

# Your GPC/SEC Toolbox

Have you got what you need for your polymer analysis?

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Applications Engineer

LC Columns and Consumables Technical Support

March 21, 2023



# Why Determining MW and MWD is important?

The primary goal of GPC is to discover the MW distribution

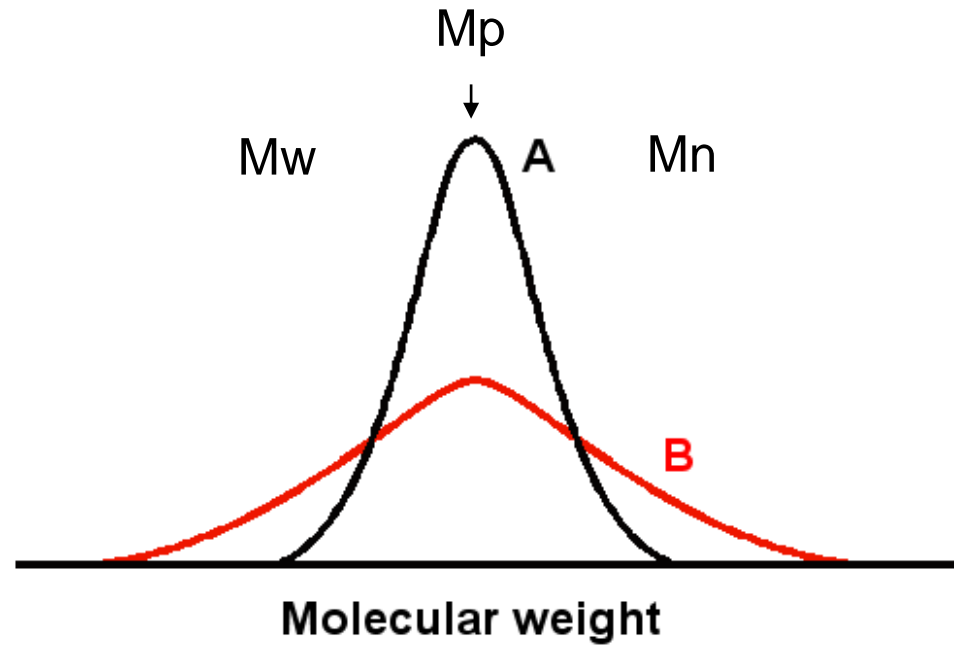
- Samples of synthetic polymers *always* contain polymer chains with a range of chain lengths
- One way to describe the length of the polymer chains is in terms of an average molecular weight – the average of all the chain lengths in the sample

*However...*

- Different samples of the same polymer can have the same average chain length, but very different distributions of chain lengths depending on the method of production
- In polymer science, it is the molecular weight *distribution* that is important

# Effect of MW and Polydispersity on a Polymer

Sample having same  
molecular weight but different  
distributions



	Strength	Toughness	Brittleness	Melt Viscosity	Chemical Resistance	Solubility
Increasing Mw	+	+	+	+	+	-
Decreasing distribution	+	+	-	+	+	+

# Sample Examples

## Why GPC/SEC is done

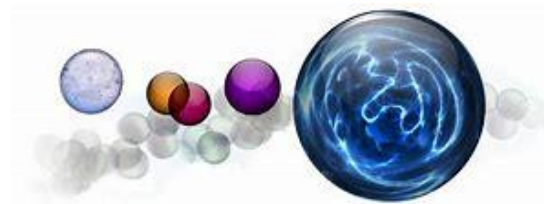
- **Plastics**

- Mol wt dictates polymer strength, flexibility, and physical properties



- **Water soluble polymers**

- Mol wt impacts viscosity, surfactant effects, dissolution, and chemical characteristics

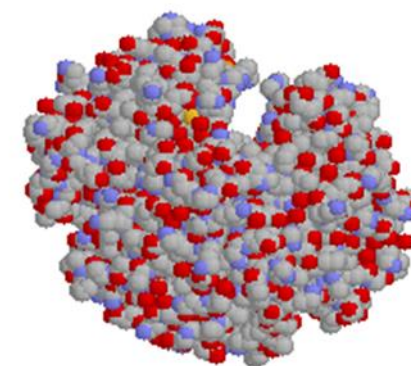


- **Sample cleanup**

- Separates target molecules from large molecules that fragment in MS and cause interference

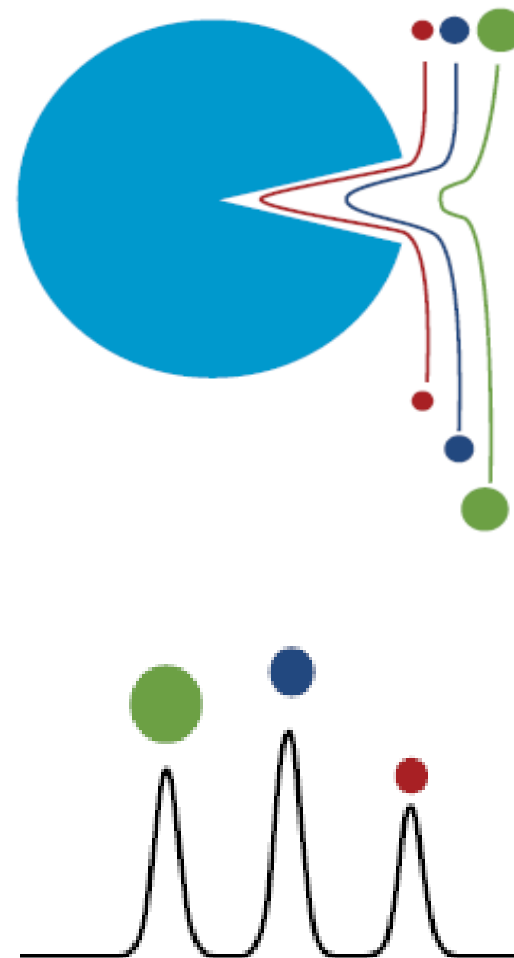
- **BioMolecules**

- Mol wt is often known
- Can be run on intact molecules
- Aggregation can be dangerous



# GPC/SEC Separation Mechanism

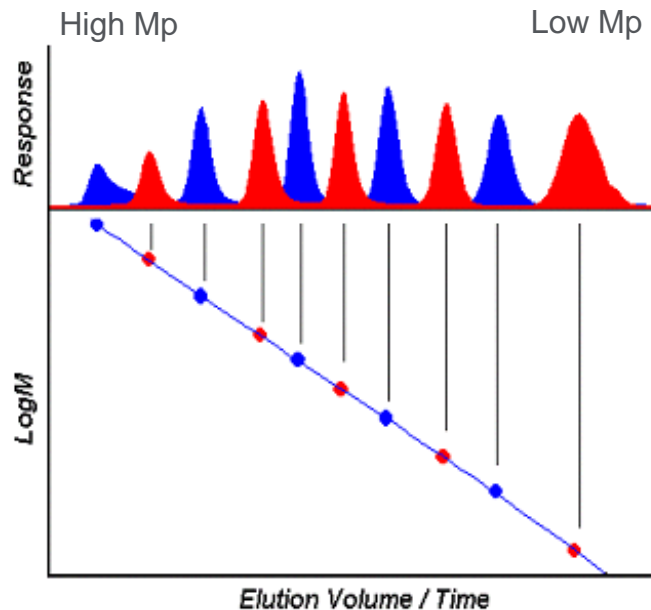
- A GPC/SEC column is packed with porous beads of controlled porosity and particle size
- Sample is prepared as a dilute solution in the eluent and injected into the system
- Large molecules are not able to permeate all pores and have a shorter residence time in the column
- Small molecules permeate 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



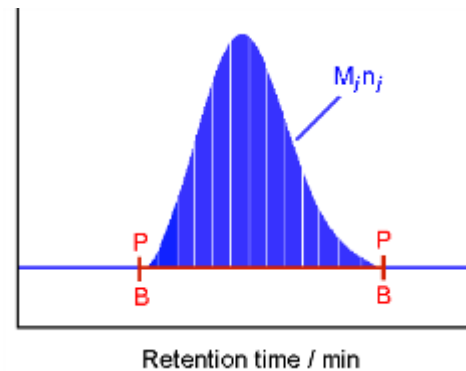
# Conventional GPC/SEC Workflow

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

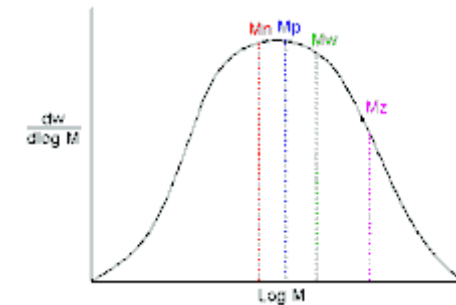
Chromatogram and plot of narrow standards



GPC sample chromatogram



Molecular weight distribution

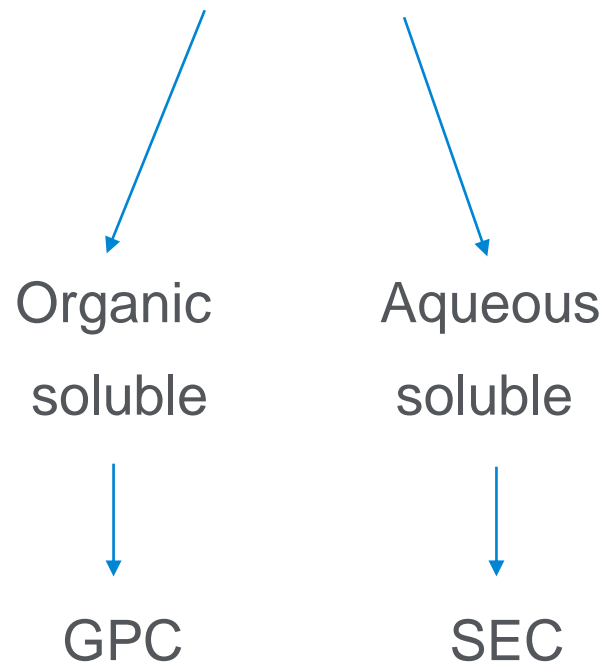


# Polymer Sample Type

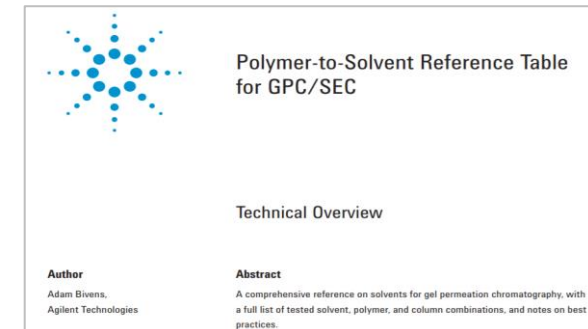
## Solvent considerations

### Questions that you need to ask

What type of polymer do I have?



An Agilent technical guide detailing most common solvent suggestions for polymer samples:



Polymer-to-solvent Reference Table – publication number:  
5991-6802EN

# Solvents

- Selecting a solvent system is one of the first steps in developing a GPC method
- Some polymer samples are easy to dissolve, some are much harder
- Some polymers may require elevated temperature for dissolution
- The Agilent range of GPC/SEC columns are available with phase chemistries that are optimized for all types of solvents that may be required: aqueous and organic, polar and nonpolar solvents.




# Criteria for Solvent Selection

## Successful solvent choice

Polymers are often employed due to their strength and toughness. Aggressive solvents and long dissolution times often required while ensuring:

- The solvent must be able to fully solubilize the sample
- **Must have true sample solubility** to avoid non-size exclusion effects
- Compatibility with columns
- It must permit adequate detection (for example, refractive index, UV cut off)
- Safety (toxicity, elevated temperature)

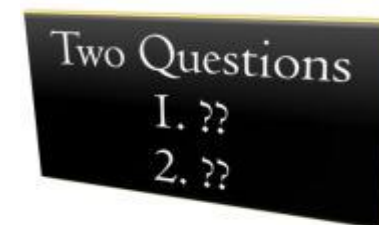
Solvent Polarity		Solvent
Low	6.0	Perfluoralkanes
	7.3	Hexane
	8.2	Cyclohexane
	8.9	Toluene
	9.1	Ethyl acetate
	9.1	Tetrahydrofuran (THF)
	9.3	Chloroform
	9.3	Methyl ethyl ketone (MEK)
	9.7	Dichloromethane
	9.8	Dichloroethane
	9.9	Acetone
	10.0	o-Dichlorobenzene (o-DCB)
	10.0	Trichlorobenzene (TCB)
	10.2	m-Cresol
	10.2	o-Chlorophenol (o-CP)
	10.7	Pyridine
	10.8	Dimethyl acetamide (DMAc)
	11.3	n-Methyl pyrrolidone (NMP)
	12.0	Dimethyl sulphoxide (DMSO)
High	12.1	Dimethyl formamide (DMF))

# Two Important Questions

## Solvent considerations

Question 1: What solvent is your sample soluble in?

Type	Typical Solvents
Organic	<ul style="list-style-type: none"><li>• THF</li><li>• Chloroform</li><li>• Toluene</li><li>• TCB/ODCB</li></ul>
Mixed or polar organic	<ul style="list-style-type: none"><li>• THF/water</li><li>• DMF</li><li>• NMP</li></ul>
Aqueous	<ul style="list-style-type: none"><li>• Water</li><li>• Buffer in water</li><li>• Water/methanol (up to 50%)</li><li>• Water/other organic</li></ul>



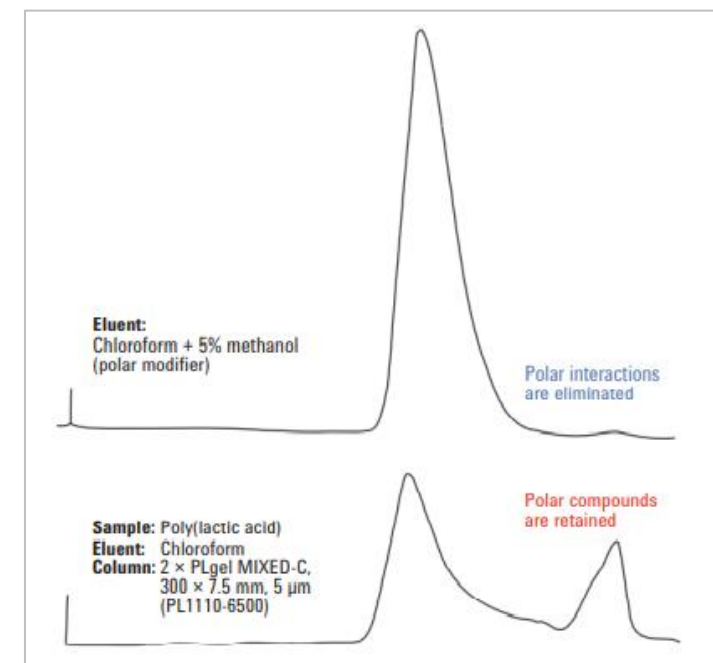
### Additives can be employed to:

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

# Successful Solvent choice

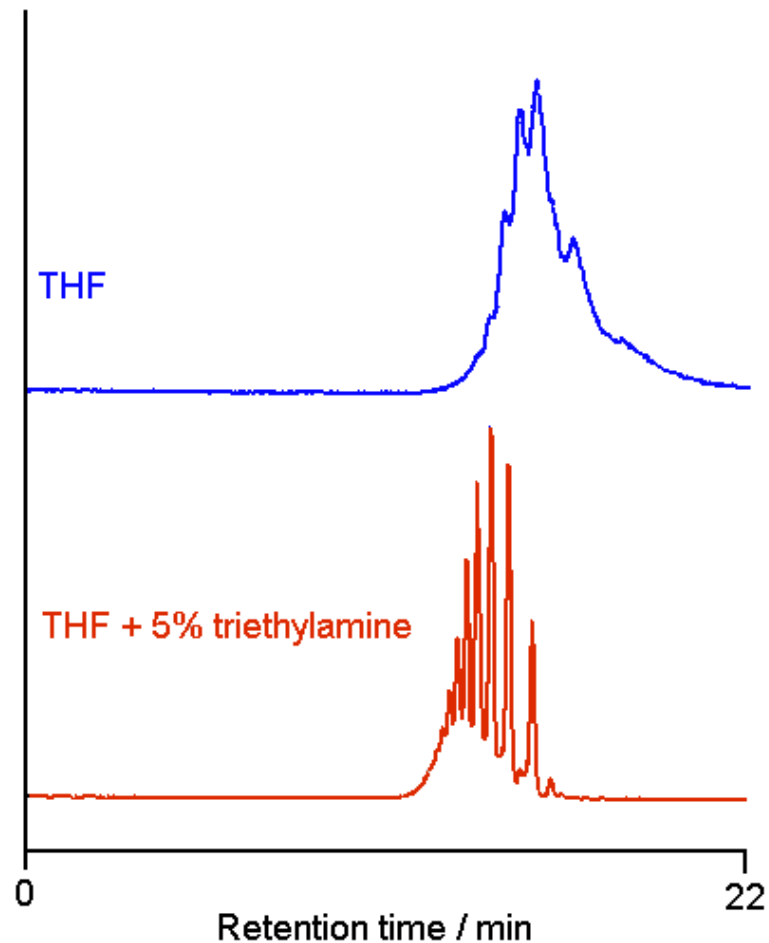
## Tips for use of additives:

- Addition of salts to aqueous and polar organic solutions is the preferred method for eliminating polar interactions by 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 5% methanol addition to the eluent

# Eluent Modification in Organic GPC



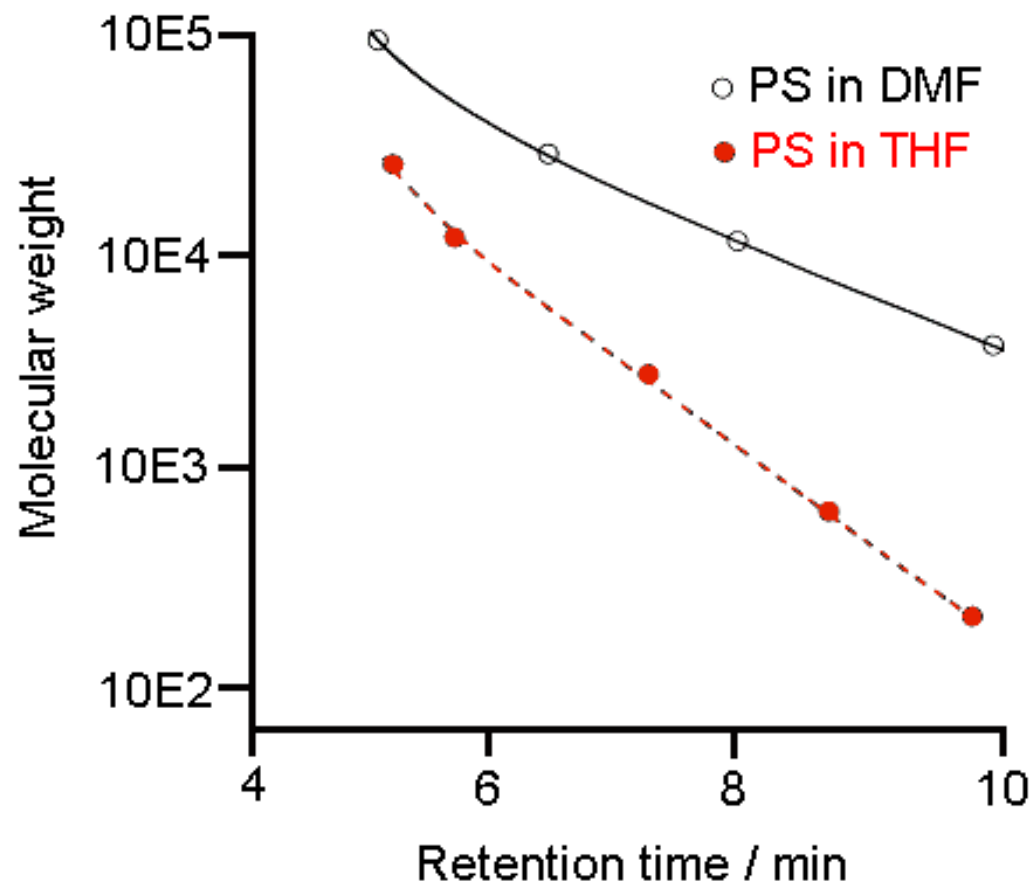
## Hostavin N30

Polymeric UV stabilizer containing secondary amine groups

Column: 2 x Plgel, 3  $\mu$ m, MIXED-E  
Flow rate: 1.0 mL/min  
Detector: PL-ELS 1000

# Solvent Choice for Calibration Standards

Need to maintain GPC/SEC mechanism



PS/DVB columns are excellent in many solvents, but remember that although the column may be used in certain solvents this does not mean SEC will occur – the example here is polystyrene standards running in DMF.

Column: PL1110-6525 PLgel, 5  $\mu$ m, 500 Å  
300 x 7.5 mm

# Selecting a GPC/SEC Column

Points to consider when making a column choice:

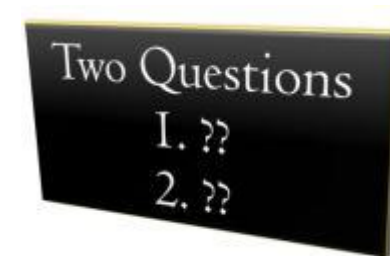
- Is an organic or aqueous eluent being used?
- What is the expected molecular weight range for your sample?
- What type of column options are there?
- What are your **key** requirements for your GPC/SEC analysis?

# Two Important Questions

## Choosing a GPC/SEC column

Question 2: What is the expected molecular weight range of your polymer sample?

Mol Wt	Mol Wt Range (g/mol or Da)
High	Up to several millions
Intermediate	Up to hundreds of thousands
Low	Up to tens of thousands
Very low	A few thousand



# Column Chemistries

PLgel 10  $\mu\text{m}$  10E3 A and 10E6 A particles with their rigid pore structure

## Polymer chemistries

Common types:

Polymethacrylate packings

Polyester copolymers

DVB, divinylbenzene

PS-DVB, polystyrene divinylbenzene

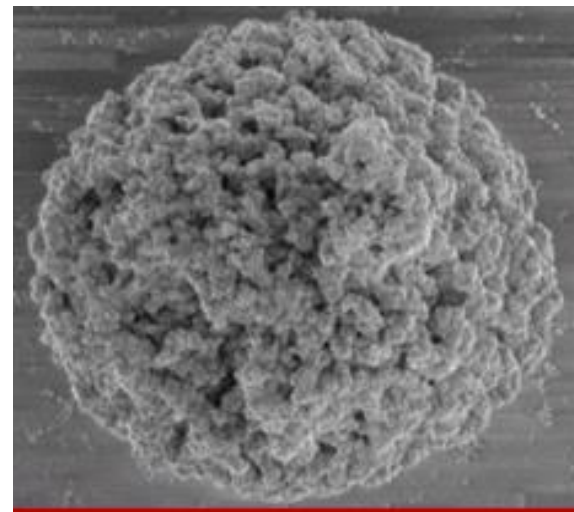
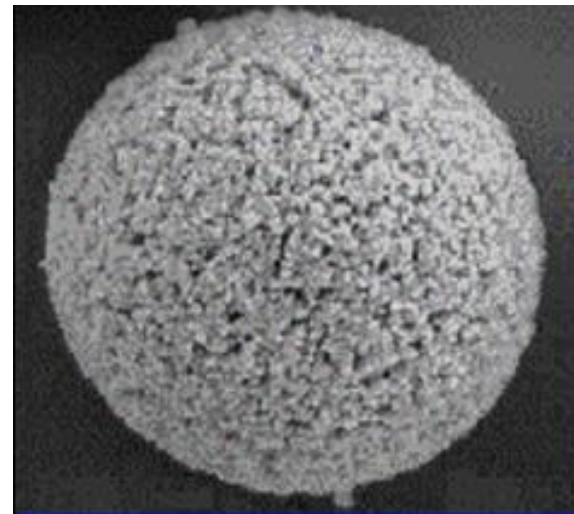
## Silica chemistries

Common types:

Diol

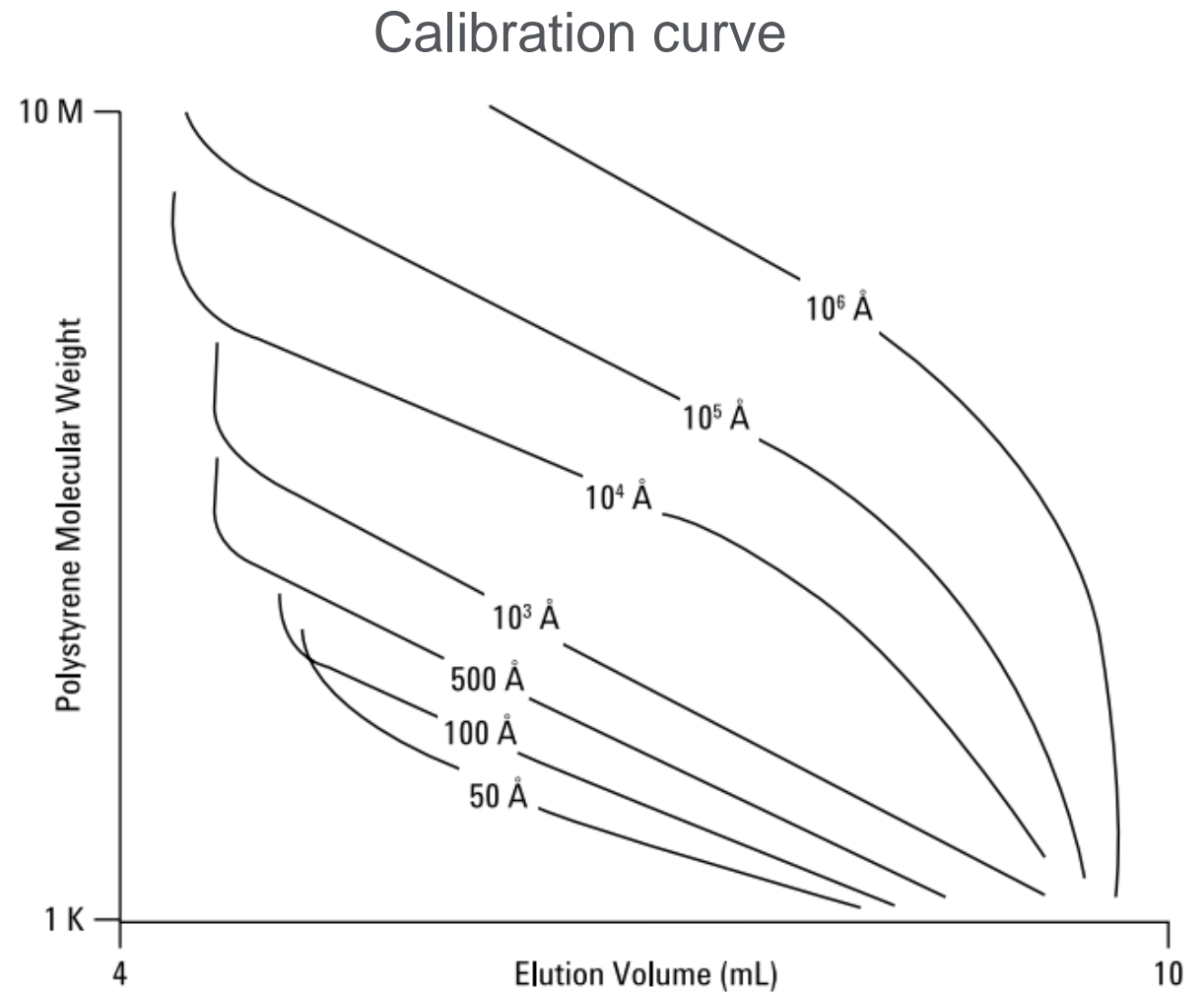
Surface-modified hydroxyl

Surface-modified polymeric



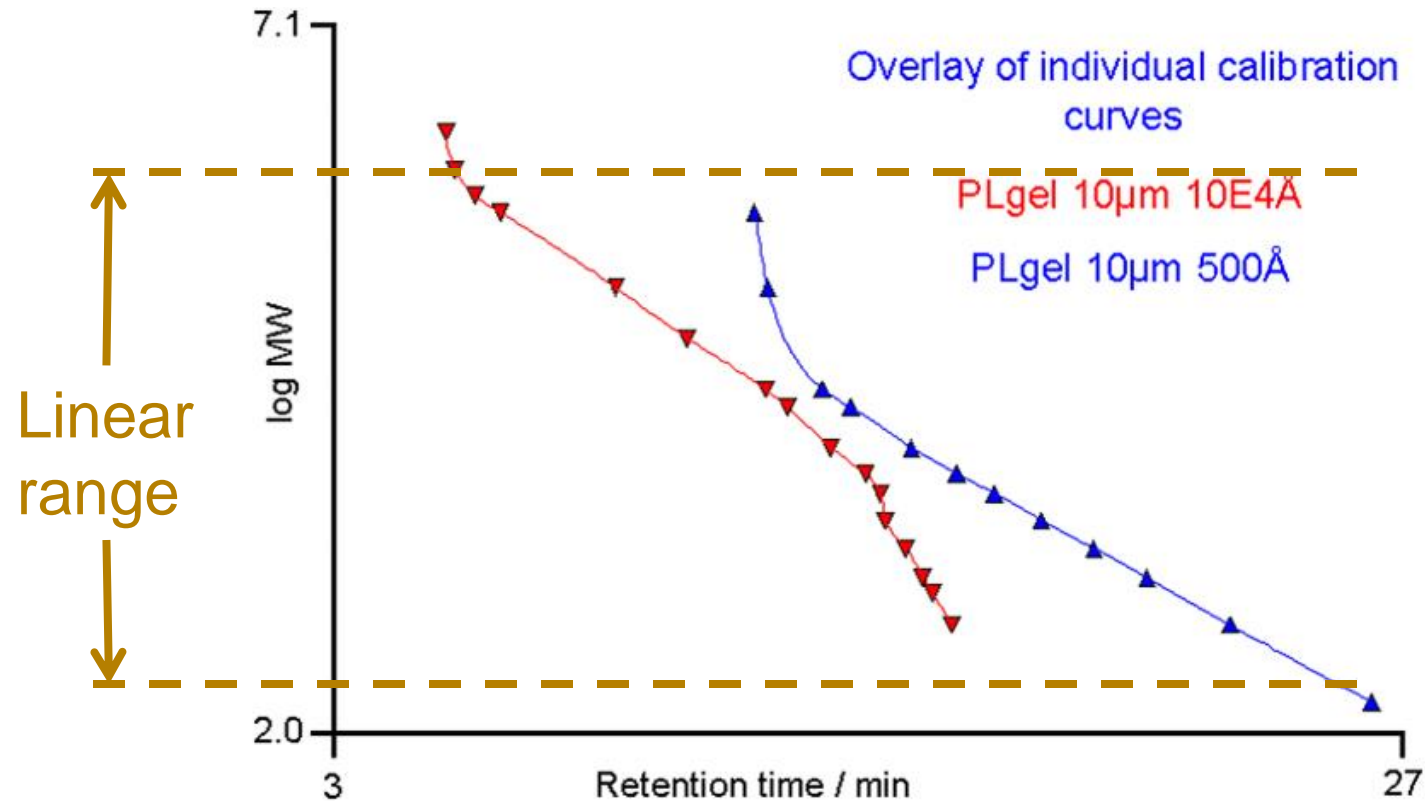
# Column Types: Individual Pore Size

- All particles have the same pore size
- Good separation, but narrow range of mol wt
- Very nonlinear curve; linear only over a narrow mol wt range
- Oldest technology, but still popular, and useful for separating very small and very large compounds
- Wider mol wt range possible by combining different columns in a series, but you need to select carefully so you not to have a column 'mismatch'



PLgel individual pore size calibration plots

# Increasing the Resolving Range

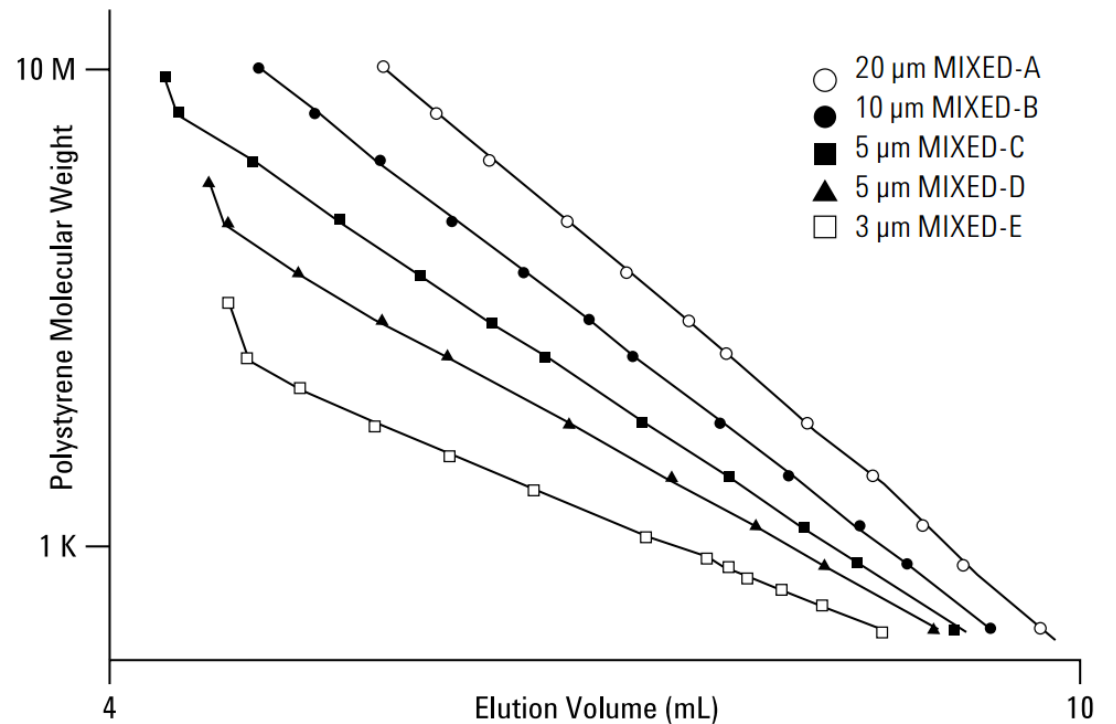


- Individual columns can be coupled in series
- Need linear calibration ranges to complement without overlap

# Column Types: MIXED

- Individual pore size particles are mixed together/blended to make a linear curve
- Very wide ranges possible, but only a small amount of separation of each mol wt
- Linear curve makes chromatogram easy to read and analyze
- Most popular technology, well established and widely used
- Columns in series of same type are still linear

Column family: PLgel

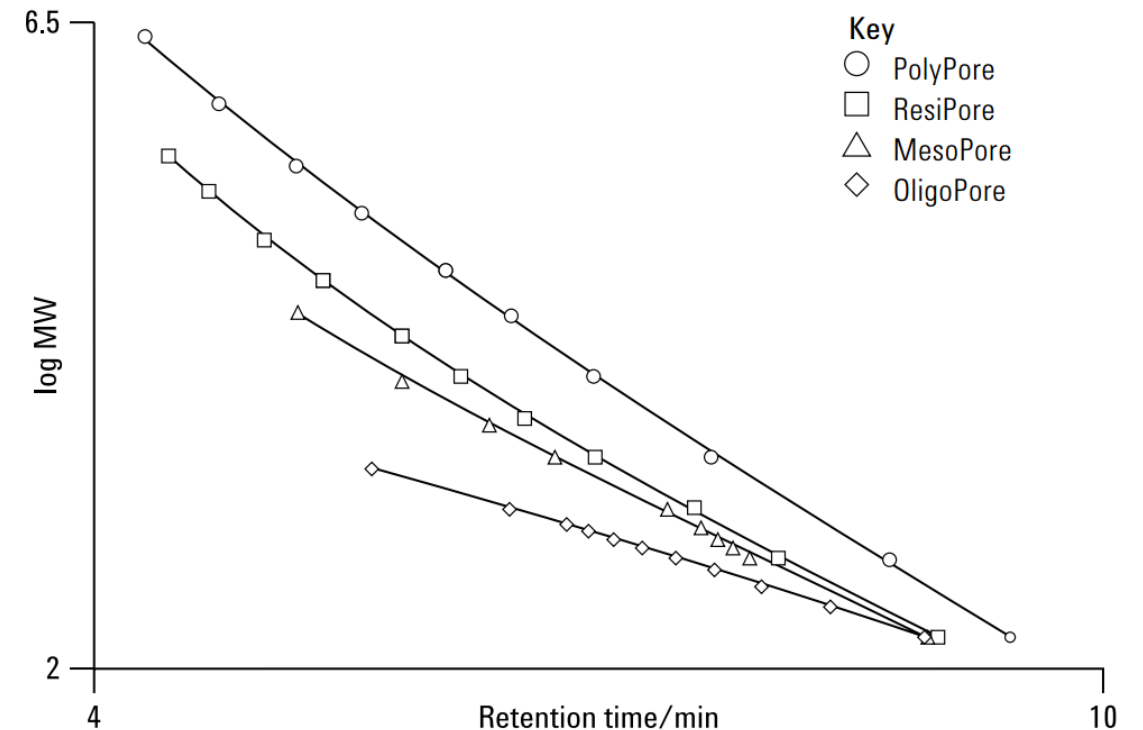


PLgel MIXED calibration plots

# Column Types: Multipore Particle

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

Column family: PlusPore



PlusPore calibration plots

# Agilent Columns for Polymer Applications Using Organic Solvents

## PLgel and PlusPore series

- Cover all molecular weight ranges
- High pore volume
- High efficiency
- Maximize resolution

## Unequalled solvent compatibility

- Easy transfer between polar and nonpolar eluents
- Outstanding physical rigidity
- Provides extended lifetimes that minimize downtime

Typical application areas include: polystyrenes, polyolefins, polycarbonates, polyurethanes, polysiloxanes, epoxy resins, polyester resins, silicone fluids, polyolefin waxes, prepolymers, resins, polyols

Available in multiple particle size options and column sizes ranging from narrow-bore to preparative



# Agilent Columns for Polymer Applications Using Polar Organic or Aqueous Solvents

## Polargel series

- Medium polarity surface
- High mechanical stability
- For use in intermediate and polar solvent combinations

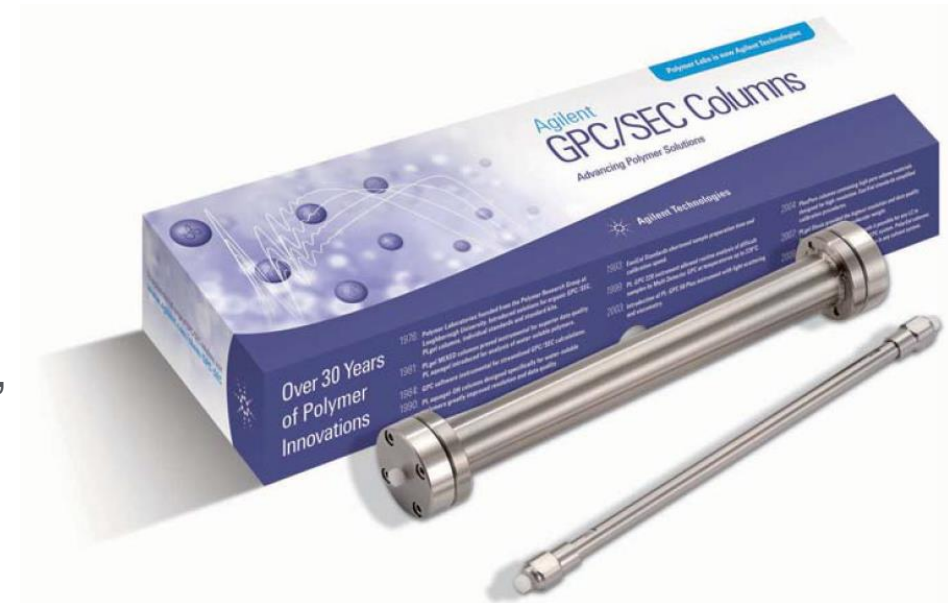
Typical application areas include resins, starches, acrylics, biopolymers, polysaccharides, polyamides

## PL aquagel-OH series

- Chemically and physically stable
- “Neutral” surface
- High performance analyses
- Neutral, ionic and hydrophobic moieties

Typical application areas include PEG, PEO, polysorbate, celluloses, dextran, and acrylamide

Available in multiple particle size options and columns sizes ranging from narrow-bore to preparative

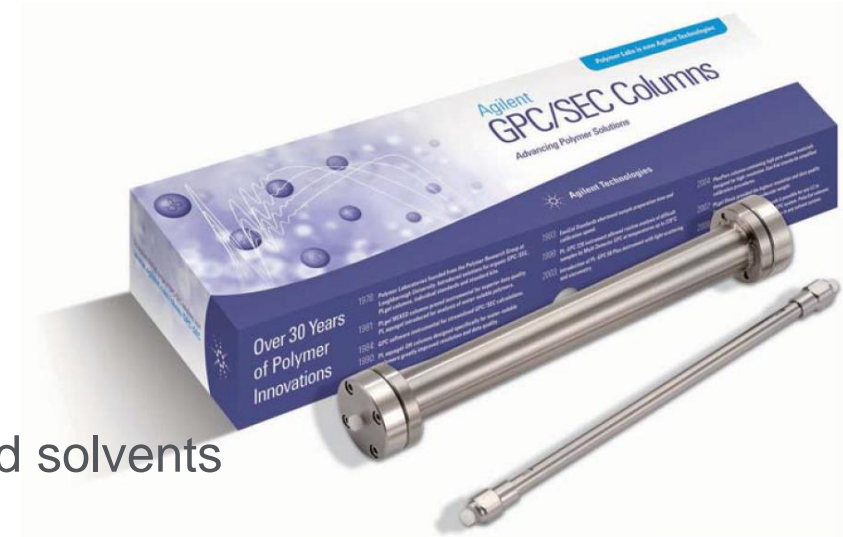


# Agilent Columns for Specialty Applications

## PL HFIPgel

- Chemically and physically stable
- Multiporous packing, optimized separation range
- For use in hexafluoroisopropanol, HFIP, and related polar fluorinated solvents

Typical application areas include polyesters, polyamides (nylon), polyethylene terephthalate (PET), and poly(lactic-co-glycolic acid) (PLGA)



## PLgel Olexis

- 13  $\mu\text{m}$  particles providing stability and resolution with no shear degradation
- Wide resolving range
- Excellent for use at very high temperatures
- Optimized for analysis of polyolefins and performance polymers

### Column dimensions

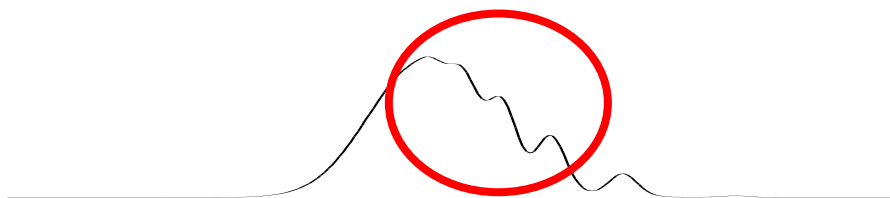
HFIPgel: 4.6 x 250 mm and  
7.5 x 300 mm

Olexis: 7.5 x 300mm

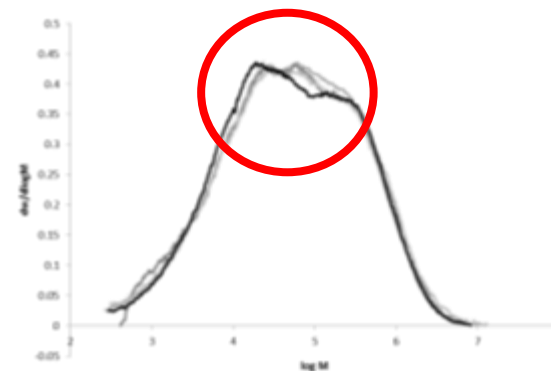
# GPC Column Selection

## Key Requirement

Resolution is too low



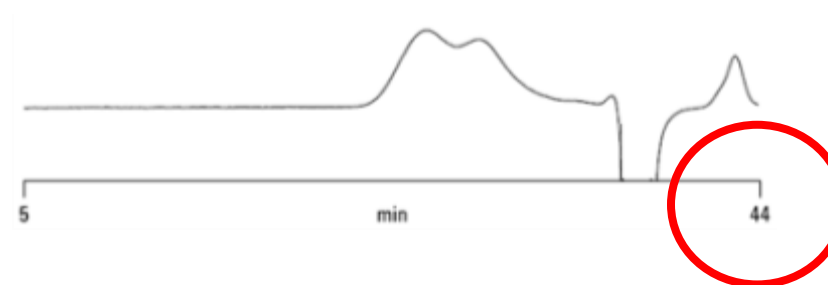
Results are not reproducible



Peak shapes are poor



Run times are too long



# GPC Column Selection

## Ways to improve resolution

Running two columns in a series using different pore sizes

- 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 pore volume increases the resolution

Use a packing with a smaller particle size

- Decreasing the particle size increases column efficiency

# GPC Column Selection

## How many GPC/SEC columns to use

More than one column is typically used

More columns = improved resolution

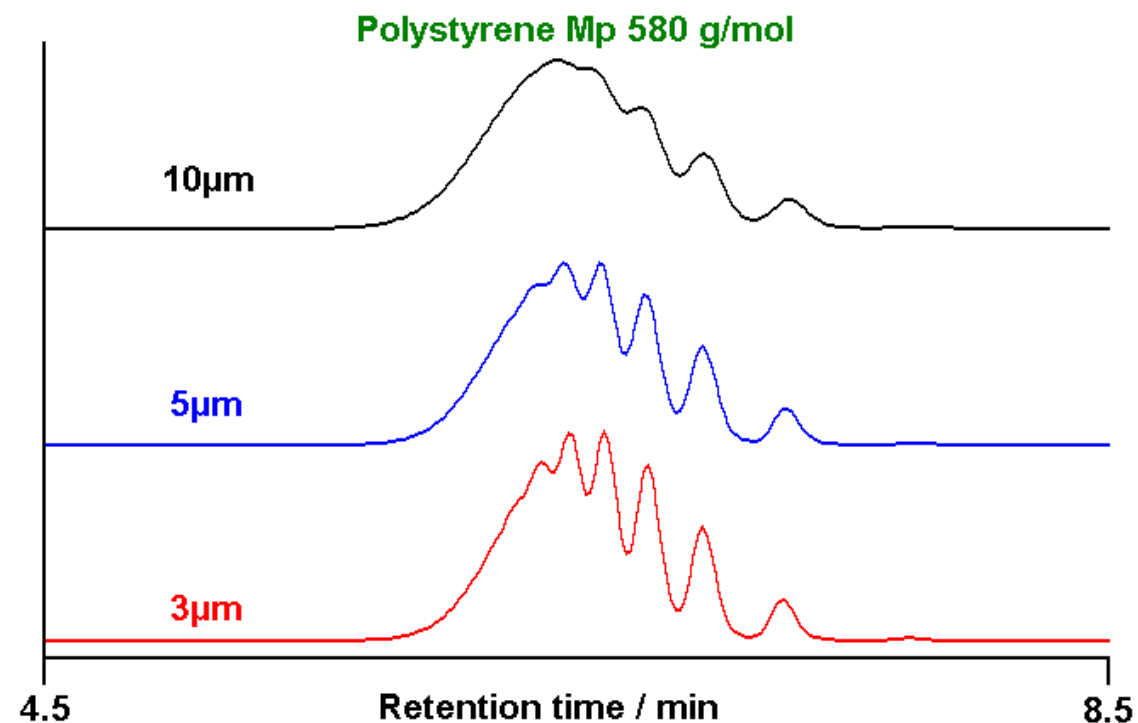
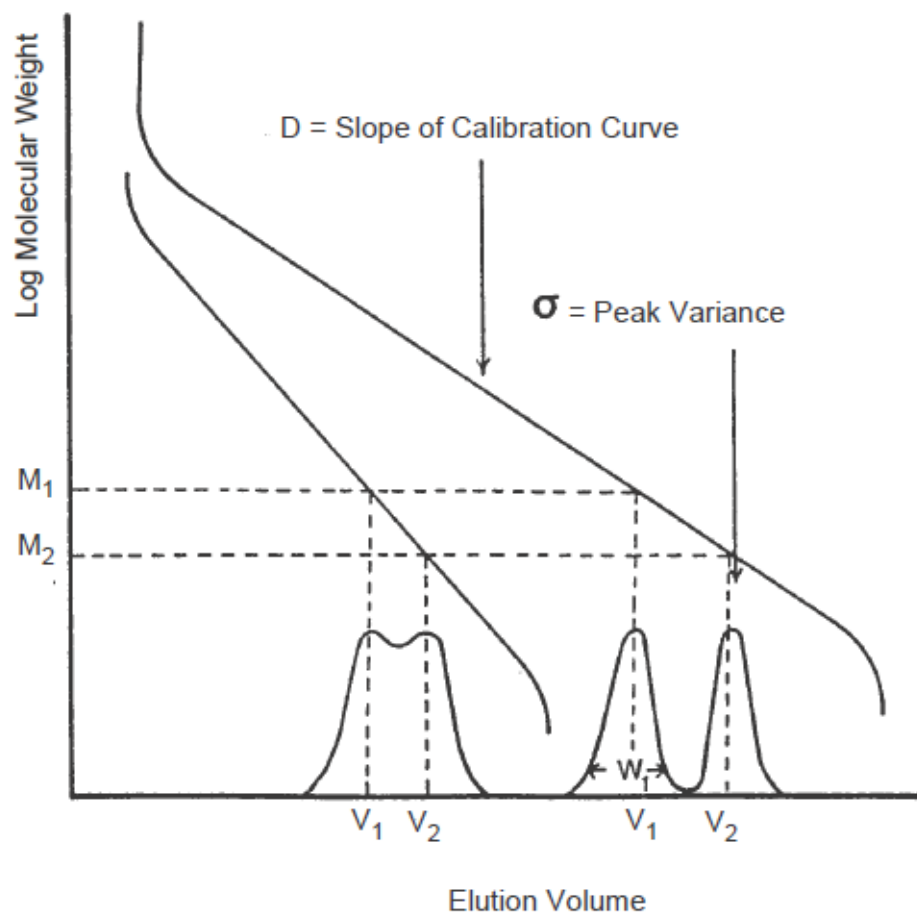
- The greater the particle size of the media in the column (which is dependent on the expected molecular weight of the samples), the lower the resolution. More columns will be required to maintain the quality of the results.
- For higher molecular weight samples, larger particles are necessary to reduce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



# Resolution Too Low

## Column length and particle size



Eluent: THF  
Flow rate: 1.0 mL/min  
Inj vol: 20  $\mu\text{L}$   
Detector: DRI

# GPC Column Selection

## Effect of column length on resolution

Columns: 1 x PLgel, 10  $\mu$ m, MIXED-B, 7.5 x 300 mm  
p/n PL1110-6100  
3 x PLgel, 10  $\mu$ m, MIXED-B, 7.5 x 300 mm  
p/n PL1110-6100

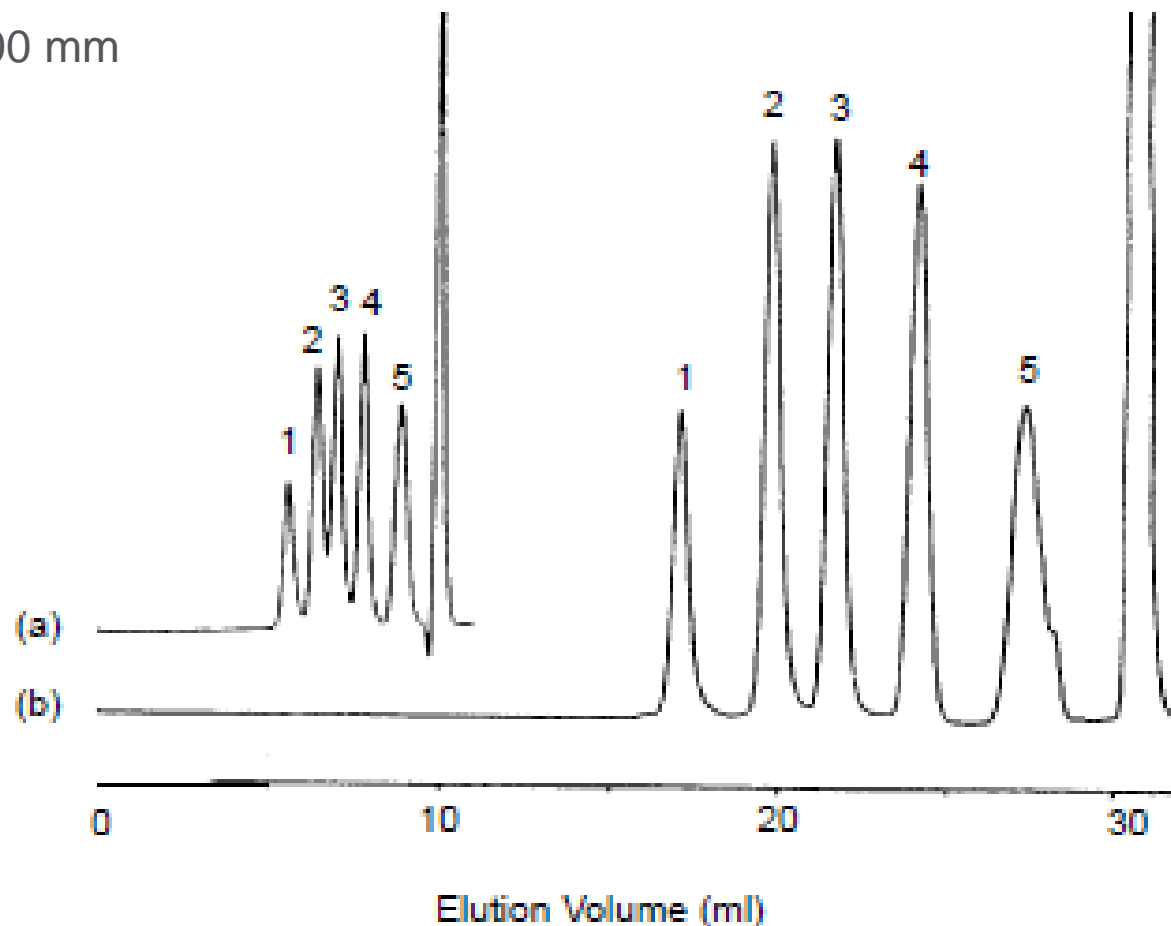
Eluent: THF

Flow rate: 1 mL/min

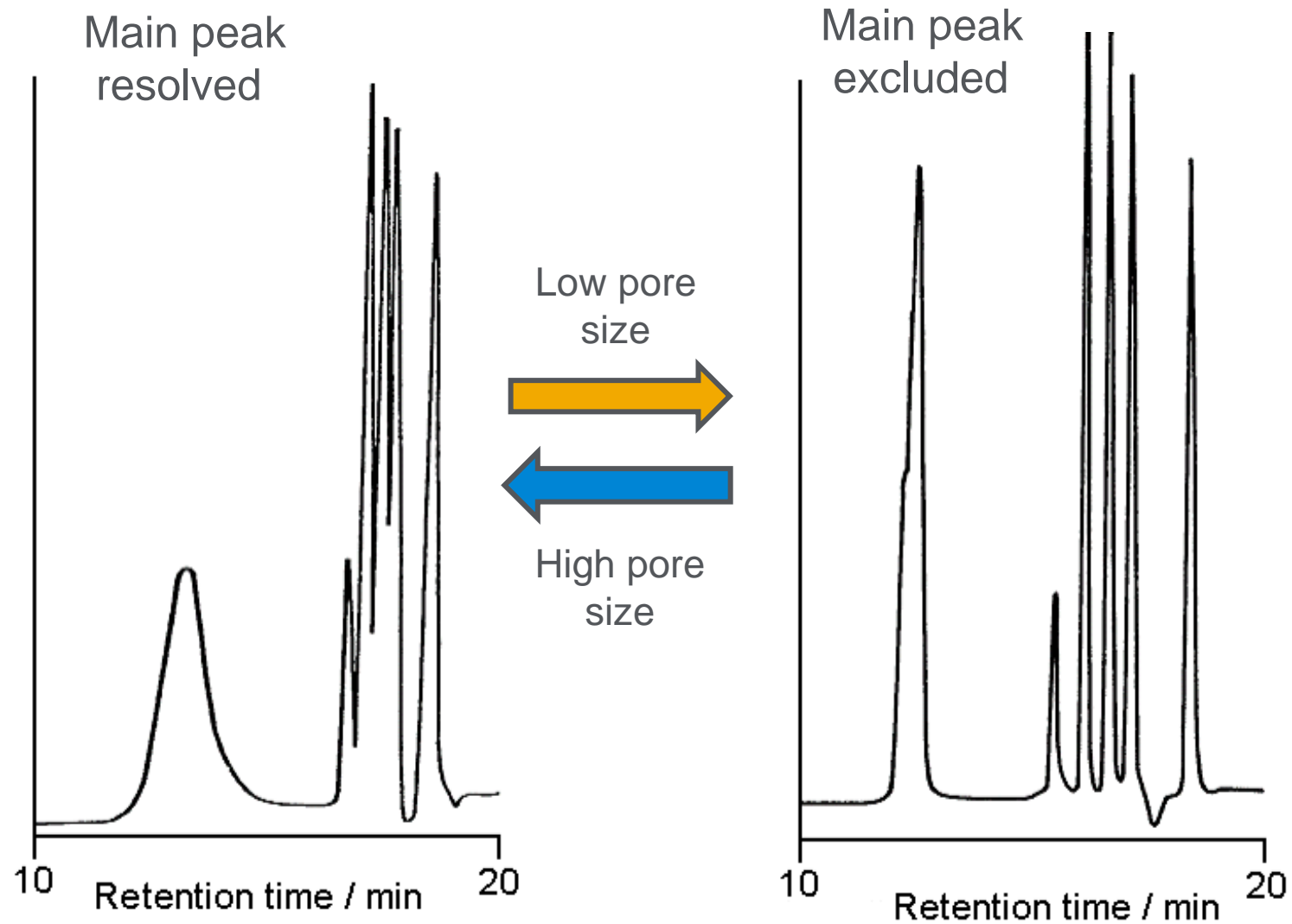
Detector: RI

Polystyrene standards  
Easical

1. 3,040,000
2. 330,000
3. 66,000
4. 9200
5. 580

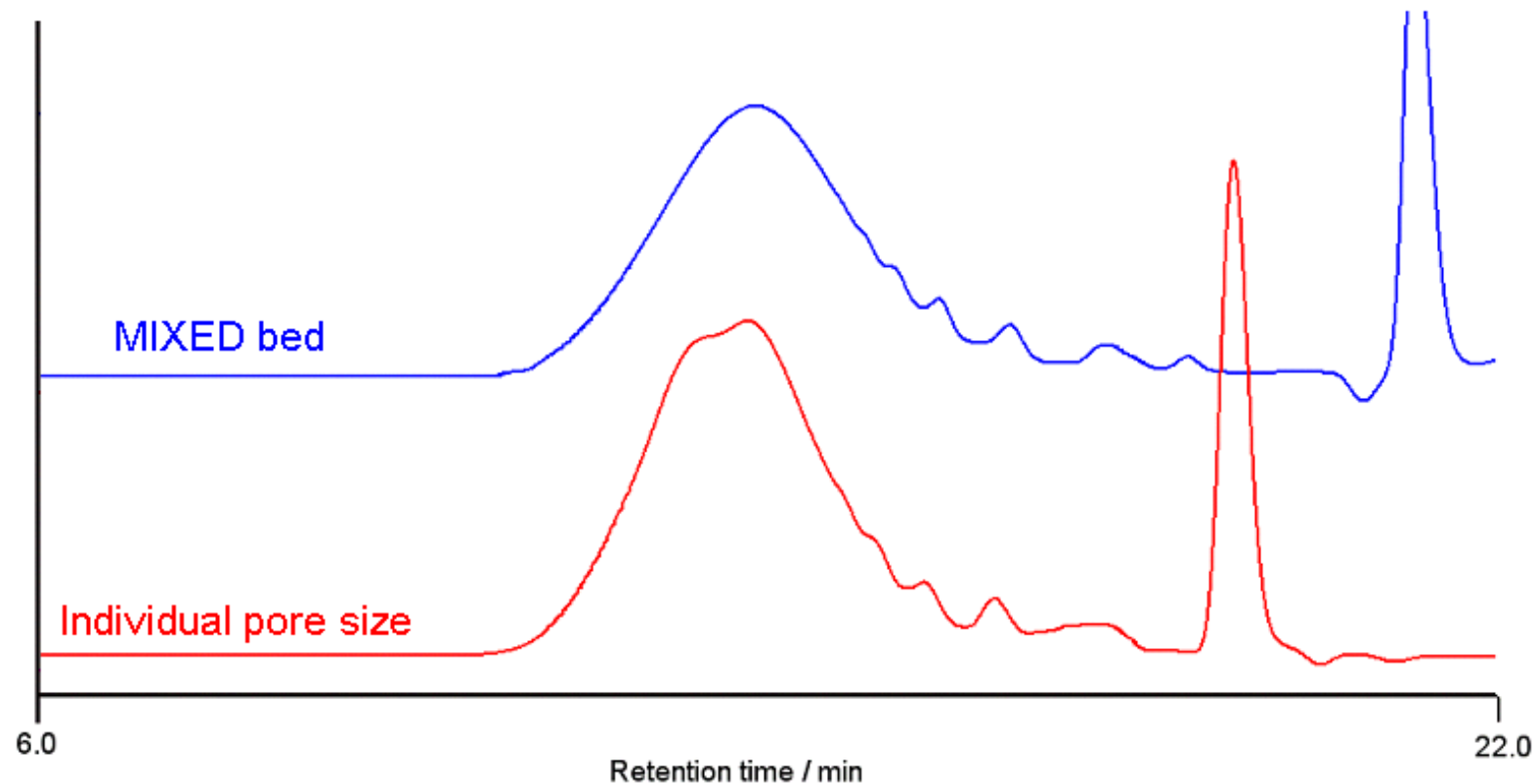


# Resolution: Pore Size Selection

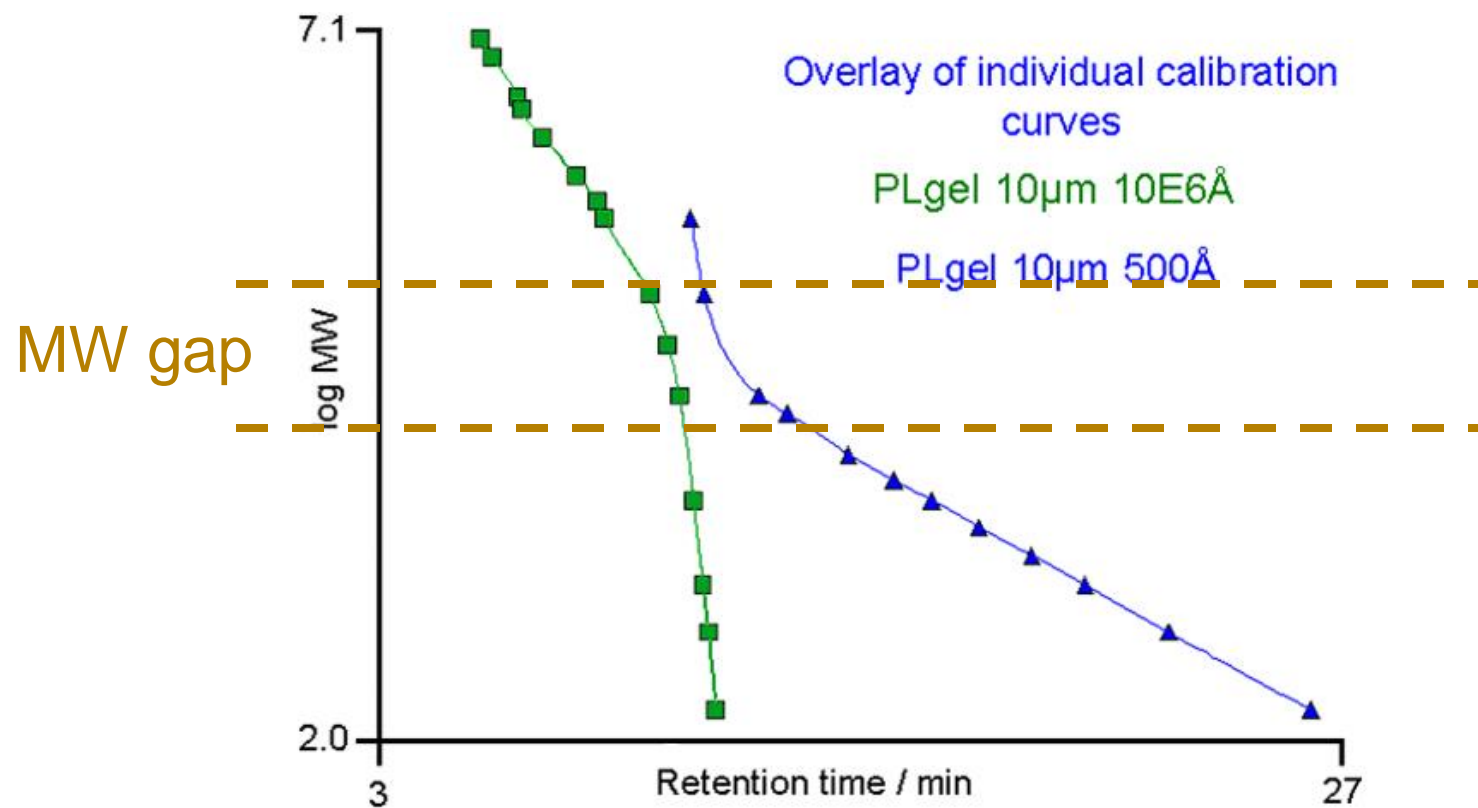


# Results Not Reproducible

Individual pore versus MIXED for our sample



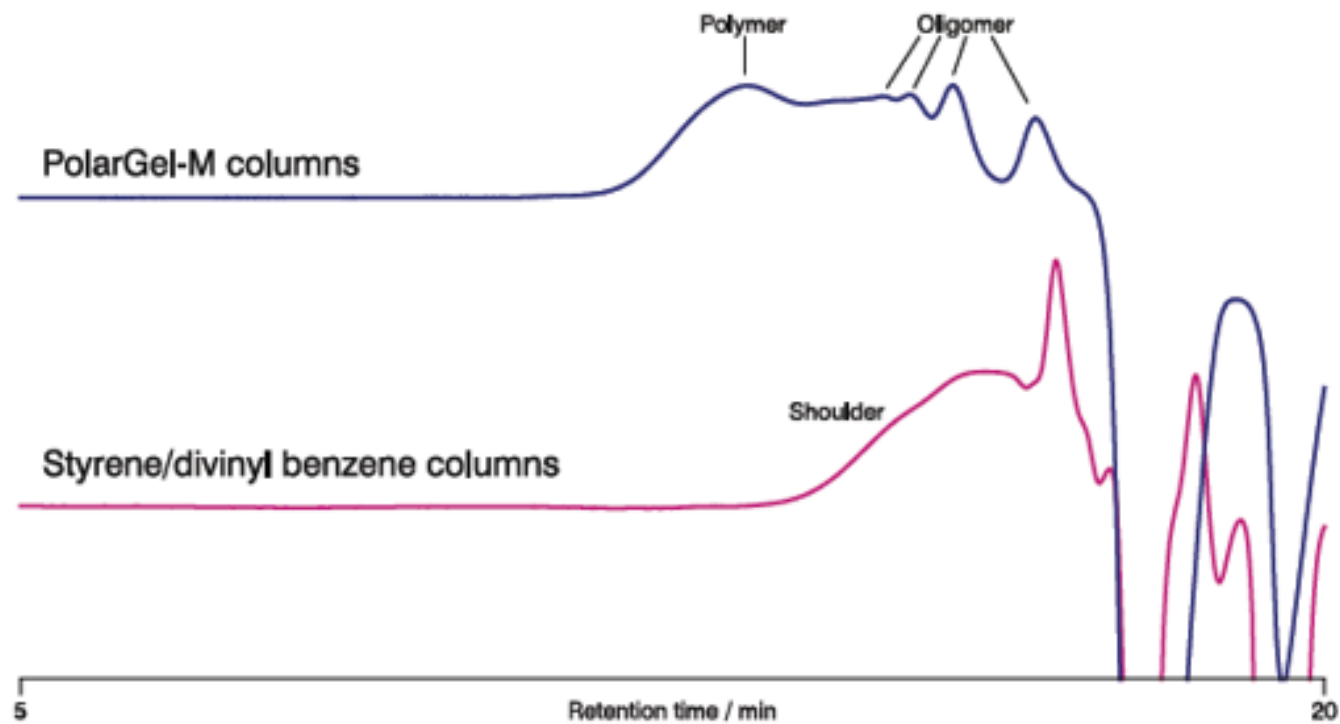
# Wrongly Coupled Columns



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

# Correct Column Choice

Peak shapes of polar compounds improved using Polargel



# Run Time Too Long

## Rapide columns for fast trend analysis

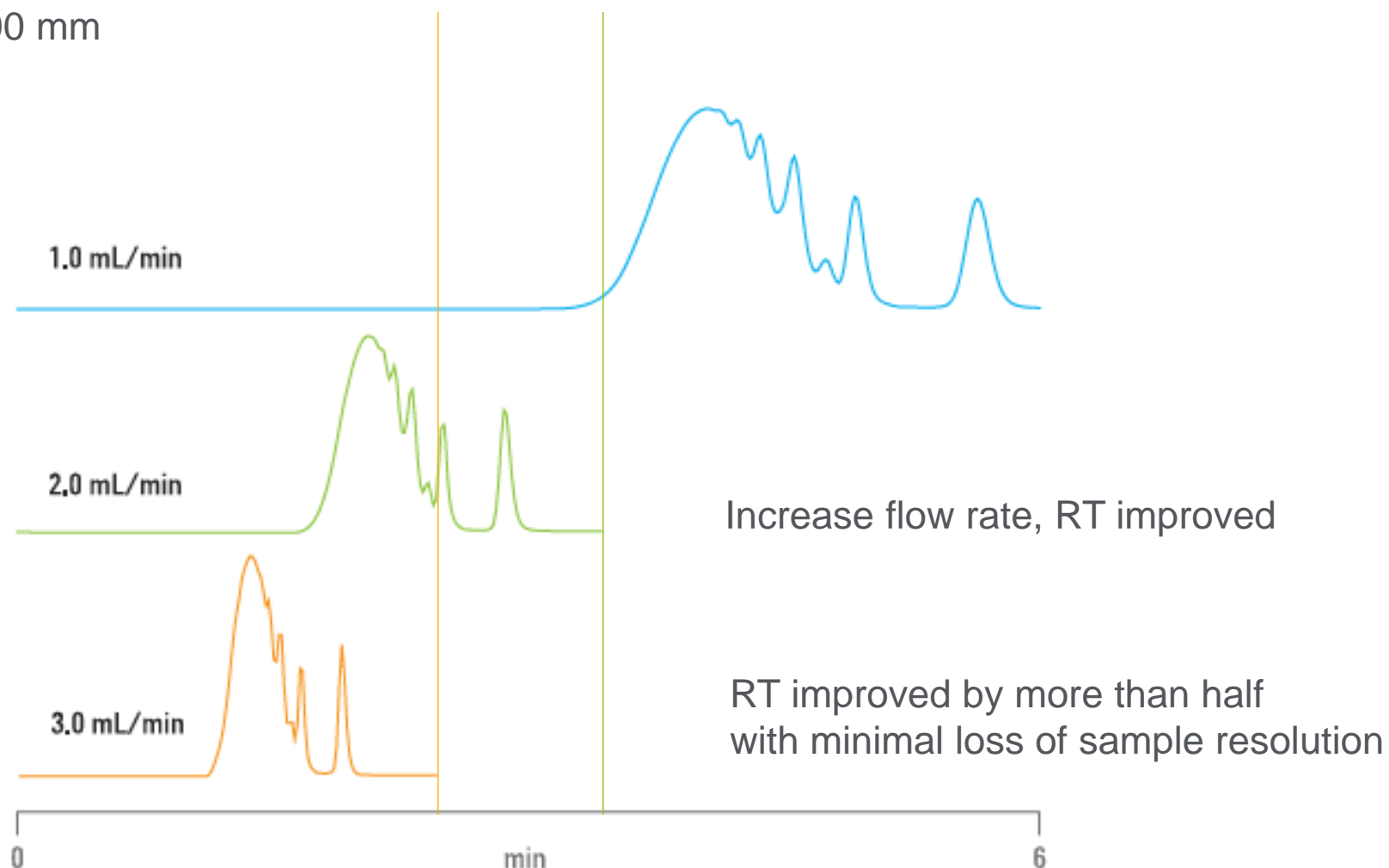
Column: PL Rapide L, 10 x 100 mm

Sample: Epoxy resin

Eluent: THF

Flow rate: As noted

Detector: UV, 254 nm



# Common Questions Around GPC/SEC Standards

What standards to use?



What is the eluent/mobile phase?

Solvent Type	GPC/SEC Standards Type
Organic	<ul style="list-style-type: none"><li>• Polystyrene (PS)</li><li>• Polymethylmethacrylate (PM)</li></ul>
Mixed or Polar Organic	<ul style="list-style-type: none"><li>• Polymethylmethacrylate (PM)</li><li>• Polyethylene glycol/oxide (PEG/PEO)</li></ul>
Aqueous	<ul style="list-style-type: none"><li>• Polyethylene glycol/oxide (PEG/PEO)</li><li>• Polysaccharide (SAC)</li><li>• Polyacrylic acid (PAA)</li></ul>

# Agilent Polymer Calibration Standard Offerings

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

- EasiVial – pre-prepared for fast and easy, accurate concentration, 12-point column calibration for organic and aqueous solvents
- EasiCal – easy 3-step process for accurate 10-point calibration, for organic solvents
- Calibration kits and individual standards – Polystyrene, PMMA, PEG/PEO, Polysaccharide



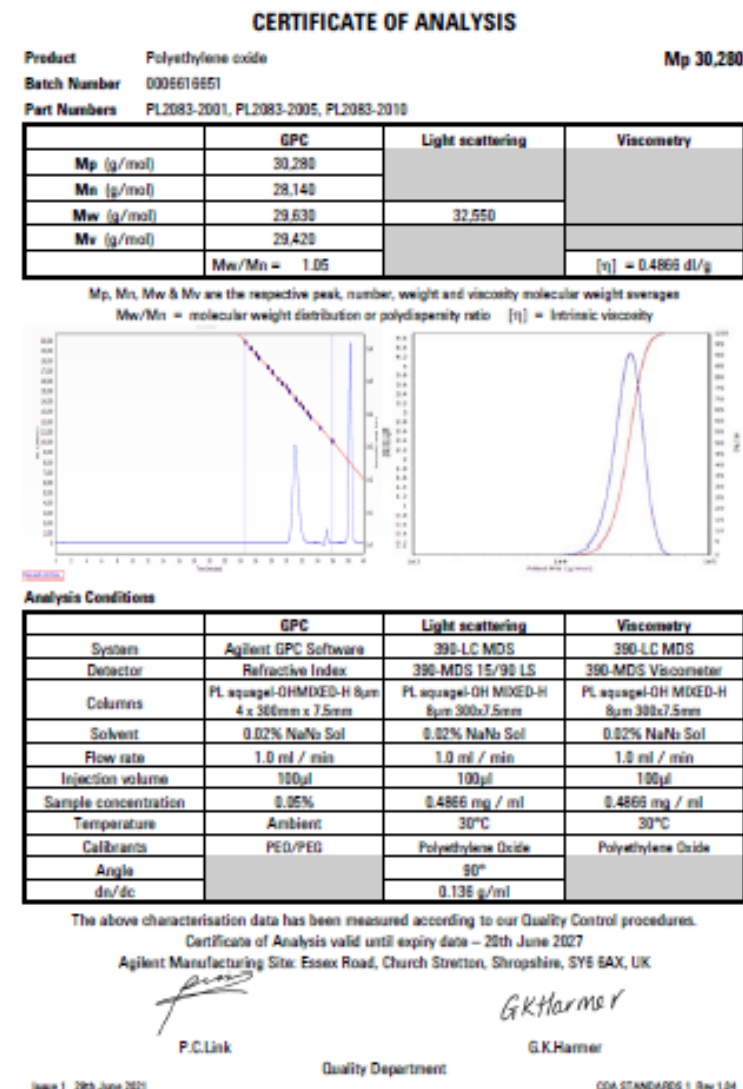
Agilent GPC/SEC Polymer Standards,  
publication number: 5994-7996EN

# 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).
- Finally, all CoAs include details of the exact method and characterization results for maximum transparency and reproducibility.

Example: Individual standard certificate



www.agilent.com



# 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

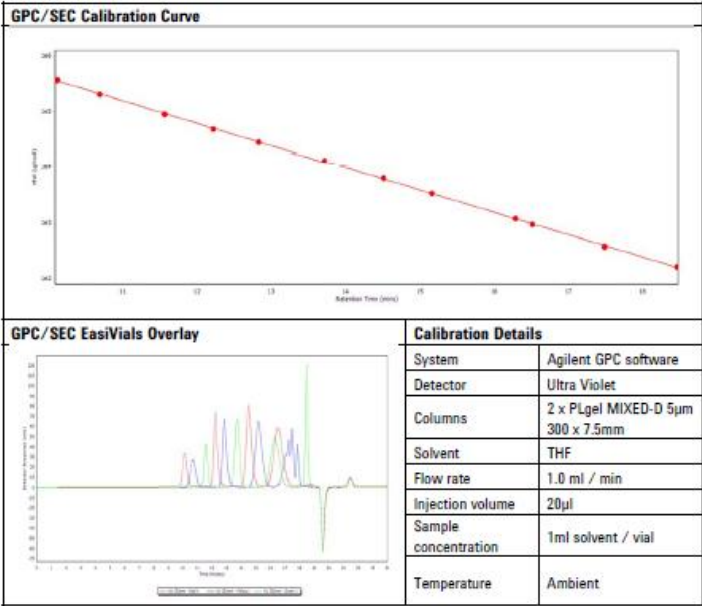
COA STANDARDS-3 Rev 2.21

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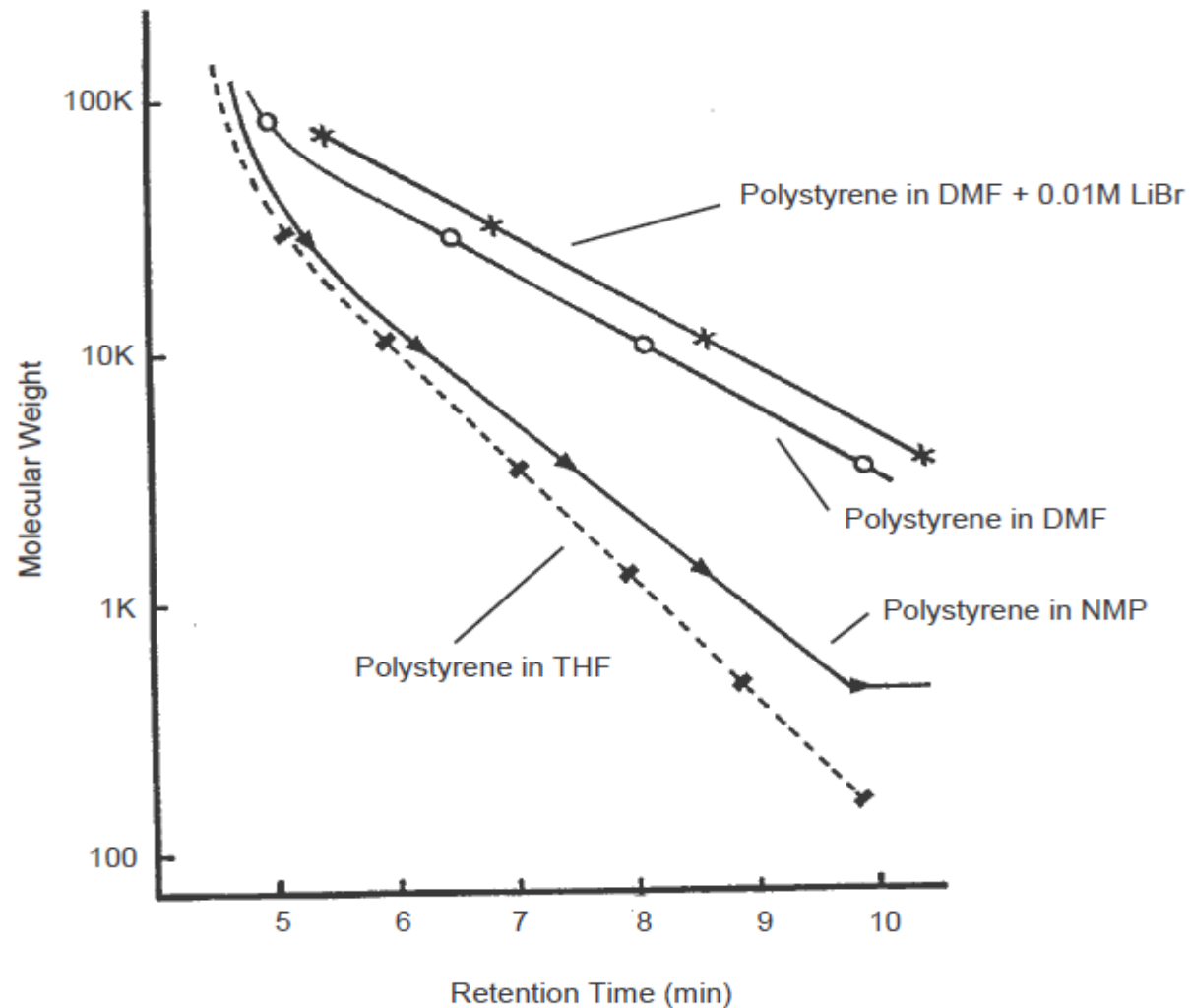
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# GPC/SEC Standards

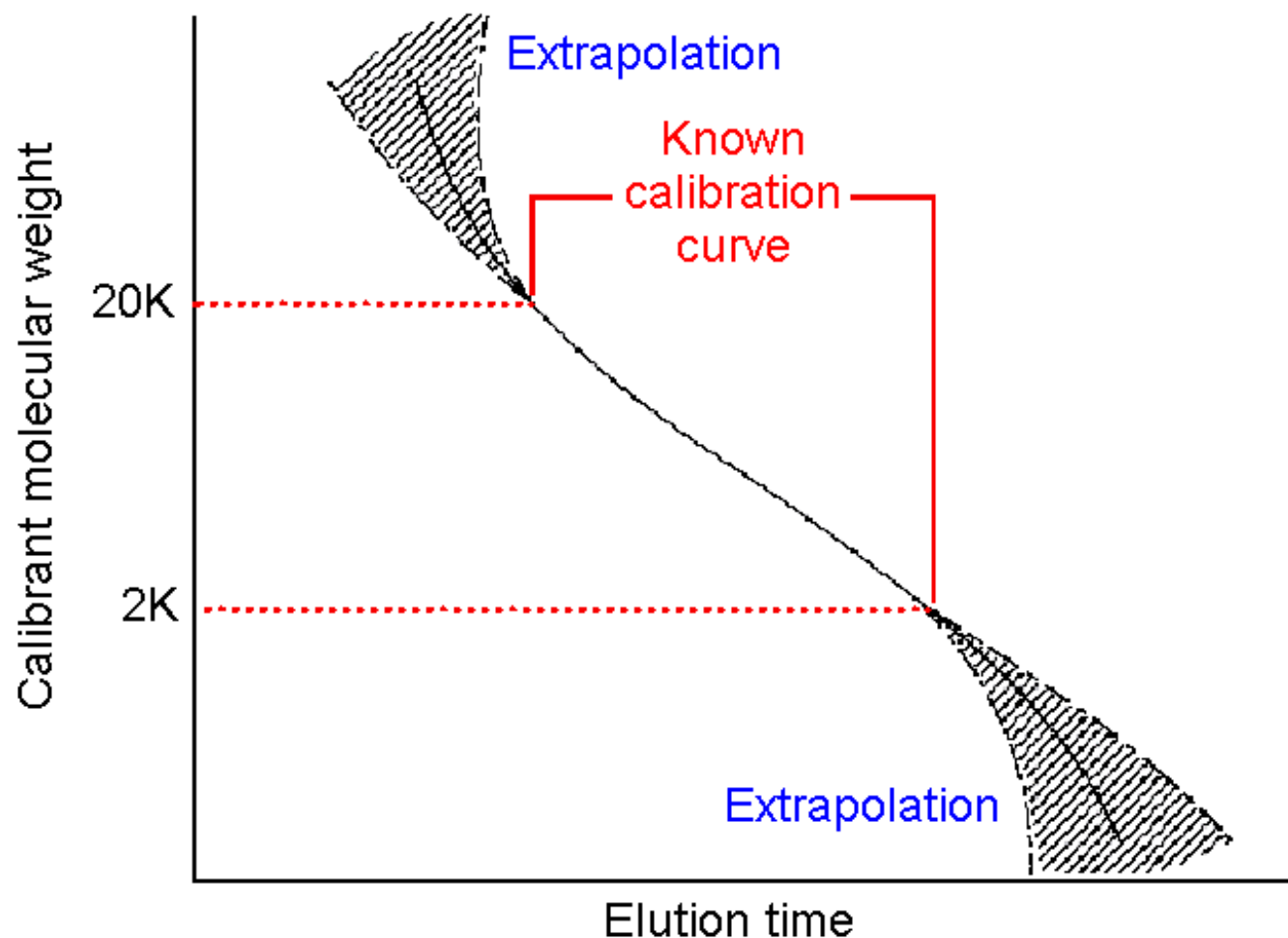
## Importance of solvent selection



Example: PS/DVB columns are excellent in many solvents, but remember that, although the column may be used in certain solvents, this does not mean SEC will occur. The example here is polystyrene standards running in NMP, DMF.

# Calibrate for the Column's MW Range

Importance for sample MW calculations

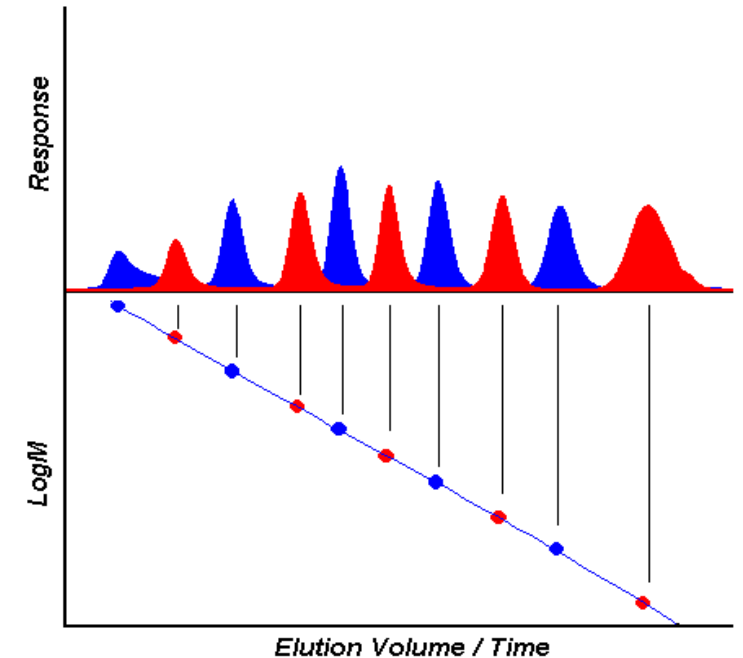


The choice of polymer standards for the column calibration should cover the full elution time region of the sample to avoid errors due to extrapolation.

# Common Questions Around GPC/SEC Standards

How often should you calibrate?

- Calibration frequency is subjective. At minimum, once a week is suggested if no major changes occur with system/columns.
- Recalibration is essential whenever a component of the system is altered or there is an eluent or column change.
- Calibrating frequently can also help identify potential issues quickly and corrective/preventative steps can then be put into effect immediately.

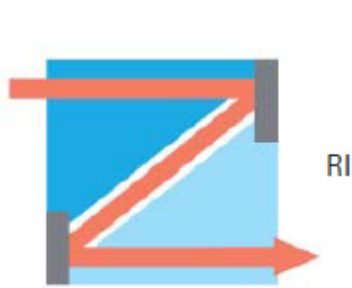


Publication number: 5991-2720EN

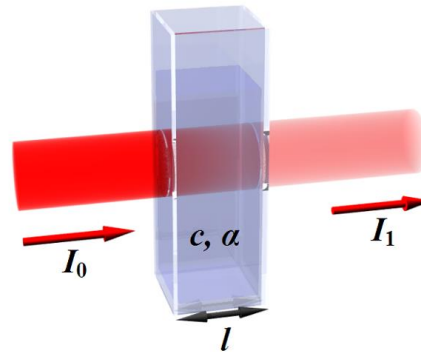
# Detectors

## Concentration detectors

Most common detectors for GPC/SEC are *concentration* detectors:



RID



UV/DAD

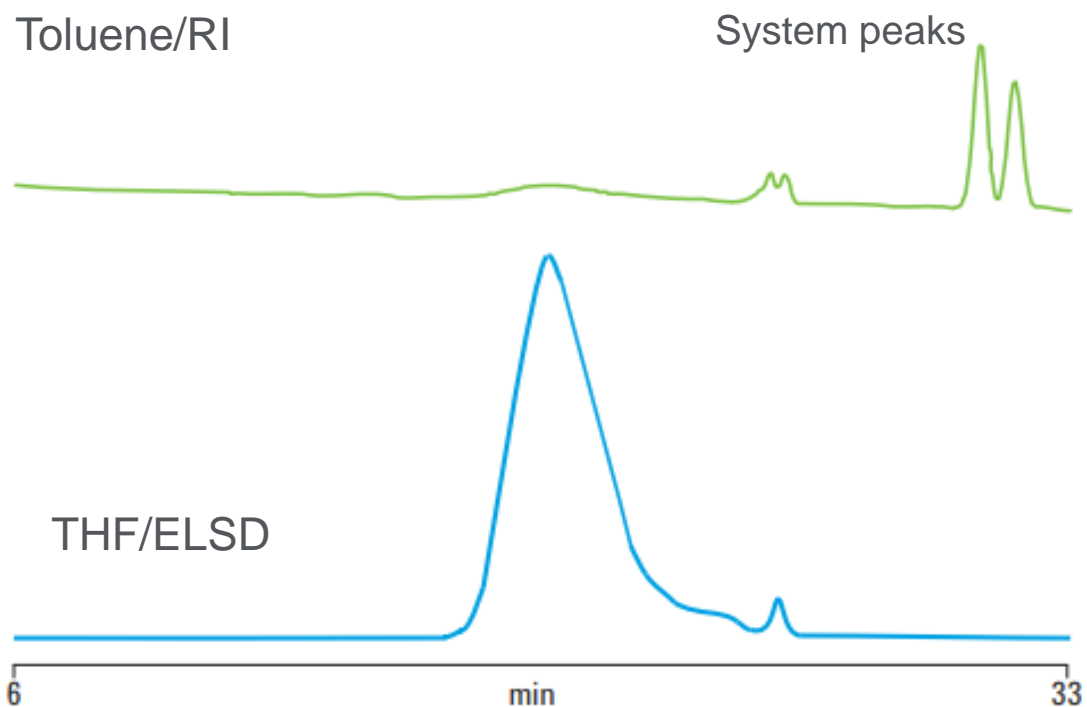


ELSD

These provide information on the amount of polymer or sample eluting from the column at any given time.

# System Detection

## Choice of solvent and detection



Column: 3 x PLgel, 5  $\mu$ m, MIXED-D  
7.5 x 300 mm, p/n PL1110-6504  
Eluent: Toluene or THF  
Flow rate: 1.0 mL/min  
Sample: Polysiloxane, 0.2% w/v  
Injection vol: 100  $\mu$ L

Application note publication number: 5990-7897EN

# 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 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

# 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 \(agilent.com\)](https://www.agilent.com/InfinityLab/LC_Supplies_Guide)

# Agilent Acquires Polymer Standards Service GmbH – PSS

## Broadening offerings for GPC/SEC polymer analysis – August 2, 2022



A Part of Agilent

Products from PSS to broaden and extend the Agilent portfolio

GPC/SEC/GFC chromatography columns

Polymer standards, reference materials, and kits

Chromatography systems and software



In the US and Canada, integration of the PSS GPC/SEC products is now complete, and columns and standards can be ordered through Agilent.

Visit Agilent website: [GPC/SEC Columns & Standards | Agilent](#)



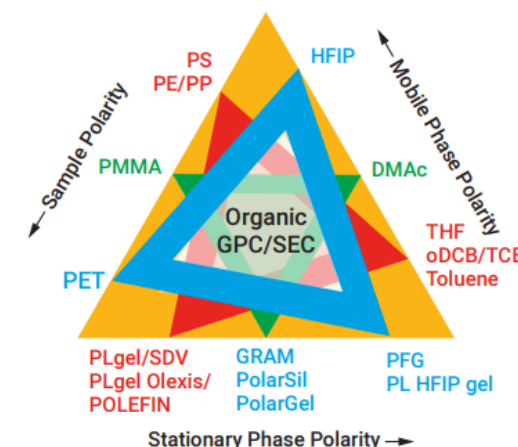
# Familiar PSS Products – Now a Part of Agilent

Column Product Name	Packing	Typical Solvents	Application Areas
SDV	Styrene divinylbenzene copolymer	THF, Toluene, TCM, DCM	Poly(styrene), poly(vinyl chloride), poly(carbonate), elastomers, resins and other
GRAM	Polyester copolymer	DMF, DMAc, NMP, DMSO	Polyurethane, polyimide, starches, cellulose, certain polyamide, other polar polymers
PolarSil	Polar modified silica	DMF, DMAc, NMP, DMSO	Low-to-medium molar mass resins and lignins
PFG	Polar modified silica	HFIP, TFE, other fluorinated solvents	Crystalline polymers, polyesters, polyamides, poly(lactides), POM
POLEFIN	Modified styrene-divinylbenzene copolymer	TCB, o-DCB, Decalin	Poly(ethylene), poly(propylene), other polyolefins

## Organic GPC Columns

- Column particle sizes: 3 µm to 20 µm
- Column sizes: analytical to preparative sizes
- Column types: Individual pore and MIXED/linear type column packings

[Organic GPC Columns | Agilent](#)



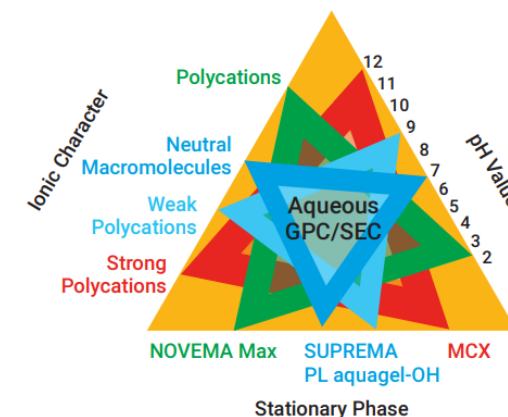
# Familiar PSS Products – Now a Part of Agilent

Column Product Name	Packing	Typical Solvents	Application Areas
SUPREMA	Modified acrylate copolymer	Water (with salts/buffers, MeOH, ACN) pH: 1.5 – 13	Neutral and anionic polymers (PEO, PEG, pullulan, dextran, poly(acrylamide), hyaluronic acid, poly(acrylic acid), carboxymethyl cellulose)
NOVEMA Max	NH-functionalized acrylate copolymer	Water, water with salt/buffer, MeOH, ACN, TFA; pH: 1.5 – 7.0	Cationic polymers, (polymeric quaternary ammonium Compounds, poly(DADMAC), poly(vinylpyridine), chitosan, poly(ethylene imine)
MCX	Sulfonated styrene-divinylbenzene copolymer	Water, water with salt/buffer, MeOH, ACN; pH: 7 – 13	Sulfonated polyanions, poly(styrene sulfonate), lignin sulfonate Modified starches, acids, alcohols, pectins
PROTEMA	Special modified silica	Water, water with salt/buffer, MeOH, ACN; pH: <7	Natural and synthetic proteins, peptides, enzymes, gelatins/collagens

Column particle sizes: 5 µm and 10 µm, 3 µm and 5 µm in PROTEMA

Column sizes: Analytical to preparative sizes

Column types: Individual pore and MIXED/linear type column packings



# Other PSS Products – Now a Part of Agilent



A Part of Agilent

Polymer standards, reference materials, and kits – many polymer options

- Individual Standards
- GPC/SEC calibration kits – conventional kits, ReadyCal
- Validation kits – ReadyValid
- Specialty polymers and particle standards

Chromatography systems, detectors, and analysis software



Agilent weblink: [GPC/SEC Standards | Agilent](https://www.agilent.com/chem/pss/gpcsecstandards)



# GPC/SEC Columns and Supplies Resources

- Agilent webpage GPC/SEC: [GPC/SEC Columns & Standards | Agilent](#)
- **Expanded** Portfolio GPC/SEC Columns and Standards: [Agilent GPC/SEC Columns and Standards Brochure](#)
- Organic GPC Columns: [Organic GPC Columns | Agilent](#)
- Aqueous SEC Columns: [Aqueous SEC Columns | Agilent](#)
- GPC/SEC Polymer Standards: [GPC/SEC Standards | Agilent](#)
- GPC/SEC User Guide: [GPC/SEC column user guide](#)
- Polymer to Solvent Reference Table: [Polymer to Solvent Reference Table](#)
- GPC Troubleshooting poster: [GPC Troubleshooting Guide](#)
- InfinityLab Supplies catalog: [InfinityLab LC Supplies \(agilent.com\)](#)
- Consumables Community: [Agilent Collection of Columns, Supplies, and Standards Resources - Consumables - Agilent Community](#)
- App finder: [Application Finder | Agilent](#)
- Agilent University: [Agilent University](#)
- 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](mailto:gc-column-support@agilent.com)

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