Optimize Your Polymer Analysis by Selecting the Ideal GPC/SEC Column

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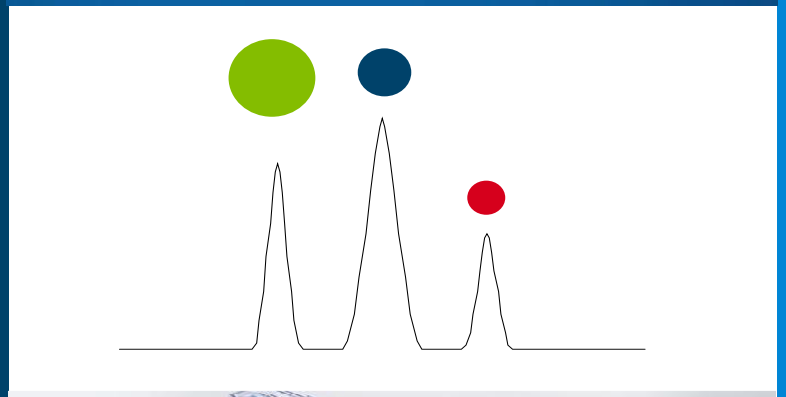
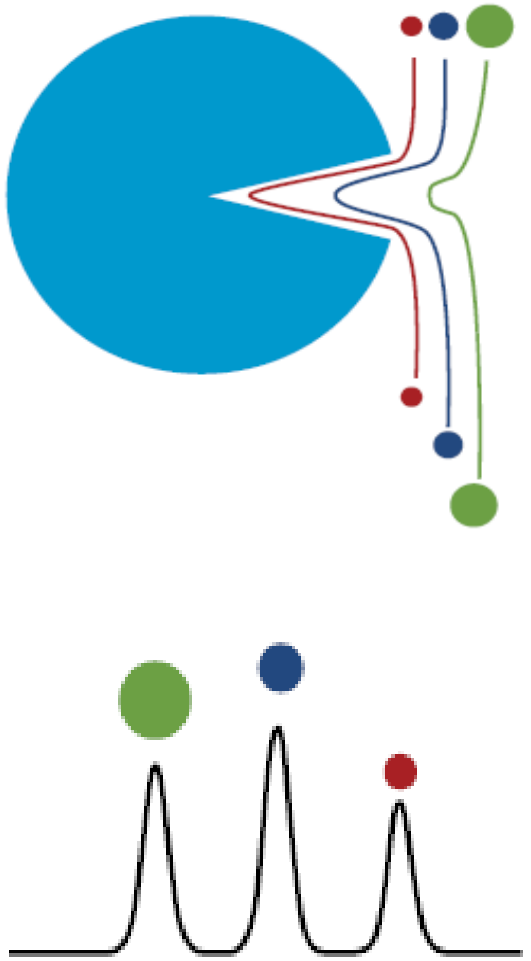


GPC/SEC Separation Mechanism

- A GPC/SEC column is packed with porous beads of controlled porosity and particle size
- Sample is prepared as a dilute solution in the eluent and injected into the system
- Large molecules are not able to permeate all pores and have a shorter residence time in the column
- Small molecules permeate deep into the porous matrix and have a long residence time in the column
- Sample molecules are separated according to molecular size, eluting largest first, smallest last

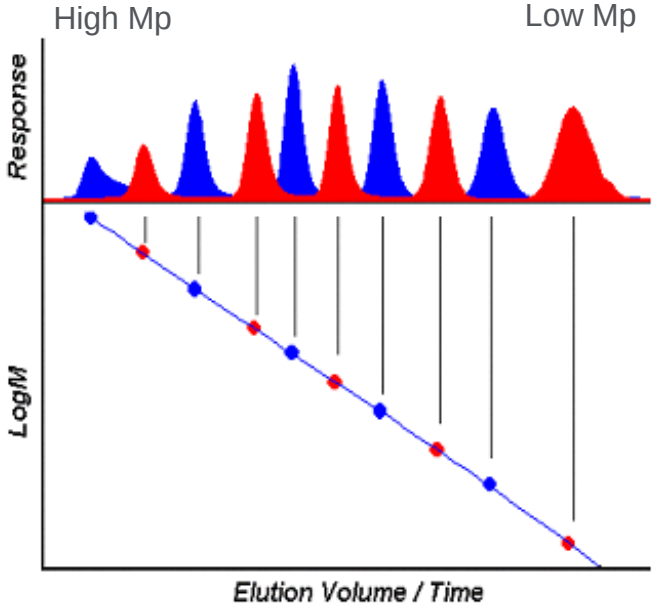


Conventional GPC/SEC Workflow

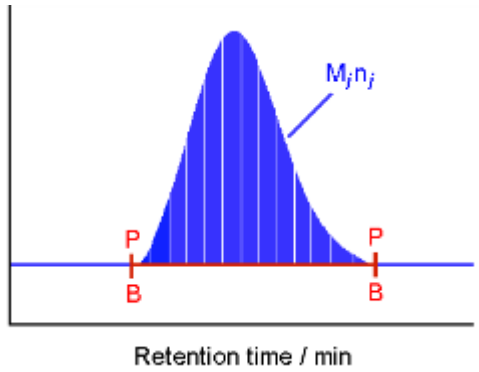
- Calibrate the GPC/SEC column with a set of narrow polymer standards
- Plot retention time (RT) versus peak log molecular weight (logM)
- Calibration is used to generate the molecular weight (averages and distribution) of unknowns on the same system/column set
- Molecular weights are relative to the standards used

If absolute molecular weights are required
Multidetector GPC/SEC

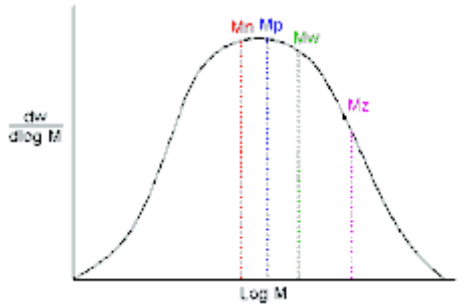
Chromatogram and plot of narrow standards



GPC sample chromatogram



Molecular weight distribution



Considerations for GPC/SEC Column Selection

Key questions to ask

- What polymer are you analysing?
- Which solvent (or solvents) is your polymer soluble in?
- What is the expected molecular weight range of your polymer?
- What is the requirement for your analysis or what would you like to improve about your existing GPC/SEC separation?
  - Resolution is important
  - Reproducibility of sample chromatography and results
  - Speed of analysis or sample throughput is something to improve on

Further considerations

- Know the properties of the sample
- Be familiar with the properties of the columns being considered
- It is important to balance polarities for the sample, solvent, and column packing

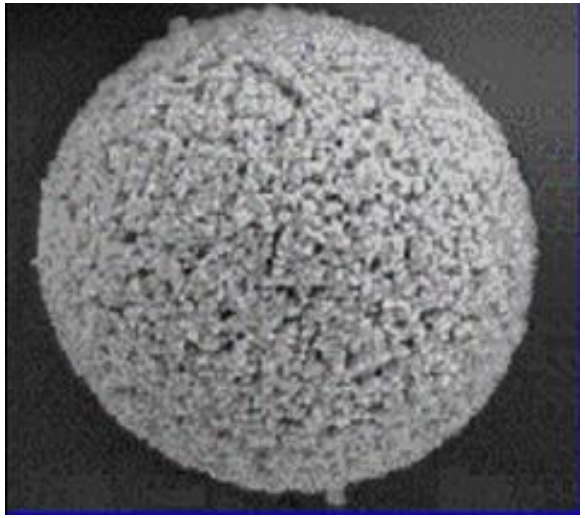
Common Column Chemistries for GPC/SEC

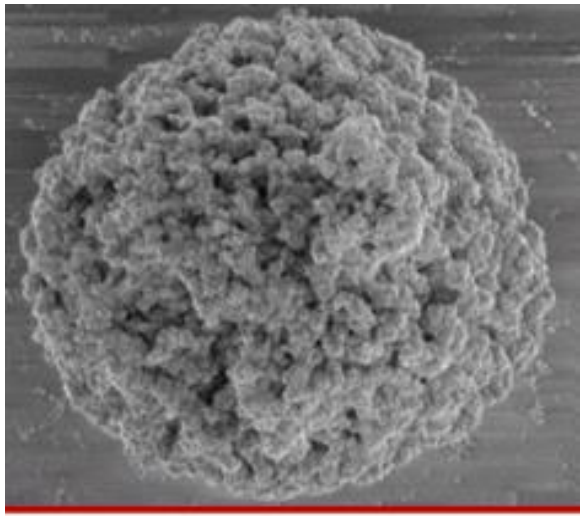
Polymer chemistries

These have a higher pore volume. You might see differences in mechanical stability between vendor packings. Due to the polarity of the stationary phase, observed interactions are reduced.

Common polymer packing types:
Polymethacrylate packings
Polyester copolymers
DVB, divinylbenzene
PS-DVB, polystyrene divinylbenzene

PLgel 10 µm 10E3 A and 10E6 A particles with their rigid pore structure





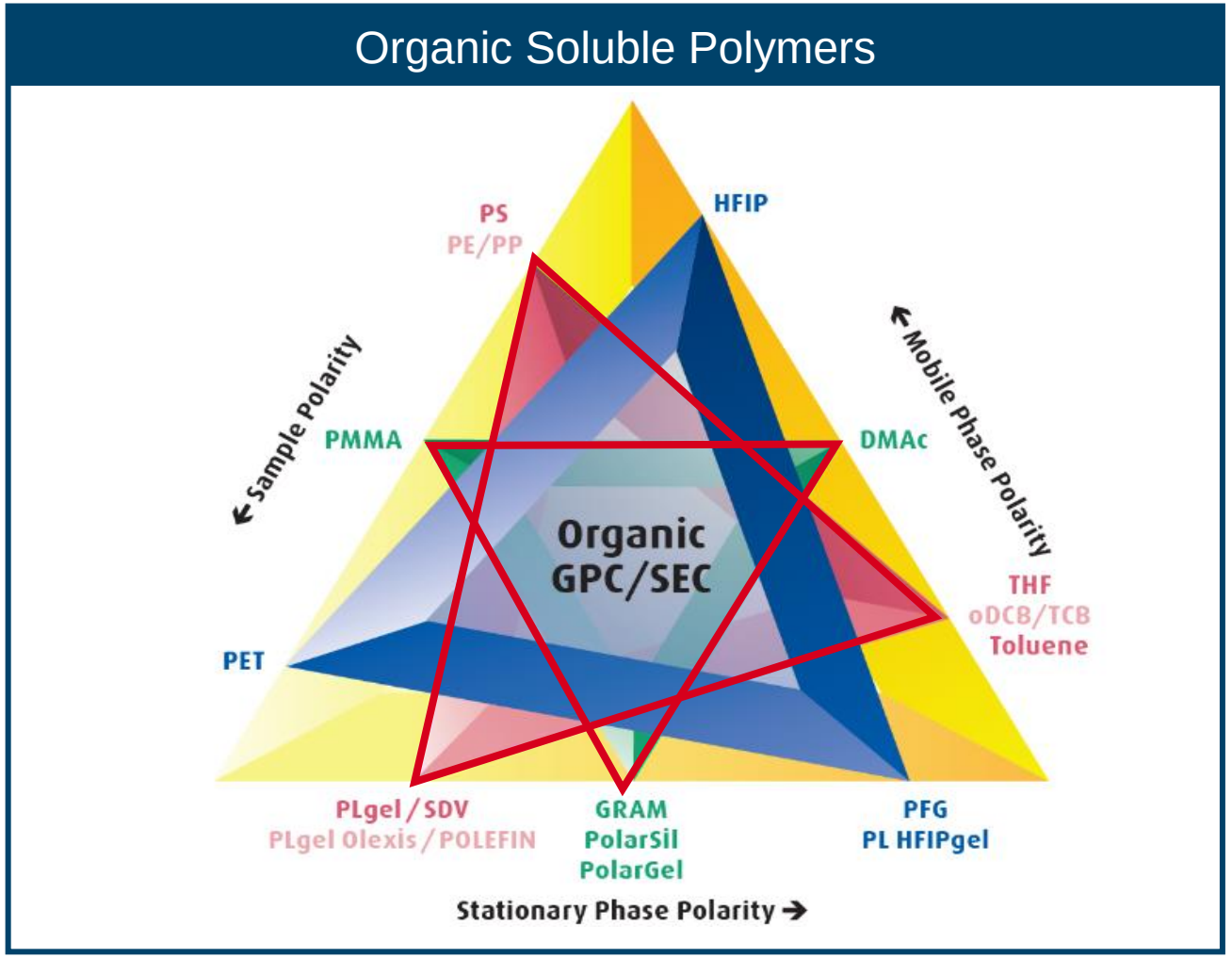
Silica chemistries

Typically have a lower pore volume versus polymeric but are mechanically stronger. They exhibit enthalpic properties due to presence of silanols.

Common silica packing types:
Diol
Surface-modified hydroxyl
Surface-modified polymeric

Agilent Magic Triangles

Selecting right column family for your workflow



Organic-soluble polymers

An equilateral triangle indicates balanced polarities, which is a requirement for obtaining a true separation process, based only on molecular size.

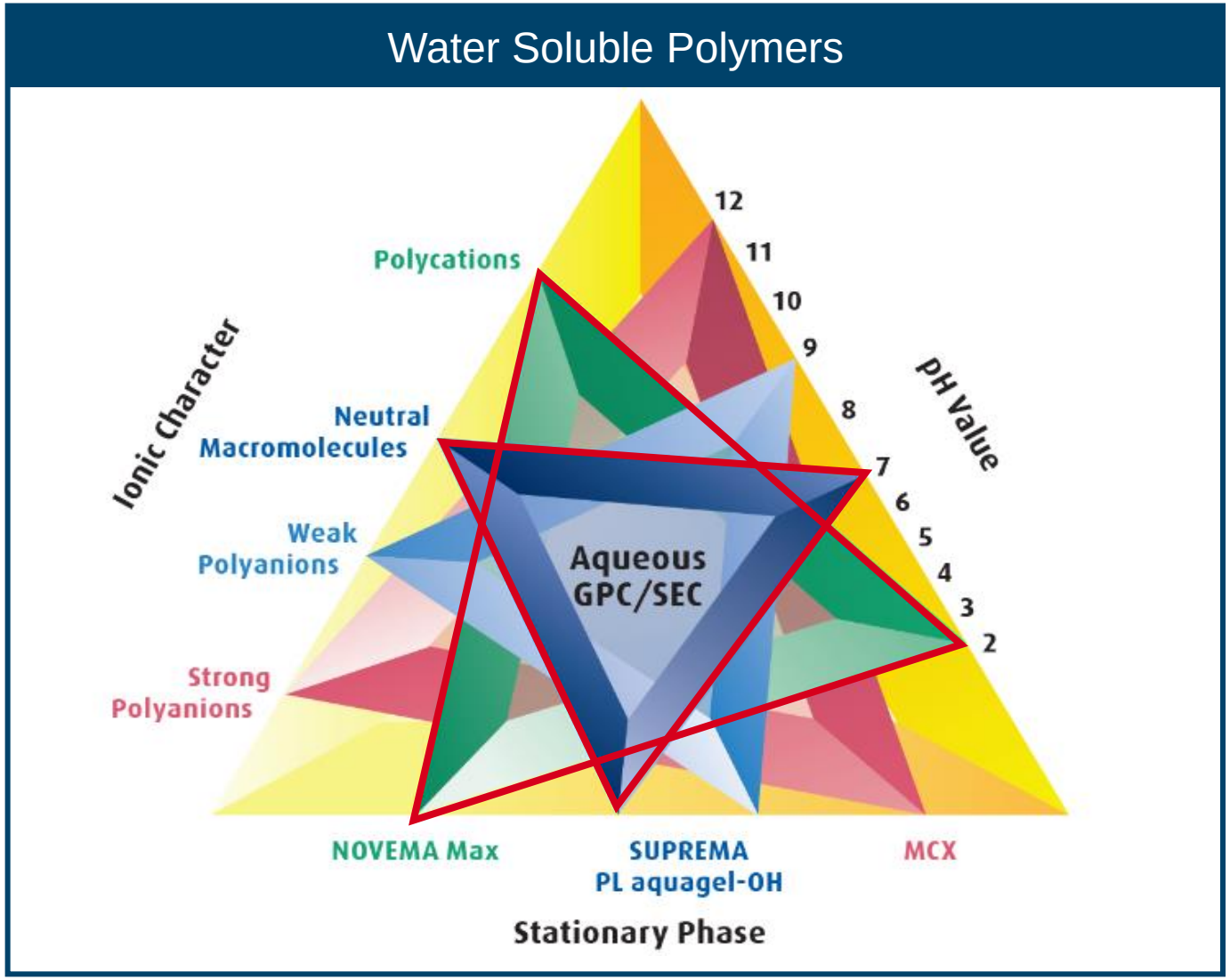
This is critical as we typically need to calibrate retention time/volume versus size/molecular mass to obtain molar mass distribution information.

It's important to consider:

- Sample polarity
- Mobile phase polarity
- Stationary phase polarity

Agilent Magic Triangles

Selecting right column family for your workflow



Water-soluble polymers

The main solvent is water, but your column choice should be made in correlation to the pH ranges at which the columns will be used.

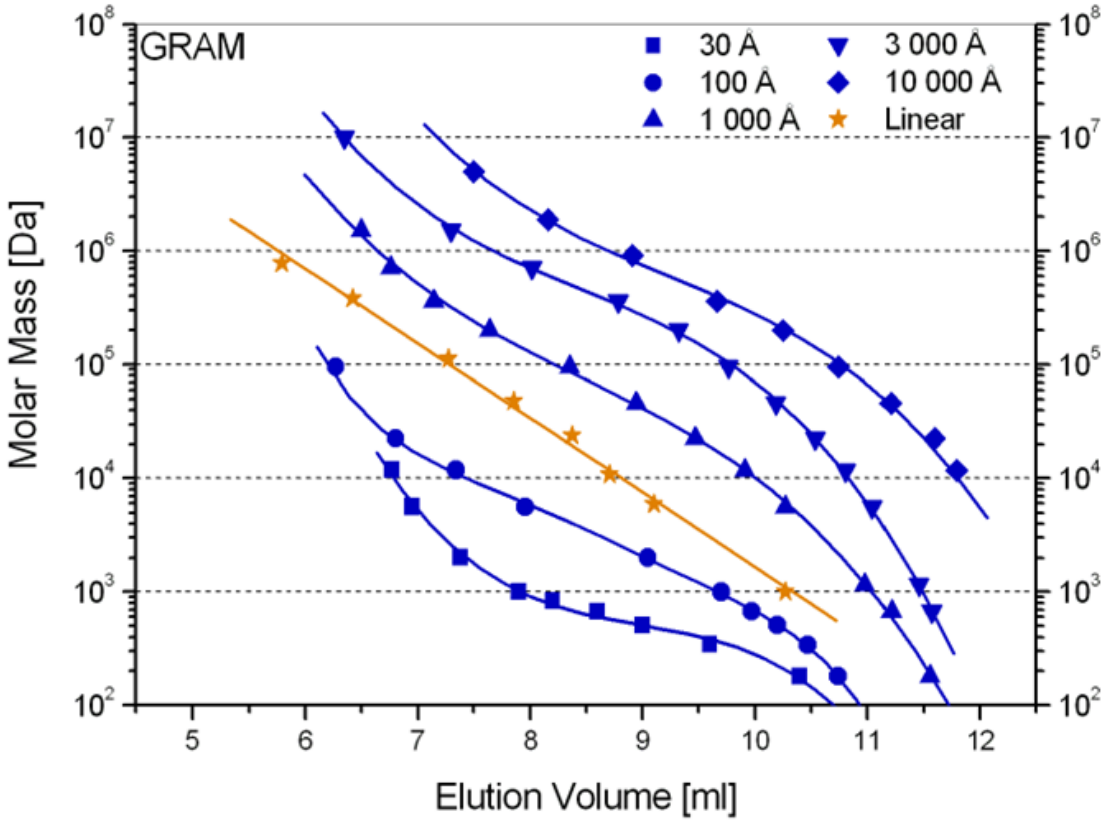
Column Selection: Porosity

Individual pore size columns: Blue calibration curves

- A calibration curve with a shallower gradient indicates a higher resolution within a molecular weight range, for example, compare 100 versus 10,000 Å curves
- MW ranges are more specific
- Combining columns of different pore sizes extends the separation range
- Combining columns of the same pore size of increases resolution

Linear/MIXED: Orange curve

- Later/newer development of columns
- Blend of different pore sizes in one column with fixed analysis range
- Use columns of the same type together
- Combining columns of exactly the same type of linear/MIXED bed columns increases resolution



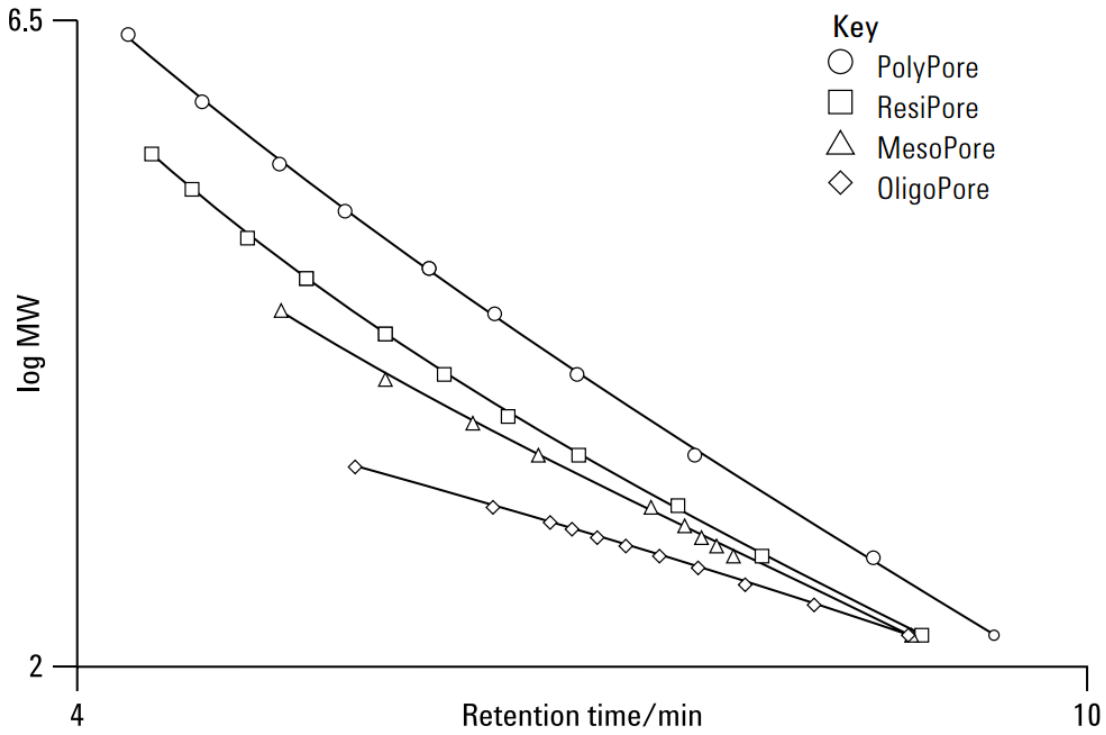
(Retention time at 1 mL/min)

Column Selection Porosity

Multipore particle

- Newest, fastest growing technology
- Each particle has multiple pore sizes
- Increased pore volume
- Highest resolution and efficiency
- Best performance for most common molecular weight ranges

Column family: PlusPore



PlusPore calibration plots

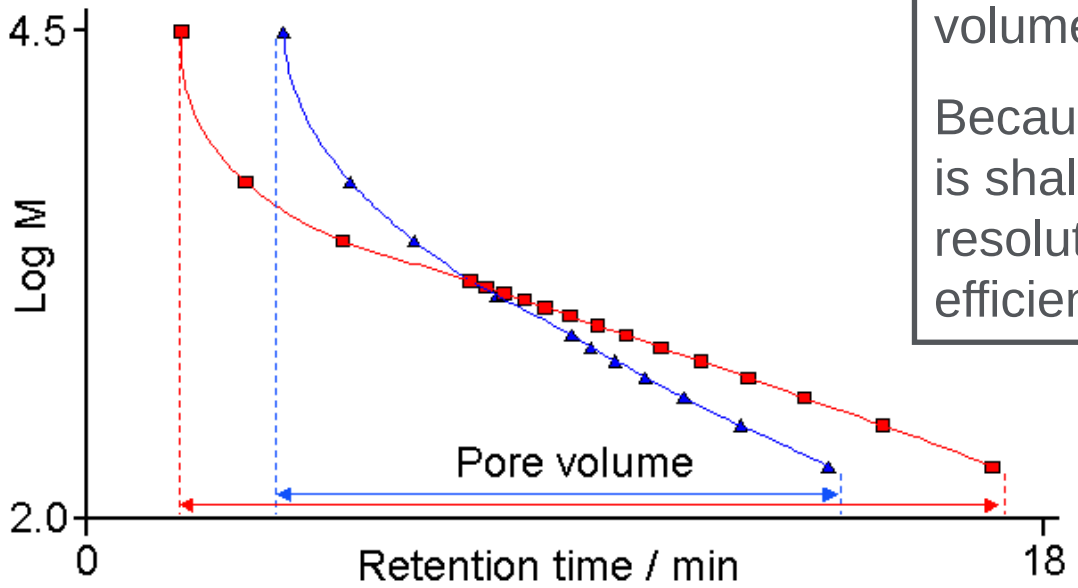
Column Selection: Porosity

Comparison of individual pore sizes and Linear/MIXED/Multipore columns. When is each type used?

Individual Pore Size	Linear/Mixed/Multipore
Narrow pore size range	Blend of different pore sizes in one column
Narrow separation range with shallow slope (higher resolution)	**Large separation range with steeper slope** (less resolution per Mw decade)
Combinations of different pore sizes extend the separation range.	Should only be used with linear/mixed/multipore of exactly the same type. Separation range is fixed
Combinations of the same pore size increase resolution	Combinations of the same linear/mixed/multipore increase resolution
	Particle size is often optimized to analysis range
When to use?	When to use?
When the user requires flexibility to add or remove pore sizes to alter analysis range and time	**The single column can be used for scouting purposes**
Use of individual large particle, large pore sizes can help with problems like “viscous fingering“	Can be used to replace individual pore column set for reducing run time when analysis time and throughtput are important
Typically used in R&D	**Typically used in QC where expected Mw range is well defined**

Individual Pore Versus MultiPorous Particle

Columns	2 x PLgel, 3 µm, 100 Å, 300 x 7.5 mm, p/n PL1110-6320 2 x OligoPore, 300 x 7.5 mm, p/n PL1113-6520
Eluent	THF
Flow rate	1.0 mL/min



▲ - PLgel
■ - Oligopore

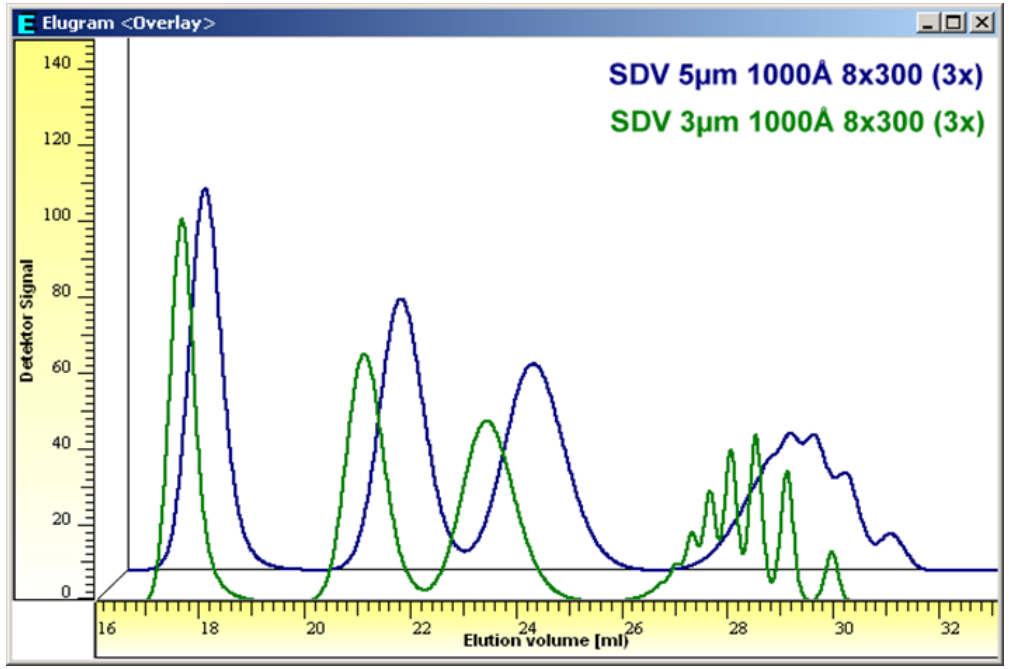
Both columns have a similar exclusion limit, but OligoPore has greater pore volume than PLgel 100 Å.

Because of this, the slope of the curve is shallower leading to greater resolution for columns of the similar efficiency.

Column Selection
Particle size

The range of particle sizes in GPC/SEC columns varies between 3 µm and 20 µm.
Generally, smaller particles mean improved resolution for the same column length or faster separation with the same resolution.

The example shows the separation of polystyrene oligomers, having increased resolution when using smaller particle sizes (green curve versus blue chromatogram).



For GPC/SEC separations of low molecular weight compounds, such as mono-, di- and triglycerides, and linear hydrocarbons, we recommend a small porosity, small particle column. An example would be PLgel 3 µm 100 Å or the Oligopore column.

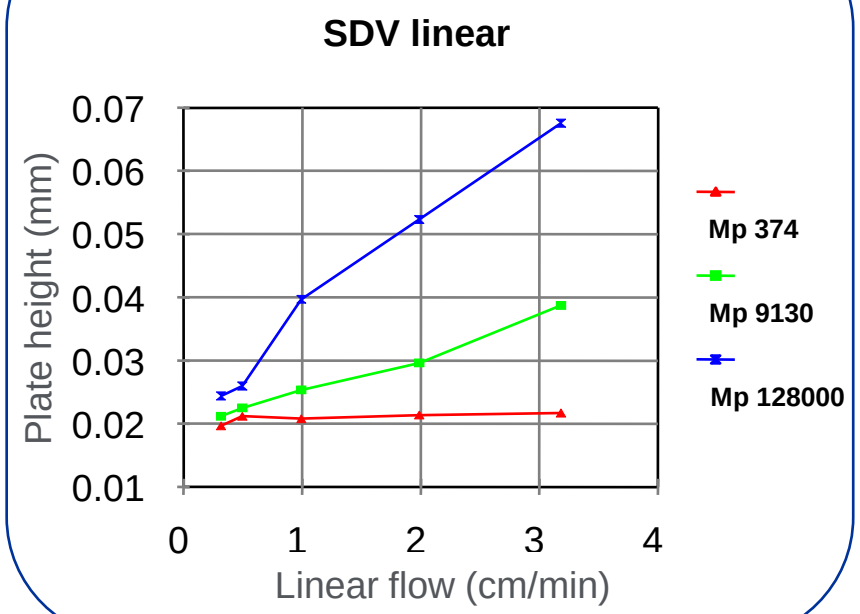
Column Selection – Particle Size

What are the limits when using smaller particle sizes ?

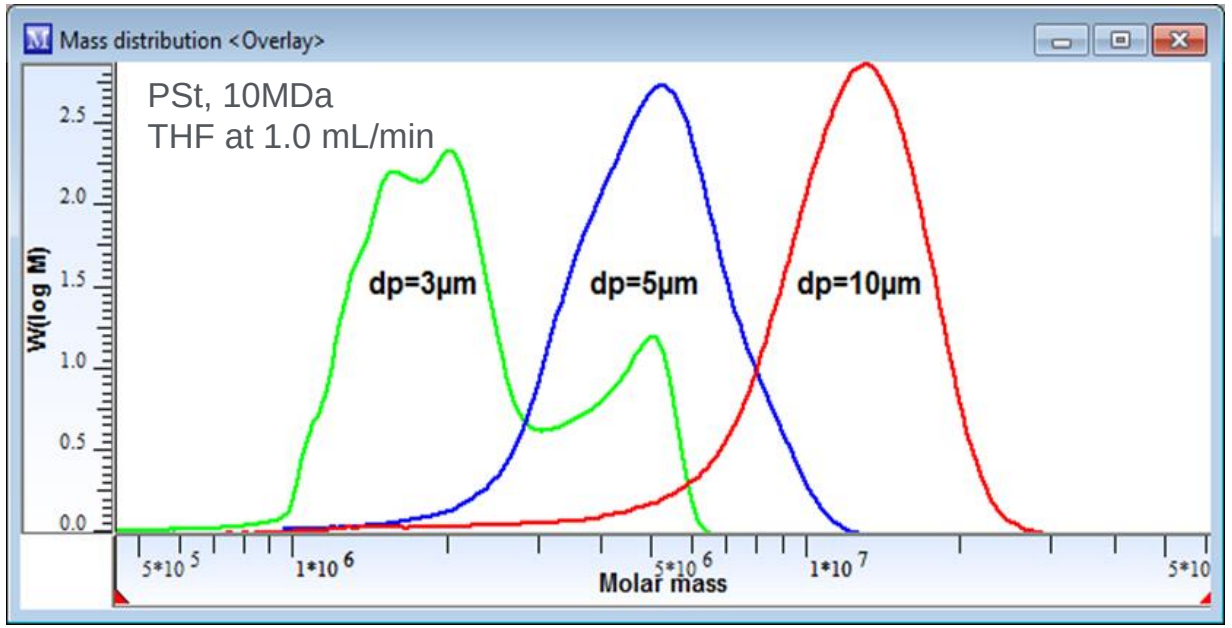
When separating larger molecules, such as polyolefins, it is important to choose a large particle size to avoid deformation or degradation of the molecule. The analysis flow rate also plays an important role and for very large molecules it is often necessary to use lower flow rates.

Large particles are also recommended for use with viscous solvents to decrease operating backpressure.
It is not recommended to mix different particle sizes in a column set.

Different molar masses



Effect of using small particle sizes on PS 10 MDa



Column Selection – Particle Size

How many GPC/SEC columns to use

More than one column is typically used
More columns = improved resolution

- The greater the particle size of the media in the column (which is dependent on the expected molecular weight of the samples), the lower the resolution. More columns will be required to maintain the quality of the results.
- For higher molecular weight samples, larger particles are necessary to reduce the danger of shear degradation of samples.

Particle Size	Number of Columns
20 µm	4
13 µm	3
10 µm	3
8 µm	3
5 µm	2
3 µm	2

Columns for Low Polarity Organic Eluents

Packing material polarity/characteristics are well matched for the analysis of organic soluble macromolecules in low-polarity eluents.

Example: THF, toluene, and chloroform, as well as sustainable eluents, ethyl acetate, 2-methyl-THF in nonlife science applications.

Suitable for the following sample types: synthetic polymers, polystyrene, polybutadiene, PMMA, PIB, silicones, epoxy resins, polyols, and more.

Smaller id columns are available if sample is limited. Use of smaller ID columns can help reduce solvent consumption and disposal costs.

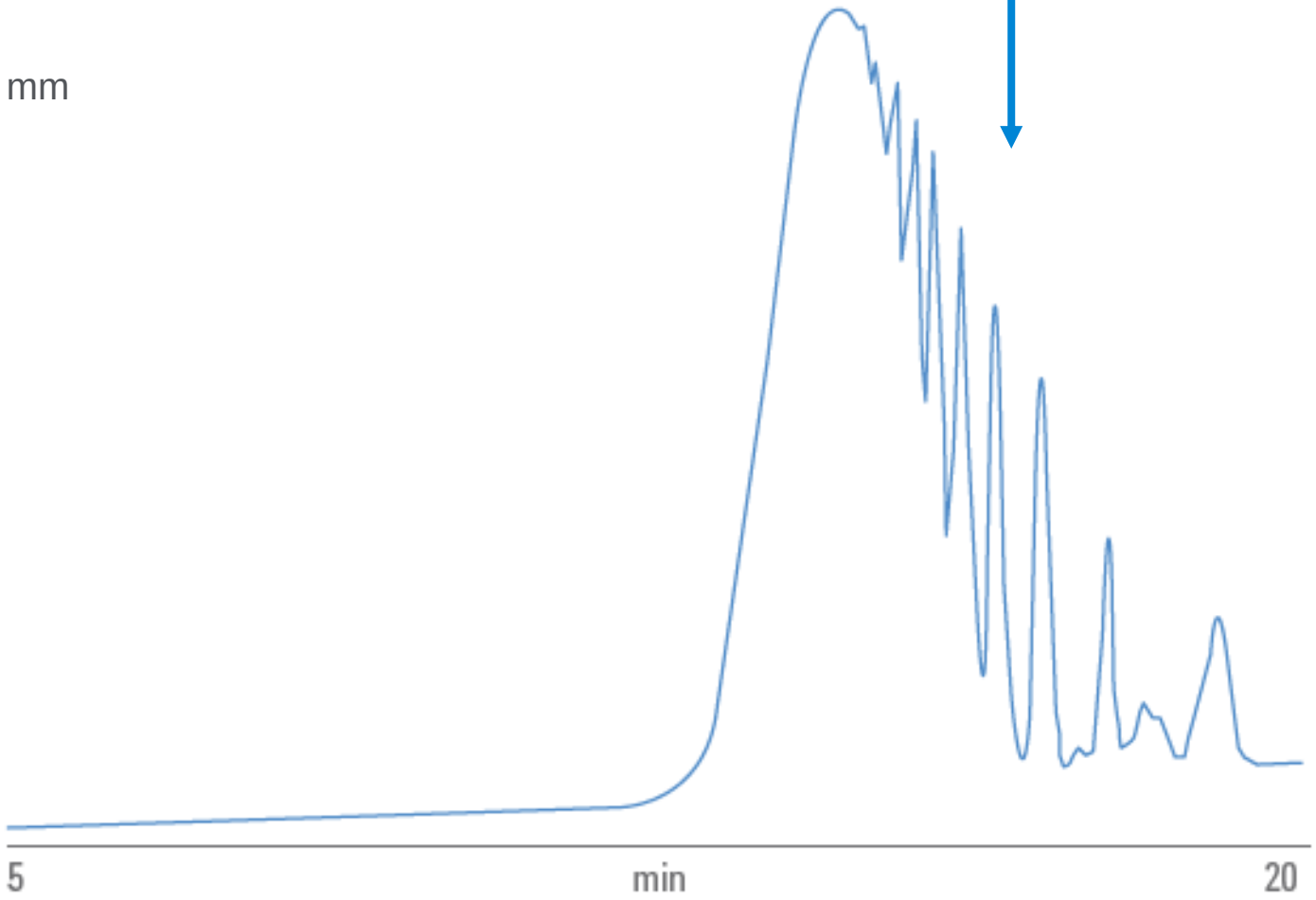
Column	Porosities	Particle Size	Column ID (mm)	Comments
PLgel*	Five MIXED gels: A to E Range: 100 to 40,000,000 Da Seven individual pore sizes: 50 to 10e6 Å Range: 100 to 10,000,000 Da	3 to 20 µm 5 and 10 µm	Analytical (7.5) Preparative (25) Micro (4.6)	Premium product line combining robustness with high resolution. Particle and pore sizes of MIXED gels are optimized according to molecular mass range to avoid shear degradation. Easy transfer between solvents giving greater flexibility of use. Preparative columns include EnviroPrep for EPA clean up methods
PlusPore	Four multipore packings: PolyPore, ResiPore, MesoPore, and OligoPore Range: 100 to 2,000,000 Da	3 to 6 µm	Analytical (7.5) Preparative (25) Micro (4.6) Minimicro (2.1)	Provide ultrahigh resolution OligoPore preparative column Minimicro columns suitable for MS
SDV*	Four MIXED gels: LIS, LIM, LXL, LUH Range: 100 to 30,000,000 Da Seven individual pore sizes: 100 to 10e7 Å Range: 100-30,000,000 Da	3 to 20 µm 3, 5, and 10 µm	Analytical (7.5) Preparative (25) Micro (4.6)	*Select columns are available for use with laser light scattering detectors to minimize start time

GPC Application

Polyester and oligomers

- 3 µm particle
- High efficiency
- Oligomeric detail

- Column: 2 x ResiPore 7.5 x 300 mm
  p/n PL1113-6300
- Eluent: THF
- Temperature: Ambient
- Flow rate: 1.0 mL/min
- Injection volume: 20 µL
- Detector: UV 254 nm



Columns for Medium Polarity Organic Eluents

Packing material polarity/characteristics are well matched for the analysis of organic soluble macromolecules in medium polarity eluents.

Example: DMF, DMAc, NMP and DMSO with salt modifiers, for example, LiBr if required, in nonlife science applications.

Suitable for the following sample types: synthetic polymers, polyurethanes, polyimides, some polyamides, cellulose, starches, amylose/amylopectin, and others.

Smaller id columns are available if sample is limited. Use of smaller ID columns can help reduce solvent consumption and disposal costs.

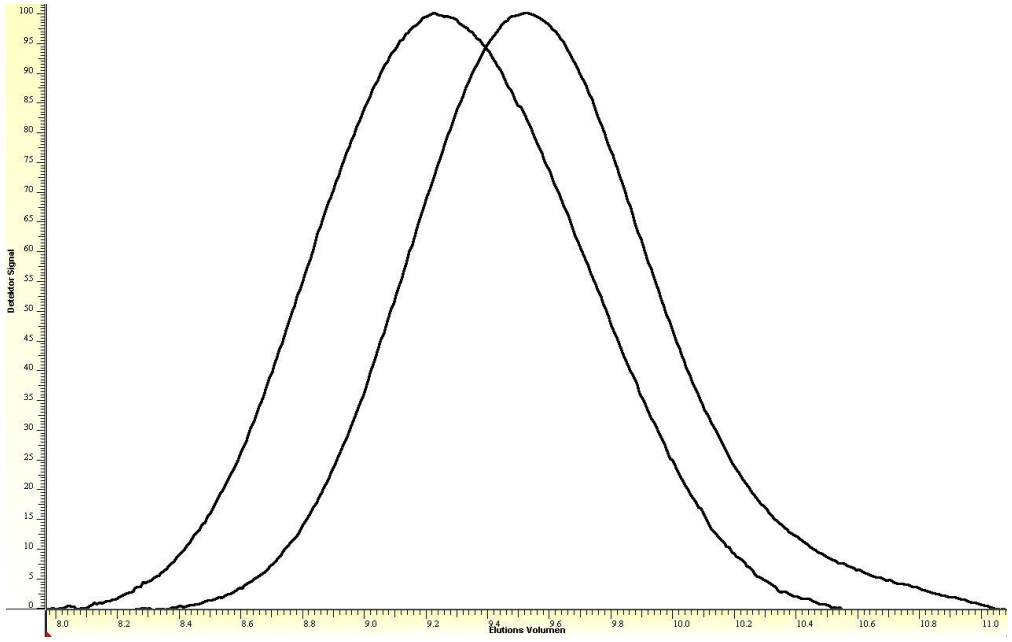
*Select columns are available for use with laser light scattering detectors to minimize start time

Column	Porosities	Particle Size	Column ID (mm)	Comments
GRAM*	One MIXED gel: LIN Range 500 to 1,000,000 Da Five individual pore sizes: 30 to 10,000 Å Range: 100 to 50,000,000 Da	10 µm 10 µm	Analytical (8.0) Preparative (20)	Polyester copolymer packing. Suitable for the analysis of very high molecular mass molecules, for example, starches. Often used with salt modifiers, such as LiBr, and can resolve the salt peak from oligomeric material. Offered in larger particle sizes for viscous eluents to reduce back pressure and extend column lifetime.
PolarGel	Two MIXED gels: M, L L range: 100 to 60,000 Da M range: 1000 to 500,000 Da	8 µm	Analytical (7.5) Preparative (25)	Suitable for the analysis of medium molecular mass molecules, lignins, and resins.
PolarSil	Two MIXED gels: LIS, LIM LIS range: 100 to 300,000 Da LIM range: 100 to 1,000,000 Da Three individual pore sizes: 100 to 1,000 Å, Range: 100 to 1,000,000 Da	5 µm 3 µm, 5 µm	Analytical (8.0) Micro (4.6)	Polar modified silica packing. Small pore, small particles are ideal for the GPC/SEC analysis of low molecular mass resins, providing high resolution oligomer separations.

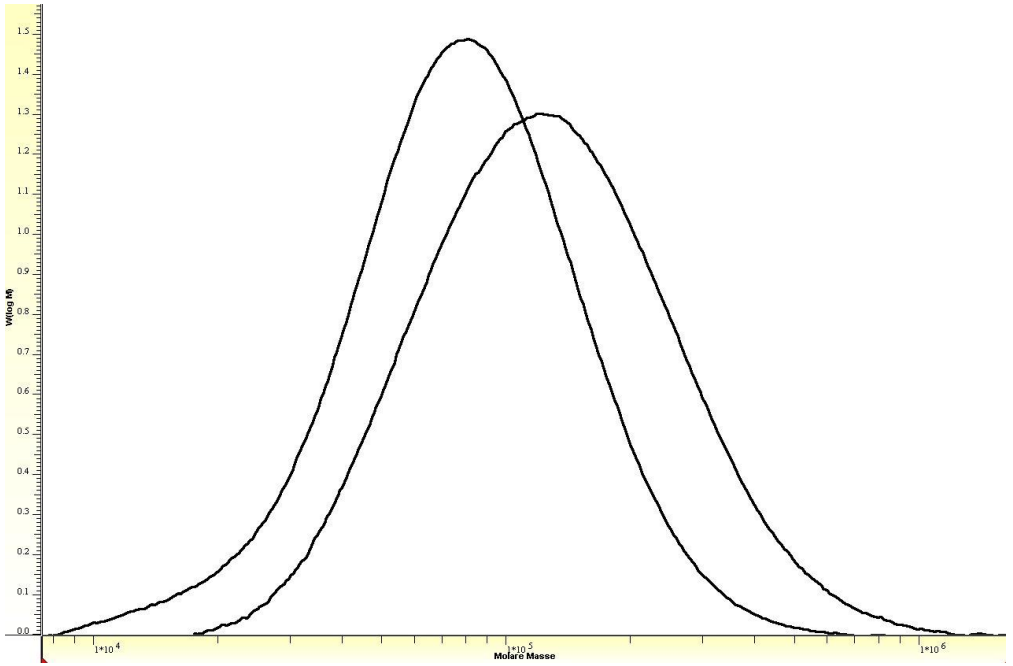
GPC/SEC Application
Hydroxypropyl cellulose

- Column: 1 x GRAM 10 µm linear, 8 x 300 mm, p/n AMA083010LIN
  GRAM 10 µm GUARD, 8 x 50 mm, p/n AMA080510
- Eluent: Dimethyl sulfoxide, LiBr 5 g/L
- Temperature: 60 °C
- Flow rate: 1.0 mL/min
- Detector: RI

Using a conventional poly(methylmethacrylate) calibration enables the calculation of the molecular weight distribution (MWD) as well as average molar mass values. Values calculated will be relative to the poly(methylmethacrylate) standards used.



Overlay of the elugrams of two HPC samples



Overlay of the molecular weight distribution of two HPC samples

Columns for High Temperature GPC/SEC

Packing material polarity/characteristics are well matched for the analysis of organic soluble macromolecules in eluents such as:

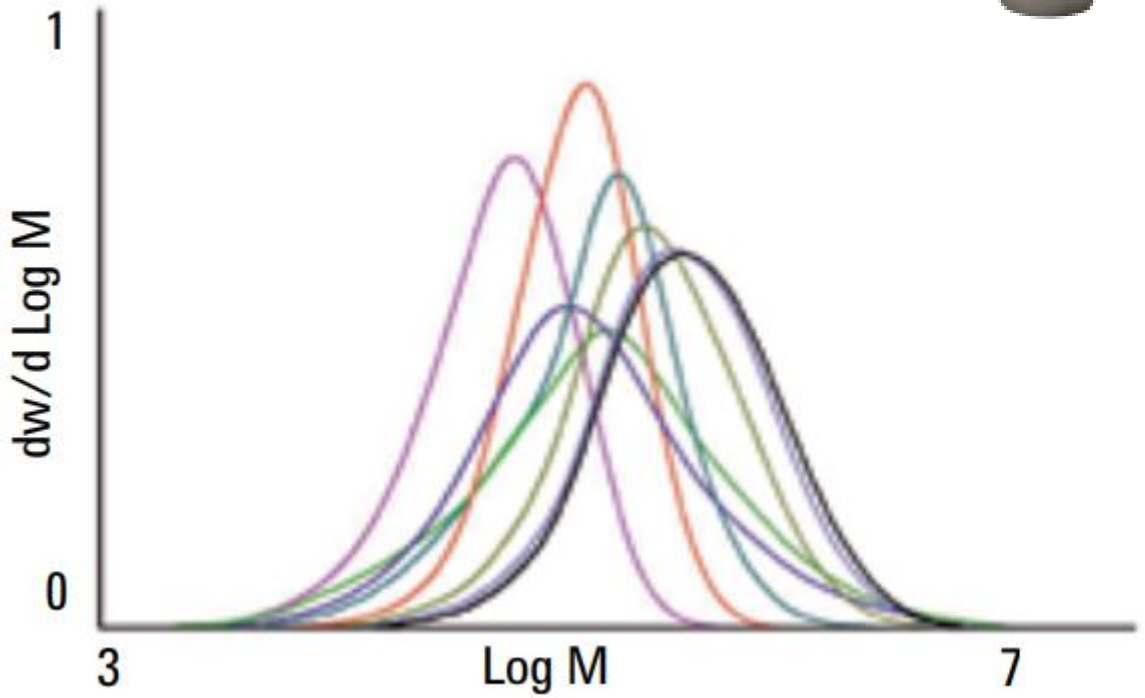
TCB, oDCB, o-Chloronaphthalene and butylal

Suitable for the following sample types: polyolefins, polyethylene, polypropylene, and high-performance polymers.

Column	Porosities	Particle Size	Column ID (mm)	Comments
PLgel Olexis	One MIXED gel Range: 2,000 to 10,000,000 Da	13 µm	Analytical (7.5)	Our premium product line. Optimized design for the analysis of polyolefins and performance polymers Particles of 13 µm provide stability and resolution, with no shear degradation Extended lifetime at very high temperatures
PLgel*	Two MIXED gels: A, B Range: 500 to 40,000,000 Da Range: 500 to 10,000,000 Da	 20 µm 10 µm	Analytical (7.5) .	MIXED A columns (20 µm) are suitable for ultrahigh molecular weight polymers *Select columns are available for use with laser light scattering detectors to minimize start time
POLEFIN	Two MIXED gels: LIM, LXL Range: 200 to 1,000,000 Da Range: 2,000 to 30,000,000 Da	 10 µm 10 and 20 µm	Analytical (8.00)	

GPC Application
Polyolefins

- Column: 3 x Olexis 7.5 x 300 mm
  p/n PL1110-6400
- Eluent: TCB + 0.15% BHT
- Temperature: 160 °C
- Flow rate: 1.0 mL/min
- Injection volume: 200 µL
- Instrument: Agilent 1260 Infinity II HT GPC system



The differential log plot shows a range of polyolefin samples analyzed on a PLgel Olexis column. They display a fully covered wide range of sample molecular weights.

Publication number: 5991-2517EN

Columns for Fluorinated Organic Eluents

Packing material polarity/characteristics are well matched for the analysis of organic soluble macromolecules, typically with a high degree of crystallinity, in fluorinated eluents, for example, HFIP, TFE in nonlife science applications.

Suitable for the following sample types: synthetic polymers, recyclates, polyesters, PET, polyamides, and polylactides.

Smaller id columns are available if sample is limited. Use of smaller ID columns can help reduce solvent consumption and disposal costs.

Column	Porosities	Particle Size	Column ID (mm)	Comments
PFG*	Three MIXED gels: LIS, LIM, LXL Range: 500 to 3,000,000 Da Four individual pore sizes: 100 to 4,000 Å Range: 100 to 3,000,000 Da	5 and 7 µm 5 and 7 µm	Analytical (8.0) Preparative (25) Micro (4.6)	Combines robustness with high resolution Small pore, small particle 5 µm for high-resolution separation of oligomers
PL HFIPgel	One multipore Range: 200 to 2,000,000 Da	9 µm	Analytical (7.5mm) Micro (4.6mm)	Optimized separation range delivers high performance with no artifacts Avoid warped calibration curves, dislocations, shoulders, and poor resolution caused by HFIP and similar solvents

*Select columns are available for use with laser light scattering detectors to minimize start time

GPC Analysis of PET (Polyethylene Terephthalate)

A series of different PET materials were analyzed. Typical analysis conditions and molecular weights are given below.

The system is calibrated with PMMA standards since these give expected SEC behavior in HFIP. Agilent conventional modules can be used to run HFIP or, alternatively, for more flexibility and safety, the high temperature GPC system, Agilent 1260 Infinity II HT GPC system is recommended.

Columns : PL-HFIPgel 300 x 7.5mm p/n PL1114-6900HFIP

Eluent : HFIP + 0.02 M NaTFAc

Temp : 40 °C

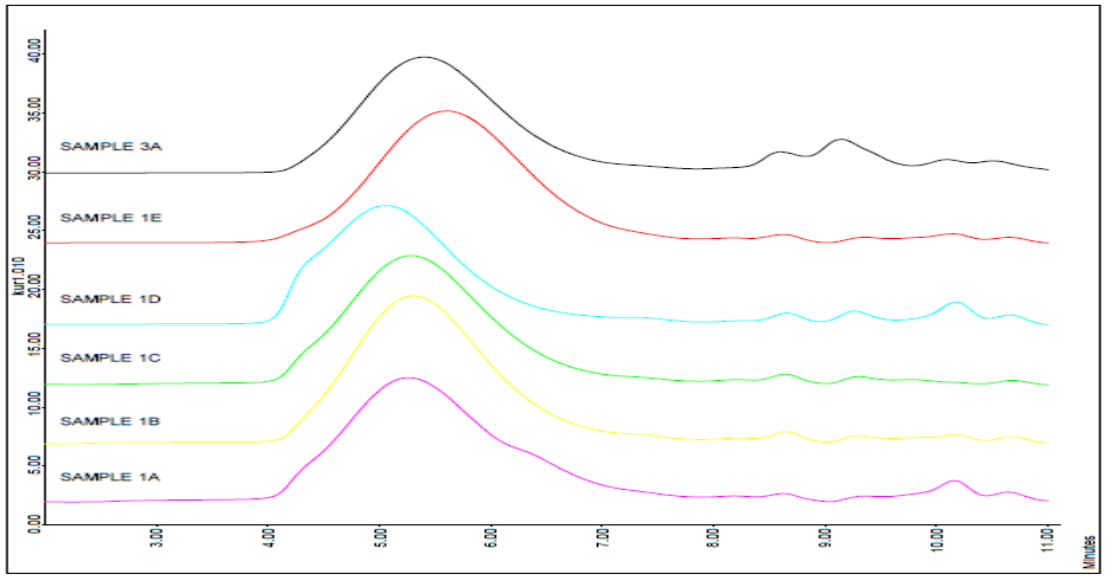
Flow rate: 1 mL/min

Detector : RI

Sample concentration: 2 mg/mL approx.

Calibrants : Polymethylmethacrylate

Mp = 51500
Mw= 64149
Mn= 227063
Mz= 119914
PD= 2.8



Columns for Aqueous Eluents: Neutral or weak Anions

Polysaccharides

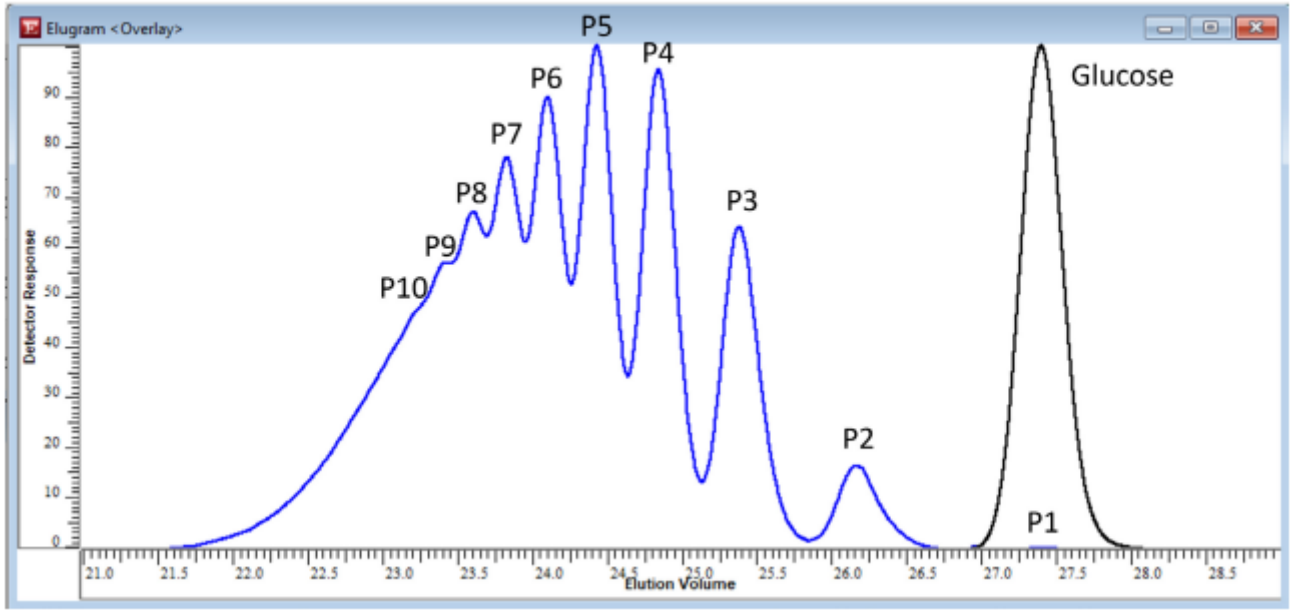
Packing material polarity/characteristics are well matched for the analysis of water-soluble neutral or anionic macromolecules in nonlife science applications. Suitable for the following sample types: synthetic polymers, cosmetics, food ingredients, APIs, polyacrylamide, hyaluronic acid, polysaccharides, dextrans, modified cellulose, and PEGs.

*Selected columns available for use with laser light scattering detectors to minimize start up time

Column	Porosities	Particle Size	Column ID mm	Comments
SUPREMA*	Four MIXED gels: LIS, LIM, LXL, LUH Range: 100 to 30,000,000 Da Six individual pore sizes 30 to 30,000 Å Range: 100 to 30,000,000 Da	5 to 10 µ 3, 5, and 10 µm	Analytical (8.0) Preparative (20) Micro (4.6)	Applicable to L25, L37, L38, and L39 USP monographs pH range stability 2-12 Compatible with organic modifiers (up to 100%) Robustness of packing material enables wider choice of analytical conditions 3 µm particle size delivers the highest resolution separations on typical GPC/SEC equipment (ultrahigh pressure not required)
PL aquagel-OH	Two MIXED gels: MIXED-H and MIXED-M Range: 200 to 10,000,000 Da Five individual pore sizes: 30 to 30,000 Å Range: 100 to 10,000,000 Da	8 µm 5, 8, and 15 µm	Analytical (7.5) Preparative (25) Micro (4.6)	The inert surface can also be used for cations pH range stability 2-10 Compatible with eluents with up to 50% methanol 15 µm particles are available for large molecule analysis

SEC Application

Low MW dextran



- Column: 3x SUPREMA 5 µm 100 Å, 8.0 x 300 mm
  p/n SUA0830051e2
  SUPREMA GUARD p/n SUA080505
- Eluent: Water w/0.05% sodium azide
- Temperature: 80 °C
- Flow rate: 0.25 mL/min
- Injection volume: 20 µL
- Detector: Refractive index (RI)

Using a reduced particle size (5 µm) and combining columns of same 100 Å pore size in a series enables high-resolution oligomeric separation.

[Saccharide and Polysaccharide Analysis
Publication number: 5994-5702EN]

Columns for Aqueous Eluents: Polycations and Strong Polyanions

Packing material: NH-functionalized acrylate copolymer. For the analysis of water-soluble cationic macromolecules in nonlife science applications.

Suitable for samples such as Poly(ethylene) imine, poly(DADMAC), chitin, chitosan, polymer quaternary ammonium compounds.

*Selected columns are available for use with laser light scattering detectors to minimize start time

Column	Porosities	Particle Size	Column ID mm	Comments
NOVEMA Max *	Three MIXED gels: LIS, LIM, LUH Range: 100 to 30,000,000 Da Four individual pore sizes 30 to 3,000 Å Range: 100 to 3,000,000 Da	5 to 10 µm 5 to 10 µm	Analytical (8.0) Preparative (20)	Recommended operating pH range of 1.5 to 7.0 due to the cationic nature of the surface. Compatibility with organic modifiers (up to 100%). Availability of large porosities. Delivers the highest resolution separations on typical GPC/SEC equipment (ultrahigh pressure not required).

Packing material: Sulfonated styrene-divinylbenzene copolymer. For the analysis of water-soluble strong polyanions (for example, sulfonates) in nonlife science applications.

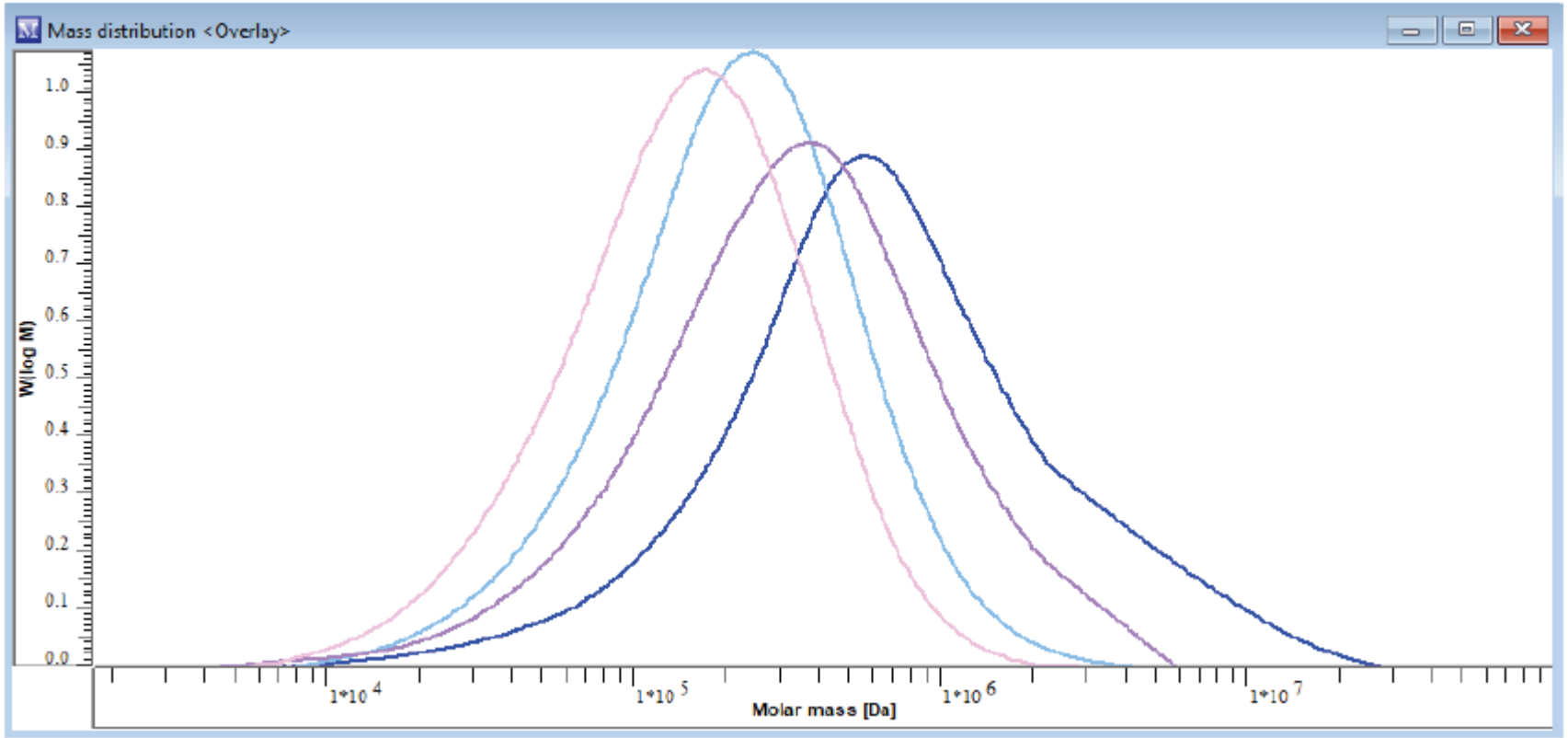
Lignins

Suitable for samples such as those used in food ingredients, poly(styrene) sulfonate, lignin sulfonate, modified starches, acids, alcohols, and pectins.

Column	Porosities	Particle Size	Column ID mm	Comments
MCX	Five individual pore sizes 100 to 10e7 Å Range: 100 to 5,000,000 Da	5 to 10 µm	Analytical (8.0) Preparative (20)	pH stability 1 to 13. Operating pH range of 7.0 to 13.0 due to the anionic nature of the surface. Widest range of compatibility with organic modifiers (up to 100%). Availability of large porosities. Deliver the highest resolution separations on typical GPC/SEC equipment (ultra high pressure not required).

GPC/SEC Application
Chitosan

- Column: 3x NOVEMA Max 10 µm ultrahigh, 8 x 300 mm, p/n NMA083010LUH
  NOVEMA Max 10 µm GUARD, 8 x 50 mm, p/n NMA080510
- Eluent: Water, 0.1 M NaCl, 0.3% volume trifluoracetic acid
- Flow rate: 1.0 mL/min
- Detector: RI



Overlay of the molecular weight distribution of four chitosan samples.

Using a conventional Pullulan calibration would enable calculation of molecular weight distribution (MWD), as well as average molar mass values. Values calculated will be relative to the Pullulan standards used

Column Selection: Additional Considerations

Guard columns/precolumns

- Help to protect the main column set
  - Filter particulates
  - Prevent unwanted chemical contamination
  - Protect against pressure spikes

Background noise affects mainly sensitive laser light scattering detectors

- Users can save themselves time by choosing columns that have been specially prepared for use with LS detectors.

- These columns can be identified by their part number. They have LS at the end and are sometimes referred to as “Lux” columns, for example:
  - PLgel MIXED-A **LS**, 7.5 x 300 mm, 20 µm, p/n PL1110-6200**LS**
  - SUPREMA **Lux**, linear ultrahigh, 8 x 300 mm, 10 µm , p/n SUA083010LUH**LS**

GPC Column Selection

Putting columns together in a series

Agilent InfinityLab

Running two columns in a series using different pore sizes

- This extends the resolving range and enables analysis of multiple attributes in one run

Running two columns in a series using the same pore size/same type

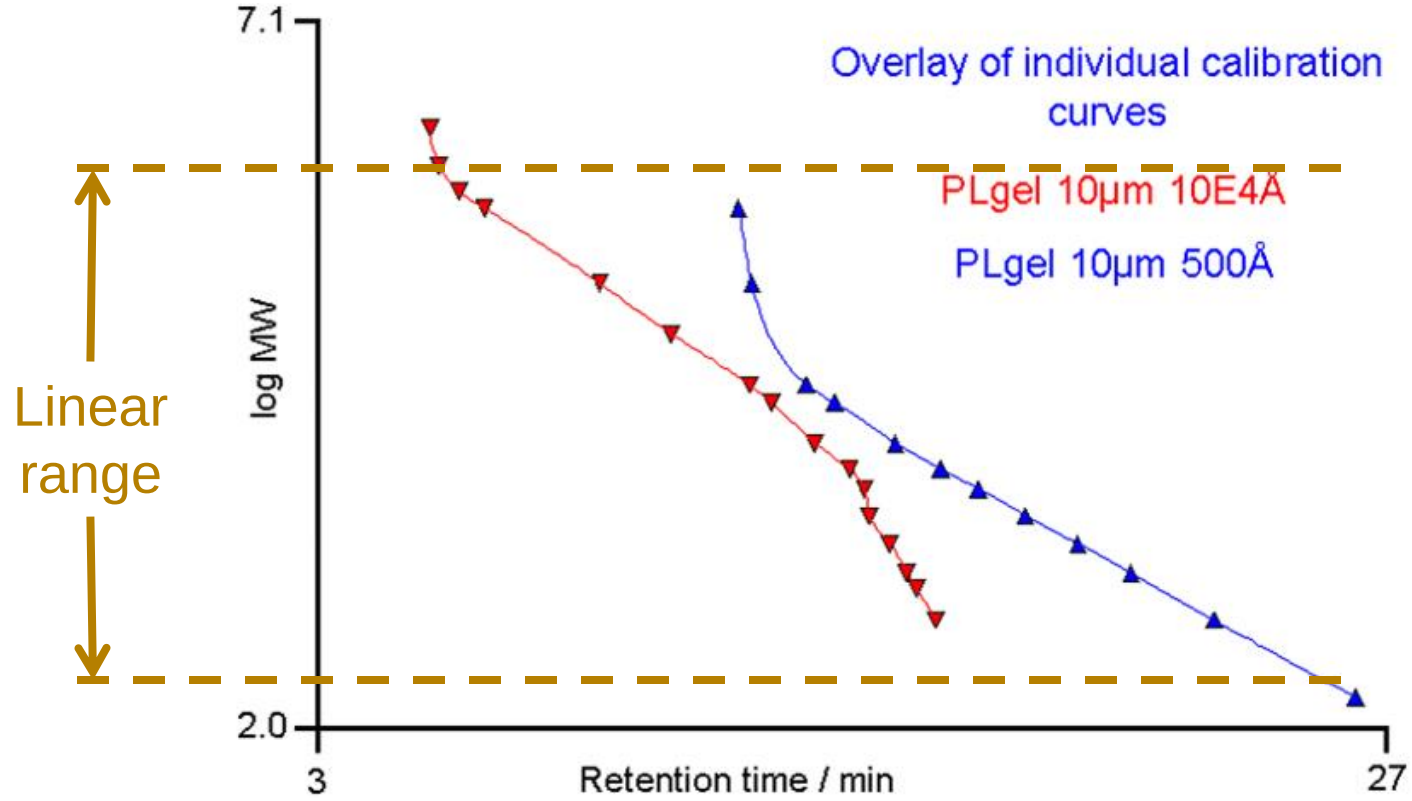
- Increasing the pore volume increases the resolution

Use a packing with a smaller particle size

- Decreasing the particle size increases the column efficiency

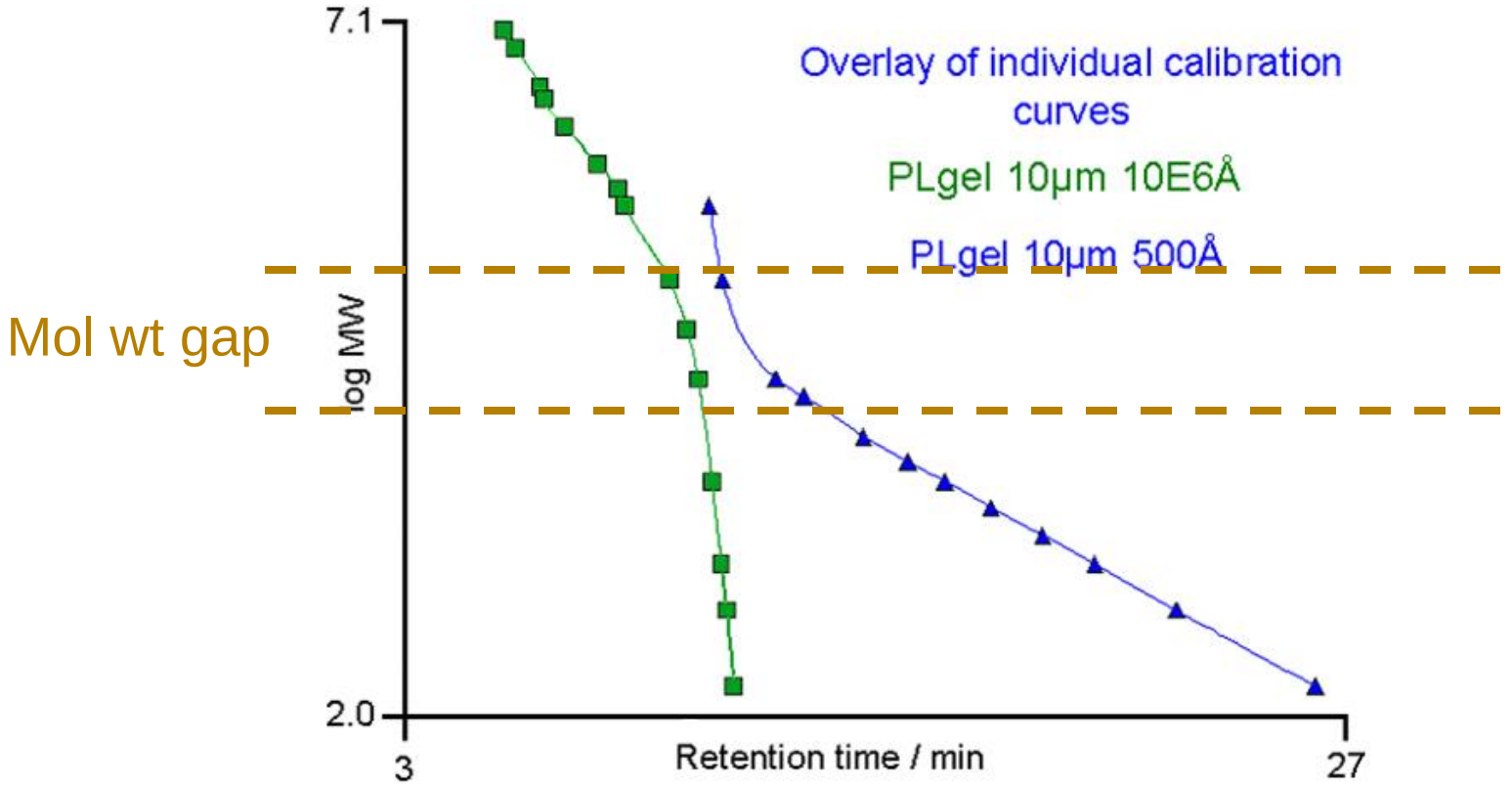
Columns in Series

Increasing the resolving range



- Individual columns can be coupled in a series
  For example, PLgel individual pore columns
- Linear calibration ranges need to complement each other without too much overlap

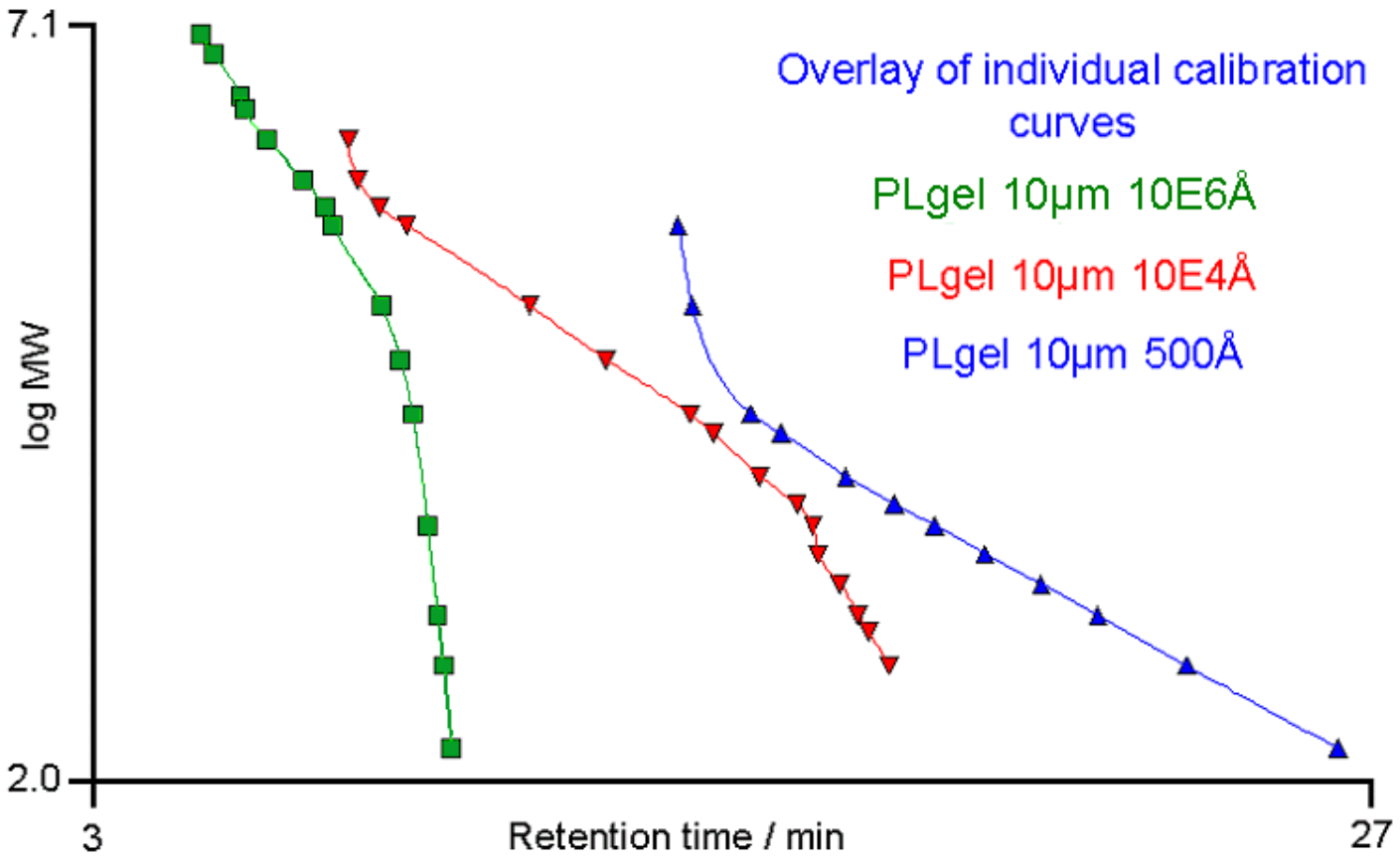
Wrongly Coupled Columns



- Molecular weight gap between linear ranges
- Changes retention and gives unusual peak shapes

Combination of Individual Pore Size Columns

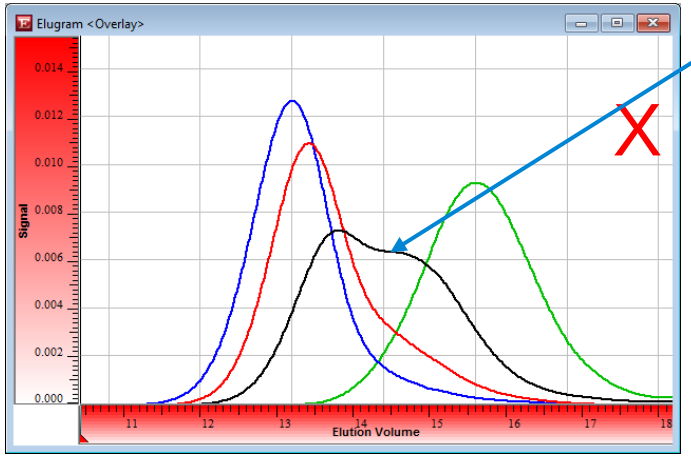
Traditional approach to increasing molecular weight operating range of column set



Column Selection: Porosity

Pore size mismatch after combining columns

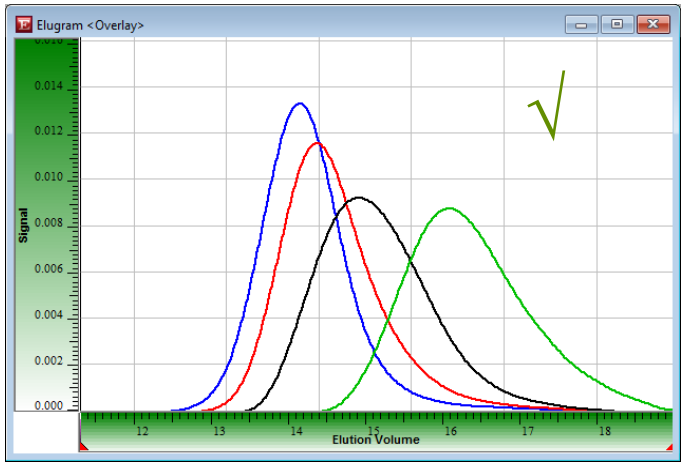
Where? Occurs when there is a sudden change in the slope of the calibration curve



Artefact caused by pore size mismatch

When can it occur?

- If there is an incorrect combination of columns from individual pore sizes
- Combination of a linear/MIXED/multipore column with a single pore size column
- Combination of two different linear/MIXED/multipore column types



Chromatograms after combining columns with matched pore sizes

How to avoid a pore size mismatch

- Use recommended combinations of individual pore sizes
- Only use linear/MIXED/multipore columns of the same type together
- Use the new GPC/SEC Column Selector tool:

https://www.agilent.com/en/selector-tools/gpc-sec-columns.html

Column Selection – Porosity

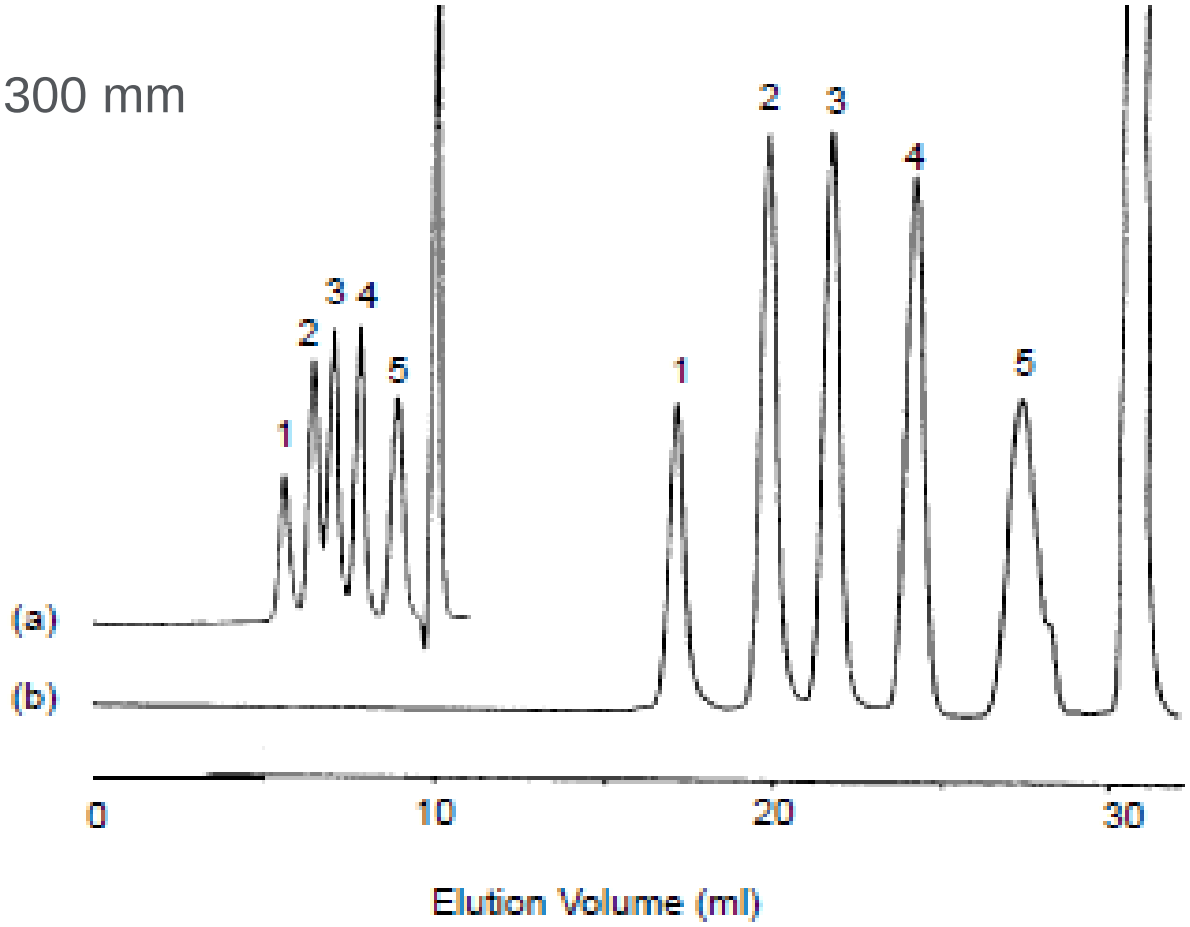
Columns of same typed used in a series

- ✓ No mismatch
- ✓ More pore volume
- ✓ Improved resolution

Columns: 1x PLgel 10 µm, MIXED B, 7.5 x 300 mm
p/n PL1110-6100
3x Plgel 10 µm, MIXED B, 7.5 x 300 mm
p/n PL1110-6100
Eluent: THF
Flow rate: 1 mL/min
Detector: RI

Polystyrene standards
EasiCal

1. 3,040,000
2. 330,000
3. 66,000
4. 9,200
5. 580



Column Selection and Importance of Solvent Choice

Criteria for solvent selection

- The factor that principally controls which type of column is selected for GPC/SEC analysis is the solvent
- Many polymers dissolve in only limited numbers of solvents
- The columns used must be compatible with the solvent of choice
- Solvent choice permits adequate detection
- Balance polarity for sample type, solvent, and column packing
- Most importantly, the size exclusion mechanism must be maintained

Solvent Selection
Sample type

What solvent is your polymer soluble in?

Type	Typical Solvents
Organic, low polarity	• THF • Chloroform • Toluene • TCB
Mixed or medium polarity organic	• THF/water • DMF • DMAc • NMP
Aqueous	• Water • Buffer in water • Water/methanol (up to 50%) • Water/other organic

Additives can be employed:

- Minimize nonsize exclusion interactions between the sample and the column
- Stabilize the solution of the polymer (ionic aggregation)

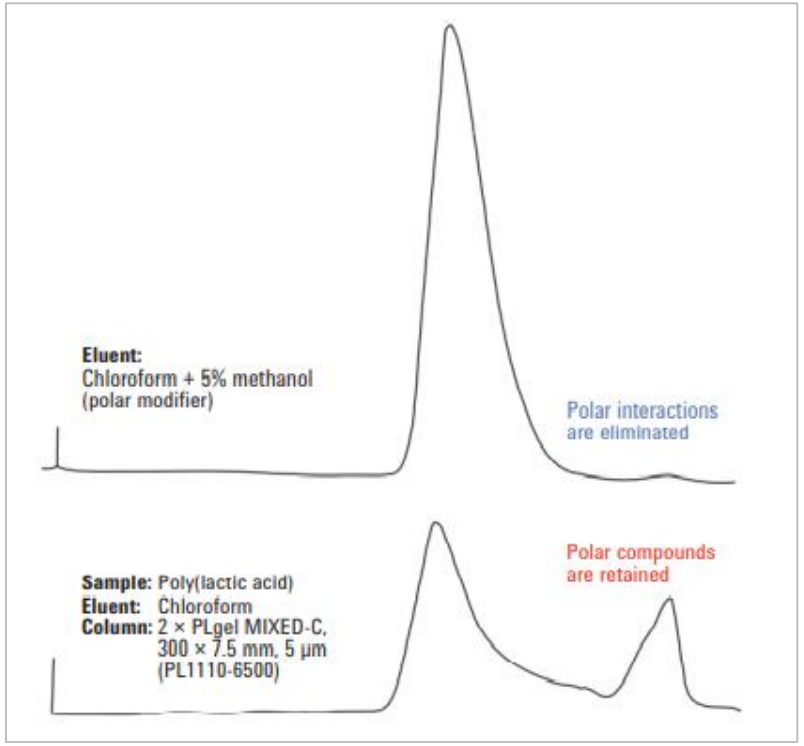
Publication number: 5991-6802EN
Polymer-to-Solvent Reference Table for GPC/SEC

Successful Solvent Choice

Agilent InfinityLab

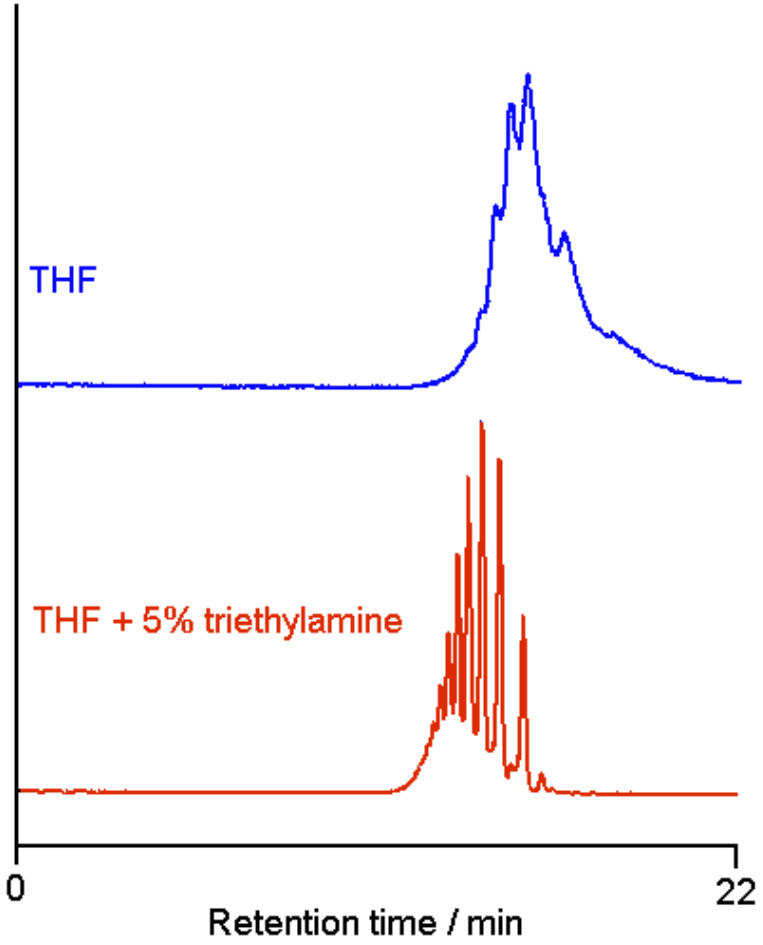
Tips for use of additives:

- Addition of salts to aqueous and polar organic solutions is the preferred method for eliminating polar interactions through electrostatic screening. **Salts should be flushed from the system after analysis.**
- For water-soluble polymers, interactions can also be minimized by addition of an organic solvent, such as methanol.
- Lewis bases, such as polyamines and polyamides, may interact with polymeric media, but this can be eliminated by the addition of an amine to the mobile phase, such as triethylamine (TEA).



Polar interactions in the lower chromatogram are eliminated with the addition of 5% methanol to the eluent

Eluent Modification in Organic GPC



Hostavin N30

Polymeric UV stabilizer containing secondary amine groups

Column: 2x PLgel 3 µm MIXED-E
7.5 x 300 mm p/n PL1110-6300
Flow Rate: 1.0 mL/min
Detector: ELSD

Common Questions Around GPC/SEC Standards

Which standards to use?

What is the eluent/mobile phase?

Solvent Type	GPC/SEC Standards Type
Organic	• Polystyrene (PS) • Polymethylmethacrylate (PM)
Mixed or Polar Organic	• Polymethylmethacrylate (PM) • Polyethylene glycol/oxide (PEG/PEO)
Aqueous	• Polyethylene glycol/oxide (PEG/PEO) • Polysaccharide (SAC) • Polyacrylic acid (PAA)

Remember, just because the standard is soluble in the eluent, it may not be the correct standard to use for your column

Agilent Polymer Calibration Standard Offerings

Which **type** of kits best suits my needs?

EasiVial/ReadyCal – preprepared for fast and easy accurate concentration, 10 to 12 point column calibration for organic and aqueous mobile phases

EasiCal – easy three-step process for an accurate 10 point calibration, for organic solvents like THF

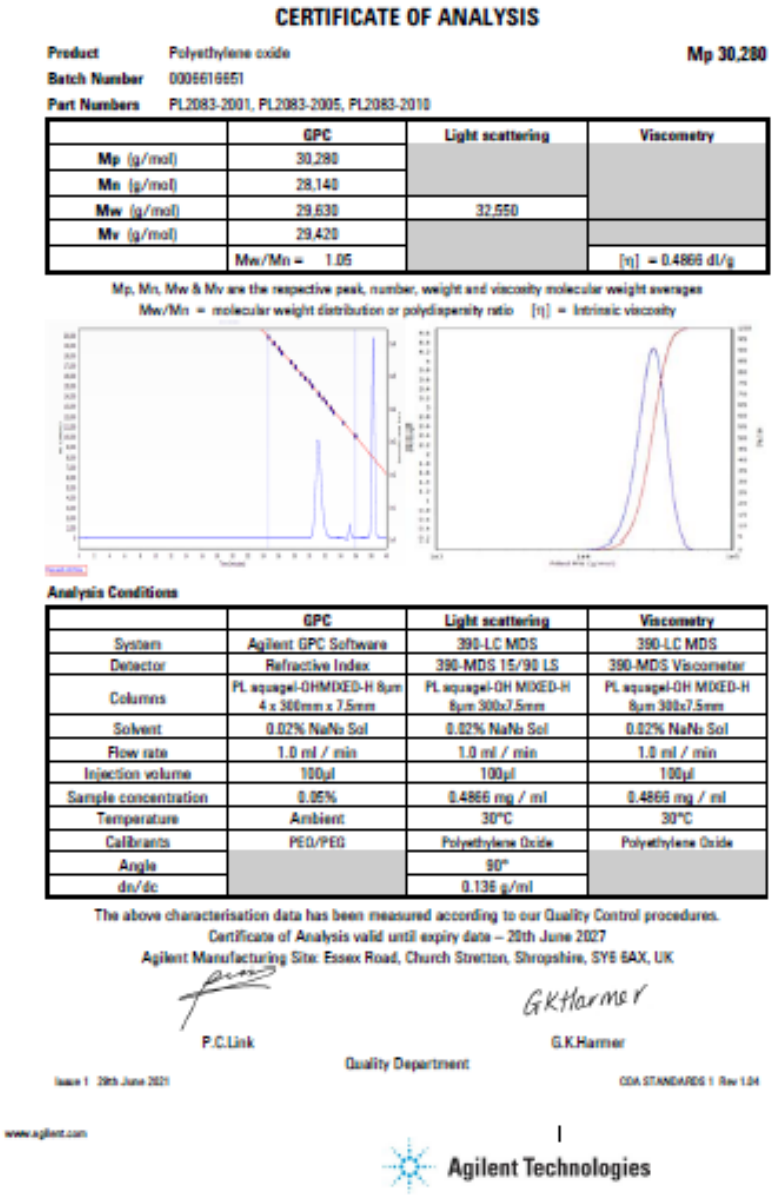
Calibration kits and individual standards – numerous polymer standard types available, covering all MW ranges for GPC and SEC columns. Individual polymer standards provided in a wide molecular weight ranges and in various quantity options.

Agilent GPC/SEC Polymer Standards, publication number: 5994-7996EN

Well Characterized Polymer Standards

Example certificates of analysis

- Agilent standards are manufactured under an ISO 9001:2008 approved quality system.
- Each standard is fully traceable with a unique batch number and provided with a complete certification of analysis (CoA).
- All CoAs include details of the exact method and characterization results for maximum transparency and reproducibility.



CERTIFICATE OF ANALYSIS

Product Polyethylene oxide — Mp 30,280

Batch Number 0006616651

Part Numbers PL2083-2001, PL2083-2005, PL2083-2010

	GPC	Light scattering	Viscometry
Mp (g/mol)	30,280		
Mn (g/mol)	28,140		
Mw (g/mol)	29,630	32,550	
Mv (g/mol)	29,420		
	Mw/Mn = 1.05		[η] = 0.4866 dl/g

Mp, Mn, Mw & Mv are the respective peak, number, weight and viscosity molecular weight averages
Mw/Mn = molecular weight distribution or polydispersity ratio [η] = Intrinsic viscosity

Analysis Conditions

	GPC	Light scattering	Viscometry
System	Agilent GPC Software	390-LC MDS	390-LC MDS
Detector	Refractive Index	390-MDS 15/90 LS	390-MDS Viscometer
Columns	PL aquagel-OH MIXED-H 8µm 4 x 300mm x 7.5mm	PL aquagel-OH MIXED-H 8µm 300x7.5mm	PL aquagel-OH MIXED-H 8µm 300x7.5mm
Solvent	0.02% NaN3 Sol	0.02% NaN3 Sol	0.02% NaN3 Sol
Flow rate	1.0 ml / min	1.0 ml / min	1.0 ml / min
Injection volume	100µl	100µl	100µl
Sample concentration	0.05%	0.4866 mg / ml	0.4866 mg / ml
Temperature	Ambient	30°C	30°C
Calibrants	PEO/PEG	Polyethylene Oxide	Polyethylene Oxide
Angle		90°	
dn/dc		0.136 g/ml	

The above characterisation data has been measured according to our Quality Control procedures.
Certificate of Analysis valid until expiry date – 20th June 2027
Agilent Manufacturing Site: Essex Road, Church Stretton, Shropshire, SY6 6AX, UK

P.C.Link — G.K.Harmer

Quality Department

Issue 1 20th June 2021 — CDA STANDARDS 1 Rev 1.04

www.agilent.com — Agilent Technologies

Example: individual standard certificate

Easivial Certificate of Analysis

CERTIFICATE OF ANALYSIS

Product Polystyrene Medium EasiVials (2ml)
Part Numbers PL2010-0301, PL2010-0302, PL2010-0700
Batch Number 0006676796

Vial Code	IV (dL/g)	Mw (g/mol) (Light Scattering)	Mn (g/mol)	Mw (g/mol)	Mw/Mn	Mp (g/mol)	Mass/vial (mg)
RED	1.0263	319,200	348,500	364,700	1.05	**364,000**	0.4
	0.2543	50,800	46,950	48,900	1.04	**49,350**	0.8
	0.0691	7,090	6,090	6,260	1.03	**6,250**	1.2
	0.0264	1,100	890	950	1.07	**935**	1.6
YELLOW	0.6739	191,900	197,000	204,100	1.04	**200,500**	0.4
	0.1757	29,960	27,600	28,250	1.02	**28,440**	0.8
	0.0503	3,920	3,190	3,310	1.04	**3,320**	1.2
	0.0262	445	410	450	1.10	**370**	1.6
GREEN	0.3849	90,300	86,150	88,350	1.03	**89,050**	0.4
	0.1095	14,330	13,250	13,530	1.02	**13,440**	0.8
	0.0329	1,370	1,100	1,170	1.06	**1,180**	1.2
	-	-	-	-	1.00	**162**	1.6**

** Due to the volatile nature of this constituent weights may vary.

Mp, Mn & Mw are the respective peak, number and weight molecular weight averages.
Mw/Mn = molecular weight distribution or polydispersity ratio.
IV is the intrinsic viscosity value

The above characterisation data has been measured according to our Quality Control procedures.
Certificate of Analysis valid until expiry date: 28th April 2027
Agilent Manufacturing Site: Essex Road, Church Stretton, Shropshire, SY6 6AX, UK

Storage:
The polymers in each vial should be stored in a cool dark place when not in use. After preparation, the polymer solutions should be stored in a cool, dark place and used within 1 week.

P.C.Link M.Griffin

Q.C. Department

Issue 1 6th May 2022 COA STANDARDS5-3 Rev 2.21

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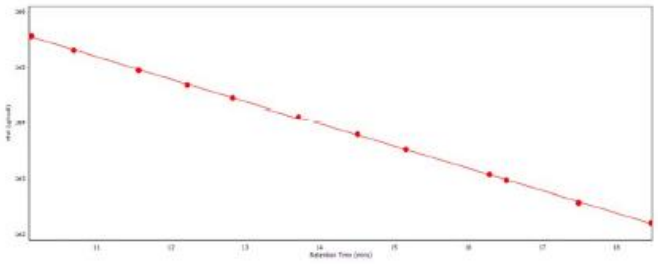
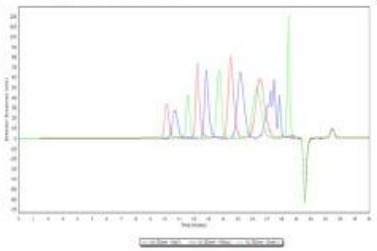
GPC/SEC Calibration Curve



GPC/SEC EasiVials Overlay



Calibration Details

System	Agilent GPC software
Detector	Ultra Violet
Columns	2 x PLgel MIXED-D 5µm 300 x 7.5mm
Solvent	THF
Flow rate	1.0 ml / min
Injection volume	20µl
Sample concentration	1ml solvent / vial
Temperature	Ambient

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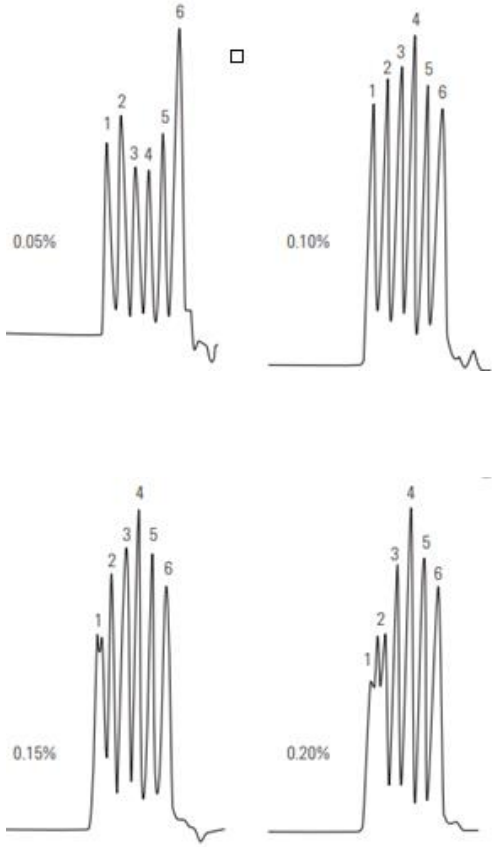
[Agilent weblink: eCertificates]

Common Questions Around SEC Standards

How are standards prepared?

- Standards should always be dissolved with the same solvent used as the mobile phase.
- Vortexing, sonicating, and shaking of standard solutions should also be avoided (these are high-shear activities and will result in a change of peak shape, retention time, and MW).
- Concentration is critically important. Band broadening due to excessive sample viscosity can occur if the standard is too concentrated. Loss of resolution is also a factor.
- The signal-to-noise ratio will be too small to reproducibly integrate with too low a concentration.



How frequently should new standards be prepared?

- Standards are typically stable in the form they are supplied in, but once made up can degrade, expedited by UV and heat. ***It is good practice to make up new standards on a weekly basis.***

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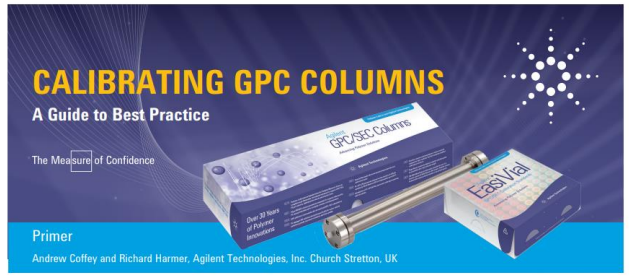
[User Guide Agilent Polymer Standards for GPC/SEC]

Common Questions Around SEC Standards



Publication number: 5991-2720EN

How often should you calibrate?

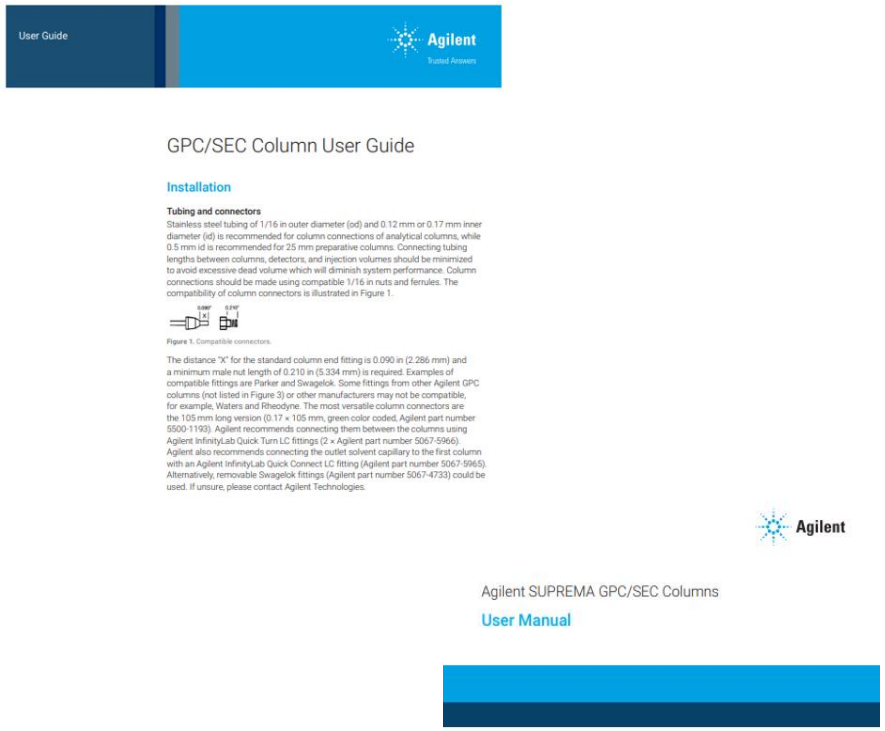
- Frequency of calibration is subjective. For continuous work, it is advisable to calibrate daily, but with the use of internal verification, a weekly calibration should be performed at minimum. *Once a week is suggested,* if no major changes occur with the system or columns.

- It is essential to recalibrate *whenever a component of the system is altered, or if there is an eluent change.*

Calibrating frequently can also help to identify potential issues and can allow you to quickly to put into place corrective/preventative steps.

Installation and Care of Your Column

See the column user guide



The user guide provides information for:

- Shipping solvent
- Technical specifications
- Installation instructions
- Column conditions
- Mobile phase and transfer guidelines
- Column care
- Troubleshooting
- Column cleaning and storage

Agilent weblink: GPC/SEC Column User Guide
Or it can be found on the product page for the column, under Product Details -> Support
Example: SUPREMA | Agilent

Before Your Analysis

Do a performance test of your column

Every new column should be tested on your instrument

LC Column Performance Report

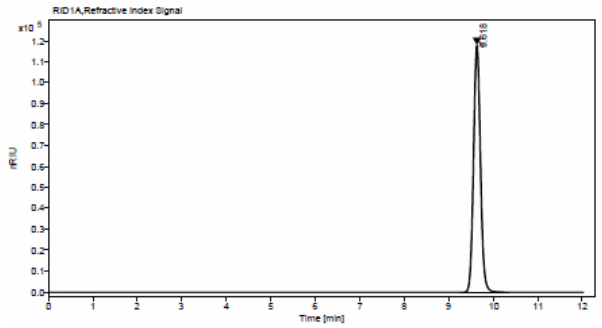
Agilent Technologies

Serial number	0006742315-197
Part number	PL1149-6800
Description	PL aquagel-OH MIXED-H 8um 300 x 7.5mm
Batch number	0006742315

Test Conditions

Mobile phase	Water containing 0.02% sodium Azide
Column pressure	19 Bar (includes system pressure)
Flow rate	1.00mL/min
Temperature	Ambient
Injection volume	2.4µl
Test mixture	Glycerol (5% in water containing 0.02% sodium azide)
LC system	Agilent LC Test system with OpenLab CDS 2

	TEST VALUES	SPECIFICATIONS
Theoretical plates 1/2 height per metre	62,992	>56,000
Theoretical plates 5 sigma per metre	55,723	>47,000
Peak asymmetry 10%	1.11	0.80 - 1.35



Operator MB

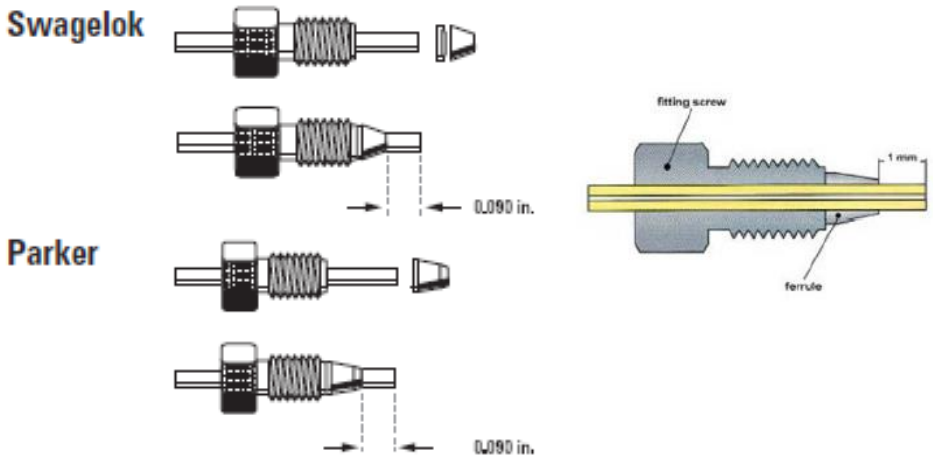
This column is shipped in water containing 0.02% sodium azide Agilent Test LC systems are optimized to minimize extra-column volume, so performance results may vary from those on systems in your lab Peak widths are dependent on integration settings, so results may also vary between data systems

Performance verification based on Agilent checkout

- Run Agilent checkout before use
  - Record the difference between your instrument and the performance report (use this as a base value)
- Perform again if the column seems to lose performance
  - Compare with the results from your first run
- Run a set of polymer standards for your column at its first use
- Perform again if the column seems to lose performance
  - Compare with the results from the first run

Column Selection – Additional considerations

Importance of proper fittings



Result of improper fittings/ferrules can lead to:

Poor chromatography
- Broad or tailing peaks
- Increased dispersion, loss of resolution

Added maintenance costs
- Leaks, added troubleshooting
- Overtightening
- Column damage

Attention and warning: The distance from the ferrule to the end of the capillary can differ:
  - Between manufacturers
  - Between different brands from same manufacturer
- Check the compatibility of the two connectors between the column and the instruments.
- If in doubt, use new connectors with the new column (or columns).
- Old connectors and the replaced column (or columns) can be stored.

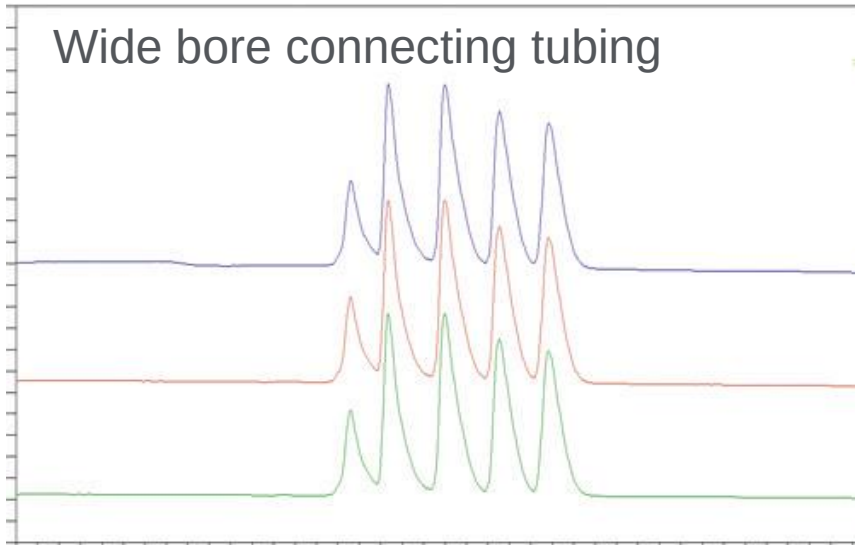
Quick Turn style fittings offer a universal solution

Use plastic connectors and ferrules, such as PEEK, with caution. If using these, be sure to check:
- Temperature limits
- Pressure ratings
- Solvent compatibility

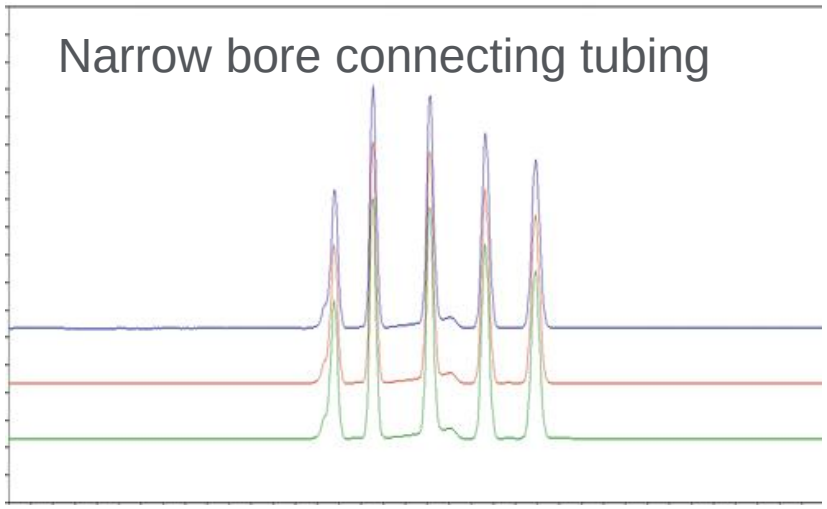
InfinityLab Quick Turn fitting
Pub number: 5991-5164EN

HPLC Fittings & Ferrules, Peek (Polyetheretherketone) Fittings | Agilent

System Tubing
Reduce dead volume



- Peak shape is poor
- Resolution between peaks is poor



- Keep tubing connections short
- Inner diameter for tubing should be as narrow as possible

Your GPC/SEC Toolbox

Consumables to have on hand

Sample preparation

- Sample filters
- Syringes

Sample handling

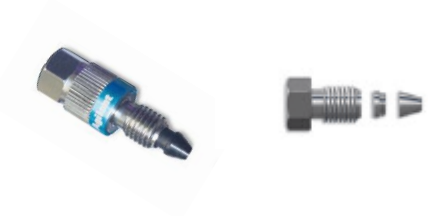
- Vials and caps

Mobile phase solvents

- Filtration
- Solvent containment and caps
- Inlet filters

System consumables

- Pump frits and piston seals
- Needle
- Needle seat
- Loop capillary
- Rotor seal
- Stainless steel capillary tubing
- Fittings/ferrules
- Waste tubing



[InfinityLab LC Supplies Guide (agilent.com)]

GPC/SEC Columns, Supplies, and Application Resources

- Agilent webpage for GPC/SEC solutions: GPC/SEC Solutions for Macromolecular Characterization | Agilent
- Agilent webpage for GPC/SEC Columns & Standards: GPC/SEC Columns & Standards | Agilent
- **Expanded** portfolio of GPC/SEC columns and standards: Agilent GPC/SEC Columns and Standards Brochure
- GPC/SEC Column Selector Tools: GPC/SEC Column Selector Tools
- Polymer-to-Solvent Reference Table: Polymer-to-Solvent Reference Table
- GPC/SEC User Guide: GPC/SEC column user guide
- GPC Troubleshooting poster: GPC Troubleshooting Guide
- Consumables Community: Agilent Community GPC SEC Solutions
- GPC/SEC eBook series: GPC/SEC Columns – What You Should Know When You Need To Analyze Polymers, Biopolymers, and Proteins

- App finder: Application Finder | Agilent
- InfinityLab Supplies catalog: InfinityLab LC Supplies Catalog
- Your local product specialists
- Webinars, upcoming and recorded: LC and LC/MS Column Webinars | Agilent

Contact Agilent Chemistries and Supplies Technical Support

Available in the U.S. and Canada, 8-5 all time zones

1-800-227-9770 option 3, option 3:

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration, and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

Option 6 for Prozyme products

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

pzi.info@agilent.com

Agilent

Trusted Answers

Agilent GPC Column Product Families For Organic-soluble Polymers

	Neutral Polymers Polycarbonate PVC	PLgel PLgel MIXED PLgel MIXED-LS* PL Rapide SDV SDV Linear SDV Lux*	– Individual pore sizes and mixed-bed or linear columns
	Polycarbonates Polyurethanes Epoxy Resins Polyester Resins Siloxanes Silicone Fluids	InfinityLab MesoPore InfinityLab OligoPore InfinityLab PolyPore InfinityLab ResiPore	– Next generation of InfinityLab GPC/SEC columns available in smaller internal diameters – New high-efficiency media with improved pore volumes – Maximizes overall separation performance in shorter run times with less solvent consumption
	Nylons Polylactides Polyesters PET	PFG PFG Lux* PL HFIPgel	– Compatible with fluorinated solvents – Available in 5 µm particles for higher efficiency
	Food Films PE PP Polymers	PLgel Olexis POLEFIN	– High-temperature GPC – Particle and pore sizes optimized for analysis of large molecules in viscous eluents under demanding analysis conditions
	Epoxy Polyurethanes Polysulfones Celluloses	GRAM GRAM Lux* PolarGel PolarSil	– Medium-polar organic solvents – PolarSil available in 3 µm particle sizes for higher efficiency

Agilent SEC Column Product Families For Water-soluble Polymers

	Dextran Saccharides Hyaluronic Acid Acrylates Acrylamides Heparin Gum	PL aquagel-OH PL Multisolvent PL Rapide Aqua SUPREMA SUPREMA Lux*	– Individual pore sizes and mixed-bed columns – SUPREMA compatible with 100% organic modifiers
	Sulfonated Polyanions Lignins	MCX	– Charged anionic polymers – Robust at high pH – Compatible with organic modifiers – Available in 5 µm particle sizes
	Chitosan Food Ingredients Cationic Polymers	NOVEMA Max NOVEMA Max Lux*	– Charged cationic polymers – Robust at low pH – Compatible with organic modifiers – Available in 5 µm particle sizes

Recommended Polymer Standards – Solvent Combinations

✓ Standard soluble in solvent (✓) Standard soluble under special conditions, temperature, additives, certain Mw ranges ✓* Standard soluble but isorefractive	Dextran	Poly(2-vinyl pyridine)	Poly(acrylic acid) Na+	Poly(α-methyl styrene)	Poly(butadiene1,2)	Poly(butadiene 1,4)	Poly(dimethyl siloxane)	Poly(ethylene glycol)	Poly(ethylene oxide)	Poly(ethylene terephthate)	Poly(ethylene)	Poly(isobutylene)	Poly(isoprene 1,4)	Poly(isoprene 3,4)	Poly(lactide)	Poly(methacrylic acid) Na+	Poly(methyl methacrylate)	Poly(propylene glycol)	Poly(styrene sulfonate) Na+	Poly(styrene)	Poly(t-butyl methacrylate)	Proteins	Pullulan
Water	✓	(✓)	✓					✓	✓							✓			✓			✓	✓
Ethanol/methanol																					✓		
Trifluroethanol															✓								
Hexafluoroisopropanol										✓					✓		✓						
Dimethylformamide				✓				✓	✓								✓			✓	✓		
Dimethylacetamide				✓				✓	✓								✓			✓	✓		
Dimethylsulfoxide	✓									(✓)							✓						✓
Tetrahydrofuran		✓		✓	✓	✓	✓*	(✓)				✓	✓	✓			✓	✓		✓	✓		
Acetone																	✓				✓		
Chloroform				✓			✓					✓			✓		✓			✓	✓		
N-Methyl-2-pyrollidone				✓													✓			✓	✓		
Trichlorobenzene				✓							(✓)	✓								✓	✓		
Dichlorobenzene				✓	✓	✓					(✓)	✓								✓	✓		
Toluene				✓	✓	✓	✓					✓	✓	✓			✓			✓	✓		
EasiCal/ReadyCal	✓							✓	✓								✓			✓		✓	✓

Expanding Conventional GPC/SEC

Addition of viscometer and light scattering detectors

Advanced detectors give a greater understanding of the analyte, as well as overcoming the limitations of conventional GPC.

GPC/SEC Technique	Molecular Weight	Molecular Size	Information
Conventional (RI or UV)	Relative to the standards used for calibration	No	Molecular weight distribution, concentration
Viscometry	More accurate from universal calibration	Yes, hydrodynamic radius (Rh)	Conformation, branching Works with copolymers
Light scattering	Absolute determination	Yes, radius of gyration (Rg), directly	Conformation, branching
Triple	Absolute determination	Yes, Rg and Rh, directly	The ultimate configuration for comprehensive polymer characterization

Agilent InfinityLab II 1260 with MDS