macmod

SMARTER CHROMATOGRAPHY

Upgrading Size-Exclusion Chromatography (SEC) applications with monodisperse fully porous particles (MFPP)

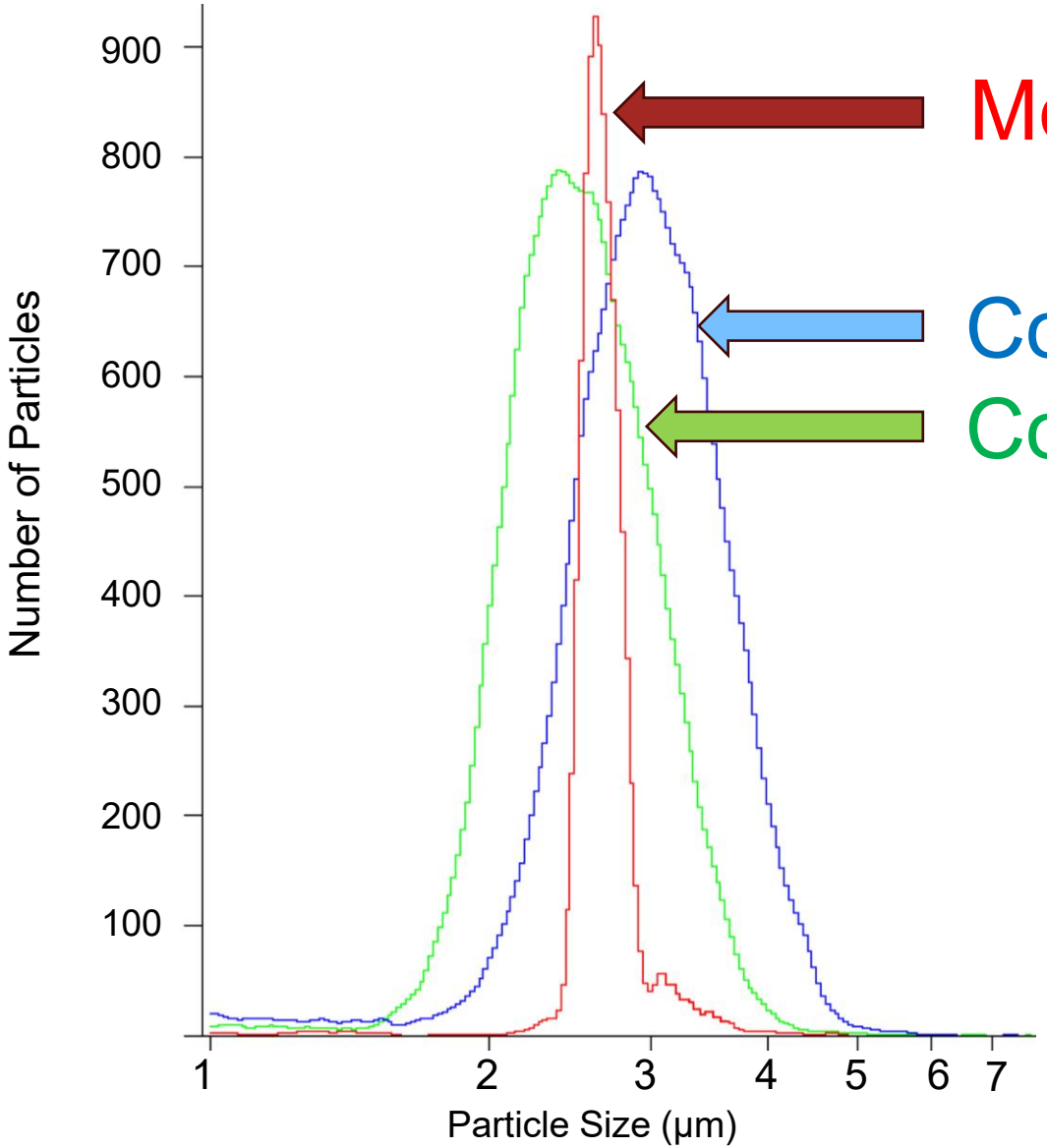
Presented by Geoffrey Faden BSc. Chem

Outline

- Introduction to Monodisperse Fully Porous Particle (MFPP) Technology
- MFPP Technology Applied to Size Exclusion Chromatography

Introduction to Monodisperse Particle Technology

Monodisperse vs. Competitor Polydisperse Comparisons

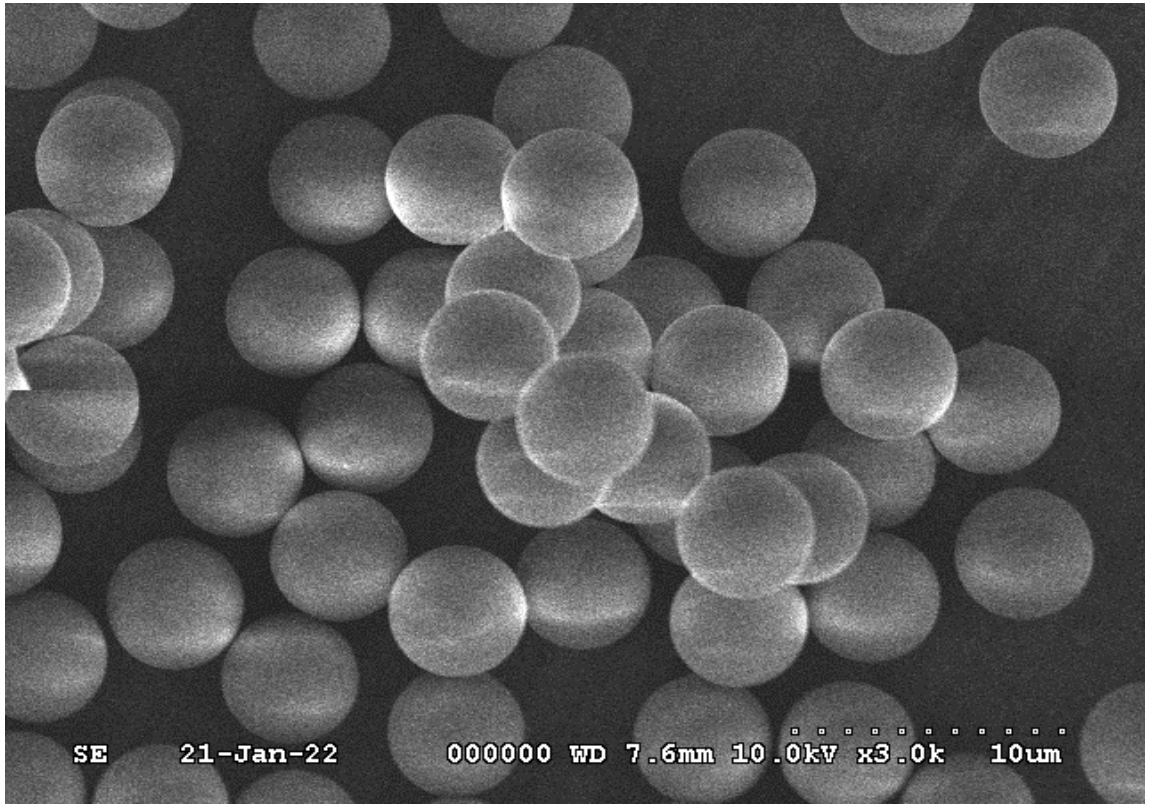


Monodisperse FPP 3 µm

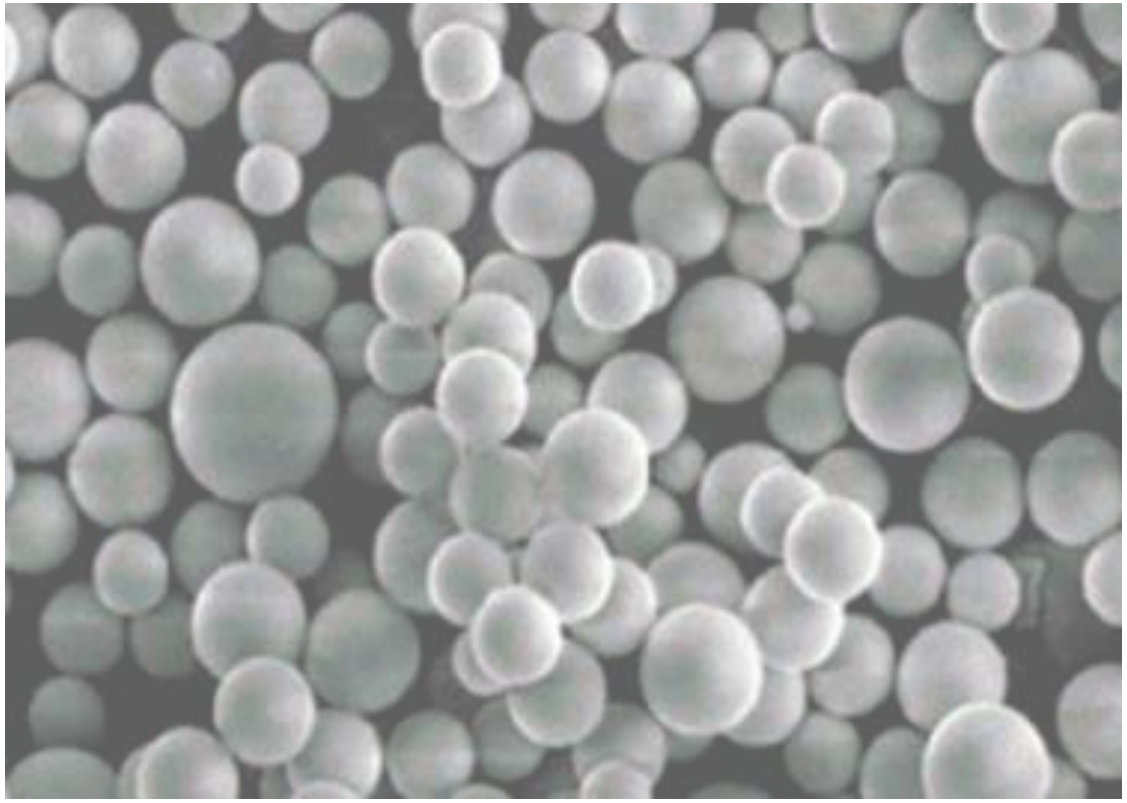
Commercial FPP A 3 µm

Commercial FPP B 3 µm

SEM - New MONOBIO Particles Technologies

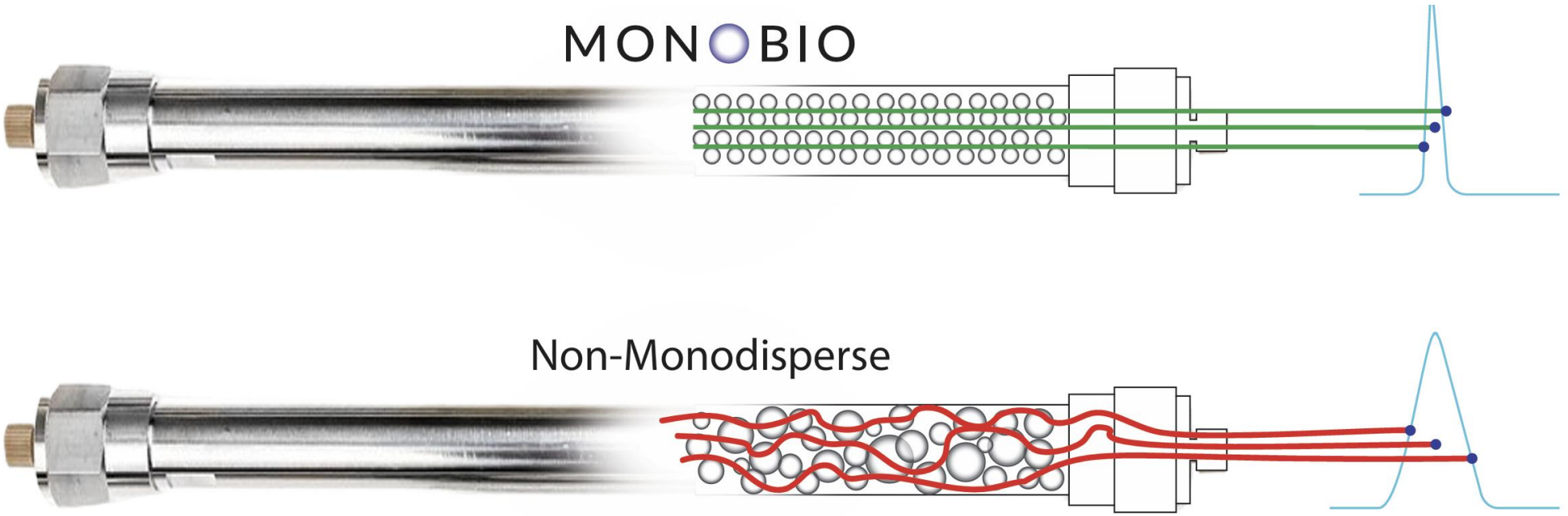


Monodisperse

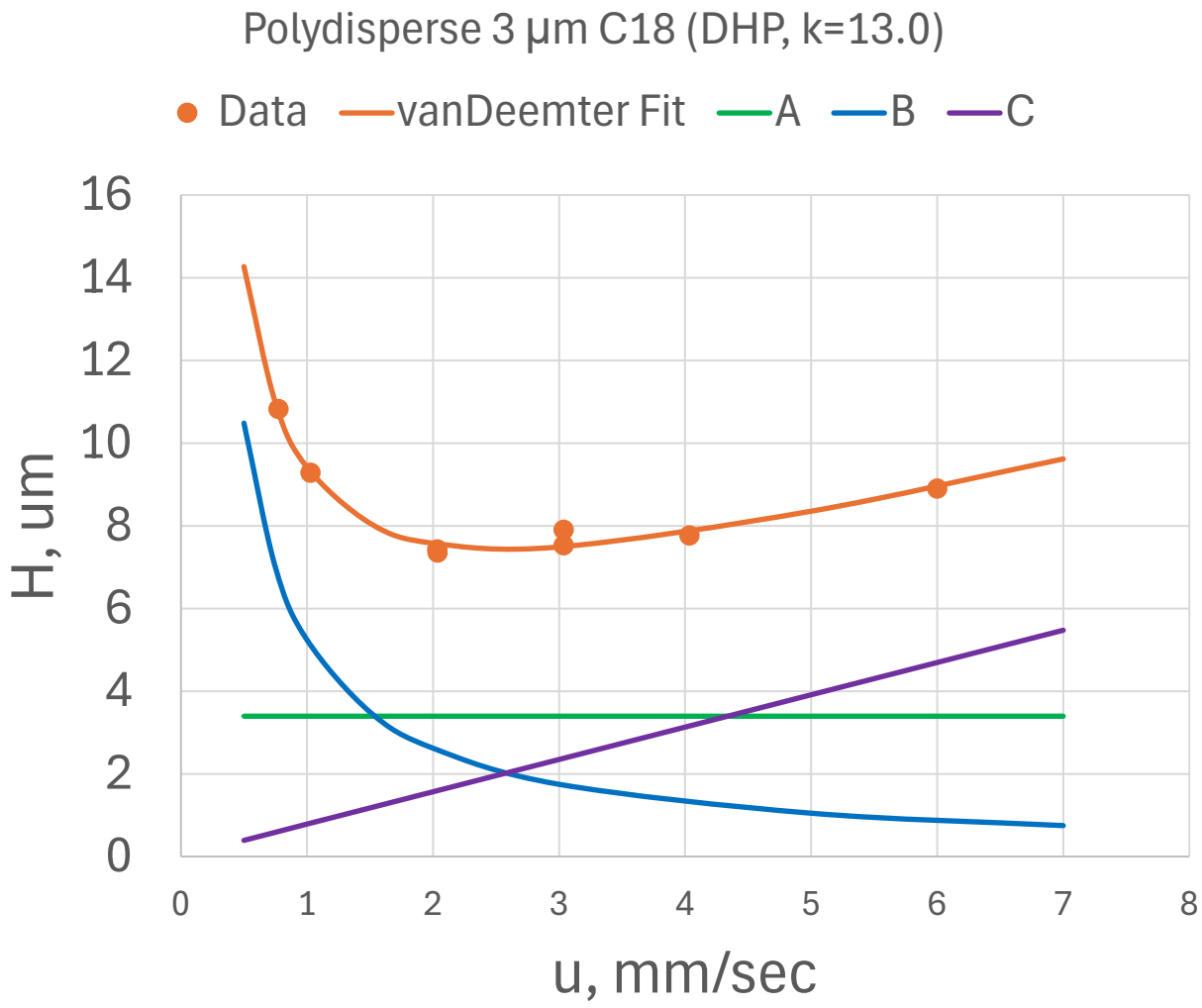


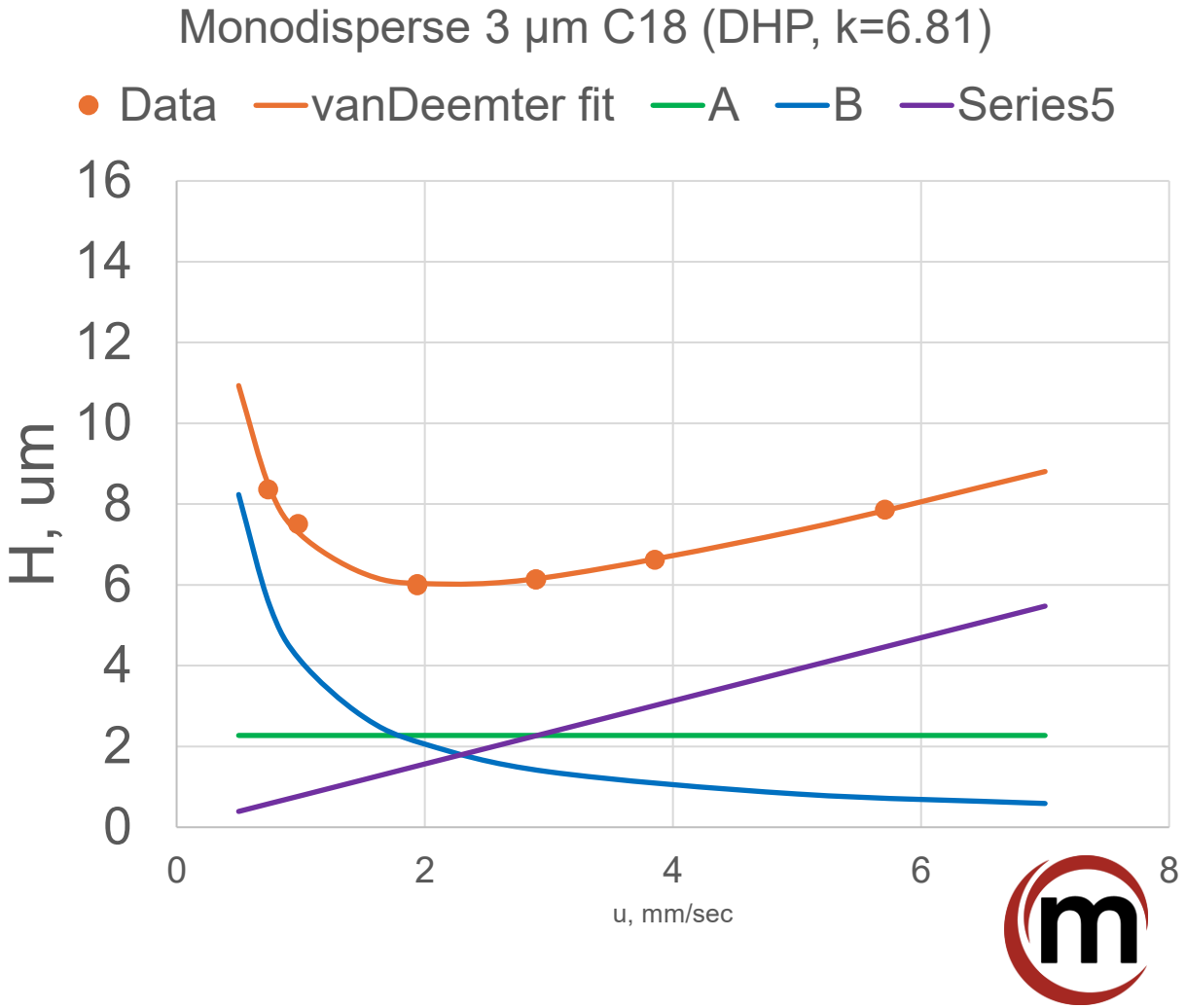
Polydisperse

More Efficient Peaks due to Narrower Particle Size Distribution

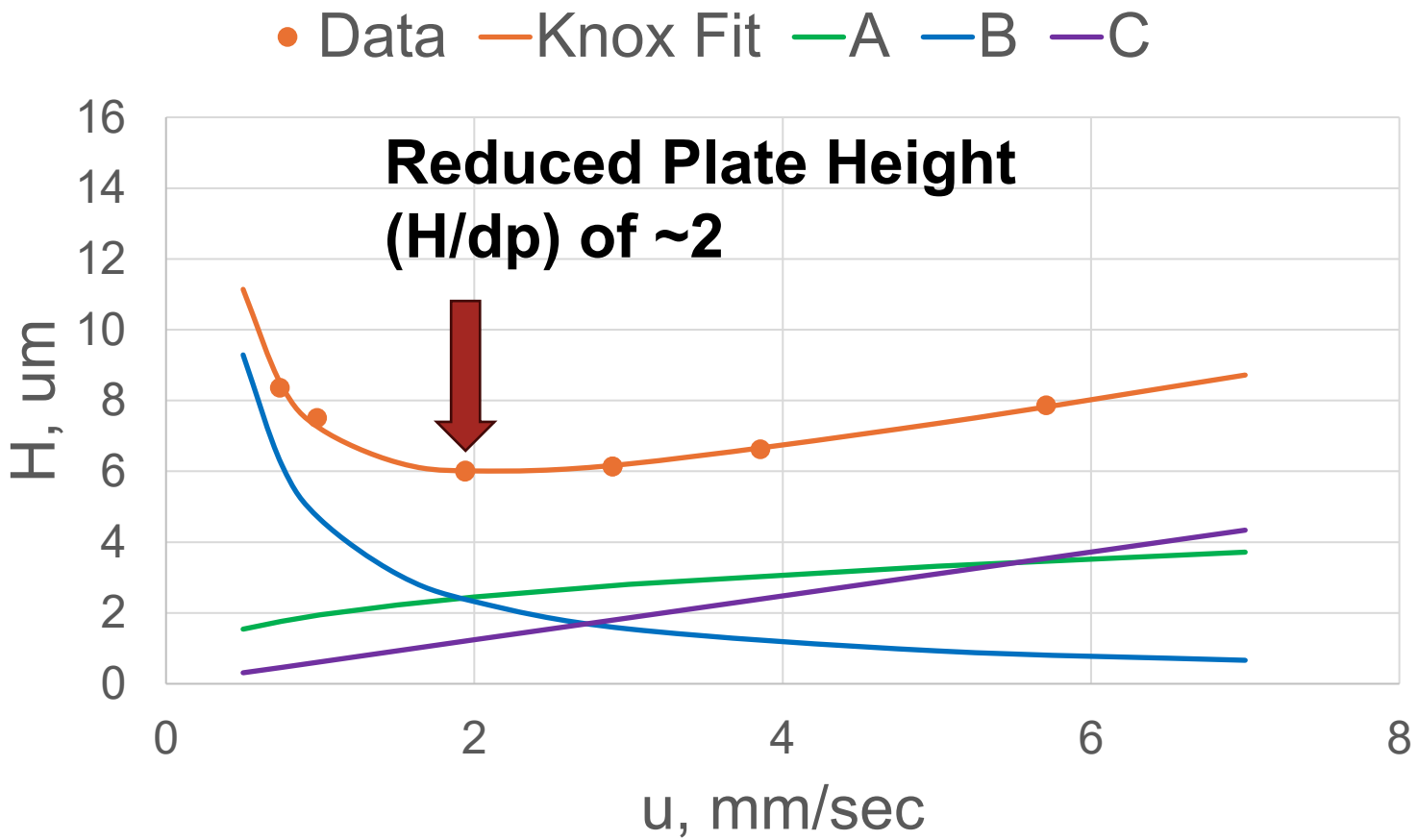


Experimental Van Deemter fits for Dihexylphthalate (DHP) of Polydisperse vs Monodisperse





Monodisperse 3 µm (3.0 mm x 50 mm) Van Deemter Curve for Dihexylphthalate (DHP)



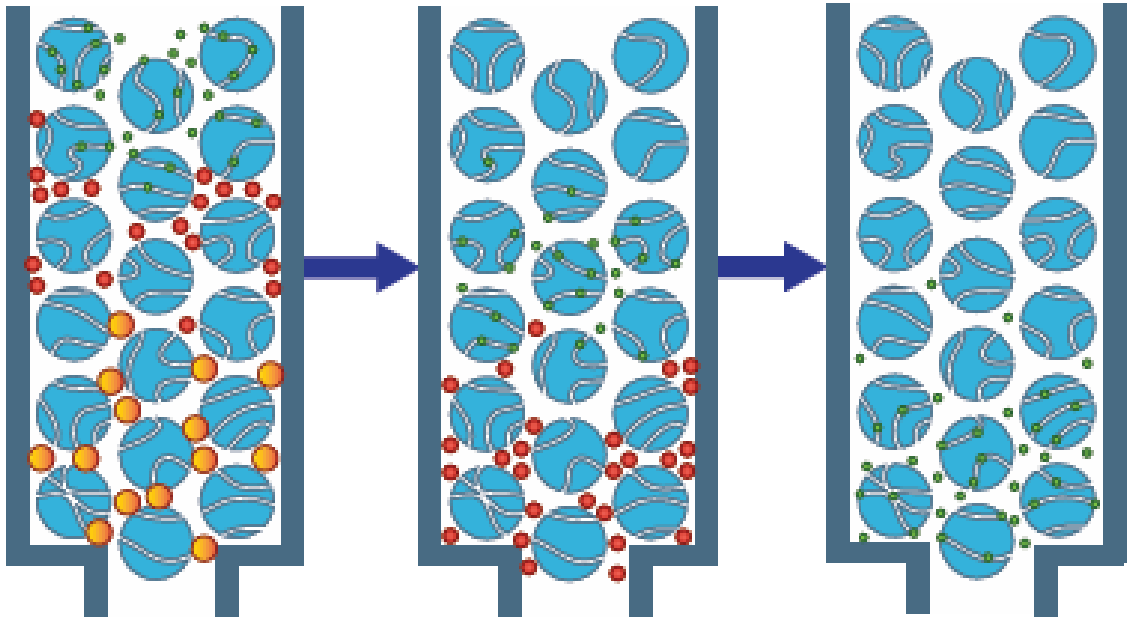
Data Generated by Merlin Bicking from ACCTA, Inc.

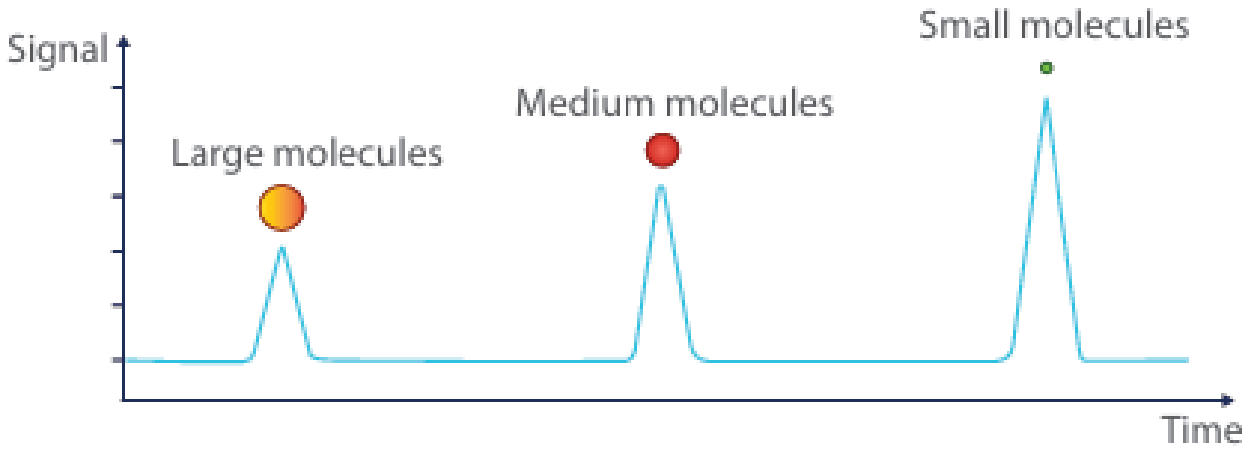

MONOBIO SEC Platform

Introducing MONOBIO!

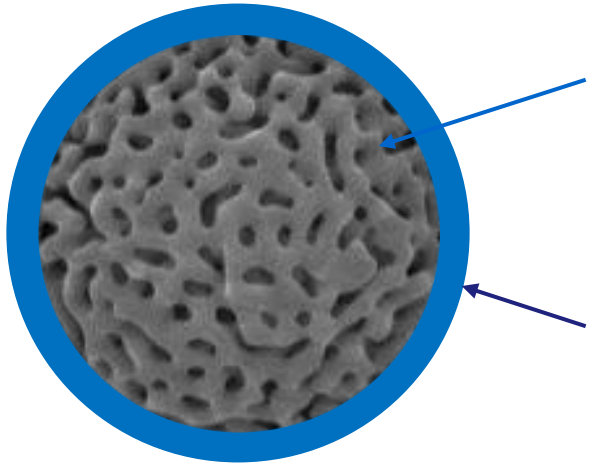
Powered By Monodisperse Particle Technology

High Level Review of SEC





MONOBIO SEC Monodisperse Columns Features



Monodisperse
High Pore-Volume
Porous Spherical
Silica-Based particles

Bonded with Proprietary Neutral Hydrophilic Layer (DIOL)

Features

- **Innovative particle technology**
- **Advanced bonding chemistry**
- **Multiple pore size options**
- **Robust column packing**
- **Good column-to-column reproducibility**

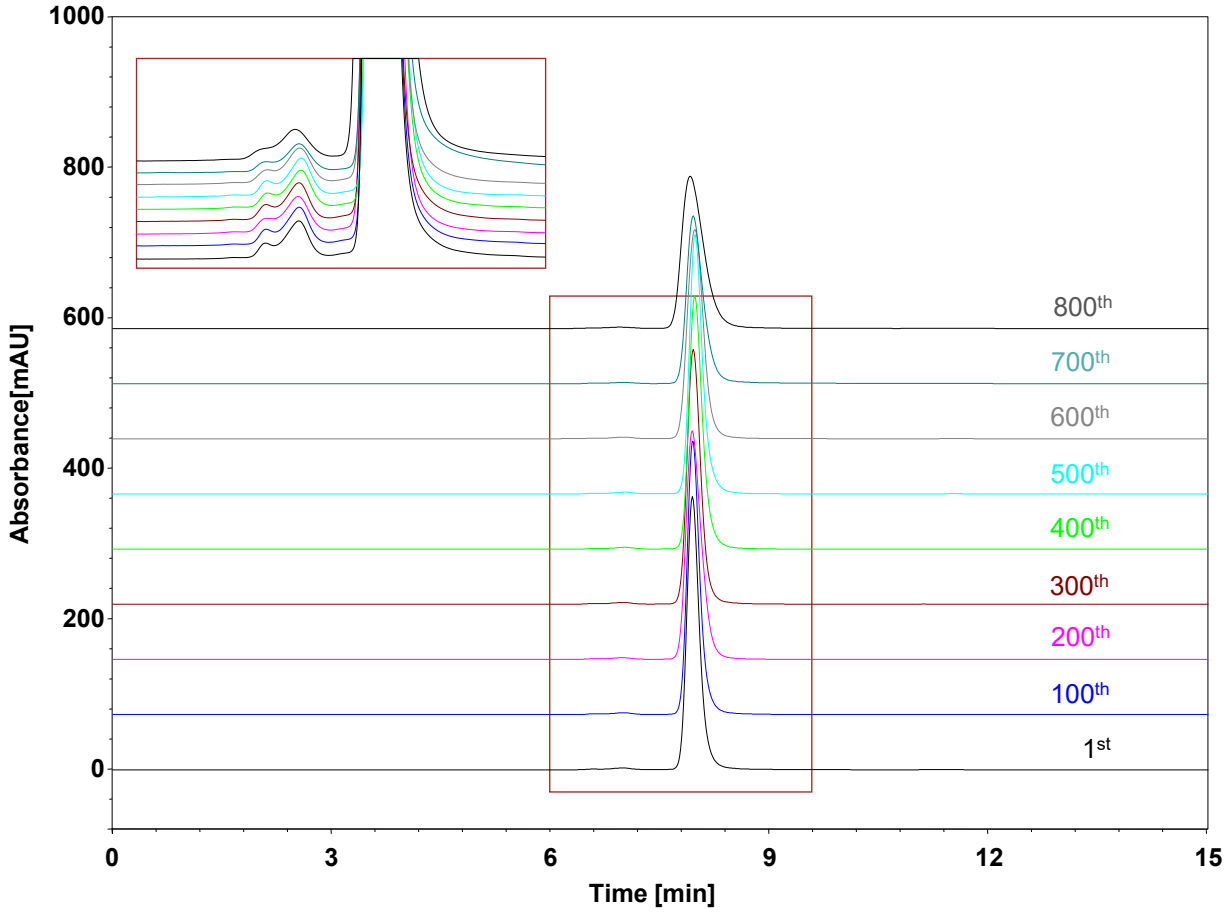
MONOBIO SEC Specifications and Column(s) Dimensions

Pore Size (Å)	Bonded Phase	Particle Composition	Particle Size (µm)	pH Range	Application Area
120	DIOL- Proprietary (DIP)	Monodisperse Fully Porous Silica Particles	1.8 3.0 5.0	2-8	Small Molecule Drugs Heparin Peptides Glycans Small Oligonucleotides
150	DIOL- Proprietary (DIP)	Monodisperse Fully Porous Silica Particles	1.8 3.0 5.0	2-8	Small Molecule Drugs Heparin Peptides Glycans Small Oligonucleotides Small Proteins
300	DIOL- Proprietary (DIP)	Monodisperse Fully Porous Silica Particles	1.8 3.0 5.0	2-8	mAbs and Aggregates
500	DIOL- Proprietary (DIP)	Monodisperse Fully Porous Silica Particles	1.8 3.0 5.0	2-8	mAbs High-order Aggregates Large Proteins Small DNA/RNA

Large Pore Size Columns

700	DIOL- Proprietary (DIP)	Monodisperse Fully Porous Silica Particles	3.0	2-8	AAV (adeno-associated viruses)
1000	DIOL- Proprietary (DIP)	Monodisperse Fully Porous Silica Particles	3.0	2-8	Aggregates and Fragments in mRNAs and Plasmids Circular RNA Rhinoviruses Adenoviruses Lentiviruses
2000	DIOL- Proprietary (DIP)	Monodisperse Fully Porous Silica Particles	3.0	2-8	Aggregates and Fragments in mRNAs and Plasmids Circular RNA Rhinoviruses Adenoviruses Lentiviruses

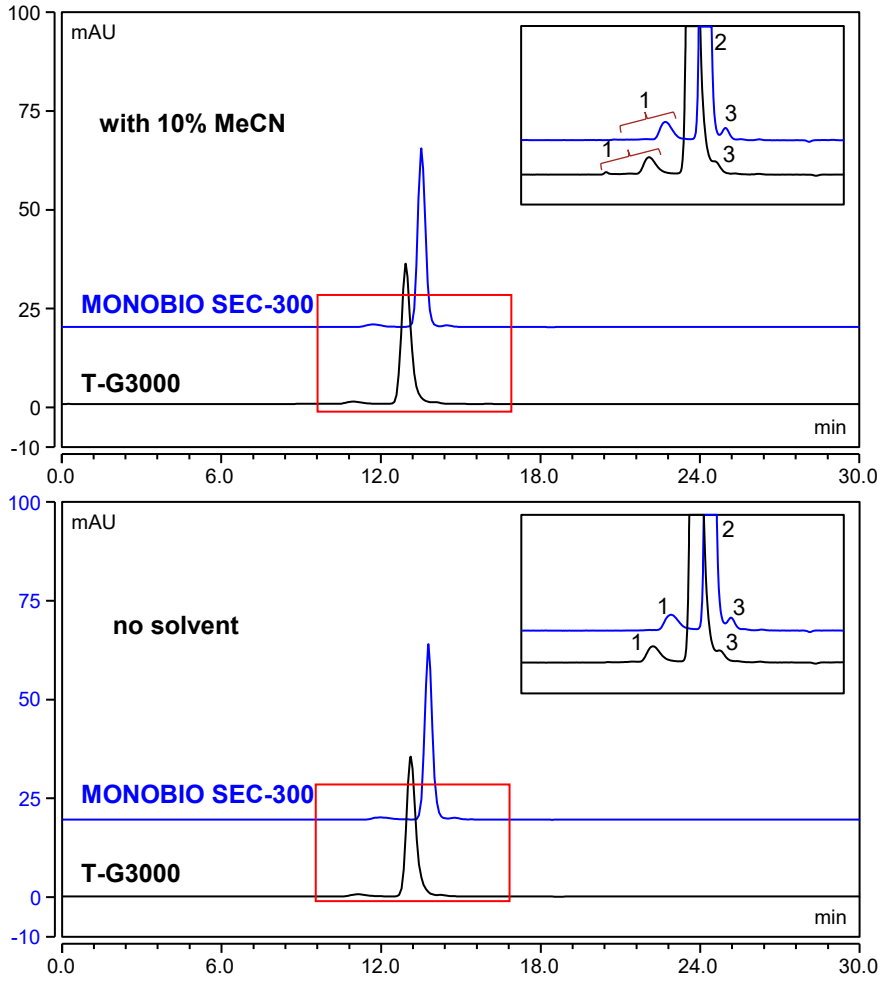
Column Lifetime – Ruggedness Test



Column:	**MONOBIO SEC-300**, 3 μm
Dimension:	7.8×150 mm
Mobile phase:	0.2 M NaCl in 20 mM Phosphate Buffer, pH 6.8
Flow rate:	0.5 mL/min
Temperature:	25 ℃
Injection:	10 μL
Detection:	UV 280 nm
Sample:	Antibody (10 mg/mL)

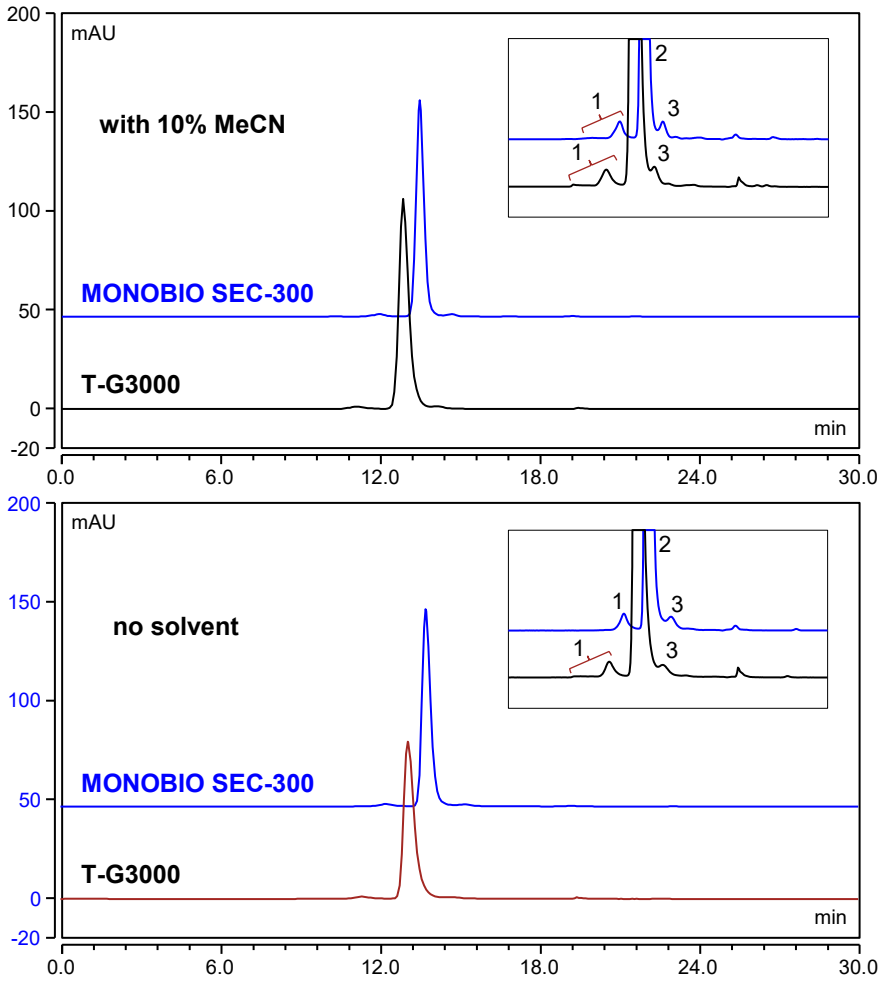
Injection	R.T. (min)	HMW (%)	Monomer (%)	Theoretical Plate (USP)
1st	7.936	1.152	98.848	9934
100th	7.941	1.167	98.833	10061
200th	7.933	1.183	98.817	6417
300th	7.946	1.192	98.808	8353
400th	7.968	1.207	98.793	8732
500th	7.971	1.205	98.795	9396
600th	7.969	1.203	98.797	6077
700th	7.946	1.148	98.852	5573
800th	7.906	1.125	98.875	3125
RSD(%)	0.26	2.49	0.03	/

Direct Comparisons to a SEC leading Brand



Column:	
Blue:	**MONOBIO SEC-300,** 5 μm
Black:	**T-G3000**, 5 μm
Dimension:	7.8×300 mm
Mobile Phase:	
Method 1:	10/90 v/v MeCN/200 mM Na_2SO_4 in 50 mM phosphate buffer, pH7.0
Method 2:	200 mM Na_2SO_4 in 50 mM phosphate buffer, pH7.0
Flow Rate:	0.6 mL/min
Temperature:	30 ℃
Injection:	5 μL
Detection:	UV 280 nm
Instrument:	Agilent Infinity II 1290 (Bio)
Sample:	mAb I (10 mg/mL, pI 7.86-8.90)
Peaks:	1. Aggregates 2. Monomer 3. Fragment

Direct Comparisons to a SEC leading Brand - ADC



Column:
Blue: **MONOBIO SEC-300**, 5 μm
Black: **T-G3000**, 5 μm
Dimension: 7.8×300 mm
Mobile Phase:
Method 1: 10/90 v/v MeCN/200 mM Na_2SO_4 in 50 mM phosphate buffer, pH7.0
Method 2: 200 mM Na_2SO_4 in 50 mM phosphate buffer, pH7.0
Flow Rate: 0.6 mL/min
Temperature: 30 ℃
Injection: 5 μL
Detection: UV 280 nm
Instrument: Agilent Infinity II 1290 (Bio)
Sample: ADC I (15 mg/mL, pI 6.78-7.16)
Peaks: 1. Aggregates 2. Monomer 3. Fragment

Bispecific Antibody

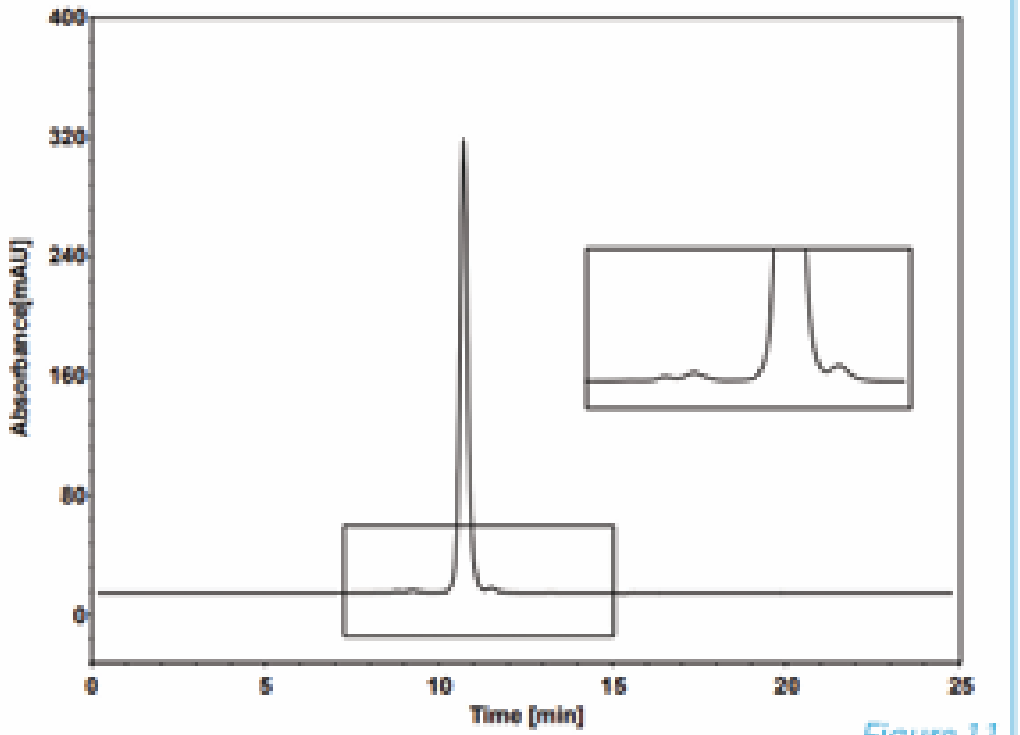


Figure 11

Column:	MBSECDIP204630
Description:	MONOBIO SEC DIP 300 Å, 1.8 µm, 4.6 x 300 mm Monodisperse HPLC Column
Mobile Phase:	100 mM Na_2SO_4 in 50 mM phosphate buffer, pH 6.8
Flow Rate:	0.25 ml/min
Temperature:	30°C
Injection:	10 µl
Detection:	UV 214 nm
Analyte ID:	Bispecific Antibody (10 mg/ml)

Trispecific Antibody

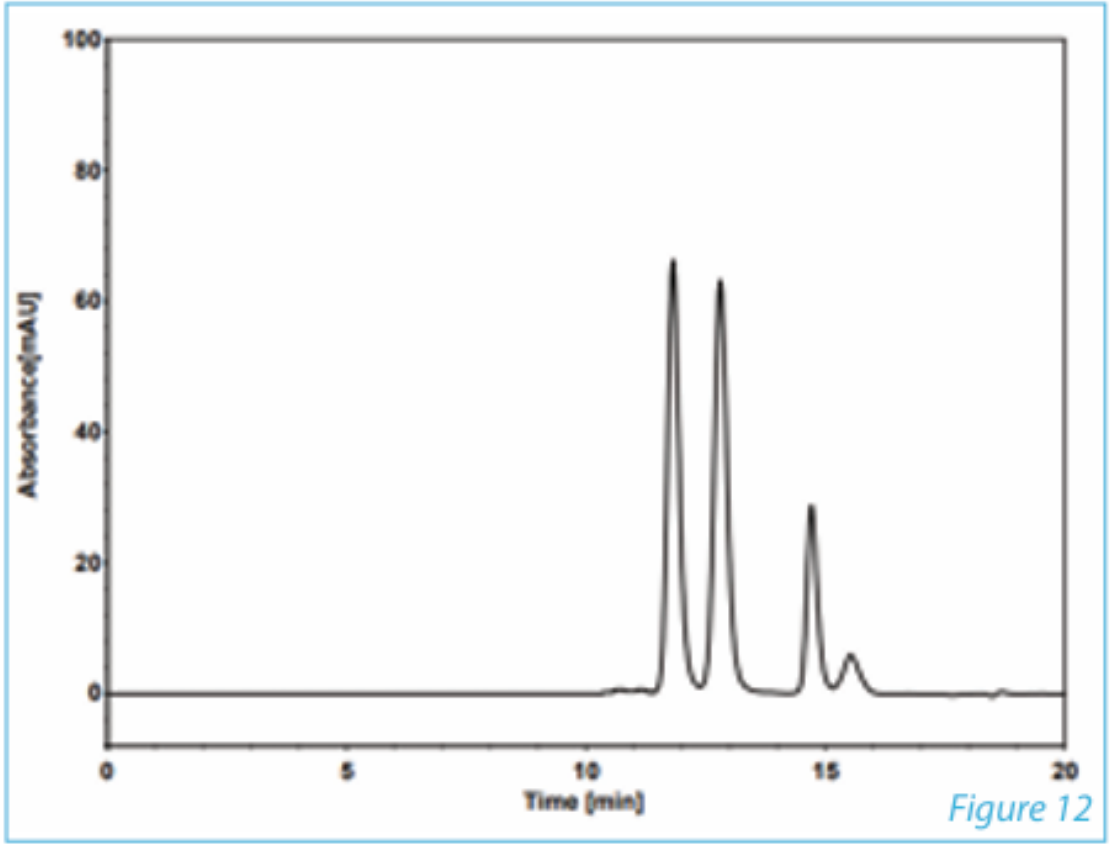


Figure 12

Column:	MBSECDIP214630
Description:	MONOBIO SEC DIP 300 Å, 3.0 µm, 4.6 x 300 mm Monodisperse HPLC Column
Mobile Phase:	90/10 v/v 300 mM NaCl in 50 mM phosphate buffer, pH 6.8/ACN
Flow Rate:	0.21 ml/min
Temperature:	30°C
Injection:	2 µl
Detection:	UV 280 nm
Analyte ID:	Trispecific Antibody (5mg/ml)

Antibody Drug Conjugate

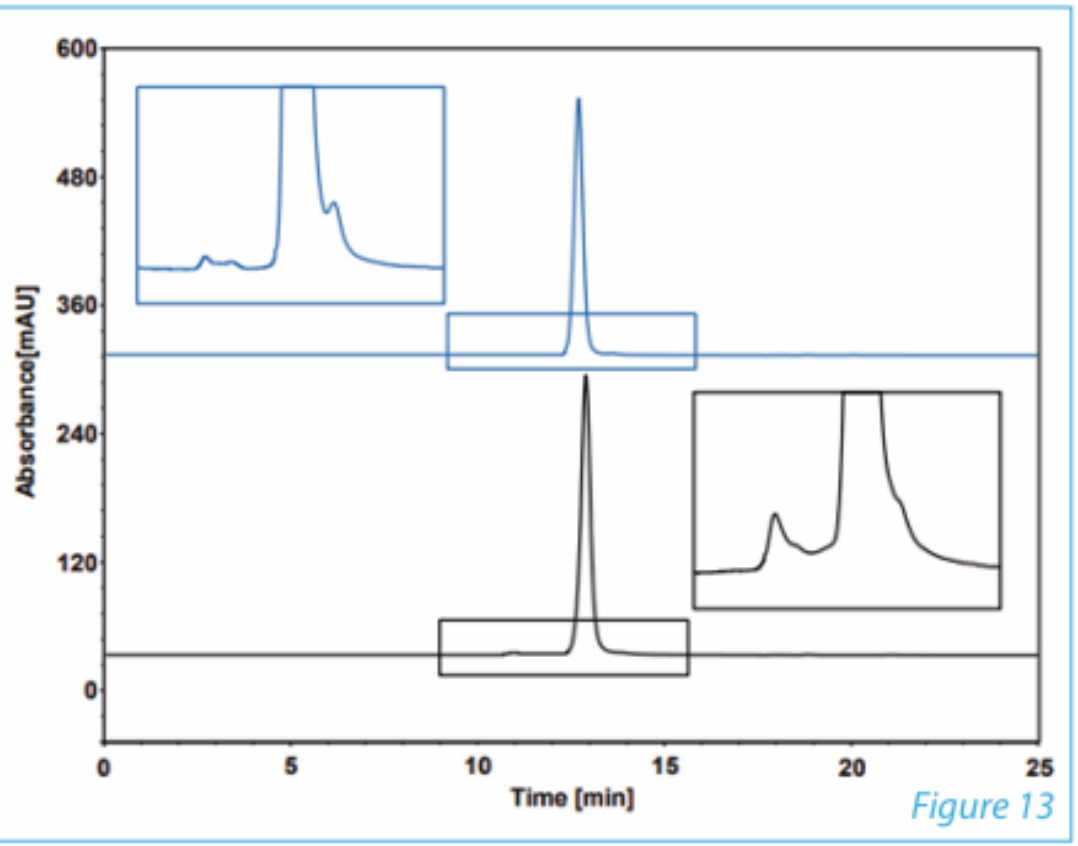


Figure 13

Column:	MBSECDIP214630
Description:	MONOBIO SEC DIP 300 Å, 3.0 µm, 4.6 x 300 mm Monodisperse HPLC Column
Mobile Phase:	90/10 v/v 300 mM NaCl in 50 mM phosphate buffer, pH 6.8/ACN
Flow Rate:	0.21 ml/min
Temperature:	30°C
Injection:	2 µl
Detection:	UV 280 nm
Analyte ID:	ADC (10 mg/ml)

Separation of mAb, Aggregates, Heavy and Light Chain Fragments

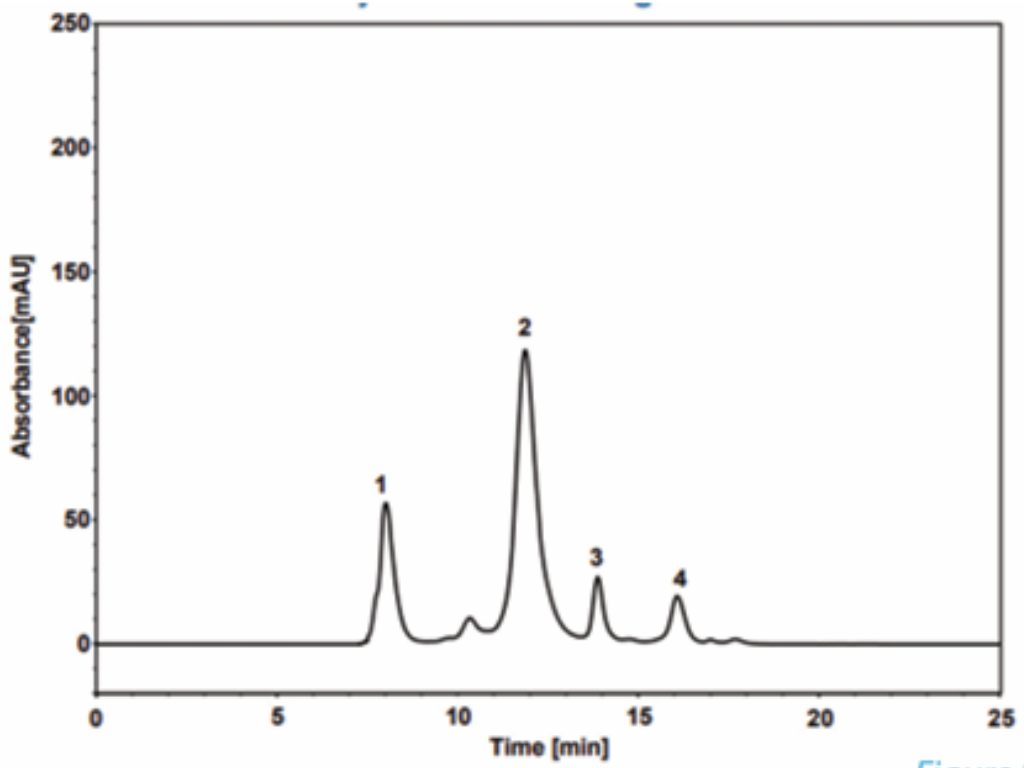


Figure 8

Column:	MBSECDIP227830
Description:	MONOBIO SEC DIP 300 Å, 5.0 µm, 7.8 x 300 mm Monodisperse HPLC Column
Mobile Phase:	300 mM NaCl in 50 mM phosphate buffer, pH 6.8
Flow Rate:	0.7 ml/min
Temperature:	25°C
Injection:	10 µl
Detection:	UV 214 nm
Analyte ID:	1. Aggregate (MW ~900 kDa) 2. mAb (MW ~ 150 kDa) 3. Heavy chain (MW ~50 kDa) 4. Light chain (MW ~25 kDa)

Big Pharma MONOBIO SEC-300 Conditions

Column: MONOBIO SEC DIP 300 Å Monodisperse HPLC Column
Particle Size: 1.8 µm
Dimensions: 4.6 mm x 300 mm

- SEC Conditions:

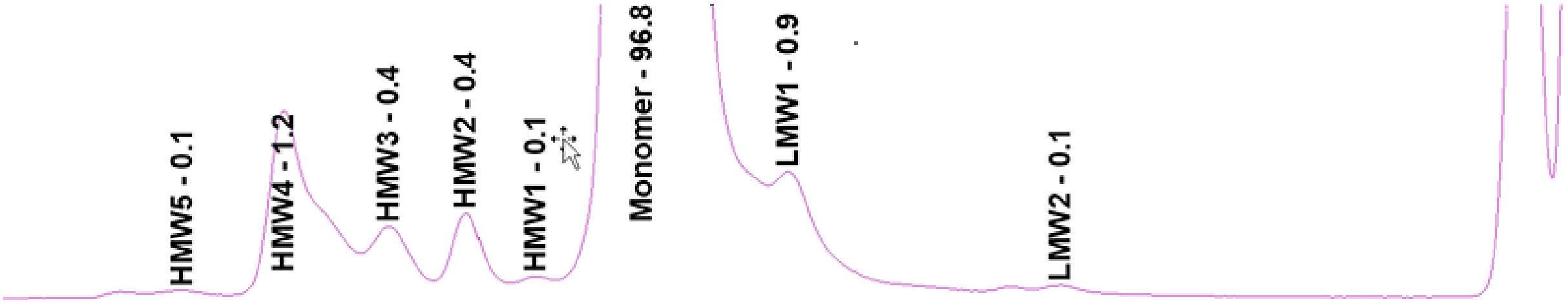
Mobile Phase - 125 mM Sodium Phosphate Monobasic Monohydrate, 175 mM Sodium Sulfate, pH 7.0, Column

Column Temp. 25°C, Flow rate: 0.2 mL/min

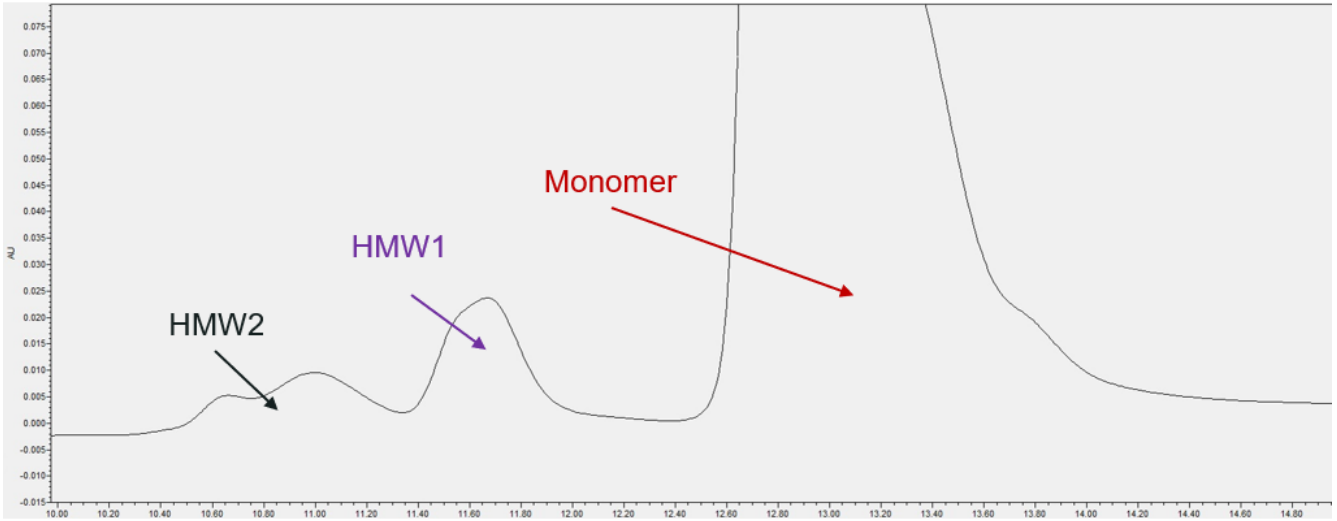
Detection: UV at 214 nm, Peak width > 0.05 min or 1 s response time. LC cycle time per injection: 30 min

Injection: 3 µL or 3 µg protein

Big Pharma SEC on HMW, LMW and Monomer

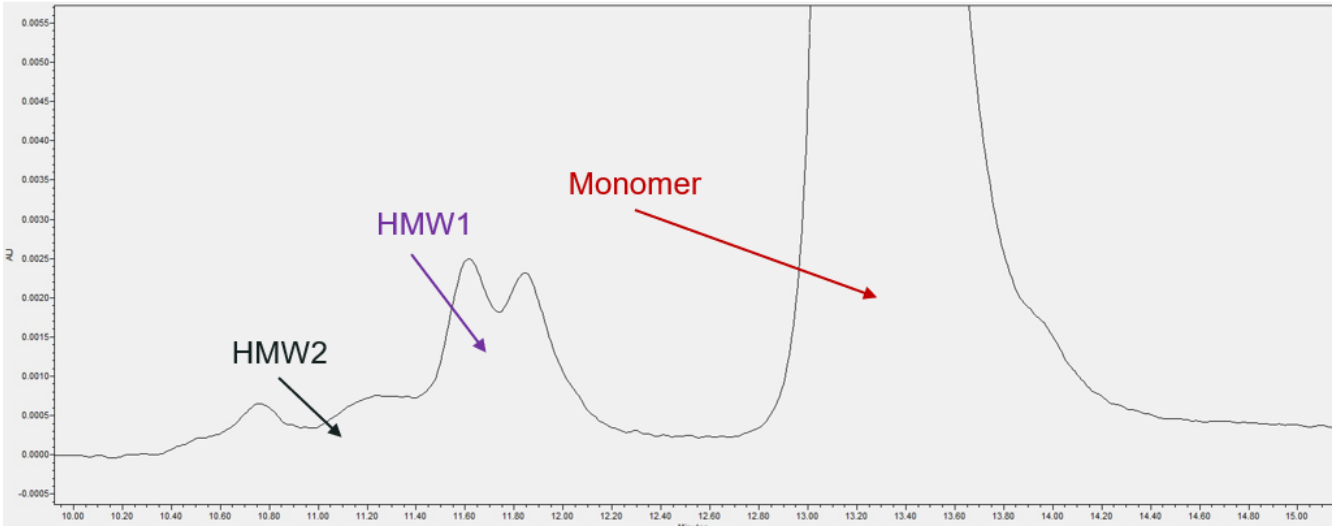


Resolution of mAb-HMW by Column



TSKgel G3000SW Column

- Column Dimensions: 4.6 x 300
- Particle Size: 2.0 µm
- Pore Size: 250 Å



MONOBIO SEC-300

- Column Dimensions: 4.6 x 300
- Particle Size: 1.8 µm
- Pore Size: 300 Å

Future Objectives

- Prove Monodisperse Particles provide better efficiency than polydisperse particles
  - Compare Van Deemter curves of polydisperse and monodisperse particle columns for a series of model proteins spanning a broad molecular weight range
  - Use experimental and simulated Van Deemter behavior to quantify relative contributions of eddy diffusion, film mass transfer, and pore diffusion in mono and poly-disperse particle systems.
- Utilize MONOBIO Columns for more challenging separations (Trial and Evaluation Columns Available)

Summary

- New Particle Platform based on Monodisperse Particle Technology can be successfully applied to SEC to increase performance for the characterization of Protein/Antibody based biologics

- Resolution, reproducibility, ruggedness, throughput and scalability are of great significance in analyzing biologics chromatographically, which impose separation challenges that MAC-MOD MONOBIO can provide advantages in terms of column technology.

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Thank you for your time
Questions?