avantor™

Technical note

BAKERBOND™ Multimode ion exchange chromatography media

BAKERBOND™ POLYABX

INTRODUCTION

PolyABx is a multimode ion exchanger which primarily functions as a weak cation exchanger over a wide pH range. Secondary anion exchange sites are due to the presence of amine groups. This multimodal functionality off ers better selectivity than conventional weak cation exchange media with equivalent capacities and is capable of separating proteins and peptides having similar isoelectric points (pI). Unique selectivity of closely related molecules is often achieved with PolyABx where conventional ion exchangers fail to provide suffi cient separation in a process environment.

The average particle size of 35 µm with narrow particle size distribution (d_{60}/d_{10} <1.4) provides high efficiency and will produce narrower elution bands of highly concentrated product. 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.

PolyABx is based on highly cross linked rigid spherical polymethacrylate particles with optimal porosity and mechanical strength. The hydrophilic backbone has low non-specifi c binding and the ability to withstand prolonged contact with all commonly used cleaning and sanitizing solutions. PolyABx can be easily packed to bed heights 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. PolyABx is manufactured under the strictest controls and testing and supported by world class quality systems and applications resources.

As a result of this unique combination of selectivity, efficiency, and the ability to maintain performance at high linear velocities, PolyABx can provide high process throughput, productivity improvements, and enable new processes.

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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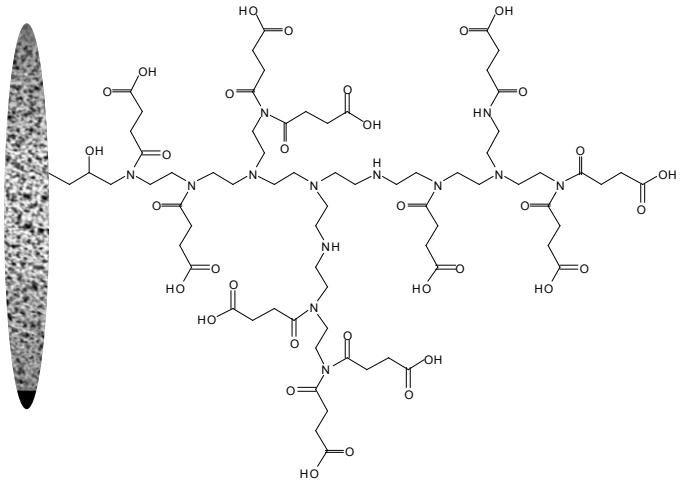
MULTIMODE FUNCTIONALITY

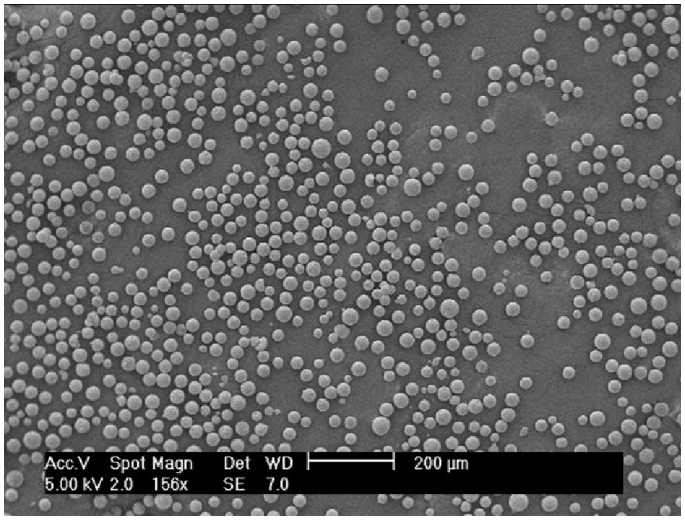
Fig. 1. Ligand structure of PolyABx



The multimode functionality of PolyABx is obtained by covalently bonding PEI to the surface of highly cross linked polymethacrylate beads and functionalizing PEI to obtain carboxylic acid groups, thereby providing a primarily weak cation exchange and secondary weak anion exchange behavior.

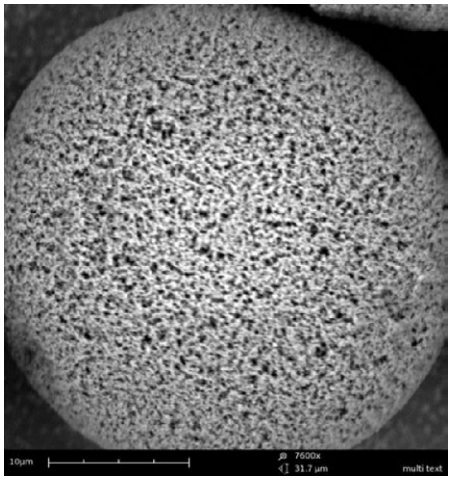
PHYSICAL CHARACTERISTICS

Fig. 2. SEM image of PolyABx at 156 magnification



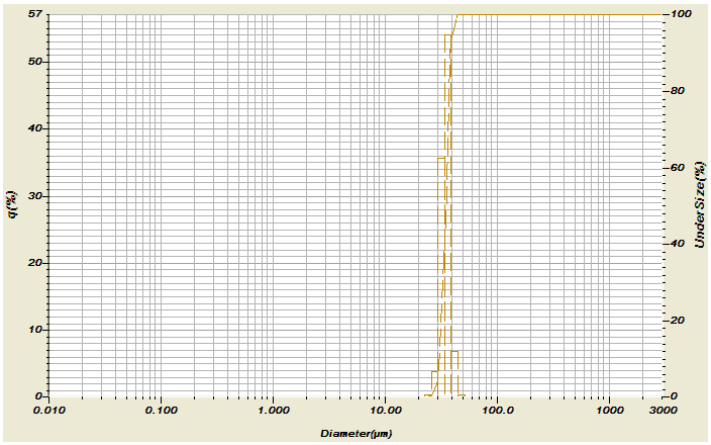
Uniform particle size distribution is evidenced in this picture.

Fig.3. SEM image of PolyABx at 7600 magnification



The picture shows excellent and uniform pore structure of the bead.

Fig.4. Particle size distribution of a typical lot of polymethacrylate beads

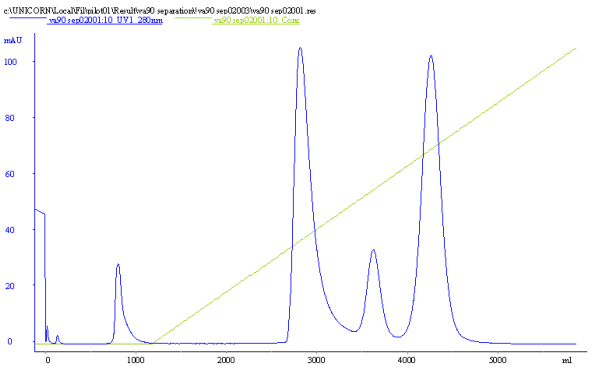


The median particle size of 35 micron and a particle size distribution of less than 1.4 (d_{60}/d_{10}) shows a total lack of fi nes.

As illustrated by the SEM picture and particle size distribution graph, PolyABx provides high column efficiency leading to narrow elution bands and therefore concentrated product fractions. The SEM picture also shows good porosity and 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 high mechanical strength allows the use of PolyABx at high linear velocities due to good mass transfer.

SEPARATION PERFORMANCE: SELECTIVITY AND CAPACITY

Fig. 5. Separation of bovine serum albumin (bsa), igg, cytochrome c and lysozyme



The unique selectivity of PolyABx is capable of separating proteins with closelyrelated isoelectric points.

Sample injection: 100 mL

Sample: 1B SA(pI4 .7), 2I gG(pI7 .2), 3C ytochrome(pI1 0.4), 4L ysozyme(pI1 1)were dissolved in binding buffer

Column: Millipore V90 165 × 90 mm ID packed with PolyABx

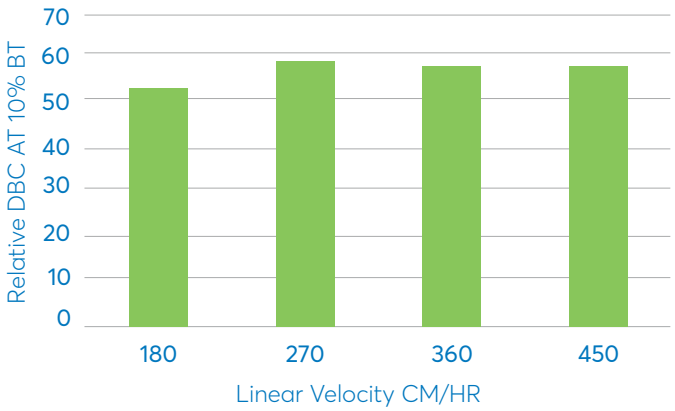
Binding Buffer: 20 mM Sodium Acetate/Acetic Acid, pH 5.5.

Elution Buffer: 1.0 M NaCl in binding buffer

Flow rate: 300 cm/hour.

Gradient: 10 CV

FIG. 6. Dynamic binding capacity of rabbitiggon polyabx at different linear velocities



Dynamic binding capacity is maintained at high linear velocities.

Rabbit IgG concentration: 1 mg/mL in Buffer A

Column: 4.6 × 100 mm.

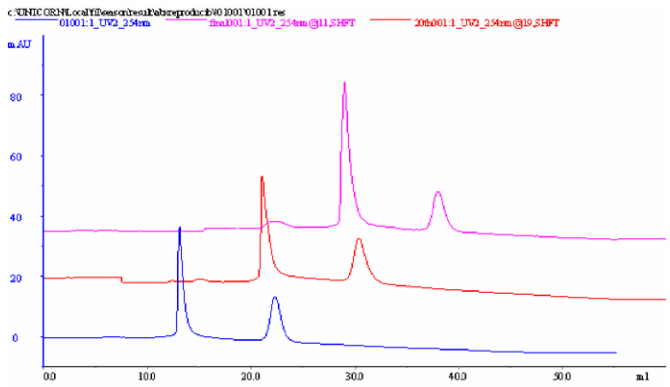
Binding buffer: 20 mM sodium acetate at pH 5.6.

Elution buffer: 1 M acetate at pH 6.0

PolyABx media provides unique selectivity and can be used to separate closely related proteins due to its unique chemistry and multimodal functionalities. As Fig. 5. illustrates, Lysozyme and Cytochrome having close isoelectric points (pI 10.4 and 11) are well resolved and can be easily separated. As expected, BSA flows through without binding while IgG is retained demonstrating the capability to separate albumin and IgG as well. This in combination with the media's high efficiency enable high column loading. Fig. 6. Illustrates that the breakthrough capacity of IgG does not change significantly over the linear velocity range of 180 to 720 cm/hour. This is due to the particle's open pore structure and rigidity facilitating good mass transfer. High resolution and the ability to maintain capacity at high linear velocities facilitate high throughput for both large and small proteins.

STABILITY AND CONSISTENCY

Fig. 7. Polyabx performance consistency over 50 cycles



Separation performance is maintained over 50 cycles.

Sample: 0.25mg IgGand0.12m glysozymein binding buffer;
Sample injection: 0.5mL

Column: 10 × 10 mL packed with PolyABx

Conditions: Linear gradient from binding buffer (0.05MMES/NaOHpH5.6) toelutionbuffer (1 M NaCl in 0.05 M MES/NaOH pH 5.6) in 5 column volumes (26 min)

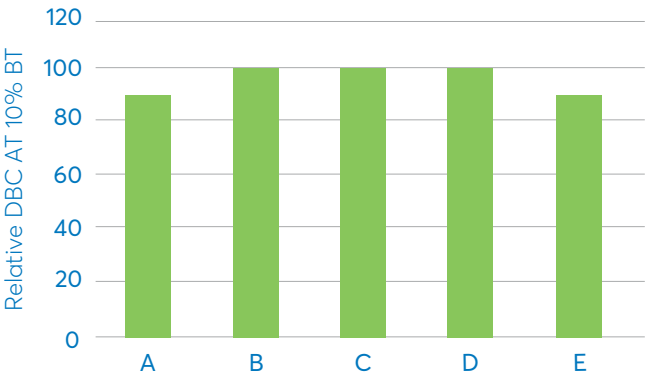
Flow rate: 1.5 mL/min

Blue: 1st injection

Red: 20th injection

Pink: 50th injection

Fig. 8. Dynamic binding capacity of rabbit igg on polyabx under various storage conditions



Dynamic binding capacity is maintained under various storage conditions.

Rabbit IgG concentration: 1 mg/mL in buffer A

Column: 4.6 × 100 mm.

Binding buffer: 20 mM sodium acetate at pH 5.6.

Elution buffer: 1 M acetate at pH 6.0

A: Initial

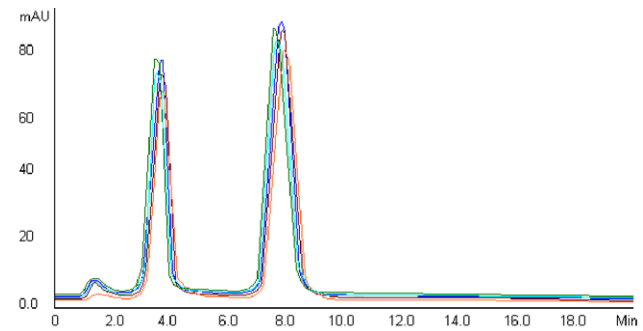
B: 24 months in storage buffer at 4 °C

C: 5 years in 0.1 N NaOH at 4 °C

D: 5 years in 0.1 N NaOH at room temperature

E: 5 years in storage buffer at 4 °C after washing with 0.5N NaOH

Fig. 9. Separation of Bovine Serum Albumen, IgG and Lysozyme.



Separation performance is maintained under various storage conditions.

Column: 0.775 × 10 cm packed with PolyABx

Buffer A: 50 mM MES, pH 6.2 and Buffer B: 50 mM MES containing 1M NaCl, pH 6.2.

Flow rate: 4 mL/min

Orange: 20% ethanol at room temperature, Pink: Phosphoric acid at room temperature

Yellow: Phosphoric acid at 4 °C, Green: Sodium hydroxide at room temperature

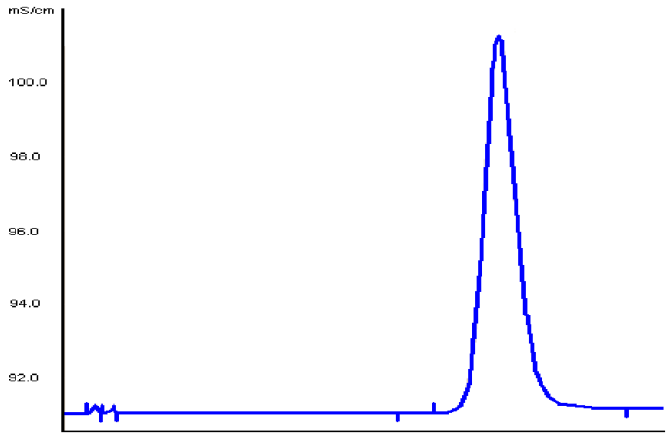
Blue: Sodium hydroxide at 4 °C

In Fig. 7., the consistent separation behavior of PolyABx was evaluated by separating an IgG and Lysozyme mixture for 50 cycles. After each separation the column was regenerated by washing with 5 CV of elution buffer. There is no measureable change in retention time of the two proteins, indicating consistent separation performance over multiple cycles

PolyABx is chemically stable and as shown in Figs 8 and 9., there is no significant change in capacity or resolution when media is subjected to various storage conditions. The former study evaluated the change in dynamic binding capacity of human IgG between initial media compared to media samples stored in different solutions for up to 5 years. Slight variations are expected within the error of the experiment. High selectivity and resolution of PolyABx are also maintained when stored at extreme pH conditions over a period of 10 months as illustrated in the 5 overlaid chromatograms where 5 different storage buffers were chosen to demonstrate consistent chromatographic behavior.

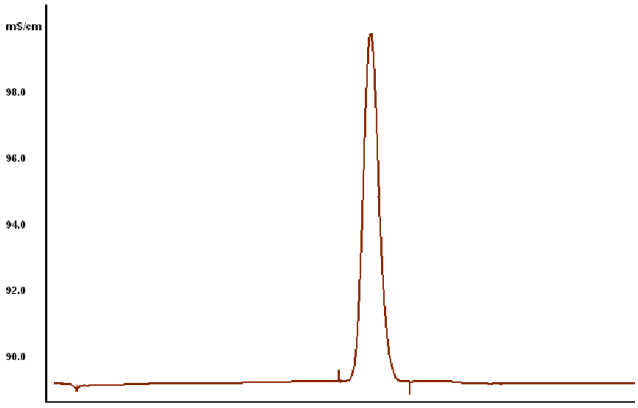
OPERATIONAL FLEXIBILITY

Fig. 10. Efficiency of column (20 cm id × 18 cm high) packed with PolyABx



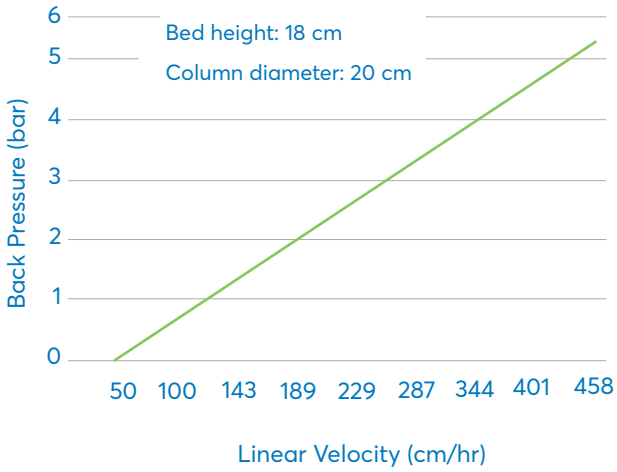
13,926 plates/meter is achieved with an asymmetry of 1.18

Fig. 11. Efficiency of column (45 cm id x 18 cm high) packed with PolyABx



13,500 plates/meter is achieved with an asymmetry of 1.20.

Fig. 12. Pressure–Flow relation



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

Fig. 13. Scale–up to a BPG column

PolyPEI can easily be packed in large columns for high throughput applications.

Repeated column packing of the media yields high efficiencies as well as low asymmetries and is reproducible from a 20 cm id to 45 cm id column, indicating easy process scalability.

The narrow particle size distribution and mechanical stability of PolyABx provides the ability to operate in conventional low pressure columns at a bed heights of up to 20 cm. Modern medium pressure columns, rated at 7 bar, can be used to pack beds up to 40 cm bed height and operate at higher linear velocities. The linear pressure–flow curve for PolyABx indicates that there is no resin compression at a bed height of 18 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.

Product ordering information

Size	Product Number
50 mL	**7586-01**
100 mL	**7586-02**
500 mL	**7586-03**
1 L	**7586-04**
5 L	**7586-05**

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