A work in process

Membrane-based chromatography is paving the way for high-throughput biopharmaceutical processing.

BY TIM N. WARNER AND SAM NOCHUMSON

In pharmaceuticals, there is tremendous pressure to bring new drugs to market quickly. Each day of delay can result in millions of dollars of lost revenue. In the case of small biotech start-ups, survival is often correlated with the burn rate as well as the speed of Phase II clinical trials. The traditional process development cycle of a new drug is complex and time-consuming. If there was a way to streamline the cycle and thereby accelerate clinical trials of new drug candidates, pharmaceutical and biotech companies would reap enormous financial benefits.

Chromatography challenges

In biopharmaceuticals, few have succeeded in developing purifications without chromatography. Chromatography is a popular purification method that exploits both the physical and chemical differences between biomolecules. Although widely used for biological molecules, chromatography is slowly evolving to meet the needs of pharmaceutical manufacturers. Currently, biopharmaceutical processors face demands for highly purified products that are free of contaminating proteins, viruses, and nucleic acids. Several steps must be used to achieve the required purity. Unfortunately, each step potentially results in a loss of valuable sample and precious time. Every advance in separation techniques brings the hope of a single robust and powerful chromatographic method.

Ion-exchange chromatography has been the workhorse of downstream processing because of its exceptional capacity. Over the decades, continual improvements in ion-exchange media have met the increasing demands for purity. One way to achieve higher resolution is to use smaller and more uniform chromatographic beads, but this solution comes with a concomitant reduction in the flow rate. One can increase the pressure at which the columns are run (as in HPLC), but this increase has its limitations. Although HPLC columns have grown larger in recent years, there are manufacturing limitations to their size. Historically, there has been a compromise between resolution and throughput. Many engineers have chosen large chromatography beads (80–100 µm) in process-scale chromatography columns to achieve faster flow rates while still maintaining sufficient resolution.

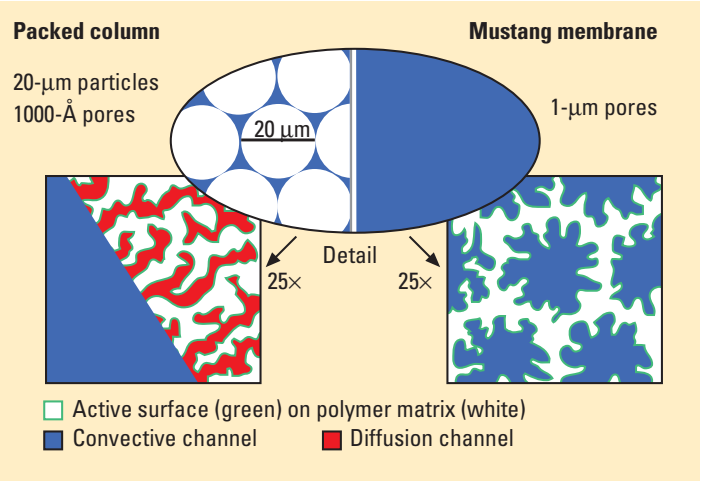

Figure 1. Diffusive difference. For resin-based chromatography columns (left), there is a long diffusion path into the internal pores of beads. By contrast, macromolecules are readily bound in membrane chromatography (right) without diffusing long distances.

Diffusion limitations

In purification science, we have often asked: What if there were no need to compromise flow rate and resolution?

Membrane chromatography may offer a solution to this seemingly absurd question. Compared with traditional chromatography, it has 100-fold higher throughput and efficiency, making it a compelling option for biopharmaceutical purification. Throughput is an important consideration in large batch sizes. Columns are sized so that there will not be a bottleneck in the downstream process. Surprisingly, a small membrane chromatography unit not only can handle the workload of a large chromatography column but also can do it much faster.

Polymeric chromatography beads present several roadblocks to fast purification that limit their efficacy. The flow rate depends on diffusion into the beads' pores, which is a rate-limiting step. The vast majority of chromatography beads' ion-exchange groups are located in internal pores. The long diffusion path restricts mass transfer, thereby limiting purification speed. Furthermore, large molecules might actually be excluded from the bead's pores (Figure 1). If polymeric bead pores are enlarged to accommodate large molecules, the bead's framework will become too fragile to withstand the high pressures needed for high flow rates. Beads with large pores also have reduced capacity because of a smaller internal surface area.

Unfortunately, even large-pore beads would have preferential flow through the spaces between the beads rather than into the pores. Because of the limited accessible surface area, beads are inefficient at purifying large biomolecules (>300 kDa). For example, large plasmid DNA vectors would

KEY TERMS: genomics, high throughput, proteomics, regulations, technique

ILLUSTRATION COURTESY OF PALL LIFE SCIENCES

bind only on the outside of beads, because they are excluded from the pores. There are fewer binding sites available for large molecules and thus a limited capacity. Furthermore, the height of a chromatography column containing traditional beads is in part determined by the residence time needed for slow diffusion into the bead pores. The column for DNA and virus capture is often sized by flow rate, not by capacity. Therefore, to process large volumes, the columns are often much larger than capacity alone would dictate.

Dynamic capacity

Ion-exchange membranes are composed of microporous materials that have been chemically modified with charged hydrophilic polymers. These polymers are cross-linked to the membrane pore surfaces. The membranes are available in both the cation-exchange sulfonic acid (S) and the anion-exchange quaternary amine (Q). The S surfaces are negatively charged and therefore capable of capturing most proteins while allowing DNA, most viruses, and endotoxins to flow through. Using the same principle, Q surfaces are positively charged and thus retain DNA, viruses, and endotoxins.

Membrane-based chromatography offers several advantages that help increase the downstream throughput. Membranes have a higher dynamic capacity than beads, allowing faster flow rates for capturing biomolecules. Studies have shown that using membranes increases dynamic capacity dramatically and improves the flow rate up to 100-fold. The pathway for binding biomolecules to a charged membrane is much shorter than that for charged bead pores. Increased throughput results directly from the convective flow that minimizes diffusion distances. DNA and protein binding is no longer limited by long diffusion times as it is in bead-based columns.

The capture efficiency (dynamic capacity) is much greater for membrane than for column chromatography. In fact, the thickness of a membrane's stack (i.e., its bed height) is not as critical as with resin-bead chromatography. Therefore, membrane chromatography units are designed with a shorter bed height than traditional columns. The column bed height is held constant so that the membrane chromatography unit can easily be scaled up as a linear function.

Dollar for dollar

To compare the costs of membrane- and resin-based chromatographic separations, we will describe the factors involved in the processing of a 2000-L biopharmaceutical sample. The results are summarized in the table.

Time consumed. One disposable membrane chromatography cartridge working at a flow rate of 50 L/min requires approximately 1 h to be tested for integrity, to be washed, and to process the 2000-L sample. By comparison, a 90-L traditional chromatography column is needed for a flow rate of 20 L/min (a larger column could be used if a higher flow rate were desired). This 90-L column requires 0.5 h for equilibration, 1.75 h for processing the 2000-L sample, and 1.75 h for cleaning. Three more hours are then needed to test resolution and cleaning efficiency and to make a microbial growth measurement. Thus, a membrane system will not only decrease the time required to process a batch but also increase the number of possible runs per day.

Buffer. Another important cost consideration is the amount of buffer used for cleaning. People are often surprised that the cost of buffer accounts for approximately half of the downstream processing costs. An average buffer cost of $5/L is used in the following calculations.

Traditionally, each wash of a column requires three to five column volumes (270–450 L) of buffer. Assuming an average equilibration, cleaning with various solutions, and storage buffers, this batch would require ~2000 L of buffer, or an expenditure of $10,000. Because of its disposability, a single membrane chromatography unit consumes only 60 L of buffer for an expense of $300 and a per-batch savings of $9700.

Labor-ious math. Labor is another significant cost in biopharmaceutical operations. Assuming two operators are needed to process the 2000-L batch, the labor costs, including overhead, are estimated at $150/h per operator. Seven hours of labor will be required for each batch (excluding the time spent packing the column). If the labor cost is $300/h (2 × $150/h), the labor cost per batch is $2100. A single-use membrane unit, however, requires only 1 h for the process and therefore costs $300 in labor—a savings of $1800 per batch.

A capital idea. Significant capital expenditures are involved in establishing traditional process-scale chromatography systems. A 90-L column represents a capital outlay of ~$200,000 for a large chromatography column and a packing station. If this cost were amortized over 1000 cycles, or 7 years, the equipment expense would be $200/cycle. The chromatography media required for a 90-L column would be approximately $70,000 if used for 50 cycles. Thus, the cost per cycle is approximately $1400. The combined column and media capital outlay is $1600 per cycle. By comparison, the outlay for an equivalent membrane chromatography unit is approximately $3500 per cycle.

Although the expense of membrane chromatography is greater in the last category, a comparison of the overall cost of media, equipment, buffers, and labor indicates that membrane chromatography offers a $9600 savings for each batch processed. (See the table on costs.)

Per-cycle costs of resin-based column and membrane-based chromatography.

Process	Media and equipment	Buffer	Labor	Total
Resin-based	$1,600	$10,000	$2,100	$13,700
Membrane-based	$3,500	$300	$300	$4,100
Savings	–$1,900	$9,700	$1,800	$9,600

Membranes can be wound into units similar to classic sterile filtration cartridges and capsules. For example, a 4 × 30 in. membrane chromatography unit has a flow rate of approximately 45 L/min. Achieving a similar flow rate with a traditional packed bed would require a >100-L column.

There is no need for cleaning and the associated cleaning validation because membrane chromatography units are discarded after each batch processing. Cleaning validation of traditional, bead-based chromatography columns can take months of costly process development time. If cleaning and reproducibility problems arise in process development, the timeline may be extended significantly. These validation problems can have a serious impact on time-to-market for pharmaceuticals. Furthermore, the direct costs of time associated with cleaning steps are hidden and easily overlooked by some biopharmaceutical manufacturers. Significant labor costs also are associated with producing large buffer volumes for column cleaning (see box, "Dollar for dollar").

The efficient capture of membrane chromatography offers significant advantages for gene therapy applications.

Capture efficiency

Membrane chromatography is most advantageous for applications requiring the processing of large volumes, in which a minor contaminant such as DNA or virus needs to be removed. Efficient binding to quaternary amines on the convective Q-membrane surfaces eliminates typical diffusion-based equilibrium. The membrane is robust and not significantly affected by changes in residence time due to accidental increases in flow rates.

The efficient capture of membrane chromatography offers significant advantages for gene therapy applications. Membrane chromatography has a much greater capacity for viruses than other chromatography media. For example, a 16-layer membrane stack could bind 10^{13} adeno-associated viruses per cubic centimeter of membrane volume, whereas traditional ion-exchange resins have a 20-fold lower capacity for virus particles. As gene therapy and vaccine products become more accepted worldwide, the pressure for production is expected to rapidly increase. Membrane chromatography's high dynamic capacity and high throughput enable efficient and economic manufacturing of gene vectors.

Tim N. Warner is senior manager of process development, BioPurification, for Pall Life Sciences (Ann Arbor, MI), and **Sam Nochumson** is laboratory manager of the SLS Bioprocessing Laboratory at Pall Membrane Technology Center (Pensacola, FL). Send your comments or questions about this article to mdd@acs.org or to the Editorial Office address on page 3. ■