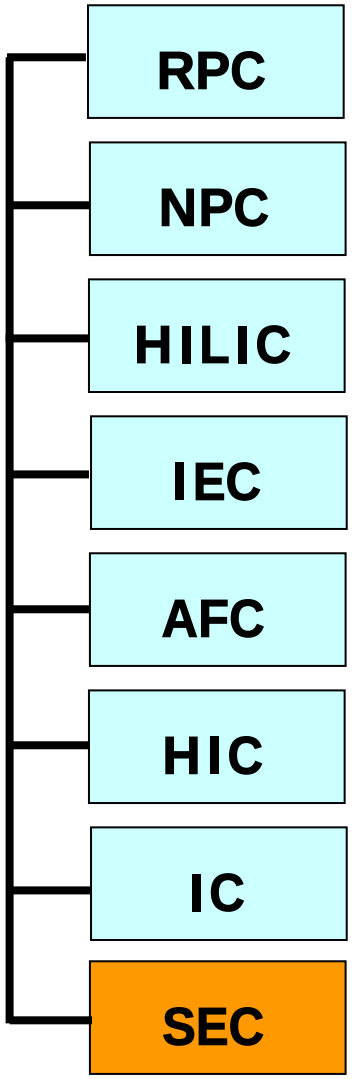
Polymer Analysis: Gel Permeation and Gel Filtration Chromatography Applications

Joe Machamer
GPC Marketing Manager

Outline

- Introduction
- Calibration Curves
- Extending Linear Range
- TSKgel GPC Columns
  - TSKgel PW
  - TSKgel Alpha and SuperSW
  - TSKgel H Series
  - TSKgel SuperMultipore
- Tosoh EcoSEC GPC System

HPLC Separation Modes



Reversed Phase Chromatography

Normal Phase Chromatography

Hydrophilic Interaction Chromatography

Ion-Exchange Chromatography

Affinity Chromatography

Hydrophobic Chromatography

Ion Chromatography

Size Exclusion Chromatography

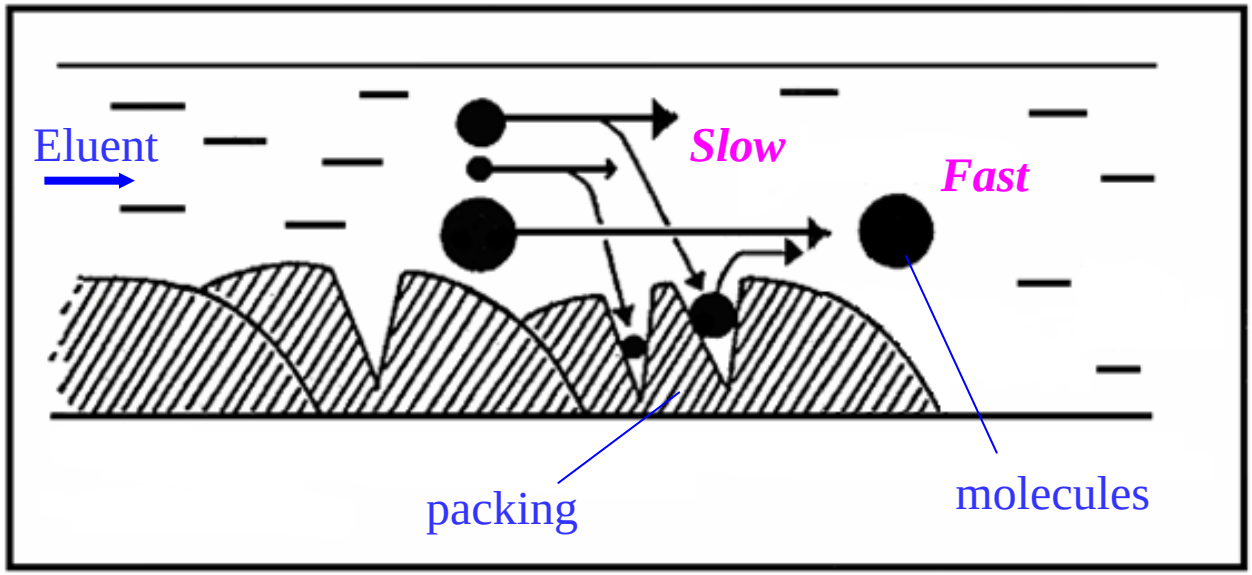
GPC: Organic GFC: Aqueous

Polymer Separation Column Types

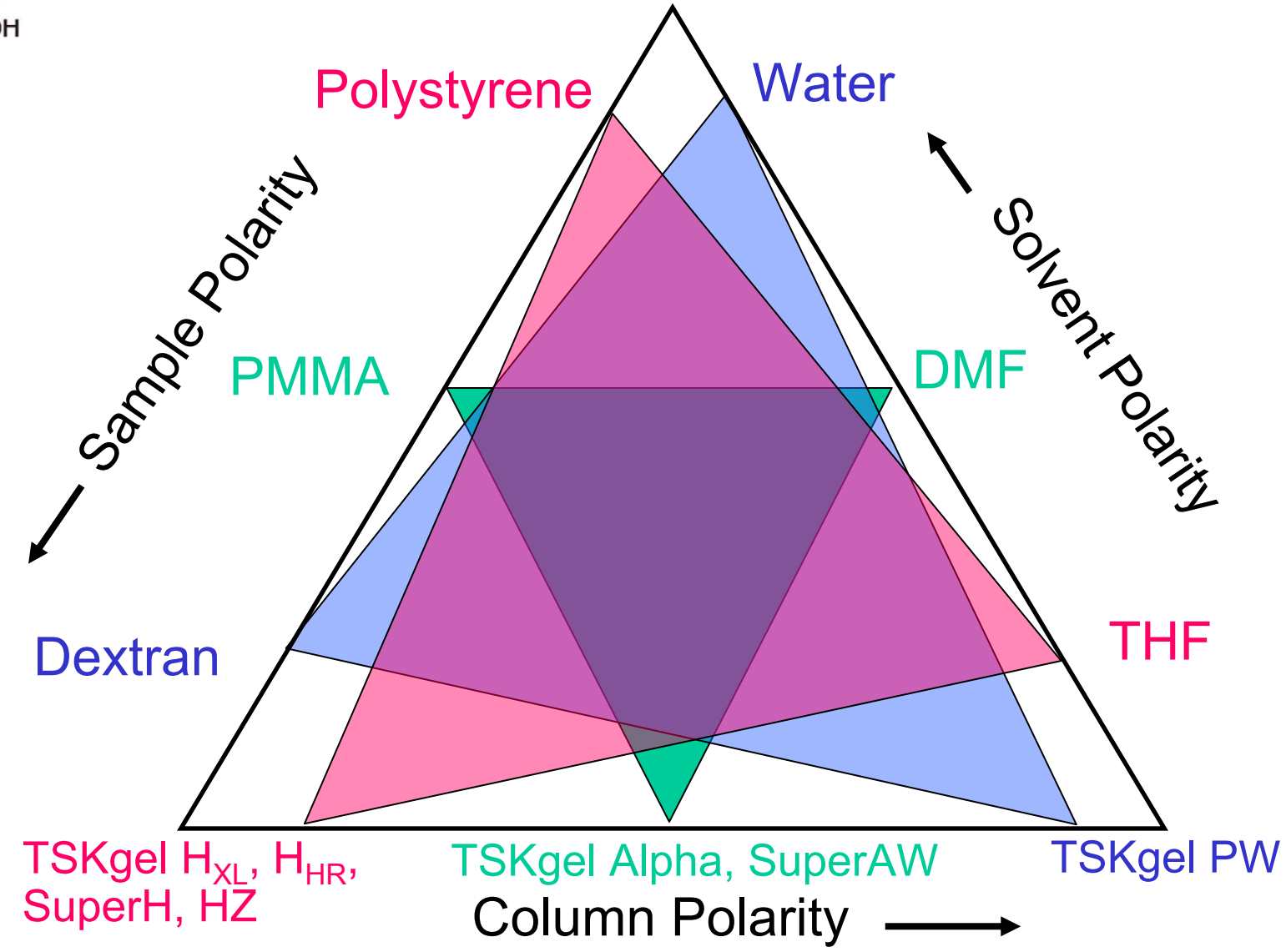
- Water-Soluble Polymer Samples
  - TSKgel PW
  - TSKgel PW_{XL}
  - TSKgel PW_{XL}-CP
  - TSKgel SuperMultiporePW
- Polar Organic-Soluble Polymer Samples
  - TSKgel Alpha
  - TSKgel SuperAW
- Organic-Soluble Samples
  - TSKgel H_{XL}
  - TSKgel SuperH
  - TSKgel H_{HR}
  - TSKgel SuperHZ
  - TSKgel SuperMultiporeHZ

SEC Separation Mechanism

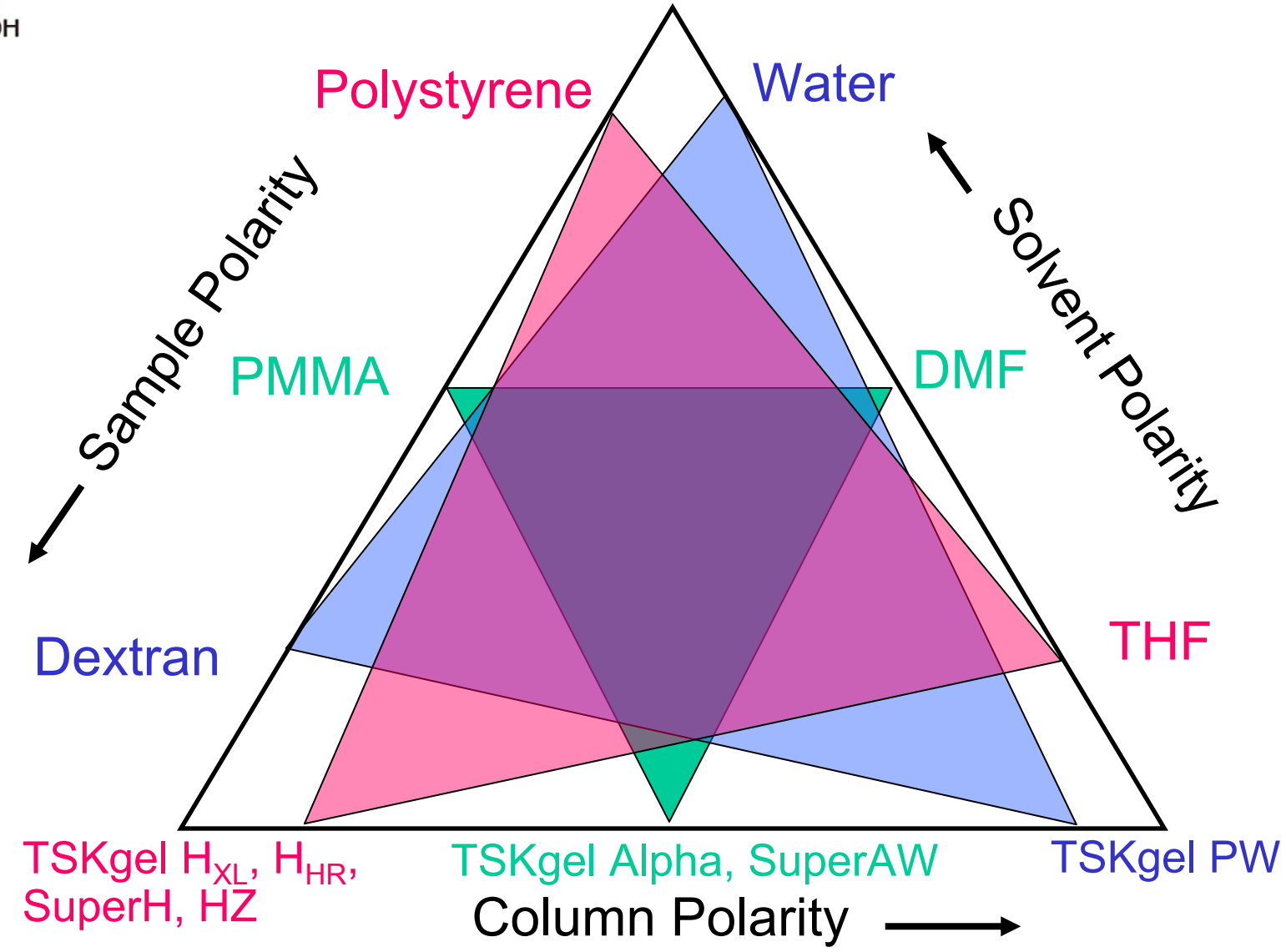
Basis-Difference in apparent molecular size with no additional interaction between column packing material and the sample



GPC Magic Triangle



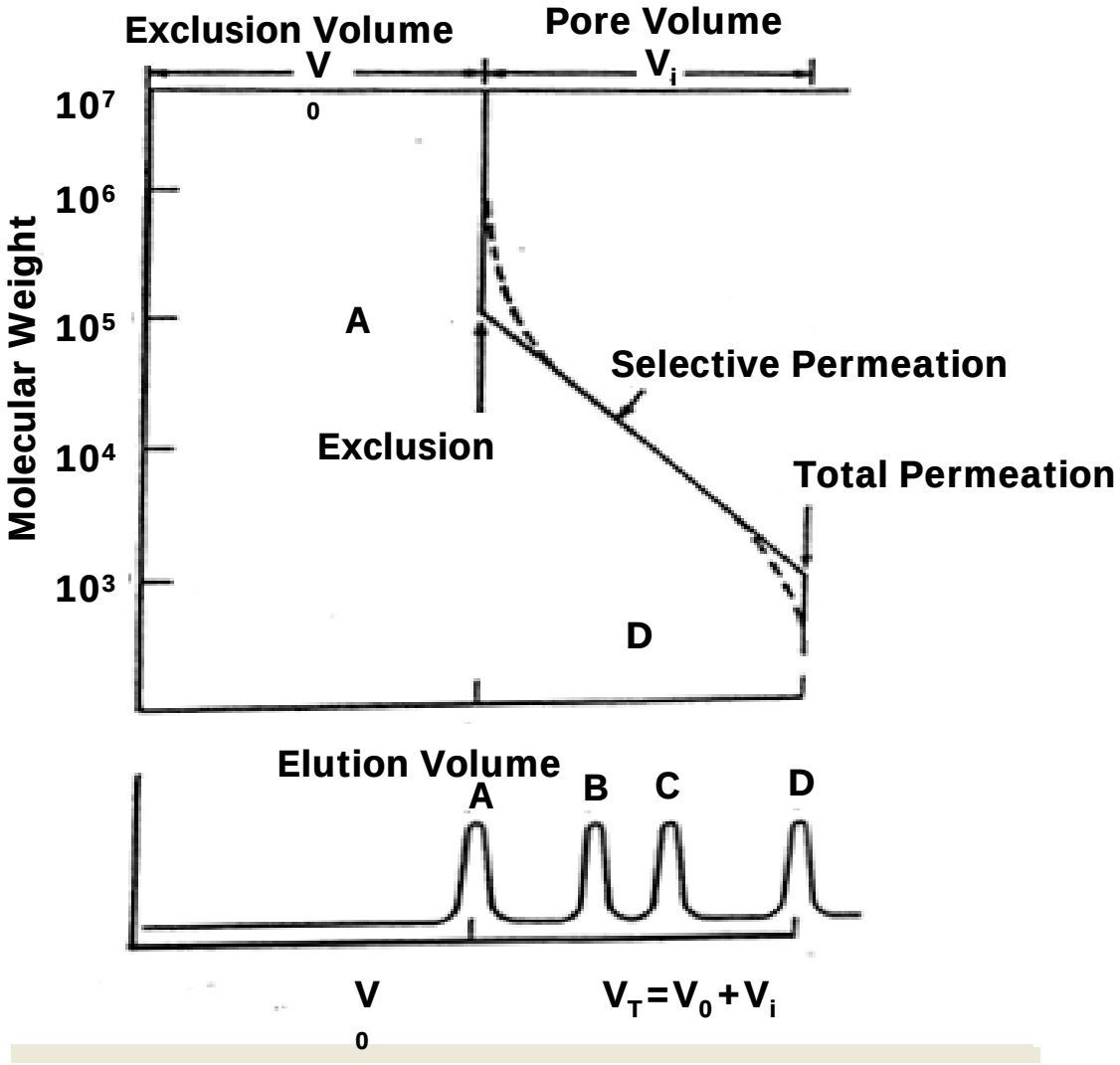
GPC Magic Triangle



SEC Mechanism - No Interaction

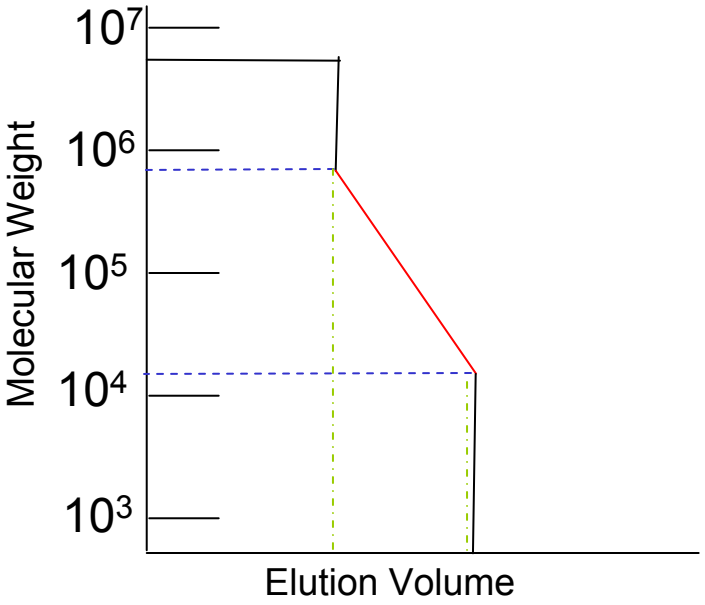
- Proper SEC requires sample, mobile phase, and column chemistry to have similar polarities
  - Interactions between sample and column chemistry induces large errors in MW determination
  - Interactions between sample and column chemistry cause polymers to appear to be smaller

Calibration Curve

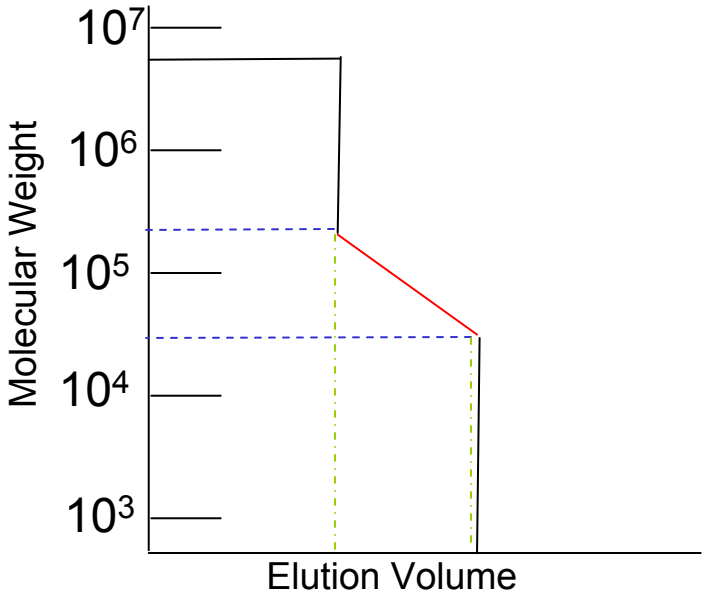


Resolution vs. Linear Range Trade-off

- Shallow calibration curve means:
  - More resolution
  - Less linear range



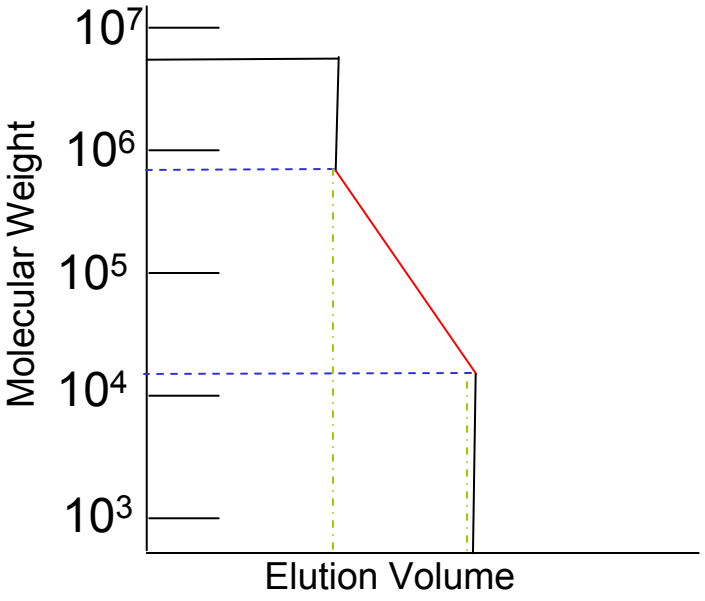
Low Resolution, Large Linear Range



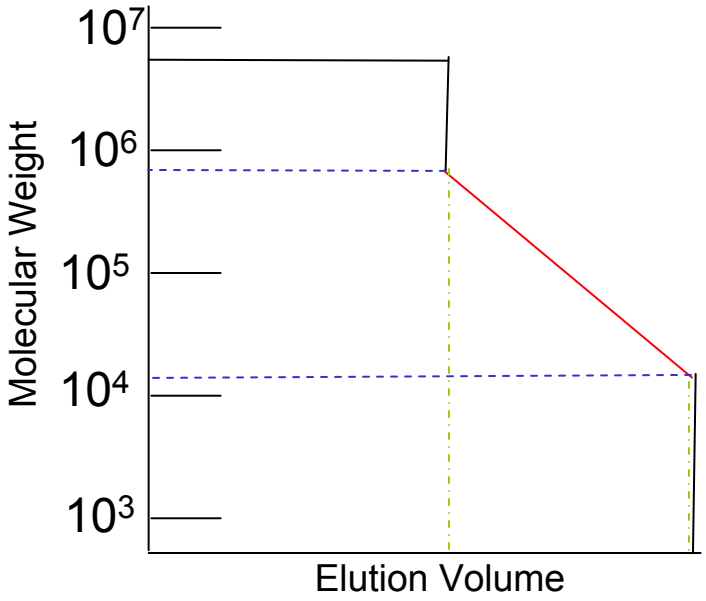
Higher Resolution, Smaller Linear Range

How To Increase Resolution

- Add more columns of same type
  - Longer Run Time
- Use smaller particle size



One column

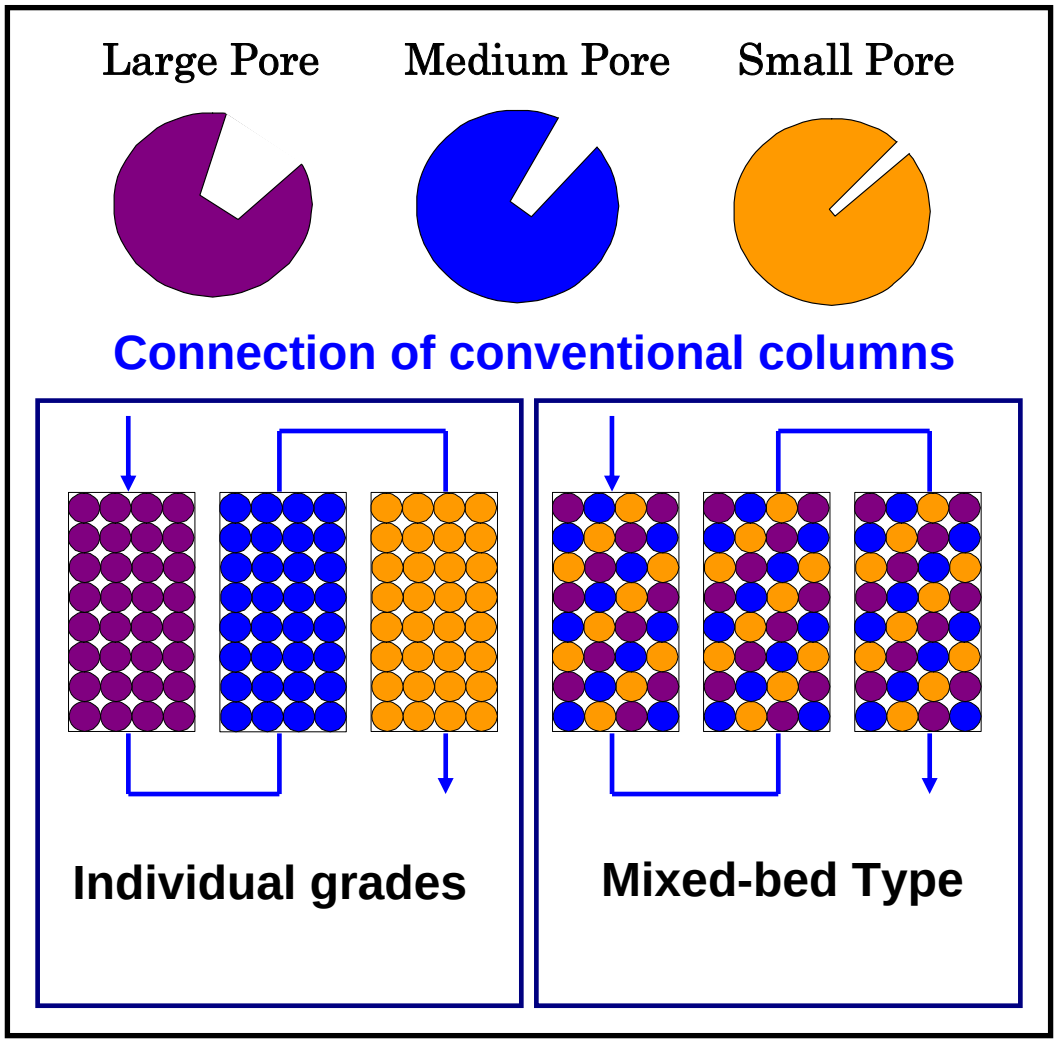


Two columns

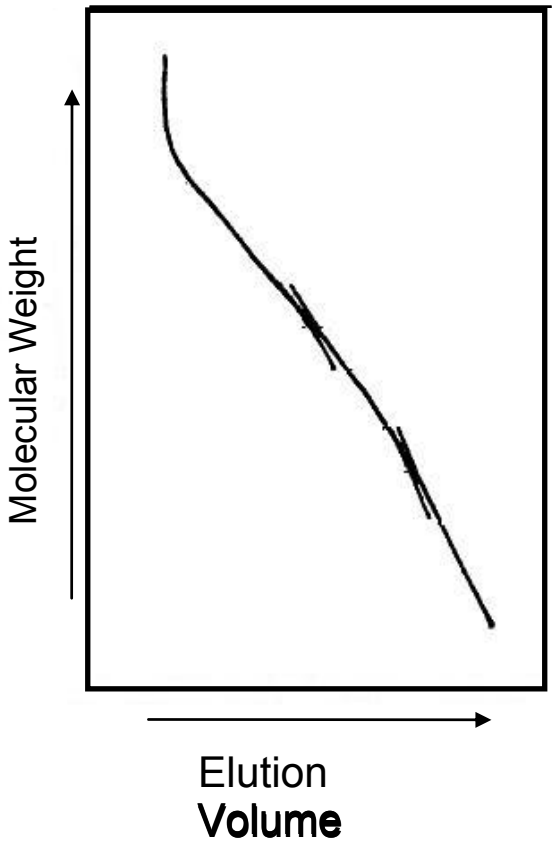
How To Increase Linear Range

- Use series of columns with different pores sizes
- Use columns contained a mixture of beads with different pore sizes

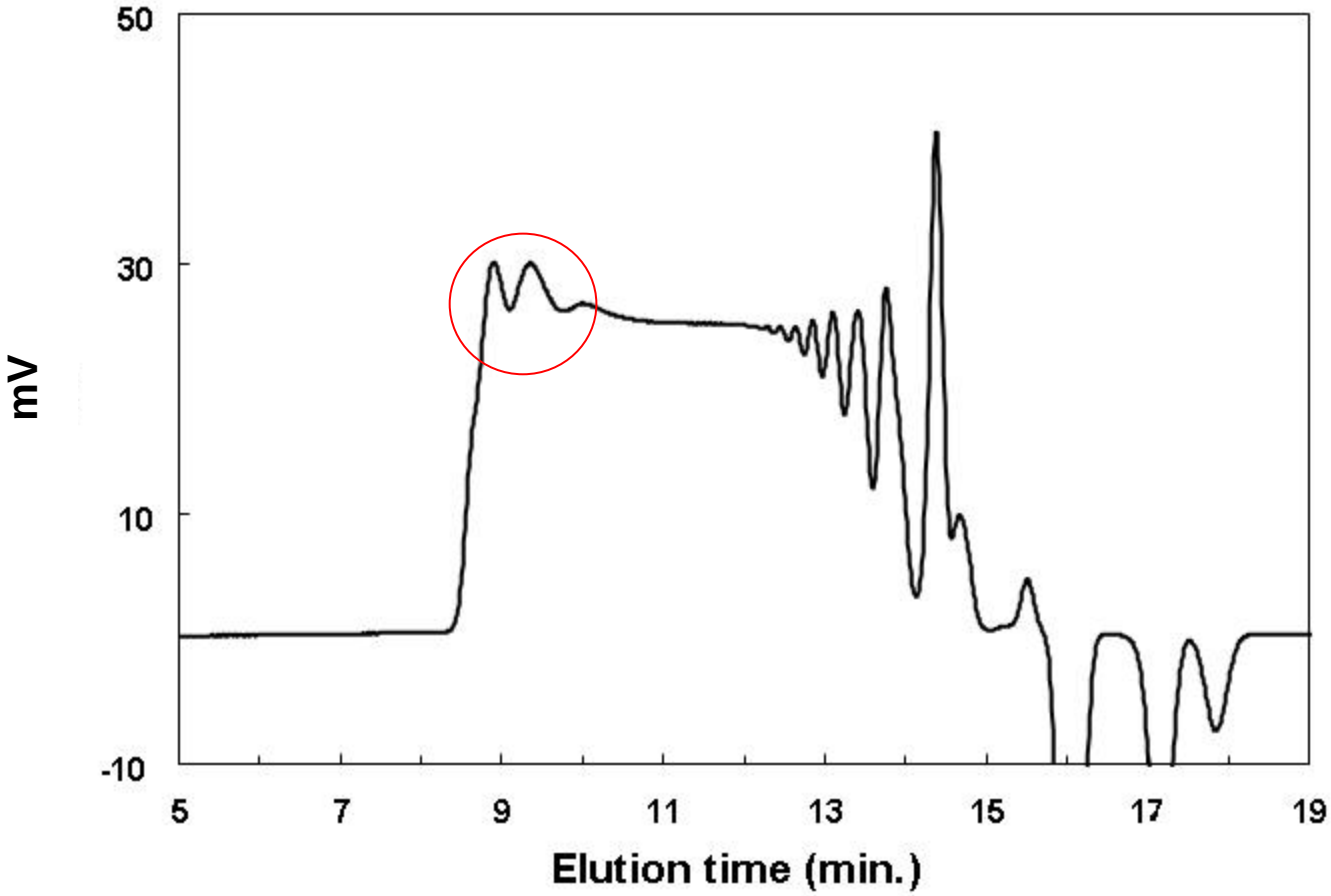
How To Increase Linear Range



Effect on Calibration Curve

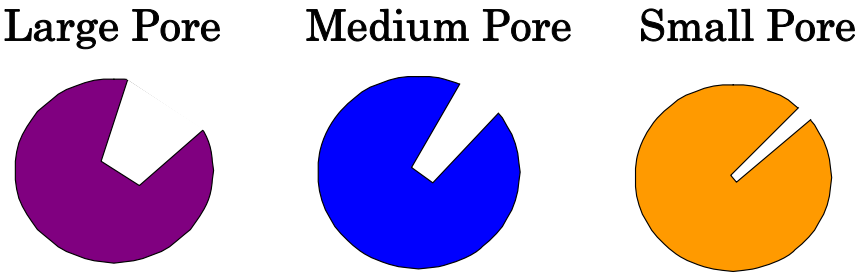


Distortion on Chromatogram

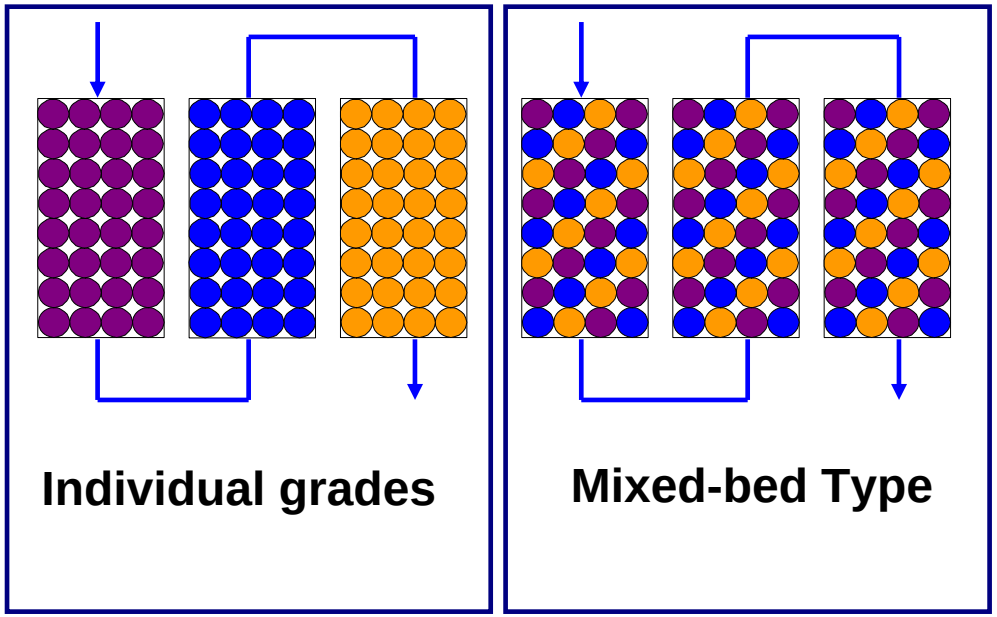


Schematic diagrams of SEC packings

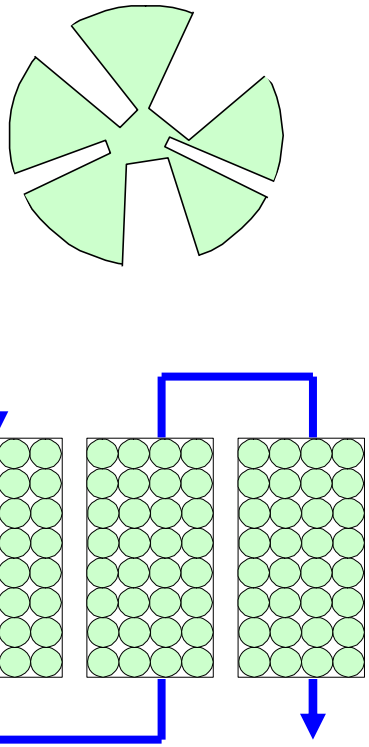
Conventional packings/columns



Connection of conventional columns

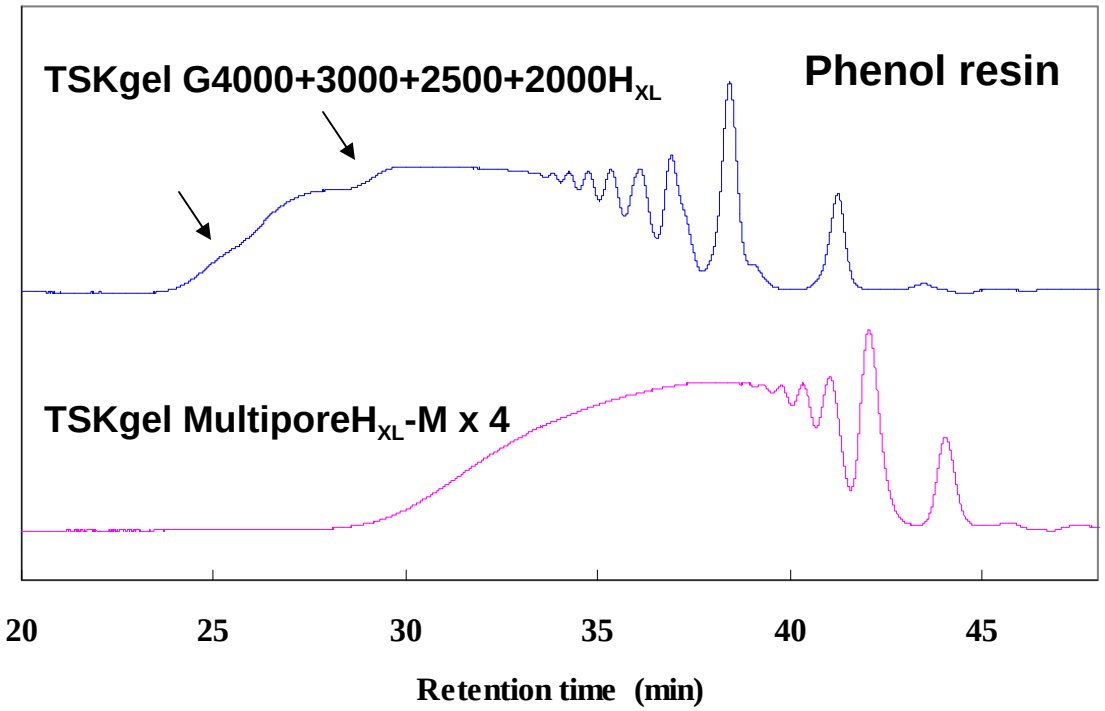


Multipore type packings



TSKgel SuperMultipore Type

Comparison of Sequential vs. Multipore Columns



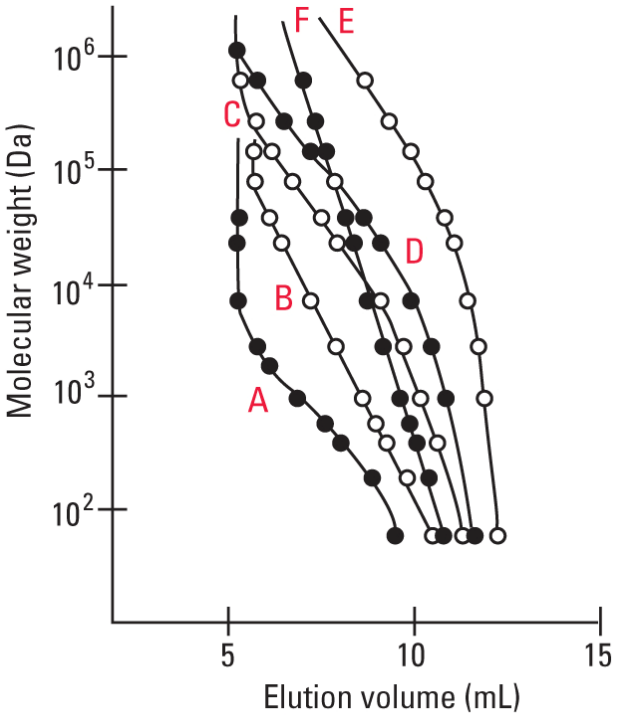
Columns:	TSKgel G4000+3000+2500+2000H$_{XL}$, 7.8mm ID x 30cm x 4
	TSKgel MultiporeH$_{XL}$-M x 4
Mobile phase:	THF
Flow rate:	1.0mL/min
Detection:	RI
Injection vol.:	100µL
Conc.:	2.0mg/mL

TSKgel PW Series Columns

- For water-soluble polymers
- Hydrophilic polymethacrylate stationary phase
- pH stability 2 to 12
- Organic stability to 20% Methanol
- Subtypes
  - TSKgel PW
  - TSKgel PW_{XL}
  - TSKgel PW_{XL}-CP
  - TSKgel SuperMultiporePW

TSKgel PW_{XL}

- Compared to TSKgel PW
  - Smaller Particle Size (6 - 13µm)
  - Higher resolution
- Pore size range <200Å to > 1000Å plus mixed bed

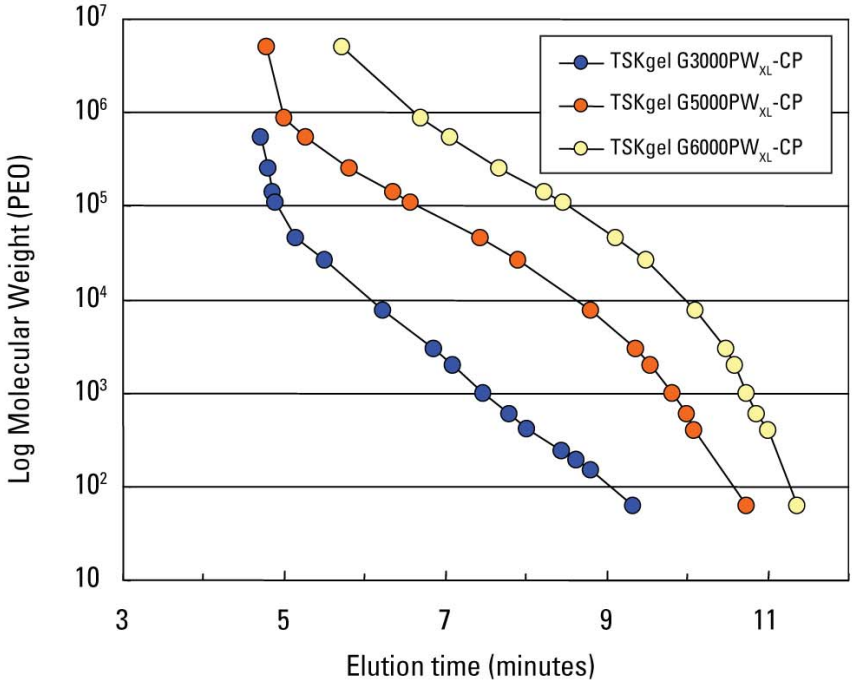


TSK-GEL PW_{XL} columns: A. $G2500PW_{XL}$, B. $G3000PW_{XL}$, C. $G4000PW_{XL}$, D. $G5000PW_{XL}$, E. $G6000PW_{XL}$, F. $GMPW_{XL}$

Mobile phase:	distilled H_2O
Flow Rate:	1.0mL/min
Detection:	RI
Samples:	polyethylene oxides (PEO) standards
	polyethylene glycols (PEG) standards

TSKgel PW_{XL}-CP

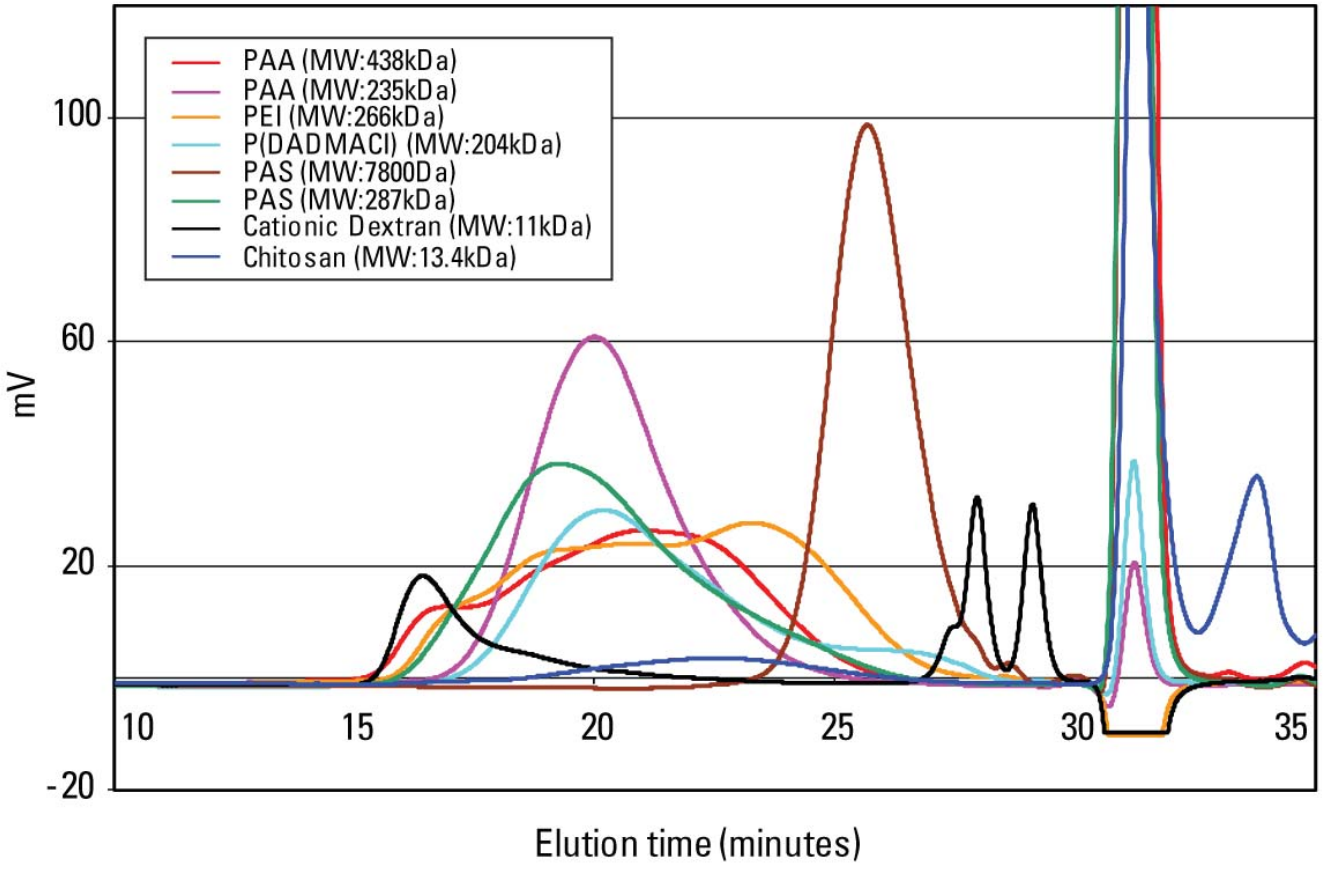
- For separation of cationic water-soluble polymers
- Cationic groups on the surface prevent adsorption of cationic polymer
- Elution under low salt conditions



TSKgel G3000PW$_{XL}$-CP, 7µm
TSKgel G5000PW$_{XL}$-CP, 10µm
TSKgel G6000PW$_{XL}$-CP, 13µm

Mobile phase:	0.1mol/L $NaNO_3$
Flow Rate:	1mL/min
Detection:	RI
Temp:	25°C
Samples:	polyethylene oxides (PEO) standards polyethylene glycols (PEG) standards

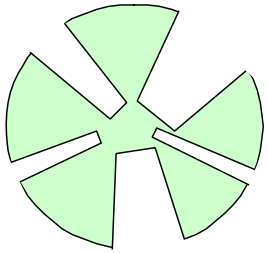
TSKgel PW_{XL}-CP Cationic Polymer Analysis



TSKgel G3000PW_{XL}-CP, 7µm, 7.8mm ID x 30cm
TSKgel G5000PW_{XL}-CP, 10µm, 7.8mm ID x 30cm
TSKgel G6000PW_{XL}-CP, 13µm, 7.8mm ID x 30cm

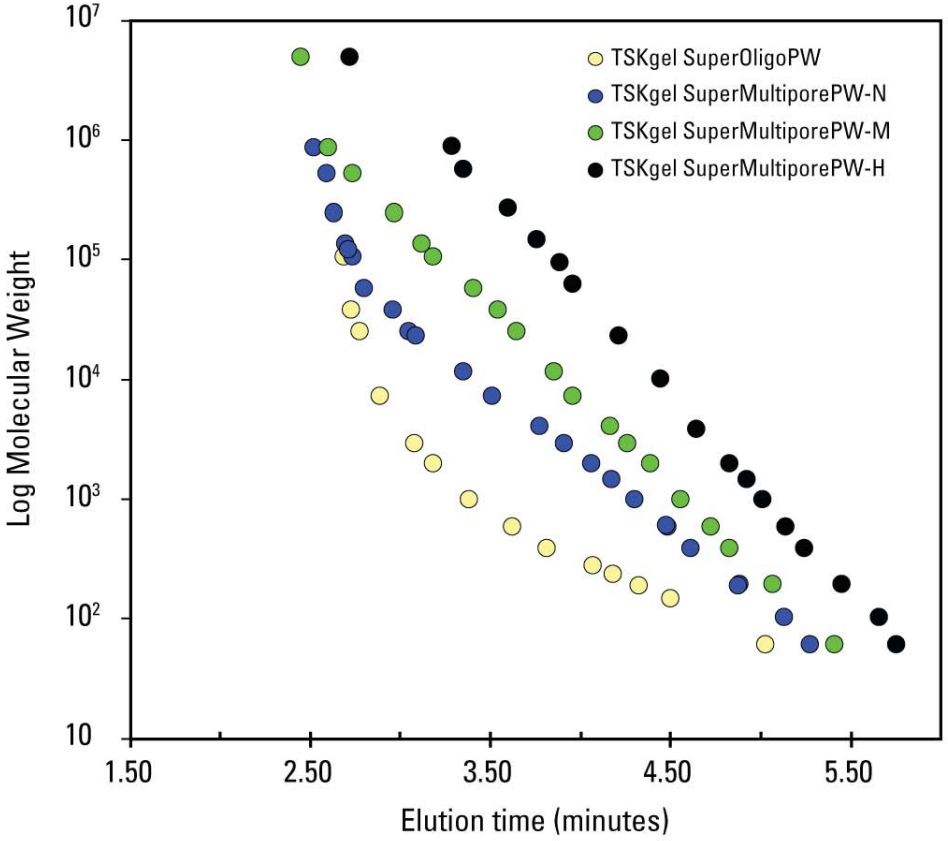
Mobile phase:	0.1mol/L $NaNO_3$
Flow Rate:	1mL/min
Detection:	RI
Temperature:	25°C
Sample Load:	3g/L, 100µL

TSKgel SuperMultiporePW



- Multipore pore morphology
- Extended linear range w/o chromatogram distortion
- Less hydrophobic than TSKgel PW_{XL} columns

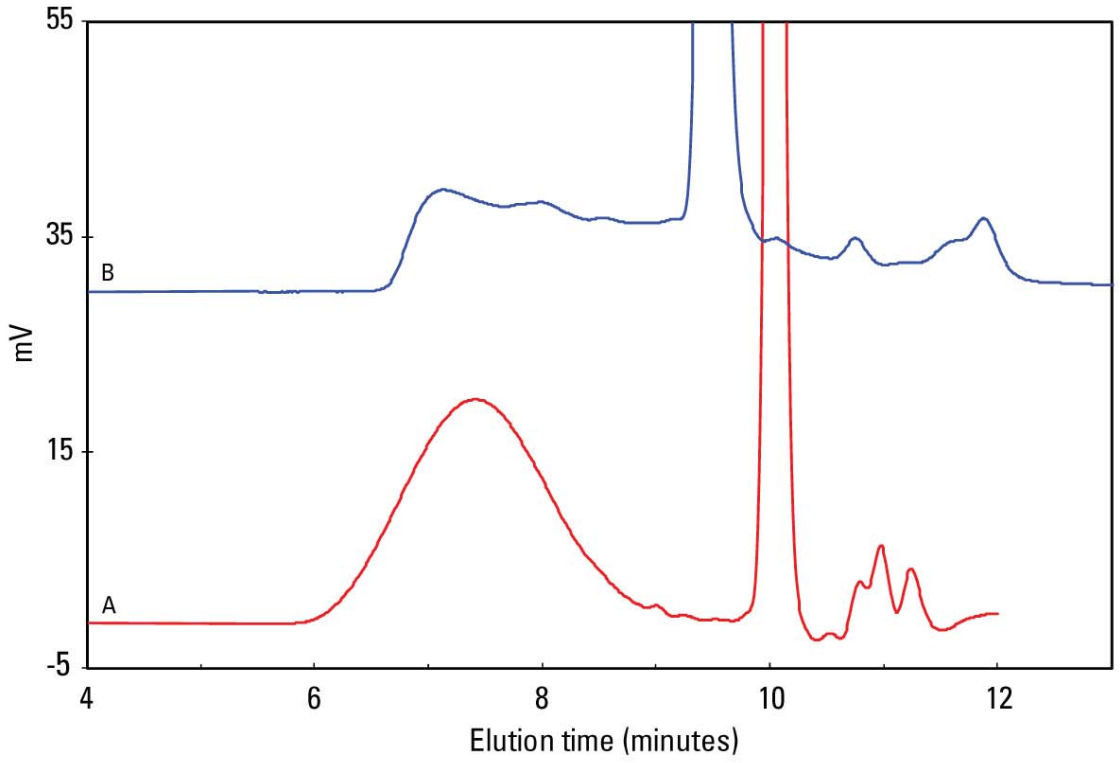
TSKgel SuperMultiporePW



TSKgel SuperOligoPW, 6.0mm ID x 15cm
TSKgel SuperMultiporePW-N, 6.0mm ID x 15cm
TSKgel SuperMultiporePW-M, 6.0mm ID x 15cm
TSKgel SuperMultiporePW-H, 6.0mm ID x 15cm

Mobile phase:	H_2O
Flow rate:	0.60mL/min
Detection:	RI
Temperature:	25°C
Samples:	PEO, PEG and ethylene glycol

PVP Analysis: TSKgel PW_{XL} vs. TSKgel SuperMultiporePW-N



Columns:	A: TSKgel SuperMultiporePW-N, 6.0mm ID x 15cm x 2
	B: TSKgel G3000PW_{XL}+G2500PW_{XL}, 6.0mm ID x 15cm x 2
Mobile phase:	100mmol/L $NaNO_3$
Flow rate:	0.60mL/min
Detection:	RI
Temperature:	40°C
Injection vol.:	20µL
Samples:	PVP(K-15)

TSKgel PW-type Applications

Sample			Column Selection: First selection	Column Selection: Second selection	Selection Criteria
Carbohydrates	polysaccharides		TSKgel GMPW$_{XL}$	TSKgel G5000PW$_{XL}$ + G3000PW$_{XL}$	large pore size linearity of calibration curve
	oligosaccharides		TSKgel G-Oligo-PW	TSKgel G2500PW$_{XL}$ TSKgel G2000PW	resolving power
Nucleic Acids	DNA fragments	large	TSKgel G-DNA-PW TSKgel G5000PW$_{XL}$		large pore size resolving power
		medium & small	TSKgel G4000SW TSKgel G3000SW		suitable pore size resolving power
	RNA		TSKgel G4000SW TSKgel G3000SW		
	oligonucleotides		TSKgel G2500PW$_{XL}$		small pore size ionic interaction
Proteins	normal size proteins		TSKgel G3000SW TSKgel G4000SW TSKgel G2000SW	TSKgel G3000PW$_{XL}$ TSKgel G4000PW$_{XL}$	resolving power
	large proteins	low density lipoprotein	TSKgel G6000PW$_{XL}$ TSKgel G5000PW$_{XL}$		large pore size resolving power
		gelatin	TSKgel GMPW$_{XL}$	TSKgel G5000PW$_{XL}$ + G3000PW$_{XL}$	large pore size linearity of calibration curve
Peptides	large		TSKgel G3000SW TSKgel G2000SW	TSKgel G3000PW$_{XL}$	
	small		TSKgel G25000PW$_{XL}$	TSKgel G2000SW	linearity of calibration curve resolving power
Virus			TSKgel G6000PW$_{XL}$ TSKgel G5000PW$_{XL}$		large pore size resolving power
Synthetic polymers			TSKgel GMPW$_{XL}$	TSKgel G5000PW$_{XL}$ + G3000PW$_{XL}$	large pore size linearity of calibration curve low adsorption
Synthetic oligomers	nonionic and cationic		TSKgel G-Oligo-PW	TSKgel G2500PW$_{XL}$	small pore size resolving power ionic interaction
	anionic		TSKgel G2500PW$_{XL}$		

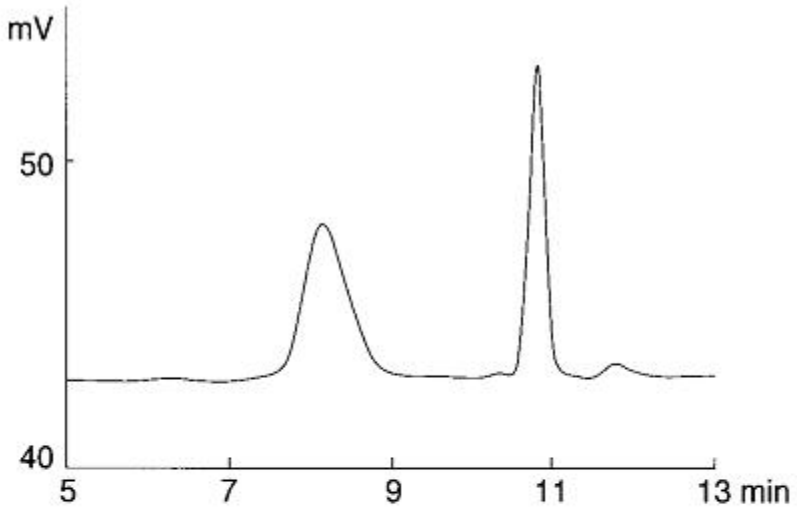
TSKgel Alpha and SuperAW Series Columns

- For Polymers of Intermediate Polarity
- Higher crosslinking than TSKgel PW series
- TSKgel SuperAW Resolution Same As Alpha, but 2x faster and 2/3 less solvent consumption
- Minimal shrink and swell in polar organic solvents
  - methanol, ACN, DMSO, IPA, THF, and HFIP
- Available in 5 discrete exclusion ranges plus a mixed bed column

TSKgel Alpha and SuperAW Applications

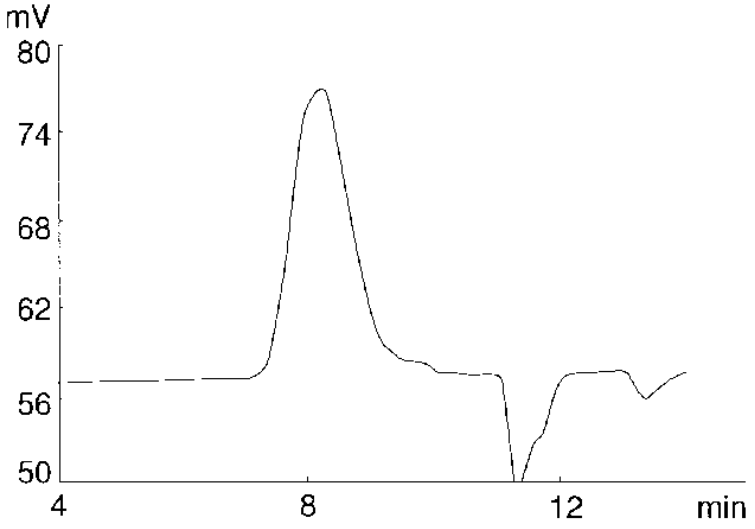
- Sodium chondroitin sulfate
- Sodium alginate
- Carboxymethyl cellulose
- Sodium polystyrene sulfonate
- Polyvinyl pyrrolidone
- Gum arabic
- Ethylhydroxy-ethylcellulose
- Vinyl alcohol/vinyl butyral copolymer
- Hydroxypropylcellulose
- Polymethyl vinyl ether
- Cellulose acetate
- N-isopropyl acrylamide
- Polyacrylonitrile
- Vinyl chloride/vinyl acetate copolymer
- Styrene/allylalcohol copolymer
- Poly (p-phenylene ether sulfone)

Solvent Flexibility



Chromatogram of Sodium Chondroitin Sulfate

Column: TSKgel SuperAWM-H (6.0mm I.D. × 15cm × 2)
Eluent: 0.2mol/L sodium nitrate
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20μL (0.5g/L)



Chromatogram of Poly (p-phenylene Ether Sulfone)

Column: TSKgel SuperAWM-H (6.0mm I.D. × 15cm × 2)
Eluent: DMF containing 10mmol/L LiBr
Flow rate: 0.6mL/min
Temperature: 40°C
Detection: Refractive index detector
Sample load: 20μL (0.5g/L)

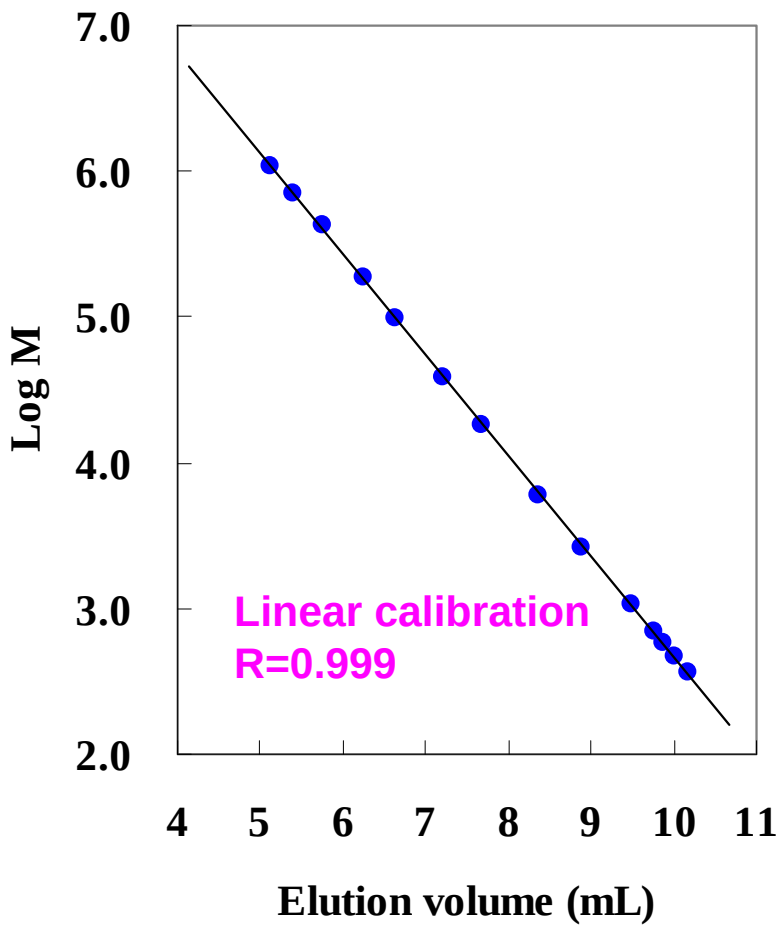
TSKgel H-Series Columns

- For GPC Analysis of Organic Soluble Polymers
- PS-DVB Matrix
- Eight pore sizes
- Range of Mixed Bed Columns
- Range of Multipore Columns

TSKgel H_{XL} and SuperHZ

- TSKgel H_{XL} (7.8mm ID x 30cm)
  - Ultra low polymer adsorption
  - Solvent can be switched one time
  - Extended linear range available with one Multipore column and five mixed-bed columns
- TSKgel SuperHZ (4.6/6.0mm ID x 30cm)
  - Smaller particle and column size versions of TSKgel H_{XL}
  - Ultra low polymer adsorption
  - Solvent can be switched one time
  - Extended linear range available with three mixed-bed columns

Characteristics of TSKgel MultiporeH$_{XL}$-M



Characteristics of linear type packing

1) The range of molecular weight for measurement is wide.

2) Calibration curve can be similar in a linear equation.

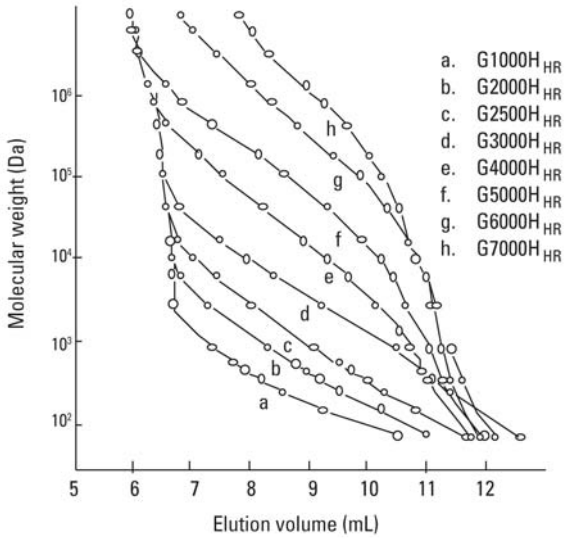
3) The distortions of chromatogram coming from the point of inflection does not appear..

More correct measurement of molecular weight

TSKgel H_{HR} and SuperH

- TSKgel H_{HR} (7.8mm ID x 30cm)
  - Solvent can be switch many times
  - Five mixed-bed columns for extended linear range
  - Stable to 140^{O}C
  - Broad Solvent Range
- TSKgel SuperH (6.0mm ID x 15cm)
  - Smaller particle and column size versions of TSKgel H_{HR}
  - 2x shorter run times than TSKgel H_{HR}

Characteristics of Individual TSKgel H_{HR} Columns



TSKgel H_{HR} series, 7.8mm ID x 30cm

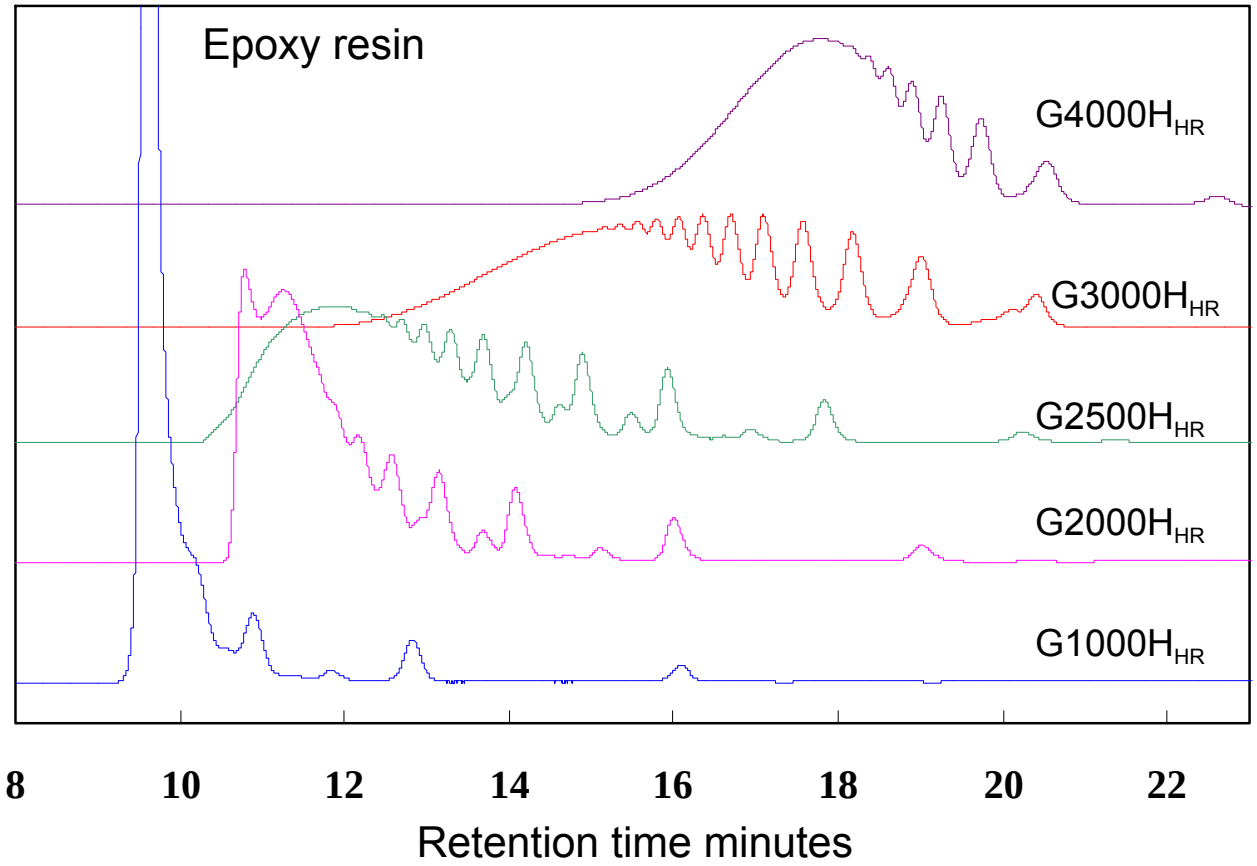
Mobile phase:	THF
Flow Rate:	1.0mL/min
Detection:	UV@254nm
Temperature:	25°C
Samples:	polystyrene standards

The range of molecular weight for measurement is narrow.

Good separation at specific range of molecular weight

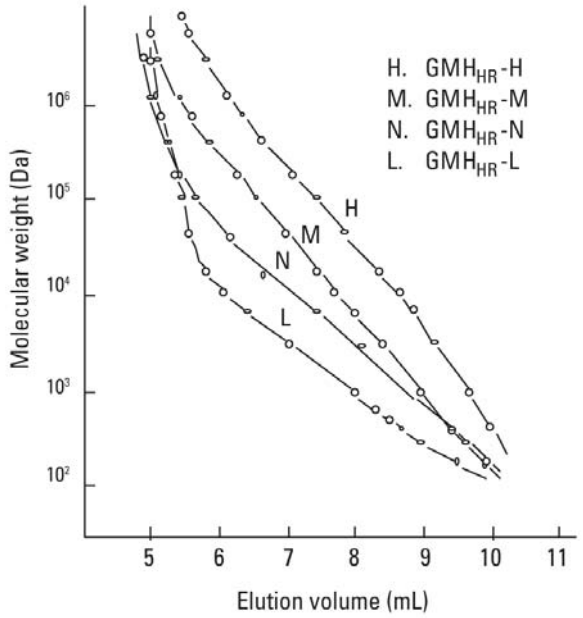
TSKgel G1000H_{HR}	Separation of solvent peaks
TSKgel G2000H_{HR}	Oligomers
TSKgel G3000H_{HR}	Low weight polymers

Chromatograms with Various TSKgel H_{HR} Columns



Columns:	TSKgel H_{HR}, 7.8mm ID x 30cm x 2
Mobile phase:	THF
Flow rate:	1.0mL/min
Detection:	RI
Injection vol.:	100µL
Conc.:	2.0mg/mL

Characteristics of Mixed Bed TSKgel H_{HR} Columns



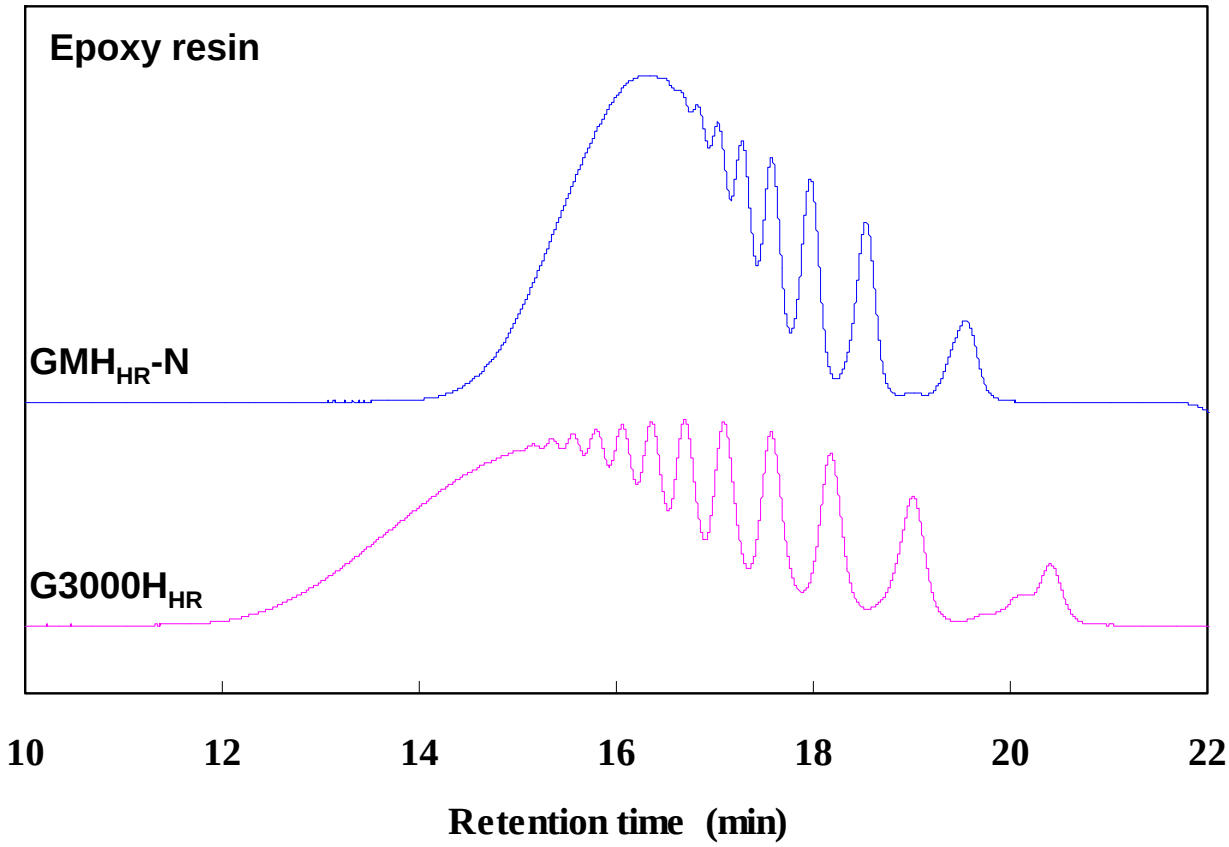
TSKgel H_{HR} series, 7.8mm ID x 30cm

Mobile phase:	THF
Flow Rate:	1.0mL/min
Detection:	UV@254nm
Temperature:	25°C
Samples:	polystyrene standards

The range of molecular weight for measurement is wide.

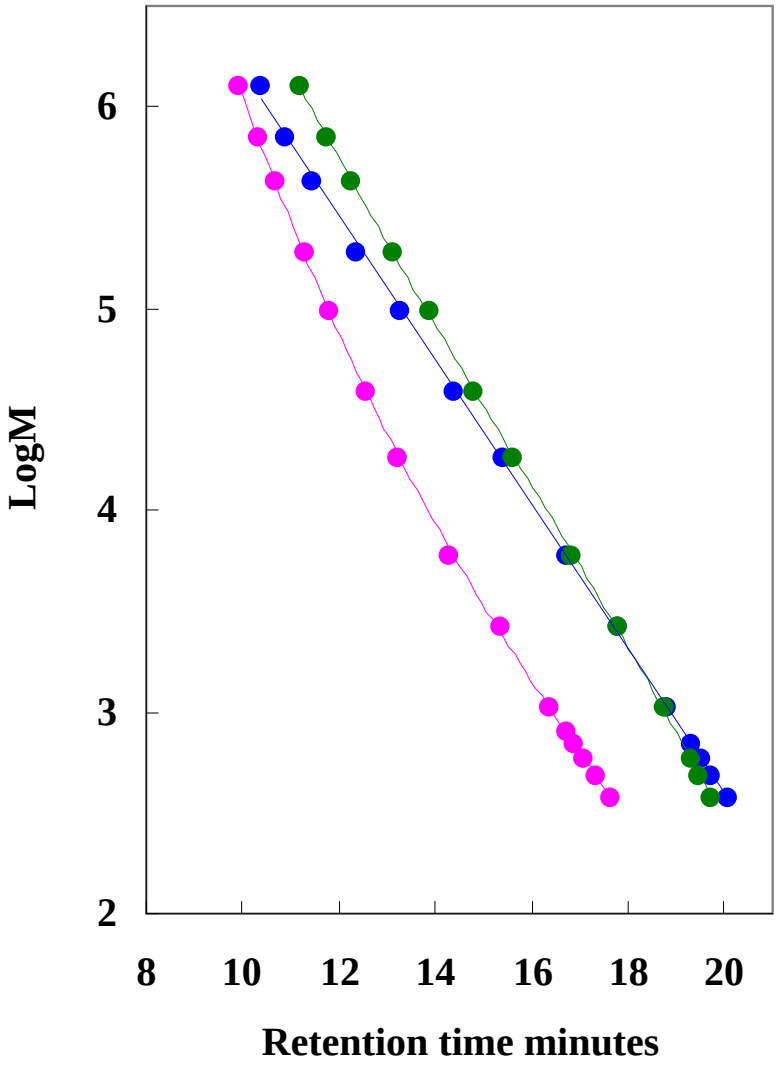
Good for screening measurement of molecular weight for unknown polymer

Comparison of Chromatograms with Individual and Mixed Type Packing



Columns:	TSKgel H_{HR}, 7.8mm ID x 30cm x 2
Mobile phase:	THF
Flow rate:	1.0mL/min
Detection:	RI
Injection vol.:	100µL
Concentration:	2.0mg/mL

Comparison of Calculated Molecular Weight with Various Type Packing



Polystyrene (NIST SRM706)

Regular type
Column: TSKgel SuperH5000+4000+3000+2000
M_n 1.27x10^5 M_w 2.65x10^5

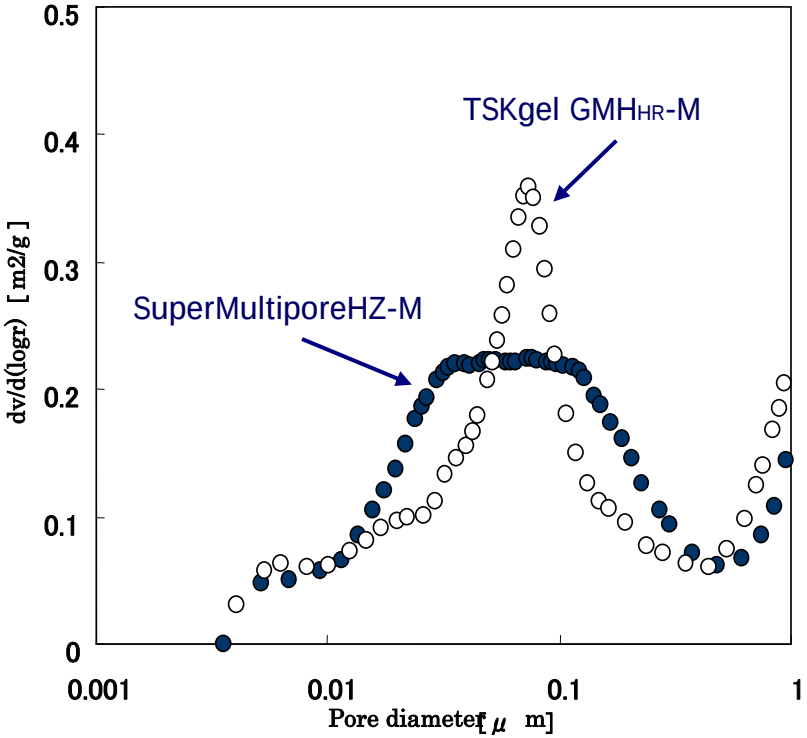
Mixed type
Column: TSKgel SuperHZ-M x 4
M_n 1.20x10^5 M_w 2.73x10^5

Linear type
Column: TSKgel SuperMultiporeHZ-M x 4
M_n 1.28x10^5 M_w 2.83x10^5

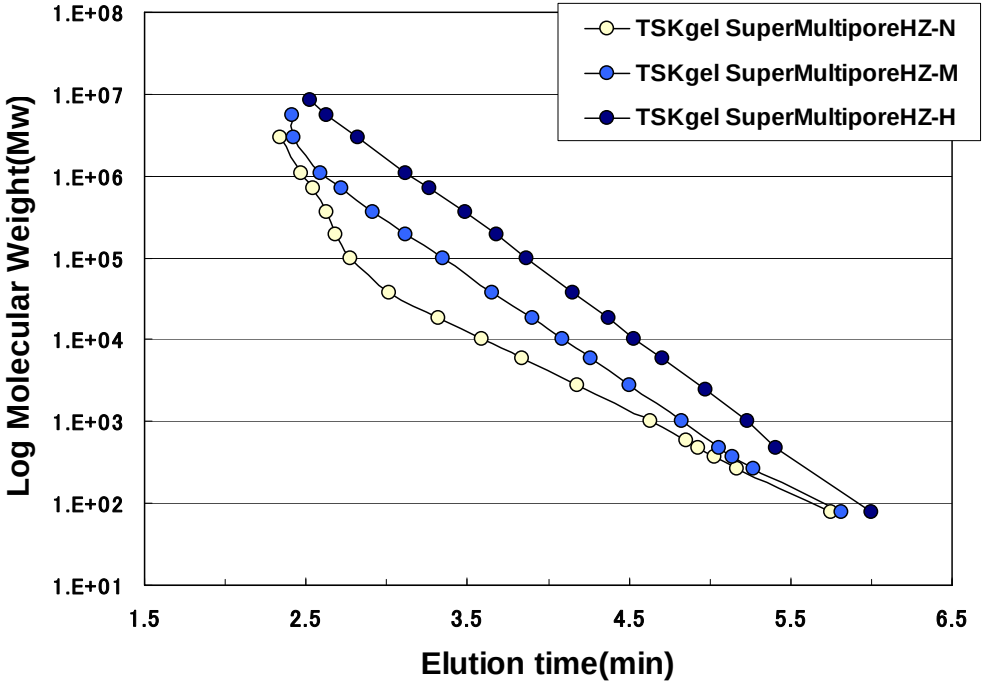
TSKgel SuperMultiporeHZ

- Ultra low polymer absorption
- Multipore pore morphology for linearity with out chromatogram distortion
- Semi-micro column size
  - Run times are 50% lower than conventional columns
  - Solvent consumption is 85% less than conventional columns

Pore characterization of TSKgel SuperMultiporeHZ-M



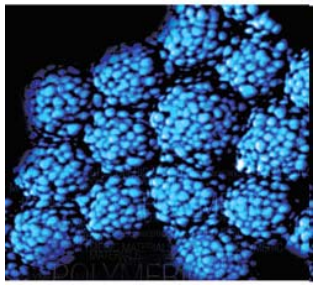
Calibration curves of TSKgel SuperMultiporeHZ columns



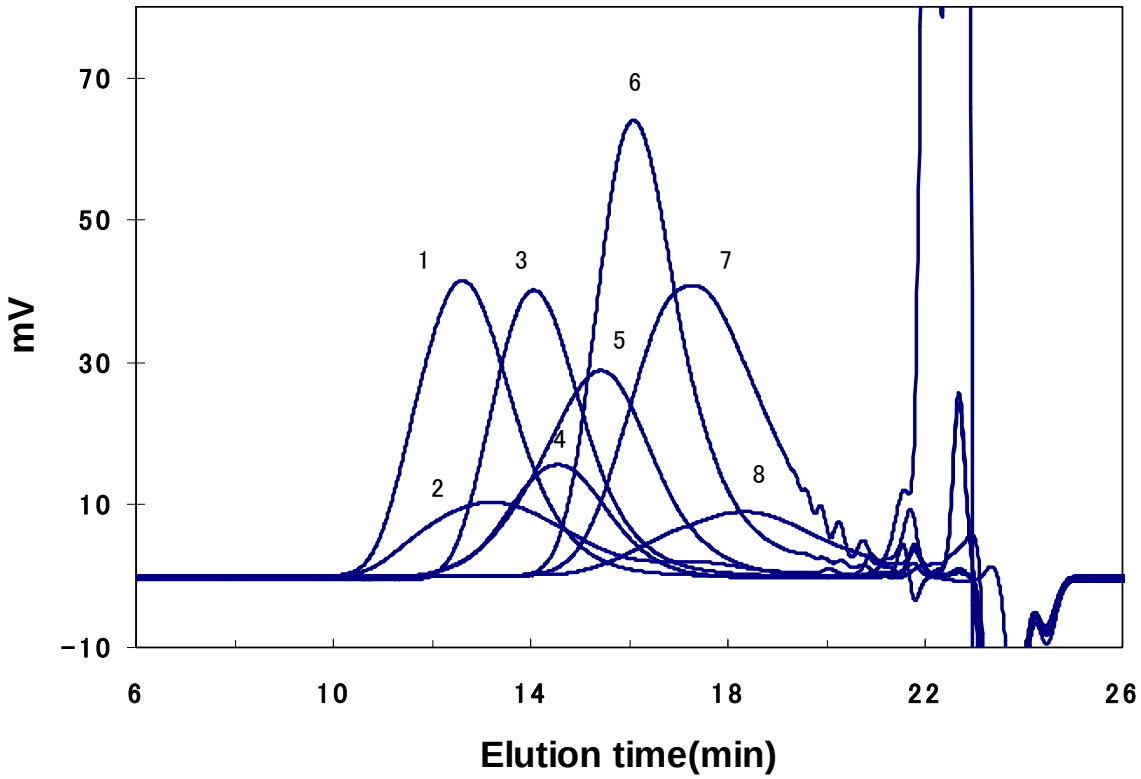
Column: TSKgel SuperMultiporeHZ, 4.6mm ID x 15cm
Mobile phase: THF
Flow rate: 0.35 mL/min
Detection: UV@254nm
Temperature: 25℃
Injection. vol.: 5µL
Samples: Std. Polystyrene

Properties of TSKgel SuperMultiporeHZ columns

	SuperMultiporeHZ-N	SuperMultiporeHZ-M	SuperMultiporeHZ-H
Base	PS/DVB	PS/DVB	PS/DVB
Particle Size (µm)	3	4	6
Exclusion Limit (MW)	120,000	2,000,000	40,000,000
Mean Pore Dia. (nm)	8	14	
Separation Range (MW)	300-50,000	500-1,000,000	1,000-10,000,000
Theoretical Plates	20,000TP/15cm	16,000TP/15cm	11,000TP/15cm
Column Size	4.6mm ID x 15cm	4.6mm ID x 15cm	4.6mm ID x 15cm



Chromatograms of various polymers on TSKgel SuperMultiporeHZ column



Column:	TSKgel SuperMultiporeHZ-H, 4.6mm ID x 15cm x 4
Mobile phase:	THF
Flow rate:	0.35 mL/min
Detection:	RI
Temperature:	40°C
Load:	10µL, 3g/L each
Sample:	1. Poly isobutylene
	2. Acrylic resin #1
	3. Polystyrene (SRM706)
	4. Polyvinylchloride
	5. Polyvinylbutylral
	6. Polycarbonate
	7. Epoxy resin
	8. Acrylic resin #2

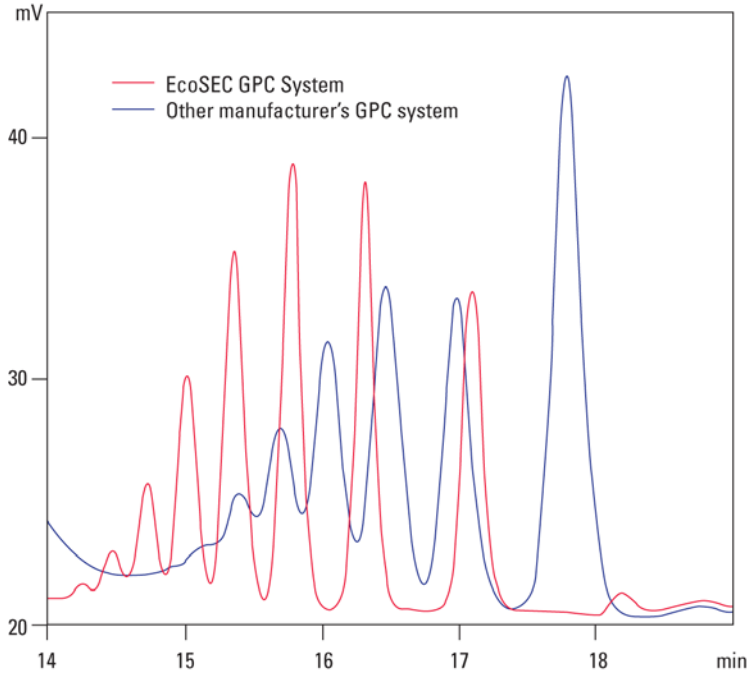
Semi-micro vs. Conventional Columns

	Conventional	Semi-micro
Diameter (mm)	7.8	4.6-6.0
Length (cm)	30	15
Flow Rate (mL/min.)	1.0	0.35
Run Time (Arbitrary)	100	50

Semi-micro columns reduce run times by 50% and flow rate by 2/3. Throughput is doubled and solvent costs drop by 1/6.

Resolution: Semi-micro vs. Conventional System

- Semi-micro system offers better resolution for semi-micro columns



TSKgel SuperHZ2000, 4.6mm ID x 15cm, × 4

Mobile phase:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temperature:	40°C
Injection vol.:	10µL (0.2mg/mL)

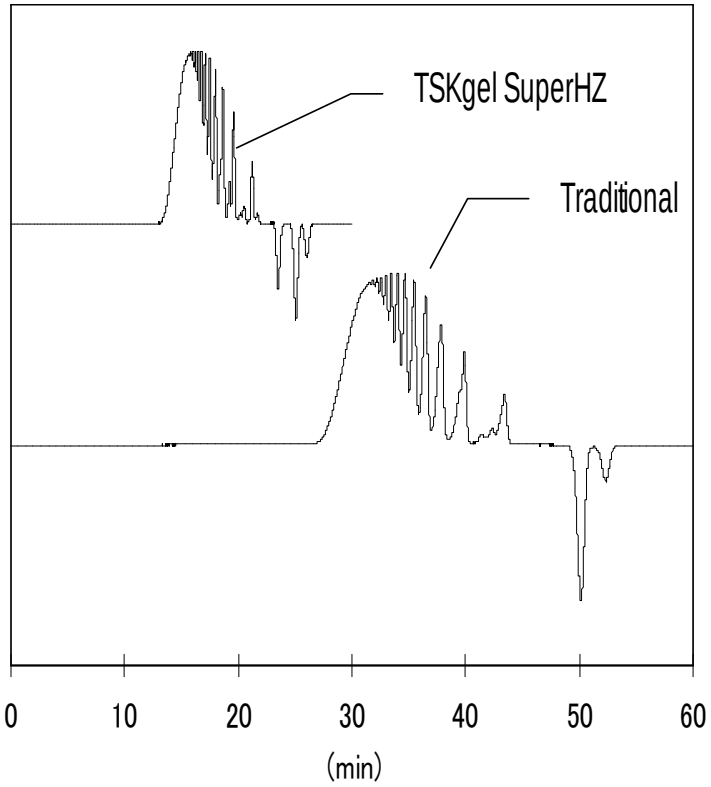
EcoSEC GPC System

Save Time and Money

- Low dead volume design and system layout
  - 7.5µL Stroke Volume
  - 2.5µL RI Cell Volume
  - Use of 0.2mm and 0.4mm tubing

Save Time and Money

- Reduce run times by 50%



Flow rate: TSKgel SuperHZ (0.35mL/min)
TSKgel H_{HR} (1.0mL/min)

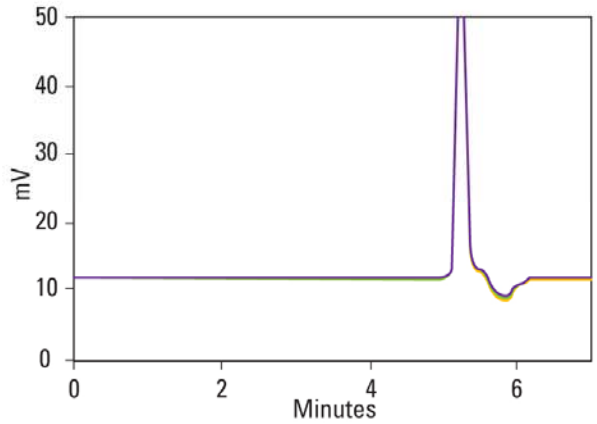
Dimensions: 4.6mm ID x 15cm*4 (TSKgel SuperHZ)
7.8mm ID x 30cm*4 (TSKgel H_{HR})

Save Time and Money

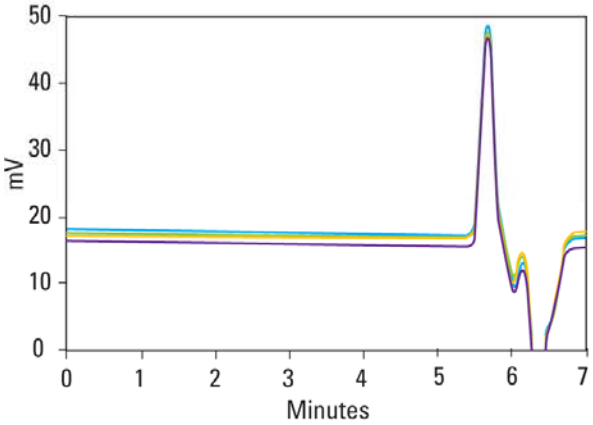
- Reduce solvent usage by 85%

Solvent	Competitor GPC (solvent + disposal)	EcoSEC GPC System (solvent + disposal)	Savings
NMP ($30/L)	$3082	$1312	$1,770.00
Chloroform ($17/L)	$1830	$779	$1,051.00
DMF ($25/L)	$2600.50	$1117	$1,463.50
HFIP ($1000/L)	$96,493	$41,193	$55,300.00

Superior Performance



EcoSEC GPC System



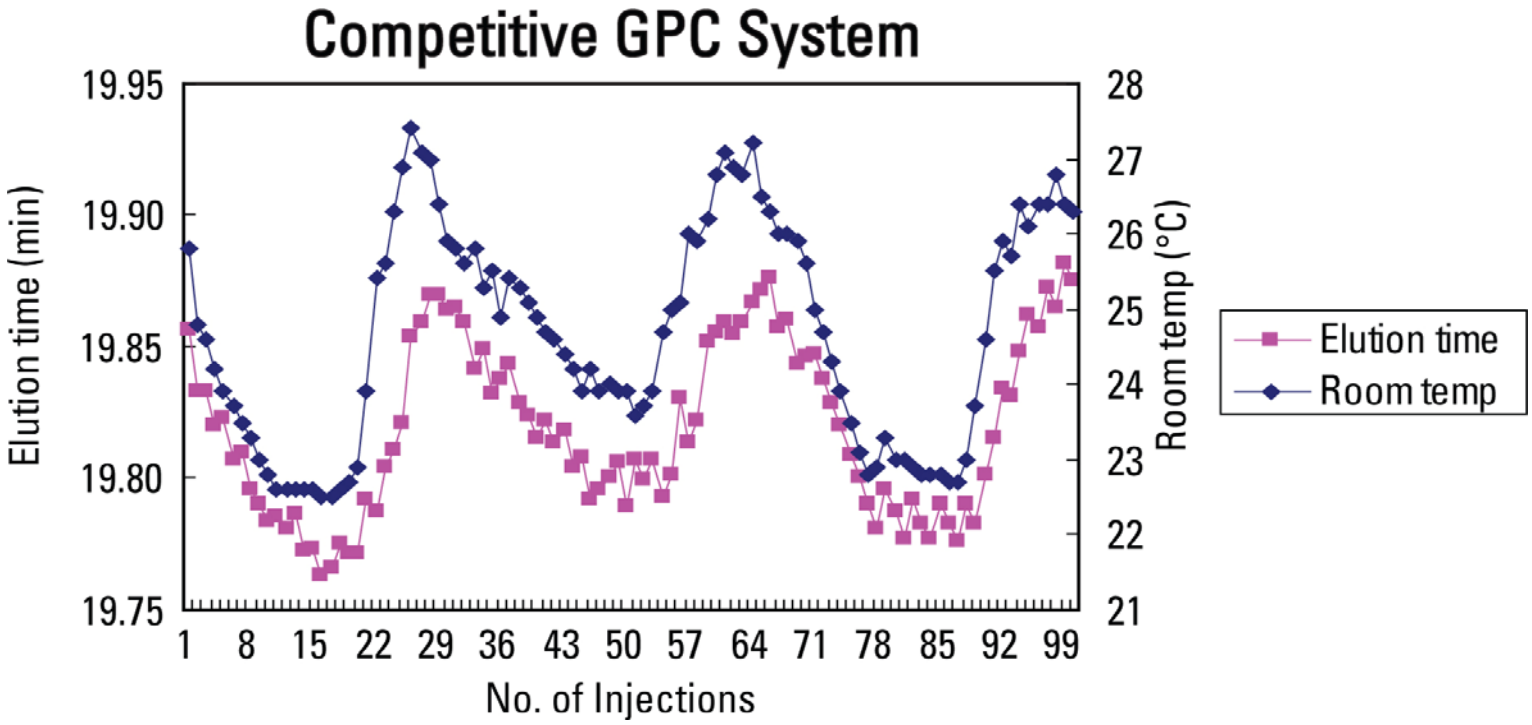
Other GPC manufacturer

TSKgel SuperMultiporeHZ-M, 4.6mm ID x 15cm

Mobile Phase:	THF
Flow rate:	0.35mL/min
Detection:	RI
Temperature:	40°C
Injection vol.:	10µL
Sample:	dicyclohexyl phthalate

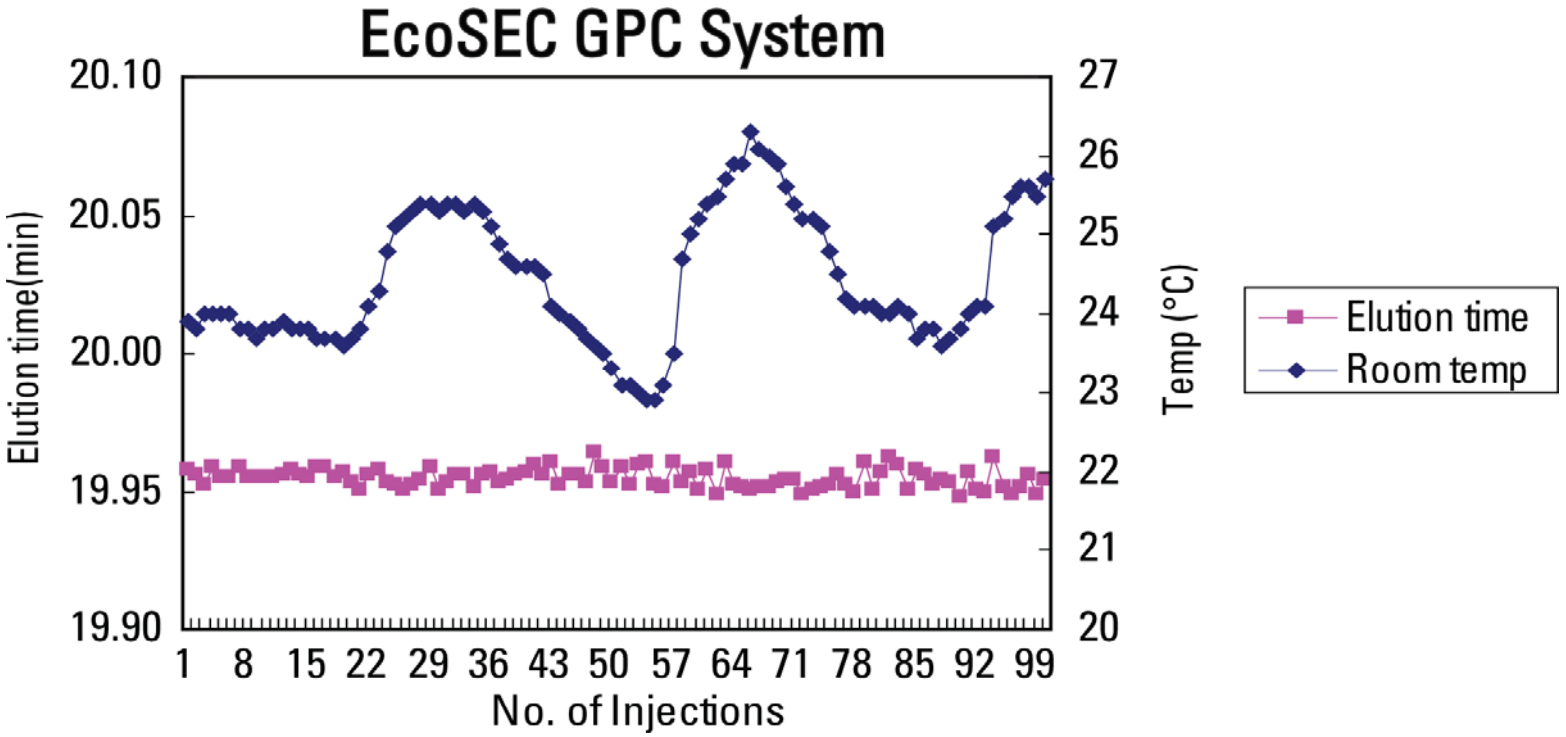
Superior Performance

- Room Temperature Affect On Retention Time
  - Conventional Instrument-No Pump Temperature Control



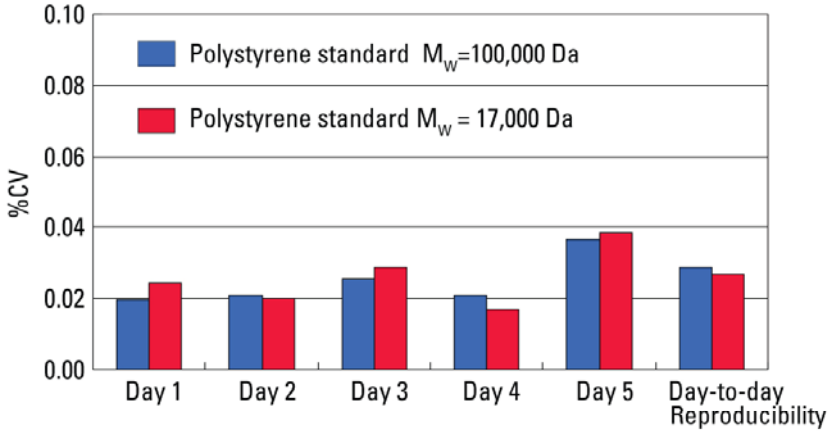
Superior Performance

- Room Temperature Affect On Retention Time
  - EcoSEC GPC System-With Temperature Controlled Pumps



Superior Performance

- Temperature Controlled Pumps
- Excellent Retention Time Precision



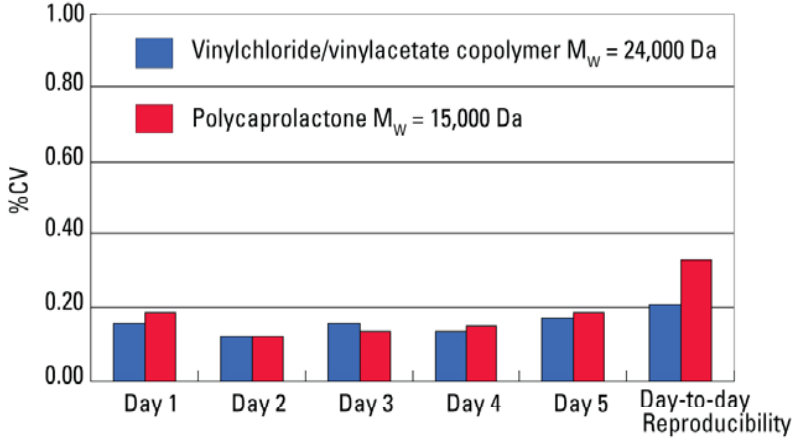
CV value less than 0.04% a day.
CV value less than 0.03% on different days.

TSKgel SuperMultiporeHZ-M, 4.6mm ID x 15cm, × 2

Mobile phase:	THF
Flow rate:	0.35mL/min
Temperature:	40°C
Injection vol.:	10µL
Samples:	polystyrene standards
# of injections:	**10/day**

Superior Performance

- Temperature Controlled Pumps
- Excellent Molecular Weight Precision



CV value less than 0.2% or less a day.
CV value less than 0.4% on different days.

TSKgel SuperMultiporeHZ-M, 4.6mm ID x 15cm, × 2

Mobile phase:	THF
Flow rate:	0.35mL/min
Temperature:	40°C
Injection vol.:	10µL
Samples:	copolymer and polyester standards
# of injections:	**10/day**

Versatility

- Column oven holds up to 8, 30cm columns

Conclusions

- SEC requires no interaction between sample molecules and column packing
- Columns with shallow calibration curves provide best resolution, but less linear range

Conclusions

- Linear range can be extended
  - Individual pore size columns in sequence
  - Mixed bed column
  - Multipore column particles
    - No distortion in chromatogram

Conclusions

- TSKgel PW Columns
  - For water-soluble polymers
- TSKgel Alpha and SuperAW Columns
  - For polar-organic soluble polymers
- TSKgel H-Series Columns
  - For organic-soluble polymers
- TSKgel SuperMultiporeHZ Columns
  - Wide linear range with no chromatogram distortion
  - Semi-micro dimensions
- Tosoh EcoSEC GPC System
  - Optimized for semi-micro columns
  - Ideal GPC system

TOSOH

Available solvent of SEC columns

TSKgel Column Type	*Shipment Solvent*	*Changeable Solvent*
SuperHZ H_{XL}	Tetrahydrofuran	Benzene,Toluene,Xylene,Chloroform,Dichloroethane,Dichloromethane
	Dimethylformamide	None
	Cyclohexane	None
	Acetone	n-Hexane, DMF, NMP, DMAC
SuperH HR	Tetrahydrofuran	Benzene,Toluene,Xylene,Chloroform,Dichloroethane,Dichloromethane
		Dimethylformamide,DMSO,Dioxan,n-Hexane,Cyclohexane,Dodecane,NMP,Acetone
		Quinoline,MEK,ODCB,Trichlorobenzene,HFIP,Pyridine,o-Chlorophenol/Chloroform
		Carbon Tetrachloride,Ethyl Acetate,Methanol/Chloroform,Ethanol,Dimethylacetamide
		1-Chloronaphthalene,Trichloroethane
SuperAW	Water	Methanol,Ethanol,Acetnitorile,Dimethylformamide,DMSO,Tetrahydrofuran,HFIP

Notes: In the case of TSKgel SuperHZ, only one way solvent change