Optimizing GPC Separations

Agilent Technologies

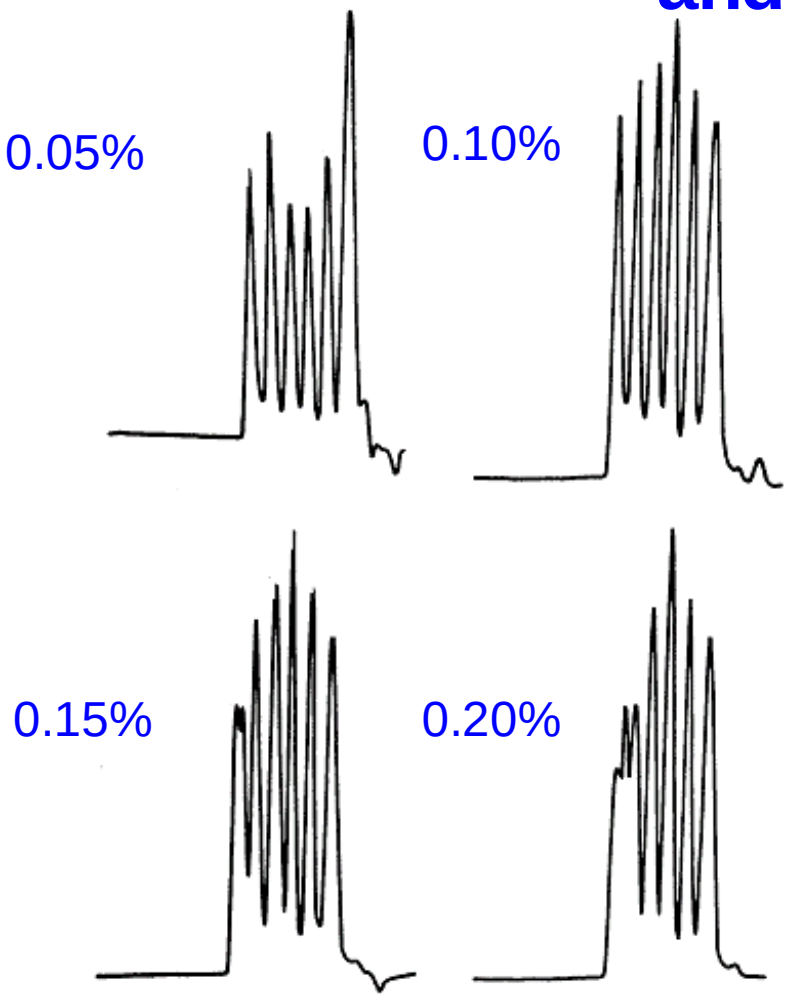
Criteria for Solvent Selection

- 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)

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



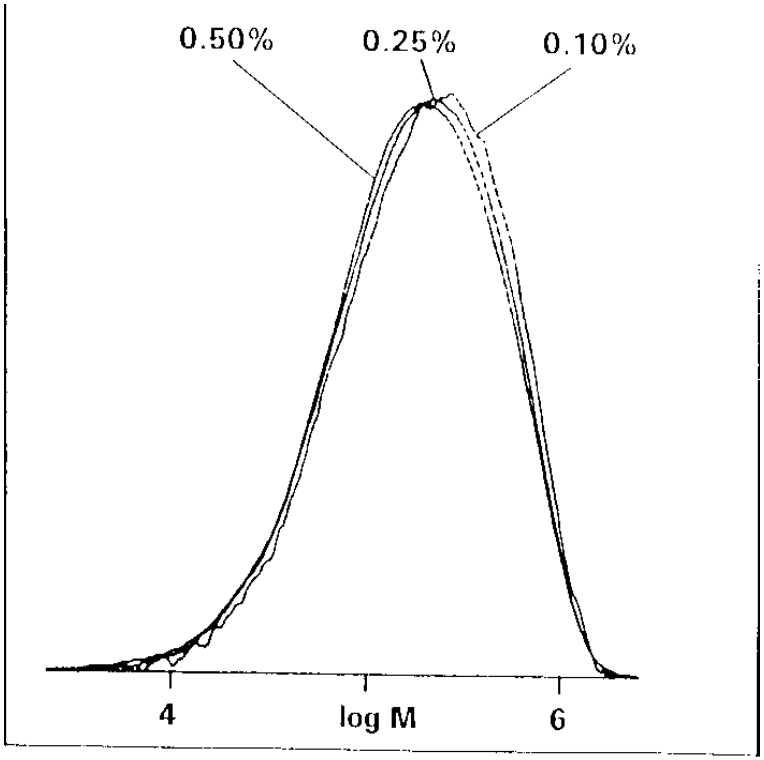
Column: PLgel 10µm MIXED-B 300x7.5mm
Eluent: THF
Flow Rate: 1.0ml/min
Detector: UV

Polystyrene standards

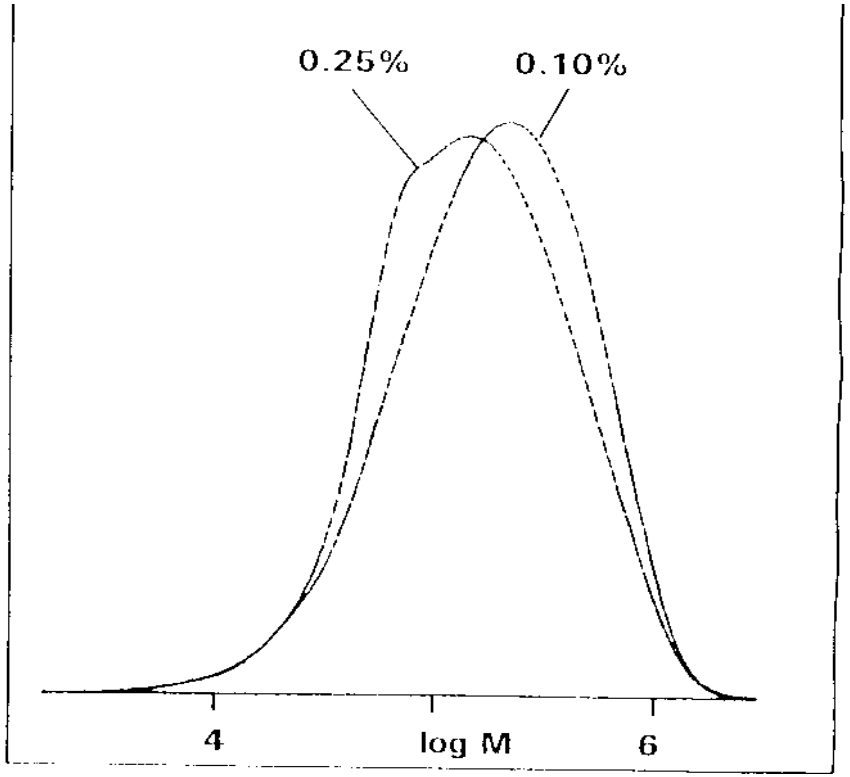
1. 8,500,000
2. 1,130,000
3. 170,000
4. 34,500
5. 5,100
6. 580

Effects of Column Loading

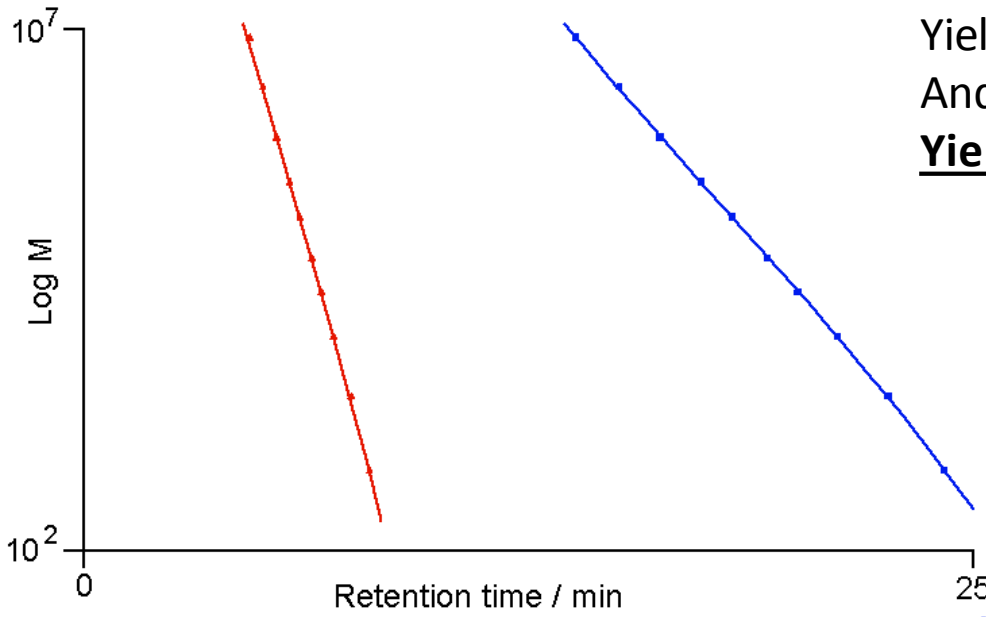
Analytical column

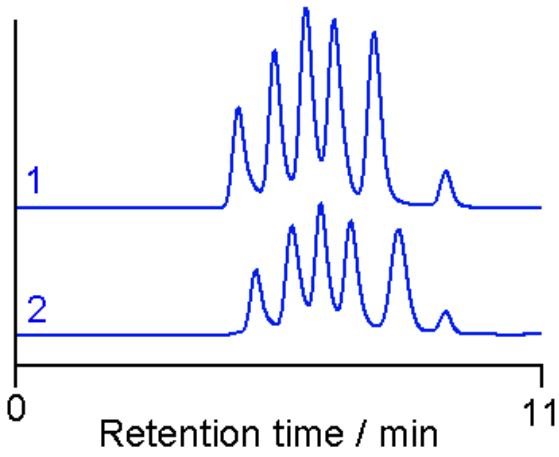


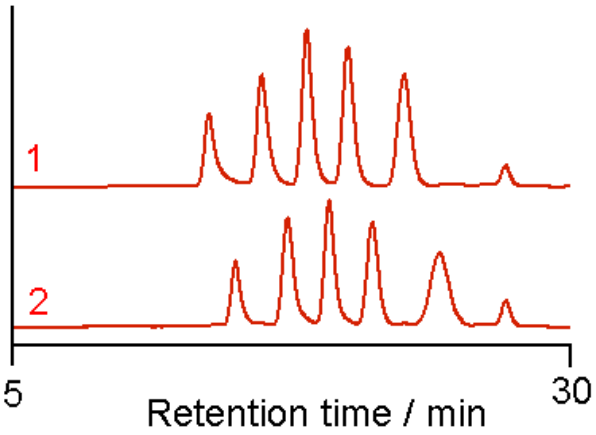
Narrow bore column



Adding Columns Adds Pore Volume and
Yields Higher Resolution
And
Yields Greater Reproducibility







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

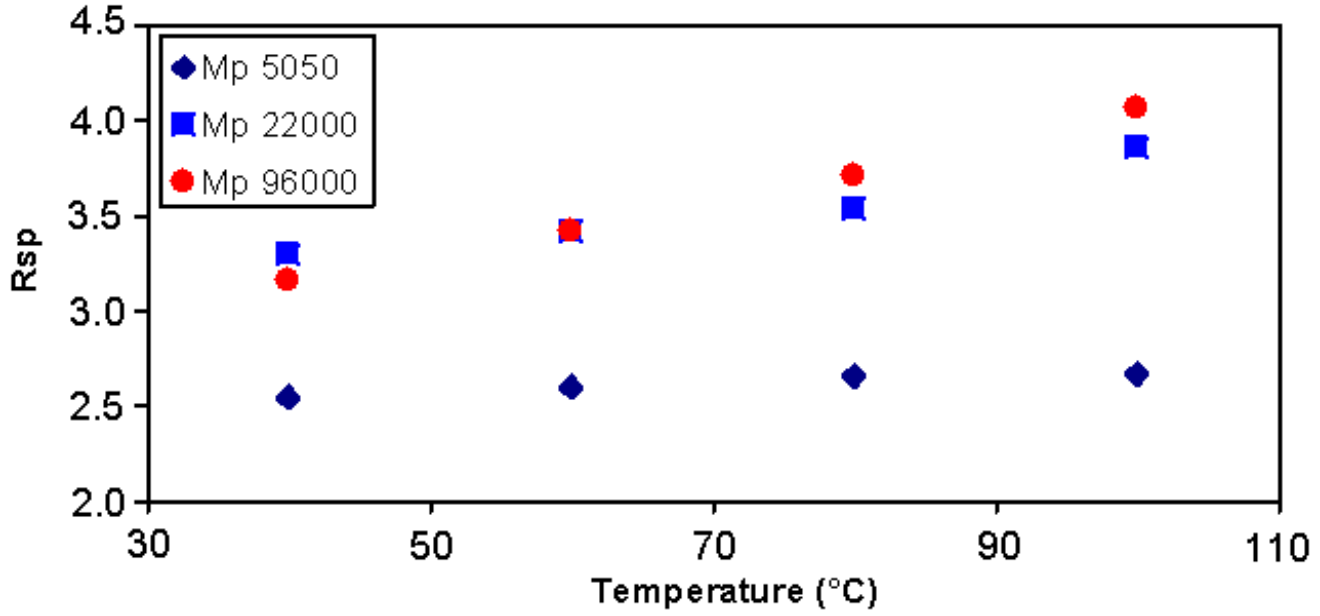
For **low MW** samples use higher concentrations and avoid larger injection volumes to maintain high resolution

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

Effect of Temperature on Specific Resolution

Column:	PLgel 5µm MIXED-D 300x7.5mm
Eluent:	Toluene
Flow Rate:	1.0ml/min
Test Probes:	Polystyrene standards

Resolution increases with temperature, more obvious for the higher MW probes



Effect of Temperature on Separations in Polar Solvents

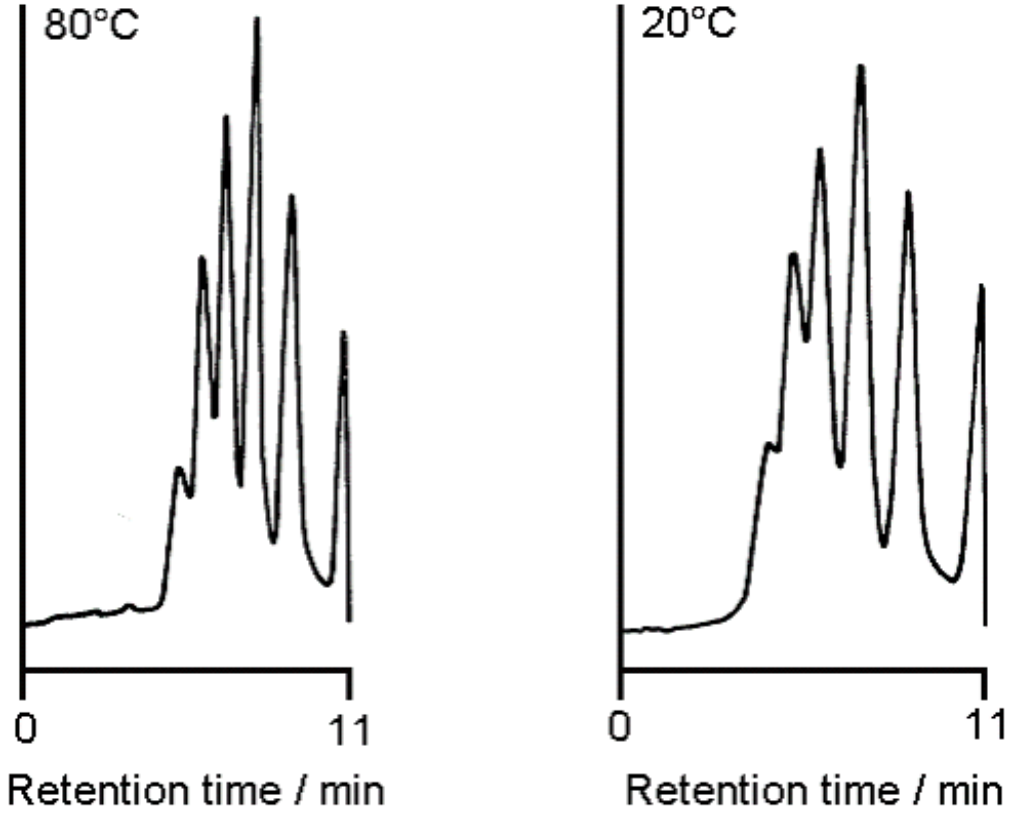
Column : PLgel 5µm MIXED-C
300x7.5mm
Eluent : DMF
Flow rate : 1.0ml/min

Increased temperature :

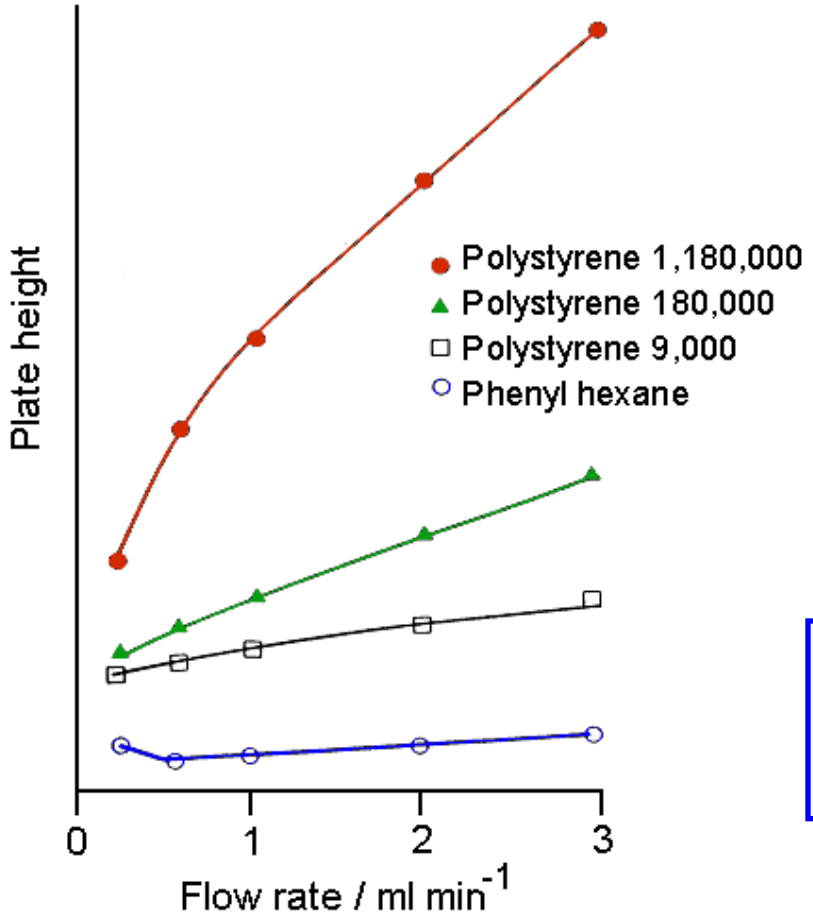
- *Reduced operating pressure*
- *Improved resolution, particularly at high MW*

PEO/PEG standards

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



Effect of Flow Rate on Column Efficiency (2)

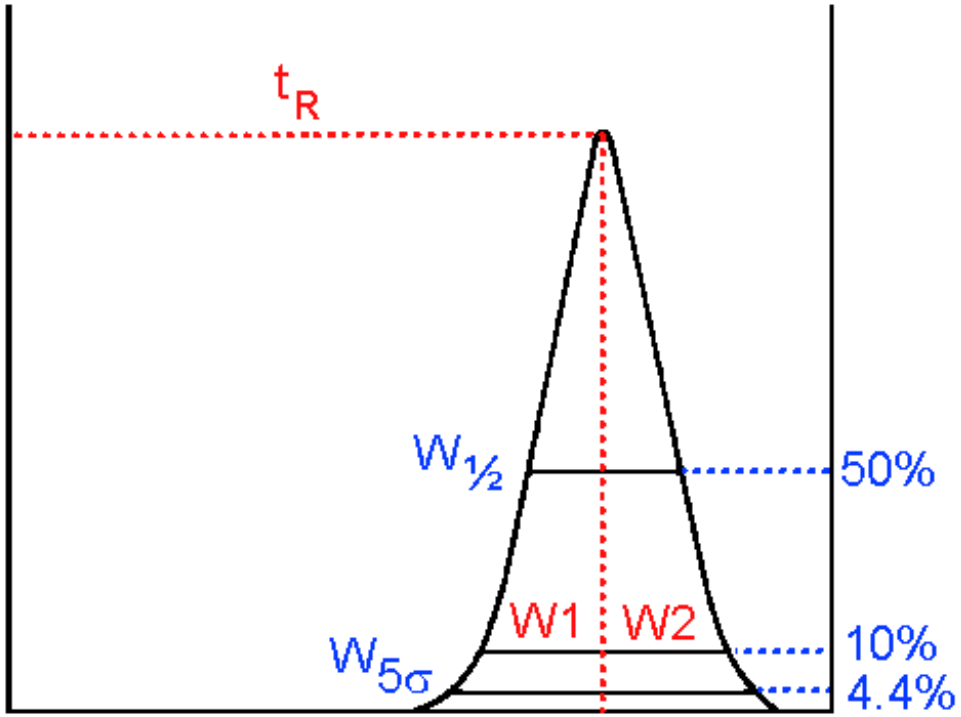


Eluent: THF

Column: PLgel 10µm MIXED-B

For high MW samples, high flow rate should be avoided, reduced flow rate may be required to improve resolution

Determination of Column Performance



t_R = retention time

$W_{½}$ = peak width at 50% peak height

W_{5s} = peak width at 4.4% peak height

L = column length in meters

Efficiency (½ height)

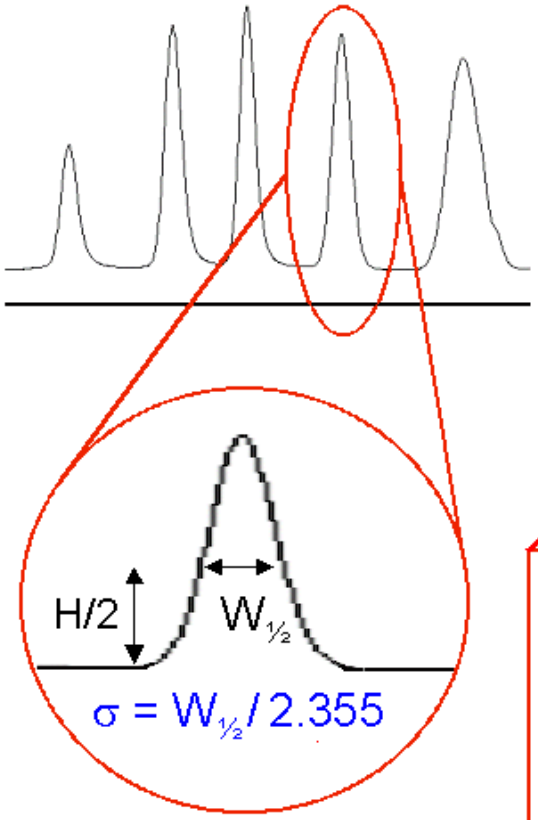
$$N=\frac{5.54(t_R/W)^2}{L}$$

Plate count efficiency (5s)

$$N=\frac{25(t_R/W_{5s})^2}{L}$$

Symmetry $=W_1/W_2$

Effect of Particle Size on Resolution



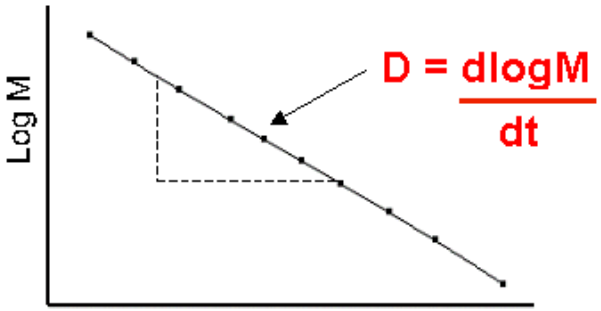
Specific resolution per decade of MW

$$Rsp = \frac{0.25}{D\,\sigma}$$

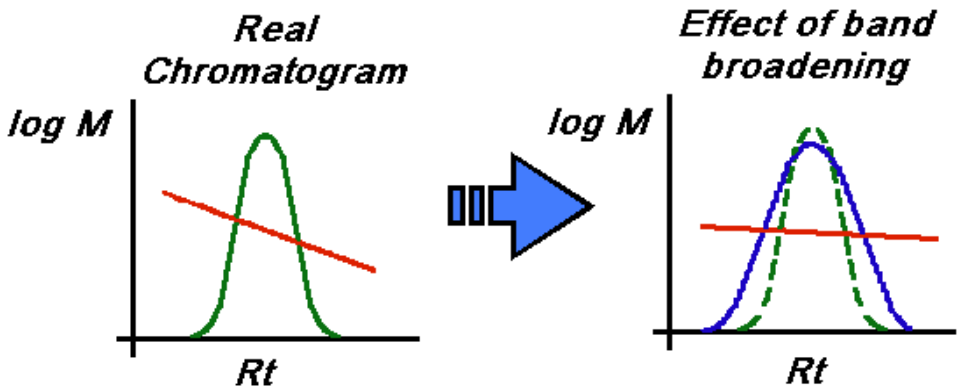
Particle Size

$$\frac{1}{W_{½h}} \alpha \text{ Efficiency (plates / m)} \alpha \frac{1}{\text{Particle size}^2}$$

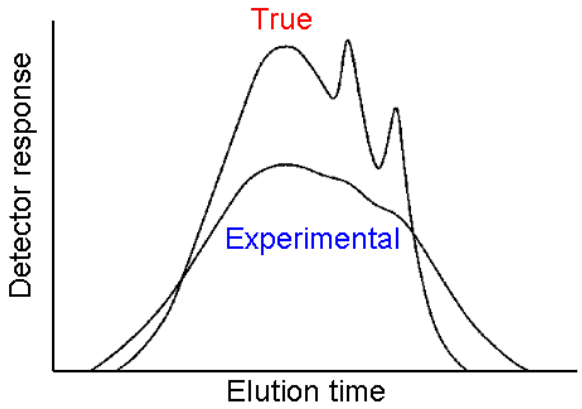
Pore volume



Effects of Band Broadening



Modern high performance GPC columns have minimised 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 minimised, especially when using very high efficiency columns.



Eluent Modification in GPC

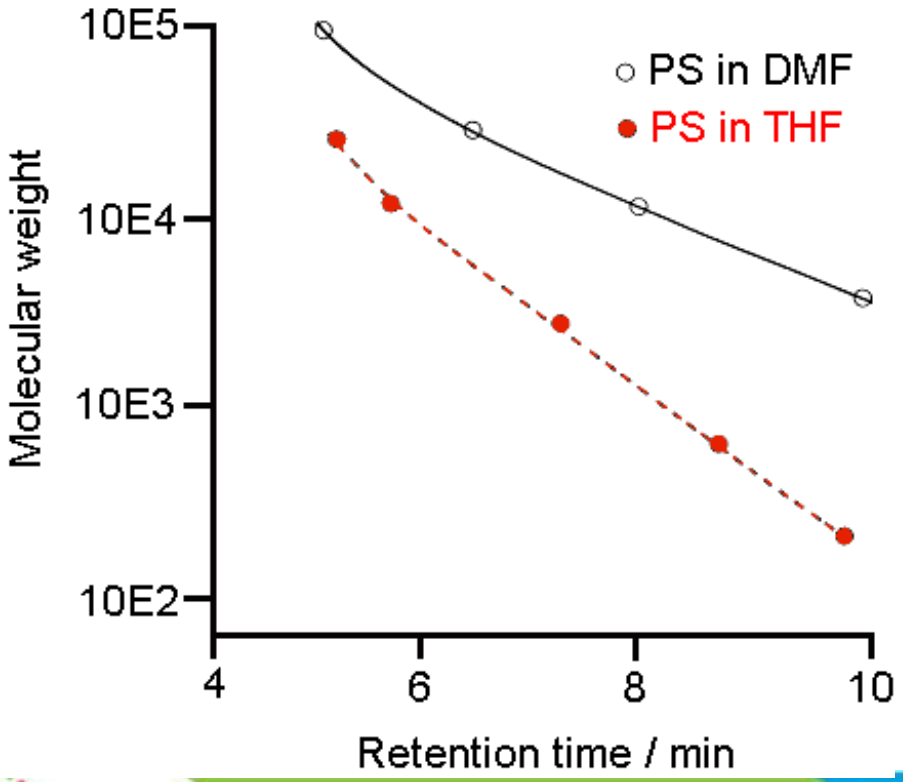
Required to:

- minimise non-size exclusion interactions between the sample and the column
- stabilise the solution of the polymer (ionic aggregation)

Possibilities:

- addition of more polar solvent to organic eluent (eg methanol or water added to THF or chloroform)
- addition of ionic compound to solvent (eg lithium salts in polar organic solvents, triethanolamine or acetic acid to THF)

Adsorption of Polystyrene Standards in DMF

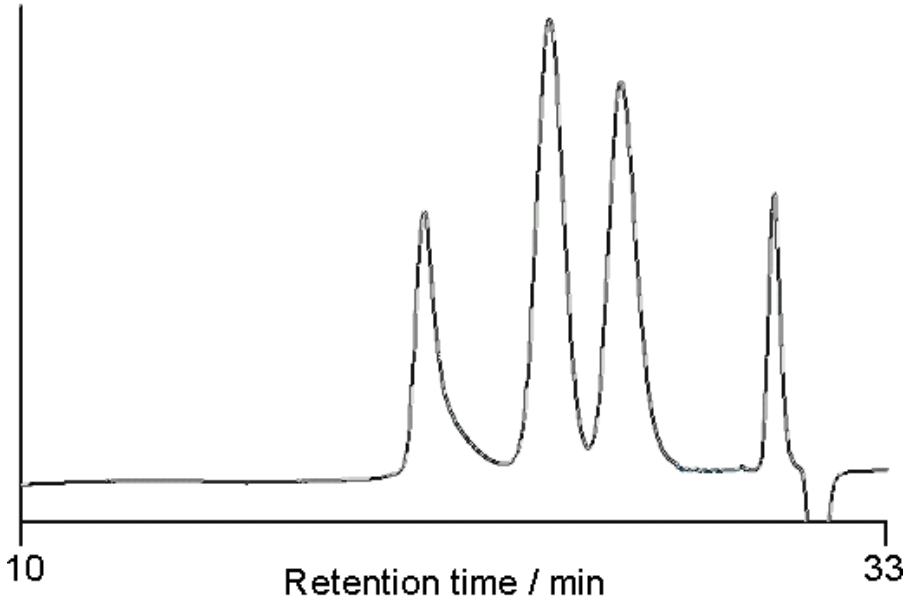


PS/DVB columns are excellent in many solvents, but remember that although the column may be used in certain solvents this does not mean SEC will occur - the example here is polystyrene standards running in DMF

Column : PLgel 5um 500Å
300x7.5mm

Polystyrene Sulfonates - ANIONIC and HYDROPHOBIC

Column:	2xPL aquagel-OH MIXED 8µm, 300x7.5mm
Eluent:	70% 0.2M $NaNO_3$, 0.01M NaH_2PO_4, pH9 30% methanol
Flow Rate:	1.0ml/min
Detector:	DRI



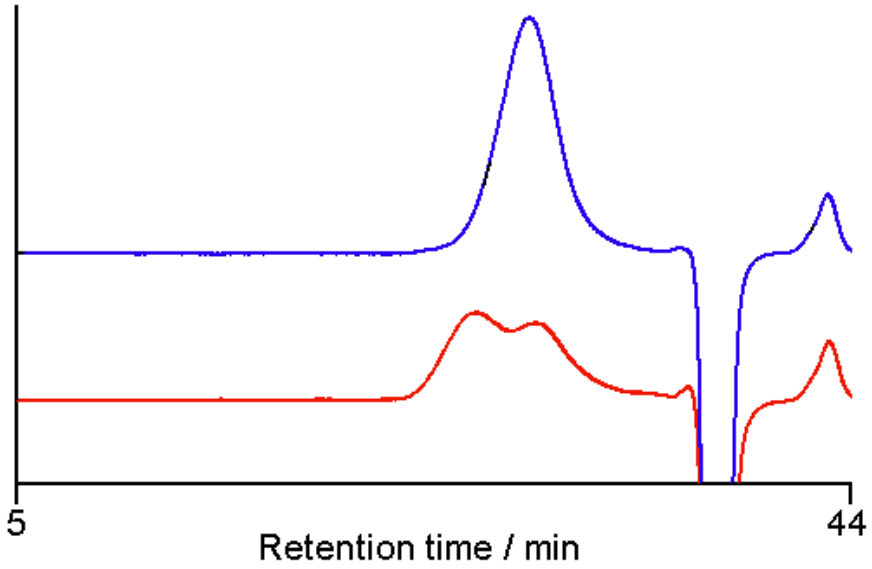
Mp values

1. 400,000
2. 35,000
3. 4,600

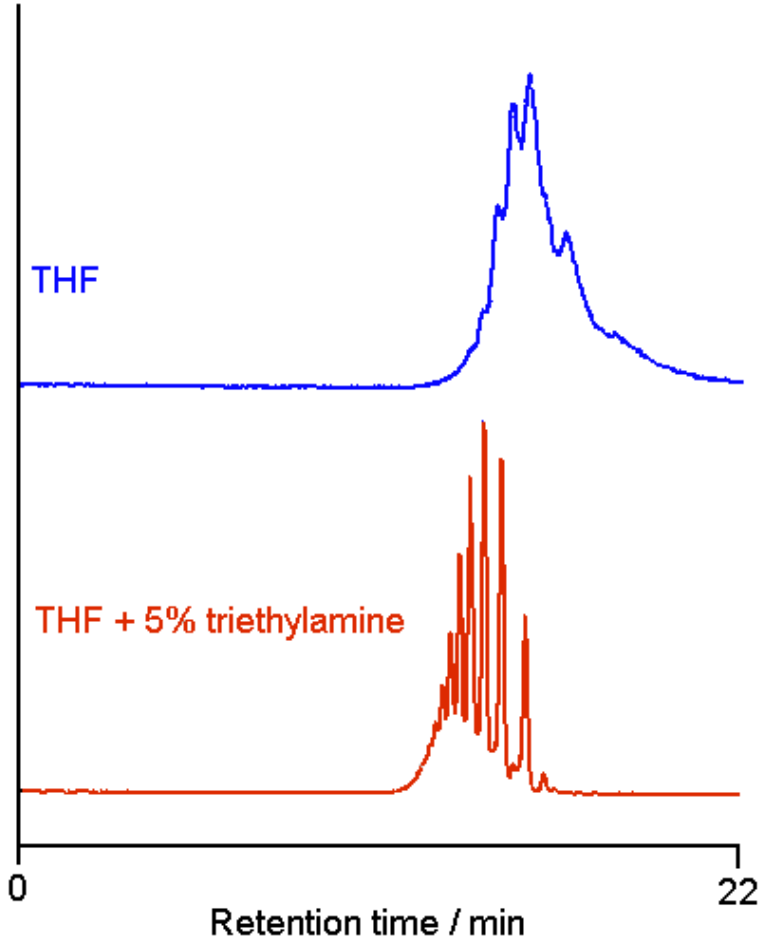
Starch Analysis

Column:	4xPLgel 20µm MIXED-A 300x7.5mm
Eluent:	DMSO + 5mM $NaNO_3$
Flow Rate:	1.0 ml/min
Temp:	80°C
Detector :	DRI

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



Eluent Modification in Organic GPC



Hostavin N30

- Polymeric UV stabiliser containing secondary amine groups

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

Analysis of Natural Rubber, RI Limitations

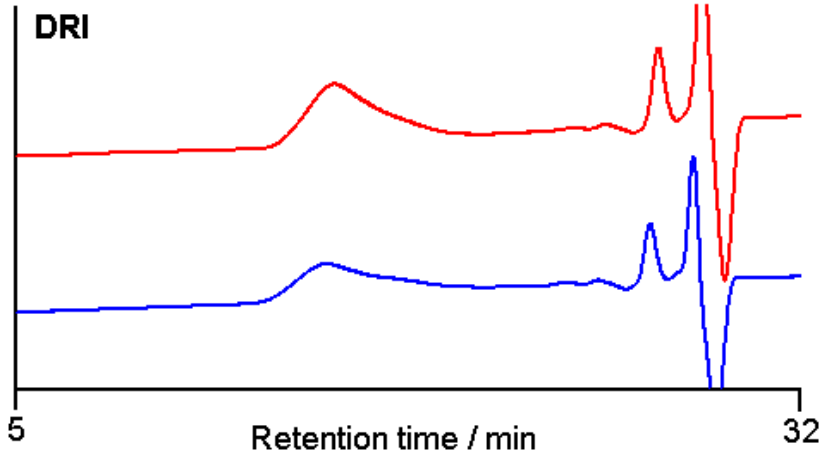
Columns 3 x PLgel 10µm MIXED-B

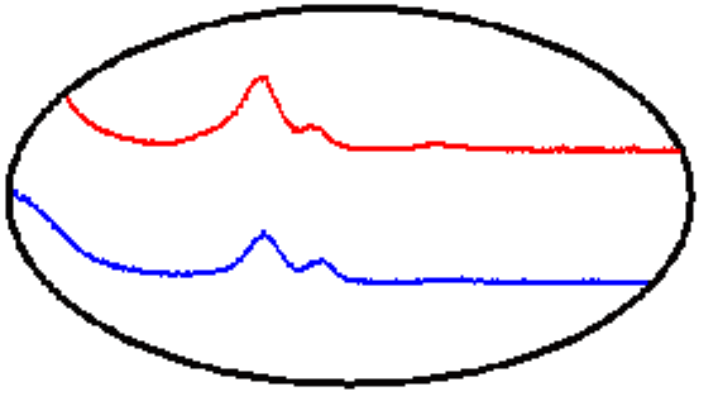
300x7.5mm

Eluent Toluene

Loading ~0.2%, 200µl

Detectors DRI at 1V FSD



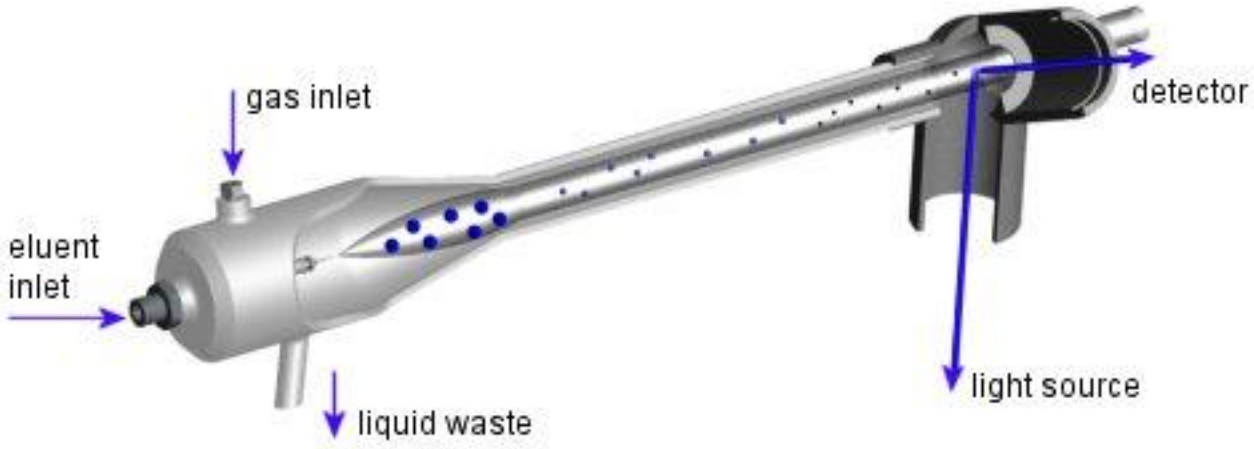


Refractive Index Values for Common Solvents

Solvent:	Refractive Index (@ 25° C)
Acetic Acid	1.37
Acetone	1.35
Acetonitrile	1.34
Chloroform (amylene)	1.44
Chloroform	1.44
Cyclohexane	1.42
0-Dichlorobenzene	1.55
Ether (Anhydrous)	1.35
Ethyl Acetate	1.37
n-Heptane	1.38
Hexanes	1.37
Isobutyl Alcohol	1.38
Methanol	1.32
Methyl tert-butyl Ether	1.36
Methylene Chloride	1.42
Methyl Ethyl Ketone	1.37
Pentane	1.35
2-Propanol	1.38
Pyridine	1.5
THF (BHT)	1.4
THF	1.4
Toluene	1.49
Trichlorobenzene	1.57
Trimethylpentane	1.38
Water	1.33

Advantages of the Varian 380 and 385 Evaporative Light Scattering Detectors

- Ability to Detect Semi-Volatile and Volatile Compounds
  - Novel Three Stage Addition of Gas Makes Detection Possible
- Post Nebulization Gas Reduces Relative Humidity Inside Evaporator Tube Which Allows for Low Temperature Evaporation of Solvents

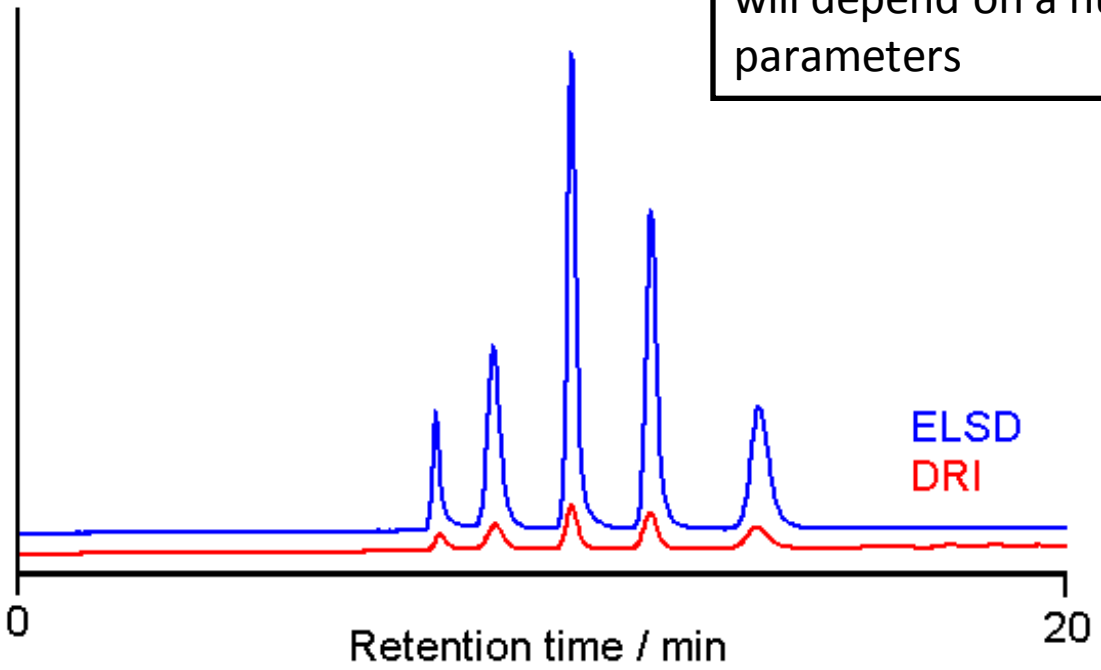


Sensitivity of DRI Versus ELS

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

Mp values

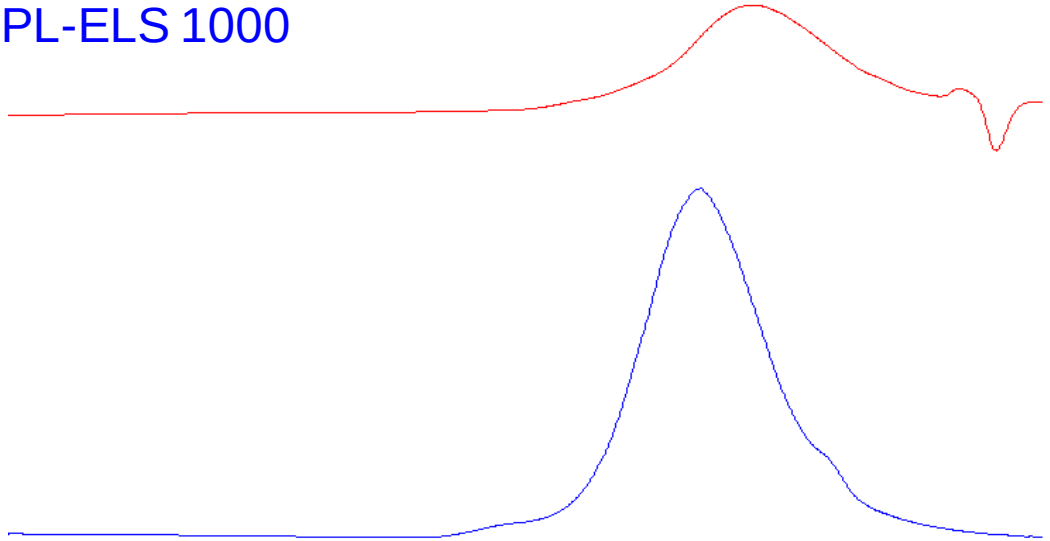
1. 7,500,000
2. 841,700
3. 148,000
4. 28,500
5. 2,930



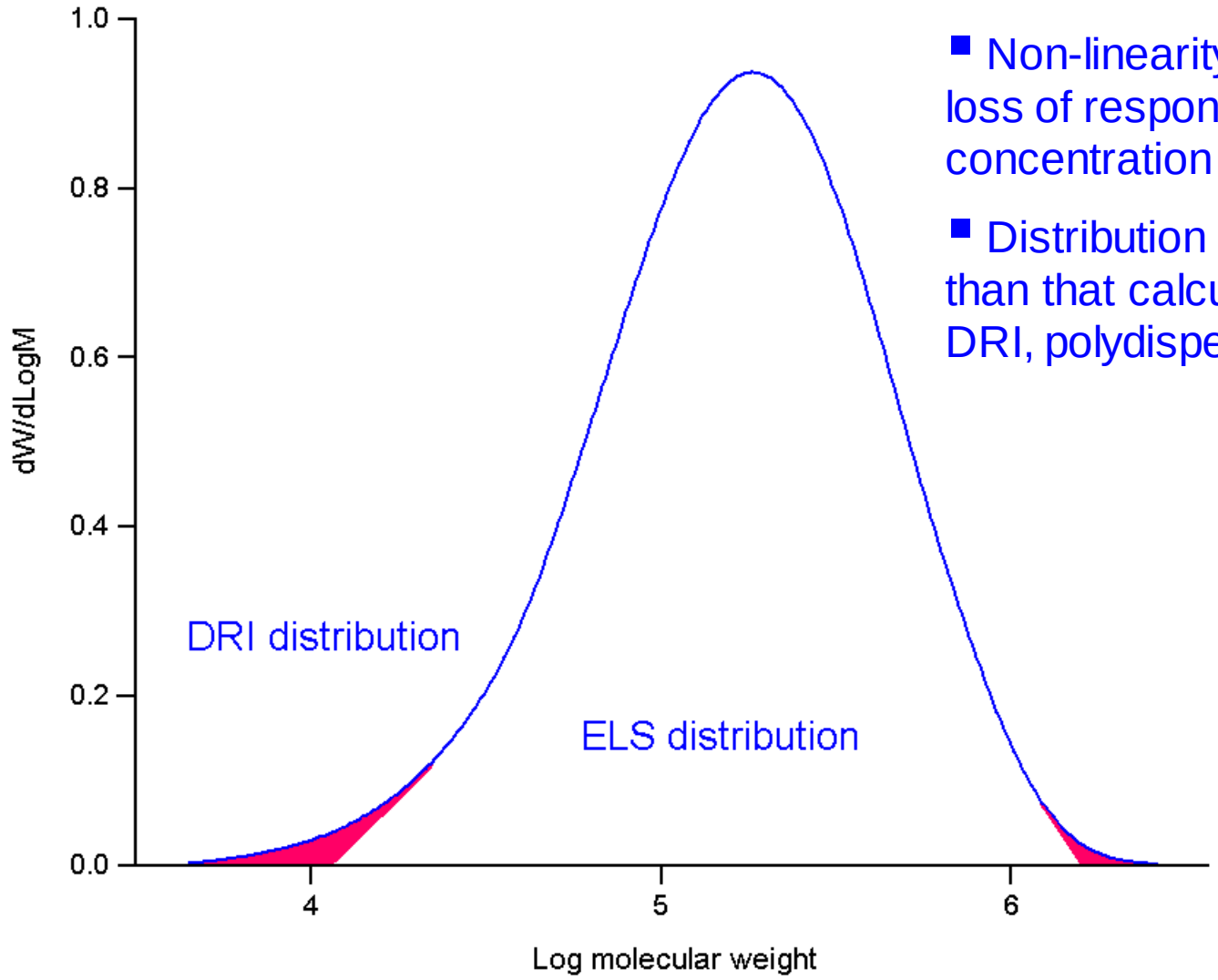
ELS is essentially independent of dn/dc, improvement in sensitivity will depend on a number of solute parameters

PVP/PVA Copolymer (Kollidon VA64)

Column:	2xPL aquagel-OH MIXED 8µm, 300x7.5mm
Eluent:	1. 70% 0.2M $NaNO_3$, 0.01M NaH_2PO_4, pH7, 30% methanol
	2. 70% 0.1M ammonium formate, 30% methanol
Flow Rate:	1.0ml/min
Detector:	1. DRI
	2. PL-ELS 1000



Effects of Non-Linearity With ELSD Detection



- Non-linearity results in loss of response for low concentration peak tails
- Distribution narrower than that calculated by DRI, polydispersity low

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

A Concentration Detector is Needed

- Can be any type that gives a response proportional to concentration
- Typically a differential refractive index detector is used
- DRI detector response proportional to concentration only
- Operation identical to conventional GPC, determines the concentration of material eluting from a GPC column

$$RI_{signal} = K_{RI} (dn/dc) C$$

Viscosity Detectors

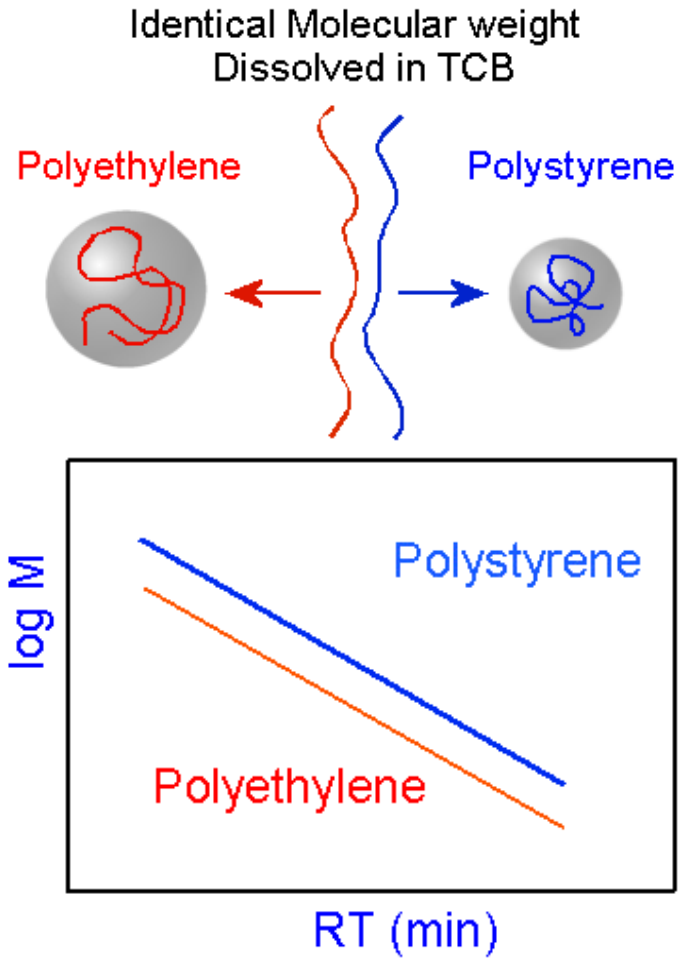
- Detector response proportional to the **intrinsic viscosity [η]** of the polymer
- Generate molecular weight values for a variety of polymer types via Universal Calibration approach
- Permits determination of branching in polymers

Light Scattering Detectors

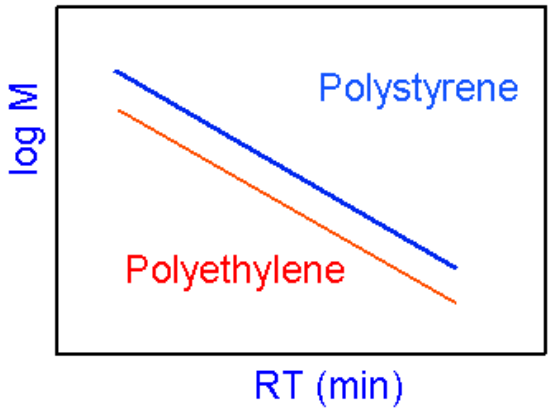
- Detector response directly proportional to weight average **molecular weight (Mw)** of the polymer
- No column calibration required
- Scattered light intensity measured at more than one angle permits determination of radius of gyration

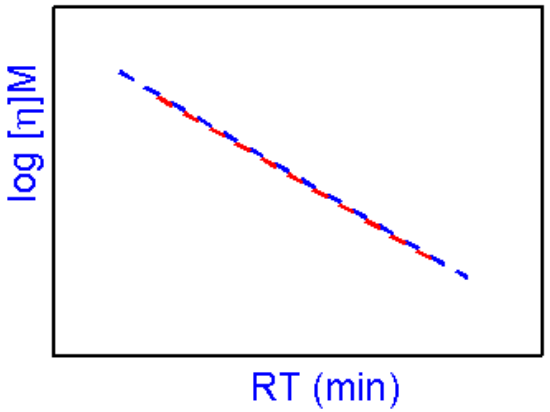
Conventional GPC

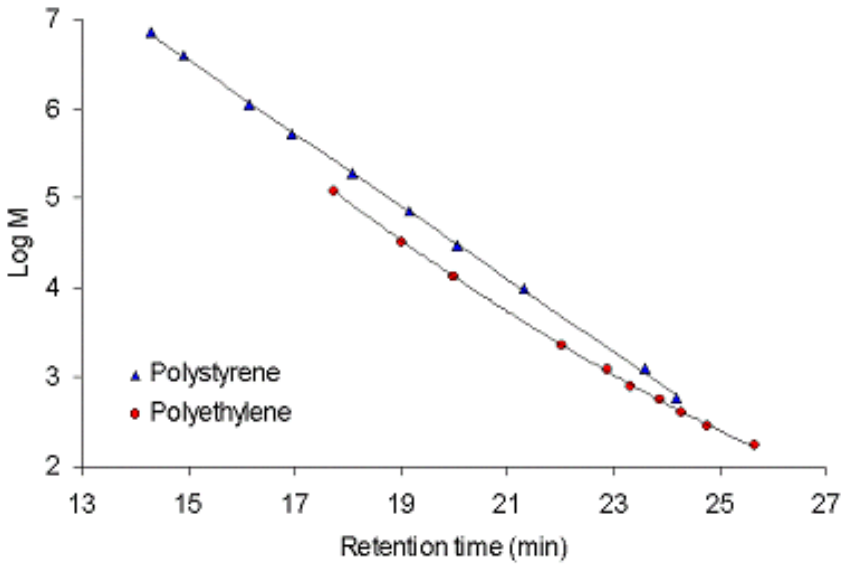
- Column separates on basis of molecular size NOT molecular weight
- Two different polymers will interact differently with solvent
- At any molecular weight, the two polymers will have different sizes in solution
- Molecular weights from conventional GPC are dependent on a comparison in size between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

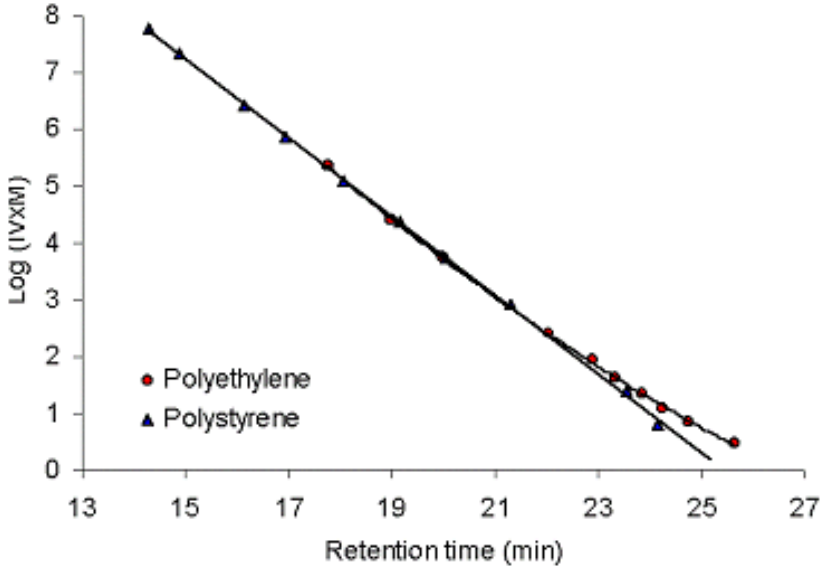


Conventional and Universal Calibration









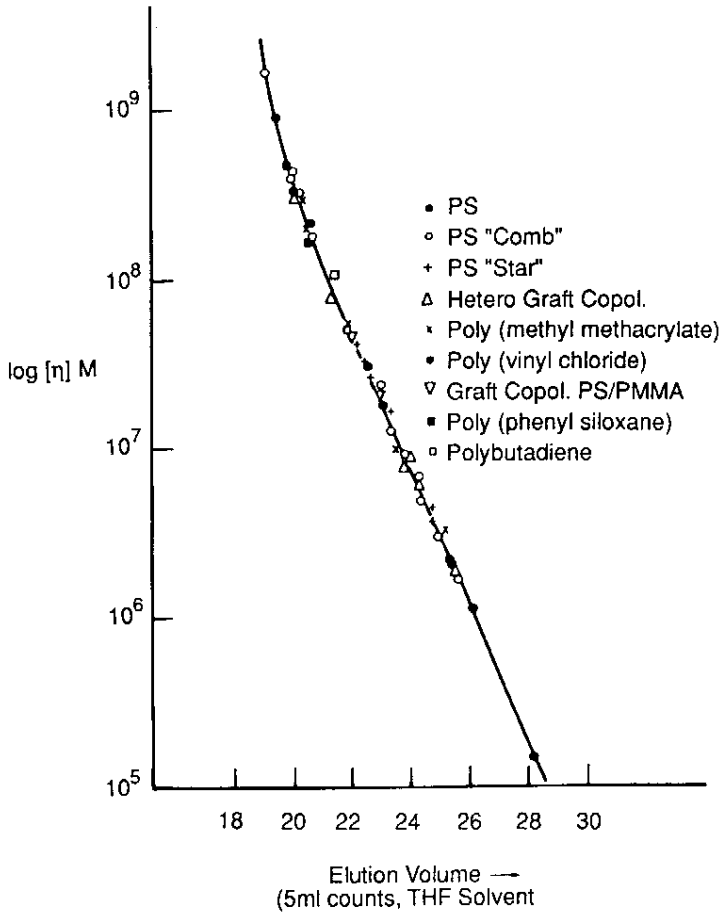
Universal Calibration

- However, if a calibration of size versus retention time could be generated then one true calibration would hold for all sample types

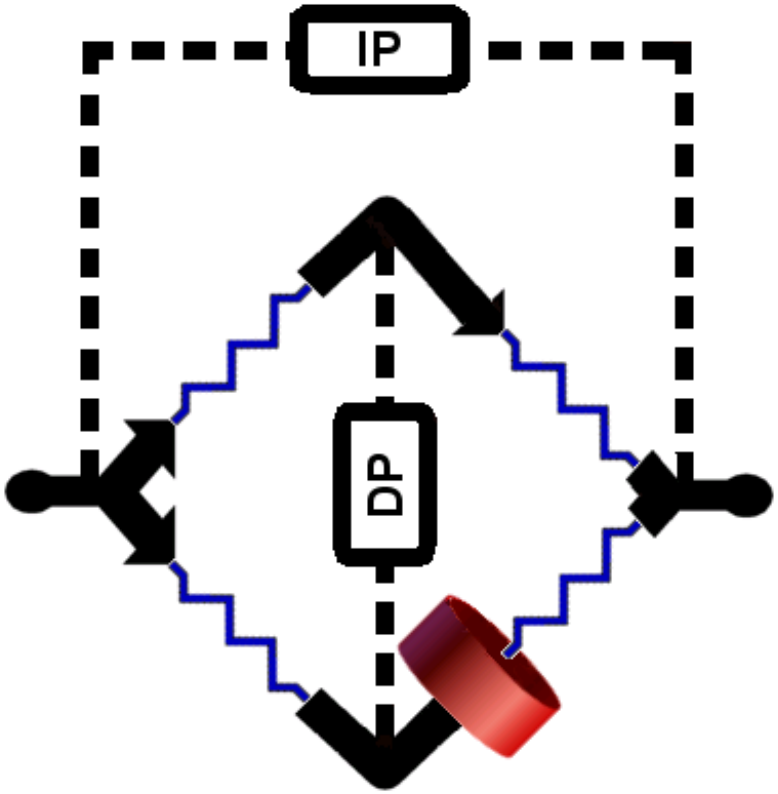
Hydrodynamic volume = [η] M

- A **Universal Calibration** plot of log[η]M versus RT holds true for all polymer types

- Can use measured intrinsic viscosity and retention time to get accurate molecular weights



Simplified Schematic of PL-BV 400 Differential Viscometer



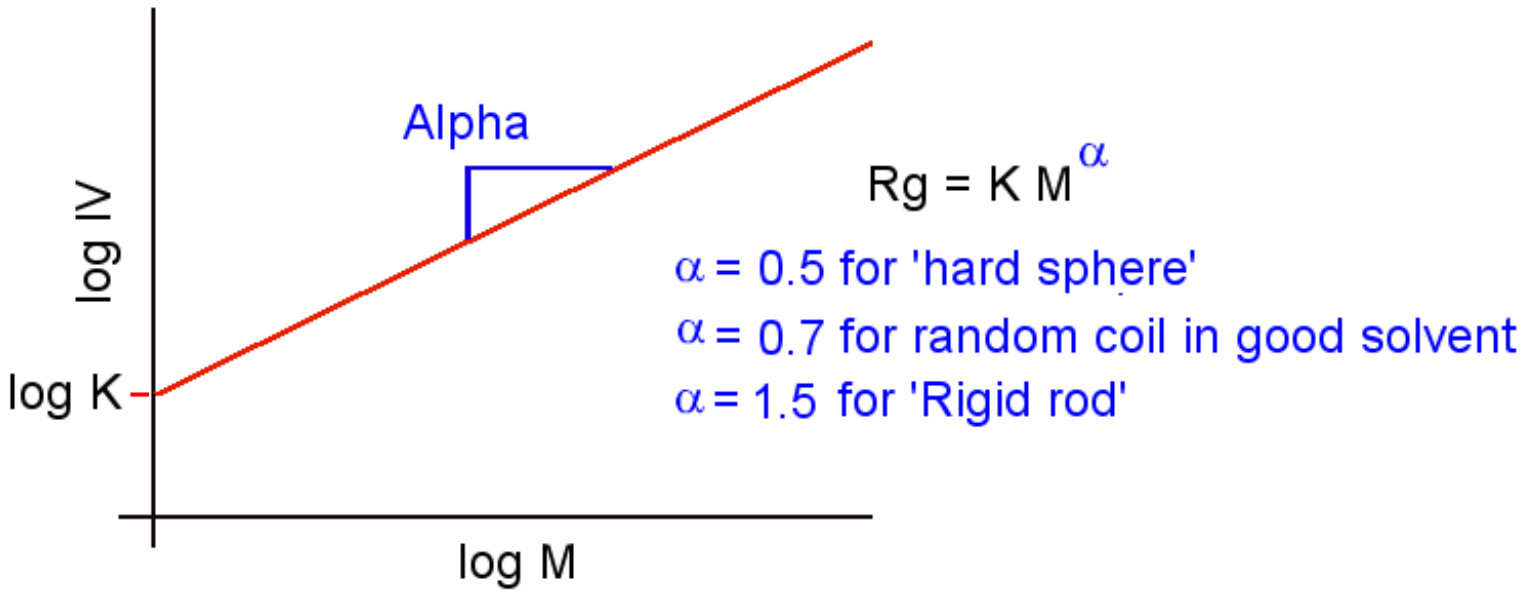
IP = Inlet pressure
DP = Differential pressure

$$\text{Specific viscosity} = \frac{4DP}{IP-2DP}$$

$$IV = \frac{\text{Specific viscosity}}{\text{Concentration}}$$

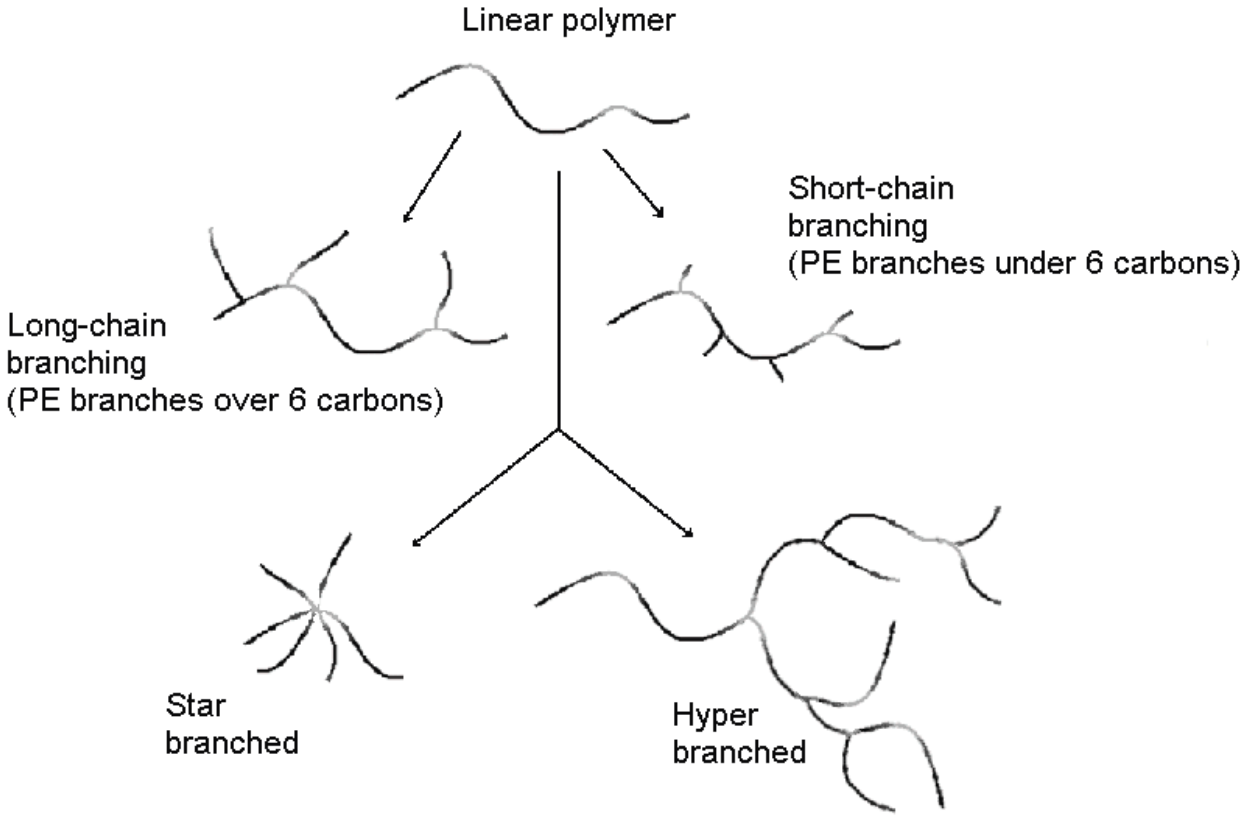
In the limit as conc. tends to zero

The Mark-Houwink Plot



- A Mark-Houwink plot of log IV versus log M should give a straight line as long as the Universal Calibration is obeyed (i.e no interactions occur)
- K and alpha vary between different solvents and polymers
- Alpha is a measure of the shape of the polymer in solution

Structural Information – 'Branching'



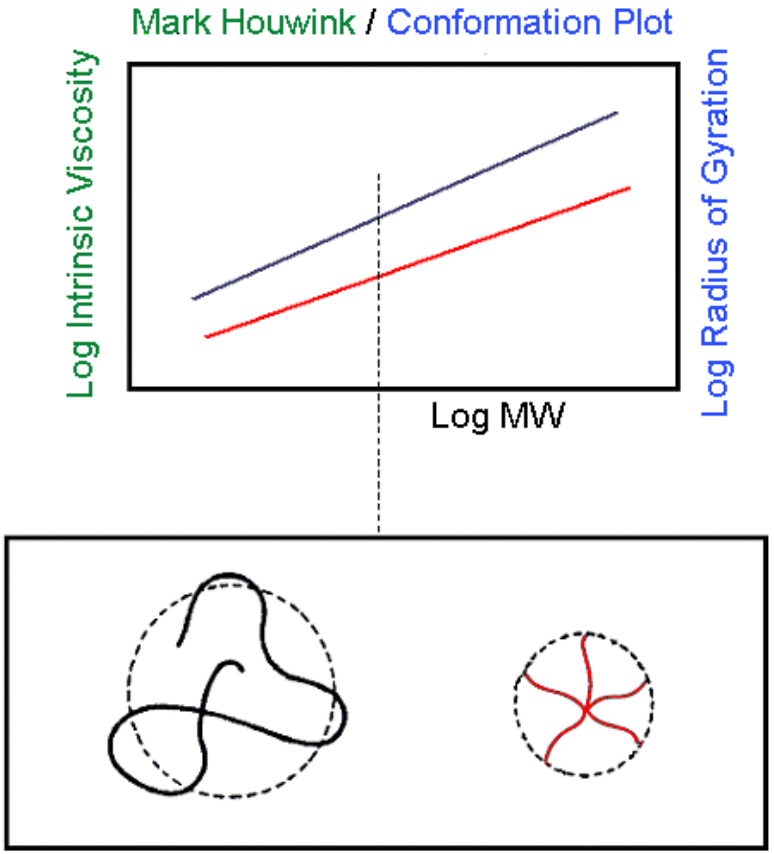
Using Viscometry and Light Scattering to Determine Branching

- **Linear** versus **branched** polymers
- Compared to a linear polymer the hydrodynamic volume of the branched molecule is smaller and so Rg will be smaller. Also, with the same mass of polymer enclosed the density will be higher producing a lower Intrinsic viscosity

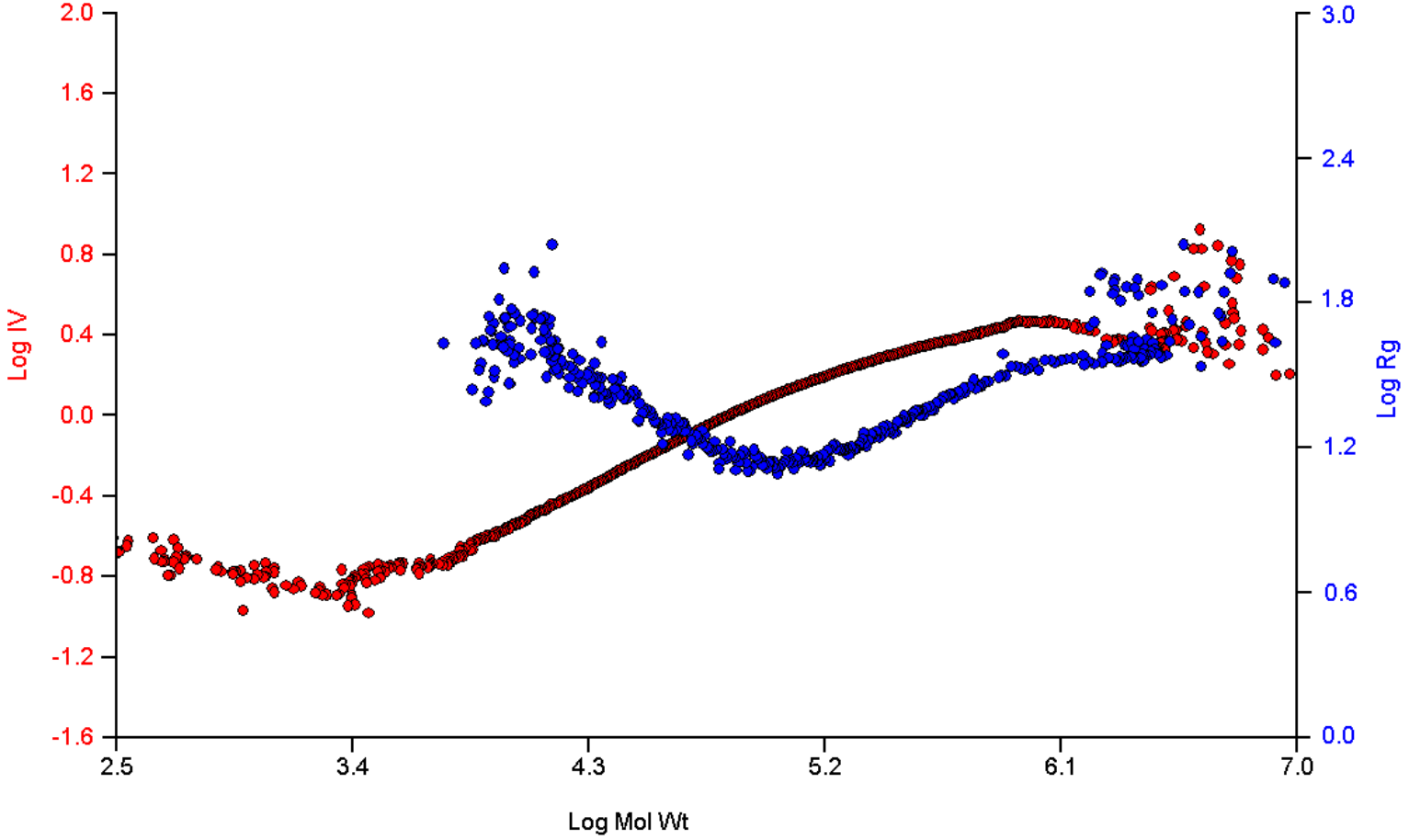
$$g = \left(\frac{\text{Rg branched}}{\text{Rg linear}} \right)$$

or

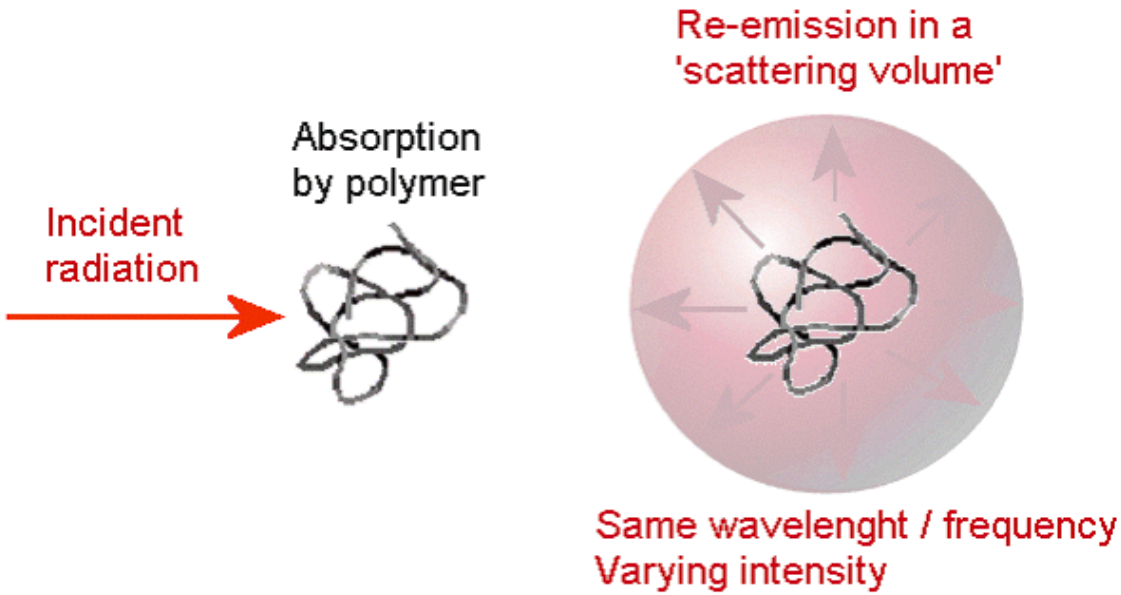
$$g = \left(\frac{\text{IV branched}}{\text{IV linear}} \right)^{1/E}$$



Mark Houwink and Conformation Plots For Branched Polyethylene NIST 1476

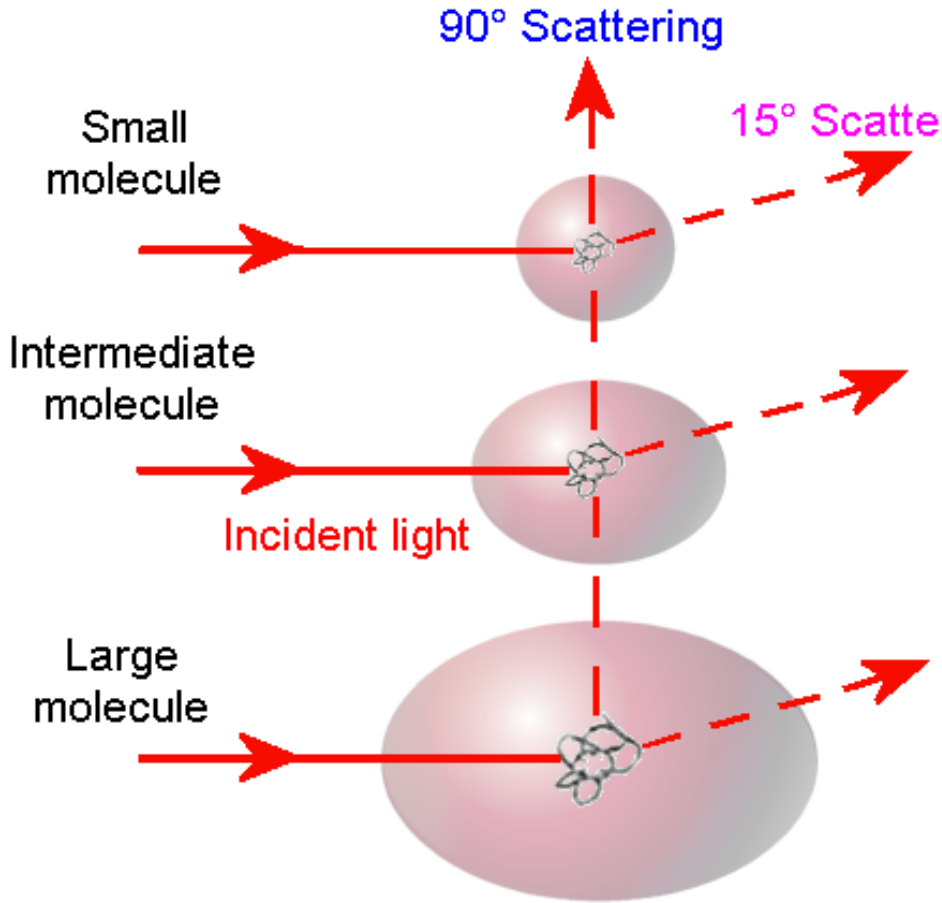


Light Scattering of Polymers



- In static light scattering, coherent incident radiation (usually a laser) interacts with components of the polymer backbone in a small scattering volume
- Excitation of the polymer chain results in re-emission of the radiation at the same wavelength and frequency but at variable intensity
- Measurement of the intensity of the scattered radiation allows the calculation of molecular weight and the chain dimensions

Effect of Polymer Size on Light Scattering



As molecule increases in size collection at 90° underestimates scattering compared to 15°

P(θ) drops below 1, can use this to estimate Rg

- Can use the difference in intensity of light scattered at two or more angles to determine Rg, the radius of gyration, by the Dissymmetry method

Static Light Scattering Equation

$$R_{(\theta)} = C\ M\ (dn/dc)^2\ P_{(\theta)}K_{(\theta)}$$

- $R_{(\theta)}$: Detector Response
- $C\ M$: Concentration X Mass
- $(dn/dc)^2$: Specific Refractive Index Increment
- $P_{(\theta)}$: Particle Scattering Function
- $K_{(\theta)}$: Light Scattering Constant

$R_{(\theta)}$ = Light Scattering Signal LS

The Light Scattering/Refractive Index Combination

$LSsig = R_{(q)} = C\,M\,(dn/dc)^2\,P_{(q)}K_{(q)}$ *Light Scattering equation*

$RI_{signal} = K_{RI}\,(dn/dc)\,C$ *Refractive Index equation*

Calculate dn/dc for sample

$$\frac{LS}{RI_{signal}} = \frac{K_{(q)}\,M\,(dn/dc)\,P_{(q)}}{K_{RI}}$$

$$\frac{LS}{RI_{signal}} \sim M$$

LS / RI Combination

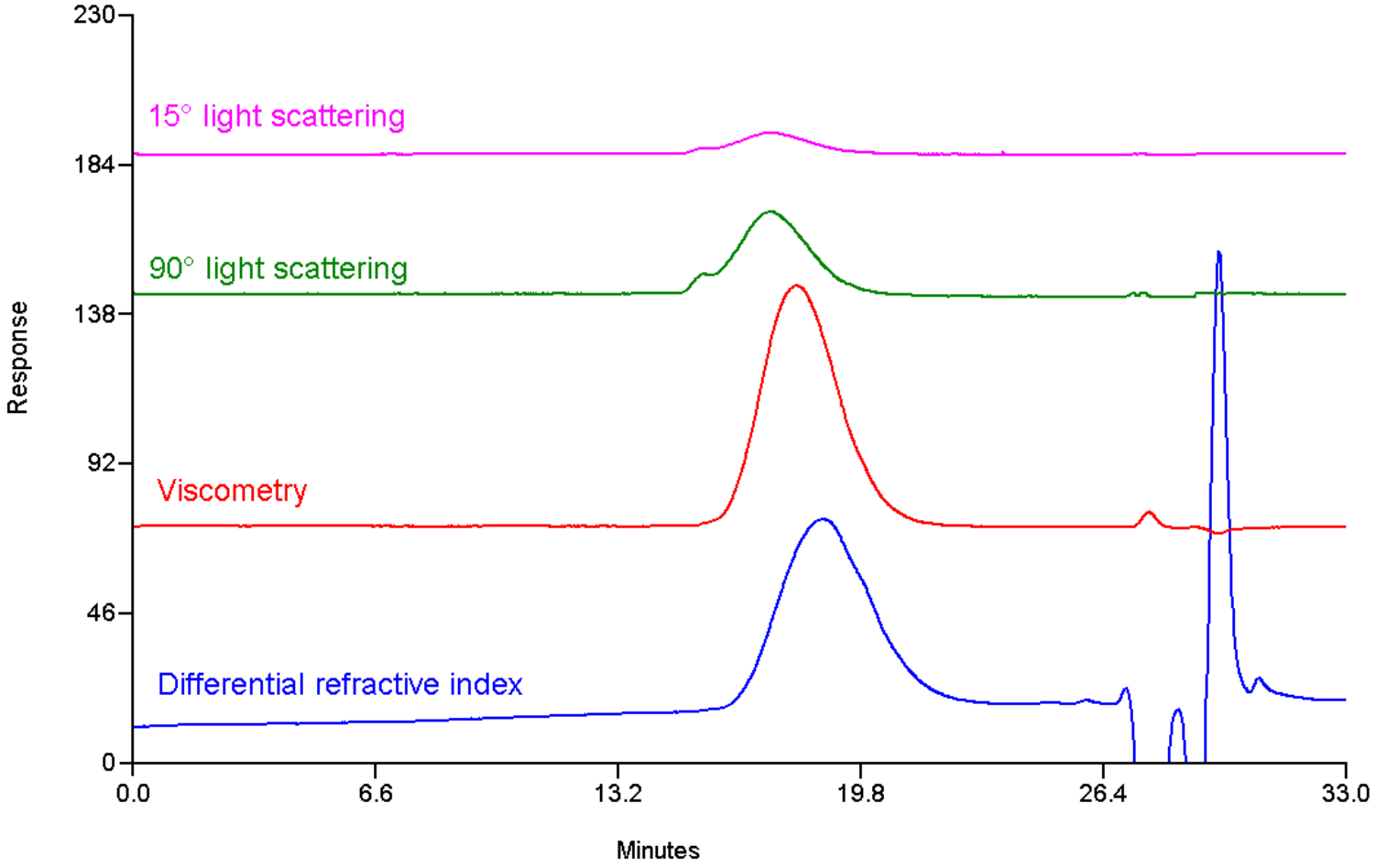
Directly Proportional to Mass

In addition radius of gyration (Rg) can be measured using combination of 15° and 90° detectors

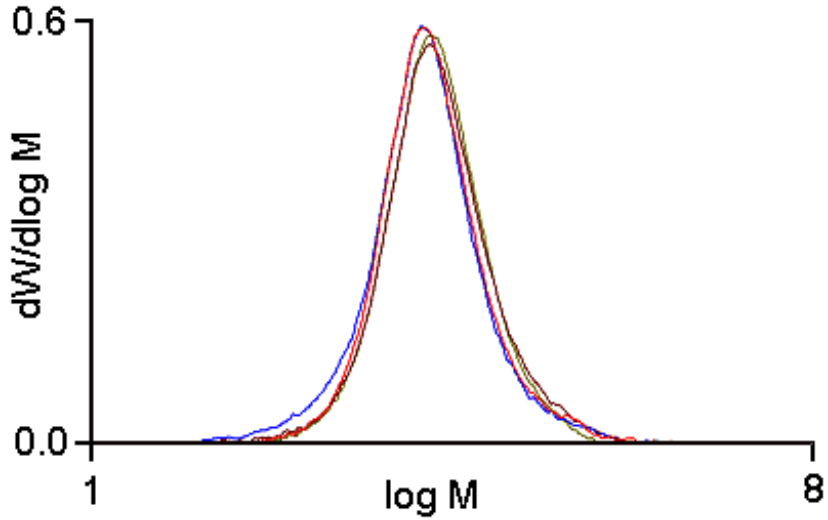
Case Study - Analysis of Polyolefins

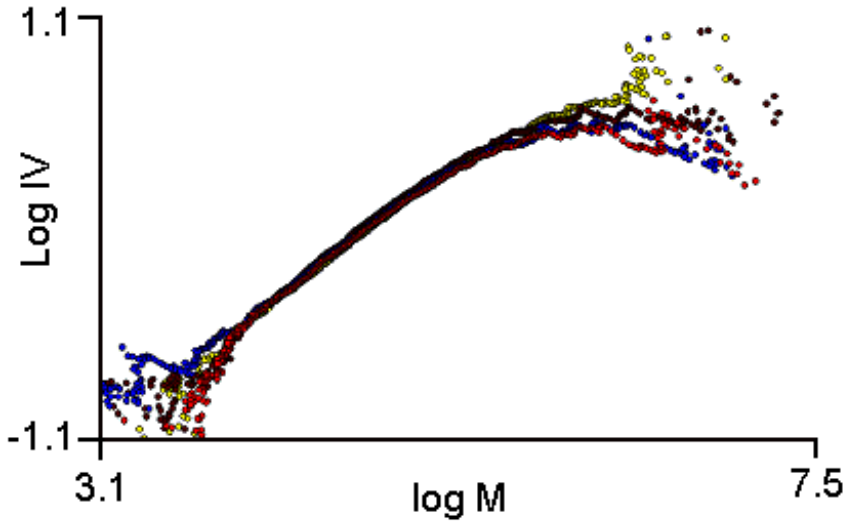
- Polyolefins are important high-tonnage engineering polymers
- Crystalline materials, only soluble at >120°C
- Structures can contain branching morphologies depending on the method of synthesis
- Long chain branching (over 6 carbons in length) can serious effect viscosity, density and processability
- Multi detector GPC is an ideal means of probing the structure of polyolefins

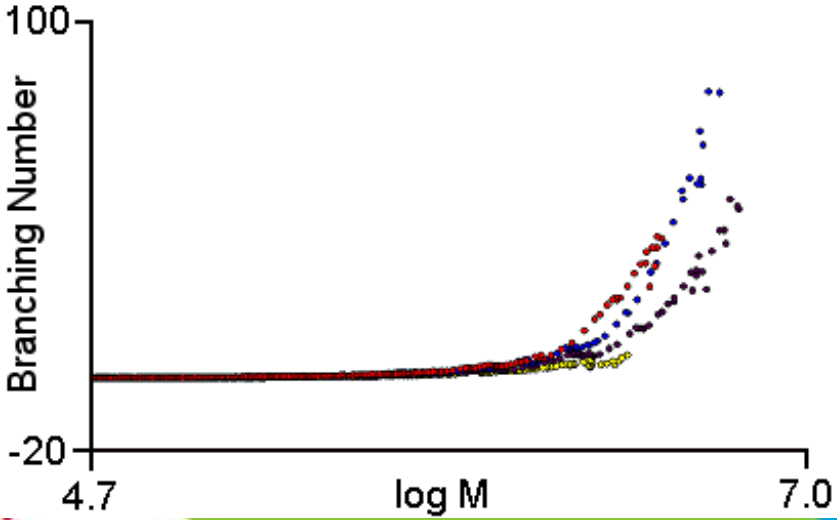
Polyethylene Triple Detection Data



PE Samples by GPC/Viscometry







The four samples had very similar molecular weight distributions but the Mark Houwink plots showed that there were some branching differences.

Summary of GPC Detector Capabilities

Method	Molecular weight	Branching?	Rg?
Conventional GPC	Relative to polymer standards used for calibration	✗	✗
GPC employing Mark-Houwink correction	Improved accuracy, rely on K and a values from literature	✗	✗
GPC-viscometry	From Universal Calibration	✓ directly from [n] measurements	✓ Yes, but indirectly
GPC-light scattering	Absolute determination, no column calibration required	✓ directly from Rg measurements	✓ when more than one angle is used
GPC-light scattering-viscometry	Accurate results	✓	✓