

Micro/Nanofiber Media for Biopharmaceutical Filtration

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Abstract

The biopharmaceutical industry is highly dependent on filtration to ensure pure raw materials, separate and purify the target compound and ensure a sterile product. The production of therapeutic compounds by biological routes is ten times more filtration intensive than standard pharmaceutical operations. Every biopharmaceutical process is different and requires a different level of filtration but, in general, the process can be broken down into fermentation, cell harvest, clarification, purification, diafiltration, virus removal and sterile filtration.

The fermentation step requires sterile air and liquids to be feed into the bioreactor. Cells are concentrated and broken apart after they have produced the desired product. The effluent is a mixture of cells, cell fragments, colloids, biopolymers and proteins. The Clarification process harvests soluble protein from the cell culture. A combination of nonwoven depth and surface filters in combination with microfiltration membranes are used. The purification step is the key operation in the process where the product molecule is separated from proteins, viruses and endotoxins. Microfiltration, ultrafiltration or nanofiltration membranes and/or chromatography are used depending on the size of the target molecule. The volume of the purified product is reduced and the buffer system exchanged in a diafiltration step. High flow membranes are generally used in this step. The concentrated product undergoes viral clearance and sterile filtration to ensure product safety.

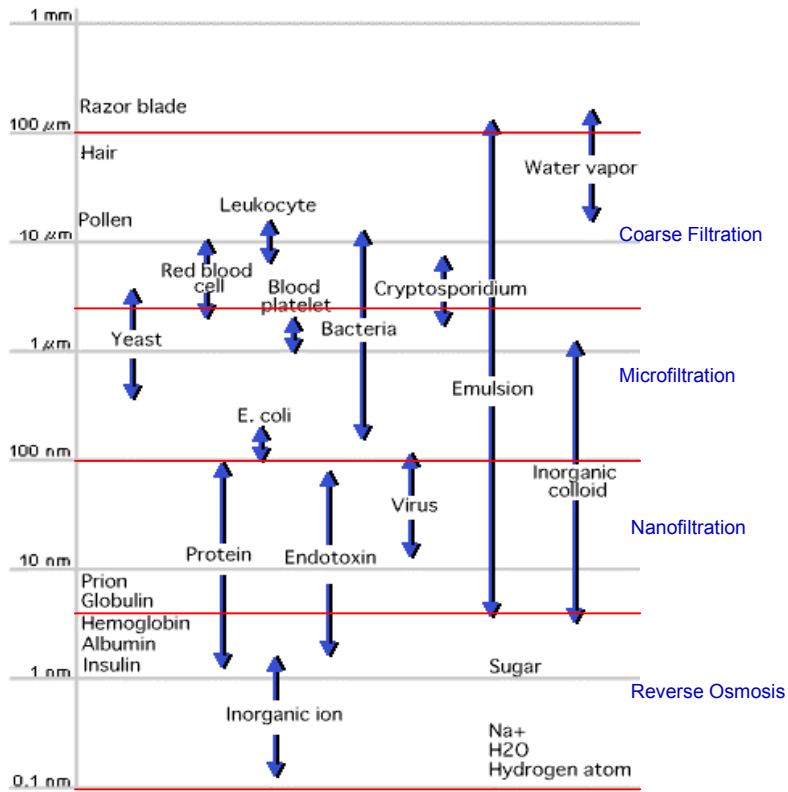
The biopharmaceutical industry uses a combination of nonwoven and membrane media for their filtration needs. Nonwoven media have been limited to the clarification and membrane prefiltration steps due to their poor performance in submicron filtration. A significant amount of research has been expended over the last few years on developing high efficiency wetlaid media using micro/nanofibers to improve their performance in submicron filtration applications. This paper will review the methods of construction, performance and cost of traditional wetlaid, drylaid and membrane media used biopharmaceutical filtration compare them with the next generation nonwoven media containing micro/nanofibers.

Keywords: Biopharmaceutical, Nonwoven, Membrane, Microfiltration, Ultrafiltration, Nanofibers, Microfiberglass

Biopharmaceutical Filtration

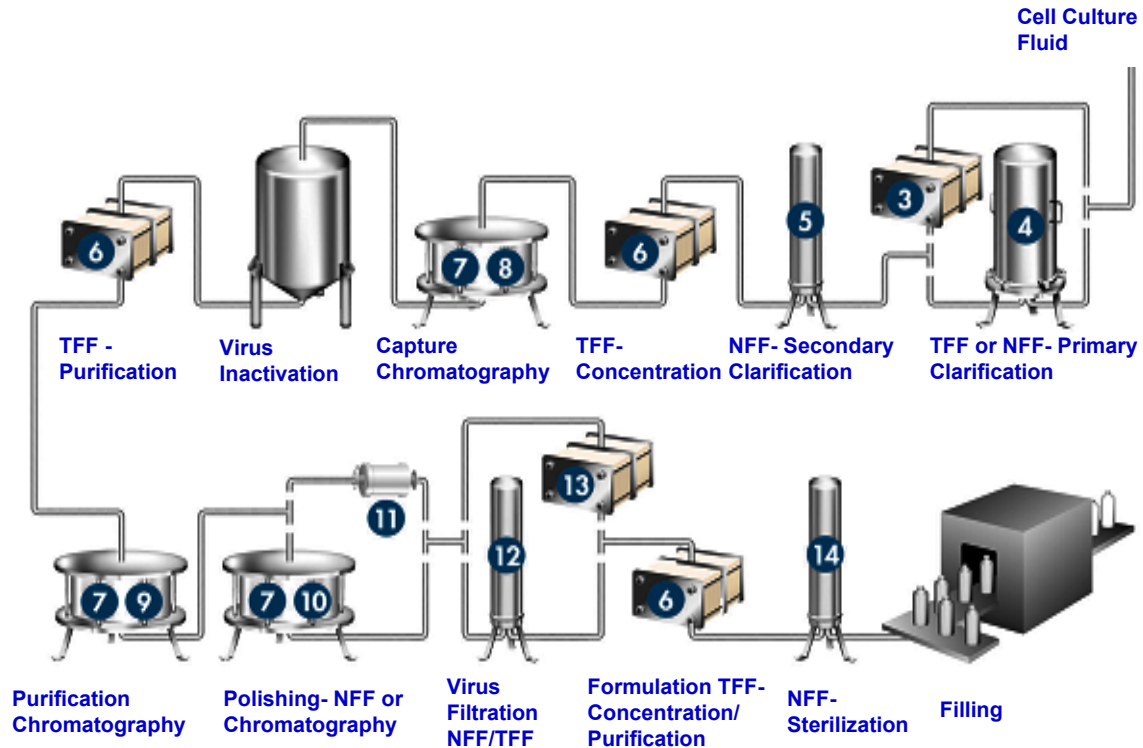
The biopharmaceutical Industry is highly dependent on filtration to ensure pure raw materials, separate and purify the target compound and ensure a sterile product. There are a wide range of products and/or contaminants below that must be removed from biopharmaceutical liquid streams. Figure 1 shows the relative size of proteins, virus, endotoxins, bacteria and colloids and the filtration class that is needed to remove them.

Figure 1



The production of therapeutic compounds by biological routes is ten times more filtration intensive than standard pharmaceutical operations. Every biopharmaceutical process is different and requires a different level of filtration but in general the process can be broken down into fermentation, cell harvest, clarification, purification, diafiltration, virus removal and sterile filtration. Figure 1 outlines a typical biopharmaceutical filtration operation.

Figure 2 Typical Biopharmaceutical Filtration Operation



Herb Lutz, Robert Shaw , Millipore Corporation

Fermentation and Cell Harvest

The fermentation step requires sterile air and liquids to be feed into the bioreactor. Cells are concentrated and broken apart after they have produced the desired product. The effluent is a mixture of cells, cell fragments, colloids, biopolymers and proteins

Clarification

The clarification process harvests soluble protein from the cell culture. Due to the high particle load and wide distribution of particle sizes after cell harvest it is more economical to break the clarification operation into several steps. The primary clarification step is the bulk removal of whole cells, cell fragments, and large particles. A series of depth or pad filters are used to remove progressively smaller and smaller particles. The effluent from this separation still contains relatively high levels of submicron particles. These particles are predominately colloidal, protein agglomerates.

A secondary clarification step removes colloids, lipids and reduces the bioburden. This step reduces the fine particle load to a level that will not plug the purification filters prematurely without removing the intended product. A combination of nonwoven depth and surface filters in combination with microfiltration membranes are used.

Purification and Diafiltration

The purification step is the key operation in the process where the product molecule is separated from proteins, viruses and endotoxins. Microfiltration, ultrafiltration or nanofiltration membranes and/or chromatography are used depending on the size of the target molecule. The target molecule may be retained or passed through the membrane filter. The volume of the purified product is reduced and the buffer system exchanged in a diafiltration step. High flow membranes are generally used in this step. Balancing efficient product recovery and processing rates is always a critical factor in diafiltration.

Viral Clearance

The concentrated product undergoes viral clearance and sterile filtration to ensure product safety. More than one type of viral clearance step is required so that viruses resistant to the first mechanism may be cleared by the second. This is needed to ensure product safety. Economical clearance with high product recovery requires careful selection and optimization. Current virus-retentive filters are ultrafiltration membranes or very tight microporous membranes. Particle retention by these filters relies heavily on size exclusion. There is no industry standardization for filter performance or ratings.

Sterile Filtration

Sterile filtration of a final purified product ensures safety by maintaining low bacterial counts and minimizes endotoxin levels. Sterile filters are usually microfiltration membranes with a 0.45 μm rating. The final filter should provide sterile filtration without altering the efficacy or potency of the product. This is best accomplished by using filters that are non-pyrogenic, with low binding and low extractables. Sterilizing filters must be integrity testable and either readily sterilized or supplied pre-sterilized. The final filter (or prefilter) combination must provide proper flow rate.

Filtration Media Types

Membranes

Microfiltration and ultrafiltration membranes are produced by a wide variety of processes. The most common process is to dissolve a polymer in a solvent and produce a liquid film on a support material. The solvent is removed by evaporation or dilution in a nonsolvent and the polymer is precipitated forming a porous structure. The concentration of polymer in the solution and the rate of precipitation determine the degree of porosity and the pore size distribution. MF membranes can be produced using many different polymers. Nylon, Nitrocellulose, cellulose acetate, Polysulfone, PVDF and polycarbonate are the most common. Each polymer produced a membrane with different characteristics. Microfiltration membranes are produced with efficiencies from 0.05 μm to 5 μm .

Ultrafiltration membranes can also be made with a range of polymers. Cellulose acetate, regenerated cellulose, polysulfone, PVDF, PPS and polycarbonate are the most common. MF membranes are generally rated using molecular weight cutoffs instead of micron ratings. They are produced with efficiencies below 0.01 μm or 1 kilodalton to 1,000 kilodalton.

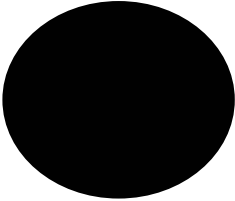
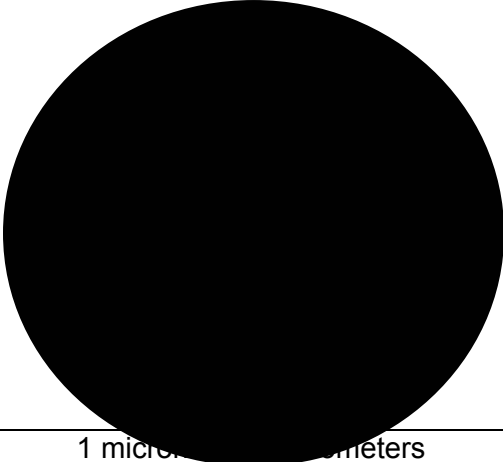
Nanofiltration membranes are used to remove low molecular weight proteins, sugars and monovalent salts. Reverse Osmosis membranes are used for the removal of monovalent

salts. Cellulose acetate and thin film composite membranes are the most common type. Cellulose acetate membrane formation is similar to UF membrane process. The membrane is then annealed to increase efficiency. The efficiency and permeability can be changed with temperature and residence time. Cellulose acetate membranes are lower cost but less efficient and lower permeability at a given pressure drop than thin film composite membranes. Thin film composite, RO membranes are produced by forming a MF membrane on a fabric. An ultrathin layer of monomer is coated on the MF membrane. The monomer solution is polymerized forming a high rejection low pressure drop membrane.

Nonwoven Media

Nonwoven media are produced by forming a mat of fibers. The fiber diameter, orientation, packing density and web weight all determine the filter media properties. The finer the fiber diameter the smaller the pores that are produced. The smaller the nonwoven web pore size the finer the filtration efficiency. In very general terms, the average fiber diameter of a nonwoven media is equivalent to the efficiency. For example, a nonwoven media with an average fiber diameter of 1 μm will have a nominal efficiency of +/- 1 μm , depending on web weight, density and uniformity. Figure 3 shows the relative dimension of nano and micro sized fibers.

Figure 3

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0.01 micron/10 nanometers	0.1 micron/100 nanometers
	
0.5 micron/500 nanometers	1 micron/1000 nanometers

Nonwoven media can be classified into two distinct types based on their method of formation. The first method is a dry laid process, which includes carded, needled, spunbond and meltblown media. The second process uses a wet laid formation, which is generally done on a paper machine. Each process produces a media with unique properties that have advantages in different applications.

Dry laid processes generally produce media with nominal ratings that are low cost and have high dirt holding capacities. The nonwovens generally are produced with polyolefin, polyester or nylon polymers and fibers. Meltblown media are one of the most versatile nonwovens for liquid filtration and the only one that can achieve submicron filtration. Meltblown media is generally composed of a continuous network of self-bonded polypropylene, polyester or nylon microfibers produced with a controlled fiber uniformity and density. The resulting media has a uniform porosity, does not shed fibers and contains no binders, adhesives or surfactants. Meltblown media have nominal ratings from 1 μm to 50 μm and when calendered or laminated into composites can have sub micron and absolute ratings. Meltblown media can also be produced using high-purity FDA-acceptable polymers.

Meltblown media are produced by blowing a thermoplastic resin from an extruder die tip with air at a high velocity onto a substrate, belt or wire, which results in a self-bonded web with relatively fine fibers. Fibers produced in a meltblown operation are generally in the 4 – 20 μm range. Using very high airflow, meltblown fibers can be produced in the 2 – 5 μm range. Meltblown media have not generally been successful in finer filtration applications due to the relatively large fibers that are produced. A significant amount of research has been expended over the last few years on developing fine diameter fibers. Submicron fibers can be produced with a meltblown process using specialized equipment and slow production rates. Generally the improvements in performance are outweighed by the higher cost.

Another method of producing higher efficiency meltblown media is to hot calender the product. In this process the meltblown media is run between two heated steel rolls or a heated steel roll and a soft rubber roll with pressure. The calendering process compresses the fibrous

structure and with enough heat and pressure can begin to form a microporous film. Calendering increases the efficiency of the media but also increases the pressure drop. Meltblown media can be calendered to absolute efficiencies below 1 μm .

Wetlaid Media

Wetlaid media are generally produced on a paper machine and with cellulose, polymeric or glass fibers. It is common for media to be produced with one or more of the different fiber types. Microfiberglass media can be produced with the broadest range of filtration capability and efficiencies due to the wide range of fibers available. Wetlaid media can be made with nominal or absolute filter ratings. They typically contain binders which can have poor chemical and thermal resistance and high extractables when compared to air laid media. Wetlaid media can also be made using FDA compliant materials. New wetlaid media have begun to compete with MF membranes in many applications. High efficiency wetlaid media have equivalent efficiency as MF membranes but with significantly higher dirt holding capacity or life.

Cellulose based media is generally lower cost with poor retention characteristics and low dirt holding capacity. Cellulose fibers are coarse and flat which produces a dense, two-dimensional structure with high-pressure drop. The addition of synthetic polymeric fibers to a cellulose sheet will significantly improve the filtration performance by opening up the structure and adding finer cylindrical fibers to the matrix which do not surface load as readily. Wetlaid media produced with 100% synthetic or glass fibers will generally result in a very three-dimensional sheet with lower pressure drop and higher dirt holding capacity.

Performance

Three different glass based media samples were made with different sized nano/micro fibers at the same basis weight. The nano/micro fiber glass media were produced with 200 nm, 500 nm and 1000 nm diameter glass fibers. Glass fibers are produced with a distribution of fiber diameters. The values used in this paper are were weight average fiber diameter. The media samples were tested for efficiency, clean water flowrate and dirt holding capacity and compared to MF membranes and calendered meltblown. Table 1 compares the rated efficiency value, resistance to airflow and the median pore size of three glass media samples.

Table 1

Ave. Fiber Size (nm)	Rating (μm)	Resistance (Pa)	MFP (μm)
200	0.2	1200	1.1
500	0.5	800	1.5
1000	1.0	400	3.5

Table 2 compares the performance of each media type at the same micron rating. The nano/micro glass fiber media has significantly higher water flux and dirt holding capacity than the MF membrane or the calendered meltblown and particle retention that is close to the MF membrane with a cost that is 40% lower.

Table 2

Media Type	Clean Water Flux	Initial Efficiency	Turbidity Reduction	Dirt Holding Capacity	Cost
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	(ml/min- cm ₂)	(%)	(%)	(g/m ²)	(Euro/SQM)
Glass	21	89	98	28	6
MF Membrane	2	93	100	1	10
Calendered MB	1	24	99	1	4

Conclusion

Glass based nonwoven media can be produced with nano/micro sized glass fibers to produce media with high efficiency. These high efficient glass media can compete with the more traditional microfiltration media, such as, membranes and calendered meltblown, in Biopharmaceutical applications.