Conventional GPC

Polymers and Molecular Weight
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

\[
\begin{align*}
\left[ \text{HO-} \right]_n + \left[ \text{H}_2\text{N-}R'\text{-NH}_2 \right]_n & \rightarrow \left[ \text{H}_2\text{N-}R'\text{-NH}_2 \right]_n
\end{align*}
\]
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.
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

<table>
<thead>
<tr>
<th>Average</th>
<th>Mn</th>
<th>Mw</th>
<th>Mz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number average</td>
<td>Mn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight average</td>
<td>Mw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z average</td>
<td>Mz</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Mn** can be correlative with polymer colligative properties, e.g., freezing point depression.
- **Mw** may be correlated with properties such as melt viscosity.
- **Mz** may be correlated with properties such as toughness.
- **Polydispersity** characterises the shape of the distribution.

Polydispersity, \( d = \frac{M_w}{M_n} \)
Defining the Molecular Weight Distribution

A molecular weight distribution can be defined by a series of average values. Except $M_p$, these are various moments of the average of the molecular weights of the distribution.

$M_p$ is the molecular weight of the peak maxima.

For any polydisperse peak:

$$M_n < M_w < M_z < M_z + 1$$
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 an 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.

<table>
<thead>
<tr>
<th></th>
<th>Strength</th>
<th>Toughness</th>
<th>Brittleness</th>
<th>Melt viscosity</th>
<th>Chemical resistance</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increasing Mw</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Decreasing</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>distribution</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Increasing Mw:
- Strength
- Toughness
- Brittleness
- Melt viscosity
- Chemical resistance
- Solubility

Decreasing distribution:
- Strength
- Toughness
- Brittleness
- Melt viscosity
- Chemical resistance
- Solubility
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 $M_w$, osmometry measures $M_n$ and centrifugation measures $M_z$.

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

![Diagram showing molecular weight distribution and techniques]

- Small molecules
- Macromolecules
- HPGPC
- Traditional GPC
- HPLC
- GC

Molecular weight / g mol$^{-1}$: $10^1$ to $10^7$
There are many ways of measuring the molecular weight of a polymer however, *there is only one technique that measures the molecular weight distribution.*

- **Gel Permeation Chromatography, GPC** (Polymer Industry)

One technique but multiple acronyms, also known as

- **Size Exclusion Chromatography, SEC** (Academia)
  and
- **Gel Filtration Chromatography, 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

Liquid Chromatograph

GPC/SEC Column

Detectors, Data Acquisition and Processing

Agilent Technologies
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

2 PHASE SYSTEM
- Aqueous Water Surfactants
- Organic Styrene DVB Peroxide Diluents

MICROSPHERE FORMATION & FUSION
- Porous particle

PARTICLE SIZING
- Refine particle size distribution
  - 3µm
  - 5µm
  - 10µm
  - 20µm
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.

![Elution Profiles Diagram](image)
Typical Calibration Curves for PLgel Individual Pore Size Columns

Eluent: THF
Flow rate: 1.0 ml/min
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

Spatula A → EasiCal PS-1 separation on 3 x PLgel 10µm MIXED-B → Spatula B

Retention time / mins

8 12 16 20 24 28 32

8 12 16 20 24 28 32

Retention time / mins
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
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.

**Plasticized PVC**

Columns: 3xPLgel 5μm MIXED-C, 300x7.5mm (PL1110-6500)

Eluent: THF
Flow Rate: 1.0ml/min
Detector: RI

![Diagram of chromatogram with labels: Sample, Dead Space, Flow Rate Marker, System Peak]
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 NaNO₃
- Flow Rate: 1.0ml/min
- Temp: 80°C
- Detector: RI

**Peaks Co-elute**

![Chromatogram of starch with peaks co-eluting](image_url)
Excluded Peaks

Main Peak Resolved

Main Peak Excluded

Low Pore Size

High Pore Size

Retention time / min
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
• 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

Hyaluronic Acid

Columns: PL aquagel-OH 60 15μm, 300x7.5mm (PL1149-6260)
         PL aquagel-OH 40 15μm, 300x7.5mm (PL1149-6240)
Eluent:  0.2M NaNO₃, 0.01M NaH₂PO₄, pH 7
Flow Rate: 1.0ml/min
Detector: RI

• 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 NaNO₃, 0.01M NaH₂PO₄, pH 7
Flow Rate: 1.0ml/min
Detector: RI

Chromatogram

LogM  100K  1M  10M  30M  100K  1M  10M  30M
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 let's take a look at ways we can improve the quality of our ‘Conventional GPC Analysis’