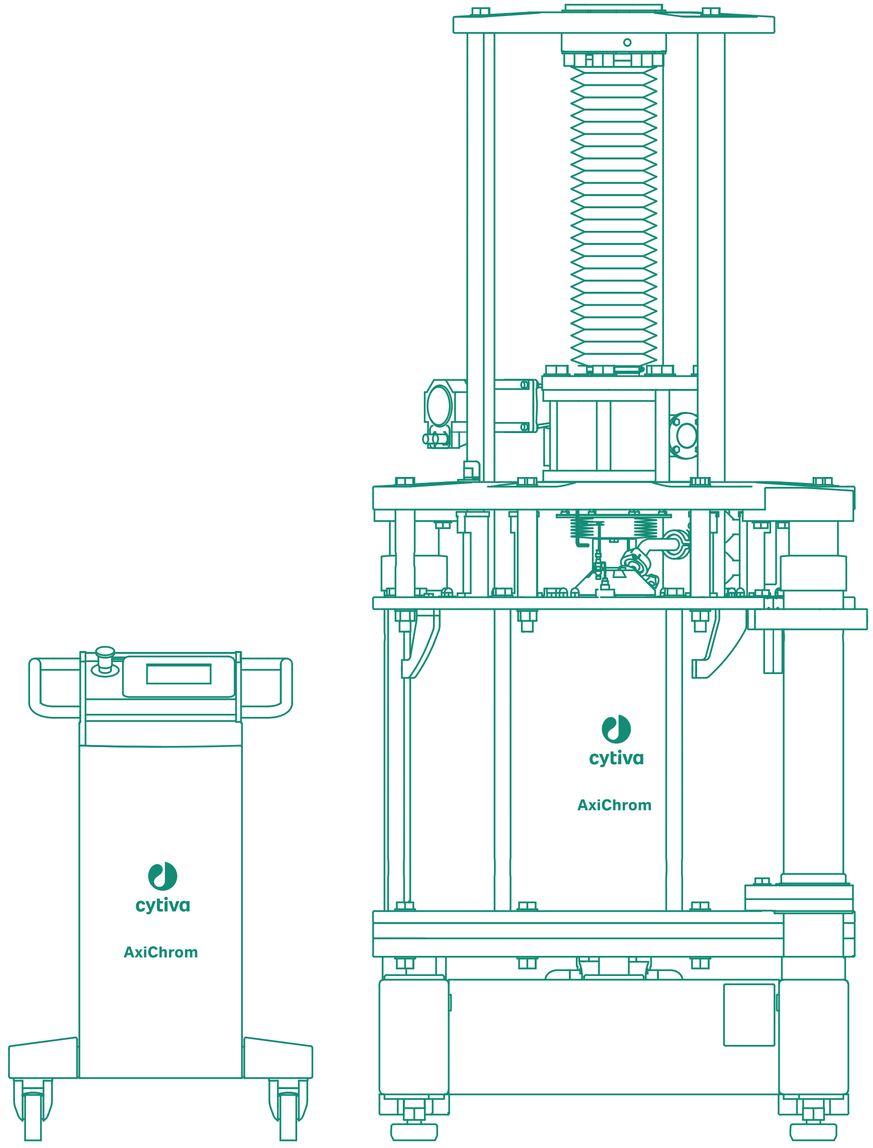
Principles and methods

Packing and maintaining process-scale chromatography columns



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Introduction

This handbook describes areas and procedures for column handling and column changeover for bioburden control. The handbook acts as guide to help you to avoid product cross-contamination by applying established routines and procedures to prepare the column for packing and for new process runs.

Chromatography column packing operations typically involve steps such as resin slurry preparation, column precleaning, column packing, cleaning in place (CIP), unpacking, and column/resin storage. Each of these steps is time-consuming and have significant effect on overall process performance. In addition, columns need to be cleaned and maintained between campaigns to mitigate risks of accumulating biofilms, bioburden, vegetative and spore contamination, or product and process cross-contamination.

Bioburden control, cleaning to avoid product carry-over (batch-to batch or product to product), or cleaning of resin are important parts of column and resin handling, all of which are discussed in this document.

Symbols, acronyms, and terminology used in this handbook

Symbols



Highlights troubleshooting advice to help analyze and resolve difficulties.



Denotes mandatory advice and gives a warning when special care should be taken.

Highlights chemicals, buffers, and equipment

Provides an outline of the experimental protocol.

Acronyms and abbreviations

API	Active pharmaceutical ingredient
BnOH	Benzyl alcohol
CFU	Colony-forming units
cGMP	Current good manufacturing practice
CIP	Cleaning in place
COP	Cleaning out of place
DEAE	Diethylaminoethyl
EPDM	Ethylene propylene diene monomer
HW	Hardware
mAb	Monoclonal antibody
GMP	Good manufacturing practice
HETP	Height equivalent to a theoretical plate
i.d.	Inner diameter
IPA	Isopropanol (isopropyl alcohol)
LAL	Limulus amebocyte lysate
NaOH	Sodium hydroxide
PA	Polyamide
PAA	Peracetic acid
PE	Polyethylene
PM	Preventive maintenance
PMMA	Poly(methyl methacrylate)
PP	Polypropylene
PVC	Polyvinyl chloride
Q	Quaternary ammonium
S	Methyl sulfonate
SIP	Sanitization in place
SP	Sulfopropyl
RSF	Regulatory support file
RPC	Reversed phase chromatography
SEC	Size exclusion chromatography
SIP	Sanitize/sterilize in place
SS	Stainless steel
TOC	Total organic carbon

Terminology

From ispe.org/glossary, ISPE Glossary of Pharmaceutical and Biotechnology Terminology and ich.org/home.html ICH Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients Guidance for Industry

Adapter

Used for the movable end pieces of columns. It contains a filter and flow distributor and can be used to connect tubing.

Affinity chromatography

A group of methods based on various types of specific affinities between target molecule(s)—for example, a protein and a specific ligand coupled to a chromatography resin.

Asymmetry factor (A_s)

Factor describing the shape of a chromatographic peak. Measured at a defined height of the peak; can be at the base (between tangents drawn) or at 5% or 10% of the actual peak. Peaks are known as tailing when $A_s > 1.0$ and fronting when $A_s < 1.0$. Gaussian peaks are defined as $A_s = 1.0$.

Back pressure

The pressure drop across a column and/or a chromatography system.

Bed volume

Volume of the resin bed that the adapter compresses and locks in place.

Binding buffer

Buffer, solution, or eluent used to equilibrate the column before sample loading.

Binding capacity

The maximum amount of target protein that can bind per milliliter of chromatography resin. *See* also dynamic binding capacity.

Bioburden

The number of viable contaminating organisms per product unit. The concentration of microbial matter per unit volume. Microbial matter includes viruses, bacteria, yeast, mold, and parts thereof.

Chromatogram

A graphic presentation of detector response(s) that indicates the concentration of the solutes coming out of the column during the purification, expressed in volume or time.

Chromatography

From the Greek *chroma*, color, and *graphein*, to write.

Chromatography resin

The stationary phase. The chromatography resin is composed of a porous matrix that is usually functionalized by coupling ligands to it. The matrix is in the form of particles or, rarely, a single polymer block (monolith).

Cleaning

Action of removing and dissolving or dispersing soiling from a surface. Note: Cleaning may be affected by one or more of the following means: physio-chemical (detergent action); chemical (e.g., sodium hydroxide, NaOH); biochemical (e.g., enzymes); physical (e.g., shear forces caused by brushing or hosing). Cleaning efficiency is also dependent on the length of time and temperature of application, etc.

Cleaning in place (CIP)

Internally cleaning a piece of equipment without relocation or disassembly. The equipment is cleaned but not necessarily sterilized. The cleaning is normally performed using acid, caustic-based detergent, or a combination of both, with water-for-injection (WFI) rinse.

Clean out of place

An automated or semi-automated procedure where the process equipment is disassembled and its components are placed into an agitated fluid bath of cleaning solutions. Baskets may be utilized to hold and wash smaller parts such as gaskets, clamps, valve bodies, PD pump rotors, etc. The cleaning is normally performed with a proprietary acid or caustic-based detergent, or a combination of both, with a compendial water rinse.

Column efficiency and column efficiency test

High efficiency means that the band broadening is low and that you'll obtain sharp peaks. It's often given as the number of theoretical plates (N) or as theoretical plates per meter (N/m) to an independent measure of column length in the experiment. Efficiency plays a central role in qualifying and monitoring packed bed performance. The optimal high column efficiency gives low band and peak broadening and indicates how well-packed the column is before starting purification.

Column efficiency is typically defined using two parameters:

- Peak broadening over the column is described by an equivalent number of theoretical plates.
- Peak symmetry is described by a peak asymmetry factor, A_s.

Each Cytiva resin protocol describes the procedure to determine column efficiency.

Column hardware

The column tube and adapters. Refers to all pieces of the column except for the chromatography resin and the packed bed.

Column hardware pressure

The pressure inside the column. Column hardware pressure that is too high can break the column.

Column packing

Controlled filling of the column hardware with chromatography resin compressed to a certain, predefined extent to achieve a stable particle bed.

Column volume

The geometrical volume of the column interior.

Contamination

The undesired introduction of impurities of a physical, chemical, or microbiological nature, into or onto a raw material, intermediate, or API during production, sampling, packaging, repackaging, storage, or transport.

Counter ion

Ion of opposite charge that interacts with an ion exchange chromatography resin after the column equilibration. A protein that binds to the ion exchanger displaces the counter ion. If you apply a high concentration of the counter ion, it will compete with the bound protein and elute it from the chromatography column.

Counter ligand

Substances that interact with ligands of a chromatography resin and can be displaced by a solute (e.g., protein) binding to the ligand.

Cross-contamination

The contamination of a drug substance or product by another.

Dead volume

The volume in a chromatography system, from the injector to the detector, not including the column volume. The dead volume contributes to band broadening.

Dynamic binding capacity

The dynamic binding capacity (DBC) of a chromatography resin is the amount of target molecule, that is, a protein that binds to the resin under given flow conditions before a significant breakthrough of unbound protein occurs.

Effluent

Mobile phase leaving the column, eluate.

Eluate

Mobile phase leaving the column, effluent.

Elution buffer

Buffer or solution used for elution (desorption) of bound solutes (e.g., proteins) from a column.

Elution volume

The volume of buffer or solution (eluent) required to elute the solute (e.g., a protein), known as the retention volume.

Elution time

The time required for the elution of a solute (e.g., a protein) is known as the retention time.

Equilibration

Passage of buffer or solution through the chromatography column to establish good binding conditions for the selected sample components. For example, column equilibration can establish the correct pH and ionic strength and ensure that proper counter ions or counter ligands are present.

Flow rate/flow velocity

“Flow rate” refers to the volumetric flow rate and is expressed as volume per unit time (e.g., mL/min). “Flow velocity” refers to the flow rate divided by the cross-sectional area of a chromatography column and is expressed as distance per unit time (e.g., cm/h).

Flowthrough

Chromatography flowthrough refers to material that passes through the column during sample loading without binding.

Frit

A type of deep filter often used at the top and bottom of columns.

Gel filtration

Gel filtration (GF) is size-exclusion chromatography. It separates solutes (e.g., proteins) according to size.

Gradient elution

Continuous increased or decreased concentration of a substance (in the eluent) that causes elution of bound solutes (e.g., proteins).

Hydrophobic interaction chromatography

Hydrophobic interaction chromatography (HIC) is a method based on the hydrophobic interaction between solutes (e.g., proteins) and the chromatography resin in the presence of high salt concentration.

Ion exchange chromatography

Ion exchange chromatography (IEX) is a method based on electrostatic interactions between solutes (e.g., proteins) and chromatography resin.

Ligand

The specific molecular group that couples to the matrix to functionalize the chromatography resin.

Matrix

The matrix is the nonfunctional base for the chromatography resin. The matrix has a porous structure and large surface area that you can modify with ligands to enable protein binding.

Mobile phase

The fluid (buffer or solution) carrying the solutes during chromatography. Can also be referred to as the eluent.

Particle size (dp)

The mean diameter of the spherical beads of the chromatography resin.

Passivation

A final chemical treatment/cleaning process that removes exogenous iron or iron compounds from the surface of stainless-steel (SS) piping and equipment using a mild oxidant, such as a nitric acid solution, or by “in-situ electropolishing. The purpose of passivation is to restore and/or enhance the spontaneous formation of the chemically inert surface or protective passive film.

Plate number

A measure of chromatography peak broadening and column efficiency. Also referred to as the number of theoretical plates. Scientists use the plate number of a column to measure column efficiency. The more plates a packed column has, the higher the column efficiency.

The plate number is calculated from $N = 5.545 \times (V_R/W_h)^2$ assuming a Gaussian peak, where:

- V_R = retention (elution) volume
- W_h = peak width at half peak height.

V_R and W_h must have the same unit (e.g., mL). You can substitute V_R with retention time, t_R, but then the unit of Wh must be time.

Pressure over the packed bed

The pressure drop across the packed bed when the solution passes through the column. Caused by flow resistance in the packed bed.

Product changeover

The program by which a processing area is cleared of supplies and components used in the manufacture of a previous product and then readied for production of a new product. This often includes parts changeover and/or special cleaning to eliminate cross-contamination.

Reduced plate height (*h*)

A dimensionless and thus convenient parameter for efficiency characterization is the reduced plate height h. This parameter facilitates the comparison of column efficiency irrespective of column length and particle diameter of the medium. The reduced plate height is calculated from $h = HETP/dp$, where HETP is the height equivalent to a theoretical plate and dp is the particle diameter of the chromatography resin. Optimal column efficiency typically corresponds to an experimentally determined reduced plate height of $h \leq 3$ for the porous resin employed in bioprocess chromatography. This efficiency is achieved when testing a well-packed bed with an optimized set-up of column and system under optimal test conditions.

Residence time

The time a buffer or solute spends in the column.

Sanitization

That part of decontamination that reduces viable microorganisms to a defined acceptance level; normally achieved by using a chemical agent or heat to reduce microbial levels.

Size exclusion chromatography

Chromatography method used to separate target molecules according to size, usually by isocratic elution. SEC includes both gel filtration and gel permeation chromatography. The terminology *gel filtration* is used when both stationary and mobile phases are polar while gel permeation is used for the opposite, that is, both the hydrophobic resin and mobile phase are nonpolar.

Stationary phase

Often called resin, chromatography particles, chromatography material, or chromatography medium or media.

Theoretical plate numbers per meter (N/m)

See plate number.

Wash buffer

Used in the wash step to remove any unbound or weakly bound material from a column after the sample loading.

01
Column packing

1.1 Introduction

Good column packing is essential for any chromatographic process and plays a key role in the large-scale commercial manufacture of biopharmaceuticals. A bed packed too densely might crack, which can lead to channeling and early breakthrough. A bed packed too loosely can further compress causing a liquid gap where mixing can occur.

Either instance will lead to costly process disruptions and loss of valuable product. Proper column packing eliminates such concerns and ensures a stable bed that performs according to expectations over many processing cycles. This chapter describes how to prevent failure and improve packing success by describing the considerations and methodologies for pilot- and process-scale column handling and packing.

The most important consideration for column packing is to pack the resin in the column to a correct compression rate. Correct compression of the resin means it will perform well and be stable over long periods of processing, minimizing deviations and downtime because of the column packing.

1.2 Preparations for column packing

1.2.1 Column hardware and system preparations

When packing a column with resin, the resin packing method, column type, buffers (solutions, temperature, flow rates) may all influence the result and need to be taken into consideration. From the resins used/chosen in process development, the column type/size can be chosen for the scale up. The column then sets the type/range/limitations for the packing methods, packing with the required flow/pressure or axial compression, meaning that the surrounding equipment can be set.

1.2.2 Large-scale BioProcess™ column types

AxiChrom™ columns

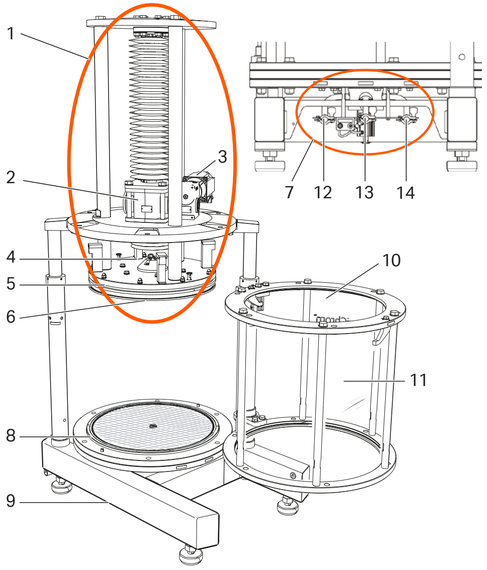
AxiChrom columns are low-pressure, axial compression chromatography columns that give you the flexibility to use for process development as well as manufacture of biopharmaceutical products. The many configuration possibilities (e.g., column diameters and packed resin bed heights gives you a great deal of flexibility for different biomanufacturing needs. This attribute of flexibility, in combination with automated packing and easy maintenance, makes the platform an excellent choice for multipurpose facilities.

A wide range of AxiChrom column sizes is available, from 50 to 2000 mm i.d. Each column has an intuitive design for easy handling and maintenance. The smaller columns, from 50 to 200 mm i.d. include a stand with a pivot design. In larger columns of 300 to 2000 mm i.d., the tube swings out allowing hoist-free handling. The novel swing-out function reduces column handling time compared to other heavy and large column series (Fig 1.1). The main components of AxiChrom columns are shown in Figure 1.2.

AxiChrom columns are controlled using Intelligent packing, which enables the creation of automated methods for packing, unpacking, priming, and maintenance. Automated methods bring you several benefits, for example, minimized hands-on time and excellent reproducibility for all column sizes. Intelligent packing is part of the UNICORN™ software for use with



Fig 1.1. AxiChrom column in swing-out mode provides easy access to bed supports and O-rings. In multipurpose facilities, changing the plastic bed supports for a new campaign is quick and hassle free.



Part	Function
1	Top unit
2	Worm gear and bellows
3	Servo motor
4	AxiChrom 300–1000 columns: Top mobile phase inlet/outlet AxiChrom 1200–1600 columns: Rinse nozzle (including top mobile phase inlet/outlet)
5	Adapter
6	Adapter bed support and distributor
7	Resin valve assembly
8	Bottom bed support and distributor
9	Column stand
10	Process chamber
11	Column tube
12	Resin valve slurry inlet
13	Resin valve bottom mobile phase inlet/outlet
14	Resin valve rinse inlet

Fig 1.2. Main components of an AxiChrom column.

ÄKTA™ chromatography systems (ÄKTA avant™ 150 system, ÄKTA pilot™ 600 system, ÄKTA process™ system) and is also possible to control with DeltaV™ software automation. For AxiChrom 300 to 2000 columns, Intelligent packing is also available through the separate AxiChrom column control unit.

When packing AxiChrom 50 to 200 columns, slurry is introduced manually and the adapter movement is driven by internal hydraulics. In AxiChrom 300 to 2000 columns, slurry is introduced via a resin valve in the center of the bottom bed support. The adapter is driven by an electric servomotor. Control of the resin valve and adapter is performed via AxiChrom column controller. The control unit also offers support for other parts of the process, for example, simple step-by-step maintenance guides, which reduces risks for operator errors.

Video training: Access free instructional videos for AxiChrom columns at https://cytiva.sourcin.com (registration required).

Classroom training: Attend Cytiva large-scale column packing (COL1) course for more hands-on training. Schedule and information can be found at cytivalifesciences.com/shop/large-scale-column-packing-col1-p-02814.

Chromaflow™ columns

Chromaflow columns are a family of convenient-to-use process-scale columns. The nozzle in the top and bottom of the column allows packing, unpacking, and cleaning when fully assembled, that is, with the lid in place. Chromaflow low-pressure columns are available in a choice of dimensions and materials. The complete range offers i.d. from 300 to 2000 mm with column tubes manufactured from cast acrylic or stainless steel (SS). All dimensions are available with variable bed heights, from 100 to 500 mm, providing a wide variety of bed volumes. All columns are pressure-rated for operation at 3 bar (0.3 MPa, 43.5 psi). The patented, pack-in-place nozzle allows process liquids to enter and exit. Manual or automated versions of the nozzles are available. The automated nozzle is controlled from the packing station or the nozzle control unit. The nozzle has three positions to facilitate the different aspects of column operation: packing, operation, unpacking and cleaning. In addition to this pack-in-place functionality, the nozzle also contains the process liquid flow path to provide a consolidated solution to the process stream handling. Bed supports are available in 316 L SS or PE. All other are manufactured from plastic or noncorrodible materials for use in low pH /high salt applications.

BPG columns

BPG columns are glass chromatography columns designed for industrial applications which demand high standards of hygiene. The column line ranges from 100 to 450 mm i.d. with bed volumes from 2 to 121 L at a pressure range up to 8 bar (116 psi, 0.8 MPa) depending on column diameter. The columns are constructed from component materials of the highest quality and withstand the harsh conditions used for cleaning in place (CIP) of packed separation resins. The columns are characterized by a hygienic design and operation. Microbial attachment and growth are hindered using calibrated precision glass, high-grade electropolished stainless steel, and an absence of dead pockets. Easy handling and efficient packing and running is made possible with the single screw adapter. All polymeric materials meet the requirements for USP class VI, as described in USP Biological Reactivity Tests, *In Vivo*.

1.2.3 Choice of column

When the application and resin are known, you should choose a column that can be packed to utilize the physical flow properties of the resin. Other considerations that you will need to consider when selecting a column for your process include column size, type of adapter (fixed or movable), material of construction for chemical resistance, pressure rating, and bed support porosity. Factors affecting column choice are described here:

Movable adapter: A column providing a variable bed height by use of a movable adapter is essentially packed the same way as a laboratory column (Fig 1.2). The resin is settled from a slurry by a liquid flow through the bed until the bed is packed and stable, at which point the flow is stopped and the top adapter is manually lowered to the resin surface. An alternative method with movable adapters is the use of axial compression packing, forcing the liquid through the bottom of the column with the adapter finally compressing the settled consolidated resin axially. This approach generally gives more consistent packing results.

Fixed adapter: Columns with fixed end pieces must be filled with the resin differently. There is no extra space for a diluted slurry so the amount of resin to pack needs to be forced into the set column volume space in one action. This method of packing is called pack-in-place (PIP) and for Chromaflow columns the resin is packed into the column at high flow rates through a special nozzle.

Pressure specification: The column pressure specification must withstand the pressure required to pack and the required highest pressure during processing.

pH: If the pH in the process is lower than 5.0 for longer periods, you should consider a column with wetted components made of plastic (a column with polypropylene [PE] sinter) due to the risk of corrosion on stainless steel at low pH applications.

High salt/chlorides: If high salt/chlorides are used for longer periods, select a plastic column (a column with PE sinter and plastic wetted components) to eliminate the risk of corrosion on SS. When salt is applied to SS columns, it is crucial to rinse with clean water for at least 5 column volumes (CV) or until the conductivity is back to zero. Alternative materials to plastic columns are higher grades of corrosion-resistant steel, discussed in Chapters 6 and 7.

Organic solvents: The acrylic tube of Chromaflow and AxiChrom columns can withstand organic solvents to some extent, check the respective operating instructions for details. Solutions of 20% ethanol and 30% isopropanol (IPA) can be used for shorter periods if followed by extensive rinsing with water. If a higher concentration of solvent is needed for the application, a SS tube is required. The static O-rings in Chromaflow and AxiChrom are made of ethylene propylene diene monomer (EPDM) or polytetrafluoroethylene (PTFE)-encapsulated Viton™. These materials can withstand up to 100% ethanol

for shorter periods and with extensive rinsing after. Another material for these O-rings could be chosen if high concentrations of solvents are to be used for long periods.

Column bed dimensions, flat wide bed vs thin high bed: AxiChrom columns incorporate an optimized distribution system to handle many resin bed sizes, from a 10 cm bed height in a 1.6 m column to 50 cm bed height in 30 cm i.d. column. The columns optimized distribution system in combination with axial compression packing gives excellent results almost regardless of bed ratio. Flat and wide beds set high demands on the distribution system being able to spread the sample in the full surface area so that it enters the bed in a plug flow (a simple model of the velocity profile of fluid flowing in a pipe, where the velocity of the fluid is assumed constant across any cross-sections of the pipe).

For modern high-flow resins, bed height may be increased with maintained residence time, which allows the use of a smaller column and minimizes floor space requirements. This means smaller columns and better resin utilization.

For Superdex™ and Sephacryl™ size exclusion chromatography (SEC), dividing the bed into two to three 30 to 50 cm high beds makes packing easier and increases the throughput as well, rather than packing a 1 m bed height into one column.

Pressure rating: Pressure rating of the column and ancillary equipment is set by the resin and its packing and process pressures.

Bed supports: Factors to consider when choosing the correct bed support for the column are:

1. Choice of bed support material

SS bed supports are a layered woven meshes ensuring robust long-term processing. They have a lifetime like the column. They are mechanically robust, easy to clean, and autoclavable.

PE bed supports are resistant to low pH and high salt concentrations, typically used in applications with protein A resins. They are single use, which eliminates the required cleaning validation for the bed supports.

2. Choice of porosity of bed support

The porosity of the mesh must not be larger than 1/3 of the mean particle diameter of the resin that will be used in the column. This is to prevent beads from clogging the net.

Sample applied to the column should be filtered so that no aggregates can block the bed supports. If the column is to be used in the first step for capture, a 20 to 50 µm bed support could be considered depending on the mean particle size of the resin (d_{50v}).

1.2.4 Column setup

The column should first be visually inspected for integrity and cleanliness as part of a preventive maintenance (PM) routine. PM protocols can be found in the operating instruction for each product, *see Chapter 6*, section 6.1.

The top and bottom bed supports should have a porosity of one-third or less that of the average particle size of the resin to prevent loss of the resin during packing. Replacement of elastomers is also recommended. If a measuring tape on the column tube is to be used for volumetric and bed height determinations, you should check the position of the tape for accuracy. Appropriate valves with the same or similar i.d. as the column outlets should be connected to the top and bottom of the column using matched i.d. gaskets. Hosing for inlet and waste lines of appropriate i.d. can then be attached to the valves as necessary for your packing method. The column can then be positioned for packing, leveled in place, and the adapter removed if applicable. The bed supports (except for pack-in-place methods) should be wetted with 20% ethanol and the air removed from under the bottom bed support. In some cases, air removal is accomplished by simply opening the bottom valve of the column briefly to waste. However, in many cases, the air must be completely suctioned out from under a net support. This is easily performed by using a small peristaltic pump connected to hosing with a half-inch sanitary connector, which is pressed gently against the net over the air pockets. For AxiChrom columns, follow the Intelligent packing wizard to prime the column.

Column and system integrity test

You should confirm operation and control of the liquid delivery system at the desired flow rates/pressures before connection to the column. To prevent loss of resin, the column should be hydrostatically tested for leaks. This can be performed by connection of an appropriate pressure gauge between the top valve and adapter inlet of the column. With the bottom column valve closed, the column is filled with water, then air purged from the column and adapter via the top valve and waste lines. Water from a pressure vessel or appropriate pump is then used to purge connecting hosing to the top valve and carefully increase the hydrostatic (fluid) pressure within the column until it reaches the desired level. The top valve is then quickly closed to isolate the column, and the reading on the pressure gauge recorded, after 15 min, every minute for 5 min. A rapid drop (> 0.068 bar/min [1.0 psi/min,0.007 MPa/min]) in the pressure indicates a leak in the column, which must be fixed before packing. If the exterior of the column and floor is dried just prior to testing, this leak can be quickly pinpointed by the appearance of water on or under the column.

It is extremely important to remember that column “pressure” testing should NEVER be performed by pressurizing an empty column with air!

1.2.5 Packing equipment setup

You should gather equipment selected for packing the column in the packing room, check for compatibility with functional power outlets, and assess the operational status and hosing connection requirements. Ancillary equipment needed for packing is dependent on the specific packing method but in general will include a liquid delivery system (LDS, pump, or pressure vessel), tankage, shutoff valves, and hosing. The internal diameter of the tank outlet, valves, and hosing to the LDS should be large enough to avoid cavitation during packing at high flow rates. Top and bottom column valves will be necessary to purge air

from the column prior to packing, control flow direction, and isolate the packed column. A pressure gauge should be mounted on the top valve of the column in line with the pump outlet. This gauge will register the summation of the pressure from the packed bed and the gauge.

The gauge pressure is defined as the pressure due to the empty column and downstream hardware and should be minimized by using hosing and other ancillary equipment with an i.d. large enough to prevent flow restriction at the maximal packing flow rate to be used.

In addition to the fluidics, you can use equipment for control of automated systems and data acquisition from inline monitors. Since this equipment must be protected from moisture, you should position it to minimize exposure to leaks, spills, or splashes during packing operations but it should still be easily accessible to you. Alternatively, use equipment rated to be used in these environments. Placement of other equipment should then be based on accessibility to essential stationary items such as power and air outlets, lighting, drains, and access doors. Hosing and cable connections to this equipment should not be allowed to present a to hazard your mobility. Ergonomic placement of the buffer tank near the pump, the pump near the column, and the column near the waste drain will minimize hosing requirements and facilitate column packing and operation.

Resin handling

For large-scale biomanufacturing, you may require equipment for resin handling to generate and accurately transfer large quantities of resin slurry in an automated manner between storage vessels and the column. The equipment must be gentle enough to prevent excessive shear forces that could lead to resin degradation, clogged nets, and increased back pressure.

Cytiva offers automated equipment for resin handling, including the Media Wand™ media handling unit and the BioProcess™ resin mixer. For smaller volumes, a simple Media stirrer can be used. The resin mixer can be connected directly to the AxiChrom column controller and systems such as ÄKTA process chromatography system for fully automated handling of chromatography resins during column packing and unpacking.

1.2.6 Choice of packing method

In choosing a packing method, one of the most important considerations is the volume of sample to be processed per unit column volume per unit time (also known as throughput). The desired throughput requires an appropriate selection of resin and column as well as a packing method that will provide column bed stability during operation. The method of packing can therefore limit the throughput by dictating the maximal operational flow rate and pressure, above which bed instability will result and compromise capacity, resolution, and reproducibility. Maintaining bed stability may also require flow rate/throughput reduction when you use highly viscous sample or buffer components and/or low operational temperature.

Method considerations

The packing method you use will be dependent on the selected resin, packed bed dimensions, column design, and available liquid delivery system. Detailed and established packing methods for more popular resin types are available in many cases and should be utilized whenever possible. For AxiChrom columns, the packing methods are verified and preprogrammed into the Intelligent packing in the AxiChrom column controller and UNICORN software. Method choices may be influenced by time, equipment, and facility considerations, by the physical properties of the resin and column, and by the bed dimensions when long or short bed heights are necessary due to column availability or process requirements. The method must also employ column and equipment components in contact with fluid, which are made with materials resistant to all chemicals used in the packing, testing, and operational procedures. Except for the pack-in-place method, the selected column must be large enough to hold the entire slurry volume below the adapter set at its highest level in the column, and the adapter must be adjustable to the desired final bed height.

The packing method selected may also depend on the pressure limitations of the column or pump if less than that of the resin. A pump selected for flow packing should have speed control and accurately maintain the necessary packing flow rates (determined by constructing a pressure flow curve) with minimal slippage and pulsation near the pressure limit of the resin. Ancillary packing equipment (i.e., hosing, monitors, and valves) should have an i.d. that does not restrict the flow path, which might otherwise negatively affect attainable flow rates. When unavoidable pressure limitations require the use of suboptimal flow rates during packing, a lower quality pack can be expected.

1.2.7 Resin and buffer preparation

Resin volume determination

AxiChrom columns

The amount of resin required for packing the column, allowing for the packing factor (*PF*) required, can be calculated using the following formula:

$$V = \pi \times r^2 \times H \times PF/1000$$

Where:
V = volume of resin needed, liter
π = 3.14
r = radius of column (half the diameter), cm
H = bed height in column, cm
PF = packing factor for the resin. *See* 1.2.8 Resin slurry concentration determination.

Some resins require the volume for this calculation to be performed with its compression factor. This is due to the resins settling behavior during consolidation, settling to a higher bed under low flow than settled under gravity, and behavior in different packing solutions, for example, NaCl and other salt-containing packing solutions. *See* the packing instructions for each resin, respectively.

Chromaflow columns

The amount of resin required for packing the column, allowing for the *CF* required, can be calculated using the following formula:

$$V = \left(\pi \times r^2 \times H \times \frac{CF}{1000}\right) + X$$

Where:
V = volume of resin needed, L
π = 3.14
r = radius of column (half the diameter), cm
H = bed height in column, cm
CF = compression factor of resin
X = added volume due to the conical design of the distributor plates, in liters (*see* Table 1.1)

Table 1.1. Volumes of the dished end cells in Chromaflow 400 to 1000 columns; for larger columns see the column's operating instructions

Column inner diameter (mm)	Total conical areas volume (L)
400	0.46
600	1.4
800	3.0
1000	5.8

Table 1.2 illustrates column volumes for corresponding bed heights and Chromaflow column diameters, without taking compression into account.

Note: The volumes indicated are the total column volume and include the volume for the two conical distributor plates in the columns.

Table 1.2. Column volume (L) compensation in Chromaflow columns due to conical end cells

		Bed height (cm)						
Column diameter (cm)	**Cross-sectional area (cm²)**	**10**	**15**	**20**	**25**	**30**	**40**	**50**
40	1260	13	19	28	32	38	51	63
60	2830	30	44	58	72	86	114	143
80	5030	53	78	104	12	154	204	254
100	7850	84	124	163	202	241	320	398

1.2.8 Resin slurry concentration determination

A guideline to determining resin slurry concentration can be found online in reference 1.

Proper bed geometry is determined by several factors, all of which are predicated on accurate determination of resin slurry concentration (Fig 1.3). A successful column packing therefore begins with correct measurement of the slurry concentration. Thereafter, the required resin volume can be transferred to an empty column for packing to the target bed height at the correct level of compression. Using the correct compression factor (*CF*) allows delivery of a known amount of resin to a calibrated column volume.

When working with axial compression packing methods, the *PF* is often used instead of *CF*. When settling using flow, *PF* is determined by detection of the height of the consolidated (not yet mechanically compressed) bed. Axial compression is mostly associated with AxiChrom columns but can also be applicable for other columns such as the BPG columns. The difference between the columns is that the packing process is automated for AxiChrom columns and manual for BPG columns.

Equations to calculate *CF*, *PF*, and column volume (V_c) are shown below:

$$\text{Compression factor, } CF = L_{settled} / L_{packed}$$
$$\text{Packing factor, } PF = L_{cons} / L_{packed}$$

where

$L_{settled}$ = bed height measured after settling by gravity (cm).

L_{packed} = packed bed height (cm).

L_{cons} = consolidated bed height, that is, bed height measured after settling the resin at a given linear velocity (cm/h).

$$\text{Column volume, } V_C = L_{packed} \times A_C$$

where

A_C = cross-sectional area of the column (cm²)

Why accurate slurry concentration is important

To obtain the correct amount of chromatography resin for packing to target bed height or compression, it is important that you measure the slurry concentration correctly. For example, when packing a 50% slurry to a target bed height of 20 cm at a *CF* of 1.15, an inaccuracy of ± 2% in the slurry concentration can cause the final packed bed height to vary between 19.2 and 20.8 cm.

Appropriate bed compression and resin slurry concentration ensure:

- A stable bed, especially at high flow velocities.
- Reproducible performance between column packings and campaign products.
- Optimal column resolution (R_s) and asymmetry (A_s).
- Consistent process step throughput.

Two options are available for you to determine slurry concentration:

1. Slurry concentration determination using Tricorn™ columns

Slurry concentration can easily and accurately be determined using the Slurry concentration kit (Fig 1.4). This method uses a Tricorn 10/100 column and resin in water. Slurry is added to the column and allowed to settle.

1. Column filling: add thoroughly mixed slurry to the column up to the 10 cm mark with a pipette, and then add distilled water until the column is filled.
2. Resin settling: fill a syringe with distilled water and fit it to the top of the column, press the syringe at 5 to 10 mL/min until the liquid over the resin bed is clear, and then stop the flow.
3. Slurry concentration measurement: allow the bed to stabilize for 0 to 30 min (time for stabilization depends on resin), and then read the bed height.

2. Slurry concentration determination using settling by gravity

1. Settle the resin bed by gravity overnight, remove storage buffer by decanting, and replace with an equal volume of water or 20% ethanol.
2. Shake or stir the container until the content is homogenous. For larger volumes, use the Media handling unit.
3. Immediately fill a calibrated container, such as a 100 mL graduated cylinder (Fig 1.5), with a representative sample from the resin container and allowed to sit undisturbed overnight or until the resin has completely settled. Perform in triplicate samplings from different locations within the bulk container to normalize local concentration effects.
4. The percentage of resin in the slurry can now be read directly from the graduation on the cylinder.

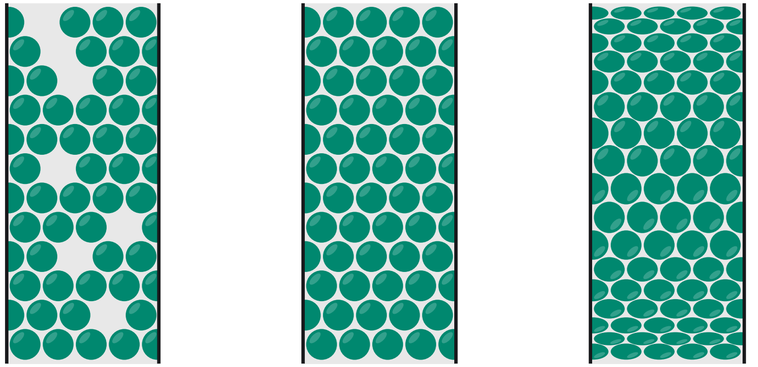


Fig 1.3. A well-packed bed (middle) generates a stable column that offers good resolution. A poorly compressed bed (left) from a non-homogenous slurry generates a less stable bed and an uneven flow through the column. A bed compression that is too high (right) generates elevated operating pressures, cracked bed, or dry areas in the bed (= wasted resin).

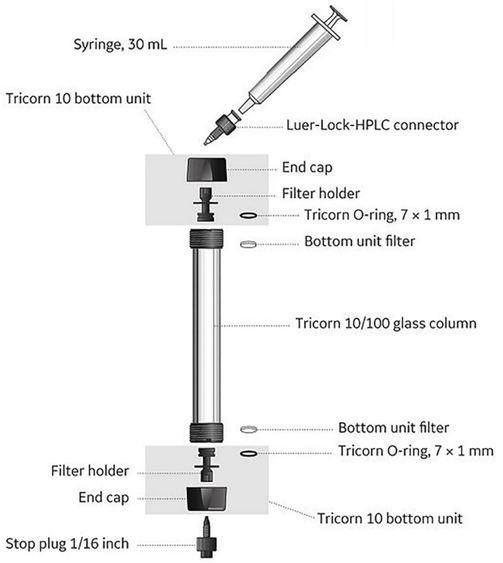


Fig 1.4. Slurry concentration is determined using the Slurry concentration kit.

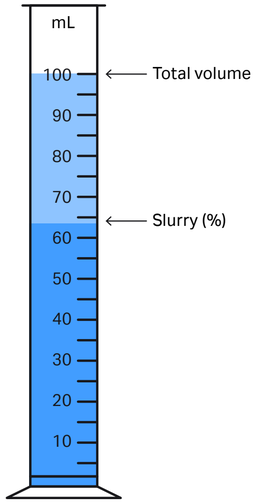


Fig 1.5. A graduated cylinder can be used for determination of slurry concentration for gravity-settled beds.

The slurry volume required for a desired column bed volume (volume of resin settled by gravity, V_{gs}) can now be calculated using the equation: 100 × V_{gs}/slurry (%). These measurements should be performed in triplicate to capture any user error or method variation, using sample from different locations within the resin slurry container to normalize local concentration effects. If not, a sample port is used from a well-stirred resin tank.

Use plastic gradient cylinders and pipettes if possible, as resin tends to adhere more to glass than to plastic surfaces.

1.2.9 Packing buffer preparation

Buffers should be prepared in a convenient location using distilled water and filtered if necessary. The buffer should be temperature equilibrated in the packing location before use. Degassing is usually not necessary if made a day in advance. The amount of packing buffer depends on the packing method used.

In flow packing or performing a pressure flow curve, the buffer can be recycled through the pump and column minimizing buffer requirements to three to four times the packed column volume. Pressure packing does not allow continual recycling and may require five to six times the column volume of buffer. For pack-in-place methods, no more than 3 column volumes (CV) of buffer are needed to prepare the slurry and fill the column. The suction and axial compression packs require only buffer to prepare the slurry and remove air from the bed supports and rinse the resin valve from resin after column filling (AxiChrom 300 to 2000 columns). Additional buffer will be needed to fill the hydraulic chamber during axial compression packs if required by the column design.

1.2.10 Test buffer and sample preparation

Testing buffer should be prepared using distilled water and filtered if necessary. All test solutions should be temperature equilibrated in the packing location before use. Degassing is usually not necessary. Required volume of testing buffer is dependent on whether pre-equilibration of the column is to be performed, how many tests are to be run, and whether the test buffer is to be recycled. Pre-equilibration may require as many as 3 CV of buffer with approximately 1 CV needed per test without recycling. The amount of sample prepared must allow initial purging of the pump and hosing between the sample container and column application valve in addition to the volume to be applied to the column per test.

For more information and details of column efficiency testing, *see* reference 2.

1.3 Column packing

1.3.1 Choosing a place to pack

An appropriate packing location within your facility should be selected based on requirements such as the availability of power, water, compressed air, and adequate drainage. Dimensions of one door to this room must allow entry of necessary tankage, column and ancillary equipment, and the room should be large enough to position these items for easy access without blocking entry and exit from the room. As scale increases, ergonomics and hardware mobility also become major concerns. In some cases, large columns will be uncrated and moved to the packing location, moved again to the testing room and/or process suite, and be stored in yet another location of the facility. A safe, facile method and accessible pathway for column mobilization to these locations should be established at the outset. This also applies to equipment that must be moved in parallel with the column and is especially important when you pack and operate large columns in a temperature-controlled environment in which space and accessibility are limited.

Wherever packing or column maintenance is performed, operation of special equipment such as a hoist or crane may be necessary and require special room features such as higher ceiling clearance.

1.3.2 Column packing methods

Many packing methods or techniques for column packing exist and certain columns are designed to be packed using one or more of these methods. Combinations of methods are also reported. Here, the principle of the columns will only be described briefly, while most information on equipment can be found in the operating instructions for the respective products.

Every packing method contains one or more steps that are critical for the success of the entire pack. You should rehearse column packing methods beforehand as there is no time to refer to the instructions when this must be carried out. Many column packing steps must be carried out in sequence in a matter of seconds. This becomes especially important when many operators are involved. Everyone needs to know exactly what to do and when.

Column packing method based on pressure-flow properties

A pressure flow curve using the chosen resin, column, and hardware should be prepared at approximately the desired bed height with buffer viscosity and temperature conditions chosen for packing. Assuming your system is not hardware limited, the information obtained from this data curve will allow appropriate selection of the optimal flow velocity for flow and suction packing methods, or pressure for constant pressure packing.

The *CF* obtained under these packing conditions is also extracted from this curve, allowing calculation of the amount of resin needed to obtain the desired bed height with good accuracy and reproducibility.

Information regarding the *CF* of the resin is also critical for hydraulic and suction packs—the final adapter position based on resin should be calculated for a successful pack. With the pack-in-place method where adapter position is fixed, a known *CF* allows delivery of the correct amount of resin into the column, thus removing an important variable in developing packing methodology.

A pressure flow curve is performed first using only packing buffer in the column to establish the pressure contribution of the empty column, system, and hardware. This will be subtracted from the pressure-flow curve with the resin in the column to obtain the pressure-flow curve representative of the packed bed only.

The amount of resin used is approximated based on the desired bed height and the homogeneous slurry added to the prepared column to gravity-settle overnight. The bed height of the settled resin, L_{gs}, is critical for determining the *CF* and should be accurately determined and plotted against zero flow velocity. When L_{gs} > 30 cm, physical compression of the resin at the bottom of the gravity-settled bed may produce an artificially low L_{gs} value for softer resins, which should not be used for *CF* calculations. In this case, the slurry in the column is adjusted to a known volume, S_{Vp}, and the percentage of slurry determined by settling a sample of the slurry in a 100 mL graduated cylinder, *see* reference 2. The L_{gs} is then calculated as follows:

$$L_{gs} = \frac{S_{Vp} \times 100}{Slurry\ (\%) \times A_c}$$

Where

L_{gs} = bed height of the settled resin

S_{Vp} = slurry volume

A_c = cross-sectional area of the column (cm^2)

The resin is then re-slurried and the adapter bolted in place. Air is purged from the column and system lines and a flow rate of < 50 cm/h (based on resin type) is established to settle the resin again. When the bed has stabilized, the flow rate, pressure, and bed height are recorded. The flow (or pressure) is then increased in increments appropriate for the resin type and flow rate as well as pressure and bed height recorded after bed stabilization at each step until the pressure reaches a limiting value. If the pressure limitation is not due to bed over-compression, the optimal flow rate and CF for packing may not be achievable with your system. However, the information from a pressure flow curve is still quite useful for appraising the capabilities of your system, making resin calculations, and establishing expectations.

See reference 3 for details on how to pack resins based on pressure-flow measurements in BPG columns.

Interpreting a pressure-flow curve

To remove hardware contributions and obtain the pressure drop across the packed bed, pressure values obtained from a pressure-flow curve generated with the "empty" column should first be subtracted from pressure values recorded with the resin-filled column. Flow velocities are then plotted against pressure with the resulting curve becoming parabolic to the pressure axis at the highest flow velocities attainable (Fig 1.6). This provides a guideline for the maximal flow velocity (V_{crit}) that should not be exceeded in your packing protocol due to physical perturbation of the semispherical shape of the beads comprising the chromatographic resin. Recommended flow packing rates for which a stable, quality pack can be obtained, are generally 70% to 85% of V_{crit}.

From the pressure-flow curve data, the bed height at the selected packing flow rate can be obtained and used to determine the compression factor, which is obtained by dividing the bed height of the gravity-settled resin by the bed height at the selected packing flow rate. The parameters for packing should target the recommended *CF* ± 2%.

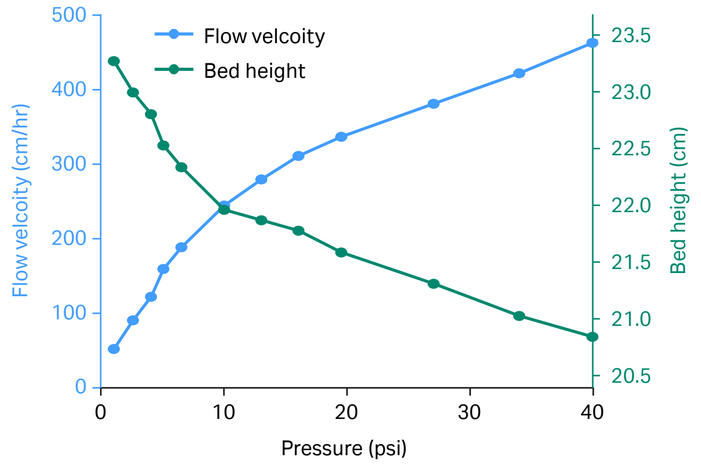


Fig 1.6. Open bed pressure flow test performed on a Q Sepharose™ Fast Flow resin measuring bed height and pressure of the settled resin at different flow rates.

Mechanical axial compression packing (Intelligent packing, AxiChrom columns)

The axial compression packing method requires a minimal amount of equipment. It is useful for situations where rapid, repetitive, and highly reproducible packing is important (Fig 1.7). It has been used successfully with a wide range of resin types such as Superdex, Sephacryl, Sepharose FF and HP, Capto™, and MabSelect™ family and gives good results with relatively noncompressible resin types such as SOURCE™ resin. In this method, hydraulic pressure or mechanical force is used to drive the column adapter into the column containing the resin slurry. The resin builds up on the bottom bed support within the column as the packing buffer exits through the bottom valve. The rate of adapter descent controls the density of the bed as it forms. As the adapter touches the bed, mechanical compression of the resin establishes the final packed bed configuration.

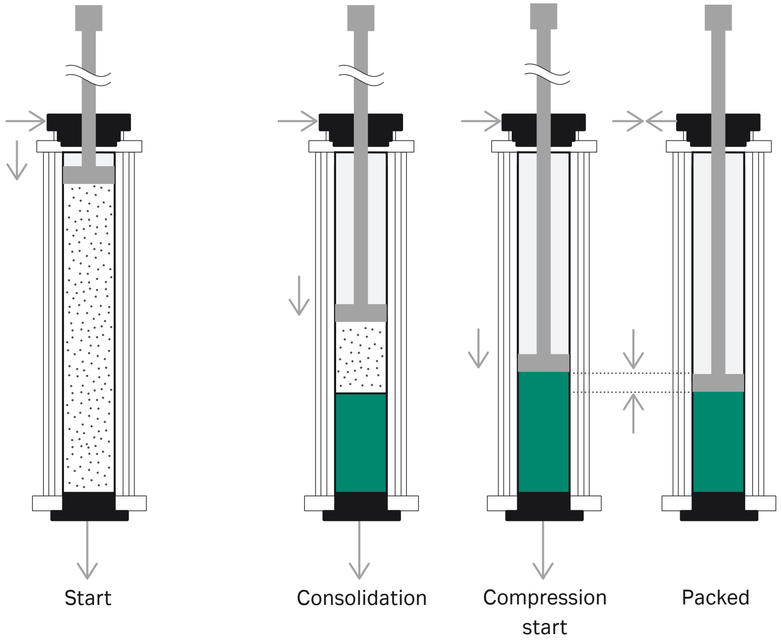


Fig 1.7. Intelligent packing method in AxiChrom columns. The adapter is mounted to the column tube and the wizard is started (**Start**). The adapter moves down, forcing packing liquid out through the bottom bed support. The resin forms a consolidated bed (**Consolidation**). When the adapter contacts the consolidated bed surface, the operator initiates bed compression in the UNICORN wizard (**Compression start**). Compression occurs according to a predetermined *PF*. The target bed height is attained (**Packed**).

Column packing in large AxiChrom columns

In AxiChrom 300 to 2000 columns, slurry is introduced via a resin valve in the center of the bottom bed support and the adapter is driven by an electric servomotor. The bipositional resin valve enables filling, packing, and unpacking without adjusting the assembled column. After the column is primed, the adapter rises from its lowest position and the column fills with slurry via the resin valve. The slurry volume is calculated automatically from the target bed height, slurry concentration, and *PF*. As an electric servomotor controls the movement of the adapter, its position is monitored with millimeter accuracy.

When the correct slurry volume has been drawn into the column, the adapter starts to move downwards and packing buffer is forced out through the bottom bed support and bed consolidation starts. The time to complete consolidation (i.e., when the adapter reaches the bed) is automatically calculated (as for the AxiChrom 50 to 200 columns) allowing you to carry out other tasks in the meantime.

As the adapter meets the consolidated bed, it is detected by the Intelligent packing wizard. When this occurs, you can confirm the bed detection. Compression of the resin starts, and a graphical interface is shown on the control screen of UNICORN control software or AxiChrom column controller. This graphical interface assists the operator in finishing the packing, giving a well-packed bed. When the adapter symbol is within the range of approved packing factors and bed height limits, you can end the packing.

Depending on the AxiChrom column controller version and installed control software, there are additional alternatives for packing the AxiChrom 300 to 2000 columns. You must select one of the alternatives for packing before the packing process can start.

The three alternatives for packing are:

1. Automatic bed detection confirmation and stop at the set packing factor.
2. Automatic bed detection confirmation and stop at the set bed height.
3. Confirm bed detection and manually stop packing at the preferred bed height or packing factor.

If selected in the UNICORN wizard, Intelligent packing will automatically run a packed bed evaluation test after the packing. For large AxiChrom columns, automatic methods for priming and unpacking can also be created with the Intelligent packing wizard.

Constant flow or pressure (BPG columns)

Constant flow packing is a two-step method that is simple and applicable to all types of resin. Assuming a suitable liquid delivery system is available, the main requirements are that the column must have a movable adapter and a pressure rating which allows the optimal packing flow rate to be obtained.

In the first step, a low flow rate is used to form a loosely packed bed from the slurry. This allows the adapter to be lowered most of the distance towards its final packed position with little or no expansion of the bed during travel time.

In the second step, the flow rate is increased to a predetermined optimal value to obtain the desired packing density. When flow is stopped, the proximity of the adapter to the bed minimizes the time necessary to successfully reposition the adapter to stabilize the packed bed.

☞ An alternative second step is to mechanically compress the bed to its final height while keeping the bottom valve opened and the top valve closed.

Pack-in-place (Chromaflow columns)

Pack-in-place is a packing technique used to pack columns where the stationary phase (chromatography resin) is introduced and packed in the column via special nozzles, thus eliminating the need to remove and replace the adapter every time the column is packed and unpacked. This method is only applicable to Chromaflow columns. The same procedure could be used to pack a variety of chromatography resins in Chromaflow columns.

Chromaflow columns have two nozzles in the center of each screen. The nozzles have three positions: run, pack, and unpack. In the run position, the nozzle tip is aligned with the screen and no liquid or slurry can pass through the nozzle to the column chamber. This is the position used when the column is packed and in operation. In the packing position, the nozzle is partially inserted into the column revealing a few rows of holes directed radially out from the center. Through these holes, it is possible to spray liquid or slurry when the column is being filled and primed with water, packed, or unpacked. In the unpack position the nozzle is fully inserted into the column revealing a passage from the inside of the column to the slurry outlet port. During unpacking, packing buffer or slurry is sprayed into the column to break up the bed. Excess slurry is expelled through the slurry outlet and into a collection tank. For packing, the packing flow rate is first set by pumping slurry into the slurry inlet and out via the slurry outlet in the top nozzle when this is in the run position, where there is an open passage between inlet and outlet. Then, the pump is momentarily stopped, the top nozzle is put in the pack position, and the bottom mobile phase is opened. When the pump is started, slurry is being sprayed into the column while the excess liquid is expelled through the bottom screen which will act like a filter retaining the particles. The bed will build up from the bottom and when the correct amount of resin has been transferred from the slurry tank to the column the pump is stopped, the nozzle is quickly retracted, and the bottom mobile phase is closed. Preferably, the packing flow rate is chosen so that the bed is over-compressed, meaning the correct amount of resin has been packed into the column before the bed reaches the top screen. This ensures that the top of the bed is well-packed and that the nozzle does not leave an imprint in the bed.

Chromaflow columns are available with external hydraulics. These columns are best packed by packing the column as described above, without the need to achieve the high compression. In this case, a 5% compression from flow is sufficient. The rest of the compression is achieved by the hydraulic cylinders. The reason for packing the column as usual at first is that the bed supports are conical and a pure axial compression pack may result in too little resin in the center of the column.

1.3.3 Verified packing protocols

In the next three sections, we provide you with Cytiva verified protocols for packing key resins in different large-scale columns. For more information about resin types not covered in this handbook, refer to cytiva.com or the specific resin instructions.



When packing BPG and AxiChrom columns, *PF* is used in the packing procedure to calculate the packed bed height after the consolidation step. *CF* is used in the resin preparation step to calculate the resin volume needed to pack a desired bed height. Because Chromaflow columns are pack-in-place columns, they have no registered consolidated bed heights and the *CF* is used throughout the packing process. *See* section 1.2.7.

1.4 Packing MabSelect PrismA™ chromatography resin in large-scale columns using verified methods

MabSelect PrismA resin is a protein A affinity resin with a genetically engineered protein A-derived ligand coupled to an optimized high-flow agarose base matrix. The ligand provides enhanced alkaline stability and binding capacity for mAbs and many bispecific antibodies (bsAbs) via binding to the Fc region as well as the VH3 region of the variable heavy chain.

The MabSelect resin family of affinity resins continues to expand, and now includes MabSelect VH3 resin (with selectivity only for the VH3 region of mAbs to allow efficient separation of antibody fragments and bispecific antibodies), and MabSelect VL resin (with an engineered protein L ligand for binding of antibody fragments and bsAbs containing the kappa light chain). Refer to the specific resin instructions for details on column packing.

This section shows you the packing procedures for packing in your AxiChrom, BPG, or Chromaflow columns.

1.4.1 How to pack high-flow agarose resins such as MabSelect PrismA resin

The bed height of a gravity-settled bed differs from the bed height of a bed settled at low flow (consolidated). Therefore, the *CF* must be distinguished from the *PF*. In water, for example, the consolidated bed height (at 60 cm/h) is higher than the gravity settled bed height. For MabSelect PrismA resin, the *PF* in this example would be 1.18 while the *CF* would be 1.1.

MabSelect resins settle differently in different solutions. Adding NaCl to the packing slows the settling of the resin beads and allows them to settle less tightly. As little as 1 mM NaCl changes the consolidated and gravity-settled bed height by more than 10%. It is important to measure the resins in correct solutions to minimize the effect on settling. In pilot-scale columns, NaCl is used as packing buffer to minimize wall effects on the packing result and bed stability.

Water is the preferred packing solution for MabSelect PrismA resins in AxiChrom 50 to 1600 columns; however, you can use other solutions such as storage solution or decanted storage solution.

Table 1.2 shows typical *PF*s for MabSelect PrismA in different solutions if the recommended packing solution cannot be used. The listed *PF*s are also relevant for large-scale AxiChrom columns with an i.d. of 300 mm or larger, at a bed height of 20 cm.

Table 1.2. Typical *PF* for MabSelect PrismA resin in different solutions for optimal bed performance where the bed is consolidated at 60 cm/h

Solution	Packing factor (*PF*)
Water	1.18
20% ethanol	1.18
10 mM NaCl	1.20
400 mM NaCl	1.20
2% benzyl alcohol (BnOH)	1.18*

* 2.0% v/v BnOH is compatible with the acrylic material used in AxiChrom columns at up to 24°C and for up to 1 yr.

You will need a variable *PF* depending on bed height to optimize the bed and flow performance of MabSelect PrismA in the larger AxiChrom columns ≥ 300 mm. Figure 1.8 shows *PF*s for MabSelect PrismA packed in AxiChrom 300 to 1600 with a bed height of 10 to 30 cm for packing in water.

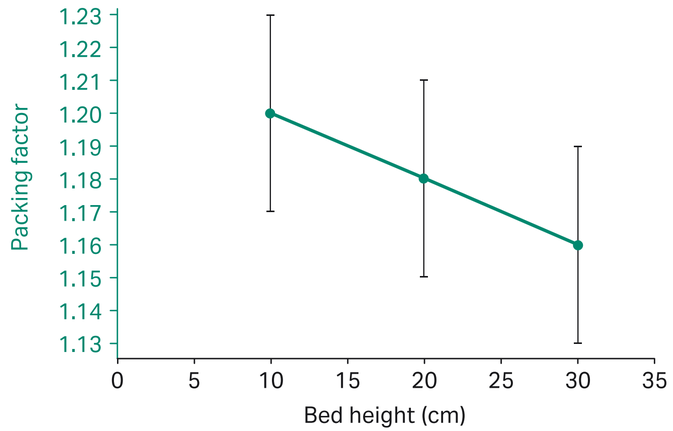


Fig 1.8. *PF* interval for AxiChrom 300 to 1600 columns packed with MabSelect PrismA resin to different bed heights in water with a consolidation velocity of 60 cm/h. The *PF* varies in relation to bed height and is calculated as y = −0.002x + 1.22 (where y is the calculated *PF* and x the packed bed height). For each bed height, the acceptable interval for the packing factor is ± 0.03.

Slurry preparation

Start by calculating the resin volume (V) needed to pack the desired bed height (*see* 1.2.7 Resin slurry concentration determination). In this step, use *CF* 1.1, which is the *CF* in 20% ethanol for the gravity-settled resins. To achieve the correct amount of chromatography resin for packing to target bed height or compression, it is important that you measure the slurry concentration correctly. Measure slurry concentration with a Tricorn 10/100 column. We offer a slurry concentration kit with all the materials required for determination of slurry concentration.

1.4.2 Packing MabSelect PrismA in AxiChrom columns

You may use both 10 and 20 µm bed supports (SS and PE) in AxiChrom columns with MabSelect PrismA resin.

If you are working with a crude feed, the 20 µm bed support is recommended.

When packing AxiChrom 50 to 200 columns with ÄKTA chromatography systems, Intelligent packing control is managed by UNICORN system control software. Intelligent packing of AxiChrom 300 to 2000 columns is performed by AxiChrom column controller, a separate unit that comprises a touchscreen-operated user interface. On ÄKTA process chromatography system, Intelligent packing is performed from the system's UNICORN software.

Chromatography column packing methods are created by entering values for the packing variables (e.g., column, resin, slurry concentration, target bed height) in the Intelligent packing wizard. The *PF* given in the Intelligent packing wizard is dependent on entered packing variables and the packing solution. To pack the column, start the chosen method in the UNICORN software and follow the instructions.

When packing AxiChrom 50 to 200 columns, the slurry is introduced into the column manually and adapter movement is driven by internal hydraulics. For ÄKTA chromatography systems with Intelligent packing support for AxiChrom 50 to 200 columns, a slurry recipe can be generated with packing solution recommendations. The recipe is valid for slurry concentration measurements performed with resin in 20% ethanol that has settled by gravity. The method controls the flow rate of hydraulic fluid to drive the adapter and packing of the bed.

1.4.3 AxiChrom column packing results

AxiChrom columns packed with the methods outlined above were tested for plate number, asymmetry, pressure-flow properties, and stability.

Table 1.3 provides examples of efficiency and stability results for MabSelect PrismA resin packed in AxiChrom columns ranging from 50 to 1600 mm i.d. These results show that the verified packing methods used give excellent packing AxiChrom columns. The stability test showed that the beds were stable when running in water at the velocities given.

Pressure-flow curves provide you with a simple, yet effective illustration of column performance in terms of the maximum operating velocity at which the purification process can be run. These curves also show you the magnitude of the back pressure in the system at a certain liquid velocity. AxiChrom columns can use the full flow potential of MabSelect PrismA resin, even at large column diameters (Fig 1.9 to 1.14).

Table 1.3. Column efficiency data for different packings of MabSelect PrismA resin in different AxiChrom columns at different bed heights

Column	Bed height (cm)	Average plates/m[1]	Reduced plate height (*h*) range[1]	Asymmetry factor (A_s) range[1]	Flow velocity for stability test (cm/h)[2]	Change after stability test (%)	
						h	A_s
AxiChrom 50	10	10 900	1.4–1.6	1.1–1.1	600	-2	0
	20	11 000	1.5–1.5	1.0–1.0	300	7	1
	40	11 400	1.4–1.5	0.8–0.9	150	5	2
AxiChrom 100	10	12 000	1.3–1.4	1.1–1.1	600	7	4
	20	12 100	1.3–1.4	1.0–1.1	300	5	3
	40	10 900	1.4–1.6	1.0–1.1	150	3	2
AxiChrom 140	20	12 300	1.3–1.4	1.0–1.1	300	3	4
AxiChrom 200	10	11 200	1.4–1.5	1.2–1.3	600	5	6
	20	11 700	1.4–1.4	1.1–1.1	300	4	5
	40	11 100	1.4–1.5	1.0–1.1	150	-2	4
AxiChrom 300	10	10 000	1.6–1.7	1.3–1.4	600	-3	-6
	20	11 000	1.5–1.6	1.1–1.2	300	-3	-4
	30	10 800	1.4–1.6	1.0–1.3	200	7	-7
AxiChrom 600[3]	20	10 300	1.5–1.5	1.1–1.1	300	-2	3
	30	10 100	1.5–1.6	1.1–1.1	200	5	4
AxiChrom 1000[3]	10	11 700	1.4–1.5	1.2–1.4	NA	NA	NA
	20	11 100	1.5–1.6	1.1–1.2	300	3	3
	30	10 200	1.5–1.8	0.9–1.1	NA	NA	NA
AxiChrom 1600[3]	10	10 900	1.4–1.7	1.2–1.4	550	-2	-1.5
	20	10 400	1.5–1.7	1.1–1.2	300	0	6
	30	10 000	1.6–1.8	1.1–1.2	200	-2	0

[1] Test performed at near optimal test conditions. Average and ranges of upflow and downflow tests for at least three packings.
[2] Stability tests were run once for each bed height/resin/column combination in water for 16 h at the given velocity.
[3] Results from studies performed on MabSelect PrismA base matrix.

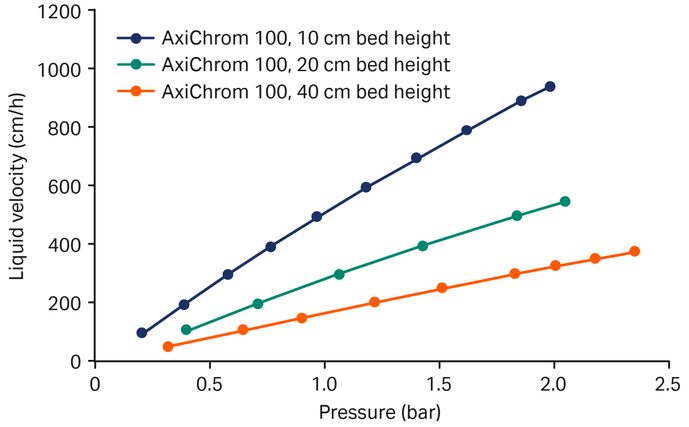


Fig 1.9. Pressure flow curves for MabSelect PrismA resin packed in AxiChrom 100 column at different bed heights. The results are normalized to 10, 20, and 40 cm bed height in water at 20°C. Tubing and system pressure are excluded.

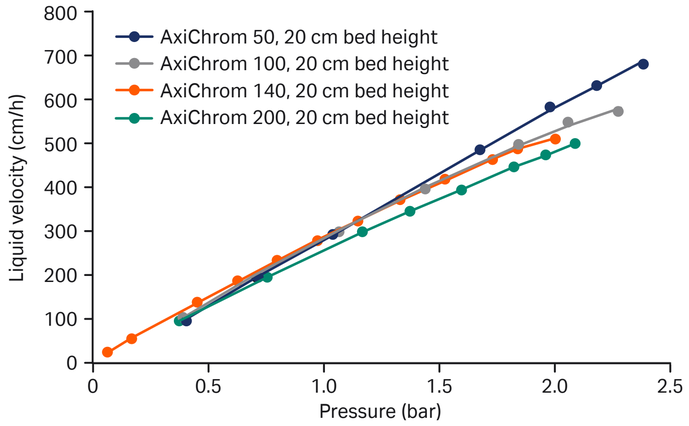


Fig 1.10. Pressure-flow curves for MabSelect PrismA resin in AxiChrom 50, 100, 140, and 200 columns at 20 cm bed height. The results are normalized to 20 cm bed height in water at 20°C. Tubing and system pressure are excluded.

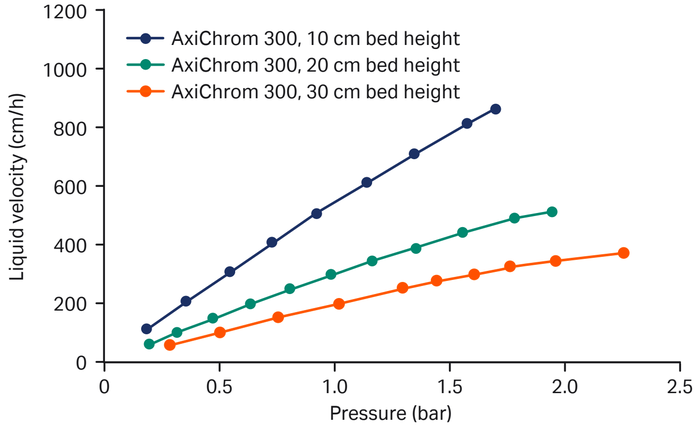


Fig 1.11. Pressure-flow curves for MabSelect PrismA resin packed in AxiChrom 300 column at different bed heights. The results are normalized to 10, 20, and 30 cm bed height in water at 20°C. Tubing and system pressure are excluded.

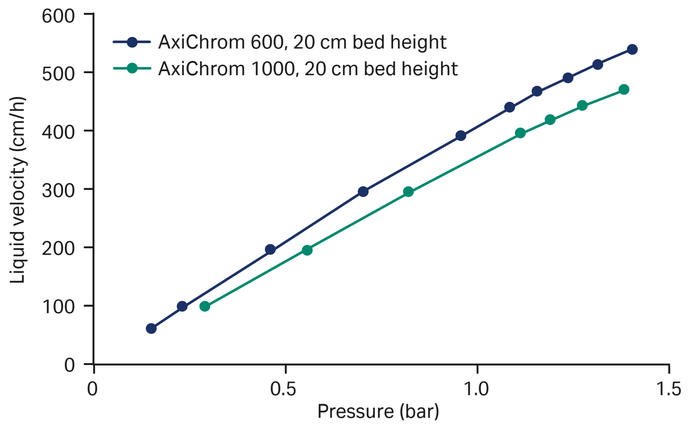


Fig 1.12. Pressure-flow curves for the MabSelect PrismA resin at 20 cm bed height in AxiChrom 600 and 1000 columns. The results are normalized to 20 cm bed height in water at 20°C. Tubing and system pressure are excluded.

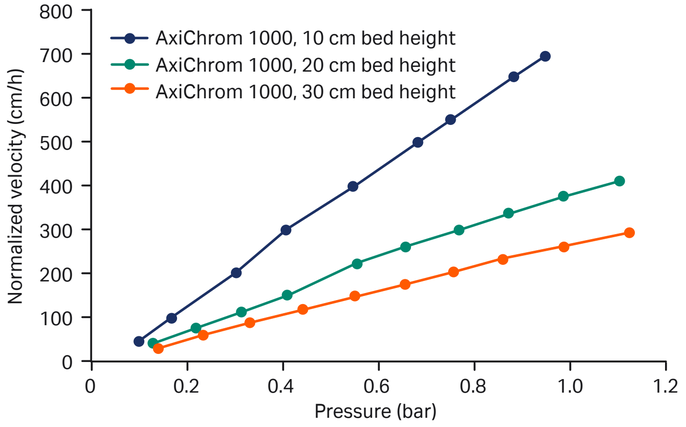


Fig 1.13. Pressure-flow curves for the MabSelect PrismA base matrix in AxiChrom 1000 column at 10, 20, and 30 cm bed height at the optimal compression for each bed height. The results are normalized to 20 cm bed height in water at 20°C. Tubing and system pressure are excluded.

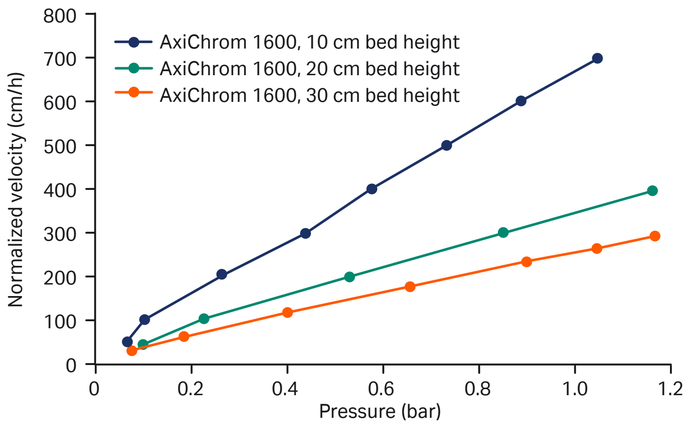


Fig 1.14. Pressure-flow curves for the MabSelect PrismA resin in AxiChrom 1600 column at 10, 20, and 30 cm bed height at the optimal compression for each bed height. The results are normalized to 20 cm bed height in water at 20°C. Tubing and system pressure are excluded.

1.4.4 Packing MabSelect PrismA resin in BPG columns

Column and system preparation for BPG column at 20 cm bed height

A detailed description of column preparation is available in the BPG instructions. The packing pump should be as pulsation-free as possible. Screw or rotary lobe pumps are the most suitable for this task and multiheaded diaphragm pumps are satisfactory.

Ensure that the column has no visible scratches in the glass tube and that the adapter moves smoothly in both upward and downward directions before packing. In addition, there should be no difficulty in tightening the adapter O-ring to the column inner wall. Follow the steps below to prepare the BPG column:

Required packing parameters for packing in BPG columns at 20 cm height:

Slurry and packing solution:	20% ethanol
Slurry concentration:	60% (± 2)
Consolidation velocity:	30 cm/h (21.2 L/h for BPG 300)
Consolidation time:	50 min (± 5)
Mechanical compression (packing factor):	1.13 (± 0.01)

1. Place a new 10 µm net on both adapter and bottom end piece.
2. Level the column using a spirit level.
3. A pressure relief valve should be used for safety reasons, especially against pressure spikes. Position this valve on the pump outlet and add a pressure gauge on the adapter.
4. Mount one 4-port-2-way valve on bottom inlet and one on top of the pressure gauge, 10 mm i.d. for BPG 300 column and 6 mm i.d. for BPG 100 and 200 columns.

Resin preparation

Dilute the resin to the desired concentration using 20% ethanol.

Packing the column

1. Set the pressure alarm or pressure relief valve according to the pressure specification (4 bar). Purge the system and tubing from air.
2. Purge the net of trapped air by draining packing solution through the column outlet until about 2 cm of solution remains in the column. Close the bottom valve. If air is still trapped under the end-piece net, add more packing solution, and connect a tube to the suction side of a pump. Start the pump and place the pump inlet tube on the bottom net and extract the remaining air.
3. Add the slurry to the column and, if needed, additional packing solution to about 40 cm. Mix the resin and the packing solution to a homogeneous slurry. Note! The available height to allow the adapter to be inserted into a 50 cm column tube (for filling slurry) is 40 cm. Use a longer column tube when packing beds higher than 20 cm (75 cm and 95 cm tubes are available).
4. Rinse the wall from particles and let the resin settle until there is about 1 cm of clear liquid on top of the slurry. This reduces the risk of particles sticking between the O-ring and the column wall, which can cause the column to leak.
5. Insert the adapter and secure it to the column tube. Lower the adapter to the surface of the slurry and allow some clear liquid to pass the O-ring. Tighten the adapter O-ring.
6. Make sure the column top valve is open. Slowly move the adapter down until no air bubbles can be seen leaving the top valve.
7. Start the pump and adjust the settling velocity to 30 cm/h. Shift the top valve into the column and immediately open the bottom valve and lead the liquid to waste.
8. Run the settling flow until the bed is completely consolidated. Note the consolidated bed height and calculate the packed bed height using the *PF* (1.13) in 20% ethanol. The packed bed height is the ratio between the consolidated bed height and the packing factor. Use a marker to indicate the packed bed height on the column.
9. Stop the flow and close the top valve. Loosen the O-ring so that the adapter can be moved while pushing the liquid out through the bottom. Lower the adapter smoothly downwards to the consolidated bed and into it further compressing it to the indication mark for the compressed final height (step 8). Excess packing solution is removed through the bottom of the column during the complete adapter lowering process.

Your column is now ready for use.

1.4.5 BPG column packing results

Examples of efficiency and stability results for MabSelect PrismA are shown in Table 1.4. Good plate numbers and asymmetry factors were achieved.

Table 1.4. Column efficiency data for MabSelect PrismA resin packed in a BPG column with 300 mm i.d.

Column	Bed height (cm)	Average plates/m[1]	Reduced plate height (h) range[1]	Asymmetry factor (A_s) range[1]	Flow velocity for stability test (cm/h)[2]	Change after stability test (%)	
						h	A_s
BPG 300	20	8000	1.7–2.8	1.2–1.5	300	6	3

[1] Test performed at near optimal test conditions. Average and ranges of upflow and downflow tests for at least three packings.
[2] Stability tests were run once for each bed height/resin/column combination in water for 16 h at the given velocity.

The pressure-flow curve for the resin in BPG 300 column is shown in Figure 1.15. The pressure-flow profile for MabSelect PrismA resin in BPG 300 column at 20 cm bed height shows that you can run the resin at 300 cm/h with a pressure of less than 2.0 bar over the bed.

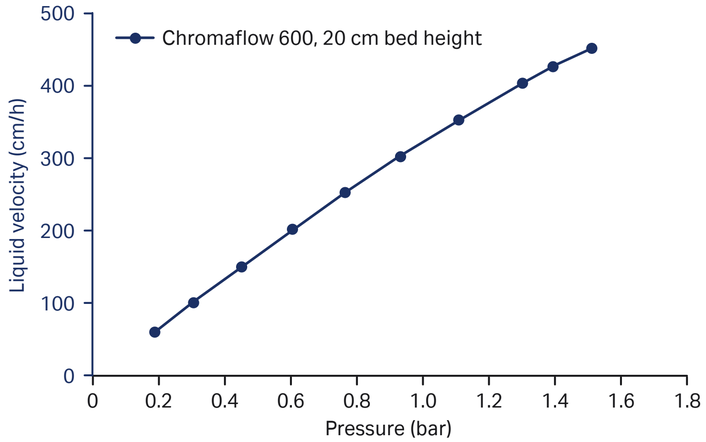


Fig 1.15. Pressure/flow rate study on MabSelect PrismA resin packed at 20 cm bed height in a BPG 300 column. The results are normalized to 20 cm bed height in water at 20°C. Tubing and system pressure are excluded.

1.4.6 Packing MabSelect PrismA resin in Chromaflow columns

Required packing parameters for Chromaflow 600 column at 20 cm bed height

Pressure:	Chromaflow packing station 100 start pressure = 5.5 bar (79.8 psi, 0.55 MPa). Pressure is kept at 3 bar (43.5 psi, 0.3 MPa) once column pressure has reached that level.
Packing solution:	purified water
Stop position:	5 mm from adapter screen
Slurry concentration:	~ 50%

This scaled-up method used to pack Chromaflow 600 column is based on a standard Chromaflow packing procedure (i.e., via nozzles in the top and bottom bed supports, and without removing the lid or adapter). This method makes use of the optional Chromaflow packing station, which includes a control panel with pneumatically actuated diaphragm pumps and valves, and a tank to hold the slurry.

Use the solution in which the resin is delivered or a decanted solution, as 10% to 20% ethanol in the slurry gives a good packing result. If the delivery solution is decanted, replace it with water.

We do not recommend Chromaflow 1000, 1400, or 2000 columns due to limitations in flow restriction/capacities for packing MabSelect PrismA resin. Instead, we recommend AxiChrom columns for use with MabSelect PrismA resin because they utilize the resin's pressure and flow properties to produce stable, well-performing packed beds.

To avoid trapping air bubbles in the column when packing, additional slurry is required for the extra volumes in tank and tubing. Add the slurry to the slurry tank and stir the resin until trapped air bubbles are removed and the slurry has a good consistency.

Dilute the suspension to about 50% slurry concentration.

Column preparation

It is important that the supply air flow rate follows the specification of the Chromaflow packing station (1000 L/min for pack 100 packing station) and that the supply air pressure into the packing station is 6 to 7 bar (87 to 101.5 psi, 0.6 to 0.7 MPa).

1. Set up the column according to the Chromaflow columns operating instructions).
2. A pressure relief valve (adjusted to the operating pressure limit of the column) should be used for safety reasons. Position this valve on the slurry inlet top (SIT), with the waste tubing connected to the slurry tank. Place a pressure gauge on the mobile phase top (MPT) to record the pressure during packing. Mount one 3-port, 2-way valve on top of the pressure gauge and one on the mobile phase bottom (MPB). The top valve should lead in two directions: one side into the system and one to waste for purging the tubing. On the bottom valve, one side leads to the system and a 1.5″ to 2″ tubing leads to waste (for packing). Part of the MPB waste tubing should be placed above the outlet valve to prevent air from entering through the MPB.
3. Connect appropriate tubing (i.d. 1″ or 1.5″) and tanks to the column and packing station. If a flow meter is used, place it between the SIT and the packing station.
4. Level the adapter to the desired bed height. Remember to loosen the nuts on the adapter rods to allow the adapter to be raised or lowered. Flush the adapter rods with 20% ethanol as lubrication.
5. Prime the column, packing station, and tubing with water according to the Chromaflow columns operating instruction.

Column packing

Packing Chromaflow columns is a rapid procedure compared with other packing procedures and it is therefore important to thoroughly read the packing instructions and practice the packing steps in advance of the packing.

1. Set both nozzles in run position to prime the top nozzle tubing with slurry. Lead the slurry outlet top (SOT) tubing back to the slurry tank and secure it. Stir the slurry to keep it homogeneous, select slurry and SIT on the packing station, open the slurry tank, and start the packing pump.
2. As the aim of this procedure is to prime the tubing and allow the pump speed to be set, the chromatography column is bypassed at this stage. Increase the pump flow until the pressure on Chromaflow packing station pack 100 is 5.5 bar/79.8 psi/0.55 MPa (or about 101 L/min).
3. When the tubing is primed and the flow rate set, set the SIT/slurry inlet bottom (SIB) valve to the position between SIT and SIB on the Chromaflow packing station to block the flow during step 4, while maintaining the pump pressure/correct flow rate for the next step. If a flow meter with flow totalizer function is used, reset the flow totalizer.
4. Move the top nozzle down into the packing position.
5. Simultaneously open the bottom mobile phase valve to waste and turn the SIT/SIB valve to SIT on the packing station, this is preferably done by two operators. The column then starts to fill with slurry and the bed builds up slowly from the bottom as excess liquid exits via the MPB.

   Column pressure should not exceed the operating pressure limit of the column (i.e., 3 bar, 45 psi, 0.3 MPa). If this pressure is reached, gently decrease the packing flow so that the pressure remains just below 3 bar. Typically, the final pressure in the column is 2 to 3 bar (29–43.5 psi, 0.2–0.3 MPa) depending on the viscosity of the packing solution, column diameter, and bed height.
6. Stop the packing pump when the bed is 0.5 cm from the top bed support by setting the SIT/SIB to the position between SIT and SIB, as described in step 3. Once the flow is stopped, the bed will expand to meet the adapter.

   If a nontransparent (e.g., stainless steel) column tube is used, stop the packing flow when the calculated volume of slurry has been introduced into the column. Check the volume in the slurry tank or use a volume totalizator.
7. Immediately move the top nozzle back to the run position. The bed will then expand until it reaches the adapter.
8. Close the MPB valve when the pressure in the column is between 0.3 and 0.1 bar (4.35 to 2.97 psi, 0.03 to 0.01 MPa). If a flow totalizer is used, register the total slurry volume pumped through the column.
9. Use packing solution to rinse residual resin from the tubing and the top nozzle. Pump the packing solution through the top nozzle back into the slurry tank.
10. Close the slurry tank and empty the tubing between the tank and packing station.
11. Equilibrate the packed bed up flow to remove any trapped air in the column.

Figure 1.16 shows the column pressure and flow velocity used when packing a 20 cm bed height in Chromaflow 600 column. The graph shows that the pressure is kept constant at 3 bar after 60 s.

1.4.7 Chromaflow column packing results

The efficiency results for MabSelect PrismA resin packed in Chromaflow 600 column are shown in Table 1.5. Good plate numbers and asymmetry factors were achieved.

Table 1.5. Column efficiency data for different packings of MabSelect PrismA resin in a Chromaflow column with 600 mm i.d.

Column	Bed height (cm)	Average plates/m[1]	Reduced plate height (*h*) range[1]	Asymmetry factor (A_s) range[1]	Flow velocity for stability test (cm/h)[2]	Change after stability test (%)	
						h	A_s
Chromaflow 600	20	8000	1.8–2.3	0.9–1.1	300	9	6

[1] Test performed at near optimal test conditions. Average and ranges of upflow and downflow tests for at least three packings.

[2] Stability tests were run once for each bed height/resin/column combination in water for 16 h at the given velocity.

The pressure-flow curve for Chromaflow 600 is shown in Figure 1.17. The pressure-flow profile for MabSelect PrismA in Chromaflow 600, at 20 cm bed height, shows that the resin can be run at 300 cm/h with a pressure of less than 2.0 bar (29 psi, 0.2 MPa) over the bed.

1.4.8 Testing the performance of the packed column

Your process-scale packed columns must perform with a high degree of efficiency over many processing cycles (i.e., display high stability). The efficiency of a packed column can be expressed in terms of height equivalent to a theoretical plate (HETP) and asymmetry factor (A_S).

This test should be repeated regularly to monitor the state of the bed throughout the working life of the column. 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. You should set the requirements for the test in accordance with test conditions and the goal of the purification. The test is further described in reference 2.

Test conditions used in the performance study described in the application note were:

Sample:	2% v/v acetone or 0.8 M NaCl (AxiChrom 50 to 200)
Sample volume:	1% of the column volume (V_c)
Test velocity:	30 cm/h for AxiChrom, BPG, and Chromaflow columns
Eluent:	Water or 400 mM NaCl (AxiChrom 50 to 200)

To compare the performance of columns packed with chromatography resin of different particle diameters, the reduced plate height (*h* = HETP/average bead diameter [dp]) is typically used. As a guideline, a value of *h* < 3.0 is considered good at optimal test conditions.

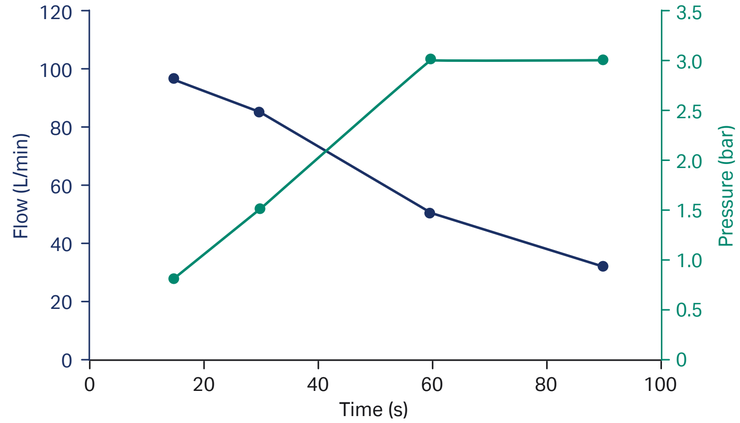


Fig 1.16. Column pressure and flow velocity during packing of a 20 cm bed height in Chromaflow 600 column using Chromaflow packing station pack 100.

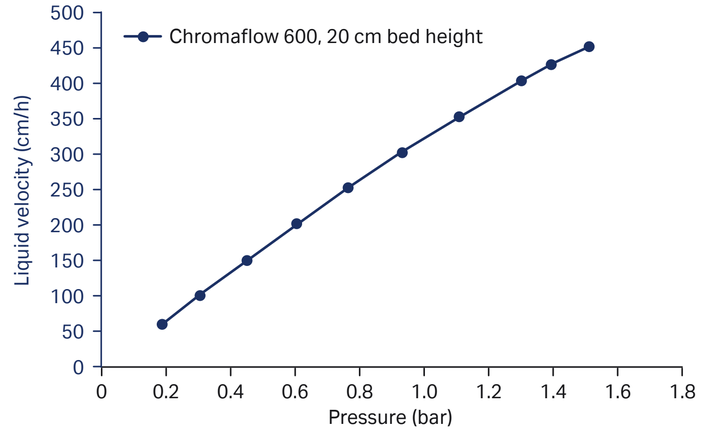


Fig 1.17. Pressure-flow curve for the MabSelect PrismA resin at 20 cm bed height in Chromaflow 600 column. The packed bed compression was 1.08. The results are normalized to 20 cm bed height in water at 20°C. Tubing and system pressure are excluded.

1.4.9 Efficiency tests at different flow velocities

Efficiency tests were run at different flow velocities. Figure 1.18 shows a van Deemter plot of the results. The asymmetry factor is stable at the different flow velocities. The reduced plate height increases with the flow velocity, which is in accordance with the van Deemter equation. In this study, optimal results were achieved at 20 to 40 cm/h.

When running at higher flow velocities, the asymmetry factor and reduced plate height continued to show a linear correlation, which means that you can run the efficiency test at any flow velocity.

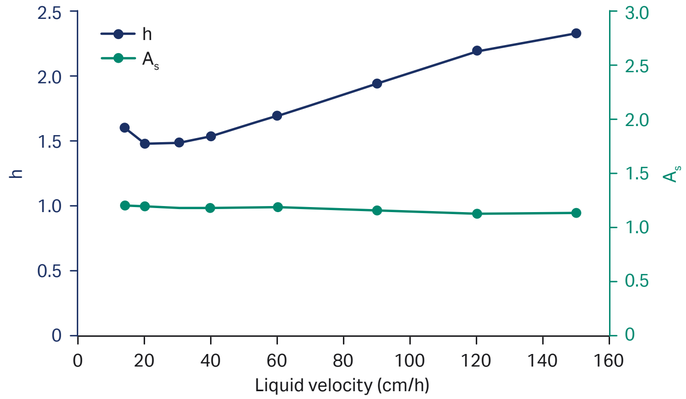


Fig 1.18. van Deemter plot for AxiChrom 300 column packed with MabSelect PrismA resin at 20 cm bed height.

1.5 Packing Capto S ImpAct resins in large-scale columns using verified methods

Capto S ImpAct resin is a strong cation exchange resin for polishing of mAbs and a wide range of other biomolecules. This section shows you the packing procedures for packing in your AxiChrom, BPG, or Chromaflow columns.

Flow characteristics for different columns and bed heights for packing with Capto S ImpAct resin are are given in Table 1.6.

Table 1.6. Expected flow velocities for different columns packed with Capto S ImpAct resin and bed heights (measured with 150 mM NaCl at 20°C)

Column type	Column diameter (mm)	Bed height (cm)	Flow velocity (cm/h)
AxiChrom	≤ 1000	10	440*
AxiChrom	≤ 1000	20	220*
AxiChrom	≤ 1000	30	165*
AxiChrom	≤ 1000	40	110*
BPG	≤ 300	40	110
Chromaflow	600	20	150

* Verified for columns up to 600 mm.

The bed height of a gravity-settled bed differs from the bed height of a bed settled under low flow (consolidated). Therefore, you will need to separate the *CF* from the *PF*. For example, for a 20 cm bed in 150 mM NaCl when consolidated at 60 cm/h, the *PF* is 1.20 and the *CF* is 1.23 for Capto S ImpAct resin. This information is helpful when the amount of gravity-settled resin needed for a specific column size is calculated (amount of gravity settled resin needed to obtain a bed with a certain *PF* [e.g., 1.20] is calculated as column volume (V_c) × *CF* [e.g., 1.23]).

The equation to calculate *CF, PF*, and column volume is found in 1.2.7 Resin slurry concentration determination.

Slurry preparation

When preparing the slurry, start by calculating the chromatography slurry volume (V) needed to pack the desired bed height. The slurry concentration can be determined in several ways. However, to get an accurate slurry concentration determination of Capto S ImpAct resin in salt-containing solutions, use the method described below. When calculating the slurry volume (V), use CF = 1.24, which is valid when the resin is in storage solution.

The slurry concentration determined by the method below corresponds to the gravity-settled concentration in water.

$$V = A_c \times \frac{L_{packed} \times CF}{C_{slurry}}$$

where

A_c = cross-sectional area of the column (cm^2)
C_{slurry} = concentration of the slurry

Capto S ImpAct is supplied in 20% ethanol with 200 mM sodium acetate. Before packing, transfer the resin to the saline packing solution (150 mM NaCl) as described in the packing instructions for the relevant column.

1.5.1 Packing of Capto S ImpAct resin in AxiChrom columns

When packing AxiChrom 50 to 200 columns for use with ÄKTA chromatography systems, Intelligent packing control is managed by the UNICORN control software. For AxiChrom 300 to 1600 columns, Intelligent packing is performed by AxiChrom column controller, or from the UNICORN software on ÄKTA process chromatography system.

The general considerations for packing MabSelect PrismA chromatography resin by Intelligent packing in AxiChrom applies also to packing of Capto S ImpAct resin, *see* section 1.3.2 Column packing methods for details.

Packing variables and recommended packing buffers are provided in Table 1.7.

Table 1.7. Packing variables for packing of Capto S ImpAct resin in AxiChrom columns

Packing variable	AxiChrom 50 to 200	AxiChrom 300 to 1600
Packing solution	150 mM NaCl	
Packing speed/velocity	60 cm/h	
Packing factor	Bed height 10 cm: 1.22 ± 0.02 Bed height 40 cm: 1.16 ± 0.02 Bed height 30 cm: 1.18 ± 0.02 Bed height 40 cm: 1.16 ± 0.02	
Flow conditioning	3 CV at 440 cm/h for 10 cm bed height 3 CV at 220 cm/h for 20 cm bed height 3 CV at 165 cm/h for 30 cm bed height 3 CV at 110 cm/h for 40 cm bed height	Optional for AxiChrom 300: 3 CV at 220 cm/h for 20 cm bed height 3 CV at 110 cm/h for 40 cm bed height

CV = column volumes

1.5.2 AxiChrom column packing results

The results for Capto S ImpAct resin in column sizes ranging from 100 to 600 mm were very similar, showing that the verified packing methods available in Intelligent Packing give equal results, independent of column size and bed height. We performed a stability test on the AxiChrom, BPG, and Chromaflow columns used in this study according to the following conditions.

Bed efficiency evaluation: conditions used in the evaluation of AxiChrom, BPG, and Chromaflow columns

Sample:	2% v/v acetone in 150 mM NaCl or 0.8 M NaCl (AxiChrom 50 to 200 columns)
Sample volume:	1% of the bed volume (V_C)
Flow velocity:	30 cm/h for AxiChrom and BPG columns, 20 cm/h for Chromaflow columns
Eluent:	150 mM NaCl or 0.4 M NaCl (AxiChrom 50 to 200 columns)

Stability test conditions used:

150 mM NaCl or water for 16 h at stated flow for each test.

Using the conditions defined above, we show that the resin beds were stable when running in water at velocities given in Table 1.8.

Table 1.8. Column efficiency data for Capto S ImpAct resin in AxiChrom columns

Column	Bed height (cm)	Average plates/m	Reduced plates height (*h*) range	Asymmetry factor (A_s) range	Flow velocity for stability test (cm/h)	Change after stability Test (%)	
						h	A_s
AxiChrom 100	20	12 000	1.6 to 1.7	1.1 to 1.2	250	-1	-3
AxiChrom 300	10	13 000	1.5 to 1.6	1.3 to 1.4	500	-6	4
AxiChrom 300	20	12 000	1.5 to 1.7	1.1 to 1.2	250	-1	2
AxiChrom 300	40	11 000	1.7 to 2.0	1.1 to 1.2	125	2	8
AxiChrom 300	30	11 000	1.7 to 1.8	1.0 to 1.2	167	1	3
AxiChrom 600	20	13 000	1.4 to 1.5	1.1 to 1.2	250	1	3

Pressure-flow curves provide a simple, yet effective illustration of column performance in terms of the maximum operating flow velocity at which the purification process can be run. These curves also show the magnitude of the back pressure in the system at a certain flow velocity and for different bed heights. Pressure-flow curves for Capto S ImpAct resin packed in AxiChrom 300 column to different bed heights are shown in Figure 1.19.

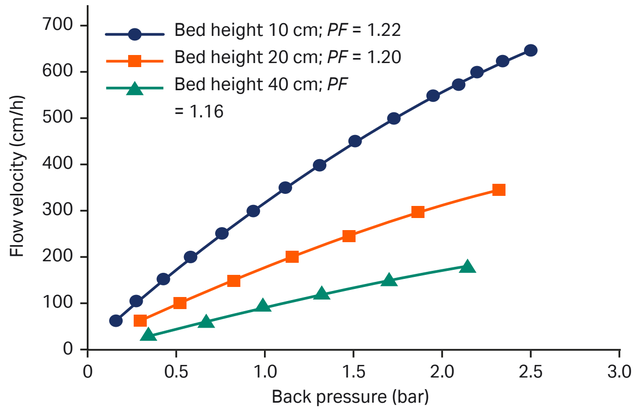


Fig 1.19. Pressure-flow curves for Capto S ImpAct resin in 150 mM NaCl packed in AxiChrom 300 column to different bed heights using different packing factors. Capto S ImpAct resin can be run at 220 cm/h with a back pressure of less than 3 bar (43.5 psi, 0.3 MPa) for bed heights up to 20 cm.

1.5.3 Packing Capto S ImpAct resin in BPG columns

Capto S ImpAct resin can be packed in BPG 100 to 300 columns. The bed height interval recommended for BPG 100 to 300 columns is 10 to 40 cm. In BPG columns with small diameters (BPG 100), the increased influence of the wall support means that salt solution is required for packing. We therefore recommend that Capto S ImpAct resin is packed with 150 mM NaCl in BPG 100 and BPG 300 columns. The packing factor for packing of Capto S ImpAct resin to various bed heights in BPG columns is 1.14.

Chromatography resin preparation

You can perform equilibration of the resin to the packing solution by using the BPG column as a "filter". Pour the resin into the column, mount the adapter, tighten the adapter O-ring, move the adapter down and compress the bed slightly, connect the pump, and wash the resin with at least 3 CV of the packing solution. Unpack and resuspend the slurry and pack according to the method below.

Equilibration is critical for Capto S ImpAct resin in BPG columns as the delivery solution contains 200 mM sodium acetate in 20% ethanol, and the recommended packing solution is 150 mM NaCl.

Column and system preparation

A detailed description of column preparation is available in the BPG column operating instructions. The packing pump should be as pulsation-free as possible. Screw and rotary lobe pumps are the most suitable for this task but multiheaded diaphragm pumps are also satisfactory.

Ensure that the column has no visible scratches in the glass tube and that the adapter moves smoothly in both upward and downward directions before packing. In addition, you should not find tightening of the adapter O-ring to the column inner wall difficult.

1. Place a new 10 µm net on both the adapter and the bottom end pieces.
2. Level the column with the aid of a spirit level.
3. A pressure relief valve should be used for safety reasons, especially against pressure spikes. Position this valve on the pump outlet and add a pressure gauge to the adapter.
4. Mount one 4-port, 2-way valve on the bottom inlet and one on top of the pressure gauge, i.d. 10 mm for BPG 300 and i.d. 6 mm for BPG 100.

Packing

Packing variables for packing BPG 100 to 300 columns

Packing solution	150 mM NaCl
Packing flow velocity	60 cm/h
Packing factor	Bed height 40 cm: 1.14
Flow conditioning	Optional for BPG: 3 CV at 110 cm/h

1. Set the pressure alarm or pressure relief valve according to the pressure rating of the column. Purge the system and tubing of air.
2. Purge the end piece net from trapped air by draining some packing solution through the column outlet. Leave about 2 cm of solution in the column and close the bottom valve. If air is still trapped under the end piece net, add more packing solution, and connect tubing to the suction side of a pump. Start the pump and place the tubing on the bottom net and extract any remaining air.
3. Add the slurry to the column and, if needed, additional packing solution (150 mM NaCl) to about 40 cm. Mix the resin and the packing solution to a homogeneous slurry.

   The available height to allow the adapter to be inserted into a 50 cm column tube (for filling slurry) is 40 cm. Use a longer column tube when packing beds higher than 20 cm: 75 cm and 95 cm tubes are available.

4. Rinse the wall from particles and let the medium settle until there is about 1 cm clear liquid on top of the slurry to reduce the risk of particles sticking between the O-ring and the column wall, which can cause leakage.
5. Insert the top adapter and secure it to the column top flange. Lower the adapter to the surface of the slurry and allow some clear liquid to pass the O-ring. Tighten the adapter O-ring.
6. Make sure that the column top valve is open. Slowly move the adapter down until no air bubbles can be seen leaving the top valve.
7. Start the pump and adjust the settling velocity to 60 cm/h (4.7 L/h for BPG 100 and 42.4 L/h for BPG 300 column). Shift the top valve into the column and immediately open the bottom valve and lead the liquid to waste.
8. Run the settling flow until the bed is completely consolidated. Note the consolidated bed height and calculate the packed bed height. For BPG 100 and 300 columns, *PF* = 1.14 in 150 mM NaCl for bed heights up to 40 cm.

   ☞ The packed bed height is the ratio between the consolidated bed height and the *PF*. Use a marker pen to indicate the packed bed height on the column.

9. Stop the flow and close the bottom valve. Loosen the O-ring and lower the adapter down to 1 cm above the settled bed and seal the adapter O-ring. Shift the top valve to waste and use the adapter to mechanically compress the bed to the mark on the column (step 8). Excessive packing solution is removed through the adapter tube.

   ☞ Compressing Capto S ImpAct resin in BPG columns, especially the larger BPG 300, is physically demanding.

   ☞ Do not use extension rods on the adapter height adjuster to compress the resin.

   ☞ To increase the performance and stability of the bed, flow condition the column downwards for 5 CV and upward for 5 CV at 110 cm/h with packing solution.

10. Connect the pump to the top of the column. Purge the system and tubing by running the mobile phase to waste by bypassing the column inlet with the 4-port valve. Start at a low flow velocity (approximately 60 cm/h).
11. Shift the top valve to direct the flow to the column and immediately open the bottom mobile phase to waste or connect it to the buffer tank for recirculation.
12. Increase the flow to 110 cm/h and run the column at this flow for 5 CV.
13. Slowly decrease the flow for 2 min to avoid disturbance of the conditioned bed.
14. Exchange the mobile phase connections. Connect the tubing from the pump to the bottom valve and open the top valve to waste or to the buffer tank for recirculation.
15. Repeat steps 10 through 13 with upward flow to complete the conditioning procedure.
16. Test the packing at the optimal test velocity (20 to 30 cm/h).

1.5.4 BPG column packing results

The packing method described in 1.5.1 Packing Capto S ImpAct resin in AxiChrom columns works well for Capto S ImpAct in different sizes of BPG columns at different bed heights. The stability test (*see* 1.5.2 AxiChrom column packing results) showed that the bed was stable in water at velocities given in Table 1.9.

Table 1.9. Column efficiency data for Capto S ImpAct resin packed in a BPG column with 100 and 300 mm i.d.

Column	Bed height (cm)	Average plates/m	Reduced plate height (*h*) range	Asymmetry factor (A_s) range	Flow velocity for stability test (cm/h)	Change after stability test (%)	
						h	A_s
BPG 100	40	10 000	1.7 to 1.8	1.0 to 1.1	110	-4	1
BPG 300	20	11 000	1.5 to 1.7	1.0 to 1.3	250	-2	10
BPG 300	40	10 000	1.5 to 2.0	1.0 to 1.5	110	-4	-3

The pressure-flow curves for Capto S ImpAct at 40 cm bed height in BPG 100 and BPG 300 are shown in Figure 1.20. Capto S ImpAct shows linear behavior in these columns.

1.5.5 Packing Capto S ImpAct resin in Chromaflow columns

The method described in this section has only been verified for packing of Capto S ImpAct resin in Chromaflow 600 column to a bed height of 20 cm. As the pump is stopped 5 mm from the adapter, as seen in the packing method, lower beds will not be compressed sufficiently. Moreover, the extreme velocities needed to efficiently pack a shorter bed cannot easily be achieved using standard equipment.

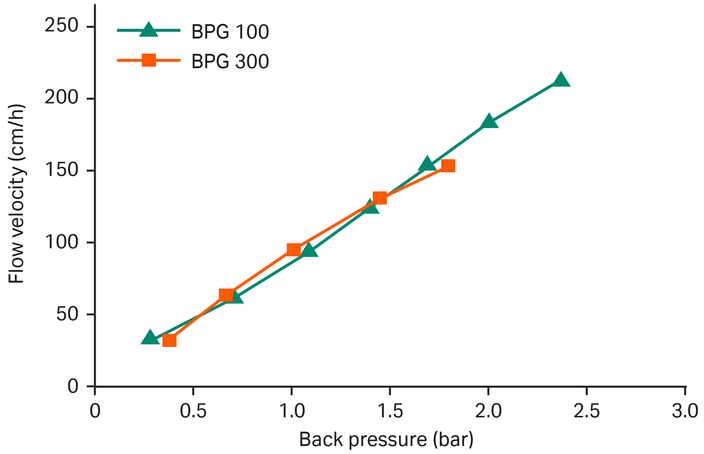


Fig 1.20. Pressure-flow curves for Capto S ImpAct in 150 mM NaCl packed to 40 cm bed height (*PF* = 1.14) in BPG 100 and BPG 300. System/tubing pressure is excluded.

To pack Capto S ImpAct resin in Chromaflow 600 column, the slurry is introduced from the top nozzle using Chromaflow packing station 100.

The recommended operational flow velocity for Capto S ImpAct resin in Chromaflow 600 column at 20 cm bed height is 150 cm/h.

The flow capacity of Chromaflow packing station pack 100 is required for packing Capto S ImpAct resin in Chromaflow 600 column.

Chromatography resin preparation

The recommendation is to use 150 mM NaCl as packing solution for Chromaflow columns and that this packing solution is used throughout the whole procedure.

Water can potentially be used as packing solution, but compression and flow velocities have not been verified.

To avoid the trapping air in the column when packing, additional slurry is required for the extra volumes in the tank and tubing. Add the slurry to the slurry tank and stir the resin. Dilute the suspension to a slurry concentration of about 50%.

Column and system preparation

For more detailed description about the column and packing station preparation, *see* the operating instructions for Chromaflow columns (28962232) and Chromaflow packing station (29046228). In this procedure, standard Chromaflow equipment is used for the connections on the column and the packing station.

It is important that the supply air flow rate follows the specification of Chromaflow packing station pack 100 (1000 L/min) and that the supply air pressure into the packing station is 6 to 7 bar (87 to 101 psi, 0.6 to 0.7 MPa).

1. Set up the column according to the Chromaflow column operating instructions, 28962232.
2. A pressure relief valve should be used for safety reasons. Position the pressure relief valve on the slurry inlet top (SIT). Place a pressure gauge on the mobile phase top (MPT) to record the pressure during packing. Mount a 3-port, 2-way valve on top of the pressure gauge and the mobile phase bottom (MPB). The top valve should lead in two directions: one side into the system and one to the waste for purging the tubing. On the bottom valve, one side leads to the system and a 1.5" to 2" tubing leads to the waste (for packing). Part of the MPB waste tubing should be placed above the outlet valve to eliminate air entering through the MPB.
3. Connect appropriate tubing (i.d. 1" or 1.5") and tanks to the column and packing station. If a flow meter is used, place it between the SIT and the packing station.
4. Level the adapter to the desired bed height. Remember to loosen the nuts on the adapter rods to allow the adapter to be raised or lowered. Flush the adapter rods with 20% ethanol as lubrication.
5. Prime the column, packing station, and tubing with water according to the Chromaflow column operating instructions, 28962232.

Packing

Packing Chromaflow columns is a rapid procedure compared with other packing procedures and, therefore, it is important to thoroughly read the packing instructions and go through the packing steps in advance of starting the packing.

1. Set both nozzles in run position to prime the tubing with slurry. Lead the slurry outlet top (SOT) tubing back to the slurry tank and secure it. Stir the slurry to keep it homogeneous, select slurry and SIT on the packing station, open the slurry tank and start the packing pump.
2. The initial flow velocity should be at least 1400 cm/h, corresponding to an air pressure on the packing station of at least 2.5 bar (36.3 psi, 0.25 MPa) for packed bed heights of 20 and 30 cm.
3. When the tubing is primed and the flow velocity set, set the SIT/slurry inlet bottom (SIB) to the position between SIT and SIB to block the flow during step 4 while maintaining the correct flow rate for the next step.
4. Move the top nozzle down into the packing position.
5. Open the bottom mobile phase valve to waste and turn the SIT/SIB valve to SIT on the packing station. This procedure can be facilitated if performed by two operators. The column starts to fill with slurry and the bed builds up slowly from the bottom as excess liquid exits via the MPB.

☞ Column pressure should not exceed the operating pressure limit of the column (i.e., 3 bar, 43.5 psi, 0.3 MPa). If this pressure is reached, gently decrease the packing flow so that the pressure remains just below 3 bar. Typically, the final pressure in the column is 2 (29 psi, 0.2 MPa) to 3 bar (43.5, 0.3 MPa) depending on the viscosity of the packing solution, column diameter, bed height, and so forth.

6. Stop the pump when the building bed is 5 mm from the top bed support by setting the SIT/SIB to the position between SIT and SIB, as described in 3. Once flow is stopped, the bed will expand to meet the adapter.

☞ If a nontransparent column tube is used, stop the packing flow when the calculated volume of slurry has been introduced into the column. Check the volume in the slurry tank or use a volume totalizator.

7. Immediately move the top nozzle back to the run position.
8. Close the MPB valve when the pressure in the column is between 0.3 and 0.1 bar (4.4 to 1.45 psi, 0.03 to 0.01 MPa).
9. Use packing solution to rinse residual resin from the tubing and the top nozzle. Pump the packing solution through the top nozzle back into the slurry tank.
10. Close the slurry tank and empty the tubing between the tank and packing station.
11. Pump liquid upwards through the column until the air is expelled.

1.5.6 Chromaflow column packing results

The packing method described in section 1.5.5 Packing Capto S ImpAct resin in Chromaflow columns works well for Capto S ImpAct in different sizes of Chromaflow 600 columns at 20 cm bed heights, *see* Table 1.10. The stability test (*see* 1.5.2 AxiChrom column packing results for details) showed that the bed was stable in water at velocity given in the table.

Table 1.10. Column efficiency data for Capto S ImpAct packed in Chromaflow 600 column

Column	Bed height (cm)	Average plates/m	Reduced plate height (*h*) range	Asymmetry factor (A_s) range	Flow velocity for stability test (cm/h)	Change after stability test (%)	
						h	A_s
Chromaflow 600	20	9 500	1.9 to 2.0	1.1 to 1.2	200	2	-2

Figure 1.21 shows the increase in pressure and decrease in flow velocity when packing a 20 cm bed height in the Chromaflow 600 column.

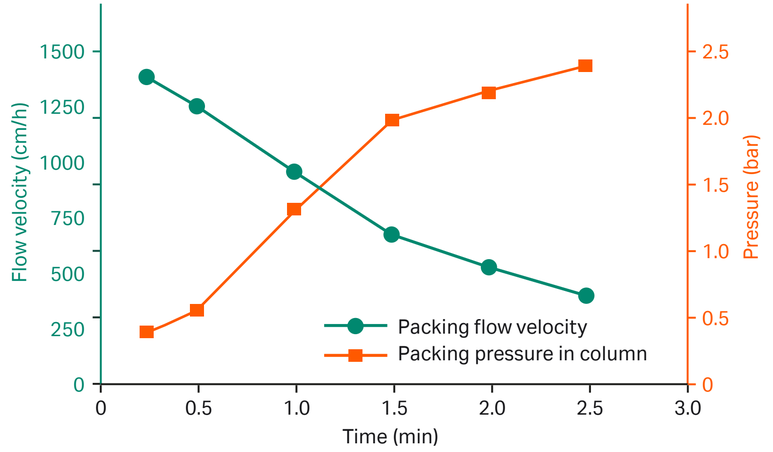


Fig 1.21. Column pressure and flow velocity during packing of a 20 cm bed height in Chromaflow 600 using Chromaflow packing station pack 100.

We also ran efficiency tests at different flow velocities—Figure 1.22 shows a van Deemter plot of the results. The asymmetry factor is stable at the different flow velocities. The reduced plate height increases with the flow velocity and optimal result in this study is achieved at 10 to 40 cm/h. When running at higher flow velocities, the asymmetry factor and reduced plate height continue to behave linearly, indicating that the efficiency test can be run at an increased flow velocity without significantly changing the results.

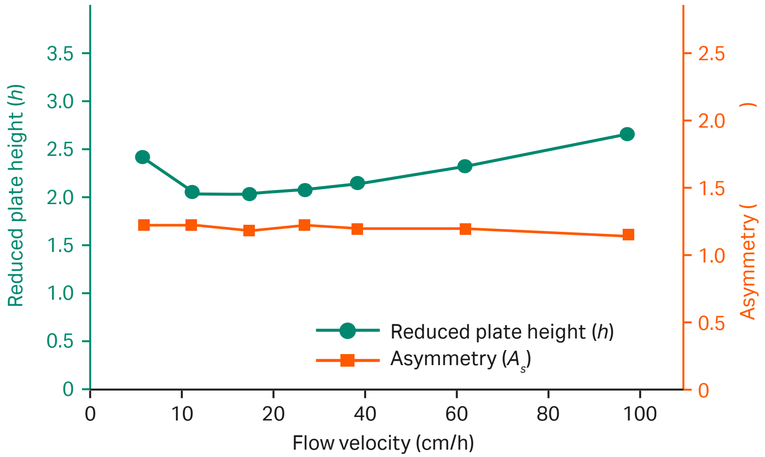


Fig 1.22. Reduced plate height and asymmetry values at different flow velocities for Capto S ImpAct packed to a bed height of 20 cm in a Chromaflow 600 column (*CF* = 1.10). These values should be regarded as examples. However, similar behavior can be expected for packed beds in other columns such as AxiChrom and BPG columns.

1.6 Packing Capto S, Q, and DEAE ion exchange (IEX) resins in large-scale columns using verified methods

Capto S, Capto Q, and Capto DEAE resins are strong cation, strong anion, and weak anion exchange chromatography resins, respectively designed for capture at high capacity. This section shows you how to pack these resins in your AxiChrom, BPG, or Chromaflow columns.

The equation to calculate *CF, PF,* and column volume is found in 1.2.7 Resin slurry concentration determination.

Properties of Capto S, Q, and DEAE resins in various packing solutions

Capto IEX resins settle quickly in both water and in 20% ethanol. When using these solutions, remember that tubing and nozzles must be rinsed directly after packing to prevent clogging of the flow path. Adding salt to packing solutions slows the settling of the resin beads and allows them to settle less tightly. Consequently, it is difficult to measure slurry concentration by gravity with salt-based solutions. However, the slurry concentration method described below allows quick and accurate determination of slurry concentration.

When Capto S, Capto Q, and Capto DEAE resins are settled at 30 to 60 cm/h, the consolidated bed height will be 5% to 15% higher in salt solution than in water or 20% ethanol. The effect is almost the same for 1 mM NaCl as for 0.5 M NaCl and can be compensated for by using different packing factors. Table 1.11 shows packing factors for water and a variety of other packing solutions.

Table 1.11. Typical packing factors for Capto S, Capto Q, and Capto DEAE resins in different solutions for optimal bed performance where the bed is consolidated at 30–60 cm/h

Solution	Capto Q	Capto S	Capto DEAE
Water	1.10	1.10	1.10
20% ethanol	1.10	1.10	1.10
10 mM NaCl	1.17	1.17	1.17
20% ethanol with 200 mM sodium acetate	N/A	1.20	N/A

Slurry preparation

When preparing the slurry, start by calculating the chromatography slurry volume (V) needed to pack the desired bed height. The slurry concentration can be determined in several ways but to get an accurate slurry concentration of Capto S resin in its storage solution or Capto IEX resins in salt-containing solutions, use the method described below. When calculating the slurry volume (*V*) use the compression factor in water/20% ethanol (*CF* = 1.07).

The slurry concentration determined by the method below corresponds to the gravity-settled concentration in water.

Chromatography slurry volume, $V = \frac{A_c \times L_{packed} \times CF}{C_{slurry}}$

where

A_c = cross-sectional area of the column (cm^2)
C_{slurry} = concentration of the slurry

Capto Q and Capto DEAE resins are supplied in 20% ethanol and Capto S in 20% ethanol with 200 mM sodium acetate. Before packing, transfer these resins to the packing solution as described in the packing instructions for the relevant column.

1.6.1 Packing of Capto IEX resins in AxiChrom columns

When packing AxiChrom 50 to 200 columns for use with ÄKTA chromatography systems, Intelligent packing control is managed by the UNICORN control software. For AxiChrom 300 to 1600 columns, Intelligent Packing is performed by AxiChrom column controller, or from the UNICORN software on ÄKTA process system.

The general considerations for packing MabSelect PrismA chromatography resin by Intelligent packing in AxiChrom applies also to packing of Capto IEX resins, *see* 1.4 Packing MabSelect PrismA chromatography resin in large-scale columns using verified methods

1.6.2 AxiChrom packing results

You can see examples of efficiency and stability results for Capto S, Capto Q, and Capto DEAE resins packed in AxiChrom columns in Table 1.12. The results for Capto S resin in column sizes ranging from 50 to 1600 mm are similar. Similar results are also seen for Capto Q and Capto DEAE resin in AxiChrom 400 and 600 columns. Different bed heights also give comparable result (*see* Capto Q resin in Table 1.12), showing that the verified packing methods available in Intelligent packing in the UNICORN wizard give you similar results independent of column size and bed height. The stability test showed that the beds were stable when running in water at the flow velocities given in Table 1.12.

Table 1.12. Column efficiency data for different packings of Capto S, Capto Q, and Capto DEAE resins in different AxiChrom columns

Resin	AxiChrom column	Bed height (cm)	Average plates/m*	Reduced plates height (*h*) range*	Asymmetry factor (A_s) range*	Flow velocity for stability test (cm/h)†	Change after stability test (%)†	
							h	A_s
Capto S	50	20	6900	1.4–1.5	1.1–1.2	700	1	2
Capto S	300	20	7200	1.5	1.0–1.1	700	-4	4
Capto S	600	20	6600	1.7	1.2	700	6	-1
Capto S	1000	20	6700	1.5–1.7	1.1–1.2	700	4	6
Capto S	1600	20	5100	2.0–2.5	1.2–1.4	700	-3	-5
Capto Q	400	20	7400	1.4–1.6	1.1–1.2	700	2	6
Capto Q	400	40	7300	1.4–1.6	1.1–1.2	450	5	2
Capto DEAE	600	20	7200	1.4–1.7	1.1–1.2	700	4	4

* Test performed at optimal test conditions. Average and ranges of up-flow and down-flow tests for at least three packings.

† Stability tests were run once for each bed height/resin/column combination in water for 16 h at given velocity.

Pressure-flow curves provide a simple, effective illustration of column performance in terms of the maximum operating velocity at which the purification process can be run. AxiChrom columns can utilize the full liquid velocity of Capto resins, even at large column diameters (Fig 1.23).

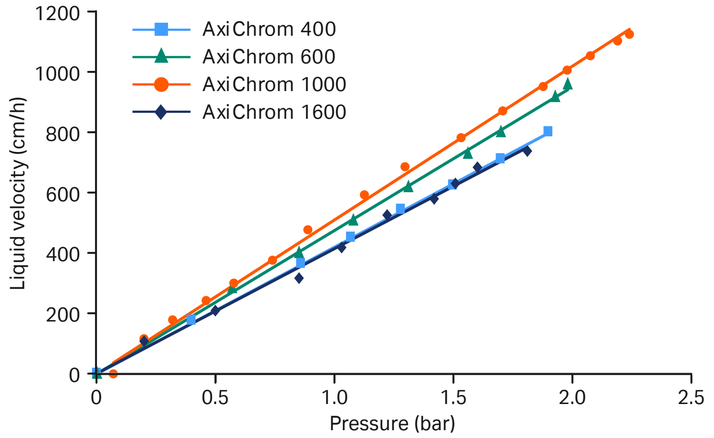


Fig 1.23. Pressure-flow curve in water at 20°C for Capto S resin, 20 cm packed bed height, in different AxiChrom columns. Capto resins can be run at 700 cm/h with a back pressure of less than 3 bar (43.5 psi, 0.3 MPa). System/tubing pressure is excluded.

1.6.3 Packing of Capto IEX resins in BPG columns

Capto S, Capto Q, and Capto DEAE resins can be packed in BPG 100 to 300 columns. BPG 450 column is, however, not recommended for Capto IEX resins because of its low-pressure limit. Capto S, Capto Q, and Capto DEAE resins are packed with 10 mM NaCl in BPG 100 column and with water in BPG 300 column. Because of the increased influence of the wall support, salt solution is required in columns with small diameters. The PF varies with the choice of packing solution and therefore the PF will be 1.10 in water for BPG 300 and 1.17 in 10 mM NaCl for BPG 100.

Equilibration is critical for Capto S resin in BPG 300 column as the delivery solution contains salt, and the recommended packing solution is water. Measure the conductivity of the solution leaving the column during the equilibration and equilibrate until the conductivity is zero.

Column and system preparation

A detailed description of column preparation is available in the BPG operating instructions (29193223). The packing pump should be as pulsation-free as possible. Screw and rotary lobe pumps are the most suitable for this task but multiheaded diaphragm pumps can also be used.

1. Place a new 23 µm net on both adapter and bottom end piece.
2. Level the column with the aid of a spirit level.
3. A pressure relief valve should be used for protection, especially against pressure spikes. Position this valve on the pump outlet and mount a pressure gauge on the adapter.
4. Mount one 4-port, 2-way valve on the bottom inlet and one on top of the pressure gauge, 10 mm i.d. for BPG 300 columns and 6 mm i.d. for BPG 100 columns.

Packing

1. Set the pressure alarm or pressure relief valve according to the pressure rating of the column. Purge the system and tubing of air.
2. Purge the end-piece net of trapped air by draining some packing solution through the column outlet. Leave about 2 cm of solution in the column and close the bottom valve. If air is trapped under the end-piece net, add more packing solution and connect a tubing to the suction side of a pump. Start the pump and place the tubing on the bottom net and extract any remaining air.
3. Add the slurry to the column and, if needed, additional packing solution to about 40 cm (if a 20 cm bed is packed). Mix the resin and the packing solution to a homogeneous slurry.

   ☞ The available height for the adapter to be inserted into a 50 cm column tube (for filling slurry) is only 40 cm. Use a longer column tube or packing extension tube when packing beds higher than 20 cm: 75 cm tubes are available.
4. Rinse the wall from particles and let the resin settle until there is about 1 cm of clear liquid on top of the slurry. This reduces the risk of particles sticking between the O-ring and the column wall, which can cause column leakage.
5. Insert the adapter and secure it to the column top flange. Lower the adapter to the surface of the slurry and allow some clear liquid to pass the O-ring. Tighten the adapter O-ring.
6. Make sure the column top valve is open. Slowly move the adapter down until no air bubbles can be seen leaving the top valve.
7. Start the pump and adjust the settling velocity to 30 cm/h (2.3 L/h for BPG 100 and 21 L/h for BPG 300 column). Shift the top valve into the column and immediately open the bottom valve and lead the liquid to waste.
8. Run the settling flow until the bed is completely consolidated. Note the consolidated bed height and calculate the packed bed height using *PF* = 1.17 in 10 mM NaCl for BPG 100 and *PF* = 1.10 in water for BPG 300 column. The packed bed height is the ratio between the consolidated bed height and the *PF*. Use a marker pen to indicate the packed bed height on the column.
9. Stop the flow and close the bottom valve. Loosen the O-ring and lower the adapter down to 1 cm above the settled bed and seal the adapter O-ring. Shift the top valve to waste and use the adapter to mechanically compress the bed to the mark on the column (step 8). Excessive packing solution is removed through the adapter tube.

   ☞ Compressing Capto resins in BPG columns, especially the larger BPG 300 column, is physically demanding. Do not use extension rods on the adapter height adjuster to compress the resin. To increase the performance and stability of the bed, flow condition the column downwards for 5 CV and upward for 5 CV at 3 bar (43.5 psi, 0.3 MPa), or at the maximum flow if 3 bar cannot be achieved. This can be performed with water or packing solution.
10. Connect the pump to the top of the column. Purge the system and tubing by running the mobile phase to waste by bypassing the column inlet with the 4-port valve. Start at a low flow velocity (approximately 100 cm/h).
11. Shift the top valve to direct the flow to the column and immediately open the bottom mobile phase to waste or connect it to the buffer tank for recirculation.
12. Increase the flow until a pressure of 3 bar is reached or to the maximum flow/pressure if 3 bar cannot be achieved. Run the column at this pressure for 5 CV.
13. Slowly decrease the flow for 2 min to avoid disturbance of the conditioned bed.
14. Exchange the mobile phase connections. Connect the tubing from the pump to the bottom valve and open the top valve to waste or to the buffer tank for recirculation.

15 Repeat steps 10 through 13 with upward flow to complete the conditioning procedure.

16. Test the packing at the optimal test velocity (20 to 30 cm/h).

1.6.4 BPG column packing results

Examples of efficiency and stability results for Capto S, Capto Q, and Capto DEAE resins packed in BPG 100 and BPG 300 columns are shown in Table 1.13. The results for Capto S, Capto Q, and Capto DEAE resins in BPG 300 column at 20 cm bed height are very similar.

Generally, the packing method works well for the three resins in different sizes of BPG columns at different bed heights. The stability test showed that the bed was stable in water at velocities given in Table 1.13.

Table 1.13. Column efficiency data for different packings of Capto S, Capto Q, and Capto DEAE resins in BPG 100 and BPG 300 columns

Resin	BPG column	Bed height (cm)	Average plates/m*	Reduced plate height (*h*) range*	Asymmetry factor (A_s) range*	Flow velocity for stability test (cm/h)†	Change after stability test (%)†	
							h	A_s
Capto S	100	20	5300	1.6–2.5	1.2–1.4	700	3	6
Capto S	300	20	6000	1.6–1.9	1.1–1.2	700	6	1
Capto S	300	30	6000	1.4–2.4	1.1–1.5	500	3	6
Capto Q	300	20	7000	1.4–1.6	1.1–1.1	700	2	1
Capto DEAE	300	20	6100	1.5–2.0	1.1–1.2	700	5	4

* Test performed at optimal test conditions. Average and ranges of up-flow and down-flow tests for at least three packings.

† Stability tests were run once for each bed height/resin/column combination in water for 16 h at given velocity.

The pressure-flow curves for 20 cm bed height in BPG 100 column are shown in Figure 1.24. These curves show linear behavior and the three resins have the same pressure-flow properties. The pressure flow profile for BPG 100 column shows that the resin can be run at 700 cm/h with a back pressure of less than 3 bar (43.5 psi, 0.3 MPa).

1.6.5 Packing Capto IEX resins in Chromaflow columns

To achieve the highest *CF*, a low initial packing flow rate is used, as the particles settle more tightly at a lower flow rate. When the bed is a few centimeters from the adapter screen, the packing flow rate is increased to the maximum flow the recommended packing station can produce without reaching the column pressure limit.

For Capto DEAE, Q, and S IEX resins, beds of 20 cm and above work well in Chromaflow columns. The extreme flow rates needed to efficiently pack a shorter bed cannot easily be achieved using standard equipment.

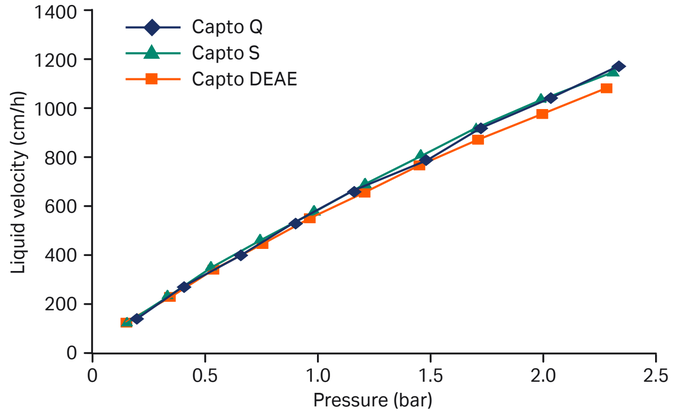


Fig 1.24. Pressure-flow curve in water at 20°C for Capto Q, Capto S, and Capto DEAE resins, 20 cm packed bed height, in BPG 100 column. The three resins have nearly the same pressure-flow properties. System/tubing pressure is excluded.

The packing method described is for a Chromaflow 600 column using a pack 100 packing station. The pack 100 flow capacity enables packing of Capto resins as described for operational flow velocities of up to 500 cm/h compared with when packing an AxiChrom column where 700 cm/h is achieved. For higher operational flow velocities, the larger pack 200 packing station is required.

With larger column diameters, higher flows are required from the packing station to achieve sufficient bed compression. A Chromaflow 800 column requires a pack 200 packing station or larger while a Chromaflow 1000 column requires a pack 400 and a specially designed column with a larger nozzle bore size to achieve the required packing flow rate. For Chromaflow columns larger than 1000, axial compression is needed, which requires a specially designed column. Contact your local Cytiva representative for advice.

Chromatography resin preparation

The recommendation for packing Chromaflow columns is to use the solution in which the resin is delivered or a decanted solution, as 10% to 20% ethanol in the slurry gives a good packing result. If the delivery solution is decanted, then replace it with water.

You can avoid trapping air bubbles trapped in the column when packing, additional slurry is required for the extra volumes in tank and tubing. Add the slurry to the slurry tank and stir the resin. Dilute the suspension to about 50% slurry concentration.

Column and system preparation

For more detailed description about the column and pack station preparation, *see* Chromaflow columns instructions for use and Chromaflow packing stations instructions for use.

It is important that the supply air flow rate follows the specification of the Chromaflow packing station (1000 L/min for Pack 100 packing station) and that the supply air pressure into the packing station is 6 to 7 bar (87.0 psi, 0.6 to 0.7 MPa).

1. Set up the column according to the Chromaflow columns instructions for use.
2. A pressure relief valve (adjusted to the operating pressure limit of the column) should be used for safety reasons. Position this on the slurry inlet top (SIT), with the waste tubing connected to the slurry tank. Place a pressure gauge on the mobile phase top (MPT) to record the pressure during packing. Mount one 3-port, 2-way valve on top of the pressure gauge and one on the mobile phase bottom (MPB). The top valve should lead in two directions: one side into the system and one to waste for purging the tubing. On the bottom valve, one side leads to the system and a 1.5″ to 2″ tubing leads to waste (for packing). Part of the MPB waste tubing should be placed above the outlet valve to prevent air from entering through the MPB.
3. Connect appropriate tubing (i.d. 1″ or 1.5″) and tanks to the column and packing station. If a flow meter is used, place it between the SIT and the packing station.
4. Level the adapter to the desired bed height. Remember to loosen the nuts on the adapter rods to allow the adapter to be raised or lowered. Flush the adapter rods with 20% ethanol as lubrication.
5. Prime the column, packing station, and tubing with water according to the Chromaflow operating instruction.

Packing

Packing Chromaflow columns is a rapid procedure compared with other packing procedures and it is therefore important to thoroughly read the packing instructions and go through the packing steps in advance of the packing.

1. Set both nozzles in run position to prime the tubing with slurry. Lead the slurry outlet top (SOT) tubing back to the slurry tank and secure it. Stir the slurry to keep it homogeneous, select slurry and SIT on the packing station, open the slurry tank, and start the packing pump.
2. The initial flow rate should be as low as possible while maintaining an even stroke rate. The pressure on the packing station should be 1.5 to 2 bar (21.8 to 29.0 psi, 0.15 to 0.2 MPa), which corresponds to the flow rates shown in Table 1.14.
3. When the tubing is primed and the flow rate set, set the SIT/slurry inlet bottom (SIB) to the position between SIT and SIB to block the flow during step 4 while maintaining the correct flow rate for the next step.
4. Move the top nozzle down into the packing position.
5. Two operators should simultaneously open the bottom mobile phase valve to waste and turn the SIT/SIB valve to SIT on the packing station. The column then starts to fill with slurry and the bed builds up slowly from the bottom as excess liquid exits via the MPB.
6. When the bed has reached the Lx (Table 1.14), increase the packing flow quickly to the compression step pressure (on packing station).

   Column pressure must not exceed the operating pressure limit of the column (i.e., 3 bar [43.5 psi, 0.3 MPa]). If this pressure is reached, gently decrease the packing flow so that the pressure remains just below 3 bar. Typically, the final pressure in the column is 2 bar (29.0 psi, 0.2 MPa) to 3 bar depending on the viscosity of the packing solution, column diameter, bed height, and so forth.

Table 1.14. Parameters for packing of Capto S, Capto Q, and Capto DEAE IEX resins in Chromaflow 600 using Pack 100 packing station

	Packed bed height (cm)	
	20	**30**
Initial step		
Flow rate (L/min)	20	20
Pack station pressure (bar)	1.5 to 2.0	1.5 to 2.0
Lx (cm)*	16	25
Compression step		
Flow (L/min)	700	50
Pack station pressure (bar)	6.0 (87 psi, 0.6 MPa [max.])	4.5 (65.5 psi, 0.45 MPa)

* Bed height at which the flow should be increased.

If a nontransparent column tube is used, increase the packing flow when about 75% of the calculated volume of slurry has been introduced and stop the packing when all calculated volume of slurry is introduced into the column. Check the volume in the slurry tank or use a volume totalizator.

7. Stop the packing pump when the bed is 1 to 2 mm from the top bed support by setting the SIT/SIB to the position between SIT and SIB, as described in step 3. Once the flow is stopped, the bed will expand to meet the adapter.
8. Immediately move the top nozzle back to the run position.
9. Close the MPB valve when the pressure in the column is between 0.3 and 0.1 bar (between 4.4 psi, 0.03 MPa and 1.45 psi, 0.01 MPa).
10. Use packing solution to rinse residual resin from the tubing and the top nozzle. Pump the packing solution through the top nozzle back into the slurry tank.
11. Close the slurry tank and empty the tubing between the tank and packing station.
12. Pump liquid upflow through the column until the air is expelled.

1.6.6 Chromaflow column packing results

Column efficiency results for Capto S, Capto Q, and Capto DEAE resins packed in Chromaflow 600 column are shown in Table 1.15. Both 20 and 30 cm bed heights give good plate numbers and asymmetry factors. In addition, the stability test shows that the bed is stable when running in water at the velocities given in the table.

Table 1.15. Column efficiency data for different packings of Capto S, Capto Q, and Capto DEAE resins in Chromaflow columns

Medium	Chromaflow column	Bed height (cm)	Average plates/m*	Reduced plates height (*h*) range*	Asymmetry factor (A_s) range*	Flow velocity for stability test (cm/h)†	Change after stability test (%)† *h*	Change after stability test (%)† A_s
Capto S	600	30	4600	2.0–2.4	1.0–1.3	500	1	5
Capto Q	600	20	6800	1.4–1.9	1.1–1.4	500	1	9
Capto DEAE	600	20	5600	1.3–2.6	1.0–1.5	500	6	5

* Test performed at optimal test conditions. Average and ranges of up-flow and down-flow tests for at least three packings.

† Stability tests were run once for each bed height/resin/column combination in water for 16 h at given velocity.

The pressure-flow curve is shown in Figure 1.25. As the optimal compression factor is difficult to achieve in standard pack-in-place columns, the maximum flow velocity that can be run through the packed bed is limited. The highest operating velocity recommended for this type of column is 500 cm/h compared with AxiChrom columns, where 700 cm/h could be used for a 20 cm bed. Note that bed efficiency and bed stability are very good, provided this 500 cm/h guideline is met.

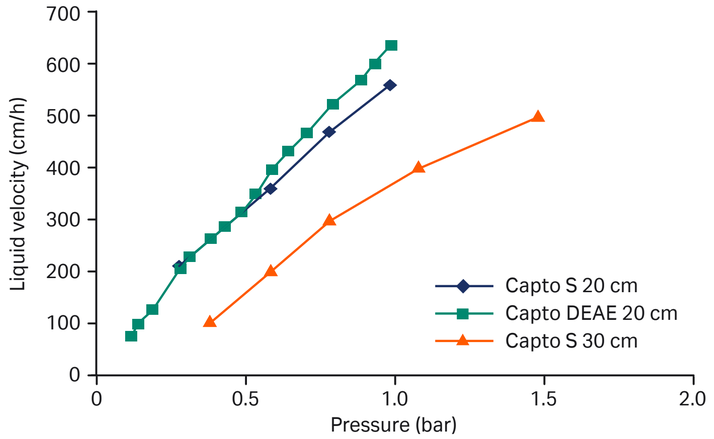


Fig 1.25. Pressure-flow curve in water at 20°C for Capto S and Capto DEAE, 20 cm packed bed height and Capto S 30 cm packed bed height in Chromaflow 600 column. As the optimal compression factor is difficult to achieve in standard pack-in-place columns, the maximum flow velocity that can be run through the packed bed is limited. The highest operating flow velocity recommended for this column is 500 cm/h compared with in AxiChrom columns where 700 cm/h could be used for a 20 cm bed height.

1.7 Post-packing considerations

1.7.1 Bed relaxation

The bed is often non-homogeneously compressed, both axially and radially, immediately after packing. The structure and the nature of the variations depend on what type of column it is and how it has been packed.

Compression gradients in the bed created during packing must reach equilibrium before the final properties of the gradients are set and steady.

Wait at least 15 min before starting the HETP test on the bed after packing. Some rigid resins such as Capto chromatography resins often require longer than 15 min.

Flowing the bed at a low flow velocity, for example 30 cm/h, can usually be considered as letting the bed relax. Therefore, the equilibration phase of the HETP test can usually be counted into these 15 min, if the bed is equilibrated at these flow velocities.

1.7.2 De-aeration, equilibration

Directly after packing, running the column in upflow mode is recommended for most packing techniques to purge any trapped air in the top of the column. This is usually done as the first step in column equilibration for the HETP test. First, run a small volume upflow to purge air and then run in the direction most commonly used (downflow mode) to equilibrate the column for the HETP test.

1.7.3 Extra-mechanical compression

This can be a useful tool if the test results indicate that the bed is under-packed or if it is known that the target compression was not achieved. Adding a few extra percent compression normally works well. Be careful not to damage the threads on rods and screws, since a lot of force is applied, especially on large columns. It is not suitable for Chromaflow columns, for example, but sometimes still required though.

Observe that this methodology can be used for the initial packing settings. It is quite common for short and wide beds to need extra high compression for optimal performance since it increases the pressure drop and thereby improves the liquid distribution. Therefore, when packing beds in very large columns having an aspect ratio (D/L) above 10, increase the packing or compression factor by 0.02 or 0.03 to improve performance.

1.7.4 Flow conditioning

This is a very useful tool to sometimes achieve better results after a pack. There are many examples where results improve drastically after this procedure. It may be possible to remove double peaks and many other defects. Flow conditioning means that liquid is run through the column and bed at a high enough rate to shift the structure of it. It also can help in removing air that remains after packing.

1. Normally, 3 CV should be run at a flow rate high enough to just cause a small head space in the column or to reach the pressure rating of the column or the resin.
2. Make sure that the flow rate is ramped both up and down during about 2 min to avoid exposing the bed to any shocks.
3. Best results are often achieved if flow conditioning is run in both directions, even if only one is sufficient in many cases.
4. If results seem to indicate that the top of the bed is more compressed than the bottom, downflow conditioning is often most useful as this shifts stresses downwards in the bed.

Depending on resin type, flow conditioning is usable due to the resin properties, rigidity, and particle size distribution.

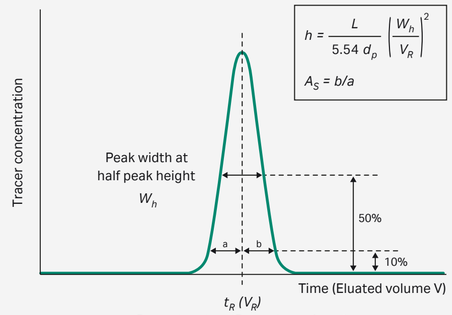
1.7.5 Column efficiency testing

Testing plays a central role in the qualification and monitoring of packed bed performance. Even though it cannot be used as a single parameter to predict purity and recovery, it is a quick way to test the column and equipment performance before starting the purification process. This test can also be used in-between runs to check for changes of the bed integrity.

Efficiency testing is the analysis of the residence time distribution for a tracer substance passing through the column. Typical test signals applied to the column are pulse or step signals.

To characterize the chromatography column without interference, tracer substance and eluent conditions are selected such that chemical interactions with the resin and disturbances of the fluid flow are avoided.

The most common type of test signal applied is a Pulse test. A small volume of a tracer substance is added to the liquid flow close to the column inlet and the broadening of this pulse is analyzed when measured as a chromatographic peak at the column outlet. A typical pulse response and its evaluation are summarized in Figure 1.26.



$$h = \frac{L}{5.54\ d_p}\left(\frac{W_h}{V_R}\right)^2$$

$$A_S = b/a$$

The relative peak width is defined as number of (theoretical) plates (N), height equivalent of a theoretical plate (HETP) or preferably as reduced plate height (h):

$$N = \frac{\mu_f^{\ 2}}{\sigma^2} \approx 5.54\left(\frac{V_R}{W_h}\right)^2$$

$$HETP = \frac{L}{N}$$

$$h = \frac{HETP}{d_p} = \frac{L}{d_p}\frac{\sigma^2}{\mu_1^{\ 2}} \approx \frac{L}{d_p}\frac{1}{5.54}\left(\frac{W_h}{V_R}\right)^2$$

The asymmetry factor, A_S, describes the deviation from an ideal Gaussian peak shape and is calculated from the peak width at 10% of peak height:

$$A_S = b/a$$

Definitions

Plate number	N
Mean residence time[1]	μ_1
Variance[1]	σ^2
Retention time[2]	t_R
Retention volume[2]	V_R
Peak width at half peak height	w_h
Bed height	L
Particle diameter	d_p
Height equivalent of a theoretical plate	$HETP$
Reduced plate height	$h = HETP/d_p$
Asymmetry factor	A_s

1 First and second moments in residence time distribution (RTD) curve derived from integration of tracer signal.

2 Retention time (retention volume) corresponding to time (or eluted volume) at maximum peak height.volume) at maximum peak height.

Fig 1.26. Overview of pulse test with calculations and definitions.

Column efficiency is typically defined in terms of two parameters:

- Peak broadening over the column is described by an equivalent number of theoretical plates (equilibrium stages).
- Peak symmetry is described by a peak asymmetry factor, A_s.

Specifications for qualification of optimal column efficiency may be defined as:

Reduced plate height, $h \leq 3$

Asymmetry factor, A_s $0.8 < A_s < 1.8$

These specifications rely on the use of optimized test conditions and parameters including:

- An inert tracer substance to avoid interaction with the resin.
- Optimal liquid velocity enabling highest theoretical efficiency
- Low external volume.
- A low sample volume in the pulse test (1% of V_c)

Specifications for column qualification as well as test procedures should always be reviewed regarding specific application needs as well as practical constraints that may not allow for application of optimal test conditions. The specification for reduced plate height and asymmetry may very well be different for different purification steps in the process. In addition to the efficiency test described in this application, additional parameters could be used to track the process (e.g., pressure of the bed and retention volume of the product).

Choosing a test method

In theory, the best column efficiency achievable in terms of reduced plate height is typically h = 1.5 to 2 when using porous chromatography resin used in bioprocess applications. This theoretical efficiency is derived from a van Deemter analysis and reflects the convective and diffusive dispersion effects within the packed bed under optimized test conditions (i.e., liquid velocity). Additional sources of peak broadening are caused by packed bed heterogeneity, imperfections in column fluid distribution, system effects, or sample volume. To set appropriate specifications for chromatography column testing (and also for troubleshooting purposes) you should control these individual sources of band broadening that define the baseline for the theoretical efficiency.

Test equipment

A packed column may be moved to a new location such that equipment used for packing need not be the same as that used for evaluation. A suitable liquid delivery system must be provided to operate at the required testing flow rate, which may be significantly less than that used for packing. A method for accurate sample delivery must be provided that minimizes dilution and distortion of the sample zone before it is applied to the column. A good rule of thumb is that V_{ext} of the test system should not be more than 0.1% of V_c to minimize dilution. The use of reducers and unnecessary hardware should also be avoided to preserve the sample zone shape. This requires a system allowing sample application as close to the column bed as possible (minimal tubing length and i.d.) with a brief or no interruption of the flow rate. A separate sample pump and 4-port, 4-way valve at the top of the column are often used for this purpose, but also optimizing sample application with the used ÄKTA process system, by priming the sample past the column valve optimizing a closed setup for packing and evaluation. This is the default testing setup for Intelligent packing for the AxiChrom 300 to 1600 columns. The monitoring flow cell should also be connected to the column exit with minimal tubing i.d. and length, and the flow cell volume chosen to avoid excessive dilution of the sample zone eluting from the column.

Test solutions

The test sample selected should not interact with the resin and should be small enough to have complete access to the interstitial pore volume, V_i. The sample must have physical properties which can be monitored such as conductivity for salt or UV for acetone. The choice of sample may also be limited if the column is for use at production scale. Salts such as sodium chloride (NaCl) are the most used test samples. Solutions of NaCl are inexpensive, easy to prepare and may already be approved for use in many purification processes. However, be aware that salt ions will interact with and be repulsed by the charged ligands immobilized on ion exchange resin when water is used as the mobile phase. This interaction alters the shape of the sample zone such that it may no longer reflect the quality of the packed column. This effect is also observed to a lesser extent with resin whose base matrix contains a small number of charged residues. Minimizing this artifact can be accomplished by the addition of 400 mM NaCl to the mobile phase and using a sample salt concentration of 800 mM. The salt in the mobile phase then “masks” the charged groups on the resin to the salt in the sample.

For the use of NaCl as a tracer, a minimum of 400 mM NaCl (conductivity of 35 to 40 mS/cm) in the eluent must be used to suppress charge interaction effects between the tracer and the chromatography resin (otherwise, you may observe misleading test results).

An example of the disturbance of peak symmetry when using a tracer substance that interacts with the resin is seen in Figure 1.27. The bed was tested with a sample containing 1% acetone and 1 M NaCl (sample volume 1% V_C). The sample concentrations were kept constant while the conductivity in the eluent was changed. The UV-pulse asymmetry was between 1.2 and 1.4 in all runs. The conductivity pulse was strongly affected by the mobile phase conductivity. If the conductivity of the eluent is too low, a leading peak will be observed.

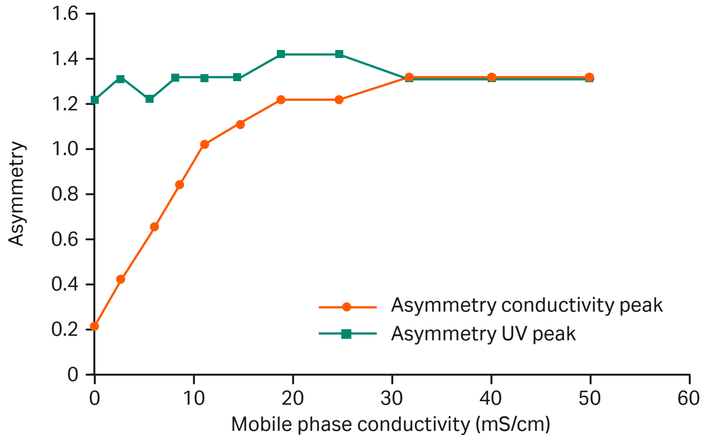


Fig 1.27. Effect of the mobile phase conductivity on the asymmetry of the conductivity peak in pulse evaluation of a packed bed of Sepharose XL resin.

Liquid velocity

Peak broadening and therefore chromatographic efficiency are strongly dependent on the liquid velocity applied during the test. The relationship between peak broadening and liquid velocity is described theoretically by the van Deemter equation (1):

$$HETP = A + \frac{B}{u} + C \times u$$

where

A is related to eddy dispersion

B is related to molecular diffusion

C is related to mass transfer resistance

u is liquid velocity (cm/h)

Testing equipment setup

You should try to minimize external column effects from unnecessary testing hardware when packing the column. However, test hardware may be preassembled for convenience if it does not interfere with the packing process. Desired packing flow rates may not be attainable due to pressure limitations from flow restriction caused by placing a small i.d. testing flow cell on the column outlet before packing. However, a testing flow cell with the same or larger i.d. than the outlet tubing would not produce this problem. In general, testing (or evaluation) of your column will require a suitable liquid delivery system capable of reasonably accurate delivery of the desired sample volume and testing flow rate. If you perform sample application and elution using the same pump, the setup should minimize dilution and distortion of the sample zone before application to the column. The setup requires that the volume of the flow path between the undiluted sample and the column bed should be made as small as practical using minimal tubing i.d. and lengths, avoiding the use of unnecessary or oversized valves, adapters, or gauges. If a separate pump is used for sample application, it should be connected directly to the top valve of the column so that sample can be introduced with minimal dilution with brief or no interruption of the mobile-phase pump.

The flow cell used to monitor the elution of the test sample should be connected near the bottom valve of the column exit using minimal tubing i.d. and length. The recommended operational flow rate range for the flow cell should be compatible with the testing flow rate to be used. More than one flow cell can be used, but be aware that changes in their relative position to each other and to the column will result in slight differences in relative peak positions. Equipment required for test data acquisition should be positioned for accessibility and necessary cable connections, as well as minimal exposure to spills and splashing.

Using AxiChrom columns with Intelligent packing, these considerations have already been considered for, optimizing the testing methods and system connections to give good results with the column packing connections.

When a suitable optimized setup for efficiency testing cannot be implemented, acceptance criteria must be adjusted accordingly. This could be the case, for example, when forced to run an efficiency test at low liquid velocity in a system designed for high throughput processing.

As a rule of thumb, a setup for efficiency testing of typical columns used in bioprocess chromatography should have an external volume smaller than 5% of the column volume and preferably less than 3% to achieve optimal column efficiency. We strongly recommend that you determine the external volume in the system before testing the bed efficiency. Also, a record of the retention volume in an efficiency test should be kept comparing retention volume to the nominal retention volume of the packed bed and column only. The retention volume for an inert tracer substance is calculated from the bed voidage εb intraparticle porosity ε_p, and size exclusion coefficient K_e (K_e = 1 for small molecule tracer substance):

$$V_R/V_c = \varepsilon_b + (1-\varepsilon_b)\,\varepsilon_p K_e$$

If the retention volume is significantly larger than expected, you should observe possible interactions between the tracer and the chromatography resin, as well as the holdup volume of the test system external to the column. When the test setup has been decided and a column has been packed, we recommend that you run three successive tests to measure the repeatability of the method.

Transition analysis

Instead of running a pulse test, a step function of tracer substance can be applied to the column. The resulting breakthrough curve can then be analyzed to derive column efficiency (Fig 1.28).

Transition analysis may be employed as a method for column qualification due to restrictions in available hardware. In addition, transition analysis can also be used as a tool for online column efficiency monitoring during processing (for example, when following step changes in conductivity). However, consideration should be given to parameters and prerequisites for robust column efficiency monitoring (as outlined above) such as tracer inertness, stability of fluid density and viscosity, and especially fluid velocity. Consequently, efficiency data obtained by online process monitoring typically differs from data obtained during initial column qualification by the pulse method.

The mathematical relationship between pulse function *E(t)* and the residence time distribution defined by a step function *F(t)* is

$$E(t) = \frac{dF(t)}{dt}$$

The differentiate function in UNICORN evaluation software can be utilized for this operation. As an example, the pulse function *E(t)* has been plotted below for the step function shown. In a second step, the calculated pulse function can be analyzed.

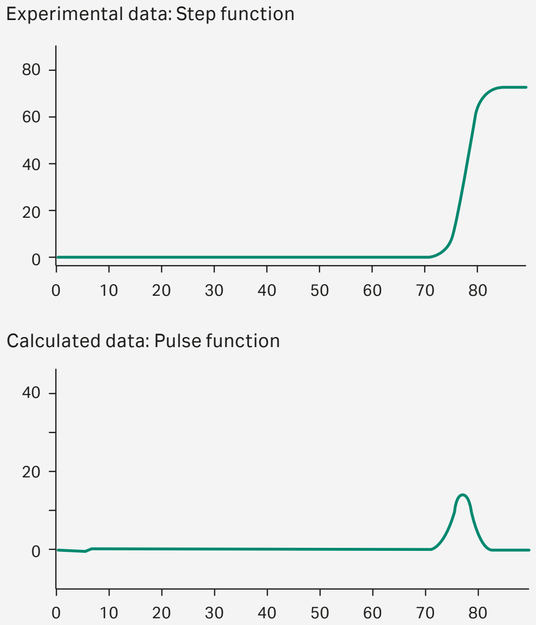


Fig 1.28. Evaluation of column efficiency for a step function obtained in transition analysis.

1.7.6 Column efficiency evaluation, when and how often?

Evaluation of bed performance is required to ensure the packed column performance. You should perform the evaluation before any processing to ensure results from a well performing bed. An evaluation might also be performed before storage or unpacking to evaluate the beds stable performance over time. We recommend that you perform regular bed evaluation in between process runs with pulse test or in-process measurements of transitions occurring in the process. This gives a better understanding of the bed behavior throughout the campaign and for long-term use.

1.7.7 Bed stability evaluation

Using recommended or verified packing methods for a certain combination of resin and resin or column combination will give you a robust bed that can repeatedly show performance for long purification campaigns. However, you cannot foresee all different velocities, times, buffers (viscosity), or flow-direction changes, hence more information of what the packed bed can tolerate is important to evaluate.

The simplest way of stability testing is running/recirculating water or equilibration buffer at the highest process flow rate or approximately 30% above for 2 to 16 h followed by a bed performance evaluation to ensure long-term bed stability. Upflow and downflow can also be performed to further stress the bed.

An alternative, but more costly approach, is to perform mock runs to simulate the complete process with all buffers, valve switches, flow direction changes, and CIP, but without sample application. This method simulates a real run but without protein sample that might be a complex mixture to evaluate bed behavior for.

1.7.8 Sanitization

Sanitization in place (SIP) is important for robust long-term purification campaigns. Bioburden events and investigations are costly and should be prevented by handling of the equipment, column, resin, and buffers in a preventive way. More on cleaning and sanitization in Chapter 3.

1.7.9 Storage

A packed column might be stored for some time until taken into production. Check the chemical resistance of each resin before choosing a storing solution. There are generally two solutions that most columns and resin can be stored in, the most common being 20% ethanol. All resins, except those with S or SP ligands, can be stored in 20% ethanol in bulk form or in packed beds. Resins with S and SP ligands should be stored in 20% ethanol + 200 mM sodium acetate to avoid autocatalysis of the base matrix. More on storage in Chapter 7.

1.7.10 Column unpacking

Unpacking most columns is quite easy, but at large scale the resin and buffer volumes makes manual unpacking difficult. Chromaflow columns are unpacked using the packing station. AxiChrom columns are unpacked with a relaxation and flush-out method controlled by the Intelligent packing approach. In BPG columns, the adapter is manually moved to the top and liquid is added to form slurry.

All columns can be unpacked to approximately 50% slurry, which is optimal for repacking. The key is to use only a fraction of 1 CV to create the slurry, the rest needs to be used for flushing. In most cases, use 0.7 CV to break up the bed or form the slurry and 0.3 CV to flush and clean. More detailed unpacking methods are described in each column's user manual. Unpacking can in most cases be carried out using any buffer. We recommend that you first equilibrate the resin in the storage buffer and then add more of the same buffer to create the slurry.

1.8 Troubleshooting your column packing

Troubleshooting your column packing might be required from time to time. Hopefully the problem with the column is identified before processing, which will negate having to investigate lot differences. Issues arising during processing can be bed instability and performance deterioration, head space formation or increased process pressure by resin and bed support clogging by process precipitate or resin fines.

This section gives you a brief overview of what to investigate if your column underperforms or fails entirely.

Common troubleshooting areas for column packing

Investigating column performance issues might require looking into several different aspects of the column packing that can affect the result. Start by asking yourself if anything has been performed differently since the last packing.

What can affect your packing results?

- Test method
- Packing method
- Preparation and status of the hardware
- Preparation and status of the resins
- Air

The potential cause of the problem you are having with your packed column is described in Table 1.16, which provides the starting point for a root-cause investigation.

Table 1.16. Common causes of problems with packed columns and suggested areas for investigation

	Cause of problem				
Problem	**Test method**	**Packing method**	**HW condition**	**Resin condition**	**Air**
Deviation in bed height		×		×	
High pressure		×	×	×	
Efficiency or asymmetry not approved	×	×	×	×	×
Stability - Performance changes/deteriorate from run to run		×	×	×	×
Process run deviations		×	×	×	×

Column packing specific parameters to investigate are:

- Axial compression range and velocity.
- Flow/pressure, level, and evenness.
- Hydraulic pressure, level, and evenness.
- Slurry concentration, accuracy, and concentration.
- Packing solution, viscosity/temperature.

Column hardware and accessories to investigate are:

- Column not leveled properly.
- Clogged bed supports.
- Calibrated and controlled accessories.
- Column pressure integrity.
- Pressure relief valve flow meter, pressure gauge functionality.
- Suitable dimensions.
- Tubing i.d. and lengths.
- Bed aspect ratio—high thin bed or low wide.
- Pressure after column.
- System external volumes.

Resin troubleshooting:

- Inaccuracy in determination of slurry conc. or slurry volume.
- Fouled resin.
- Broken beads, fines.
- Unhomogeneous slurry and/or packing solution.

Bed instability:

- Column packed too loosely or too hard.
- Packed bed exposed to excessively high flow velocity.
- Insufficient CIP method.
- Clogged bed supports.
- Column packed with excessively high slurry concentration.

1.9 References

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02

Column changeover

This chapter describes areas and procedures for column handling and column changeover for bioburdencontrol. Here, you will find out how to avoid product cross-contamination by applyingestablished routines and procedures to prepare your column for packing and new process runs.

2.1 Column changeover considerations

Column changeover is the process of preparing a chromatography production column for a new purification campaign. The column changeover process includes controlled handling, different stages of cleaning, sanitization, and sometimes also maintenance activities to prevent contamination of a drug product. Column changeover is a preventive procedure performed at scheduled intervals depending on the column manufacturer recommendations and process demands. It may involve changing wetted components at certain intervals. The rationale for changeover is mainly to prevent elastic polymer (elastomer) breakdown, but also includes maintaining equipment, cleaning, and removal or replacement of all product contact surfaces. Regular changeover procedures prevent carryover but, most importantly, also ensure that the purification processing will continue to work with a lower risk of bioburden, fouling, or clogging of the bed supports or resins. A bioburden incident can lead to a lengthy and costly stop in production, followed by a quality assurance investigation to find the root cause. This is costly and can have a significant commercial impact.

According to the ISPE glossary, (product) changeover is defined as “The program by which a processing area is cleared of supplies and components used in the manufacture of a previous product and then readied for production of a new product. This often includes parts change over and/or special cleaning to eliminate cross-contamination.”

Most procedures require validation in commercial manufacturing to provide bioburden/contamination-free handling: This includes several steps to ensure that the required level of cleanliness needed for the next purification campaign is achieved.

The steps in column product changeover, cleaning, and sanitization can include, but are not limited to the following. Validation is required for the steps indicated by an asterisk (*);

- Decontamination of resin and column from the previous campaign.
- Unpacking of the resin from the column.
- Disassembly and change of wetted components.
- Empty column cleaning and parts cleaning out of place (COP)*.
- Pre-treatment of wetted components.
- Mounting of wetted components.
- Column assembly and integrity test.
- Empty column cleaning-in-place (CIP).
- Column packing and packed bed evaluation.
- Sanitization in place and/or CIP before column transfer to production/sign off for reclassification of production area*.

2.2 Vendor contribution

Establishing changeover procedures and practices minimizes cross-contamination between successive products and prevents microbiological contamination and malfunction.

Cytiva columns are designed to support changeover procedures by:

- Clearly identifying process wetted components in supporting documentation.
- Use of sanitary design features to make cleaning simple and to facilitate sanitization for bioburden control, as is the case for AxiChrom large-scale chromatography columns (Fig 2.1).
- Preventing airborne and mechanical transfer by equipment training and designing closed systems for resin handling and column packing.

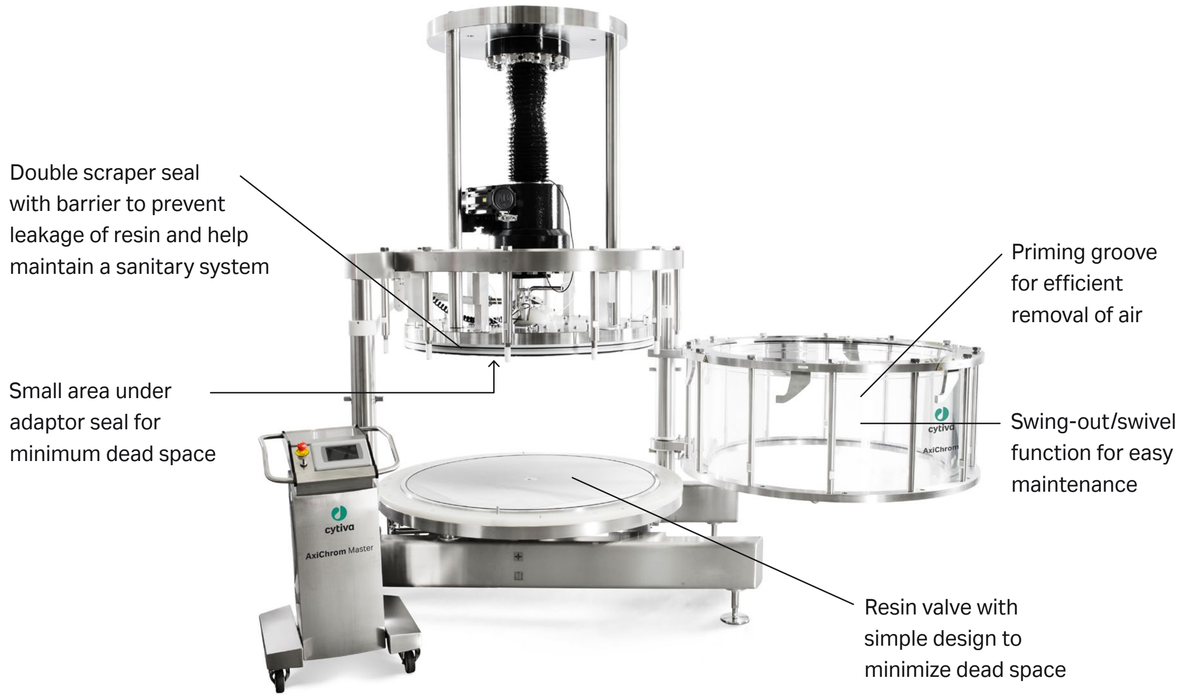

Fig 2.1. The sanitary design of AxiChrom columns minimizes the risk of bioburden event.

2.3 Demands from regulatory authorities

Regulatory authorities set high standards regarding bioburden control due to the potential impact on public health (1). Current good manufacturing practice (cGMP) requires defined cleaning, maintenance, and sanitization intervals for prevention of contamination of the active pharmaceutical ingredient (API)/ intermediate or malfunctioning equipment. The potential impact of a bioburden event can be very costly so minimizing this risk saves money and reduces the risk on the finished pharmaceutical product. Following cGMP and setting your own "best practice standards" improves process security and API quality.

☞ GMP guidance is provided in the US FDA Code of Federal Regulations, (CFR) 211.67 (2): "(a) Equipment and utensils shall be cleaned, maintained, and, as appropriate for the nature of the drug, sanitized and/or sterilized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements."(2).

☞ "Validation of cleaning procedures should reflect actual equipment usage patterns. If various APIs or intermediates are manufactured in the same equipment and the equipment is cleaned by the same process, a representative intermediate or API can be selected for cleaning validation. This selection should be based on the solubility and difficulty of cleaning and the calculation of residue limits based on potency, toxicity, and stability" (3).

Some guidance warns against using the same equipment for highly potent APIs. Dedicated equipment or disposables can be required to be used for, for example, the production of erythropoietin (EPO), as it can be hard to show that no carryover can occur (3). A cleaning risk assessment based on the actual product or a representative API using scientific data based on validated assays should be performed to generate and justify the acceptance criteria for the cleaning/part exchange/disposable usage.

The guidance on cleaning depends not only on the used equipment but also on the facilities in which the biopharmaceutical is produced. Adequate space to prevent mix-up of parts is required and adequate written procedures and controlled flow of materials and persons to prevent contamination must be documented.

Regional demands set the standard on how to handle equipment in a clean-room environment for drug production. Each region in which a biopharmaceutical is sold might have different demands for biopharmaceutical production approval and therefore different criteria might apply to the production and changeover procedures. However, the International Committee for Harmonization (ICH) and the Pharmaceutical Inspection Co-operation Scheme (PIC/S) regard cGMPs and best practices as region-free, which enables the use of similar best practices in several different regions.

You should consider the impact of your processes and cleaning on the equipment to help decide which recommendations to follow and at what frequency.

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03

Column life-cycle scenarios

The column lifecycle starts with delivery from the manufacturer and ends with decommissioning from production use. In between these two milestones, moves between different classified areas, and changeovers between different purification processes are made. Prior to these steps, the column is uncrated, installed, and commissioned. At the end of commissioning, cleaning is performed before turnover for GMP applications. Stages which involve product contact may be classified as GMP critical and include packing with used resin and processing. With the correct maintenance procedures, a column can be used for multiple products over a long lifespan.

3.1 New columns

When incorporating a new column in your process, an established procedure for cleaning can smooth the transition from the warehouse to your production or column packing suite. Cleaning of both internal and external surfaces is important for the removal of any particulates. Most columns are delivered either empty or filled with a sanitary solution, typically 20% ethanol.

External surfaces should be cleaned with 70% isopropanol or 70% ethanol. If your column is fitted with an acrylic tube, you will need to wipe excess solution away to avoid damage. The highest recommended concentration for longer contact with acrylic tubes is 20% for ethanol and 30% for isopropanol.

Alternative surface cleaning agents for acrylic materials are, as recommended in reference 1:

(a) Aqueous sodium hypochlorite (NaOCl) [CAS 7681-52-9]
(b) Aqueous hydrogen peroxide, 3% to 20% (H2O2) [CAS 7722-84-1]
(c) Aqueous chlorine dioxide, up to 2% (ClO2) [CAS 10049-04-4]
(d) Aqueous peracetic acid, up to 5% (CH3CO3H) [CAS 79-21-0]

Guidelines from the user manual for cleaning the integral parts of a new column should be followed. Further validation of the CIP procedure is the user's responsibility, but the guidelines contained in the user manual should be consulted as a baseline for these practices. Column design aspects might result in different cleaning protocols depending on movable adapters, valve design, and column configurations. The operating instructions for large-scale AxiChrom columns, for example, provide a list of recommended CIP solutions for the columns.

Compatibility of the chemical used with all materials it contacts must be evaluated.

3.1.1 Chemical compatibility: common mistakes

It is relatively common for acrylic tubes to haze and crack from mistreatment. The most common cause for this is the mix up of different solutions in the spray bottles and/or mixing up of their instructions for use. A good practice is to make sure you wipe off any excess sprayed on chemical from surface although this may be difficult for less-accessible column parts.

Chlorides can cause corrosion of stainless-steel (SS) column tubes and bed supports since they are in constant contact with the process fluids and requires regular rinsing to avoid long contact times with chlorides and chlorides in combination with low pH.

AxiChrom columns can be customized and configured for improved chemical resistance with higher-grade SS surfaces. Bed supports, connection tubing, and the column tube can be made from AL6XN, ASTM 32205 Duplex, or Hastelloy® on request. Standard SS components in contact with process fluids are made from 316L or ASTM 32205 Duplex.

If using long contact time of chlorides > 400 mM as well as chlorides > 400 mM in combination with low pH, ReadyToProcess™ prepacked, single-use columns or columns with polyethylene (PE) bed supports and polypropylene (PP) tubing should be considered to reduce the risk of roughing and corrosion.

If SS is required due to incompatibility of the process chemicals with for example, acrylic column tubes, a frequent passivation protocol can be implemented as preventive maintenance. Passivation treatment is described in detail, *see* Local passivation of bed support ring in 6.5.4 Bed supports. PE bed supports are also an excellent alternative to SS bed supports that are difficult to clean onsite.

3.2 Packed columns

A column already in the production suite would have gone through the exterior cleaning initially. Exterior cleaning/wiping should be performed at regular intervals to maintain exterior surface cleanliness.

Note: Wipe off excess of cleaning agent since depending on concentration and type of solution, it might damage the column material. The most common effects are cracking and hazing of acrylic column tubes subjected to higher concentrations of ethanol or isopropanol used for external surface cleaning.

3.3 Multipurpose columns

A multipurpose column is used for different molecule processes including different solutions and resins. To ready a column after finalization of a campaign, procedures to prepare this column for a new campaign need to be validated to prevent carryover from the previous processing solutions/molecules/resin to the new process. Risk assessments should justify the level of cleaning and/or change of critical components in contact with the process stream.

Following unpacking and decontamination, considerations need to be made before disassembling the column for elastomer/part exchange. The area where the column is handled, gowning of staff, treatment, and handling of spare parts are important for bioburden control and should be considered.

3.4 Single-product columns

A single product column is used with the same resin type and the same upstream feed material. While this is the most common scenario the risk of, and requirements for batch carryover still needs to be considered. Bioburden control is equally important regardless of the column used. Working area, gowning, and spare part handling are also critical when working with bioburden control.

3.5 Prepacked columns

To limit the amount of cleaning validation in downstream processing, a different column and system format can be used. A disposable prepacked column is delivered ready to use. The benefit with a prepacked column is that the user can remove multiple activities and by that save time. A prepacked column can be used for multiple cycles with CIP and sanitization between cycles using the same procedures as a conventional packed column. To make changeover easy, a prepacked column should not be used to purify multiple products.

☞ When performing changeover with prepacked columns, replace the prepacked column and flow path to remove risk for carryover from one process/campaign to another.

☞ ReadyToProcess columns are prepacked columns packed by validated methods for consistent performance. These columns fulfill industrial demands for security of supply, robust performance, and regulatory support. More information on ReadyToProcess columns can be found in on the solution pages on our website at cytivalifesciences.com.

3.6 Reference

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04

Cleaning and sanitization

Columns used in cGMP environments need to maintain very high standards of hygiene. Sanitization is required to ensure bioburden control. Cleaning out of place (COP) and in place (CIP) are required for robust long-term chromatography process steps.

4.1 What is clean?

The limit for what is clean depends on the actual API risk assessments and this depends on:

- Potency of the molecule
- Possible carryover
- Guidance by authorities

Both 21 CFR210/211 and the ICH Q7 regulations give guidance on cGMP. The ICH Q7 guidelines focus on cGMP for the API and provide you with guidance on manufacturing the API under an appropriate system for managing quality.

Cleaning and sanitizing are essential elements of any process to ensure that biological contamination is kept within acceptable limits. During a normal operation cycle, you may need to clean for the following reasons:

- Commissioning of a new column.
- Before and after servicing of the column.
- During normal process cycles with a packed column.
- Before storing a column with or without a packed bed.
- After storage in preparation for packing and running or storage of the packed column.
- Remediation after contamination.
- Before decommissioning and removal from the process environment.

4.2 Cleaning instructions

You should clean disassembled column parts with a validated procedure, which requires clear instructions with enough details to be performed correctly. Instructions need to include:

- How to clean — Wiping, or scrubbing, direction, order, and duration of cleaning, and tools used.
- Detergent — Type and concentration.
- Water — Type, temperature, amount.
- Hold times — Dirty and clean hold times.

The level of cleaning should have a numerical limit based on, but not limited to:

- Therapeutic dosage levels.
- Toxicity.
- Solubility of the residue (detergent).
- Batch size and nature of other products made using the same equipment.



Pay special attention to areas that can be harder to clean such as cramped spaces, O-ring grooves, cavities, and bed supports. It is hard to be sure that used elastomers have been sufficiently cleaned, so they are mostly replaced. Bed supports also require special attention due to their complex structure. *See* section 5.4.4 for more on bed support cleaning.

4.3 *In situ* cleaning methods

Assembled column hardware cleaning and packed bed cleaning should be included in the cleaning validation. Cleaning validation should be based on the biomolecule(s)' physiochemical properties, in-house procedures, and risk assessment. The established cleaning procedures should be determined from an investigation of many parameters relative to the effectiveness of the cleaning. Some of these parameters are listed here:

- Contact time
- Type of cleaning agent
- Concentration
- Procedures
- Material compatibility
- Analysis methods, e.g., total organic carbon, TOC
- Visual inspection
- Chemical clearance tests

As a starting point, use the recommendations from user manuals or sanitization application notes. Refer to the equipment's operating instruction or the resin regulatory support file (RSF) for recommendations on cleaning procedures/methods. Also, you should consider column hardware material chemical compatibility to CIP and sanitization solutions. To facilitate choice of chemical agents for cleaning, chemical compatibility data is available. For additional support, consult relevant documentation that demonstrates procedures for effective reduction of bioburden and endotoxins, as well as chemical clearance (1–5).

4.4 Challenging parts and equipment

Some column hardware parts are more complex in structure or have a limited chemical compatibility and might require some additional cleaning considerations.

O-ring cleaning can be done with soaking and wiping. The optimal soaking agent is determined in the risk assessment and can be 1 M NaOH for 1 h or alternatively, sprayed until dripping in 70% isopropanol and then wiped off with a lint-free cloth.

The cavities in which O-rings sit may be cleaned/brushed with a small soft brush. However, never brush O-rings due to risk for scratch creation for bacterial growth promotion.

Nozzles may be soaked in cleaning solutions after which the O-rings are remounted. Alternatively, nozzle tips with pre-mounted O-rings can be used, which reduces the risk of damaging the O-rings during assembly of the nozzle tip.

The SS bed supports can be cleaned with different methods and mostly require the use of a third-party supplier of a cleaning service. Use PE sintered bed supports to reduce the need for cleaning of the bed supports. PE sintered bed supports are disposable and exchanged instead of cleaned, removing the demand for cleaning validation. For handling of stainless-steel components and bed supports *see* also reference 6.

Each column has its own characteristics and details can be found in the respective column's manual and documentation or at cytiva.com. Important information can be pressure rating, materials of construction, chemical resistance, and spare parts lists. Each pilot-scale or production-scale column has a recommended sanitization study procedure available, which describes methods used to clear the column and packed bed from microbiological content (9–12).

Important considerations for empty column sanitization and packed bed sanitization include the complete removal of air bubbles and air pockets. A first step is to have all column parts cleaned in a sufficient manner and not have any different surface films or surface charges that lead to entrapment of air.

When packing the resin in the column, trapping air bubbles in the column should be avoided via the resin supply, or mobile phase tubing. Ensure priming of these process lines prior to packing. Air bubbles can be trapped or cause cracking in the resin bed, which elevates the risk of trapping contamination/bioburden in the air pockets.

Special attention is needed for the sintered polyethylene (PE) bed support for Chromaflow and AxiChrom columns. Follow the wetting procedure, *see* the operating instructions for AxiChrom columns to ensure proper wetting of the bed supports and removes the risk of trapping air in the bed support structure.

Column and bed support priming are crucial steps to perform to ensure a column free of air bubbles. Priming is performed differently depending on column type. The priming process is performed manually for Chromaflow columns and is automated for AxiChrom columns and its control unit, or together with UNICORN software in ÄKTA process chromatography system.

4.5 Resin sanitization/cleaning of a packed bed

Precleaning and in-process cleaning are important procedures that you should use as preventive actions to maintain the process performance of the resin, as well as for avoidance and control of bacterial contamination. The procedure for regular commercial manufacturing should be validated to ensure that the desired effect is reached. Important aspects to consider for the sanitization and cleaning protocols are the frequency interval, balancing resin chemical tolerance, ligand stability/binding capacity, and purity for the process, that is, how many cycles can a cleaning agent or sanitization agent be used on the resin until the resin lifespan is reached?

The frequency of your cleaning/sanitization depends on the results of the risk assessment, the composition of the sample, and the purification step type, for example, chromatography capture, intermediate purification, or polishing step. The earlier steps in a process may require more cleaning/sanitization due to the higher amount of process-related impurities. The latter stages of the purification process on the other hand result in a cleaner sample requiring less cleaning/sanitization intensity. However, at later stages with a "purer" load, other effects such as sample precipitation may present you with a significant cleaning challenge.

How often you deploy cleaning/sanitization also depends on the degree of contamination growth, growth promotion of process solutions, and time between cycles, runs, and campaigns. The longer the intervals, the longer the time for potential contaminants and biofilm to grow from a clean column or proliferate from a dirty column. Evaluation of the dirty hold time (DHT) and clean hold time (CHT) is required for cleaning validation. DHT is defined as the time between the end of manufacturing and the beginning of the cleaning process. CHT is generally considered to be the time between the completion of cleaning and the initiation of the subsequent manufacturing operation.

The evaluation of the cleaning process performance is critical for the success of your process success. The efficiency and frequency of the procedure needs to be determined to minimize the risk of contamination growth and resin fouling.

You should perform cleaning-in-place of resins at regular intervals to avoid bacterial growth that can affect the performance and properties of the bed and affect the quality of the product. Most resin can be cleaned in place using 0.1 to 0.5 M NaOH (3). A fouled column can also benefit from being run in an up-flow direction (a flow opposite to the process flow direction) during CIP, which ensures so that impurities do not move through the entire bed rather taking the shortest path route through column.

To flush the NaOH from the column, use a salt solution—preferably a buffered one —to achieve neutral pH. A suitable buffer is 250 mM Tris buffer or 200 mM sodium acetate at ~ pH 7.0. After this, wash with water and return the resin to storage buffer. The required concentrations are resin dependent and, for anion exchangers, a higher buffer concentration is required. Evaluate suitable equilibration buffers depending on the resin type you are using.

Protein A resins that are sensitive to alkali treatment such as MabSelect™ resin should be cleaned in place using another buffer. Use 10 to 20 mM NaOH and a contact time of 15 min. Immediately wash using the equilibration buffer described above (1). Also, you can add ethanol, 1-propanol, or 2-propanol to the NaOH solution to increase the log reduction of, for example, Bacillus subtilis spores, but will reduce the resin lifetime.

For details of resin chemical compatibility and recommended CIP/sanitization solutions, *see* the regulatory support file (RSF) for each resin, respectively.

4.5.1 Chemical tolerance of protein A resins

An industry survey published highlighting the importance of resin selection—not only for its capacity and resolution—but also for its sanitization robustness towards sanitization solutions has been published (7). Protein A ligand resins have lower sanitization chemical tolerances than, for example, ion exchangers. These ligands are more prone to bioburden events and are more difficult to remediate, from, for example, spore-forming bacteria. Recent advances in ligand development and chemical agent investigations have revealed alternatives for longer resin lifetimes for the effective reduction of spore-forming bacteria. Studies have shown that MabSelect SuRe™ and MabSelect PrismA chromatography resins can be sanitized with the oxidizing agent peracetic acid (PAA [2, 8]). MabSelect PrismA has an additionally increased NaOH tolerance up to the use of 1 M NaOH increasing the resin's general lifespan during continuous cleaning cycles.

Studies have shown that the oxidizing agent peracetic acid (PAA) is highly efficient in reducing bacterial spores. a more than 6-log reduction of *Bacillus subtilis* spores was obtained after a 15 min treatment with 30 mM PAA or after a 30 min treatment with 20 mM PAA (Fig 4.1). PAA is a strong oxidizing agent that might be expected to have a negative impact on the performance of chromatography resins, especially those containing protein ligands. However, studies have shown that PAA at limited contact (two to three times during the lifespan of MabSelect SuRe resin) provide a good safeguard against spore-forming bacteria, with low impact on resin performance and lifetime (3).

MabSelect PrismA resin—performance over repeated CIP cycles

We have evaluated the lifetime performance of MabSelect PrismA during repeated CIP cycles (5). The lifetime study with antibody-containing sample demonstrated that the dynamic binding capacity and performance of MabSelect PrismA in terms of mAb elution pool volume and purity are stable over the 100 purification cycles. CIP was performed using 0.5 M NaOH at a contact time of 15 min after each cycle (Fig 4.2). The lifetime study shows that MabSelect PrismA can withstand a NaOH concentration of 0.5 M for 300 cycles (Fig 4.3), and a NaOH concentration as high as 1.0 M for 150 cycles (Fig 4.4). The results show that the protein A ligand of MabSelect PrismA is stable and that it withstands repeated and effective CIP procedures with high concentrations of NaOH as cleaning agent over a long resin working life.

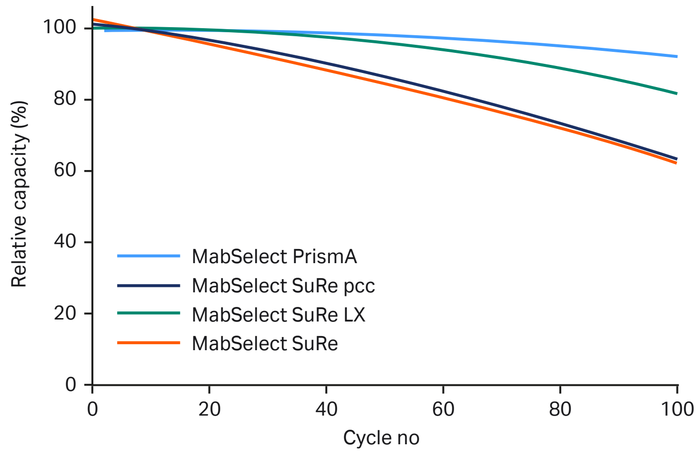

Fig 4.2. Relative remaining DBC at 10% breakthrough ($QB_{10\%}$) determined using MabSelect platform resins on ÄKTA pcc™ system for 100 cycles. CIP was performed with 0.5 M NaOH, 15 min/cycle.

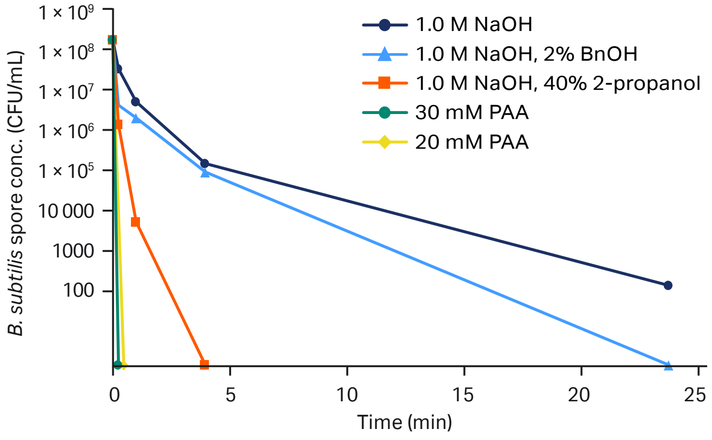

Fig 4.1. *B. subtilis* spore challenge test in 50% MabSelect SuRe resin slurry, showing sporicidal effects of 20 and 30 mM PAA at 15 to 30 min contact time.

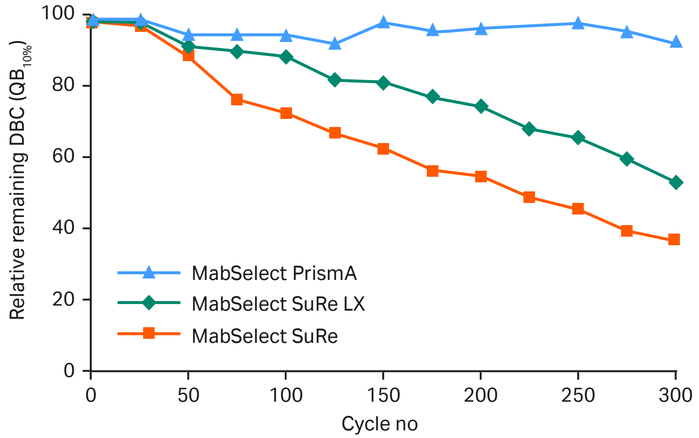

Fig 4.3. Relative remaining capacity ($QB_{10\%}$) for 300 cycles, including CIP with 0.5 M NaOH for 15 min/cycle.

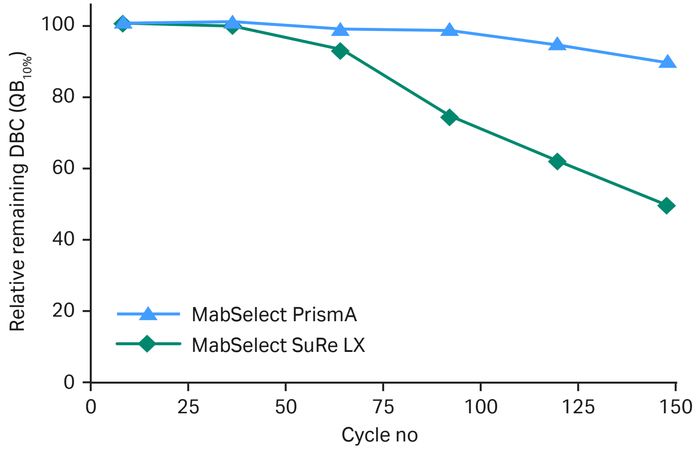

Fig 4.4. Relative remaining capacity ($QB_{10\%}$) for 150 cycles, including CIP with 1.0 M NaOH for 15 min/cycle.

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05

Bioburden control

Bioburden control requires an overall view of all parts of the processes. If one or more parts of the equipment in your process are not evaluated for bioburden control, the risk of bioburden increases. Classification of column handling area, gowning of staff, handling, and treatment of spare parts are areas to consider, as well as column packing; how can that be made more controlled and closed?

5.1 Gowning

Gowning requirements are different depending on the different classes of clean rooms. The USP 797 desribes standards and suggested changes on proper gowning. The clean room gowning room design and donning procedure workflow is also important for contamination control.

Refer to USP Healthcare Quality and Safety, General Chapter <795>, <797>, <800>, and <825> (*see* usp.org):

<795> Pharmaceutical Compounding – Nonsterile Preparations
<797> Pharmaceutical Compounding – Sterile Preparations
<800> Hazardous Drugs – Handling in Healthcare Settings
<825> Radiopharmaceuticals – Preparation, Compounding, Dispensing, and Repackaging

5.2 Treatment and handling of spare parts

Spare parts to be mounted in the column in a preventive maintenance procedure require precleaning procedures to assure their condition when mounted. Set standard procedures for spare part cleaning should be used and if required, you will need to determine appropriate level of cleaning with risk assessments. Each part should be traceable (wetted process contact parts and pressure retaining parts) and documented.

5.3 Elastomer treatment

A common practice prior to installation is to clean the elastomers and spare parts with recommended solutions for reduction of bioburden. When columns have been assembled, CIP is performed normally with 1 M NaOH for 1 to 16 h depending on requirements and risk assessments.

Spray O-rings with 30% isopropanol or 20% ethanol solution and wipe them dry. Spray again with the same solution for lubrication at assembly. Follow the recommended service procedures for elastomer exchange to ensure they are installed correctly to maintain the integrity of the column. For parts in contact with acrylic surfaces, do not use concentrations above 20% ethanol or 30% isopropanol.

Always use gloves and proper protection for vegetative contamination protection. Spray gloves and parts touched or worked upon to minimize contamination.

Open environment soaking may also be used for high-risk handling and operation. Spare-part soaking requires special equipment for controlled handling of chemicals.

5.4 Bed supports

The bed support structure of your column is difficult to clean. You can clean stainless steel bed supports to reduce bioburden in an ultrasonic batch with 0.8 to 1.0 M NaOH at an elevated temperature (up to 50°C) or electropolishing. You may use plastic sinters as an alternative to cleaning procedures for reusable bed supports; these are available for pilot and process columns, are single use and can be discarded and exchanged at regular intervals rather than cleaned. For handling of stainless steel and plastic bed supports for AxiChrom and Chromaflow columns, *see* 6.5.4 Bed supports and reference 1.

Large bed supports can be more challenging to handle, requiring the use of lifting devices.

5.5 Column packing considerations for bioburden control

5.5.1 Column handling area

Due to the nature of column packing, unpacking, and general maintenance, the industry trend is to isolate these activities from the manufacturing suite. This means a separate packing room with a different environmental class is required due to the nonsterile resin material and open handling of it. To mitigate the risks in column packing, the concept of closed column packing has been developed.

5.5.2 Closed column packing

Classical handling of resin in column packing results in handling of the resin and slurry in an open environment, all the way from the delivery container to the slurry tank (Fig 5.1). Stirring is performed in the slurry tank to achieve homogenous resin slurry to be transferred to the column for packing. Airborne particles and organisms in the clean room or packing suite may contaminate the resin slurry. To minimize the risk for viable contamination, closing the environment for the resin is beneficial. You can transfer the resin slurry from the containers to a closed system using the Media handling unit and Media Wand from Cytiva. If you are using the resin mixer from Cytiva, this should be sanitized and connected to the column prior to resin pooling. Alternatively, the resin handling can be performed in a separate room transferring the resin slurry to a “closed” slurry tank in the classed environment (2).

Storing the resin in disposable bags can eliminate several open handling steps. Resin stored in plastic bags can be aseptically connected to a column and easily be packed in, for example, AxiChrom process columns. Using this closed column packing approach significantly lowers contamination risks.

A different approach is to preclean the resin slurry in the slurry vessel by subjecting it to the cleaning solution for the appropriate contact time. Resin should be packed in the same cleaning solution, as used for cleaning of the slurry vessel and column components.



Fig 5.1. Resin mixer from Cytiva connected to an AxiChrom 450/500 column for packing in a closed system.

5.6 Removing biofilm and spores

For removal of spores and biofilms, harsher treatment is required. CIP with, for example, NaOH alone is not sufficient; mechanical scrubbing with peroxide solutions is often required (3). For suggested handling of a bioburden event, *see* section 4.5.

5.7 Contamination response

Occurrence or a reoccurrence of a bioburden event going above the set warning limits needs to be addressed, for example, performing a sanitization in place. Having pre-established, thorough procedures to find the root cause is preferable and the following are tips to let you get your column back in service:

Ensure that the material treated tolerates the cleaning agent. Make sure that disinfectants such as peroxide are rinsed away after standard cleaning.

You might not detect the bioburden issue be detected in standard liquid samples such as in the final rinse after cleaning. In this case, spot swabbing with mini swabs is required to ensure removal of bioburden.

1. Remove the bioburden by autoclave, peroxide scrub, soaking in disinfectant, etc. In most cases, mechanical force is needed and can be the starting point together with a chemical treatment during and after scrubbing. Alternatively depending on setup, superheated steam or superheated buffer can be used (4).
2. After the focused bioburden cleaning, use standard cleaning procedures and standard testing (TOC, swab, limulus amebocyte lysate [LAL], and colony-forming units [CFU]) to release the column for use.

If there is any biofilm present, mechanical treatment in combination with a disinfectant will be required. Use a soft brush for scrubbing with peroxide solutions.

Cleaning of parts in biofilm or bioburden contact

Scrub the column tube and distribution system parts with a soft brush. If biofilm is present or suspected, scrub with a peroxide solution on the affected parts before NaOH soaking. Discard elastomers for new ones. Be careful with the brushing and use a brush that does not harm the surface of the part to be cleaned. Rougher surfaces promote vegetative growth.

5.8 References

1. Application note: Handling of stainless-steel column parts in sensitive environments. Cytiva, CY14505-16Jun20-AN. https://cdn.cytivalifesciences.com/api/public/content/digi-14457-pdf
2. Application note: Using Xcellerex mixing system as slurry tank when packing chromatography columns. Cytiva, CY13440-14May20-AN. https://cdn.cytivalifesciences.com/api/public/content/digi-16552-pdf
3. Murphy M, Bell BL, Moshi J, Grassi R, Forng RY, Salaman A et al. Microbiological Control for Affinity Capture Chromatography Processing: An Industry Perspective. *PDA J Pharm Sci Technol*. 2018 Mar-Apr;72(2):213-221. doi:10.5731/pdajpst.2017.008045
4. Application note: Virus purification using superheated buffer in place (SBIP) for packed bed chromatography. Cytiva, CY13344-11May20-AN. https://cdn.cytivalifesciences.com/api/public/content/digi-15818-pdf

06

Product changeover and preventive maintenance

This chapter describes different product changeover scenarios and the main actions to perform. Consider which aspects of these scenarios apply and understand that a real case can be more complex and requires a risk-based approach.

6.1 Preventive maintenance

You should perform preventive maintenance (PM) according to the vendor recommendation but also based on a thorough risk assessment of the environment, process type, sanitization effectiveness, and cleaning chemicals. Based on site requirements, Cytiva can provide PM agreements, that allow for parts replacement on a periodic schedule completed by a trained engineer.

A changeover procedure should be controlled and documented. Parts should be inspected, cleaned, reassembled, or replaced. The assembly needs to be re-inspected and all new parts should be supplied with documentation covering regulatory requirements. AOF, and USP Class VI. These parts should be traceable via a LOT number.

Where you need to perform maintenance depends on the plant´s layout and working environment but our recommended best practice is to have a separate room for column packing and column maintenance. For large, immobile columns, the changeover procedure is performed in the purification plant under a temporary lower clean-room classification. You need to perform the changeover in a controlled manner to minimize bioburden. To avoid bioburden risks, you should handle the column in the same clean room classification as production. A higher standard for gowning may be required due to the open handling of the column interior and parts than during production.

6.2 General maintenance schedule for process-scale columns

Table 6.1 provides recommendations for the frequency of regular maintenance procedures for columns in continuous use. Exchange elastomers on a periodic schedule. This prevents the elastomers curing and cracking which can promote bioburden. The recommendation is to change all elastomers at the same time.

Table 6.1. Recommendations for the frequency of regular maintenance procedures for process-scale columns in continuous use

Procedure	Frequency[1]
Change all O-rings	At least once every 2 yr
Clean O-ring grooves	Every time O-rings are changed
Change liquid in flushing channels	Every 2 mo and preferably before each packing
Check all seals for leakage	Regularly
Change O-rings and seals on moving parts (e.g., adapter and nozzles)	Every 5 to 10 packings, more frequently if required by process requirements, or at product changeover
Clean stainless-steel bed supports	Every 5 to 10 packings, more frequently if required by process requirements, or at product changeover
Replace plastic bed supports	Every 5 to 10 packings, more frequently if required by process requirements, or at product changeover

[1] Frequency of interval is a recommendation on wear and tear for normal use for the material properties. If you require longer intervals when, for example, the column is used with a resin with life span longer than 2 yr, you may need to perform specific risk assessment to ensure optimal performance.

- Have the maintenance schedule and actions documented in a logbook supplemented by maintaining completed PM records and logging copies of material certification for parts exchanged. Parts may be revised over their life span for which a change control notification (CCN) reference is needed.
- We recommend that any time the column is unpacked, you should perform an inspection of the column parts and elastomers. For multiproduct columns, you will have to replace elastomers. Alternatively, if repacking is required several times a year, the dynamic operations of the column and processing may wear some parts that require more regular inspection/replacement.
- Spare parts to be mounted in the column in a PM procedure require precleaning procedures to assure their condition when mounted. Set standard procedures for spare part cleaning and if required, determine appropriate level of cleaning with risk assessments. Each part should be traceable (wetted process contact parts and pressure retaining parts) and documented.
- After column reassembly, always perform an integrity test (leak test) of the column to ensure it holds its rated pressure.

6.3 Single-product column

6.3.1 Maximum resin lifetime is reached

When your resin reaches its maximum resin life, you should unpack and discard the expended resin and clean the column. All soft parts in contact with the product should be inspected and considered for changeover since exposure to common buffers used in chromatographic steps can lead to the curing and cracking of elastomers, which can promote bioburden growth. The bed supports should also be inspected, cleaned, or swapped out if made of PP. *See* 6.5.4 Bed supports for suggestions on how to clean larger bed supports.

6.3.2 In-process bed performance test failure

Failure of in-process bed performance (continuous monitoring of process performance of the packed bed by transition analysis during process purification runs) may be handled by additional CIP or a harsher CIP regimen. The next step is to repack the same resin, or exchange the resin to new unused resin, including column and bed support cleaning to re-establish good performance. If the old resin is to be reused, investigate resin condition before repacking so that a disqualified result of the packing will not be due to fouled resin or fines.



Process debris and precipitation can result in clogged bed supports. Long-term use can also result in resin fines moving into the bed an getting trapped in the bed support. Confirming clean bed supports and clean resin are important parameters to determine the root cause for a failed bed performance test. Excessive pressure on the bed can be alleviated by cleaning of the bed supports, *see* 6.5.4 Bed supports.

A successful repack and blank run may rule out the column packing operation as contributing to the failed result. Change the elastomers before performing the repack. In general, when the column is unpacked and opened, perform an intentional inspection to look at product parts replace if necessary.

6.4 Multiproduct column

A multiproduct column requires validated procedures and risk assessments, but the procedures may differ from the single-product column. A multiproduct column can have, for example, product-specific bed supports. This is to ensure that despite cleaning, the risk of transferring resin from one product to another is minimized.

- Always discard used elastomers before handling new ones to remove the risk of reusing the old elastomers between products. Used elastomers and new elastomers shall be handled separately.
- Product or process bed supports or disposable polyethylene (PE) bed supports need to be replaced for each new product process.
- Level of CIP or COP on non-elastomers should be determined by a risk assessment.

The risk for cross-product contamination may be higher since there are different products produced in the same column or plant. The risks for carryover shall be minimized and preferably removed.

Consideration for the cross-contamination risk and cleaning regimen should dictate the extent to which product parts in contact with the product are replaced or cleaned.

6.5 Preparing and performing product changeover

6.5.1 Overview for previously used columns

The following checklist can be referred to when unpacking or packing resin from a used column:

1. CIP and decontamination after processing. Follow the validated protocols for CIP and SIP to remove dangerous biological or chemical substances from the packed bed in the column.
2. Readiness of production suite if used during column changeover or move column to separate packing suite or room.
3. Unpacking: The procedure of getting the resin out from the column, either for storage, repacking, or to discard it if it is at its end of life.
4. Column disassembly: The process of removing adapter and/or column tube to reach the column interior and product contact surfaces to remove old elastomers and inspect column components. It is also important to dispose of residual resin during this step.
5. Clean or change bed supports: SS bed supports are most often reused and have different alternatives for cleaning. Larger bed supports often require third-party contractors. Consider the following cleaning methods:
   - Electropolishing
   - Ultrasonic cleaning
   - *In situ* cleaning
   - High-pressure cleaning

   Plastic bed supports should not be reused. Discard and replace.
6. Wetted components and elastomer exchange: Clean or change all parts that have been in contact with the process. Cleaning risk assessment should identify the component items that require COP and/or exchange.



Scrub column parts with a soft brush, that is, the inner surface of the column tube and the distribution system. For removal of biofilm if present, scrub with a peroxide solution on the affected parts before NaOH soaking. Do not scrub elastomers; replace with new ones. Use a brush that does not harm the surface of the part to be cleaned. Use the right tools for scrubbing to avoid scratching surfaces, which will promote vegetative growth.

Spray new elastomers with 70% ethanol or isopropyl alcohol (IPA) and wipe them dry with a particulate-free cloth. Alternatively, for high-risk environments, soak elastomers in 1 M of NaOH for at least 1 h. Make sure the elastomers are rinsed thoroughly to remove NaOH before assembled into the column with the aid of 20% ethanol. Use controlled parameters for soaking. Refer to the column's chemical compatibility for different parts.

The column tube is often made from acrylic material (poly[methyl methacrylate], PMMA), which does not withstand higher concentrations of alcohol for long periods of time. Ensure to minimize the exposure time of the acrylic column tube to alcohol-based cleaning liquids and wipe away any excess cleaning solution to avoid damage to the tube.

7. Column assembly: Assemble the column according to the instructions provided by the manuals to be able to prime and perform an integrity test (pressure test) to ensure operating pressure of the column. Work as cleanly as possible to avoid bioburden. Ensure a clean-room environment, special gowning, and spray with ethanol or IPA to minimize the risk of promoting vegetative contamination into the open column and parts. You should develop a detailed procedure for the site/application describing a preferable working order and method for aseptic handling during cleaning and assembling of the column parts.
8. Assembled column cleaning: An empty column should be cleaned before packing the resin in the column.

   The procedure can be dependent on the column design, that is either a fixed vs movable adapter, and is also dependent on the intended type of packing procedure. With regards to the liquid cleaning agents used, take the following into account:

   - Material compatibility.
   - Concentration, contact time, and procedure.
   - Sampling after cleaning to have proof of cleaning method functionality, for example, TOC sample from washing, without disassembling the column.
9. Column release for packing, or for empty storage.
10. Pack resin per recommended procedure for the used column type. Consider closed column packing for bioburden control and minimizing risk for resin contamination.
11. Packed bed performance qualification, see reference 1 for the complete method.
12. Sanitization or CIP of the packed column for process or for storage

    Use the recommended procedure from the manufacturer for packed bed sanitization and cleaning. The sanitization solution needs to be both column and resin compatible. Consider the following:

    - Material compatibility.
    - Resin compatibility.
    - Concentration, contact time, procedure.
    - Sampling after cleaning to have proof of cleaning method functionality, e.g., for example, TOC sample from flushing.

    Consider that resin is not a sterile material and may require sanitization to have a safe and clean and sanitized start to the next production cycle and to extend its lifespan.
13. Equilibrate to storage solution before process or for storage. Storage solution requirements are both resin and column dependent. Storage conditions outside vendor recommendation needs to be validated to not interact/damage either resin or column HW components. The most damaged HW components are the column tube which are often subjected to high solvent concentrations, halides, low pH, air contact, or combinations of a solvent and an organic acid. Consider the storage information in the RSF and validate the concentration, contact time, and procedure as well as the exchange frequency combined with or without CIP.
14. Release of column: Column ready for use in processing.
15. Move to production suite or storage.

6.5.2 Overview for new columns

For a new column delivered from your vendor, there are some initial handling steps that are required that differ from working with the packed column previously described in section 6.5.1.

1. Arrival, uncrating.
2. Move column to column packing suite or production area including external surface wiping in passages to classified environments.
3. [1]Column disassembly to exchange elastomers and perform cleaning procedure of parts before assembly.
4. Column assembly if point 3 is performed.
5. Continue from step 8 in section 6.5.1.

[1] Depending on your requirements.

6.5.3 AxiChrom and Chromaflow columns: changeover tips

Here, we provide tips for some of the critical steps when performing PM on AxiChrom and Chromaflow process-scale columns. These steps affect the final assembled columns' integrity related to bioburden control and risk for contamination.



AxiChrom columns: Disassemble the bottom assembly first, so that components that may be dropped when working on the adapter assembly will not damage the fragile bed support. Similarly, assemble the adapter assembly before working on the bottom assembly.



Column and bed support priming is critical to avoid growth promoting areas that are inaccessible to sanitization and CIP solutions due to air pockets. Perform priming according to recommended procedures to remove all air from the column.

- Regularly (at least every 3 mo) change the liquid (20% ethanol) between the adapter seals. This preventive action maintains the bacteriostatic environment in those areas. Alternative solutions that can be used for sanitization of said compartments and for adapter movement in cleaned column tubes are 100 mM PAA. This instantly sanitizes the contacted area while the adapter is moved along the column tube.
- Complex structures such as the nozzle tips can be removed and soaked in cleaning solution. The only area however that is in contact with process streams is the tip of the nozzle and the closest O-ring. The other parts are in contact with the resin for packing and unpacking.
- The nozzle tip for both AxiChrom and Chromaflow columns can also help in clearing the column interior, for example, all the way up in the dead leg by the adapter.

A full PM procedure is a service that is available from Cytiva on request.

6.5.4 Bed supports

A column consists mainly of the tube, the distribution system, and the bed support. Different types of bed support are used in chromatography columns, the most common being multilayer woven stainless-steel screens, PP, or polyamide (PA) nets, and sintered polypropylene. The difference between nets and sinters is that nets consist of an ordered array of woven threads while the sinters are sintered plates from randomly ordered strands of plastic fibers without a defined structure. The polypropylene, PP nets, PA nets, and PP sinters are considered disposable and should be exchanged rather than cleaned. This results in a more efficient way of minimizing bioburden carryover

The risk of bed support clogging depends on process composition and packing methodology. Regularly generating empty column pressure curves is a recommended method for detecting degradation of bed support performance over time. You should start with an empty column pressure-flow measurement at column commissioning or new bed support installation to obtain a baseline. Before repacking or when appropriate, perform the empty column pressure-flow measurement again to track the trend of clogging over time.

In-process pressure trends, such as increasing equilibration pressure between cycles, can also indicate bed support clogging. In this case, however, you will also need to take resin clogging into consideration when evaluating the CIP effectiveness, composition, contact times, and intervals in a CIP regimen.

Process-specific bed supports should be considered in multiproduct facilities to remove the need for validation, ensure product purity, and to reduce costs.

If you need to change the bed support material, there are guides available for converting AxiChrom columns between different bed support materials SS and PE, *see* the instructions for column bed conversion for AxiChrom 300 to 1600 columns and 50 to 200 columns.

Bed support cleaning

When removing bed supports, cleaning/decontamination must first be performed according to internal validated procedures to remove any biological hazards. This is usually performed using NaOH or another approved method. After that, the bed supports can be cleaned out of place to remove biological residual resin particles and remaining cleaning agents using ultrasonic cleaning, electropolishing, or a combination of soaking and spray washing.

Equipment required for the different methods of bed support cleaning:

- An ultrasonic bath that can soak the full surface area of the bed support.
- Electropolishing unit.
- A basin or tray large enough for the bed support and with a min. depth of 30 cm (with support rails for better draining) or a rotary device to turn the bed support during cleaning.
- Purified water according to min USP 24 standard for water (1).
- Spray bottles.
- Chemicals as required by the specific procedure, that is, NaCl, ethanol, NaOH, or passivation chemicals such as citric acid.
- Support material in chloride-free plastic (i.e., polypropylene).
- High-pressure cleaner with all components in contact with the water in ceramic, SS, ethylene propylene diene monomer (EPDM) rubber or other approved materials in order not to contaminate the bed support. Also consider the hygienic standard in the cleaner and its accessories. The recommended power of the high-pressure cleaner should be approx. 5 kw at 175 to 200 bar (17.5 to 20 MPa, 2538 to 2900 psi) with a 15° to 25° flat spray nozzle.
- Infradryer, min. 2 kW.
- Protective clothing, gloves, and eye protection.

Note: The following methods are suggestions and recommendations and are not validated methods.

Ultrasonic cleaning of bed supports

Ultrasound is a suitable method for cleaning and can be used for all SS bed supports. This method requires an ultrasonic bath that fits at least half the screen.

Industrial-scale ultrasonic baths are available for purchase from third parties. We use these at our Uppsala and Marlborough labs for columns up to the 1000 mm scale although larger columns are also available. Ultrasound and 1 M NaOH at 50°C are used to dislodge any potential resin from the mesh layers and sanitize them from any vegetative organism.

Ultrasonic cleaning may require consultation with a contractor to perform this work, which could require offsite transport of the bed supports if an ultrasonic bath is not made available onsite.

Bed support cleaning procedures and frequency are dependent upon the applications as well as load or foulant attributes, cycling, column cleaning procedures, and other operating and process parameters.

Example procedure:

1. The screens should be placed with the resin side facing downward, either flat on the bottom in a plastic beaker or in a tray deigned for the bath. In the large bath, the screens are hung vertically.
2. Make sure the screens do not directly contact any steel surfaces in the bath as they may be scratched when the bath vibrates.
3. Fill the bath or the beaker/tray using new 1 M NaOH solution and run the screen at least 15 min on each side in the small bath or before a 90° rotation. If available, the process is run at an elevated temperature of 50°C. For bed supports previously in contact with reversed phase chromatography (RPC) resin, NaOH should be replaced by 20% ethanol or propanol and should not be run at an elevated temperature.
4. Wash the screens and repeat the method using water. Rinse thoroughly.

Electropolishing of bed supports

Electrolyte polishing is a method where a stainless-steel surface is polished by an electrolyte liquid and direct current. Normally a mixture of sulfuric acid and phosphoric acid is used. This method removes the ferrite on the surface, which makes the surface more resistant to corrosion.

Visual inspection of particle and break-down product removal, including packed bed evaluation has shown very good results showing restored performance of the bed support. There are several portable cleaning units available on the market.

Electropolishing is also an excellent choice for dealing with stainless-steel rouging (corrosion).

High-pressure rinsing

High-pressure rinsing is a simple easier available cleaning procedure using a high-pressure cleaner in combination with cleaning and neutralizing chemicals. A rinsing basin and cleaning room is required for this procedure.

Pass over the course side/support mesh surface area of the bed support with a high-pressure liquid stream. If needed, the resin retaining side can also be rinsed, but the risk is that any unrinsed resin bead may be further forced deeper into the mesh.

Always wear protective clothing, gloves, and eye protection when handling the bed supports. Always place the bed support on surfaces protected with chloride-free plastic (i.e., PP, not PVC) to protect it from scratches, biological contamination, and chloride ions.

High-pressure rinsing using a basin

1. Place the bed support with the fine mesh (the side facing the bed in the column) facing downwards in a large enough basin with a drain and with a height that allows for soaking it entirely. Support the bed support on plastic covered supports on the outer ring. Do not support it on the center bed retainer ring.
2. Ensure that the bed supports are drained from water but not dry.
3. Spray the mesh with 5% to 10% phosphoric acid (~ 1 L/m^2) to create a low pH which will aid in breaking up clogged resin particles.
4. Gently flush the mesh with purified water from the coarse side to remove most of the acid and then use the high-pressure cleaner to remove the resin particles. Work in a systematic pattern with overlapping movements pushing the contaminants forward with the spray nozzle at a slight angle toward the surface and 20 to 50 mm from the mesh until the entire mesh surface is covered.
5. Spray the mesh with 0.1 M NaOH (~ 1 L/m^2) to neutralize any remaining acid and leave for 10 min, then flush with water (high pressure). Repeat step until water is no longer discolored.
6. Turn the bed support and inspect it for remaining resin particles. The removal of all trapped resin particles cannot be guaranteed, and a small amount may still be trapped within the mesh.
7. Inspect the bed support for signs of rouging, dents, and scratches. Remove the rouging with the de-rouging procedure, and treat the scratches with the local passivation procedure, *see* below.

De-rouging procedure for stainless-steel mesh bed supports

Some processes require the bed supports to be regularly treated for rouging due to exposure to low pH and/or chlorides. The treatment for rouging is passivation and this can be performed with different acids and concentrations depending on the level or rouging. Acetic acid and phosphoric acid are the most used passivation solutions.

1. Clean the bed support according to the cleaning procedure.
2. Remove most of the water in the mesh by placing it vertically for approximately 30 min. Take care to avoid denting the bed support and make sure it is placed on a soft plastic support surface. The upper rim is protected where it is supported. Alternatively, use clean, oil-free compressed air. Blow gently until most of the water is removed.

3. Soak the entire mesh with 10% to 25% phosphoric acid. Pour onto the mesh until it is completely soaked. Leave for 30 to 60 min. Start with 10% phosphoric acid for 30 min and increase the strength and exposure time if required.
4. Inspect the mesh and check that all rough has been removed.
5. Wash thoroughly along the outer ring and toward the center to remove the acid with purified water and check the conductivity on the wastewater until the conductivity is the same as for the incoming water or below 100 µS/cm.
6. Soak the bed support in 0.1 M NaOH for 20 min for neutralization.
7. Wash the bed support and the basin until the conductivity on the wastewater is the same as for the incoming water or below 10 µS/cm.

Local passivation of bed support ring

If your bed support is scratched or dented or exposed to iron contamination, the thin passive layer can be destroyed locally and this can lead to corrosion. Through a local passivation procedure, the corrosion products (iron oxides) can be removed (after physically removing any visible contaminant) and the passive film can be restored.

1. Apply 7.0 M nitric acid (concentrated acid 65%, diluted with 50% purified water) on spotted or damaged areas with a sponge.
2. Contact time should be 60 to 80 min with the surface remaining wet.
3. Wash the passivated areas thoroughly with purified water.
4. Wipe with smooth lint-free paper.

☞ Perform the passivation in a well-ventilated area, making sure to always use rubber gloves, protective clothing, and eye protection. Purified water defined by USP 24 (1) should be used in all steps of the passivation procedure since any chloride ions will destroy the process.

☞ Citric acid is becoming more common for passivation due to its many advantages. More standards are including citric acid passivation in its recommendations.

For passivation standards *see* references 2 to 6.

6.5.4 Drying and storing of SS bed supports

The bed support should be stored clean and dry. It is difficult to remove all the water from the very fine mesh since it will act as a soaking paper. The five or seven-layer (depending on column) construction will keep the water encapsulated if not removed with the aid of gravity and heat.

1. Lift the bed support from the basin, place it vertically on a soft, plastic covered surface in order not to scratch or dent the rim and let the excess water run off.
2. Move the bed support to a support structure with plastic supports above an infra heater (min 2 kW).
3. Dry the bed support above the infra heater for 24 h.
4. Place the bed support on a plastic pallet, cover it with a chloride free plastic wrap and store at 20°C to 35°C, 50% to 95% humidity.

6.5.5 Visual inspection and drainage test

There are several ways to assess the success of cleaning. One is to simply look over the screen with a microscope and look for trapped particles. Check at least 10 different areas, especially those that are close to a weld. However, with bigger bed support surface areas, you may need additional means for evaluating the cleaning quality. You can finally perform a pack and see if packing results are restored after cleaning.

An alternative assessment method is the drainage test, where often only the bottom screen can be evaluated. Finally, pack the column and see if packing results are restored after cleaning. The drainage test is outlined below.

Drainage test

1. The screens are mounted into the column, which is filled with water to approximately 22 cm height. The adapter must be taken out of the column to allow air to pass into it.
2. Connect a wide, short hose or simply use no hose, and open/remove the mobile phase bottom (MPB) valve to start the test.
3. As the liquid level reaches for example 20 cm, start the stopwatch.
4. Record the time it takes for the liquid level to move for example every 5 cm. Compare times with the new screen measurement.
5. Make sure the same hose is used in all experiments since this can contribute significantly to the pressure drop and thereby the drain rate.
6. If the drain time increases, it is a clear sign that the screens are being blocked. The differences are sometimes small, and it can be a question of a few seconds. An increase of ~ 5% can be a sign that the screens are clogged or close to being clogged. Note that an increase in drain time does not necessarily mean that the screens need to be cleaned or replaced, but this should be considered.



Regularly generating empty column pressure curves is a recommended method for detecting degradation of performance over time. Start with an empty column pressure flow at column commissioning or new bed support installation as a baseline. Before packing or when appropriate, perform a pressure flow of the empty to track the trend of clogging over time. After the bed supports have been cleaned, performance should be compared to the baseline to prove cleaning effectiveness.

6.6 References

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07

Chemical compatibility of columns

The column HW components and wetted material must withstand the chemicals needed for the sanitization and cleaning of the resin to avoid affecting process or product quality in terms of unwanted particles or substances in the product eluate.

Chemical compatibility lists are supplied by the manufacturer. Most often, the sub-supplier's statement on chemical compatibility are listed, and you should confirm the chemical compatibility of the chemicals used in the specific process.

7.1 Chemical compatibility: column hardware

The information in Tables 7.1 and 7.2 has been collected from several published sources, not from individual tests on column components. It should be used only as a guide. The effect of chemicals will generally be more severe at higher temperatures. Note also that the combined effects of agents have not been considered in this table. The applicable chemical resistance depends on the configuration of the column and the associated materials of construction.

Table 7.1. Chemical compatibility, AxiChrom columns

Chemical	Concentration[1]	Max. contact time	Comments	Temperature limits[2]	CAS no.[3]
Acetic acid	25%	3 h	Cleaning in place (CIP)	2°C to 30°C	64-19-7
Acetone	2%	1 h	Efficiency test	2°C to 30°C	67-64-1
Ammonium sulfate	2 M[4]	5 h	Adsorption	2°C to 30°C	7783-20-2
BnOH[5]	2.0% w/w[6]	12 mo	Storage	2°C to 24°C	100-51-6
Ethanol	20%	12 mo, max. 0.5 bar	Storage	2°C to 24°C	64-17-5
Ethanol	70%	3 h	CIP	2°C to 30°C	64-17-5
Ethanol/acetic acid	20%/10%	3 h	CIP	2°C to 30°C	64-17-5/64-19-7
Guanidine HCl	6 M[7]	5 h	CIP	2°C to 30°C	50-01-1
HCl	0.1 M (pH 8.0)[8]	1 h	CIP	2°C to 30°C	7647-01-0
IPA	30%[9]	1 h	CIP	2°C to 30°C	67-63-0
Phosphoric acid	5%	8 h	For passivation of SS bed supports	2°C to 30°C	7664-38--2
1-Propanol	20%	16 h	Wetting plastic bed supports	2°C to 30°C	71-23-8
NaCl	0 to 3 M[10]	3 h	Purification, CIP	2°C to 30°C	7647-14-5
NaOH	1 M (pH 14.0)	24 h, room temp. to 30°C	CIP	2°C to 30°C	1310-73-2
NaOH	10 mM (pH 12.0)	12 mo	Storage	2°C to 30°C	1310-73-2
NaOH/ethanol	1 M/20%	3 h	CIP	2°C to 30°C	1310-73-2/64-17-5
Sodium sulfate	1 M[4]	3 h	Adsorption	2°C to 30°C	57-13-6
Aqueous buffers typically used in chromatography	10 to 250 mM, pH 2.0 to 10.0	24 h	Equilibration, adsorption, elution	2°C to 30°C	

[1] When a concentration is given as a percentage, this is v/v, except in the case of BnOH.
[2] Do not exceed the operating temperature specifications for the individual column size.
[3] CAS no.: Registration number assigned by the Chemical Abstract Services (CAS), American Chemical Society.
[4] pH in these solutions depends on the pH of the buffer, which can vary between 3 and 13.
[5] The use of a high ionic strength buffer may contribute to a slower rate of dissolution of BnOH and “salting out” of the solvent component. Both cases will lead to elevated concentrations and is therefore not recommended for columns with acrylic column tubes.
[6] Take care when preparing solutions for both accuracy and allowing sufficient time for the BnOH to fully dissolve.
[7] Not for use with columns containing wetted components of SS.
[8] We do not recommend pH below 4.0 for SS columns.
[9] Applies only to acrylic column tubes.
[10] For columns containing wetted SS components, we recommend using max. 1.0 M NaCl.

7.1.1 Resistance to NaCl

Depending on configuration, AxiChrom and Chromaflow columns can be equipped with wetted components of SS and must therefore be appropriately maintained when exposed to NaCl during chromatographic processes. While the SS bed supports have a high degree of resistance to corrosion, they may corrode on extended exposure to NaCl. The degree of susceptibility varies according to conditions of use. Low pH in combination with NaCl, high temperature and damage to the steel surface all increase the risk of corrosion.

If a SS surface is damaged (scratches, impact marks etc.) you should clean and passivate it before use in your operation. Your Cytiva representative can provide guidance on suitable cleaning and passivation procedures if required.

We recommended that you rinse with at least 5 CV of water to remove NaCl from SS surfaces. Water is the preferred rinse solution because of the solubility of NaCl in water. Columns should be stored with solutions free from chloride ions.

Table 7.2. Chemical compatibility, Chromaflow columns

Chemical	Concentration[1]	Max. contact time	Comments	Temperature limits[2]	CAS no.[3]
1-propanol		16 h	For wetting plastic bed supports		71-23-8
Acetic acid	25%	3 h	CIP	2°C to 30°C	64-19-7
Acetone	2%	1 h	Efficiency test	2°C to 30°C	67-64-1
Ammonium sulfate	2 M[4]	5 h	Adsorption	2°C to 30°C	7783-20-2
BnOH	2.0% (w/w)[5]	12 mo	Storage	2°C to 24°C	100-51-6
Ethanol	20%	12 mo and max. 0.5 bar (7.25 psi, 0.05 MPa)	Storage	2°C to 24°C	64-17-5
Ethanol/ acetic acid	20%/10%	3 h	CIP	2°C to 30°C	64-17-5/6 4-19-7
Guanidine hydrochloride	6 M[6]	5 h	CIP	2°C to 30°C	50-01-1
Hydrochloric acid[7]	0.1 M (pH = 1.0)	1 h	CIP	2°C to 30°C	7647-01-0
Isopropanol	30%	1 h	CIP	2°C to 30°C	67-63-0
Phosphoric acid	5%	8 h	For passivation of stainless steel (SS) bed supports	2°C to 30°C	7664-38-2
1-Propanol	20%	16 h	Wetting plastic bed supports	2°C to 30°C	71-23-8
Sodium chloride	0 to 3 M[8]	3 h	Purification, CIP	2°C to 30°C	7647-14-5
Sodium hydroxide	1 M (pH = 14)	24 h	CIP	2°C to 30°C	1310-73-2
Sodium hydroxide	0.01 to 0.1 M (pH = 12 to 13)	12 mo	Storage	2°C to 30°C	1310-73-2
Sodium hydroxide/ ethanol	1 M 20%	3 h	CIP	2°C to 30°C	1310-73-2/64-17-5
Sodium sulfate	1 M[4]	3 h	Adsorption	2°C to 30°C	7757-82-6
Urea	8 M[4]	5 h	Purification, CIP	2°C to 30°C	57-13-6
Aqueous buffers commonly used in chromatography	10 to 250mM, pH 2 to 10	24 h	Equilibration, adsorption, elution	2°C to 30°C	

[1] When a concentration is given as a percentage, this is v/v, except in the case of BnOH, *see* footnote 5.
[2] Do not exceed the operating temperature specifications for the individual column size.
[3] CAS no.: Registration number assigned by the Chemical Abstract Services (CAS), American Chemical Society.
[4] pH in these solutions depends on the pH of the buffer, which can vary between 3 and 13.
[5] Take care when preparing solutions for both accuracy and allowing sufficient time for the benzyl alcohol to fully dissolve.
[6] Not for use with columns containing wetted components of stainless steel.
[7] pH below 4.0 for stainless steel is not recommended.
[8] For columns containing wetted SS components, we recommend max. 1.0 M NaCl.

Avoid using the following chemicals in process columns:

- Extreme oxidizers.
- Fluorine and halogenated compounds.
- Chlorinated solvents (such as methylene chloride).
- Esters.
- Aromatic hydrocarbons (such as toluene).
- Alcohols at concentrations higher than those specified in the table above.
- Salt in combination with pH below 4. Always wash the column with at least 5 CV of pH-neutral solution (water) between and after use of salt and low pH buffers.

7.2 Resin chemical compatibility

The resin chemical compatibility is more thoroughly investigated for some chemical solutions and results from the studies are available in the regulatory support file, RSF, of the resin. The RSF states which chemical the resin withstands and to what concentrations and contact times. The RSF gives recommendations on sanitization and CIP solutions to use for regulatory or intermittent cleaning of the resin before and during batch run cycles.

More information about compatibility with various chemicals for sanitization and CIP are found in the references below.

7.3 References

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08
Column storage

8.1 Storage of packed columns and recommended storage solutions

Most chromatography resins are delivered in 20% ethanol or optionally, 2% BnOH (including 0.2 M sodium acetate for some cation exchangers). Shelf-life studies are performed during resin development. Cytiva cannot define a storage time for used resin and we therefore recommend that you perform your own binding capacity test or other relevant test after storage according to your own protocol.

Storage solutions for packed columns are generally recommended to be the same as the shipping solution, normally 20% ethanol or 20% ethanol with 0.2 M sodium acetate. Alternative storage solutions for other resins than those with attached protein ligands are NaOH (1, 2). Using other storage solutions requires functional validation and column HW compatibility validation. Check the available chemical resistance of each resin and columns before choosing a different storing solution than the recommended.

Always store resin and systems in 20% ethanol, 2.0% v/v BnOH (3), or 10 mM NaOH over shorter periods to prevent bacterial growth, provided the resin, system, and column components are stable under the storing conditions. For some resins, you will need to add 0.2 M sodium acetate as a stabilizer, although not together with BnOH for acrylic column tubes. For details on the recommended storage solutions for resins, (1–3). For detailed resin storage data, see each respective resin's regulatory support file (RSF).

When you use BnOH as a storage solution, it is critical to store the column under a controlled temperature environment below 24°C. Note that concentrations of BnOH higher than 2.0% v/v are not recommended for columns with acrylic column tubes. Also do not use any organic salt/acid to stabilize the pH in the BnOH solution since this will damage the acrylic tube.

Organic salts/acids such as sodium citrate buffer (for pH stabilization) in combination with BnOH lower the solubility. This results in salting out and local concentration of the BnOH, which leads to a hazy, rougher, and damaged surface of the acrylic tube. Refer to the chemical resistance chart for each column to avoid damage to the column caused by inappropriate storage conditions.

Change the storage solution regularly, at least once every 3 mo since liquid exchange and liquid circulation also prevents vegetative growth. For columns in storage rooms, additional pumping equipment like a peristaltic pump can be required to exchange liquids during storage. Remember to change the storage solution in the rinse channel—the compartment between the adapter seals—to maintain the sanitary barrier.

Before storing a packed bed or resin long term, we recommend that you clean the resin in place before equilibrating it in storage solution.

Storing columns in the cold room puts column integrity at risk because of the higher corrosion risk and higher column leakage risk. This is because many components made of different materials respond in different ways to decreased temperature.

Ensure that when moving columns in and out of cold rooms, that nuts and bolts are tightened to recommended torque settings and released according to the specific column's operating manual. This will prevent damage to the column.

Inspect the column regularly for surface humidity, rouging, and corrosion. Preferably, also perform additional exterior cleaning to ensure that no salts or chloride ions remain on the column's SS components. Investigate if the resin, packed in the column can be stored in room temperature with controlled temperature and humidity.

Valves and nozzle compartments not used during processing or storage can be stored sealed in 20% ethanol or 2.0% v/v BnOH.

8.1.1 Before storage

The following procedure is recommended before long-term storage of a column with i.d. ≥ 30 cm. For column-specific recommendations, *see* the relevant column operating instructions.

1. Perform sanitization in place according to your recommended cleaning procedure (If the column will be filled with 20% (v/v) ethanol as the storage solution (*see* step 3), the column should be rinsed with a suitable buffer that to give neutral pH. The buffer must not contain any chlorides as these may cause corrosion.
2. Pump a suitable storage solution into the column using an amount of storage solution corresponding to five times the present column volume.
3. Close the bottom valve, disconnect the bottom tubing, and fit blind caps on all available inlets/outlets in the bottom of the column.
4. Disconnect all tubes connected to the top inlet. If the tubes are not disconnected; a vacuum may form in the next step. For AxiChrom 300 to 2000 columns, keep the top tubing connected.
5. Fit a blind cap on the top inlet.

8.1.2 During storage

Regardless of length of storage, it is critical to maintain the column sanitary conditions. Exchange the storage solution at regular intervals to maintain the solutions bacteriostatic properties.

8.1.3 After storage

Qualify the packed bed for use. Equilibrate the bed for at least 5 CV and then perform a column evaluation according to preset standards (1). Take necessary action such as SIP/CIP to take the column into production again.

8.2 Storage of empty columns

To avoid bacterial/fungal growth during storage, the column should be clean and dry. You should store in a controlled environment without temperature changes and air particles. Prevent condensation build-up in the process and in column storage. Condensed air inside a closed volume such as an empty column can lead to fungal growth.

The short-term storage recommendation is to store columns > 30 cm i.d. fully primed with a bactericidal/bacteriostatic storage solution. The storage solution is recommended to be changed every 3 mo to maintain its bacteriostatic effect depending on type of storage solution.

Long term-storage recommendations for columns > 30 cm i.d: Before storage perform a complete cleaning and PM procedure. You should store the column preferably without the adapter in the column tube, which will let air pass and reduce the risk for condensation in the process chamber. The column should be stored open, covered with a sheet providing air passage but stopping moist and particulates. Smaller columns, depending on column design, may be stored dry without, for example, the adapter O-ring to have interchangeability with the air inside and outside of the column.

For empty, assembled columns, store with a bed height that is as low as possible. If filled with liquid, less storage solution is required.

Make sure all parts of the column are flushed or filled with storage solution before carefully placing blind flanges on every inlet and outlet. When columns are to be stored dry, wash with 20% ethanol and leave the columns as open as possible until it dries out. Do not cap, as temperature fluctuations may cause condensation inside the column.

8.2.1 Storage conditions

Keep air humidity and air temperature differences as low as possible to prevent condensation and corrosion.

- Maintain the storage area at a constant temperature < 24°C if the storage solution used in the column is 2.0% (v/v) BnOH or 20% ethanol.
- Make sure the relative humidity is between 0% and 95%, noncondensing for column storage and between 0% to 80% noncondensing for storage of AxiChrom columns.
- Acrylic tube columns must be protected from sunlight, and other sources of UV light.
- Do not expose the column to chlorides.

Storage of an empty column

Before storage: Perform a complete cleaning and elastomer replacement.

During storage:

- Dry storage: Stable controlled environments preventing condensation.
- Wet storage: Completely primed and filled column with storage solution. Exchange storage solution according to risk-assessment intervals or at least every third month

A column or system that has been in storage for some time should be completely flushed and inspected for corrosion and bacterial growth. Clean the equipment in place before putting the column into use. Make sure all flow paths in the column and system are cleaned.

8.2.2 Storage solutions and conditions for empty columns

Storage solutions for empty columns are typically 20% ethanol, 0.01 M NaOH, or 2.0% (v/v) BnOH under controlled temperature and humidity environment.

Note that concentrations of BnOH higher than 2.0% v/v are not recommended for columns with acrylic column tubes. Furthermore, do not store above in 20% ethanol or 2.0% v/v BnOH above 24°C.

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Related literature

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	Code number
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Affinity Chromatography - Vol. 2: Tagged Proteins	CY13980
Affinity Chromatography - Vol. 3: Specific Groups of Biomolecules	CY13979
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Use of BnOH as a shipping and storage solution for chromatography resins, CY13376-12May20-AN.
Lifetime performance study of MabSelect PrismA during repeated cleaning in place cycles, CY14006-02Jun20-AN.
Chromatography column packing—MabSelect PrismA resin, cytivalifesciences.com/solutions/bioprocessing/knowledge-center/column-packing-for-mabselect-prisma-resin.
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Packing Capto S ImpAct using verified methods, CY27610-2FEB22-PD.
Packing Capto S, Capto Q, and Capto DEAE in production-scale columns, CY13253-08May20-AN.
Develop your column packing methods based on pressure flow, CY18964-12Oct21-PD.
Collaborating to address the bioburden challenge, CY15143-12Jul20-PT.

Ordering information

Ordering information

Product	Quantity	Product code
AxiChrom columns		*See* cytiva.com/axichrom
Chromaflow columns		*See* cytiva.com/chromaflow
BPG columns		*See* cytiva.com/bpg

Resins

MabSelect PrismA for protein A affinity capture		
MabSelect PrismA	25 mL	17549801
MabSelect PrismA	200 mL	17549802
MabSelect PrismA	1 L	17549803
MabSelect PrismA	5 L	17549804
MabSelect PrismA	10 L (made to order)	17549805
MabSelect PrismA in BnOH	1 L (made to order)	17549823
MabSelect PrismA in BnOH	5 L (made to order)	17549824
MabSelect PrismA in BnOH	10 L (made to order)	17549825

Capto Q, S, and DEAE for IEX		
Capto Q	25 mL	17531610
Capto Q	100 mL	17531602
Capto Q	1 L	17531603
Capto Q	5 L	17531604
Capto Q	10 L	17531605
Capto Q	60 L	17531660
Capto S	25 mL	17544110
Capto S	100 mL	17544102
Capto S	1 L	17544103
Capto S	5 L	17544104
Capto S	10 L	17544105
Capto S	60 L	17544160
Capto DEAE	25 mL	17544310
Capto DEAE	100 mL	17544302
Capto DEAE	1 L	17544303
Capto DEAE	5 L	17544304
Capto DEAE	10 L	17544305
Capto DEAE	60 L	17544360

Capto S ImpAct for IEX		
Capto S ImpAct	25 mL	17371701
Capto S ImpAct	100 mL	17371702
Capto S ImpAct	1 L	17371703
Capto S ImpAct	5 L	17371704
Capto S ImpAct	10 L	17371705
Capto S ImpAct	60 L	17371760

Accessories

Slurry concentration kit		29096100
Media Wand 50 resin handling unit		28922767
Media Wand 100 resin handling unit		28922764
Media handling unit		28922769
AxiChrom column controller		29717831
Resin mixer 500 L ASME		29404714
Resin mixer 1250 L ASME		29404715
Resin mixer 2000 L ASME		29404716

Chromatography systems		
ÄKTA process DCS		29692670
ÄKTA process CFG		29729595
ÄKTA avant 25		28930842
ÄKTA avant 150		28976337
ÄKTA pilot 600S		29274325
ÄKTA pilot 600R		29743856

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