



Technology solutions simplifying chromatography operations

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Technology solutions simplifying chromatography operations

Chromatography operations typically involve steps such as resin slurry preparation, column packing, cleaning, unpacking, and resin storage. While each of the aforementioned steps can potentially be time-consuming and have significant effect on overall process performance, there are many tools available from GE Healthcare that address the challenges associated with each step. This application note describes how modern tools and technology solutions can help facilitate chromatography operations for an effective and productive protein purification process.

Introduction

Commonly, bulk chromatography resins are supplied in 20% ethanol or other suitable solution for transport and storage of the resin in a swollen state. Before column packing, the resin might need to be transferred to a packing solution and prepared as a homogenous slurry, which is essential for optimal column packing and effective chromatographic purification. While handling small amounts of chromatography resin can be managed manually, manual handling of large amounts of chromatography resin can be cumbersome, time-consuming, and even unsecure if aseptic handling is required.

Chromatography columns need to be packed quickly and reliably at all scales, while demanding minimal set-up and operator time. Packed beds must perform efficiently, reliably, and reproducibly over many processing cycles. The process must be robust and scalable, and the purification platform (column, resin) should be designed to minimize scale-up risks and facilitate achieving purity and yield in final manufacturing scale that is comparable with the process in development scale.

GE Healthcare provides tools and technologies that help facilitate effective chromatography unit operations (Fig 1). Here, we describe how these tools and technical solutions can be integrated for automated operations in closed or functionally closed fashion. Closed or functionally closed systems can help reduce the number of process steps,

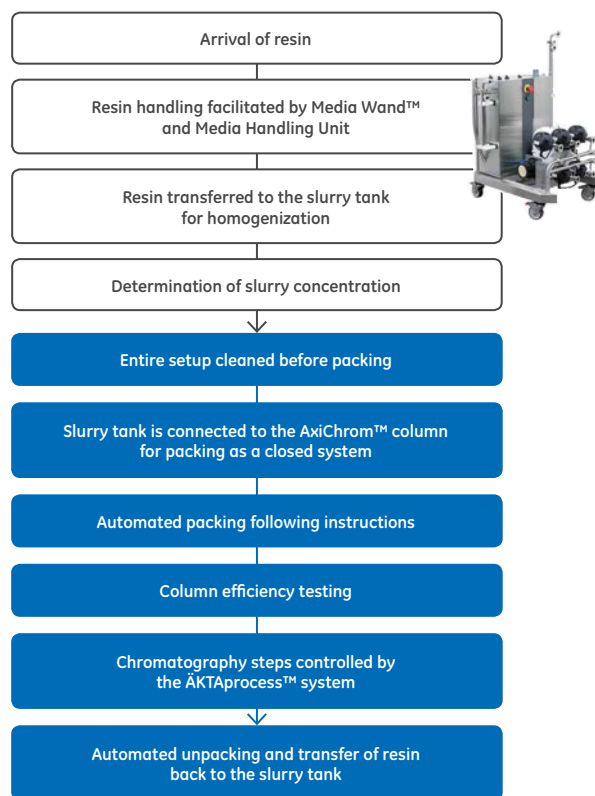


Fig 1. Chromatography unit operation workflow, with blue boxes indicating possibility of closed system operations.

which in turn, can help reduce floor space (facility overhead) requirements and shorten overall process time. Automated workflows in closed system operation reduce bioburden by decreasing manual handling between process steps.

Slurry preparation

Chromatography resins are delivered in transport solutions and containers of varying sizes. Resin transfer into packing solution and slurry preparation are starting points of the chromatographic purification workflow. Slurry can be prepared manually, mechanically, or by using the Media Wand slurry mixing and transfer tool. Shaking gives good results, but is often not practical for larger volumes. For stirring, a soft stirrer without sharp edges is preferred. Connected to the Media Handling unit, Media Wand can be used to remove the transport solution and resuspend the resin in packing solution directly in the container (Fig 2). The resin is transferred to the slurry tank in one operation, which makes Media Wand suitable for large-scale packing (Fig 3).



Fig 2. Media Wand connected to the Media Handling unit.

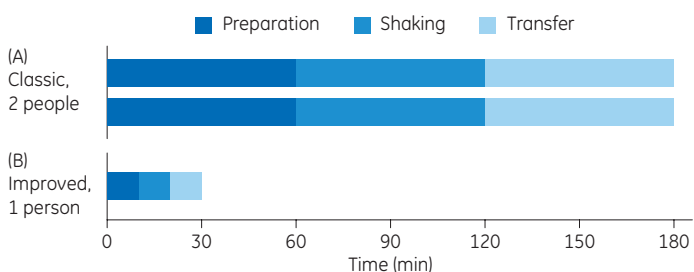


Fig 3. Compared with (A) a classic approach, the use of (B) Media Wand and Media Handling unit offers a simplified workflow for time-savings to be achieved in slurry preparation for packing of larger columns. Time estimates are for slurry preparation from large-scale resin volumes of typically more than 50 L.

Slurry tanks

Slurry tanks from GE Healthcare support different steps around packing and unpacking of resins, and are an essential part of the performance of the packed column (Fig 4). The slurry tanks help generate homogenous slurry and thus play a critical role in ensuring a well-packed bed and a robust and efficient purification process. Using a slurry tank can help avoid excessive shear forces that can otherwise lead to resin degradation, clogged nets, and increased back pressure. The slurry tank supports all packing methodologies, including pressure packing of brittle resins. The slurry tank can be directly connected to the column for aseptic transfer of the slurry for column packing in a closed manner. The slurry tank and its procedure of use are designed to support minimal resin loss.



Fig 4. A slurry tank, with control panel and wheels, is easily moved for simplified and more secure packing.

Single-use mixer systems

As single-use alternatives to the slurry tanks, Xcellerex™ XDM and XDUO mixing systems can be used for slurry preparation for smaller columns, up to 400 mm in diameter depending on bed height and slurry concentration (Fig 5) (1). The single-use mixer bag can be aseptically connected to the chromatography column using ReadyCircuit™ tubing assemblies and ReadyMate™ connectors.



Fig 5. XDM Quad single-use mixing systems, with powerful motors and magnetically locked impellers, effectively mix even highly viscous materials.

Determination of slurry concentration

To obtain the correct amount of chromatography resin for packing to target bed height or compression, it is important to measure the slurry concentration correctly. Considering a target bed height of 20 cm, at a compression factor of 1.15, packed using 50% slurry, an inaccuracy of $\pm 2\%$ in slurry concentration can cause the final packed bed height to vary between 19.2 and 20.8 cm. Inaccurate determination of slurry concentration can impact both performance and throughput, and be the main factor causing poor purification outcomes.

Slurry concentration can accurately be determined using GE Healthcare's Slurry Concentration Kit.

Column packing

Historically, column packing has been a manual process (using flow or pack in place methodology). Some major challenges with manual column packing are varying packing success rate and bed instability. Also, modern high-flow resins were found to be less compatible with the hardware design of traditional columns. After evaluating the challenges with existing equipment and packing methods, a completely new column concept was developed based on axial compression methodology and preprogrammed and verified packing methods.

With AxiChrom columns, packing is straightforward and the need of extra equipment is limited. Through automated packing using verified methods, less time is spent on packing and repacking. Verified methods also facilitate scaling or the packing process. The use of a patented axial compression and a patented liquid distribution system allow accurate and reproducible control of the packing procedure (Fig 6 and 7). In addition, axial compression provides the possibility to successfully pack more types of resins, especially modern chromatography resins, and enables achieving desired outcomes in a reproducible manner.

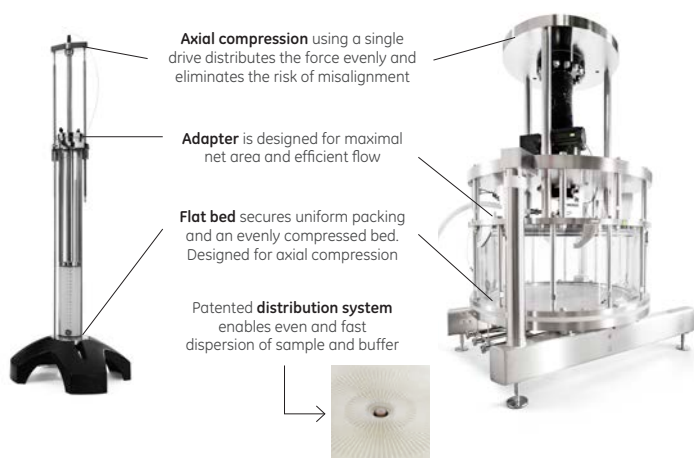


Fig 6. Together with AxiChrom column design features, automated packing using verified methods and axial compression ensure optimally packed beds.

AxiChrom columns are available with inner diameters ranging from 50 to 1600 mm. When packing *AxiChrom 50 to 200 columns* for use with ÄKTA™ systems, Intelligent Packing control is managed by the UNICORN™ system control software. For *AxiChrom 300 to 1600 columns*, Intelligent Packing is performed by the AxiChrom Master, a separate unit that comprises a touchscreen-operated user interface, or from the UNICORN software on the ÄKTAprocess system. Packing methods are created by entering values for the packing variables in the Intelligent Packing wizard. The packing factor given in the Intelligent Packing wizard is dependent on entered packing variables and the packing solution. Column packing is initiated simply by choosing a method in the UNICORN software and following the instructions.

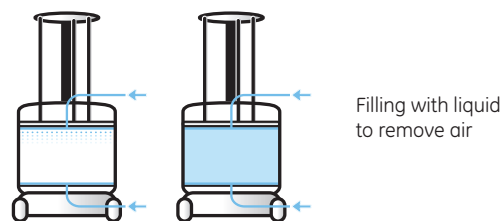
AxiChrom columns are easy to operate. Even the largest sizes can be handled securely by a single operator. The step-by-step interface guides users through key packing, unpacking, and maintenance steps. Previous experience or extensive training is not necessary, making it easy to quickly move columns between projects and/or locations, while maintaining reliable and reproducible process performance. In addition to helping avoid operator-error, these intuitive aids also allow for maximized column use.

Depending on column size, ÄKTA avant, ÄKTApilot™, ÄKTAprocess, or BioProcess™ Modular will be the chromatography system of choice. AxiChrom 300 to 1600 columns can be integrated into a higher level automation system, such as the DeltaV™ or Siemens control systems. This integration is managed using the available PROFIBUS™ connection on the AxiChrom Master.

The slurry tank can also be controlled by the AxiChrom Master to further automate the packing procedure.

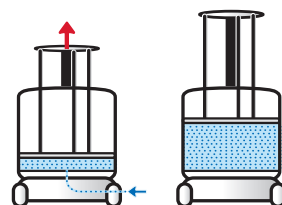
If selected in the UNICORN wizard, Intelligent Packing will automatically run a packed bed evaluation test after packing.

(A) Priming the column



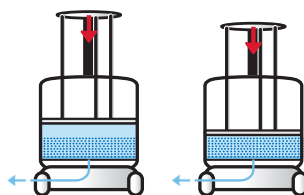
Filling with liquid to remove air

(B) Syringe-like filling



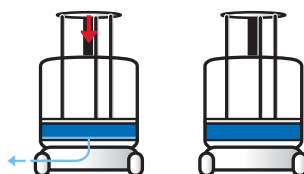
Suction of calculated, exact amount of resin, while avoiding stress on resin

(C) Resin consolidation



Slow and controlled adapter movement for optimal bed formation

(D) Bed detection and compression



Slow and controlled axial compression

Fig 7. Brief overview of automated column packing.

Column efficiency testing

A well-packed bed is characterized by a high degree of bed stability and resolution. For a well-packed bed, the resin needs to be distributed homogeneously in the packed column to ensure that the mobile phase travels through the bed in a uniform manner (Fig 8). A well-packed bed minimizes zone broadening and ensures optimum performance in terms of resolution. The resin also needs to be appropriately compressed, which ensures stability, especially at high flow velocities, and reproducibility of performance from one column packing to another.

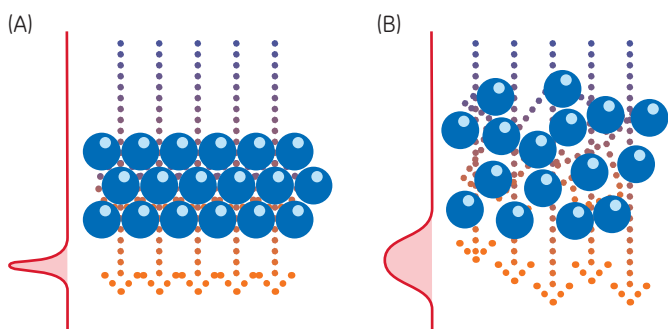


Fig 8. (A) A well-packed bed, generating a stable column that offers good resolution. (B) A poorly packed bed from a non-homogenous slurry, generating a less stable bed and an uneven flow through the column. Chromatograms are shown on the left.

The separation efficiency of a packed column can be expressed in terms of the number of theoretical plates per meter bed (N/m) and asymmetry factor (A_s). A column with a high number of theoretical plates will generate narrower peaks at a specific retention time compared with a column with a lower number. The A_s describes the shape of the peak. The larger the A_s value, the more asymmetrical the peak, and this can have a negative impact on separation quality. Column efficiency tests should be performed after packing and repeated regularly to monitor the state of the bed throughout the working life of the column (Fig 9). If the test results are to be comparable over time, conditions such as fluid velocity (cm/h), liquid pathway, sample composition, and elution buffer should be kept constant (2).

The most commonly used methodologies for efficiency testing are pulse efficiency tests and transition analysis, both of which can be carried out in an automated fashion via the UNICORN software installed on ÄKTA chromatography systems.

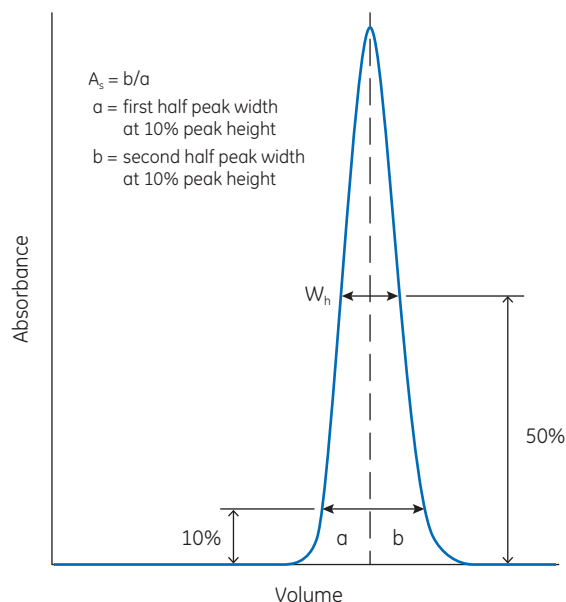


Fig 9. Peak width (W_h) and shape give indications on separation efficiency of a column.

Chromatography process

AxiChrom columns are developed for use with a wide range of resin types, from traditional chromatography resins to high-flow resins such as the Cipto™ and MabSelect™ product families (3–5). Combining an AxiChrom column with a high-flow resin can help drive towards lean biomanufacturing and operational excellence, bringing more speed and better economy to downstream processing.

Irrespective of experience or location, users can expect the same good separation efficiency when scaling up (6). AxiChrom columns enable smooth and predictable technology transfer between departments and sites or to contract manufacturing organizations.

Column unpacking and maintenance

For AxiChrom columns, Intelligent Packing, available through the UNICORN wizard or AxiChrom Master, also allows methods for column unpacking and cleaning to be created. Minimizing resin loss is taken into account.

The *swing-out* and *swivel* features on the AxiChrom column make maintenance convenient, secure, and effective, as they remove much of the extra work needed for disassembly (Fig 10).

The construction materials of AxiChrom columns are resistant to most chemical agents used in chromatography, including solutions effective in column cleaning, sanitization, and storage. Challenge testing shows that AxiChrom columns can be efficiently sanitized using 1 M NaOH (7). Column cleaning-in-place (CIP) is facilitated by CIP templates in the UNICORN software.

The column design allows easy access to all relevant parts, and concise interactive software instructions increases uptime. As an example, disassembly, changing all wetted parts, and reassembly only take a few hours for AxiChrom 600 (time can vary depending on setup).



Fig 10. The swing-out and swivel functions of AxiChrom columns simplify maintenance.

Fast Trak services

Our global Fast Trak team provides a broad range of process development support for biomanufacturing. Additionally, Fast Trak offers training and education services in the same area. Practical project support and advice from Fast Trak can help you plan, implement, and document processes from start-up to clinical manufacturing, while training your personnel at the same time. Additionally, our Fast Trak team of bioprocessing experts can support you in the optimization and troubleshooting of existing chromatography unit operations or in the design of new efficient and cost-effective processes.

Conclusion

Chromatography unit operation involves many steps that can be individually optimized for a simplified workflow. GE Healthcare provides tools and technologies to facilitate efficient chromatography unit operations. Integration of standalone tools into workflow solutions enables automated operations in closed or functionally closed systems to reduce contamination risk and floor space requirement, while shortening the overall process time. Let our experts guide you along the way. We support you with a range of services and solutions, from training programs to the design and construction of your production facility.

References

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Ordering information

Product	Product code
Media Wand 50	28922764
Media Wand 100	28922767
Media Handling Unit	28922769
Slurry tank	29010656
Slurry Concentration Kit	29096100
XDM Quad mixing system	29048373
AxiChrom 50/300/Glass/20SS	28901831
AxiChrom 50/500/Glass/20SS	28901841
AxiChrom 70/300/Glass/20SS	28901840
AxiChrom 70/500/Glass/20SS	28901847
AxiChrom 100/300/Glass/20SS	28903274
AxiChrom 100/500/Glass/20SS	28903276
AxiChrom 140/300/Glass/20SS	28907702
AxiChrom 140/500/Glass/20SS	28943927
AxiChrom 200/300/Glass/20SS	28907703
AxiChrom 200/500/Glass/20SS	28943928
AxiChrom 50/300/Glass/20PE	29016534
AxiChrom 70/300/Glass/20PE	29016537
AxiChrom 100/300/Glass/20PE	29016536
AxiChrom 140/300/Glass/20PE	29016535
AxiChrom 200/300/Glass/20PE	29016533

AxiChrom column configurations not listed above are ordered via the sales configurator.
 For more information on ÄKTA chromatography systems, visit gelifesciences.com/aktadesign
 For more information on Fast Trak services, visit gelifesciences.com/fasttrak

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