membrane.

The above properties of SeleXtrac™ membranes make them very suitable for organic/salt separation as well as for separating similar organic compounds that may be difficult to achieve with other separation methods.

Effects of Membrane Supports

Effects of the types of membrane support and their structure on the rates of transport through supported PPG SeleXtrac™ membranes are shown in Table C-3. For the Celgard® flat sheet membranes, increase in NaOH concentration in the strip solution had no effects on the overall mass transfer coefficients (Celgard® 2400 and 2500). The mass transfer resistance due to the boundary layer on the strip side was thus not significant due to the rapid chemical reaction between PNP and NaOH. Doubling the membrane thickness resulted in half of the transport rate (Celgard® 2402). Permeation through the PPG layer was, therefore, likely the rate limiting step, partly due to slow diffusion through the viscous polymeric liquid and partly due to vigorous mixing in both compartments of the stirred cells. It is not clear why the Celgard® 2500 was twice as fast as the 2400 with only small increases in pore size and porosity based on the data from the membrane supplier. With the assumption that the overall mass transfer coefficients obtained with flat sheet membranes in stirred cells are practically the permeation rates of PNP through the PPG SeleXtrac™ membrane, PNP diffusion coefficient in the PPG layer can be obtained by (see Appendix)

Membrane Support	NaOH (moles/L)	Mass Transfer Coeff. (10-4 cm/s)
Celgard® 2400	0.1	3.4
	0.5	3.5
Celgard®2500	0.1	7.0
	0.5	7.0
Celgard® 2402	0.1	1.7
Gore-Tex® 0.02	0.1	3.4
Gore-Tex® 0.2	0.1	12.4

Feed: 4000 mg/L PNP in 20% KCl solution, pH = 4-5
Membrane: PPG-4000 in various membrane supports
Strip Solution: NaOH (room temperature)

Table C-3 Effects of support characteristics on PNP transport through PPG SeleXtrac™ membranes

$$D = (K_f / K_p) \cdot (l\,\tau / \varepsilon) \qquad (5)$$

Where:
l: membrane thickness
τ: tortuosity
ε: porosity

The different transport rates obtained for various flat sheet membranes could thus be due to the differences in their physical characteristics. For Celgard® 2400, l is equal to 25 mm or 25 x 10-4 cm and ε = 0.38. If we assume the tortuosity τ for Celgard® 2400 of about 2 [19], τ for Celgard® 2500 must then be 1.2 to account for the doubling in rate.. Similarly, the tortuosity for Gore-Tex® 0.02 µm would be 1.1, which is not unreasonable considering the very open structure of the Gore-Tex® membranes [20]. However, the tortuosity for Gore-Tex® 0.2 µm would be only 0.6 to account for the very high transport rate. For the tortuosity of this Gore-Tex® membrane to be 1, that of Celgard® 2400 must be at least 3.6, unless higher porosity and larger pore size also exerted some non-obvious secondary effects on the transport rates in PPG SeleXtrac™ membranes. Note that these comparisons assume that the pores of different membranes were completely filled with the liquid polymer, which was likely the case. It is clear from the results obtained in this section that characteristics of membrane supports such as material, thickness, pore size, pore structure, and porosity have strong effects on SeleXtrac™ transport rates.

Effects of Operating Conditions

Key operating parameters that impact the SeleXtrac™ performance are temperature, characteristics of the feed solution as well as of the stripping fluid (such as gas vs. liquid; pH, ionic strength, % organic solvent), and to some extent the circulating flow rates through the membrane module. Temperature has a very strong effect on the rate of permeation through the membrane, primarily due to the significant increase in the diffusivity as a consequence of lower viscosity of the polymeric liquid. The characteristics of the feed solution and the strip solution can affect the transport rates as well as the obtained selectivity. Since only neutral compounds can permeate the membrane at a practical rate, pH of solution can be adjusted to manipulate the charged characteristics of compounds in solution for the desired selectivity, either extracted from the feed into the liquid membrane or stripped from the membrane into the strip solution. Likewise, volatile compounds can be selected over less volatile ones by using gas as the stripping fluid instead of liquid. Subsequent reactions or physical removal (e.g., adsorption) of the permeating compounds provide another way to drive the transport process. Sufficient circulating flow rates through the membrane module are necessary to minimize the transport resistances due to the boundary layers at the membrane surfaces. Some of these manipulations are evident in the applications discussed below.

Process Applications

The SeleXtrac™ technology has been used successfully to remove and concentrate organics from several actual aqueous waste streams. We will describe the process application for removing p-nitrophenol, carboxylic acids, and methyl tertiary butyl ether (MTBE) from contaminated water.

Wastewater Treatment

Phenolics (p-nitrophenol) Waste

This waste stream was generated from a chemical manufacturing plant and typically contains p-nitrophenol (PNP) as the major organic contaminant at <1 wt%. The waste stream also contained high salt level (~20 wt%, mainly KCl). Existing treatment options for this stream includes PNP removal followed by destruction or biodegradation. The feasibility of using a SeleXtrac™ membrane process to remove PNP from the waste solution in a concentrated form for recycle was investigated.

The pKa value for PNP is 7.2. Thus at pH around 5 or lower, all dissolved PNP molecules will be in the neutral (non-ionized) state and partition strongly into the liquid membrane phase (Kp from 100 to over 500 with PPG-4000 as the liquid polymer). In order to concentrate the permeating PNP, the strip solution was held at alkaline pH (pH>10) to convert PNP into its ionized form, which was blocked from going back to the feed side by the hydrophobic liquid.

Various radial flow commercial membrane modules had been previously tested in the laboratory. Batch results obtained with 2.5 and 4 inch modules using a simulated PNP-containing feed solution were already reported (18). Here we described the results obtained with a 4 inch module tested at the plant using an actual waste stream.

A number of batch runs were first conducted to check out the physical integrity of

the PPG SeleXtrac™ membrane and the module performance regarding PNP removal. About 100 L of the PNP waste solution at pH 4 was processed per run. The same strip solution (0.5 N NaOH) was used for the whole campaign. The feed solution was circulated on the shell side at 11.4 to 45.4 L/min (equivalent to NRe of ~1 to 4), and the strip solution on the lumen side at a fixed flow rate of 9.5 L/min. Inlet pressure on the feed side was only 0.9 psi (6.2 kPa) at the highest flow rate, and was higher on the lumen side, 7.5 psi (51.7 kPa), which is still relatively low. The SeleXtrac™ module was found very effective for PNP removal. PNP concentration in the feed solution typically dropped from several thousand mg/L (ppm) to a few ppm in 1-2 hours. PNP concentration in the strip solution built up from zero initially to over 20,000 ppm after seven runs. As discussed above, extraction of the overall mass transfer coefficient, Kf, from batch data is quite complex and requires a great deal of approximation. However, rough estimates from the fit to the linear portion of the data show that Kf was not sensitive to flow rates in the tested range and was about 2.8 x 10^{-4} cm/s at 29°C and 4.5 x 10^{-4} cm/s at 41°C at an average PNP concentration of ~2000 ppm. These values are in good agreement with earlier laboratory results for a similar 4 inch radial flow module using clean, simulated PNP solutions. Note that the feed-side mass transfer coefficient ranged from 3.8 to 6.7 x 10^{-3} cm/s at room temperature, which is more than an order of magnitude higher than K_f.

Continuous runs were next conducted using the above module at a feed flow rate of 18.9 L/min (NRe ~1.7) and strip flow rate of 9.5 L/min. Steady state was typically achieved within 5 minutes of starting the feed flow. The degree of PNP removal from the feed solution through the membrane module remained constant throughout each run. The results obtained for two types of commercial fibers (X-10 and X-20) are shown in the table below. It is noting that PNP removal ranged from ~30 to over 50% for a single pass. This is quite impressive considering the relatively high flow rate of 18.9 L/min and the compact size of the module (4 x 28 inches). The feed (shell) side mass transfer coefficient kf at this flow rate was estimated to be 4.69 x 10^{-3} cm/s, from which the membrane coefficient km was found to be about 5.1 x 10^{-6} cm/s for the X-10 fibers, and 3.6 x 10^{-6} cm/s for the X-20 fibers. The higher km values for the less porous X-10 fibers are probably an artifact of the high PNP concentrations in the feed solution for the X-10 runs, which overestimates the drop of Kp in this high concentration range. The highest PNP concentration in the data used to obtain the correlation for Kp was only 3000 ppm (14).

Fiber Type	PNP in Feed, mg/L		PNP Removal per Pass	km x 10^6 cm/s	K_f at 2000 ppm PNP X 10^4 cm/s
Inlet	Outlet				
X-10					
(30% porous)	7000	5080	27%	4.8	9.5
	5068	3195	37%	5.5	10.6
X-20					
(40% porous)	2048	1212	41%	3.8	7.8
	671	342	49%	3.6	7.4

Table C-4 PNP removal from a waste stream

Note that the true membrane coefficient, kmKp, is over two orders of magnitude higher than km, since the partition coefficient Kp varies from 200 to 600 for the PNP/PPG system. At an average PNP concentration of 2000 mg/L, the overall mass transfer coefficients from these continuous runs were found (using Eq. 1) to be about 10 x 10^{-4} cm/s for the X-10 fibers and 7.6 x 10^{-4} cm/s for the X-20 fibers, which are substantially higher than the batch values obtained above and the values obtained earlier with simulated waste solutions (18). It is very encouraging that in the preferred continuous mode of operation and with an actual waste stream, the SeleXtrac™ membrane process performed even better than expected. Additional pilot testing is being conducted with various polyglycol polymers and fiber types that differ in pore size, porosity, and wall thickness to optimize the process with respect to both transport rates and liquid membrane/module stability.

Based on the rate data obtained with the PPG-4000 Celgard® X20-400 hollow fiber membrane, we estimated that a membrane area about 15,000 ft^2 is required to remove over 99.99% of PNP in a 15 gal/minute waste stream at 60°C (to get below the 200 ppb needed to meet the regulation discharge). On the basis of 6 to 10 dollars per ft^2 of membrane installed cost (21), the required membrane area translates into a maximum of $150,000 for the entire membrane module. This is contrasted with an alternate adsorption treatment using activated carbon, which costs from $500,000 to $1 million a year for the carbon. Since the membrane module is likely to last at least a year or so, the membrane process looks extremely attractive, especially if the recovered PNP from the waste could be recycled for some payback value. While PNP is a particularly attractive case, it should be emphasized that the ability to control rate through control of the membrane structure can permit the application of this approach to many cases where the rates based on existing membrane supports are much lower than for PNP.

Carboxylic Acids Waste Stream

This waste stream was generated from a chemical manufacturing plant. The organic content of the waste comprises mainly C2-C6 monobasic carboxylic acids (about 3800 mg/L organics or 2000 mg/L TOC). The stream also contains 1% nitric acid (pH 1.2) and trace metals and is at 60-70°C. Our study involved using the membrane process to simultaneously remove and concentrate the carboxylic acids from the waste solution to facilitate further treatment. At pH 1.2, all the carboxylic acids in solution are in their neutral (uncharged) state and would permeate through the membrane. Nitric acid and metal ions also present in the solution exist as charged species, hence can not go through the membrane. The development of the SeleXtrac™ membrane process to remove the organic acids leading to significant reduction in the TOC of the solution is described below.

Compound	Mass Transfer Coefficient (K_f, cm/s)	Partition Coefficient (Kp)	$K_f Kp$ x 10^5
Formic Acid (C_1)	0.055 x 10^{-4}	0.45	1.22
Acetic Acid (C_2)	0.10 x 10^{-4}	0.65	1.54
Propionic Acid (C_3)	0.33 x 10^{-4}	not measured	
Butyric Acid (C_4)	0.42 x 10^{-4}	7.2	0.58
Valeric Acid (C_5)	1.6 x 10^{-4}	24.6	0.65
Caproic Acid (C_6)	4.0 x 10^{-4}	83.0	0.48

Feed side: 0.1-5wt% acid in 20wt% KCl, pH = 2
Strip side: 0.1N NaOH
Room Temperature

Table C-5 Transport of Carboxylic Acids Through Celgard® 2400/PPG-4000 SeleXtrac™ Membrane

FLAT SHEET MEMBRANES Partitioning and transport rates of C1-C6 carboxylic acids were measured individually using the stirred cell with flat sheet membranes. The results (Table C-5) were obtained with the PPG impregnated membrane sandwiched between a feed

solution containing each organic acid and a 0.1 N NaOH strip solution to neutralize the acid. Note that the feed solutions also contained 20% KCl, which imposed a very high osmotic pressure difference across the membrane and would rapidly destabilize conventional SeleXtrac™ membranes employing low molecular weight organic solvents (12,13). The transport rates were found to correlate with the number of carbons in the carboxylic acids. Shown in Table C-2, caproic acid (C6) was the fastest followed by smaller acids in the order of size. K_f for caproic acid was actually over 70 times higher than for formic acid. The strong correlation between the overall mass transfer coefficients and the corresponding partition coefficients suggests that the membrane resistance was probably a controlling factor.

The relative rates of transport observed for the individual carboxylic acids seem to hold for the mixture in the waste solution with caproic acid permeating through the membrane first, followed by valeric acid and butyric acid. Because of this characteristic of the process, the rate of organic removal steadily decreased as its concentration in the waste solution dropped. It was found, however, that up to 90% organic reduction the transport process could be approximated by two average rates. Linearized plot of concentration vs. time gives two rate constants: one termed Kf initial is valid up to 50-60% organic removal, the other is K_f final and is good up to about 90% removal. K_f final is roughly half the value of K_f initial. This mathematical approximation allows the membrane area required for any degree of TOC removal to be estimated in a simple but accurate fashion.

Higher temperatures significantly enhanced the rate of TOC removal. This is an advantageous feature of the membrane process since the waste stream was at 65°C at the plant. Shown in Table C-6 for SeleXtrac™ (Celgard® 2500/PPG-4000) membrane, the rate went up 2.6 times as temperature increased from 25 to 65°C. The effects of temperature on the transport rates with SeleXtrac™ membrane were investigated in more detail by Ho et al. (16) with p-nitrophenol as the permeant. In that study, two opposing effects were found to occur with increasing temperature: the partition coefficient decreases but is accompanied by an increase in the diffusion rate through the membrane. Assuming the diffusivity is proportional to temperature and viscosity-1, as in the Wilke-Chang equation (22), the increase in diffusivity was almost seven-fold from 25 to 65°C, which can more than account for the 2.6-fold increase in the overall transport rate. This strong effect comes mainly from the drastic drop in viscosity of the polyglycol at higher temperatures.

Temperature °C	K_f initial ($\times 10^4$ cm/sec)	K_f final ($\times 10^4$ cm/sec)
25	2.4	1.4
47	3.1	1.7
65	6.3	3.5

Table C-6 Effect of temperature on rate of TOC removal using a SeleXtrac™ (PPG-4000/2500) membrane

HOLLOW FIBER MEMBRANES Several small modules (256 cm^2 membrane area) were prepared in our laboratory for testing with the CA waste solution. The ability of the hollow fiber membranes to remove the organic acids from the solution was shown with 10% sodium carbonate (soda) solution, pH 9.8, as the strip solution to neutralize the organic acids permeating through the membrane. At the end of the run, TOC in the waste had dropped from the initial value of 2000 ppm to about 50 ppm, equivalent to over 97% TOC reduction. Organic content in the soda solution built up to about 6%, which is equivalent to over 15-fold concentration.

A large module was purchased from a supplier for the scaleup study. It contained 9000 fibers (polypropylene X20-240), with a membrane area of 3.72 m^2, thus a scaleup factor of over 100-fold compared to the small modules. A total of eight runs were made with the large module, five at room temperature and three at 60°C. Two liters of fresh CA solution was used for each run. The same 2L of 10 wt% soda solution was used as the strip solution throughout the campaign. The strip flow rate was maintained constant at 2.65 L/min; its pH was 9.85 initially, dropping gradually to 9.22 after eight runs due to the accumulation of the permeating carboxylic acids. The pH of the feed solution hardly changed after each run (from 1.28 initially to 1.30-1.33) during which 92 to 94% organic removal from the feed side occurred. These pH results are consistent with neither nitric acid nor salts being transported through the membrane at any significant extent.

The effects of feed flow rates on transport rates at 25°C and 60°C were investigated. It is clear that for this large module the liquid film mass transfer on the feed side (lumen) was very efficient at room temperature with the maximum transport rate achieved at the Reynolds number of about 6. The initial mass transfer coefficient was, however, only about 0.3 x 10^{-4} cm/s, which is less than one half of that obtained with the small modules. This lower value probably reflects the poor liquid distribution on the shell side given the large number of fibers packed in this shell-tube configuration. More recent work with a different waste stream using radial flow hollow fiber modules gave much better scale-up results (16). The new radial flow design was introduced recently by a supplier for better fluid distribution on the shell side.

The transport rates increased substantially with temperature. At 60°C, the rates were 3 to 4 times higher than at room temperature (Kfi = 1 x 10^{-4} cm/s). This relationship seems to hold for the initial as well as the final mass transfer coefficient. With the large module, some transport of water through the membrane could be observed from the feed side to the strip side due to the osmotic pressure difference. At room temperature, a volume increase of about 50 ml was observed on the strip side over a three-hour period, which translates to a water flux of about 0.4 ml/hr-cm^2. The water flux at 60°C was about 0.9 ml/hr-cm^2. These relatively small amounts of water transport did not seem to affect the efficiency of the process.

PROCESS ECONOMICS CONSIDERATION Since this study was at an exploratory stage, no detailed cost analysis was conducted. However, we did look into the approximate capital cost. For membranes, the cost is based on the total membrane area required, which is inversely proportional to the overall transport rate. As presented earlier, the organic acid removal rate was quite rapid up to about 60%, followed by a slower rate up to about 90%. While removal over 97% was achievable, the final rate was much lower. Thus, the targeted organic removal has a major impact on the membrane cost.

Consider a waste stream with a flow rate of 385 gallons/min (1457 L/min) and a required 90% TOC reduction. Based on the highest mass transfer coefficients obtained (Celgard® 2500 at 65°C, Table C-3), a total membrane area of 150,000 ft^2 (about 14,000 m^2) would be needed. With approximately \$10/ft^2 membrane area for the largest membrane module (about 1500 ft^2) from Hoechst-Celanese (18), the capital cost for the membrane portion would be around \$1.5 million. For handling the soda strip solution (assumed ~ 6 wt% organics), a process option evaluated by the plant involved thermal destruction of the organics and recycle of the salts.

This would add another $1.2 million. Thus, the total capital cost would be around $3 million, considering additional auxiliaries needed. This cost was judged promising since it was about one half of the estimated cost for an alternate biotreatment process considered by the plant. Also, on the basis of $/gpd (gal per day of waste solution), the capital cost estimated for the SeleXtrac™ based process (~$5/gpd) seems to compare well with pervaporation, which ranges from $5-8/gpd for the easy cases (hydrophobic, volatile organics) to over $100/gpd for the more difficult ones (hydrophilic, water soluble organic mixtures) (4).

With the membrane cost constituting a major portion of the total capital cost, the membrane module's lifetime is obviously a major factor in the overall economics. Other key areas for step-change cost improvements include a) increasing the organic concentration in the strip solution, which reduces the liquid volume going into the thermal treatment step, b) recycling the recovered organics to regain some value rather than destroying them, which incurs additional cost, and c) selecting a polymeric liquid containing functional groups that exhibit much higher selectivity for carboxylic acids than PPG does, such as sulfoxide or phosphine oxide (6), thus substantially increasing the transport rates, especially for the smaller carboxylic acids.

Methyl Tertiary Butyl Ether (MTBE) Removal

The use of MTBE as a gasoline additive has generated a serious, widespread groundwater contamination problem in the US, especially in California. The primary source of contamination in groundwater is leaking underground fuel tanks (LUFTs). There are thousands of such sites in California, some resulting in nearby groundwater concentrations of MTBE as high as 6,000,000 ppb. Groundwater MTBE migrated from LUFTs to drinking water supplies. The current maximum contaminant level of MTBE in drinking water ranges from 5 ppb (recommended) to 13 ppb (acceptable).

In addition to the currently investigated technologies for removal and/or destruction of MTBE in groundwater, such as oxidation, air stripping and activated carbon adsorption, membranes could provide an effective means for this treatment. The key issue is clearly that of economics: the process has to be cost effective, as well as practical on a large scale. These organics have relatively high solubilities, low Henry's constant (23), low sorption and low biodegradability, such that common treatment technologies such as air stripping, granular activated carbon or bioremediation are not very cost-effective (24,25).

The diversity of the contamination situations, which range from levels at low ppb to thousands of ppm, will require combination of complementary technologies to achieve the best economical approach for each situation. Membranes could play a key role in this combined approach. For instance, activated carbon adsorption tends to be very efficient at treating organic contaminants at very low levels, since carbon binds tightly to many organics, and the lower the contaminant level the more water volume can be treated for the same amount of carbon. Membranes, however, tend to be more cost effective at percent or high ppm levels, because they work by removing a certain fraction of the initial contaminant level; the same amount of membrane area (thus the same cost) will be required to remove, say, 90% of the initial contaminant amount, be it 1% or 0.01%.

LIQUID POLYMER SELECTION Based on our previous work with existing commercially available liquid polymers, silicone 350 cst (Si-350) and polybutylene glycol

MW 4800 (PBG-4800) represent the two best ones for the removal of methyl tertiary butyl ether (MTBE) from contaminated water. These are non-toxic, have extremely low solubility in water, and have been shown to be stable as SeleXtrac™ membranes in contact with pure water. Quantitatively, two main parameters are typically used to characterize a new liquid polymer with respect to its utility in the SeleXtrac™ membrane configuration. One is the partition coefficient; the other is the transport rate. The partition coefficient K_p, defined as (MTBE in polymer/MTBE in water) at equilibrium, is a direct measure of the polymer's affinity for extracting MTBE from water. For both polymers at room temperature, the partition coefficient was found to be about 9, which is quite good considering the high solubility of MTBE in water.

For measuring transport rates, we used our standard stirred glass cell with a flat sheet membrane. This system allows tight control of the membrane preparation and the operating conditions, as well as accurate determination of the membrane area. The results are shown in Figure C-10. It is clear that MTBE accumulated more rapidly in the strip solution for the silicone membrane than for the PBG one. Figure C-10 also shows the fit of a mathematical model for extracting the overall mass transfer coefficient, K_f, from the concentration-time data. K_f is a quantitative measure of transport rate. As can be seen, MTBE transport rate with Si-350 is more than two times faster than that with PBG-4800 (K_f = 9.65 x 10^{-4} cm/s and 4.24 x 10^{-4} cm/s, respectively).

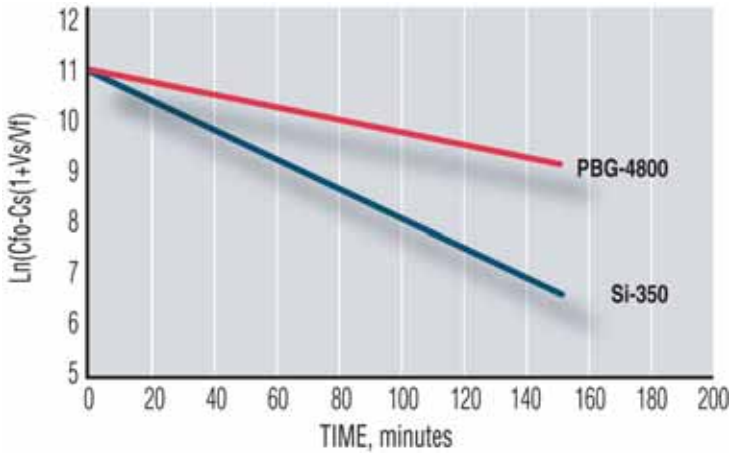


Figure C-10 Transport of MTBE across supported liquid membranes

HOLLOW FIBER MODULE TESTING With silicone selected as the liquid polymer of choice for this application, we next studied the use of SeleXtrac™ membrane in a process for MTBE removal from contaminated water. A SeleXtrac™ membrane hollow fiber membrane module (MiniKros® Sampler) whose small size and compact design makes it very convenient for laboratory testing was used. The hollow fiber SeleXtrac™ membranes are of ultrafiltration type, made of polysulfone and having a 400,000 molecular weight cutoff. The characteristic dimensions of this size module are shown in Table C-7.

Spectrum Module	*Active Fiber Length*	*12.3 cm*
MiniKros®, PS/400K	*Number of Fibers*	*288*
Shell/Tube	*Fiber ID*	*0.05 cm*
Configuration	*Fiber Wall Thickness*	*0.008 cm*
	Membrane Area	*680 cm^2*
	Membrane Porosity	*~ 0.5*
	Cross-Sectional Area	*0.565 cm^2*

Table C-7 Characteristics of Spectrum Modules Used

In order to assess the utility of SeleXtrac™ membranes for removal of MTBE from water, two membrane modules were used. One was a standard, uncoated, i.e. membrane pores empty; in the other, the pores were filled with Si-350 using a centrifugal method to apply the polymer to the membrane. About 2.75 g of silicone was loaded on the coated module. Studies using hollow fiber membranes (HFM) under pervaporation indicate that this is a viable option for removing MTBE from an aqueous solution (26).

Time (min)	MTBE in Feed (ppm)
1	799.7
5	739.7
7	703.7
11	638.7
17	571.7
25	487.7
38	357.7
47	292.7
56	240.7
67	188.7
81	139.7
102	85.5
138	37.5
190	11.6

Feed (lumen): 0.1% MTBE in water circulating at 430 ml/min;
Volume: 958.7 ml
Strip (shell): nitrogen sweep at 5 L/min

Table C-8 MTBE Removal with Silicone-Coated SeleXtrac™ Module

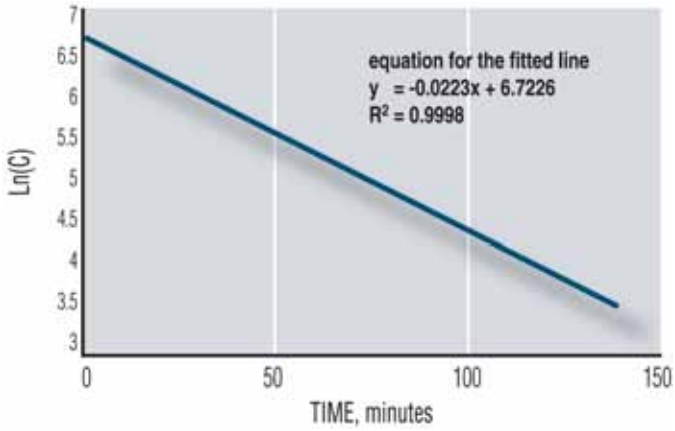


Figure C-11 MTBE transport with silicon coated SeleXtrac™ module

Shell Side N_2, L/min	Lumen Side Feed, ml/min	K_f, cm/s Silicone Coated	K_f, cm/s Silicone Uncoated
5	210	3.94E-04	2.6E-04
5	280	4.68E-04	
5	430	5.23E-04	2.6E-04
5	510	5.71E-04	
5	775		2.2E-04
5	1020	6.07E-04	

Table C-9 MiniKros® Sampler (PS 400K, 680 cm²)

The SeleXtrac™ membrane system was operated in a pervaporation mode, with a gas (nitrogen or air) sweeping on one side of the membrane and MTBE-containing water circulating on the other side. The water circulating flow rates were varied to determine the significance of liquid film mass transfer resistance versus the liquid membrane resistance. The gas sweeping flow rate was deliberately set very high to minimize the gas film resistance. Most of the runs were made with the water circulating through the lumens of the fibers, which represents a more well-defined fluid flow situation. A few runs were made with the water circulating through the shell side for comparison.

In a typical run, MTBE concentration in the water was monitored as a function of time at a certain circulating flow rate. A set of data for one run is shown in Table C-8.

Figure C-11 shows the excellent data fit using a simple transport model. The transport rate expressed as the magnitude of the overall mass transfer coefficient, K_f, for a particular run can be easily calculated from the slope of the fitted line.

Table C-9 summarizes all the results obtained for the two modules, one uncoated (control sample) and one coated (SeleXtrac™ test model), with water through the lumen. It can be seen that the transport rates clearly increased at higher water circulating rates, from about 4 x 10^{-4} cm/s at 200 ml/min to 6 x 10^{-4} cm/s at 1000 ml/min. This typically means that the liquid-side mass transfer resistance (as supposed to membrane resistance) is important in this flow regime and needs to be considered during scaleup. The transport rates started to level off above 500 ml/min, corresponding to a linear velocity of about 15 cm/s and a Reynolds number of about 80.

It is interesting that the uncoated module actually exhibited lower transport rates (2.2-2.6 x 10^{-4} cm/s) than the SeleXtrac™ module. This was unexpected since the presence of the liquid polymer instead of just open pores should retard the transport of MTBE through the membrane, which can occur by volatilization. We suspected that water vapor may have condensed inside the open pores of the un-coated

fibers, effectively creating a water film that MTBE had to diffuse through. Actually, based on an estimated MTBE diffusivity in water of 8.67 x 10^{-6} cm^2/s and the known membrane physical characteristics, the resulting mass transfer coefficient through a water-filled membrane would be 2.7 x 10^{-4} cm/s, which is quite close to the measured values. Incidentally, we also observed a significant amount of moisture collected in the tubing on the gas side during the runs with the un-coated module, but none with the coated ones. Thus the liquid polymer coating results in a double benefit; it not only forms a physical barrier to ensure separation between the contaminated water and the other side, but also unexpectedly enhances the overall transport rate by preventing possible condensation of water vapor in the membrane pores.

We have also tested earlier with two other modules in which water was circulating on the shell side and gas sweeping through the lumen. At the highest flow of 1000 ml/min, the obtained mass transfer rate ranges from 5.7 to 7.5 x 10^{-4} cm/s, which is comparable to the 6.1 x 10^{-4} cm/s shown in Table C-9. For well-engineered designs, such as radial flow or cross flow, the mass transfer on the shell side can be better than in the lumen. However, with the simple shell and tube configuration, the mass transfer on the shell side tends to drop significantly upon scaleup due to poor contact between the circulating liquid and the large number of fibers in big modules. This aspect will need to be carefully considered in the module design for the eventual large-scale operation.

A follow-up study by an independent investigator (Keller, University of California at Santa Barbara) evaluated the SeleXtrac™ membrane process with a Spectrum pilot module. The membranes used in this study were about 31cm (12.3 in.) long and the module has an overall external diameter of about 6.5 cm (2.5 in.). Based on the information provided by Spectrum Laboratories, Inc., each module contained 5,414 polysulfone (PS) fibers, with a molecular weight cutoff of 400 kD. The outside surface area is 3.5 m^2, while the inside surface area is 2.7 m^2. The fibers were arranged along the length of the HFM module and were coated with Si-350. The experimental setup was similar to that described above, with the MTBE solution flowing upward through the lumen side of the module and the sweep gas flowing counter-currently downward on the shell side.

An aqueous solution containing ~50-100 ppm MTBE was used as the feed solution and a pressurized or vacuum sweep gas passed through the shell side. Liquid flow rates were in the range from 0.5 to 8 L/min. The results indicate that MTBE can be readily removed from the solution using these membranes, and that K_f is a function of liquid and gas flow rates, as well as the overall operating pressure. The highest Kf (3.86 x 10^{-4} cm/s) was obtained under high vacuum conditions (0.23 atm), compared to 2.3-3.3. x 10^{-4} cm/s for the pressurized sweep gas or low vacuum conditions (0.73 atm).

Separation of Similar Organics

The SeleXtrac™ membranes can be used to not only recover but also separate organic compounds based on differences in their physical properties such as hydrophobicity, hydrogen-bonding capability, and degree of dissociation as indicated by their pKa's. The example here demonstrates the separation achieved between p-nitrophenol (pKa=7.1) and benzoic acid (pKa=4.25) based on the difference in the pKa's by varying the solution pH. The results are shown in Table C-10. As pure component at pH several units below their pKa's, these compounds

pass through the membrane at fairly high rates. As a mixture at a solution pH of 5.7, PNP exists in solution mainly in the non-ionized form whereas benzoic acid is practically all ionized. PNP was found in this case to readily pass through the membrane, as expected, resulting in about 99% PNP removal from the feed solution overnight. Benzoic acid, however, was virtually blocked by the membrane: overnight only trace amount of benzoic acid (<1% of feed concentration) was detected in the strip solution. It should be noted that this remarkable separation was accomplished in the presence of very high ionic strength (20wt% KCl), which would render ion-exchange methods practically inoperable for this separation. To further demonstrate the effect of pH and ionization on the transport rates, the same experiment was repeated but this time at solution pH of 2.6, which is below both pKa's. Overnight, both PNP and benzoic acid were virtually gone from the feed, having been transported completely to the strip side, and the measured transport rates for the two as a mixture were fairly comparable to those obtained with pure components.

Solution	pH	Membrane Permeability* Benz. Acid	PNP
Pure components	<pKa	4.0	4.7
Mixture (2000 ppm PNP, 1700 ppm Benz. acid, 20% KCl)	2.6	3.3	5.1
Same as above	5.7	0.0	5.97

* Membrane permeability unit: 10^{-4} cm/sec

Table C-10 Separation of p-Nitrophenol and Benzoic Acid

Flavor Recovery/Modification

General Approaches

Based on the transport characteristics of Spectrum's SeleXtrac™ technology, we identified three general ways that the technology can be utilized in the beverage/flavor area:

A. Selective transport of complex flavor profiles directly from a natural source (such as peels slurry or extract) into the liquid of choice (such as a finished beverage) across the membrane, thus bypassing many intermediate steps while preserving the natural complex flavor. We have demonstrated this in the laboratory with peels from lemon and grapefruit, as well as with wines and grape juice.

B. Selective removal of objectionable components from a finished product or flavor source. This applies to the removal of the vegetative flavor in O'Doul's discussed on page 101.

C. A tool for product development by food/beverage/flavor companies taking advantage of the membrane's capability to impart complex flavors into a target solution without carrying along other components like sugars, proteins, ions, etc.

The next section describes the work done on improving the flavor/taste of low-alcohol beers using approaches (a) and (b).

Low Alcohol Beer Applications

As mentioned above, polyglycol SeleXtrac™ membranes can transport esters very rapidly, much faster than ethanol. Other membranes that have similar properties are SeleXtrac™ silicone (polydimethylsiloxane) and dense silicone membranes, and asymmetric hydrophobic pervaporation membranes. With an appropriate pickup fluid (strip) on one side of the membrane in contact with a regular or concentrated beer on the other side, sufficient flavor components could pass through the membrane while little ethanol does, resulting in a strip solution that has full beer flavor but with little alcohol, essentially the desired compositions for a low alcohol beer (<0.4% ethanol).

BENCH-SCALE TESTS Stirred cells with flat sheet membranes were used for rapid feasibility test of the approach and evaluation of key operating parameters affecting the process. These include membrane type, temperature and feed beer type. For these feasibility tests the concentrations of key flavor compounds (e.g., aldehydes, esters and higher alcohols) in the treated solutions were used to judge the effects of various operating parameters. To be qualified as low-alcohol (or non-alcohol) beers, the final ethanol concentration has to be below 0.5%.

EFFECTS OF LIQUID MEMBRANE TYPE The SeleXtrac™ membranes used in this test were polypropyleneglycol (PPG, MW 4000), polybutyleneglycol (PBG, MW 4800) and silicone (polydimethylsiloxane, MW 12500). Michelob Light beer was used as the sample feed solution and HPLC grade water was used on the strip (permeate) side. Representative compounds of three types of organic molecules in the solutions were analyzed to determine their relative transport rates through the membranes: alcohols (C2 through C5), esters (ethyl acetate and amyl acetate), and an aldehyde (acetaldehyde). Table C-11 shows the results as the permeate to feed ratios for these compounds after 24 hr operation. A value of 1 indicates equal concentration between permeate and feed, i.e., equilibrium was reached. The lower the ratio for a compound, the slower its transport across the membrane.

Compound	Permeate/Feed Ratio		
	PPG	PBG	Silicone
acetaldehyde	0.4	0.1	0.4
ethyl acetate	1.1	1.1	1.1
isoamyl acetate	1.1	1.1	1.1
amyl alcohols	1.0	0.8	1.0
isobutyl alcohol	0.8	0.3	0.7
propanol	0.6	0.2	0.5
ethanol	0.3	0.1	0.2

Table C-11 Beer extraction with various SeleXtrac™ membranes.

It's clear that the two esters (ethyl acetate and isoamyl acetate) permeate very rapidly through these membranes, reaching equilibrium in less than 24 hr. Of the alcohols, the relative permeation rates are: C5 > C4 > C3 > C2 (i.e. amyl alcohol > butyl alcohol > propanol > EtOH). Only the amyl alcohol appears to have reached equilibrium after 24 hours. Acetaldehyde permeation rate lies between those of ethanol and propanol. These results show that the polyglycols and silicone membranes tested could be used for separating esters from ethanol.

EFFECT OF TEMPERATURE The relative transport rates of the organic compounds through the SeleXtrac™ (PBG-4800/Celgard® 2500) membrane as a function temperature were studied. Table C-12 shows the permeate/feed ratios for these compounds at 21°C and 4°C after 24 hr exposure. The lower temperature appears to enhance the selectivity of other components over ethanol. The drawback of low temperature operations would be slow transport rates.

Compound	Permeate/Feed	
	21°C	4°C
acetaldehyde	0.1	0.1
ethyl acetate	1.1	0.6
isoamyl acetate	1.1	1.3
amyl alcohols	0.8	0.3
isobutyl alcohol	0.3	0.1
propanol	0.2	0.03
ethanol	0.1	0.01

Table C-12 Permeate to feed ratios for key compounds in membrane-treated beers at two temperatures.

PILOT-SCALE OPERATIONS To enable the preparation of sufficient beer solution for taste testing, a large pilot unit was installed and operated at the A-B pilot brewery. The initial approach was to impart flavor components from regular beers into O'Doul's to improve its taste and aroma. This was done by contacting the O'Doul's with a regular beer separated by the SeleXtrac™ membrane. The operation was done in a batch mode, i.e., the two solutions were circulated across the membrane for various times. The second approach was to remove the objected "beany" or vegetative flavor from the O'Doul's. This was done by using just water on the other side of the membrane to pick up the bad flavor. In this case, the O'Doul's was fed continuously through the membrane module in a single pass while the water was circulated.

FLAVOR ADDITION APPROACH Several pilot runs were made in a second campaign in which the air introduction was minimized and several types of feed beer were used. Figure C-12 shows typical concentrations for various key components in the treated O'Doul's as a function of run time. As shown, levels of ester compounds and higher alcohols increased significantly while that of ethanol remained low. Based on a sensory testing with an expert seven-person panel, the treatment was found to add top notes to the O'Doul's. However, while one member gave the membrane-treated sample the highest score in preference tasting, the rest gave it rather poor scores in comparison to the control O'Doul's. Thus there was not a consensus whether the flavor-enhanced beer would be a better product, probably due to the complex interactions between the new flavors and those already in the O'Doul's.

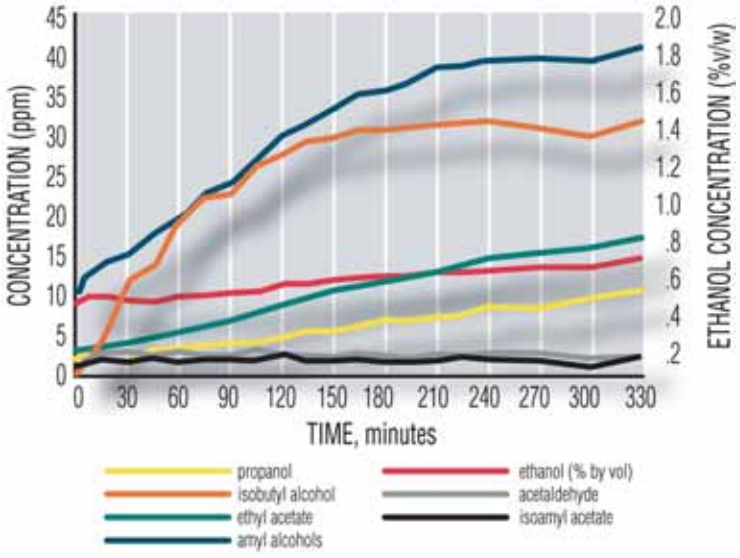


Figure C-12 Transport of key beer components from an English Ale feed beer across a treated hollow fiber membrane into O'Doul's® pickup solution

FLAVOR REMOVAL APPROACH The above approach had focused on improving O'Doul's flavor by selectively adding more "beery" flavor from a regular beer. However, it was also noticed that the objectionable "beany" flavor of O'Doul's was gone from the membrane-treated products. Apparently, while flavors were moving from the feed source across the membrane into the O'Doul's, there was simultaneous movement of the unappealing vegetative flavor in the opposite direction.

To test this hypothesis, the membrane process was operated with O'Doul's on one side and only carbonated water on the other side. With a feed solution pumped through the shell side of the module at about 500 ml/min and carbonated water as the pick-up fluid circulated through the lumen side of the module at a rate of 3 liters/min, a tasting panel found no beany note present in the treated O'Doul's. When the feed flow rate was raised to 1000 ml/min, however, the panel did detect some residual beany note in the treated O'Doul's. This simple process resulted in a significantly preferred O'Doul's with strong consensus from the panel.

Additional pilot-scale testing of various liquid polymers led to polybutylene glycol (PBG-4800) as the best for this application in terms of membrane stability in the cold room and effectiveness of the beany flavor removal. Unfortunately, this polymer has not been approved by the Food and Drug Administration for use in food processing. A PBG's close cousin (polypropylene glycol) and one silicone liquid (200 Fluid from Dow Corning) are approved for use in food processing, but PPG is not stable in contact with cold water (high solubility) and silicone is not effective for the vegetative flavor removal. Consequently, obtaining FDA approval of PBG-4800 for use in low-alcohol beer processing appears to be the only path for commercializing the membrane technology for this application.

PROCESS DESIGN AND COST CONSIDERATIONS Cost estimates for the SeleXtrac™ membrane process have not been done. One piece of relevant information is that the membrane cost for 50 modules is about $500,000 ($10,000 per module). So if the modules lasted only one year instead of the typical two to five years for this type of operation, the membrane cost alone would amount to an additional $1 per barrel of the beer produced. According to our A-B collaborators, an added cost of 1 to 2 dollars per barrel would be preferable, while $5/barrel would be too high. While we did not pressure test this criterion, it certainly indicates that the membrane process for this application can be economically viable. One major upside is that the membrane area required could be much lower than estimated based on the beer flow rate of 300 ml/min. Our modeling calculations and data obtained with other systems have shown that, at this low flow rate through the shell side, the liquid film mass transfer is severely limiting the overall rate. The correlation obtained for liquid transport on the shell side of Hoechst-Celanese radial flow modules (16) shows that the mass transfer coefficient varies to the 0.4 power of liquid flow rate: NSh = 1.28 NRe0.4Sc1/3. Thus, the transport rate can increase several fold by raising the flow rate from 300 ml/min to, say, 3 L/min or higher and stacking the modules in series to obtain the needed residence time.

Analytical Applications

General Methodology

To date, the analytical applications of SeleXtrac™ membrane technology are much less developed than process applications. However, based on the capability of SeleXtrac™ technology to selectively extract organic compounds from a complex mixture, it is not difficult to come up with many interesting and enabling analytical applications. The availability of many natural as well as synthetic polymeric liquids of diverse extractive properties and the ease of membrane preparation make SeleXtrac™ technology particularly attractive here. The general approach is to expose the complex mixture to the membrane, which extracts the compound of interest that can then be analyzed without the interference of the complex matrix. The analysis can be direct via a detector on the other side of the membrane, or through a sweeping fluid (mobile phase) that carries the extracted compound to the appropriate detector. Two specific examples will be discussed below, one for each type of detection.

Another potential usage of SeleXtrac™ membrane technology for analytical purposes is sample preparation. One could utilize the SeleXtrac™ technology to extract a particular organic compound from a complex mixture and concentrate it in the liquid polymer. The support matrix in this case can be membranes but could also be porous materials, porous beads or tubing. This mode of application is similar to the popular solid phase extraction (SPE) method utilized by analytical chemists. While not as robust as (SPE), one advantage of SeleXtrac™ membrane technology for certain applications is that the liquid polymer along with the dissolved organic compound could be completely removed from the support matrix with an appropriate solvent, thus accomplishing 100% recovery rapidly.

Direct Analysis in Complex Mixture

Detection of Phenol in Urine

This example covers the application in which small molecules are extracted rapidly from a complex mixture, possibly in concentrated form, so very low detection limits can be attained without the intermediate time-consuming sample preparation. The sample can be water, urine, blood plasma, or any liquid compatible with the liquid membrane. Contact time and pH of the sample can be adjusted to maximize the extraction of the organic compound of interest into the liquid membrane. Since ionic compounds will not be extracted by the liquid membrane, this method is effective for extracting metabolites from urine or blood plasma leaving proteins and salts behind. The injection liquid on the other side of the membrane can also be pH adjusted to maximize the extraction of the organic compound from the liquid membrane. This added separation increases the overall selectivity of the method.

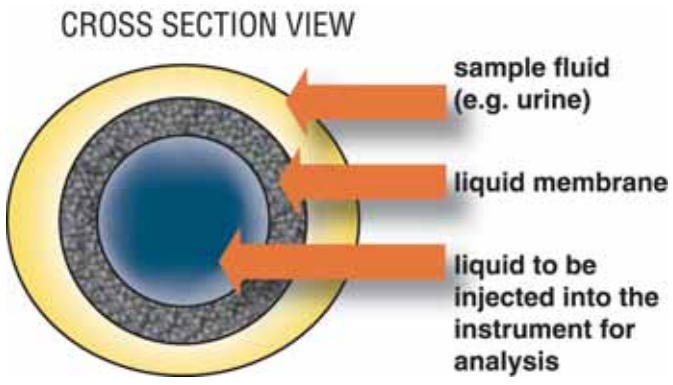


Figure C-13 Schematic diagram for direct analysis of a trace chemical component in a complex mixture

In the determination of phenol in urine as an example was demonstrated in principle using the stirred cell described in the experimental section. Urine solution at pH neutral or lower was in contact with a PPG-polyproylene liquid membrane and an alkaline solution (either NaOH or an amine buffered solution) was on the other side of the membrane. The experiment showed that phenol was selectively extracted into the alkaline solution without the accompanying proteins and salts. Phenol analysis was then done either with simple spectrometric light absorption or using HPLC. For an actual analytical set-up,10 ml of urine could be passing through a liquid membrane loop. The phenol getting into the liquid membrane can then be extracted into an HPLC 0.1 ml injection loop by the alkaline buffered solution, thus accomplishing both sample cleanup and concentration.

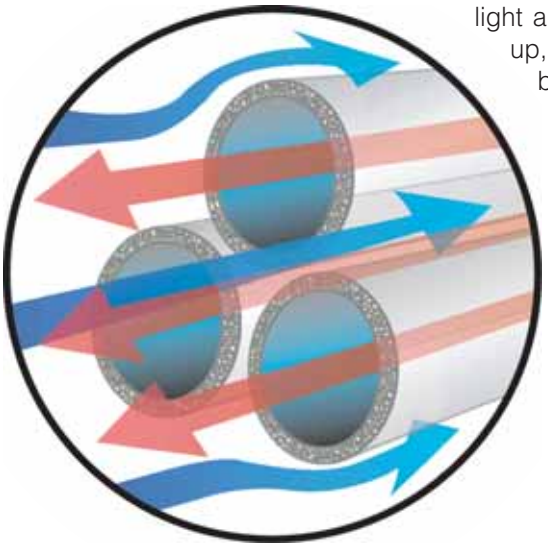


Figure C-14 Schematic diagram of HPLC setup for analysis with a liquid membrane sample loop

Over the past few years HPLC-mass spectrometry interface using electrospray techniques has become a powerful tool for combining the identification capability of mass spectrometry with the separating capability of HPLC. This has enabled analytical chemists to directly identify unknown compounds present in complex mixtures. An important limitation of this approach is the compatibility of the HPLC mobile phase with the electrospray process. The SeleXtrac™ membrane technology method could potentially solve some of these problems since the injection solution can have any buffer that is compatible with the electrospray process.

Field Detection of a Target Enzyme

In this example the presence of a particular enzyme in a complex mixture is detected by the action of the enzyme on an added substrate that releases a colored product. Normally the color generated will be either masked by the matrix or difficult to measure accurately. SeleXtrac™ membrane technology can be used to extract the colored compound from the matrix enabling more accurate detection.

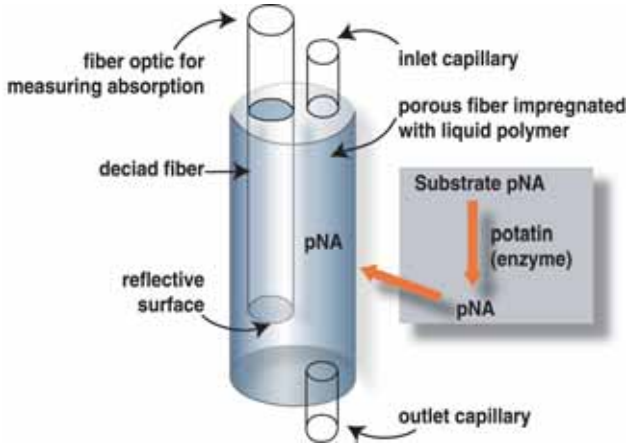


Figure C-15 Schematic diagram for detection of potatin directly in crude plant samples

Figure C-15 shows the schematic diagram for the detection of potatin, a tyrosine kinase in plants. A fiber optic probe is inserted in the lumen of a hollow fiber liquid membrane, with sample inlet and outlet capillaries at each end of the hollow fiber for introducing the strip solution. A chromogenic enzyme substrate such as peptide-paranitroaniline (P-PNA) is typically added to a field sample to detect the presence of potatin that would release the colored PNA from the peptide. The hollow fiber membrane, polypropylene impregnated with PPG, will extract PNA from the sample solution surrounding the membrane. The fiber optic probe encased in the hollow fiber can be used to detect and quantify the amount of PNA present in the strip solution, which correlates with the original amount of PNA released in the sample.

Biomedical Applications

There has not been any experimental work done in this area, even though the potential for various biomedical applications of SeleXtrac™ membrane technology has been recognized and discussed with several technical groups. The objective of this section is to briefly summarize the main thoughts for future reference.

In-situ Monitoring

Similar to the use of SeleXtrac™ for analytical applications, the possibility of extracting specific compound from a complex mixture to the other side of the membrane for detection leads to the idea of in-situ monitoring of the target compounds in animals or humans. One can envision a hollow fiber membrane device not unlike kidney dialysis unit but whose fibers are impregnated with the appropriate liquid polymer. Blood or urine can circulate through the lumens of the fibers and an appropriate strip solution on the shell side of the fibers will bring the picked-up target compound(s) to a detector for the analysis. Thus the detection is not intrusive and can be both quantitative and continuous. The possibility of detecting phenol present in urine has already discussed in the analytical section. Similarly, phenol or other metabolites of drugs or drugs themselves in the blood stream could, in principle, be monitored continuously after injection or taken orally.

Detoxification

Whereas kidney dialysis simply allows the rather nonselective removal of ions and small molecules from the blood stream, SeleXtrac™ devices could be used to selectively extract larger and more complex organic compounds that are considered toxins in the blood stream, thus accomplishing the detoxification objective using the same procedure. In tandem with kidney dialysis, SeleXtrac™ membranes can lead to treatment of diseases that is not currently possible.

Controlled Delivery

The controlled delivery idea comes from the recognition that the liquid polymer supported in a solid matrix can store certain drug compounds that release slowly into the surrounding blood or tissues. Or the liquid membrane can act as a selective barrier that meters the drug at a controlled rate to the body.

Acknowledgement

The development of the SeleXtrac™ membrane technology received great intellectual as well as financial support from many organizations and the author's colleagues. In particular, the author would like to acknowledge his colleagues: Drs Philip Brodsky and Philip Needleman for their enthusiastic management support, P. Wayne Sheridan for his first-rate technical support and collaboration throughout the development effort, and Drs. Robert Orth and Leanna Levine for their contributions to the analytical applications development. Great appreciation is extended to the late Dr. Alan Michaels for his invaluable intellectual contribution to the discovery, understanding, and development of this technology. Lastly, the interest and technical collaboration from Spectrum Laboratories have made a difference in solving numerous practical problems associated with the commercialization of this technology.

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The ABCs Of Cell Culture

Bioreactor Design

Introduction

Most pharmaceutical fermentation products are derived from bacteria and fungi grown in stirred tank bioreactors ranging in size up to tens of thousands of liters. Most of these older processes employ microbial cells.

However, many products can be produced by mammalian cells. Mammalian cells are significantly different and much more difficult to grow than microbial cells.

Some of the disadvantages of using mammalian cells include: slow growth, sensitivity to shear and interfacial forces, complex nutritional requirements and anchorage-dependency.

With the advent of recombinant DNA techniques and the emergence of the biotechnology industry, new technology has had to be developed to meet the requirements of these new processes.

The most successful developments have been the manufacture of monoclonal antibodies by *in vitro* mammalian cell culture using hollow fiber membrane modules.

Monoclonal Antibodies

All mammals produce antibodies as part of their immune response system. Antibodies are also called immunoglobulins (Igs). They are large glycoprotein molecules composed of polypeptide chains linked by disulfide bonds. Five main types of circulating antibodies are known: IgG, IgA, IgM, IgD, and IgE.

IgGs are the most abundant immunoglobins in human serum. They provide defense against bacteria, viruses, and bacterial toxins. The molecular weights of IgGs are about 150,000 Daltons.

Monoclonal antibodies are produced commercially in large volume bioreactors.

The major uses of monoclonal antibodies are for:

in vivo diagnostics to detect human and animal health problems; contamination of foods, drugs, or water; and the presence of plant pathogens

Therapeutic drugs and drug delivery agents

Purification of high-value molecules by affinity chromatography

Miscellaneous diagnostics or catalysts

Bioreactors

A large number of technologies and types of bioreactors have been developed to address the problems associated with growing mammalian cells. These include modified stirred tanks (including airlift); porous and nonporous microcarrier materials; entrapment by microencapsulation techniques; fluidized beds; porous ceramic matrices and membrane bioreactors. All have been used in applied research and process development and some have been scaled-up for production.

As discussed above, with the advent of recombinant DNA techniques and the emergence of the biotechnology industry, it was assumed that production of the new class of pharmaceuticals would use these same bioreactors. When the first recombinant products were produced in bacteria, it was discovered that many of

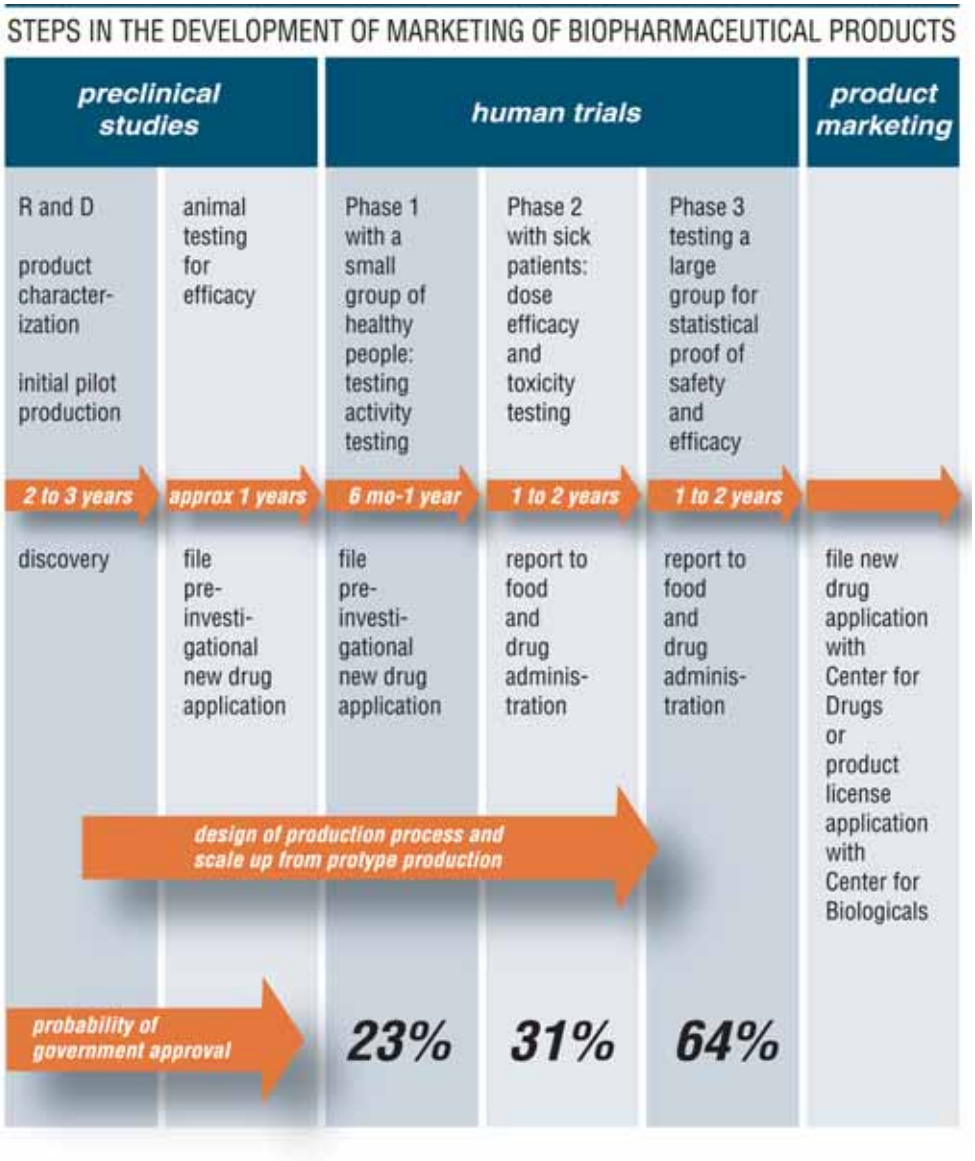


Figure D-1 Biopharmaceutical Development Steps

them were not biologically active. This has led to the development of new technology for mammalian cells.

Bioreactors can be generally classified into two categories: batch systems and exchange systems.

Batch Bioreactor Systems

The simplest form of bioreactor is the single batch-fed type in which cells are inoculated into a growth chamber in a fixed volume of culture medium. The cells are allowed to expand over time to a desired level and then the cells or cell product(s) are harvested. With single batch fed systems, there is no culture medium exchange from the start-up to the termination of the culture.

The single batch fed bioreactor is usually simple to operate, and because of less manipulation steps poses relatively low risk from contamination. However, the method often gives less than optimal cell expansion due to nutrient deprivation, and the accumulation of inhibitory catabolic waste products, such as ammonium ion, lactic acid and other growth inhibitors. Often there is inadequate oxygenation- and pH control, which adversely affect the quality and quantity of harvested cells.

Exchange Bioreactor Systems

Another type of bioreactor operation is one in which nutrients consumed by cells as they expand are replenished by the exchange of nutrient-depleted culture medium with fresh culture medium. Medium exchange may be accomplished by either sequential batch exchange or continuously.

For both the batch and continuous exchange modes, medium exchange is best performed when the growing cells are confined in a small compartment of the system, with the bulk medium perfused either unidirectional or by recirculation from a large medium reservoir. The restriction of cells to a small volume of medium, but allowing them access to the total nutrient available in a large volume of perfusing culture medium in the bioreactor is best achieved by the use of semipermeable membranes. A greatly simplified schematic of such a system is shown in Figure D-2.

Since operation in the continuous mode allows the continuous diffusion or bulk transfer of medium and products between the perfusing medium compartment and the cell compartment, the efficiency of exchange is highest when there is maximum membrane surface area per unit volume across which medium and products can flow. Referring to figure D-2, cells are gently pumped through the inside diameter of the hollow fiber membrane tubes. Cells are retained in the lumen of the membranes to be recirculated back to the reservoir. Spent media, secreted proteins and unwanted metabolites permeate the membrane structure and enter the shell side of the module to be harvested. As media is depleted, fresh media is added to maintain bioreactor volume.

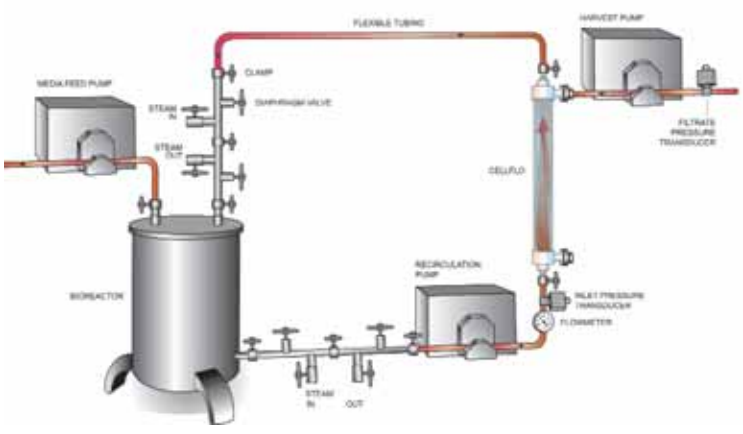


Figure D-2 Schematic of exchange type bioreactor system using Spectrum's CellFlo Module

Bioreactor Design Considerations

The main components of a continuous exchange system using hollow fiber membrane modules include the reservoir, pumps, valves, the gas exchanger and the module.

The Hollow Fiber Module

Several factors must be considered in the design of the membrane module. These include:

The pore size of the membrane

Other physical characteristics of the membrane, such as lumen diameter, length, etc.

The ability to support attachment of anchorage-dependent cells

Ability to sterilize the module

Biocompatibility of the materials used

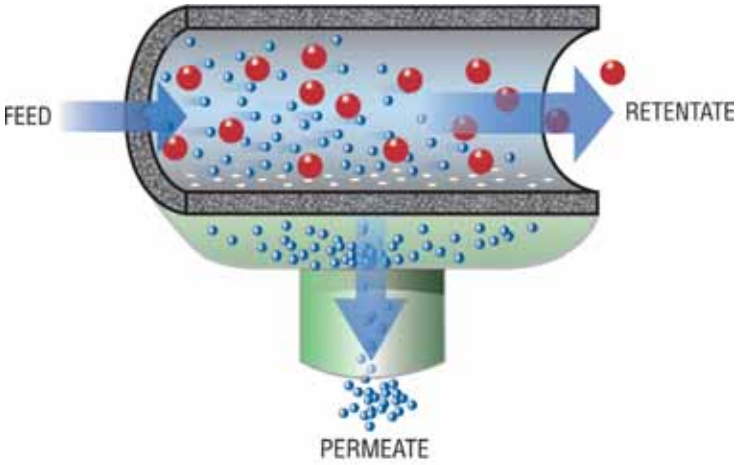


Figure D-3 Schematic of a hollow fiber membrane showing cross flow filtration

The latter four characteristics above are established in the basic design of the module. However, a variety of membrane pore sizes are available to fit the needs of the user.

As shown in figure D-3, the medium flows through the lumen of the hollow fibers that separate the cells from the medium. Nutrients and oxygen are supplied from the perfusing medium via diffusion and/or bulk transfer through the wall of the hollow fibers. Products produced by the cells traverse in the opposite direction. The membrane wall material and its porosity will therefore greatly influence how well cells expand in the module.

The Gas Exchanger

An adequate supply of oxygen is critical for proper growth and productivity of mammalian cells. Most commonly, commercial production of products from mammalian cells is carried out in stirred tanks that were originally designed to support bacteria and yeast. Oxygen delivery to these stirred tanks is usually accomplished by sparging directly into the liquid media. The combination of vigorous stirring and gas sparging will deliver adequate levels of oxygen to bacteria and yeast.

Mammalian cells, however, do not have a cell wall and usually cannot withstand the same levels of stirring and sparging. Also, serious problems with foaming can occur in sparged cultures containing serum or high levels of proteins.

In addition oxygen demand increases significantly in perfusion mammalian cell culture over that for bacteria and yeast.

The Spectrum CellGas™ module is designed as an internal gas exchange device to be used for increased oxygen demand and to eliminate foaming and shear related problems characteristic of sparging.

CellGas™ is a modified hollow fiber module without the “shell” of the standard hollow fiber module. When mounted properly within the bioreactor, it allows for maximum exposure of the gas exchange surface of the hollow fibers to the liquid contents of the bioreactor.

A more detailed description of CellGas™ is included in a later section.

The Pumps

The pumping device, other than its output capacity, must generate very low heat, yield no leachables and be autoclavable in the parts exposed to medium or product.

The Medium Reservoir and Tubing

The most important considerations regarding the reservoir and tubing is that they be constructed of material that does not yield leachables into the culture medium and are autoclavable.

Sterilization

Sterilization by autoclaving is generally preferred over other methods. However gas sterilization (ethylene oxide) or irradiation may be used.

Conversion of Existing Batch Systems to Continuous Perfusion Systems

The long history of pharmaceutical production in fermenters and a lack of bioreactors that are custom designed for mammalian cells, has led to interest in converting existing stirred-tank bacterial fermenters into mammalian cell bioreactors.

Most microbial fermenters are designed for batch operation. Batch operation of a microbial fermenter is economical due to high cell densities (>10^9 cells/ml), rapid doubling times (apx. 20 min for many bacteria) and to the hardy nature of the cells, which allows the use of aggressive agitation and gassing. Typical microbial batch operations last 5 to 10 days. In this short period of time, vast quantities of product can be produced.

Mammalian cells, on the other hand, grow to densities 10-1000 times lower than bacterial cells, even under optimal conditions. They have doubling times in the range of 18-36 hours, much more complex nutritional requirements than microbial cells and they are far more sensitive to minor perturbations in their environment. A mammalian cell culture operated in a 5 to 10 day batch mode will reach reasonable densities only near the end of the run, so product yields are generally low. Variations of batch operation (such as fed-batch or semi-continuous batch) can improve the run length, cell density and productivity, but are still far from optimal.

Therefore, optimization of bioreactor designs and culture processes for mammalian cells has become a high priority within the biopharmaceutical industry. Optimization efforts share common goals: maximizing cell density; cell viability; productivity per cell and culture length. To achieve these goals, all aspects of the culture process must be examined and carefully controlled. Factors to be considered include: cell line construction; culture media formulations; feeding strategies; bioreactor system design and process control strategies.

There are three compelling reasons to convert bacterial fermenters into mammalian cell bioreactors:

There is a long history of use of stirred tanks and their design and operation are well understood.

There are a large number of fermenters being used for bacterial production that can be converted to mammalian cell production, resulting in enormous capital equipment savings.

Many custom designed mammalian cell culture bioreactors have been reported as being non-scalable for use as production systems.

Modifications to existing fermenter designs to convert them to mammalian cell bioreactors include tank design, impeller design, impeller drive design, gas transfer system design and pH and DO controller designs. In addition to hardware designs, changes sometimes are made to the culture process itself.

Optimization of mammalian cell production requires a high degree of control over the extracellular environment and the ability to run batches for several weeks. Control of the extracellular environment is possible with continuous perfusion culture. In perfusion culture, fresh media is continuously delivered to the cells and waste products are removed. Under these conditions mammalian cells grow to densities as much as ten-fold higher than in batch or fed-batch culture, leading to proportional increases in production.

The higher cell densities also result in reduced serum dependence, thus lowering operating and purification costs.

Available Options for Conversion to Perfusion Operation

A chemostat-like environment can be achieved by simply pumping the contents of the bioreactor to a harvest bottle while also feeding, however this technique removes cells and dilutes the culture, severely limiting the cell density and thus, total production. The optimal continuous perfusion process is one where the cells are retained within the bioreactor, while fresh media is added and waste media is removed, allowing higher cell densities and production.

Retention of cells within the bioreactor requires a separation process of some type. Methods for converting fermenters to continuous perfusion bioreactors include the use of disc filters, spin filters, flat sheet filters and hollow fiber filters. Due to the cell density and the presence of cell debris, filters used for continuous perfusion clog or foul after a period of time. As a result, in order to maintain continuous perfusion, the filter must be replaceable while the system is operating.

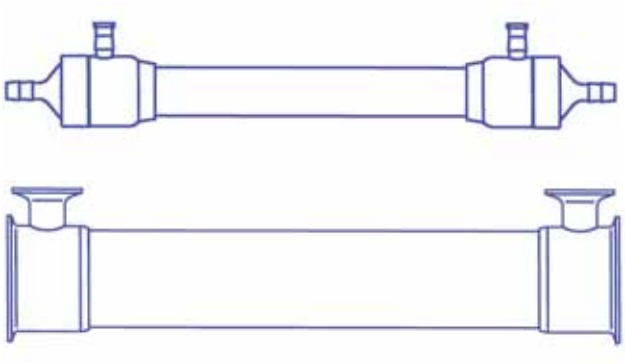
A perfusion filtration system can be mounted inside or outside the bioreactor.

Spin filters and hollow fiber filters have the proper dimensional characteristics to fit within most bioreactors. Flat sheet and hollow fiber filters can be mounted outside the bioreactor and connected to the bioreactor via an external recirculation loop. There are advantages and disadvantages to both types of setups. Placement of the filter inside the bioreactor eliminates the need for pumps to move the bioreactor contents through the filter and return them to the bioreactor. The disadvantage of this setup is that there is no access to the filter once the culture is started. If there are any problems with the filtration setup (such as clogging), the bioreactor must be shut down, ending the production run. In addition, spin filters are specialized devices that require modifications to the impeller and headplate of the bioreactor that can require custom engineering.

External filtration setups on the other hand, connect to the bioreactor through existing ports and do not require specialized hardware. They do require that the cells be pumped through an external loop. The major advantage of an external perfusion filter is it can be changed if it becomes clogged (or other problems occur), allowing long-term continuous operation.

Optimal performance of a perfusion filtration system requires efficient retention of cells within the bioreactor, a convenient filtration system that can be readily changed when it becomes clogged and a membrane porosity sufficiently large to allow removal of the desired secreted product as part of the perfusion process. Low product residence time minimizes exposure to proteases, reduces the potential for feedback inhibition and improves overall product stability. Of the various filtration options available, microporous hollow fiber filters best meet the requirements for optimal performance of a perfusion filtration system. They have a high filter surface area to volume ratio, are compact, easily incorporated into a fluid loop configuration and can be changed during a run if necessary. A filter with 0.2 um to 0.45 um pore size will allow high flux rates, large volume throughput and the continuous harvest of spent media containing cell-free product. Since the harvest is cell free, the initial cell separation step is eliminated, thus, simplifying downstream purification. In addition, a continuous cell-free harvest is compatible with existing continuous affinity purification techniques.

CellFlo™ Technology for Cell Recycle Perfusion of Animal Cell Bioreactors



Process volumes > 10,000 liters.
Diameters: 18.5 to 70 mm
Total Lengths: 276 to 689 mm

Figure D-4 Schematic of CellFlo™ hollow fiber cross flow module used for production scale process volumes and continuous external loop, cell-recycle perfusion of deep tank reactors.

Spectrum has developed the CellFlo™ line of hollow fiber cell separators specifically for the conversion of stirred or airlift bioreactors to continuous perfusion. CellFlo™ modules utilize large internal diameter membranes, allowing recirculation of cells without damage and reducing plugging with cell debris.

Manufactured from non-cytotoxic USP XXI class VI materials, Spectrum CellFlo™ modules are supplied non-pyrogenic by LAL testing. They can be sterilized by autoclaving and are priced to be disposable.

Single use disposability eliminates the costs and risks associated with cleaning and rinsing, simplifies validation and ensures consistent optimum performance and protein passage.

CellFlo™ modules are available in a variety of surface areas from 800 cm^2 to 3.3 m^2.

Results Obtained Using CellFlo™ Hollow Fiber Cell Separators for Converting Bioreactors to Continuous Perfusion

Recent comparisons of fed-batch versus continuous perfusion operation of a 40L-airlift bioreactor for the production of monoclonal antibodies showed several differences between the two modes of operation. Both the product concentration and daily production increased 5-7 fold in perfusion culture (figures D-5 and D-6). Cumulative production also increased proportionally (D-7). In perfusion culture, the production per liter of media consumed was 2-5 fold higher than in batch culture, thus reducing the overall media cost per gram of product produced (Figure D-8). In addition, the ability to produce 5-10 fold more product from the existing bioreactor reduced the length of the production run required to produce a given quantity of product. Perfusion can also reduce capital equipment costs by extending the capacity of existing bioreactors and reducing the size of new bioreactors.

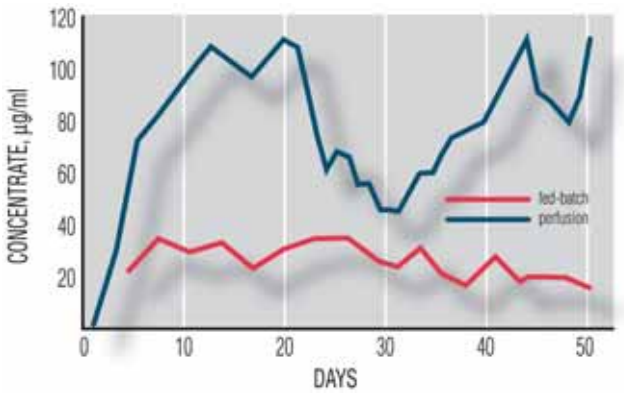


Figure D-5 Comparison of antibody concentration in continuous perfusion reactor vs. fed batch reactor

Although there are a wide variety of different bioreactors being used for mammalian cell culture, the setup and operation of a perfusion loop does not differ significantly from one bioreactor type to another.

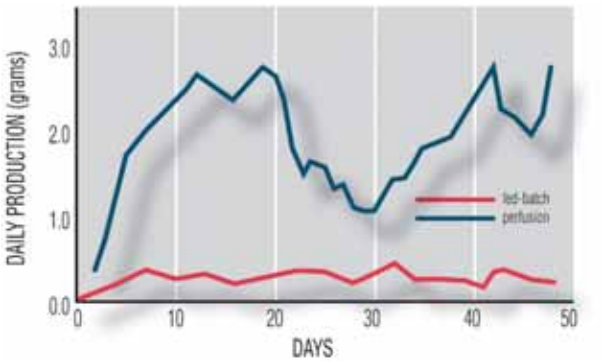


Figure D-6 Comparison of antibody production in continuous perfusion reactor vs. fed batch reactor

Converting a Batch Bioreactor to Perfusion Using CellFlo™ Cell Separators

Virtually any stirred or airlift bioreactor can be converted to perfusion operation using CellFlo™ cell separators. The conversion process and the materials used differ slightly from one reactor type to another and with increasing bioreactor size. The essential components required are three fluid metering pumps, a CellFlo™ cell separator, tubing, valves and connectors. The hardware setup and connections differ somewhat between systems and with or without a steam source.

Although there are several different options for operation, a basic system is presented that illustrate the general operation of the CellFlo™ conversion system.

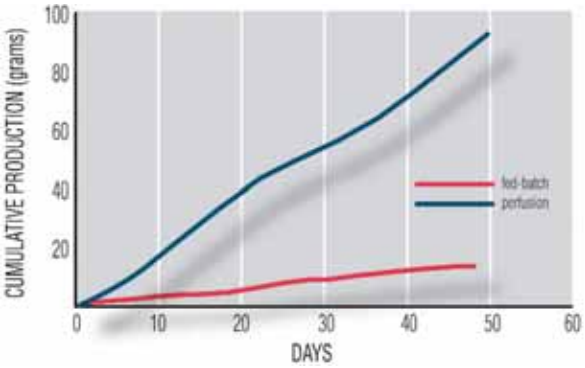


Figure D-7 Comparison of cumulative production in continuous perfusion reactor vs. fed batch reactor

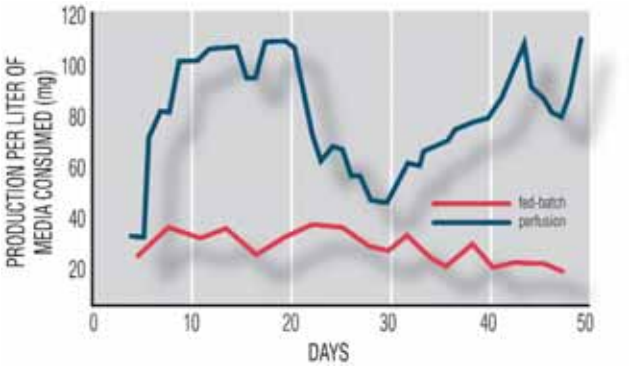


Figure D-8 Efficiency of continuous perfusion reactor vs. fed batch reactor in terms of production per liter of media consumed

Bioreactor with Steam Supply for Sterilization – The Basic System Setup

A basic system setup is illustrated in figure D-9. The essential components are the bioreactor, tubing for recirculation, the CellFlo™ module, sanitary valves for aseptic connection of the loop to the bioreactor, a media feed line, a harvest line and three pumps. The system illustrated is one of numerous possible setup configurations, depending upon the needs and desires of the end-user.

This setup was designed to meet GMP requirements and to simplify the setup and on-line changing of CellFlo™ modules.

The setup shown in figure D-9 provides maximum flexibility in the connection of the perfusion loop and for changing of CellFlo™ modules during a continuous culture. As detailed in figure D-9, the dual valve sets at the outlet and inlet to the bioreactor permit sterile changing of the entire perfusion loop, including all tubing. The dual valve sets at the inlet and outlet of the CellFlo™ module permit sterile replacement of just the CellFlo™ module.

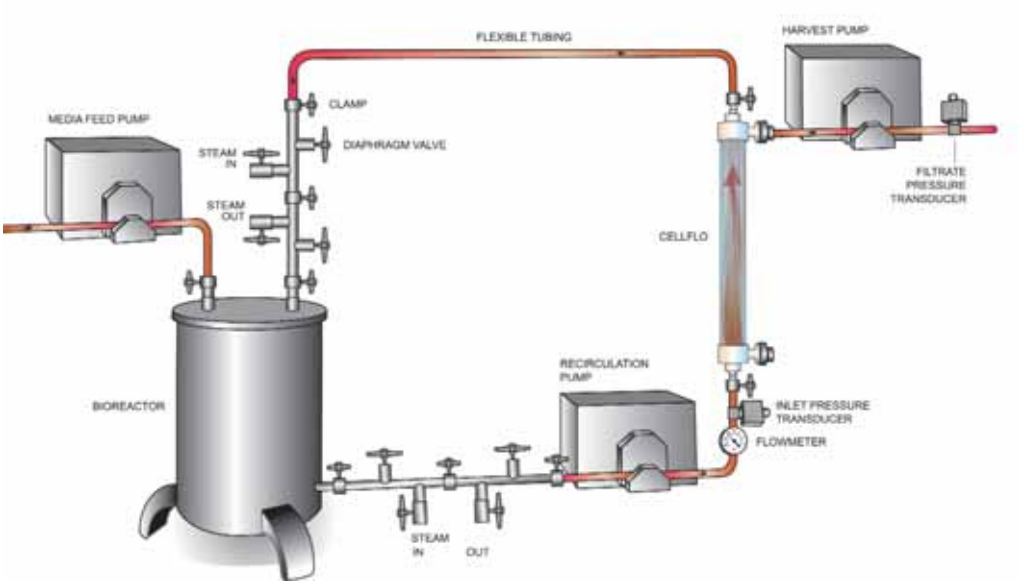


Figure D-9 Schematic of continuous perfusion reactor using the CellFlo™ module

Component Parts of the Basic Bioreactor System Design are listed below:

1. BIOREACTOR. Any stirred or airlift will do. Bioreactor size will affect the size of the tubing and the CellFlo™ module used but not other components.

2. DIAPHRAGM VALVE. Sanitary valve with access port for steam inlet.

3. SMALL DIAPHRAGM VALVE. Valve for steam inlet or outlet at the connection point.

4. DIAPHRAGM VALVE. Second valve to serve as a sterile disconnection point for the entire perfusion loop.

5. SMALL DIAPHRAGM VALVE. For steam inlet or outlet.

6. FLEXIBLE PUMP TUBING (suitable for extended operation).

7. FLOWMETER with digital readout (optional-to monitor the recirculation rate.)

8. DIAPHRAGM VALVE. For on-line sterile connection of replacement CellFlo™ Modules.

9. SMALL DIAPHRAGM VALVE. Steam inlet outlet for sterile connection.

10. DIAPHRAGM VALVE. Second valve for sterile connection of replacement CellFlo™ Modules.

11. SMALL DIAPHRAGM VALVE. Second steam inlet outlet valve.

12. CELLFLO™ MODULE. The size of the CellFlo™ module will depend upon the size of the bioreactor, the serum content and desired module life.

13. PRODUCT HARVEST LINE.

14. PRESSURE TRANSDUCER (optional). For monitoring transmembrane pressure. This transducer is useful for determining when the filter is fouling and needs to be changed.

15. DIAPHRAGM VALVE. For sterile connection of the top end of the CellFlo™ module.

16. SMALL DIAPHRAGM VALVE. Steam inlet/outlet for sterile connection.

17. DIAPHRAGM VAIVE. Second valve for sterile connection of the top end of the CellFlo™ module.

18. SMALL DIAPHRAGM VALVE. Second steam inlet/outlet for sterile connection.

19. FLEXIBLE PUMP TUBING.

20. DIAPHRAGM VALVE. For sterile connection of the upper end (bioreactor inlet) of the perfusion loop.

21. SMALL DIAPHRAGM VALVE. Steam inlet/outlet for sterile connection.

22. DIAPHRAGM VALVE. Second valve for sterile connection of perfusion loop.

23. SMALL DIAPHRAGM VALVE. Second steam inlet/outlet for sterile connection.

24. MEDIA INLET TUBING LINE.

CellGas™ Module

The CellGas™ module is designed as an internal gas exchange device to be used when traditional means of oxygenation are insufficient and/or cause foaming or shear related problems. As pictured in figure D-10, CellGas™ is a modified hollow fiber module without the “shell” of the standard hollow fiber module. When mounted within the bioreactor, it allows for maximum exposure of the gas exchange surface of the hollow fibers to the liquid contents of the bioreactor.

The efficiency of the CellGas™ module is determined by the gas concentrations at and immediate to-the gas exchange surface on the outside of the hollow fibers. The higher the concentration differential of any gas across this surface, the higher the gas transfer rates.

Thus, the gas exchange rates are affected by controllable variables on both the inside and outside of the fiber bundle. The primary variables on the inside of the

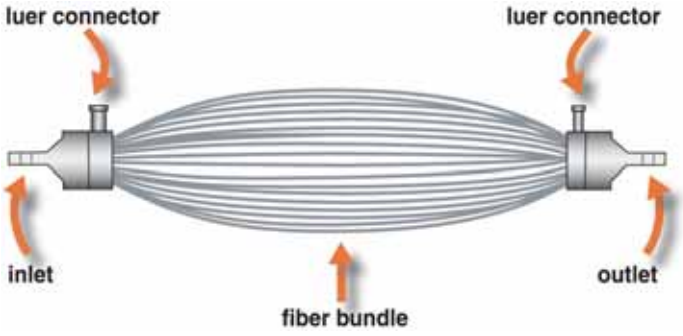


Figure D-10 Schematic of CellGas™ oxygenator

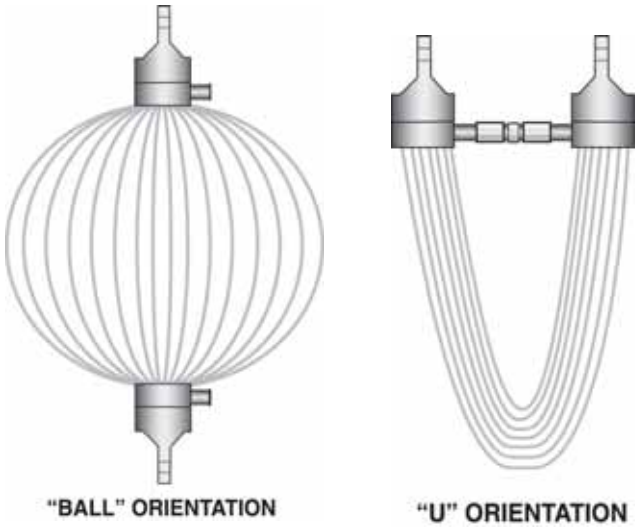


Figure D-11 CellGas™ oxygenator configurations

fibers are the feed gas(es) concentration and the gas flow rate. On the outside, the primary variables are the dissolved gas(es) concentration and the liquid flow rate over the surface of the fibers. Gas flow rate, impeller speed and CellGas™ module orientation then become the controllable variables.

The module has two endcaps that contain the potted fibers and form the gas inlet and outlet for the module. The fiber bundle is composed of unsupported, individual, hydrophobic fibers of varying lengths and numbers in accordance with the surface area rating of the module.

The CellGas™ module can be steam sterilized in place inside the bioreactor.

In general there are two recommended orientations of the CellGas™ Module. Figure D-11 shows the "Ball" orientation and the "U" orientations.

Cellmax® Capillary Cell Culture

Conventional Cell Culture Technologies

Cell culture in the laboratory, as contrasted with applications that are oriented more toward the process level previously described is a tool used primarily for the production of laboratory scale cells and cell products. For many applications, conventional cell culture technology has proven inefficient, cumbersome, costly and labor-intensive, all too often hindering rapid progress in the laboratory.

Cells have a natural tendency to grow in three dimensions. Two-dimensional substrates, such as T -flasks and roller bottles, are not designed for layered cultures. As cells pile up, cells in lower layers can quickly perish from slow diffusion of oxygen, nutrients and metabolic waste.

Nutrient medium must be frequently replaced, and cell populations must be minimized to prevent overcrowding and layering. Medium replacement causes sudden, frequent changes in the microenvironment immediately adjacent to the cells. These changes often prevent the accumulation of beneficial cell secreted growth factors.

In addition, autocrine and conditioning factors are lost when cells are fed and the pH of the growth medium can become very acidic which is non-physiological.

Suspension Cultures

In suspension cultures, cells are continuously agitated in nutrient medium. Some cells are too fragile to tolerate this environment. Culture density is also severely limited. A high-density culture does not permit adequate agitation, feeding and oxygenation of cells due to the higher viscosity of the medium. In order to continually divide and function, most cells also require costly medium comprised of 5-20% serum or serum proteins. Moreover, this technology requires that the desired cell secreted products be concentrated from very large volumes of medium.

Capillary Cell Culture

To overcome the disadvantages above, Spectrum developed CellMax® to duplicate the *in vivo* process as closely as possible. This will produce the optimal pericellular environment for growth as well as maximal secretion and accumulation of cell product.

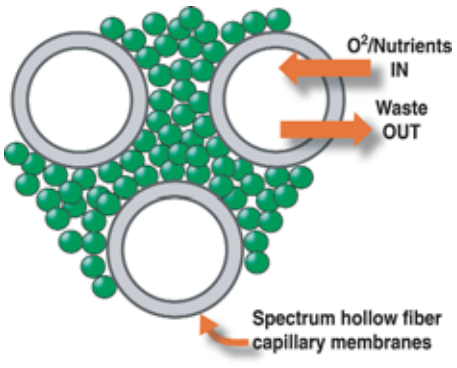


Figure D-12 Schematic of CellMax® configuration with cells

In the CellMax®, cells are grown on and around a network of hollow fiber artificial capillaries encased in a cartridge shell. Cells suspended in tissue culture medium are inoculated into the extra-capillary space of the cartridge. These cells settle onto the outer surface of the capillaries as shown in figure D-12.

Cells remain in the extra-capillary space during culture, protected from the shear of the rapidly perfusing, continually circulating medium. Since the flow rates for each cartridge can be independently controlled, both new and ongoing cultures can be maintained simultaneously.

Nutrients and oxygen in the circulating medium readily diffuse through the capillary walls to nourish the cells. Metabolic waste products similarly diffuse away from the cells and are diluted in the circulating medium.

To remain viable, cells require a significant amount of oxygen. The entire CellMax® system-operates in a standard CO_2 incubator where oxygen from the incubator atmosphere diffuses through the walls of the system's silicone tubing.

Advantages of Capillary Cell Culture

Metabolic waste and inhibitory factors are dialyzed away from the cells

Autocrine and conditioning factors concentrate around the cells reducing serum requirements. In addition, the long term culture, stable microenvironment and cell specific autocrine factors allow cells to resemble tissue and organs

pH of growth medium is stable and physiological

Large surface area for cell attachment and provision of oxygen and nutrients

Secreted products (protein & antibodies) concentrated in the small volume (10 ml to 60-ml) extracapillary space (ECS)

Large cell numbers (up to 5 x 10^{10} can be conveniently handled

Endotoxin load reduced

Cells can be cultured for over 6 months. Cells reach confluence and stay attached and alive

Closed system allows safer handling of pathogenic cultures

High cell densities (10^8/mL) permit developmental interactions to become observable in cell co-cultivation cultures

Stable microenvironment allows *in vivo* like cell growth

Advantages of capillary cell culture over older methods are shown in the table on the next page.

Advantage	Description
Size	The Cellmax® has a surface area equivalent to eight large tissue culture flasks. The CellMax® cartridge also has a surface area equivalent to two roller bottles.
Space	The CellMax® system occupies the same incubator space as a single large flask. In addition, the CellMax® unit fits inside a standard incubator – roller bottles do not. A CellMax® Quad has the equivalent surface area of eight roller bottles and fits inside a standard cabinet incubator – the eight roller bottles do not.
Cost	One CellMax® cartridge can last up to six months of continuous culturing. Regular tissue culture flasks need to be changed monthly. Over time, the CellMax® system uses 20% less culture media than regular tissue culture flasks or roller bottles.
Convenience	CellMax® artificial capillary cartridges are ready to use right out of the package. All that is needed are cells and media. Roller bottles require a temperature-controlled room that is inconvenient and expensive. The CellMax® System can be placed in easy-to-use cabinet incubators.
Time	Changing the media for a CellMax® artificial capillary system takes only five minutes. This minimizes manipulation of the cell culture and system contamination. The CellMax® system reduces cell seeding culture time from hours to minutes!

Table D-1 Advantages of the CellMax® System over Flask or Roller Bottle Culturing

Representative Applications of the CellMax® Cell Culture System

Lymphocyte Expansion

Repeated culture and harvest of primary lymphocytes can be achieved with the CellMax® Capillary Cell Culture System. Modules are available for the expansion of lymphocytes up to 10^{10} per module. This method can be used to produce and collect significant amounts of lymphocytic growth factors. CellMax® modules have also been used to increase the efficiency of viral transductions.

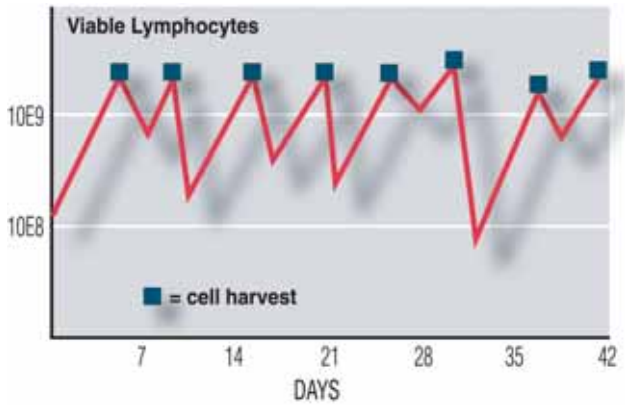


Figure D-13 Culture and harvest of primary lymphocytes with CellMax® Capillary Cell Culture System

Endothelial Cell Culture

Spectrum has developed several Capillary Cell Culture Cartridges specifically designed for the culture and study of endothelial cells under flow. The system provides a means to evaluate endothelial cell function in a more physiological environment when compared to other methods.

As a result, typical cell morphology is maintained, allowing the investigator to generate results that more closely reflect the *in vivo* situation.

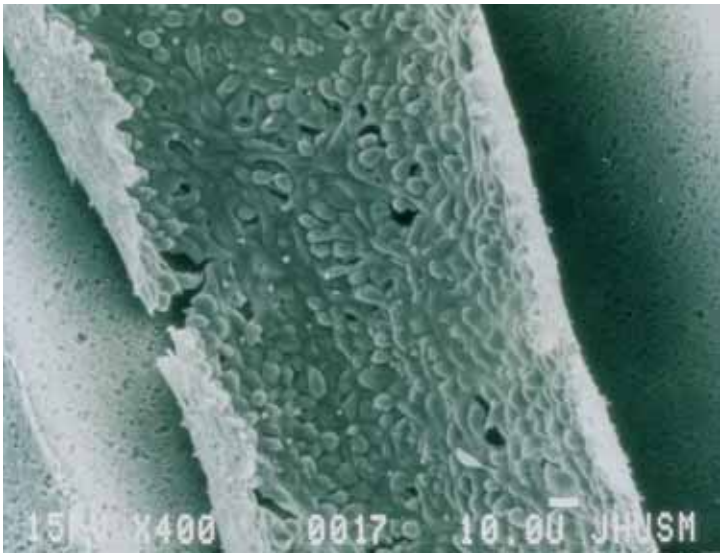


Figure D-14 Culture and study of endothelial cells with CellMax® cell culture system

CellMax® provides a reproducible level of shear force that emulates venous or arterial shear conditions. In addition, the microporous nature of the capillaries creates an ideal format for co-cultures with perivascular cells, such as smooth muscle or brain neuroglia.

Figure D-14 is a scanning electron micrograph of endothelial cells cultured in a CellMax® Endothelial Module under conditions of arterial shear stress of 13-15 dynes/cm^2. Individual fibers were sectioned longitudinally revealing an adherent monolayer of cells attached to the lumenal surface of a polypropylene capillary. As an artifact of preparation, one edge of the monolayer has partially detached from the inner capillary surface revealing the contiguous nature of the endothelial layer.

Monoclonal Antibody Production

The CellMax® Artificial Capillary system is the method of choice for the in-vitro production of 50 mg to several grams of monoclonal antibody. A cellulosic fiber with a MWCO of 30kd is used. This permits the inhibitory factor TGF beta to freely diffuse away while trapping the higher molecular weight immunoglobulins along with the hybridoma cells in the extra capillary space.

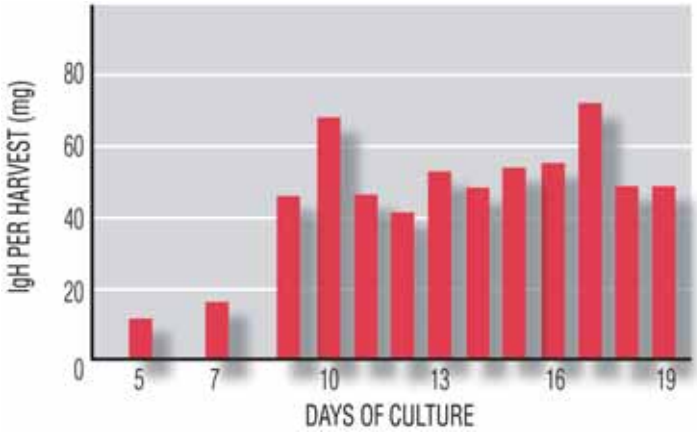


Figure D-15 High Titer Monoclonal Antibody Production using the Spectrum CellMax® System

Cell density can become higher than 10^8/ml and antibody concentration is in the range of 0.5 mg to 5 mg per ml. Harvest volume is only 10 to 20 ml and each cartridge will produce antibody for up to 4-6 months. Serum requirements can be reduced by as much as 5 fold and the adaptation to serum free medium is facilitated. Chimeric and non-murine antibodies can be easily produced and biological contaminants such as lipids, endotoxins, proteins or viruses are reduced or eliminated. Purification is simplified and overall yields are improved.

Secreted Protein Production

Current methods for the production and harvest of secreted biologicals such as growth factors, recombinant proteins and antibodies involves the use of inefficient in-vitro culture systems or animals. Spectrum artificial capillary cell culture systems permit the culture of large numbers of cells (up to 5 x 10^{10} cells) within a small volume extra capillary space (10 ml to 100 ml). Selection of the appropriate fiber molecular weight cut-off can trap the secreted product in this small volume while allowing lower molecular weight nutrients and waste products to diffuse into the circulating medium. Both total concentration of product and product secreted per cell can be increased by a factor of 10 or more using the CellMax® system. The reduced harvest volumes and the use of reduced serum or serum free mediums simplify post-harvest purifications.

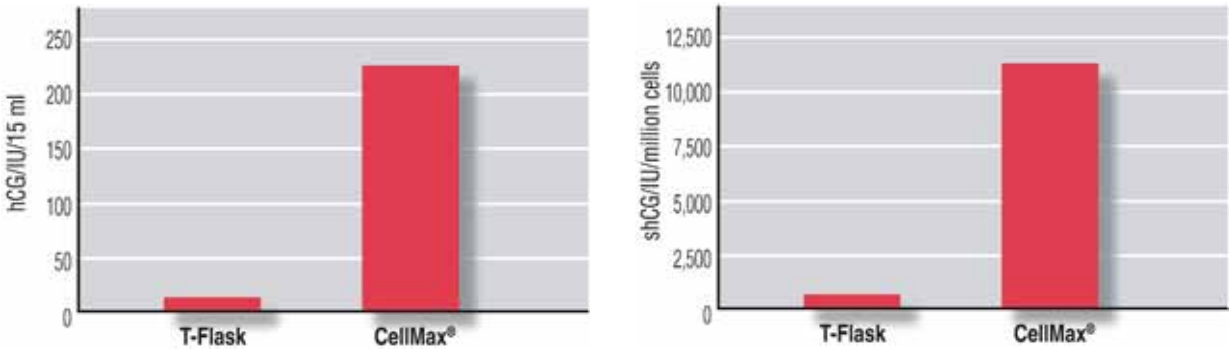


Figure D-16 BeWo Choriocarcinoma hCG Production – T-Flask vs. Spectrum CellMax® Artificial Capillary Cell Culture

The CellMax® Capillary Cell Culture System

Referring to figure D-17, culture medium is drawn from the medium reservoir bottle (A) through silicone rubber tubing (B). The adjustable pump compresses the thick walled tubing (C) causing the medium to flow through the oxygenator (D) where oxygen and carbon dioxide are exchanged. The medium flows into the artificial capillary cartridge (F) at the inlet end port (E). As shown in the enlarged inset, medium flows through the lumen of each fiber within the artificial capillary bundle where it perfuses the cells growing on the outside of the capillaries with fresh nutrients and oxygen, and removes metabolic waste products. There is no direct medium flow within the extracapillary space between the capillaries (enlarged inset). The medium is then returned to the reservoir bottle.

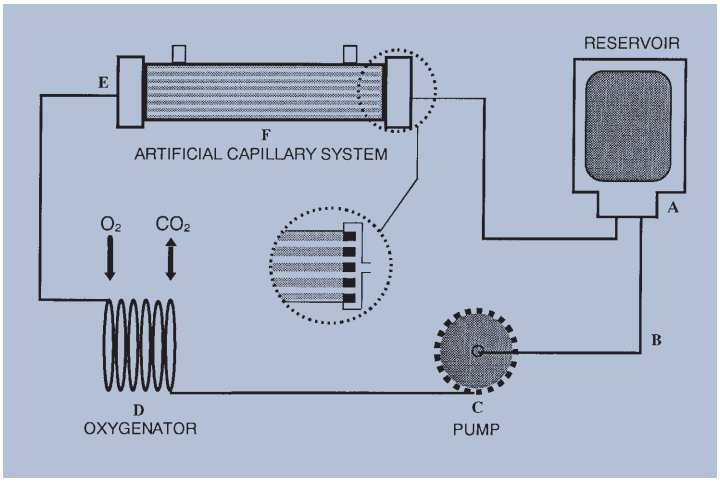


Figure D-17 Schematic of operation of the CellMax® Capillary Cell Culture System

CellMax® Systems

Single Station Cell Culture System

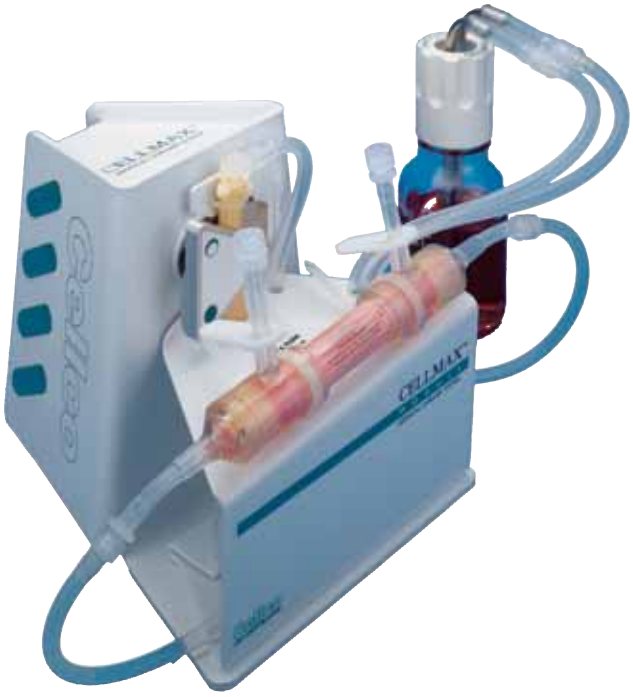


Figure D-18 CellMax® single station cell culture system

The CellMax® single station cell culture system is designed to support cell culture in one capillary cartridge. The system has a positive pressure displacement pump system that can generate high culture medium flow rates without the use of a peristaltic pump. Peristaltic pumps can cause particulates to shed from the wall of the pump tubing, clogging the pores of the fibers and shortening cartridge life.

With this system monoclonal antibodies have been produced for up to six months in the same cartridge. The CellMax® system also supported a glioma cell line for over two years of continuous culture.

The single station system features three flow rates and takes up about the same area in an incubator as a T225 flask. The system comes complete with all components required to operate one independent culture module, and permits the culture of up to 5 x 10^9 cells per module and produce 5 to 50 mg of monoclonal antibody per harvest.

Four Station Cell Culture System

The Spectrum CellMax® QUAD Cell Culture System is designed for the culture of a variety of mammalian cell types.

Figure D-19 CellMax® Quad four station cell culture system

It features a proprietary positive pressure displacement pumping mechanism that permits long term culture in artificial capillary modules but has 12 precisely controlled flow rates. The range of available flow rates is especially useful for endothelial cell studies under conditions of defined shear stress.

The system comes complete with all components to simultaneously operate up to four separate, independent culture modules. The system-allows the simultaneous culture of several cell lines, or for repetitive studies of a single cell type in one system.

Cell Implant for Drug Screening

New technology has been developed by the National Cancer Institute for the screening of anti-cancer compounds. This *in vivo* method of identifying compounds with potential chemotherapeautic activity against cancer and HIV is now routinely used by many researchers.

The technology allows human cells to be implanted into host animals and subsequently harvested en masse. While in the host, cells can be exposed to potential chemotherapeutic agents and the impact of that exposure can be measured after retrieval.

As shown in figure D-20, target cells are macroencapsulated in biocompatible hollow fibers that are then implanted into the subcutaneous or intraperitoneal compartments of laboratory rodents. After selecting a subset of compounds with the hollow fiber encapsulation assay, the activity of the compounds is confirmed using the classical testing models currently used for evaluating these types of chemotherapeutic agents.

This methodology results in significant savings of time, labor, amount of compound required and the number of animals used in the evaluation of the potential therapeutic activity of these candidate compounds.

The hollow fiber assay is a unique *in vivo* model permitting the simultaneous evaluation of compound efficacy against six cell lines at subcutaneous (s.c.) and peritoneal (i.p.) sites (Hollingshead, et al. Life Sci. 57; 131, 1995).

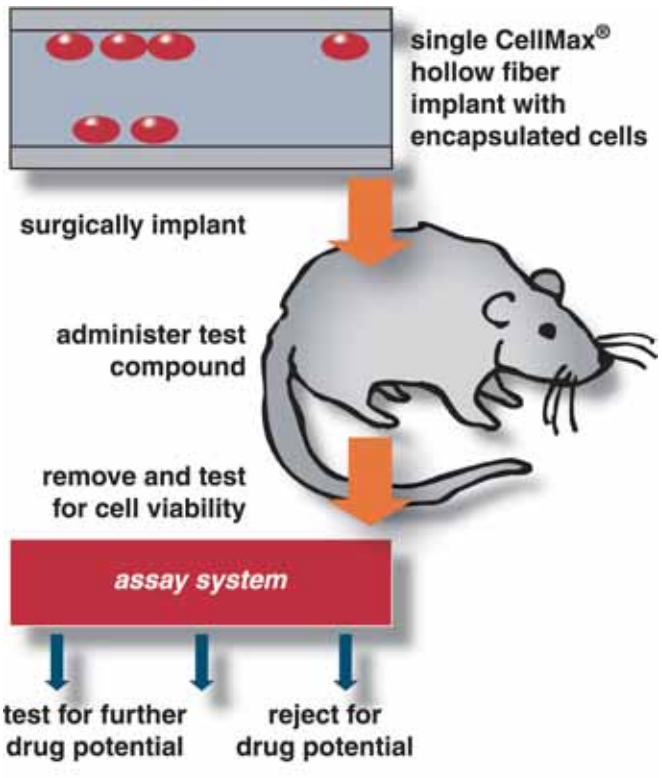


Figure D-20 Procedure for CellMax® implant assay

As an example, this model was used to investigate the relationship between cell density and compound activity. Additionally, the model was adapted for mechanism of action studies. A racemic mixture of flavorpiriodol (YZ149, a literature standard and a cyclin-dependent kinase inhibitor) was used. HCT116 cells (human colon carcinoma) were loaded into hollow fibers at cell densities of 1.0, 5.0 or 7.5 x 10^6 cells/mL, and implanted s.c. or i.p. into nude mice. YZ149 was dosed daily by i.p. bolus at 6, 12, 18 and 25 mg/kg on days 3 through 6 after implantation. Cell growth was determined by the MTT dye conversion assay. In controls, increasing cell density decreased net cell growth with maximal cell growth at 1 x 10^6 cells/mL, indicating the compound is more active against the higher cell density. After dosing, cells were removed from fibers and changes in expression of cell cycle related proteins such as cdk's, cyclins and Rb were monitored by Western analysis. The protein changes were correlated with *in vivo* growth inhibitory activity.

These data demonstrate that the efficacy of a compound is a function of cell density, suggesting that the degree of stringency for drug activity can be targeted with the manipulation of cell density using this assay.

Additionally, the model was adapted for mechanism of action studies. This technology is now used routinely by the National Cancer Institute of NIH for testing compounds for anti-cancer activity. It is also being employed to look for antiviral compounds for HIV.

CellMax® implants can also be used with established cancer cell lines, virus Infected cells, hematopoletic cells, bacteria, fungi and specific organ cells (liver, kidney etc.). The membrane capsules are non-porous to immune system cells, viruses and mycoplasma.

CellMax® Implants have a biocompatible inner and outer surface.

CellMax® Implants have the following advantages over the current xenographic model: 10 day assay versus 60 day assay; smaller assay variability; fewer animals needed; uses small amounts of test compounds; multiple cells lines can be tested in the same animal model and they are compatible with different cell lines.

When the Spectrum CellMax® Implant *in vivo* assay is used to screen compounds with poor pharmacokinetics they are rejected and those compounds with pro-drug activity are accepted.

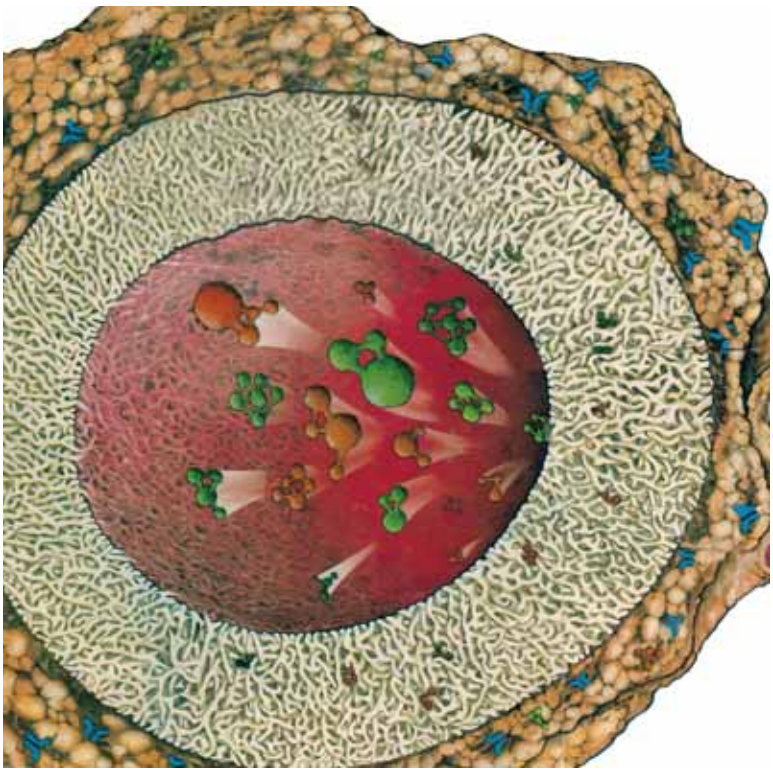


Figure D-21 Cells growing within the lumen of a CellMax® hollow fiber membrane

ABCs Of Liquid Chromatography

Introduction

Liquid Chromatography is a tool used by Biologists, Microbiologists, Biochemists, Chemists, and others interested in separating or purifying proteins or other biological molecules. In principle the method is quite straightforward. One simply injects a test sample into a flowing stream of solvent (the mobile phase), another stream passes through a bed of media in a column (the stationary phase). As the sample passes through the column the components of the sample spend time attached to the stationary phase and time free in the mobile phase. Since these times are different for the different molecules, the components of the sample exit the column at different times. The separated components of the sample are then collected as they exit the column.

In practice, however, development of the technique can require some patience. The stationary phase is made of small, spherical beads, usually between 10 and 100 µm in diameter. These beads need to be packed into the chromatography column so the material is stable and without voids. The art of packing the column can be difficult to learn. It may be the most difficult part of the separation. Fortunately, once a column is properly packed it can provide several years of use. There may also be some trial-and-error effort involved in the selection of the mobile phase.

The three most commonly used methods in Low Pressure Liquid Chromatography are Gel Permeation Chromatography (GPC), also called Gel Filtration Chromato-graphy, Ion Exchange Chromatography (IEC), and Affinity Chromatography (AC). These three methods are similar, though the stationary phase and the mobile phase used may be different, but they all can use the same pump, column, monitor, and fraction collector.

Gel Permeation Chromatography (GPC) is used to separate protein molecules by size or molecular weight. The stationary phase is usually beads made of agarose or polyacrylamide. The beads have small pores through which the smaller molecules of the sample can move. It is difficult for the larger molecules to enter the pores. As the molecules pass through the column, the smaller molecules take a longer, more tortuous path into and out of the pores on the beads while the larger molecules take a more direct route around straight through the column. In a GPC separation the larger molecules elute before the smaller molecules.

If a molecule is larger than the size of the pores in the beads, it will not be able to enter any of the beads. Any of these large molecules that are excluded from the beads elute first, followed by the smaller molecules that can get into the beads. This simple binary separation, of molecules that are too big from molecules that are not too big, is performed commonly enough to get it's own name. It is referred to as Size Exclusion Chromatography. It is simply a specialized application of GPC and is commonly used to desalt protein solutions.

Since GPC separates molecules based primarily on their size and not on their chemical characteristics, the chromatograms are generally run with no change in solvent during the chromatogram (an isocratic separation). Many other types of chromatography make use of a change in solvent to control the separation. These solvent gradients can be gradual changes in solvent (usually produced with a dedicated solvent mixer) or they can be step gradients that can be produced by simply switching the pump inlet from a flask of one solvent to a flask of another.

Figure E-1 A typical chromatography system

Ion Exchange Chromatography (IEC) is used to separate biomolecules based on their net charge. The stationary phase used is either a resin with a positively charged functional group attached (an anion exchange resin) or a resin with a negatively charged functional group attached (a cation exchange resin). Which type of resin is used depends on whether the molecules to be separated are negatively charged (anionic) or positively charged (cationic). As the sample components pass over the stationary phase, those appropriately charged are attracted to and bound by the charged resin. If the binding is weak, the chromatogram may be run isocratically with the less highly charged molecules eluting first followed by the more highly charged. If the binding is strong, as is usually the case, the bound sample components must be eluted from the column by changing the composition of the mobile phase; usually a sharp, step gradient is used for this. The change in solvent releases the bound biomolecules and allows them to pass through the column.

Affinity Chromatography (AC) makes use of active ligands attached to agarose beads to bind specific proteins and antibodies. The ligands are generally tailored to the specific protein(s) or antibody(s) to be purified and may be proteinaceous themselves. As in IEC, the sample components to be purified bind to the stationary phase as the sample passes through the column. A properly designed affinity ligand results in very tight binding of only the protein to be purified, requiring a change in mobile phase to elute it from the column.

A typical low pressure chromatographic system is shown in figure E-1. Performance and selection criteria for each of the components are discussed in the remainder of this chapter.

The Column

Important considerations in the selection of the column are the method of separation, the sample size, the required resolution, and the solvents to be used.

Unlike Affinity Chromatography and IEC, GPC is commonly used both for the purification of biomaterials as well as for their characterization. In analytical GPC, the molecular weight of an unknown can be determined. To make this determination, a calibration chart of the volume to wash a molecule through the column vs. the logarithm of its molecular weight is made for several known compounds. This should produce a linear calibration plot. An unknown can then be applied to the column and its elution volume used to determine its molecular weight.

Analytical GPC can provide very accurate results. Best results are obtained when the pore size of the packing media is similar to the size of the unknown. The pore size distribution is usually provided by the manufacturer of the media as a “linear fractionation range” indicating the range of molecular weights over which a linear relationship between the elution volume and the log of the molecular weight can be expected. The length of the column affects the precision of the molecular weight determination; the resolution of the elution volume will increase with the column length.

When doing preparative GPC, one needs to carefully trade off the resolution of a longer column against the increased dilution of the separated material that it causes. The total volume of a peak increases with the square root of the column length. If the peak volume increases for the same amount of material

being purified, dilution occurs. Increasing the column length also linearly increases the time required for a separation.

GPC is usually done in long, small diameter columns. For analytical work, a column diameter of 6 to 25 mm is usually suitable. Column lengths can range from 10 to 200 cm. A sample size of about 1 to 2% of the total column volume generally produces good analytical results. As mentioned above, there are other factors that will affect the results, including the length of the column, the media selected and the size of the proteins. Samples sizes of up to 20% of the column volume can be used for preparative separations with columns as large at 15 cm in diameter and as long as 200 cm.

IEC sample sizes can be larger than those used for GPC, especially in the case of strong binding. As the sample is loaded onto the column, the active sites on the resin nearest the column inlet will fill with the charged components of the sample. As more and more sample is loaded the boundary between the filled resin sites and the available resin sites will move toward the column outlet. When all of the active sites on the resin are filled, some of the material that should be bound will begin to pass out of the column. If an appropriate detector is in the column outlet, this breakthrough volume can be determined and recorded for future runs.

After all of the sample has been loaded into the column, an appropriate change in solvent can be made to elute the bound material from the column. It is not necessary to fill all of the active sites, but ensure that there are enough sites available to bind all of the sample. Generally the solvent change involves a solution that contains solutes that bind more tightly to the resin. This allows the new solutes to displace the material that is being purified.

Because the resolution in this type of IEC is mostly independent of the column flow characteristics, short, large diameter columns are usually used. Short, large diameter columns can be used at higher flow rates than long, small diameter columns of the same volume and ion exchange capacity. This reduces the time required for the separation.

The sample loading and operation considerations for Affinity Chromatography are similar to those for IEC. One generally has better initial knowledge of the column's capacity. Because of the high cost of the media, column sizes are usually small, but any short, large diameter column is suitable.

When selecting a column, be sure that the column has a non-clogging bed support that does not broaden or mix the bands as they elute. If a pump is being used and the bed support becomes clogged, the pressure rating of the column may be exceeded causing it to leak or crack. When using gravity flow, the clogging of the bed support may stop flow completely in the column. In either case clogging requires packing the column again. Some non-clogging bed supports broaden the bands as they elute from the column. This results in a degradation of the resolution that was desired.

Many of the chemical interactions used in chromatography are temperature dependent. Choose a column that can be fitted with a water jacket. This allows the use of a temperature controlled water bath to maintain the temperature of both the column packing and the liquid flowing through it.

Another, often overlooked consideration is the compatibility of the column material with the solvents to be used. In biological laboratories, most chromatography

processes are done with rather benign aqueous buffers. Generally almost any material (except for many metals) can be used without risk. Spectrum manufactures a line of columns with less expensive polypropylene endplates for these applications. Other scientists use less benign solvents in their separations, including organic solvents and concentrated acids and bases. More inert materials are required in these applications. Spectrum manufactures a line of columns with more inert PTFE endplates for these users. The only wetted materials in these columns being the PTFE endplates and the borosilicate glass bodies.

A glass column is frequently superior to a column made of metal or other opaque material, since it allows the condition of the media to be observed. Voids or separations which may develop can be easily discovered and corrected. The transparency of a glass column also makes it easier to layer the sample on the top of the media bed without dismantling the column.

Spectra/Chrom® columns all feature transparent glass bodies and non-clogging bed supports. They are available with either polypropylene endplates for lower cost or PTFE endplates for additional chemical compatibility. They are available in diameters ranging from 0.6 cm (about 0.25 inch) to 15 cm (about 6 inches) and in lengths to 200 cm.

The Pump

Low Pressure Liquid Chromatography can be performed using only gravity to supply the energy to drive the mobile phase through the column. As long as the solvent container is located above the top of the column hydrostatic pressure can move fluid through the column. Step gradients can be generated using two or more solvent jugs and switching the column inlet from one to another. This method is inexpensive, simple and pulse free. Drawbacks to using this method are:

As the fluid level falls, the hydrostatic pressure decreases resulting in a decreasing flow rate during the separation.

Gradients can only be step-gradients.

The separation takes longer because of the low pressures involved (a fluid level 3 feet above the top of the column is less than 2 psi).

A peristaltic pump is an instrument that forces fluid along tubing by using rollers to sequentially squeeze the tubing against a platen, trapping the fluid in small packets between the rollers. The rollers continuously move the packets of fluid across the platen releasing one downstream as each roller lifts off the platen. As each packet of fluid is released a small pressure pulse is generated. This pulse is a small sudden drop in pressure caused by the increase in tubing volume that occurs when the pump roller lifts off of the tubing. Under some conditions, these pressure pulses can disturb the top of the column packing. These pulses may be dampened by using a length of soft tubing between the pump and the column minimizing their effect on the column packing.

The big advantage of a peristaltic pump over a piston pump is that the fluid is confined inside a piece of tubing. No other parts of the pump are ever in contact with the mobile phase. By changing the size and composition of the pump tubing, the

available flow rates and chemical compatibility of the pump can easily be customized to a particular application.

Figure E-2 Spectrum MP-2 Peristaltic Pump

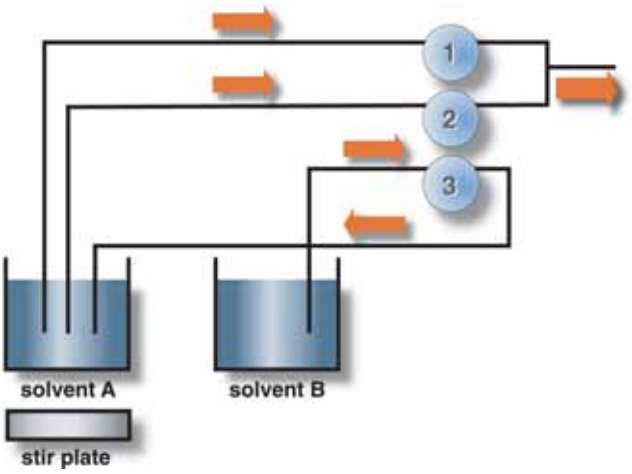


Figure E-3 Generated a linear gradient with 3 Pump Heads

Some peristaltic pumps have additional features such as flow rate calibration and digital display of the selected flow rate. They are promoted as precise and accurate after user calibration. Calibration involves measuring the volume delivered at a particular flow rate for a fixed period of time. The flow rate of a pump is dependent not only on the speed of the pump motor, but also on the operating pressure, elasticity, and exact inside diameter of the tubing. To maintain the quoted accuracy of these pumps, they need to be calibrated every time the tubing is changed, and periodically as the tubing in the pump ages. The digital display is no more useful than a simple knob, since it is just displaying the flow rate that has been set.

Most low pressure separations are very forgiving in terms of pump flow rates. Ion exchange and affinity separations, where a step gradient is used to effect elution, does not require exact flow rates, since nothing will elute from the column until the step is made. The flow rate is of more significance for gel filtration separations, since the elution process is continuous. Even when doing gel filtration chromatography, knowing the flow rate to better than ±3% is of little value.

The Spectra/Chrom MP-1 & MP-2 Peristaltic Pumps (figure E-2) are general purpose peristaltic pumps. The pump motors are fitted with digital tachometers to maintain motor speeds within 1% of the user set point. The only flow rate changes that occur are caused by the tubing and the column. Up to three pump heads can be mounted on the pump drive. This allows the user to easily generate both linear and non-linear continuous gradients (figure E-3).

The UV Monitor

The UV Monitor is the heart of the chromatography system. It is an instrument that is able to detect when a component of the sample is eluting from the column because bio-molecules absorb UV radiation. It monitors the transmittance of a narrow beam of ultraviolet radiation passing through the effluent stream. A flowcell with two tubing fittings is connected in the effluent stream. The flowcell has two quartz windows for the ultraviolet beam to intersect the effluent stream and exit to strike a photocell or photodiode. The signal is then converted to an absorbance and displayed in Absorbance Units (AU).

When radiation is passed through a substance in solution the radiation is absorbed depending upon the absorbancy, and the concentration of the substance in the solution. If L_0 is the light entering the solution and L is the light leaving the solution, then the transmittance (T) is defined as:

$T = (L/L_0)$

Absorbance is defined as:

$A = \log_{10} (1/T)$

$A = \log_{10} (L_0/L)$

Because this is a log function AU is dimensionless. One AU is equal to a reduction of the incident radiation by a factor of 10, while 2 AU is a reduction of the radiation by a factor of 100. A more useful equation is the Beer-Lambert law, which states the absorbance, is equal to:

$A = a\ k\ l$

Where: a is the absorbtivity in liter/mol•cm,
k is the concentration in mol/liter, and
l is the path length in cm through the solution.

This is a linear equation rather than logarithmic, and it is easier to use to determine concentrations. Modern UV Monitors display results in AU.

The variable wavelength, the scanned array, and the fixed wavelength are the three basic types of UV Monitors. The first two are very expensive and are used in most high performance liquid chromatography (HPLC). Most biological molecules are detected at two wavelengths (280 nm and 254 nm) so fixed wavelength monitors such as the Model 280 UV Monitor from Spectrum are most often used for low pressure liquid chromatography.

The variable wavelength monitor uses a Deuterium light source to generate a continuous spectrum from about 190 nm to 400 nm. A diffraction grating is then used to select the wavelength to be used for detection. Drawbacks to this system are the mechanical linkage to the grating and the resolution of the grating/optical system. There may be "play" in the linkage between the knob that is used to set the wavelength so that the center wavelength may not always be repeated. The user must approach the selected wavelength from the same direction each time to minimize this affect. The grating/optical system has a bandwidth as high as 20nm. This means that if the user selects 254 nm as the center wavelength the monitor will detect absorbance as low as 245 nm and as high as 265 nm. If the user is attempting to quantify two different components of the sample and the absorbance profile at the selected wavelength is changing, the ratio of the areas will give an erroneous indication of the relative quantities of the two components. A discrete wavelength monitor used at 254 nm would give the correct quantities since it has a bandwidth of 0.1 nm at 254 nm.

Scanned array monitors also use Deuterium lamps and diffraction gratings, but in

these monitors the position of the diffraction grating is fixed. The diffraction grating is used to disperse the radiation from the Deuterium lamp onto an array of photodiodes instead of just one photodiode. This eliminates the need to rotate the grating, removing one of the drawbacks of the variable wavelength monitor. A scanned array monitor usually has 256 diodes in the array. The bandwidth is about 1 nm, which makes this instrument useful for quantization.

A scanned array monitor is very rarely required in low pressure liquid chromatography, but is a useful tool in analytical HPLC. It is able to produce a 3 axis display of absorbance plotted against wavelength and time facilitating the identification of unknown compounds in a sample. These very computer controlled monitors have features that allow both the chromatogram and the individual spectra to be observed in various formats. They also include algorithms that can be used to identify samples components from their empirical spectra. The scanned array monitor is ideal for work involving the identification of unknowns, but they generally add unneeded complexity to preparative low pressure liquid chromatography.

Discrete wavelength detectors are the ideal detector for low pressure liquid chromatography. They are compact, inexpensive, and easy to use. They provide an output in Absorbance Units based on the quantity of a compound in the effluent stream. This type of detector generally uses a low pressure Mercury arc lamp as its light source providing a radiation line at 254 nm. Other longer wavelengths can be generated using this line to excite rare earth phosphors.

When selecting a discrete UV Monitor one important feature to require is a display of the output. This enables a quick determination of the status of the chromatogram. Another time saving and frustration reducing feature is an autozero. With this feature the user can press a button to zero the baseline on the absorbance range to be used. Monitors without this feature must be manually zeroed at the highest available range and then switched to lower ranges, re-zeroing at each step, until the desired absorbance range is reached.

Changing the wavelength can be a time consuming chore on some discrete monitors. Some instruments require replacing lamps and/or filters which usually requires removing and replacing the cover, then waiting for an extended time for the baseline to settle down. Select an instrument in which changing wavelengths is a simple step requiring no tools and very little time.

Extremely sensitive absorbance ranges are not generally needed for preparative chromatography. Because proteins and most other interesting biomolecules have broad and deep UV absorbance bands, most low pressure liquid chromatography is done at sensitivities ranging from 0.1 to 1.0 AU.

Instruments with usable ranges more sensitive than 0.01 AU full-scale are usually quite expensive. (Some less expensive instruments include more sensitive ranges, but have noise figures that would preclude operation at these more sensitive ranges.) Two specifications that are of more significance than the lowest absorbance range available are the detection limit and the noise level of the monitor. It is important that the wavelength and the measurement conditions are specified with these parameters. Typically the noise at 254 nm with a dry flowcell is much less than the noise that will be experienced at 280 nm with solvent flowing through the flowcell.

If the UV Monitor is the heart of the chromatography system, then the flowcell is the heart of the UV Monitor. The design of the flowcell is critical to the performance of the UV Monitor. When selecting a UV Monitor for low pressure liquid chromatography

make sure that flowcells are available to span all of your liquid chromatography needs. Four parameters to consider in selecting a flowcell for a particular separation are:

What is the range of specific absorbtivity and concentration of the components of the sample to be separated?

What is the flow rate that will be used?

What is the size of the sample?

Will gradient separations be used?

The absorbtivity and concentration of the sample determine the path length of the flowcell that should be used. Most UV Monitors use 5 mm as the path length for their standard flowcell, and this is satisfactory for most applications. If the concentration and absorbtivity will be very low, then a 10 mm flowcell may be required. As the Beer-Lambert law shows, this will double the sensitivity of the monitor. If the absorbtivity and concentration is very high, then a short path length flowcell, such as 2 mm path length flowcell may be needed. This increases the absorbance range by a factor of 2.5.

Preparative liquid chromatography may be done with flow rates that are quite high, as high as several liters per minute. You should be sure to select a flowcell that will handle the highest flow rates you plan on using. For a given pressure, a short path length flowcell will generally have a lower flow rate than a long path length flowcell. Larger columns may have pressure limits as low as 15 psi, limiting the pressure available at the flowcell to something less than 15 psi. A graph of the pressure drop vs. flow rate can tell you if a particular flowcell can accommodate your anticipated flow.

If the volume of the sample is small, then a flowcell with a large internal volume may affect the resolution of the separated components. If the flowcell is to be used in HPLC then this is an especially important factor. Flowcells with internal volumes of less than 2 microliters per millimeter path length are sometimes necessary to preserve resolution. Flowcells that are designed for low pressure liquid chromatography and preparative liquid chromatography have large internal volumes, which are small, compared to the preparative sample size, but still allow for the higher flow rates that are necessary.

One of the most important considerations in selecting a UV Monitor and flowcell is whether there is the possibility of gradient separations being used. With a two component gradient the different absorbtivity of the solvents can cause the baseline to change considerably. This makes it difficult to accurately determine the area of peaks when quantitating the results. In some cases the chromatogram will go off scale. Select a UV Monitor that contains dual flow path flowcells. Not only does this give a stable reference when doing isocratic separations, but flowing the gradient through the reference path of the flowcell prior to the column will subtract baseline changes caused by the solvent.

Figure E-4 Model 280 UV Monitor

The Spectrum Model 280 UV Monitor (see figure E-4) has been designed with all of

the features that a discrete wavelength UV Monitor should have. It has a digital display of the output, an autozero, and three dual flow path prep flowcells of 2 mm, 5 mm, and 10 mm available (the 5 mm path length flowcell is included with the monitor). In addition it has an event mark pushbutton allowing the user to place positive going spikes on the chromatogram to signal events such as injection. It is compact and has an optional bracket that allows mounting on a mast close to the column exit. It comes complete with both 254 nm and 280 nm capability. To change wavelengths it is merely necessary to flip the filter holder. No tool is required and the time expended is less than 10 seconds. The Model 280 has a fast warm-up with drift becoming negligible on the 0.05 absorbance range within 10 minutes after turning on the power. The Model 280 UV Monitor is the best value on the market for a low cost state of the art monitor for low pressure Liquid Chromatography.

The Fraction Collector

Fraction collection is the art of dividing the liquid eluting from the column to maintain the separation of the sample that occurred in the column. The separated components are collected in individual containers (usually test tubes). The scientist collects these fractions for further study as in analytical chromatography or uses them as a step toward an end product as in preparative chromatography.

A typical modern fraction collector is shown in figure E-6. It can not only collect fractions by volume, but also collects by time or drop. Using the absorbance signal from a UV Monitor, the modern fraction collector can be programmed to discriminate against the peaks detected by the monitor so that only the desired parts of the sample are collected. The effluent that is of no interest is diverted into some other container or to waste.

There are four basic types of fraction collectors. The simplest is just a set of valves attached to a manifold that are sequenced to divert the effluent into large containers. A rectilinear fraction collector, moves collection vessels under the effluent stream. The X-Y fraction collector moves the effluent stream over the collection vessels. The round fraction collector moves both the tubes and the effluent stream. All four of these basic fraction collectors have their advantages and disadvantages and have options that enhance their utility for specific applications.

Which Type Fraction Collector Should Be Used?

A number of questions need to be answered before selecting which of the four types of fraction collector to use.

What is the size of the fractions to be collected?

What is the flow rate of the effluent stream?

How many fractions need to be collected?

Is band spreading important?

Is cross contamination of samples important?

Are the peaks to be collected in separate tubes?

Usually the only time one uses valves to collect fractions is in large scale preparatory chromatography where the sample is very large and the time that the peaks of interest will elute from the column is precisely known. The fraction size may be many liters in size, and most of the time only one peak is collected. The valve(s) can be controlled by a sequential timer that starts either when the mobile phase is started (if the sample has been layered on top of the column), or when the sample is injected into the flowing mobile phase stream. The only other fraction collector that can collect extremely large fractions is the X-Y. This is accomplished with an option that replaces the normal test tube rack with an array of funnels connected to tubing that leads to large collection vessels. This type of scheme is used when collecting more than three or four large peaks.

For moderate sized fractions the other three fraction collectors can be used although the researcher needs to consider certain features of the round and the X-Y fraction collector that might be detrimental to the collection desired.

Because the Round fraction collector moves the drop head along the radius of the round test tube rack and the X-Y over the whole test tube rack, the tubing coming from either a valve or the detector must be long enough to accommodate the traverse. As the peaks travel along this extra length of tubing they can spread out reducing the resolution that was obtained coming out of the column. For very large fractions this may not be a problem, but for small fractions one must keep the length of tubing between the column outlet and the collection tube to a minimum.

Another problem with Round and X-Y fraction collectors is their tendency to "fling" hanging drops when they are moving from one tube to another. This causes drops from one peak to be deposited in tubes meant for other peaks. The manufacturers of these types of fraction collectors have tried all types of design schemes to prevent this problem but nothing works. The only solution is a stationary drophead, which only the Rectilinear fraction collector has.

The X-Y fraction collector is the only system that can collect very small fractions. This is because it can be programmed to deposit the peaks in a microplate. Of course the problem of the drops falling where they are not supposed to can be a serious problem.

The Rectilinear fraction collector has another useful feature. The user can remove the test tube racks as the peaks are collected without interrupting the rest of the collection.

Time, Drop or Volume?

If the flow rate is low enough (less that 6 ml/min), the best method of collection for most separations is counting drops. This approach eliminates variations in collection volume caused by changes in pumping speed, or changes to the flow rate due to level changes in the solvent vessel when using gravity flow. There will still be changes in the volume collected caused by different size drops due to the different physical properties of components of the sample. In most cases these will be minimal. The Spectrum CF-1 Rectilinear fraction collector has a drop counter as standard equipment.

For higher flow rates the time collection mode is usually used. As the name implies, effluent is collected into each collection vessel for the same time period. The user should select a collection time so that with constant pumping speed the fraction collector will change collection vessels when they are filled to 75% so there will be no danger of them overflowing.

Some fraction collectors (the Spectrum CF-1) allow collection by volume. In this mode the fraction collector is controlled by the pump, changing tubes when a pre-set number of pulses from the pump indicate that the desired collection volume has been reached. This is very useful when collecting equal aliquots of a liquid in a series of test tubes to be used, for instance, as an incubation media. When using this collection method with a column separation there will still be changes to the volume collected in the test tubes due to the changing characteristics of the column during the separation.

Requirement	Type of Fraction Collector			
	VALVES	RECTILINEAR	X-Y	ROUND
Very Large Fractions > Liter	l			
Large Fractions > 75 ml	l		l	
Standard Fractions < 75 ml		l	l	l
Small Fractions < 50 µl			l	
High Flow Rate > 100 ml/min	l		l	
Flow Rates < 100 ml/min		l	l	l
More Than 3 Fractions		l	l	l
No Cross Contamination of Peaks		l		
Minimum Band Spreading		l		

Table E-1 Requirements and the Fraction Collectors that will adequately satisfy them

Custom Fraction Collector

When a simple fraction collector is used to collect the separated parts of a sample, the size of the fraction will remain relatively constant throughout the collection. The test tubes containing the peaks will have the same amount of liquid in them as the test tubes containing the liquid from between the peaks. If the fractions fill the tubes to 75%, some of the peaks will probably be diluted with non-peak effluent. If the fractions are kept small to keep the peaks as pure as possible one may run out of tubes.

A "smart" fraction collector such as the Spectrum CF-1, using the signal from a UV Monitor, presents the scientist with four options: (1) Collect peaks and non-peaks at the same volume, (2) Collect peaks at one volume and non-peak effluent at another volume, (3) Collect only the peaks, shunting the non-peak effluent either to waste or a large collection vessel, (4) Collect only the peaks of interest. With the CF-1 Fraction Collector one can customize the collection and retain in memory up to four different collection protocols.

Time Windows

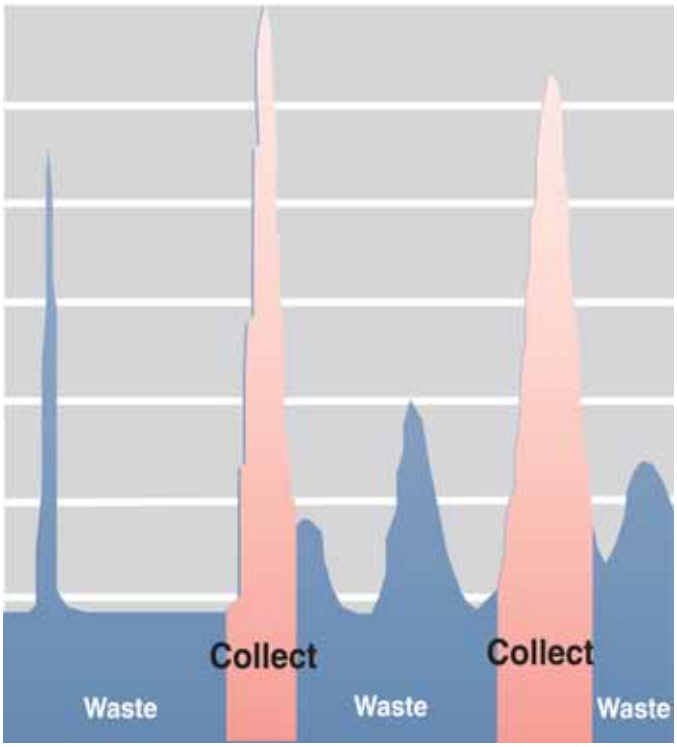


Figure E-5 Time Windows

Most smart fraction collectors utilize time windows to allow the user to customize a collection. The CF-1 provides up to 10 time windows, which allow the user to select intervals from the start of a separation in which the fraction collector will:

Collect all the effluent in the test tubes.

Collect only when the UV detector signal indicates that there is a peak eluting from the column.

Shunt all of the effluent to waste or a separate container.

The time windows and peak detector, used with the signal from the UV Monitor, will allow the user to collect only the peaks of interest. Figure E-5 shows five time windows used to collect two peaks while shunting the unwanted effluent and the other peaks to waste. The peak detector is used in the slope mode in this example.

NOTE. It is always a good idea to collect the "waste" rather than letting it flow into a drain on the off chance that a valuable sample may have inadvertently been included in the waste either through a UV Monitor or Fraction Collector malfunction or a programming error. If all the peaks are collected and the separation is isocratic, the "waste" is pure solvent and may be recycled.

Peak Separation

There are three sensing modes in which smart fraction collectors may use the signal from a UV Monitor to identify peaks. They are level sensing, slope of the leading edge of the peak, and both level and slope sensing.

Most smart fraction collectors have at least level sensing. With this sensing mode the fraction collector will determine that a peak is present anytime the UV Monitor signal is above a level preset by the user. The big disadvantage to this method is that baseline drift can cause the fraction collector to think there is a peak present. When using two or more solvents with different absorbtivity during a gradient separation, the base line can change significantly. For this reason level sensing is almost useless unless the separation is isocratic. Most gradient separations require that the fraction collector recognize a peak by the slope of the UV Monitor signal.

The Spectrum CF-1 Fraction Collector has a sophisticated slope detector as well as a level detector. Not only does the user have the option of selecting whether the peak signal has a fast or slow rise time, but also whether the peaks will be large (over 50% of full scale), narrow or broad (can enter the expected range of widths of

the peaks). Specifying these criteria insures that all of the peak will be collected. Since during lengthy separations peaks that take a long time too elute the column is broader than fast eluting peaks, the user can specify that the fraction collector adjust the criteria to expect some broadening.

If a user wants to collect peaks in the highest concentration possible it is necessary to be able to use both the slope and level peak sensing together. In this mode the user sets the slope appropriate for the desired peaks, and then the level is set 10% to 15% above the baseline. This will eliminate the portion of the peak in the tail that is not very concentrated while retaining the main body of the peak.

The Intelligent Fraction Collector

Using a fraction collector with the features of the Spectrum CF-1 (see figure E-6), gives the scientist the opportunity to collect fractions in a way best suited to each separation. The fraction collector can be programmed to collect one peak or one hundred peaks; all the non-peak effluent or none of the non-peak effluent. Peaks can be collected at one volume while non-peak effluent can be collected at another. Using a time window the void volume from the column can be shunted to waste so as not to use collection tubes unnecessarily. Test tubes from 10 mm diameter to scintillation vials of 28 mm may be used.

Figure E-6 CF-1 Fraction Collector

The Spectrum CF-1 is easy to program and can retain four different programs for future use. It has an easy to read LCD display that leads the user through the programming sequence. The membrane key-pad allows the selection of one of four options displayed each step of the programming sequence. At each step help is available by pressing the “help” key.

A mast package which allows the mounting of a column and a UV Monitor is available as an option. This allows the effluent flow path to be as short as possible, keeping band spreading to an absolute minimum.

The Spectrum CF-1 is a low priced unit that will satisfy all possible collection requirements.

Appendix

Glossary of Terms

ABSOLUTE -A degree of filtration, usually referring to 100% removal of rigid particles greater than a stated size.

ABSORB -To take up or drink in, as a sponge imbibes water.

ACTIVATED CARBON -Carbon activated by high temperature steam or carbon dioxide to form a material of high adsorptive capacity.

ADSORB -Attracting and holding a gas, vapor, or liquid on the surface of a solid.

ADSORBENT -A solid material that adsorbs, such as clay, carbon, activated alumina, etc.

AMORPHOUS -Non-crystalline, having no determinable form.

ANGSTROM (Å) - Unit of measure (10^{-10} m)

ANISOTROPIC - Materials that vary from one side to the other, asymmetric or non symmetric.

AREA -The surface exposed to the flow of a fluid.

ASBESTOS - A natural group of magnesium silicate materials found in fibrous form.

ASYMMETRIC – See anisotropic.

ATTRITION - Loss of material due to wear caused by rubbing or friction.

BACKWASH - To reverse flow of air, liquid, etc., through the filtration media to effect solids removal.

BAFFLE - A plate protecting filter elements from the velocity of flow entering vessel.

BIND - To adhere to another object.

BLIND SPOTS - A place in the filter media where no filtration takes place.

BLINDING - Reducing or shutting off of flow due to closing of pores in the filter media

BLOWDOWN - The use of pressure to remove liquids and/or solids from a vessel.

BRIDGING - Particles being removed arch over individual openings in the filter media or between the individual filter septa.

BUBBLE POINT - The point at which gas pressure applied to a membrane overcomes the surface tension of a fluid wetting the membrane. The bubble point correlates to pores size, as the pore size increases the bubble point decreases.

CAKE - Solids deposited on the filter media.

CARTRIDGE - Self contained filter media. Usually applied to dead-ended filters.

CASTING - Process for making a membrane. Typically a polymer mix is allowed to form a membrane as solvents evaporate out of the mix. May be a wet or a dry process.

CE - Cellulose ester. Common material used in manufacture of membranes.

CONCENTRATE - The unfiltered solution on the upstream side of a filter that becomes more concentrated as filtration progresses.

CELLULOSE - fibrous material of vegetable origin.

CLARITY - Clearness of a liquid measured by the amount of contaminants remaining.

CLAY - A natural occurring material usually being activated and used as an absorbent.

COMPRESSIBILITY - Degree of physical change in a filter cake when subjected to pressure.

CONCENTRATION GRADIENT – the force generated by atomic valence.

CONCENTRATION POLARIZATION - The formation of a thin layer of material not able to pass through the filter pores on the upstream side of a filter. Acts to inhibit permeate flow.

CONTACT ANGLE – the angle between the perpendicular at which water contacts a filter surface and the perpendicular to the filter surface.

CROSS FLOW FILTRATION - Filtration method in which the flow path of the fluid being filtered is parallel to the membrane surface so that fouling agents and particles are swept away by the flow. Recirculation flow keeps material from being driven into the membrane while trans-membrane pressure drives materials that are able to pass through the membrane.

CYCLE - Filtration interval; length of time filter operates before cleaning.

DALTON OR "D" - Unit of measure for the molecular size of molecules. One Dalton refers to the size of one hydrogen atom.

DEAD ENDED FILTRATION - method perpendicular to the filter surface in which there is no recirculation or bypass. As fluid enters the filter it is either passed or retained by the filter media.

DELTA P (ΔP) - The difference in pressure between two points in a system.

DENSITY – mass per unit volume (in filtration terms a measure of compactness or thickness.

DEPTH FILTRATION – Filter media with many paths to entrap particles.

DIAFILTRATION - A filtration method where molecules are being removed or washed by the addition of solvent directly to the solute being purified to maintain a constant volume.

DIALYSIS - The use of concentration gradient to separate solutes of differing sizes across a semipermeable membrane.

DIALYSATE - The solution (buffer) used for receiving the solutes which diffuse (dialyze) through a dialysis membrane.

DIFFUSION – the passage of a fluid through the intermolecular structure.

DIFFUSIONAL FLOW - The rate of gas diffusion flow from the high-pressure side to the low-pressure side of a filter.

DIATOMACEOUS EARTH - Absorbent earth.

DIFFERENTIAL PRESSURE - The difference in pressure between two given points.

DOCTOR KNIFE (BLADE) - A scraper for filter cake removal. Normally found on drum type filters. Also, a knife for forming thickness of cast membrane filters.

EDGE TYPE FILTER - A filter that entraps particles on the edges of the medium.

EFFLUENT - The discharged liquid from a filter.

ELEMENT - Usually another term for septum.

FILTER - A device used to remove contaminants.

FILTER AID - Diatomaceous earth, etc.

FILTER MEDIA - Material mounted on a support for separation of particulates or molecules from liquids or gases.

FILTRATE - The portion of material that has passed through the filter element and appears on the downstream side of the filter. The liquid that has passed through the filter. See also permeate.

FILTRATION - The process of removing particles from a fluid.

FINES - Particles smaller than a specified size.

FLAT WIDTH - The measurement of the tubing width in a flat position (1/2 x the circumference). Diameter = (2 Flat Width) x 3.14

FLOW RATE - The amount of material passing a given point per unit of time. Typically measured in gallons per minute or hour, or liters per minute or hour.

FLUX - The amount of filtrate flow rate per unit area. Usually stated as, for example, liters per hour per square meter.

FULLER'S EARTH – Clay, a hydrous aluminum silicate.

GEL LAYER - A layer of fouling agents, particles or both that builds up on the upstream side of the filter to inhibit flow. See also concentration polarization.

GPH - Gallons per hour.

GPM - Gallons per minute.

GSFD - Gallons per square foot per day.

HYDROPHILIC - Water wetting. To have an affinity for water.

HYDROPHOBIC - Water rejecting. To repel water.

INTEGRITY TEST - A test designed to ensure that filter pore size has not been compromised by any form of physical damage.

ISOTROPIC - Material having a uniform physical structure through the material. In the case of a membrane, the pore size is uniform from one surface to the other surface.

LEAF - A support for the filter medium.

LIQUID - Material to be filtered.

LUMEN - The cylindrical open space in the middle of a hollow fiber.

M - A unit of measure for molar concentration, given to be moles of material per liter.

MEMBRANE - A thin, porous material with a specific pore size that allows the passage of solutes with sizes smaller than the pores and the retention of solutes with sizes larger than the pores.

MESH - Number of openings in a lineal inch of wire or woven cloth.

MICROMETER (MICRON) - Unit of length: 10^{-6} meters.

MODULE - A term for a membrane and its housing. Often used to describe a hollow fiber cross flow unit.

MOLE - The fundamental Standard International (SI) unit used to measure the mass of a given substance containing a specific number (Avagadro's number) of entities. Used to determine the molar mass of a given substance.

MOLECULAR WEIGHT - The sum of the atomic weights of all the atoms in a molecule.

MOLECULE - The smallest unit of matter, consisting of two or more atoms bonded together, that retains all the physical and chemical properties of that substance.

ml or mL - A unit of measure for volume, milliliter.

mm - A unit of measure for distance, millimeter.

MWCO - "Molecular Weight Cut Off" is an indirect determination of the pore size of a membrane. The membrane will retain 90% of spherical, uncharged target macromolecules that have a molecular weight equal to the membrane MWCO.

NEGATIVE PRESSURE - Vacuum or suction.

NORMAL – Perpendicular to a surface.

PARTICLE SIZE DISTRIBUTION - The distribution obtained from a particle count grouped by specific micron sizes.

PARTICLE - A non-soluble piece of material suspended in solution or air.

PERMEATE - The portion of a solution that passes through the membrane and appears on the downstream side of a filter. See also filtrate.

PORE - An open passageway through any filter element.

PORE DENSITY - The number of pores per unit area of a membrane surface.

PORE SIZE - Approximate diameter of individual pores.

PORE SIZE DISTRIBUTION - Range of pore sizes within a membrane.

POROSITY - The fraction of a filter membrane that is open for flow. Usually expressed as a percentage.

PPB or ppb - part per billion, a measure of particle or substance concentration.

PPM or ppm - part per million, a measure of particle or substance concentration.

PSI - A unit of measure for pressure, pounds per square inch.

PVDF - polyvinylidene difluoride.

PTFE polytetrafluoroethylene

PREFILT - Material to be filtered.

RECYCLE - The return of filtered liquid for another filtering.

RC - regenerated cellulose

RECIRCULATION RATE - The amount of flow which passes through the lumen of the hollow fibers in a cross-flow filter.

RETENTATE - The solution or solid containing large molecules that is retained by the membrane.

RPM - A unit of measure for centrifugal force, revolutions per minute.

SEPTUM - The material mounted on a support; support for filter aids.

SOLVENT - A liquid into which other materials are contained or dissolved.

SOLUTE - Substances dissolved or suspended in a solvent.

SKINNED - Used to describe a membrane that has a relatively dense surface with a much less dense underlying structure.

SYMMETRICAL - Having the same physical characteristics from one side to the other. The pores have the same diameter from one surface of the membrane to the other.

SURFACE AREA - The area of a filter available for filtration, usually measured in cm^2 or ft^2.

SUPERNATANT - Liquid above settled solids.

TANGENTIAL FLOW - Flow parallel to the upstream membrane surface. Also known as cross flow.

TANGENTIAL FLOW FILTER - A filter with the fluid flowing parallel to the surface of the membrane.

THRUPUT - The amount of solution that passes through a filter or membrane before it plugs.

TRANS-MEMBRANE PRESSURE - In a cross flow filter, the average of the inlet and outlet pressures less any backpressure on the filtrate. TMP -[(Pin + Pout/2] - Pfiltrate

µL - A unit of measure for volume, microliter.

WETTABILITY - The ability of water to wet out a membrane.

VOIDS - The openings in a medium or filter cake.

Trademarks

This chemical resistance chart is intended for use as a guide, not as a guarantee of chemical compatibility. Variables in temperature, concentrations, durations of exposure and other factors may affect the use of the product. It is recommended to test under your own conditions.

The following codes are used to rate chemical resistance:

R . . . . . .Recommended
L . . . . . .Limited Exposure
NR . . . . . .Not Recommended
U . . . . . .Unknown

Chemical Resistance

Membrane Compatibility Table

	Cellulose Ester (CE) /Mixed Cellulose (ME)	Regenerated Cellulose (RC)	Polysulfone (PS) /Polyethersulfone (PES)	Polypropylene (PP)	Polyvinylidene Diflouride (PVDF)
Acetic acid (diluted-5%)	L	R	R	R	R
Acetic acid (med conc-25%)	NR	R	R	R	R
Acetic acid (glacial)	NR	R	R	R	R
Acetone	NR	R	NR	R	L
Acetonitrile	NR	R	NR	R	L
Ammonium hydroxide (diluted)	NR	R	R	R	R
Ammonium hydroxide (med conc)	NR	L	R	R	R
Amyl acetate	NR	R	NR	R	R
Amyl alcohol	L	R	L	R	R
Aniline	NR	R	NR	R	R
Benzene	NR	R	L	R	R
Benzyl alcohol	NR	R	NR	R	L
Boric acid	R	R	R	R	R
Brine	R	R	R	R	R
Bromoform	NR	R	NR	R	R
Butyl acetate	NR	R	NR	R	R
Butyl alcohol	L	R	R	R	R
Butyl cellosolve	NR	L	NR	U	R
Butylraldehyde	NR	R	NR	R	R
Carbon tetrachloride	NR	R	NR	R	R
Cellosolve	NR	L	R	R	R
Chloracetic acid	NR	R	NR	R	R
Chloroform	L	R	L	R	R
Chromic acid	NR	NR	NR	L	R
Cresol	NR	R	NR	R	NR
Cyclohexane	L	R	L	R	R
Cyclohexanone	NR	R	NR	R	L
Diacetone alcohol	NR	R	NR	R	R
Dichloromethane	L	R	L	R	R
Dimethyl formamide	NR	L	NR	R	NR
Dimethylsulfoxide	NR	R	NR	R	L
1,4 Dioxane	NR	L	L	R	R
Ethers	NR	R	NR	L	L
Ethyl acetate	NR	R	NR	R	R
Ethyl Alcohol	L	R	R	R	R
Ethyl alcohol (15%)	R	R	R	R	R
Ethyl alcohol (95%)	L	R	L	R	R
Ethylene dichloride	NR	R	NR	L	R
Ethylene glycol	L	R	R	R	R
Ethylene oxide	NR	L	R	R	R
Formaldehyde (2%)	L	R	R	R	R
Formaldehyde (30%)	L	R	R	R	R
Formic acid (25%)	NR	R	R	R	R
Formic Acid (50%)	NR	R	R	R	R
Freon®	R	R	R	R	R

	Cellulose Ester (CE) /Mixed Cellulose (ME)	Regenerated Cellulose (RC)	Polysulfone (PS) /Polyethersulfone (PES)	Polypropylene (PP)	Polyvinylidene Diflouride (PVDF)
Gasoline	R	R	L	R	R
Glycerine	R	R	R	R	R
Glycerol	R	R	R	R	R
Hexane	R	R	R	R	R
Hexanol	L	R	R	R	R
Hydrochloric acid (diluted-5%)	R	R	R	R	R
Hydrochloric acid (med conc-25%)	NR	NR	R	R	R
Hydrochloric acid (con-37%)	NR	NR	R	L	R
Hydrofluoric acid (25%)	NR	L	L	NR	R
Hydrogen peroxide (30%)	R	R	R	R	R
Iodine solutions	NR	NR	NR	R	R
Isobutyl alcohol	R	R	R	R	R
Isopropanol	L	R	R	R	R
Isopropyl acetate	NR	R	NR	R	R
Isopropyl alcohol	L	R	R	R	R
Isopropyl ether	L	R	R	L	R
Jet Fuel 640A	R	R	R	R	R
Kerosene	R	R	R	R	R
Lactic acid	R	R	R	R	R
Methyl acetate	NR	R	NR	R	R
Methyl alcohol	L	R	L	R	R
Methyl alcohol (98%)	L	R	R	R	R
Methyl cellosolve	L	L	R	R	R
Methyl Chloride	NR	R	NR	R	L
Methyl ethyl ketone	NR	R	NR	R	L
Methyl formate	NR	L	NR	R	R
Methyl isobutyl ketone	NR	R	NR	R	L
Methylene chloride	L	R	L	R	R
N-Methyl-2-Pyrrolidone	NR	R	NR	R	R
Mineral spirits	R	R	R	R	R
Monochlorobenzene	L	R	NR	L	R
Nitric acid (diluted-5%)	L	R	R	R	NR
Nitric acid (med conc-25%)	NR	NR	R	R	NR
Nitric acid (6N)	NR	N	R	L	R
Nitric acid (conc-70%)	NR	NR	NR	NR	NR
Nitric acid (concentrated)	NR	NR	R	NR	L
Nitrobenzene	NR	L	NR	NR	R
Nitropropane	NR	L	NR	L	R
"Oils, mineral"	R	R	R	R	R
Pentane	R	R	R	R	R
Perchloric acid (25%)	NR	L	NR	NR	R
Perchloroethylene	NR	R	NR	L	R
Petroleum based oils	R	R	R	R	R
Petroleum ether	R	R	R	R	R
Phenol (0.5%)	R	R	R	R	R

	Cellulose Ester (CE) /Mixed Cellulose (ME)	Regenerated Cellulose (RC)	Polysulfone (PS) /Polyethersulfone (PES)	Polypropylene (PP)	Polyvinylidene Diflouride (PVDF)
Phenol (10%)	NR	R	L	R	R
Phosphoric acid (25%)	NR	L	R	R	R
Potassium hydroxide (1N)	L	L	NR	R	R
Potassium hydroxide (25%)	NR	R	R	R	R
Potassium hydroxide (50%)	NR	NR	R	R	R
Propanol	R	R	R	R	R
Pyridine	NR	R	NR	R	L
Silicone oil	R	R	R	R	R
Sodium hydroxide (0.1N)	L	R	R	R	R
Sodium hydroxide (diluted-5%)	NR	L	R	R	R
Sodium hydroxide (25%)	NR	L	R	R	R
Sodium hydroxide (conc-50%)	NR	NR	R	R	R
Sodium Hydroxide (Concentrated)	NR	NR	R	R	R
Sodium Hypochlorite	R	R	R	L	R
Sulfuric acid (diluted-5%)	L	R	R	R	R

	Cellulose Ester (CE) /Mixed Cellulose (ME)	Regenerated Cellulose (RC)	Polysulfone (PS) /Polyethersulfone (PES)	Polypropylene (PP)	Polyvinylidene Diflouride (PVDF)
Sulfuric acid (med conc-25%)	NR	L	R	R	R
Sulfuric acid (6N)	NR	L	R	R	R
Sulfuric Acid (concentrated)	NR	NR	R	NR	L
Tetrahydrofuran	NR	R	NR	R	R
Toluene	R	R	L	R	R
Trichloroacetic acid (25%)	NR	NR	R	R	R
Trichlorobenzene	NR	R	NR	R	R
Trichloroethane	L	R	L	R	R
Trichloroethylene	R	R	R	R	NR
Triethylamine	NR	R	NR	L	R
Turpentine	NR	R	NR	R	R
Urea	R	R	R	R	R
Urea (6N)	NR	R	NR	R	R
Water	R	R	R	R	R
Xylene	NR	R	NR	R	R

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Figure A-5 Filtration and Bioprocessing Chart

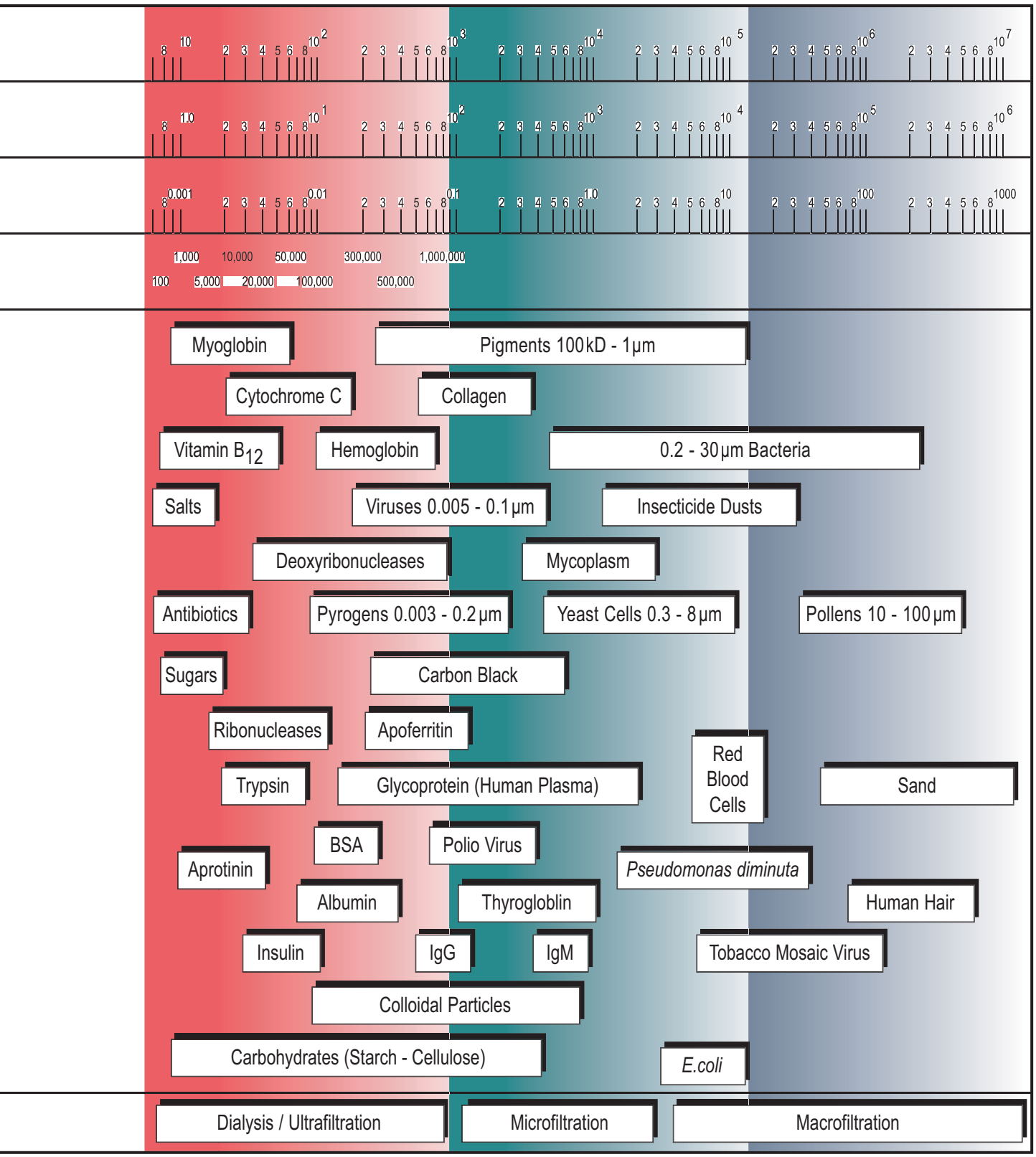
Hollow Fiber Tangential Flow Filtration

Spectra/Por® Dialysis Membranes

SeleXtrac® Molecular Extraction

CellMax® Cell Expansion

Angstroms (A)	log scale	
Nanometers (nm)	log scale	
Microns (µm)	log scale	
Daltons (MW)	no scale	
Relative Sizes of Common Materials		
Separation Process		


0.001
0.01
0.1
1.0
10
100
1000
1,000
10,000
50,000
300,000
1,000,000
100
5,000
20,000
100,000
500,000
Myoglobin
Pigments 100kD - 1µm
Cytochrome C
Collagen
Vitamin B12
Hemoglobin
0.2 - 30µm Bacteria
Salts
Viruses 0.005 - 0.1µm
Insecticide Dusts
Deoxyribonucleases
Mycoplasm
Antibiotics
Pyrogens 0.003 - 0.2µm
Yeast Cells 0.3 - 8µm
Pollens 10 - 100µm
Sugars
Carbon Black
Ribonucleases
Apoferritin
Red Blood Cells
Trypsin
Glycoprotein (Human Plasma)
Sand
BSA
Polio Virus
Aprotinin
Pseudomonas diminuta
Albumin
Thyrogloblin
Human Hair
Insulin
IgG
IgM
Tobacco Mosaic Virus
Colloidal Particles
Carbohydrates (Starch - Cellulose)
E.coli
Dialysis / Ultrafiltration
Microfiltration
Macrofiltration