SP Sepharose™ High Performance

SP Sepharose High Performance is a BioProcess™ chromatography medium (resin) with a well-deserved reputation as a highly successful cation ion exchange medium for purifying a wide range of biomolecules. It shares an impressive list of operational characteristics that includes:

- High-resolution, high-capacity separations with high recovery
- Reliable and reproducible
- High chemical stability for effective CIP/sanitization
- Available in different convenient prepacked formats, such as PreDictor™ 96-well filter plates, PreDictor Robocolumn™, HiTrap™, HiScreen™, and HiPrep™ columns
- Easy to scale up

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Please read these instructions carefully before using the products.

Safety

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

1 BioProcess™ media

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

2 Medium characteristics

SP Sepharose High Performance is a strong cation exchanger based on rigid, highly cross-linked, beaded agarose with a mean particle size of 34 µm. The ion exchange group is a sulphopropyl group, see Figure 1, which remains charged and maintains consistent ionic capacity over the entire working range, pH 4 to 13. The ionic exchange groups are coupled to the base matrix through chemically stable ether bonds.

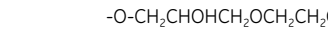

Fig 1. Partial structure of SP Sepharose High Performance

The high ionic capacity of SP Sepharose High Performance is illustrated by the titration curve in Figure 2. Total capacity is 0.15–0.20 mmol/ml medium. Dynamic capacity is in the range 50–100 mg/ml medium, for example, binding capacity for Ribonuclease is approx. 55 mg/ml medium (column diameter: 5 mm, bed height: 5 cm, sample concentration: 5 mg Ribonuclease/ml, and buffer: 100 mM sodium acetate, pH 6.0).

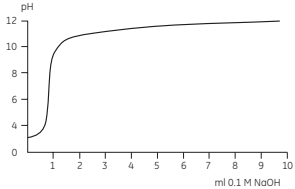

Fig 2. Titration curve. Approx 5 ml of SP Sepharose High Performance medium in 50 ml 1 M HCl..

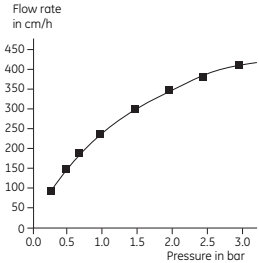

Fig 3. Pressure/flow rate curves for SP Sepharose High Performance. BPG 200/500 column, bed height 10 cm, distilled water.

SP Sepharose High Performance has excellent physical and chemical stabilities, see Table 1. Strong oxidizing agents, however, should be avoided. This medium combines good kinetics with high physical stability to give excellent performance even at flow rates up to 100 cm/h in columns with 10–15 cm bed height. The pressure/flow rate curve is shown in Figure 3.

Table 1. Characteristics of SP Sepharose High Performance

Matrix	6% spherical, cross-linked agarose
Functional group	$CH_2CH_2CH_2SO_3^-$, sulphopropyl
Total ionic capacity	0.15 to 0.20 mmol (H^+)/ml medium
Average particle size (d_{50v}) [1]	34 µm
Recommended operational flow velocity	Up to 150 cm/h
Binding capacity	Approx. 70 mg BSA/ml medium
pH stability [2]	
Working range	4 to 13
Cleaning-in-place	3 to 14
Working temperature	4°C to 30°C
Chemical stability	1 M sodium hydroxide, 1 M acetic acid, 8 M urea, 6 M guanidine hydrochloride, 30% acetonitrile, 30% isopropanol, 70% ethanol and 2% SDS
Avoid	Oxidizing agents, cationic detergents
Storage	20% ethanol, 0.2 M sodium acetate

[1] d_{50v} is the median particle size of the cumulative volume distribution.

[2] Working range: pH interval where the medium can be operated without significant change in function.

Cleaning-in-place: pH stability where the medium can be subjected to cleaning-in-place without significant change in function.

3 Method optimization

The aim of designing and optimizing an ion exchange separation process is to identify conditions that promote binding of the highest amount of target molecule, in the shortest possible time with highest possible product recovery and purity. Design of the method in laboratory scale.

For certain proteins, dynamic binding capacities increase at increased conductivity and this is pH dependent. Therefore, scouting of both pH and conductivity for optimal dynamic binding conditions on SP Sepharose High Performance is recommended. Flow velocity can also be included in the scouting.

Elution of protein can either be done by use of salt, pH or a combination of both. For optimization of the elution, sample load, flow velocity and gradient volume should be considered. The three factors are interrelated and best results will be obtained using:

- Maximized sample load with respect to dynamic binding capacity.
- Maximized flow velocity with respect to system constraints and media rigidity.
- The gradient volume that provides the best resolution with maximized sample load and maximized flow velocity.

The use of PreDictor plates is preferentially included in the method development. The PreDictor plates are 96-well filter plates pre-filled with chromatography media, which can be used for rapid screening of chromatographic conditions in small scale. The suggested workflow with PreDictor plates is shown in Figure 4, where a large design space can be explored prior to further experiments in packed column formats, such as prepacked HiScreen columns.

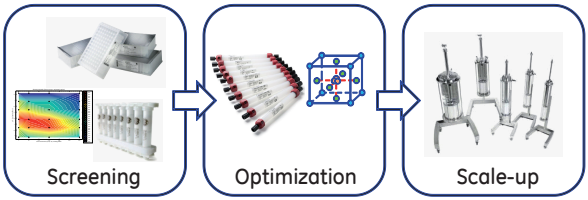

Fig 4. The recommended workflow is described in the figure. It starts with screening of conditions in high throughput formats, followed by optimization, preferably by applying Design of Experiments (DoE), in small columns and finally scale-up to large columns.

Table 2. The experimental conditions to consider when designing and optimizing the process.

Phases	Activity	Conditions to consider
1. Equilibration of column and sample preparation	Equilibration of column and adjustment of sample	• pH • Conductivity • Column volume • Column bed height • Particle content • Temperature
2. Sample application	Manual or automatic application onto the column	• Flow rate • Sample pH • Sample conductivity • Upward/downward flow
3. Wash	Wash out unbound material with clean binding buffer	• Flow rate • Upward/downward flow • Buffer choice (normally same as column equilibration buffer)

Phases	Activity	Conditions to consider
4. Elution	Elute the material from the column either with salt or by change in pH	• Sample load • pH • Conductivity • Flow rate • Upward/downward flow

For more information about method development and optimization, consult the handbooks, *Ion exchange Chromatography & Chromatofocusing: Principles and Methods*, (11-0004-21) and *High throughput process development with PreDictor plates*, (28-9403-08).

4 Scale-up

After optimizing the method at laboratory scale, the process can be scaled up. Scale-up is typically performed by keeping bed height and flow velocity constant while increasing bed diameter and volumetric flow rate. However, since optimization is preferentially performed with small column volumes, in order to save sample and buffer, some parameters such as the dynamic binding capacity may be optimized using shorter bed heights than those being used in the final scale. As long as the residence time is kept constant, the binding capacity for the target molecule remains the same.

Other factors, such as clearance of critical impurities, may change when column bed height is modified and should be validated using the final bed height. The residence time is approximated as the bed height (cm) divided by the linear flow velocity (cm/h) applied during sample loading.

Procedure

Step	Action
1	Select bed volume according to required binding capacity. Keep sample concentration and gradient slope constant.

Step	Action
2	Select column diameter to obtain the bed height (10 to 40 cm) from method optimization. ***Note:*** *The excellent rigidity of the high flow base matrix allows for flexibility in choice of bed heights.*
3	The larger equipment used when scaling up may cause some deviations from the method optimized at small scale. In such cases, check the buffer delivery and monitoring systems for time delays or volume changes. Different lengths and diameters of outlet tubing can cause zone spreading on larger systems. Check also the compatibility of the hardware and chromatography media pressure limits with expected pressure during packing and operation.

5 Packing HiScale™ and XK columns

Introduction

The following instructions are for packing HiScale 16/20, HiScale 26/20, XK 16/20 and XK 26/20 with 10 cm bed height.

For more details about packing HiScale columns, see instructions *HiScale columns (16, 26, 50) and accessories* (28-9674-70).

For more details about packing XK columns, see Instruction 28-9920-23.

Materials needed

- SP Sepharose High Performance
- HiScale column or XK column
- HiScale packing tube
- Plastic spoon or spatula
- Glass filter G4
- Vacuum suction equipment
- Filter flask
- Measuring cylinder
- Distilled water

Equipment

- Chromatography system, such as ÄKTA™ system, or a stand-alone pump such as Pump P-900, depending on the flow rate required, can be used for packing.
- Pressure monitor

Equilibrate all materials to room temperature.

Preparation of the slurry

To measure the slurry concentration, let the medium settle in 20% ethanol at least overnight in a measuring cylinder or use the method for slurry concentration measurement described in application note 28-9259-32. This method can also be used for HiScale and XK columns.

Washing the medium

Mount a glass filter funnel onto a filtering flask. Suspend the medium by shaking and pour into the funnel and wash according to the following instructions:

Step	Action
1	Wash 5 times with 5 ml distilled water/ml medium.
2	Gently stir with a spatula between additions.
3	Move the washed medium from the funnel into a beaker and add distilled water to obtain a 50% slurry concentration.

Packing preparations

Step	Action
1	Mount the packing reservoir at the top of the column and rinse with distilled water.
2	Mount filter and bottom piece on the column.
3	Wet the bottom filter by injecting 20% ethanol through the effluent tubing.
4	Mount the column and packing reservoir vertically on a laboratory stand. Rinse them with distilled water.
5	Apply distilled water 2 cm over the column end piece and put a tubing clamp on the effluent tubing.
6	Pour all the separation media slurry into the column and packing reservoir and top up carefully with distilled water.

Packing procedure

Step	Action
1	Connect the pump outlet to the inlet on the packing reservoir and open the column outlet.
2	Pack the column with distilled water at a constant flow (see Table 3, Step 1) until the medium bed is stable.
3	Adjust the flow rate to 2x the final one (see Table 3, Step 2) and decrease it step-wise until the pressure signal is 480 ± 20 kPa. Pack the column at the flow rate which gives 480 ± 20 kPa for 45 minutes.
4	Dismount the packing reservoir.
5	Carefully fill the rest of the column with distilled water to form an upward meniscus at the top and insert the flow adapter. The adapter should be adjusted down to the bed surface.
6	Continue packing the column at 480 ± 20 kPa for 6 minutes.
7	Mark on the column the position of the bed surface, stop the pump, close the column outlet and adjust the adapter to the bed surface and then push the adapter a further 3 mm.

Table 3. Packing parameters

Column	Sedim.[1] medium (ml)	Slurry (ml)	Height (mm)	Step 1 (ml/min)	Step 2 (kPa)	Final flow rate (ml/min)
HiScale or XK 16/20	25	50	100	1.0	480±20	~12
HiScale or XK 26/20	66	132	100	2.5	480±20	~30

[1] Sedimented medium volume = 1.25 × Packed medium volume.

6 Evaluation of column packing

Intervals

Test the column efficiency to check the quality of packing. Testing should be done after packing and at regular intervals during the working life of the column and also when 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). These values are easily determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 0.8 M NaCl in water as sample and 0.4 M NaCl in water as eluent.

For more information about column efficiency testing, consult the application note *Column efficiency testing* (28-9372-07).

Note: *The calculated plate number will vary according to the test conditions and it should only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc. will influence the results.*

Sample volume and flow velocity

For optimal results, the sample volume should 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 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} = mean diameter of the beads (cm)

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

The peak should be symmetrical, and the asymmetry factor as close to 1 as possible (a typical acceptable range could be $0.8 < A_S < 1.8$).

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.

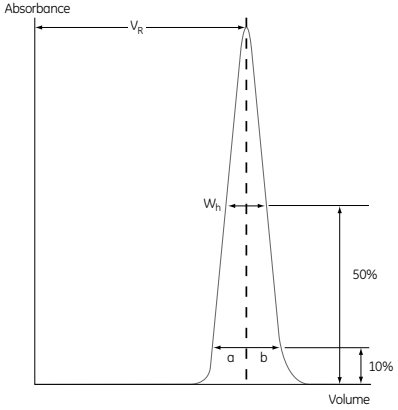

Fig 5. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

7 Maintenance

For best performance from SP Sepharose High Performance and to maximize the life time of the media, follow the procedures described below.

Equilibration

After packing, and before a chromatographic run, equilibrate with start buffer by washing with five bed volumes or until the column effluent shows stable conductivity and pH values. The equilibration step can be shortened by first washing with a high concentration buffer to obtain approximately the desired pH value and then washing with start buffer until the conductivity and pH values are stable.

Regeneration

After each separation, elute any reversibly bound material with a high ionic strength solution (e.g., 1 to 2 M NaCl in elution buffer). Regenerate the medium by washing with five bed volumes of start buffer or until the column effluent shows stable conductivity and pH values.

Cleaning-in-place (CIP)

Regular CIP prevents the build-up of contaminants in the packed bed and helps to maintain capacity, flow properties and general performance.

A specific CIP protocol should be designed for each process according to the type of contaminants present.

Precipitated, hydrophobically bound proteins or lipoproteins	Wash with 1 M NaOH solution with reversed flow direction. Contact time depends on the type of contaminant present.
Ionically bound proteins	Wash with 2 M NaCl with reversed flow direction. Contact time 1 to 2 h.
Lipids and very hydrophobic proteins	Wash with 2 to 4 column volumes of 0.5% non-ionic detergent (e.g., 1 M acetic acid) with reversed flow direction. Contact time 1 to 2 h. Alternatively, wash with 2 to 4 column volumes of up to 70% ethanol [1] or 30% isopropanol with reversed flow direction. Contact time 1 to 2 h.

[1] Specific regulations may apply when using 70% ethanol since the use of explosion-proof areas and equipment may be required.

Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5 to 1 M NaOH with a contact time of 1 h is recommended. The CIP protocol for precipitated, hydrophobically bound proteins or lipoproteins sanitizes the medium effectively.

Storage

Store in 20% ethanol, 0.2 M sodium acetate, at a temperature of 4°C to 30°C.

After storage, equilibrate with at least five column volumes of start buffer.

8 Ordering information

Product	Quantity	Code No
SP Sepharose High Performance	75 ml	17-1087-01
	1 l	17-1087-03
	5 l	17-1087-04
	10 l	17-1087-05

SP Sepharose High Performance is supplied in suspension in 20% ethanol, 0.2 M sodium acetate. For additional information, please contact your local GE Healthcare representative.

Related products

Product	Quantity	Code No
PreDictor RoboColumn SP Sepharose HP	8 × 200 µl	28-9861-04
PreDictor RoboColumn SP Sepharose HP	8 × 600 µl	28-9861-93
HiTrap SP HP	5 × 1 ml	17-1151-01
HiTrap SP HP	5 × 5 ml	17-1152-01
HiScreen SP HP	1 × 4.7 ml	28-9505-15
HiPrep SP HP 16/10	1 × 20 ml	29-0181-83
Tricorn™ 5/100 column	1	28-4064-10
Tricorn 10/100 column	1	28-4064-15
HiScale 16/20	1	28-9644-41
HiScale 16/40	1	28-9644-24
HiScale 26/20	1	28-9645-14
HiScale 26/40	1	28-9645-13
HiScale 50/20	1	28-9644-45
HiScale 50/40	1	28-9644-44

Accessories

Product	Quantity	Code No
Tricorn Glass Tube 5/100	1	18-1153-06
Tricorn Packing Connector 5-5	1	18-1153-21
Tricorn Packing Equipment 10/100	1	18-1153-25
Packing tube 20 (HiScale 16)	1	28-9868-16
Packing tube 40 (HiScale 16)	1	28-9868-15
Packing tube 20 (HiScale 26)	1	28-9803-83
Packing tube 40 (HiScale 26)	1	28-9645-05
Packing tube 20 (HiScale 50)	1	28-9802-51
Packing tube 40 (HiScale 50)	1	28-9645-06

Literature

Product	Code No
Data File: Q Sepharose High Performance, SP Sepharose High Performance	18-1172-88
Handbook: Ion Exchange Chromatography& Chromatofocusing, Principles and Methods	11-0004-21
Handbook: High throughput process development with PreDictor plates	28-9403-58
Instructions: Tricorn Empty High Performance Columns	28-4094-88
Instructions: HiScale columns (16, 26, 50) and accessories	28-9674-70
Application note: Column efficiency testing	28-9372-07

For local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com/bioprocess

First published Jan. 2011

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Limited
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Japan Corporation
Sanken Bldg. 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan

18-1060-26 AG 12/2014 a2040