cytiva

Butyl **Sepharose** 4 Fast Flow
Octyl **Sepharose** 4 Fast Flow

Hydrophobic interaction resin

Instructions for Use

Butyl Sepharose™ 4 Fast Flow and Octyl Sepharose 4 Fast Flow form part of the Cytiva resin range for hydrophobic interaction chromatography (HIC).

These instructions contain information about resin characteristics, column packing, and maintenance.

Table of Contents

Read these instructions carefully before using the products.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

1 Introduction

BioProcess™ resins

Butyl Sepharose 4 Fast Flow and Octyl Sepharose 4 Fast Flow belong to the BioProcess resins. BioProcess chromatography resins are developed and supported for production-scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production-scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

Description

The base matrix, Sepharose 4 Fast Flow, is a cross-linked, 4% agarose derivative with excellent kinetics, making them ideal for process scale applications, particularly during initial capture and intermediate stages of a separation process when high flow rates are required.

The high physical and chemical stabilities of the matrix prevent bed compression and formation of fines, and allow efficient maintenance procedures for increased resin life time. A typical pressure/flow curve is shown below for Butyl Sepharose 4 Fast Flow.

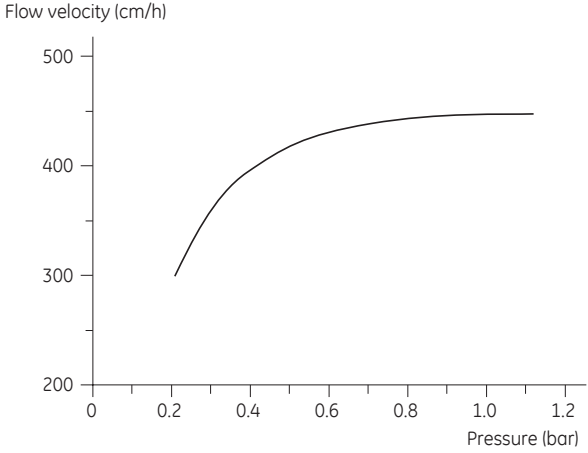


Fig 1. Typical pressure/flow curve for Butyl Sepharose 4 Fast Flow in an XK 50/30 column, bed height 15 cm; mobile phase 0.1 M NaCl.

Table 1. Characteristics of Butyl Sepharose 4 Fast Flow.

Matrix	Cross-linked agarose, 4%, spherical
Type of ligand	Butyl: R-O-CH_2-CH(OH)-CH_2-O-$(CH_2)_3$-CH_3
Particle Size, d_{50v} [1]	~ 90 µm
Ligand concentration	~ 40 µmol Butyl/mL resin
pH stability, operational [2]	3 to 13
pH stability, CIP [3]	2 to 14
Chemical Stability	Stable to commonly used aqueous buffers, 1 mM HCl, 1.0 M NaOH[4], 30% isopropanol, 70% ethanol, 6 M guanidine hydrochloride
Autoclavability	20 min at 121°C in distilled water pH 7, 5 cycles

Pressure/flow characteristics	150 to 250 cm/h at < 0.1 MPa in a XK 50/60 column with 5 cm diameter and 25 cm bed height (at 20°C using buffers with the same viscosity as water).[5] [6]
Operating temperature	4°C to 40°C
Delivery conditions	20% ethanol

1 Median particle size of the cumulative volume distribution.

2 pH range where resin can be operated without significant change in function.

3 pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

4 1.0 M NaOH must only be used for cleaning purposes.

5 The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.

6 Pressure/flow test performed on the base matrix.

Table 2. Characteristics of Octyl Sepharose 4 Fast Flow

Matrix	Cross-linked agarose, 4%, spherical
Type of ligand	Octyl: R-O-CH_2-CH(OH)-CH_2-O-$(CH_2)_7$-CH_3
Particle Size, d_{50v} [1]	~ 90 µm
Ligand concentration	~ 5 µmol Octyl/mL resin
pH stability, operational [2]	3 to 13
pH stability, CIP [3]	2 to 14
Chemical Stability	Stable to commonly used aqueous buffers, 1 mM HCl, 1.0 M NaOH[4], 30% isopropanol, 70% ethanol, 6 M guanidine hydrochloride
Autoclavability	20 min at 121°C in distilled water pH 7, 5 cycles
Pressure/flow characteristics	150 to 250 cm/h at < 0.1 MPa in a XK 50/60 column with 5 cm diameter and 25 cm bed height (at 20°C using buffers with the same viscosity as water).[5] [6]
Operating temperature	4°C to 40°C
Delivery conditions	20% ethanol

1 Median particle size of the cumulative volume distribution.

2 pH range where resin can be operated without significant change in function.

3 pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

[4] 1.0 M NaOH must only be used for cleaning purposes.

[5] The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.

[6] Pressure/flow test performed on the base matrix.

Method design and optimization

The main purpose of optimizing a chromatographic step is to reach the predefined purity level with highest possible product recovery by choosing the most suitable combination of the critical chromatographic parameters. In process chromatography, in contrast to analytical or small-scale preparative chromatography, this has to be accomplished as quickly and economically as possible. This means finding the conditions that give the highest possible productivity and process economy.

Recommendations for optimizing the critical operational parameters which affect the maximum utilization of a HIC step can be found in the handbook (11001269), available from your local Cytiva office.

Resin screening

Table 3, on page 6 shows the kits that help with screening and selection of resin.

Table 3.

Screening kit	Description
PreDictor™ HIC Screening	Phenyl Sepharose 6 Fast Flow (low sub)
High Hydrophobicity	Capto™ Butyl
	Phenyl Sepharose Fast Flow (high sub)
	Capto Phenyl (high sub)

Screening kit	Description
PreDictor HIC Screening Low Hydrophobicity	Butyl-S Sepharose 6 Fast Flow Octyl Sepharose 4 Fast Flow Butyl Sepharose 4 Fast Flow Capto Octyl
HiTrap™ HIC Test Kit 7×1 mL columns	Phenyl Sepharose 6 Fast Flow (high sub) Phenyl Sepharose 6 Fast Flow (low sub) Phenyl Sepharose High Performance Butyl Sepharose 4 Fast Flow Butyl-S Sepharose 6 Fast Flow Octyl Sepharose 4 Fast Flow

2 Column packing guidelines

General

Purifying biological macromolecules by HIC is a typical high selectivity technique where the difference in retention for the molecules to be separated can be substantial at any specific ionic strength.

Therefore, relatively short columns can be used if the selectivity of the adsorbent is exploited in an optimal way. Typical bed heights range from 3 to 15 cm, which will minimize back pressure and allow high throughput.

Recommended columns

Lab-scale columns:

- Tricorn™ 5/50 (5 mm i.d.) for bed volumes 0.2 to 1.1 mL at bed heights between 0.8 to 5.6 cm.

- Tricorn 5/100 (5 mm i.d.) for bed volumes 1.2 to 2.1 mL at bed heights between 5.8 to 10.6 cm.
- Tricorn 5/150 (5 mm i.d.) for bed volumes 2.1 to 3.1 mL at bed heights between 10.8 to 15.6 cm.
- Tricorn 10/50 (10 mm i.d.) for bed volumes up to 4.4 mL at bed heights up to 5.6 cm.
- Tricorn 10/100 (10 mm i.d.) for bed volumes 3.6 to 8.4 mL at bed heights between 4.6 to 10.6 cm.
- Tricorn 10/150 (10 mm i.d.) for bed volumes 7.6 to 12.6 mL at bed heights between 9.6 to 15.6 cm.
- XK 16/20 (16 mm i.d.) for bed volumes up to up to 31 mL at bed heights up to 15.5 cm.
- XK 26/20 (26 mm i.d.) for bed volumes up to 66 mL at bed heights up to 12.5 cm.
- XK 50/20 (50 mm i.d.) for bed volumes up to 274 mL at bed heights up to 14 cm.
- XK 50/30 (50 mm i.d.) for bed volumes up to 559 mL at bed heights up to 28 cm.
- HiScale™ 50/20 (50 mm i.d.) for bed volumes up to 393 mL at bed heights up to 20 cm.
- HiScale 50/40 (50 mm i.d.) for bed volumes 274 to 785 mL at bed heights between 14 to 40 cm.

Large-scale columns:

- AxiChrom™, i.d. 50 to 200 mm, bed volumes up to 16.7 L, bed heights up to 50 cm
- AxiChrom, i.d. 300 to 1600 mm, bed volumes up to 1005 L, bed heights up to 50 cm

- BPG, variable bed, glass columns: inner diameters from 100 to 450 mm, bed volumes up to 130 L, bed heights max 58 cm.
- Chromaflow™, variable and fixed bed columns. Inner diameters from 280 to 2000 mm.

Packing XK columns

Material needed

- Butyl Sepharose 4 Fast Flow or Octyl Sepharose 4 Fast Flow
- XK 16/20 column
- HiLoad™ Pump P-50
- Gradient maker
- Injection valve (LV-3 or LV-4)
- Graduated cylinder or beaker
- Vacuum flask and pump
- 5 mL syringe
- Glass rod
- Packing buffer

Note: *The packing buffer must be the same as the binding buffer. See , for buffer recommendations. High viscosity buffers must not be used during packing. If such buffers are required for the separation, equilibrate the column in the high viscosity buffer at a reduced flow rate when packing is completed.*

Preparing the resin

Step	Action
1	Equilibrate all material to room temperature.
2	Sepharose Fast Flow HIC resins are supplied preswollen in 20% ethanol. Decant the ethanol solution and replace it with packing buffer to a total volume of 32.5 mL (75% settled resin: 25% buffer).
3	De-gas the slurry under vacuum.

Assembling the column

Details of the column parts can be found in the instructions supplied with the column. Before packing, make sure that all parts, particularly the nets, net fasteners and glass tube, are clean and intact.

Step	Action
1	Connect the column bottom end piece to a pump or syringe.
2	Submerge the end piece in buffer and fill it using the pump or syringe. Make sure that there are no air bubbles trapped under the net.
3	Close the tubing with a stopper and attach the end piece on the column.
4	Flush the column with buffer, leaving a few mL at the bottom.
5	Attach the column vertically on a laboratory stand.

Packing the column

These instructions are based for packing Sepharose Fast Flow HIC resin in the recommended XK 16/20 Column. To modify these instructions for columns of different dimensions, refer to Appendix A.

Step	Action
1	Pour the resin slurry into the column in one continuous motion. Pouring down a glass rod held against the wall of the column helps prevent the introduction of air bubbles. Fill the remainder of the column with buffer.
2	Wet the column adapter by submerging the plunger end in buffer, and drawing buffer through with a syringe or pump. Make sure that all bubbles have been removed. Disconnect the pump or syringe.
3	Insert the adapter into the top of the column at an angle, taking care not to trap air under the net. Tighten the adapter O-ring to give a sliding seal on the column wall.
4	Fit a syringe barrel to the sample application valve and connect the valve between the adapter and the pump. With the valve in the sample application position, slide the adapter down into the column. This will displace all air in the tubing as far as the sample application valve. Switch the valve a few times to remove any trapped bubbles. Continue inserting the adapter until it reaches the resin slurry. Tighten the O-ring and lock the adapter in position.

Step	Action
5	Open the bottom outlet of the column and start the pump. Pack the resin at a flow rate of 12 to 14 mL/min until the bed height is constant (normally 4 to 5 minutes).
6	Stop the pump, close the column outlet, loosen the adapter O-ring to give a sliding seal and reposition the adapter on the surface of the resin bed. Press the adapter into the surface of the resin an additional 1 to 2 mm.
7	Lock the adapter in position, open the column outlet and start the pump at the column packing flow rate.

If the bed continues to pack, repeat step 5. When the resin bed is stable, the column is packed, equilibrated and ready for use.

Equilibration

To equilibrate, pump approximately 100 mL of start buffer through the column at a flow rate of 2.5 mL/min. The column is fully equilibrated when the pH and/or conductivity of the effluent is the same as the start buffer.

Sample preparation

The amount of sample that can be applied to the column differs considerably, depending on the ligand concentration of the resin, the nature of the sample, and on start buffer conditions. See *Table 1, on page 4* and *Table 2, on page 5* for capacity guidelines.

High ligand concentration does not necessarily correspond to high capacity for adsorption of protein, but a high ligand concentration can encourage multipoint attachment of proteins which otherwise can have difficulty adsorbing to lower ligand concentrations. A moderate ligand concentration allows selective binding of the protein of interest by adjustment of the binding buffer concentration.

The sample must be dissolved in start buffer. Alternatively the sample can be transferred to start buffer by dialysis or by buffer exchange using a HiTrap Desalting or a PD-10 Desalting column. The viscosity of the sample must not exceed that of the buffer. For normal aqueous buffer systems, this corresponds to a protein concentration of approximately 50 mg/mL.

Before application the sample must be centrifuged or filtered through a 0.45 µm filter to remove any particulate matter.

Operating flow rates

The flow rate used for sample binding and subsequent elution will depend on the degree of resolution required, but is normally within the range of 2.5 to 5 mL/min. A lower flow rate gives a better resolution.

Binding

The binding of proteins to hydrophobic resins is influenced by:

- the structure of the ligand (for example carbon chain or an aromatic ligand)
- the ligand concentration
- the ionic strength of the buffer
- the salting-out effect (see The Hofmeister series below)
- the temperature

Salts that cause salting-out (for example ammonium sulphate) also promote binding to hydrophobic ligands. The column is equilibrated and the sample is applied in a solution of high ionic strength. A typical starting buffer is 1.7 M $(NH_4)_2SO_4$, which is just below the concentration employed for salting out proteins.

Hydrophobic interactions are weaker at lower temperatures. This must be taken into account if chromatography is performed in a cold room.

Elution

Bound proteins are eluted by reducing the strength of the hydrophobic interaction. This can be done by:

- reducing the concentration of salting-out ions in the buffer with a decreasing salt gradient (linear or step)
- increasing the concentration of chaotropic ions in the buffer with an increasing gradient (linear or step)
- eluting with a polarity-reducing organic solvent (for example ethylene glycol) added to the buffer
- eluting with detergent added to the buffer

The Hofmeister series

	← Increasing precipitation ("salting-out") effect
Anions:	PO_4^{3-} SO_4^{2-} CH_3COO^- Cl^- Br^- NO^{3-} ClO^{4-} I^- SCN^-
Cations:	NH^{4+} Rb^+ K^+ Na^+ Cs^+ Li^+ Mg^{2+} Ba^{2+}
	Increasing chaotropic ("salting-in") effect →

Increasing the salting-out effect strengthens hydrophobic interactions; increasing the chaotropic effect weakens hydrophobic interactions.

A suggested starting gradient is a linear gradient from 0 to 100% B with:

Buffer A: 50 mM phosphate buffer, pH 7.0 + 1.7 M $(NH_4)_2SO_4$ [1]

Buffer B: 50 mM phosphate buffer, pH 7.0

Packing process-scale columns

General packing procedures

Columns can be packed in different ways depending on the type of column and equipment used. Always read and follow the relevant column instruction manual carefully. Sepharose 4 Fast Flow based resins are easy to pack since their rigidity allows the use of high flow velocities, see *Figure 1, on page 4*.

General parameters for large-scale packing:

- Preferred packing solution: 10% to 20% ethanol
- Resin slurry concentration: 50%
- Packing pressure: 0.1 MPa (1 bar, 14.5 psi)
- Packing flow velocity: 200 to 300 cm/h

Two types of packing methods are described:

- Pressure packing (for columns with adapters)
- Chromaflow packing

How well the column is packed will have a major effect on the result of the separation. It is therefore very important to pack and test the column according to the following recommendations.

[1] When working with proteins which have a tendency to aggregate, start with a lower $(NH_4)_2SO_4$ concentration to avoid protein precipitation.

Begin the packing procedure by determining the optimal packing flow rate. Guidelines are given below for determining the optimal packing flow rates for columns with adapters and fixed bed heights.

Determining optimal packing flow rates

The optimal packing flow rate is dependent on column size and type, resin volume, packing solution, and temperature. The optimal packing flow rate must therefore be determined empirically for each individual system.

To determine the optimal packing flow rate, proceed as follows:

Step	Action
1	Calculate the amount of resin needed for the slurry (this is especially important for columns with fixed bed heights). The quantity of resin required per liter packed volume is approximately 1.15 L sedimented resin.
2	Prepare the column exactly as for column packing.
3	Begin packing the resin at a low flow velocity (30 cm/h).
4	Increase the pressure in increments and record the flow rate when the pressure has stabilized. Do not exceed the maximum pressure of the column, or the maximum flow rate for the resin.
5	The maximum flow rate is reached when the pressure/flow curve levels off or the maximum pressure of the column is reached. Stop the packing and do not exceed this flow rate. The optimal packing flow rate/pressure is 70% to 100% of the maximum flow rate/pressure.

Step	Action
6	Plot the pressure/flow curve as in *Figure 1, on page 4* and determine the optimal packing flow rate.

The operational flow rate/pressure must be < 70% of the packing flow rate/pressure.

Packing AxiChrom columns

AxiChrom columns are packed using mechanical axial compression packing. For more information regarding the AxiChrom columns and packing procedures see below documentation:

- *AxiChrom Columns* (28929041)
- *AxiChrom 50, 70 and 100 columns, Operating instructions* (28933108)
- *AxiChrom 140 and 200 columns, Operating instructions* (28943123)
- *AxiChrom 300-1600 columns, Operating instructions* (29065430)

Pressure packing of BPG columns

BPG glass columns are supplied with a movable adapter. They are packed by conventional pressure packing by pumping the packing solution through the chromatographic bed at a constant flow rate (or back pressure).

Step	Action
1	Pour some water (or packing solution) into the column. Make sure that there is no air trapped under the bottom net. Leave about 2 cm of liquid in the column.

Step	Action
2	Mix the packing buffer with the resin to form a 50% to 70% slurry. (Sedimented bed volume/slurry volume = 0.5 to 0.7). Pour the slurry into the column. Insert the adapter and lower it to the surface of the slurry, making sure no air is trapped under the adapter. Secure the adapter in place.
3	Seal the adapter O-ring and lower the adapter a little further into the slurry to fill the adapter inlet with packing solution.
4	Connect a pump and a pressure gauge, then start packing at the predetermined packing flow rate (or pressure). Keep the flow rate (or pressure) constant during packing and check the pressure at the column inlet. Never exceed the pressure limit for column or resin.
5	When the bed has stabilized, mark the bed height on the column tube, close the bottom valve and stop the pump. The bed starts rising in the column. Loosen the O-ring and lower the adapter to 0.5 to 1 cm from the bed surface.
6	Seal the O-ring, start the pump and continue packing. Repeat steps 5 and 6 until there is a maximum of 1 cm between bed surface and adapter when the bed has stabilized.

Step	Action
7	Close the bottom valve, stop the pump, disconnect the column inlet and push the adapter down to approximately 3 mm below the mark on the column tube without loosening the adapter O-ring. The packing solution will flush the adapter inlet. Remove any trapped air by pumping liquid from the bottom (after the inlet tubing and the bottom valve have been properly filled).

Packing Chromaflow columns

Prepare the column for packing as described in the *Operating instructions*.

This section describes how to pack from the top, or from below.

To pack from the top, follow these instructions:

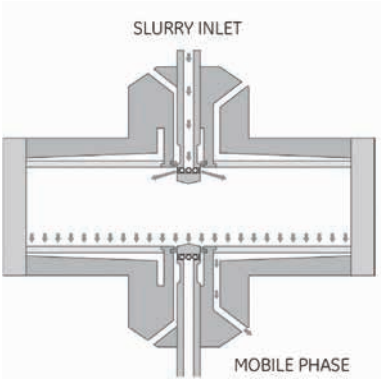
Step	Action
1	Set the top nozzle to the pack position (mid-position).
2	Fully retract the bottom nozzle (run position).
3	Make sure that the top mobile phase is closed.
4	Open the bottom mobile phase.

Step	Action
5	Open Inlet **(C)** and start the packing pump. Adjust the flow to achieve the required packing conditions for the selected resin. Monitor column pressure and the outlet flow rate in order to record column packing parameters. Remember to stir the resin slurry during packing to prevent it from settling.
6	Continue pumping until the column is fully packed and the pump stalls due to buildup of resin in its pipelines. Turn off the packing pump.
7	Fully retract the top nozzle to its run position. Close Outlet **(C)**. Open Inlet **(B)** from the water/buffer tank and open Outlet **(D)**. The pump must now be restarted to rinse the top slurry lines. If the nozzle is full of liquid when in the packing position, make sure that the waste slurry outlet is open before retracting the nozzle.
8	To clean-in-place, exchange the buffer tank for wash/ buffer tank containing cleaning solution.

To pack from below, carry out the same procedure for the connections and flow path via the bottom nozzle. The column is now ready to equilibrate and test.

Note: *It is also possible to use a slightly different packing method where the amount of resin is packed into the column causing compression of the bed. When all resin has entered the column the pump is stopped, the top nozzle is retracted, the bottom mobile phase valve closed, and the resin is allowed to decompress within the column.*

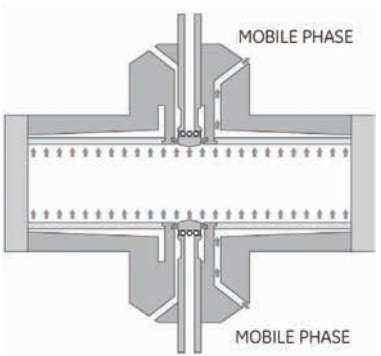
The illustration below shows the principles of operating Chromaflow columns.



Packing position

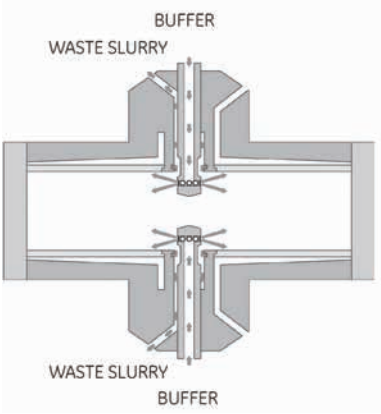
The top nozzle is extended part of the way (mid position) into the column. The bottom nozzle is fully retracted.

Slurry enters the column via the top nozzle and excess liquid exits via the bottom mobile phase outlet. After packing, the slurry lines are isolated from the mobile phase and can be cleaned independently from the rest of the column.



Running position

The bottom and top nozzles are retracted. Mobile phase enters the column directly into an annulus, immediately behind the bed support. The annulus is cut through at an angle to make sure that linear flow rate is kept constant during distribution of the mobile phase across the bed.



Unpacking position

In this position, both bottom and top nozzles are fully extended into the column, thereby exposing a third passage through which resin leaves the column.

Cleaning solution can be pumped through the nozzles and sprayed into the column. In this way the column is easily and effectively cleaned without exposing the interior or the resin to the environment, or without dismantling the column.

3 Evaluation of packed column

Introduction

The packing quality needs to be checked by column efficiency testing. The test must be done after the packing and at regular intervals during the working life of the column and also when the separation performance is seen to deteriorate.

Column efficiency testing

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). The values are easily determined by applying a test sample such as 1% acetone solution or sodium chloride to the column.

Note: *Use a concentration of 0.8 M NaCl in water as sample and 0.4 M NaCl in water as eluent.*

The calculated plate number is depended on the test conditions and must only be used as a reference value. It is important that the test conditions and the equipment are the same so that the results are comparable.

Note: *Changing the solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, chromatography system, etc., influence the results.*

For more information about column efficiency testing, consult the application note (28937207).

For optimal column efficiency results, the sample volume must be approximately 1% of the column volume and the flow velocity 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for the column use.

Sample volume and flow velocity

For optimal column efficiency results, the sample volume must be approximately 1% of the column volume and the flow velocity 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for the column use.

Method for measuring HETP and A_S

Calculate HETP and A_S from the UV curve (or conductivity curve) as follows:

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

L = bed height (cm)

N = number of theoretical plates

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

V_R = volume eluted from the start of sample application to the peak maximum.

W_h = peak width measured as the width of the recorded peak at half of the peak height.

V_R and W_h are in the same units.

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height, h, is calculated as follows:

$$h = \frac{HETP}{d_{50v}}$$

d_{50V} = Median particle size of the cumulative volume distribution (cm)

As a guideline, a value of < 3 is very good.

The peak must be symmetrical, and the asymmetry factor as close to 1 as possible. A typical acceptable range could be 0.8 < A_S < 1.5.

A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use.

Peak asymmetry factor calculation:

$$A_s = \frac{b}{a}$$

a = ascending part of the peak width at 10% of peak height

b = descending part of the peak width at 10% of peak height

The Figure below shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_S values are calculated.

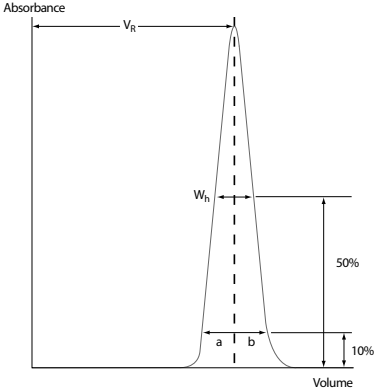


Fig 2. A typical test chromatogram showing the parameters used for HETP and A_S calculations.

4 Maintenance

Regeneration

For best performance from the resins, bound substances must be washed from the column after each chromatographic cycle.

Wash with 2 bed volumes of water, followed by 2 to 3 bed volumes of starting buffer.

To prevent a slow build up of contaminants on the column over time, it is possible that more rigorous cleaning protocols have to be applied on a regular basis.

Cleaning-in-place (CIP)

Cleaning-in-place (CIP) is the removal of very tightly bound, precipitated or denatured substances from the purification system generated in previous purification cycles. If such contaminants accumulate on the column, they can affect the chromatographic properties of the column. If the fouling is severe, it can also block the column, increasing back pressure and reducing flow rate.

The following are suggested methods to remove strongly bound hydrophobic proteins, lipoproteins, and lipids:

- Wash the column with 4 to 10 bed volumes of up to 70% ethanol or 30% isopropanol followed by 3 to 4 bed volumes of water. Apply gradients to avoid air bubble formation when using high concentrations of organic solvents.
- Alternatively, wash the column with 1 to 2 bed volumes of 0.5% nonionic detergent followed by 5 bed volumes of 70% ethanol to remove the detergent, and 3 to 4 bed volumes of water.

CAUTION
70% ethanol can require the use of explosion-proof areas and equipment.

To remove other contaminants the following method is suggested:

- Wash the column with 4 bed volumes of 0.5 to 1.0 M NaOH at 40 cm/h, followed by 2 to 3 bed volumes of water.

The CIP protocols given above must be used as guidelines when formulating a cleaning protocol specific for the raw material used. The frequency of CIP will depend on the raw material applied to the column, but it is recommended to use a CIP procedure at least every 5 cycles during normal use. Depending on the nature of the contaminants, it can be necessary to use a combination of different protocols. If fouling is severe, it can be necessary to further optimize the protocols. During CIP flow direction through the column must be reversed.

Sanitization

Sanitization is the reduction of microbial contamination in the column and related equipment to an acceptable minimum. A specific sanitization protocol must be designed for each process according to the type of contaminants present. The following is a recommended protocol.

Wash the column with 0.5 to 1.0 M NaOH at a flow velocity of approximately 40 cm/h, contact time 30 to 60 minutes.

Sterilization

To sterilize Butyl Sepharose 4 Fast Flow or Octyl Sepharose 4 Fast Flow, dismantle the column and autoclave the resin for 20 minutes at 121°C.

Storage

Store Butyl Sepharose 4 Fast Flow and Octyl Sepharose 4 Fast Flow in 20% ethanol at 4°C to 30°C, to avoid microbiological growth.

5 Reference information

This chapter lists product codes for products, handbooks, and data files that relate to Butyl Sepharose 4 Fast Flow and Octyl Sepharose 4 Fast Flow. For further information, see *cytiva.com*

Product	Quantity	Product code
Butyl Sepharose 4 Fast Flow	25 mL	17098010
	200 mL	17098001
	500 mL	17098002
	5 L	17098004
	10 L	17098005
Octyl Sepharose 4 Fast Flow	25 mL	17094610
	200 mL	17094602
	1 L	17094603
	5 L	17094604
PreDictor Screening High Hydrophobicity	6 µL	28992392
PreDictor Screening High Hydrophobicity	50 µL	28992397
PreDictor Screening Low Hydrophobicity	6 µL	28992395
PreDictor Screening High Hydrophobicity	50 µL	28992398
HiTrap HIC Test Kit	7 x 1 mL	28411007
HiTrap Butyl FF	5 x 1 mL	17135701
	5 x 5 mL	17519701
HiTrap Octyl FF	5 x 1 mL	17135901
	5 x 5 mL	17519601
HiPrep 16/10 Butyl FF	20 mL	17509601
HiPrep 16/10 Octyl FF	20 mL	17509701
Tricorn 5/50	1	28406409
Tricorn 5/100	1	28406410
Tricorn 5/150	1	28406411
Tricorn 10/50	1	28406414
Tricorn 10/100	1	28406415

Product	Quantity	Product code
Tricorn 10/150	1	28406416
XK 16/20	1	18877301
XK 26/20	1	18100072
XK 50/20	1	28988952
HiScale 50/20	1	28964445
HiScale 50/40	1	28964444

Related literature		Product code
Handbook	*Hydrophobic Interaction Chromatography and Reversed Phase Chromatography: Principles and Methods*	11001269
Data files	HiTrap HIC Selection Kit	17114321
	HiScreen Prepacked Columns	28930581
	Butyl Sepharose 4 Fast Flow	18102017
	BPG 100, 140, 200, 300, 450	18111523
	Chromaflow	18113892
	AxiChrom Columns	28929041
	Media Wand Media handling Unit	28923101
Instructions	Predictable scale-up through column design and robust packing methodology	28949052
	Constant Flow Packing method	29001795
	Pack-in-place packing procedure	29001797

The complete range of Sepharose Fast Flow resins includes other HIC resins as well as resins for ion exchange and affinity chromatography. Further information is available upon request.

Appendix A

Converting to columns of different dimensions

Flow rates

Flow rates quoted in this instruction are for an XK 16/20 column. To convert flow rates for columns of different dimensions:

1. Divide the volumetric flow rates (mL/min) quoted by a factor of 2 (the cross-sectional area in cm^2 of the XK 16/20) to give the flow velocity in cm/min.
2. Maintain the same flow velocity and calculate the new volumetric flow rate according to the cross-sectional area of the specific column to be used

$$\text{Flow velocity} = \frac{\text{Volumetric flow rate}}{\text{Column cross-sectional area}}$$

Volumes

Volumes (buffers, gradients, etc.) quoted in this instruction are for an XK 16/20 column that has a bed volume of 20 mL (bed height x cross-sectional area). To convert volumes for columns of different dimensions, increase or decrease in proportions to the new column bed volume.

$$\text{New volume} = \text{Old volume} \times \frac{\text{New bed volume}}{\text{Old bed volume}}$$

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