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Handbook of Size Exclusion Chromatography
and Related Techniques
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Chapter 1

1 Introduction to Size Exclusion Chromatography

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Size exclusion chromatography (SEC), the technique that is the subject of this monograph, is the generic name given to the liquid chromatographic separation of macromolecules by molecular size. It has been taken to be generally synonymous with such other names as gel permeation chromatography (GPC), gel filtration chromatography (GFC), gel chromatography, steric exclusion chromatography, and exclusion chromatography. The "gel" term generally connotes the use of a nonrigid or semirigid organic gel stationary phase whereas SEC can pertain to either an organic gel or a rigid inorganic support. Despite this, the term GPC is commonly used interchangeably with SEC. In this chapter we shall focus on high-performance (or high-pressure) SEC, which requires the use of rigid or semirigid supports to effect rapid separations, lasting typically 20 minutes to one hour. (More recently, a series of high-throughput SEC columns have been introduced by several vendors. While these columns are not capable of the same degree of quantitative discrimination as the analytical SEC column, they offer a nominal five minute analysis time for comparative purposes.)

The primary purpose and use of the SEC technique is to provide molecular weight distribution (MWD) information about a particular polymeric material.

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The graphical data display typically depicts a linear detector response on the ordinate vs. either chromatographic elution volume or, if processed, the logarithm of molecular weight on the abscissa. One may ask, if SEC relates explicitly to molecular *size*, how can it directly provide molecular *weight* information? This arises from the relationship between linear dimension and molecular weight in a freely jointed polymeric chain (random coil): either the root mean square end-to-end distance or the radius of gyration is proportional to the square root of the molecular weight (1). It follows that the log of either distance is proportional to (one-half) the log of the molecular weight.

1 THE SEC EXPERIMENT AND RELATED THERMODYNAMICS

A stylized separation of an ideal mixture of two sizes of macromolecules is presented in Fig. 1. In the first frame, the sample is shown immediately after injection on the head of the column. A liquid mobile phase is passed through the column at a fixed flow rate, setting up a pressure gradient across its length. In the next frame the sample polymer molecules pass into the column as a result of this pressure gradient. The particles of the stationary phase (packing material) are porous with controlled pore size. The smaller macromolecules are able to penetrate into these pores as they pass through the column, but the larger ones are too large to be accommodated and remain in the interstitial space as shown in the third frame. The smaller molecules are only temporarily retained and will flow

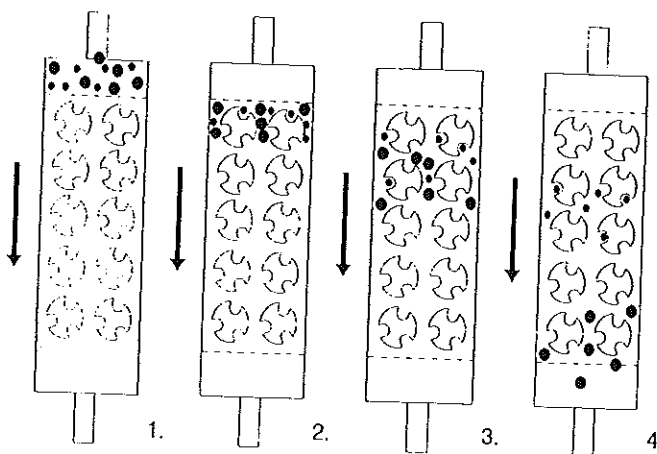


Figure 1 SEC separation of two macromolecular sizes: (1) sample mixture before entering the column packing; (2) sample mixture upon the head of the column; (3) size separation begins; and (4) complete resolution.

down the column until they encounter other particles' pores to enter. The larger molecules flow more rapidly down the length of the column because they cannot reside inside the pores for any period of time. Finally the two molecular sizes are separated into two distinct chromatographic bands as shown in the fourth frame. A mass detector situated at the end of the column responds to their elution by generating a signal (peak) for each band as it passed through, whose size would be proportional to the concentration. A real SEC sample chromatogram would typically show a continuum of molecular weight components contained unresolved within a single peak.

If a series of different molecular weight polymers was injected onto such a column they would elute in reverse size order. It is instructive to consider the calibration curve that would result from a series of molecular weights such as those depicted in Fig. 2. Here the molecular weight is plotted on the ordinate and the retention volume (V_r) on the abscissa. The left-hand edge of the chart represents the point of injection. The retention volume labelled V_0 is the void volume or total exclusion volume. This is the total interstitial volume in the chromatographic system and is the point in the chromatogram before which no

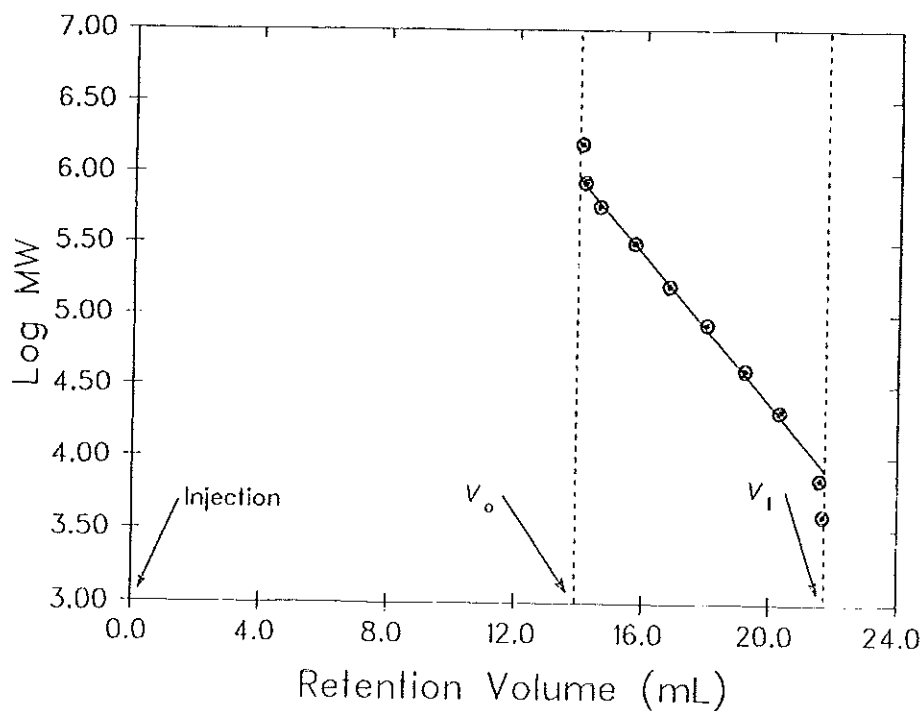


Figure 2 Typical SEC calibration curve: logarithm of molecular weight vs. retention volume.

polymer molecule can elute. The total permeation volume (V_t) represents the sum of the interstitial volume and the total pore volume. It is the point at which the smallest molecules in the sample mixture would elute. All SEC separation takes place between V_o and V_t . This retention volume domain is called the selective permeation range. In this figure the largest and smallest molecular weight species are too large and small, respectively, to be discriminated by this column and thus appear at the two extremes of the selective permeation range.

The capacity factor, k' , is an index used in chromatography to define the elution position of a particular chromatographic component with respect to the solvent front, which in the case of SEC occurs at V_t . Because all macromolecular separation in SEC occurs before V_t , k' is negative. In all other forms of liquid chromatography k' is positive. One consequence of this difference is that separation in SEC occurs over one column set volume (in the selective permeation range) whereas in other forms of high-performance liquid chromatography (HPLC) separation may occur over many column volumes. Thus components in a mixture analyzed by other HPLC forms are commonly baseline-resolved while SEC separations of macromolecules tend to be broad envelopes. It should be noted that it is not necessary to separate polymer molecules by the number of repeat units in order to determine the molecular weight distribution. (It is possible to resolve very low molecular weight components if a sufficient number of small pore size columns are utilized.) To understand how these differences come about one must consider the thermodynamics of chromatographic processes.

For any form of (gas or liquid) chromatography one can define the distribution of solute between the stationary and mobile phases by an equilibrium (2). At equilibrium the chemical potentials of each solute component in the two phases must be equal. The driving force for solute migration from one phase to the other is the instantaneous concentration gradient between the two phases. Despite the movement of the mobile phase in the system, the equilibrium exists because the solute diffusion into and out of the stationary phase is fast compared to the flow rate. Under dilute solution conditions the equilibrium constant (the ratio of solute concentrations in the stationary and the mobile phases) can be related to the standard Gibbs free energy difference between the phases at constant temperature and pressure:

$$\Delta G^\circ = -RT \ln K \quad (1)$$

and

$$\Delta G^\circ = \Delta H^\circ - T \Delta S^\circ \quad (2)$$

where ΔH° and ΔS° are the standard enthalpy and entropy differences between the phases, respectively. R is the gas constant and T is the absolute temperature.

In other modes of liquid chromatography (LC) the basis of separation involves such phenomena as partitioning, adsorption, or ion exchange, all of which are energetic in nature since they involve intermolecular forces between the solute and stationary phase. In such cases the free energy can be approximated by the enthalpy term alone since the entropy term is negligible and the equilibrium constant is given by

$$K_{LC} \simeq \exp(-\Delta H^\circ/RT) \quad (3)$$

The typical exothermic interaction between the solute and stationary phase leads to a negative enthalpy difference and hence a positive value for the exponent in Eq. (3). This, in turn, leads to an equilibrium constant greater than one and causes solute peaks to elute later than the solvent front.

In SEC the solute distribution between the two phases is controlled by entropy alone; that is, the enthalpy term is here taken to be negligible. In SEC the equilibrium constant becomes

$$K_{SEC} \simeq \exp(\Delta S^\circ/R) \quad (4)$$

The entropy, S , is a measure of the degree of disorder and can be expressed as (3)

$$S = k \ln \Omega \quad (5)$$

where k is the Boltzmann constant and Ω is the number of equally probable micromolecular states. The relative ability of a small and a larger macromolecule to access an individual pore greater in size than the larger molecule is depicted in Fig. 3. Here the number of ways in which the individual molecules can occupy space within the pore is given by the number of grid positions (representing

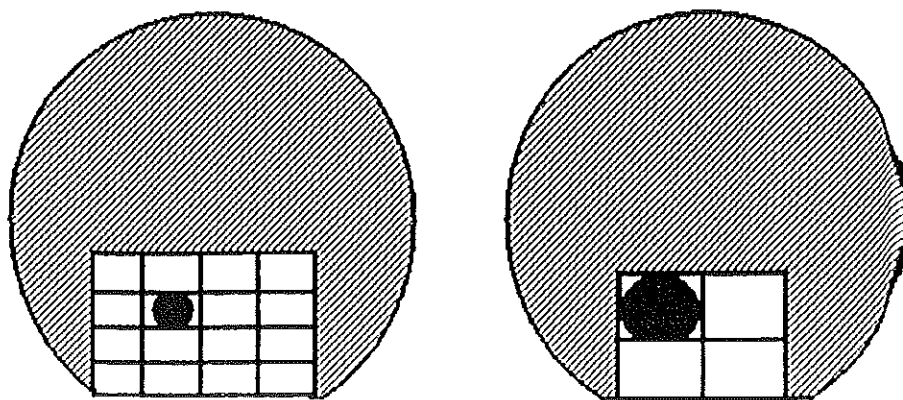


Figure 3 Entropy of macromolecular retention in a pore: the smaller molecule on the left has four times as many possibilities for retention as the molecule on the right.

individual states) allowed to them. The smaller molecule is retained longer within the pore than the larger one because its number of equally probable states is greater (and hence it possesses a larger entropy). Yet because the number of equally probable states is much smaller inside the pore than in the interstitial space for an individual molecule, solute permeation in SEC results in a *decrease* in entropy. This results in a negative exponent in Eq. (4). K_{SEC} is less than one and solutes elute before the solvent front. SEC is also inherently temperature independent, in contrast to the other liquid chromatographic separation phenomena, as can be seen by comparing Eqs (3) and (4). (Temperature does in fact have an indirect effect on SEC separations through its influence on the viscosity of polymeric solutions. The viscosity determines the mass transfer rate of polymer molecules into and out of the pores of the packing material and hence the elution of the sample.)

2 EXPERIMENTAL CONDITIONS FOR SEC

2.1 System Overview

A typical SEC system is essentially a specialized isocratic high-performance liquid chromatograph. An idealized schematic is presented in Fig. 4. First a solvent reservoir, typically 1–4 L in size, is filled with the SEC mobile phase. It is commonly sparged with helium or treated ultrasonically in order to degas it and prevent air

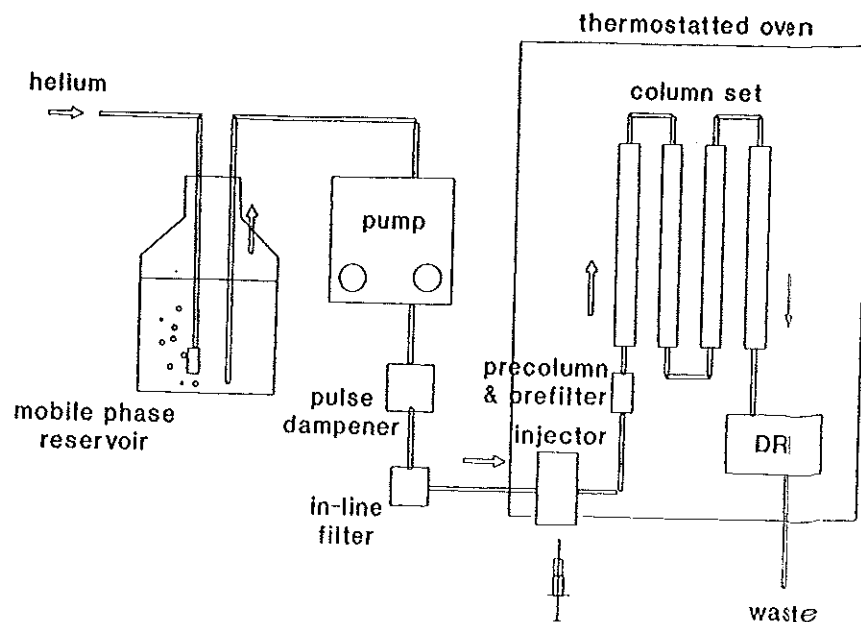


Figure 4 Schematic representation of a generic size exclusion chromatograph.

bubbles from entering the detector downstream. A high-pressure pump capable of operating at pressures up to 6000 psi forces the mobile phase through line filters and pulse dampeners to the sample injector where an aliquot of dilute polymer solution (prepared using the same mobile phase batch as contained in the reservoir) is introduced.

The sample, which initially exists as a narrow band in the system, is then carried through the precolumn and the analytical column set where molecular size discrimination occurs. The discriminated sample elutes from the column set and passes through a universal detector, which generates an electrical (mV) signal proportional to the instantaneous sample concentration. The sample and mobile phase then exit the detector and are carried to a waste container while the electrical signal is transmitted to an integrator, recorder, or computer for display and/or further processing.

2.2 Universal (Concentration) Detectors

The most common type of universal detector by far is the differential refractive index (DRI) detector. (Here, the word "universal" denotes the ability to respond to all chemical functionalities.) It senses differences in refractive index between a moving (sample-containing) stream and a static reference of mobile phase using a split optical cell. It responds well (at a moderate concentration level) to most polymeric samples provided that they are different in refractive index from the mobile phase in which they are dissolved. Despite the temperature independence of the SEC separation phenomenon, the DRI is highly temperature sensitive as a result of the strong temperature dependence of refractive index. Thus one normally maintains the DRI in a constant temperature oven along with the columns and injector (as in Fig. 4). The temperature chosen is at least 5–10°C above ambient.

It is generally assumed that the DRI's response is equally proportional to polymer concentration in all molecular weight regimes. Unfortunately this assumption breaks down at low molecular weights (less than several thousand atomic mass units (amu)) where the polymer end-groups represent a non-negligible portion of the molecules' mass and do change the refractive index. The DRI is also very sensitive to backpressure fluctuations due to variations in flow rate caused by the pump. This effect (especially of reciprocating piston pumps) is compensated for by the use of pulse dampeners as shown in Fig. 4.

Other common types of concentration detectors are the ultraviolet (UV) and infrared (IR) detectors. Neither are truly universal detectors, but they are able to respond to a variety of individual chemical functional groups (chromophores) provided that these functional groups are not contained in the mobile phase. The IR detector is slightly more sensitive than the DRI detector while the UV detector is several orders of magnitude more sensitive. The last is most commonly employed for polymers containing aromatic rings or regular backbone

unsaturation while the IR detector has been used largely to characterize polyolefins. Other less commonly utilized concentration detectors include the fluorescence, dielectric constant, flame ionization, and evaporative light scattering detectors.

2.3 Mobile Phase and Temperature

The mobile phase should be chosen carefully to fit certain criteria: it must completely dissolve the polymer sample in a continuous solution phase (non- θ condition), it must be low enough in viscosity in order for the SEC system to operate in a normal pressure range, and it must effectively prevent the polymer molecules from interacting energetically with the stationary phase (for example, through adsorption). Failure to achieve even one of these criteria would result in the inability of the system to properly characterize the sample. Temperature is a useful parameter to adjust when one or more of these conditions have not been met but where one is constrained to use a particular mobile phase. Certain polymers (for example, polyesters and polyolefins) may achieve dissolution only at elevated temperatures. The viscosity of inherently viscous mobile phases may also be lowered by raising the temperature.

The analysis of polymers containing one or more formal, like charges in every repeat unit (i.e., polyelectrolytes) incurs one additional requirement of the mobile phase. When solubilized in water, the repulsion of like charges along the polyelectrolyte chain causes it to take on an extended conformation (4). In order for normal SEC to be performed on a polyelectrolyte in an aqueous medium, its conformation must be made to reflect that of a random coil (Gaussian chain). This counteracting of the "polyelectrolyte effect" is generally accomplished by sufficiently raising the ionic strength with the use of simple salts and sometimes with concomitant pH adjustment. The former provides counterions to screen the like polymeric charges from one another and permits the extended chain to relax. The latter is used to neutralize all residual acidic or basic groups. (When fully charged these groups are no longer available to participate in hydrogen bonding interactions with the stationary phase.)

For example, it has been demonstrated that normal SEC behavior can be obtained for poly(methyl vinyl ether-co-maleic acid) with the use of a mobile phase consisting of a pH 9 buffer system (prepared from tris(hydroxymethyl)-aminomethane and nitric acid) modified with 0.2 M LiNO₃ (5). Halide salts should be completely avoided as they tend to corrode the stainless steel inner surfaces of the SEC system, which in turn causes injector fouling and column contamination.

2.4 Stationary Phases

When selecting an optimum stationary phase there are additional criteria to be met: the packing material should not interact chemically with the solute (i.e., the

sample), it must be completely wetted by the mobile phase but should not suffer adverse swelling effects, it must be stable at the required operating temperature, and it must have sufficient pore volume and an adequate range of pore sizes to resolve the sample's molecular weight distribution. For high-performance SEC, either semirigid polymeric gels or modified, rigid silica particles are typically used.

Columns are available from a number of vendors packed with monodisperse or mixed-bed pore size particles. The latter are useful for building a column set that will discriminate (usually on a log-linear basis) at least four molecular weight decades (i.e., several hundred to several million amu). For rigid particles it is also possible to design a column set consisting of individual columns of different, single pore sizes yielding a calibration curve log-linear in molecular weight if the pore size and total pore volume of each column type are known (6). Typical available pore sizes range from 60 to 4000 Å. High-performance packing materials generally have particle sizes in the range of 5 to 10 μm with efficiencies of several thousand theoretical plates per 15-cm column.

For organic mobile phases, the most common column packings are crosslinked (with divinylbenzene) polystyrene gels or trimethylsilane-derivatized silica. For aqueous mobile phases the most common are crosslinked hydroxylated polymethacrylate or poly(propylene oxide) gels (7) or glyceryl (diol) derivatized silica (8). In general, rigid packings have several advantages over semirigid gel packings: they are tolerant of a greater variety of mobile phases, they equilibrate rapidly on changing solvents, they are stable at the elevated temperatures required to characterize certain polymers, and their pore sizes are more easily defined, which facilitates column set design. Silica-based rigid packings are prone to adsorptive effects, however, and must be carefully derivatized to react away or screen labile silanol groups. An overview of typical column packing/mobile phase combinations has been recently published by Yau *et al.* (9). The reader is referred to comprehensive discussions of SEC stationary phases covered in Chapter 2 (semirigid polymeric gels) and Chapter 3 (modified, rigid silica) of this monograph.

2.5 Sample Size and Mobile Phase Flow Rate

Sample size is defined by both the volume of the aliquot injected as well as by the concentration of the sample solution. Use of excessively large sample volumes can lead to significant band broadening, resulting in loss of resolution and errors in molecular weight measurement. As a rule of thumb, sample volumes should be limited to one-third or less of the baseline volume of a monomer or solvent peak measured with a small sample (10). The optimum injection volume will be a function of the size and number of the columns employed but will generally range between 25 and 200 μL .

Sample concentration should be minimized consistent with the sensitivity of the concentration detector employed. The use of high sample concentrations can result in peak shifts to lower retention volumes and band broadening due to "viscous fingering" or spurious shoulders appearing on the tail of the peak. These phenomena are likely related to a combination of causes including chain entanglements and an inability to maintain the equilibrium between solute concentrations inside the pores and in the interstitial space. These effects are particularly problematic for high molecular weight polymers (of the order of one million amu). Optimum sample concentrations may range from 0.1% for high molecular weight samples to greater than 1.0% for low molecular weight samples.

Another unwanted viscosity effect, the shear degradation of high molecular weight polymers at high flow rates, which results in erroneous (larger) retention volumes and (lower) molecular weights, is avoided by minimizing the flow rate. In addition, the use of high flow rates can result in considerable loss of column efficiency because, under such conditions, mass transfer or diffusion in and out of the pores is not fast enough vis-à-vis the solute migration rate along the length of the column. Thus, flow rates in the general vicinity of 1 mL/min are most commonly employed for sets of SEC columns and represent a good compromise between analysis time and resolution. For single column separations, a flow rate of 0.5 ml/min is commonly used. The reader is referred to Chapter 5 (aqueous SEC) and Chapter 6 (nonaqueous SEC) of this monograph for comprehensive discussions of sample size and flow rate optimizations.

3 CALIBRATION METHODOLOGY AND DATA ANALYSIS IN SEC

In modern high-performance SEC there are only four commonly employed calibration methods. Three of these can be utilized in conjunction with a single (i.e., concentration) detector SEC system: direct (narrow) standard calibration, polydisperse or broad standard calibration, and universal calibration. The fourth type of SEC calibration requires the use of a second, molecular weight sensitive detector connected in series with the concentration detector (and in front of it in the case of the DRI). The purpose of calibration in SEC is to define the relationship between molecular weight (or typically its logarithm) and retention volume in the selective permeation range of the column set used and to calculate the molecular weight averages of the sample under investigation.

3.1 Direct Standard Calibration

In the direct standard calibration method, narrowly distributed standards of the same polymer under analysis are used. The retention volume at the peak maximum of each standard is equated with its stated molecular weight. While this is the

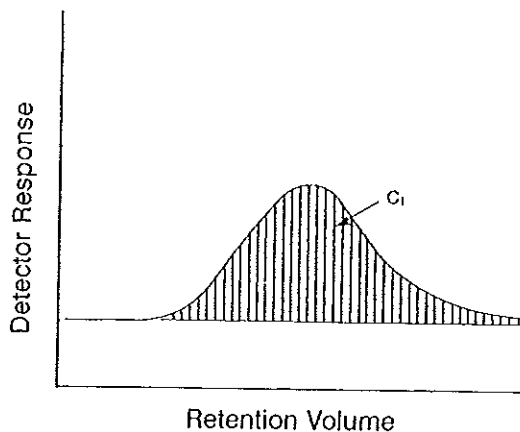


Figure 5 Time-sliced peak output from a concentration detector (DRI).

simplest method it is generally restricted in its utility owing to the lack of availability of many different polymer standard types. It also requires a sufficient number of standards of different molecular weights so as to completely cover the entire dynamic range of the column set or, at least, the range of molecular weights spanned by the samples' molecular weight distributions. Narrow standards currently available include polystyrene, poly(methyl methacrylate), poly(ethylene), (used for nonaqueous GPC), and poly(ethylene oxide) or poly(ethylene glycol), poly(acrylic acid), and polysaccharides (used in aqueous GPC) are common commercially available standards. It is instructive to study the mechanism of narrow standard calibration since all of the other methods are based upon it. A thorough review of this subject has been provided by Cazes (11).

In this approach, the raw chromatogram obtained as output from the concentration detector is divided into a number of time slices of equal width as depicted in Fig. 5. For a polydisperse sample the number of time slices must be greater than 25 for the computed molecular weight averages to be unaffected by the number of time slices used. (Most commonly available SEC data programs utilize a minimum of several hundred time slices routinely for each analysis.) An average molecular weight is assigned to each time slice based upon the calibration curve and it is further assumed for computational purposes that each time slice is monodisperse in molecular weight. A table is constructed with one row assigned to each time slice. The following columns are created for this table: retention volume, area (A_i), cumulative area, cumulative area percent, molecular weight (M_i), A_i divided by M_i , and A_i times M_i . The area column and the last two factors are also summed for the entire table.

Once this data table has been completed it is possible to compute the molecular weight averages or moments of the distribution. The most common

averages defined in terms of the molecular weight at each time slice and either the number of molecules, n_i , or the area of each time slice are as follows:

Number average:

$$\bar{M}_N = \frac{\sum_i n_i M_i}{\sum_i n_i} = \frac{\sum_i A_i}{\sum_i A_i/M_i} \quad (6)$$

Viscosity average:

$$\bar{M}_V = \left[\frac{\sum_i n_i M_i^{1+a}}{\sum_i n_i M_i} \right]^{1/a} = \left[\frac{\sum_i A_i M_i^a}{\sum_i A_i} \right]^{1/a} \quad (7)$$

where, a is the Mark-Houwink exponent.

Weight average:

$$\bar{M}_W = \frac{\sum_i n_i M_i^2}{\sum_i n_i M_i} = \frac{\sum_i A_i M_i}{\sum_i A_i} \quad (8)$$

“Z” average:

$$\bar{M}_Z = \frac{\sum_i n_i M_i^3}{\sum_i n_i M_i^2} = \frac{\sum_i A_i M_i^2}{\sum_i A_i M_i} \quad (9)$$

The dispersity or polydispersity, D , is given by the ratio of the weight to the number average molecular weight and is a measure of the breadth of the molecular weight distribution. The SEC number, viscosity, weight, and “Z” averages correspond to those obtained classically by osmometry, capillary viscometry (intrinsic viscosity), light-scattering photometry, and sedimentation equilibrium methods, respectively. The viscosity average molecular weight approaches the weight average as the Mark-Houwink exponent, a (described in Sec. 3.4 of this chapter), approaches one. (See the subsequent discussion concerning universal calibration.) The “Z” and weight average molecular weights are most influenced by the high molecular weight portion of the distribution whereas the number average is influenced almost exclusively by the low molecular weight portion. Narrow standards employed in this calibration method are ideally monodisperse but practically must have dispersities less than 1.1.

3.2 Band Broadening Measurement and Correction

It is important to review the molecular weight distribution generated for symmetric and unsymmetric band broadening that will result in non-negligible errors in computed molecular weight averages. An American Society for Testing and Materials (ASTM) method describes a procedure to calculate the magnitude of these effects and to correct the molecular weight averages (12). It is necessary to know both \bar{M}_W and \bar{M}_N for each standard of the entire series of narrow standards

used. The symmetric band broadening factor, Λ , is calculated for each standard according to

$$\Lambda = \frac{1}{2} \left[\frac{\bar{M}_N(t)}{\bar{M}_N(u)} + \frac{\bar{M}_W(u)}{\bar{M}_W(t)} \right] \quad (10)$$

The skewing or unsymmetric factor, sk , is calculated according to

$$sk = \frac{\Phi - 1}{\Phi + 1} \quad (11)$$

where

$$\Phi = \frac{\bar{M}_N(t)\bar{M}_W(t)}{\bar{M}_N(u)\bar{M}_W(u)} \quad (12)$$

and t and u refer to the true and uncorrected moments. Under ideal conditions, $\Lambda = 1$ and $sk = 0$ and no corrections are necessary. Practically this is never the case but if these values are 1.05 and 0.05 or less, respectively, then the resulting corrections are small and can be ignored. If, on the other hand, they are larger than these values, the sample's distribution moments may be corrected according to

$$\bar{M}_N(t) = \bar{M}_N(u)(1 + sk)\Lambda \quad (13)$$

and

$$\bar{M}_W(t) = \frac{\bar{M}_W(u)}{(1 - sk)\Lambda} \quad (14)$$

A description of the correction for band broadening of the entire molecular weight distribution is beyond the scope of this introduction to SEC but the interested reader is referred to the technique described by Tung (13,14). A better approach is to employ sufficiently good experimental practices so as to obviate the need for band spreading corrections altogether. This has been demonstrated when sufficiently long column lengths and low flow rates are used (15).

3.3 Polydisperse or Broad Standard Calibration

In the polydisperse standard method one employs a broadly distributed polymer standard of the same chemical type as the sample. The sample and the standard are frequently the same material. The main requirements of this technique are that the MWD of the standard must span most if not all of the sample's dynamic range and that two moments of the standard's distribution, \bar{M}_N and either \bar{M}_W or \bar{M}_V , must be accurately known as a result of ancillary measurements. This method is particularly useful when narrow MWD standards and molecular weight sensitive detectors are unavailable and universal calibration is impractical due to lack of

information regarding appropriate Mark-Houwink coefficients and/or the inability to perform intrinsic viscosity measurements.

Balke, Hamielec et al. described a computer method to determine a calibration curve expressed by

$$V_e = C_1 - C_2 \log_{10} M \quad (15)$$

where V_e is the elution (or retention) volume and M is the molecular weight (16). Their original method involved a cumbersome, simultaneous search for the constants C_1 and C_2 , which was prone to false convergence. Revised methods featured a sequential, single-parameter search (17,18). These methods rely on the fact that the dispersity, D , is a function of the slope, C_2 , alone. Arbitrary values are first assigned to the two constants. The resulting calibration equation is iteratively applied to the time slice data while the slope value is optimized to minimize the difference between the true and computed dispersities. Once the slope has been determined it is fixed and the intercept, C_1 , is optimized to minimize the difference between the true and computed moments (either individually or their sum).

3.4 Universal Calibration

Benoit and co-workers demonstrated that it is possible to use a set of narrow polymer standards of one chemical type to provide absolute molecular weight calibration to a sample of a different chemical type (19,20). In order to understand how this is possible, one must first consider the relationship between molecular weight, intrinsic viscosity and hydrodynamic volume, the volume of a random, freely jointed polymer chain in solution. This relationship has been described by both the Einstein-Simha viscosity law for spherical particles in suspension

$$[\eta] = C \left(\frac{V_h}{M} \right) \quad (16)$$

and the Flory-Fox equation for linear polymers in solution

$$[\eta] = \Phi \left(\frac{\langle s^2 \rangle^{3/2}}{M} \right) \quad (17)$$

where $[\eta]$ is the intrinsic viscosity, V_h is the hydrodynamic volume, $\langle s^2 \rangle^{1/2}$ is the root-mean-square radius of gyration of the polymer chain, and C and Φ are constants (21). If either equation is multiplied by M , the molecular weight, the resulting product, $[\eta]M$, is seen as proportional to hydrodynamic volume. (Note that the cube of the root-mean-square radius of gyration is also proportional to volume.) Benoit and co-workers plotted this product versus elution volume for a number of chemically different polymers investigated under identical SEC

conditions and found that all points lay on the same calibration curve (19,20). This calibration behavior was said to be "universal" for all the polymer types studied.

In actual practice one would establish the following relationship

$$[\eta]_1 M_1 = [\eta]_2 M_2 \quad (18)$$

where the subscripts 1 and 2 refer to the standard and sample polymers, respectively. Even if the intrinsic viscosities are known or can be measured for each standard, it is unlikely that the value of intrinsic viscosity would be known for each time slice in the molecular weight distribution of the sample polymer. Thus, Eq. (18) must be further modified to make it more useful. This can be accomplished with the use of the Mark-Houwink equation

$$[\eta] = KM^a \quad (19)$$

where the coefficient, K , and exponent, a , are known as the Mark-Houwink constants. These constants are a function of both the polymer and its solvent environment (including temperature). If the constants are available from the literature or can be determined for the sample polymer using narrow fractions in the SEC mobile phase, then one can substitute the Mark-Houwink term for $[\eta]$ into Eq. (18) to yield

$$\log_{10} M_2 = \frac{1}{1+a_2} \log_{10} \frac{K_1}{K_2} + \frac{1+a_1}{1+a_2} \log_{10} M_1 \quad (20)$$

which is an expression for the sample molecular weight in terms of the standard molecular weight and both sets of Mark-Houwink constants.

3.5 Molecular Weight Sensitive Detectors

It is possible to add a second molecular weight sensitive detector to an SEC system in order to provide a direct means of absolute molecular weight calibration without the need to resort to external standards. These detectors represent refinements in classical techniques such as light-scattering photometry, capillary viscometry (for intrinsic viscosity), and membrane osmometry for on-line molecular weight determination. Yau has published a review of this subject with comparisons of the properties and benefits of the principal detectors currently in use (22). The present discussion will be restricted to light-scattering and viscometry detectors. The reader is referred to Chapter 4 of this monograph for a comprehensive discussion of molecular weight sensitive detectors.

3.5.1 Low Angle Laser Light Scattering Detection

The low angle laser light scattering detector (LALLS or LALS) was originally developed by Kaye (23,24) and was formerly marketed by Chromatix and LDC

Analytical. Two models, the KMX-6 and the CMX-100, are no longer commercially available. Although the former was said to be capable of a small scattering angle variation, both units were essentially fixed, low angle photometers. Overviews of the basic operating principles were provided by McConnell (25) and Jordan (26). A low angle laser light scattering detector is still offered, however, by Viscotek in the Triple Detector Array (see below).

The working equation for the determination of the weight average molecular weight by light scattering (using unpolarized light), due to Debye, is

$$\frac{Kc}{\Delta R_\theta} = \frac{1}{\bar{M}_w P(\theta)} + 2A_2C \quad (21)$$

where the constant, K , is given by

$$K = \frac{2\pi^2 n^2}{N_0 \lambda^4} \left(\frac{dn}{dc} \right)^2 \quad (22)$$

and N_0 is Avogadro's number, n is the refractive index of the solution at the incident wavelength λ , and A_2 is the second virial coefficient, a measure of the compatibility between the polymer solute and the solvent. The term dn/dc is known as the specific refractive index increment and reflects the change in solution refractive index with change in solute concentration. The term ΔR_θ is called the excess Rayleigh ratio and represents the solution ratio of scattered to incident radiation minus that of the solvent alone. The particle scattering function, $P(\theta)$, which is the angular dependence of the excess Rayleigh ratio, is defined by

$$\frac{1}{P(\theta)} = 1 + \frac{16\pi^2}{3\lambda^2} \langle s^2 \rangle \sin^2(\theta/2) \quad (23)$$

where $\langle s^2 \rangle$ is the mean-square radius of gyration of the polymer chain. The Debye equation [Eq. (21)] is actually a virial equation which includes higher power concentration terms; these higher terms can be neglected if the concentrations employed are small.

In the classical light scattering experiment one solves the Debye equation over a wide range of angles and concentrations for unfractionated polymer samples. The data are plotted in a rectilinear grid known as a Zimm plot in which the ordinate and abscissa are $Kc/\Delta R_\theta$ and $[\sin^2(\theta/2) + kc]$, respectively, where k is an arbitrary constant used to adjust the spacing of the data points (27). The Zimm plot yields parallel lines of either equal concentration or angle. The slope of the $\theta = 0$ line yields $\langle s^2 \rangle$ while that of the $c = 0$ line yields A_2 . The intercept of either of these lines is \bar{M}_w . One of the major problems associated with classical light scattering experiments relates to the effect of dust: if the entire solution contained in the large cell volume typically used is not kept scrupulously free of dust, large scattering errors can result.

The LALLS device developed by Kaye provides three significant changes that make it amenable as an SEC molecular weight detector: an intense, monochromatic light source (a HeNe laser, $\lambda = 632.8$ nm) is used, the cell volume is reduced to $10 \mu\text{L}$ and the scattering volume to $0.1 \mu\text{L}$ (26), and the single scattering angle employed is in the range of $2-7^\circ$. The net result is that the device is extremely sensitive; it can readily distinguish scattering due to an individual dust particle flowing through the cell from that due to the sample, and the angular dependence is removed from the Debye equation. The latter follows from the fact that the value of $\sin^2(\theta/2)$ for a small angle is essentially zero. Under this condition the Debye equation becomes

$$\frac{Kc}{\Delta R_\theta} = \frac{1}{\bar{M}_w} + 2A_2C \quad (24)$$

or

$$\bar{M}_w = \frac{1}{Kc/\Delta R_\theta - 2A_2C} \quad (25)$$

and \bar{M}_w can be obtained at a single finite concentration provided that A_2 is known from the literature or is determined from the slope of Eq. (24) using a series of concentrations. However, the removal of the angular variability from the LALLS detector means that it cannot be used to determine molecular size, that is, $\langle s^2 \rangle$.

The SEC/LALLS experiment is then conducted as follows. The LALLS and concentration detectors are connected in series after the SEC column set and interfaced with the computing system. Time slice data from both detectors is acquired, as shown in Fig. 6, so as to have corresponding time slices in each distribution. In order to accomplish this the time delay between the detectors must be accurately known. The instantaneous concentration in either detector, c_i , may be computed using

$$c_i = \frac{mA_i}{V \sum_i A_i} \quad (26)$$

where m is the sample mass injected, V is the effluent volume passing through the cell in the time of a single time slice, and A_i is the area of a concentration detector time slice. If one assumes that each time slice is sufficiently narrow so as to be monodisperse, then the instantaneous molecular weight is determined using Eq. (25). This data collectively constitute the absolute molecular weight distribution calibration.

It is generally acknowledged that LALLS used either as a stand-alone light-scattering photometer or as an SEC detector provides accurate values for \bar{M}_w . Yet in 1987 a number of independent workers reported that the ability of SEC/LALLS to accurately determine \bar{M}_N was dependent on the polydispersity of the sample: the

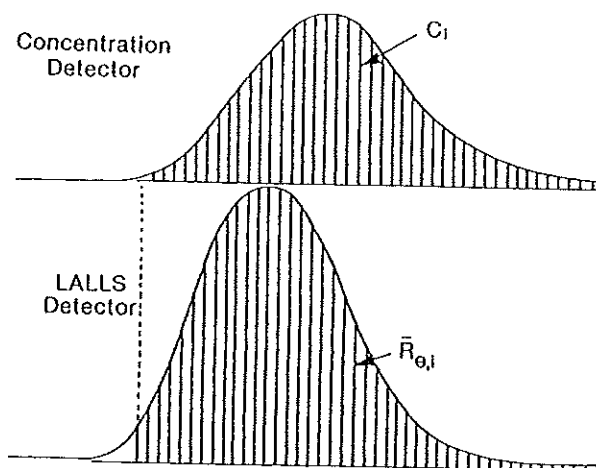


Figure 6 Overlay of time-sliced peak output from a dual (DRI/LALLS) detector system.

greater the polydispersity, the poorer the estimate of \bar{M}_N (28–30). In performing SEC/LALLS on high molecular weight poly(vinyl pyrrolidone), Senak *et al.* (28) demonstrated that this phenomenon is caused by the lack of sensitivity of the LALLS detector toward the low molecular weight portion of a broad distribution ($D = 6.0$). As shown in Fig. 7, the DRI detector is still responding (the shaded area) in a region where the LALLS detector is not. As discussed by Hamielec *et al.*,

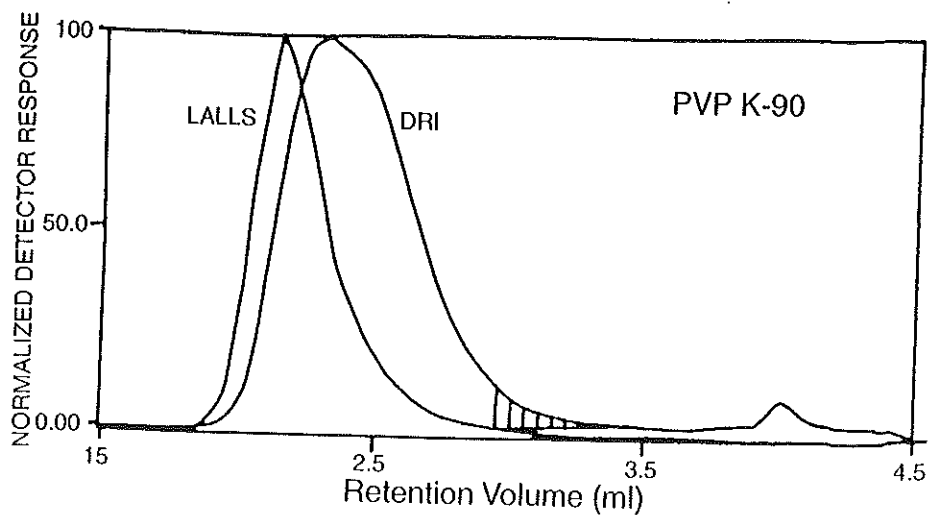


Figure 7 Relative sensitivity of a LALLS vs. a DRI detector for a broadly dispersed sample of poly(vinyl pyrrolidone).

an electronic switching device and a technique for optimizing the signal-to-noise ratio of the LALLS detector throughout the LALLS chromatogram is needed to improve its utility (31).

The LALLS detector coupled to an SEC has also been reported to be useful in measuring the relative amount of branching of a branched relative to a linear polymer of the same chemical type (32-34). The parameter of interest is g_M , defined by Zimm and Stockmayer (35) as

$$g_M = \frac{\langle s^2 \rangle_b}{\langle s^2 \rangle_l} = \frac{[\eta]_b}{[\eta]_l} M \quad (27)$$

or the ratio of the mean-square radii of gyration of a branched to a linear polymer at a constant molecular weight and, through the Flory-Fox equation [Eq. (17)], the ratio of their intrinsic viscosities (35). The measured quantity in the SEC/LALLS experiment, however, is g_V , the branching index at constant elution volume: the ratio of molecular weights of branched to linear polymers. It has been shown that the Mark-Houwink equation [Eq. (19)] can be used to convert g_V to g_M to give

$$g_M = g_V^{a+1} = \left(\frac{M_l}{M_b} \right)_V^{a+1} \quad (28)$$

where a is the Mark-Houwink exponent of the linear polymer (32,33). In principle, the variation in the branching index can be determined as a function of molecular weight provided that the exponent, a , is known. Complications may arise if there is significant band broadening in the SEC system and/or if the samples are highly polydisperse as previously discussed. It must be emphasized that the ability of the SEC/LALLS to produce branching information is strictly due to the discrimination of molecular size by the SEC column set since LALLS has no molecular size capability itself.

3.5.2 Multi-Angle Laser Light Scattering Detection

The multi-angle laser light scattering detectors (MALLS or MALS) developed and produced by Wyatt Technology Corp. (Santa Barbara, California), (the models DAWN B and DAWN F, and currently the EOS), unlike LALLS, have the ability to measure scattered light at either 15 (23-128°) or 18 (5-175°) different angles depending upon the model selected (36,37). In addition, these data can be obtained simultaneously using an array of detectors. The mathematics employed is essentially based upon Eqs (21) to (23). One of the capabilities of this instrument is the determination of polymer radius of gyration distribution when used as an on-line SEC detector. Used off line this instrument is capable of producing Zimm plots supplying weight-average molecular weight, radius of gyration, and second virial coefficient information. The ability of MALLS to make this measurement

accurately for very large and very small polymer molecules has been disputed (38,39). Other MALLS instruments are available from Polymer Laboratories (Shropshire, U.K.) which offers a dual angle (15° and 90°), which is also available with a dynamic (quasielastic) light scattering detector as an option, and from Brookhaven Instruments (Holtville, New York, U.S.A.) who offers an array of seven detectors in their MALLS unit. For a complete discussion of MALLS the reader is referred to Chapter 21.

3.5.3 Right-Angle Laser Light Scattering Detection and Triple Detection

At the 1991 International GPC Symposium (San Francisco, California) M. Haney of Viscotek Corp. introduced a new laser light scattering detector (RALLS), which operates at a fixed angle of 90° (40). Because the particle scattering function, $P(\theta)$, cannot be neglected at this angle (for large molecules), this device must be used in conjunction with another molecular weight sensitive detector (that is, a viscosity detector) and a concentration detector in order to yield absolute molecular weight information. An iterative calculation is performed on each chromatogram time slice using a simplified form of the Debye equation [Eq. (21)], the Flory-Fox equation [Eq. (17)] and the particle scattering function equation [Eq. (23)]. The convergence condition used is no further change in either molecular weight, radius of gyration, or $P(\theta)$. Viscotek claims an inherently better signal-to-noise ratio (due to lower noise) for the RALLS detector vs. either LALLS or MALLS operating at close to 0°. The use of a three detector array such as RALS, viscosity, and RI (as a concentration detector) is referred to as "Triple Detection." The current configuration of the Triple Detection instrument includes RALS, LALS and viscosity as molecular weight sensitive detectors. Also offered in this design are RI and UV as universal or concentration dependent detectors.

3.5.4 Viscometric Detection

An alternative type of molecular weight sensitive detector is the on-line viscometer. All of the current instrument designs depend upon the relationship between pressure drop across a capillary through which the polymer sample solution must flow and the viscosity of that solution. This relationship is based upon Poiseuille's law for laminar flow of incompressible fluids through capillaries:

$$\eta = \frac{\pi \Delta P r^4 t}{8 V l} \quad (29)$$

where η is the absolute viscosity, ΔP is the observed pressure drop, t is the efflux time, and r , l , and V are the radius, length, and volume of the capillary, respectively. In a capillary viscometer operating at ambient pressure, one can define the relative viscosity, η_r , as the ratio of the absolute viscosities of solution to solvent, which is equal to the ratio of their efflux times at low concentrations.

Yet when such a capillary is used as an SEC detector, the flow time is constant and the relative viscosity becomes

$$\eta_r = \frac{\eta}{\eta_o} = \frac{\Delta P}{\Delta P_o} \quad (30)$$

the ratio of the solution to solvent pressure drops. Since the intrinsic viscosity, $[\eta]$, is defined as

$$[\eta] = \lim_{c \rightarrow 0} \left(\frac{\ln \eta_r}{c} \right) \quad (31)$$

one can combine Eqs (30) and (31) to give

$$[\eta] = \frac{\ln(\Delta P/\Delta P_o)}{c} \quad (32)$$

provided that c is very small. (It is generally less than 0.01 g/dL under SEC conditions.)

Thus an on-line viscosity detector is capable of providing intrinsic viscosity distribution information directly using time slicing analogous to laser light-scattering detection. In order to act as a molecular weight detector, however, one must either obtain the Mark-Houwink constants in order to use the Mark-Houwink equation or possess a set of molecular weight standards that obeys the universal calibration behavior. If both intrinsic viscosity and absolute molecular weight information are available for each time slice, the Flory-Fox equation may be employed to generate a similar distribution for the mean-square radius of gyration (22).

A single capillary detector developed by Ouano (41) and further advanced by Lesec and colleagues (42-44) and Kuo *et al.* (45) has been internally incorporated into the Millipore/Waters model 150 CV SEC system. Chamberlin and Tuinstra developed a single-capillary detector that was directly incorporated within a conventional DRI detector (46,47). Haney developed a four-capillary detector with a Wheatstone bridge arrangement, which was commercialized by Viscotek Corp. (48,49) and further evaluated by other workers (50,51). A dual, consecutive capillary detector developed by Yau (22) (and also commercialized by Viscotek Corp.) was said to be superior to the other designs because it was better able to compensate for flow rate fluctuations: its series arrangement would cause the two capillaries to be simultaneously and equally affected, thus exactly offsetting any disturbance.

4 GENERAL REFERENCES

The interested reader is referred to several additional general references for supplemental information on the principles of SEC separations and selected applications. The first four (52-55) are compilations of papers presented by leading authorities at various International GPC Symposia sponsored by Waters Associates (Milford, Massachusetts). The next two volumes (56,57) are introductory books published by two other HPLC/SEC vendors. Finally, an early monograph edited by J. J. Kirkland (58) contains an excellent introductory chapter on GPC (SEC). Although all of these books are relatively old, they nevertheless contain valuable information that is still applicable and useful today.

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