Packing strategy for Eshmuno® and Fractogel® resins at process scale on IsoPak™ columns

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Introduction

IsoPak™ columns are known to provide fast, clean and reproducible packing and unpacking at process scale. Due to the unique valve design, the slurry is directly pumped into the column, enabling the column to be packed and unpacked without removing the top adaptor. As a result, the operation is contained and operator exposure to the chromatographic resin is minimized. Although manufacturers are familiar with packing IsoPak™ columns, there are no specific packing procedures for the IsoPak™ columns with Merck resins.

The aim of this study was to define a packing procedure for Eshmuno® and Fractogel® based bead resins at process scale by carrying out several packing strategies on an IsoPak™ 440 mm column. Impact of packing pressure, slurry concentration and packing buffer on bed stability and packing quality were assessed.

This poster presents the outcomes of the study and offers some tips and tricks to enhance packing quality when combining IsoPak™ technology with Fractogel® or Eshmuno® resins.

Objectives

- Define a packing procedure allowing acceptable packing quality of an IsoPak™ 440 mm column. Eshmuno® A and Fractogel® SO_3^- resins were selected for the study, the following packing attributes were targeted:
  - Eshmuno® A resin: asymmetry between 0.7 to 1.8 and more than 1000 plates/meter ; compression factor of 10-12% at process scale
  - Fractogel® SO_3^- resin: asymmetry between 0.8-1.8 and more than 2000 plates/meter ; compression factor of 25-30% at process scale
- Evaluate the flow rate impact on the bed back pressure and bed stability.

Material

Hardware:

- IsoPak™ chromatography process column 440mm with 10 µM bed support
- Slurry Transfer Skid (STS)
- K-Prime® 40-II chromatography system
- Tanks: slurry tank and buffer tank
- Quattroflow™ 1200S pump
- Floor scale
- Magnetic flow meter and Conductivity meter
- Standard accessories for column packing including tubing, valves and pressure gauges, beakers and graduated cylinders.

Resins:

- Eshmuno® A resin, a rigid, high-capacity, acid and alkaline resistant Protein A affinity chromatography resin with a hydrophilic polymer base matrix based on polyvinylether.
  Fractogel® SO_3^- (M) resin, a strong cation exchanger with cross-linked polymethacrylate modified base beads according to the tentacle technology.

Methods

Prepacking activities

Before starting Fractogel® and Eshmuno® resin packing studies, new bed supports were installed. For the Eshmuno® resin study, preventative maintenance was performed on the IsoPak™ valves. For the Fractogel® resin study, preventative maintenance was performed on the bottom valve after the second column packing. For both studies, the column bed height was adjusted to 20 cm and the column was leveled. The column, STS, and chromatography skid were connected, the top adjuster seal pressurized from 3 to 3.3 bar and the top bed support dried with compressed air.

Before the first packing run, the pressure of the empty column was measured with Water Reverse Osmosis (WRO). Results can be seen here below:

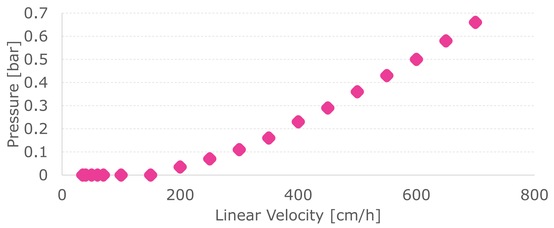

Figure 1. Pressure drop versus linear flowrate of IsoPak™ 400 mm column prior use

As resins were stored in a preservative solution, resins were first washed several times with the packing buffer by performing resuspension and settling steps in a slurry tank. The packing buffer was also exchanged before starting a new packing trial depending on packing strategy (conductivity adjustment). Slurry concentration and weight were both measured from the slurry tank before and after packing for settled bed volume and compression factor calculation.

Packing activities

Air was removed from the column bottom adaptor and tubing by displacement with packing buffer. The slurry was sent to the column through the bottom IsoPak™ valve using pressure control. Note: the slurry was resuspended before starting any packing activities and maintained in solution manually or using the STS recirculation pump during packing. Packing flow rates were also measured for information. Finally each packed bed was conditioned in both up-flow and downflow directions for 10 minutes or 2 column volumes at 100 cm/h.

Packing qualities were assessed using 2% acetone in packing buffers. Packed bed back pressure and stability were also assessed at various buffer conditions and flow rates.

Table 1. Packing buffers summary

Buffer	Buffer Concentration	Medium	Packing
Purified water	N/A	Eshmuno A®	#1,#2, #3, #4
NaCl	150 mM	Fractogel® EMD SO_3^- (M)	#1
	415 mM		#2, #3
	570 mM		#4
	110 mM		#5

Results

Eshmuno ® A resin

Table 2. Eshmuno® A packing study results

Packing	Slurry Concentration [%]	Resin compression [%]	Packing pressure [bar]	Packing Quality	
				Plate number N/m]	Asymmetry
#1	28	13	3	9523	1.26
#2	32	8	2.4	10000	1.33
#3	55	11	3	7353	1.70
#4	32	8	3	9091	1.32

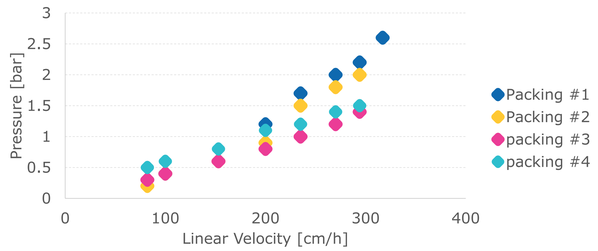

Figure 2. Eshmuno® A bed pressure flow curve (water)

Eshmuno® A resin can easily be packed with a packing pressure between 2.4 and 3 bar. Reducing slurry concentration seems not to impact packing quality. Lower slurry concentration could not be tested due to tank size limitation. No further bed compression was noticed when increasing up to 300 cm/h linear flow velocity. Using Purified water as packing buffer did not impact the packed bed stability. No correlation between resin compression and bed pressure was observed during this study.

Fractogel® EMD SO_3^- (M) resin

Table 3. Fractogel® EMD SO3- (M) packing study results

Packing	Slurry Concentration [%]	Resin compression [%]	Packing pressure [bar]	Packing quality	
				Plate number [N/m]	Asymmetry
#1	56	17	3	4184	1.16
#2	51	23	3	4367	1.05
#3	43	25	3	4348	0.94
#4	39	27	3	3759	0.99
#5	37	17	3	N/A	N/A

The packing quality of the Fractogel® EMD SO_3^- (M) resin was found to be acceptable regardless of the slurry concentration and the resin compression. However, the lower the resin compression, the lower the process flow rate range due to resin recompression. It should also be noticed that a packing pressure above 3 bar cannot be reached due to hardware pressure constraints.

The bed compression for Packing #1 using the recommended 150 mM NaCl packing buffer was insufficient showing packed bed instability over 200 cm/h and a bed compression below the Fractogel® compression recommendation of 25% at process scale. It has been decided to increase the buffer ionic strength in order to act on the Fractogel® tentacle technology (tentacular ligand chains experience less repulsive force and more freedom to move). It has been shown that increasing the buffer salt concentration up to 415 mM and 570 mM helps increasing the resin compression without acting on the packing pressure. As a result, the packed beds were also more stable at higher flowrate.

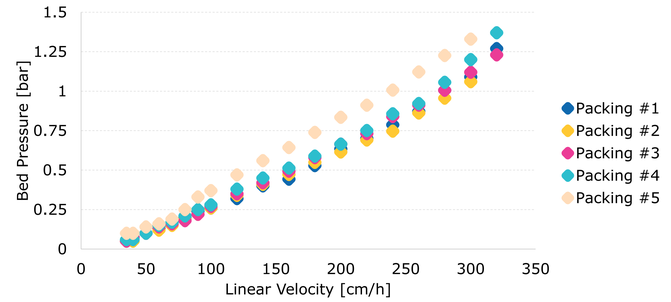

Figure 3. Pressure vs flow for Fractogel® EMD SO_3^- resin packed into an IsoPak™ 440 mm column

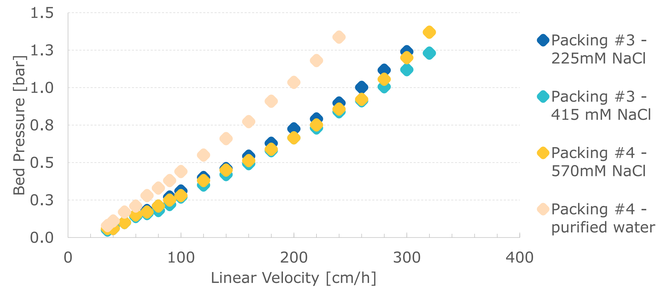

Figure 4. Pressure vs flow for Fractogel® EMD SO_3^- resin packed into an IsoPak™ 440 mm column for various buffers

In general, increased resin compression led to a slight increase in resin back pressure for a defined linear velocity (Fig. 3). However packing #5 showed unusually high back pressure despite its reduced compression. Potential bed support clogging could not be excluded for this condition (blank pressure not checked after experiments).

After packing #3 and packing #4, the packed beds were rinsed with lower salt concentration buffer solution in order to estimate the resin back pressure at various linear velocities (Fig. 4). As expected, the resin back pressure increased after being conditioned at lower salt concentration. Indeed, salt concentration directly impacts the ionic forces (repulsion) on the ligands for Fractogel® resin.

Conclusion/Recommendations

Results demonstrate that it is always possible to achieve packing quality within the targets for Eshmuno® resin regardless of the packing pressure or slurry concentration when using IsoPak™ columns. Fractogel® resin tests also included packing buffer effects, and results demonstrate that regardless of packing buffer the packing quality was always within targets. However, the final compression factors were often lower than recommended for Fractogel® resin, leading to potential bed instability at process flow rate. Increasing the packing buffer conductivity can enhance Fractogel® resin bed stability when packing an IsoPak™ column at a fixed bed height. Particular attention should be paid to pressure control during column packing and processing, especially during buffer change and product loading.