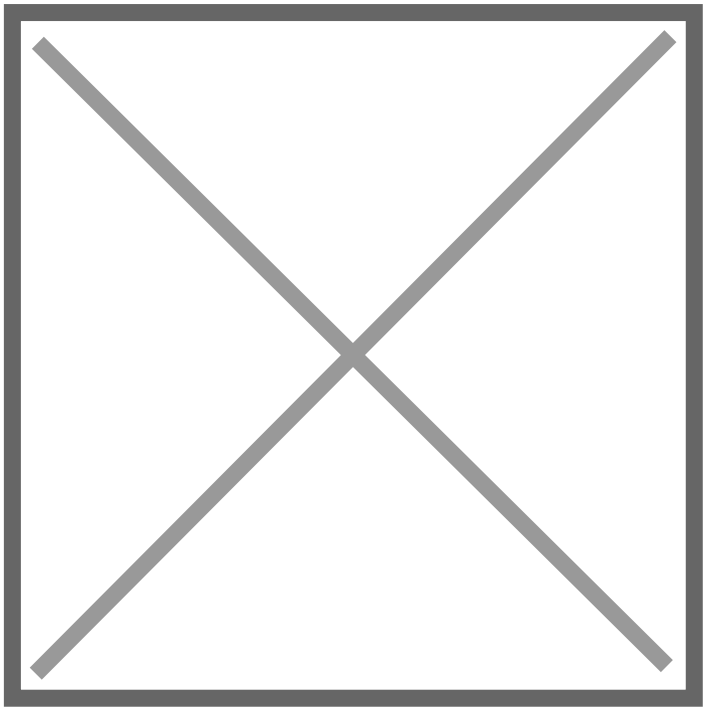


Chromatograpy Advisor

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An industry overview

Chromatography is one of the most powerful purification tools available to manufacturers, and its use can be seen across a wide array of industries. As a fundamental tool to detect, separate and purify substances, chromatography processes have been implemented into a number of manufacturing schemes. It is used to remove contaminants from foods and beverages, to separate unreactive complex hydrocarbons for petroleum applications, and to purify air and water.

For the biopharmaceutical industry, advanced chromatography technologies are helping to solve some of the tremendous capacity challenges that face drug manufacturers. To meet the rising demand for biotherapeutics that are free of contaminating proteins, viruses, nucleic acids, enzymes, and enzyme inhibitors, processors need to be aware of the ever expanding capabilities of chromatography for drug purification.

This article is the first in a series that examines some of the latest trends and technologies in process chromatography, particularly for biopharmaceutical manufacturers. This introduction provides a brief overview of some of the latest advances, and future columns will offer a more concentrated focus on specific areas of interest.

Customizing chromatography

There is a huge diversity of recombinant-based therapies that are being developed from different sources, from mammalian and non-mammalian animal cells, to transgenic milk, and from plant sources like corn and tobacco. This diversity requires customized chromatography processes in order to achieve the highest efficiency possible. In some cases a drug purification process can incorporate up to six separate chromatography steps. Fortunately, new innovations and advanced engineering can help manufacturers tailor the right solution for specific applications.

For manufacturers of recombinant therapies, column chromatography is the most common-and most powerful-purification method for downstream processing. A variety of methods are employed, including affinity, ion exchange, hydrophobic interaction, and, to a lesser extent, immobilized metal affinity and gel filtration. As the number of drugs requiring high-doses and long-term administration continues to rise, there has been strong pressure to increase volume throughput at faster rates to meet demand. Column chromatography assemblies have become larger to handle increased capacity; currently the largest columns used in manufacturing are over two meters in diameter, and possibly even larger columns may be required at some manufacturing facilities.

Column packing evolves into a science

A number of innovations have taken place in column chromatography over the past ten years, particularly in the area of automation and process control. New precision controllers for mobile phase delivery and data acquisition, as well as advanced processing systems, have improved reliability and reproducibility.

One area where automation has advanced the science of column chromatography has been in packing procedures. Packing media into chromatography columns was once considered a skilled, labor-intensive art that required extensive operator training to maintain consistency. However, even with the best-trained personnel, manual packing leads inevitably to variances that can compromise batch reproducibility. For the development of any regulated drug product, reproducibility is critical, and in cases where column packing is not up to par performance parameters such as product purity and yield are affected. In the worst case scenarios, batches can be ruined, resulting in huge cost overruns and production downtime.

Column packing experienced a breakthrough with the introduction of pack-in-place automation in which a separate packing station suspends and then pumps media into fully-assembled columns. Pack-in-place promotes a homogeneous flow and packing of media into columns, and it leads to a better resolution of product from contaminants and recovery of eluted products in a more concentrated state. It also ensures that packed beds are more stable, improving consistency in batch-to-batch separations.

Another benefit of pack-in-place automation has been to make processing more hygienic since operators utilize fully contained systems. All packing and Clean-in-Place (CIP) procedures take place without removing the top column assembly, eliminating the risks of contamination during the process, and in some cases preventing harm to operators when processing bio-hazardous materials.

With the demands of higher throughputs, column repacking takes place more often, putting further pressure on operations to perform column packing more consistently. Today, over 70 percent of new column chromatography operations utilize pack-in-place systems.

Improving process control and monitoring

Recent innovations in hydraulic control of column packing procedures have helped improve consistency and reliability, especially for Flow Compression Packing procedures. Hydraulic linear actuators have been incorporated onto column systems to carefully regulate the compressive axial force that is applied to media beds. A pressure gauge monitors the hydraulic compression in a column, locking the column end cell in place when it has reached the level of the formed bed. Controlling the level of compression to be the same each time a column is packed helps ensure optimal bed density and maximizes the performance of media during packing procedures. It also improves batch-to-batch reproducibility.

One of the most important developments in column chromatography is the use of ultrasound technology to monitor the density of packed media beds inside columns, which heretofore has been mostly guess work. Still in development, the use of ultrasound-aided column packing is poised to become commercialized, and it has the potential to offer operators an accurate diagnostic tool to measure how successfully a column has been packed, which has a direct correlation to its performance.

Ultrasound detectors are sensitive to bed compression, mobile phase composition, and the presence of soluble components in the chromatography bed. Ultrasound can detect "real time" bed formation data that can be recorded and verified before a column is used. When used along with dynamic feedback control from a packing station, it can help ensure consistent and correct packing before a column is used.

Membranes offer dynamic throughput

Over the past several years there have been a number of developments in an alternative technology, ion exchange membrane chromatography. For many applications, membrane chromatography offers significant advantages in terms of speed and process economics. This technology utilizes microporous membranes with active chemistries on the membrane surfaces; this allows the active chemistry groups to be available for immediate binding. Membranes offer high dynamic throughputs because of their pore structure, which is chemically modified with charged, hydrophilic polymers on an open pore structure that provides much greater accessible surface areas for biomolecular binding than typical chromatography resins. Convective flow pores enable fast and efficient mass transfer as the fluid flows directly through the membrane pores, with no diffusional flow restrictions.

Membranes have proven successful at process scale capture and removal of DNA, proteins, and viruses. A disposable, pre-packed membrane column can remove contaminants at 100 times the speed of resin-based column chromatography. In column chromatography, the surface area available for binding is mostly contained within the pore structure of the resin and is available only through diffusional forces. Large molecules and viruses cannot diffuse into these pores, and are limited to binding on the outer available surface area of the resin. Membranes, with no diffusional flow limitations, have a ten- to 100-

fold higher capacity per unit volume than resins for DNA, viruses, plasmids, etc. Membranes also offer linear scalability, a critical consideration for manufacturers moving a product from laboratory to pilot and to production scale processing.

Flexible disposable or reusable formats

Membrane chromatography is available in disposable capsule or cartridge formats. Smaller laboratory scale devices are used for proof of concept studies, and for protocol optimization. For manufacturers this makes their use extremely flexible, since they can be added to existing process cycles, usually as a polishing step. Since membrane capsules are disposable, there is no cleaning or cleaning validation, which significantly speeds processing and reduces labor and buffer costs.

Solution for gene vectors and viruses

Membrane chromatography is much more efficient at capturing large molecules than column chromatography resins, and this is especially advantageous for large volume processing where a small mass of plasmid DNA or virus needs to be captured. Membranes are increasingly being seen as an enabling technology in the purification of genetic therapy vectors. Positively charged Q membranes efficiently bind plasmid DNA up to 23 kb in size, while the rapid processing times offered by membrane chromatography allow efficient purification of unstable large plasmids.

Q chemistry membranes have demonstrated binding efficiencies of 1013 adenovirus particles per ml bed volume of membrane, with smaller results also being demonstrated in adeno-associated virus and lentivirus purification studies

DNA and viral clearance breakthrough in manufacturing

Membrane chromatography has also demonstrated effective removal of model viruses such as porcine parvovirus, hepatitis A virus, murine leukemia virus, and pseudorabies virus at removal efficiencies of 104 and 107.

In one illustrative case where membrane chromatography has been implemented successfully as a post-purification polishing step, FDA and EMEA (the European Medicines Evaluation Agency) recently approved the incorporation of membrane chromatography to purify AldurazymeÂ® (larondiase), the enzyme replacement therapy manufactured by BioMarin Pharmaceuticals.

When used in conjunction with a membrane-based size exclusion ultrafiltration process, membrane chromatography provides an orthogonal method of viral clearance, as stipulated by FDA and EMEA. This exempts BioMarin from DNA lot testing, marking a regulatory milestone in the production of protein-based drugs. In addition to saving an average of $2,000 per batch in processing, BioMarin has significantly reduced the possibility of a failed batch, which would be extremely costly, with human health implications.

Wide-scale adoption of processes similar to this hold great promise for saving biopharmaceutical companies tens of millions of dollars in the manufacture of monoclonal antibodies and recombinant protein drugs. It is a unique yet telling example of how new chromatography technologies are helping manufacturers cope with processing and regulatory challenges.

In future columns we will analyze in closer detail some of the new and improving innovations that chromatography is bringing to the marketplace.

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Note: This article has earlier appeared in Bioprocess International.