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# Gel Permeation Chromatography Basics and Beyond eSeminar June 6, 2012

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#### Content

- Overview of GPC/SEC
  - What is it? Why do we use it?
  - Polymers
- Molecular Weight Distribution
- Key Column selection criteria
  - Particle type, Column type
  - Solvent selection
  - Calibration Standards
- Effect of Concentration, Particle Size, and Injection Volume
- GPC Detectors
- Resources



#### **Terminology**

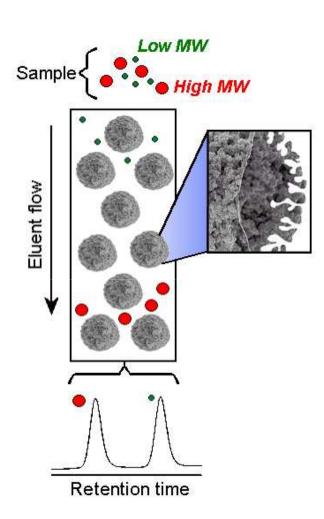
**GPC** - Gel Permeation Chromatography

**SEC – Size Exclusion Chromatography** 

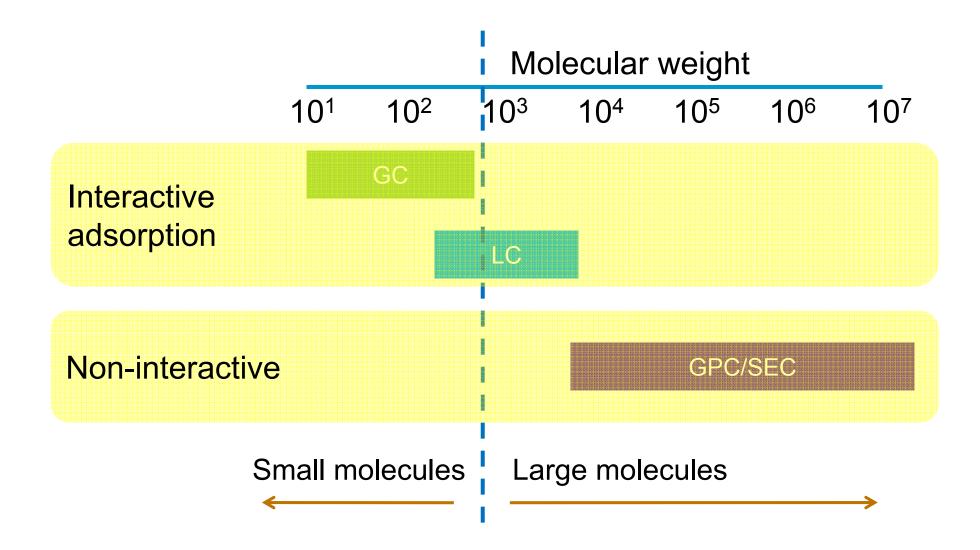
**GFC – Gel Filtration Chromatography** 

#### What is GPC/SEC?

- The GPC column is packed with porous beads of controlled porosity and particle size
- Polymer is prepared as a dilute solution in the eluent and injected into the system
- 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



#### When to use GPC



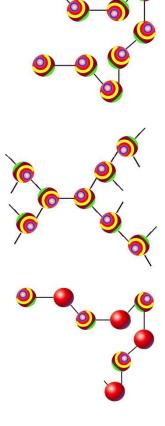


### What are Polymers?

Polymers are long chain molecules produced by linking small repeat units (monomers) together

Polymers can be varied in lots of ways, for example;

- Chemical Structure of Monomer Unit
- 3D Structure
- Different Monomer Units
- Length of polymer chains
- Distribution of polymer chain lengths



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### **Most Common Examples**



Polystyrene



Polyethylene



Nylon

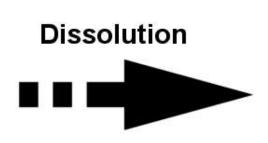
Polyvinylchloride, PVC



### **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

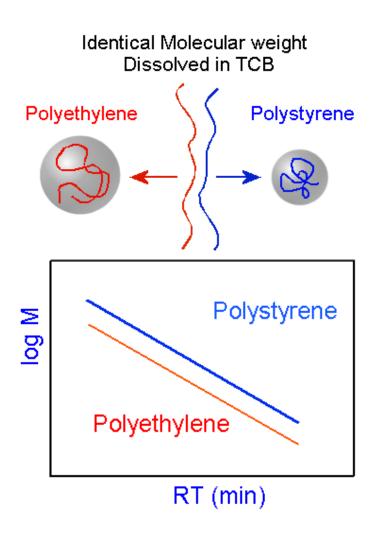






#### **Conventional GPC**

- Two different polymers will interact differently with solvent
- Column separates on basis of molecular size NOT molecular weight
- At any molecular weight, the two polymers will have different sizes in solution
- Molecular weights from conventional GPC are dependent on a comparison in size between the standards and the sample



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### **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

# The Primary Goal of GPC is to Discover the MW Distribution

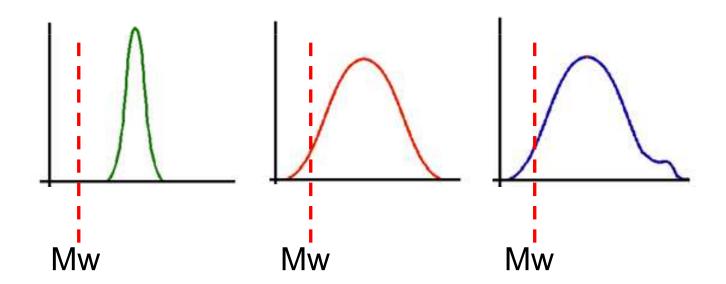
- Samples of synthetic polymers *always* contain polymer chains with a range of chain lengths
- One way to describe the length of the polymer chains is in terms of an average molecular weight, i.e the average of all the chain lengths in the sample

#### HOWEVER....

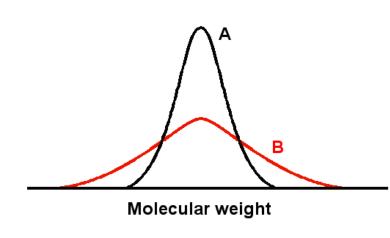
- Different samples of the same polymer can have the same average chain length but very different distributions of chain lengths depending on the method of production
- In polymer science it is the molecular weight distribution that is important

#### **Molecular Weight Distribution**

- Polymers samples contain mixtures of different chain lengths
  - Polydispersity
- Molecular weight (Mw) is an average
- Samples can have same molecular weight but different polydispersity
- Both are equally important



#### Effect of Mw and Polydispersity on a Polymer



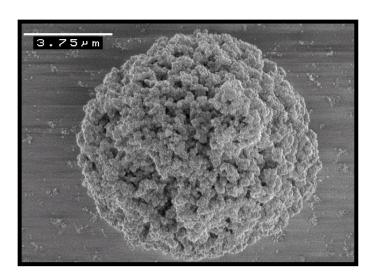
- As the broadness of the distribution decreases, the strength and toughness of the polymer increases
- However as the broadness of the distribution decreases, the polymer becomes more difficult to process
- GPC can provide key information to predict the processability and material properties of a polymer

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

#### What Are GPC Columns Made Of?

Silica Packings = Mechanically Strong 'Typically 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



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#### In General, GPC Column Specifics

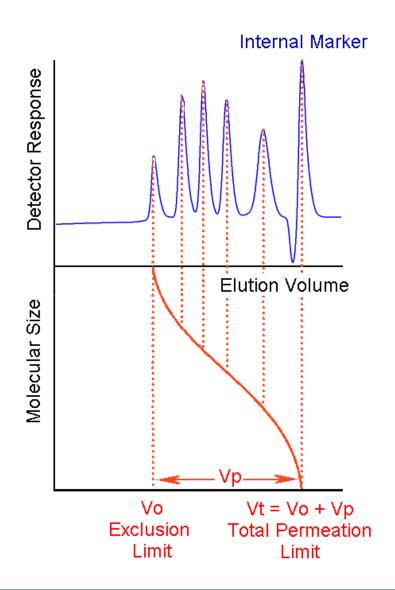
- Columns are packed with porous particles, controlled pore size and particle size
- Columns are produced by slurry packing technique, packed at pressures well in excess of 3000psi
- Column dimensions typically 7-8mm i.d., 250-600mm in length
- Exclusion volume (Vo) Upper MW limit (also known as void volume)
- Total permeation volume (Vt) Lower MW limit
- Pore volume (Vp) Working resolving range of MW

$$Vp = Vt - Vo$$

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- As a result of the GPC separation mechanism, polymer molecules elute from the column in order of size in solution
- Largest elute first, smallest elute last
- The separation is purely a physical partitioning, there is no interaction or binding
- The separation is isocratic
- If polymer molecules have the same molecular dimensions, they will co-elute by GPC and may not be separated by this technique
- The calibration curve describes how different size molecules elute from the column

#### **Elution Profiles**



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#### Column Selection: what do I need to know?

- GPC Column selection depends on:
  - Molecular weight of sample
  - Polydispersity
  - Presence of additives
  - Solvents required
  - Temperature required
- Helpful to know the properties of the sample

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#### **Further Criteria for Column Selection**

- The factors that govern which type of column is selected for a GPC experiment are the anticipated MW of the sample as well as the solvent the sample is soluble in
- Many polymers 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
- The properties of each range that must be considered when selecting them for an application

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#### Column Selection - Solvent

Solvent determination very simple

"What does the polymer dissolve in?"

- Organic most common: THF, Toluene, CHCl3, MeCl
- Polar organic or organic/aqueous mixtures DMF, DMAc,
- DMSO,
- Aggressive solvents/temperatures TCB, ODCB, NMP
- Aqueous water, water/buffer, some small %organic

# Particle Technology – what is available to choose from?

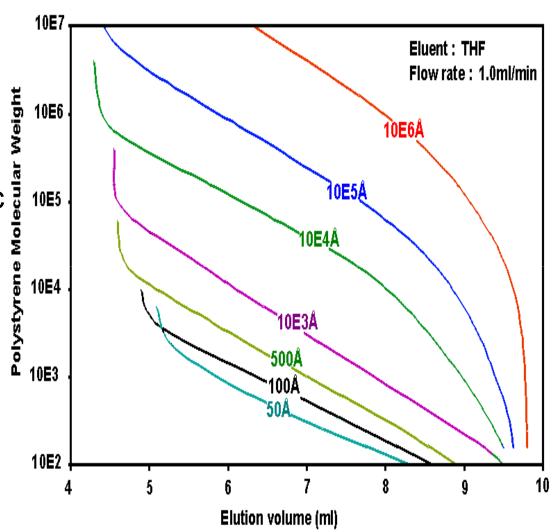
Individual Pore columns

Mixed Particle columns

Mixed Pore columns

#### **Individual Pore Technology**

- Particles are polymerized to have a specific pore size, ex 5um 10E4A
- Provides for a very specific MW operating range for the column
- Linear region is only over that specific MW range





#### **Mixed Particle Technology**

- Blend of Individual Pore Sized Material in the Same Column
- Designed to be Linear Across an Extended Molecular Weight Range
- Column Selection is Dictated by Molecular Weight Range of Polymer
- Further Resolution is Gained by the Subsequent Addition of an Identical Column Type

### **Benefits of Mixed Particle Technology**

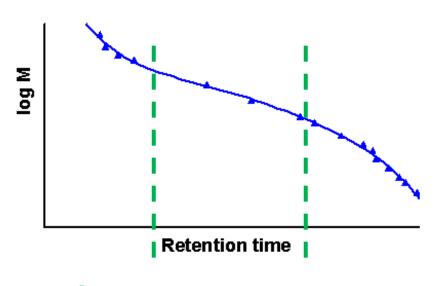
Greatly simplified column selection

Optimized columns for each application area

No artifacts due to column mismatch

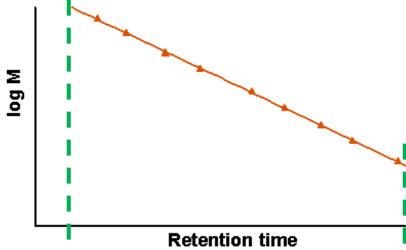
Simply add another column of the same type for greater resolution

### Individual Pore Size vs MIXED



PLgel 5µm, 10<sup>4</sup>Å

Good resolution but only over a limited Mw range



PLgel 5µm MIXED-D

Good resolution over a much wider Mw range

#### **Mixed Pore Technology**

- Produced by a novel polymerisation procedure
- Range of Pore Sizes with in an Individual Bead
- Not blended materials columns contain only one type of material
- Newer type of GPC media based on styrene / divinyl benzene
- Designed to achieve near linear column calibrations
- High pore volume materials compared to conventional GPC media



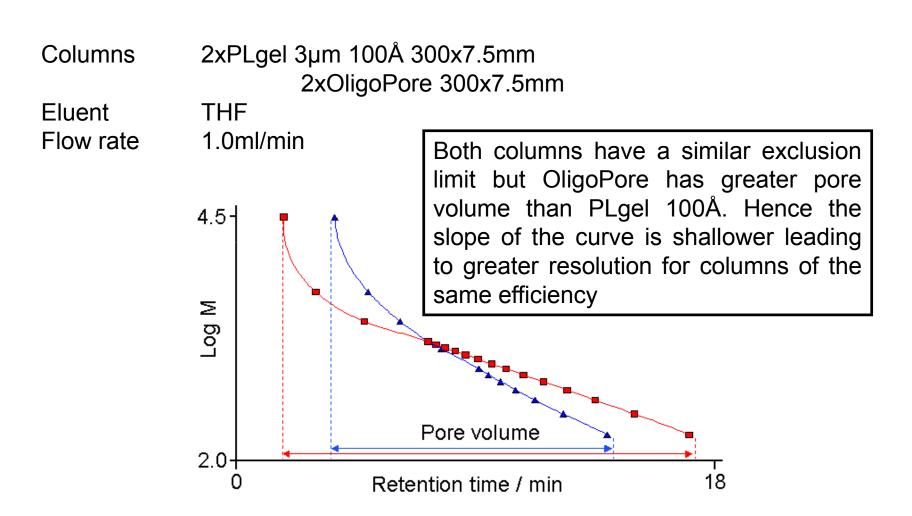
### **Benefits of Mixed Pore Technology**

Similar to Mixed Particle Bed Technology

Higher Pore Volume Leads to Increased Resolution

Ability to Transfer into High Polarity Solvents

#### **Effect of Increased Pore Volume**

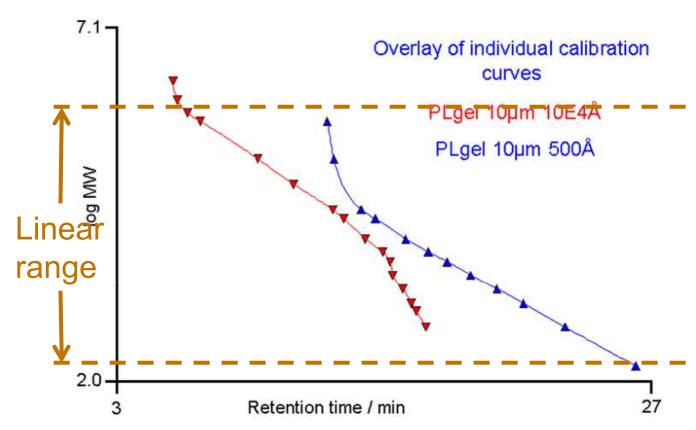


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### **Column Selection – How Many Columns?**

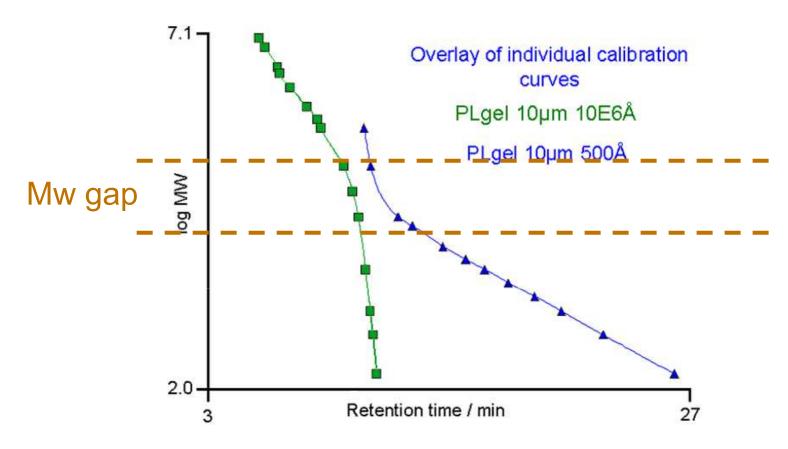
- More than one column typically used
  - More columns = better resolution
  - Also increases analysis time
- 20µm particle size 4 columns
- 13µm, 10µm three columns
- 8μm, 5μm and 3μm two columns
- Higher Mw tends to need more columns

### Increasing the Resolving Range



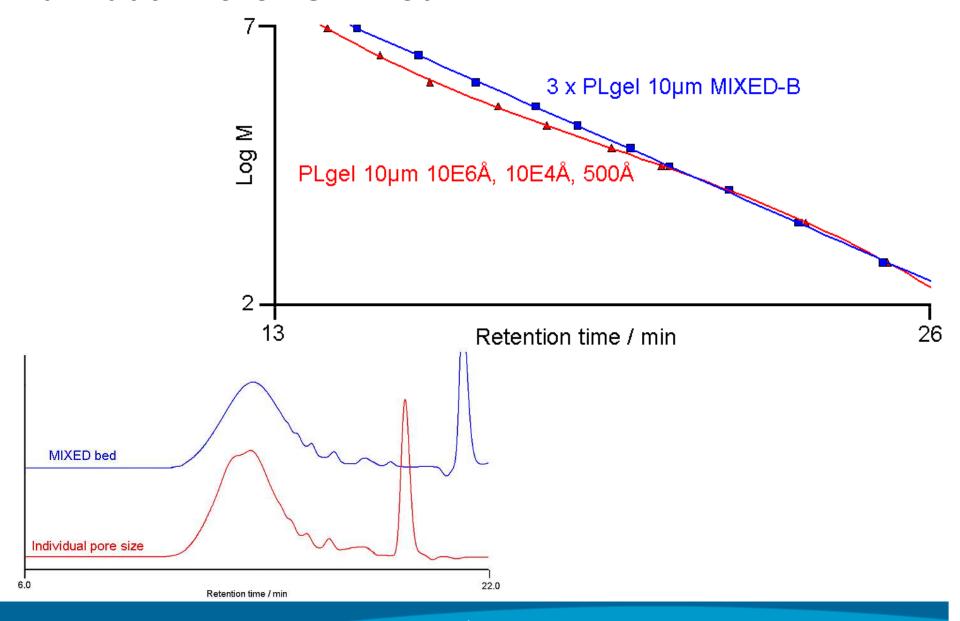
- Individual columns can be coupled in series
  - PLgel and PL aquagel-OH
- Need linear calibration ranges to complement without overlap

### **Wrongly Coupled Columns**



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

#### **Individual Pore vs Mixed**

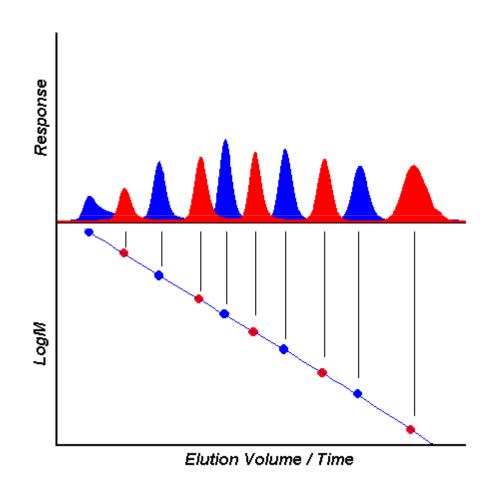


#### **Calibration Standards**

- GPC separates according to size
- Common detectors do not give Mw information
- How is Mw information obtained?
- Using calibration standards
- Known molecular weights against which unknowns are compared

# Calibration of GPC Columns Using Narrow Standards

- Chromatograph a series of well characterised,narrow polydispersity polymer standards
- Plot peak retention time (RT) versus peak log molecular weight (logM)
- Fit the data using a mathematical function (e.g. polynomial order 1,2,3, etc)
- The calibration curve will be characteristic of the GPC column set used



# Curve Fitting for Narrow Standards Calibration

#### **Polynomial**

All data points fitted with one function of the form

Log M = A + B(t) Linear (1st order)

Log M = A + B(t) + C(t<sup>2</sup>) Quadratic (2nd order)

Log M = A + B(t) + C(t<sup>2</sup>) + D(t<sup>3</sup>) Cubic (3rd order)

#### **Cubic spline**

Sets of points fitted with a series of cubic equations

#### Point to point

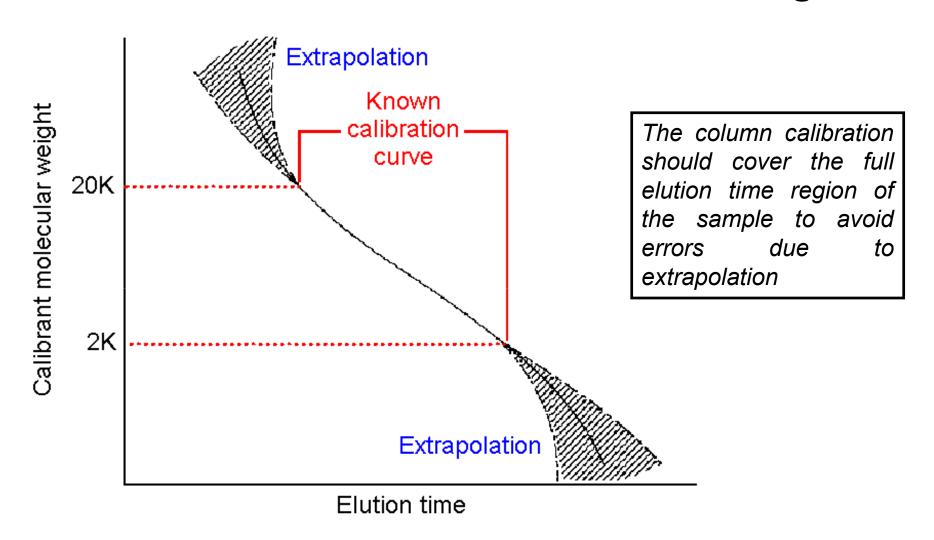
Points fitted with a series of linear equations

#### **Standard Selection**

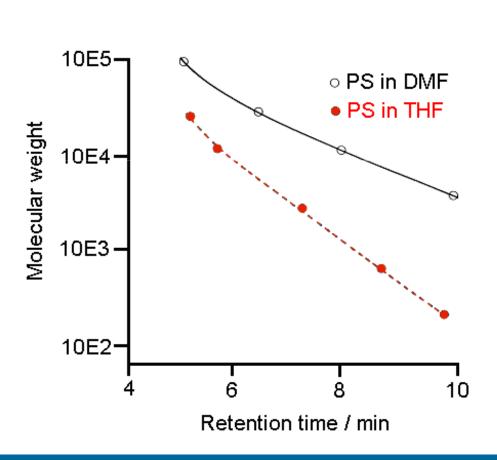
- Standards chosen by solvent type
- Ideally similar structure to the sample
- There are several popular standards

Standard	Solvents		
Polystyrene	THF, Toluene, Chloroform, TCB		
Polymethylmethacrylate	MEK, ethyl acetate, acetone		
Polyethylene	THF, Toluene		
PEG/PEO	Aqueous, DMF, DMSO, NMP		
Pullulan polysaccharide	Aqueous		
Polyacrylic acid	Aqueous		

### **Errors Due to Limited Calibration Region**



### **Adsorption of Polystyrene Standards in DMF**



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 : 500Å

PLgel 5um 300x7.5mm

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### **Sample Concentration**

- The viscosity of the polymer solution is dependant on both the molecular weight and the concentration
- A high viscosity in the separation zone leads to reduced mass transfer and band broadening
- This results in decreased resolution and in extreme cases peak splitting

# Sample Loading for GPC, General Guidelines

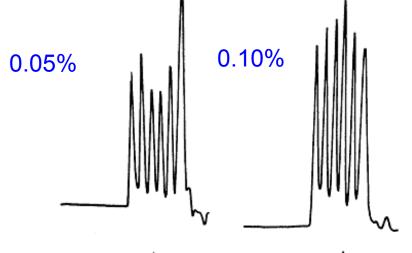
viscosity = MW \* concentration

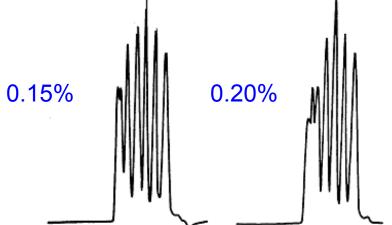
For **high MW** samples use lower concentration and if detector response requires it, increase injection volume

For **low MW** samples use higher concentrations and avoid larger injection volumes to maintain high resolution

MW	Conc (%)	lnj vol (ul)
<50,000	0.20-0.50	20-50
50,000 - 500,000	0.10-0.20	50-200
>500,000	.01-0.10	50-200

# Effect of Concentration on Peak Shape and Resolution





Column: PLgel 10µm MIXED-B

300x7.5mm

Eluent: THF

Flow Rate: 1.0ml/min

Detector: UV

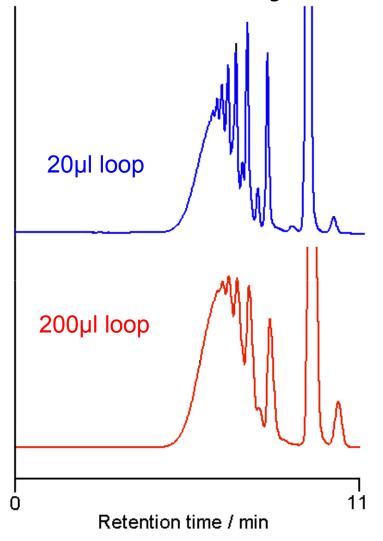
#### Polystyrene standards

1. 8,500,000 4. 34,500

2. 1,130,000 5. 5,100

3. 170,000 6. 580

#### **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

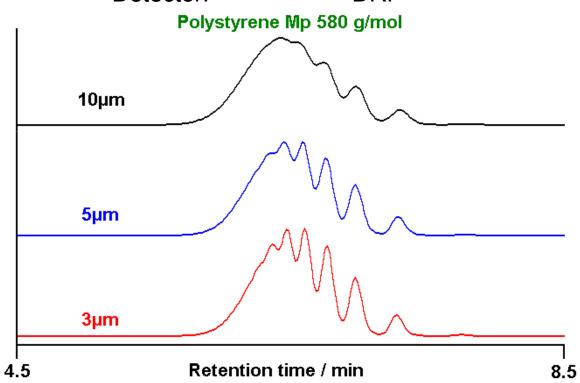
Injection loop is a major contribution to system dead volume, use reduced injection volume and increase concentration to maintain sensitivity

#### **Effect of Particle Size on Resolution**

Eluent: THF

Flow Rate: 1.0ml/min

Inj Vol: 20µl Detector: DRI



#### **Common Detectors Used**

Differential Refractive Index Detector (DRI)

UV Detector (UV)

Evaporative Mass Detector (ELSD).

### **Sensitivity of DRI Versus ELSD**

2 x PLgel 5µm MIXED-C 300x7.5mm Columns THF Eluent Flow rate 1.0ml/min **ELSD** is essentially 0.1%,  $20\mu$ l Loading dn/dc, independent of improvement in sensitivity will depend on a number of solute Mp values parameters 1. 7,500,000 2. 841,700 3. 148,000 4. 28,500 **ELSD** DRI 5. 2,930 0 20 Retention time / min

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#### **Molecular Weight Sensitive**

- These are GPC detectors that give a response directly related to the molecular weight of the material eluting from the GPC column
- By using molecular weight sensitive detectors, you can get information that is not available from conventional GPC
  - Molecular weights that aren't dependent on the chemistry of your standards and samples
  - The determination of 'structural information' about the polymer in solution

### **Viscosity Detector**

- Detector response proportional to the intrinsic viscosity [η] of the polymer
- Permits determination of branching in polymers

#### **Light Scattering Detectors**

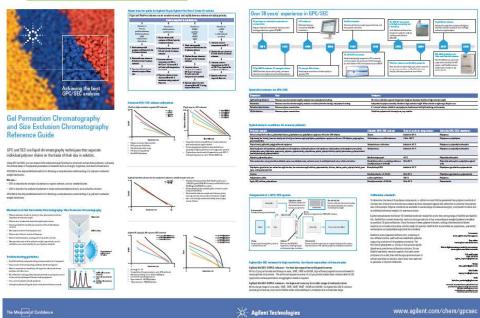
- Must be used with a concentration detector, typically DRI detector
- No column calibration required
- Detector response directly proportional to weight average molecular weight (Mw) of the polymer

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#### **Further Information**



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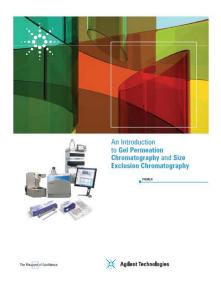


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Measure of Confidence Agilent Technologies

#### 5990-6868EN



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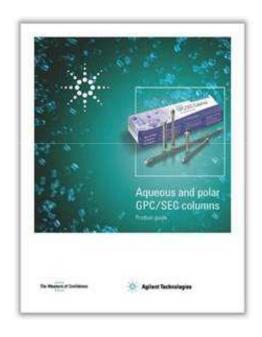
#### **Product Guides**

# Organic GPC/SEC Columns



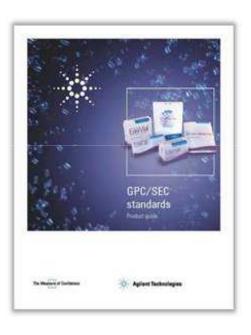
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# Aqueous and Polar GPC/SEC Columns



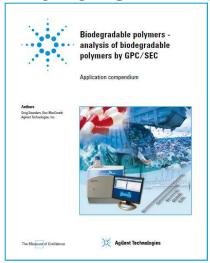
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#### **Standards**



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#### **Further Information**







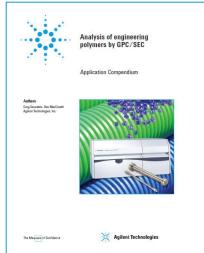


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## Thank you for your attendance!

