Conventional GPC

Polymers and Molecular Weight

GPC On Tour, Barcelona,
28th February 2012

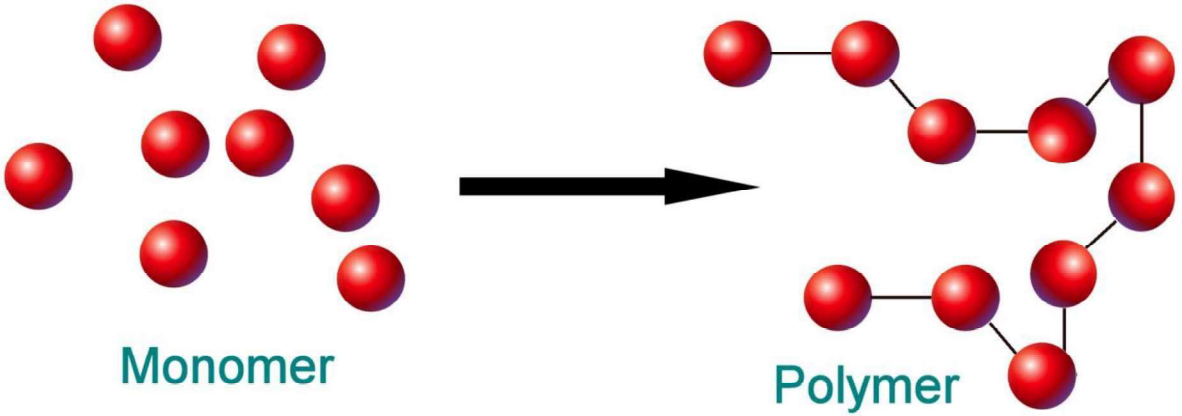
What are Polymers?

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

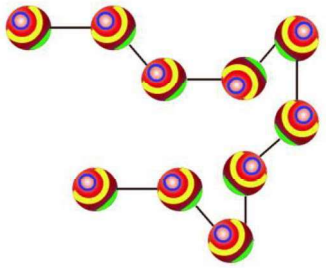
Polymers exhibit very different physical properties compared to the monomers, dependent on the length of the polymer chains

The presence of small amounts of very long or very short chains can have drastic effects on properties of the material

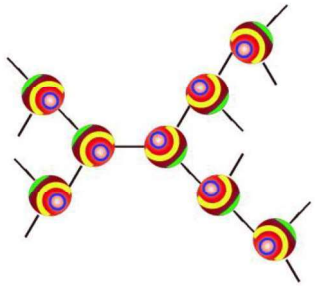


Variations in Polymers

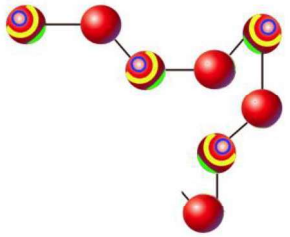
They can be varied in lots of ways, for example;



- Chemical Structure of Monomer Unit
- 3D Structure



- Different Monomer Units
- Length of polymer chains



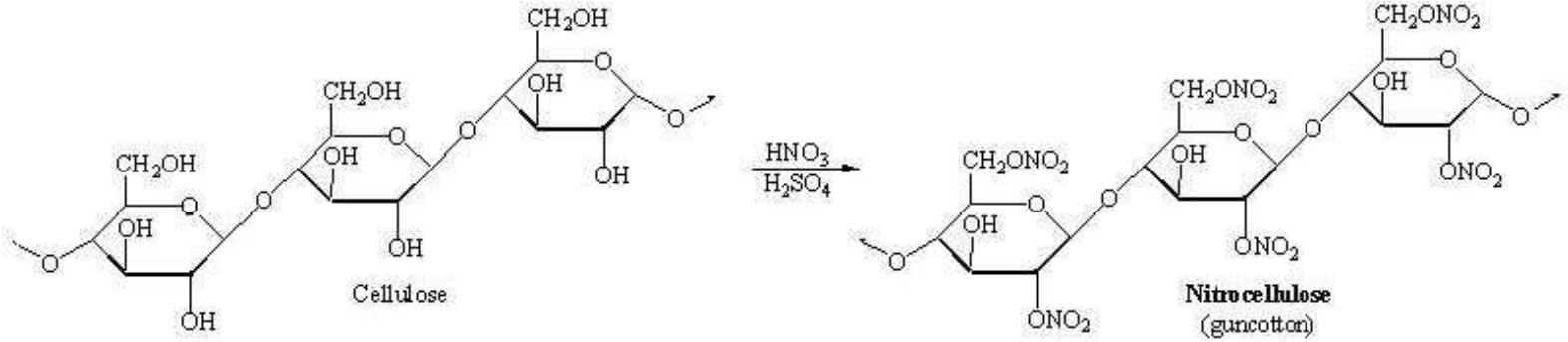
- Distribution of polymer chain lengths

Example 1 - Nitrocellulose

First synthetic polymer made in the 1890's

Hard, strong when set, durable when in moulding

Soon to be renamed gun cotton…!



Example 2 - Nylon

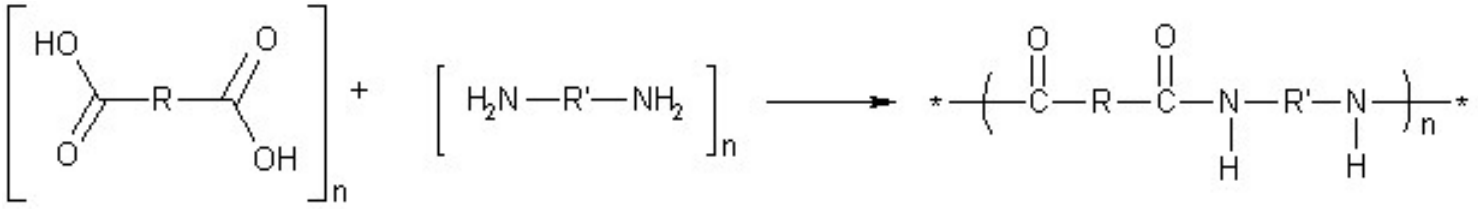
New York – London (NY-Lon)

1935 – Dupont Chemical Co.

Replaced silk in military parachutes

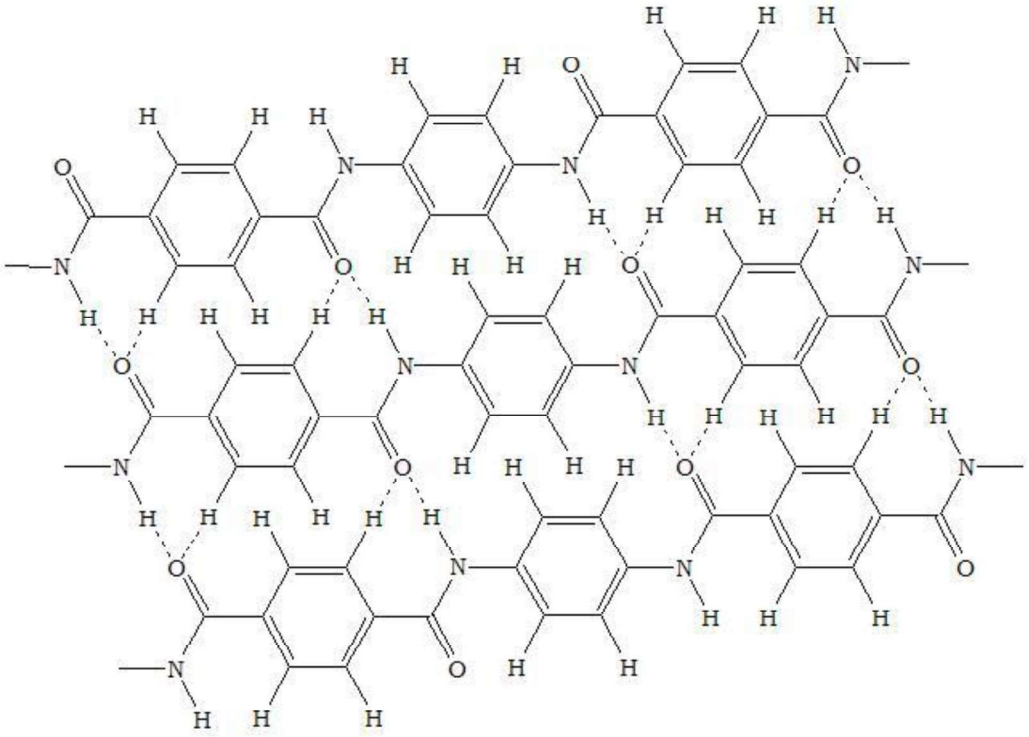
First product was nylon fibred toothbrush

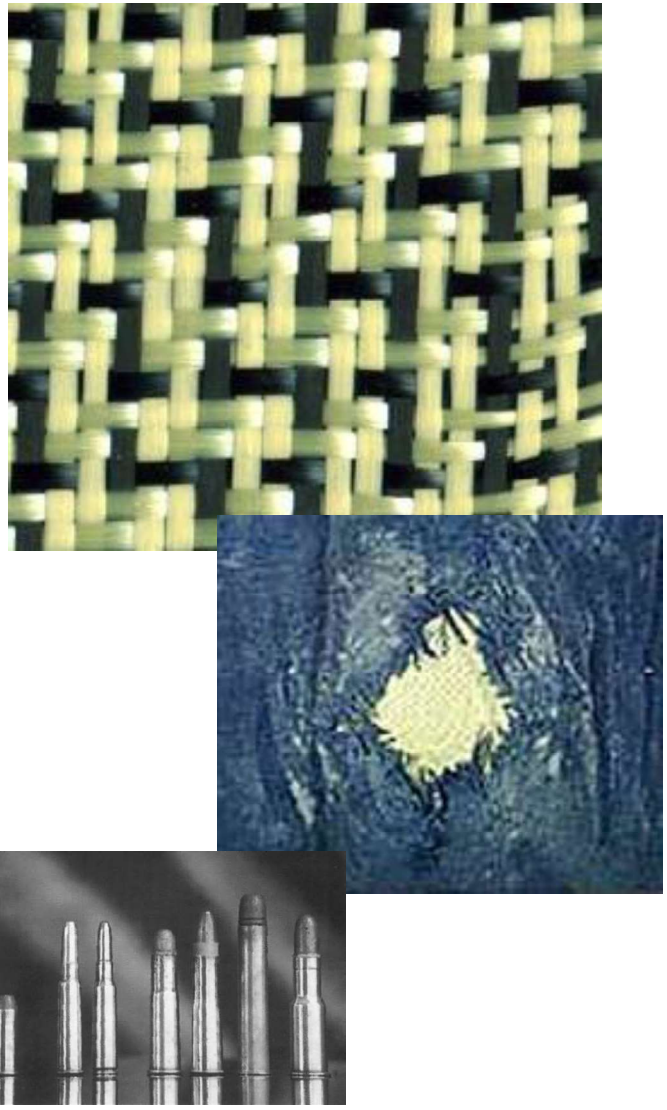
Tights came in the 1950's



Example 3 - Bullet-proof vests – Kevlar®

Strong inter-chain linkages make Kevlar bullet proof





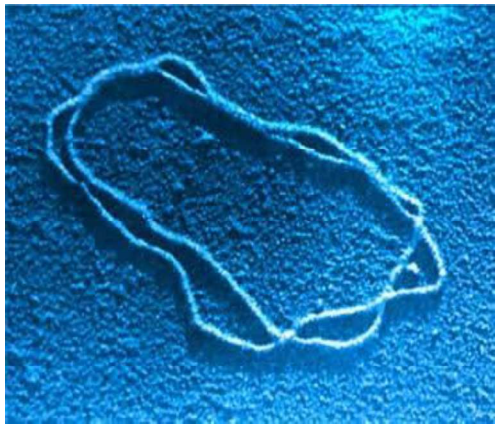
Example 4 – DNA, Deoxyribonucleic Acid

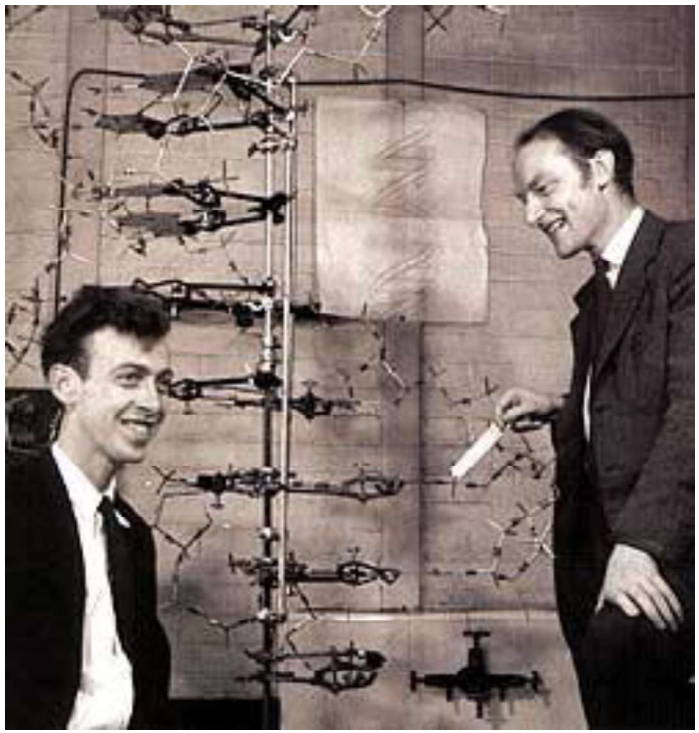
Longest natural occurring polymer

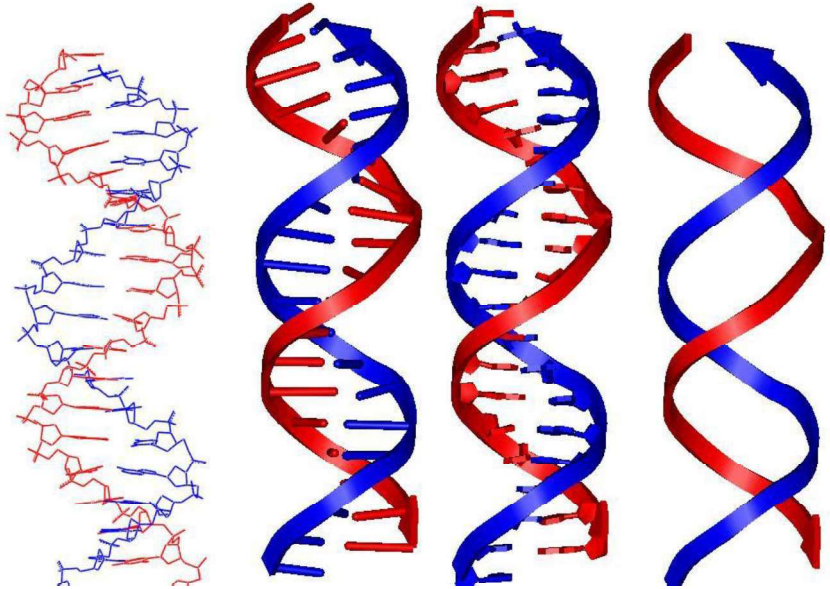
DNA in lungfish is 36 meters long per cell

DNA in humans is about 1 meter long per cell

Double helix, spiral, symmetry

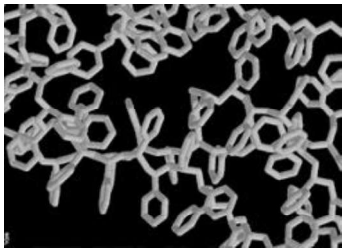






Common Polymers

Polystyrene PS



Polyethylene PE, HDPE

Polyvinylchloride PVC, UPVC

Nylon

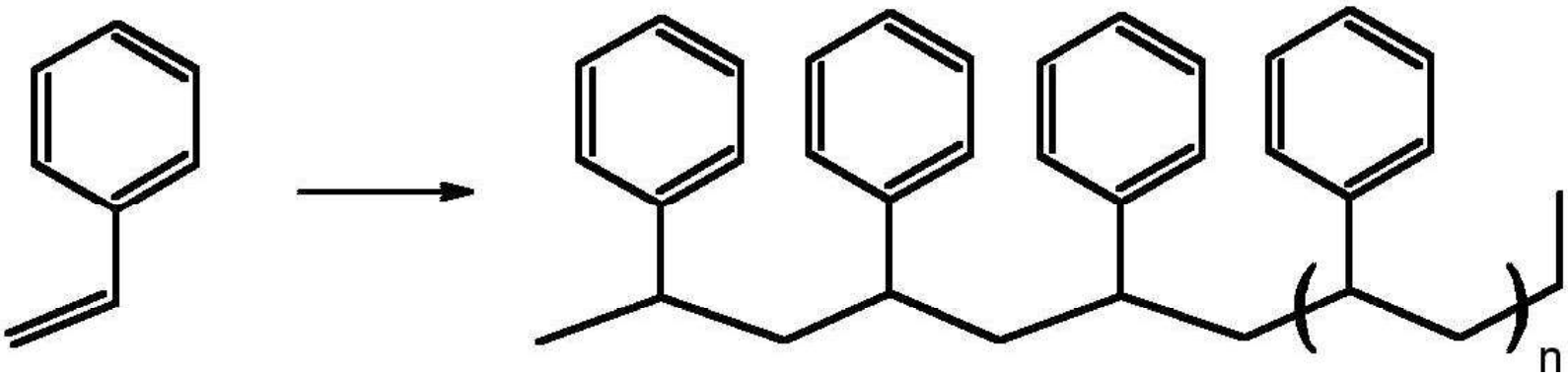
Molecular Weight

The molecular weight of a polymer is a way of describing how long the polymer chains are

Each monomer has a molecular weight (often called the formula weight)

Adding the monomers together to make polymers increases the molecular weight

The longer the chains, the higher the molecular weight



Effect of Molecular Weight

For example, let's look at hydrocarbons

Very short chain hydrocarbons are the predominant component of petrol – liquid at room temperature

Longer chain hydrocarbons are present in various waxes such as candle wax – soft, pliable and easy to melt

Polythene is a very long chain hydrocarbon – tough, strong and very resistant to heat and solvents

Polymer Molecular Weight Distributions

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….

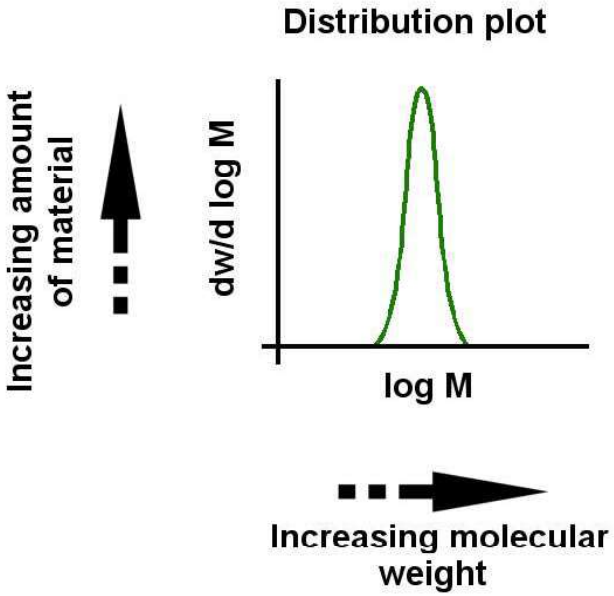
Different samples of the same polymer can have the same average chain length but very different distributions of chain lengths depending on the method of production

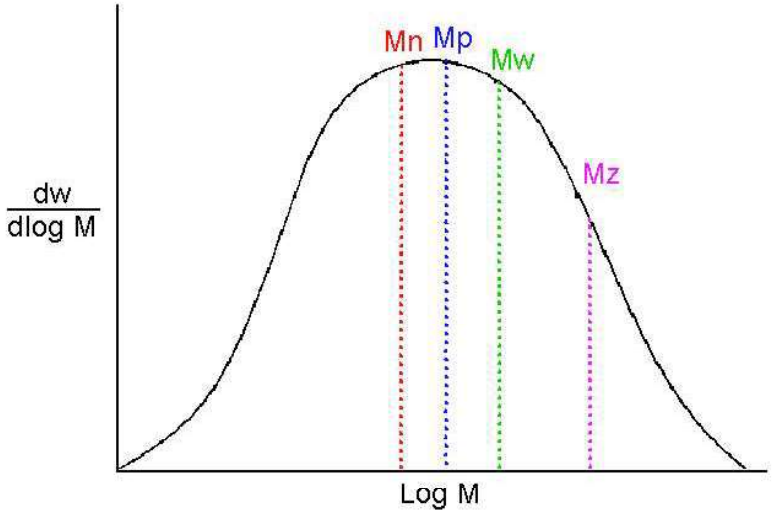
In polymer science it is the molecular weight *distribution* that is important

Molecular Weight Averages by GPC

Number average Mn	Mn can be correlative with polymer colligative properties, e.g. freezing point depression
Weight average Mw	Mw may be correlated with properties such as melt viscosity
Z average Mz	Mz may be correlated with properties such as toughness
Polydispersity, $d = \frac{Mw}{Mn}$	Polydispersity characterises the shape of the distribution

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

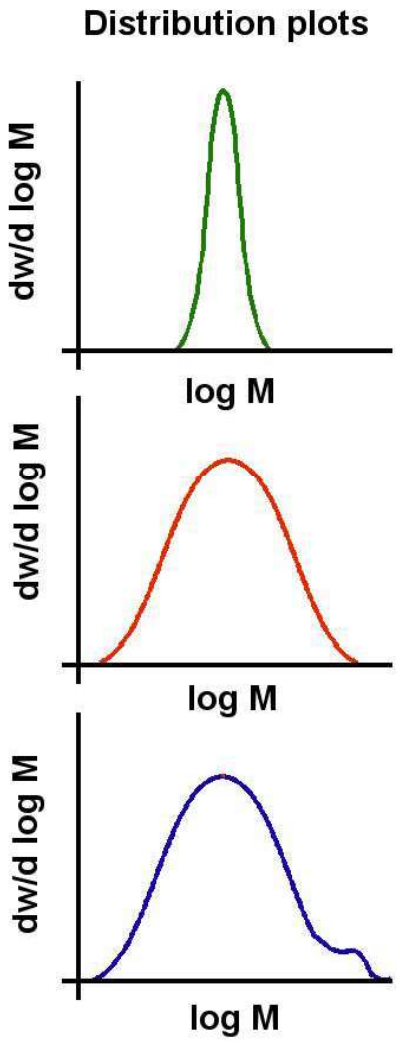
Shape of Distributions

Even for the same type of polymer, each of these distributions will describe a polymer that behaves differently

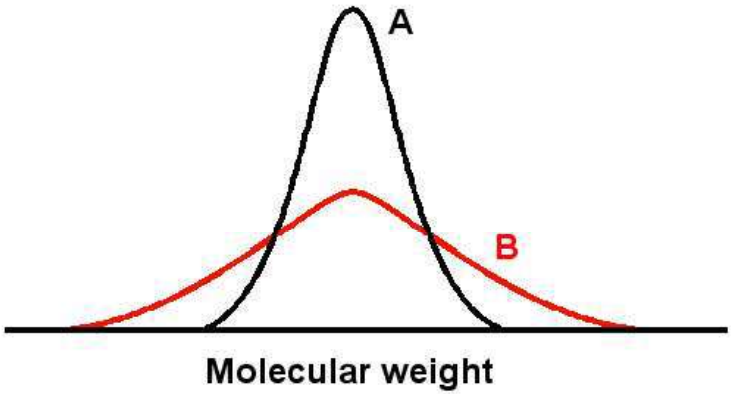
The red and green plots are for low and high polydispersity materials

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



Effect of 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 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	+	+	-	+	+	+

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

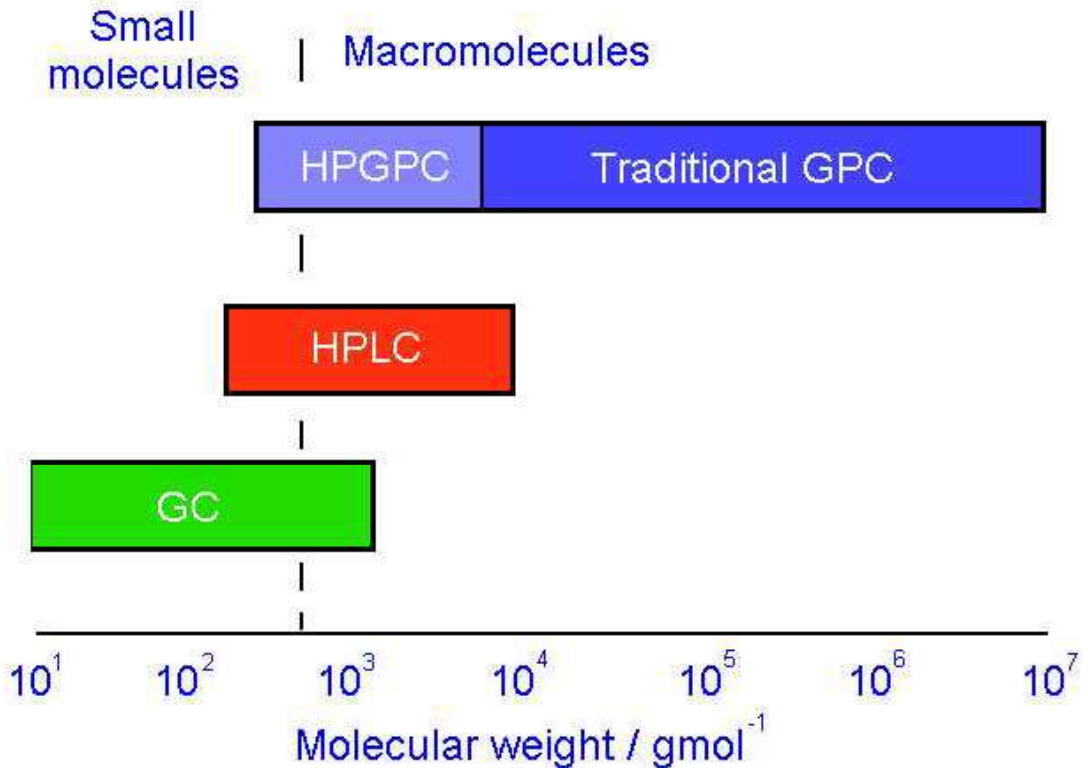
Gel permeation chromatography (sometimes called size exclusion chromatography) is a method of measuring molecular weights

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

So where in Chromatography is GPC

Interactive adsorption, partition, ion exchange, etc

Non-interactive GPC, SEC, GFC



Nomenclature

There are many ways of measuring the molecular weight of a polymer however, ***there is only one technique that measures the molecular weight distribution***.

- **G**el **P**ermeation **C**hromatography, **GPC** (Polymer Industry)

One technique but multiple acronyms, also known as

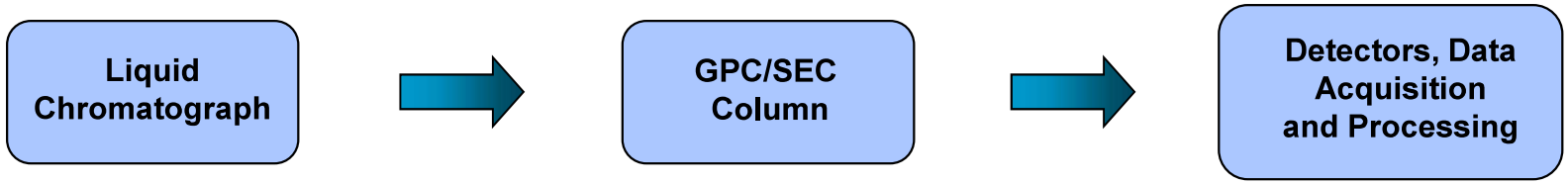
- **S**ize **E**xclusion **C**hromatography, **SEC** (Academia)

and

- **G**el **F**iltration **C**hromatography, **GFC** (Protein/Pharma)

What is a GPC/SEC System?

- A simple isocratic LC system fitted with a GPC/SEC column is a GPC/SEC system!
- Mode of separation only difference to other HPLC methods
- Specialist detectors can be used to determine properties of the samples investigated
- Special GPC/SEC software required to perform analysis



Simple isocratic system
Provides solvent flow

Column separates sample
Into components

Detectors measure the properties of eluted sample, data system provides analysis

Additional Components Used in GPC

Concentration detectors

- Differential refractometer (RI)
- Ultraviolet absorbance (UV)
- Evaporative light scattering or mass detector (ELS, EMD)
- Infra-red (IR)

Molecular weight sensitive detectors

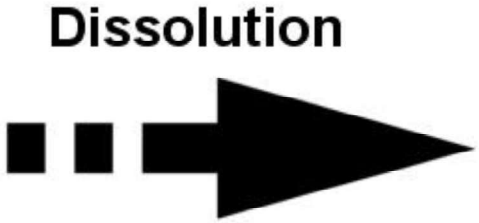
- Viscometry
- Light scattering

Additional systems

- Online degasser
- Autosampler
- Column oven
- Additional specific detectors

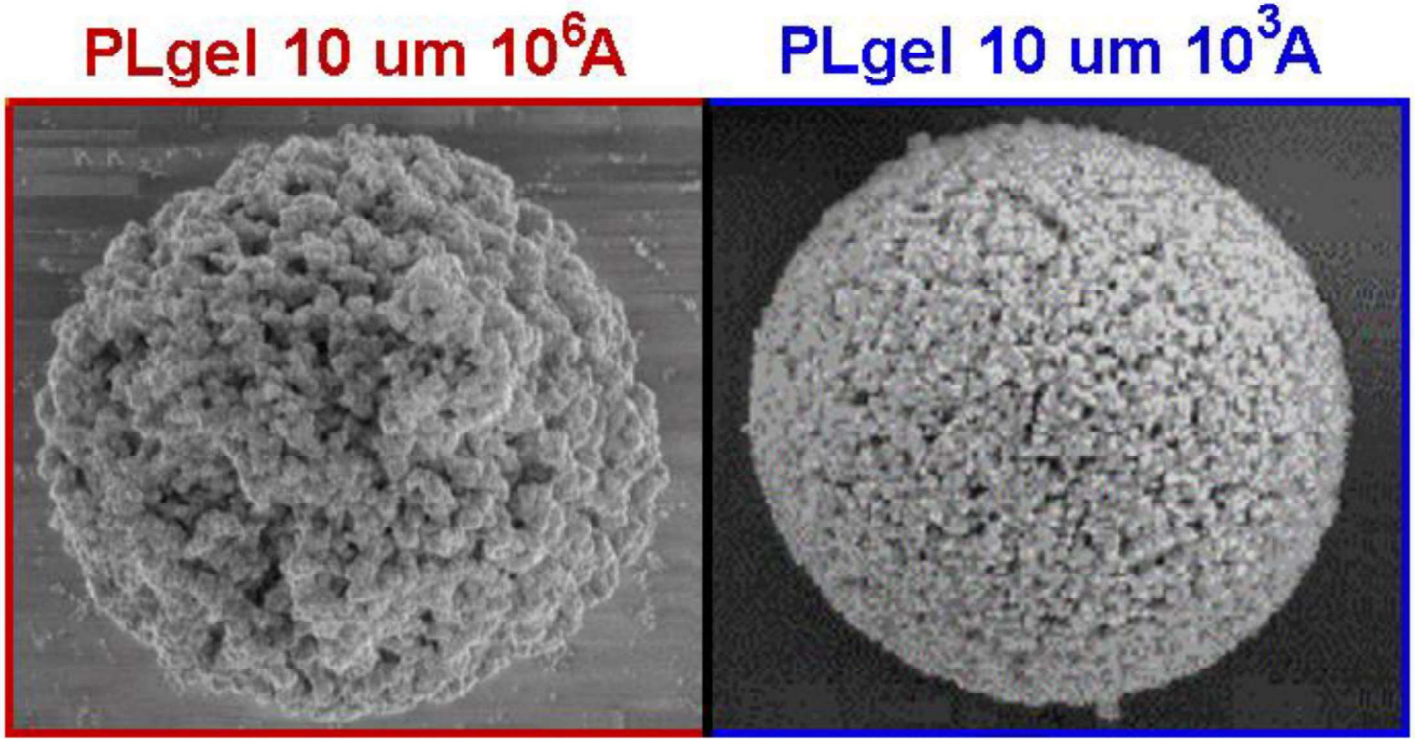
Polymer Molecules in Solution

- 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 entropic effects all but the most rigid of polymer chains curls up in solution to form a ball like shape



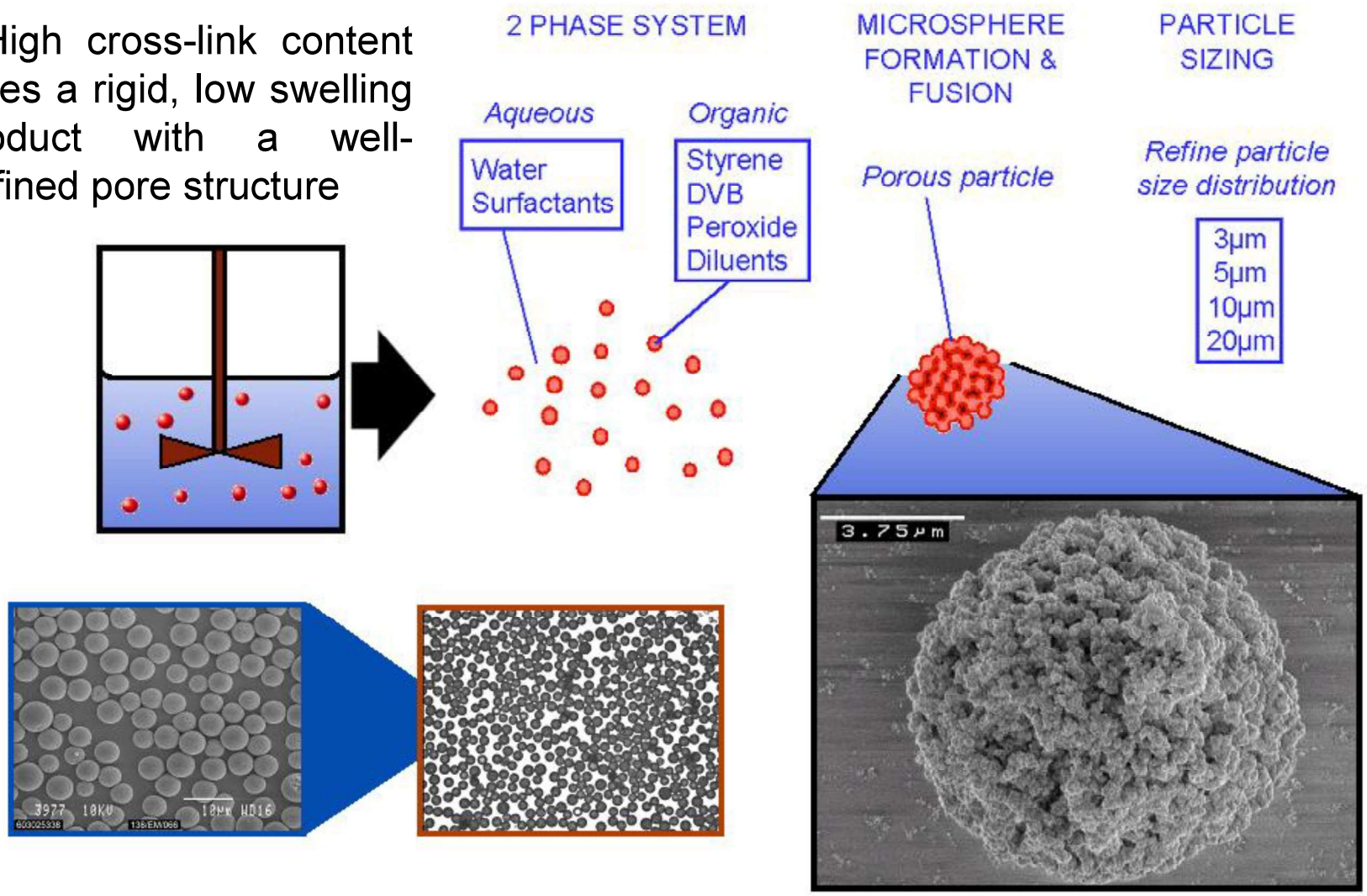
GPC Column Packings

- GPC columns are packing with cross-linked, insoluble beads, typically co-polymers of styrene and divinyl benzene for organic GPC
- These beads have a rigid pore structure that remains intact in the presence of solvent

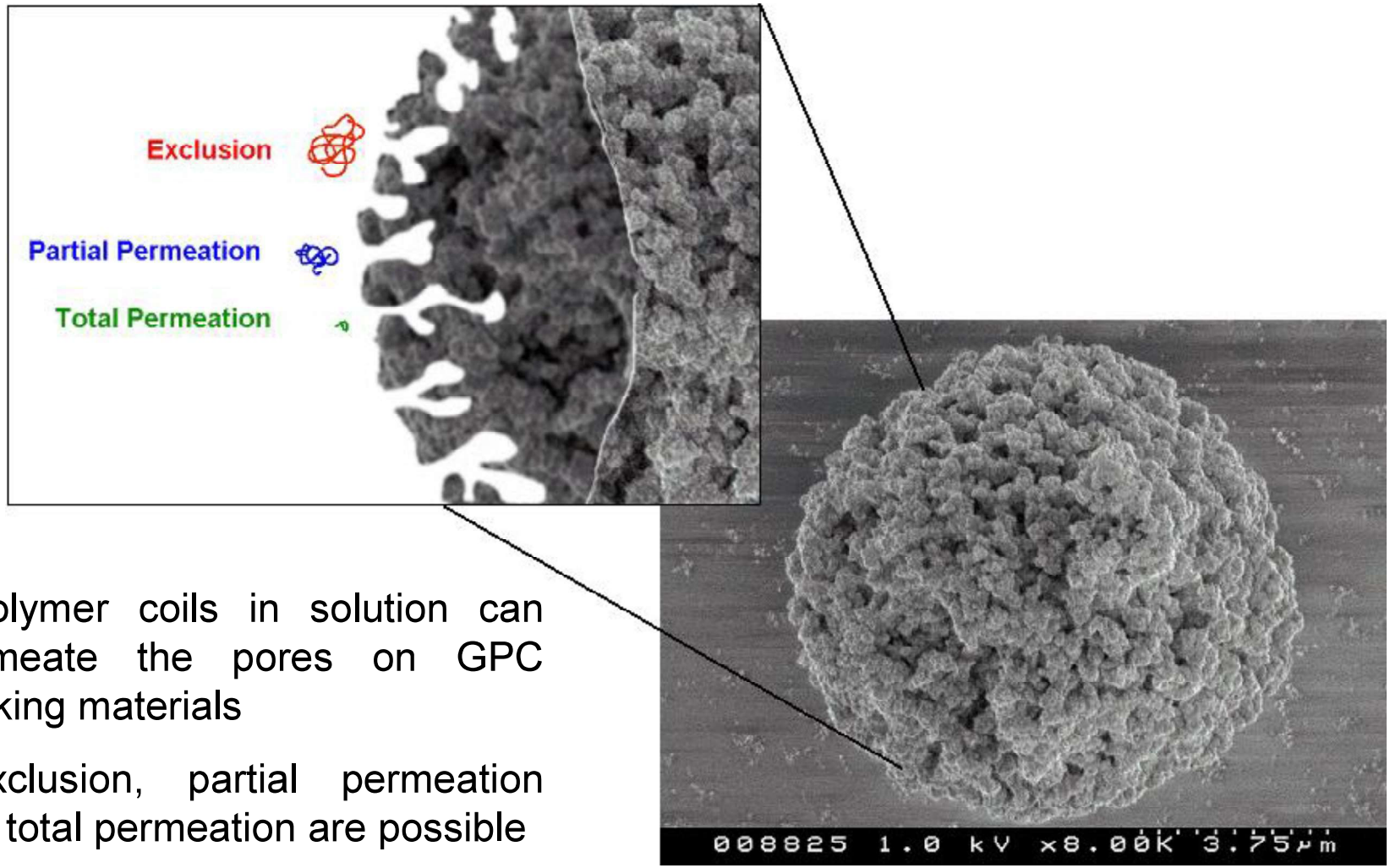


Synthesis of Porous Beads

- High cross-link content gives a rigid, low swelling product with a well-defined pore structure



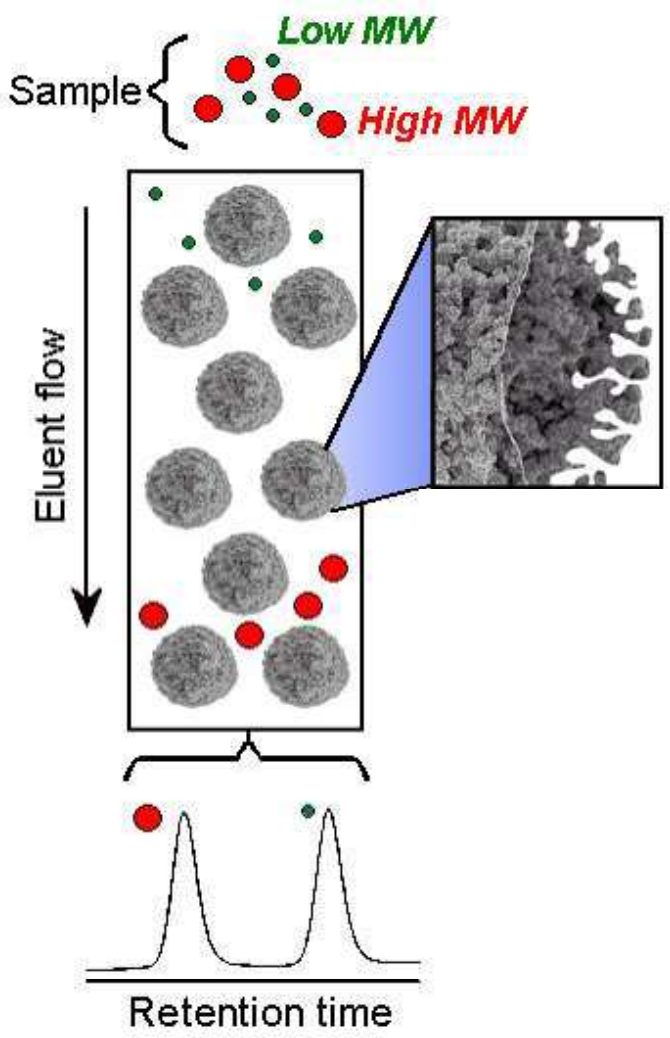
Permeation of Polymer Molecules



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

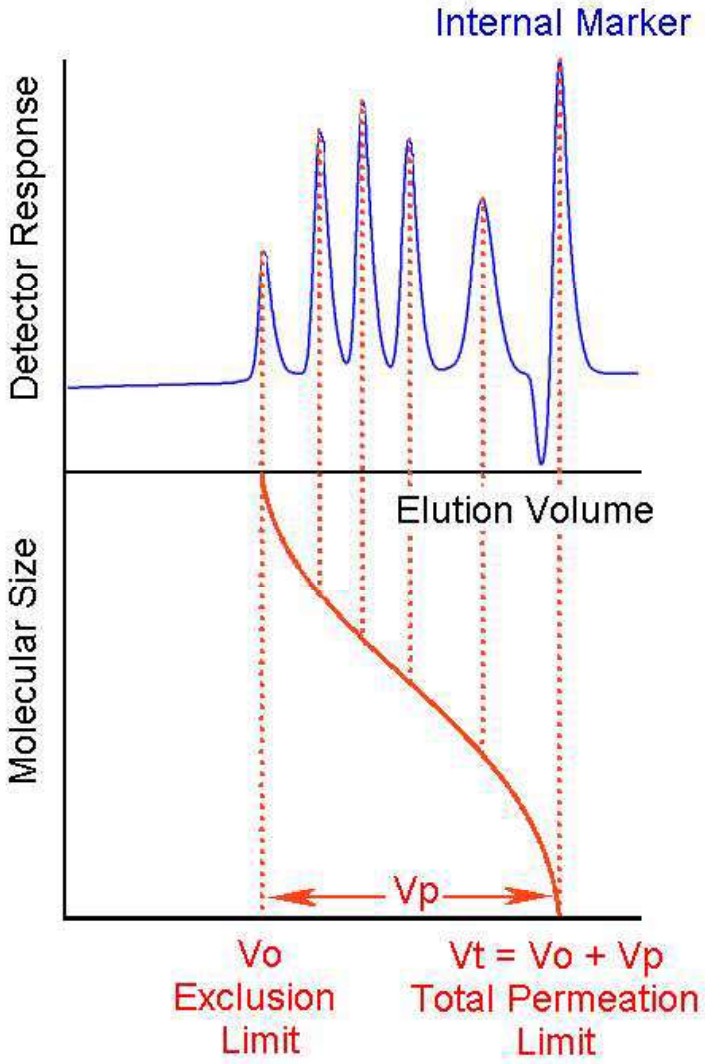
GPC Separation Mechanism

- 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

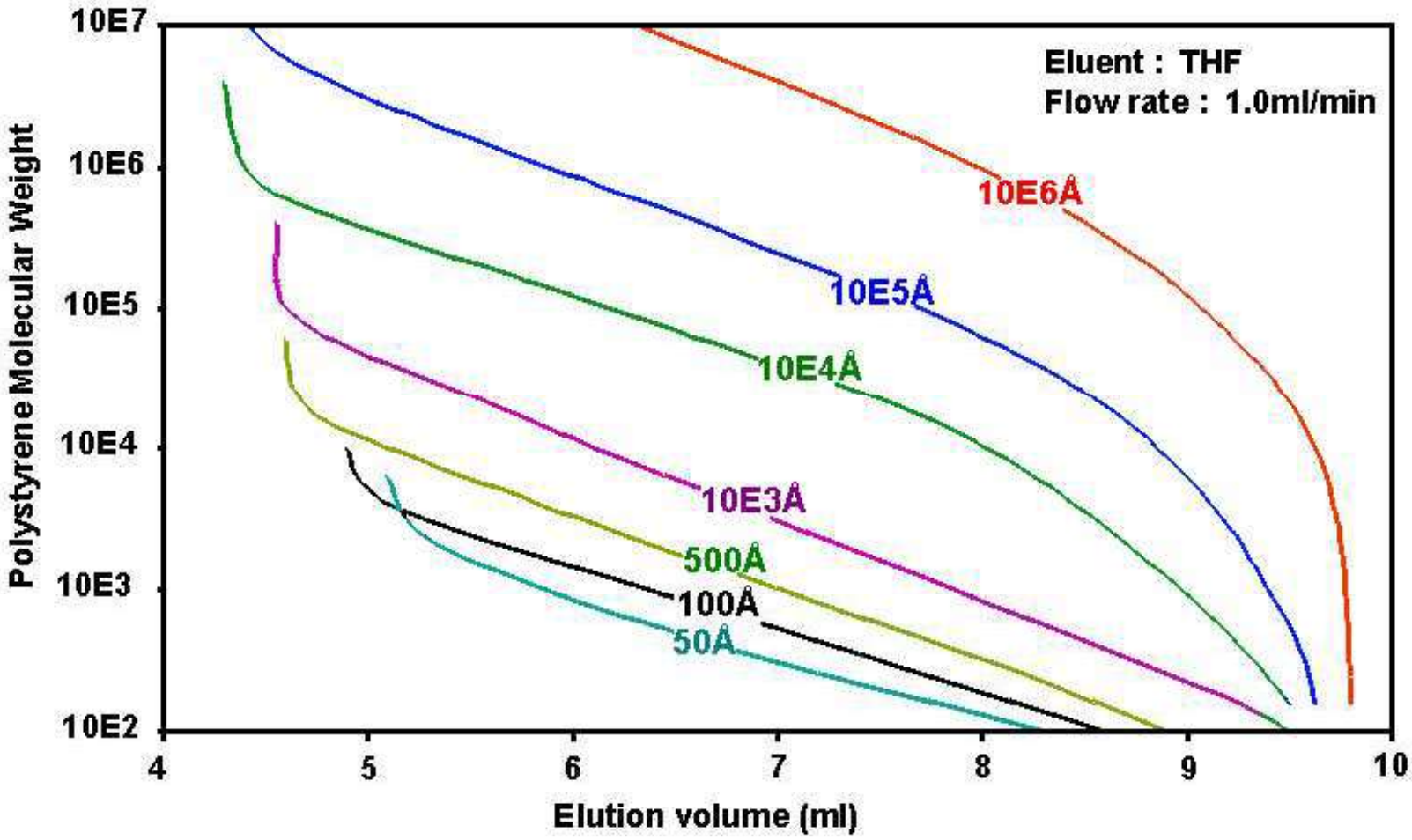


Elution Profiles

- 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

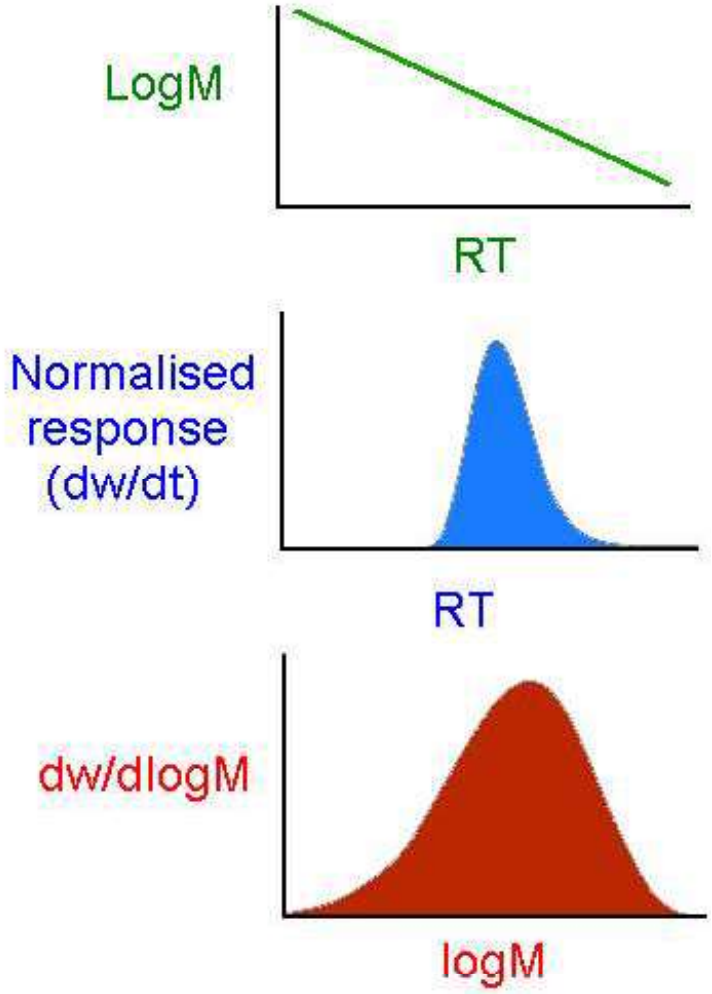


Typical Calibration Curves for PLgel Individual Pore Size Columns

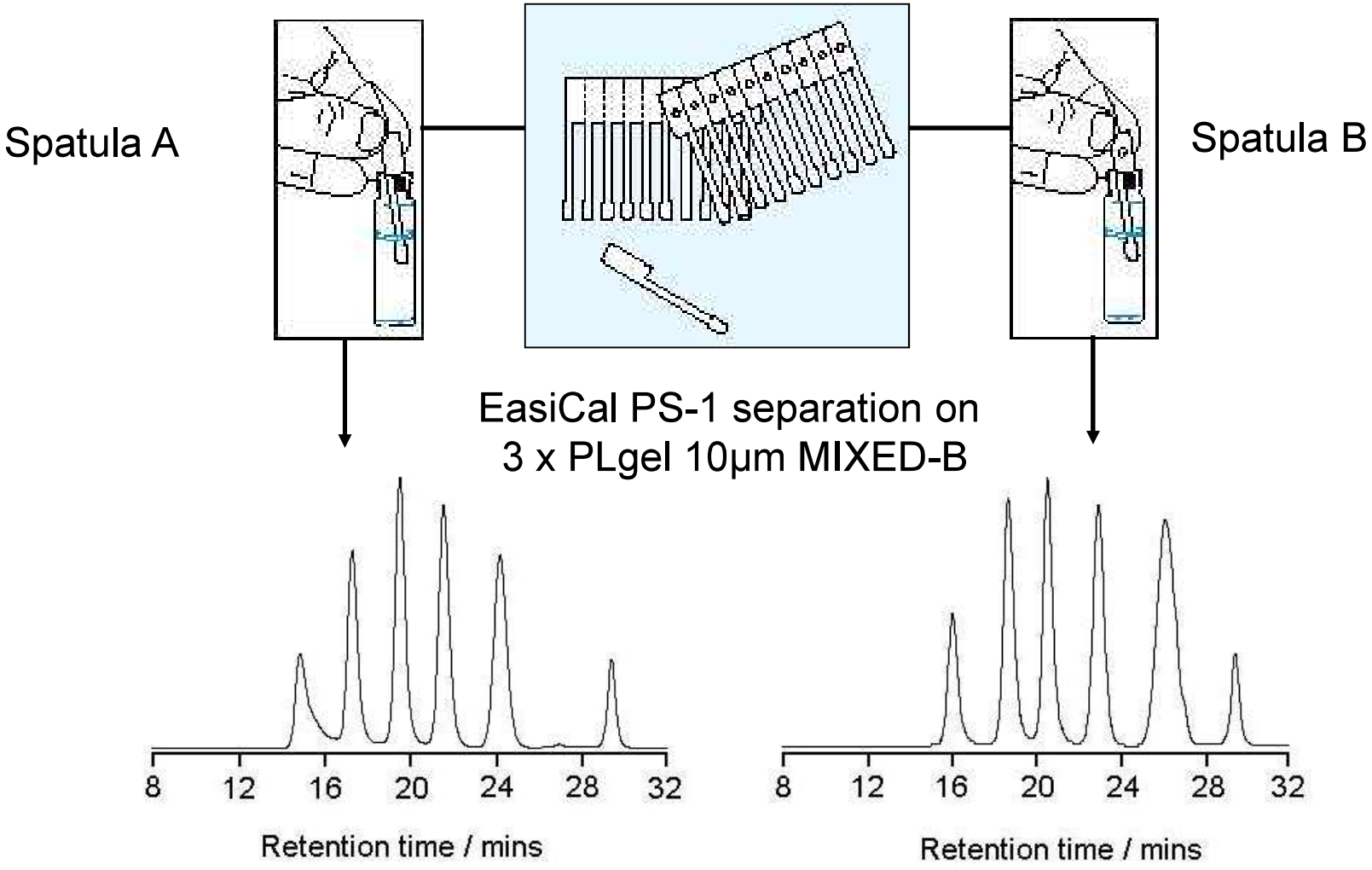


Determination of Polymer Molecular Weight Distribution by GPC

- Produce a GPC calibration curve for the column set relating log M to retention time (RT)
- Chromatograph the polymer sample
- Normalise and integrate the GPC response versus retention time plot for the polymer sample
- Convert retention time to logM via the GPC calibration curve
- Present a logM distribution plot and calculate molecular weight averages (Mn, Mw) for the distribution



EasiCal Pre-prepared Calibrants



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*

Must be extremely well characterised

Most commonly used polymer calibrants

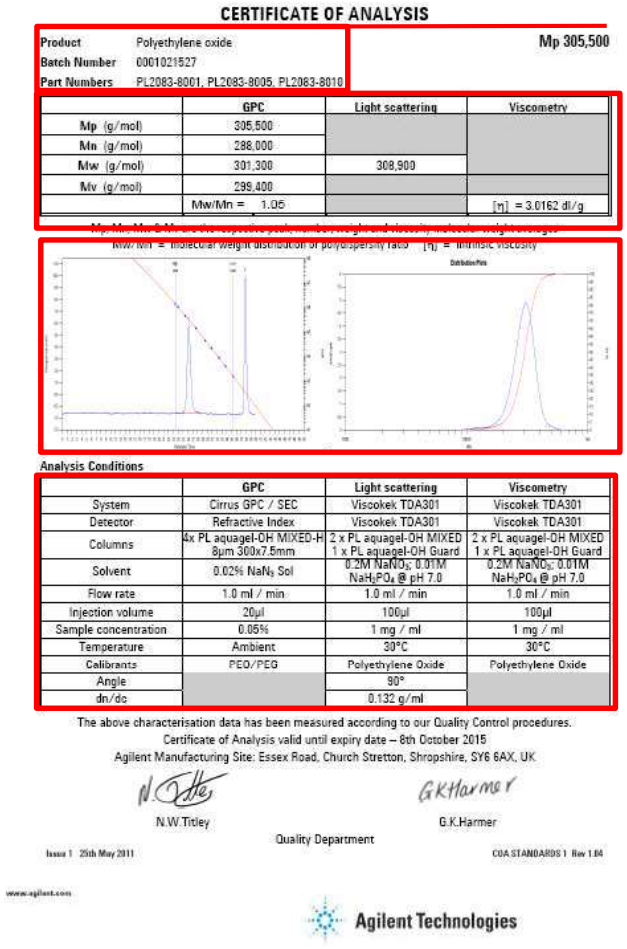
Polystyrene - THF, toluene, chloroform, TCB

Polymethyl methacrylate - MEK, ethyl acetate, acetone, DMF

Polyethylene oxide/glycol - aqueous eluents, DMF, DMSO

Example Certificates of Analysis

Individual standard



CERTIFICATE OF ANALYSIS

Product: Polyethylene oxide — Mp 305,500
Batch Number: 0001021527
Part Numbers: PL2083-8001, PL2083-8005, PL2083-8010

Batch details

	GPC	Light scattering	Viscometry
Mp (g/mol)	305,500		
Mn (g/mol)	288,000		
Mw (g/mol)	301,300	308,900	
Mv (g/mol)	299,400		
	Mw/Mn = 1.05		[η] = 3.0162 dl/g

Analysis details

Mp, Mw, Mn, Z-Mv are the respective peak number, weight and viscosity molecular weight averages
Mw/Mn = molecular weight distribution or polydispersity ratio [η] = intrinsic viscosity

GPC data

Analysis Conditions

	GPC	Light scattering	Viscometry
System	Cirrus GPC / SEC	Viscokek TDA301	Viscokek TDA301
Detector	Refractive Index	Viscokek TDA301	Viscokek TDA301
Columns	4x PL aquagel-OH MIXED-H 8µm 300x7.5mm	2 x PL aquagel-OH MIXED 1 x PL aquagel-OH Guard	2 x PL aquagel-OH MIXED 1 x PL aquagel-OH Guard
Solvent	0.02% NaN_3 Sol	0.2M $NaNO_3$; 0.01M NaH_2PO_4 @ pH 7.0	0.2M $NaNO_3$; 0.01M NaH_2PO_4 @ pH 7.0
Flow rate	1.0 ml / min	1.0 ml / min	1.0 ml / min
Injection volume	20µl	100µl	100µl
Sample concentration	0.05%	1 mg / ml	1 mg / ml
Temperature	Ambient	30°C	30°C
Calibrants	PEO/PEG	Polyethylene Oxide	Polyethylene Oxide
Angle		90°	
dn/dc		0.132 g/ml	

Results

The above characterisation data has been measured according to our Quality Control procedures.
Certificate of Analysis valid until expiry date – 8th October 2015
Agilent Manufacturing Site: Essex Road, Church Stretton, Shropshire, SY6 6AX, UK

N.W.Titley — G.K.Harmer
Quality Department

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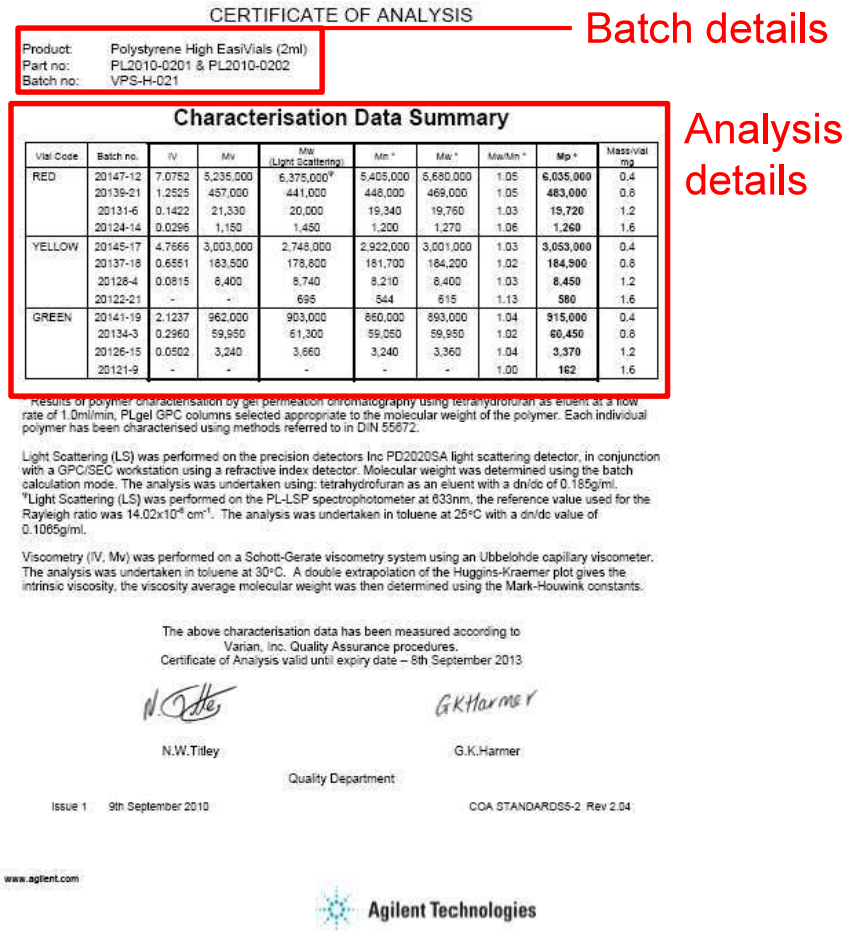
EasiVial



CERTIFICATE OF ANALYSIS

Product: Polystyrene High EasiVials (2ml)
Part no: PL2010-0201 & PL2010-0202
Batch no: VPS-H-021

Batch details

Characterisation Data Summary

Vial Code	Batch no.	IV	Mv	Mw (Light Scattering)	Mn *	Mw *	Mw/Mn *	Mp *	Mass/vial mg
RED	20147-12	7.0752	5,235,000	6,375,000[¥]	5,405,000	5,680,000	1.05	6,035,000	0.4
	20139-21	1.2525	457,000	441,000	448,000	469,000	1.05	483,000	0.8
	20131-6	0.1422	21,330	20,000	19,340	19,760	1.03	19,720	1.2
	20124-14	0.0295	1,150	1,450	1,200	1,270	1.06	1,260	1.6
YELLOW	20145-17	4.7666	3,003,000	2,748,000	2,922,000	3,001,000	1.03	3,053,000	0.4
	20137-18	0.6551	183,500	178,800	181,700	184,200	1.02	184,900	0.8
	20128-4	0.0815	8,400	8,740	8,210	8,400	1.03	8,450	1.2
	20122-21	-	-	695	544	615	1.13	580	1.6
GREEN	20141-19	2.1237	962,000	903,000	860,000	893,000	1.04	915,000	0.4
	20134-3	0.2960	59,950	61,300	59,050	59,950	1.02	60,450	0.8
	20126-15	0.0502	3,240	3,660	3,240	3,360	1.04	3,370	1.2
	20121-9	-	-	-	-	-	1.00	162	1.6

Analysis details

Results of polymer characterisation by gel permeation chromatography using tetrahydrofuran as eluent at a flow rate of 1.0ml/min, PLgel GPC columns selected appropriate to the molecular weight of the polymer. Each individual polymer has been characterised using methods referred to in DIN 55672.

Light Scattering (LS) was performed on the precision detectors Inc PD2020SA light scattering detector, in conjunction with a GPC/SEC workstation using a refractive index detector. Molecular weight was determined using the batch calculation mode. The analysis was undertaken using: tetrahydrofuran as an eluent with a dn/dc of 0.185g/ml.
[¥]Light Scattering (LS) was performed on the PL-LSP spectrophotometer at 633nm, the reference value used for the Rayleigh ratio was 14.02×10^{-6} cm^{-1}. The analysis was undertaken in toluene at 25°C with a dn/dc value of 0.1065g/ml.

Viscometry (IV, Mv) was performed on a Schott-Gerate viscometry system using an Ubbelohde capillary viscometer. The analysis was undertaken in toluene at 30°C. A double extrapolation of the Huggins-Kraemer plot gives the intrinsic viscosity, the viscosity average molecular weight was then determined using the Mark-Houwink constants.

The above characterisation data has been measured according to
Varian, Inc. Quality Assurance procedures.
Certificate of Analysis valid until expiry date – 8th September 2013

N.W.Titley — G.K.Harmer
Quality Department

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Calibration Methods for Conventional GPC

Aim : to produce a mathematical model for log M versus retention time

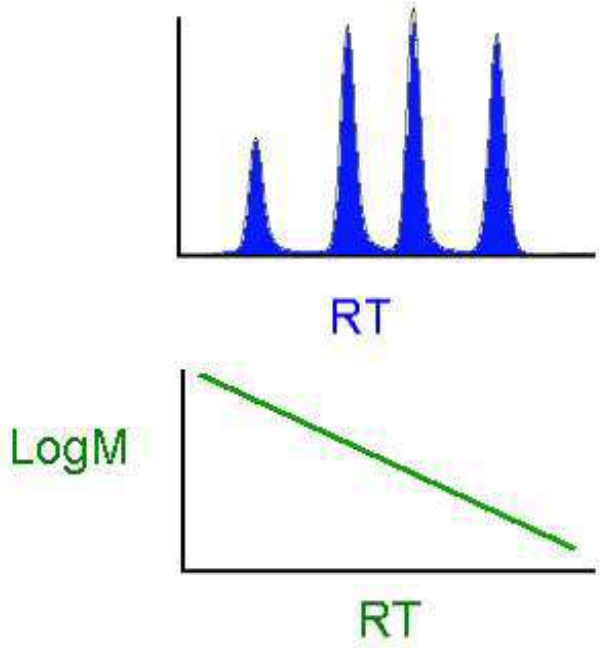
Narrow standards

Broad standards
(rarely used now)

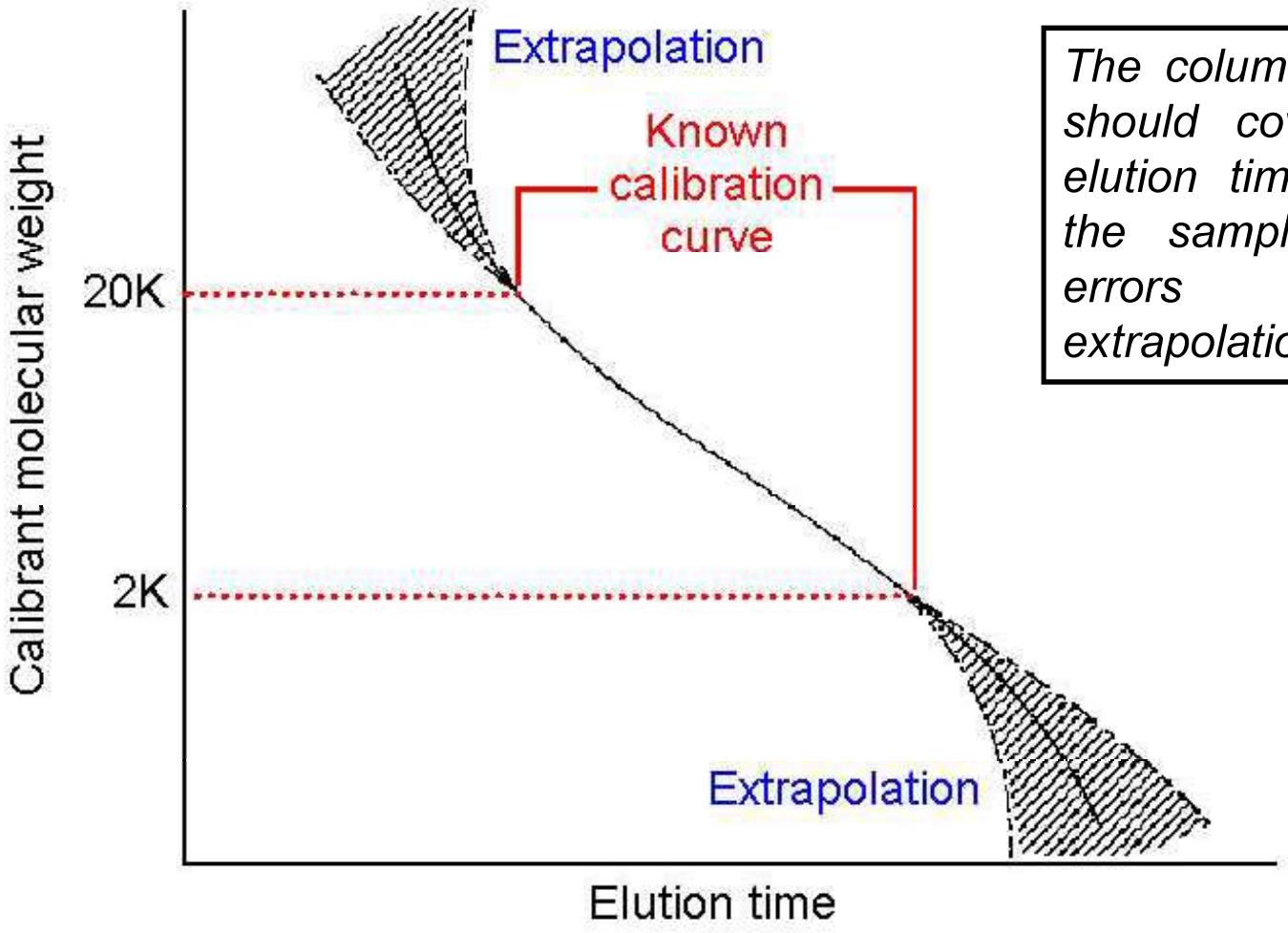
- Hamielec
- Broad on Narrow
- Integral

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



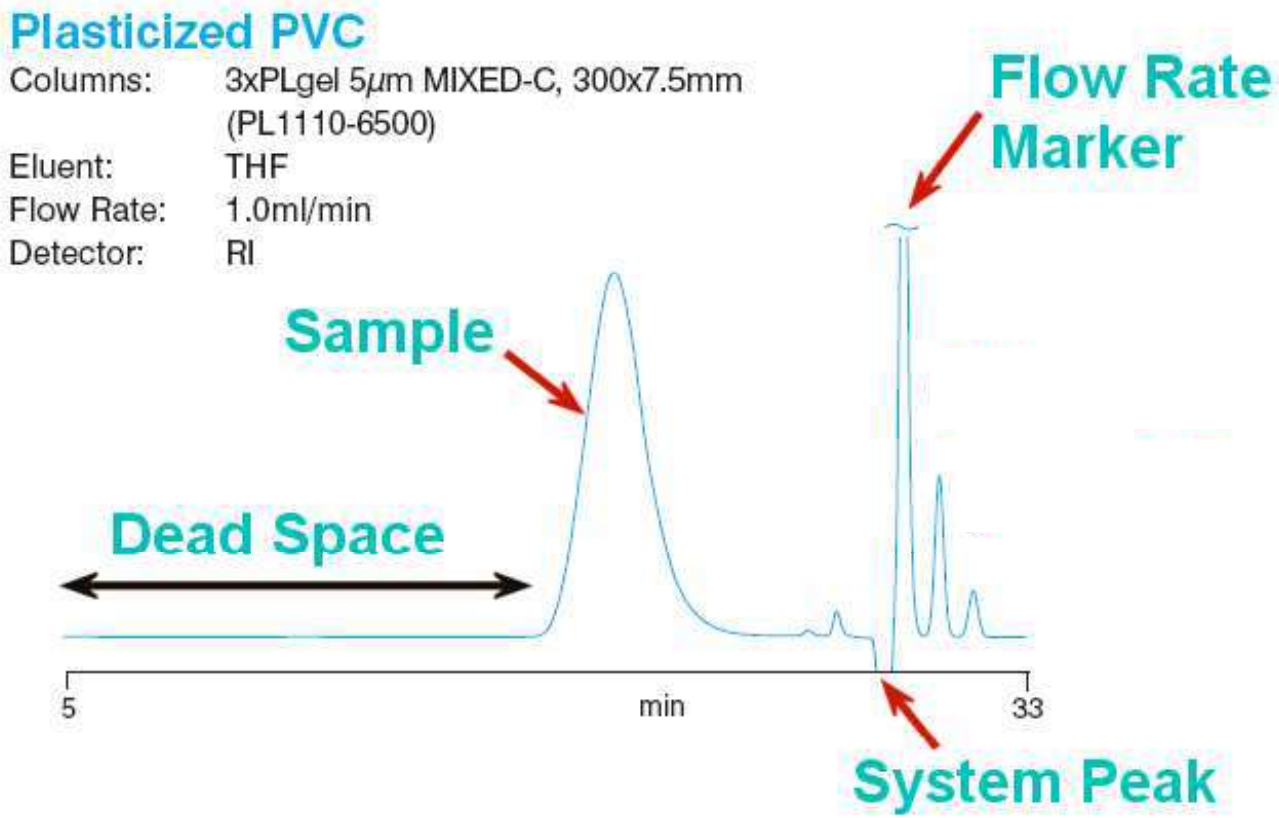
Errors Due to Limited Calibration Region



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

Interpreting Chromatograms

- The data obtained in a GPC experiment will be in the form of a chromatogram showing detector response as a function of retention time
- There are fundamental parameters that are present on all chromatograms

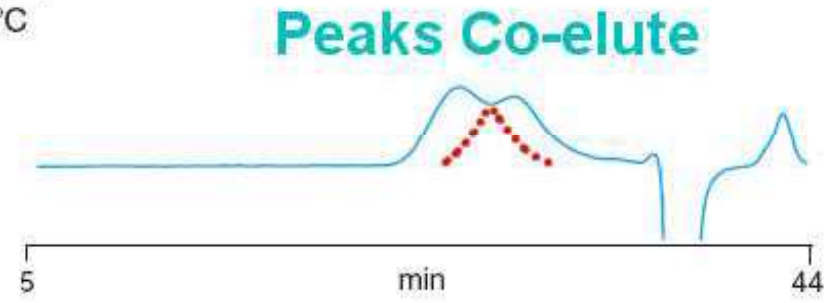


Peak Separation

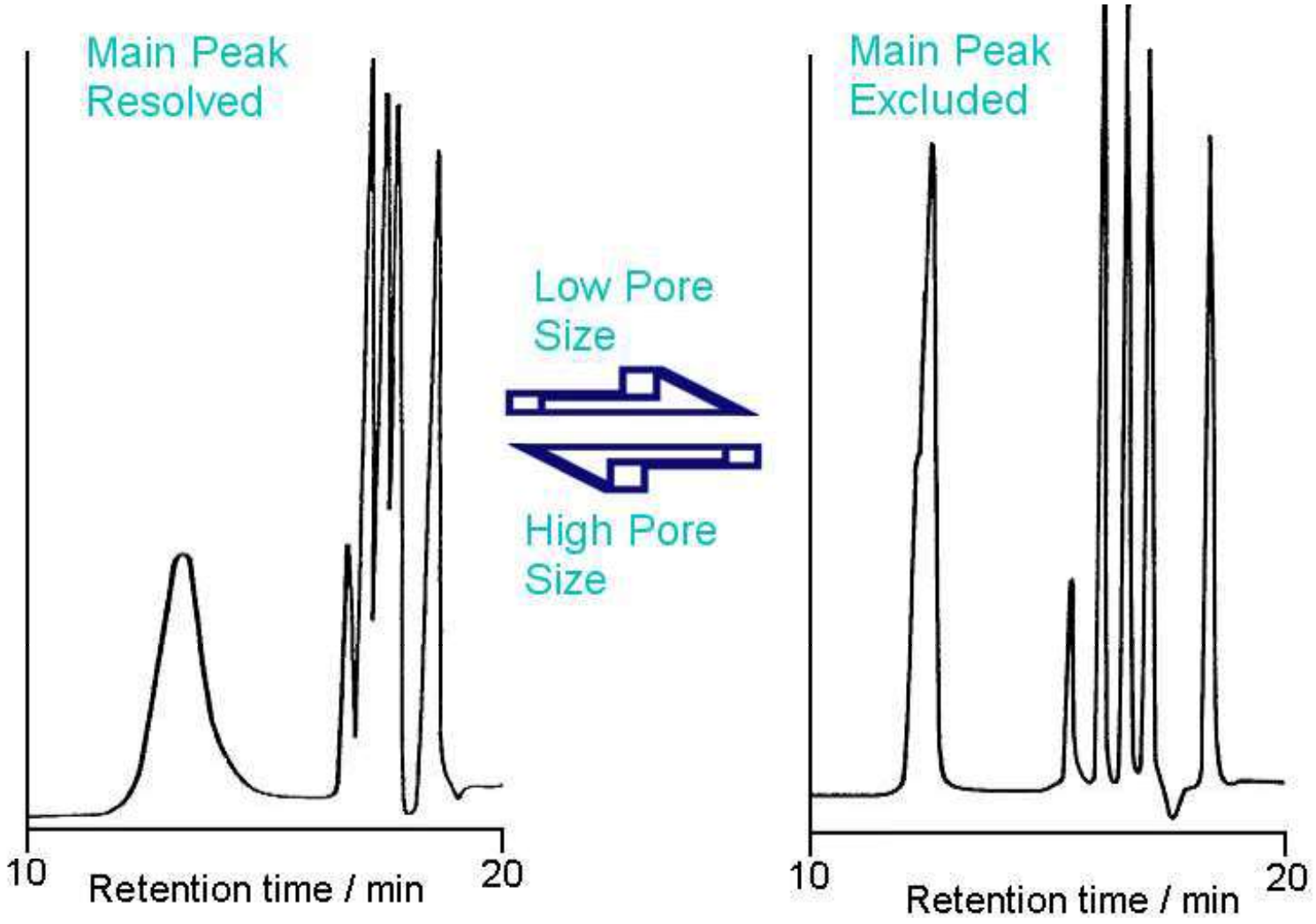
- Peak separation in GPC is dependent upon resolution and on molecular size
- If two samples have different molecular sizes, then they will be separated to baseline assuming there is sufficient resolution
- However, if samples are the same molecular size, then they cannot be separated by GPC as the mechanism of SEC is based upon size

Starch

Columns:	4xPLgel 20μm MIXED-A, 300x7.5mm (PL1110-6200)
Eluent:	DMSO + 5mM $NaNO_3$
Flow Rate:	1.0ml/min
Temp:	80°C
Detector:	RI

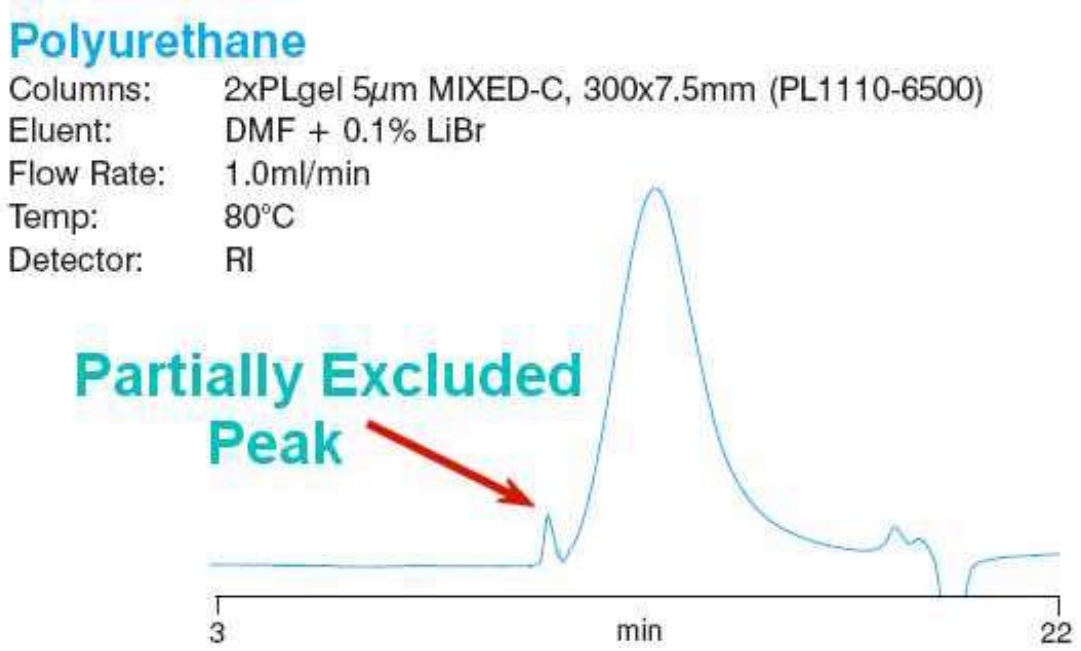


Excluded Peaks



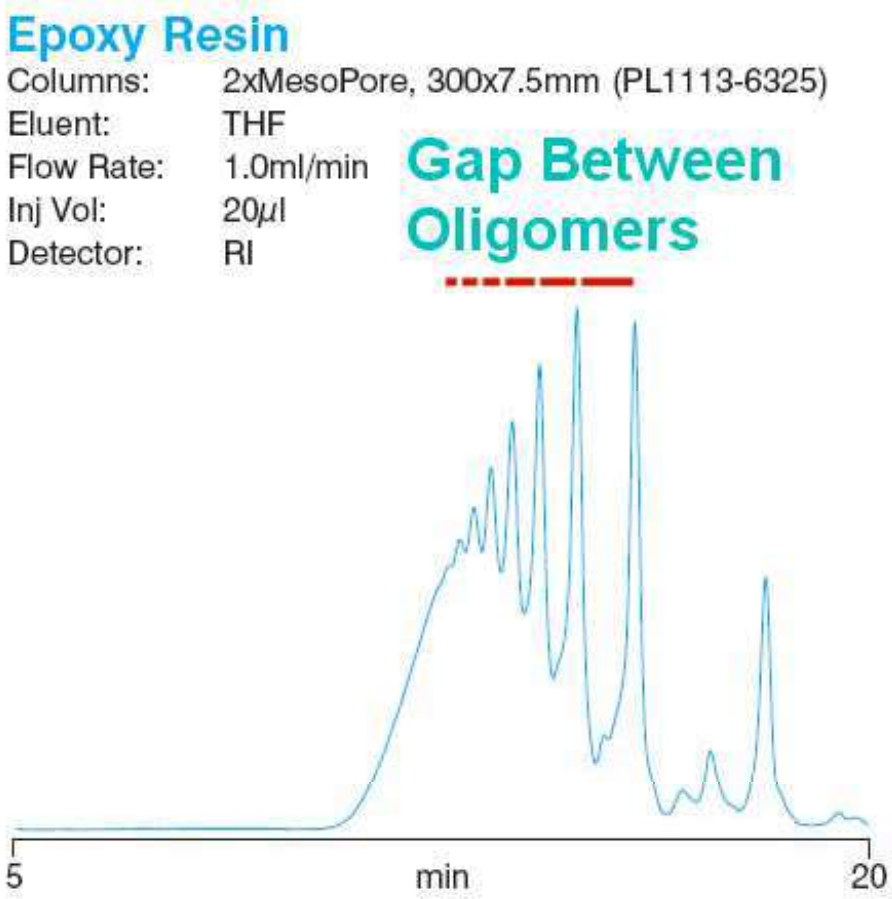
Partial Exclusion

- The dead space of the separation will be around half of the total elution volume
- Peaks eluting close to this volume may be partially excluded
- Look for sharp peaks at the front of your chromatograms



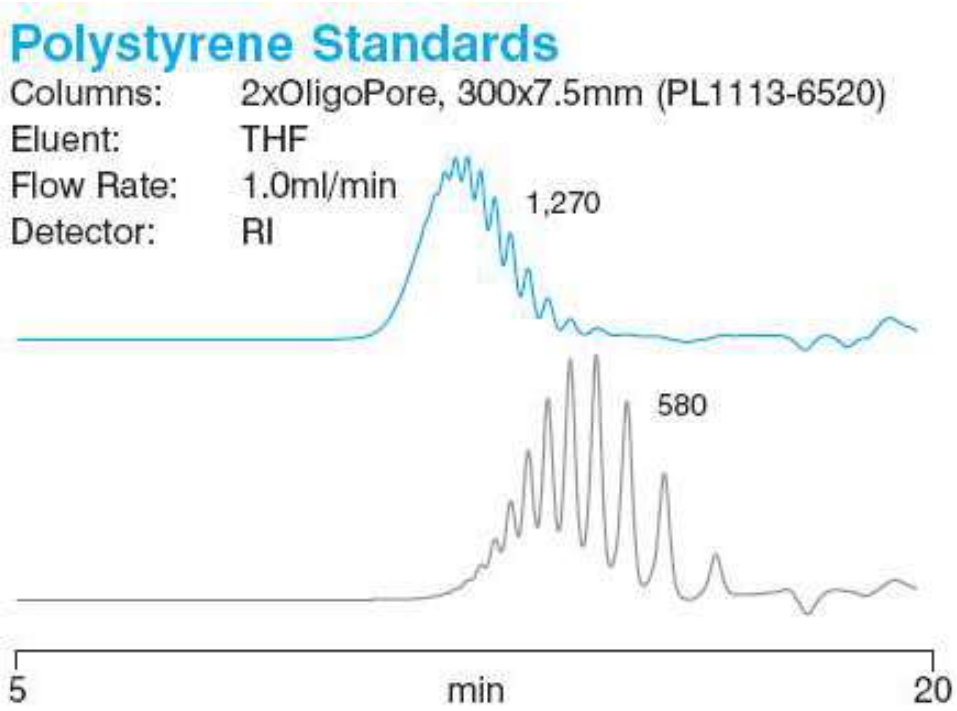
Oligomeric Resolution

- In GPC, the relationship between molecular weight and retention time is logarithmic
- As a result, peaks equidistant in molecular weight elute closer together with increasing molecular weight
- This is a classic way to tell a separation is based on SEC

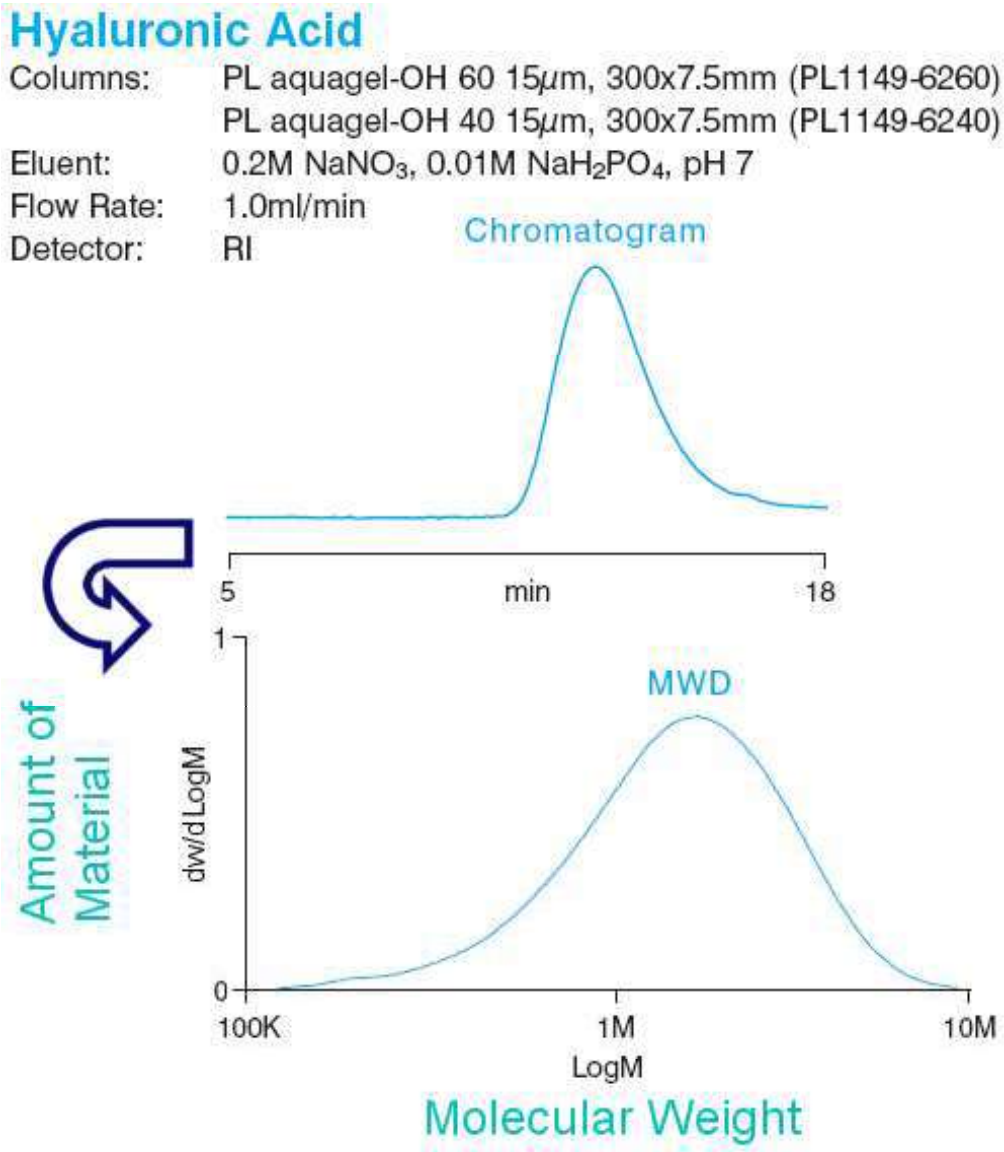


Oligomeric Resolution

- With some columns it is possible to calibrate the column using the oligomers
- The molecular weights of the initiator fragment and the repeat unit of the polymer must be known



Interpreting Molecular Weight Distributions

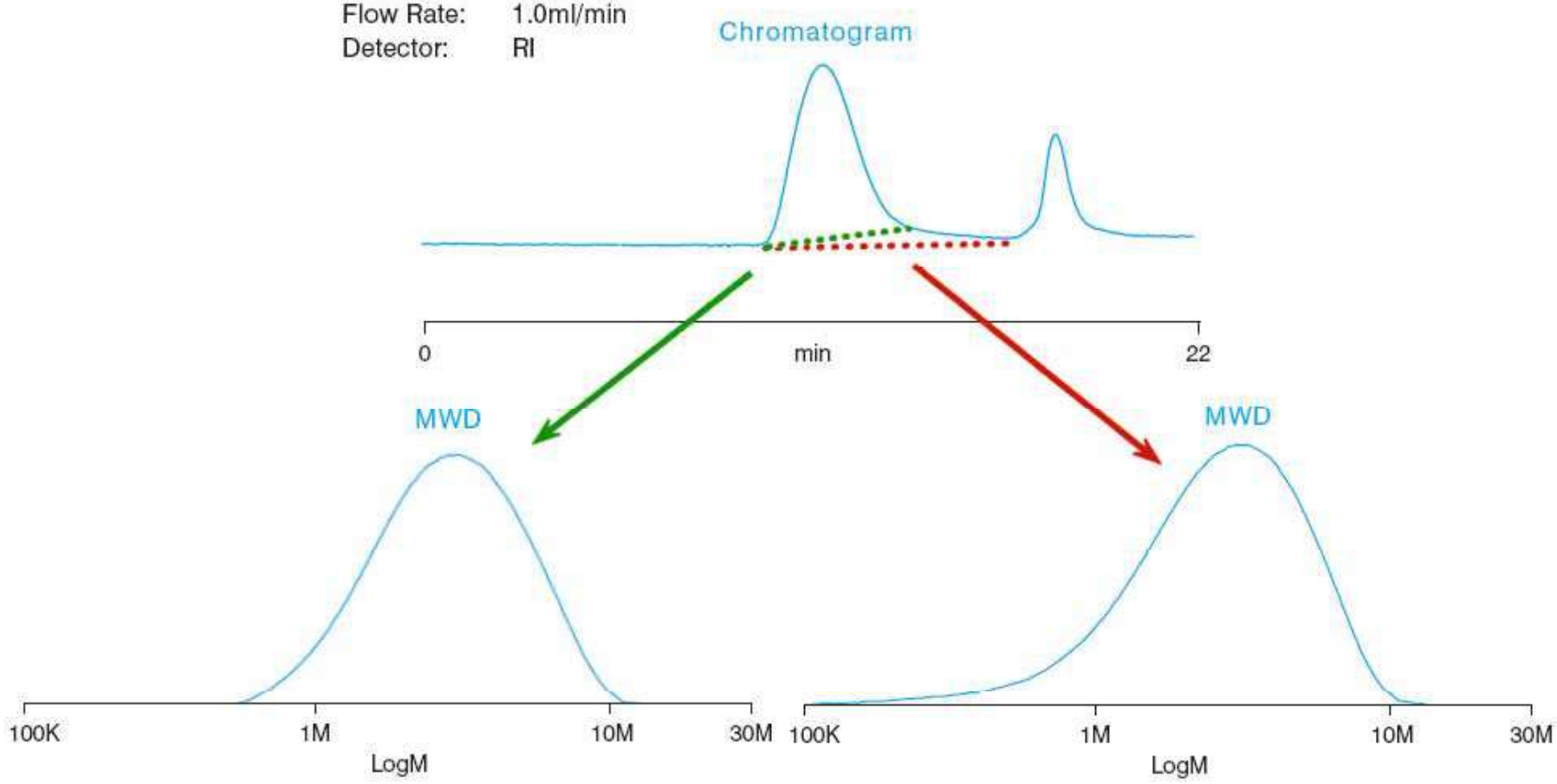


- The molecular weight distribution shows the amount of material present as a function of the molecular weight
- The MWD looks a bit like a 'mirror image' of the chromatogram

Effect of Baseline Position

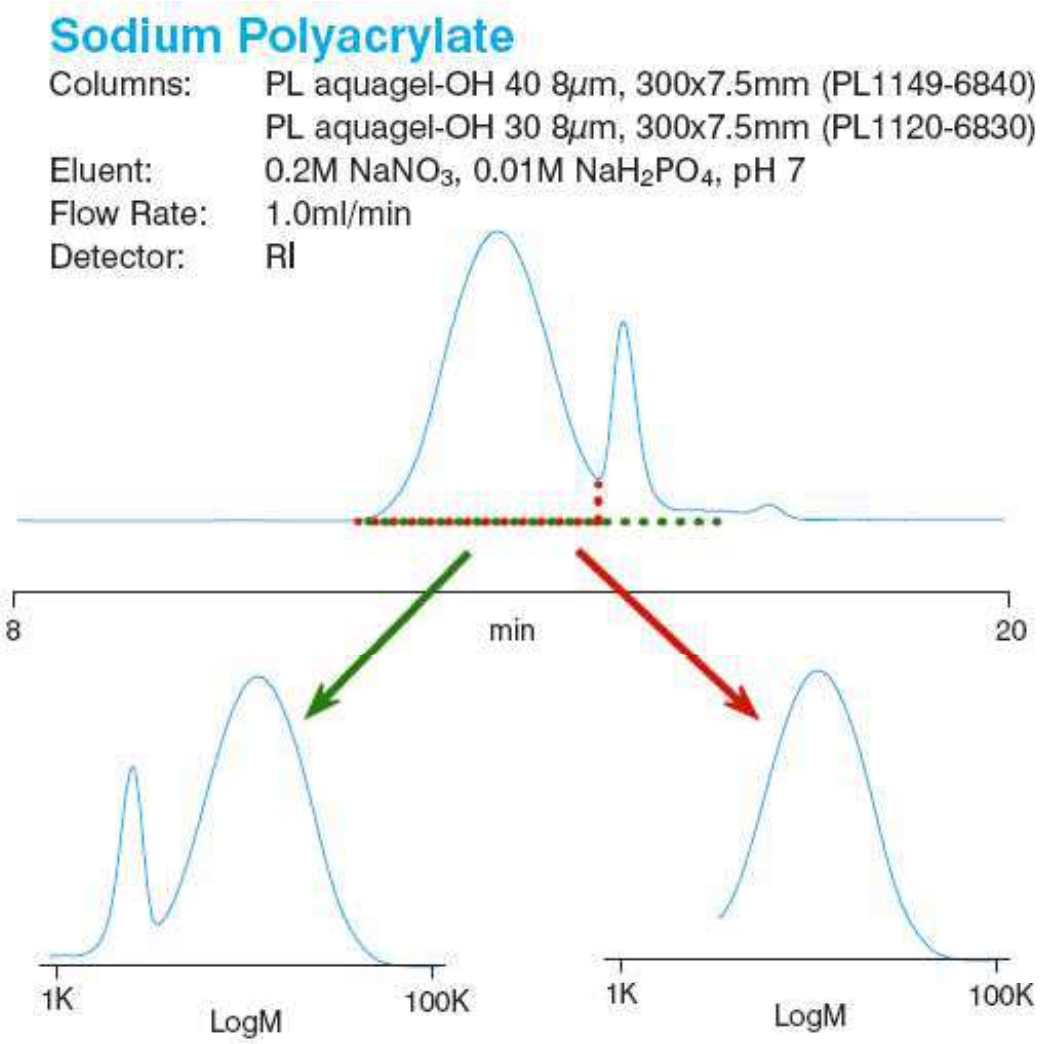
Polyacrylamide

Columns: PL aquagel-OH 60 15μm, 300x7.5mm (PL1149-6260)
PL aquagel-OH 40 15μm, 300x7.5mm (PL1149-6240)
Eluent: 0.2M $NaNO_3$, 0.01M NaH_2PO_4, pH 7
Flow Rate: 1.0ml/min
Detector: RI



Effect of Baseline Position

- The whole peak should be analysed to get a true reflection of the sample
- The peak should go down to the baseline on either side
- Leaving out components of the peak will leave an 'incomplete' MWD



Conventional GPC

Now lets take a look at ways we can improve the quality of our ‘Conventional GPC Analysis’