GPC/SEC Practical Tips and Tricks

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Section 1: Introduction

Goals

- Brief introduction to GPC/SEC
- Highlight considerations for column and solvent selection
- Examples of problematic chromatography
- Propose possible solutions
- Components of a GPC

Nomenclature

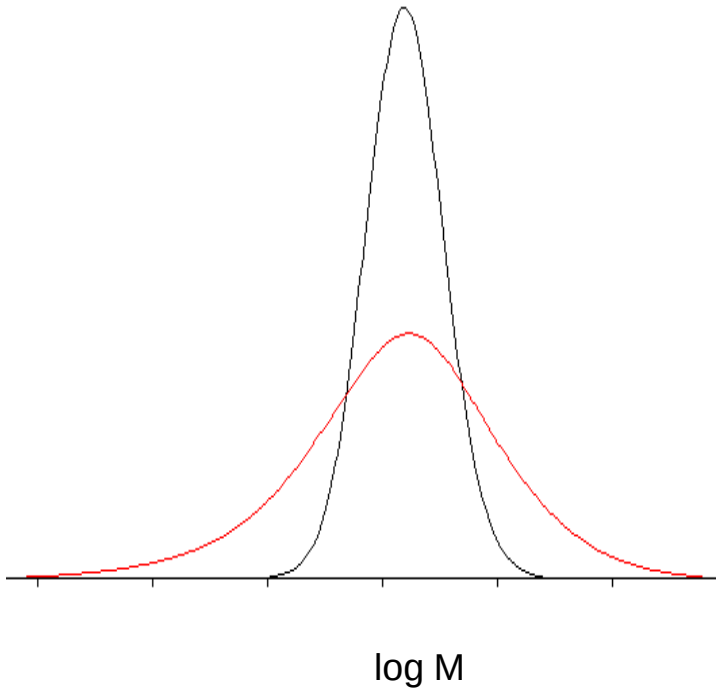
Gel Permeation Chromatography	GPC
Size Exclusion Chromatography	SEC
Gel Filtration Chromatography	GFC

All refer to the separation based on size or hydrodynamic volume in solution of a polymer or biopolymer in solution

Why do GPC?

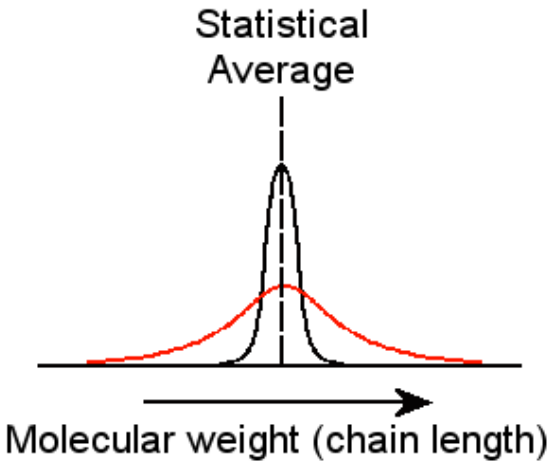
- GPC is the best technique for characterizing polymer molecular weight distribution
- GPC provides key information to predict the process-ability and material properties of a polymer….
- Often MW is not enough information

MWD determined by GPC



Both Samples = Mp 100,000

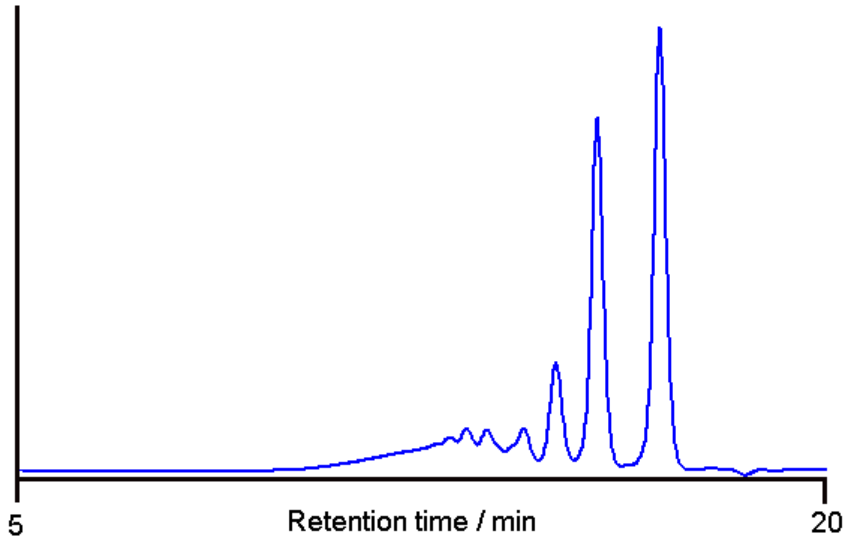
Understanding the Molecular Weight Distributions of Polymers



- The MW distribution controls many physical properties
- As distribution decreases the strength and toughness of the polymer increases
- However distribution decreases the polymer becomes more difficult to process
- GPC provides key information to predict the processability and material properties of a polymer

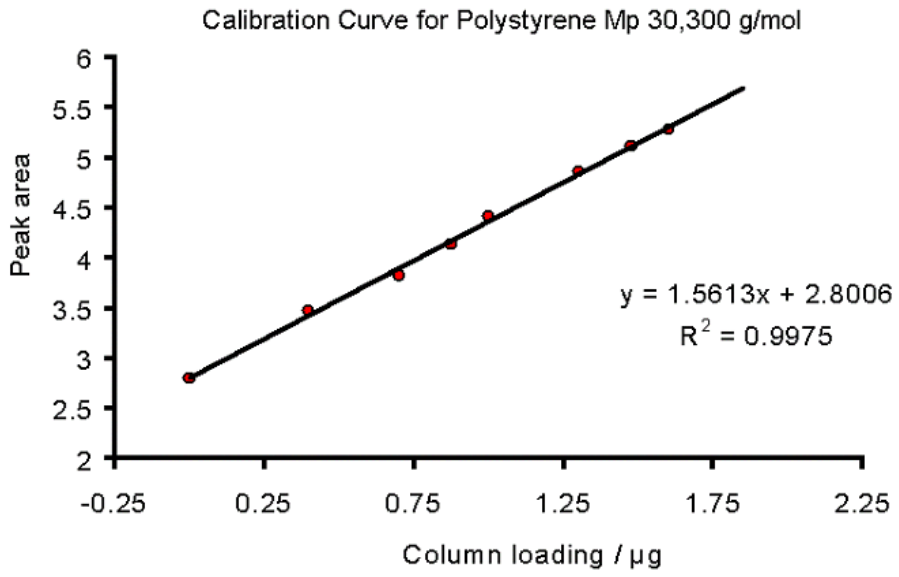
	Strength	Toughness	Brittleness	Melt viscosity	Chemical resistance	Solubility
Increasing Mw	+	+	+	+	+	-
Decreasing distribution	+	+	-	+	+	+

Why do GPC?



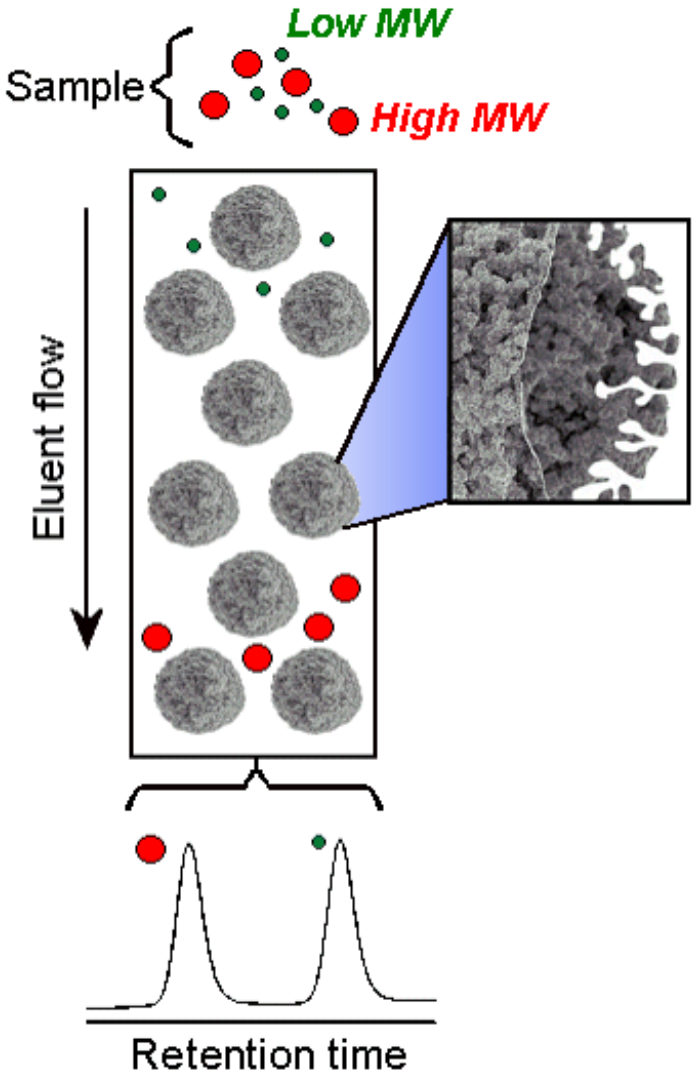
Isolation…

…Quantitation

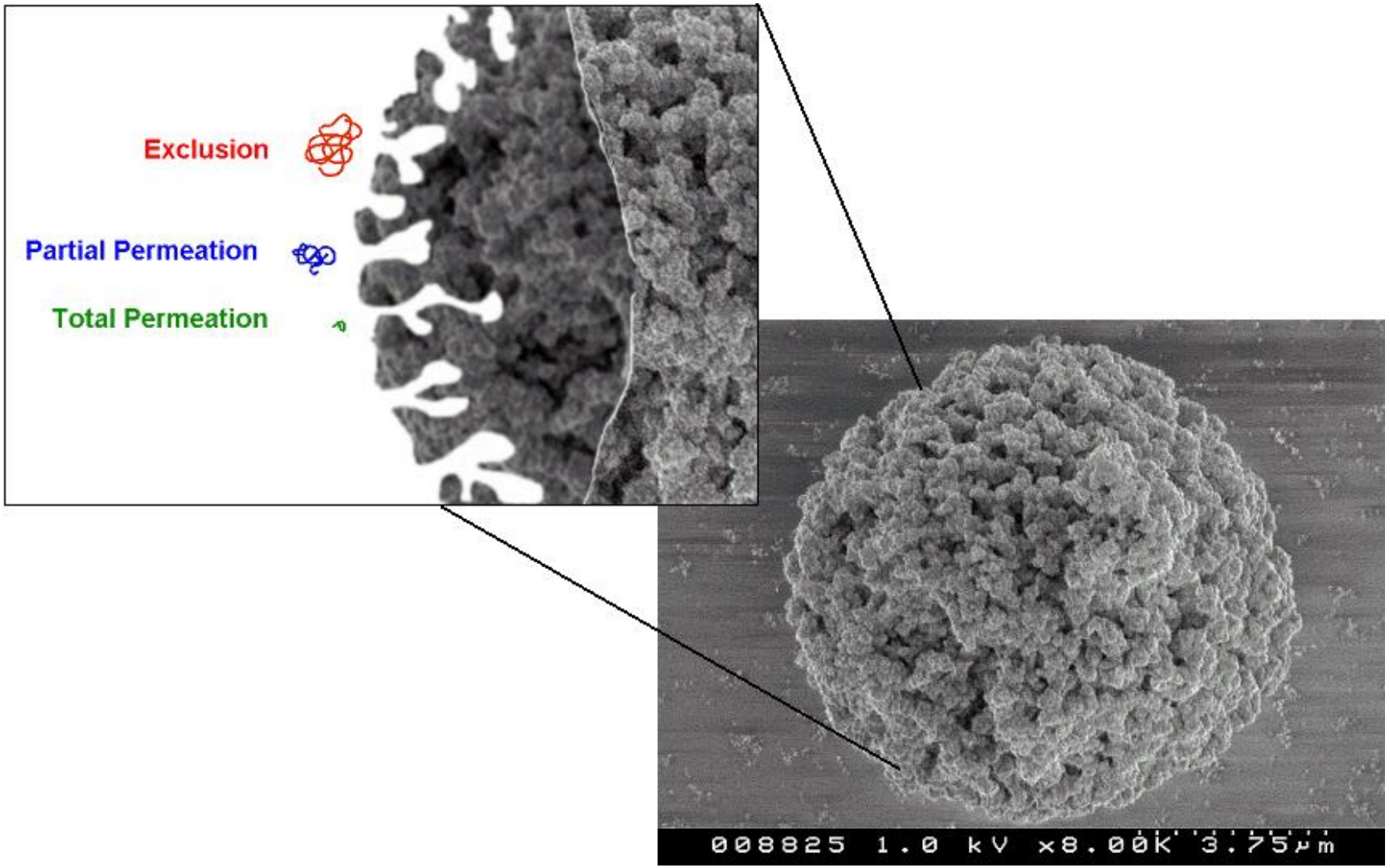


GPC Separation Mechanism

- Polymer is introduced onto the column
- The 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



Size Exclusion Mechanism



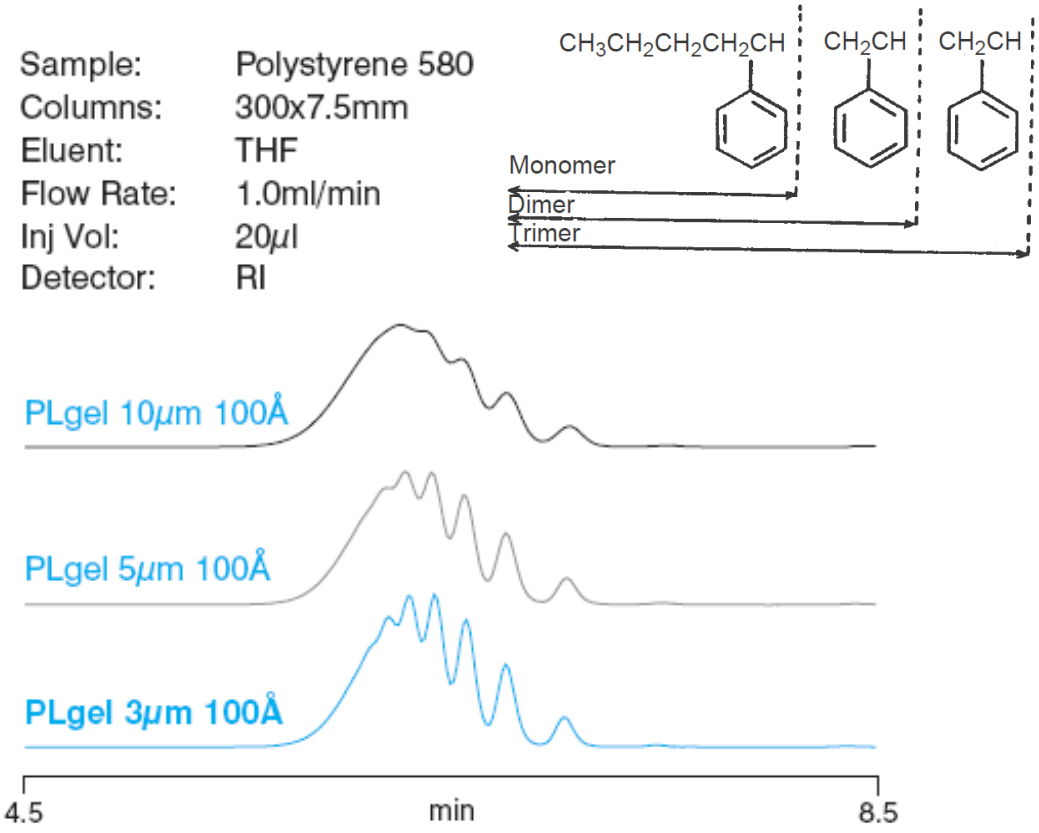
Section 2: Column Selection

- The columns perform the separation
- The choice and care of columns is critical to good chromatography
- Column selection criteria include
  - **mw range**
  - **pore size (related to mw range)**
  - **solvent compatibility**
  - **particle size**
  - **column volume**

- Let’s Look at examples

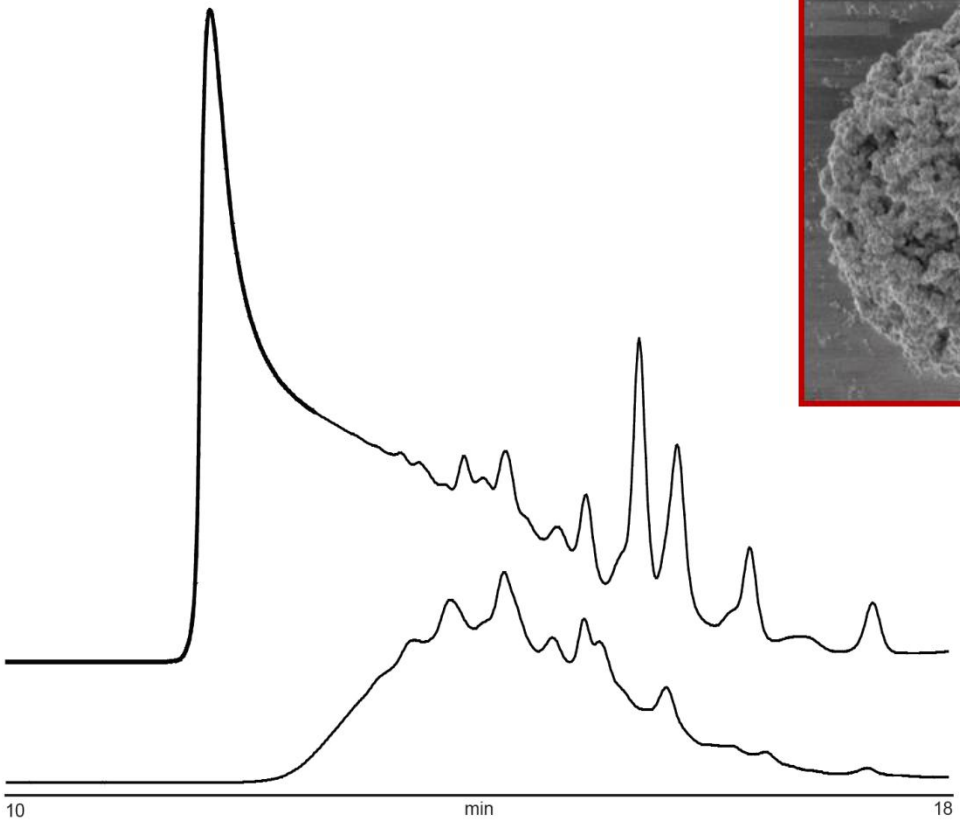
Effect of Particle Size on Resolution

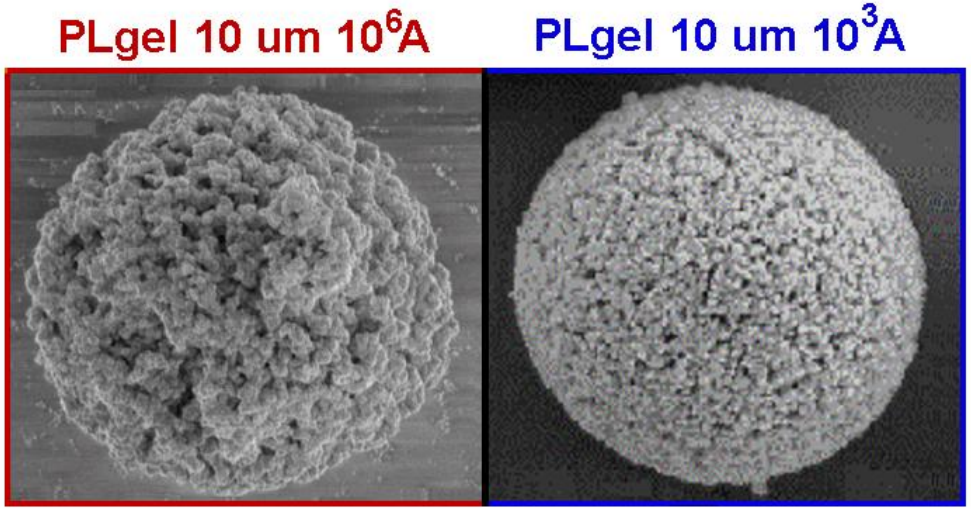
- Smaller particle size leads to greater efficiency and resolution
- Smaller particle size also leads to shear degradation
- Therefore only use small particle sizes for relatively low molecular weight separations
- High molecular weight separations require large particle sizes



Effect of Pore Size

Much of polymer is too large to fit inside the pores. Therefore the polymer is excluded…





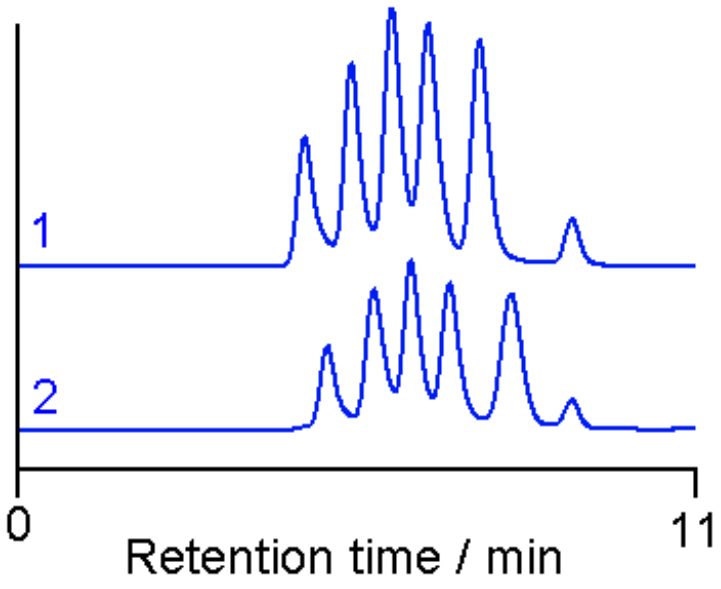
Effect of Column Length on Resolution

Eluent: THF (stabilized)
FlowRate: 1.0 ml/min
Detector: UV
Calibrants: PL EasiCal PS-1

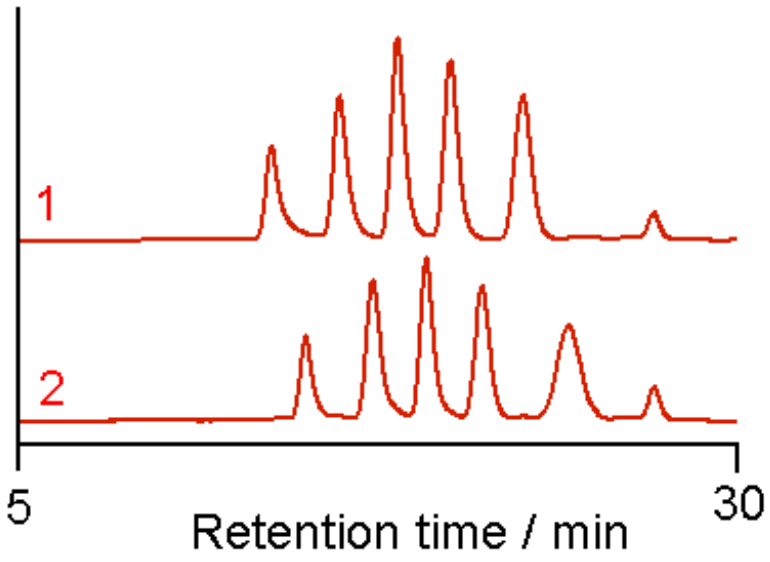
Mp values

Injection 1	*Injection 2*
1. 7500000	6. 2560000
2. 841700	7. 320000
3. 148000	8. 59500
4. 28500	9. 10850
5. 2930	10. 580

1 x PLgel Column



3 x PLgel Column



Section 3: Solvent Selection

- Sample solubility
- Solvent/column compatibility
- Avoid non-size exclusion effects (match solvent, column and polymer polarity)
- Compatible detection (RI iso-refractive solvent/sample, UV cut off)
- Safety (eg toxicity, elevated temperature, etc)

- **Let's look at examples**

Typical Range of Solvents Used (with PLgel)

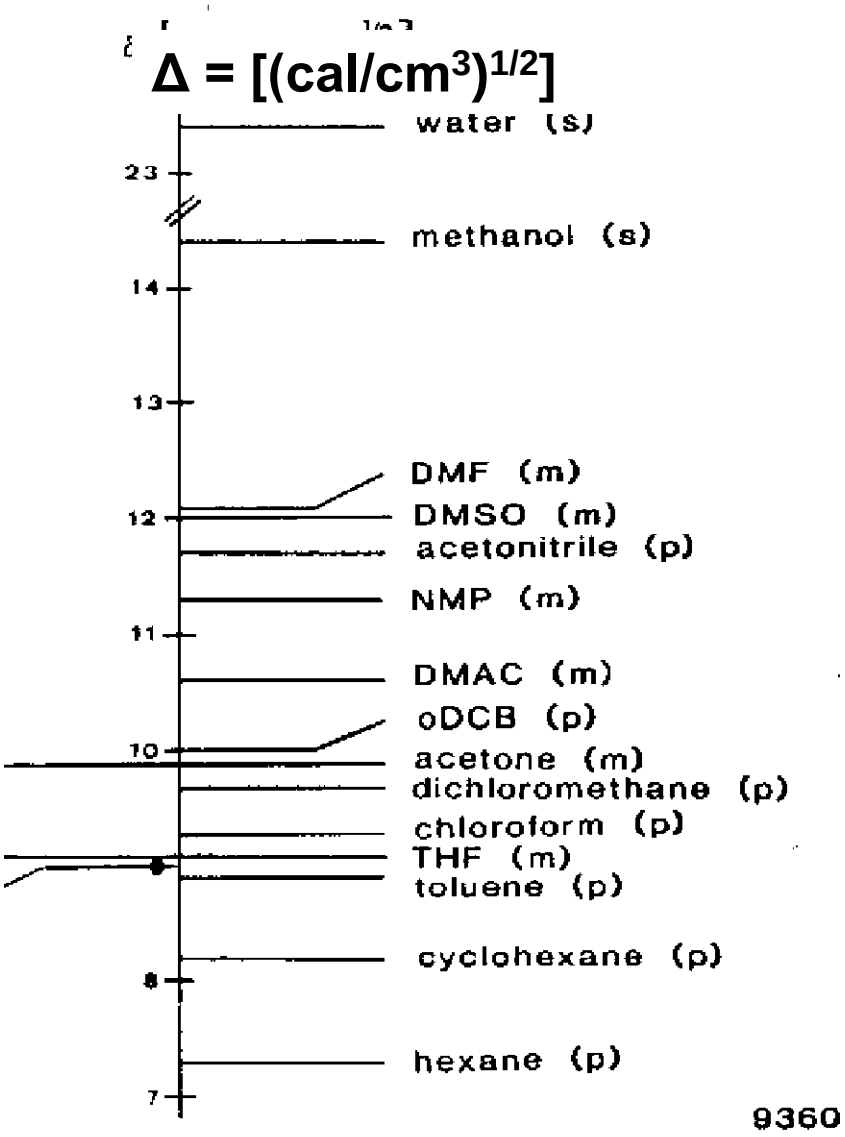
(ascending order of polarity)

Solvent	Polarity	Viscosity (cP)	Boiling point (° C)
Hexane	7.3	0.33	86
Cyclohexane	8.2	1.00	81
Toluene	8.9	0.59	110
Ethyl acetate	9.1	0.45	77
Tetrahydrofuran	9.1	0.55	66
Chloroform	9.3	0.57	61
Methyl ethyl ketone	9.3	0.40	80
Dichloromethane	9.7	0.39	40
Dichloroethane	9.8	0.79	83
Acetone	9.9	0.32	56
Dichlorobenzene	10.0	1.26	180
Trichlorobenzene	10.0	1.89	213
m-Cresol	10.2	16.90	202
o-Chlorophenol	10.2	4.11	175
Dimethyl acetamide	10.8	0.77	166
n-Methyl pyrrolidone	11.3	1.65	202
Dimethyl sulphoxide	12.0	1.10	189
Dimethyl formamide	12.1	0.90	153
Hexafluroisopropanol	12.2	1.02	58

Polarity Considerations

Interactions are typically avoided when the polarity of the solvent is with in +/- 2 units of the packing media polarity

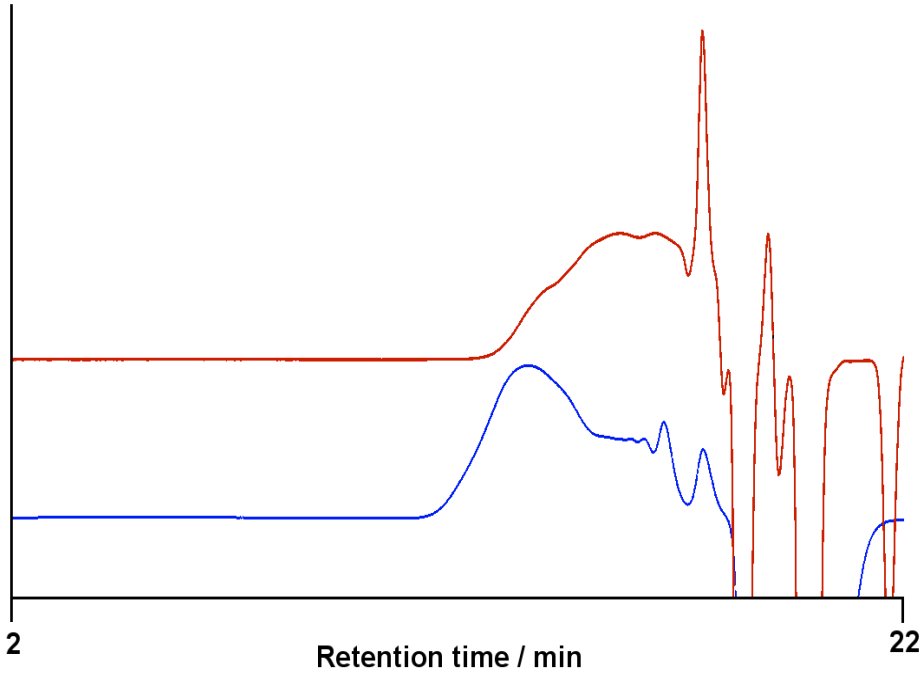
PS/DVB based GPC columns



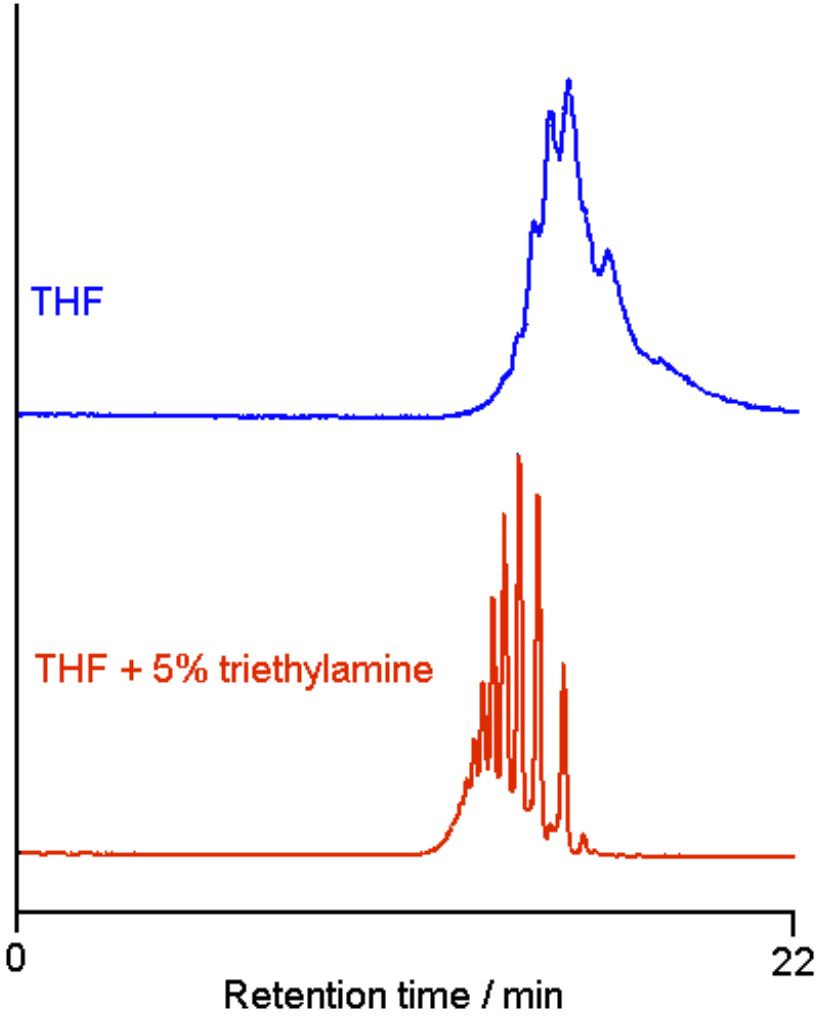
Improper column chemistry

- Phenol Formaldehyde resin analyzed on a PS/DVB column (red) and PolarGel (blue)
- PS/DVB column interactions due to low surface polarity had strongly affected the chromatography

Samples:	P-F resins
Columns:	2 x PolarGel, 2 x PS/DVB
Injection:	100µl
Eluent:	DMF + 0.1 LiBr
Temperature:	50ºC
Flow rate:	1.0ml/min
Detector:	DRI



Non-SEC Interaction controlled with modifiers



Hostavin N30

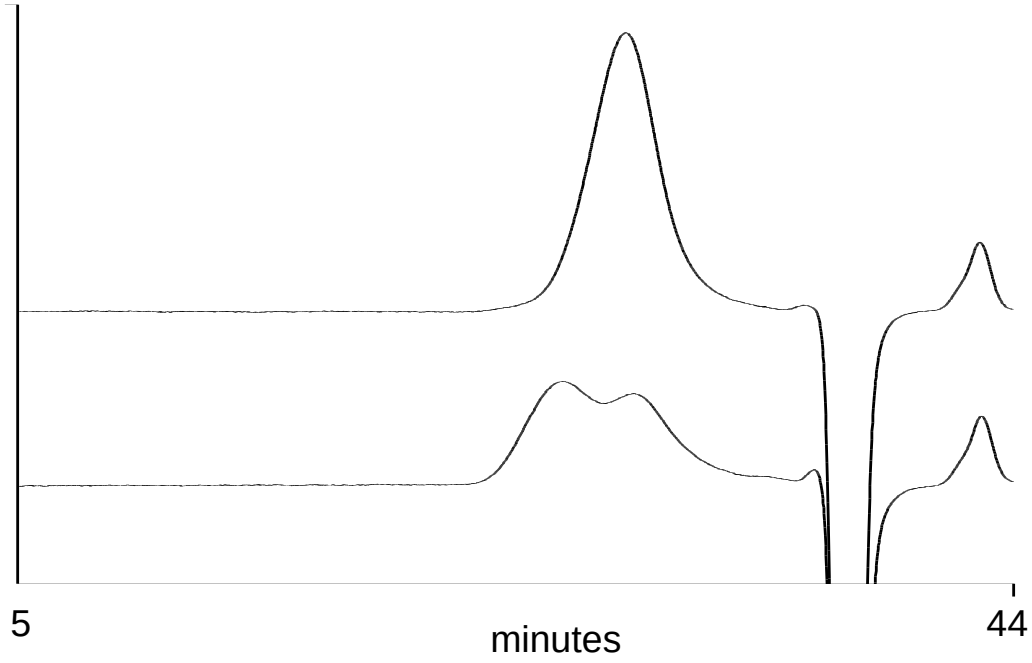
- Polymeric UV stabilizer containing secondary amine groups

Column: 2xPLgel 3µm MIXED-E
Flow Rate: 1.0ml/min
Detector: PL-ELS 1000

Aggregation controlled with modifiers

Columns :	4 x 20µm MIXED-A 300x7.5mm
Eluent :	DMSO + 5mM $NaNO_3$
Flow rate :	1.0 ml/min
Temperature :	80°C
Detector :	DRI

Addition of salt is often required for polar organic solvents to suppress ionic interaction effects



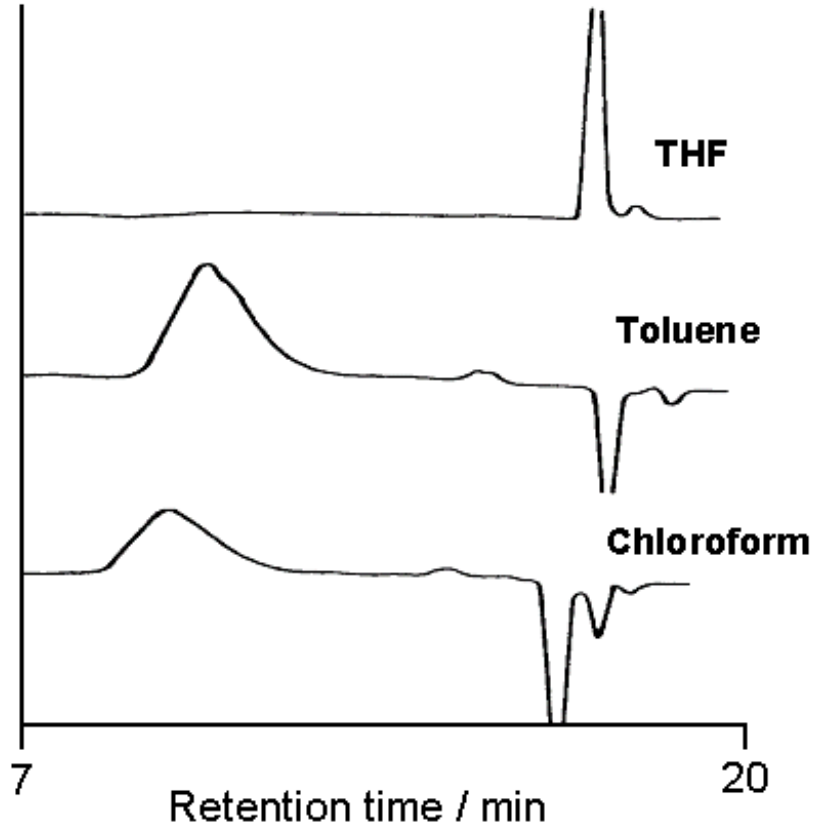
Solvent Selection with DRI Detectors

Polydimethylsiloxane (PDMS) is soluble in several common GPC solvents.

PDMS has a refractive index of 1.407 and therefore it is iso-refractive with THF

Toluene (n = 1.496) and chloroform (n = 1.444) give good DRI signals and are therefore preferred solvents for GPC of PDMS polymers when DRI is the detector of choice.

Columns:	PLgel 5 µm 104Å+ 500Å
Flow Rate:	1.0 ml/min
Detector:	DRI



Poor Column Lifetime

Degraded by materials in the mobile phase

- Use high purity HPLC grade solvents
- Filter eluent with 0.02 – 0.45 um filter prior to use
- Use THF & TCB stabilized with antioxidant (BHT)

Deterioration can occur due to contaminant build-up on the column

- This can be avoided by using guard column which can be discarded or by pre-filtering the sample solution

Follow the User Guide Recommendations

- Avoid incompatible solvents and exceeding temperature and pressure range

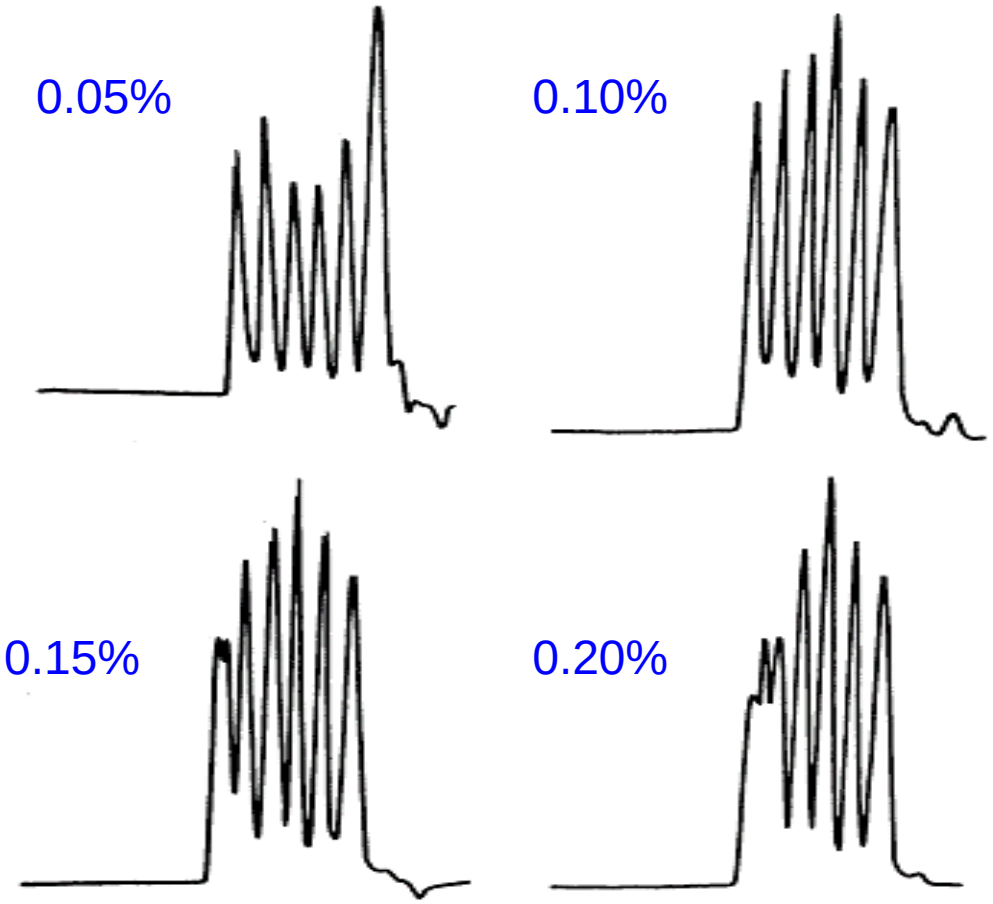
Section 4: Sample Preparation

- Use an aliquot of the mobile phase to prepare the solution
- Sample concentration depends on molecular weight (high MW lower concentration)
- For polymers, avoid high shear stirring
- Dissolution time and temperature will depend on molecular weight and crystallinity of polymer
- Filter samples to remove insoluble material (0.5 - 1.0um filters)

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

Effect of Concentration on Peak Shape and Resolution



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

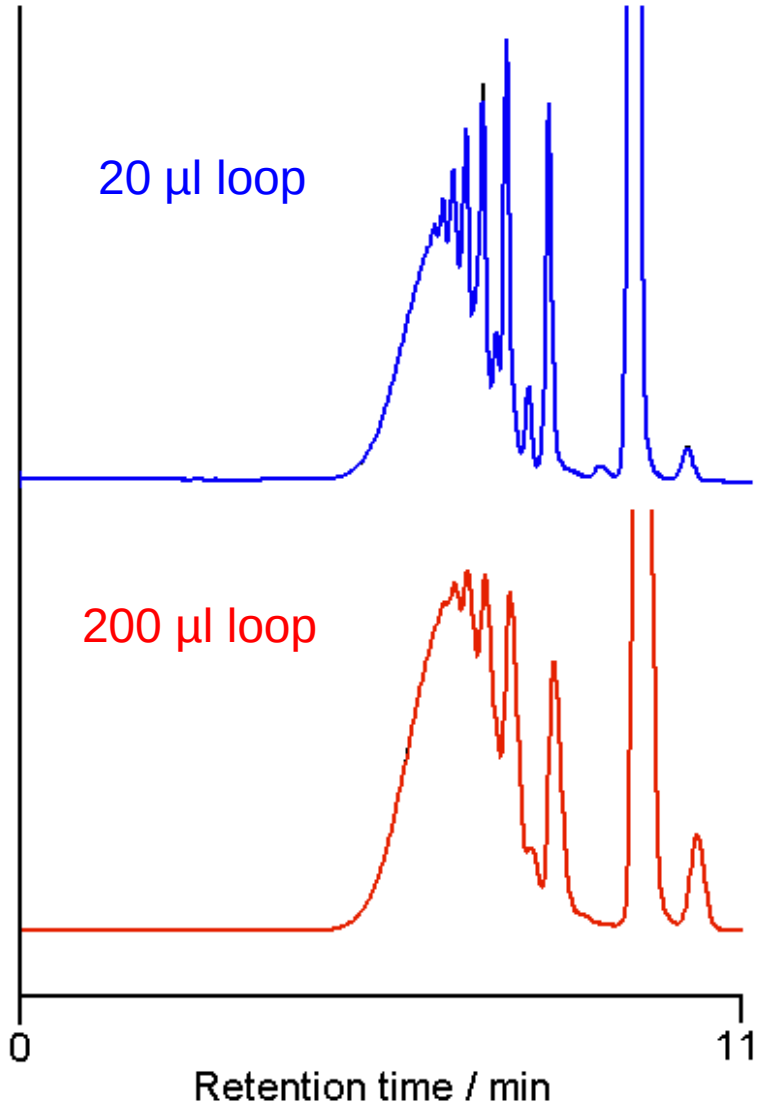
Polystyrene standards

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

Injection Volume

- GPC columns have a relatively large volume (typically 300 x 7.5 mm)
- Injection volumes for GPC can therefore be higher than for HPLC
- As a rule of thumb, 20-50 µl per 300 x 7.5 mm column length will have little effect on band broadening
- Minimize injection volume for high efficiency separations (e.g. 3 µm columns) to avoid band broadening which will decrease resolution
  - 3 µm Particle Size: 20 µl injection
  - 5 µm Particle Size: 50 to 100 µl injection
  - 10-20 µm Particle Size: 100 to 200 µl injection

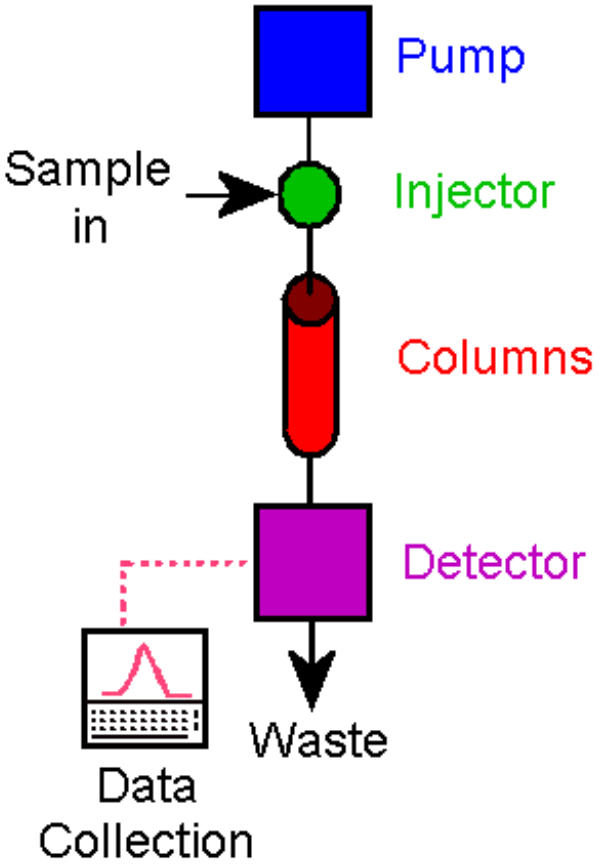
Effect of Injector Loop Size on Resolution



Column :	PLgel 3 µm MIXED-E 300 x 7.5 mm
Eluent :	THF
Flow rate :	1.0 ml/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

Section 5: Components of a GPC System:



Pump: delivers flow down the column

Injection valve: Allows us to introduce our samples

GPC column set: Performs the separation

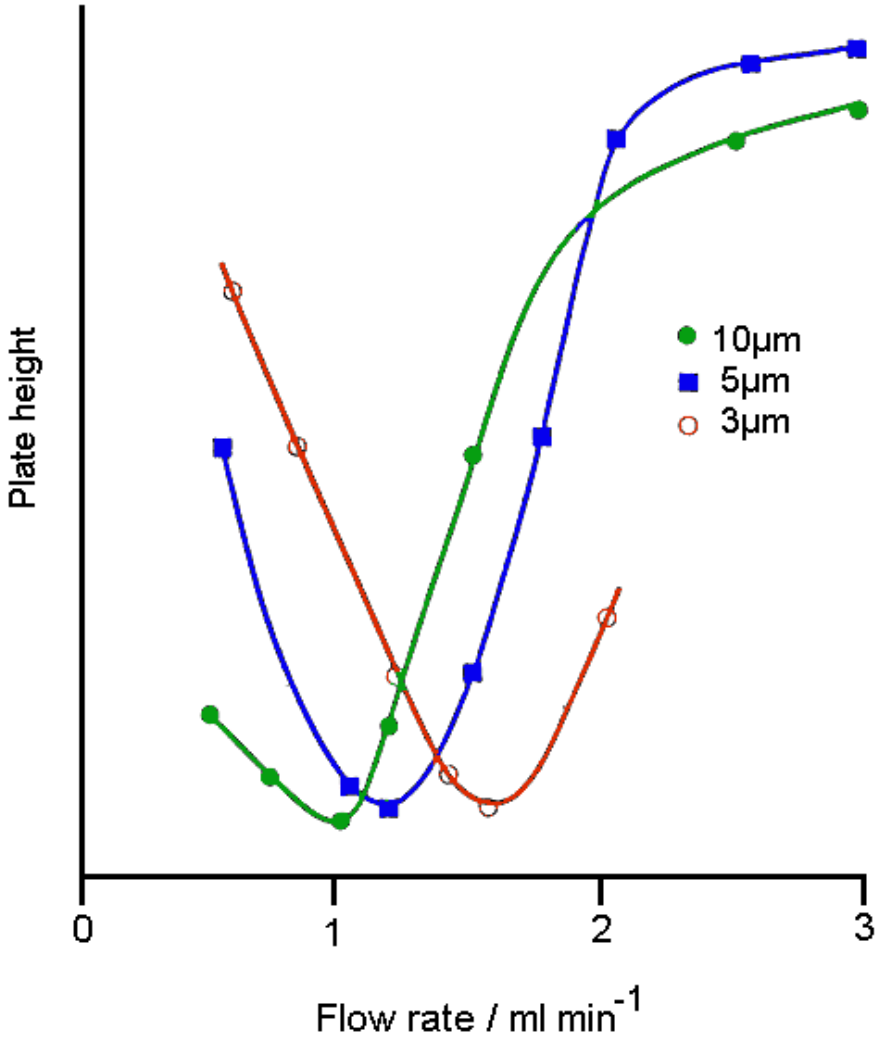
Detector: detects the material leaving the columns

Optional extras: autosamplers, degassers, etc.

Pump

- Isocratic pump (single channel)
- GPC/SEC is *always* isocratic
- Pulseless or low pulse flow required to ensure good detector baselines
- Service typically includes replacement of worn check valves and piston seals: same service as any LC

Flow Rate and Efficiency

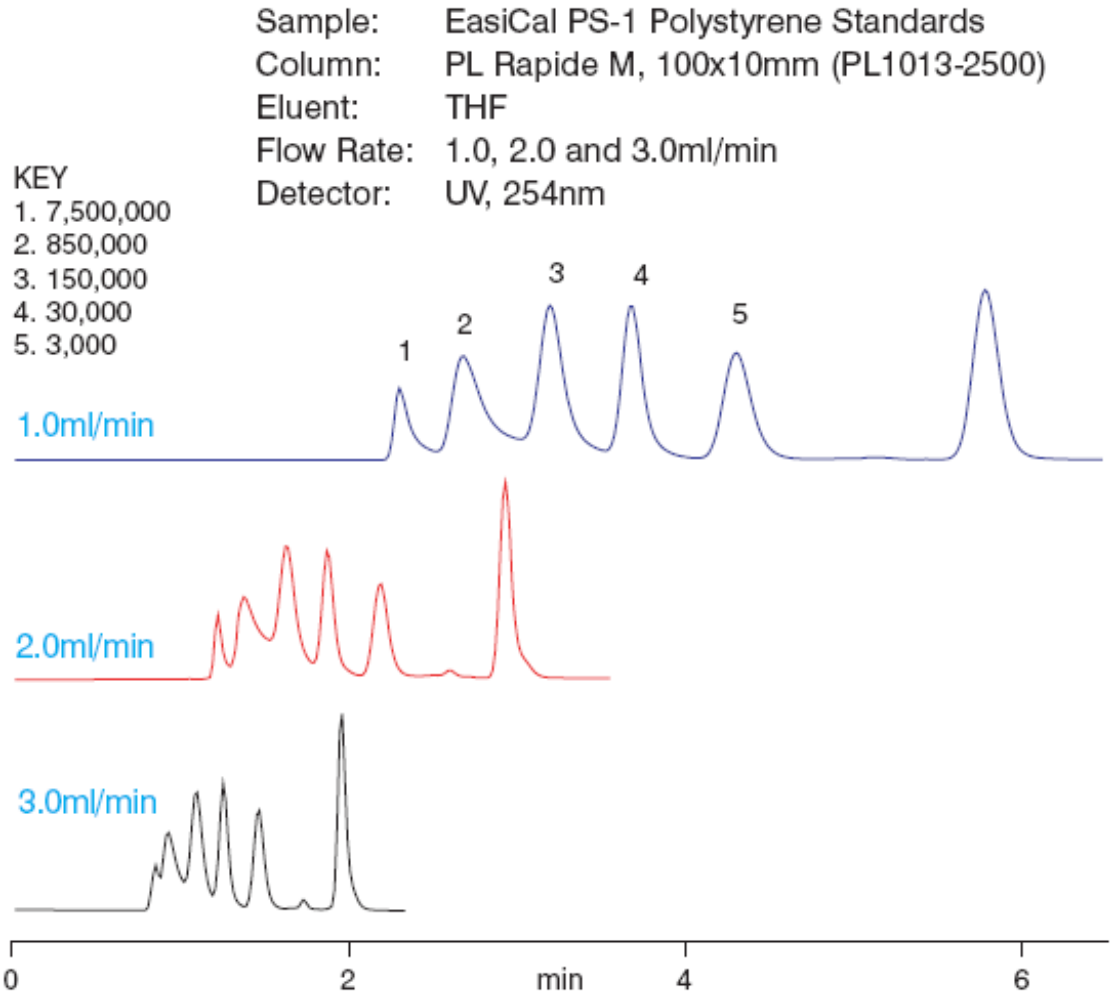


Eluent :	THF
Columns :	PLgel 100Å
Test probe :	ODCB

Optimum flow rate may differ with column particle size

Effect of Flow Rate on Resolution

- Flow rate strongly affects resolution
- Every column has an optimum flow rate, as in all LC systems
- However in GPC the mass transfer effect is much more prominent



Column Ovens

- Ovens are used to heat and maintain the temperature in a GPC separation
- They come in a range of specifications, from low temperature all the way up to very high temperatures (25 – 220 o C)
- Temperature can be critical in GPC for stability, solubility and to reduce back pressure
- Some GPC experiments are impossible without working at elevated temperature (polyolephins in TCB)

Why use Elevated Temperature?

GPC applications employing elevated temperature generally fall into three categories :

- To reduce solvent viscosity for improved chromatography
- To achieve and maintain sample solubility
- To provide a stable thermal environment for detectors

Effect of Temperature on Separations on Viscous Solvents

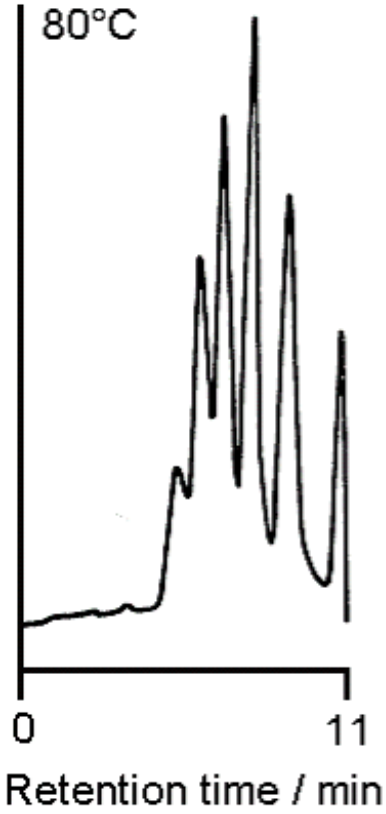
Column : PLgel 5 µm MIXED-C
300 x 7.5 mm
Eluent : DMF
Flow rate : 1.0 ml/min

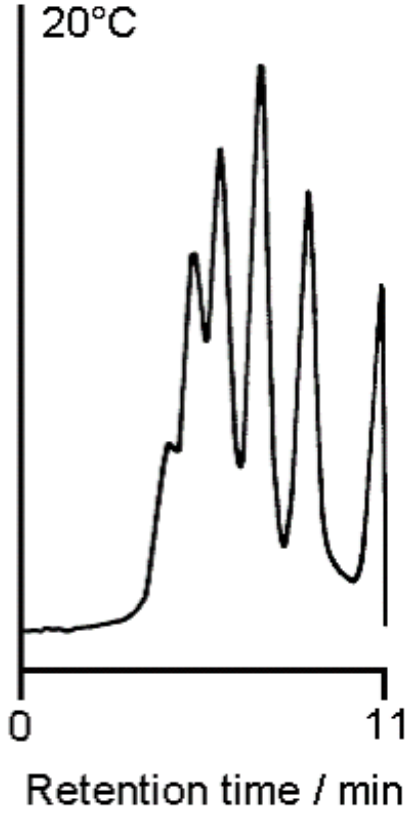
Increased temperature :

- *Reduced operating pressure*
- *Improved resolution, particularly at high MW*

PEO/PEG standards

990,000	252,000
86,000	18,000
4,800	200

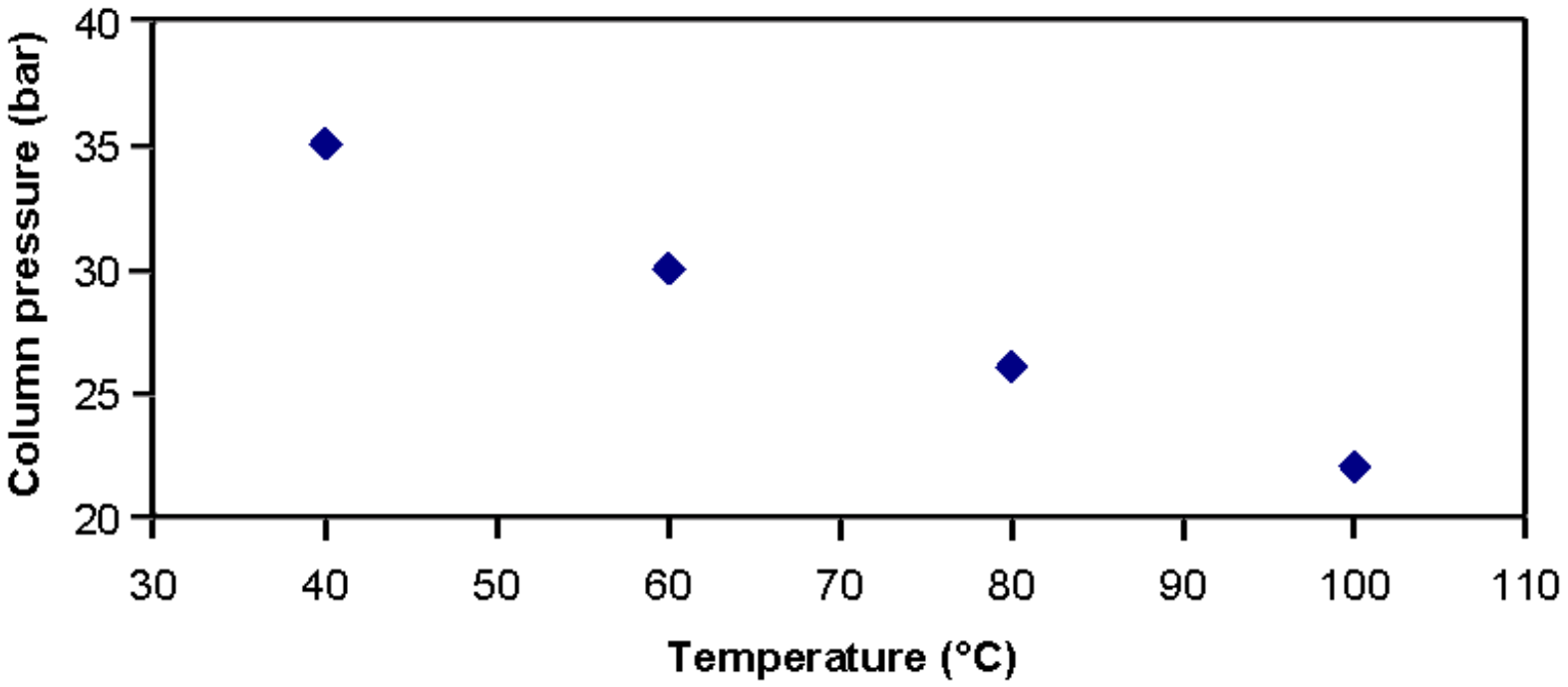




Effect of Temperature on Column Pressure

Column	PLgel 5 µm MIXED-D 300 x 7.5 mm
Eluent	Toluene
Flow rate	1.0 ml/min

Column pressure falls as temperature increases due to reduced viscosity



Concentration Detectors for GPC

There are several concentration detectors that are used in conventional GPC

- Differential refractive index (DRI)
- Ultraviolet (UV)
- Infrared (IR)
- Evaporative light scattering (ELSD)

Information Rich Detectors for GPC

- Static Light Scattering Detection
- Viscometry
- FTIR

Band Broadening

Large dead volumes

- Always use LDV end fittings and connectors
- Minimize lengths and diameters of tubing wherever possible

Mobile phase is too viscous

- May need to increase operational temperature

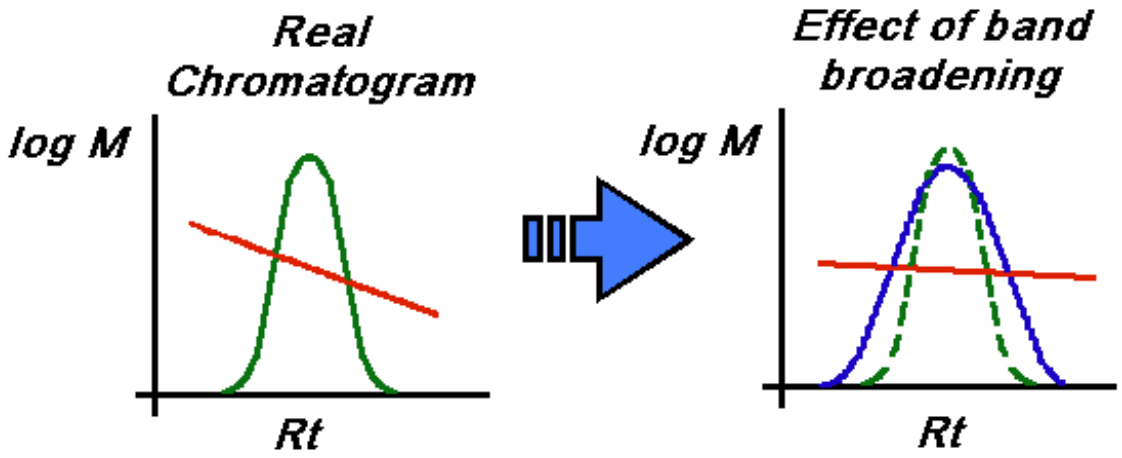
Detector cell(s) volume is too large

- If possible, use a smaller cell volume

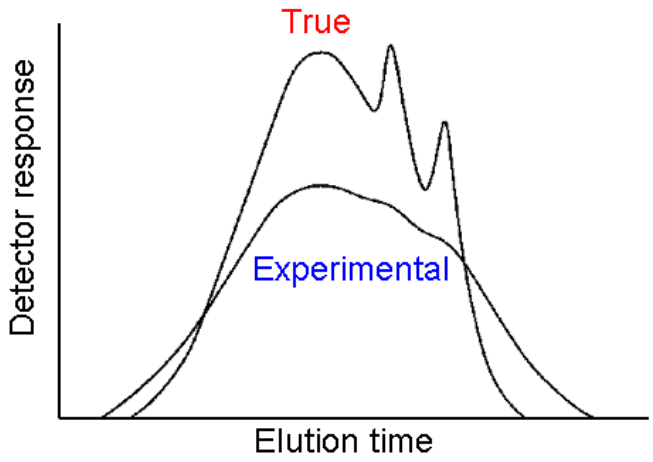
Column is not performing

- Repair or replace the column

Effects of Band Broadening



Modern high performance GPC columns have minimized the effect of band broadening in the separation. However poor system design with large amounts of dead volume can still cause loss of resolution. System dead volume should be minimized, especially when using very high efficiency columns.



Questions