Technical note

J.T. Baker® BAKERBOND® PolyPEI™ multimode weak anion exchange chromatography resin

INTRODUCTION

Utilizing a unique surface chemistry that combines primary, secondary, and tertiary weak anionic exchange sites, BAKERBOND® PolyPEI™ multimode anion exchange chromatography 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® PolyPEI™ resin provides better selectivity than conventional weak anion exchange media with equivalent capacities. It is capable of separating proteins and peptides having similar isoelectric points (pI). 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® PolyPEI™ resin can deliver greater efficiencies and higher purity profiles to biopharma chromatography schemes.

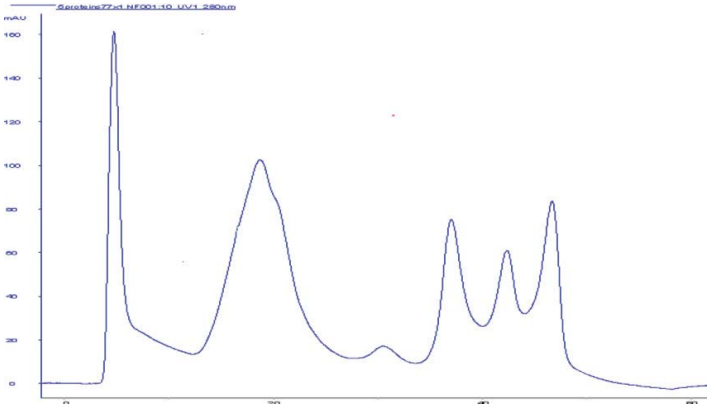
FEATURES

- 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® PolyPEI™ anion exchange 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, BSAB-Lactoglobulin B, and B-Lactoglobulin A having close isoelectric points (pI 4.7, 5.34, and 5.21) are well resolved and can be easily separated.

Fig.1. Separation of Proteins on PolyPEI



Column: 0.77 × 10 cm

Buffer A: 50 mM TRIS pH 8.0

Buffer B: Buffer A plus 1 M NaCl, pH 8.0

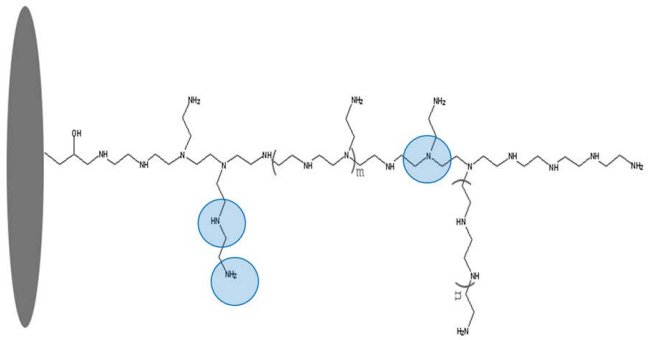
Linear gradient from 0 to 100% B in 10 CV

Flow rate: 1.2 mL/minute

Sample volume: 1.5 mL.

Protein	pI	MW	Sample conditions
Lysozyme	10.0	14.7 kD	0.4 mg/mL
Human IgG	7.0	150.0 kD	4.0 mg/mL
BSA	4.7	66.4 kD	2.5 mg/mL
B-Lactoglobulin B	5.3	18.3 kD	1.0 mg/mL
B-Lactoglobulin A	5.2	18.4 kD	1.0 mg/mL

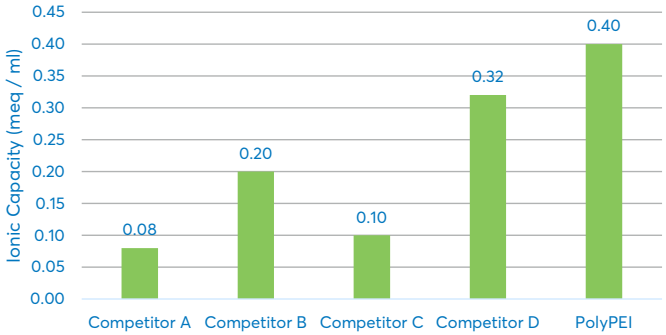
The ability to discern these minute molecular differences is driven by the resin's multimode functionality. The multimode functionality of PolyPEI™ is obtained by covalently bonding Polyethyleneimine (PEI) to the surface of highly cross linked polymethacrylate beads. Polyethyleneimine contains primary, secondary and tertiary nitrogens, which each function as weak anionic exchange sites.



Tertiary, secondary and primary amine anion exchange groups

HIGH IONIC CAPACITY

Our BAKERBOND® line of polymeric resins were all designed with higher ionic capacities than the industry leading ion exchange and hydrophobic interaction chromatography (HIC) resins. The high ionic capacity increases the salt gradient needed to separate similar molecules, thus improving selectivity. This also enables operations at high salt concentrations. A comparison of ionic capacity between BAKERBOND® PolyPEI™ and several other leading competitive hydrophobic interaction resins can be seen below.

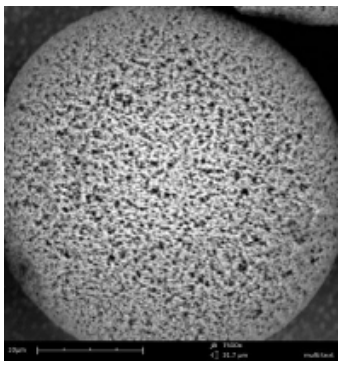


EASE OF USE

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 below, BAKERBOND® PolyPEI™ 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 PolyPEI™ at high linear velocities due to good mass transfer.

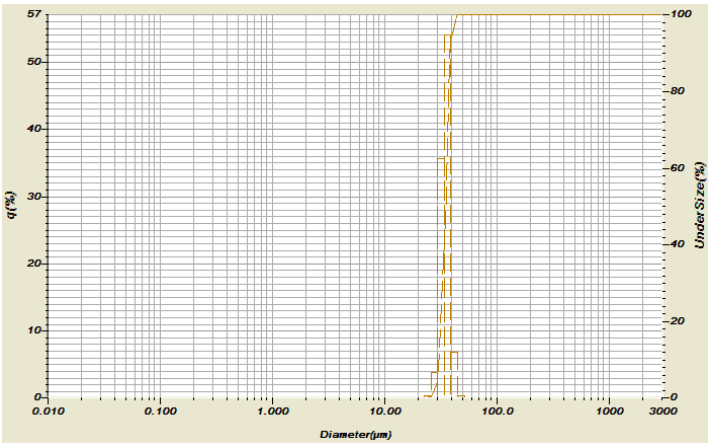
Fig.2. SEM image of polmethacrylate spherical beads at 2,000 magnification



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

A 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



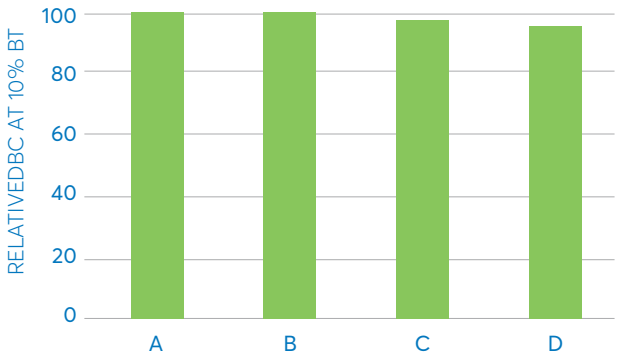
A median particle size of 35 micron and a particle size distribution of less than 1.4 (d_{60}/d_{10}) show a lack of fines and result in high efficiency at low back pressure.

The hydrophilic backbone of BAKERBOND® PolyPEI™ has low non-specific binding and the ability to withstand prolonged contact with commonly used cleaning and sanitizing solutions. PolyPEI™ 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 is supported by world class quality systems and application resources.

STABILITY AND CONSISTENCY

The performance of BAKERBOND® PolyPEI™ 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 BSA after prolonged storage under both acidic and basic conditions. Product performance was evaluated by measuring the dynamic binding capacity of BSA. Similarly, the purification behavior of PolyPEI™ showed little to no change in its ability to separate a mixture of lysozyme, rabbit gamma globulin, and alpha lactalbumin after subjecting the column to a comparable range of storage conditions

Fig.4. Relative Dynamic Binding Capacity (DBC) of BSA under various storage conditions



Column: 7.75 × 100 mm

Binding buffer: 20 mM Sodium Acetate, pH 6.2

Elution buffer: 1 M Sodium Acetate, pH 6.2.

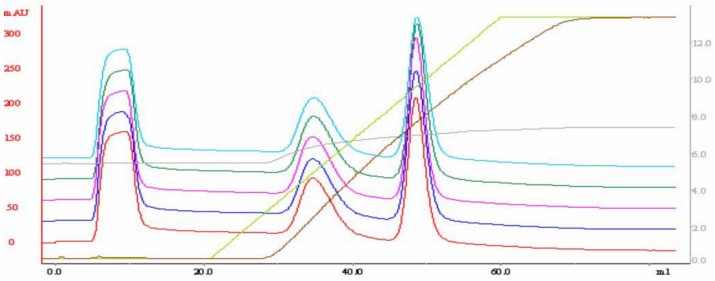
A: Fresh lot in Sterilant buffer at room temperature

B: in 10mM H3PO4 at room temperature for 4.5 years

C: in 0.1N NaOH at room temperature for 4.5 years

D: in Sterilant buffer at low temperature for 4.5 years

Fig.5. Chemical Stability of PolyPEI



The unique selectivity of PolyPEI is not affected by extreme pH conditions over extended periods of time.

Column: 10 × 100 mm, sample injected: 5 mL

Binding buffer: 20 mM Sodium Acetate, pH 6.2

Elution buffer: Binding buff er plus 1 M NaCl, pH 6.2 Sample: lysozyme, rabbit gamma globulin and alpha lactalbumin

Red: PolyPEI for 10 months in storage solution at 4 °C

Blue: PolyPEI for 10 months in storage solution at room temperature

Pink: PolyPEI for 10 months in 10 mM phosphoric acid

Green: PolyPEI in 0.1 NaOH at room temperature for 10 months

Cyan: PolyPEI washed dynamically with 0.5 M NaOH for 48 hours at 1.0 mL/min.

OPERATIONAL FLEXIBILITY

BAKERBOND® PolyPEI™ scales easily to the production environment with reproducible results in columns ranging from 20 cm 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 illustrated in Fig.6, the linear pressure-flow curve for BAKERBOND® PolyPEI™ 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 of polymethacrylate

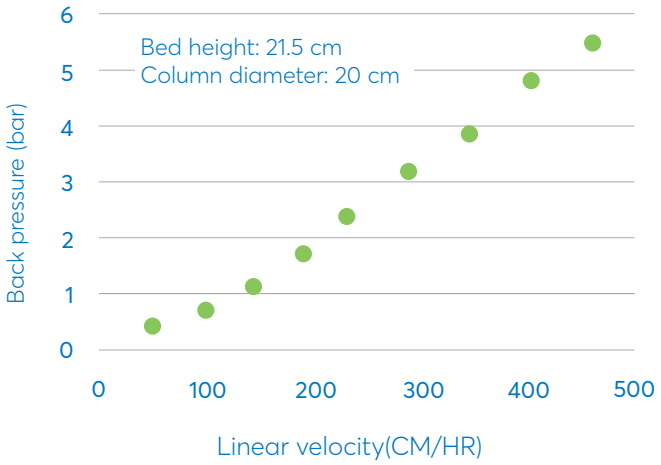


Fig.7. Efficiency of column (20 id x 21.5 cm high) packed with polymethacrylate based media

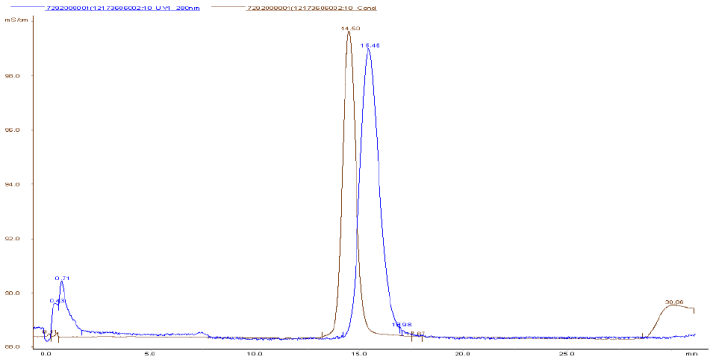
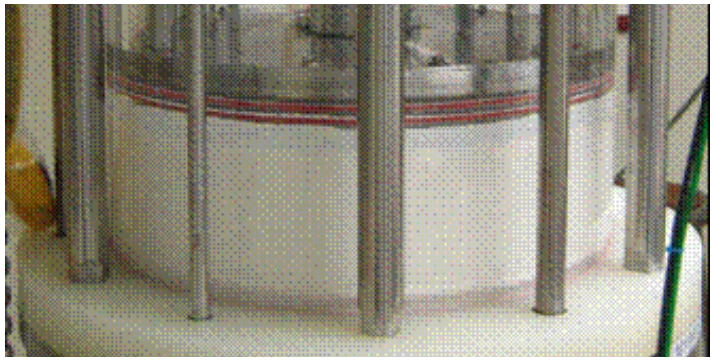


Fig.8. Scale – up to a 45 cm ID Column



CONVENIENT PACKAGING

BAKERBOND® PolyPEI™ 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)	**6059-07**
5 mL Lab Columns (5-pack)	**6059-25**
50 mL	**7585-01**
100 mL	**7585-02**
500 mL	**7585-03**
1 L	**7585-04**
5 L	**7585-05**

PROCESS AND APPLICATION SUPPORT

Avantor has deep expertise in process chromatography optimization and can work with you to help ensure that BAKERBOND® PolyPEI™ 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	Weak Anion Exchanger
Functional Group	$-CH_2CH_2NH_2$, $(-CH_2CH_2)_2NH$, $(CH_2CH_2)_3N$
Ion Exchange Capacity	0.3 – 0.5 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 also be stored in 20% ethanol or 0.1 M NaOH for up to 5 years.

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AUSTRIA
VWR International GmbH
Graumanngasse 7
1150 Vienna
Tel.: +43 1 97 002 0
info.at@vwr.com

BELGIUM
VWR International bv
Researchpark Haasrode 2020
Geldenaaksebaan 464
3001 Leuven
Tel.: +32 (0) 16 385 011
vwr.be@vwr.com

CANADA
VWR International
2360 Argentia Road
Mississauga, Ontario L5N 5Z7
Tel.: +1 800 932 5000
Canada_Orders@vwr.com

CHINA
VWR (Shanghai) Co., Ltd
Bld.No.1, No.3728 Jinke Rd,
Pudong New District
Shanghai, 201203- China
Tel.: 400 821 8006
info_china@vwr.com

CZECH REPUBLIC
VWR International s. r. o.
Veetee Business Park
Pražská 442
CZ - 281 67 Stříbrná Skalice
Tel.: +420 321 570 321
info.cz@vwr.com

DENMARK
VWR International A/S
Tobaksvejen 21
2860 Søborg
Tel.: +45 43 86 87 88
info.dk@vwr.com

FINLAND
VWR International Oy
Valimotie 9
00380 Helsinki
Tel.: +358 (0) 9 80 45 51
info.fi@vwr.com

FRANCE
VWR International S.A.S.
Le Périgares – Bâtiment B
201, rue Carnot
94126 Fontenay-sous-Bois cedex
Tel.: 0 825 02 30 30* (national)
Tel.: +33 (0) 1 45 14 85 00 (international)
info.fr@vwr.com
* 0,18 € TTC/min + prix appel

GERMANY
VWR International GmbH
Hilpertstraße 20a
D - 64295 Darmstadt
Tel.: 0800 702 00 07* (national)
Tel.: +49 (0) 6151 3972 0 (international)
info.de@vwr.com
*Freecall

HUNGARY
VWR International Kft.
Simon László u. 4.
4034 Debrecen
Tel.: +36 52 521130
info.hu@vwr.com

INDIA
VWR Lab Products Private Limited
No.139. BDA Industrial Suburb,
6th Main, Tumkur Road, Peenya Post,
Bangalore, India – 560058
Tel.: +91 80 28078400
vwr_india@vwr.com

IRELAND
VWR International Ltd
Orion Business Campus
Northwest Business Park
Ballycoolin
Dublin 15
Tel.: +353 (0) 1 88 22 222
sales.ie@vwr.com

ITALY
VWR International S.r.l.
Via San Giusto 85
20153 Milano (MI)
Tel.: +39 02 3320311
info.it@vwr.com

KOREA
VWR International ~
17, Daehak 4-ro, Yeongtong-gu,
Suwon-si, Gyeonggi-do
Tel.: +82 31 645 7256
saleskorea@avantorsciences.com

THE NETHERLANDS
VWR International B.V.
Postbus 8198
1005 AD Amsterdam
Tel.: +31 (0) 20 4808 400
info.nl@vwr.com

MEXICO
VWR International, S.de R.L. de C.V.
Km. 14.5 Carretera
Tlalnepantla-Cuautitlán
Col. Lechería
Tultitlán Edo. de México
CP 54940
Tel.: +52 (55) 5005 0100
vwrmx@vwr.com

MIDDLE EAST & AFRICA
VWR International FZ-LLC
Office 203, DSP Lab Complex,
Dubai Science Park,
Dubai, United Arab Emirates
Tel: +971 4 5573271
Info.mea@vwr.com

NORWAY
VWR International AS
Brynsalleen 4,
0667 Oslo
Tel.: +47 22 90 00 00
info.no@vwr.com

POLAND
VWR International Sp. z o.o.
Limbowa 5
80-175 Gdansk
Tel.: +48 58 32 38 200
info.pl@vwr.com

PORTUGAL
VWR International -
Material de Laboratório, Lda
Centro Empresarial de Alfragide
Rua da Indústria, nº 6
2610-088 Amadora
Tel.: +351 21 3600 770
info.pt@vwr.com

SINGAPORE
VWR Singapore Pte Ltd
18 Gul Drive
Singapore 629468
Tel: +65 6505 0760
sales.sg@vwr.com

SPAIN
VWR International Eurolab S.L.U.
C/ Tecnología 5-17
A-7 Llinars Park
08450 - Llinars del Vallès
Barcelona
Tel.: +34 902 222 897
info.es@vwr.com

SWEDEN
VWR International AB
Fagerstagatan 18a
163 94 Stockholm
Tel.: +46 (0) 8 621 34 00
kundservice.se@vwr.com

SWITZERLAND
VWR International GmbH
Lerzenstrasse 16/18
8953 Dietikon
Tel.: +41 (0) 44 745 13 13
info.ch@vwr.com

UK
VWR International Ltd
Customer Service Centre
Hunter Boulevard - Magna Park
Lutterworth
Leicestershire
LE17 4XN
Tel.: +44 (0) 800 22 33 44
uksales@vwr.com

UNITED STATES
VWR International, LLC
100 Matsonford Road
Building One Suite 200
Radnor, PA 19087
Tel.: +1 800 932 5000
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