Technical note

J.T. Baker® BAKERBOND® Poly Hi-Propyl™ mixed mode hydrophobic interaction chromatography (HIC) resin

INTRODUCTION

Utilizing unique surface chemistry that combines both hydrophobic and weak anionic exchange sites, BAKERBOND® Poly Hi-Propyl™ hydrophobic interaction chromatography (HIC) media provides unique selectivity to enable higher purification performance across a wide range of biopharmaceuticals.

Designed and manufactured by Avantor to the high standards established by our J.T. Baker® brand, the BAKERBOND® Poly Hi-Propyl™ resin provides better selectivity than conventional HIC media that enables separation of proteins and peptides having similar hydrophobicity. Its fully porous and crosslinked methacrylate spherical beads provide excellent mechanical and chemical stability and are optimized for maximum capacity, high mass transfer, and relatively low backpressure. The resin is provided in a non-hazardous, non-flammable storage solution through Avantor's global supply chain.

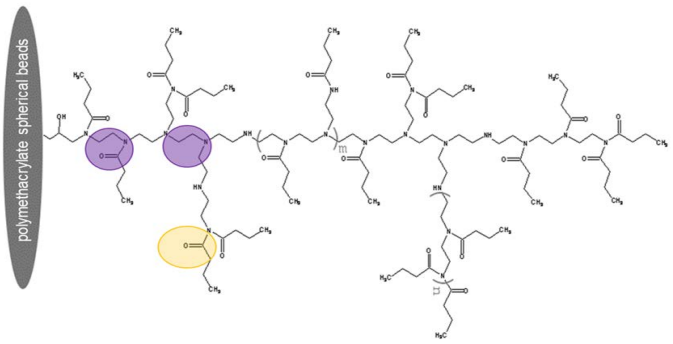
Used with Avantor's proven J.T. Baker® family of process chromatography buffers and additives, the BAKERBOND® Poly Hi-Propyl™ resin can deliver greater efficiencies and higher purity profiles to biopharma chromatography schemes.

FEATURES

- Unique selectivity delivered by its proprietary mixed mode functionality
- High ionic capacity to improve separation and enable operation at high salt concentration
- Spherical polymethacrylate beads enable uniform packing and provide mechanical stability and chemical resistance for ease-of-use
- Delivery in a non-hazardous, non-flammable buffer solution to eliminate burdensome handling requirements.

UNIQUE SELECTIVITY

BAKERBOND® Poly Hi-Propyl™ hydrophobic interaction chromatography (HIC) resin is inherently well suited to discriminate between extremely similar molecules. Its unique surface chemistry is able to discern minute differences in primary structure resulting in a unique selectivity, improved separation performance, and higher efficiency downstream operations. As illustrated in Fig.1. molecules with similar hydrophobicities such as Cytochrome C, Myoglobin, Lysozyme,

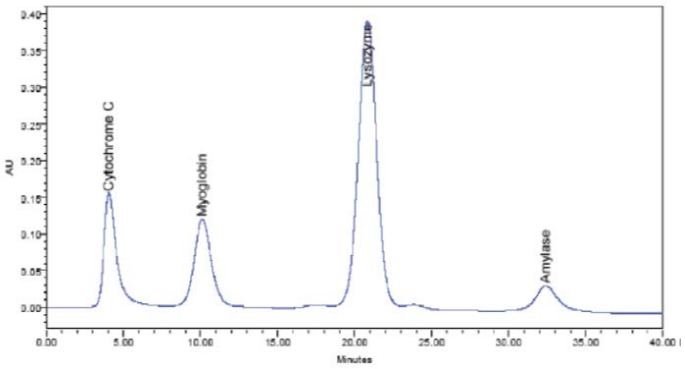


Tertiary, secondary amine anion exchange groups

Propyl group provides hydrophobic functionally

The mixed mode functionality of Poly Hi-Propyl is obtained by covalently bonding PEI to the surface of highly cross linked polymethacrylate beads and functionalizing PEI with propyl groups to obtain hydrophobic sites, thereby providing hydrophobic interaction with secondary weak anion exchange behavior.

Fig.1. Separation of proteins using typical hydrophobic conditions



Excellent selectivities are being achieved with Poly HI-Propyl

Column: 10 cm × 0.46 cm

Sample: Cytochrome C (0.77 mg/mL), Myoglobin (2.31 mg/mL), Lysozyme (2.31 mg/mL), Amylase (3.08 mg/mL)

Buffer A: 25 mM sodium phosphate in 1.7 M ammonium sulfate at pH 7

Buffer B: 25 mM sodium phosphate at pH 7

Gradient: 0 to 100 % B in 40 minutes

Flow rate: 1 mL/min

Injection volume: 100 mL.

The ability to discern these minute molecular differences is driven by the resin's mixed mode functionality. Hydrophobic sites result from propyl groups which are covalently linked to nitrogens on the Polyethylenimine (PEI) ligands. Weak anion exchange sites are due to remaining free amino groups. This proprietary surface chemistry enables enhanced purification where conventional HIC media often fail to provide sufficient separation in a process environment.

HIGH IONIC CAPACITY

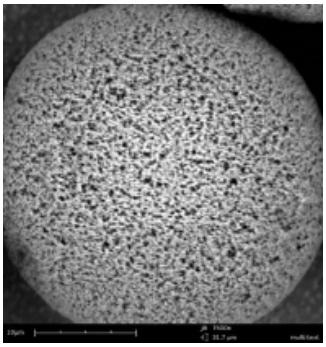
The BAKERBOND® line of polymeric resins were all designed with higher ionic capacities than the industry leading ion exchange and hydrophobic interaction chromatography (HIC) resins. BAKERBOND® Poly Hi-Propyl™ contains both hydrophobic and weak anionic exchange sites. The weak anionic exchange sites create a unique resin functionality and provided an ionic capacity not typically presence among hydrophobic interaction resins.

EASE OF USE

Our BAKERBOND® line of polymeric resins are based on a highly cross linked rigid spherical polymethacrylate media that provides optimal porosity, high mechanical strength, and excellent chemical resistivity delivered with a consistent narrow particle size distribution. These features enable robust resin application, narrow elution bands for concentrated product fractions, and provide consistency and stability to downstream operations.

As illustrated in Fig.2, BAKERBOND® Poly Hi-Propyl™ has an average pore size of 500 Å for an exclusion limit of 1×10^6 dalton. This open porosity coupled with the bead's mechanical strength allows for the use of Poly Hi- Propyl™ at high linear velocities due to good mass transfer.

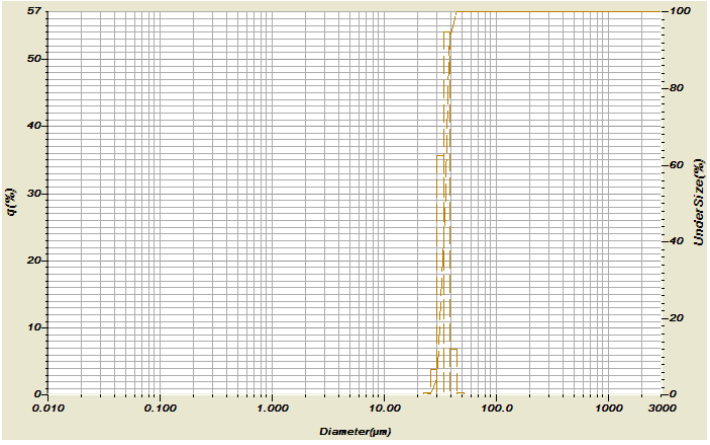
Fig.2. SEM image of polmethacrylate spherical beads at 2,000 magnification



The average pore size of the beads is 500 A, providing on exclusion limit of $1x10^6$ dalton

As seen in Fig.3, the small average particle size of 35 µm combined with a narrow particle size distribution (d_{60}/d_{10}) produces narrow elution bands of highly concentrated product for improved efficiency. This increases resolution and decreases pool volumes compared to media based on larger particles. High efficiency in combination with high selectivity enables higher loading levels while maintaining separation.

Fig.3. Particle size distribution of a typical lot of polymethacrylate beads



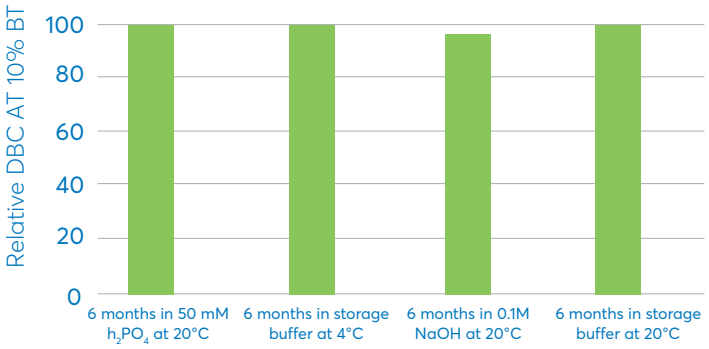
A median particle size of 35 micron and a particle size distribution of less than 1.4 (d_{60}/d_{10}) show a lack of fines and result in high efficiency at low back pressure.

The hydrophilic backbone of BAKERBOND® Poly Hi-Propyl™ has low non-specific binding and the ability to withstand prolonged contact with commonly used cleaning and sanitizing solutions. Poly Hi-Propyl™ media can be easily packed to bed heights of up to 40 cm and operated in the linear velocity range of up to 500 cm/hr using conventional columns with pressure ratings of 2 to 10 bar. Our entire line of BAKERBOND® chromatography resins, buffers, and additive are manufactured under the strictest controls and are supported by world class quality systems and application resources.

STABILITY AND CONSISTENCY

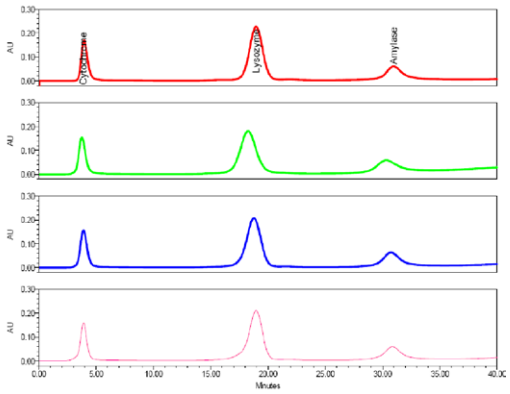
The performance of BAKERBOND® Poly Hi-Propyl™ remains consistent across a broad range of storage conditions. As illustrated in Fig.4 and Fig.5, little to no change was identified in protein binding capacity after prolonged storage across a wide range of pH's and temperatures. Product performance was evaluated by measuring the dynamic binding capacity of rabbit IgG. Similarly, the purification behavior of Poly Hi-Propyl™ showed little to no change in its ability to separate a mixture of Cytochrome C, Lysozyme, and Amylase after subjecting the column to a comparable range of storage conditions.

Fig.4. Dynamic binding capacity of rabbit igG at various storage conditions



Dynamic binding capacity is maintained under various storage conditions.

Fig.5. Separation Performance at various storage conditions for six monthsAmylase



Poly Hi-Propyl is stable at a high and low pH for extended periods of time.

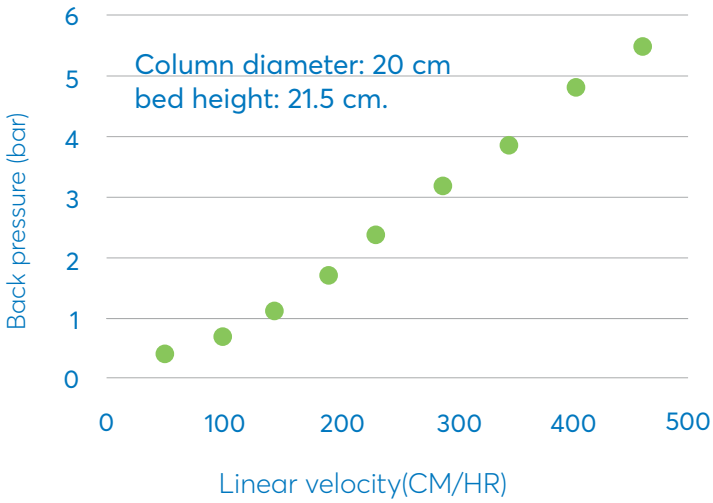
Red: media stored in shipping buffer at 8 °C.
Green: media stored in shipping buffer at 23 °C
Blue: media stored in 0.1 M NaOH at 23 °C.
Pink: media stored in 50 mM H3PO4 at 23 °C

OPERATIONAL FLEXIBILITY

BAKERBOND® Poly Hi-Propyl™ scales easily to the production environment with reproducible results in columns ranging from 20 to 45 cm in internal diameter. The narrow particle size distribution and mechanical stability of the media maintains conventional low pressure column performance at bed heights up to 20 cm. Modern medium pressure columns, rated at 7 bar, can be used to pack beds up to 40 cm in height and enable operation at high linear velocities.

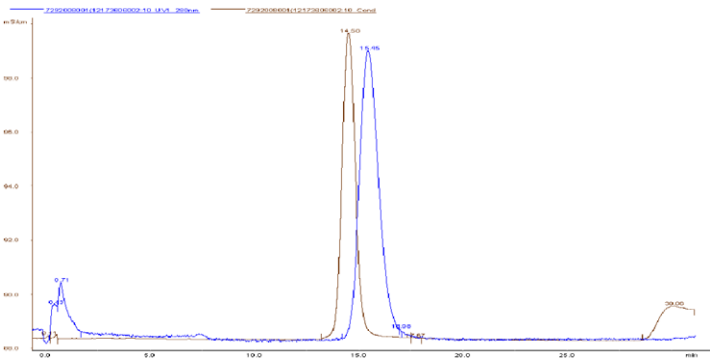
As shown in Fig.6, the linear pressure-flow curve for BAKERBOND® Poly Hi-Propyl™ as seen in Fig.6, indicates that there is no resin compression at a bed height of 21.5 cm and a column diameter of 20 cm. In addition, the media does not need to be defined prior to the initial packing or subsequent packings, reducing the consumption of buffers and simplifying operations.

Fig.6. Pressure-Flow relation



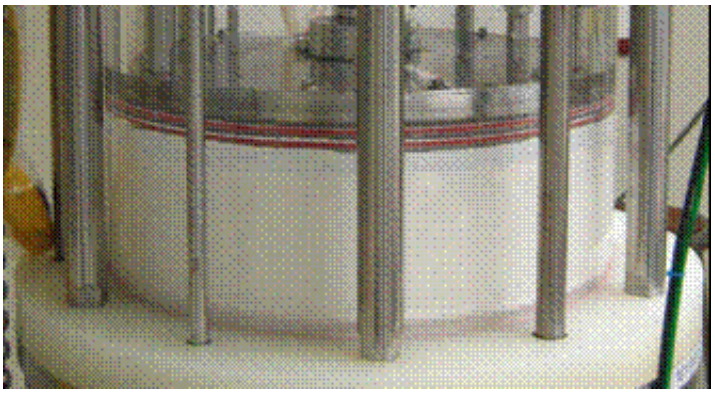
The linear behavior of the pressure-flow curve indicates no resin compression.

Fig.7. Efficiency of column (20 id × 21.5 cm high) packed with polymethacrylate based media



The conductivity peak shows an efficiency of 11,061 plates/meter at an asymmetry of 1.15.

Fig.8. Polymeric media packed in a 45 cm id Column



Hi-Propyl can easily be packed in large columns for high throughput applications.

CONVENIENT PACKAGING

BAKERBOND® Poly Hi-Propyl™ resin comes conveniently packaged in a non-hazardous, non-flammable storage buffer, eliminating many of the burdensome shipping, handling, and storage requirements common among chromatography resins.

Product ordering information

Size	Product Number
1 mL Lab Columns (5-pack)	**6064-07**
5 mL Lab Columns (5-pack)	**6064-25**
50 mL	**7588-01**
100 mL	**7588-02**
500 mL	**7588-03**
1 L	**7588-04**
5 L	**7588-05**

PROCESS AND APPLICATION SUPPORT

Avantor has deep expertise in process chromatography optimization and can work with you to help ensure that BAKERBOND® Poly Hi-Propyl™ delivers the improved performance you need in downstream processing. Technical support from our scientists and application specialists is available from our multiple global research and innovation centers.

Product Information Summary

Characteristics

Functionality	Primarily a weak cation exchanger with weak anion exchange sites	
Functional Group	-NH-C(=O)-CH_2 CH_2COOH	
Ion Exchange Capacity	Cation exchange: 0.15 – 0.25 H+meq/mL	Anion exchange: 0.10 – 0.20 Cl meq/mL
Median Particle Size	35 µm	
Particle Size Distribution (d_{60}/d_{10})	< 1.4	
Median Pore Size	500 Å	
Exclusion Limit	1 × 10^6 Daltons	
Operating pH Range	4.5 – 14.0	
Cleaning pH Range	1.0 – 14.0	
Chemical Stability	All commonly used aqueous buffers, sodium hydroxide, acetic acid, phosphoric acid, guanidine hydrochloride, up to 100% ethanol, methanol, or 2-Propanol.	
Shipping Solvent	Media is shipped as 1:1 slurry in acetate buffer containing benzyl alcohol at pH 4.5 and can be stored for up to 5 years at 4 – 15 °C. The media can be stored in 20% ethanol or 0.1 M NaOH for up to 5 years.	

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