



TSK-GEL High Resolution Size Exclusion Chromatography Column Technology for Organo- Soluble Polymers

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Outline

- SEC column characteristics
 - *Peak broadening*
 - *Resolution*
 - *Column arrangement*

- Historical perspective

- Semi-micro technology

- Multipore technology

- Column selection

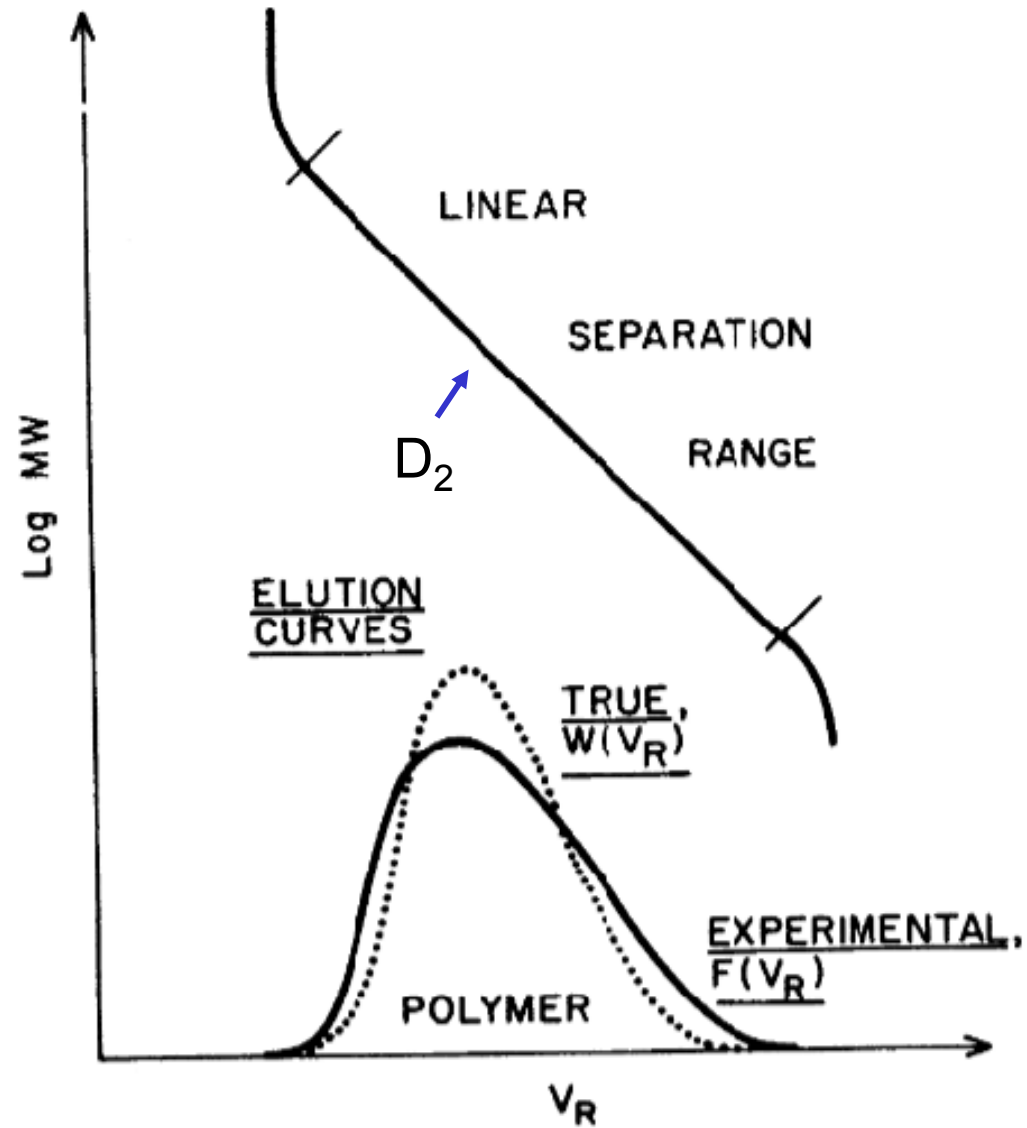
- SEC columns available from Tosoh

- Trends in high-performance SEC columns

- Conclusions



Peak Broadening in SEC Columns



W. W. Yau et al., Modern Size Exclusion Liquid Chromatography, Wiley, 1979



MW Errors Caused by Peak Broadening in SEC

MW errors (M^*) caused by peak broadening⁽¹⁾, in which M_n will be underestimated and M_w will be overestimated:

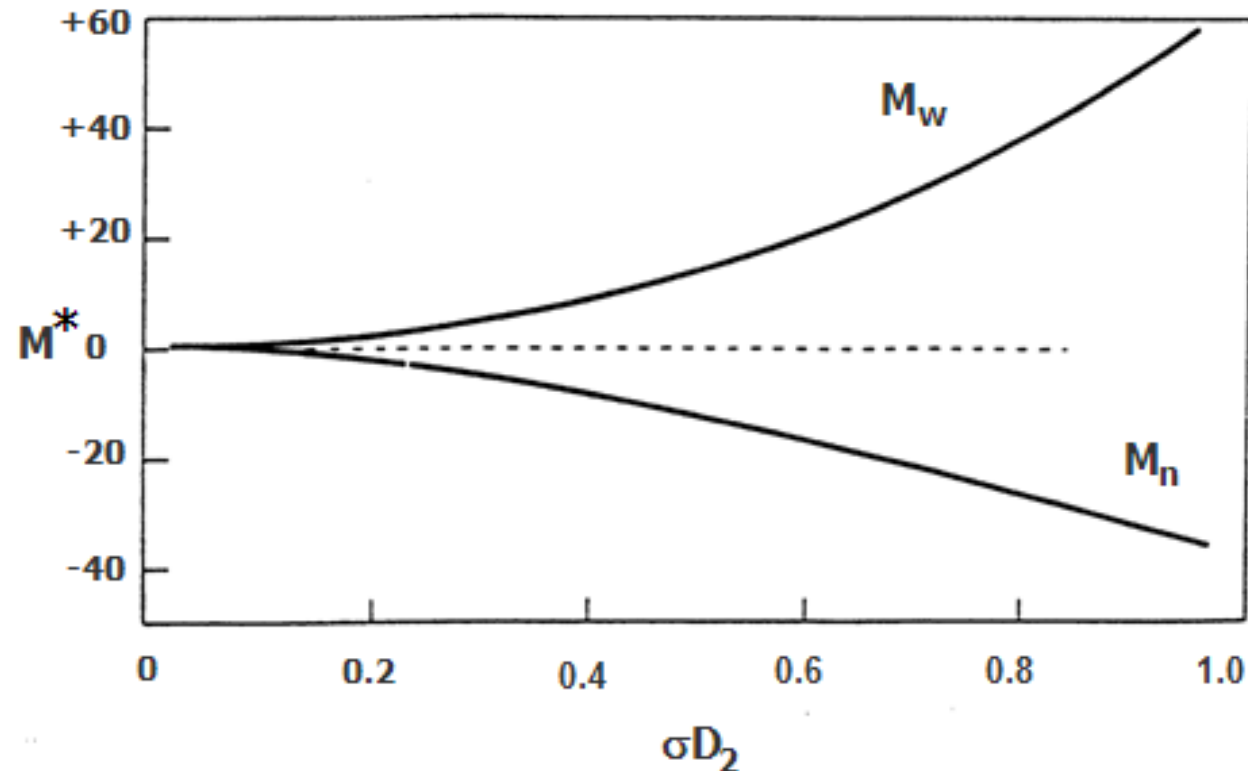
$$M_n^* = e^{-\frac{1}{2}(\sigma D_2)^2} - 1$$

$$M_w^* = e^{+\frac{1}{2}(\sigma D_2)^2} - 1$$

⁽¹⁾ W. W. Yau et al, *Modern Size Exclusion Liquid Chromatography*, Wiley, 1979



MW Inaccuracy Caused by Peak Broadening



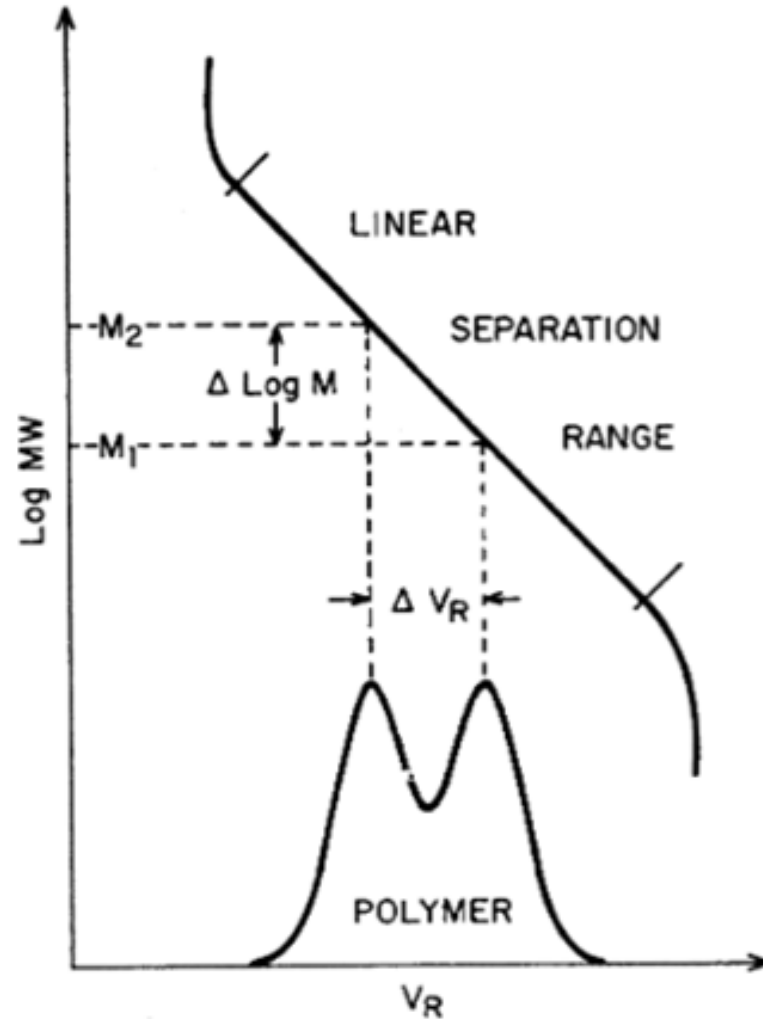
For typical high-performance columns and SEC systems, would like to have $\geq 10^4$ theoretical plates to keep MW peak broadening error $< 2\%$ ($\sigma D_2 \leq 0.2$). With high performance columns, this level can be realized by using $\leq 5\mu\text{m}$ packings in $\geq 15\text{cm}$ columns.

W. W. Yau et al., Modern Size-Exclusion Liquid Chromatography, Wiley, 1979



Resolution in SEC

$$(\sigma^2)_{\text{total}} = (\sigma^2)_{\text{inj}} + (\sigma^2)_{\text{tubing}} + (\sigma^2)_{\text{column}} + (\sigma^2)_{\text{detector}}$$



$$R_s = \frac{\ln(M_2/M_1)}{2D_2(\sigma_1 + \sigma_2)} \approx \frac{\Delta \ln M}{4\sigma D_2}$$

W .W. Yau et al., Modern Size-Exclusion Liquid Chromatography, Wiley, 1979.



Column Arrangement

**TSKgel SuperMultiporeHZ-M +
TSKgel SuperHZ1000
Polystyrenes, RI Detection**

Time (min)	M _w	Coefficient
9.45	706,000	A = 0.059
10.93	96,400	B = 0.192
12.70	5,970	C = 1.430
15.35	474	D = 4.493
15.70	370	
16.22	266	
Correlation Coefficient = 0.994		

**TSKgel SuperHZ1000 +
TSKgel SuperMultiporeHZ-M
Polystyrenes, RI Detection**

Time (min)	M _w	Coefficient
9.44	706,000	A = 0.059
10.90	96,400	B = 0.192
12.66	5,970	C = 1.419
15.30	474	D = 4.579
15.65	370	
16.17	266	
Correlation Coefficient = 0.994		

Cubic curve fit $At^3 + Bt^2 + Ct = D$



Column Arrangement

**TSKgel SuperMultiporeHZ-M +
TSKgel SuperHZ1000
RI, Terethane 1000
poly(tetramethylene ether glycol)**

Time (min)	Peak	M _n	M _w	M _z
13.20	1	2130	3320	5010
14.70	2	685	688	691
14.93	3	552	554	556
15.16	4	445	447	448

**TSKgel SuperHZ1000 +
TSKgel SuperMultiporeHZ-M
RI, Terethane 1000
poly(tetramethylene ether glycol)**

Time (min)	Peak	M _n	M _w	M _z
13.21	1	2180	3190	4750
14.71	2	680	684	688
14.86	3	546	548	550
15.10	4	443	445	447



Historical Perspective of SEC Columns for Synthetic Polymers

- Introduced 1964
- Tailor-made
 - Particle size
 - Pore size
 - Porosity
 - Particle size uniformity
- Inert surface
 - $\Delta H = 0$
 - $\delta \sim 9.1$ (THF 9.5)
- High porosity
 - % porosity > 72%



Semi Micro and Conventional SEC Columns

	Semi-micro column	Conventional column
Particle size (μm)	3 – 10	5 – 13
Column size (mm ID x cm)	6.0 x 15 4.6 x 15	7.8 x 30
Theoretical plates/column	8,000 – 16,000	8,000 – 16,000
Std. flow rate (mL/min)	0.6 (6.0 x 15 column) 0.35 (4.6 x 15 column)	1.0
Analysis time/column (min)	6	12
Solvent consumption/column (mL)	3.6 (6.0 x 15 column) 2.1 (4.6 x 15 column)	12
Sample injection volume (μL)	5 (6.0 x 15 column) 2 (4.6 x 15 column)	50

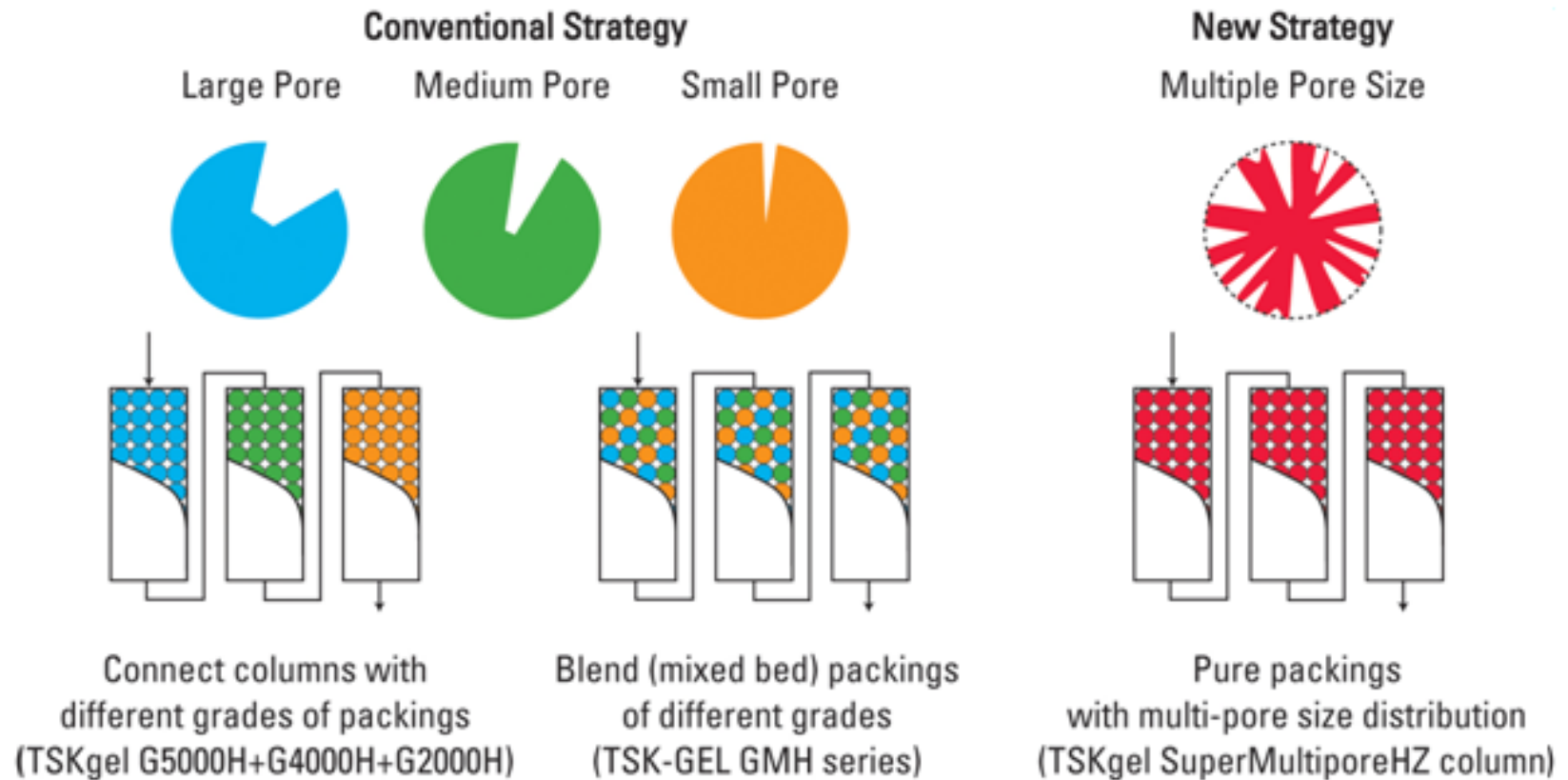


Multi-pore Technology

- Single pore column / Individual columns
 - Limited separation range
 - Necessary to connect columns with different separation range
 - Inflection point
- Mixed bed column / Linear columns
 - Wide separation range
 - Linear calibration curve
 - Inflection point
- Multipore columns
 - Wide separation
 - Linear calibration curve
 - No inflection point



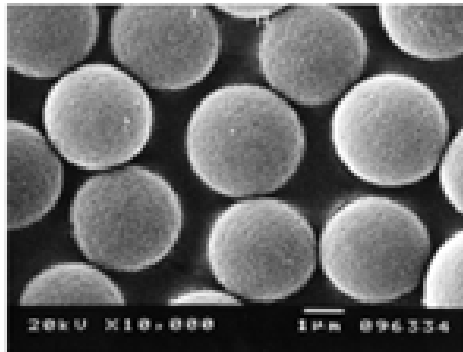
Multi-pore Particle Technology



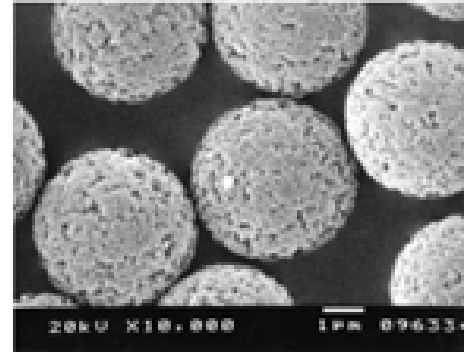


SEM Pictures of Monodisperse Multi-pore Particles

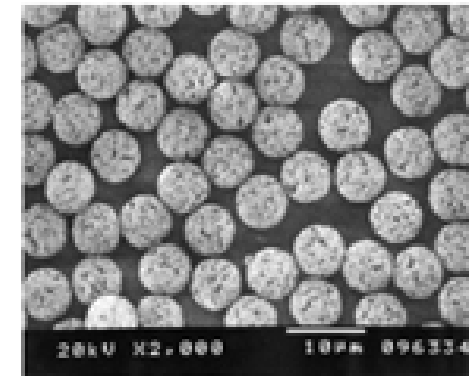
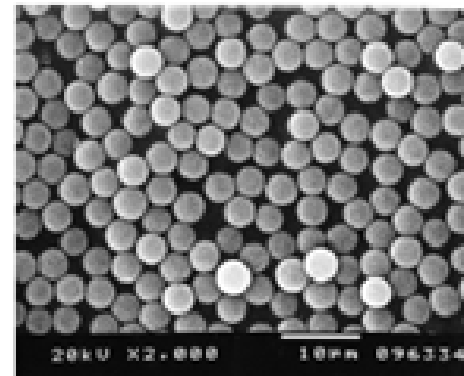
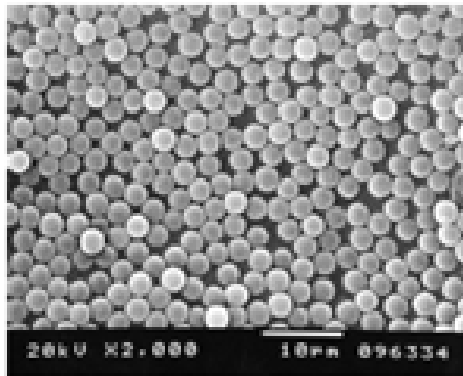
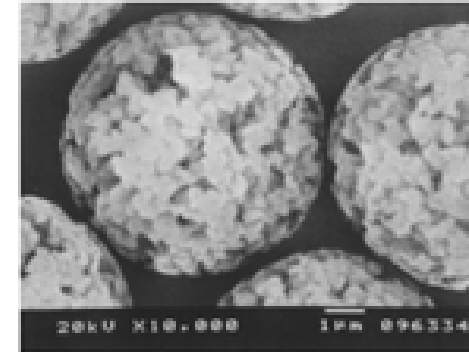
TSKgel
SuperMultiporeHZ-N,
3 μ m



TSKgel
SuperMultiporeHZ-M,
4 μ m

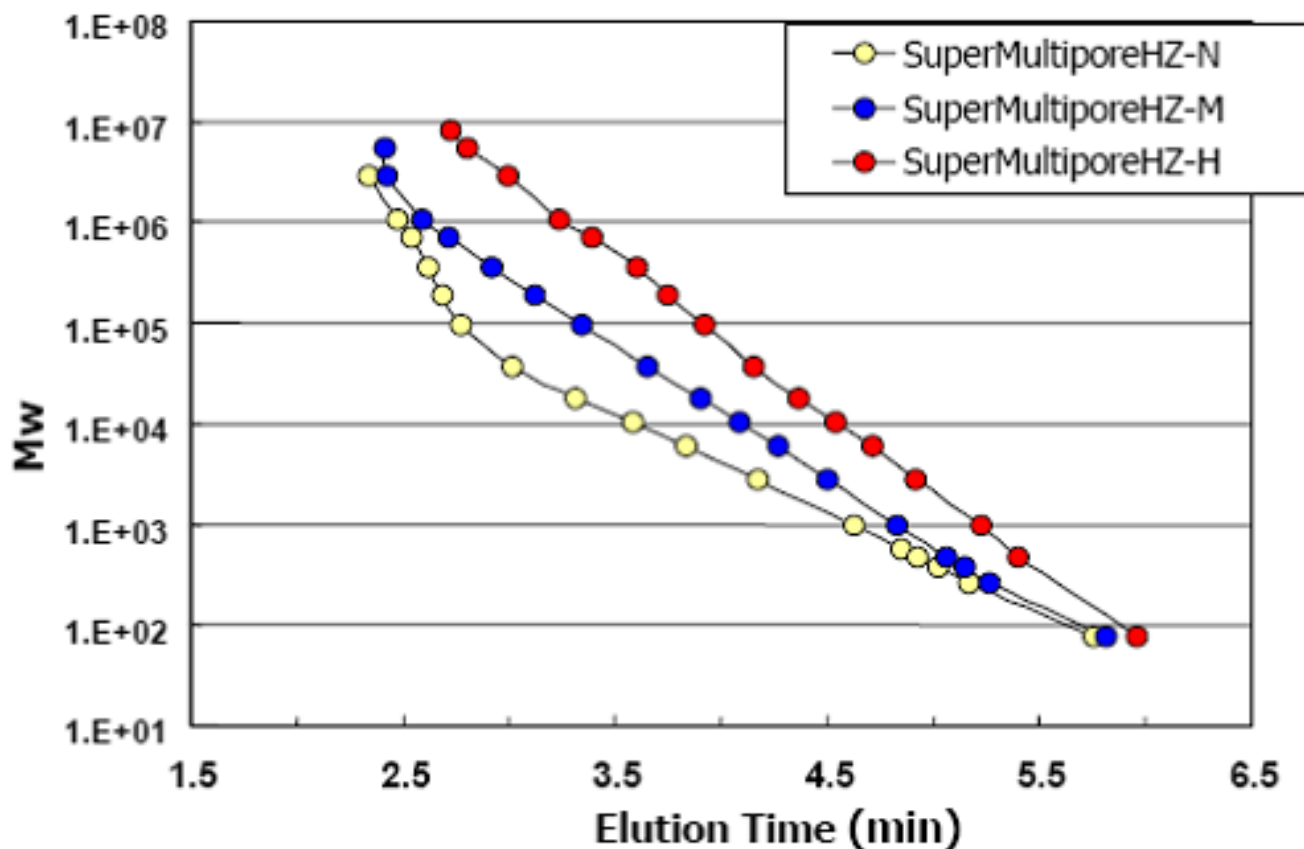


TSKgel
SuperMultiporeHZ-H,
6 μ m





Calibration Curves of TSK-GEL SuperMultiporeHZ Columns

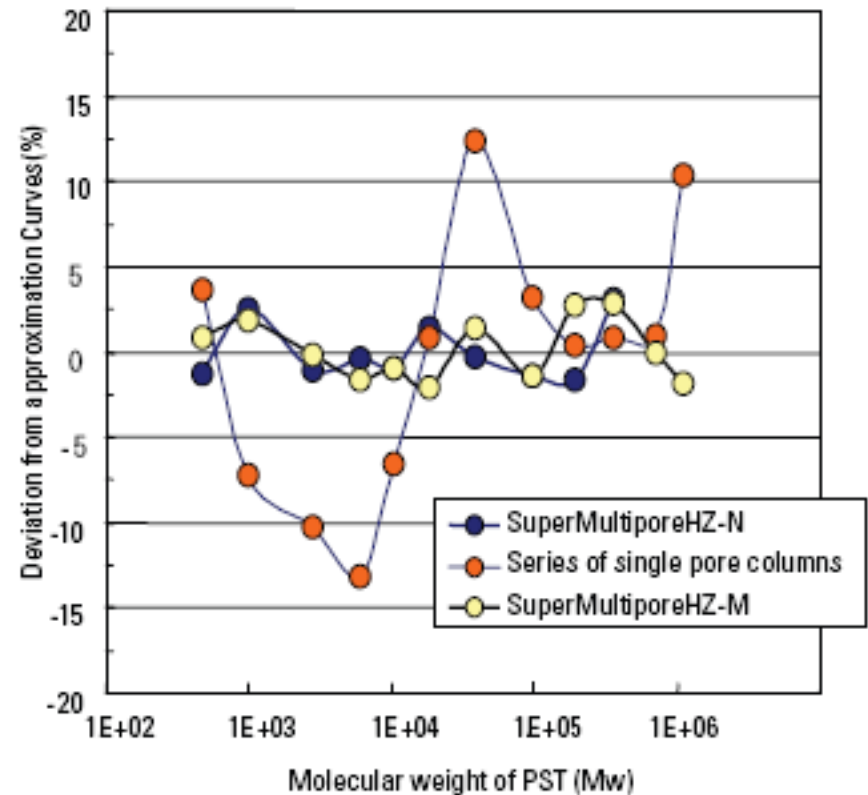
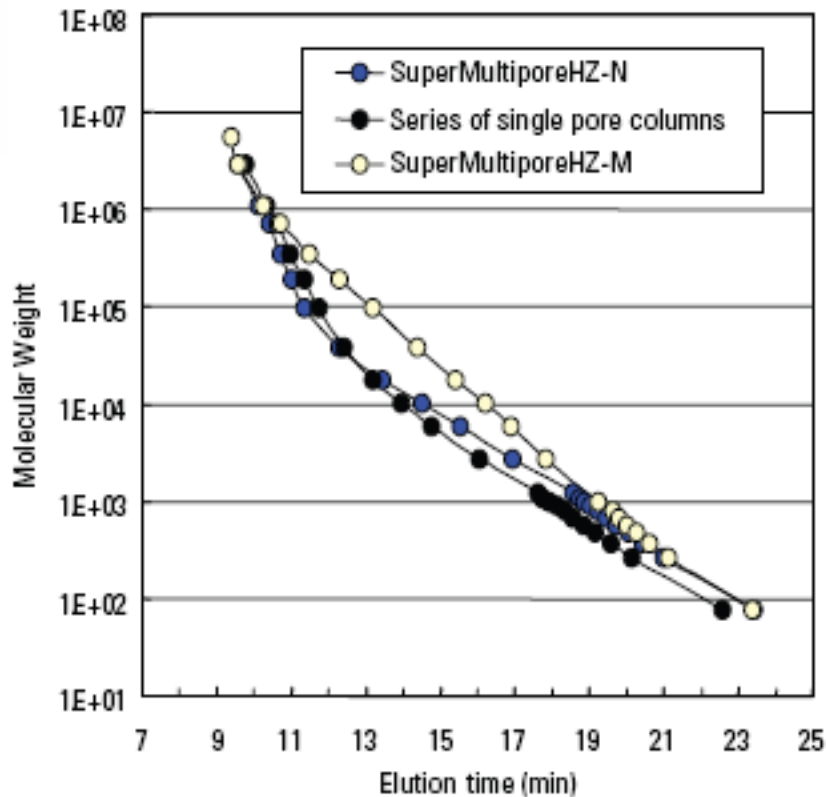


TSKgel SuperMultiporeHZ-N, 4.6mm ID x 15cm, 3 μ m
TSKgel SuperMultiporeHZ-M, 4.6mm ID x 15cm, 4 μ m
TSKgel SuperMultiporeHZ-H, 4.6mm ID x 15cm, 6 μ m

Mobile phase: THF
Flow rate: 0.35mL/min
Temperature 25°C
Detection: UV@254nm
Sample: PStQuick polystyrene standards



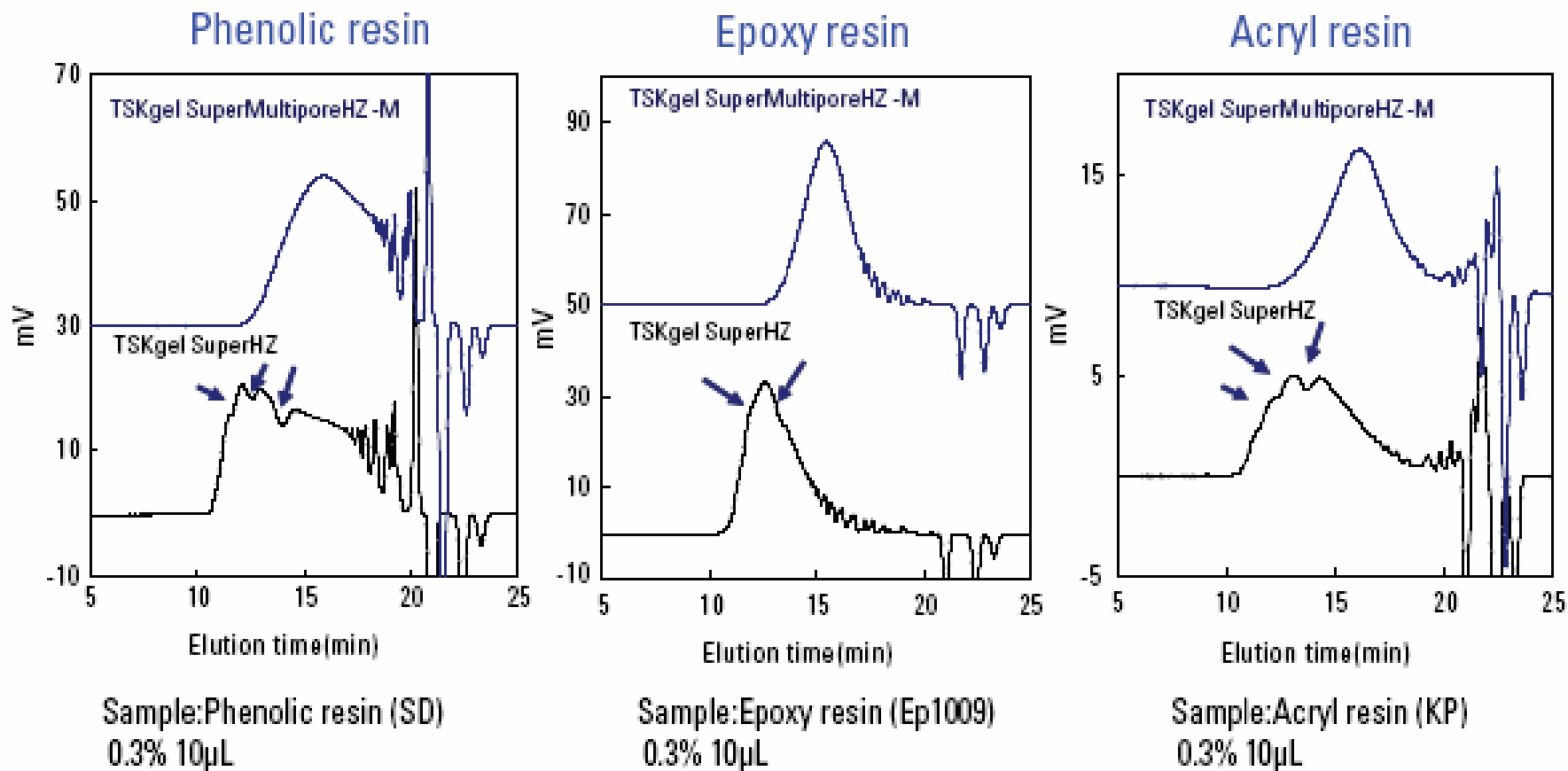
Inflection Point Origin



Column size: 4.6mm ID x 15cm x 4
Mobile phase: THF
Temperature: 25°C
Detection: UV@254nm (UV-8020 microcell)
Flow Rate: 0.35mL/min
Sample: PStQuick STD, 10 μ L



TSKgel SuperMultiporeHZ-M and a Mixed Bed Column



Columns: TSK-GEL SuperMultiporeHZ-M, 4.6mm ID x 15cm x 4
TSK-GEL SuperHZ4000, 3000, 2500, 2000, 4.6mm ID x 15cm x 4
Mobile phase: THF Flow Rate: 0.35mL/min
Temperature: 40°C Detection: RI (HLC-8120GPC)

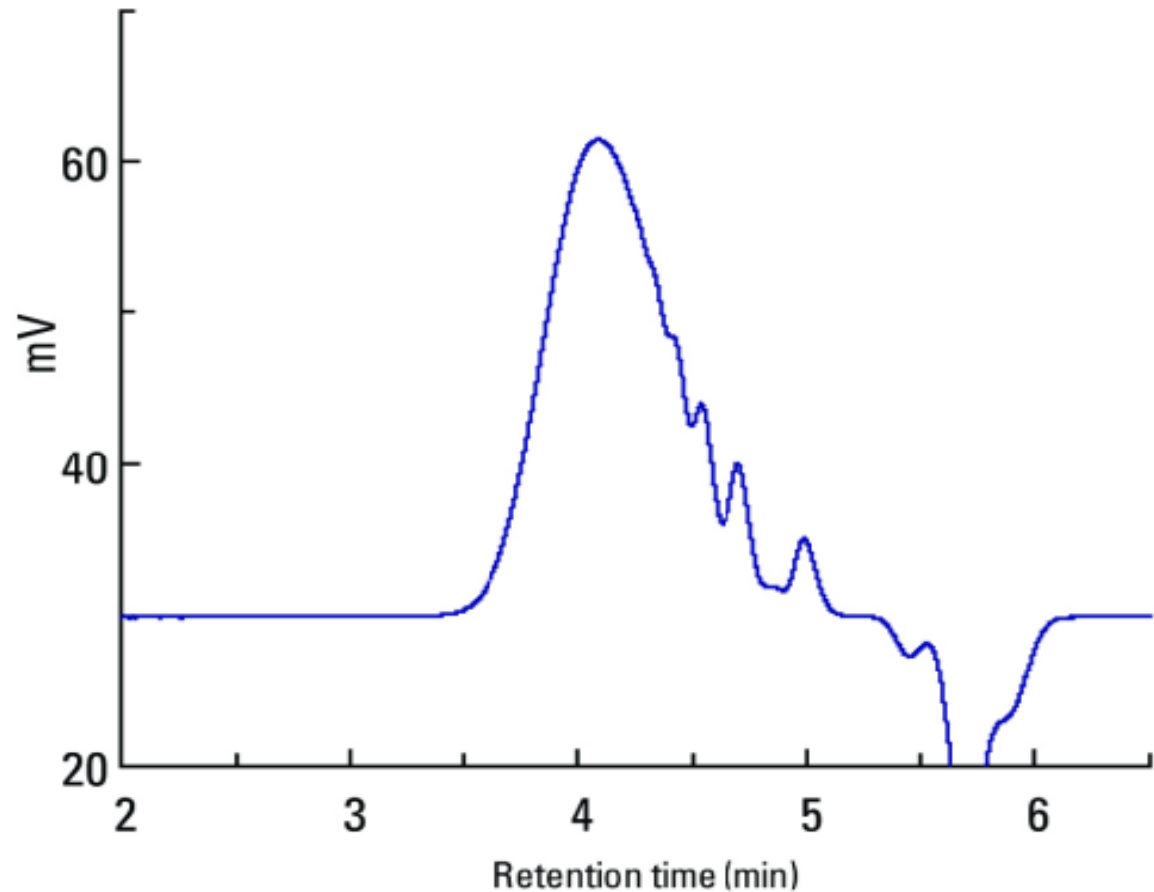


Physical Properties of TSK-GEL SuperMultiporeHZ Columns

	TSK-GEL		
	SuperMultiporeHZ-N	SuperMultiporeHZ-M	SuperMultiporeHZ-H
Base material	Poly(styrene divinyl /benzene)	Poly(styrene divinyl /benzene)	Poly(styrene divinyl /benzene)
Particle size	3 μ m	4 μ m	6 μ m
Max. exclusion limit	120,000	2,000,000	40,0000
Mean pore size	80Å	140Å	N/A
MW separation range	300-50,000	500-1,000,000	1,000-10,000,000
Theoretical plates per column	20,000	16,000	11,000
Column size (Analytical)	4.6mm ID x 15cm	4.6mm ID x 15cm	4.6mm ID x 15cm



Example Chromatogram, Epoxy Resin, 6 minutes



Column: TSKgel SuperMultiporeHZ-N, 4.6mm ID x 15cm, 3 μ m
Mobile phase: THF Temperature: 40°C
Detection: RI Injection: 5 μ L
Sample: epoxy resin Flow rate: 0.35mL/min



SEC Columns Available from Tosoh

Product	Feature
TSK-GEL H_{HR} type	5μm, 30cm Conventional GPC columns, mixed bed column
TSK-GEL SuperH type	3μm, 6mmID x 15cm The first semi-micro column, high-speed, high-resolution
TSK-GEL Multipore H_{XL}	5μm, 30cm Multi-pore
TSK-GEL SuperHZ type	3μm, 4.6mm ID x 15cm Semi-micro SEC column High resolution, high durability
TSK-GEL SuperMultiporeHZ type	3μm, 4.6mm ID x 15cm Semi-micro SEC column Multi-pore, high-speed



Column Selection Guide

Narrow polydispersity (< 2 pd) sample

1. If approximate MW known, select single-pore size column.
Add additional columns to increase resolution.
2. If approximate MW is unknown, narrow it down with
TSKgel SuperMultiporeHZ column

Broad polydispersity (> 2 pd) sample

1. Use TSKgel SuperMultiporeHZ-M column.
Add more columns to increase resolution.



Trends in High Performance SEC

Column Properties

- Increased accuracy and precision
Mn and Mw < 1.5%
- Increased ruggedness
Column lifetime > 6 mo
- Faster analysis and high throughput
< 5 min (not including sample preparation or data workup)

SEC Instrumentation

- Increased sensitivity
- High-precision temperature stability
- Low dead-volume RI detector
- Accurate and precise flow rate



Conclusions

Multi-pore Technology

- Extended linear calibrations are available
- Smoother chromatograms without inflection
- Reduces analysis times by 50% as compared to conventional columns
- Mobile phase consumption reduced by 1/6



Acknowledgements

- Bruce Kempf, Technical Services, Tosoh Bioscience LLC
- Hiroshi Tomizawa, Column Marketing Manager, Tosoh Corporation
- Dr. Howard Barth, Consultant, Polymer Characterization, Wilmington, DE

For more information visit our website at:
www.tosohbioscience.com