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Chromatography Advisor # 3

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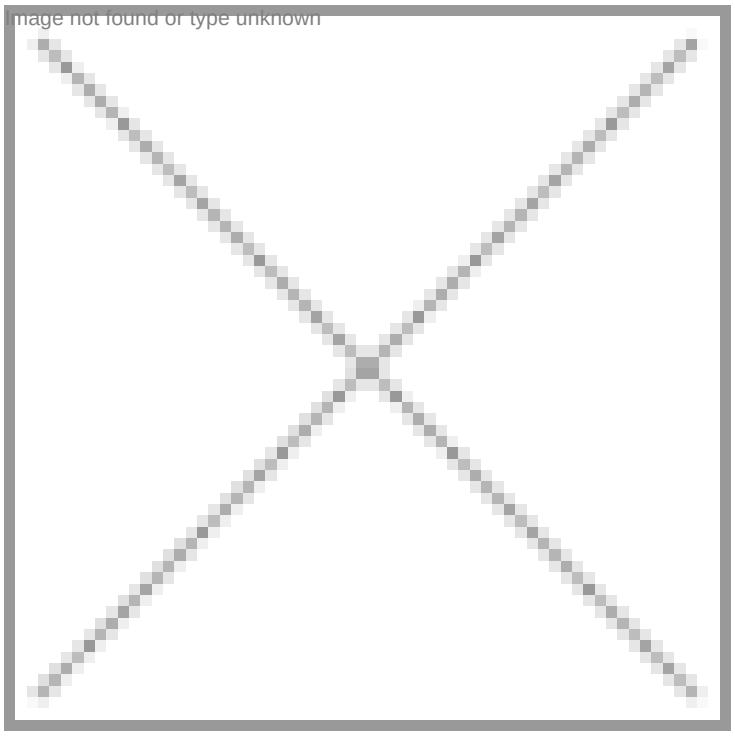

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Capturing large biomolecules with membrane chromatography

The manufacture of genetic therapies requires processing highly purified protein or plasmid preparations that are free of contaminating proteins, viruses, nucleic acids, enzymes, and enzyme inhibitors. In order to meet these needs, quick methods for separating and purifying large molecular weight proteins and genetic vector preparations are essential. Traditional chromatography presents a number of inefficiencies given the large size and weight of the target molecules and slow diffusional flow rates. This advisor takes 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.

Removal of virus from flow through (log10)	
MuLV	>4.73
PRV	>4.13
BVDV	0.40
PPV	>6.98
HAV	>3.84

Capacity limitations of resin-bead chromatography

Column chromatography presents several roadblocks to fast purification that ultimately limit its efficacy. Purification flow rates depend on diffusion into the pores of resin-beads, and since the vast majority of a resin's ion exchange groups are located within these internal bead pores, biomolecules have a long and restrictive diffusion path. Beads have a limited accessible surface area that makes them inefficient for purifying large biomolecules (>200kb) such as plasmids and viruses. Binding of these larger molecules occurs only on the outside of resin-beads 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. The column for DNA and virus capture is usually sized by flow rate and not by capacity. Therefore, in order to process large batch volumes, resin-bead columns are often much larger than capacity alone would demand. In scale-up, new hardware is an expensive endeavor, and column packing can also be labor intensive and unreliable.

Porous membranes speed flow rates

Membrane chromatography is better able to purify large biomolecules because it offers a three-dimensional structure with open (0.8um) pores. The large pore size creates channels for the immediate availability of all active chemistry groups on the membrane surface, allowing high binding capacities of large particles such as plasmids, DNA, and viruses, with almost no diffusional limitations. Due to the multitude of large pores with high internal surface area, capacity can be 10 times greater and flow rates 100 times faster than diffusion-limiting resin-bead chromatography

Figures A and B demonstrate the difference in surface areas for binding between packed columns and membrane ion exchange. In the packed column (Figure B, left) the white areas represent resin-beads and blue areas represent flow paths. On the membrane (Figure B, right), the blue area greatly exceeds the white, illustrating the 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) that has been chemically modified with charged, hydrophilic polymers. These polymers are cross-linked to the internal and external membrane pore surfaces to produce either sulfonic acid ("S") or quaternary amine ("Q") surfaces. "S" surfaces have negatively charged ion exchange groups able to capture most proteins while allowing most DNA, most viruses, and endotoxin to flow through unimpeded. Using the same principal 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 these microporous PES membranes enables high mass transfer and biomolecular binding to large surface areas by direct fluid convection. DNA, virus, endotoxin and large protein binding to membranes is 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 1013 adenovirus particles per ml bed volume of membrane.

As Figure C shows, membrane chromatography has demonstrated effective removal of model viruses such as porcine, parvovirus, hepatitis A virus, murine leukemia virus, and pseudorabies virus at removal efficiencies between 104 and 107. Model viruses range in size and complexity. If a manufacturer's data demonstrate removal of these model viruses, 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 challenging and expensive. By contrast, scale-up in membrane chromatography is a straightforward linear procedure because capacity is directly proportional to the membrane surface area (volume) and hence to the size of the membrane unit, where the bed height or number of membrane layers is held constant. With a large or small membrane unit, there is a direct correlation between flux (flow rate/pressure) and size. This makes it easy to scale-up to batch sizes of 10,000-20,000 liters or more.

Moreover, because the capture efficiency (dynamic capacity) is much greater with membrane chromatography, the thickness of a membrane's stack (i.e. its bed depth) is not as critical as with resin-bead chromatography. As a result, membrane stacks are much flatter beds and smaller volumetrically, making them flexible and more easily scaled-up, while still providing for dynamic binding capabilities. For example, a membrane stack of 16 layers (~1cm thick) can bind as many as 1013 Adeno-

associated virus per milliliter of packed bed (i.e. 1ml of membrane volume).

The ability of new 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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Note: This article has earlier appeared in Bioprocess International.