

Packing Study: 6% Cross-linked Agarose Resin

VERDOT



Agarose Bead
Technologies

6pEXCL agarose resin

- Bead homogeneity with narrow size distribution for high resolution
- High chemical tolerance
- High flow rate applications
- Commercial scale GMP manufacturing
- Regulatory support file

InPlace Column

- Automated method-driven packing
- Scalable design, 20 cm - 200 cm
- Compatible with all resin types
- Ergonomic operation
- On-site packing support
- 21 CFR Part 11 and USP VI Compliant

Process-scale packing study of ABT 6pEXCL agarose resin using VERDOT InPlace™ Chromatography Column

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Agarose Bead Technologies® (ABT) collaborates closely with customers through the customized resin service to develop optimal solutions tailored to specific needs. 6pEXCL is a custom resin composed of 6% highly cross-linked agarose with a bead size of 60-75 µm. The narrow size distribution of this resin ensures high resolution, while the customized cross-linking imparts the necessary flow properties to meet and exceed customer requirements.

VERDOT® InPlace™ Chromatography Columns are designed and manufactured for GMP process scale downstream operations (Figure 1). The innovative design provides reproducible scalability from pilot scale through clinical trials to commercial manufacturing.

In this collaborative study, we present the results of packing ABT 6pEXCL agarose prototype resin in the VERDOT InPlace Chromatography Column.

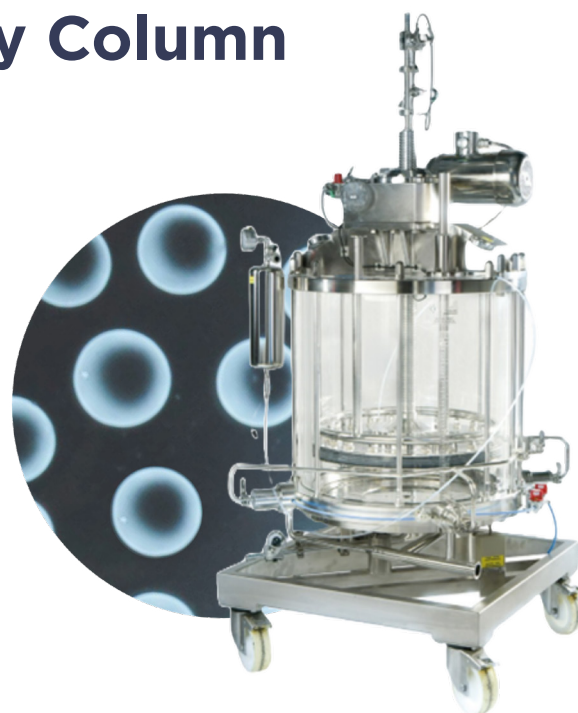


Figure 1. InPlace™ Chromatography Column with 6pEXCL agarose resin

A quick and straightforward packing protocol was used to evaluate a range of conditions and packing factors.

The insights gained from this packing study will enable both companies to provide technical column packing guidance for this base-bead type.

Materials and Methods

Equipment

The InPlace Chromatography Column D200 was mounted with filters with 20 µm absolute porosity, which is suitable for resin particle size of 60-75 µm. For motorized columns, the Control Console precisely regulates the speed and position of the top flow adaptor for accurate bed consolidation and compression. The variable height adaptor position is controlled with millimeter accuracy.

Column preparation and slurry transfer

After leveling and priming the column including the slurry manifold and process connections, both the top and bottom process valves were closed and the slurry valves were opened. The 50% slurry (v/v with deionized water) was prepared in an external tank and transferred into the column by raising the top adapter at 300 cm/hr. Once the slurry was completely transferred into the column, the slurry valves were closed.

Packing with axial compression

With the top process valve closed and the bottom process valve opened, downward movement of the adapter was initiated at 300 cm/hr to consolidate the bed. During final bed compression, the linear speed was reduced to 60 cm/hr.

Final bed compression was calculated using a Packing Factor (PF). The PF is defined as:

$$PF = \frac{\text{Consolidated Bed Height (at 300 cm/hr)}}{\text{[Final Bed Height]}}$$

Results

Several packing factors were tested from a range of 1.10 to 1.15, with the best results obtained with a packing factor of 1.15 (Table 1 and Figure 2).

Table 1. Packing Study

Consolidated Bed Height (cm)	20.6
Final Bed Height (cm)	17.9
Bed Volume (L)	7.6
Asymmetry	1.05
rHETP	7.6
HETP Flow Rate (cm/hr)	100
Buffers	Deionized Water

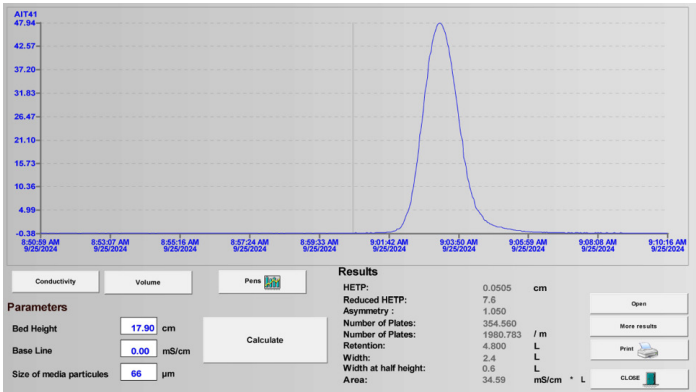


Figure 2. Asymmetry and HETP conductivity peak

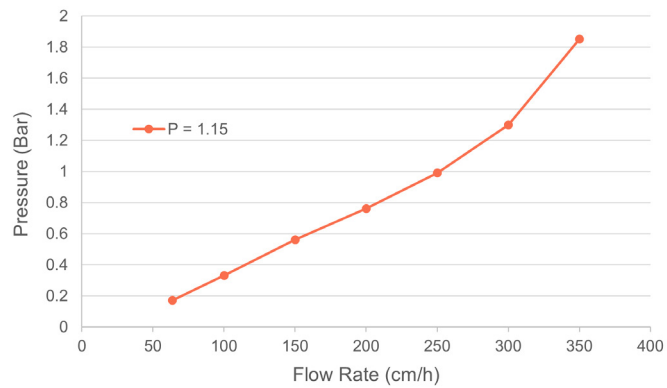


Figure 3. Pressure vs. flow curve

Conclusion

ABT 6pEXCL agarose resin was very easy to pack and to unpack in the VERDOT InPlace Chromatography Column. The low shear design of the column slurry valves ensured the integrity of the resin throughout the packing study. The axial compression at 300 cm/hr with final compression at 60cm/hr resulted in quick and straightforward packing operations. Packing factor of 1.15 appears to be sufficient and provides a low pressure/flow ratio, remaining under 2 Bar at 350 cm/hr (Figure 3). Although the results of the packing study are well within acceptance criteria, additional studies will be performed to assess optimal packing conditions for this chromatography resin.

VERDOT and Agarose Bead Technologies® value technical collaborations in order to determine optimal packing protocols for scaling-up to GMP manufacturing.