



# Biomacromolecule Separation using Sepax Monomix MC SEC Bulk Media

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## Introduction

Monomix MC SEC bulk media are highly crosslinked spherical resins made of polymethylacrylate. These rigid resins are narrowly dispersed particles with particle size selection of 10, 15, 30 and 60  $\mu\text{m}$  and pore size selection of 500 and 1000  $\text{\AA}$ . **Figure 1.** shows SEM images of 10, 15, and 60  $\mu\text{m}$  Monomix MC SEC beads with 1000  $\text{\AA}$  pore size. These resins have proprietary hydrophilic surface functional groups that minimize non-specific binding. Monomix MC SEC resins possess several key benefits: a broad pH tolerant range (1-14), elevated operating temperature (up to 80°C), high operating pressure (up to 20 bar), monosized particle ( $D_{90}/D_{10} < 1.3$ , low column back pressure, high theoretical plate number), smooth surface and highly spherical (for easy column packing).

Monomix MC SEC bulk media are highly stable over a variety of operational conditions. They are compatible with many commonly used organic solvents and aqueous buffers. Compared to silica-based SEC bulk media, they are more stable at extreme pH (1- 14) condition. Compared to agarose bulk media, they have more rigid backbones so they can resist high column back pressure and thus be operated at high flow rate. Additionally cleaning and CIP are user friendly and effective and thus elongate resin life. Overall, they can increase purification productivity and save purification cost. They have demonstrated SEC mechanism-based applications in proteins, polysaccharides, VLP, and other biomacromolecules.

## Separation of Four Protein Standards

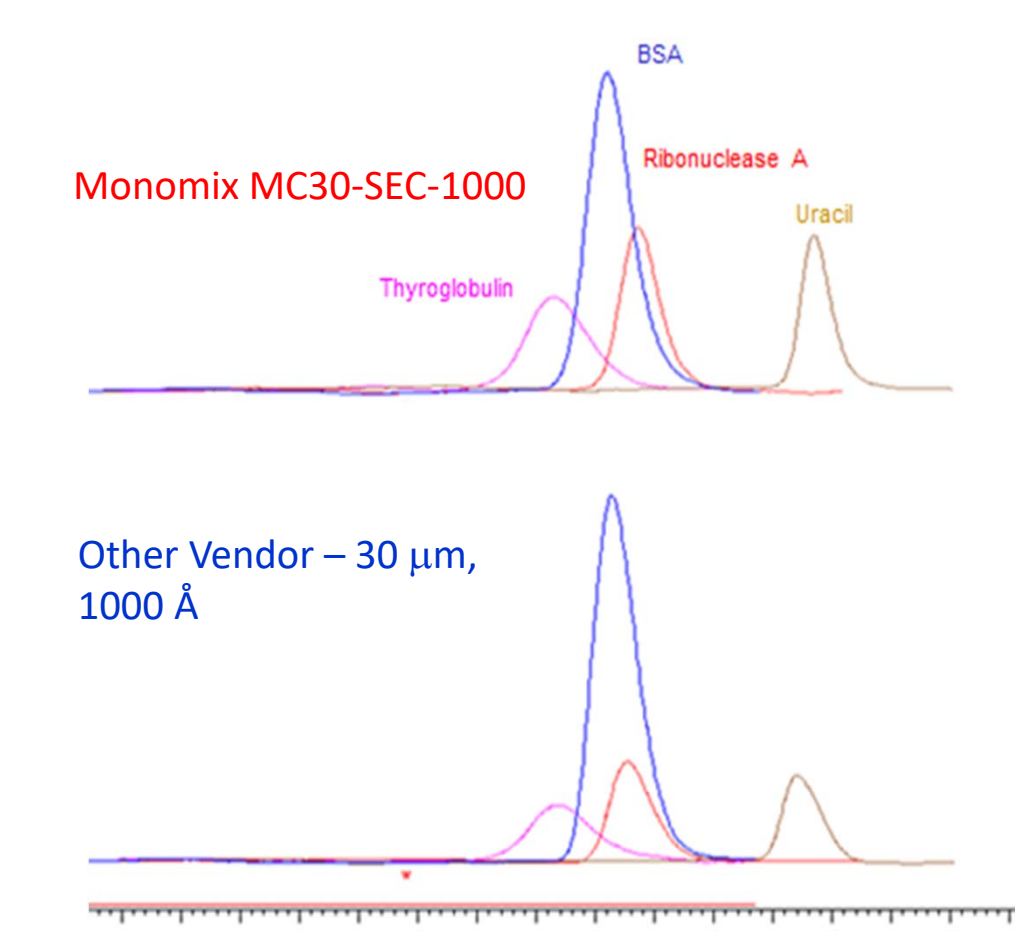
Resin product	Monomix MC10 SEC	Monomix MC15 SEC	Monomix MC30 SEC	Monomix MC60 SEC
Matrix	Polymethacrylate, rigid, porous microspheres			
Average Particle Size ( $\mu\text{m}$ )	10.0 $\pm$ 1.0	15.0 $\pm$ 1.5	30.0 $\pm$ 3.0	60.0 $\pm$ 6.0
Particle Size Distribution ( $D_{90}/D_{10}$ )	$\leq 1.3$			
Average Pore Size ( $\text{\AA}$ )	500, 1000	500, 1000	1000	1000
Specific Pore Volume (mL/g)	$\geq 1.0$			
Max Operation Pressure (MPa)	20	20	10	10
Operation Temperature ( $^{\circ}\text{C}$ )	$\leq 80$			
pH Working Range	2-12			
pH Cleaning Range (CIP)	1-14			
Storage Conditions	2-30 $^{\circ}\text{C}$ , 20% ethanol			
Compatible Solvent	Compatible with many commonly used organic solvents and aqueous solution.			
CIP and Regeneration	0.1-1.0 M NaOH, 20% ethanol, 30% isopropanol, 30% acetonitrile, 2% sodium lauryl sarcosinate, 20% isopropanol/0.01 M HCl, 1 M acetic acid, 8 M urea, 6 M guanidine hydrochloride			

## Resin Technical Properties

Product Name	Monomix MC60 SEC-1000 Monomix MC30 SEC-1000 Monomix MC15 SEC-1000 Monomix MC10 SEC-1000
Matrix	Surface hydrophilized polymethacrylate
Color	White
Physical Form	Narrowly dispersed, porous spheres
Average Particle Size ( $\mu\text{m}$ )	60 $\pm$ 6.0 30 $\pm$ 3.0 15 $\pm$ 1.5 10 $\pm$ 1.0
Particle Size Distribution	$D_{90}/D_{10} \leq 1.3$
Average Pore Size ( $\text{\AA}$ )	1000
PEO or PEG MW Separation (Dalton/mol)	500 - 1 x 10 <sup>6</sup>
Dextran MW Separation (Dalton/mol)	1 x 10 <sup>4</sup> - 1 x 10 <sup>6</sup>
Globular Protein MW Separation (Dalton/mol)	4 x 10 <sup>4</sup> - 5 x 10 <sup>6</sup>
Maximum Operating Pressure (Bar)	10 10 20 20
Operation Temperature ( $^{\circ}\text{C}$ )	$\leq 80$
pH Working Range	2 - 12
pH Cleaning Range (CIP)	1 - 14
Storage Conditions	2 - 30 $^{\circ}\text{C}$ , 20% Ethanol
Compatible Solvent	Compatible with many commonly used organic solvents and aqueous solution
CIP and Regeneration	0.1 - 1.0 M NaOH, 20% ethanol, 30% isopropanol, 30% acetonitrile, 2% sodium lauryl sarcosinate, 20% isopropanol/0.01 M HCl, 1 M acetic acid, 8 M urea, 6 M guanidine hydrochloride
Change in Bed Volume	Minor change due to change(s) of pH or ion strength

## Characteristics of Sepax Monomix MC SEC-1000

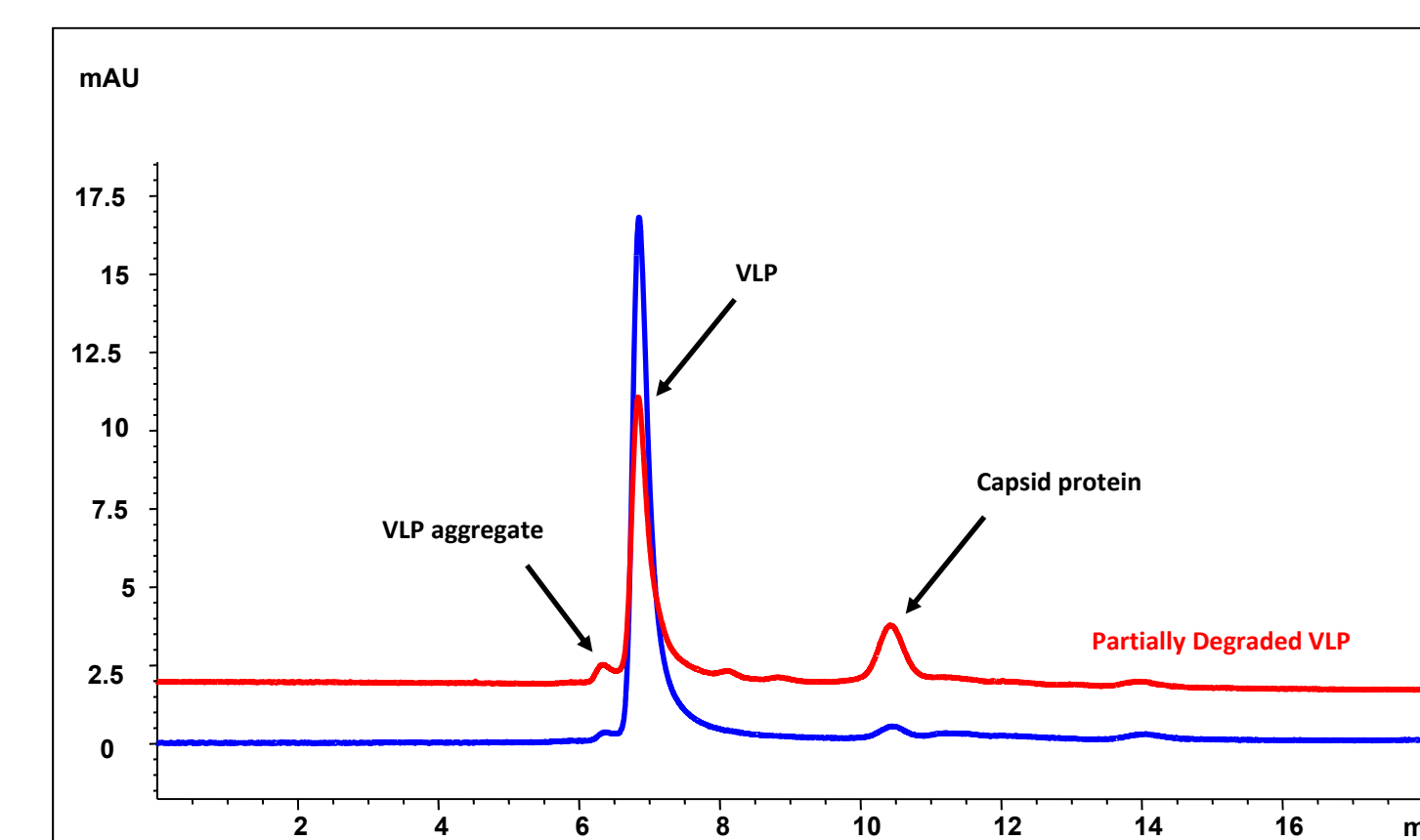
**Figure 5.** The Monomix MC30 SEC-1000 column better separated four proteins than the Other Vendor – 30  $\mu\text{m}$ , 1000  $\text{\AA}$  column.



Resin: Monomix MC30 SEC-1000 (30  $\mu\text{m}$ , 1000  $\text{\AA}$ )  
Column: FPLC 10 x 500 mm, AA  
Instrument: Sepax FPLC Generik HP36  
Mobile Phase: 150 mM sodium phosphate, pH 7.0  
Flow Rate: 1.5 mL/min (115 cm/h)  
Detector: UV 214 nm  
Samples: Thyroglobulin (1.0 mg/mL), BSA (1.0 mg/mL), Ribonuclease A (1.0 mg/mL), Uracil (0.25 mg/mL)  
Injection Volume: 200  $\mu\text{L}$

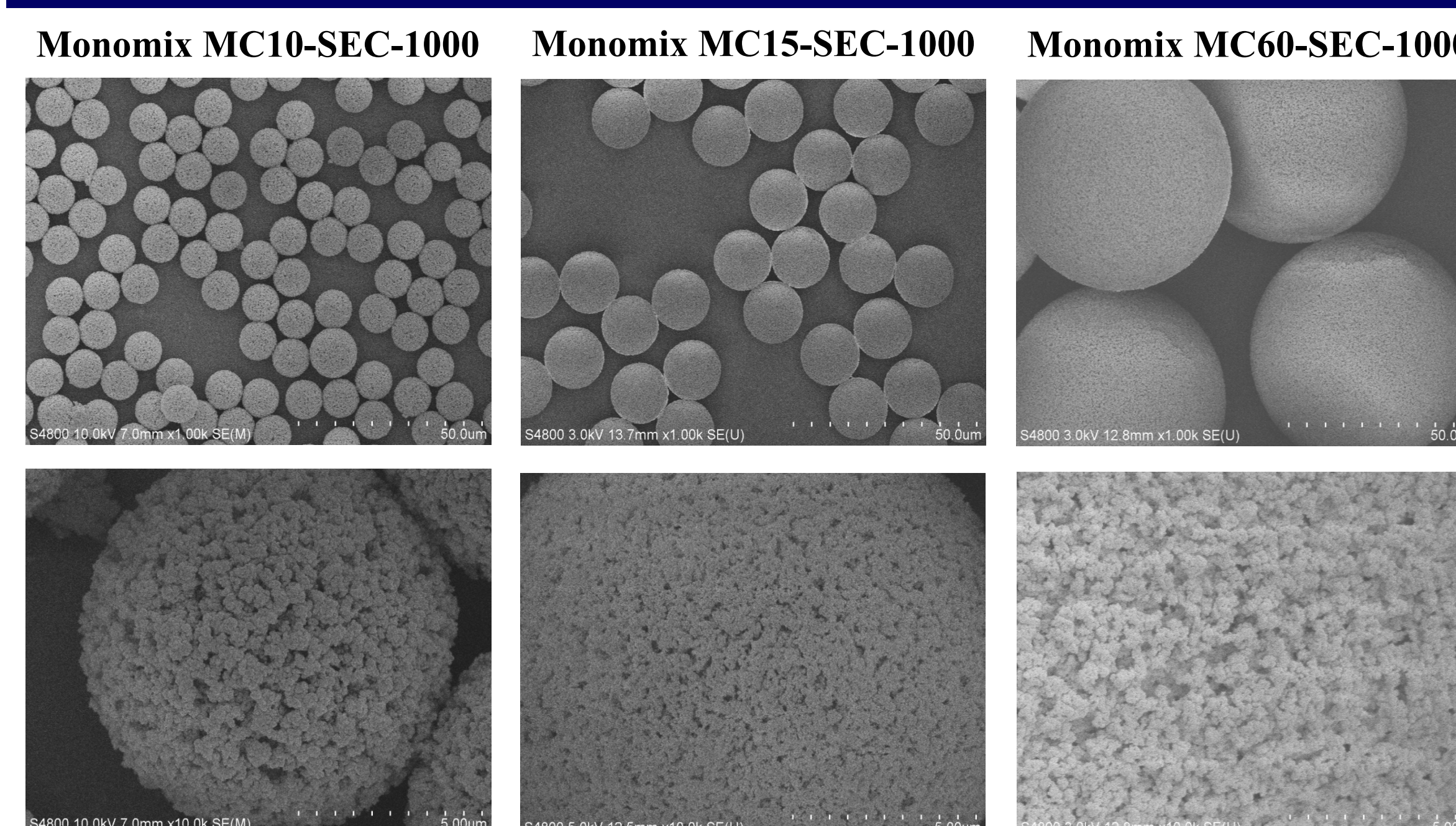
## Crude VLP Separation

**Figure 6.** Monomix MC10 SEC-500 column shows good separation of VLP, VLP aggregate and capsid protein. In partially degraded VLP sample intensity of VLP decreases while intensity of VLP aggregate and capsid protein increase. Capsid protein elutes at 10.43 min/ 8.45 mL, so its molecular weight is between BSA (8.24 mL) and Ribonuclease A (8.83 mL) [data not shown].



Resin: Monomix MC10 SEC-500 (10  $\mu\text{m}$ , 500  $\text{\AA}$ )  
Column: 7.8 x 300 mm (Stainless Steel)  
Instrument: Agilent 1260  
Mobile Phase: 20 mM sodium phosphate + 150 mM NaCl (pH 7.0)  
Flow Rate: 0.81 mL/min (100 cm/h)  
Detector: UV 280 nm  
Column Temperature: 25  $^{\circ}\text{C}$   
Sample: Crude VLP, ~80 nm  
Injection Volume: 100  $\mu\text{L}$

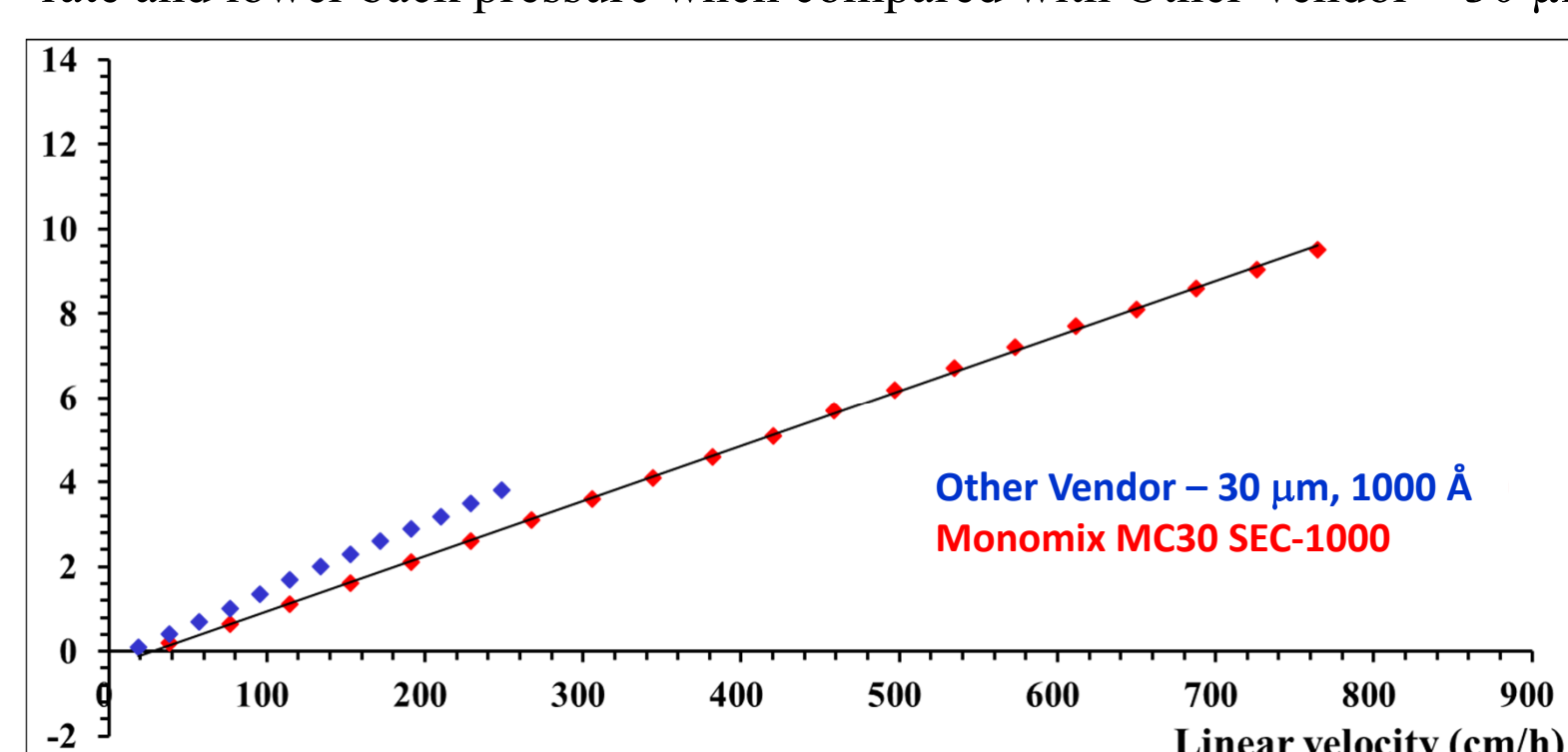
## SEM Analysis on Particle Morphology



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

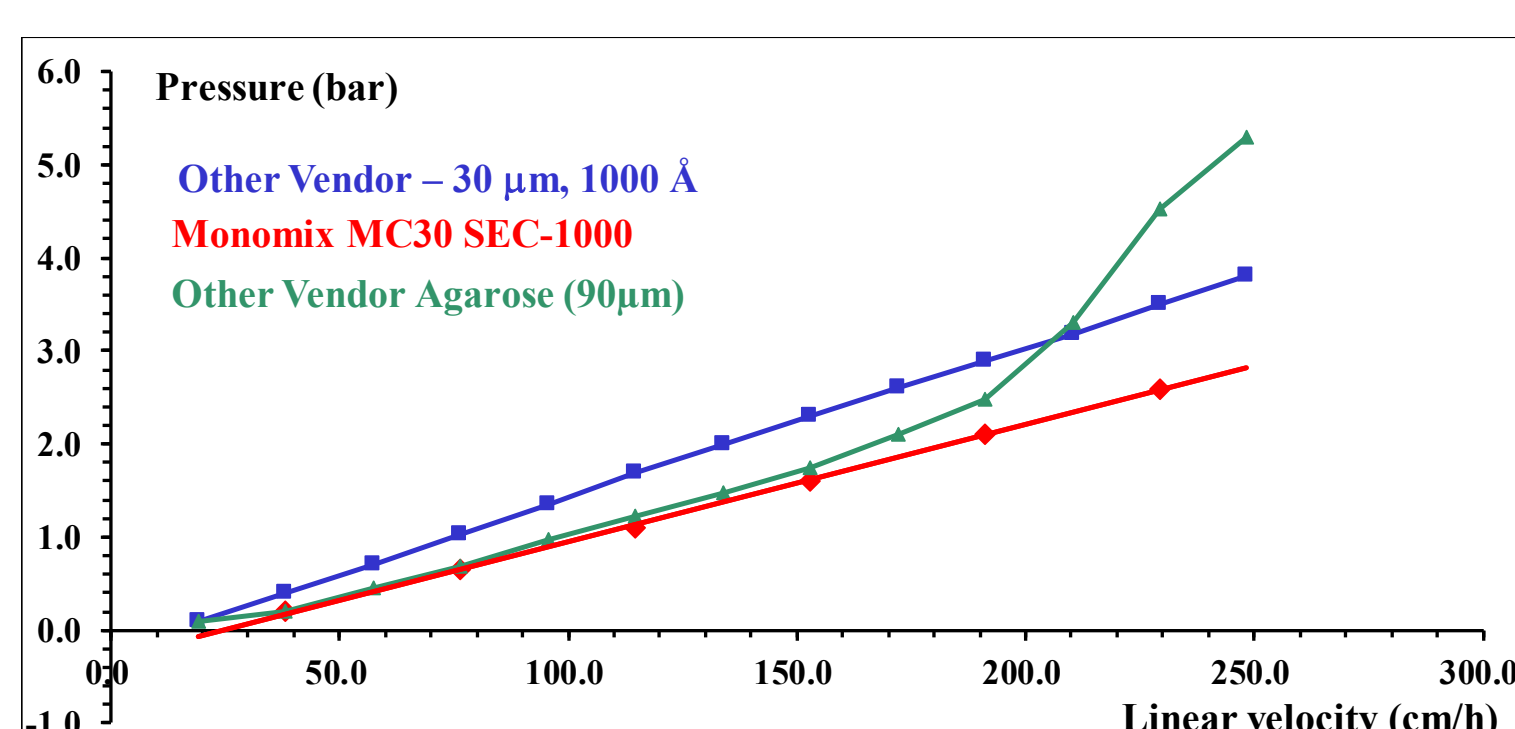
## Flow Rate-Back Pressure Test

**Figure 2.** Monomix MC30 SEC-1000 shows a linear relationship. The back pressure was under 10 bar at 760 cm/hour when tested in a 10 x 450 FPLC column. Monomix MC30 SEC-1000 can operate at a higher flow rate and lower back pressure when compared with Other Vendor – 30  $\mu\text{m}$ , 1000  $\text{\AA}$ , polymethacrylate resin.



Resin: Monomix MC30 SEC-1000 (30  $\mu\text{m}$ , 1000  $\text{\AA}$ )  
Column: FPLC 10 x 500 mm, AA  
Instrument: Sepax FPLC Generik HP36  
Mobile Phase: 150 mM Sodium Phosphate, pH 7.0  
Column Temperature: 25  $^{\circ}\text{C}$

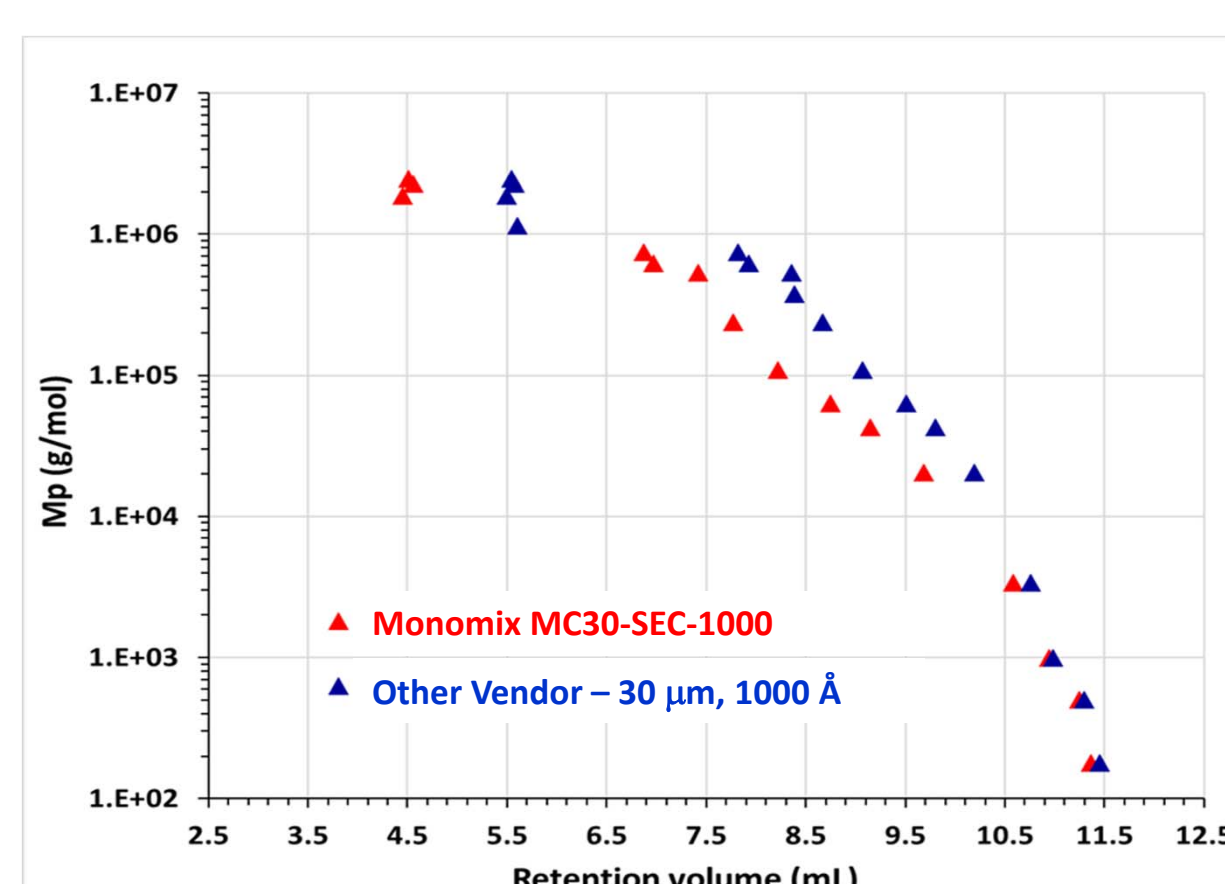
**Figure 3.** The back pressure of Monomix MC30 SEC-1000 was lower than the Other Vendor – 30  $\mu\text{m}$ , 1000  $\text{\AA}$ , polymethacrylate resin. Monomix MC30 SEC-1000 was close to the Other Vendor Agarose (90  $\mu\text{m}$ ) resin at low linear velocity, when tested in a 10 x 450 FPLC column. At high linear back pressure of velocity the Other Vendor Agarose (90  $\mu\text{m}$ ) suddenly increased, but the back pressure of Monomix MC SEC 30-1000 increased linearly, with respect to the flow rate of up to 760 cm/hour.



Resin: Monomix MC30 SEC-1000 (30  $\mu\text{m}$ , 1000  $\text{\AA}$ )  
Column: FPLC 10 x 500 mm, AA  
Instrument: Sepax FPLC Generik HP36  
Mobile Phase: 150 mM Sodium Phosphate, pH 7.0  
Column Temperature: 25  $^{\circ}\text{C}$

## Calibration Curve with Dextran

**Figure 4.** Monomix MC30 SEC-1000 exhibits a similar dextran exclusion molecular weight (indication of pore size) as the Other Vendor – 30  $\mu\text{m}$ , 1000  $\text{\AA}$ , polymethacrylate resin.

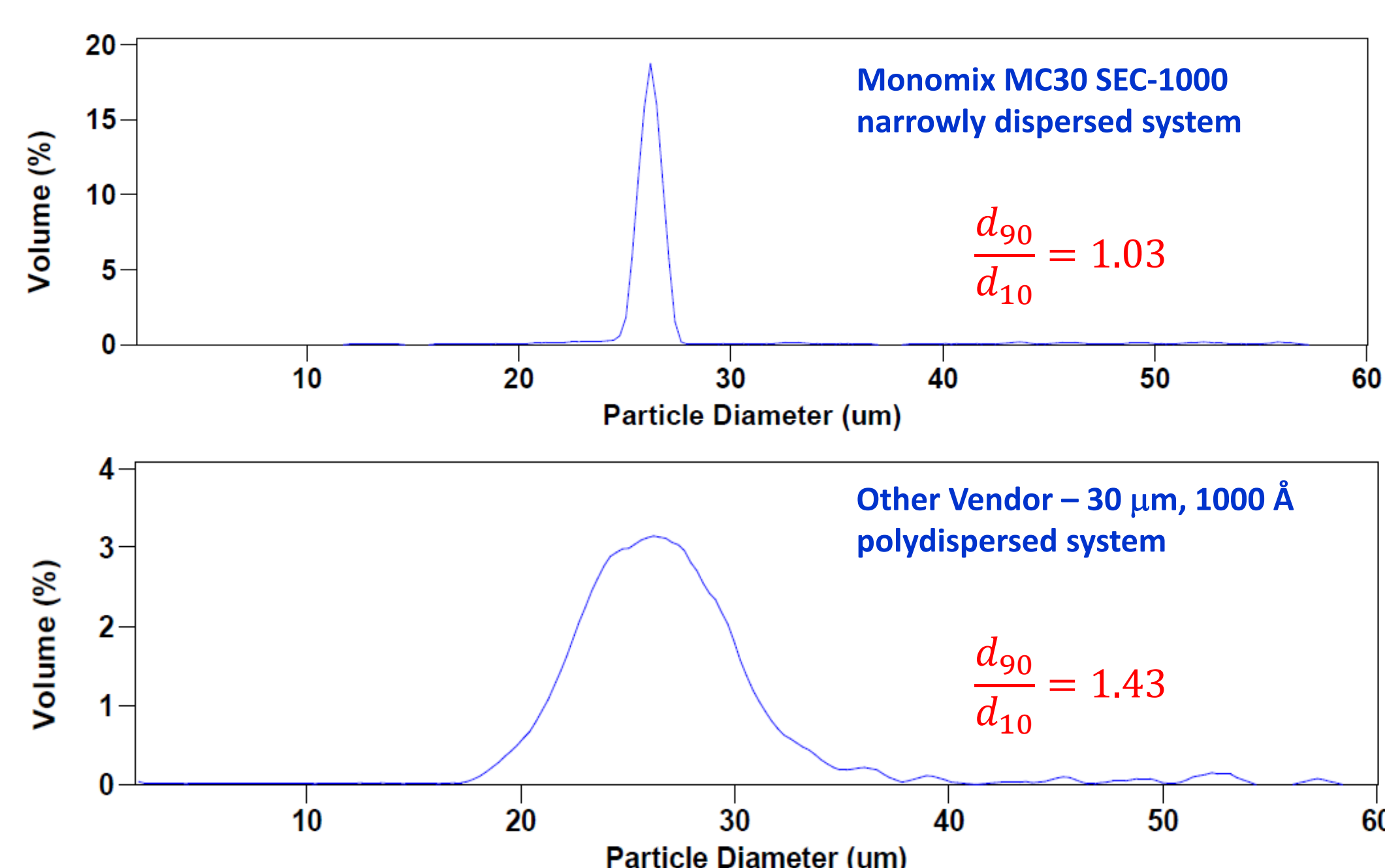


Resin: Monomix MC30 SEC-1000 (30  $\mu\text{m}$ , 1000  $\text{\AA}$ )  
Column: 7.8 x 300 mm (Stainless Steel)  
Instrument: Agilent 1260  
Mobile Phase: 150 mM Sodium Phosphate, pH 7.0  
Flow Rate: 1.0 mL/min (125 cm/h)  
Detector: RI  
Column Temperature: 25  $^{\circ}\text{C}$   
Sample: 10 mg/mL  
Injection Volume: 10  $\mu\text{L}$

## Features

- Monomix SEC resins are narrowly dispersed particles
  - Well controlled pore structure
  - Wide pH range
- Rigid beads can be operated at higher flow rates and higher pressure
  - High dynamic capacity and high loading capacity
  - High separation efficiency and resolution
- Negligible non-specific binding for high recovery of biological sample

## Benchmarking Against Other Vendor, Particle Size Distribution



## Monomix MC SEC Bulk Media Order Information

Resin	Particle Size	Pore Size	Part Number
Monomix MC10 SEC-500	10 $\mu\text{m}$	500 $\text{\AA}$	280110500
Monomix MC10 SEC-1000	10 $\mu\text{m}$	1000 $\text{\AA}$	280110950
Monomix MC15 SEC-500	15 $\mu\text{m}$	500 $\text{\AA}$	280115500
Monomix MC15 SEC-1000	15 $\mu\text{m}$	1000 $\text{\AA}$	280115950
Monomix MC30 SEC-1000	30 $\mu\text{m}$	1000 $\text{\AA}$	280130950
Monomix MC60 SEC-1000	60 $\mu\text{m}$	1000 $\text{\AA}$	280160950

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.

## Conclusion

Monomix MC SEC resins provide a wide variety of solutions to cover both analytical needs and preparative purification needs. Monomix MC SEC bulk media can be used for separation of proteins, VLP and other biomacromolecules. Monomix MC SEC process chromatography was successfully scaled up at the customer site and its performance met customer's expectations.

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