A guide to multi-detector
gel permeation
chromatography

Agilent Technologies

Contents

Introduction

This guide follows on from An Introduction to Gel Permeation Chromatography and Size Exclusion Chromatography, also published by Agilent Technologies.

The previous booklet discussed the development, theory, and practice of gel permeation chromatography (GPC) and size exclusion chromatography (SEC) using a single detector, commonly a differential refractive index detector. This is generally referred to as conventional GPC/SEC. In this guide, we expand the GPC/SEC experiment by using additional detectors to deliver multi-detector or triple detection GPC/SEC.
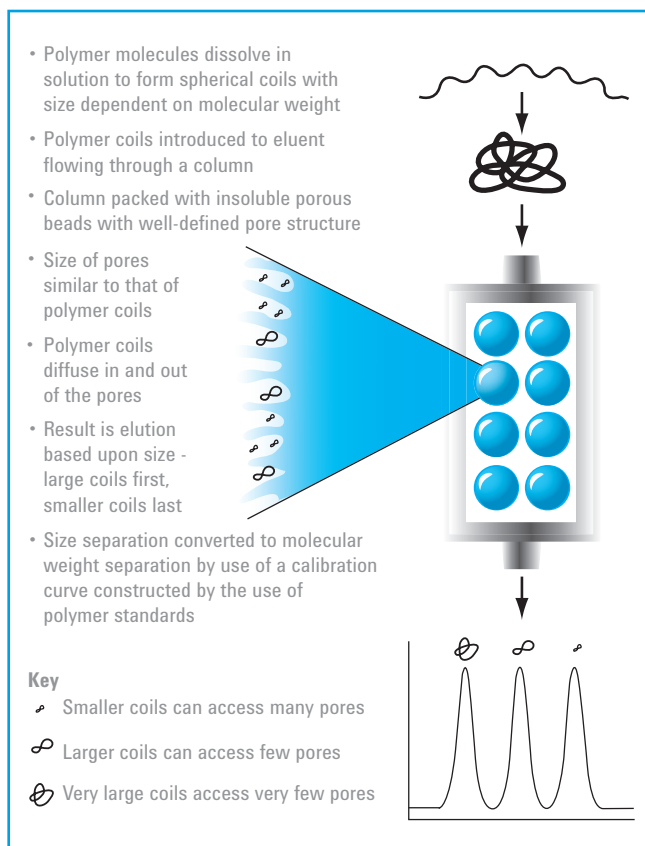
In this booklet, the terms GPC and SEC are used to describe the same chromatography process with the different acronyms being used by different industries, but generally speaking when analysts discuss GPC and SEC they are referring to the same type of chromatographic analysis. The International Union of Pure and Applied Chemists (IUPAC) prefer the term SEC for experiments of this type, but GPC is still in common use.

Conventional GPC with a single concentration detector such as a refractive index detector, and using a calibration curve generated from narrow molecular weight polymer standards, is a fairly simple technique. However, multi-detector GPC is far more complex and at its most fundamental involves a large amount of mathematics. Here we provide a general overview of the techniques of multi-detector GPC. A list of references at the back of the guide offers further reading for those requiring a more comprehensive discussion of these techniques.

A note on names

In this guide we use the term GPC to cover GPC and SEC. 'Conventional GPC' describes any GPC experiment that employs just a single concentration detector, where molecular weights are calculated with reference to a calibration of log molecular weight against retention time. 'Multi-detector GPC' is used throughout the guide to describe a GPC experiment that uses a concentration detector along with either a viscometer, a light scattering detector, or in the case of triple detection, both. We do not use the term to describe a GPC experiment that employs more than one concentration detector.

For convenience the GPC/SEC mechanism is summarized below.

- Polymer molecules dissolve in solution to form spherical coils with size dependent on molecular weight
- Polymer coils introduced to eluent flowing through a column
- Column packed with insoluble porous beads with well-defined pore structure
- Size of pores similar to that of polymer coils
- Polymer coils diffuse in and out of the pores
- Result is elution based upon size - large coils first, smaller coils last
- Size separation converted to molecular weight separation by use of a calibration curve constructed by the use of polymer standards

Key

- Smaller coils can access many pores
- Larger coils can access few pores
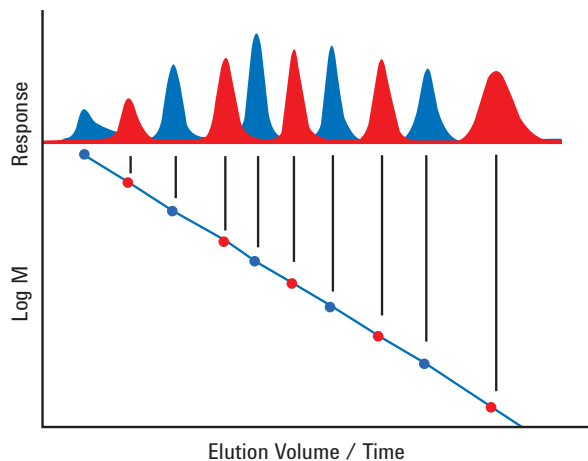- Very large coils access very few pores

Why do multi-detector GPC/SEC?

To address this question we need to first understand the limitations of conventional GPC/SEC for the analysis of polymer molecular weights.
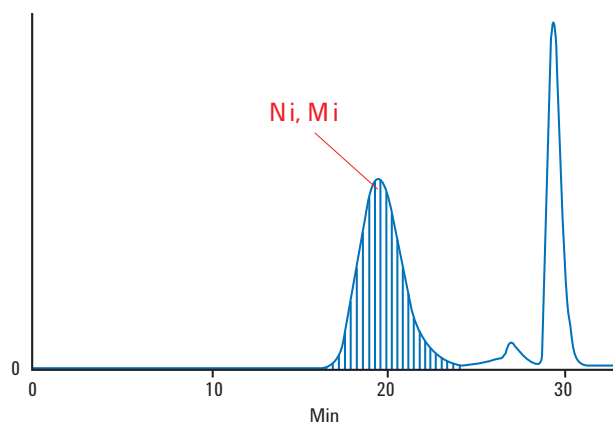
Conventional GPC with a single concentration detector

The detector in conventional GPC is typically a differential refractive index detector, but could also be a UV or an evaporative light scattering detector. During the analysis, the GPC column separates the polymer molecules on the basis of their size in solution, and the detector determines the amount of material eluting from the column as a function of retention time. This is converted to molecular weight by the use of a calibration curve – a graph that relates retention time (time of elution from the column) to the molecular weight of the polymer. The calibration curve is itself generated by the elution behavior of a series of polymer standards of known molecular weight. During the calculations the peak for the sample eluting from the column is divided into 'slices', and the retention time of each slice is used to determine its molecular weight by reference to the calibration curve. The area or height of the slice allows the percentage of the whole sample in the slice to be determined, and from the percentage and molecular weight data the molecular weight distribution is calculated.

Although very popular as an analytical tool for the determination of the molecular weight and molecular weight distribution of polymers, conventional GPC does have some limitations, as discussed overleaf.

Typical chromatograms for two mixtures of standards and resulting calibration curve

A peak sliced during GPC calculations

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A comparative technique

Conventional GPC employing a single concentration detector is actually a comparative technique. During the analysis the detector tells you how much material elutes from the column at any given time. That is then converted into a molecular weight and then into a molecular weight distribution by reference to a calibration curve of molecular weight as a function of retention time. If the standards used in the calibration and the sample are of the same chemistry, then accurate molecular weights are obtained. However, if the standards and the sample vary chemically, the results are only comparative.
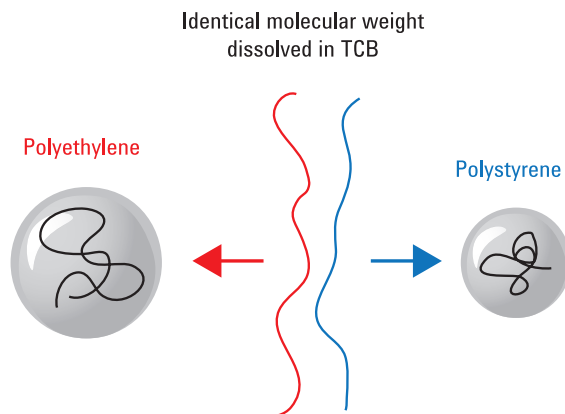
Why is this? Remember, a GPC column separates on the basis of size in solution, not molecular weight, as near-spherical polymer coils move down the column. When conventional GPC calculations are performed, it is assumed that a certain size in solution corresponds to a certain molecular weight as shown in the calibration, with the retention time being dependent on that molecular size. However, different types of polymers with different chemistries coil up in solution to form spheres of differing size. As a result, if the standards used to create the calibration are significantly chemically different to the samples under analysis, the size to molecular weight relationship for the standards and sample will be different and therefore the wrong molecular weight values will be calculated for the samples.

For example, in trichlorobenzene, polyethylene at any given molecular weight coils up to form a sphere that is a lot larger in solution than a polystyrene molecule of the same molecular weight.

As a consequence, if polystyrene standards are used to calibrate a conventional GPC system for the analysis of polyethylene, all the results calculated for the polyethylene samples will be too high as the molecule elutes from the column as if it were a polystyrene molecule of much higher molecular weight.

The correct molecular weight is only obtained from conventional GPC if the standards and samples are of the same chemistry. This is the reason for the wide variety of narrow standards available. Unfortunately, for the majority of users of GPC it is not possible (or economical) to obtain standards that match the chemistry of their own samples.

For some analysts, obtaining only a relative molecular weight distribution is adequate, as differences between batches of product can still be observed. However, for others the lack of accurate molecular weights can be a severe limitation of conventional GPC.

Comparison of sizes of two polymers in a common solvent

Information poor

Conventional GPC delivers relatively limited information. The column separates polymer molecules on the basis of their size in solution, and the detector determines the concentration of the molecules eluting from the column. No other information about the behavior of the polymers in solution is revealed, and their size, although crucial to the separation mechanism, is not measured in the analysis. Comparatively speaking, conventional GPC is not an information-rich technique.

What does multi-detector GPC/SEC provide?

Multi-detector GPC employing a concentration detector with a viscometer, a light scattering detector or both addresses the limitations of conventional GPC. Multi-detector GPC allows:

• the calculation of molecular weights that are not dependent on the chemistry of any standards used in calibration, and

• the determination of other polymer properties that cannot be measured by conventional GPC/SEC.

GPC and SEC with viscometry

The most common form of multi-detector GPC employs a concentration detector with a viscometer. The viscometer measures the solution viscosity of materials as they elute from the column. Combining this with concentration information from the other detector provides significant insight about the behavior of polymer molecules in solution.

What is a viscometer?

A viscometer used in GPC is a device for measuring the viscosity of a solution containing a polymer sample relative to viscosity of the solvent alone. In most standard designs this is done by forcing the solution and a reference solvent through narrow capillaries and measuring a pressure drop. This is converted to a viscosity by use of Poiseuille's Law, which relates the pressure drop across a capillary to the viscosity of the fluid in the capillary.

In standard fluid dynamics notation

$$\Delta P = \frac{128\mu L Q}{\pi\, d^4}$$

or

$$\Delta P = \frac{8\mu L Q}{\pi\, r^4}$$

where

ΔP is the pressure drop

L is the length of capillary

μ is the dynamic viscosity
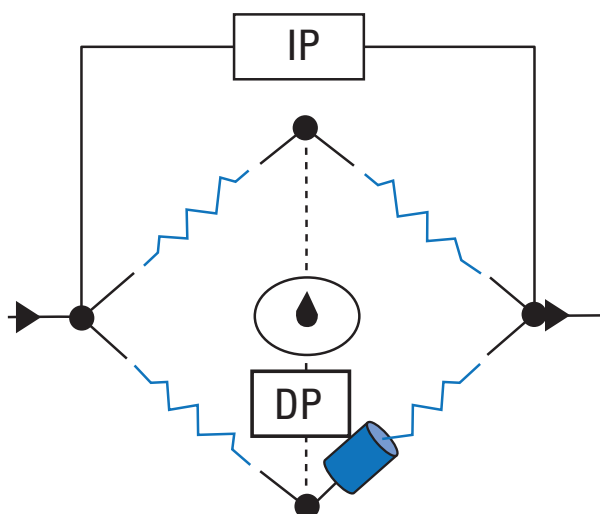
Q is the volumetric flow rate

r is the radius

d is the diameter, and

π is the mathematical constant.

The four-capillary bridge viscometer

The industrial standard design for viscometers for GPC is known as the four-capillary bridge design. This is a fluidic equivalent of the electrical circuit known as a Wheatstone bridge.

The viscometer consists of four linked capillaries in two flow paths, both of which branch from the fluid line out of the GPC columns. The two flow lines are independent but join after the two capillaries, to flow to waste. Both flow paths are identical except for the placement of a delay column after the first capillary on one of the flow paths. This is a large internal volume column packed with glass beads. A pressure is measured across the entire bridge known as the inlet pressure (IP). A second pressure measurement is made between the two flow paths, known as the differential pressure (DP).

During operation, flow from the column or other detectors reaches the split point and divides to move down the two paths, flowing equally through the first capillaries on each flow path. The sample then continues toward the second set of capillaries. However, this is where the asymmetry of the bridge comes into play. On one of the flow paths the sample moves into the next capillary, whereas on the second flow path the sample enters the hold-up column placed before the capillary and is delayed. In this way the bridge becomes unbalanced – on one side the sample is moving through a capillary, on the other side the capillary contains only solvent due to the sample being retained in the hold up column. This results in a pressure differential measured as the DP value, which is related to the viscosity of the sample. The IP and DP values are then used to measure the specific viscosity of the sample by a simple relationship.

$$\eta sp = 4DP/(IP-2DP)$$

The value measured by the viscometer is the specific viscosity of the sample. However, in GPC the concentrations of sample on-column are very low so this can be converted to the intrinsic viscosity

$$[\eta] = \eta sp \,/concentration$$

as the concentration tends to zero.
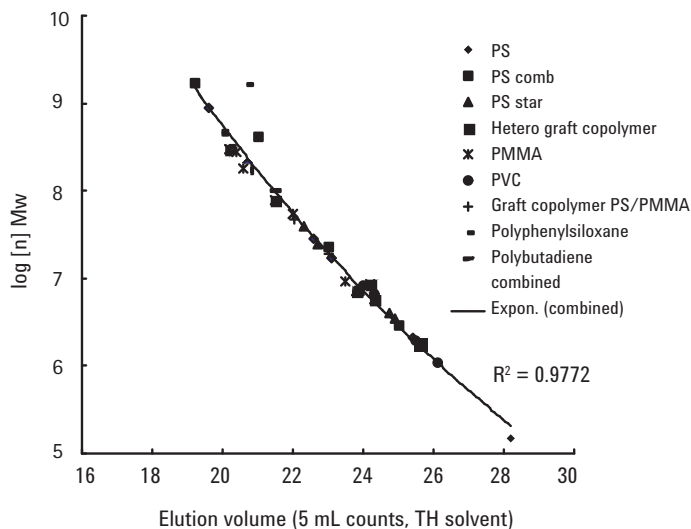

Instrument calibration

The first step in the analysis of any sample is the instrument or detector calibration. In this step, the response of the detectors is calibrated to a known property by the use of a narrow standard. In GPC with viscometry, we calibrate the response of the concentration detector to a known concentration and the viscometer to a known intrinsic viscosity. Any time delay between the detectors, known as the inter-detector delay (IDD), is also determined at this point.

Intrinsic viscosity in GPC and SEC

The benefit of measuring the intrinsic viscosity in GPC is that it allows the determination of molecular weights via the Universal Calibration. This is an approach that permits the calculation of accurate molecular weights regardless of the chemistry of the standards employed in the calibration.

The Universal Calibration makes use of the fact that the intrinsic viscosity and the molecular weight are related to the size of the molecules in solution by

hydrodynamic volume = k x molecular weight x intrinsic viscosity.

Example universal calibration

Hydrodynamic volume is a measure of molecular size and k is a constant. Therefore, if a calibration curve is generated for a set of standards plotting log (intrinsic viscosity x molecular weight) versus retention time, that is equivalent to plotting log size vs. retention time. And since the column separates on the basis of size in solution, the same calibration curve will be generated regardless of the standards employed – a Universal Calibration, as shown above.

The result is that any calibrants can be used to generate the calibration, as long as they perform pure SEC in the column and are not retained due to interactions.

When analyzing samples, the concentration and specific viscosity of a slice of a peak are determined from the response of the concentration detector and viscometer, respectively. The specific viscosity is converted into intrinsic viscosity, and this value along with the retention time is compared to the calibration curve to generate a true molecular weight for the sample.

The Mark-Houwink plot

The use of a viscometer also allows the assessment of the Kuhn-Mark-Houwink-Sakurada plot (often referred to as just the Mark-Houwink plot). This is a plot of the relationship between molecular weight and intrinsic viscosity, which is generally of the form

$$[\eta] = K M^{\alpha}$$

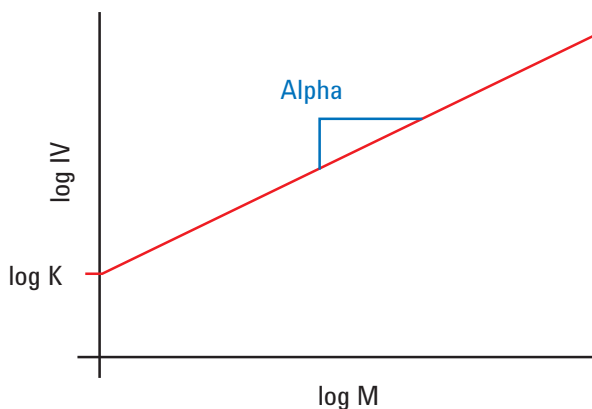where $[\eta]$ is intrinsic viscosity, M is molecular weight and α and K are the Mark-Houwink constants.

Rearranging this into a log form gives

$$\log [\eta] = \log K + \alpha \log M.$$

Plotting this relationship gives a straight line with intercept of log K and a slope of α.

This is a useful relationship because the values of K and α are constant for a given solvent and polymer combination, and because the value of α gives information about the dynamic behavior of the polymer molecules in solution. For most polymers the value of α ranges between 0.5 and 0.8, with lower values indicating a more compact dense structure (following the 'hard sphere' model), 0.7-0.8 being expected for a random coil polymer in a good solvent, and increasing values indicating a larger, more solvated structure (the limiting value of 2.0 is indicative of the 'rigid rod' model, where most of the components of the molecule are far from the centre of gravity, similar to that expected for a spinning rod structure in solution).

The Mark-Houwink relationship is thus an excellent way of probing the solution behavior of polymer molecules.

Viscometry in practice

Viscometry is a relatively simple technique to perform. Solvents and samples do not need to be prepared in any special way, as long as the instrument is purged correctly. Viscometers will generally operate well under most conditions without special attention. However, care must be taken when transferring viscometers into different solvents, especially viscous solvents where flushing at vastly reduced flow rates may be required.

GPC with viscometry works well for most samples of appreciable molecular weight, and can be used to analyze copolymers and other blends as long as concentrations are accurately known.
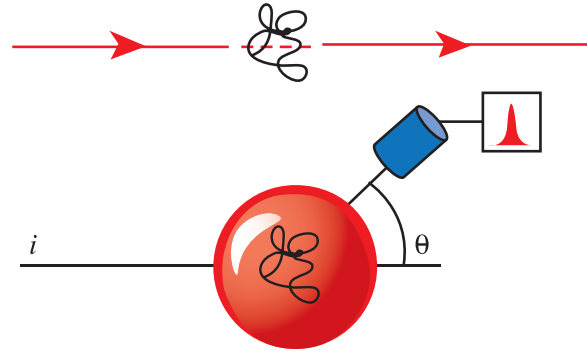
Viscometry summary

GPC employing viscometry is an excellent technique for measuring molecular weights of samples that are not the same chemistry as the standards available. It also works well for copolymers and can be used to probe the behavior of polymer molecules in solution through the Mark-Houwink plot.

α = <0.5 for 'hard sphere'
α = 0.7 for random coil in good solvent
α = ~2.0 for 'rigid rod'

Example Mark-Houwink plot

GPC and SEC with light scattering and triple detection

Static light scattering in GPC involves irradiating the material eluting from a column with a laser beam and measuring the intensity of scattered light that results. A more complex technique than viscometry, static light scattering does have some practical limitations. However, the combination of a GPC separation with a concentration detector and a light scattering detector can reveal a great deal of information about polymer molecules in solution.

The light scattering experiment

The confusing world of light scattering

Light scattering is used to describe any detector that relies on the scattering of radiation to perform a measurement. Unfortunately for the analyst new to this area, there are many very different detectors that at their heart measure a fundamental scattering of light, including such instruments as static light scattering detectors, dynamic light scattering detectors and evaporative light scattering detectors. Although they share a name, these instruments are actually performing very different measurements and are used for different types of analysis. When we use the term here of 'light scattering' we are referring to static or classical light scattering, also known as Rayleigh light scattering.

What is a static light scattering detector?

A static light scattering detector comprises a sample cell, a laser beam, and one or more detectors to collect the scattered laser light. The detectors are set at some angle to the incident beam depending on the design of the detector.

During operation the laser beam irradiates the sample and the detector collects the resulting scattered light from relaxation of the molecule. The intensity of scattered light is measured as the Raleigh ratio Rθ, the excess scattering of the sample and solvent combination over that of the solvent alone. The Raleigh ratio is directly proportional to the molecular weight of the solute molecule scattering the light.

The equation that relates Rθ is complex and takes the following form.

$$\underbrace{}_{\substack{P_q \text{, the} \\ \text{scattering function}}} \qquad \underbrace{}_{\substack{\text{Virial} \\ \text{expansion}}}$$

$$\frac{K^*c}{R_\theta} = \left(\frac{1}{Mw}\right) P_\theta \qquad + 2A_2c + 3A_3c^2 + \dots$$

$$\text{Where } K^* = 4\pi^2 n_0^2 \left(\frac{dn}{dc}\right)^2 \lambda_0^{-4} N^{-1} = K\left(\frac{dn}{dc}\right)^2$$

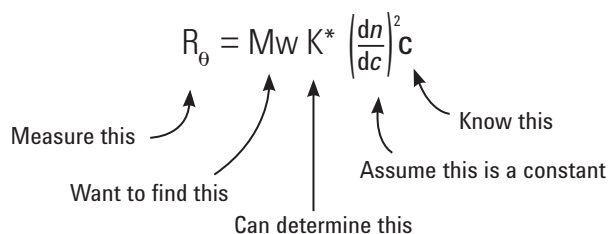$$P_\theta = \left(\frac{16\pi^2}{3\lambda^2}\right) <s^2>_z \sin^2\left(\frac{\theta}{2}\right)$$

Mw is the weight average molecular weight, λ the wavelength of the light, θ the angle of collection, $<S^2>$ the root-mean-square end-to-end distance of the polymer, n_0 the refractive index of the solvent, N is Avogadro's number and (dn/dc) the refractive index increment. A_2 and A_3 are second and third virial coefficients.

The form of this equation can be greatly simplified. For any polymer and solvent combination the refractive index increment (dn/dc) is considered a constant. The virial expansion relates to intermolecular interactions and under the dilute conditions of GPC can be considered zero. Pθ, the scattering function, is equal to one if the molecule is below a certain fundamental size of around 10 nm. Under these conditions the equation simplifies to

$$R_q = Mw \, K^* c$$

where $K^* = (dn/dc)^2 K$.

So the response from the detector is directly proportional to the molecular weight, a constant K, and the concentration.

$$R_\theta = Mw \, K^* \left(\frac{dn}{dc}\right)^2 c$$

Measure this ⟵ R_θ

Want to find this ⟵ Mw

Can determine this ⟵ c

Assume this is a constant ⟵ K^*

Know this ⟶ $\left(\frac{dn}{dc}\right)^2$

It is clear from this equation that the value of dn/dc is of vital importance in light scattering calculations. In addition, it is evident that the response is directly proportional to the molecular weight, so low-molecular-weight samples or low dn/dc sample and solvent combinations will lead to poor light-scattering data.

Refractive index increment - dn/dc

The refractive index increment dn/dc is the degree by which the refractive index of a solution varies as the concentration of solute changes. The refractive index increment is a very important term in light scattering calculations as the intensity of scattered light is directly proportional to the square of dn/dc. Generally, the dn/dc of a polymer and solvent combination is considered a constant (although it may vary at low molecular weights). Good quality light scattering data is generally only obtained in solvents where the dn/dc is an appreciable value. It is possible for the dn/dc to be zero (in which case no light scattering response will be seen) or negative.
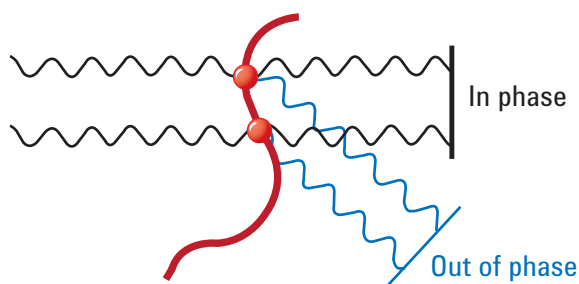
Polymer	Solvent	Refractive index increment dn/dc (g/mL)
Epoxy resin	Tetrahydrofuran	0.1869
Polybutadiene	Tetrahydrofuran	0.1295
Polycarbonate	Tetrahydrofuran	0.1855
Polymethylmethacrylate	Tetrahydrofuran	0.0830
Polystyrene	Tetrahydrofuran	0.1845
Polyvinyl chloride	Tetrahydrofuran	0.1010
Polyurethane	Tetrahydrofuran	0.1469
Dextran	Water buffers	0.1470
Hydroxyethyl starch	Water buffers	0.1354
Polyacrylamide	Water buffers	0.1420
Polyethylene oxide	Water buffers	0.1350

Source: American Polymer Standards Corporation (APS)

During light scattering calculations it is assumed that the value of dn/dc remains a constant across the sample. Light scattering is therefore not a good technique for analyzing copolymers or blends where the composition of the sample and therefore the dn/dc may vary across the molecular weight distribution.
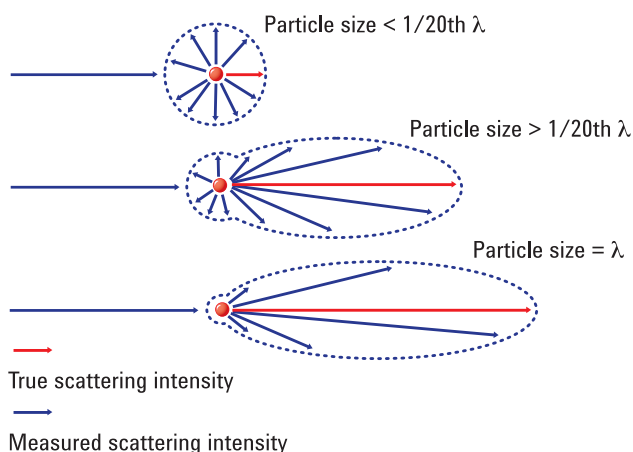
Angular dependency of light scattering

The intensity of scattered light is not necessarily equal in all directions. When the molecule under investigation is small, less than 1/20 the wavelength of the incident laser beam, the molecule acts as a point and scatters light equally in all directions. However, as the molecule grows in size relative to the incident laser it begins to act as a collection of points each individually scattering light. As a result, destructive interference reduces the amount of scattered light at the higher angles, causing Pθ, the scattering function, to drop below one.

Interference in light scattering of large molecules

As a result of the reduction in scattering intensity, molecular weights are underestimated at high angles as the molecule under investigation increases with size.

Particle size < 1/20th λ

Particle size > 1/20th λ

Particle size = λ

True scattering intensity

Measured scattering intensity

Scattering volumes of different size molecules

This is known as dissymmetry. The true intensity regardless of molecular size can only be obtained at the zero angle (that is at the angle of incidence), but this cannot be measured due to the presence of the laser beam, the majority of which is not scattered by the molecule. There are various ways of addressing dissymmetry to obtain accurate molecular weights, as described below.

Dissymmetry and radius of gyration

One benefit of the dissymmetry affect is that if the scattered light is simultaneously recorded at more than one angle, then the reduction in scattering at higher angles compared to lower angles can be used to estimate the size of the molecule. The measured value is the radius of gyration Rg, the root-mean-square average distance of the components of the molecule from the centre of gravity. The value of Rg is typically the same order of magnitude as the hydrodynamic radius. In order for Rg to be obtained from light scattering, measurements are required at more than one angle and the molecule investigated must be large enough to create angular dissymmetry, which generally means it must have an Rg greater than 10 nm. This corresponds to a polystyrene molecule in a good solvent of Mw around 200,000 g/mol.

Analyzing light scattering data

There are many ways to analyze light scattering data, depending on the design of the instrument and the number of angles involved in the analysis. The analysis methods all try to address the issue that more accurate intensities and therefore molecular weights are obtained the closer the measurement angle is to the zero angle, but the greater the noise in the data. At higher angles the data is cleaner and therefore more precise, but due to dissymmetry the accuracy of the result can be lower.

The following approaches are commonly encountered in an attempt to address these issues.

Instrument calibration

The first step in the analysis of any samples is the instrument or detector calibration. The response of the detectors is calibrated to a known property by the use of a narrow standard. In GPC with light scattering, we calibrate the response of the concentration detector to a known concentration and the light scattering detector to a known molecular weight. Any time delay between the detectors, known as the inter-detector delay (IDD), is also determined at this point.

Right angle light scattering – RALS

This approach uses only a single detector positioned at 90 ° to the incident beam and effectively ignores the dissymmetry issue. Accurate molecular weights are obtained if the molecule creates no dissymmetry, but at large molecular sizes an increasing underestimation in molecular weight occurs. For example, the following data is for a 2,000,000 g/mol polystyrene standard in tetrahydrofuran (THF).
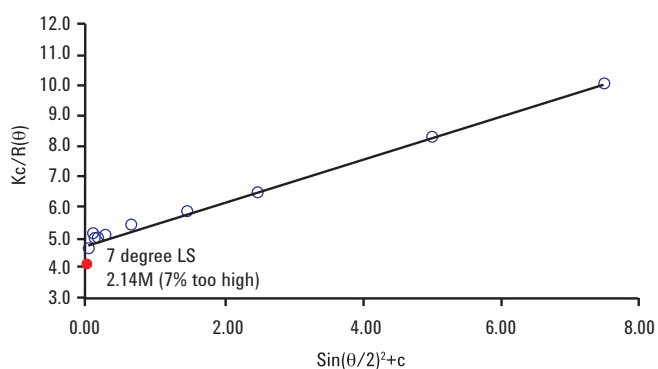
Measuring the intensity at 90 ° only (the red point), there is a considerable underestimation in the molecular weight due to dissymmetry.

Light scattering data for a 2,000,000 g/mol standard single angle analysis

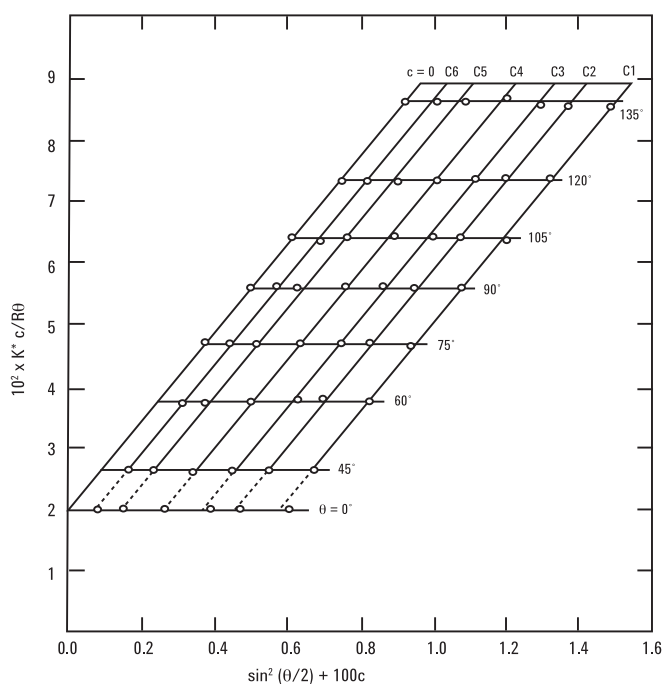### Low angle light scattering – LALS

This method involves collecting the light scattering data at as close to the zero angle as possible. In theory this gives the most accurate result, but this approach suffers from the poor precision of measurements at low angles due to the presence of the incident beam and the sensitivity of lower angles to dust and other contaminants. For example, the following data is for a 2,000,000 g/mol polystyrene standard in THF. Measuring the intensity at 7 ° only (the red point), there is a considerable error in the molecular weight due to the precision of the measurement.

Light scattering data for a 2,000,000 g/mol standard low angle analysis

Multi-angle light scattering –
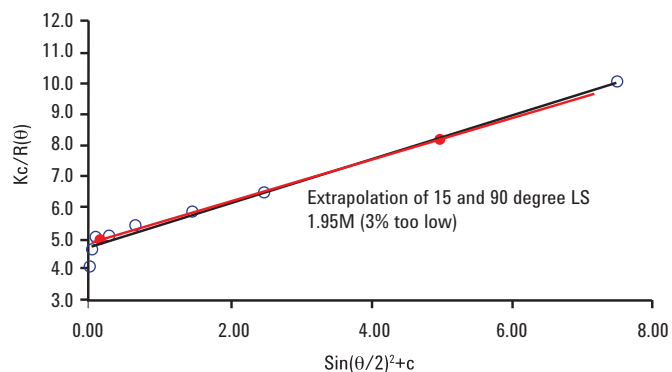## Zimm and partial Zimm approaches

The classical way of analyzing light scattering data is the 'batch' approach known as the Zimm method. This involves creating a plot of $K^*c/R\theta$ versus $\sin^2(\theta/2) + c$, and then performing a double extrapolation to zero angle and zero concentration. From this plot, the molecular weight is obtained from the intercept and the radius of gyration from the slope.

Typical Zimm plot

This method requires that measurements are made at many different angles and concentrations, and is therefore a batch calculation method. In a flow method it is not possible to measure the scattering at many concentrations, and so a partial Zimm analysis must be performed instead. In partial Zimm analysis multiple angles are used but only at a single concentration. The drawback of measurement at multiple angles can be that the flow cell in the system has to be quite large, which can lead to band broadening due to extra-column dispersion.

It is possible to perform the analysis using a partial Zimm method on any number of angles of data, as long as at least two are used, and in many cases accurate results can still be obtained for the sample. For example, the following data is for a 2,000,000 g/mol polystyrene standard in THF.
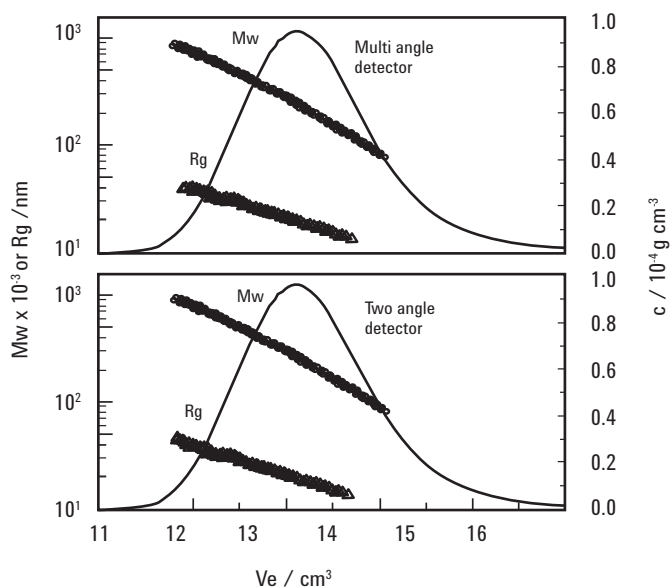
Measuring the intensity at many angles (the black line) gives a molecular weight of 2,000,000 g/mol. Measuring at only two angles, 15 ° and 90 ° (the red line) gives a molecular weight of 1,950,000 g/mol, which is within the chromatographic error normally associated with a GPC separation (about 3% for Mw measurements).

Light scattering data for a 2,000,000 g/mol standard dual angle extrapolation analysis

Multi-angle light scattering – the dissymmetry approach

The dissymmetry method is another way of analyzing dual-angle or three-angle data, where one of the angles is 90 °. In this approach, the responses from the different angles are compared and a ratio of intensities created. From this information the size of the molecule scattering the light is estimated, and a value for Pθ, the scattering function, is calculated. This is then used to correct the response from one of the angles, typically the 90° angle, to give the true intensity of scattered light.

In many cases the results from the dissymmetry method are very close to those obtained on a multi-angle system by the partial Zimm approach, providing that the angles are well spaced, that one is reasonably low and the data quality is good. The graph below for polystyrene in THF shows how closely the data matches between a multi-angle system and a two-angle system collecting at 15 ° and 90 °.

Comparison of partial Zimm and dual angle analysis

The conformation plot

In an analogous manner to the Mark-Houwink relationship in viscometry, a conformation plot of the relationship between molecular size (Rg) and molecular weight can be established using the following equation,

$$Rg = KM^{\nu}$$

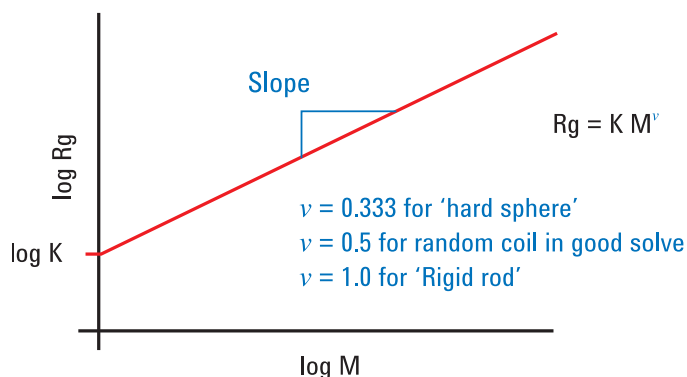where Rg is the radius of gyration, M is molecular weight and ν and K are constants.

Rearranging this into a log form gives

$$\log Rg = \log K + \nu \log M.$$

Plotting this relationship gives a straight line with intercept of logK and a slope of ν.

This is a useful relationship because the values of K and ν are constant for a given solvent and polymer combination, and because the value of ν provides information about the dynamic behavior of the polymer molecules in solution. The value of ν should range between 0.3 and 1.0, with lower values indicating a more compact dense structure (following the 'hard sphere' model), 0.5 being expected for a random coil polymer in a good solvent. Increasing values indicate a larger more solvated structure (the limiting value of 1.0 is indicative of the 'rigid rod' model, where most of the components of the molecule are far from the centre of gravity, similar to that expected for a spinning rod structure in solution).

The conformation plot is similar to the Mark-Houwink plot, although the value of ν differs from that of α as one is a size-related term and the other density related. Also, the conformation plot is less useful for samples with a reasonable number of chains below 10 nm in size, as no Rg measurements can be made by static light scattering in this region due to the lack of angular dissymmetry. However, both plots are an excellent way of probing the solution behavior of polymer molecules.

Example conformation plot

Triple detection

Triple detection is the name given to the GPC/SEC analysis of polymers employing a concentration detector, a viscometer and a light scattering detector. In this approach, the molecular weights are calculated as in GPC with light scattering, but intrinsic viscosity is also determined. The advantage of this method is that all the data is available, opening up the investigation of the sample by the Mark-Houwink plot or the conformation plot. It is thus possible to use the intrinsic viscosity to estimate the size of the molecule under analysis and therefore correct the calculated molecular weight. Triple detection is considered the most advanced form of multi-detector GPC.

Light scattering in practice

Light scattering requires a more stringent approach compared to other forms of GPC. Solvents and samples must be rigorously clean of dust and insoluble material, as these particulates will scatter light extremely strongly, especially at lower angles. Filtering of solvents through 0.02 µm filters is a requirement for obtaining good quality data.

Light scattering is particularly suited to higher molecular weight polymers and those combinations of polymer and solvent that give appreciable dn/dc values. In the calculations it is assumed that the dn/dc value remains constant across the elution volume of the polymer, therefore light scattering is most often applied to the analysis of homopolymers. The largest benefit of light scattering is that it provides molecular weights independent of a column calibration, and so obtaining data for analysis can be quick compared to other techniques.

Light scattering summary

GPC/SEC employing light scattering is an excellent technique for measuring molecular weights of samples, and does not require any column calibration. However, obtaining good quality light scattering data requires stringent laboratory practice and the response from the detector is very dependent on molecular weight and dn/dc. Due to the latter, light scattering does not work well for copolymers. However, for many samples GPC with light scattering can be used to probe the behavior of polymer molecules in solution through the conformation plot. Triple detection takes advantage of all the benefits of light scattering and viscometry, and is considered the most advanced form of multi detector GPC.

Polymer branching

In multi-detector GPC, branching is assessed by investigating changes in molecular size or intrinsic viscosity as a function of increasing molecular weight. In all cases for polymers of the same chemistry, branched molecules always have lower Rg and IV values than linear analogs due to the presence of branch points.

Branching calculations can be performed in all methods on either the intrinsic viscosity (measured or calculated) or radius of gyration (measured or calculated) data. The quality of the branching results will depend on the quality of the source data (intrinsic viscosity or radius of gyration). Contraction factors are determined from the Mark-Houwink plot (log intrinsic viscosity versus log MW) or conformation plot (log radius of gyration versus log MW) using the following relationships.

Radius of gyration contraction factor

Intrinsic viscosity contraction factor

$$g = \left(\frac{\text{Rg branched}}{\text{Rg linear}} \right)_{MW} \qquad g' = \left(\frac{\text{IV branched}}{\text{IV linear}} \right)$$

$$\text{where } g = g'^{\,(1/\varepsilon)}$$

ε (structure factor) = 0.5 - 1.5, typically 0.75

The value of g (directly or taken from the value of g′ and an estimation of the structure factor, typically 0.75) is used along with the branching repeat unit (the molecular weight of the monomer multiplied by 1,000) to obtain branching numbers using a branching model. An example is the number average ternary-branching model.

$$g = [(1+B_n/7)^{1/2} + 4B_n/9\pi]^{-1/2}$$

where n = branches per 1,000 carbons.

Branching numbers are expressed as number of branches per 1,000 carbons, due to the fact that many of these calculations were created for polyolefin samples that only contain carbon in the backbone. If the polymer in question is not polyethylene then the actual branching number may not be directly meaningful. However, comparison between samples is still possible.

Real-world applications with the Agilent 1260 Infinity Multi Detector Suite (MDS)

The 1260 Infinity MDS is an integrated detector suite designed as a simple add-on to any liquid chromatography system allowing all forms of GPC analysis to be performed. A versatile instrument with a range of detector options, the 1260 Infinity MDS is the ultimate tool for polymer analysis. Irrespective of your GPC requirements, the 1260 Infinity MDS can be tailored to meet your needs.

• If you have an existing LC, simply add the 1260 Infinity MDS with viscometer, light scattering detector or both to your current system, to completely characterize your polymers.

• If you have a range of different systems running a variety of solvents, the 1260 Infinity MDS is versatile and adaptable, and can be conveniently switched between systems as required.

• If you only require one detector, then the 1260 Infinity MDS can be configured to provide a standalone viscometer to obtain polymer structure information, or a light scattering detector for absolute molecular weight and branching studies.

For the complete system, simply combine the viscometer and the light scattering detector with the already existing RI detector, for a wealth of information and complete polymer characterization.

1260 Infinity Multi Detector Suite - Perform GPC/SEC with any LC System

Analysis of polystyrene stars by viscometry

There has been increasing interest in the synthesis of star-branched polymers due to their unusual flow and viscosity properties compared to linear analogs. Star-branched polymers are constructed with several 'arms' radiating from a central core, either by preparing the individual arms and attaching them to a central molecule - the 'arms first' approach - or by growing the polymer arms from a central core - the 'core first' approach. Many commercial polymers can be constructed with a star-branched morphology relatively easily, but their characterization is still a challenge to the analytical chemist. GPC employing a concentration detector (typically a refractive index detector) combined with a viscometer can be used to measure not only the molecular weight of the materials but also to investigate the star-branched structure.

A series of PS star-branched polymers was analyzed, which had been synthesized by a 'core first' approach giving theoretical 5-, 14- and 21-arm structures.

Conditions

Samples:	Polystyrene star-branched polymers
Columns:	2 x PLgel 5 µm MIXED-C, 300 x 7.5 mm
Eluent:	THF
Flow Rate:	1.0 mL/min
Temperature:	40 °C
Detector Temp:	All detectors set at 40 °C
Detector Train:	1260 Infinity MDS incorporating Viscometer and DRI

Samples supplied by Prof. Haddleton, University of Warwick
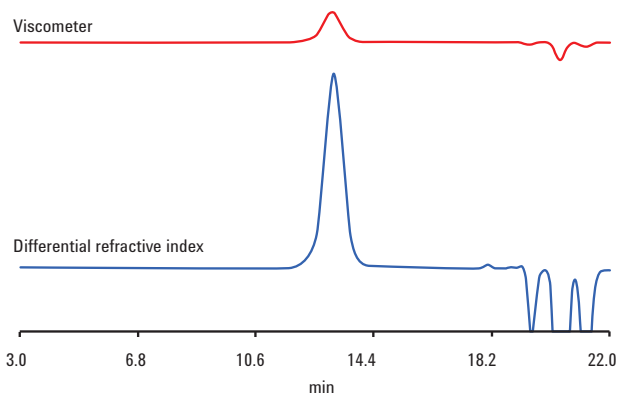

Figure 1. *Viscometer and refractive index detection of the 14-arm, star-branched polystyrene*

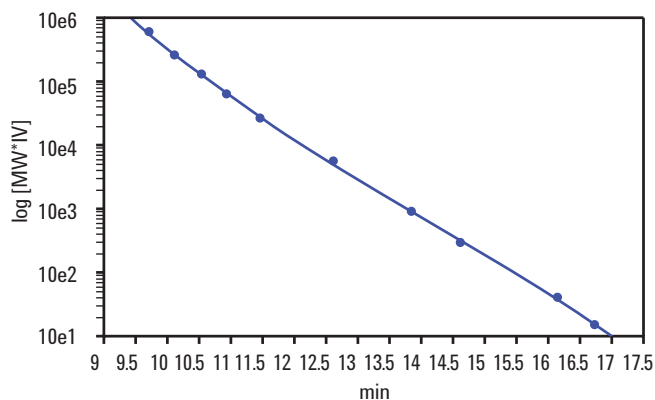Figure 1 shows the dual-detection chromatogram of the 14-arm polymer.

Figure 2. *Universal calibration curve using polystyrene standards*

The universal calibration approach was used to calculate the molecular weight averages for the star-branched polymers. The universal calibration curve was generated using linear PS standards with narrow polydispersity and is shown in Figure 2.

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Table 1. Molecular weight averages and weight average viscosities for star-branched polymers

Sample	Molecular weight average (g/mol)						PD	IVw
	Mp	Mn	Mw	Mz	Mz+1	Mv		
5 arm	56,120	10,460	64,856	98,594	134,877	46,292	6.20	0.28
14 arm	27,436	26,812	29,310	32,425	36,542	28,687	1.10	0.10
21 arm	149,752	111,377	157,884	201,225	256,977	141,293	1.42	0.21

Based on the linear regions in the Mark-Houwink plots for the stars, the intrinsic contraction factor g′ was calculated.

Based on this calibration, the molecular weight averages and weight average intrinsic viscosity (IVw) calculated for the PS star-branched polymers are given in Table 1.

Mark-Houwink plots of log intrinsic viscosity as a function of log molecular weight were calculated for the PS star-branched polymers and for a broad PS material that was known to contain no branching. Figure 3 shows an overlay of the Mark-Houwink plots obtained, indicating that increasing the number of arms on the star-branched PS resulted in a decrease in intrinsic viscosity at any given molecular weight when compared to the broad linear PS. However, for the 21-arm star-branched polymer, the change in intrinsic viscosity relative to the linear PS varied strongly with the molecular weight.

From the g′ data, the radius of gyration contraction factor g was calculated, where a = 1.104, p = 7, and b = 0.906 (Weissmüller et al. 1997).
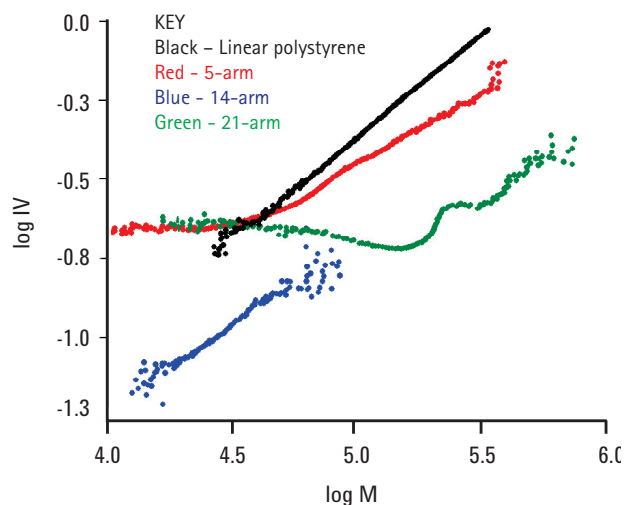

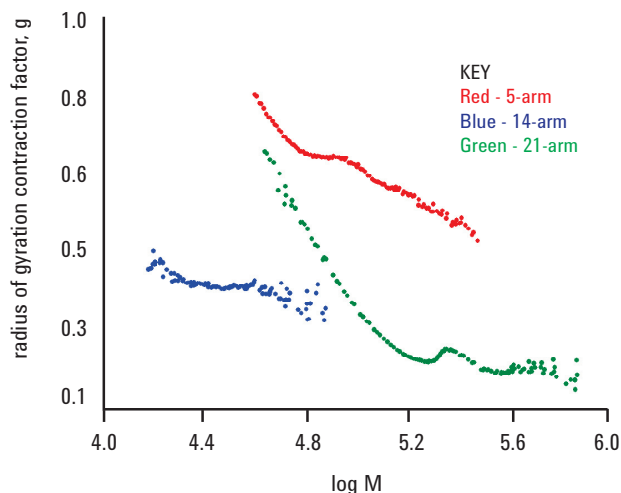**Figure 3.** Overlaid Mark-Houwink plots for different star-branched polymers

Figure 4. *Overlay of Rg contraction g plots for different star-branched polymers*

Figure 5. *Functionality f plots for the different star-branched polymers*

Figure 4 shows an overlay of Rg contraction g plots for 5-, 14- and 21-arm star-branched polymers. Using the calculated g values and the functionality f for the stars, the theoretical number of arms was calculated using the model in the equation 1, based on an assumption that the arms were 'random', that is, polydisperse in molecular weight (Burchard 1977, 1983).

$$g = \frac{3f}{(f+1)^2}$$

Figure 5 shows an overlay of f for the stars as a function of the log molecular weight. The random model gave a prediction of f, the number of arms, which was in good agreement with the value expected from the synthesis. However, for all of the star-branched polymers, especially the 21-arm PS, the calculated value of f increased sharply with molecular weight, indicating that a considerable portion of the sample at low molecular weight contained components with fewer than expected arms.

The variation in the functionality f with molecular weight provided valuable insight into the mechanism of the 'core first' approach used to synthesize the materials.

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Analysis of epoxy resins by viscometry

Epoxy resins are complex materials manufactured by the reaction of a 'resin' with a 'hardener'. This produces a cross-linked polymer that is extremely strong, tough and may be used to bond materials together. The resin component of the mixture is an epoxide pre-polymer, typically produced by the reaction of an epoxide with bisphenol-A. The resulting short-chain material contains pendant –OH and epoxide functionalities and is polar in nature. The molecular weight of the epoxide chains in the resin is important as it influences the curing rate of the final epoxy resin formulation when mixed with the hardener, however, accurate molecular weights are difficult to obtain due to the complex, inhomogeneous nature of the epoxide polymer chains.

GPC assesses the molecular weight distribution of polymers, a property that influences many physical characteristics such as the curing time of epoxy resins. Using a viscometer in combination with a differential refractive index detector has the advantage of allowing the determination of accurate molecular weights for structurally complex polymers and co-polymers via the Universal Calibration approach. Using this methodology, the same molecular weights are determined for samples regardless of the standards used to create the calibration.

Conditions

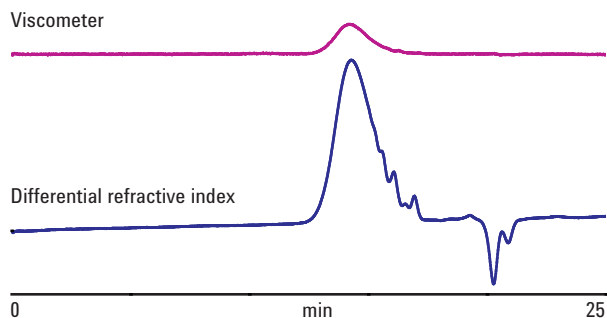|	
Samples:	Epoxide pre-polymers
Columns:	2 x PolarGel-L, 300 x 7.5 mm
Eluent:	Dimethyl formamide + 0.1% LiBr
Inj Vol:	200 µL
Flow Rate:	1.0 mL/min
Detector Temp:	All detectors set at 60 °C
Detector Train:	1260 Infinity MDS incorporating viscometer and DRI

Figure 6. Overlaid multi-detector chromatogram for an example of epoxide pre-polymer

Epoxide pre-polymers are polar in nature and can be run in polar organic solvents such as dimethyl formamide using a set of PolarGel-L columns. The 1260 Infinity MDS was chosen as part of the system as it is capable of multi-detector GPC in polar solvents.

Figure 6 shows an example overlaid multi-detector chromatogram for the sample of one of the epoxide pre-polymers. The appearance of oligomers resolved by the PolarGel-L columns is clearly apparent.
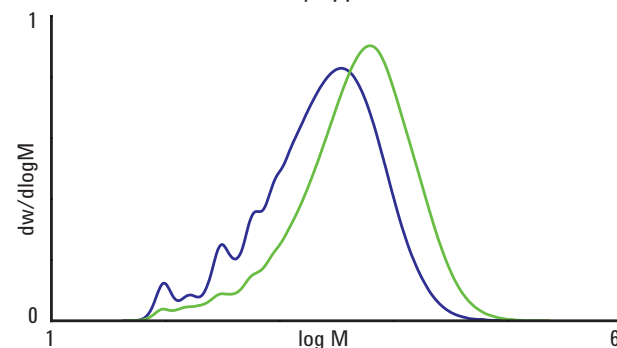

Figure 7. Overlaid multi detector molecular weight distributions of two samples of epoxide pre-polymer

Figure 7 shows an overlay of the accurate molecular weight distributions of the two samples under investigation. As can be seen, they have very different distributions indicating that the materials are two different grades of epoxide pre-polymer that will display differing hardening rates when mixed with the hardener component of the epoxy resin mixture.
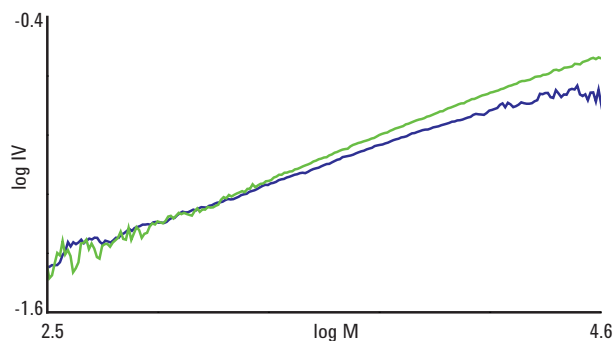

Figure 8. Overlaid Mark-Houwink plots for the two samples of epoxide pre-polymer

Figure 8 shows the overlaid Mark-Houwink plot of log intrinsic viscosity as a function of log molecular weight for the two samples. Both grades of epoxide pre-polymer display a similar relationship only showing deviation at high molecular weight.

This would indicate that the molecular dimensions of the two materials are approximately the same at low molecular weight; however, as the molecular weight increases there is a structural disparity between the two materials, most likely due to a change in the chemistry of the samples.

Analysis of polyvinyl butyral by triple detection

Polyvinyl butyral (PVB) is a resin prepared from polyvinyl alcohol by reaction with butyraldehyde. PVB is an important material that has a wide range of uses in applications that require strong binding, optical clarity, excellent surface adhesion, toughness and flexibility. The most well known application of PVB is in laminated safety glass, for example, in the windscreens of vehicles. In such products, PVB is used as an interface layer between two sheets of toughened glass. The interface layer absorbs the energy of impacts, binding broken shards of glass together and distributing the force of the impact over a wide area. The use of laminated glass has greatly reduced injuries resulting from automobile accidents. As it is the viscoelastic properties of PVB that contribute to its performance in glass laminates, the molecular weight distribution of the material must be closely monitored to ensure optimum performance.

Gel permeation chromatography (GPC) with triple detection, employing a light scattering detector and viscometer, may be used to determine accurate molecular weights for polymers such as PVB. Three PVB materials were analyzed using these techniques, one of which did not perform as expected in application testing.

Conditions

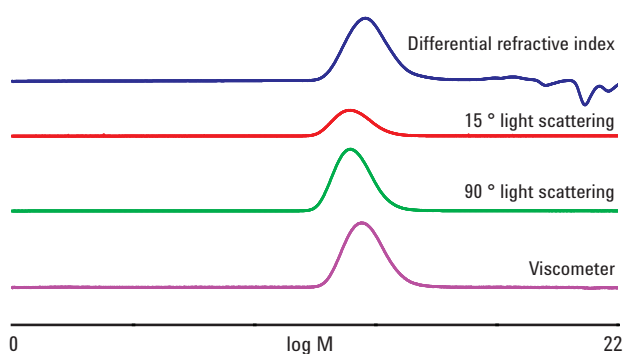|	
Samples:	Polyvinyl butyrals
Columns:	2 x PLgel 5 µm MIXED-C, 300 x 7.5 mm
Eluent:	Tetrahydrofuran (stabilized)
Inj Vol:	100 µL
Flow Rate:	1.0 mL/min
Detector Temp:	All detectors set at 40 °C
Detector Train:	1260 Infinity MDS incorporating dual-angle light scattering, viscometer and DRI

Figure 9. Overlaid multi-detector chromatogram for an example of polyvinyl butyral

Figure 9 shows an example overlaid multi-detector chromatogram for a sample of PVB. All of the samples exhibited strong signals that were fairly broad in all the detectors, indicating the high polydispersity of the materials.
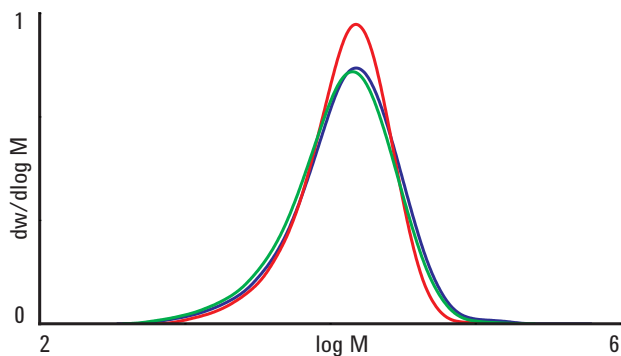
Figure 10. Overlaid triple detector molecular weight distributions of three polyvinyl butyrals

Figure 10 is an overlay of the molecular weight distributions of three PVBs. Two of the samples displayed similar molecular weights with only slight differences across the distributions. However, the third sample showed an appreciable difference in molecular weight distribution, especially in the high molecular weight region where there was less material present than the other samples. This change was thought to be responsible for the change in the performance of the material in comparison to the other polymers.
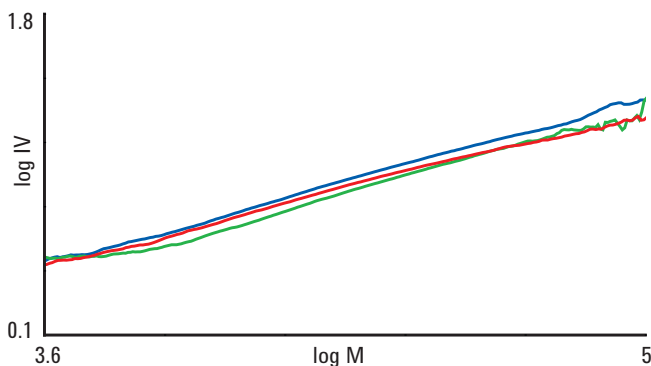
Figure 11. Overlaid Mark-Houwink plots for three samples of polyvinyl butyral

Figure 11 shows the overlaid Mark-Houwink plot of log intrinsic viscosity as a function of molecular weight. All of the samples had a similar relationship between increasing molecular weight and increasing intrinsic viscosity, indicating that the PVBs were of a comparable structure. Therefore, the difference in molecular weight observed was as a result of the synthesis methods rather than any changes to the nature of the polymers themselves.

Analysis of polybutadiene by triple detection

Polybutadiene is an elastomer – a polymer that exhibits elasticity. Elasticity is the ability to deform under external stress but return to the original form after removal of the stress. Polybutadiene is a synthetic rubber manufactured from the monomer 1,3-butadiene. It exhibits 80% recovery after stress, one of the highest values of a synthetic material. It is commonly used to coat electronic assemblies due to its extremely high electrical resistivity. Polybutadiene displays a high wear resistance, low heat build-up after repeated flexing and a low rolling resistance, making it suitable for applications such as tires. It is often used in combination with other materials to produce rubber blends with differing properties.

The 1260 Infinity MDS with triple detection determines accurate molecular weights for polymers for which narrow standards are not available, such as polybutadiene.

Conditions

Samples:	Polybutadienes
Columns:	2 x PLgel 5 µm MIXED-C, 300 x 7.5 mm
Eluent:	Tetrahydrofuran (stabilized)
Inj Vol:	100 µL
Flow Rate:	1.0 mL/min
Detector Temp:	All detectors set at 40 °C
Detector Train:	1260 Infinity MDS incorporating dual-angle light scattering, viscometer and DRI

Polybutadiene is soluble in tetrahydrofuran (THF) and, as it is a synthetic elastomer, it does not tend to contain the cross-linked gel fractions that can be found in natural rubbers. THF is therefore an ideal solvent for this analysis.
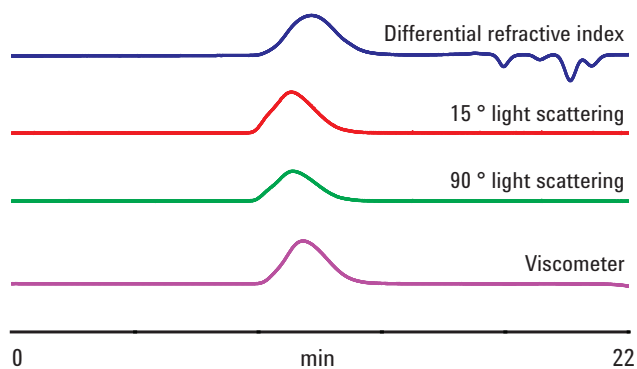

Figure 12. Overlaid multi-detector chromatogram for a sample of polybutadiene.

Figure 12 shows an overlaid multi-detector chromatogram for a sample of polybutadiene. The polymer delivered strong, fairly broad signals in all the detectors, indicating the high polydispersity of the materials.
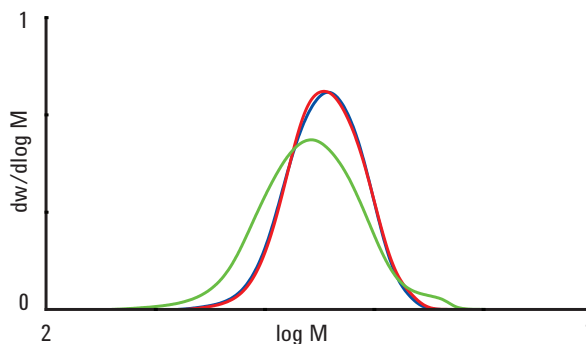

Figure 13. Overlaid triple detector molecular weight distributions of three samples of polybutadiene

Figure 13 is an overlay of the molecular weight distributions of the three polybutadiene samples. Two of the samples displayed similar molecular weights with only slight differences across the distributions. However, the third sample showed an appreciable difference in molecular weight distribution, especially at high molecular weight where there was slight exclusion of the sample. This change was thought to be responsible for the difficulty in processing this material.
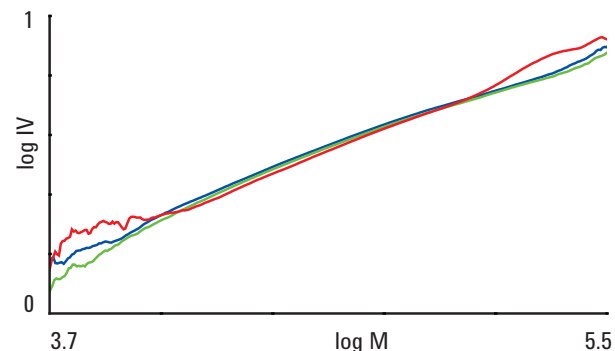

Figure 14. Overlaid Mark-Houwink plots for three polybutadiene samples

Figure 14 shows the overlaid Mark-Houwink plot of log intrinsic viscosity as a function of molecular weight. All of the samples gave the same relationship between increasing log intrinsic viscosity and increasing log molecular weight. This indicates that the polymers were structurally identical and, therefore, that the difference in molecular weight observed resulted from the synthesis methods employed rather than any changes to the nature of the polymers themselves.

Analysis of bromostyrene by triple detection

The structure of many common types of polymer may be modified by either post-polymerization reaction or by the use of modified monomers, producing materials with new and novel properties. Understanding and assessing the effect of these modified structures on the behavior of polymers in various applications is crucial to developing novel uses for such materials. Polystyrene is one of the most commonly seen polymers, used primarily for the manufacture of packing materials. Modified forms of polystyrene may be produced by the use of novel styrene monomers.

Triple detection can be used to determine accurate molecular weights for polymers for which narrow standards are not available, and to assess their solvation properties.

Conditions

Samples:	Polystyrene and polybromostyrene
Columns:	2 x PLgel 5 µm MIXED-C, 300 x 7.5 mm
Eluent:	Tetrahydrofuran (stabilized)
Inj Vol:	100 µL
Flow Rate:	1.0 mL/min
Detector Temp:	All detectors set at 40 °C
Detector Train:	1260 Infinity MDS incorporating dual-angle light scattering, viscometer and DRI

Figure 15 shows an example overlaid multi-detector chromatogram for the sample of polybromostyrene. The polymer gave strong signal in all the detectors that were fairly broad, indicating the high polydispersity of the material.
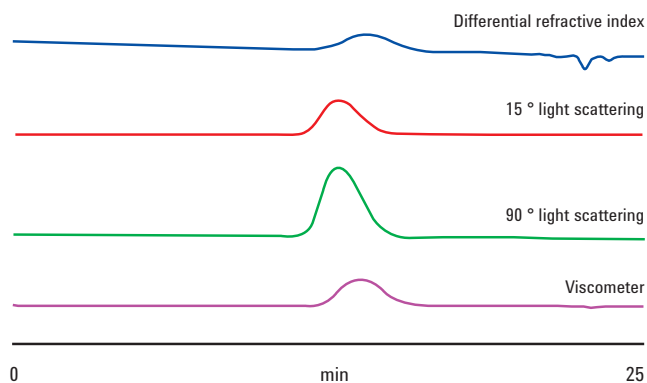

Figure 15. Overlaid multi-detector chromatogram for an example of polybromostyrene

Figure 16 shows an overlay of the molecular weight distributions of the two samples under investigation. As can be seen, the polybromostyrene was considerably higher in molecular weight than the polystyrene sample.
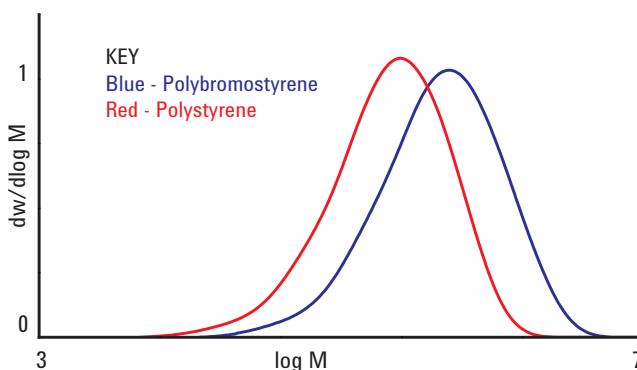

Figure 16. Overlaid triple detector molecular weight distributions of the two samples

Figure 17 shows the overlaid Mark-Houwink plot of log intrinsic viscosity as a function of molecular weight for the two samples. Compared to the polystyrene, the polybromostyrene shows a marked shift of the Mark-Houwink plot to lower intrinsic viscosity values at any given molecular weight. This indicates that polybromostyrene is smaller in solution than polystyrene across the molecular weight range, a result of changes in the level of interaction between the polymer coils and the solvent. The plot parallels that of polystyrene, indicating that the structure is identical across the range of molecular weight, as expected when a modified monomer unit is used to create the new polymer.
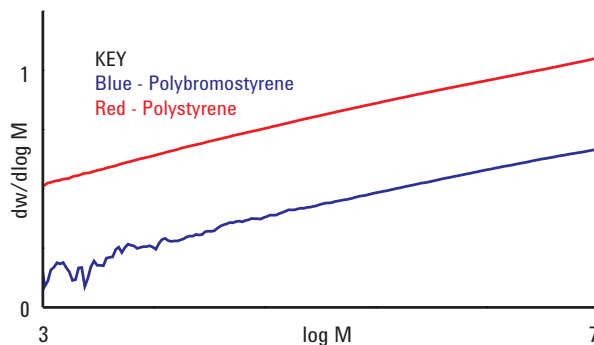

Figure 17. Overlaid Mark-Houwink plots for two samples

Conclusions

Multi-detector GPC/SEC with a viscometer or light scattering detector (or both) is a powerful technique for improving both the accuracy and information content of the experiment. The inclusion of viscometry or light scattering also addresses the limitations of conventional GPC that uses a concentration detector alone.

GPC employing viscosity detection with a concentration detector allows the calculation of accurate molecular weight via the Universal Calibration. The technique can also provide insights into the behavior of molecules in solution via the Mark-Houwink plot, revealing details of structural changes to the polymer backbone. Viscometry is a simple technique to perform and routinely gives good quality data, and so is an excellent choice for the analyst looking to improve the accuracy and quality of their GPC results with the minimum effort.

GPC with light scattering detection and a concentration detector is a very powerful method for analyzing polymers, and is capable of generating molecular weights not reliant on any form of column calibration. In addition, light scattering provides an insight into the structural properties of polymer molecules, especially their size in solution. Although more experimentally stringent than GPC employing viscosity detection, the use of light scattering is becoming more prevalent due to the wealth of information this technique can provide.

Triple GPC detection employing a viscometer and a light scattering detector with a concentration detector is the most versatile form of the analysis. Triple detection benefits from the advantages of viscometry and light scattering, and is the technique of choice for the serious polymer analyst.

Further reading suggestions

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