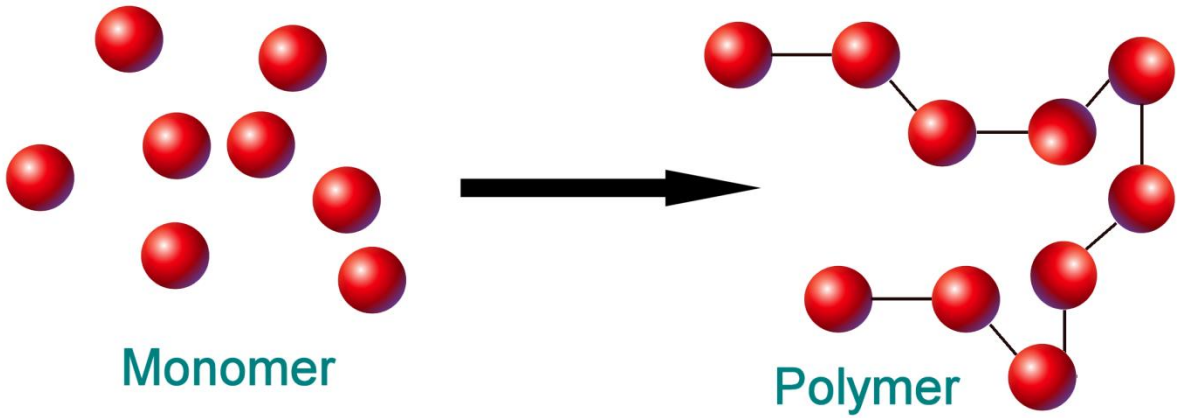
Maximizing Performance Through GPC Column Selection

Agilent Technologies

What Are Polymers?

- Polymers are long chain molecules produced by linking small repeat units (monomers) together
- There are many ways to link different types of monomer to form polymers
- Polymers exhibit very different physical properties compared to the monomers, dependent on the length of the polymer chains
- The presence of small amounts of very long or very short chains can have drastic effects on properties of the material



The Primary Goal of GPC is to Discover the Mw Distribution

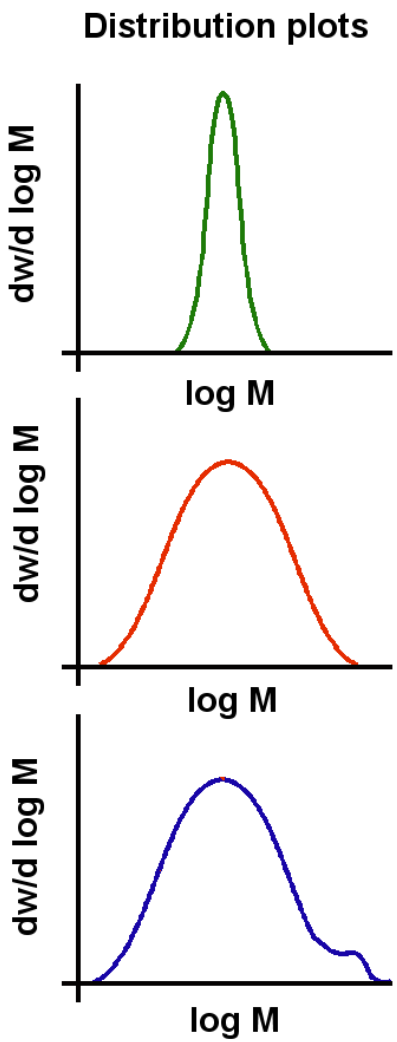
- Samples of synthetic polymers *always* contain polymer chains with a range of chain lengths
- One way to describe the length of the polymer chains is in terms of an average molecular weight, i.e the average of all the chain lengths in the sample

HOWEVER….

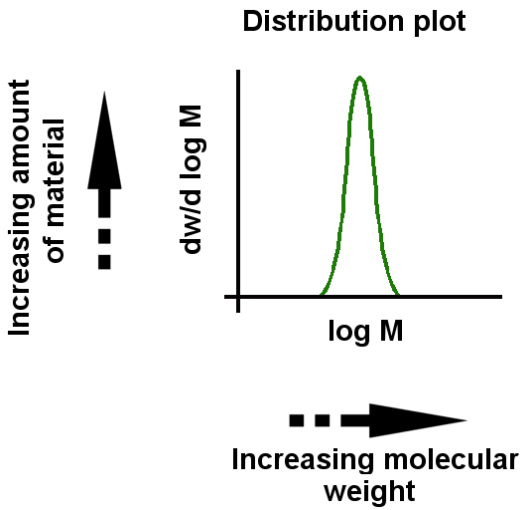
- Different samples of the same polymer can have the same average chain length but very different distributions of chain lengths depending on the method of production
- In polymer science it is the molecular weight *distribution* that is important

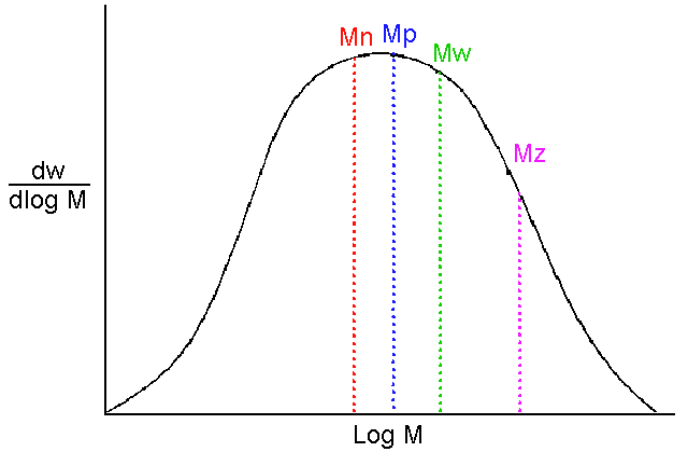
Why is the Mw Distribution Important?

- Even for the same type of polymer, each of these distributions will describe a polymer that behaves differently
- The red and green plots are for low and high polydisperity materials
- The blue plot shows a high polydispersity material with a additional high molecular weight component
- Describing these distributions is not easily, especially if they are complex



Defining the Molecular Weight Distribution

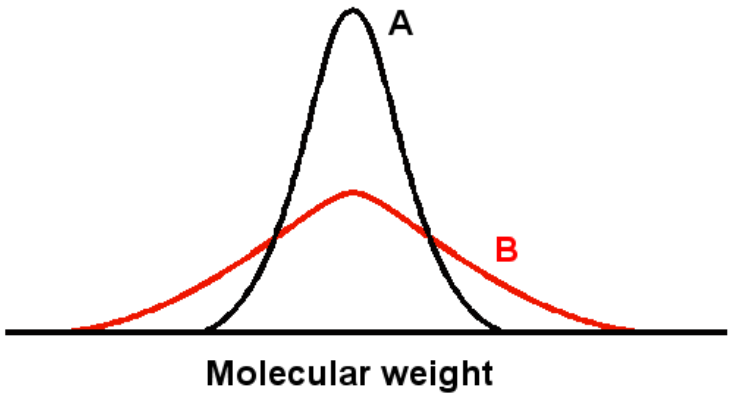




- A molecular weight distribution can be defined by a series of average values
- Except Mp, these are various moments of the average of the molecular weights of the distribution
- Mp is the molecular weight of the peak maxima
- For any polydisperse peak:

Mn<Mw<Mz<Mz+1

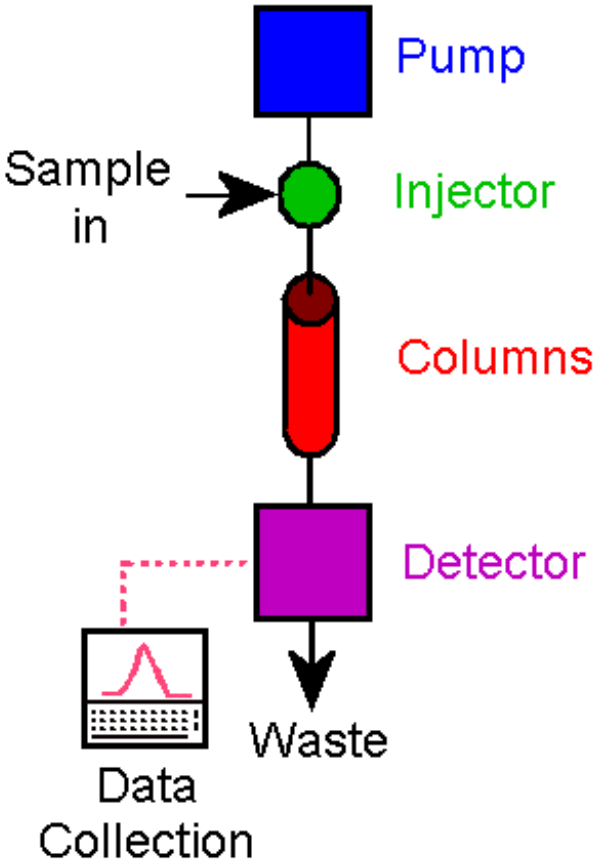
Effect of Mw and Polydispersity on a Polymer



- As the broadness of the the distribution decreases the strength and toughness of the polymer increases
- However as the broadness of the the distribution decreases the polymer becomes more difficult to process
- GPC provides key information to predict the processability and material properties of a polymer

	Strength	Toughness	Brittleness	Melt viscosity	Chemical resistance	Solubility
Increasing Mw	+	+	+	+	+	-
Decreasing distribution	+	+	-	+	+	+

What Equipment is Needed to do GPC



1. Reciprocating piston ***pump*** delivers eluent from reservoir at a constant volumetric flow rate. GPC is always an isocratic separation

2. Two position ***injection valve*** permits introduction of sample solution without interrupting solvent flow. GPC tends to use larger injection volumes than HPLC (typically up to 200ul)

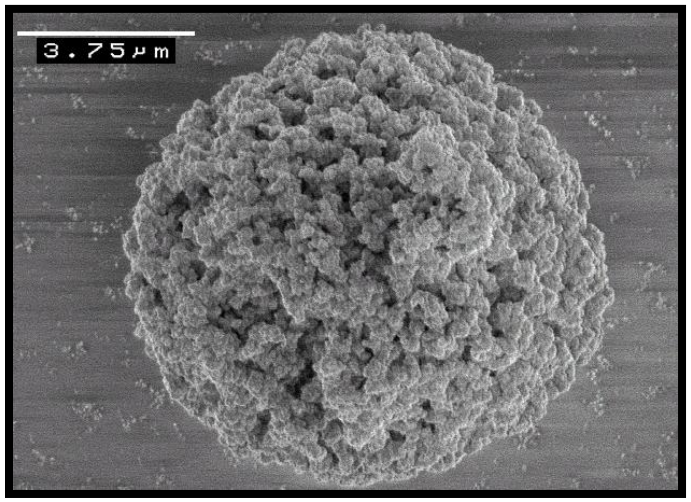
3. ***GPC columns*** perform a separation based on the molecular size of polymer molecules in solution. Resolution and/or resolving range is increased by use of multiple column systems

4. ***Detector*** responds to concentration of polymer molecules eluting from the GPC columns

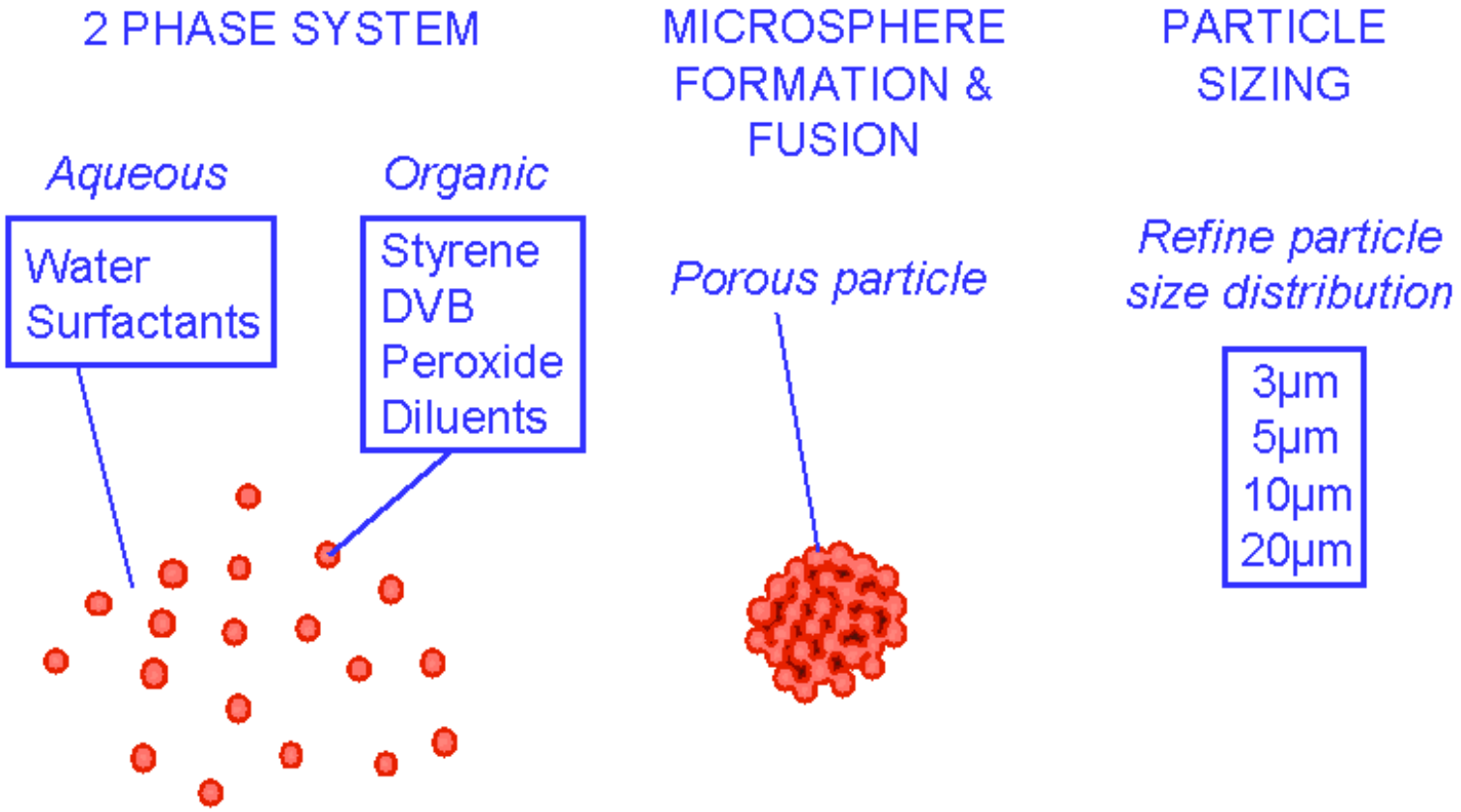
What Are GPC Columns Made Of?

Silica Packings = Mechanically Stronger However Exhibit Enthalpic Properties Due to Presence of Silanols. Also Tyically Have Lower Pore Volumes

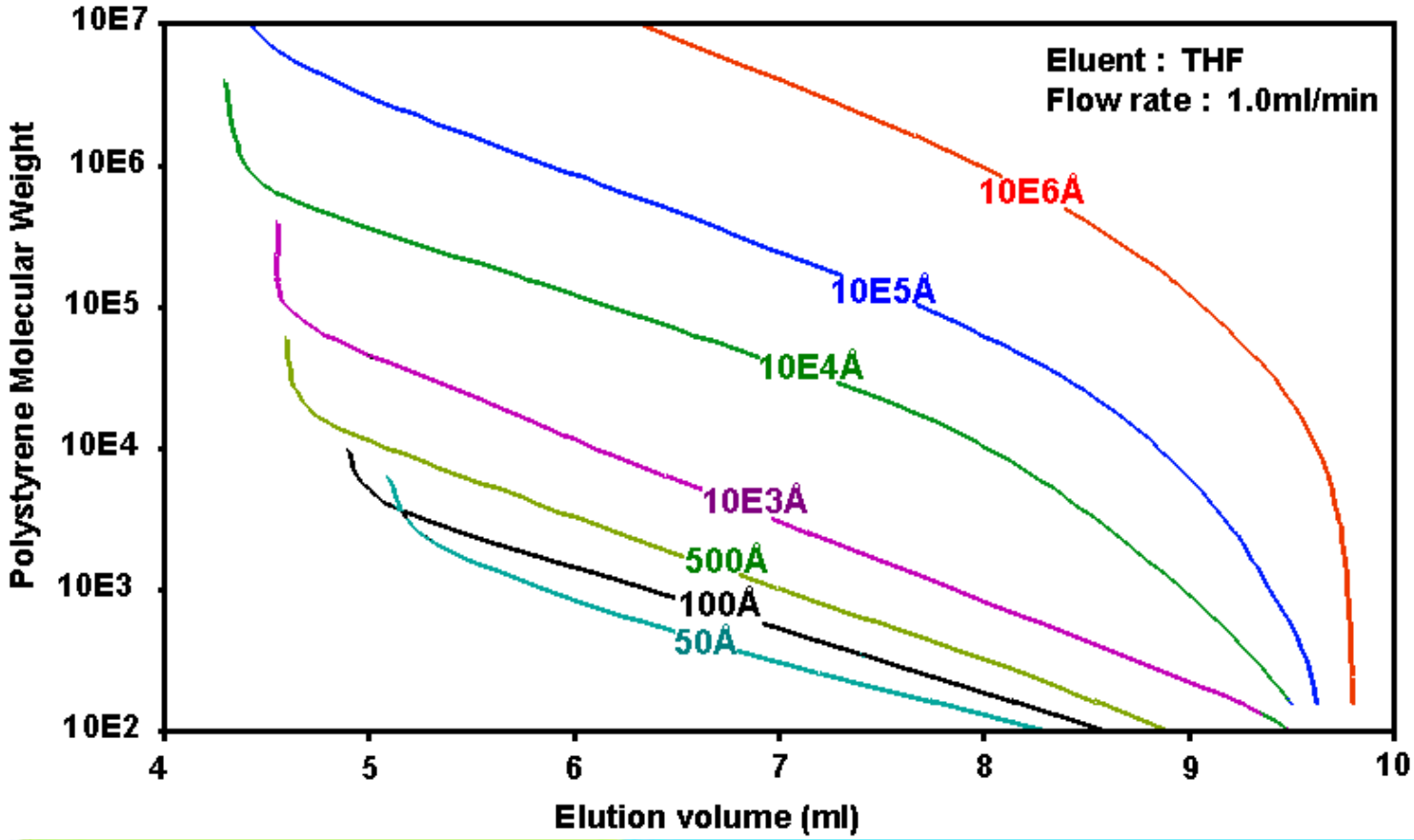
Polymeric Packings = High Pore Volume and Vendor Specific Differences in Mechanical Stability. Due to Polarity of Stationary Phase Observed Interactions are Reduced



Porous Particles Produced by Suspension Polymerization



Individual Pore Size Column Calibration Curves



Column Types

- The factor that principally controls which type of column is selected for a GPC experiment is the solvent
- Many polymer dissolve in only very limited numbers of solvents
- The columns used must be compatible with the solvent of choice
- Most importantly, the size exclusion mechanism must be maintained
- Agilent have two main ranges of GPC column for organic solvents
- The properties of each range that must be considered when selecting them for an application shall be presented

Styrene/Divinyl Benzene Columns - PLgel

PLgel is a highly crosslinked porous polystyrene/ divinylbenzene matrix which is recognized as a market leader in GPC column technology.

Manufactured and packed exclusively by Polymer Laboratories since 1976, PLgel has very special features:

- High pore volume and high efficiency for maximum resolution
- Unequalled solvent compatibility for transfers between polar and non-polar organic eluents
- Outstanding physical rigidity for extended lifetimes especially at high temperatures and in aggressive solvents

Solvent Compatibility

PLgel columns are routinely supplied in toluene*, however, they can be transferred easily and rapidly between solvents of varying polarity by the User. In organic GPC, sample to column interaction can occur occasionally and eluent modification can be used to eliminate the effects. PLgel columns are the ideal choice for these analyses, as they easily tolerate eluents in the pH range 1-14, as well as up to 10% water, in a miscible organic solvent.

Temperature Stability to 220°C

Elevated temperature is used in GPC either to reduce eluent viscosity (eg polar solvent applications), or to maintain sample solubility (eg polyolefin applications). PLgel columns can be used at temperatures up to 220°C and operating pressures up to 150 bar (2200psi).

PLgel Solvent Compatibility

- Columns can be used in a wide range of solvents
- Transfer between solvents is possible assuming care is taken
- The column user guide details the procedure

	Solvent Polarity	Solvent	PLgel Compatibility
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 pyrolidone (NMP)	✓
↓	12.0	Dimethyl sulphoxide (DMSO)	✓
High	12.1	Dimethyl formamide (DMF))	✓

PL Aquagel-OH Columns

Aqueous size exclusion chromatography (SEC) is widely used for the determination of molecular weight distributions of a variety of synthetic and naturally occurring water soluble polymers, and separations of oligomers and small molecules. The requirement to eliminate ionic and hydrophobic effects makes aqueous SEC very demanding.

The PL aquagel-OH series of columns provides a chemically and physically stable matrix for reliable aqueous SEC separations.

High Performance PL aquagel-OH Columns for Aqueous SEC

PL aquagel-OH columns are packed with macroporous copolymer beads with an extremely hydrophilic polyhydroxyl functionality.

The 'neutral' surface and the capability to operate across a wide range of eluent conditions provide for high performance analyses of analytes with neutral, ionic and hydrophobic moieties or combinations thereof.

PL aquagel-OH Features:

- pH range 2 - 10
- compatible with organic solvent, up to 50% methanol
- mechanical stability up to 140 bar (2000psi)
- low column operating pressures
- 8µm columns > 35,000 plates/m
- 15µm columns > 15,000 plates/m
- 5µm columns > 55,000 plates/m

PL aquagel-OH 8µm Analytical Columns

- PL aquagel-OH MIXED-H and M 8µm columns, high resolution over a very wide range of molecular weight, simplifying column selection and providing a versatile analytical system.
- PL aquagel-OH 30 8µm high performance columns are ideal for relatively low molecular weight separations, combining low exclusion limits, high pore volume and high column efficiency for maximum resolution.
- PL aquagel-OH Individual Pore Size 8µm columns for high performance separations across the MW range 10,000 to >10,000,000.

PL aquagel-OH 15µm Analytical Columns

- PL aquagel-OH 15µm columns for the analysis of very high molecular weight polymers. Where molecular shear degradation is a real consideration, the larger particle size and larger frit porosity permit the analysis of high viscosity polymers in the range from 1M up to 100M.

PL aquagel-OH 5µm Analytical Columns

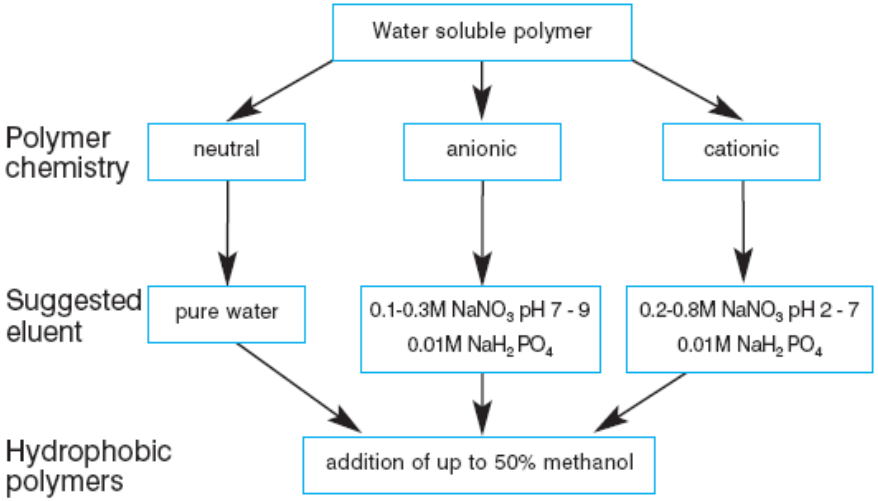
- In addition, Varian Polymer Laboratories has launched an ultra-high resolution PL aquagel-OH 20 5µm column for very high resolution analyses.

Optimizing Conditions for Aqueous SEC with PL aquagel-OH Columns

Due to the complex nature of water soluble polymers, it is often necessary to modify the eluent in order to avoid sample-to-sample and sample-to-column interactions which can result in poor aqueous SEC separations. The excellent stability of the PL aquagel-OH packing material allows the eluent to be modified to suit the polymer, while retaining the high column efficiency. For ionic interactions, the eluent can be modified by the addition of salt and/or the adjustment of pH. For water soluble polymers with hydrophobic character, only the addition of a weak organic solvent (methanol) is required to inhibit hydrophobic interactions.

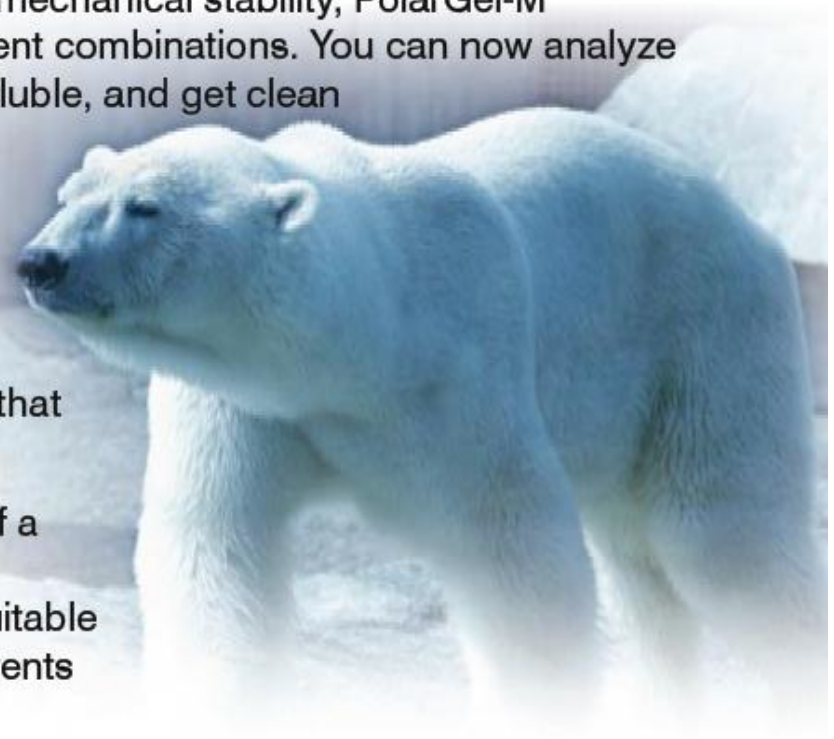
This versatility means that PL aquagel-OH columns can be used to analyse a wide range of neutral, polar, anionic and cationic samples.

Guide to Eluent Selection for PL aquagel-OH Applications



PolarGel Columns

Using its intermediate polarity surface and high mechanical stability, PolarGel-M operates with a wide range of solvents and solvent combinations. You can now analyze polar polymers that are not necessarily water soluble, and get clean chromatograms, time after time.



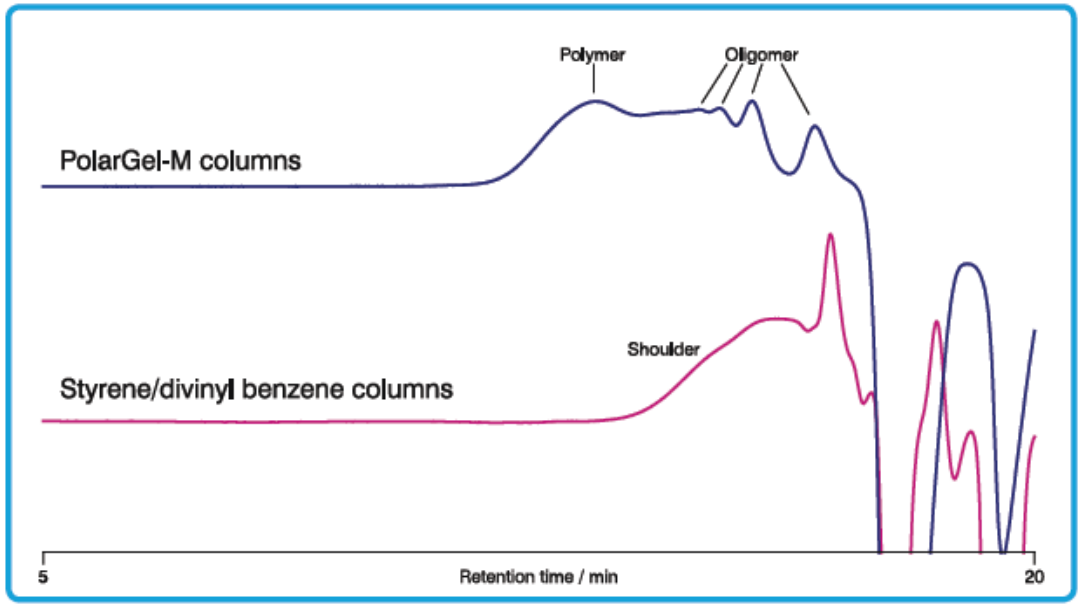
The PolarGel-M column has an intermediate polarity surface and high mechanical stability. It is capable of operation in a wide range of solvents and solvent combinations, greatly enhancing the ability to analyze polar polymers that are not necessarily water soluble.

PolarGel-M (300 x 7.5 mm) has the advantage of a polarity between PLgel (for organic GPC) and PL aquagel-OH (for aqueous GPC), making it suitable for applications in polar solvents and mixed solvents that fall between these systems.

Column	*Particle size / µm*	*Resolving range (PEG/PEO in water)*
PolarGel-L	8	Up to 30,000
PolarGel-M	8	Up to 2,000,000

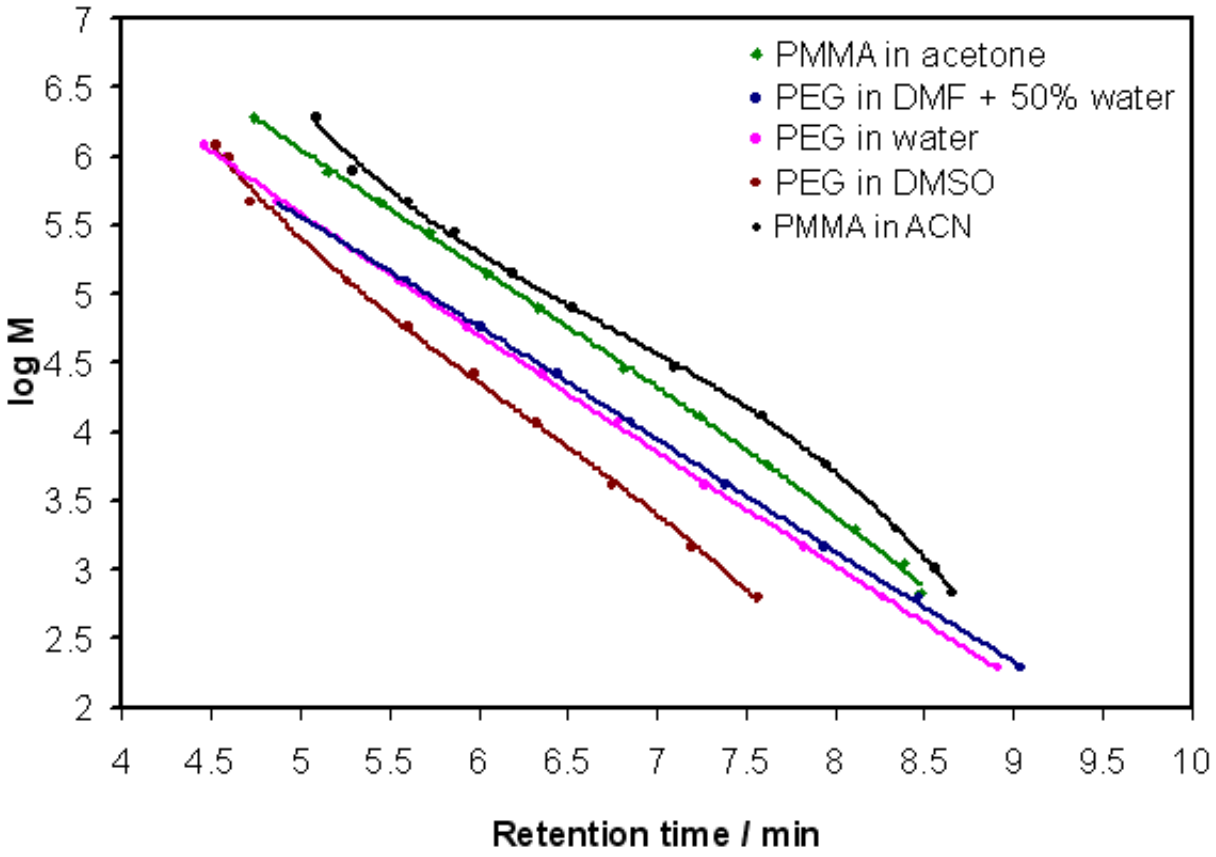
Peak Shapes of Polar Compounds

In this example, phenol formaldehyde resin is analyzed on PolarGel-M columns and traditional styrene/divinyl benzene columns in dimethyl formamide. Both column sets perform well, but the highly polar-OH groups on the resin interact strongly with the styrene/divinyl benzene columns, resulting in an unusual peak shape with numerous shoulders and some fronting. PolarGel-M columns give a much better peak shape, clearly showing the polymeric and oligomeric regions. For highly polar materials, PolarGel-M columns are definitely superior.



Superior polar performance from PolarGel-M.

PolarGel-M Calibrations

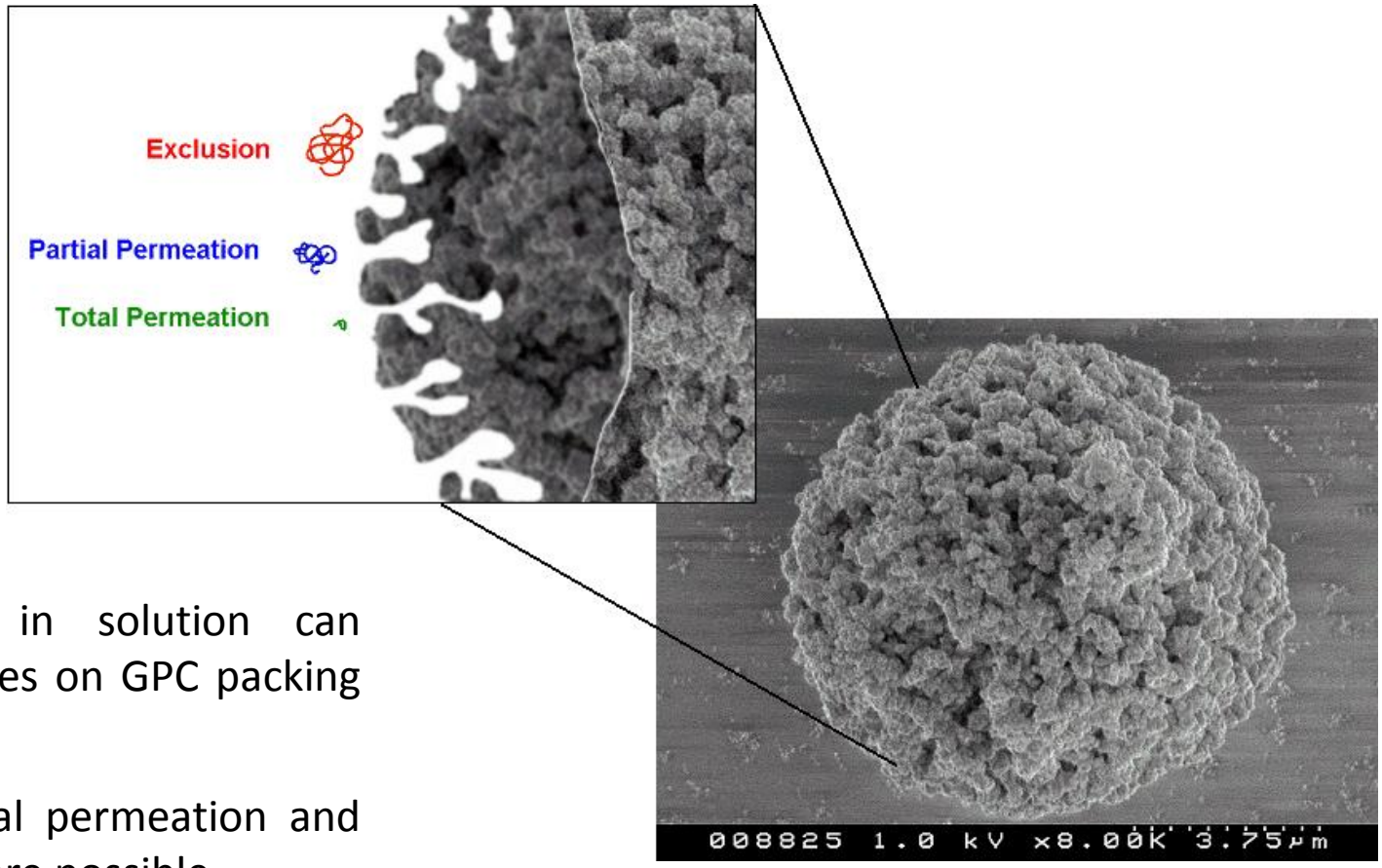


Polymer Behavior in Solution

- GPC is based on the behaviour of polymer molecules in solution
- In the solid state polymers can be considered like spaghetti – a confusing mass of intertwined chains
- In solution, polymer molecules are discrete entities
- Due to entrophic effects all but the most rigid of polymer chains curls up in solution to form a ball like shape

Dissolution

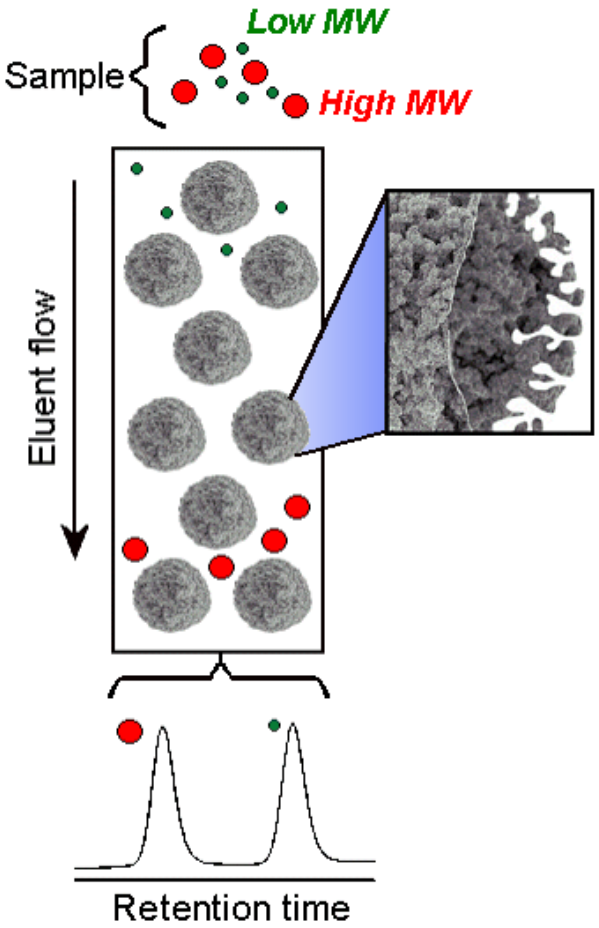
Permeation of Polymer Molecules



- Polymer coils in solution can permeate the pores on GPC packing materials
- Exclusion, partial permeation and total permeation are possible

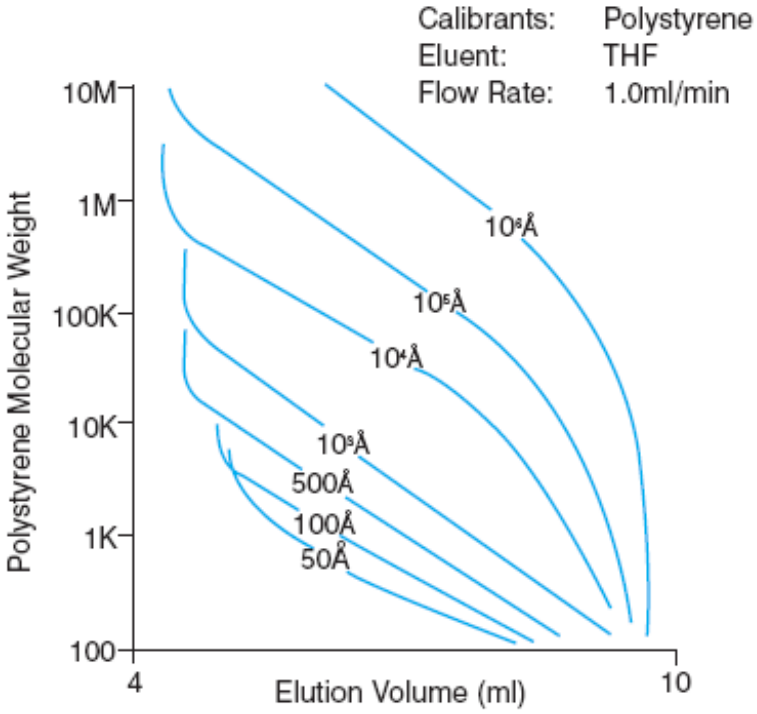
GPC Separation Mechanism

- Polymer is prepared as a dilute solution in the eluent and injected into the system
- The GPC column is packed with porous beads of controlled porosity and particle size
- Large molecules are not able to permeate all of the 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
- Polymer molecules are separated according to molecular size, eluting largest first, smallest last



PLgel Individual Pore Size Columns

Calibration Curves

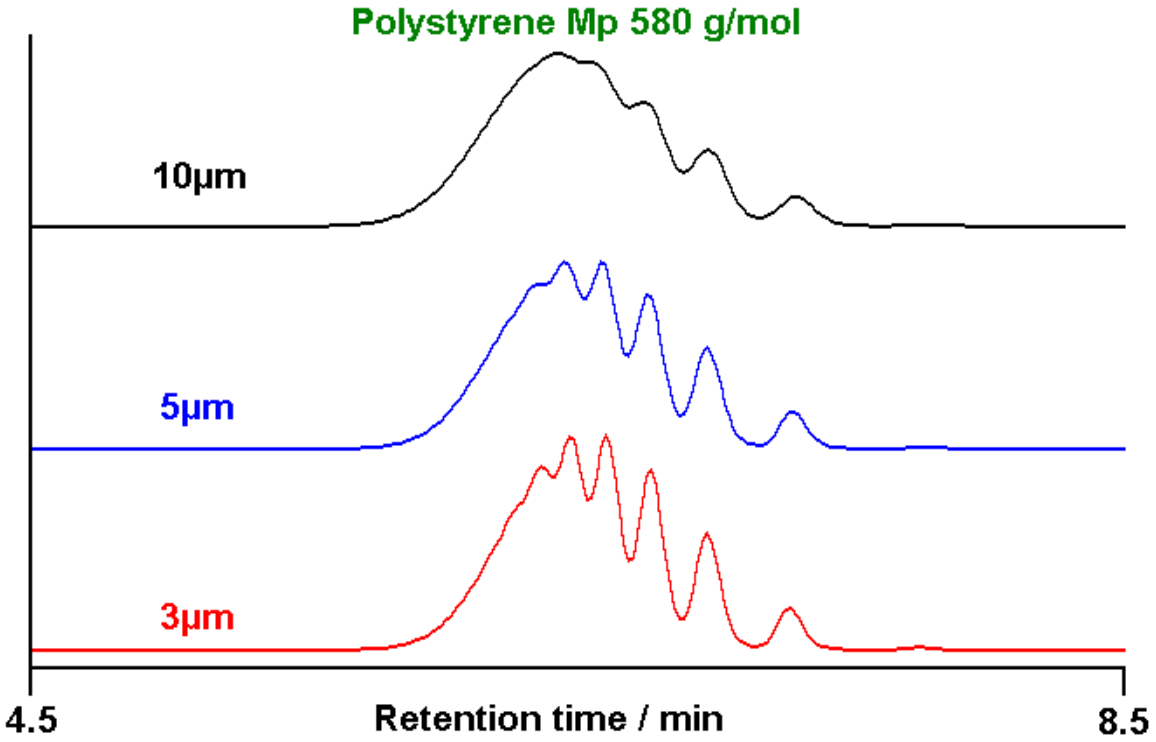


Specifications

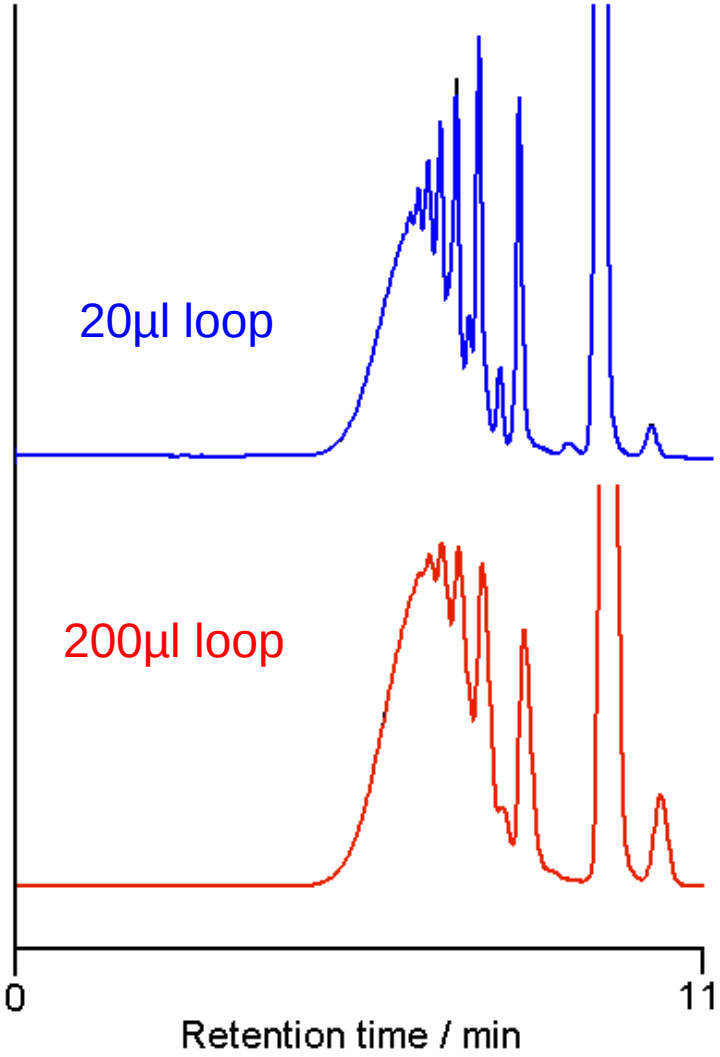
Column Type		Effective MW Range (PS)	Guaranteed Efficiency (p/m)
PLgel 3µm	100Å	up to 4,000	>100,000
PLgel 5µm	50Å	up to 2,000	>60,000
PLgel 5µm	100Å	up to 4,000	>60,000
PLgel 5µm	500Å	500-30,000	>60,000
PLgel 5µm	10^3Å	500-60,000	>50,000
PLgel 5µm	10^4Å	10,000-600,000	>50,000
PLgel 5µm	10^5Å	60,000-2,000,000	>50,000
PLgel 10µm	50Å	up to 2,000	>35,000
PLgel 10µm	100Å	up to 4,000	>35,000
PLgel 10µm	500Å	500-30,000	>35,000
PLgel 10µm	10^3Å	500-60,000	>35,000
PLgel 10µm	10^4Å	10,000-600,000	>35,000
PLgel 10µm	10^5Å	60,000-2,000,000	>35,000
PLgel 10µm	10^6Å	600,000-10,000,000	>35,000

Effect of Particle Size on Resolution

Eluent:	THF
Flow Rate:	1.0ml/min
Inj Vol:	20µl
Detector:	DRI



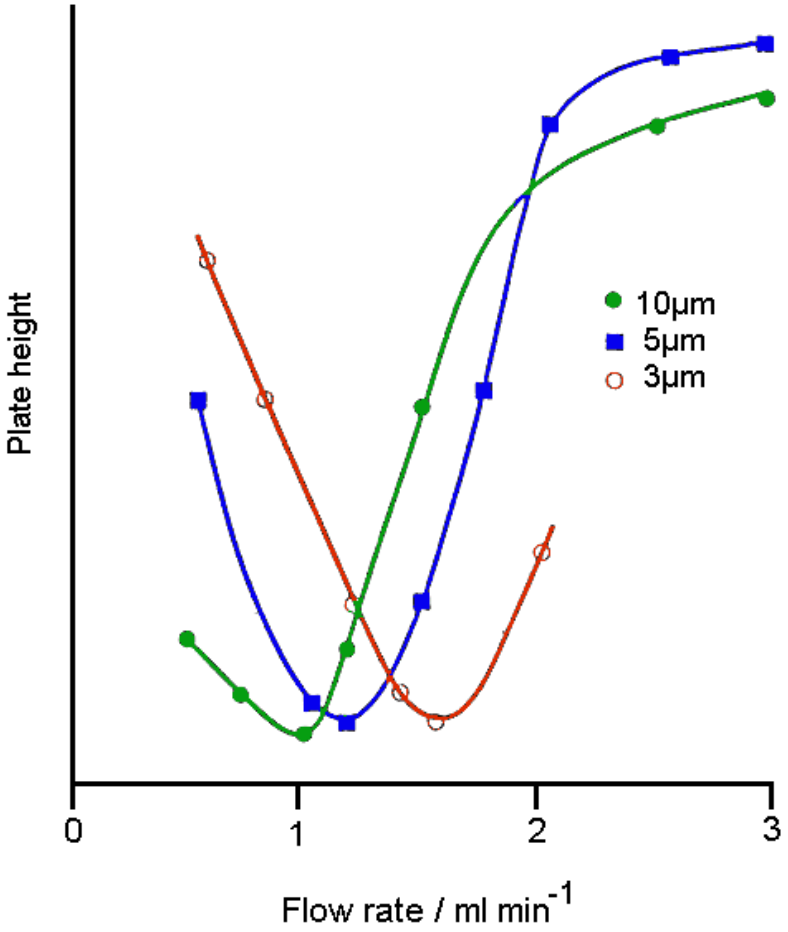
Effect of Injector Loop Size on Resolution



Column:	PLgel 3µm MIXED-E 300x7.5mm
Eluent:	THF
Flow Rate:	1.0ml/min
Sample:	Epikote 1001 epoxy resin

Injection loop is a major contribution to system dead volume, use reduced injection volume and increase concentration to maintain sensitivity

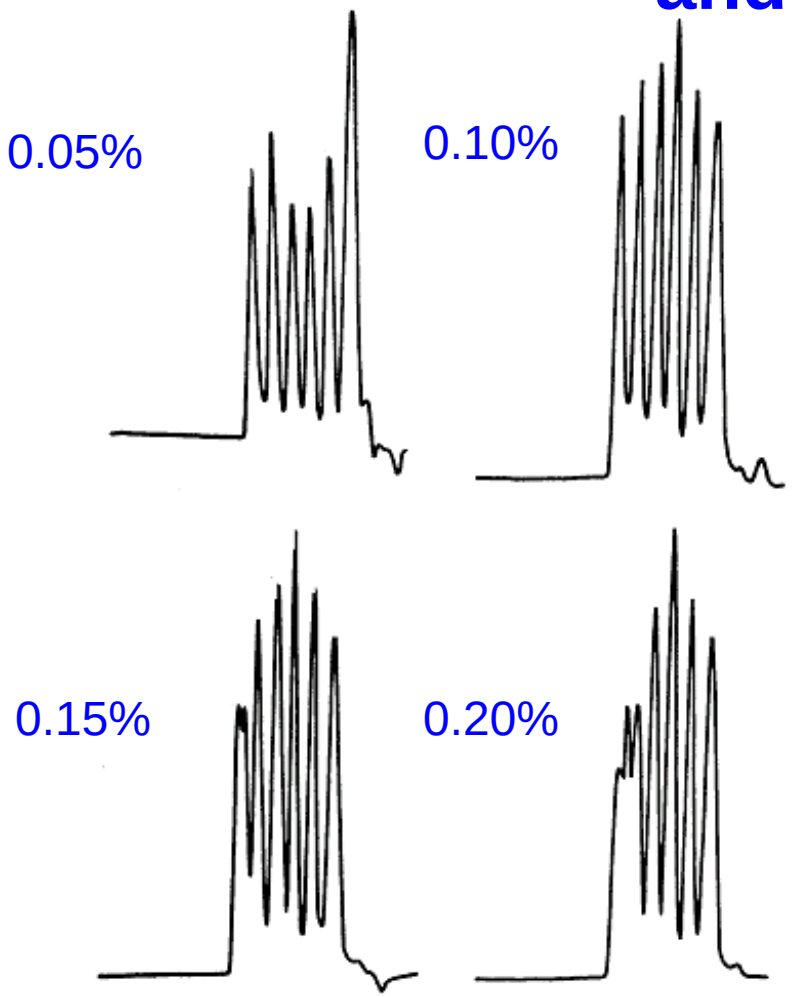
Effect of Flow Rate on Column Efficiency (1)



Eluent: THF
Column: PLgel 100Å
Test Probe: ODCB

Optimum flow rate for small molecule separations is around 1.0ml/min

Effect of Concentration on Peak Shape and Resolution



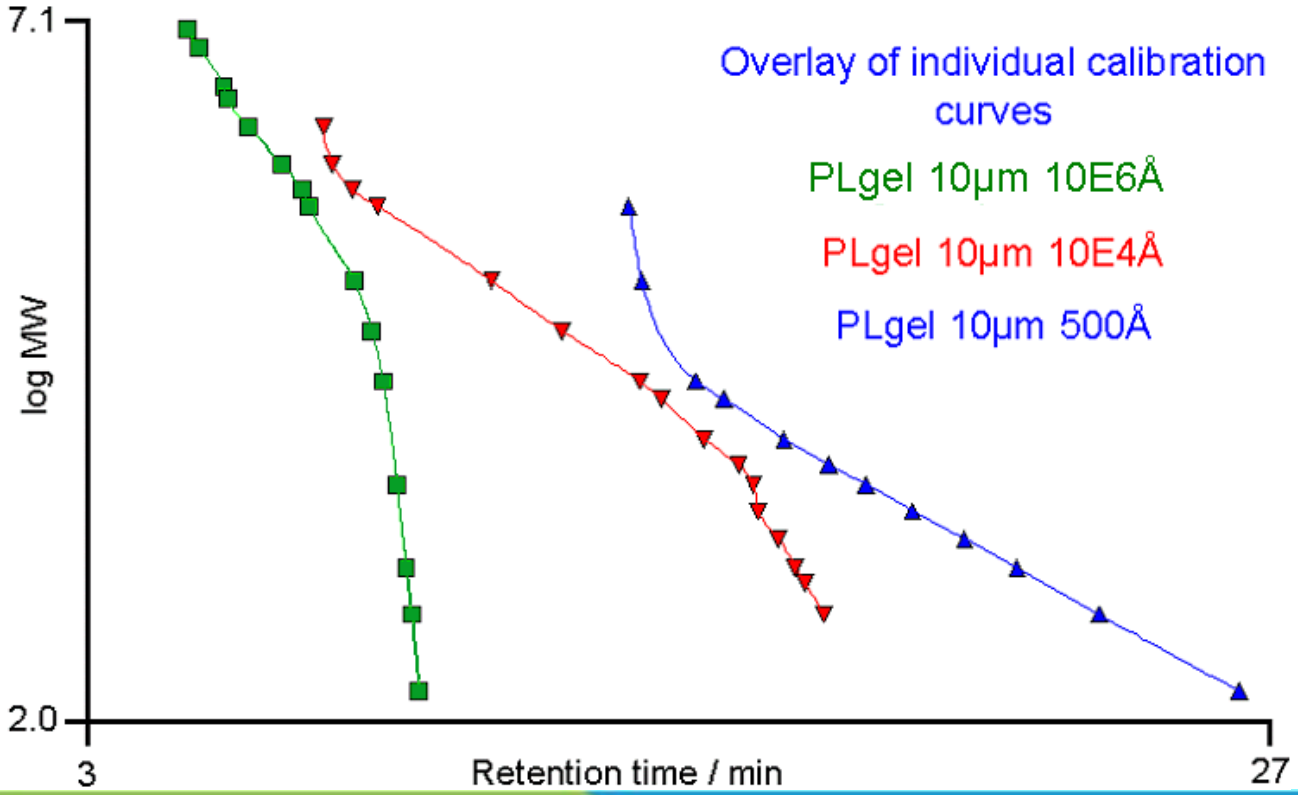
Column: PLgel 10µm MIXED-B 300x7.5mm
Eluent: THF
Flow Rate: 1.0ml/min
Detector: UV

Polystyrene standards

1. 8,500,000
2. 1,130,000
3. 170,000
4. 34,500
5. 5,100
6. 580

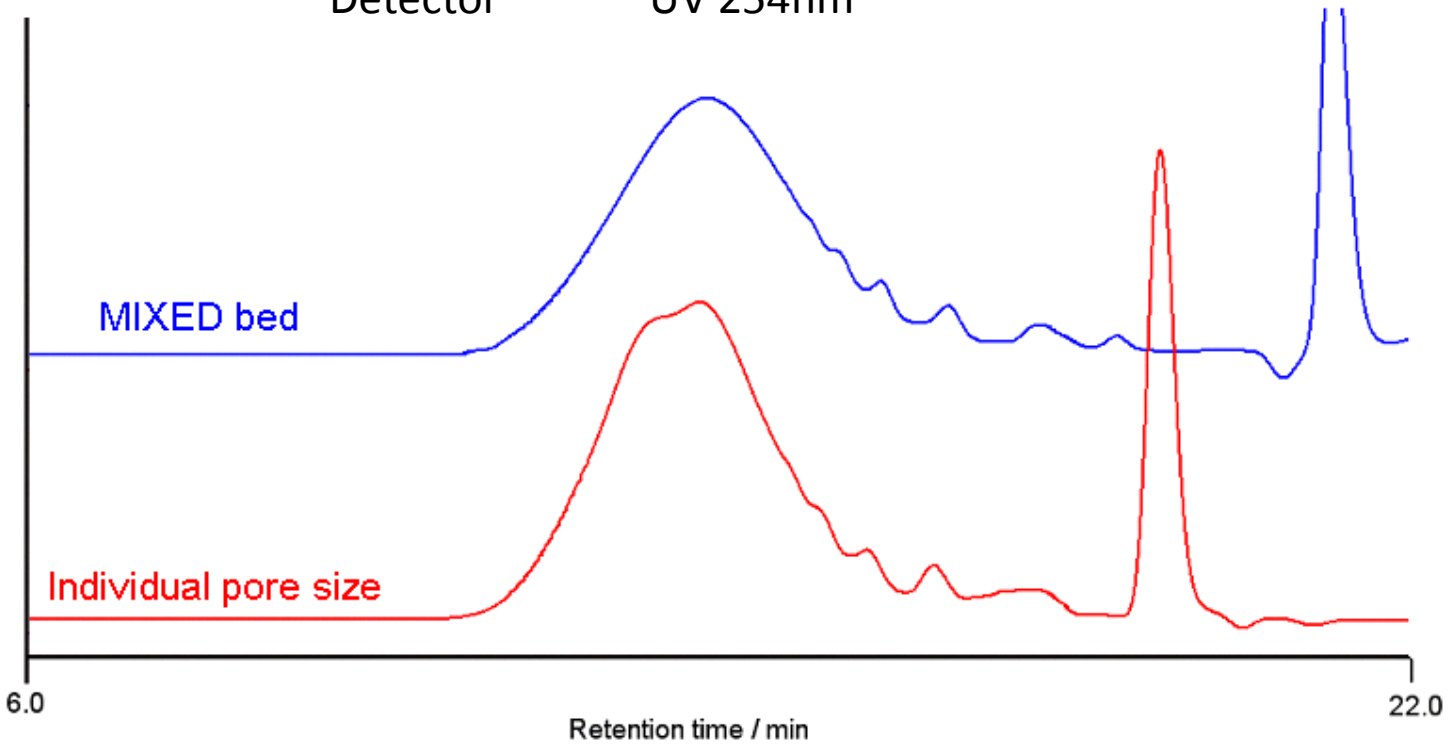
Combination of Individual Pore Size Columns

Traditional approach to increasing MW operating range of column set



Individual Pore Size Combination Versus MIXED Gel Columns - Polydisperse Sample

Eluent	THF
Flow rate	1.0 ml/min
Detector	UV 254nm



PLgel MIXED Bed columns

A significant number of GPC applications involve the analysis of polydisperse materials. The modern approach to column selection is to choose PL's MIXED gel columns, where each column contains a mixture of individual pore size materials, accurately blended to cover a specified broad range of molecular weight with a linear calibration to eliminate column mismatch. As market leaders in this field, Polymer Laboratories offers a comprehensive range of MIXED gel GPC columns designed for specific application areas.

Key Advantages of PLgel MIXED Columns Include:

- Greatly simplified column selection
- Improved confidence in the accuracy and precision of calculated data
- Optimized particle size for each application area
- Reduced replacement stock
- Elimination of mismatched column sets and spurious peaks
- Simple addition of extra column(s) for greater resolution

The calibration curves are designed to be linear over a specified molecular weight range, ensuring that the same degree of resolution is achieved across the full operating range of the column.

The particle size of the packing and the porosity of a particular MIXED gel column are carefully matched to the MW range and application, thus optimizing performance and eliminating the effects of shear degradation.

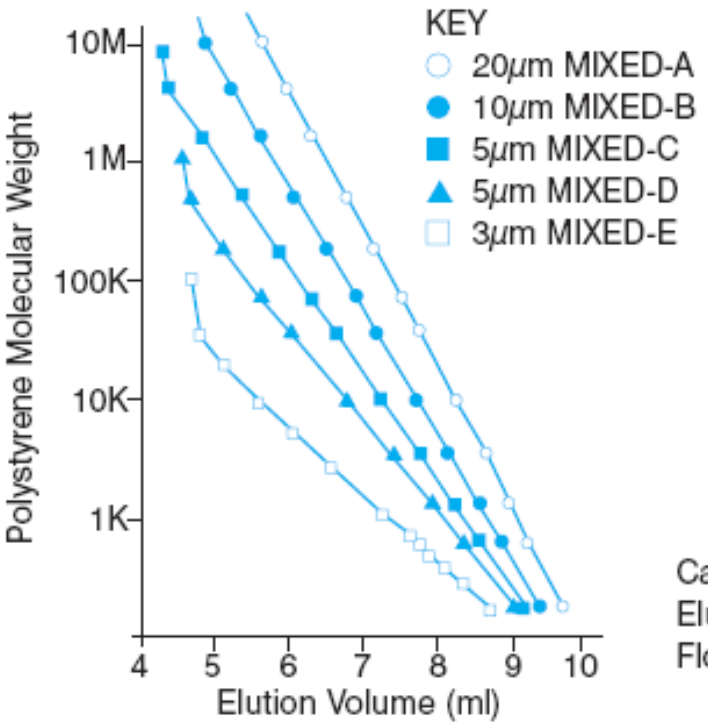
Resolution in GPC is controlled by the slope of the calibration curve and the particle size of the packing material. PL has scientifically determined the minimum number of MIXED gel columns required to perform accurate MWD determinations based on specific resolution (Rsp).

Ref: "Size exclusion chromatography columns from Polymer Laboratories", in Column Handbook for Size Exclusion Chromatography, ed. Chi-san Wu, Academic Press, 1999.

PLgel MIXED Bed Calibrations

PLgel MIXED Gel

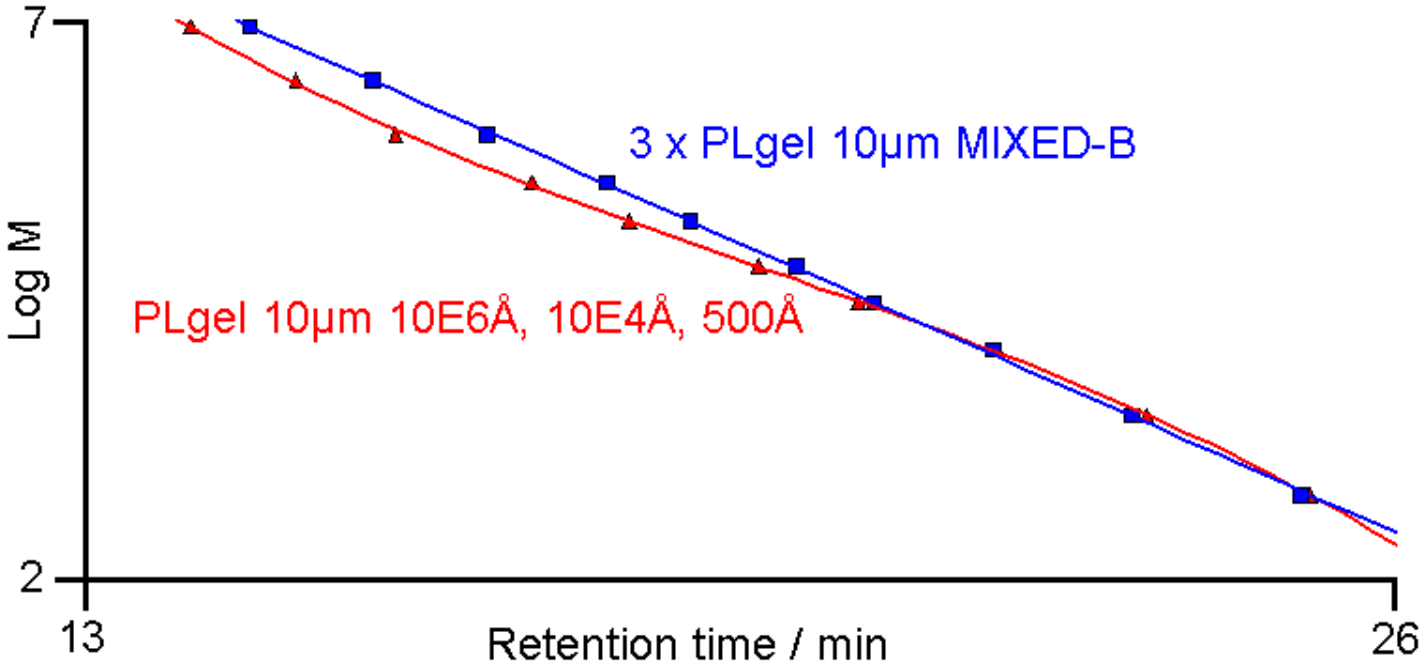
Calibration Curves



Specifications

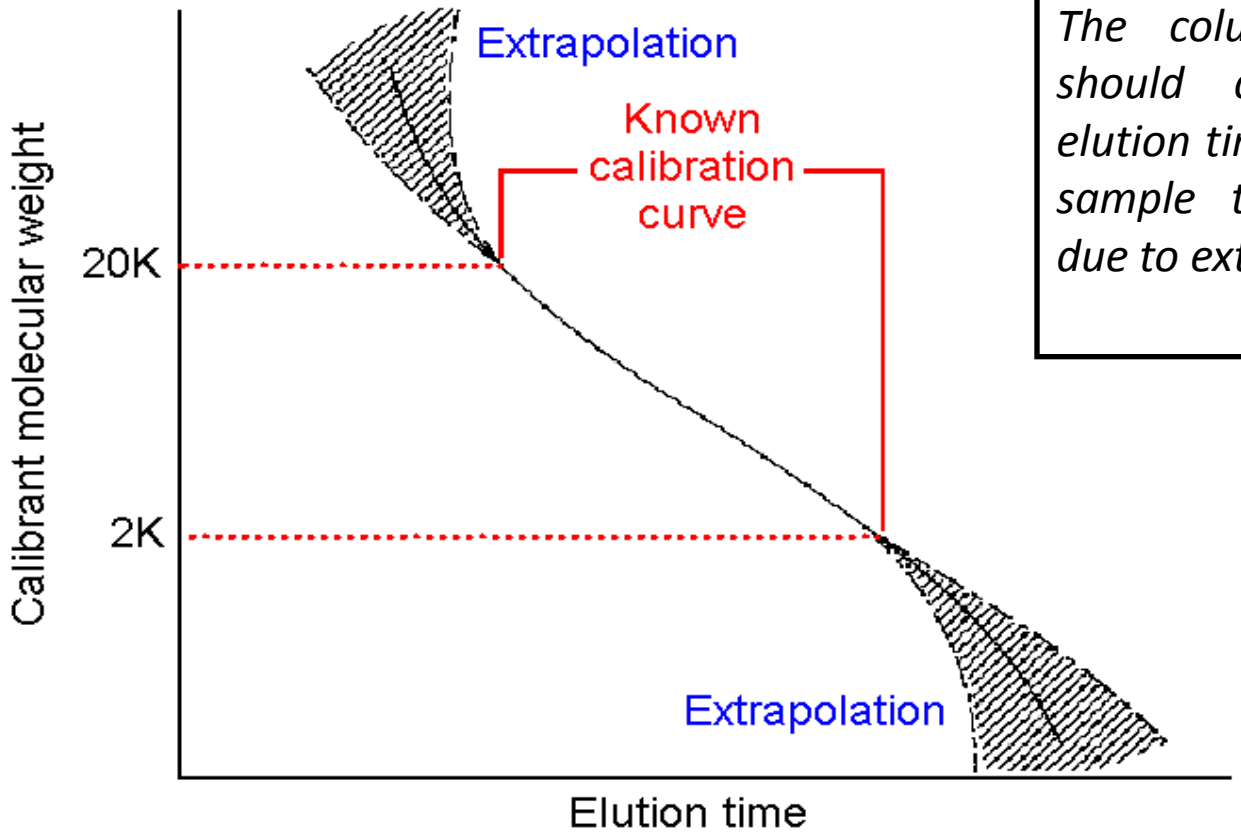
Column Type	Linear MW Range (PS)	Guaranteed Efficiency (p/m)
PLgel 20µm MIXED-A	2,000-40,000,000	>17,000
PLgel 10µm MIXED-B	500-10,000,000	>35,000
PLgel 5µm MIXED-C	200-2,000,000	>50,000
PLgel 5µm MIXED-D	200-400,000	>50,000
PLgel 3µm MIXED-E	up to 30,000	>80,000

Calibrants: Polystyrene
Eluent: THF
Flow Rate: 1.0ml/min


7
2
Log M
3 x PLgel 10µm MIXED-B
PLgel 10µm 10E6Å, 10E4Å, 500Å
13
Retention time / min
26


MIXED bed
Individual pore size
6.0
Retention time / min
22.0

Errors Due to Limited Calibration Region



The column calibration should cover the full elution time region of the sample to avoid errors due to extrapolation

PlusPore PS/DVB Columns

The PlusPore series of columns has been specifically designed for *high resolution* GPC, and represents the very latest in GPC column technology. These novel packing materials are based on the industry standard, highly crosslinked polystyrene/divinylbenzene (PS/DVB), for the widest applicability and solvent compatibility. Each is made using a novel polymerization process to produce particles which exhibit a specific, controlled pore structure for optimum GPC performance.

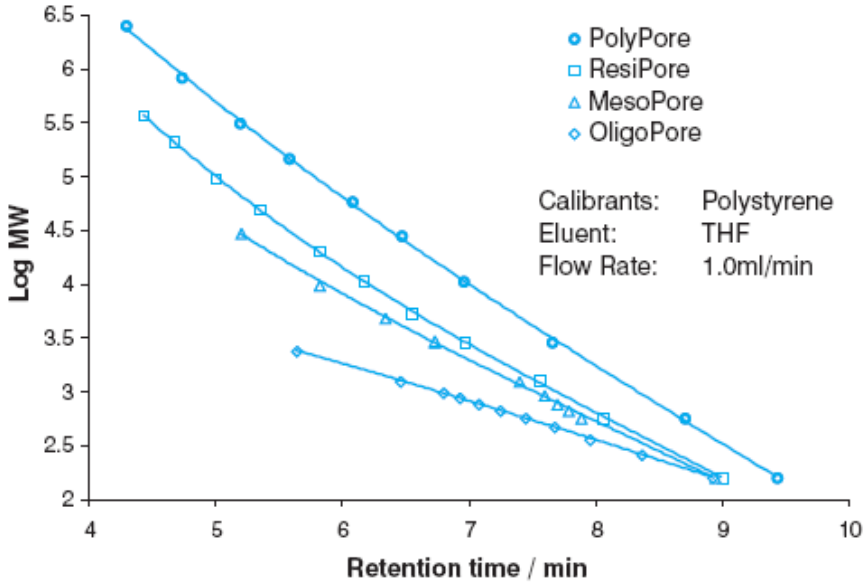
Features and Benefits of the PlusPore Range

- High pore volume, high resolution
- Wide pore size distribution
- Optimized separation range
- Full solvent compatibility
- No MWD dislocations

- **PolyPore** for the routine analysis of general polymers
- **ResiPore** for the analysis of resins and condensation polymers
- **MesoPore** for the analysis of prepolymers and low MW resins
- **OligoPore** for the analysis of oligomeric samples

PlusPore Calibrations

PlusPore
Calibration Curves



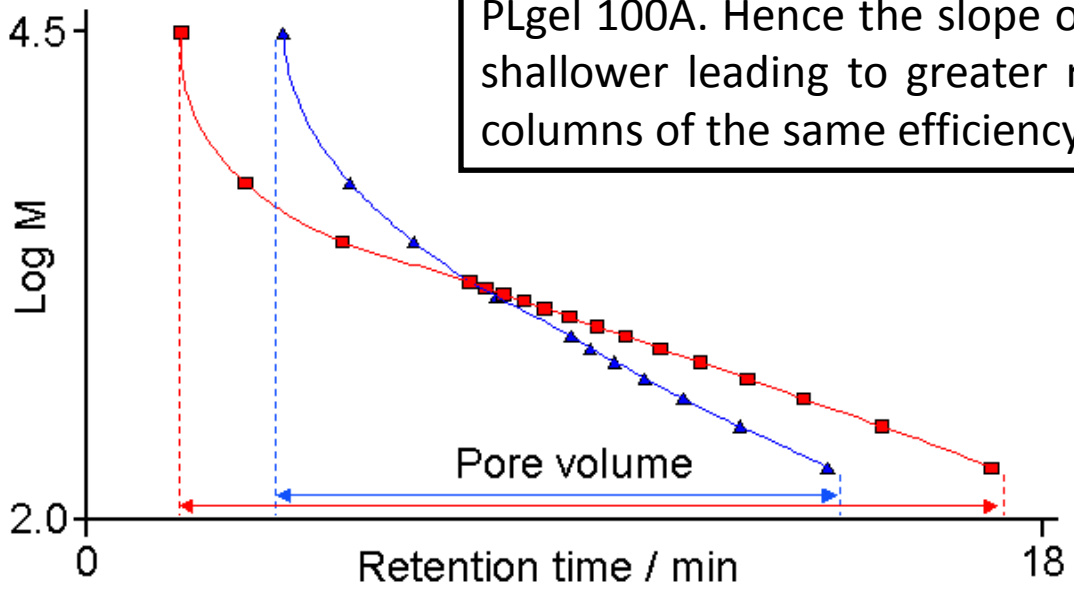
Specifications

	PolyPore	ResiPore	MesoPore	OligoPore
MW Operating Range	200-2,000,000	200-400,000	Up to 25,000	Up to 4,500
Nominal Particle Size	5µm	3µm	3µm	6µm
Typical Column Efficiency	>60,000 p/m	>80,000 p/m	>80,000 p/m	>55,000 p/m

Effect of Increased Pore Volume

Columns	2xPLgel 3µm 100Å 300x7.5mm 2xOligoPore 300x7.5mm
Eluent	THF
Flow rate	1.0ml/min

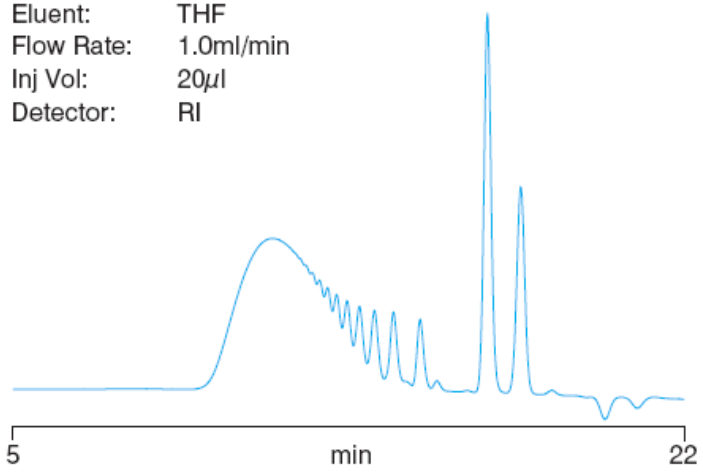
Both columns have a similar exclusion limit but OligoPore has greater pore volume than PLgel 100Å. Hence the slope of the curve is shallower leading to greater resolution for columns of the same efficiency



Examples of Resolution Using Pluspore Columns

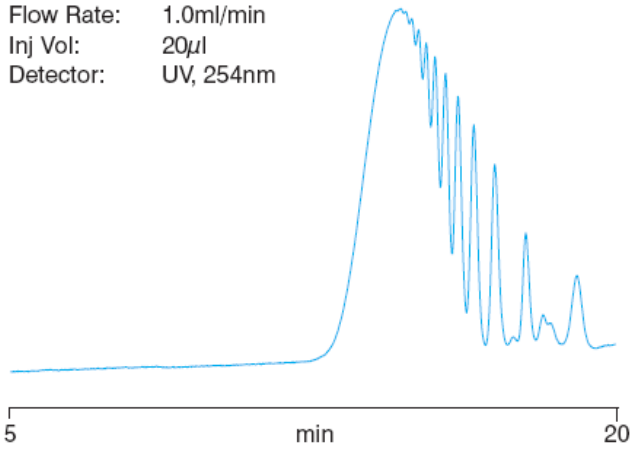
Polyurethanes

Columns: 2xMesoPore, 300x7.5mm (PL1113-6325)
Eluent: THF
Flow Rate: 1.0ml/min
Inj Vol: 20µl
Detector: RI



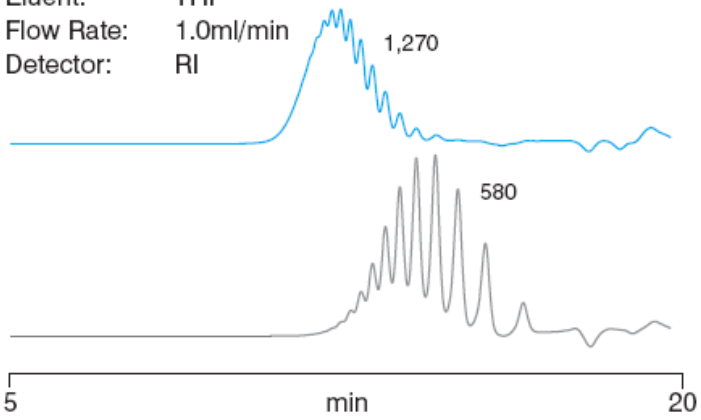
Polyester

Columns: 2xResiPore, 300x7.5mm (PL1113-6300)
Eluent: THF
Flow Rate: 1.0ml/min
Inj Vol: 20µl
Detector: UV, 254nm

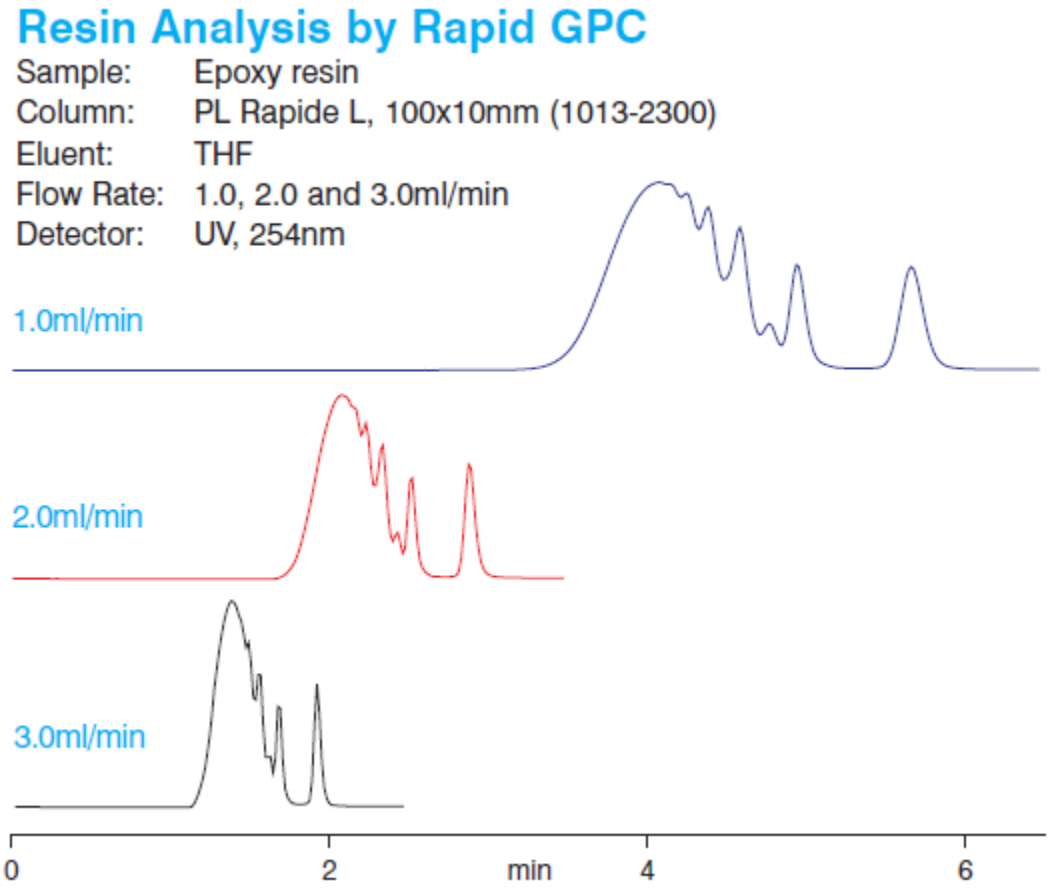


Polystyrene Standards

Columns: 2xOligoPore, 300x7.5mm (PL1113-6520)
Eluent: THF
Flow Rate: 1.0ml/min
Detector: RI



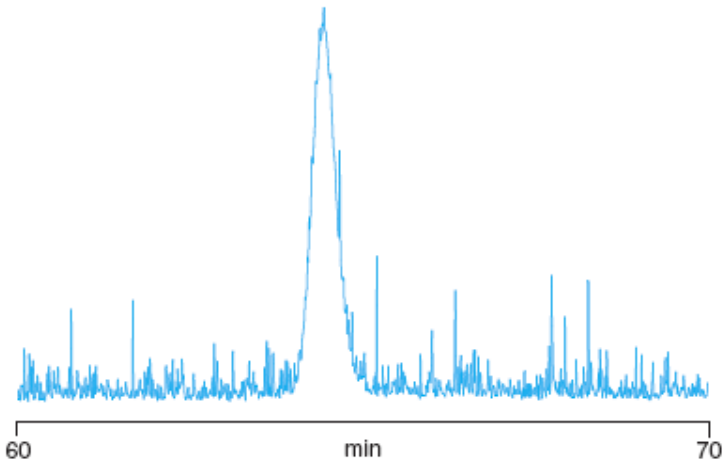
Rapide Columns Allow For Fast Trend Analysis



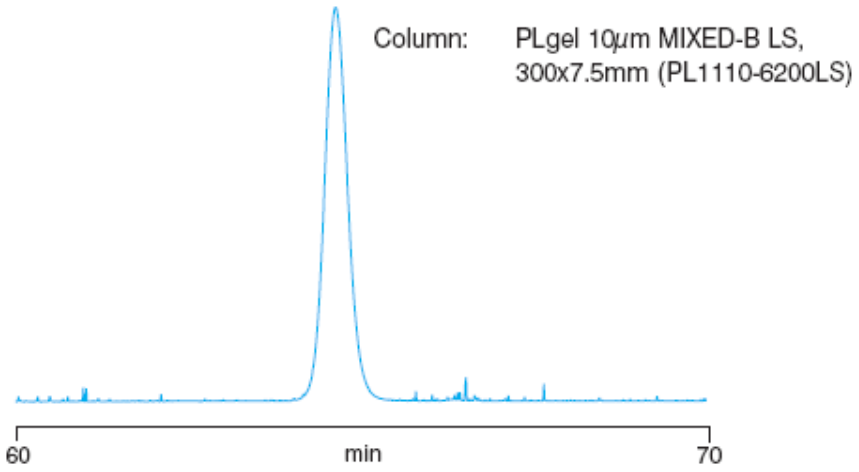
PLgel LS Columns

Polymer Laboratories has developed the PLgel LS series, a PS/DVB packing using an innovative proprietary suspension polymerization technique to virtually eliminate nano-particle leakage. A startling improvement is achieved immediately in the quality of light scattering data obtained with PLgel LS columns in place of conventional GPC columns. The light scattering chromatograms below were obtained after flushing the columns for one hour in THF at 1.0ml/min. A polystyrene standard (Mp 210,000) was injected at 1mg/ml in order to illustrate the dramatic improvement in signal to noise with the PLgel LS column.

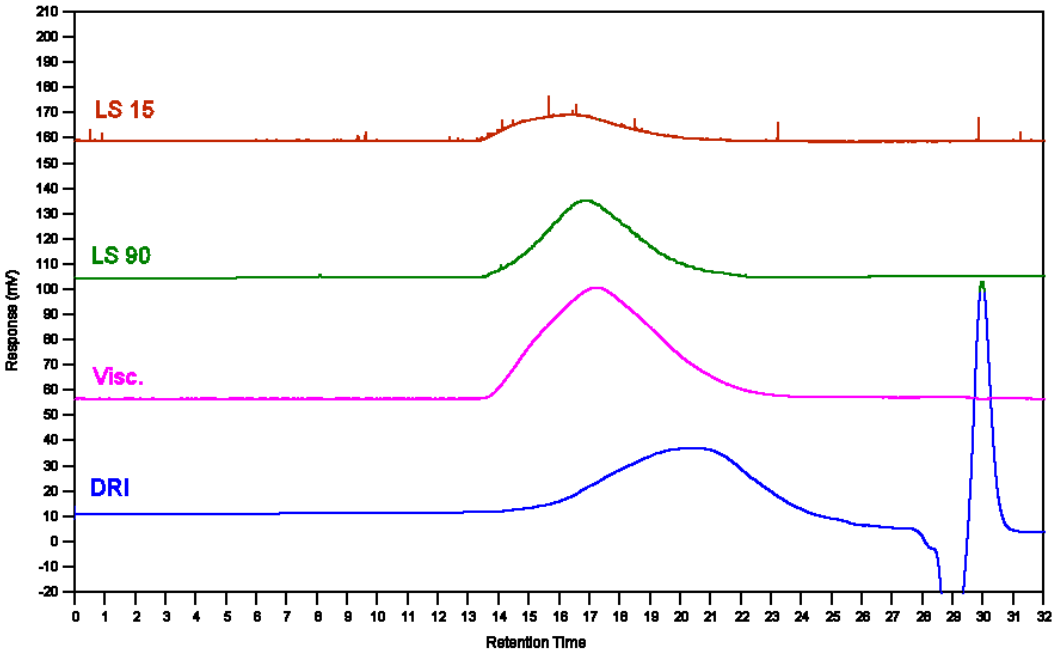
Conventional GPC Column

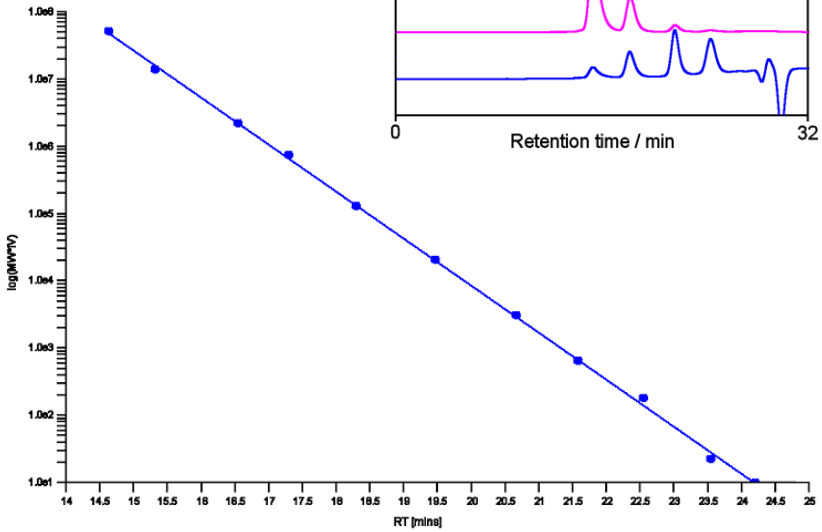


PLgel LS Column



PLgel Olexis Columns



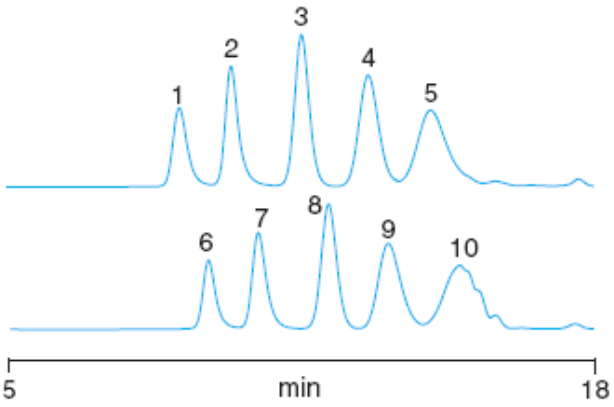


- Maximum resolution with minimum shear degradation
- The packing material is the same as the PL ‘LS’ product line, and so is especially suited for light scattering applications

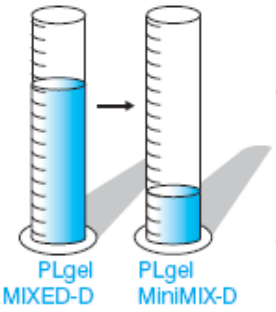
miniMIX Solvent Consumpsion

Comparison of Conventional and Narrow Bore Columns

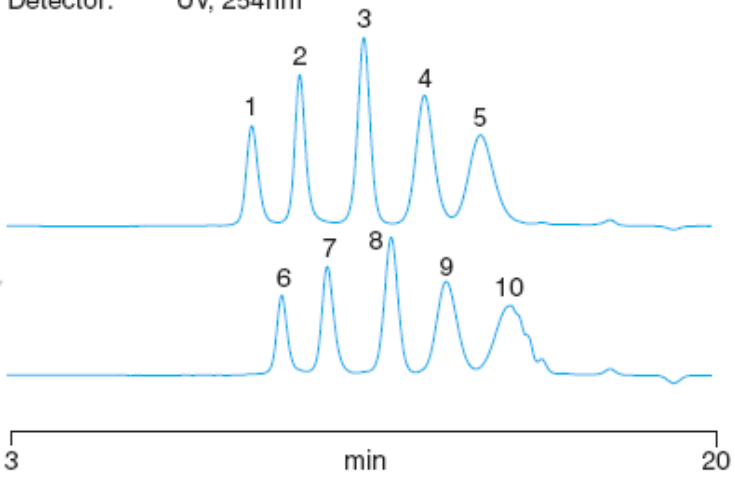
Sample: EasiCal PS-2
Columns: 2xPLgel 5µm MIXED-D, 300x7.5mm (PL1110-6504)
Eluent: THF
Flow Rate: 1.0ml/min
Inj Vol: 100µl
Detector: UV, 254nm



Comparative Solvent Consumption



Sample: EasiCal PS-2
Columns: 2xPLgel MiniMIX-D, 250x4.6mm (PL1510-5504)
Eluent: THF
Flow Rate: 0.3ml/min
Inj Vol: 20µl
Detector: UV, 254nm



KEY
1. 380,000
2. 96,000
3. 22,000
4. 5,050
5. 1,320
6. 156,000
7. 49,900
8. 11,600
9. 2,950
10. 580

Comparative Preparative Separations

Applications Include

- Deformulation of competitors' products
- Sample clean-up / extraction
- Polymer fractionation

High Performance, High Capacity

PLgel Preparative columns are packed with the same rigid, high performance media as the analytical column range. The 10μm particle size provides high column efficiency (>25,000 plates/m) for optimum resolution and loading characteristics.

PLgel 25mm ID preparative columns offer more than a 10x scale up compared to PLgel 7.5mm analytical columns. In comparison with other vendors' preparative columns, PL's increased ID and column volume permit even higher loadings per injection.

Column ID	Column Volume per 300mm Length	Minimum Scale Up
7.5mm	13	x1
19mm	85	x6
21mm	104	x8
25mm	147	x11

High Load

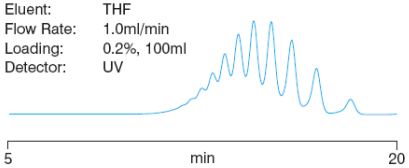
The large internal diameters of preparative columns, with their correspondingly larger bed volumes, mean that the injection volume can be significantly increased.

When fractionating low molecular weight materials, the sample concentration can also be significantly increased, enabling milligram quantities of very pure material to be isolated for further study. The actual loading is ultimately controlled by the sample and its molecular weight.

PLgel Preparative GPC columns are available in seven individual pore sizes and two MIXED gel types, and in column lengths of 300mm and 600mm. A Preparative Guard column (25x25mm) is also available.

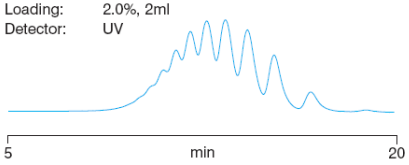
Analytical Separation

Columns: 2xOligoPore, 300x7.5mm (PL1113-6520)
Eluent: THF
Flow Rate: 1.0ml/min
Loading: 0.2%, 100ml
Detector: UV



Preparative Separation

Columns: 2xOligoPore, 300x25mm (PL1513-6520)
Eluent: THF
Flow Rate: 10.0ml/min
Loading: 2.0%, 2ml
Detector: UV



Measuring Molecular Weight

- There are many ways to measure molecular weights
- Examples include osmometry, centrifugation and batch light scattering
- Each of these methodologies gives a single measurement, and average molecular weight
- For example, light scattering measures Mw, osmometry measures Mn and centrifugation measures Mz
- Although these methods give you a molecular weight, they do not describe a distribution
- The advantage of GPC is that it is a separation technique, and as such it is the only common technique that allows the measurement of the molecular weight distribution, not just a single average value

- **Based on the Large Portfolio of Agilent We Offer the Tools to Be Successful in Any Application**