Chromatography Advisor #4

Capturing Very Large Biomolecules with Membrane Chromatography

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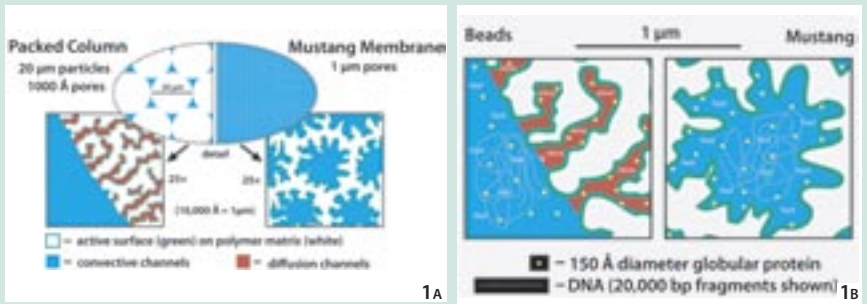
Manufacturing genetic therapies requires processing highly purified DNA/plasmid preparations that are free of contaminating proteins, viruses, nucleic acids, enzymes, and enzyme inhibitors. Quick methods for separating and purifying high–molecular-weight proteins and genetic vector preparations are essential to meet those needs. Traditional chromatography presents a number of inefficiencies with such large target molecules and slow diffusional flow rates. Here I present a closer look at the enabling technology offered by ion-exchange membrane chromatography for purification. It offers the potential to significantly accelerate cycle times and help manufacturers bring new therapies to market faster.

Figure 1: Membranes (right in both figures) have more available surface area for binding compared with packed columns (left in both figures).



Capacity Limitations of Resin Bead Chromatography

Column chromatography presents several roadblocks to fast purification that limit its efficacy. Purification flow rates depend on diffusion into resin-bead pores, and because the vast majority of a resin's ion-exchange groups are located within those internal bead pores, biomolecules face a long and restrictive diffusion path. Beads have a limited accessible surface area that makes them inefficient for purifying very large biomolecules (>200 kb) such as plasmids and viruses. Such molecules bind on only the outside of resin-bead and not within pores, leading to fewer available binding sites and limited capacity.

The height of a resin-bead column is in part determined by the residence time needed for diffusion into the beads' pores. Columns for DNA and virus capture are usually sized by flow rate and not capacity. Therefore, to process large batch volumes, resin-bead columns are often much larger than capacity alone would demand. In process scale-up, new hardware is expensive, and column packing can be labor intensive and unreliable.

Porous Membranes Speed Flow Rates

Membrane chromatography purifies large biomolecules better because it offers a three-dimensional structure with open (0.8-µm) pores. Such a large pore size creates channels for the immediate availability of all active chemistry groups on the membrane surface. This allows high binding capacities of large particles (plasmids, DNA, and viruses) with almost no diffusional limitations. Because of the number of large pores offering high internal surface area, capacity can be 10 times greater and flow rates 100 times faster than diffusion-limiting resin-bead chromatography

Figures 1A and 1B demonstrate the difference in surface areas for binding between packed columns and membrane ion-exchange chromatography. In the packed column (Figure 1B, left) white areas represent resin-beads and blue areas represent flow paths. On the membrane (Figure 1B, right), the blue area greatly exceeds the white, illustrating greater availability of surface areas for binding, which translates into a much higher flow rate.

One type of ion-exchange membrane is made of microporous polyethersulfone (PES) chemically modified with charged hydrophilic polymers. The polymers are cross-linked to internal and external membrane pore surfaces to produce either sulfonic acid ("S") or quaternary amine ("Q") surfaces. "S" surfaces have

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Process Focus: Purification

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Table 1: Virus removal by membrane chromatography

Virus	Removal from Flow-Through (log_{10})
MuLV	≥ 4.73
PRV	≥ 4.13
BVDV	0.40
PPV	≥ 6.98
HAV	≥ 3.84

negatively charged ion-exchange groups that capture most proteins while allowing the majority of DNA, viruses, and endotoxins to flow through unimpeded. Using the same principle for oppositely charged binding, "Q" surfaces are positively charged and remove negatively charged DNA, most viruses, endotoxins, and host-cell proteins.

The open, convective-pore structure of microporous PES membranes enables high mass transfer and biomolecular binding to large surface areas by direct fluid convection. DNA, viruses, endotoxins, and large proteins binding to membranes are not limited by long diffusion times (as seen in resin-based chromatography) because the flow follows directly through convective pores.

The adsorption capability of membrane chromatography has been well documented. For example, Q chemistry membranes have demonstrated binding efficiencies of 10^{13} adenovirus particles per milliliter of bed volume of membrane.

As Table 1 shows, membrane chromatography has demonstrated effective removal of model viruses — such as murine leukemia virus, pseudorabies virus, bovine viral diarrhea virus, porcine parvovirus, and hepatitis A virus — at removal efficiencies between 104 and 107. Model viruses range in size and complexity. If a manufacturer's data demonstrate their removal, it is safe to assume that the same purification protocol will effectively purge other similar viruses.

Linear Scale-Up

In resin-bead chromatography, diffusional limitations cause the maintenance of proportional flow rate and column configuration during scale-up to be difficult and expensive. By contrast, scale-up in membrane chromatography is a straightforward, linear procedure. Capacity is directly proportional to membrane surface area (volume) and hence to the size of a membrane unit, where bed height or the number of membrane layers is held constant. With a large or small membrane unit, flux (flow rate/pressure) directly correlates to size. That makes it easy to scale-up to batch sizes of 10,000–20,000 L or more.

Because capture efficiency (dynamic capacity) is much greater with membrane chromatography, the thickness of a membrane's stack (e.g., its "bed height") is not as critical as with resin-bead chromatography. Therefore, membrane stacks are much flatter and smaller volumetrically than column beds, making them flexible and more easily scaled up, while still providing for dynamic binding capabilities. For example, a membrane stack of 16 layers can bind as many as 10^{13} adeno associated viruses per mL of packed bed (i.e., 1 mL of membrane volume).

The ability of membrane chromatography technologies to handle high binding capacities and increased flow rates for DNA, viral, and endotoxin clearance is a major advantage for biopharmaceutical manufacturers previously hindered by the inefficiency of traditional chromatography in screening large biomolecules.

As gene therapy and vaccine products become more common worldwide, the pressure for production is expected to dramatically increase. Membrane chromatography's dynamic capacity and high throughput make it an enabling processing tool that can streamline production and lower overall manufacturing costs.

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