Mechanisms: Size Exclusion Chromatography

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Introduction

Size exclusion chromatography (SEC), also known as gel permeation chromatography (GPC), is a particularly valuable tool in the measurement of the molecular weights and molecular weight distributions of polymers. There are a number of variations on the basic technique to accommodate the requirements of different polymer types and to elicit additional information. This article seeks to describe the basic technique with particular comment to the often under-appreciated limitations together with detailed comment on the main variations to the basic technique. The relationship between SEC and other techniques for polymer molecular weight techniques will be summarized.

As examples of the technique, **Figure 1(A)** is an overlay plot of the computed molecular weight distributions for a range of extrusion grades of polystyrene from various producers and Figure 1(B) illustrates the variation in molecular weight for a series of poly(methylmethacrylate) (PMMA) materials polymerized under different conditions. The descriptions in this article will normally relate to the general practice in the use of SEC with polymers soluble in the normal organic solvents. Some comments will also be made on the aqueous-based SEC of processable polymers. Biopolymers, such as polypeptides and proteins are also examined using SEC, but the consideration of appropriate buffers and column packings has not been considered appropriate for separate comment.

SEC – The Basic Technique

SEC is a specific form of liquid chromatography where the only fundamental difference is in the column packing and the separation mechanism. However, the particular requirements of SEC are somewhat different to most other forms of liquid chromatography giving rise to various aspects in the choice of instrumentation, data handling and general practical philosophy.

SEC Columns and the Separation Mechanism

In SEC, the polymer molecules are separated as a function of their size in solution. The column packings are porous and the separation is achieved according to the degree of access of the polymer molecules to the pores.

The solvent in the column packing pores can be considered as the stationary phase and the interstitial solvent as the mobile phase. A distribution coefficient is established by which the time spent in the pores, or stationary phase, is dependent upon the solvated size of the polymer molecules and on the pore geometry. Since the larger molecules are more restricted in the pore volume available to them, the larger molecules spend less time in the stationary phase and are eluted first; smaller molecules are effectively retarded and elute later.

It is fundamental to SEC than no other separation mechanism (e.g. adsorption) is occurring. As a consequence, the maximum elution volume available to achieve separation is the total pore volume. Molecules which are so large that they are totally excluded from the pores will elute at a volume corresponding to the interstitial or 'void' volume while molecules which can permeate all of the pores will elute at a volume corresponding to the void volume plus the pore volume. Anything eluting at a greater volume than this must be retarded by some additional mechanism.

With consideration to the above requirements, the materials used for SEC column packings are mainly selected for their pore geometry and lack of other interaction with the polymer molecules. In many solvent systems, the potential for adsorption limits the applicability of inorganic packings and polymeric packings are the more usual choice. The bulk of all SEC work with synthetic polymers is carried out using column packings produced from polystyrene, cross-linked with divinylbenzene. In the production of these materials, the pore geometry is manipulated to give variation in pore size and hence 'tailored' to suit particular molecular mass ranges.

In addition to pore size and inertness, the normal chromatographic relationships between packing size and efficiency are applicable but, as noted later, there are limitations to using too small a packing size with the higher molecular weight polymers.

SEC column packings are normally produced with a limited polymer molecular weight applicability but it has been normal practice to use a number of different columns (with specific pore sizes) in series to give an acceptably wide molecular weight range. Typically, to cover a molecular weight range of 2000 to 2 000 000, a bank of columns such as 1×10^3 Å, 1×10^4 Å, 1×10^5 Å and 1×10^6 Å would be used.

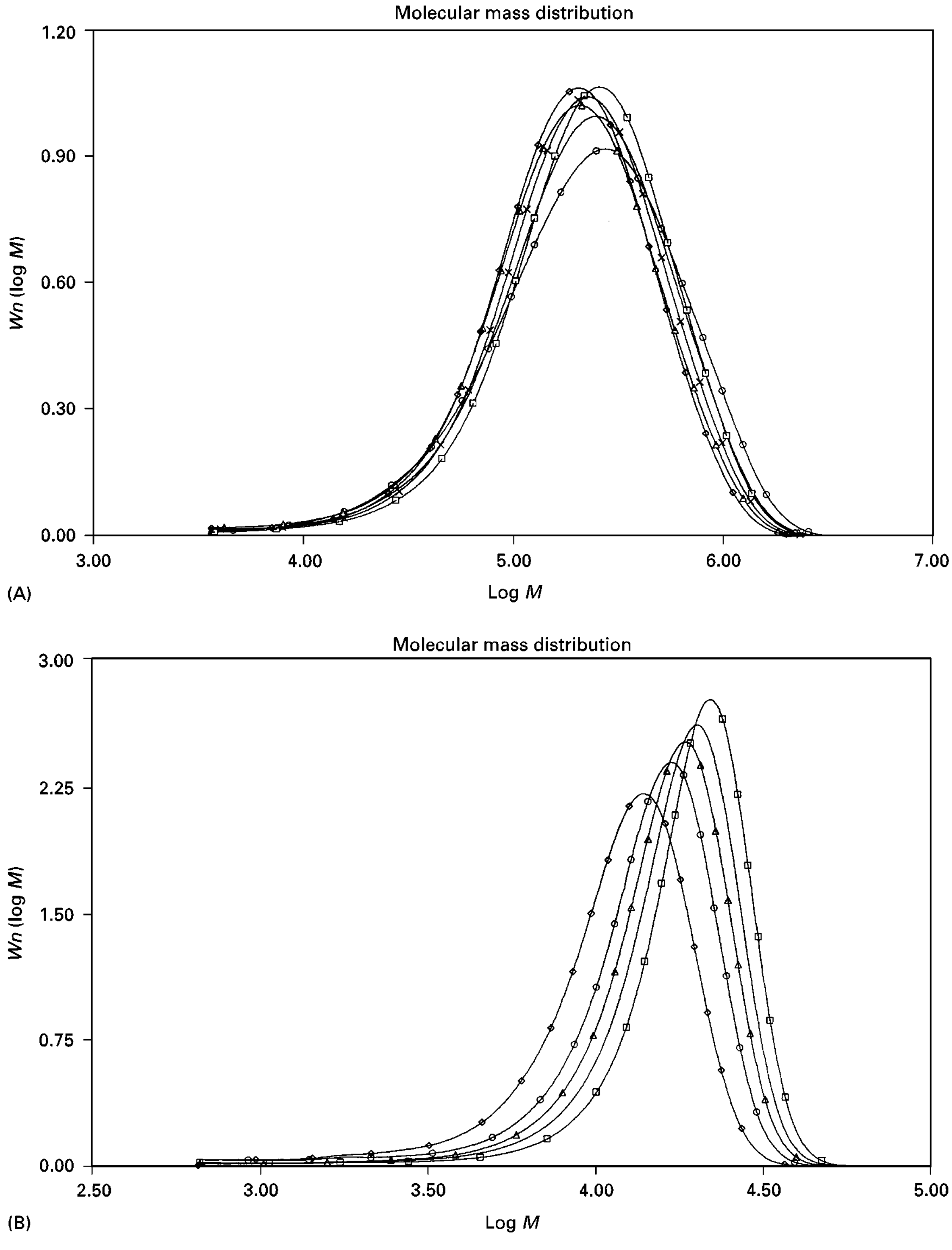

Figure 1 An overlay plot of the computed molecular weight distributions for a range of extrusion grades of polystyrene from various producers.

(The identification of pore size expressed in angstroms is common practice but potentially misleading, since the dimension refers to maximum extended polymer chain length, rather than actual pore size.)

There is potential for using an inappropriate combination of column packings and it is now becoming more common to obtain single columns which the manufacturer has filled with different porosity packings to give a wide molecular weight range. This 'mixed-bed' approach also gives an optimal combination of packing porosities such that a 'linear' calibration is obtained (see later). Even with the mixed-bed approach, a number of columns will normally need to be combined to achieve an acceptable peak capacity.

Efficiency and Resolution

The factors generally affecting column efficiency in other forms of liquid chromatography also apply to SEC, and column efficiency is calculated and expressed in the normal manner. The efficiency as calculated for a specific low molecular weight compound (e.g. an added solvent) will normally be higher than for a truly monodisperse polymer and the efficiency is normally reported for a solvent peak.

The resolution of two given monodisperse components can be sensibly expressed in a similar manner as for other forms of liquid chromatography but in SEC it is also possible to define a 'specific resolution' which relates to the theoretical resolution of two monodisperse polymers having one decade difference in molecular weight. The specific resolution will be dependent upon the gradient of the calibration (as expressed by log molecular weight versus elution volume). If the calibration is linear (on the log molecular weight scale) then the specific resolution will be constant across the molecular weight range.

Column Packing Size and Shear Effects

For some time the normal column packing particle size available has been around 10 μm but, in common with other forms of liquid chromatography, there has been a trend towards increasing the efficiency by producing smaller particle size packings. However, when working with high molecular weight polymers, the higher linear flow velocity associated with the smaller packing sizes can cause shear degradation of the polymer and the particle size must be selected to suit the molecular weight of the sample polymer. The 10 μm material is appropriate for a very wide range of molecular weights but additional efficiency can be obtained by using 5 μm packing for polymers with moderate molecular weights (ca. 10 000 to 100 000) and 3 μm packings can be used for lower molecular weights. For very high molecular weight polymers (ca. > 2 000 000) the use of larger packing size (e.g. 20 μm) should be considered. Where there is potential for shear degradation, lower solution concentrations and lower flow-rates can also reduce this unwanted effect.

SEC Pumps and Flow-Rate

Normal isocratic HPLC pumps are used for SEC work but the requirements for good control of the flow-rate are probably more severe. Since in SEC, it is an envelope of a multitude of different molecular weight peaks that is being measured, it is not possible to set an identification window and any small variation in flow rate will produce an error in the molecular weight calculation. Variation in flow-rate can be allowed for by the use of an internal marker. For a typical polystyrene, a deliberate correction of the flowrate by 0.2% produces a variation in the calculated weight average molecular weight from 308 000 to 320 000; a variation of approximately 4%.

SEC Concentration Detectors and Sample Concentration

A number of detector types for specific additional information will be considered later but the most basic SEC application requires a concentration detector to monitor the polymer as it emerges from the column.

The most common form of concentration detector encountered in SEC is a differential refractive index (DRI) detector. These detectors are a cost-effective option which are nearly universal in application but they have limited sensitivity and require a very good temperature control. Since SEC is normally used to look at bulk components (rather than trace components), the poor sensitivity is not normally a problem but some difficulties may be encountered where the differential refractive index for the polymer/solvent combination is so small that baseline noise becomes significant.

The other universal detector used in SEC is the ELSD (evaporative light scattering detector, also known as an evaporative mass detector). These units involve atomization of the eluent into a steady flow of inert gas where the solvent is removed and the remaining solute particles are detected by the light scattering they induce. It is important to distinguish between these ELSD and the light scattering from solutions considered later. The ELSD is more expensive than DRI detectors and there are potentially high running costs for the large amount of inert gas consumed. However, they are less affected by any variation in solvent composition or temperature and are more universal in the size of the response regardless of chemical composition of the polymer.

Figure 2 is a schematic diagram of a commercially available evaporative light scattering detector.

Other types of HPLC detector (such as ultra-violet, infrared or radiochemical) are also used for SEC but are more restricted in applicability and are used more as selective detectors to pick out specific components. There are latter comments with regard to the SEC of copolymers.

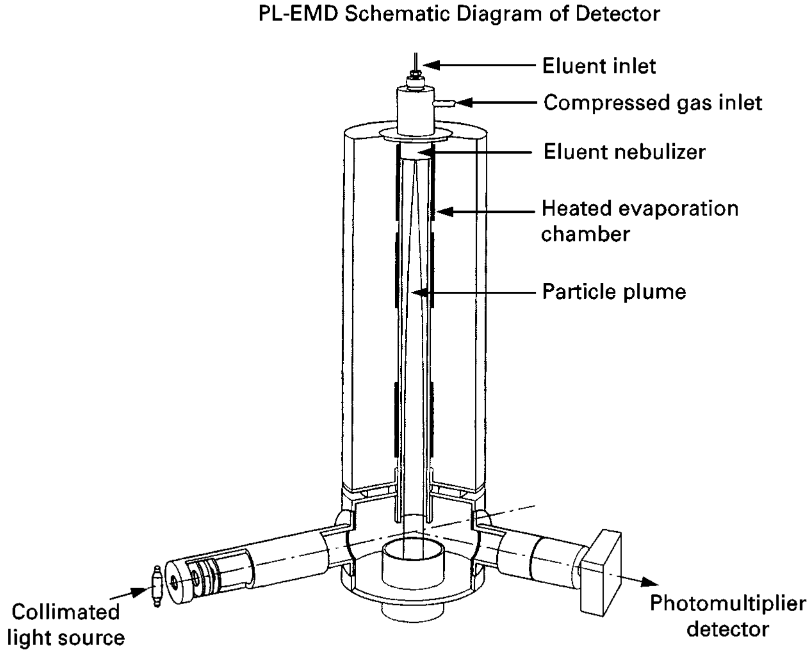

Figure 2 A schematic diagram of a commercially available evaporative light scattering detector. Printed with the permission of Polymer Laboratories Ltd, Church Stretton, Shropshire, UK.

Detector Response to Low Molecular Weight Components

The differential refractive index for a polymer/solvent combination is normally independent of molecular weight at high molecular weight. However, while it is generally known that the refractive index detector response varies with low molecular weight polymers this variation is commonly ignored. As a consequence, SEC results for polymers containing low molecular weight material often do not give an appropriate consideration to the low molecular weight components; this has important implications for many legislative requirements for defining the proportion of low molecular weight material present.

The variation in refractive index response with molecular weight is presumed to be due to the increasing influence of end groups and it seems probable that there are similar effects with most other detector types. For the evaporative light scattering detector, there is similarly the possibility that the more volatile components are lost with the solvent.

SEC Calibration and Data Handling

For SEC a calibration must be obtained by running polymers of known molecular weight. The more usual practice is to run a series of calibrants of very narrow molecular weight distribution (often described as being monodisperse) and known peak molecular weight (Mp) and obtain a calibration of log Mp versus elution volume. Alternatively, there are procedures for using broad molecular weight distribution calibrants, where the molecular weight distribution is well defined.

In practice, the most commonly used calibrants consist of narrow distribution polystyrenes, and a wide range of these is commercially available. A small range of other polymer types of narrow distribution calibrants is also available but usually with a restricted molecular weight range. The availability of well-defined broad molecular weight distribution calibrants is extremely limited.

The log Mp versus elution volume calibration is usually expressed by an equation which can be a simple linear expression or a polynomial. The software will frequently allow an excellent fit by using a high order polynomial but it is questionable whether an order of three should ever be exceeded. In practice, reasonable good fits are normally obtained using first, second or third order curves but even for third order fits, the potential for variation at low molecular weight, affecting the high molecular weight end of the distribution, is intrinsically present.

The calculation of molecular weight averages and plotting molecular weight distributions from a sample chromatogram and calibration is complex and the use of laboratory computers was introduced early to SEC. However, automatic data handling has often led to inappropriate manipulation of the data and care is always required. The whole chromato-

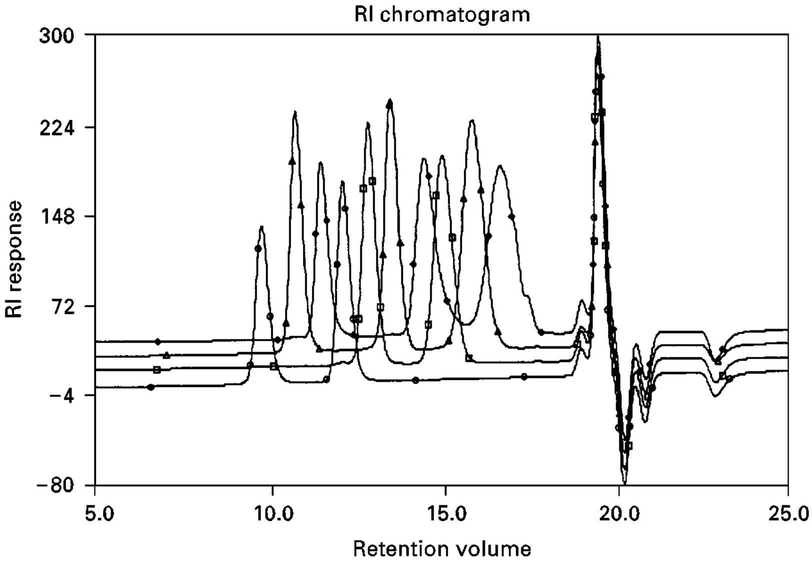

Figure 3 Chromatograms of four solutions containing 10 narrow molecular weight distribution polystyrene calibrants.

gram envelope for the polymer is used in the calculations and any minor variation in baseline placement or integration limits at the extreme ends of the distribution is likely to have significant effects on one of the calculated molecular weight averages.

Measurement of Molecular Weight Averages

All synthetic polymers and many natural polymers have a molecular weight distribution and it is usual to calculate average molecular weights from these distributions. Although there are a considerable number of averages that have previously been used, the most common are the number average, M_n, weight average, M_w, and the z-average, M_z. These are defined as follows:

$$M_n = \sum(N_i \cdot M_i)/\sum N_i$$

$$M_w = \sum(N_i \cdot M_i^2)/\sum(N_i \cdot M_i)$$

$$M_z = \sum(N_i \cdot M_i^3)/\sum(N_i \cdot M_i^2)$$

where N_i is the number of molecules of molecular weight M_i.

The molecular weight is generally calculated from the normalized chromatograms by dividing the chromatogram into slices. The area of each slice (h_i) is measured and the molecular weight (M_i) determined from the calibration curve. The molecular weight averages are then calculated as follows:

$$M_n = \sum h_i/\sum(h_i/M_i)$$

$$M_w = \sum(h_i \cdot M_i)/\sum h_i$$

$$M_z = \sum(h_i \cdot M_i^2)/\sum(h_i \cdot M_i)$$

In practice, the weight average molecular weight generally corresponds to a molecular weight near the maximum of the chromatogram and the repeatability of the measurement from SEC is normally better than for the other averages. The number average molecular weight is sensitive to minor variations at the low molecular weight end of the distribution while the z-average is more influenced by differences at the high end of the distribution.

The term ‘polydispersity’ is often used as a measure of the width of a molecular weight distribution and is usually the ratio of the weight average to number average molecular weights (M_w/M_n).

Figure 3 shows an overlay of the chromatograms for four solutions containing a total of ten individual, narrow molecular weight distribution polystyrene calibrants and **Figure 4** shows the log (molecular weight) versus retention volume calibration derived from these chromatograms.

Figure 5 shows an overlay of one of the chromatograms for one of the above calibrant solutions and a broad molecular weight distribution polymer of unknown molecular weight and **Figure 6** shows the calculated molecular weight averages and molecular distribution obtained from applying the calibration to the molecular weight computation with this chromatogram.

In Figure 6, the frequency is expressed as weight fraction per unit of log (molecular weight), *Wn*(log *M*).

The Universal Calibration Procedure

When a polystyrene calibration is applied for the measurement of a polymer other than polystyrene, the results can be expressed as the polystyrene equivalent molecular weights or it might be possible to

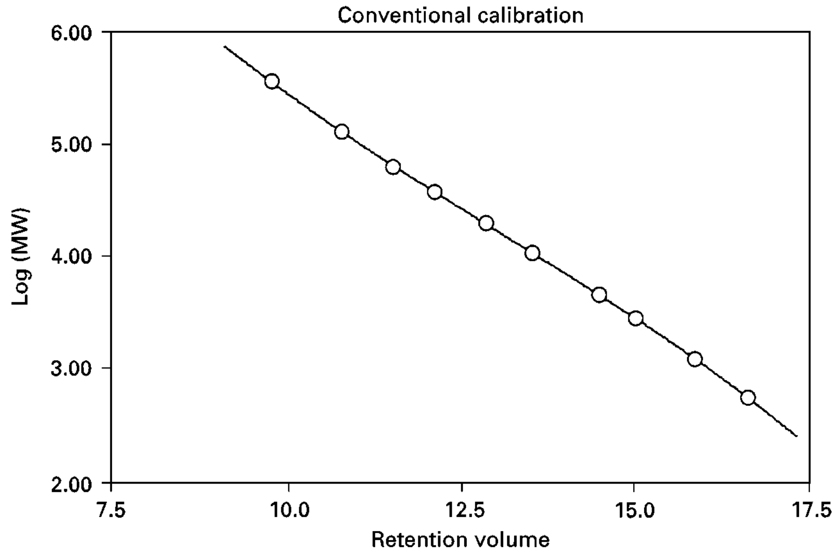

Figure 4 Log (molecular weight) versus retention volume calibration derived from chromatograms shown in Figure 3.

apply a mathematical correction to describe the true molecular weights. The most common mathematical correction, for polymer type, is known as the Universal Calibration Procedure and is based on the early empirical observation that if the calibration is expressed as the log of the product of molecular weight and intrinsic viscosity versus elution volume, then a common calibration is obtained for many polymer types (if the simple log molecular weight vs. elution volume calibrations are plotted, a series of related calibration plots are obtained).

The intrinsic viscosity factor in the universal calibration allows for the variation in solvation between different polymers. This difference in solvation is closely related to solution viscosity measurements and it is the K and alpha values from the Mark–Houwink–Sakurada equation which are used in this correction.

The K and alpha values for both the calibrant and sample polymer types in the solvent used and at the measurement temperature must be known. In practice, the literature contains a lot of data on measured K and alpha values and if appropriate values are selected with care, the computed molecular weight data can be close to the true values. However, it must be appreciated that the nature of any branching can

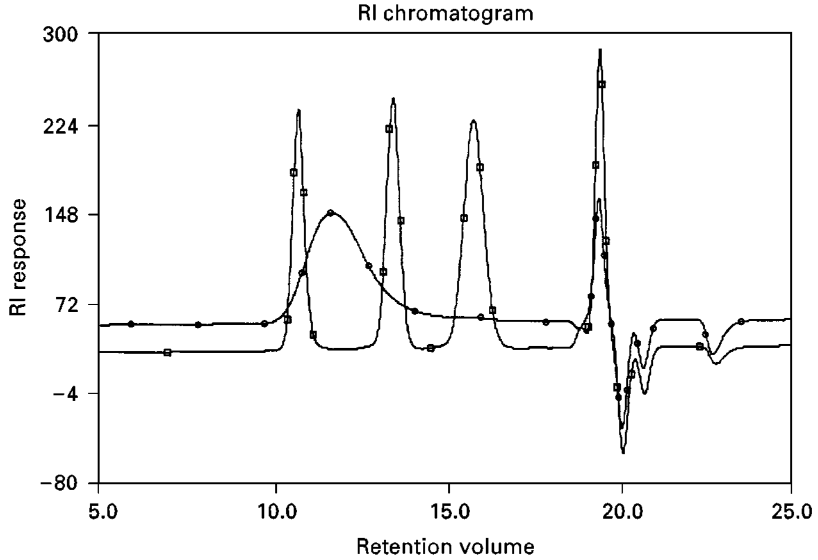

Figure 5 Overlay of one of the chromatograms for one of the above calibrant solutions and a broad molecular weight distribution of unknown molecular weight.

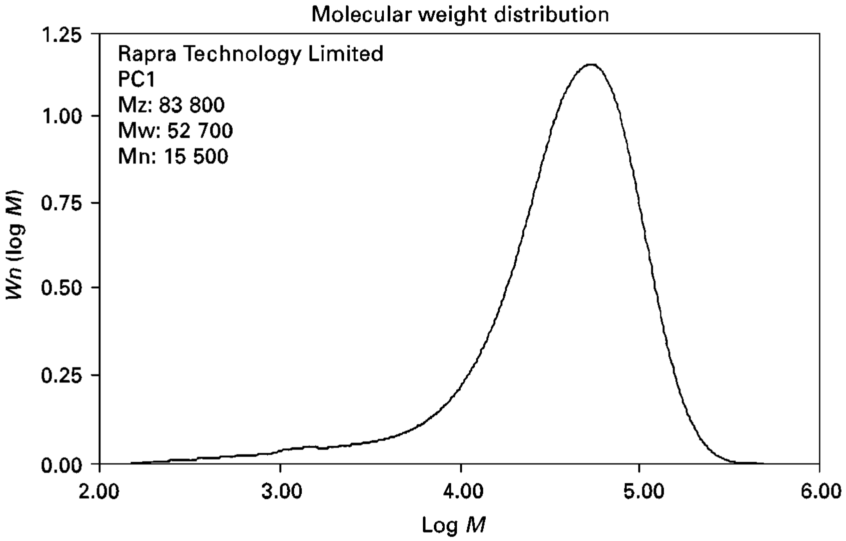

Figure 6 Calculated molecular weight averages and molecular distribution obtained from applying calibration to molecular weight computation with chromatogram in Figure 5. Frequency is expressed as weight fraction per unit of log (molecular weight), *Wn* (log *M*).

have a significant effect on these values and in the literature values were frequently obtained many years ago when the polymer structure might have been somewhat different to common materials today.

The Universal Calibration procedure appears to be appropriate for polymers which have a normal random coil configuration in solution but fails for any polymer type which has any structured conformation in solution. The philosophy behind this approach is also used in combined SEC-viscosity measurements (see later).

High Temperature SEC

In the early days of SEC, it was appreciated that this was a technique that could be used at high temperature and be applied to polymer types for which there was no room temperature solvents, particularly polyethylene and polypropylene, and appropriate commercial instrumentation was soon introduced. Despite this early appreciation of the value of high temperature SEC, it remains a difficult application.

High temperature SEC is carried out either to be able to dissolve the polymers of interest, as with polyolefins, or to reduce the viscosity of the solvents. In practice, SEC of polyolefins requries a minimum temperature of 140°C and is usually carried out with chlorinated aromatic solvents; the SEC of polyesters (such as PET) or polyamides is carried out using phenolic solvents at temperatures of at least 110°C, while SEC with polar solvents, such as dimethylformamide is carried out at temperatures around 80°C. In addition, some SEC is carried out at raised temperature to enhance the efficiency (particularly aqueous SEC).

Polyolefins tend to come out of solution if there is a cool spot in the total instrumentation, which is the main complication with these polymer types but there is potential for other temperature incompatibilities (e.g. temperature cycling on an RI detector). In addition, solvents at high temperature are likely to swell polymer components in the SEC instrumentation (e.g. pump seals) and it is desirable to dedicate the instrumentation to specific applications. With the high temperature SEC using phenolic or polar solvents, the main complications are not necessarily directly related to the high temperature.

SEC with Polar Solvents

Polar polymers normally require the use of polar solvents such as dimethylformamide (DMF), dimethylsulfoxide (DMSO), N-methylpyrrolydine (NMP) or dimethylacetamide (DMAC). Work with these solvents is carried out at raised temperatures (e.g. 80°C), to reduce the viscosity, and it is normal practice to add salts to try and suppress interactions other than SEC. SEC of polyesters and polyamides can be carried out at ambient temperature using fluorinated alcohols but the solvents are excessively expensive and of questionable toxicity.

Although the standard polystyrene-based columns are normally used for SEC with polar solvents, the polystyrene calibrants may not be applicable and the use of alternative calibrant polymers such as polyethyleneglycol/polyethyleneoxide or polymethylmethacrylate is common.

SEC with Phenolic Solvents

As noted above, the SEC of polyesters, such as polyethylene terephthalate and many polyamides is often carried out using phenolic solvents at temperatures of around 110°C or 120°C. Typical solvents are m-cresol or o-chlorophenol. It is the unpleasant nature of the solvents which is the main problem with these SEC applications but in general the problems of high temperature SEC increase with temperature.

In the author's laboratories, the SEC of polyesters appears to be reasonably straightforward but the weak DRI detector response for polyamides is problematic.

Aqueous SEC

The SEC of polymers in aqueous media has generally been considered more complex due to the frequent need for complex buffer selection and to the requirement for hydrophilic column packings. In the early days of SEC, the hydrophilic column packings available gave significant problems but this appears to have been largely overcome with the advent of high efficiency hydroxymethacrylate packings.

For most water-soluble polymers, it remains necessary to select pH and ion concentration suited to the polymer type of interest and it is not practical to have standard conditions for aqueous SEC work. However, it is usual to raise the temperature to obtain enhanced efficiency.

SEC with Selective Detectors

Fixed wavelength ultra-violet or infra-red detectors are used with a 'universal' detector (such as DRI) and appropriate software to obtain information on how specific chemical groups vary through the molecular weight distribution. However, it should be noted that appropriate spectral windows for the solvent are required to allow this approach to be used and the presence of antioxidants or other additives, in the solvent, can be a problem.

Modern diode array detectors can be used to obtain more complex information where applicable.

SEC with FTIR

It should be possible to use the rapid scanning capability of Fourier Transform Infra-Red spectrophotometers to obtain very detailed information on chemical composition but the lack of spectral windows is a severe limitation. Direct combination of SEC and FTIR has been used with tetrachloroethylene as the solvent (which has a very simple infra-red spectrum) to examine ethylenevinyl acetate (EVA) copolymers and a similar direct combination is used for polyolefin samples.

Solvent Removal Approaches

Since the main problem with direct combination of SEC with FTIR is the lack of spectral windows in the solvent, a number of researchers have studied approaches to remove the solvent prior to the FTIR examination.

Laboratory Connections Inc. have developed approaches which use either warm gas or ultra-sonication and vacuum to remove solvent from the atomized eluent and deposit the fractionated material as a thin annulus on a germanium disc. The germanium disc is then transferred to a matching rotating stage located in the infra-red instrument and spectra are recorded as the disc is rotated to mimic the chromatogram. Specialist software allows complex manipulation of the chromatography and spectroscopic data. The quality of spectra that can be obtained without any requirement to overload the chromatographic system is very good and this technique can be used for most SEC approaches and the only real limitation is the potential for losing low molecular weight material with the solvent.

SEC with Light Scattering Detection

Light scattering has long been used as a stand-alone technique for the absolute measurement of the weight average molecular weight of polymers. The classical approach to this technique was to measure the scattered light for a range of different angles and different solution concentrations such as to produce a Zimm plot which would allow determination of both the molecular weight and the shape of the molecule in solution. In the early 1970s, the introduction of laser light scattering instruments allowed the optical cell to be reduced in size to be compatible with a chromatographic detector cell and to work at a low enough angle to avoid the need for extrapolation to zero angle. These Low-Angle Laser-Light Scattering (LALLS) instruments allowed the stand-alone measurement to be carried out far more efficiently but were immediately used for direct combination with SEC such that the 'absolute' measurement of the molecular weight distribution was practical for the first time.

More recently, other instruments have been introduced which measure the scattered light at a range of angles simultaneously; these are known as Multi-Angle Laser-Light Scattering (MALLS) detectors. Instruments have also been introduced which only monitor the light scattered at a right angle (RALLS).

In addition to providing information on the absolute molecular weight distribution, SEC-light scattering is used to obtain information on branching (basically by comparing the absolute molecular weight with the apparent molecular weight for the linear polymer calibration). The response of the light scattering detector increases dramatically with molecular weight and SEC-light scattering systems are very good at examining any variation at the high molecular weight end of a distribution.

In SEC-light scattering, the solution concentration is an important parameter in the calculation and it is necessary to have accurate information of the differential refractive index for the polymer/solvent (this is a squared term in the calculation). This requirement for information on the differential refractive index is problematic for examination of copolymers.

SEC with Viscosity Measurement

As noted above, it has been empirically demonstrated that for many polymer types, a universal calibration is obtained if the log. product of molecular weight and intrinsic viscosity is used rather than simple log. molecular weight. This is utilized by combining the response of a viscosity detector and a concentration detector to give the universal calibration directly. The viscosity monitor measures the differential pressure as polymer solution travels through a capillary; detectors have been developed which use a single capillary, a pair of capillaries or four capillaries (arranged in a manner analogous to a Wheatstone bridge).

SEC-viscosity is not theoretically an absolute approach but should give the true molecular weight distribution, providing that the polymer of interest conforms to the Universal Calibration approach. As with SEC-light scattering, SEC-viscosity is valuable for obtaining information on branching. Again, the solution concentration is an important parameter in the calculation. The differential refractive index does not appear in the calculation but could produce inaccuracies in the assumed concentration and hence is also problematic for copolymers.

Commercial hardware and software is available for combining SEC-light scattering-viscosity within a single system.

Future Prospects

Although there have been suggestions that other techniques (e.g. matrix-assisted laser-desorption ionization – time-of-flight, MALDI-TOF, mass spectroscopy) might replace SEC, there seems little prospect of this in the near future.

There are new commercial integral SEC systems now available that should simplify some of the more difficult applications and make SEC combined techniques more routine. These developments should ensure that SEC is a main stream technique for the foreseeable future. There will also probably be more utilization of triple-detection (concentration, viscosity and light-scattering) for detailed characterization of specific polymer types.

See also: **II/Chromatography:** Detectors: Laser Light Scattering. **Chromatography: Liquid:** Detectors: Refractive Index Detectors; Theory of Liquid Chromatography. **III/Gradient Polymer Chromatography: Liquid Chromatography. Synthetic Polymers:** Liquid Chromatography.

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Micellar Liquid Chromatography

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Introduction

Surfactants are molecules that exist as monomers when they are at low concentrations in solution, while above their critical micelle concentration (c.m.c.) they associate to form aggregates called micelles. Two zones of different polarity exist in the molecules of surfactants: one is hydrophobic in nature, formed from one or more hydrocarbon chains; the other can be polar or even ionic. According to the nature of this second zone, surfactants are classified into three main categories: ionic (anionic and cationic), nonionic and zwitterionic (amphoteric).