Biomacromolecule Separation using Sepax PolyRP Bulk Media

Sepax Technologies, Inc.
5 Innovation Way
Newark DE 19711, USA
(877) SEPAX-US
www.sepax-tech.com

Introduction

PolyRP bulk media are highly crosslinked spherical resins made of styrene and divinylbenzene. These highly rigid resins are narrowly dispersed particles with particle size selection of 10, 15, and 30 µm and pore size selection of 100, 300, 500, and 1000 Å. These resins have abundant phenyl surface functional groups that enable hydrophobic interaction which is useful in reversed phase separation.

PolyRP bulk media are highly stable over a variety of operational conditions. They are stable to resist high temperatures up to 200°C (operating temperature up to 80°C). They are compatible with many commonly used organic solvents and aqueous buffers. PolyRP bulk media have a long lifetime. With a well-controlled polymer resin manufacturing process, PolyRP bulk media are very reproducible from batch to batch. Compared to silica based reversed phases, PolyRP bulk media are more stable at extreme pH (1- 14) with a similar separation efficiency and unique selectivity. **Figure 1** shows 10 µm PolyRP resins with pore sizes of 100, 300, and 1000 Å.

Resin Technical Properties

Resin	PolyRP 10	PolyRP 15	PolyRP 30
Matrix	Porous polystyrene/divinylbenzene microspheres		
Particle Size (µm)	10.0±1.0	15.0±1.5	30.0±3.0
Particle Size Distribution (D_{90}/D_{10})	≤ 1.3		
Average Pore Size (Å)	100, 300, 500, 1000		
Bulk Density (g/mL)	0.26 – 0.32	0.20 – 0.32	0.20 – 0.32
Specific Surface Area (m^2/g)	200 - 1000	200 - 1000	200 - 1000
Specific Pore Volume (mL/g)	0.9 – 1.4	0.9 – 2.4	0.9 – 2.4
Expansion in Methanol (vol%)	< 5	< 5	< 5
Max Pressure	15 MPa (150 bar)	10 MPa (100 bar)	10 MPa (100 bar)
Operation temperature	≤ 80 ℃		
pH range	1 – 13 for use; 1 – 14 for CIP		
Compatible Solvents	Compatible with many commonly used organic solvents and aqueous solution such as a mixture of water and acetonitrile, acetone, methanol, ethanol, n-propanol, THF; 1 M HCl, 1 M NaOH, 1 M HCl/ 90% methanol, 90% HAc, 0.45 M NaOH/40% isopropanol, 6 M guanidine		
CIP and Regeneration	Following solvents can be used alone or in combination: 0.5-1 M NaOH, 1 M HCl, 90% acetonitrile or isopropyl alcohol, 90% acetic acid, 3% TFA		
Autoclavable	20 min at 121 ℃		
Storage	2-30 ℃, 20% ethanol		

Features

- PolyRP resins are narrowly dispersed particles
- Well controlled particle size 10, 15, 30 µm
- Well controlled pore size at 100, 300, 500 and 1000 A
- Strong mechanic strength
- Wide pH operation range 1-14
- High capacity and loading
- High separation, high resolution and efficiency

SEM Analysis on Particle Morphology

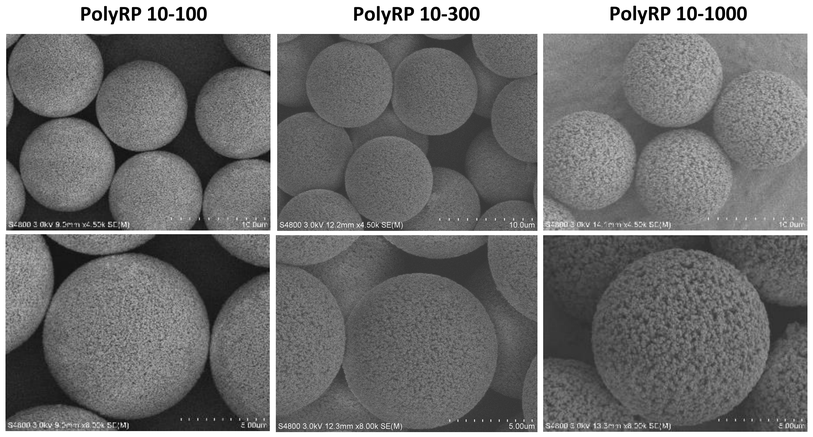


Figure 1. Rigid, spherical, monodispersed, porous microspheres. Precise control on particle morphology: bead size, pore size, surface area, pore volume.

Particle Size Distribution

Volume Statistics (Arithmetic)

Median: 9.919 µm
D(4,3): 10.27 µm
C.V.: 28.2%

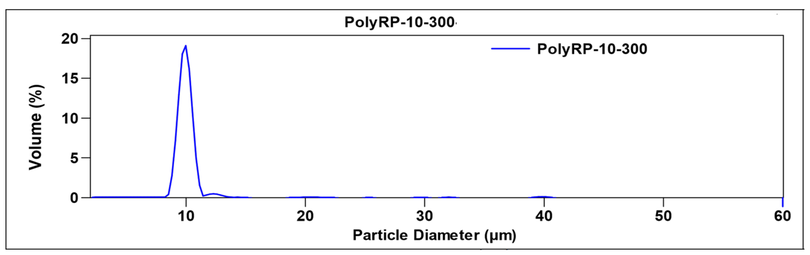
Calculations from 2.000 µm to 60.00 µm

PolyRP-10-300

d_{10}: 9.566 µm d_{50}: 9.919 µm d_{90}: 10.35 µm

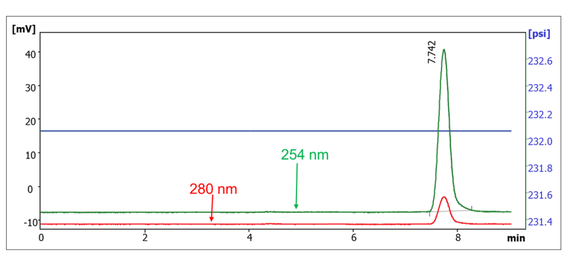
<3 µm 0.18%

$$\frac{d_{90}}{d_{10}} = 1.08$$



Evaluation of DAC Column

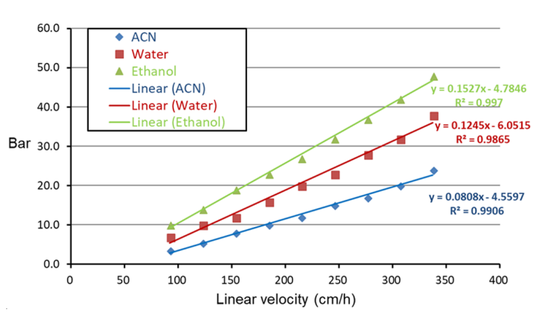
Figure 2. PolyRP 10-300 efficiency, N= 27,028/m and peak symmetry of 1.14.



Resin: PolyRP 10-300 (10 µm, 300 Å)
Column: 50 x 250 mm DAC
Mobile Phase: ACN
Flow Rate: 50 mL/min (150 cm/h)
Detection: UV 254 and 280 nm
Column Temperature: RT
Injection Volume: 500 µL
Samples: 10% Acetone in Acetonitrile (ACN)
Back Pressure: 232 Psi

Mobile Phase Impact on Back Pressure

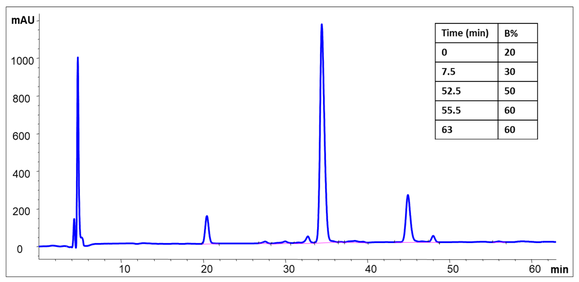
Figure 3. Net back pressure vs. flow-rate characteristics of PolyRP10-300. Typical flow rate rage is 100-600 cm/h.



Resin: PolyRP 10-300 (10 µm, 300 Å)
Column: 50 x 250 mm (DAC)

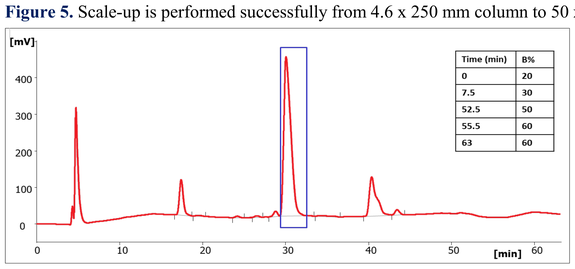
Insulin Analog Separation Using PolyRP 10-300 and Scale Up

Figure 4. PolyRP 10-300 bulk media separated crude Insulin analog based on hydrophobic interaction.



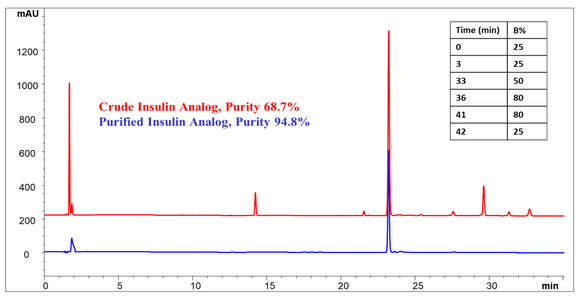
Resin: PolyRP 10-300 (10 µm, 300 Å)
Column: 4.6 x 250 mm (Stainless Steel)
Mobile Phase: *A:* 0.1% TFA - H_2O *B:* 0.1% TFA – ANC
Flow Rate: 0.67 mL/min (240 cm/h)
Detection: UV 214 nm Column
Temperature: RT
Injection Volume: 20 µL
Samples: Crude Insulin Analog, 1 mg/mL

Figure 5. Scale-up is performed successfully from 4.6 x 250 mm column to 50 x 250 mm DAC column.



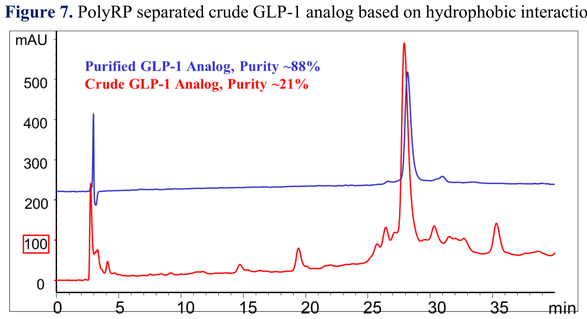
Resin: PolyRP 10-300 (10 µm, 300 Å)
Column: 50 x 250 mm (DAC)
Mobile Phase: *A:* 0.1% TFA - H_2O *B:* 0.1% TFA – ACN
Flow Rate: 80 mL/min (240 cm/h)
Detection: UV 214 nm
Column Temperature: RT
Injection Volume: 10 mL
Samples: Crude Insulin Analog, 1 mg/mL

Figure 6. Purity analysis of crude and purified insulin analog by HPLC.



Resin: GP-C18 (3 µm, 120 A)
Column: 4.6 x 150 mm (Stainless Steel)
Mobile Phase: A: 0.02 M Na_2SO_4-Triethanolamine (1%), pH 2.3, B: ACN
Flow Rate: 1.0 mL/min (360 cm/h)
Detection: UV 214 nm
Column Temperature: 40°C
Injection Volume: 5 µL
Pressure: 97-172 bar

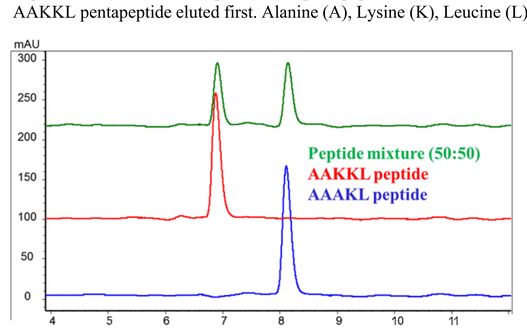
Figure 7. PolyRP separated crude GLP-1 analog based on hydrophobic interaction.



Resin: PolyRP 10-300 (10 µm, 300 Å)
Column: 4.6 x 250 mm (Stainless Steel)
Mobile Phase: *A:* 0.1% TFA - H_2O *B:* 0.1% TFA – ACN
Flow Rate: 1.0 mL/min (360 cm/h)
Detector: UV 214 nm
Column Temperature: RT
Injection Volume: 100 µL
Gradient: 0-40 min, 30-60% B

Pentapeptide Separation Using PolyRP 10-300

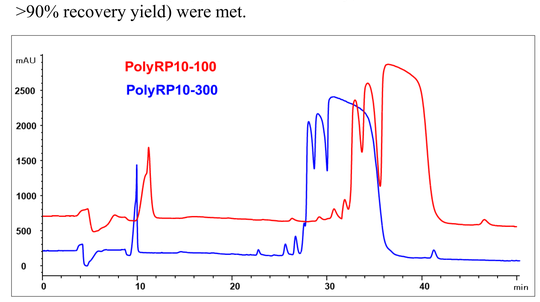
Figure 8. PolyRP 10-300 separated two pentapeptides which are different by only one amino acid. More polar AAKKL pentapeptide eluted first. Alanine (A), Lysine (K), Leucine (L).



Resin: PolyRP 10-300 (10 µm, 300 Å)
Column: 4.6 x 250 mm (Stainless Steel)
Mobile Phase: *A:* 0.1% TFA - H_2O *B:* 0.1% TFA – ACN
Flow Rate: 1.0 mL/min (360 cm/h)
Detector: UV 214 nm
Column Temperature: 25°C
Injection Amount: 4 µg
Gradient: 0-20 min, 5-50% B

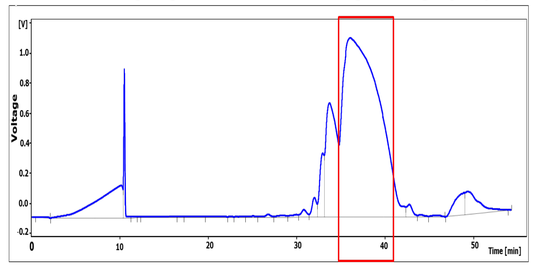
Polypeptide Separation Using PolyRP10 10-100

Figure 9. Due to a pore size impact on separation resolution, PolyRP 10-100 performed better than PolyRP10-300 for a customer's polypeptide. Additionally, the customer's demanding purification targets (>95% purity and >90% recovery yield) were met.



Resin: PolyRP 10-100 & 10-300 (10 µm, 100 Å & 300 Å)
Column: 10 x 250 mm (Stainless Steel)
Mobile Phase: *A:* 0.1% TFA -H_2O *B:* 0.1% TFA – ACN **Detector:** UV 210 nm **Column Temperature:** RT **Flow Rate:** 3.0 mL/min (230 cm/h)
Sample: Polypeptide ~3000 Da crude purity 72%

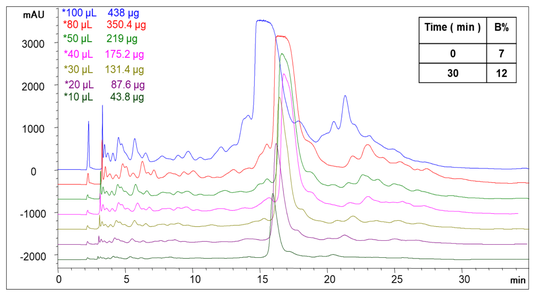
Figure 10. PolyRP10-100 process chromatography was successfully scaled up to 50 DAC and to 300 DAC (at customer site and met customer's purification targets). The customer's overall purification cost was greatly reduced due to high separation resolution and high loading capacity of PolyRP10-100.



Resin: PolyRP 10-100 (10 µm, 100 Å)
Column: 50 x 250 mm (DAC)
Mobile phase: *A:* 0.1% TFA - H_2O *B:* 0.1% TFA – ACN
Detector: UV 210 nm
Flow Rate: 40 mL/min (120 cm/h)
Sample: Polypeptide ~3000 Da crude purity 72%

ssDNA Primer Separation Using PolyRP 10-300 Bulk Media

Figure 11. PolyRP 10-300 separated 32 nucleotides ssDNA primer from smaller ssDNA primers and protein impurity. After separation, the purity increased from ~72% to 99%, and recovery yield was 91%.



Resin: PolyRP 10-300 (10 µm, 300 Å)
Column: 4.6 x 150 mm (Stainless Steel)
Mobile Phase: *A:* 100 mM TEAA (pH 7.0) *B:* ACN
Flow Rate: 0.8 mL/min (290 cm/h)
Detector: UV 260 nm
Column Temperature: 30°C
Sample: 32 nucleotides 4.38 mg/mL in water
Pressure: 38 bar

PolyRP Bulk Media Order Information

Particle Size	Pore Size	PN#
10 µm	100 Å	260110101
10 µm	300 Å	260110301
10 µm	300 Å	260510301*
10 µm	500 Å	260110501
10 µm	1000 Å	260110951
15 µm	100 Å	260115101
15 µm	300 Å	260115301
15 µm	500 Å	260115501
15 µm	1000 Å	260115951
15 µm	1000 Å	260515951*
30 µm	100 Å	260130101
30 µm	300 Å	260130301
30 µm	300 Å	260530301*
30 µm	500 Å	260130501
30 µm	1000 Å	260130951

* High loading capacity resin

Standard packing size:
1L, 5L, 10L, 25L, 50L, 100L
Additional pack sizes are available.

Additional particle and pore sizes are available.

Pre-packed stainless-steel columns for sample preparation and separation process development/ scale-up are available.

Please contact your regional sales agent for more information.

Authored by: Huiming Mao, Ke Yang, Xueying Huang, Yi Wang and Xinmei Hu

Better Surface Chemistry for Better Separation