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The advancing science of chromatography column packing

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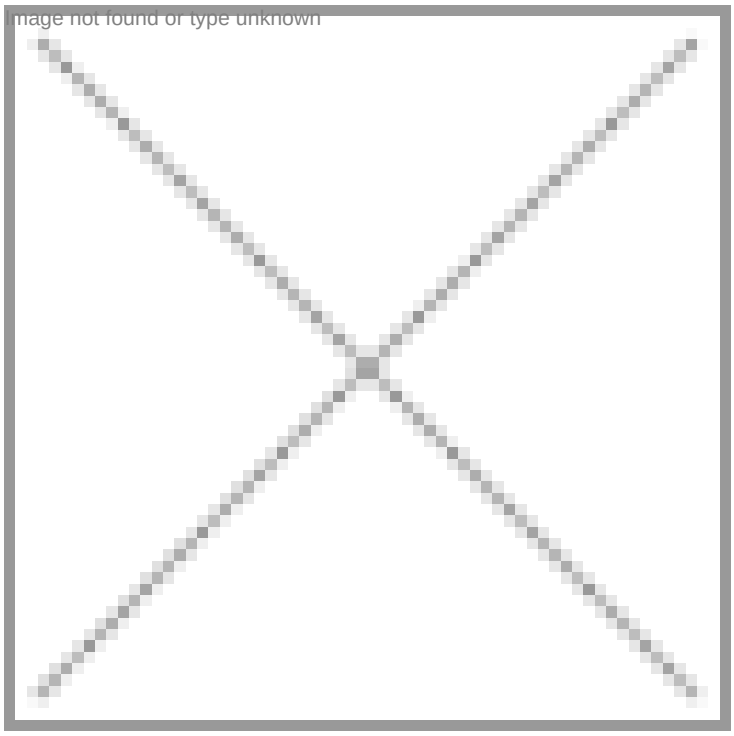

Chromatography Advisor # 2

The advancing science of chromatography column packing

Chromatography is the most powerful purification method available to drug manufacturers for the downstream processing of biotherapeutics. The number of drugs in development and commercial production has grown rapidly, especially antibody-based drugs requiring large doses and long treatment periods. For chromatography operations, this has placed unprecedented demands on speeding processing and increasing throughput-all while keeping costs down.

A critical aspect of chromatography is column packing, and advancing technologies have helped improve this important step for drug processing. This second in a series of chromatography "advisors" offers a brief examination of some of the innovations in packing methods that the marketplace has experienced, and a peak of some new trends on the horizon.

The goal for any chromatographic operation is to ensure the most optimal flow distribution possible within a column. At the same time, for all quality controllers one key question is always top-of-mind: "Is my process reproducible?"

A well-packed column bed should have a homogenous density throughout its surface area, and the space between particles- known as "interstitial volume"- should be controlled. As the liquid mobile phase enters the column the flow profile should ideally form a "plug-flow" pattern with minimal "band broadening"; broad bands during separation reflect poor resolution and performance, and it impacts on the purity of the target products being eluted.

The resin media used varies depending on the operation, and some can be quite costly. Possible failures can include density inconsistencies or local voids in the bed, which can lead to flow aberrations and irregularities and even to bed instability. In the worst case scenarios beds can crack or form a moving void as feed is being processed, ruining batches in process.

A skilled craft

It is hard to conceive that just a few years ago packing chromatography columns was largely a manual process that required extensive skills and training akin to a practiced art. Traditional column packing employed a "dry packing" or "tap packing" method. Chromatography resins were supplied dry, columns were agitated to help particles settle, and the media would then be hydrated directly in the column.

Eventually resins were supplied pre-hydrated, and "flow packing" became the new and improved procedure. Operators would pour media as a diluted slurry directly into columns from a container or conditioning tank, and the suspended media would then be stirred to ensure as much homogeneity as possible. The column's top end cell would be inserted and sealed, and liquid flow pressure applied to settle the resin particles. The end cell needed to be manipulated to remove any trapped air above the column bed, and the bed surface needed to remain undisturbed.

Given the number of manual manipulations required, reproducing flow packing procedures in a cGMP environment was typically beyond the skills of even moderately experienced operators.

Increasing automation

In the mid-1990s an automated packing method called "pack-in-place" entered the market and has since changed the manufacturing paradigm for chromatography. Pack-in-place has transformed production-scale column packing from a skilled craft into a hard science, largely eliminating the "human factor" in production.

Today, approximately 70 percent of all new process chromatography operations employ some type of pack-in-place automation, and new innovations have made monitoring and control of packing more accurate and reliable. This has improved packing reproducibility and process control, and has ensured more consistent packed bed performance.

Pack-in-place procedures are selectively calibrated to reflect different chromatographic media and adjust for the ideal rate at which packing should be performed. In this way it yields the optimal media compression within a homogenous bed to maximize column performance.

By eliminating operator handling, column packing automation removes the human variables so common with manual packing methods, and this has vastly reduced the chances of column failure. It has also improved manufacturing productivity significantly since column packing and unpacking can both take place with columns fully assembled.

Fully contained system eliminates contamination

Perhaps the greatest benefit of pack-in-place is the fact that packing operations take place in fully contained systems. Adjustable or fixed end cells are in place at ultimate bed heights, and operators form beds by pumping in slurry and simultaneously exhausting excess liquid. Since all procedures, including clean-in-place operations, take place without removing the top column assembly, this minimizes the risk of operator contact with potentially hazardous materials (for example in vaccine production), and it reduces exposure of drug products to external contamination. Overall, the procedure is more hygienic.

A key feature of automated packing is a separate slurry packing system or station (SPS), which delivers media slurries to columns during packing and unpacking. An SPS system mixes and then pumps media into columns at a controlled rate and pressure. An adjustable end cell can be set at a desired packed bed height, and media is pumped into the column to form a bed, while excess buffer is exhausted simultaneously.

Packing procedure

Packing procedures vary depending on the type media used in an operation. There are three general categories of media

used for processing biotherapies: carbohydrate-based matrices such as agarose, dextran, or cellulose; polymeric media, usually methacrylic or styrenic matrices; and rigid packings such as silica or controlled pore glass.

Packing procedures are generally divided up into four categories to accommodate the most commonly used media:

- Variable and pressure packing, which is used for moderately compressible media. The column pressure and bed height are allowed to increase slowly, and packing is complete when hydraulic pressure within the bed drops noticeably.
- Constant pressure packing, which uses more rigid media, requires that packing pressure be continuously kept at three bar throughout the packing process until the bed meets the bed support.
- Flow-compression packing, during which the piston is set at a higher bed height as slurry is pumped in. The slurry is then flowed at three bar for 15 to 30 minutes, and the piston is then moved onto the top of the bed.
- Fixed media volume packing, which is useful for costly media such as Protein A, requires bed volumes to be completely settled before slurry can be pumped into columns. Exiting mobile phase can be recycled to wash the slurry tank back into the column.

Looking ahead

Column chromatography operations have clearly benefited from the advent of pack-in-place automation, and it has helped manufacturers meet cGMP requirements as throughput demands continue to increase.

Looking ahead, a new technology is emerging to help monitor and control chromatography packing in real-time: ultrasound sensors. This new innovation, currently in beta-testing, holds great promise for optimizing packing and increasing operator control.

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