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User Guide

Eshmuno® CPX Resin

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Labscale Column Packing

Introduction

A compression factor of 1.05 - 1.09 (it corresponds to 5 to 8% compression) is recommended for packing lab scale columns with an inner diameter ≤ 1.6 cm (1.05 compression factor is to be used if packing columns have a narrow inner diameter, e.g 0.66 cm) with Eshmuno® CPX resin. This compression is lower than pilot and process scale columns which are packed at a compression factor of 1.16 (it corresponds to ~14% compression), accounting for physical differences such as wall support and ensuring optimal packing results.

Materials

- Eshmuno® CPX resin
- Graduated cylinder
- Extension tube
- Packing buffer: 150 mM NaCl or any salt buffer
- Tracer solution

Compression and Resin Calculations

A compression factor (CF) of 1.05 – 1.09 is recommended for packing Eshmuno® CPX resin in lab scale columns. An accurate determination of the slurry volume and slurry concentration are important to achieve good packing results.

Alternatively, the percentage of compression can be used to determine the right volume of resin needed for the packing.

First, the packed bed volume (PBV) is calculated as follows:

$$PBV = \text{cross sectional area} \times \text{bed height}$$

The settled bed volume (SBV) required at a given percent compression for a target packed column bed volume can be calculated as follows:

$$SBV = PBV \times CF$$

Or

$$SBV = (PBV \times 100)/(100 - \%\ compression)$$

The slurry volume required for a target bed height is equal to:

$$slurry\ volume = SBV/(slurry\ concentration) \times 100$$

Where: slurry concentration = (gravity settled volume of resin)/(total slurry volume)

The compression factor is related to the percentage of compression by the following equation:

$$CF = 1/(1-\%compression)$$

Example

Pack Eshmuno® CPX to a target bed height of 200 mm in a 10 mm i.d. column:

$$PBV = \pi \times r^2 \times h$$

$$PBV = \pi \times [0,5]^2 \times 20$$

$$PBV = 15.7\ ml$$

At 8% compression, the settled bed volume is:

$$SBV = (100 \times 15.7)/(100 - 8) = 17.1\ ml$$

Therefore, 17.1 ml of resin are needed to pack a stable bed at 20 cm bed height.

If the resin is supplied in the storage solution (20% EtOH + 150 mM NaCl, 70% slurry concentration), the volume of slurry needed is:

$$slurry\ volume = 17.1/70 \times 100 = 24.4\ ml$$

Resin Slurry Preparation

It is not recommended to pack the resin in the storage solution.

Prepare the slurry for packing as follows:

1. Generate a homogeneous resin suspension via thorough mixing and transfer the slurry into a graduated cylinder.
2. Let the resin settle under gravity for ≥ 4 hours and determine the slurry concentration.

NOTE
The settling time depends on the slurry concentration, packing solution and height of the container.

3. Remove the supernatant.
4. Add packing buffer to obtain a 50% slurry concentration.
5. Mix the resin until obtaining an homogeneous slurry.
6. Repeat steps 2 – 5 at least two additional times.
7. Refer to *Compression and Resin Calculations* to determine the required slurry volume.

Packing Procedure

1. Mark the target bed height on the column tube.
2. Install and mount the column vertically. Connect an extension tube or place a funnel with a large enough capacity on top of the column.
3. Connect the bottom of the column to the chromatography system.
4. Pump liquid through the bottom to wet the bottom bed support and fill the column with 1-2 cm of packing buffer.
5. Mix the slurry in the graduated cylinder into a homogeneous suspension.
6. Add the slurry to the column assembly. Avoid air entrapment by pouring the slurry down the column wall using a funnel or a glass rod.

7. Rinse the graduated cylinder with a few mL of water or packing buffer, mix with the leftover resin in the cylinder and add this slurry to the column. Also rinse any leftover resin from the column tube wall using water or packing buffer.
8. Ensure the column outlet is closed and connect the top flow adapter while venting air out of the inlet tube. Only lower the top adapter as much as needed to remove the air, i.e. a few millimeters into the slurry.
9. Connect the column to the chromatography system.
10. Start pumping at a low flow rate (2 mL/min) and prime the column inlet line.
11. Open the bottom outlet and make a liquid to liquid connection to the column inlet. Ensure there are no leaks or air inside the column near the top adapter.
12. Immediately pump packing buffer in the downward direction at 500 cm/h until all the resin has settled onto the packed bed and all the liquid above the packed bed is clear.

NOTE

Reduce the linear velocity of this step if the system pressure exceeds the pressure limit of the column, particularly when packing long bed heights and/or if using columns with pressure limit of ≤5 bar.

13. Stop the flow and close the bottom outlet of the column.
14. If an extension tube has been used, remove the top adapter and the extension tube and then reconnect the top adapter into the column as described above.
15. If an extension tube has not been used, open the bottom outlet of the column and move to next step.
16. Lower the top adapter to the target bed height.
17. Apply downward flow at 500 cm/h for 1 CV.

NOTE

Reduce the linear velocity of this step if the system pressure exceeds the pressure limit of the column, particularly when packing long bed heights and/or if using columns with pressure limit of ≤5 bar.

18. Check the quality of the packed bed as described below.

Packed Column Evaluation

Before use, the quality of the packing should be checked by measuring the packed column efficiency. This may also be repeated during column operation prior to re-use after storage and/or if a decrease in separation performance is observed.

The commonly used parameters to describe column efficiency are the height equivalent to a theoretical plate (HETP) and asymmetry (As).

The values for HETP and As will depend on the specific test conditions e.g. sample concentration and volume, flow rate and system tubing/pipework. It should therefore be used only as a reference and the conditions maintained the same when directly comparing specific values.

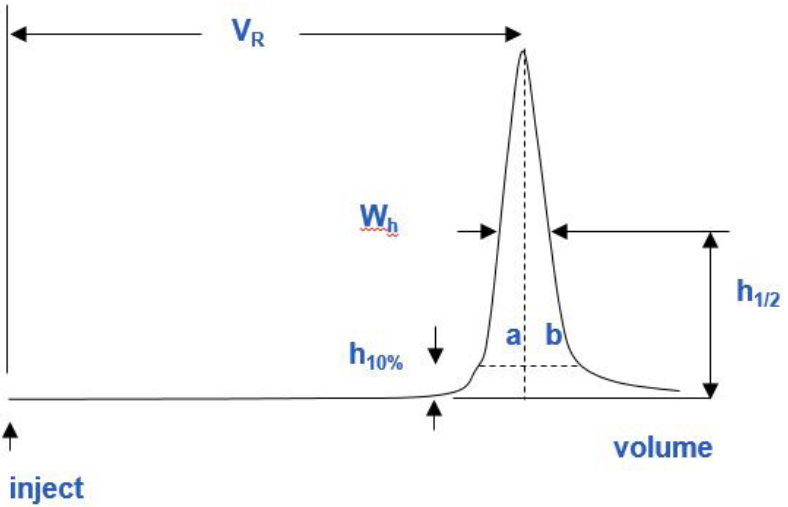
Test the column at a linear flow rate of 50 to 100 cm/h using one of the sample buffers listed here:

Sample	Mobile Phase
1 M NaCl	200 mM NaCl
Water	200 mM NaCl
2% v/v acetone in running buffer	200 mM NaCl or running buffer

NOTE

The conductivity-based test systems in this table are recommended to minimize the charge interaction of buffer ions with the functional groups of the ion exchange resin. Using other test systems may result in test artifacts (tailing or fronting).

Calculation of HETP and Asymmetry



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

$$N = 5{,}54\left(\frac{V_R}{W_h}\right)^2$$

V_R = Retention volume

W_h = Peak width at half peak height

L = Bed height

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

Where a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height

N = Number of theoretical plates

V_R and W_h are in the same units

Guideline values for packed column quality of Eshmuno® CPX resin are N > 4000/m and asymmetry values between 0.7 and 1.6 at laboratory scale.

Pilot Scale Column Packing

Introduction

A compression factor of 1.14 to 1.18 is recommended when packing Eshmuno® CPX resin at pilot scale, in columns with an inner diameter larger than 5 cm (it corresponds to 12 to 15% compression).

Materials

- Eshmuno® CPX resin
- Graduated cylinder
- Packing buffer: 150 mM NaCl or 10 mM NaOH
- Tracer solution

Compression and Resin Calculations

An accurate determination of the slurry volume and slurry concentration are important to achieve good packing results. An error here will result in an inaccurate compression of the packed bed, giving rise to high or low operating pressures and possibly poor HETP/As values.

First, the packed bed volume (PBV) has to be calculated as follows:

$$PBV = \text{cross sectional area} \times \text{bed height}$$

The settled bed volume (SBV) required at a given percent compression for a target packed column bed volume can be calculated as follows:

$$SBV = PBV \times CF$$

Or

$$SBV = (PBV \times 100)/(100 - \%\ \text{compression})$$

The slurry volume required for a target bed height is equal to:

$$\text{slurry volume} = \text{SBV}/(\text{slurry concentration}) \times 100$$

Where: slurry concentration = (gravity settled volume of resin)/ (total slurry volume).

The compression factor is related to the percentage of compression by the following equation:

$$CF = 1/(1 - \%\text{compression})$$

Example:

Column Parameters

Inner diameter (ID): 10 cm

Packed bed height (h): 20 cm

Compression factor: 1.14

$$PBV = \pi \times r^2 \times h$$

$$PBV = \pi \times [0{,}5]^2 \times 20$$

$$PBV = 1.57 \text{ L}$$

With a compression factor of 1.14 the settled bed volume is:

$$SBV = 15.7 \times 1.14 = 1.78 \text{ L}$$

So 1.78 L of resin are needed to obtain a stable bed at 20 cm bed height.

If the resin is equilibrated in the packing buffer at 50% slurry concentration, the volume of slurry needed is:

$$\text{slurry volume} = 1.78/50 \times 100 = 3.6 \text{ L}$$

Alternatively, calculate the amount of settled bed volume required at a given percentage of compression from the packed bed volume:

$$SBV = 100 \times 1.57/100 - 12\% = 1.78 \text{ L}$$

Resin Slurry Preparation

Eshmuno® CPX resin is usually supplied as a nominal 70% resin suspension in 20% aqueous ethanol, containing 150 mM NaCl.

Basic Rules for Slurry Handling

Procedure	Recommended	Strict Don'ts
Slurry Preparation	• Mixing of the sedimented slurry by use of a paddle, rod or stirrer. If mixing a settled bed, start the mixing on top of the bed. • shaking of bottled resin by hand	• permanent/intensive agitation within the settled bed • use of magnetic stirrers (the bar will crush the beads)
Unpacking	• for small diameter columns: remove the bottom adjuster if the column design allows it • for larger diameter columns: resuspend the resin within the column and pump it out	• use of magnetic stirrers to resuspend the resin within the column
Storage of Resin Slurry	• ambient temperature • use 20 % EtOH + 150 mM NaCl, or an appropriate storage solution	• freezing resin suspension • prolonged storage of resin in absence of sanitizing solution

Buffer Exchanges

Prior to packing, ethanol in the storage solution should be removed. Check your local regulations for the disposal of ethanol.

1. After allowing resin to settle in the shipping container, decant the storage solution (20% EtOH + 150 mM NaCl) once. Add packing buffer to replace the storage solution and resuspend the resin into an homogeneous solution.
2. Pour the desired amount of resin into the column or another appropriate container.
3. Perform at least 2 other buffer exchanges. For each buffer exchange, let the resin settle under gravity for >4 hours and remove the supernatant using a pump or by decantation. These steps will remove all the ethanol prior packing, and clear the potential "fines" created during shipment, resulting from base bead abrasion.
4. Once the buffer exchanges have been performed, allow the resin to settle for four hours, to have an accurate measure of the settled bed height/volume (settling for less than four hours will result in an overestimation of the amount of resin available for packing)

Packing Procedure

Different column designs can require slightly different packing options. Please consult the column manual for specifications.

Eshmuno® CPX resin can be packed with 10 µm and 20 µm bed support.

1. Add the appropriate amount of resin slurry to achieve the desired packed bed height at the recommended compression.
2. Re-slurry the resin bed by mixing with a paddle to obtain a homogeneous suspension.

3. Rinse down the walls of the column with water, so that gel particles are not trapped between the top adapter o-ring and the column wall.
4. Secure the column top, engage the seal and lower the top adapter to the surface of the liquid slurry, allowing excess liquid to escape through the inlet tubing.
5. Make sure the column inlet line is full of liquid before connecting the column inlet to the pump.
6. Open the column outlet and pack the column with the packing buffer at a starting flow rate > 300 cm/hr until the packed bed height is stable. Do not recirculate the packing buffer. Turn off the pump.

NOTE
The packing flow rate should be 20% higher than the maximum process flow rate.

7. Lower the top adapter to the desired packed bed height (this will generally be below the bed height obtained during packing). It is recommended to exhaust the liquid through the top of the column. If the resistance of the bed is too high to lower the adjuster manually to the targeted bed height, re-apply a flow at 300 cm/h in downflow mode, to recompress the bed. Once the bed is stable again, stop the flow and lower the adapter to the targeted bed height.
8. Condition the packed bed by applying flow to the column for 10 minutes in the upward flow direction at 2 bar gross pressure, followed by flow in the downward direction for another 10 minutes at 2 bar gross pressure.

Packed Column Evaluation

The quality of the packing can be checked by measuring the packed column efficiency as follows:

Run the column at a flow rate of ~150 cm/h and inject 1-2% of the packed bed volume of one of the recommended tracer solutions listed below. Monitor the conductivity or the UV absorption of the column effluent, respectively (conductivity: 1M NaCl or water as tracer; UV absorption: acetone as tracer).

The values for N/m and As will depend on the specific test conditions: sample concentration and volume, flow rate and system tubing/pipework. These values should only be used as references and these conditions maintained the same when directly comparing specific values.

Recommended test sample/buffer systems as tracer solution.

Sample	Mobile Phase
1 M NaCl	200 mM NaCl
water	200 mM NaCl
2% v/v acetone in running buffer	200 mM NaCl or running buffer

Acceptance guideline values for Eshmuno® CPX resin are N > 4000/m and Asymmetry values between 0.8 and 1,6 at pilot scale.

Large Scale Columns Packing

To pack chromatography columns having an inner diameter >45 cm, the same protocol as described in the pilot scale section should be applied when using the flow packing method. The same selection of packing buffer is recommended.

Standard Product Warranty

The applicable warranty for the products listed in this publication may be found at www.millipore.com/terms (within the “Terms and Conditions of Sale” applicable to your purchase transaction).

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