Gel Permeation Chromatography Basics and Beyond eSeminar March 13, 2013

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Technical and Applications Support
LSCA, Columns and Supplies

Content

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

Terminology

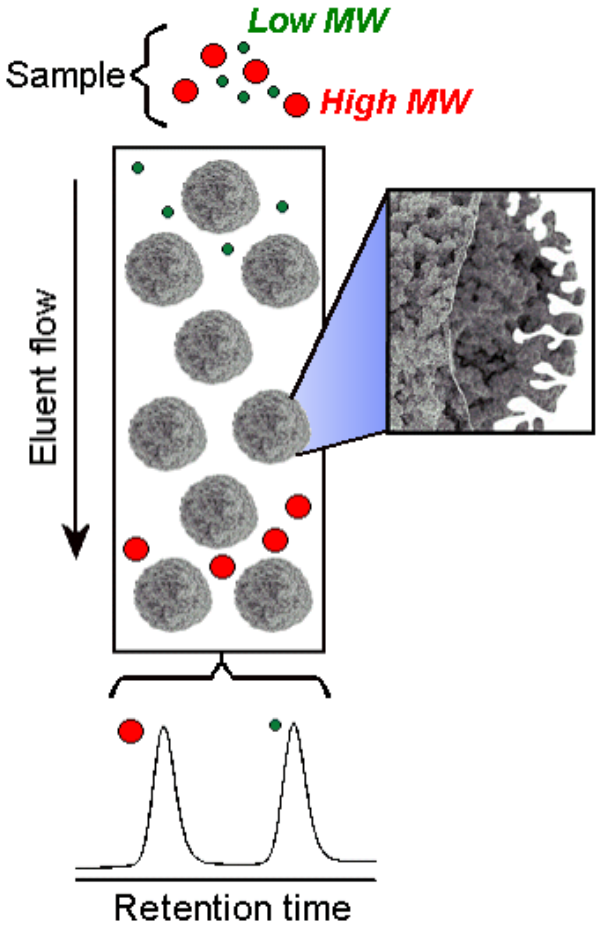
GPC - Gel Permeation Chromatography

SEC – Size Exclusion Chromatography

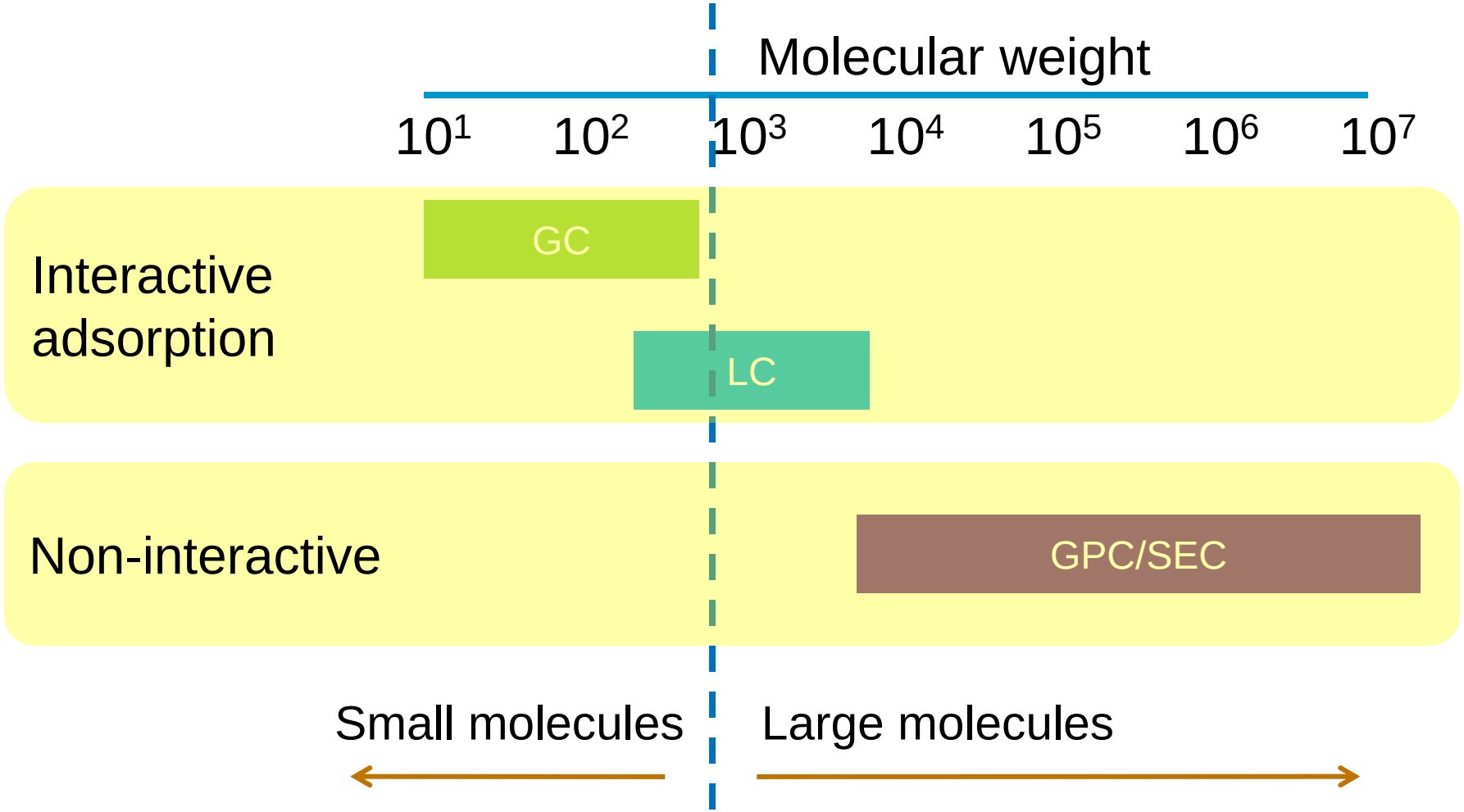
GFC – Gel Filtration Chromatography

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

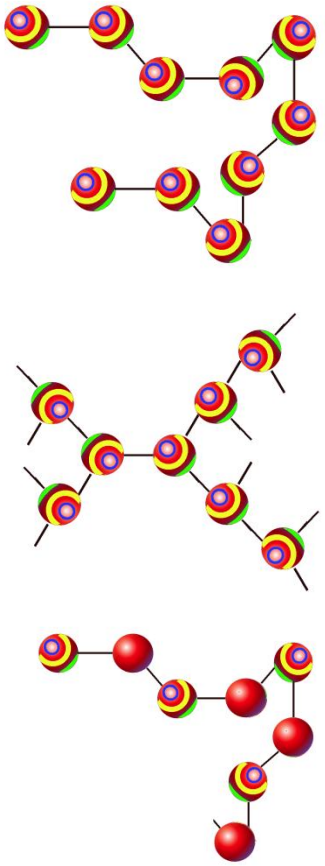


What are Polymers?

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

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

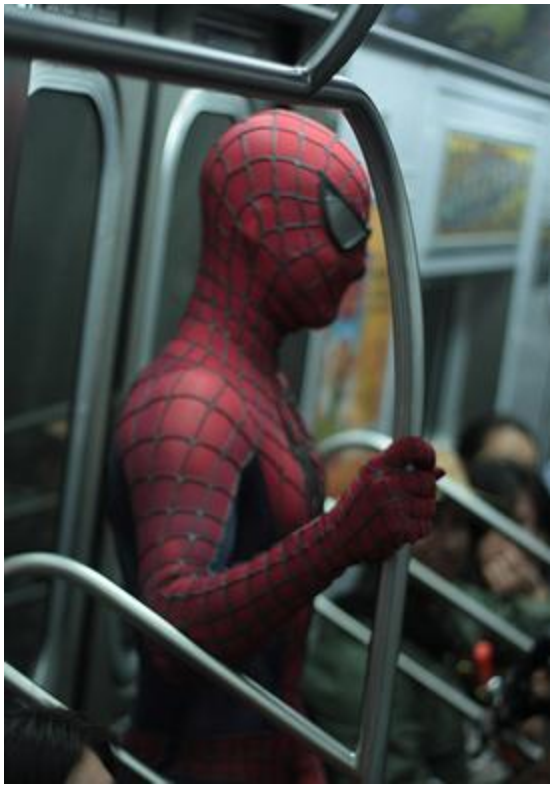
- Chemical Structure of Monomer Unit
- 3D Structure
- Different Monomer Units
- Length of polymer chains
- Distribution of polymer chain lengths



Most Common Examples

Polystyrene

Polyethylene



Nylon

Polyvinylchloride, PVC

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

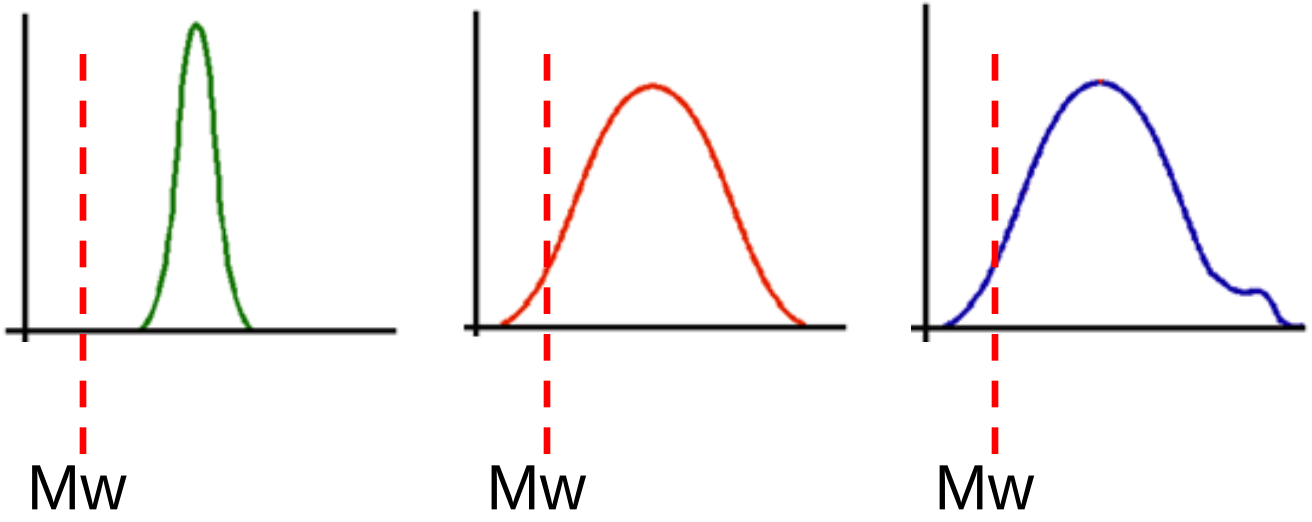
- Samples of synthetic polymers *always* contain polymer chains with a range of chain lengths
- One way to describe the length of the polymer chains is in terms of an average molecular weight, i.e the average of all the chain lengths in the sample

HOWEVER….

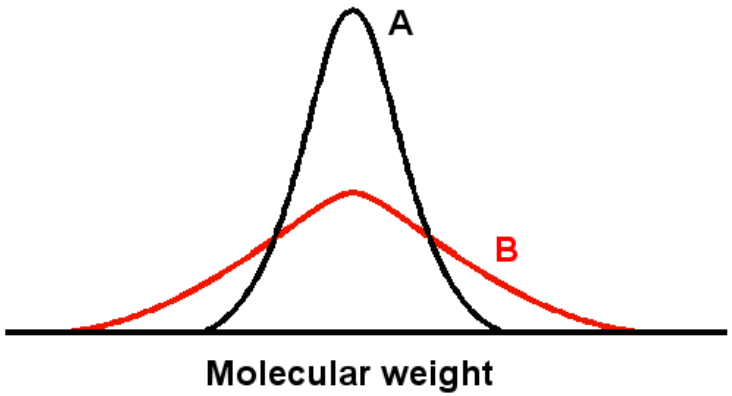
- Different samples of the same polymer can have the same average chain length but very different distributions of chain lengths depending on the method of production
- In polymer science, it is the molecular weight *distribution* that is important

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	+	+	+	+	+	+

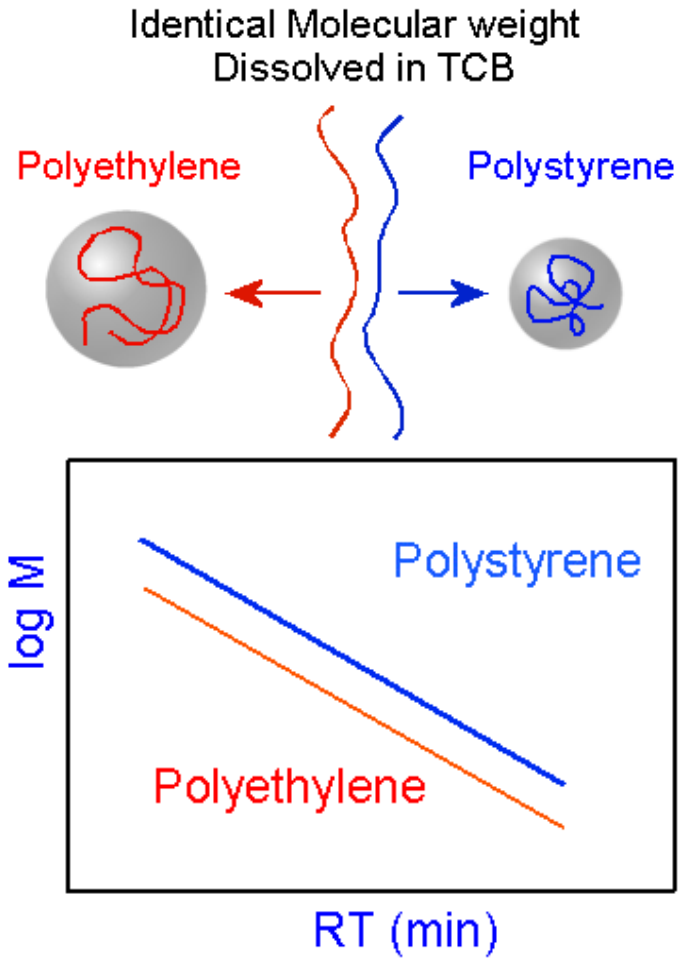
Polymer Behavior in Solution

- 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

Conventional GPC

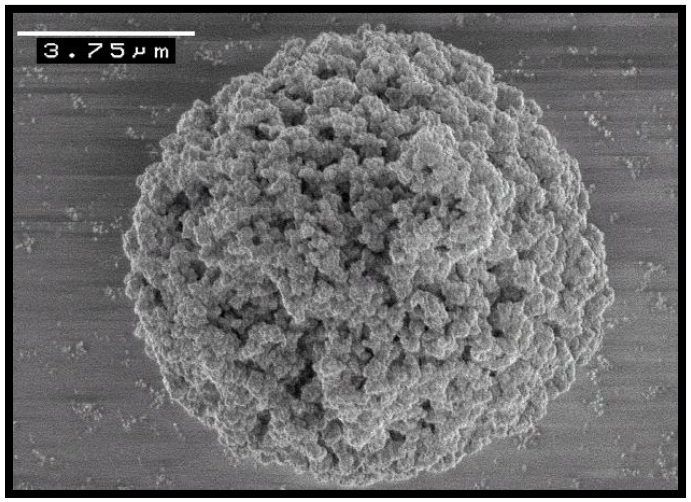
- Two different polymers will interact differently with solvent
- Column separates on basis of molecular size NOT molecular weight
- At any molecular weight, the two polymers will have different sizes in solution
- Molecular weights from conventional GPC are dependent on a comparison in size between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



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



In General, GPC Column Specifics

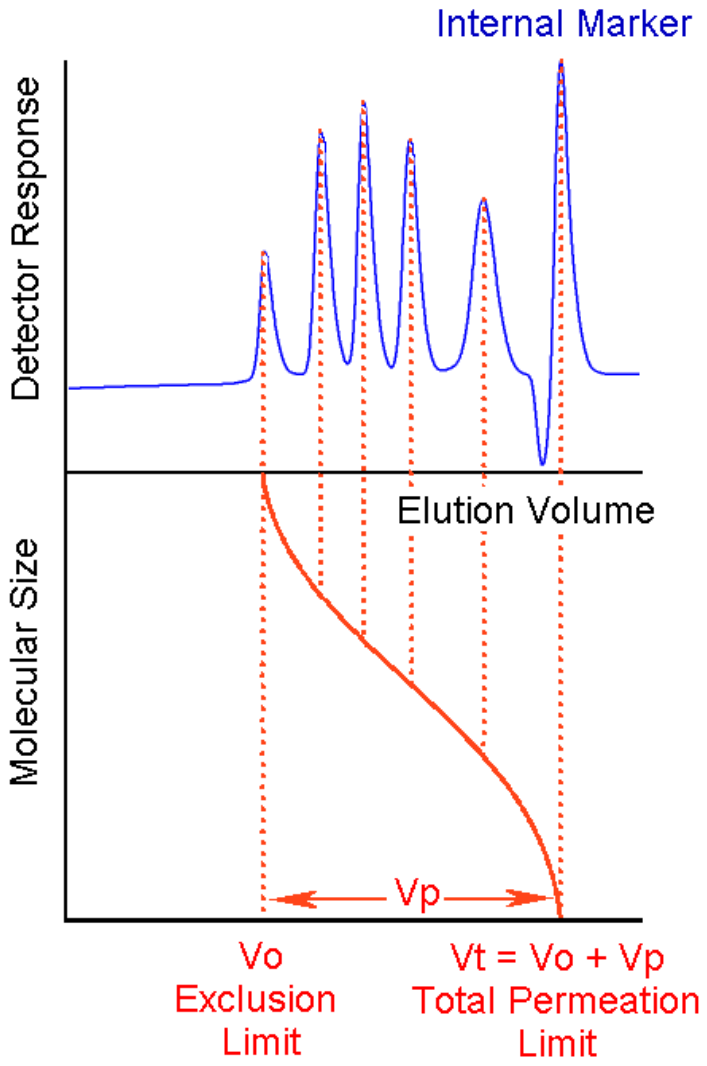
- Columns are packed with porous particles, controlled pore size and particle size
- Columns are produced by slurry packing technique, packed at pressures well in excess of 3000psi
- Column dimensions typically 7-8mm i.d., 250-600mm in length

- 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

Elution Profiles

- 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



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

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

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

Criteria for Solvent Selection

- True sample solubility (Polarity and Time dependant)
- Compatibility with columns
- Avoid non-size exclusion effects (eg adsorption by reverse phase interaction)
- Permit adequate detection (eg refractive index, UV cut off)
- Safety (eg toxicity, elevated temperature, etc)

Particle Technology – what is available to choose from?

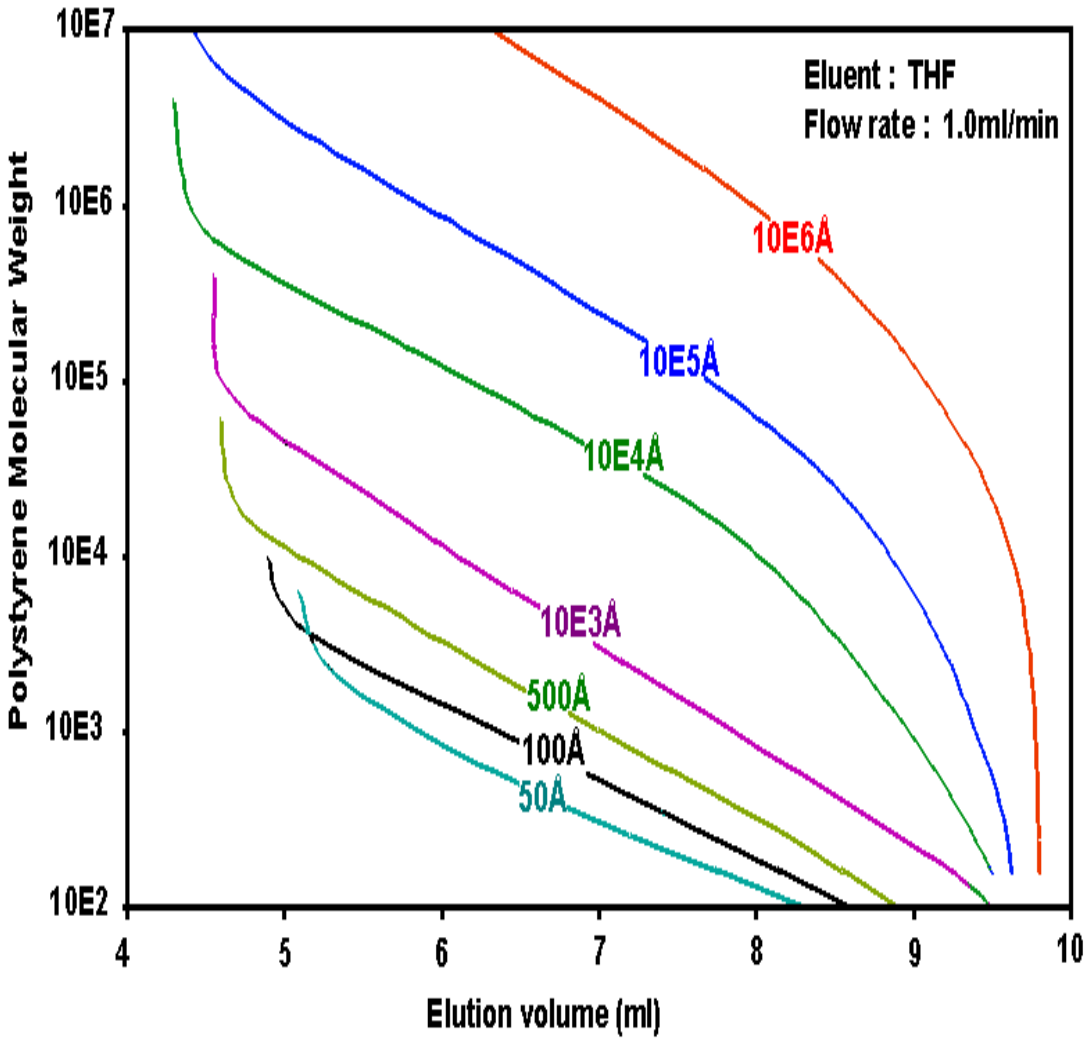
Individual Pore columns

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



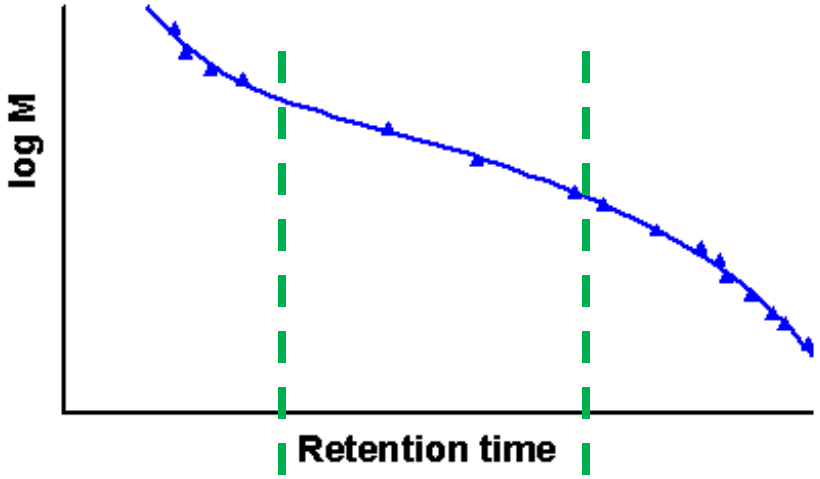
Mixed Particle Technology

- 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

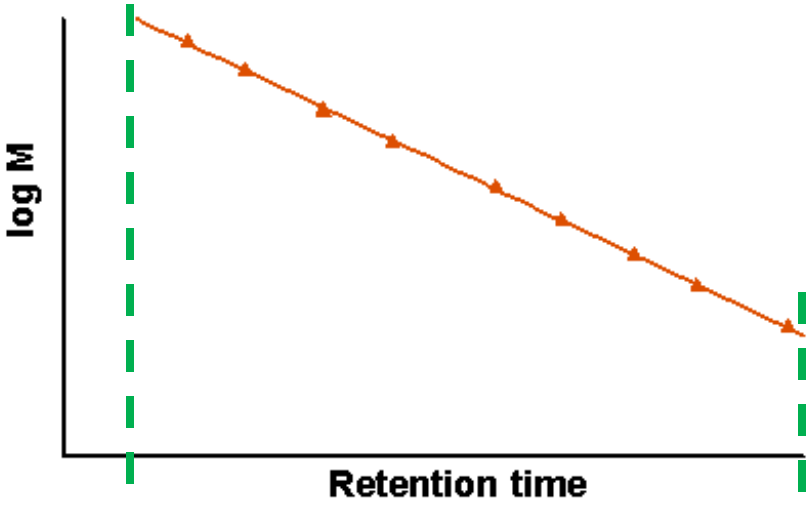
- Greatly simplified column selection
- Optimized columns for each application area
- No artifacts due to column mismatch
- Simply add another column of the same type for greater resolution

Individual Pore Size vs MIXED



PLgel 5µm, 10^4Å

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

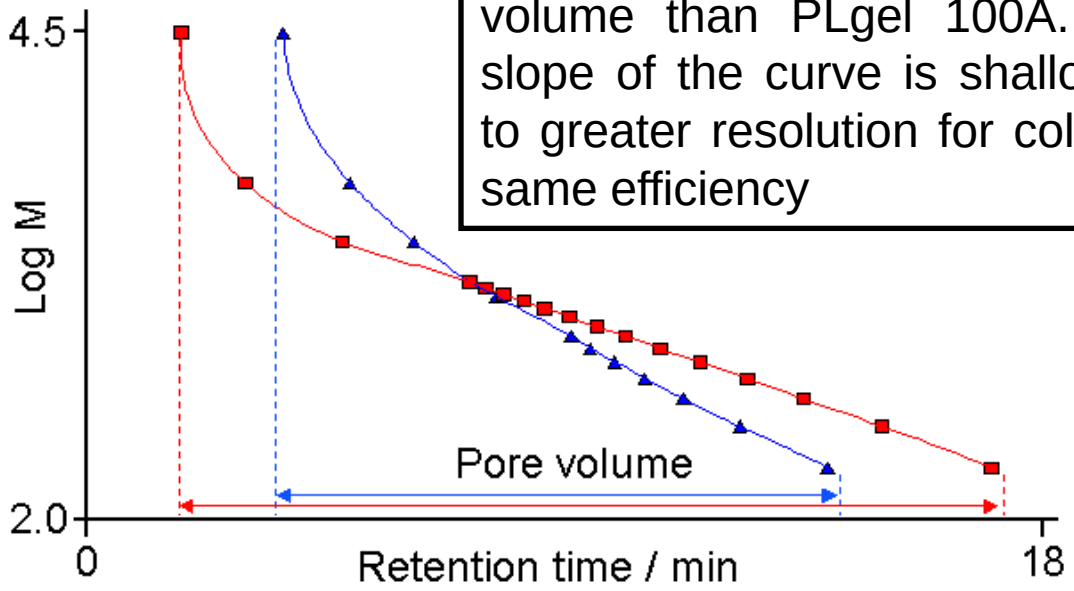
Benefits of Mixed Pore Technology

- 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

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



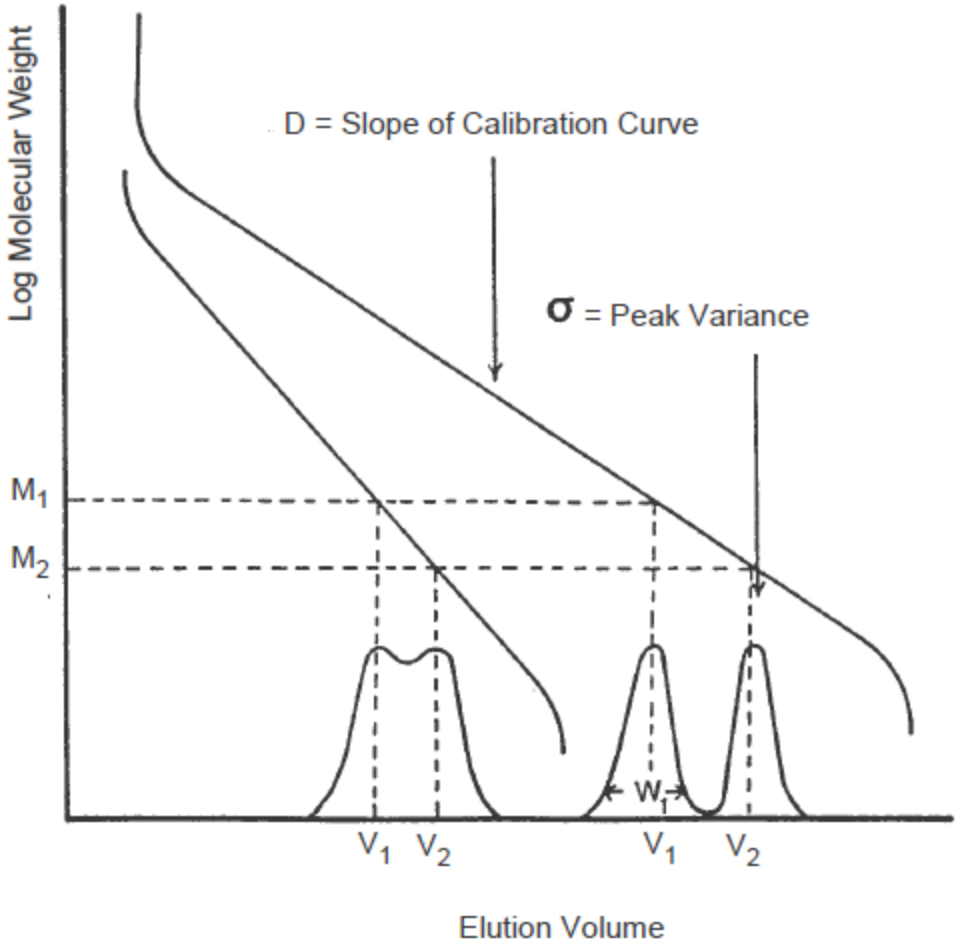
Column Selection – How Many Columns?

- More than one column typically used
  - More columns = better resolution
  - Also increases analysis time

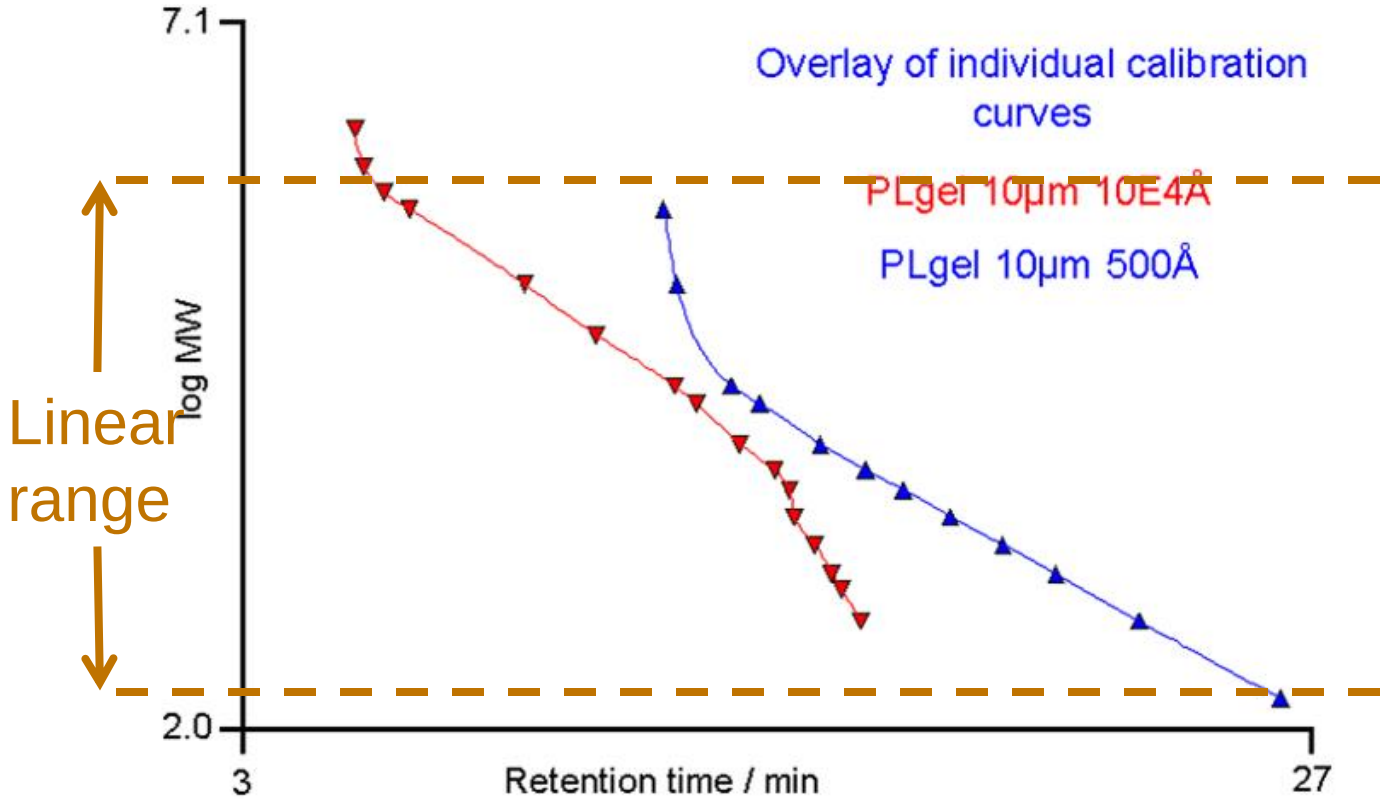
- 20µm particle size – 4 columns
- 13µm, 10µm – three columns
- 8µm, 5µm and 3µm – two columns

- Higher Mw tends to need more columns

Resolution in GPC – add a column to improve resolution

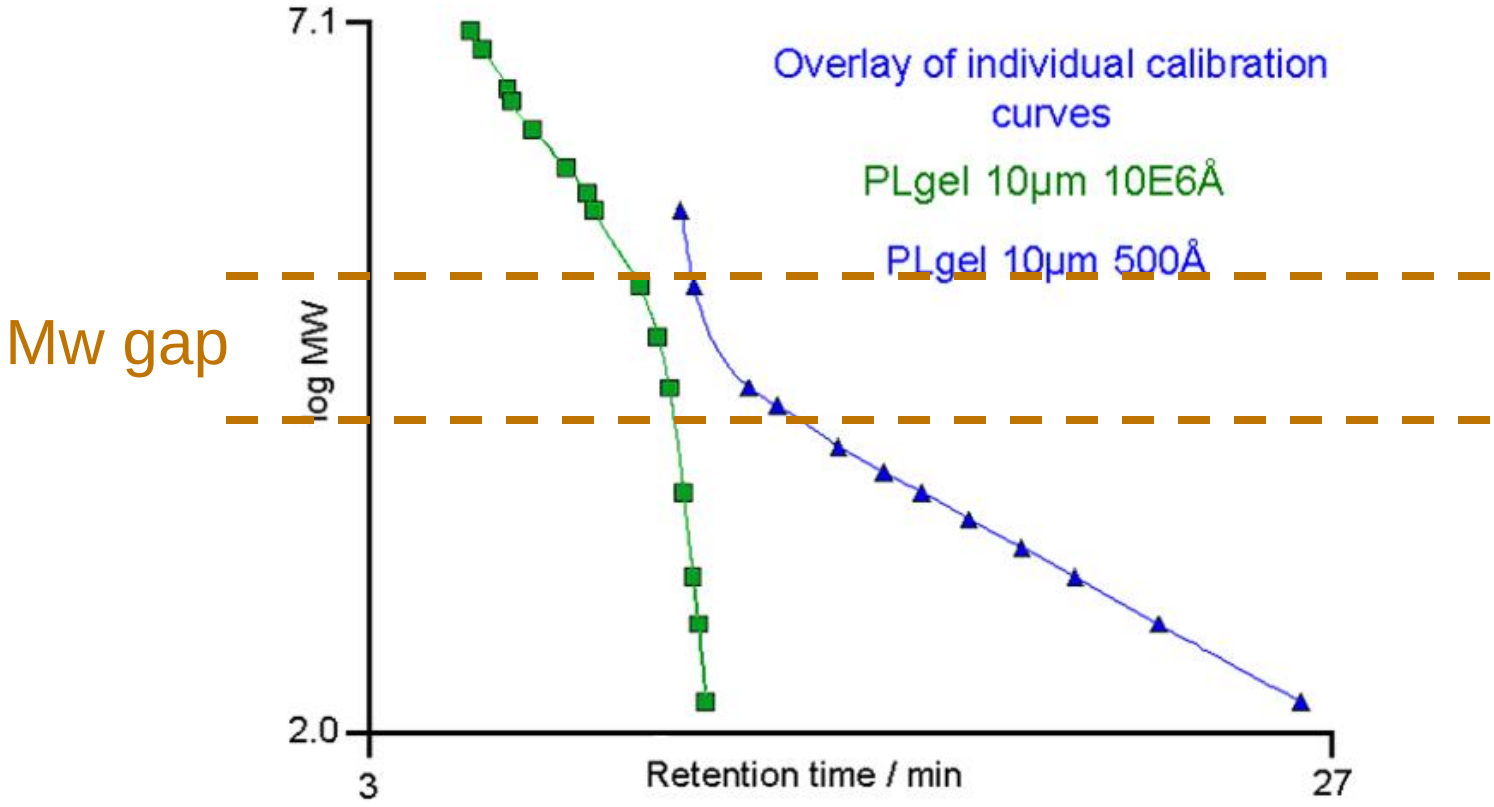


Increasing the Resolving Range



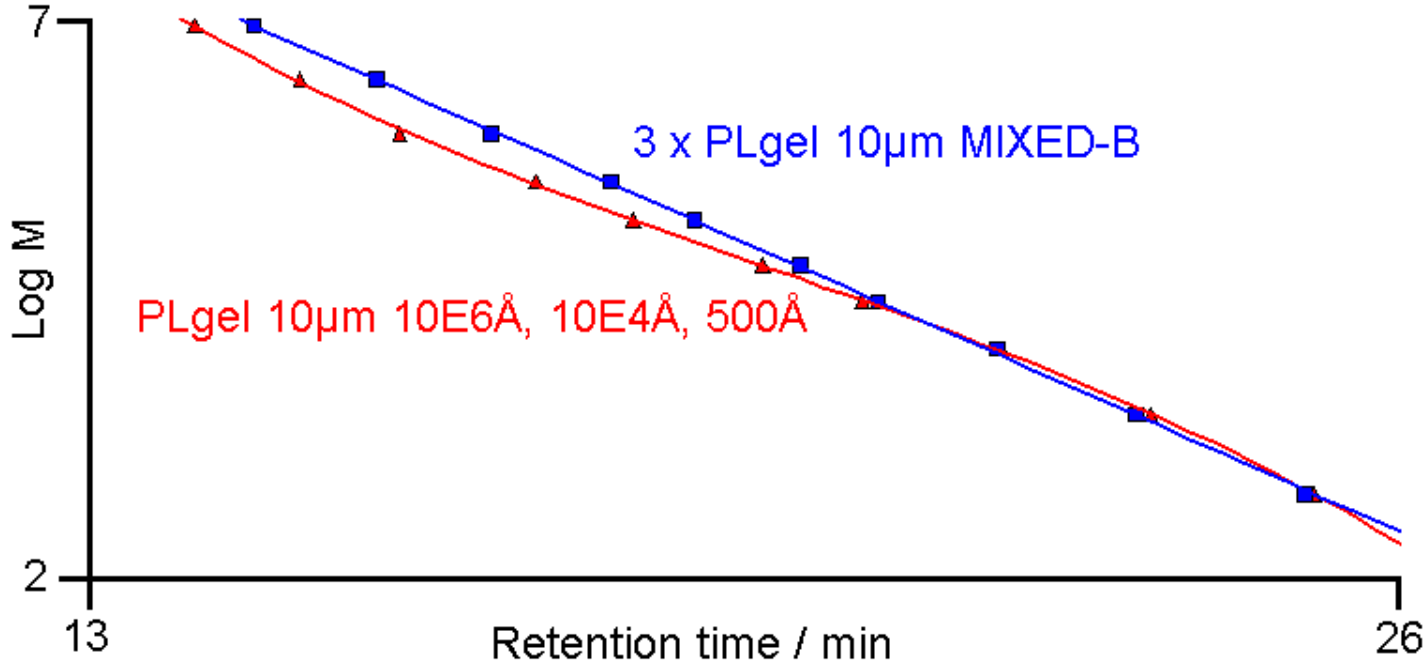
- Individual columns can be coupled in series
  - PLgel and PL aquagel-OH
- Need linear calibration ranges to complement without overlap

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

Polymer Calibrants for GPC

Mn - *number average molecular weight*

Mw - *weight average molecular weight*

Mv - *viscosity average molecular weight*

Mp ***- peak molecular weight***

Mw/Mn - *polydispersity by GPC*

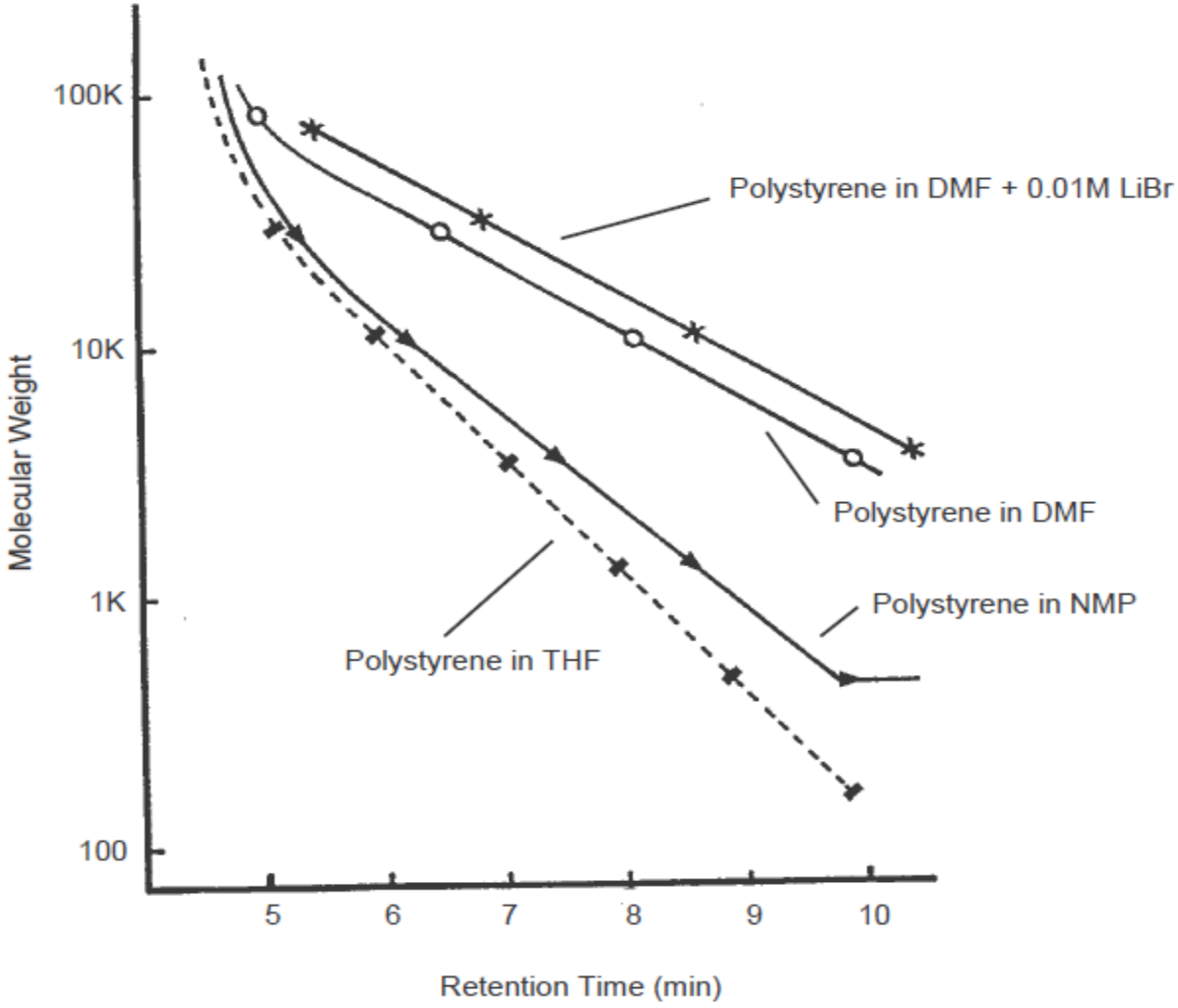
Std Must be extremely well characterized

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, DMF, DMAc
Polyacrylic acid	Aqueous

Calibrations Standards and Solvent Choice



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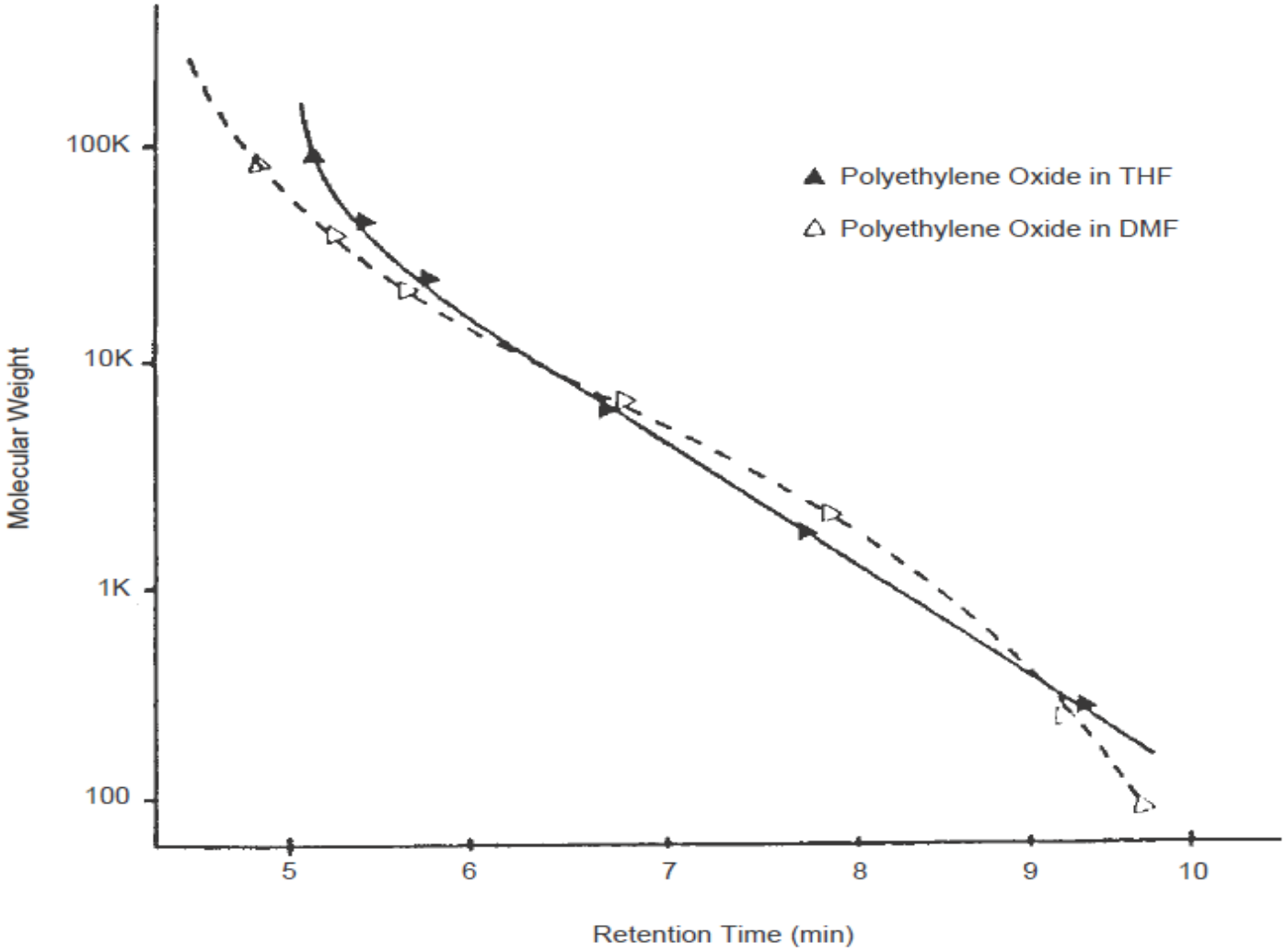
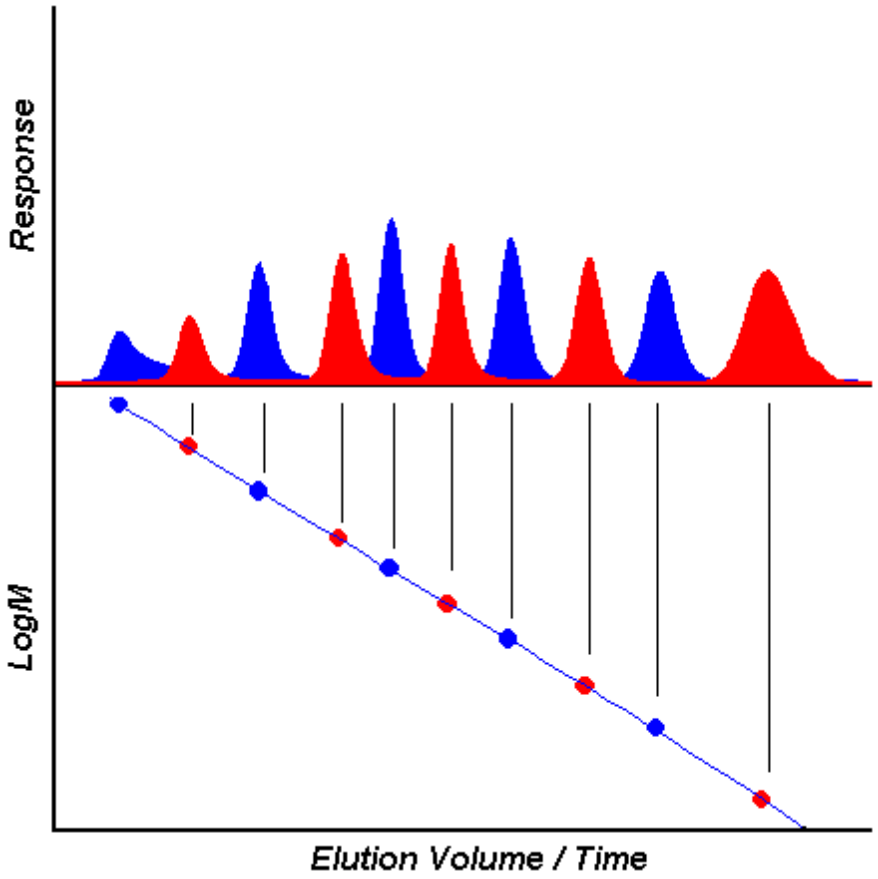
A Better Suited Polymer Standard Selection…….

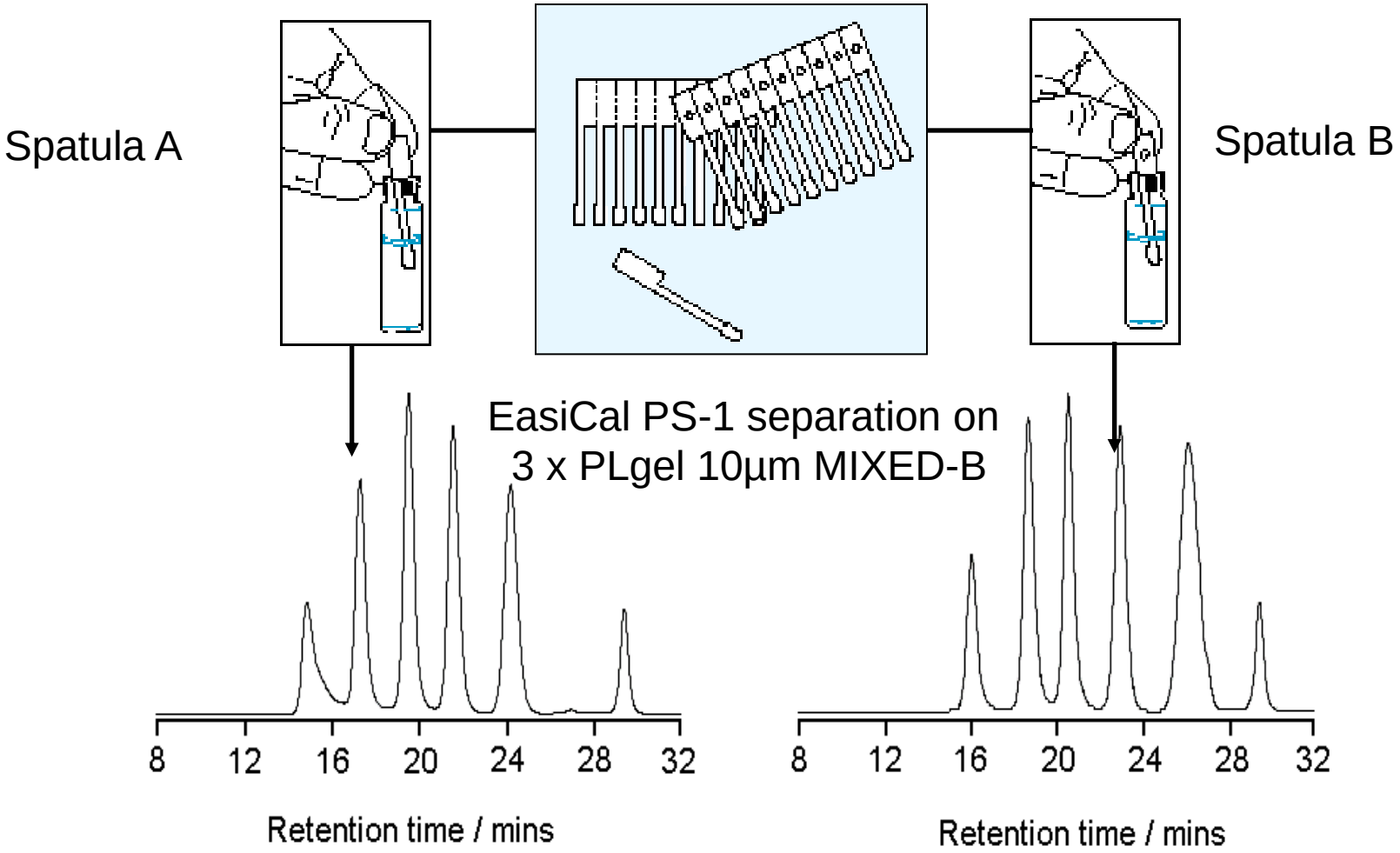


Calibration of GPC Columns Using Narrow Standards

- Chromatograph a series of well characterized,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



EasiCal Pre-prepared Calibrants



Curve Fitting for Narrow Standards Calibration

Polynomial

All data points fitted with one function of the form

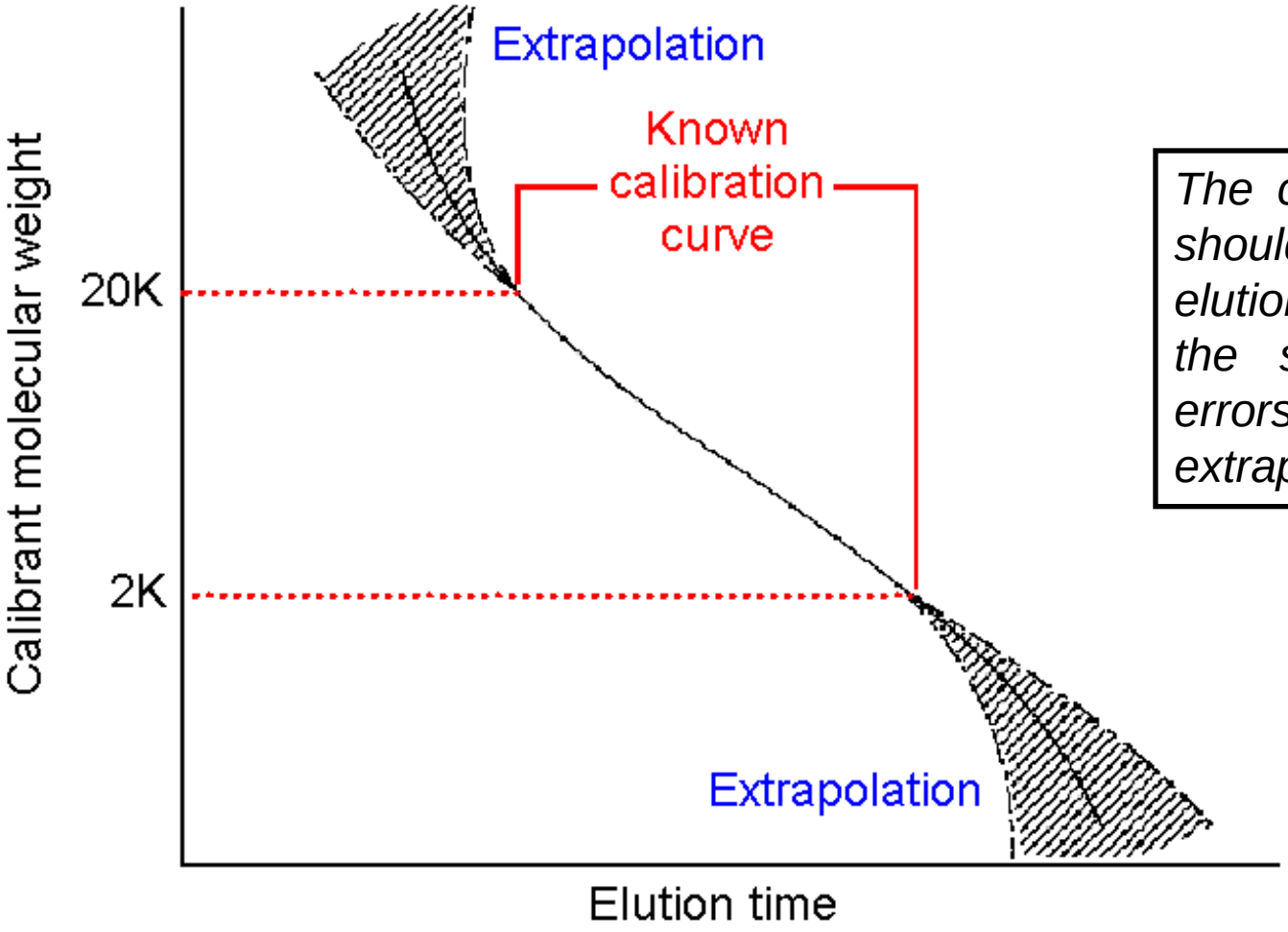
$\text{Log } M = A + B(t)$ *Linear (1st order)*

$\text{Log } M = A + B(t) + C(t^2)$ *Quadratic (2nd order)*

$\text{Log } M = A + B(t) + C(t^2) + D(t^3)$ *Cubic (3rd order)*

Column Range	Order of fit
Individual pore size	3rd
Mixed-Bed	1st
PlusPore	2nd

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

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

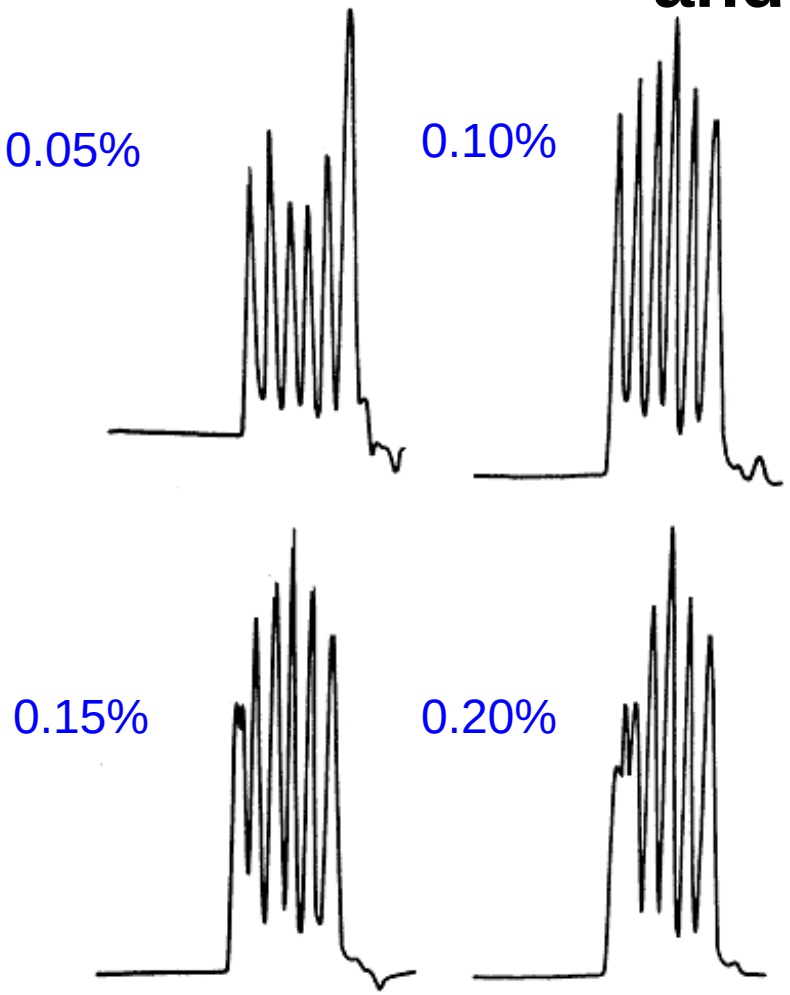
For **high MW** samples use lower concentration and if detector response requires it, increase injection volume

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

MW	Conc (%)	Inj 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

All values offered as guide only

Effect of Concentration on Peak Shape and Resolution



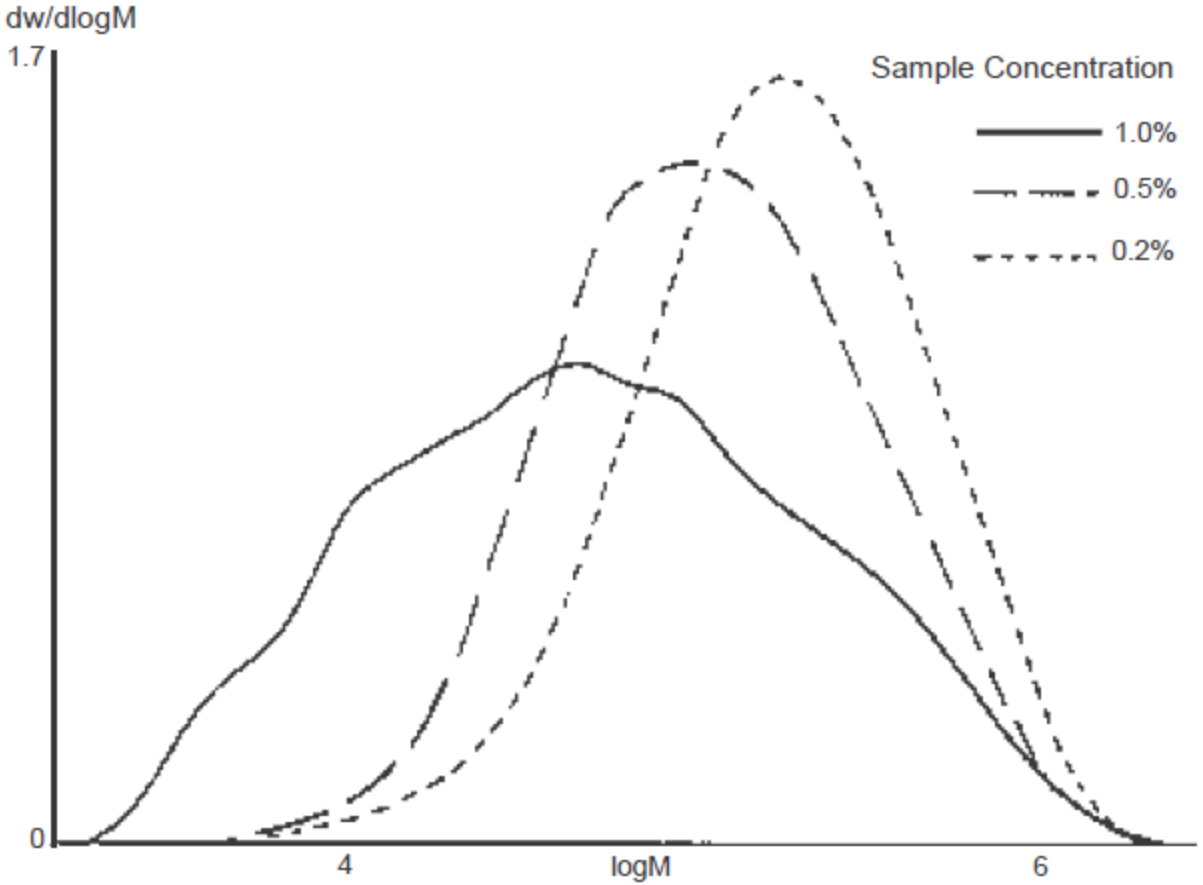
Column:	PLgel 10µm MIXED-B 300x7.5mm
Eluent:	THF
Flow Rate:	1.0ml/min
Detector:	UV

Polystyrene standards

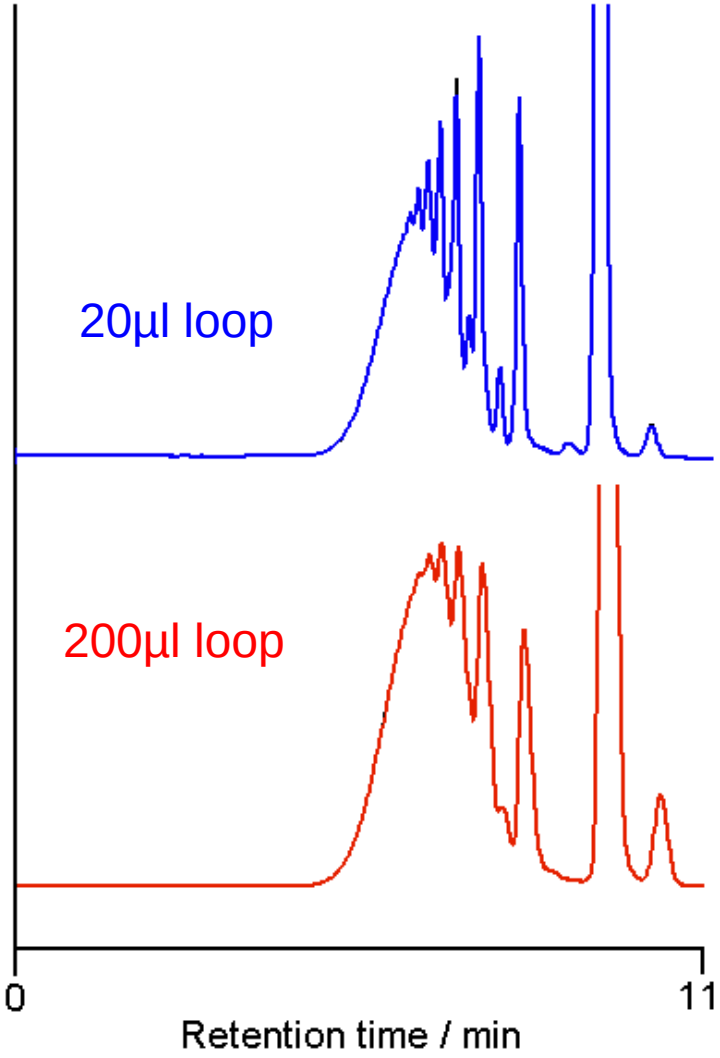
1. 8,500,000
2. 1,130,000
3. 170,000
4. 34,500
5. 5,100
6. 580

Overloading Effects

Sample:	Broad Polystyrene
Column:	2xPLgel 5µm MIXED-C, 300x7.7mm (1110-6500)
Eluent:	THF
Flow Rate:	1.0ml/min
Inj Vol:	200µl
Detector:	RI
Calibrants:	Polystyrene Standards



Effect of Injector Loop Size on Resolution

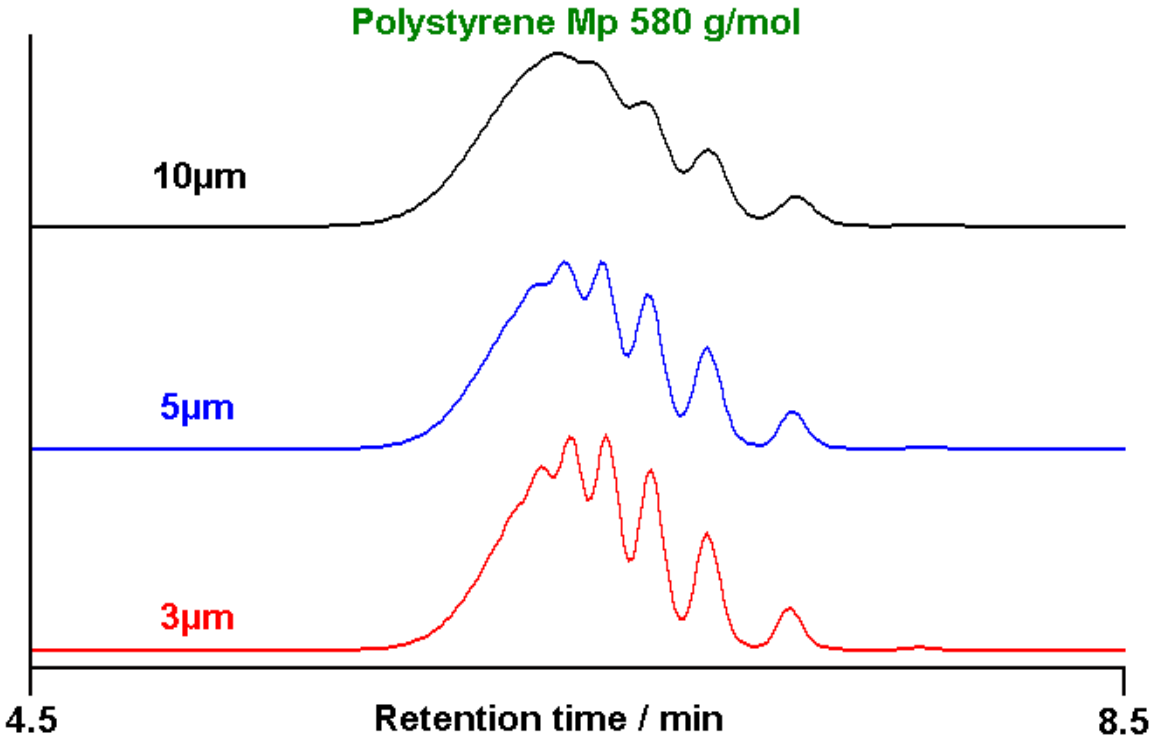


Column:	PLgel 3µm MIXED-E 300x7.5mm
Eluent:	THF
Flow Rate:	1.0ml/min
Sample:	Epikote 1001 epoxy resin

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)

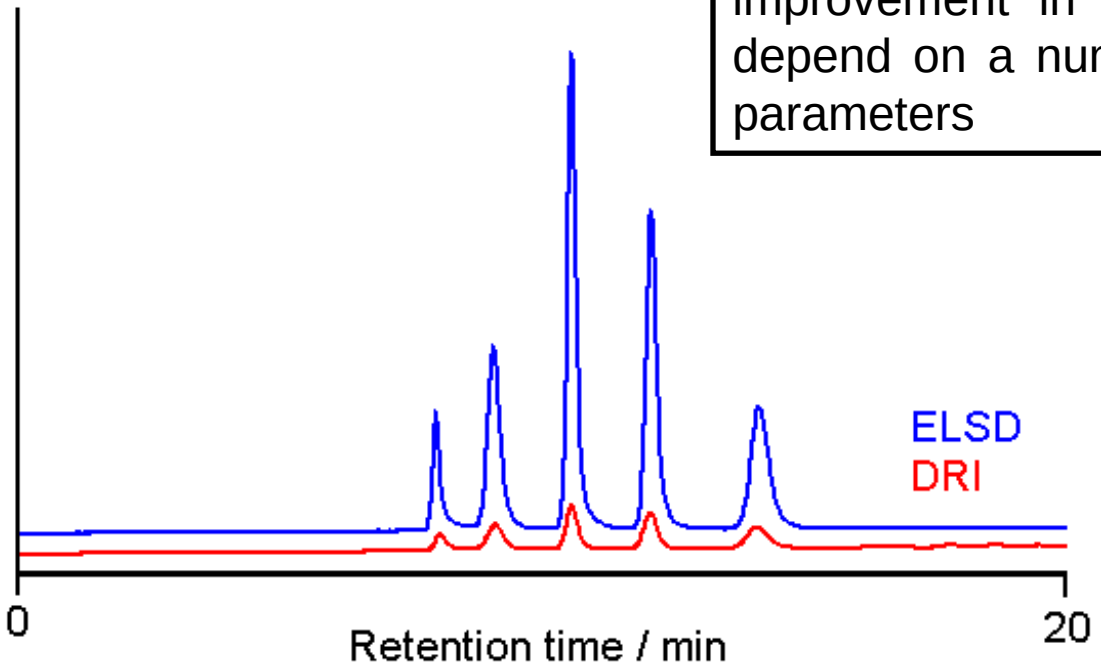
Evaporative Mass Detector (ELSD).

Sensitivity of DRI Versus ELSD

Columns	2 x PLgel 5µm MIXED-C 300x7.5mm
Eluent	THF
Flow rate	1.0ml/min
Loading	0.1%, 20µl

Mp values

1. 7,500,000
2. 841,700
3. 148,000
4. 28,500
5. 2,930



ELSD is essentially independent of dn/dc, improvement in sensitivity will depend on a number of solute parameters

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

Further Information…….
Product Guides

Organic GPC/SEC Columns

5990-7994EN

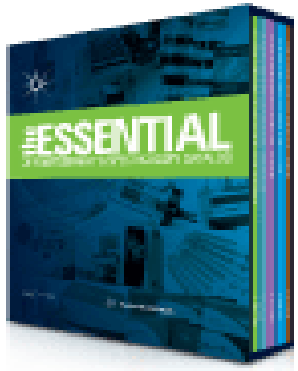
Aqueous and Polar GPC/SEC Columns

5990-7995EN

Standards

5990-7996EN

Further Information….



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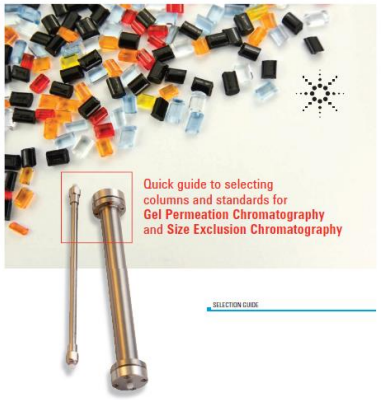

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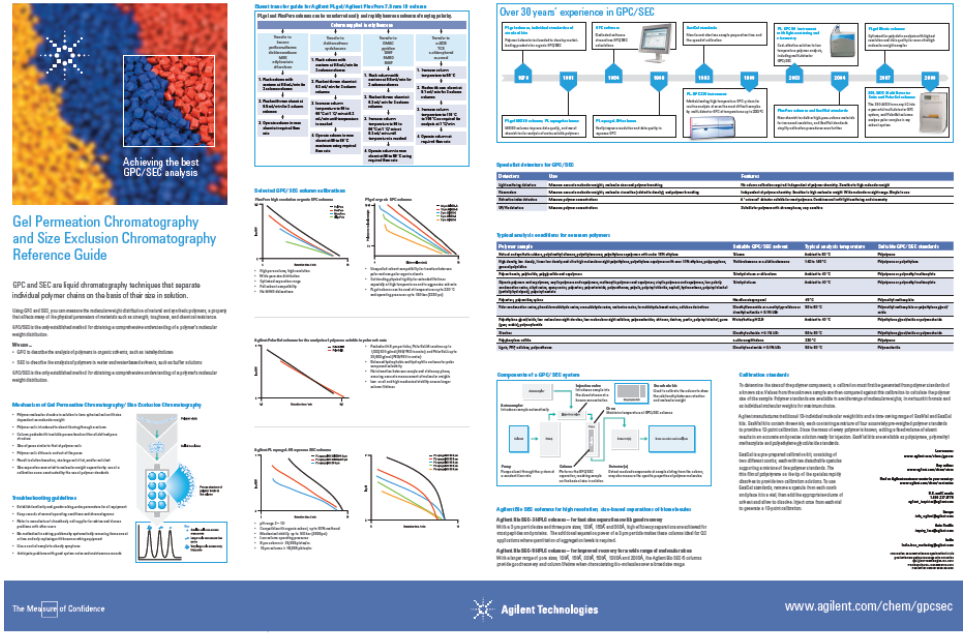

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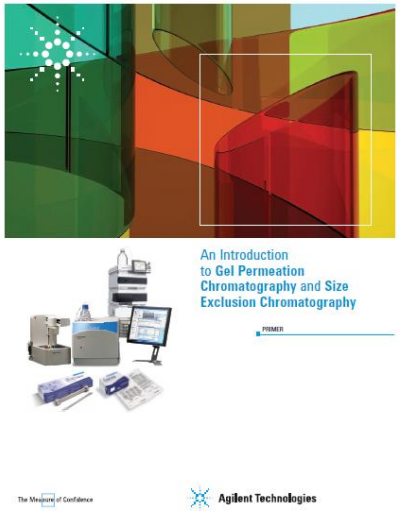

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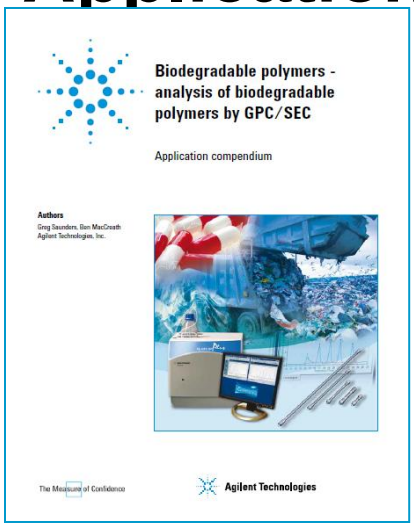
Application Compendiums



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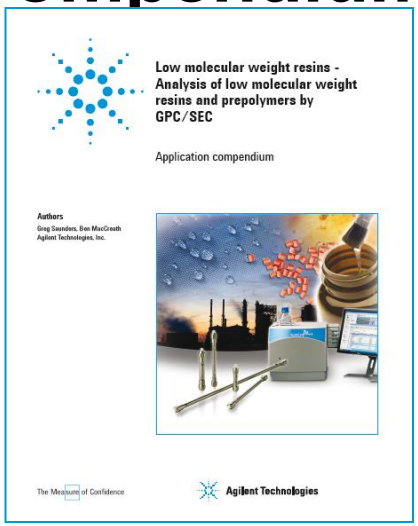
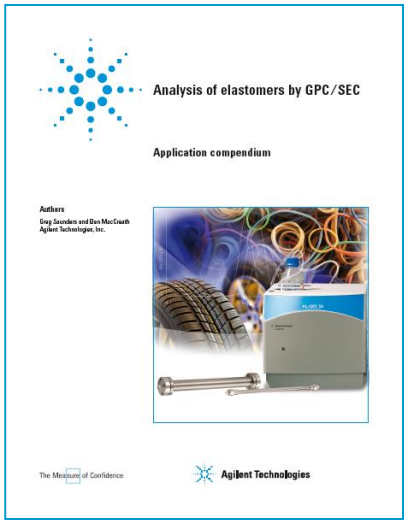


5990-6845EN



5990-6866EN

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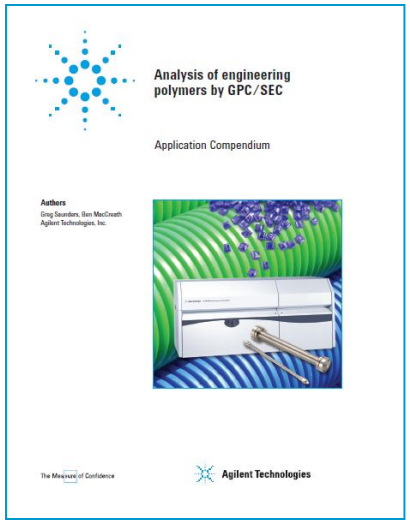

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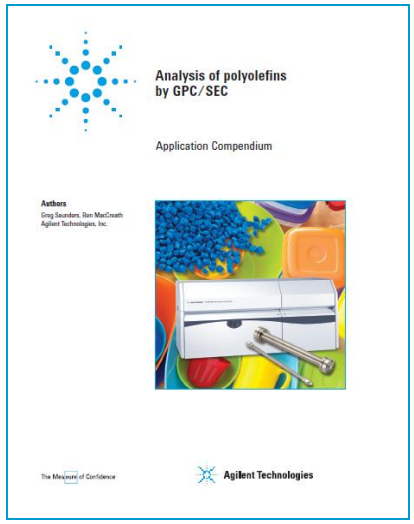

5990-6971EN

5990-7771EN

Thank you for your attendance !