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GPC Glossary**

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One of the most common and valuable tools employed for the analysis and characterization of polymers is size exclusion chromatography (SEC). The term gel permeation chromatography (GPC) is used when working with polymers soluble in polar organic or organic solvents, whereas in gel filtration chromatography (GFC) an aqueous mobile phase is used. SEC, GPC, and GFC all refer to the same chromatographic technique. GPC is normally used as an analytical procedure for separating natural and synthetic polymer molecules by their difference in size and to obtain molar mass averages (M_n, M_w, and M_z) or information on the molar mass distribution (MMD) through the application of calibration curves.

This glossary lists terms relative to GPC and polymer characterization and was compiled from various experts and resources in the area of SEC. It is meant to serve as an initial guide for those entering the field of GPC and as a point of reference for those already in the field.

Continuing with a tradition of excellence that began with the introduction of one of the first SEC HPLC columns in 1976, Tosoh Bioscience expanded our product portfolio in 2008 to include a dedicated system for GPC analysis, the EcoSEC GPC system. Excellent reproducibility of retention times, unsurpassed efficiency, and unmatched baseline stability are hallmarks of the system.

The Tosoh Bioscience website offers an all-inclusive collection of material related to the EcoSEC GPC System, including a GPC applications database for you to check to see if our laboratories have analyzed a particular polymer that is also run in your lab or that you may be analyzing at some future date. Also visit our website for dates of GPC Training Courses and Polymer Characterization Workshops we offer. If you want to learn more about the EcoSEC GPC System, you can request a lunch and learn event: your location, your applications, our cost.

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GLOSSARY

A

Absolute molar mass: The molar mass of a macromolecule determined in a calibrant-independent fashion (*e.g.*, without the comparison to known standards or reference materials via a calibration curve.) The absolute molar mass for a given macromolecule is independent of solvent and temperature conditions and typically determined using light scattering in conjunction with a concentration-sensitive detector.

Absorption: A mode of interaction chromatography where the sample components diffuse into the interior of the stationary phase. In ideal size exclusion chromatography, absorption effects are negligible.

Adsorption: A mode of interaction chromatography where the sample components are attracted to the surface of the solid stationary phase. In ideal size exclusion chromatography, adsorption effects are negligible.

Aggregation: The non-covalent accumulation, clustering, or clumping of molecules in solution.

Alternating copolymer: A copolymer comprised of two different monomers arranged alternately along the polymer chain.

^^^A-B-A-B-A-B-A-B-A-B-A-B-A^^^

Analyte: Any compound of interest in a chemical or physical analysis.

Analytical size exclusion chromatography column: A cylindrical tube constructed of stainless steel, glass, glass-lined stainless steel, PEEK, or other metallic and nonmetallic materials packed with a porous polymer medium varying in particle and/or pore size used for qualitative and quantitative analysis. Traditional size exclusion chromatography columns are 30 cm in length while semi-micro columns are 15 cm in length. The internal diameter of a column can vary; some typical values for the internal diameter are 4.6, 6.0, or 7.8 mm.

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Architecture: A term used to describe both the conformation and topology of a polymer.

Asymmetry factor: A measure of peak tailing in a chromatogram. The asymmetry factor *As* is calculated from a chromatographic peak by dropping a perpendicular line at the peak apex and a horizontal line at 10% of the peak height. From the intersection of the two lines, the distance to the front of the peak along the horizontal line (distance B) divided by the distance along the horizontal line to the tail of the peak (distance A) results in a ratio called the peak asymmetry factor. A symmetric Gaussian peak will have a value of 1 and tailed peaks will have a value greater than 1.
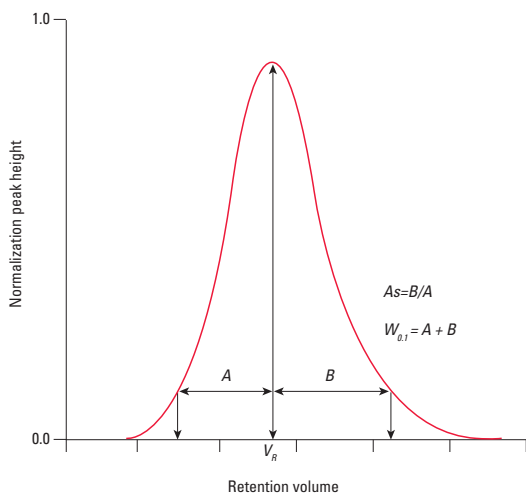
Figure 1: Peak asymmetry factor (Adapted from ref. 1)

Autocorrelation function: The average of the scattering intensities at two times, I(t) and I(t+τ), separated by a period (delay time) τ. The autocorrelation function is mathematically defined as $\langle I(t)I(t+\tau)\rangle$. In dynamic light scattering the intensity of the autocorrelation function is an indication of the time-dependent fluctuations a molecule undergoes.

B

Backpressure: The backpressure of the column is the difference in pressure between the inlet and outlet of a column, usually measured in pounds per square inch (psi). The backpressure of a system includes the amount of pressure created due to the system tubing, chromatography column, and on-line detector(s). Significant backpressure on a chromatography system can be a result of a clogged column or tubing.

Band broadening: The process that occurs in a chromatography column as the finite analyte band that is injected at the top of a column moves through the column. During this process the width of the band increases and the sample solution in the band becomes increasingly more dilute, forming semi-Gaussian bands. The broadening of solutes is typically described by the number of theoretical plates N, or the height-equivalent to a theoretical plate H.
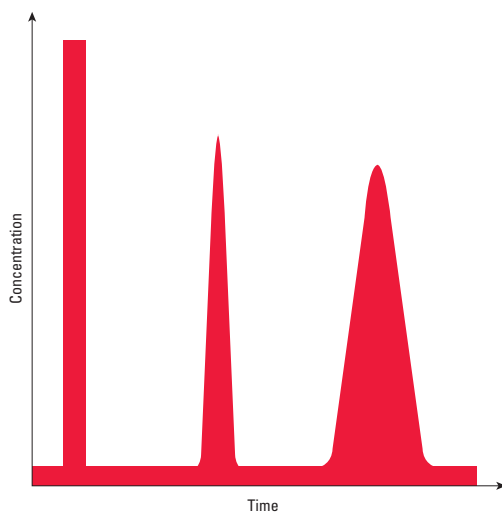
Figure 2: Broadening of a solute band as it travels through the chromatography column, forming semi-Gaussian bands

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Baseline drift: A steady movement, either positive or negative, of the baseline.

Baseline noise: Irregular fluctuations in the baseline of the chromatogram due to components of the instrument, temperature inconsistences, or variations in mixed solvent systems.

Baseline: The part of a chromatogram representing any time period during which only mobile phase is passing through the detector. The baseline of a chromatogram is defined by drawing a line from a point prior to the elution of an analyte to a point following the elution of the analyte, solvent, and system peaks. The baseline also represents the point from which integrations are made on peaks to determine peak area or peak height.

Berry plot: A variation of the Zimm plot used for the determination of molar mass and molecular size of macromolecules via light scattering. In a Berry plot the ordinate is the square root of $K*c/R(\theta)$ and the abscissa remains $\sin^2(\theta/2) + kc$. A Berry plot often provides a better fit to the data than a Zimm plot for branched polymers or for a polymer solution at good solvent-temperature conditions.
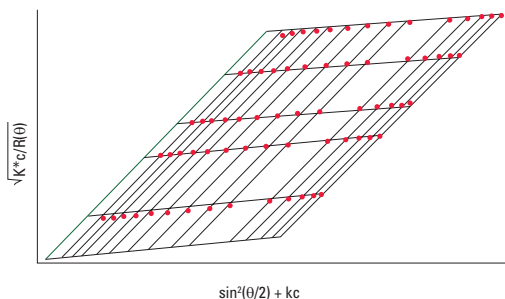

Figure 3: Berry plot, for a series of different but known concentrations measured at many angles

Biopolymer: A polymeric substance that is formed in a biological system. Biopolymers include proteins, nucleic acids, polynucleotides, polypeptides, and polysaccharides.

Block copolymer: A linear copolymer in which the different monomer units exist only in long sequences, or blocks, of the same type. Two common block copolymers are AB di-block and ABA tri-block copolymers.

   ^^^A-A-A-A-A-A-A-A-A-A-B-B-B-B-B-B-B-B-B-B^^^   (di-block)

   ^^^A-A-A-A-A-A-A-B-B-B-B-B-B-B-A-A-A-A-A-A^^^   (tri-block)

Branching index: A parameter, *g*, characterizing the effect of long-chain branches on the size of a branched macromolecule in solution. The branching index is defined as the ratio of the mean-square radius of gyration of a branched molecule $\langle r_b \rangle^2$ to that of an identical linear molecule $\langle r_l \rangle^2$ with the same relative molar mass in the same solvent and at the same temperature.

$$g = \frac{\langle r_b \rangle^2}{\langle r_l \rangle^2}$$

Branched polymer: A polymer that is composed of a main chain with one or more substituent side chains or branches. These types of polymers are characterized in terms of the number and size of the branches.

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C

Calibration curve: A plot with the retention time or retention volume on the abscissa and the log of the molar mass on the ordinate that is used for the determination of the molar mass of an unknown sample at each elution slice. Constructing a calibration curve to obtain the molar mass distribution of an unknown sample requires the following procedure: (a) narrow polydisperse standards of known molar mass are analyzed by size exclusion chromatography with a concentration-sensitive detector; (b) peak apexes of chromatograms of standards are assigned molar mass values consistent with the molar mass provided by the manufacturer and a calibration curve is constructed from the relation between retention volume and molar mass of the standards; (c) an unknown sample (which needs to be in the linear region of the calibration curve) is analyzed on the same system under the same experimental conditions as were the calibration standards; (d) elution profile of the unknown is "reflected" off the calibration curve onto the molar mass axis, to obtain the molar mass distribution of the unknown.[2]
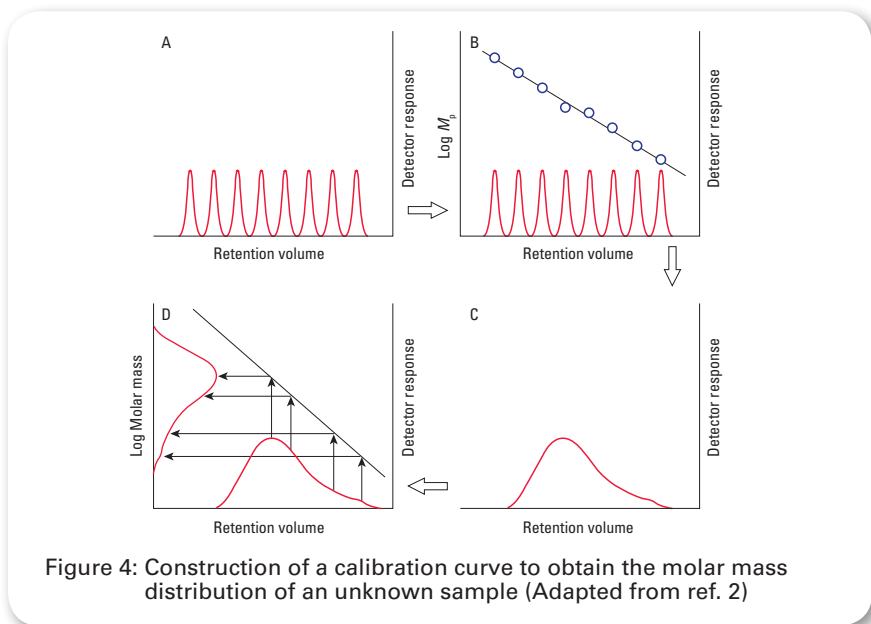
Figure 4: Construction of a calibration curve to obtain the molar mass distribution of an unknown sample (Adapted from ref. 2)

GLOSSARY

Calibration standards: A reference material with an accurately known molar mass, *e.g.* determined by absolute detection methods, that is used to create a calibration curve or calibrate a detector.

Chemical composition: The relative amounts (mole or weight fractions) of the different chemical functionalities in a polymer.

Chemical detection methods: Detectors that provide information which combines additively with that from other detectors, such as ultraviolet (UV), infrared (IR), or nuclear magnetic resonance (NMR) spectroscopy.

Chemical heterogeneity: The difference in the relative percentage of monomers among copolymeric chains of different molar mass.[3] The chemical heterogeneity affects the toughness, brittleness and biodegradability of a polymer.

Chromatogram: A plot of the detector signal versus retention time or retention volume showing the results of a chromatographic separation.

Colligative property: Properties of solutions that depend on the number of dissolved particles in solution and not on the identity of the particles.

Colloid: A homogenous mixture consisting of dispersed particles that have some linear dimension between 10^{-9} m and 10^{-6} m.

Column coupling: Connecting multiple columns of the same or various pore sizes together for optimization of a separation. The coupling of multiple columns of the same pore size can increase the resolution of the separation. Coupling columns of different pore sizes can increase the separation range and resolution. In the coupling of columns, there has been some debate over which column to place first in the series; most recently it has been shown that column order does not matter.[4]

Column length: The length (L) of a chromatography column. Typically size exclusion chromatography columns have a length of 15 or 30 cm.

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Column selection: The selection of a column for a size exclusion chromatography experiment is based largely on the required molar mass range of the separation and the nature of the sample solvent combination. Selection factors may also include column availability and column coupling.

Comb polymers: A type of copolymer that consists of a main chain with two or more three-way branch points from which linear side chains protrude.

Concentration-sensitive detectors: Detectors that measure the concentration of analyte at each slice eluting from the column. Common concentration-sensitive detectors, include differential refractive index (DRI) detectors, UV-Vis detectors, and evaporative light scattering or evaporative mass detector (ELSD or EMD).

Conformation: The spatial structure of a macromolecule in dilute solution where, depending on solvent-temperature conditions, the polymer may adopt random coil, compacted sphere, or highly extended conformations or any conformation between these.

Conformation plot: A plot of the polymeric size versus the molar mass of a polymer, with each axis on a logarithmic scale, (i.e., the plot of $\log R_G$ versus $\log M$). The slope of a conformation plot, α, is directly related to the conformation of the macromolecule in dilute solution. For a rigid rod, a one-dimensional object $\alpha=1$, for a flat disk, a two-dimensional object $\alpha=1/2$, and for homogeneous sphere of constant density, a three dimensional object $\alpha=1/3$.

Contour length: The length of the chain along the backbone of a polymer. For a chain of n backbone bonds, each of length l, the contour length is $n \times l$.

Copolymer: A polymer composed of two or more different repeat units or monomers.

Copolymer composition: The relative amounts of the different monomers or repeat units in a copolymer.

GLOSSARY

Critical overlap concentration: The upper limit of sample concentration that represents a boundary between near-infinitely dilute and semi-dilute solutions. When using molar mass-sensitive detection methods such as static light scattering or differential viscometry, accurate calculations of the molar mass of a polymer is dependent on the polymer solution being at near-infinitely dilute concentration, thus sample concentrations must be below the critical overlap concentration, c^*.

$$c^* = \frac{1}{A_2 M_w} \quad or \quad c^* = \frac{1}{[\eta]}$$

A_2: Second virial coefficient

M_w: Weight-average molar mass

$[\eta]$: Intrinsic viscosity

Crosslinking: A structure within a polymer that occurs when a bond is formed either between different polymer chains or between different parts of the same polymer chain.

Cumulative molar mass distribution: A pictorial representation of the distribution of the molar mass of a sample, *e.g.* what fraction of a sample has a molar mass within a certain range. The cumulative or integral molar mass distribution is obtained by plotting the cumulative weight fraction or the % cumulative weight fraction on the ordinate and the log of the molar mass on the abscissa.
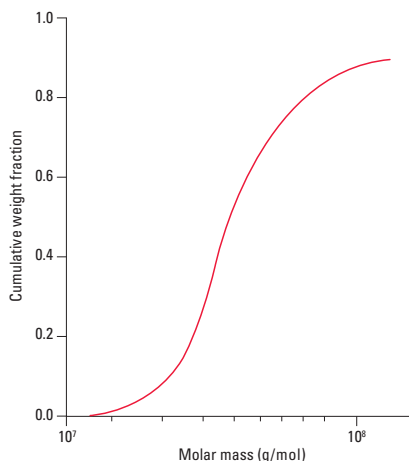
Figure 5: Cumulative molar mass distribution

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D

Deborah number: The ratio of hydrodynamic forces to Brownian forces, or the ratio of the longest relaxation time of the polymer to the convective time scale of the flow. The Deborah number (De) can be used to explain flow-induced deformation of polymers. At De < 1, polymer stretching can be considered insignificant and molecular size is the same as that at equilibrium conditions. Polymer deformation begins to occur at De=0.1 and at De=0.5, critical deformation occurs leading to highly extended, thread-like structures.

Degradation: The breaking down of the chemical structure of a given material into smaller fragments. With respect to polymers, degradation is not only a decrease in molar mass or chain length but also any deterioration of properties of a polymer which manipulates the end-use properties.

Degree of polymerization: The number of repeat units in a polymer chain.

Dendrimer: A hyperbranched or repetitively branched polymer. A dendrimer is typically symmetric around the core and often adopts a spherical three-dimensional structure. Dendrimer roots from the Greek word "dendron" meaning tree.

Detector delay volume: The volume between the first detector and all sequential detectors in a multi-detector size exclusion chromatography set-up. The delay volume is represented by the internal and external tubing that occurs between the flow cell of one detector and the flow cell of another detector. The detector delay volume must be accurately determined for a given experimental set-up in order to evaluate all detector responses with respect to the same eluting slice from the size exclusion chromatography column.

Differential molar mass distribution: A graphical representation of the molar mass distribution of a polymer in the form of a derivative. The differential molar mass distribution provides visual representation of the weight fraction of a sample within a certain molar mass range and provides information on the ultimate resolution for the entire fractionation, data collection, and analysis system by plotting the weight or weight fraction of a polymer of a given molar mass on the ordinate and the log of the molar mass on the abscissa.
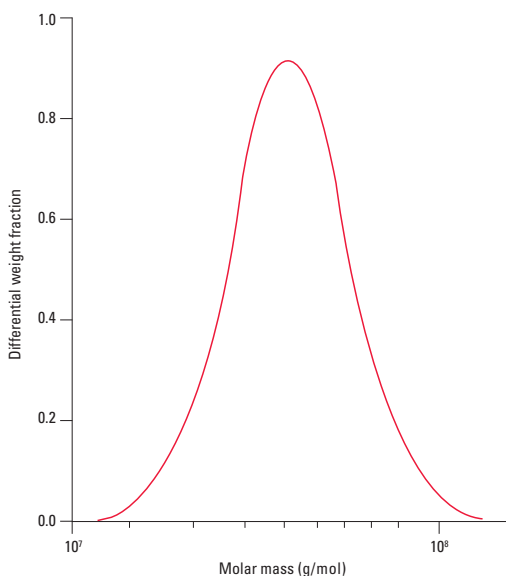

Figure 6: Differential molar mass distribution

Differential pressure transducer: A transducer that is connected to the inlet and outlet of the capillary in a viscometer that is used to monitor the pressure drop of the fluid flowing through the capillary.

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Differential refractive index detector: A deflection-type concentration-sensitive detector employing the principles of Snell's law of refraction. In this type of detector, light emitted from a source is transmitted through the flow cell of the RI detector and strikes a detector element. The flow cell is constructed in such a way that there are two chambers: (1) the reference chamber, consisting of stagnant pure solvent; and (2) the sample chamber, containing a flowing stream of analyte in the same solvent as in the reference chamber. As light passes through the reference side into the sample sides, the direction in which the light is traveling is changed *e.g.*, the path is bent. The amount of bending that takes place depends on the nature of the flow cell, the wavelength of light being used, the temperature, and the concentration of analytes in the cell. The light then strikes a mirror and reflects back through the cell and lens to the detector, which consists of either two photodiodes mounted on a single chip or of a photodiode array. The photodiodes will produce equal signals if the contents of the reference and sample chambers have the same refractive indices as each other. In contrast, if the reference and sample chambers have different refractive indices, a voltage difference will result between the photodiodes. The difference in refractive indices between the two chambers produces a voltage difference proportional to the concentration of the analyte in solution at the particular eluting slice.
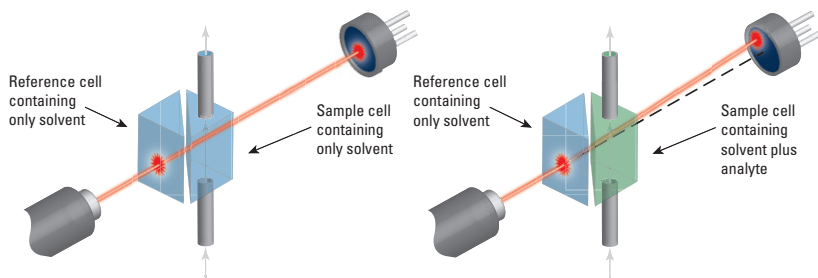

Figure 7: Differential refractive index detector flow cell

Differential viscometry: The differential viscometer uses capillaries in the form of a Wheatstone bridge to measure the resistance to flow of a polymer in solution. In differential viscometry, analyte solutions flow through one part of the detector and pure solvent flows through another part, creating a flow imbalance. The differential viscometer measures an increase in viscosity due to the presence of dissolved analytes by employing a Wheatstone bridge design through the use of capillaries and differential pressure transducers. On one side of the bridge a differential pressure (DP) transducer measures the change in pressure across the bridge, while on the other side of the bridge an inlet pressure (IP) transducer measures the pressure change (ΔP) through the bridge. The differential pressure transducer signal is proportional to the specific viscosity of the polymer solution.

Diffusion: The process whereby solute is transferred in a fluid from a point of high concentration to a point of lower concentration.

Diffusion coefficient: The rate of transfer of the diffusing substance across the unit area of a section, divided by the space gradient of concentration at the section.[5] A fundamental parameter of a molecule in solution (D_m or D_T) or in the stationary phase (D_s) that depends on the molar mass of the solute, temperature, solvent viscosity, and molar volume of the solute.

Dimensionless radii ratios: The combination of two of the four main macromolecular radii, i.e., the radius of gyration, hydrodynamic radius, visometric radius, and thermodynamic radius, R_G, R_H, R_η, and R_T respectively, in various permutations to define polymeric architecture and conformation.

Distribution coefficient: For size exclusion chromatography the distribution coefficient refers to the establishment of a thermodynamic equilibrium between the polymer in the interstitial volume and in the pore volume.[2] True size exclusion chromatography is a strictly entropy driven, temperature-independent process.

$$K_{SEC} \cong e^{\Delta S/R}$$

K_{SEC}: Distribution coefficient for size exclusion chromatography

ΔS: Change in entropy

R: Ideal gas constant

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Dual flow refractive index detector: A specific type of differential refractive index detector that contains a flow cell constructed in such a way that there are two chambers: (1) the reference chamber, containing a flowing stream of pure solvent; and (2) the sample chamber, consisting of a flowing stream of analyte in the same solvent as in the reference chamber. A dual flow refractive index detector results in increased baseline stability, reduced baseline drift and compensates for changes in the refractive index of the pure solvent over time by continuously flowing pure solvent through the reference chamber.

Reference cell containing flowing solvent

Sample cell containing only solvent

Reference cell containing flowing solvent
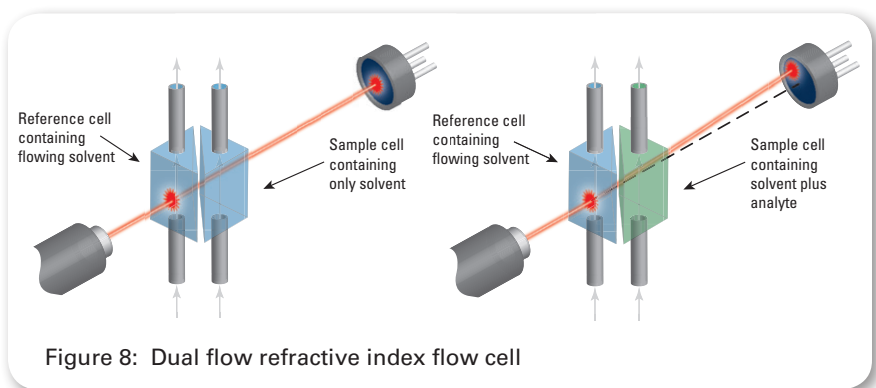
Sample cell containing solvent plus analyte

Figure 8: Dual flow refractive index flow cell

Dynamic light scattering: A type of light scattering also referred to as quasi-elastic light scattering that measures the time-dependent fluctuations of scattered light. See quasi-elastic light scattering.

E

Eddy-diffusion: The *A*-term in the van Deemter equation that arises from the multitude of pathways by which a molecule can find its way through a packed column. The value of *A* is dependent on the packing homogeneity and particle size of the column bed.

Effective radius: The radius of a polymer equal to half of the mean maximum cross-section of the fluctuating random coil.

$$R_e = \frac{(\pi n l^2/6)^{1/2}}{2}$$

R_e: Effective radius

n: Number of segments in the polymer

l: Length of one segment

Eluent: The mobile phase used to perform the separation.

Elution volume: The volume of eluent or mobile phase required to elute a solute from a column. The elution volume (V_R) of a solute is the volume from the point of sample injection to the volume corresponding to the apex of the solute peak.

End-group analysis: The determination of both the nature and the number of terminal groups in a macromolecule.

End-to-end distance: The mean distance between the ends of the chain for a random coil.

Enthalpy: The measure of the total energy of a system, ΔH. An ideal size exclusion chromatography separation is considered to be enthalpy independent as solute-stationary phase interactions are nonexistent.

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Entropy: The measure of the disorder or uncertainty associated with a system, ΔS. The separation mechanism in size exclusion chromatography is governed mainly by the entropy changes between phases, *e.g.* the mobility of the solute becomes more limited inside the pores than outside the pores of the column packing material.

Evaporative light scattering: An evaporative-type concentration-sensitive detector, also known as an evaporative mass detector (EMD), where the column effluent is nebulized to form an aerosol. The aerosol then enters a heated drift tube where the mobile phase evaporates leaving behind only particles of the analyte. The analyte particles then enter the optical cell of the detector, where they cause a light beam from a collimated light source to be scattered. The scattered radiation is then detected by a photomultiplier tube or a photodiode, providing the detector response.

Excess Rayleigh ratio: The amount of light scattered by a dilute polymer solution at a given angle, θ, in excess of that scattered by the neat solvent at the same angle, corrected for distance dependence and incident light intensity.

$R(\theta)$: Excess Rayleigh ratio

I_θ: Scattered intensity per unit scattering volume

$$R(\theta) = \frac{I_\theta r^2}{I_0}$$

I_0: Intensity of the incident radiation

r: Distance between the scattering column element and the photodetector of the light scattering unit

Excluded volume: The difference between the volume occupied by the polymer in solution, at a given set of solvent-temperature conditions, and the volume occupied at theta conditions.

F

Flory-Huggins theory: A mathematical model used to describe the thermodynamics of the formation of polymer solutions. The Flory-Huggins theory accounts for the equilibrium thermodynamic properties of polymer solutions that have large negative deviations from Raoult's Law, phase-separation and fractionation behavior, melting-point depressions in crystalline polymers, and swelling of networks.

Flow rate: The volume of mobile phase per unit time being pumped through a chromatography system. Typical flow rates for size exclusion chromatography are less than 2.0 mL/min while 1.0 mL/min is often preferred as it is the best compromise between resolution and speed. The flow rate has a significant influence on the efficiency and resolution of a size exclusion chromatography column.

Flow-induced degradation: The breaking down of high molar mass polymers occurring in the interstitial medium and at the pore boundary of a chromatographic column, as a result of high mobile phase flow rates. Flow-induced degradation leads to early elution volumes and inaccurate molar mass determination. The presence of flow-induced degradation can be confirmed or denied by comparing the molar mass averages obtained by performing the separation at multiple flow rates.

Fractals: From the term fractional dimension referring to the self-similarity of objects in space or processes in time at different scales or measurement resolution.[6] Polymers are often characterized in terms of their fractal dimension as they are not always simple one-, two-, or three-dimensional objects whose relationship between size and mass can be accurately described using the topological dimensions.

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G

Gel filtration chromatography: A branch of size exclusion chromatography discovered in 1959 by J. Porath and P. Flodin through the demonstration that columns packed with cross-linked polydextran gels could be used to size-separate various water-soluble polymers. Gel filtration chromatography (GFC) refers to the separation of molecules based on their size when an aqueous mobile phase flows through a column packed with a porous medium.

Gel permeation chromatography: A subunit of size exclusion chromatography discovered in 1964 by J.C. Moore. Moore's work involved the use of cross-linked polystyrene "gels" for separating synthetic polymers soluble in organic solvents. Gel permeation chromatography (GPC) refers to the separation of organic soluble polymers based on their size when an organic mobile phase flows through a column packed with a porous medium.

Gibbs free energy: A thermodynamic state function related to enthalpy (ΔH), temperature (T), and entropy (ΔS). In chromatography Gibbs free energy (ΔG) is used to describe the transfer of solute from the mobile phase to stationary phase through the solute distribution coefficient, K.[1] Mathematically $\Delta G = -RT \ln K$ or $\Delta G = \Delta H - T\Delta S$.

Glass transition temperature: The temperature at which the polymer undergoes the transformation from being as soft as rubber to as hard as glass.

Good solvent: A polymer is considered to be solvated in a "good" solvent when the chemical potential between the polymer and solvent is minimized, the molecule is more extensively solvated, the chain assumes a more extended configuration due to buttressing effect of the solvent, and the excluded volume is positive.[7]

Graft copolymer: A branched polymer in which the branches have a different chemical structure to that of the main chain.

Guard column: A short column, 2 cm, composed of the same packing material as the analytical column that is placed between the injector and the analytical column. The guard column is used to protect the analytical column from sample impurities.

H

High temperature size exclusion chromatography: A separation that occurs under the same mechanism as size exclusion chromatography but is performed at a temperature greater than 100 °C. High temperature size exclusion chromatography (HTSEC) systems are used to analyze polymers which are not soluble in any solvent at room temperature or whose solutions are too viscous at lower temperatures.

Hold-up reservoir: A reservoir found in differential viscometers located between two capillaries on the reference side of the detector. The hold-up reservoir allows for 50% or less of the sample entering the viscometer to be delayed, thus simultaneous measurement of the polymer solution in one part of the detector and pure solvent in the second part of the detector is possible. The polymer delayed in the hold-up reservoir must empty before another sample can be injected.

Homopolymer: Polymers composed of a single type of repeat unit or monomer.

Hydrodynamic chromatography: A solution-based separation where analytes sample the streamlines of flow in an open tube or in the interstitial volume of a column packed with non-porous material in a size-dependent manner. Separation is due to the parabolic flow velocity profile in the open tube channel, which allows small particles to be close to the walls, where the flow is stagnant, while larger particles remain near the center of the tube, where flow is the fastest. In hydrodynamic chromatography (HDC) larger analytes elute earlier than smaller ones due to the preferential sampling of the faster streamlines by the larger analytes. HDC can be observed during a size exclusion chromatography experiment when separation beyond the column exclusion limit occurs, *e.g.*, larger analytes which do not penetrate the pores of the column packing separate in a size-dependent manner rather than sampling the interstitial flow profile.[8]

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Hydrodynamic radius: R_H is the radius of an equivalent hard sphere that feels the same force due to flow as does the macromolecule or the radius of an equivalent hard sphere that has the same translational diffusion coefficient as a macromolecule. The hydrodynamic radius is also known as the Stokes radius.

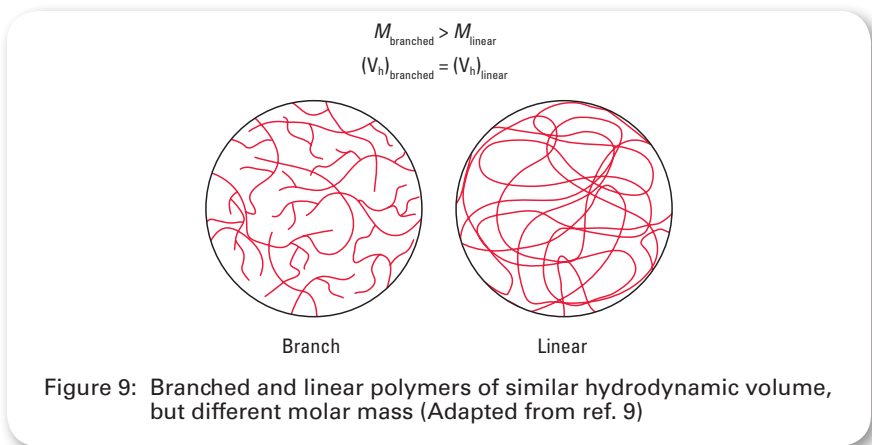$$R_H \equiv \frac{k_B T}{6\pi\eta_0 D_T}$$

k_B: Boltzmann's constant

T: Absolute temperature

η_0: Viscosity of the neat solvent

D_T: Translational diffusion coefficient

Hydrodynamic volume: The volume occupied by a polymer when it is in solution. The hydrodynamic volume V_h can vary for any given polymer depending on the molar mass and structure of the polymer as well as the solvent and temperature conditions of the polymer solution.

$M_{branched} > M_{linear}$

$(V_h)_{branched} = (V_h)_{linear}$

Branch   Linear

Figure 9: Branched and linear polymers of similar hydrodynamic volume, but different molar mass (Adapted from ref. 9)

GLOSSARY

I

Internal standard: A small amount of a known analyte added to a sample to check the reproducibility of a chromatography system. For size exclusion chromatography toluene and acetone are most often used as an internal standard or flow rate marker. Small amounts of either substance usually produce a sharp, distinct peak far away from any analyte peaks. The internal standard is added to each calibration standard and an average retention volume is determined. The retention volume of the internal standard in the individual injections of a calibration standard is then compared to the average value over all injections of all standards. The retention volumes of the polymer standards can then be corrected relative to the average. The addition of the internal standard to each sample solution then allows for correction of minor flow-rate fluctuations, due to pumping instabilities during sample analysis.

Interparticle obstruction: Obstructed diffusion of solute molecules that occurs around the particles in a size exclusion chromatography column.

Interstitial volume: The volume of mobile phase located between the packing materials or outside of the pores of the packing material in a size exclusion chromatography column.

Intraparticle obstruction: Obstructed diffusion of solute molecules that occurs within the pores of the packing material in a size exclusion chromatography column.

Intrinsic viscosity: The amount a dissolved molecule contributes to the overall viscosity of the solution. The dimensions of the intrinsic viscosity $[\eta]$ are mass/volume (*e.g.*, mL/g), so intrinsic viscosity may be thought of as "inverse density" of a molecule in dilute solution. The intrinsic viscosity is recognized as the ratio of the signal from the viscometer to the signal from the concentration-sensitive detector for the same data slice, after correction for interdetector delay, in the limit of near-infinite dilution.

$$[\eta] \equiv \lim_{c \to 0} \frac{\eta_{sp}}{c}$$

η_{sp}: Specific viscosity

c: Concentration

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L

Laminar flow: Also known as streamline flow, occurs when a fluid flows in a smooth motion in parallel layers, with no disruption between the layers.

Long-chain branching: A polymer is perceived to have long-chain branching (LCB) when the branches have lengths comparable to, or a substantial fraction of, the length of the main macromolecular backbone. Long-chain branching can influence a variety of chemical, physical, processing, and end-use properties of polymers and polymer solutions.

Longitudinal Diffusion: The B-term in the van Deemter equation that describes the diffusion of injected analyte molecules along the axis of the flow path as they are transported through the column. The contribution of the B term minimizes as flow rate increases because at higher flow rates the time available for longitudinal diffusion decreases.

Long-term noise: A steady movement, either positive or negative, of the baseline occurring over a time constant ranging from a few seconds to a few minutes.

Low-angle light scattering: A type of light scattering detector that measures the intensity of light scattered at a single angle. Low-angle light scattering (LALS) allows for the most accurate determination of the weight-average molar mass, however, no information on the size of the sample is obtained because angular dissymmetry cannot be measured using a single angle. The current generation of LALS detectors measures the light scattered at 7°, the lowest practical angle at which a photodiode can be placed. LALS detectors are ideal for polymers with a molar mass between 10^3 to $>10^6$ g/mol and a radius of gyration of 10 to 150 nm.

GLOSSARY

M

M_n: See number-average molar mass.

M_p: See peak-average molar mass.

M_v: See viscosity-average molar mass.

M_w: See weight-average molar mass.

M_z: See z-average molar mass.

Macromolecule: A very large molecule, such as a colloidal particle, protein, or polymer, composed of hundreds or thousands of atoms. The words polymer and macromolecule are used interchangeably, but the latter strictly defines the molecules of which the former is composed.

Mark-Houwink equation: An equation describing the dependence of the intrinsic viscosity of a polymer in dilute solution on its molar mass. The Mark-Houwink equation is used in the form of a Mark-Houwink plot to determine the conformation of a polymer in solution (see Mark-Houwink plot.)

$$[\eta] = KM^a$$

$[\eta]$: Intrinsic viscosity

K: Mark-Houwink constant for a given polymer type (chemistry and architecture) at a given solvent and temperature.

a: A scalar quantity which relates to the conformation of a polymer.

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Mark-Houwink plot: A plot of the intrinsic viscosity [η] versus the molar mass of a polymer, with each axis on a logarithmic scale, (*i.e.*, the plot of log [η] versus log M) used for the analysis of polymer conformation. The slope of a Mark-Houwink plot, a, is directly related to the conformation of the macromolecule in dilute solution. For a random coil at theta conditions $a=1$, for a random coil in a "good" solvent $a=0.8$, and for a hard sphere $a=0$.
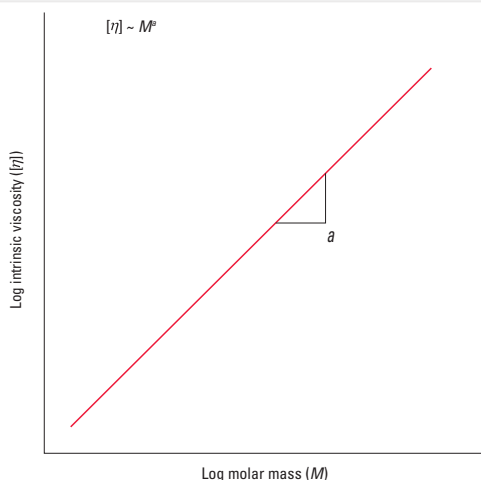

Figure 10: Generic Mark-Houwink plot

Miscibility: The ability for one liquid to mix with or dissolve in another liquid. The miscibility as well as polarity of solvents must be considered when transferring columns and chromatography systems from one solvent to another. The mixing of immiscible solvents and solvents with large polarity differences can result in poor column performance and at times can even be destructive.

Mobile phase: The solvent that moves the solute molecule through a chromatography column.

Mobile-phase mass transfer: A band broadening process that is caused by the velocity gradient profile that exists in a single flow stream. This contribution to band broadening is a result of the liquid near the surface of the column packing particle moving relatively slower than the liquid at the center of the flow stream, thus solute molecules at the center migrate faster downstream than the others.

Molar absorptivity: A measurement of how strongly a polymer absorbs light at a given wavelength. The molar absorptivity ε is specific to a polymer in a given solvent at a given temperature and at a given wavelength.

Molar mass averages: Any of a series of averages or statistical moments of the molar mass distribution (MMD), which are useful for assessing the dispersity of the distribution and certain properties of the polymer (*e.g.*, flexibility). The three most important molar mass averages are the number-average M_n, weight average M_w, and z-average M_z.
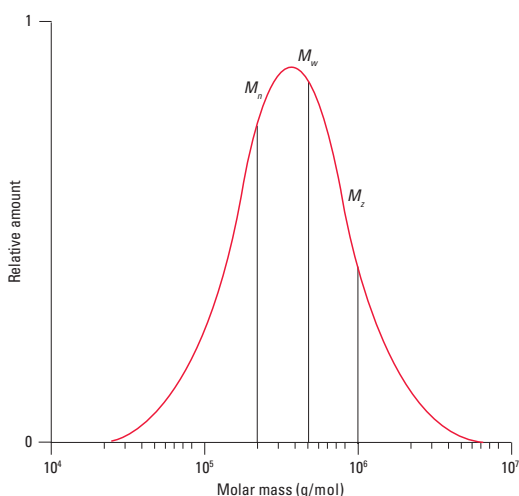

Figure 11: Molar mass distribution of a polymer showing the relative locations of the number-, weight- and z-average molar masses, M_n, M_w, and M_z respectively

Molar mass distribution: A way to characterize the different relative amounts of the various molar masses present in a polymer. The molar mass distribution (MMD) plays a critical role in determining mechanical, bulk, and solution properties. The width of the MMD is used to determine the dispersity of a polymer. There are two types of molecular weight distributions: a differential MMD and a cumulative (integral) MMD.

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Monodisperse: A polymer composed of uniform molecules with respect to molar mass *M* or radius *R*. A polymer is considered monodisperse when the polydispersity index (*PDI*) is equal to one.

Monomer: An atom or small molecule that may bind chemically to other monomers to form a polymer.

Multi-angle light scattering: A type of static light scattering that measures the intensity of scattered light over relatively long time scales and averages the results at a multiplicity of angles. Commercially available multi-angle light scattering (MALS) detectors have two-, three-, seven-, or eighteen-angle configurations. In a MALS experiment an incident beam of light, with a wavelength λ_0, passes through a flow cell. Due to the wave-like nature of light, when the laser light strikes the analyte the oscillating electric field of the laser light creates an oscillating dipole within the sample. The sample solution then scatters light in a direction perpendicular to the oscillating electric field of incident radiation. The amount of light scattered from these oscillating dipoles is then measured at each of the photodiodes or charge coupled devices (CCDs). The latter are placed at different angular positions around the sample cell and provide a response directly proportional to the intensity of scattered light they receive. From this response, the molar mass of the analyte is obtained. For analytes much smaller than the wavelength of incident light in the medium (an isotropic scatterer), the intensity of the scattered light is angularly-invariant. Conversely, for larger analytes, intramolecular interference leads to an angular dependence in the intensity of the scattered light. From this dependence, the size (radius of gyration) of the analyte is determined.

GLOSSARY

N

Network polymer: A polymer with a three-dimensional structure where each chain is connected to all others by a sequence of junction points and other chains. Such polymers are crosslinked and are characterized by their degree of crosslinking.

Non-size exclusion effects: Separation processes that may occur during a size exclusion chromatography experiment that result in the analytes not eluting strictly in a size-dependent manner. Intermolecular electrostatic interactions between the solute and the packing (*e.g.* ion exchange, ion exclusion, and ion inclusion), intermolecular electrostatic interactions, and adsorption (hydrogen bonding and hydrophobic interactions between the solute and the packing) are non-size exclusion effects that can potentially plague experiments, especially those in aqueous mobile phases. Other non-size exclusion effects that can occur are viscous fingering and concentration effects.

Number-average molar mass: The first statistical moment in the molar mass distribution. The number-average molar mass M_n is a convenient way of measuring the "average" chain length in a polymer sample. M_n correlates with a number of polymer physical properties such as brittleness and flow properties of a polymer and is defined as the mass of the sample in grams, divided by the total number of chains present.

$$M_n = \frac{\sum N_i M_i}{\sum N_i}$$

N_i: Number of molecules eluting in slice i

M_i: Molar mass of molecules eluting in slice i

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O

Obstruction factor: A factor that denotes the hindered diffusion of solute within a packed column, as compared to the unobstructed diffusion of the same solute in an open tube. The obstruction factor, γ, is highly influential on flow through porous media due to band broadening effects present due to the resistance to mass transfer.

Off-line multi-angle light scattering: In an off-line or batch mode experiment the multi-angle light scattering (MALS) detector is uncoupled from the chromatography system (*i.e.*, experiments are performed without the separation capabilities of size exclusion chromatography). A basic off-line MALS experiment consists of measuring the scattering, at a multiplicity of angles, for a series of solutions of different concentrations. The results of each concentration at each angle are plotted together in what is known as a Zimm plot (see Zimm plot). The values of $R_{G,z}$ and M_w based upon on-line and off-line MALS experiments should be equal, as unequal values can imply analyte aggregation or analyte degradation during the chromatographic separation.

Oligomer: A molecule that consists of a few (< 10) monomer units. Dimers, trimers, and tetramers are oligomers while anything higher in number of monomers is a polymer.

Optimum velocity: The velocity at which a column has maximum efficiency. The optimum velocity, u_{opt}, is the minimum sum of all three dispersion processes and represented on a van Deemter plot as the location where minimum plate height is achieved.

P

Peak area: The area measured under a chromatographic peak.

Peak-average molar mass: The molar mass of the slice eluting at the peak apex in a size exclusion chromatography chromatogram. The peak-average molar mass M_p is primarily used in assigning molar masses when constructing peak-position calibration curves based on monodisperse standards.
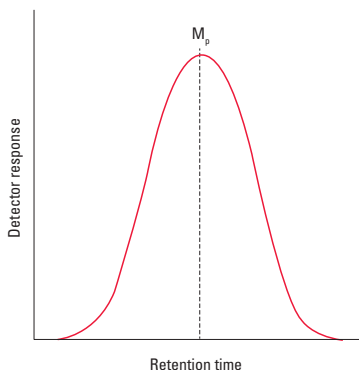

Figure 12: Molar mass distribution of a polymer showing the location of the peak-average molar mass M_p

Peak capacity: The maximum number of peaks that can be resolved within a specific range of retention volume. Compared to other modes of liquid chromatography, size exclusion chromatography has extremely low peak capacity as separations are constrained to occur within the limits of the packing pore volume. Additionally the major use of size exclusion chromatography is not to resolve and identify species but to obtain molar mass distribution information from the chromatogram, thus a large peak capacity is not required.

Peak height: The intensity or height of a chromatographic peak. The height of a chromatographic peak is measured from the baseline of the chromatogram to the apex of the peak.

Peak-position calibration: See calibration curve.

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Peak width: The breadth of a peak. The peak width W_b for a chromatographic peak is calculated by drawing tangent lines to the front and back slopes of the peak and then measuring the distance between the intersections of the tangent lines with the baseline.

Persistence length: The length over which the chain "persists" or extends in the same direction as the first statistical bond or the average projection of the end-to-end distance vector onto the first bond of the chain, in the limit of infinite chain length (*i.e.*, as degree of polymerization goes to infinity).[2]
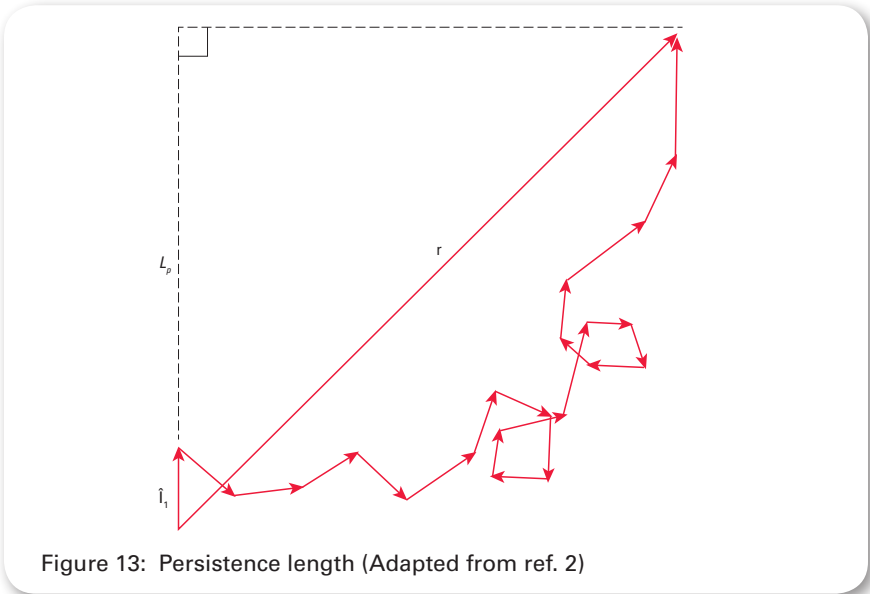

Figure 13: Persistence length (Adapted from ref. 2)

$$L_p = \langle \frac{\hat{l}_1}{l_1} \sum_{i=1}^{n} \hat{l}_1 \rangle \ \ as \ n \to \infty$$

L_p: Persistence length

$\hat{l}_1$: First bond of the chain

$\frac{\hat{l}_1}{l_1}$: Unit vector in the direction of $\hat{l}_1$

n: Degree of polymerization

Physical detection methods: Physical detection methods are those which generally combine with each other in a synergistic fashion, such as a viscometer or light scattering photometer.

Polydisperse: A measure of the breadth of the given distribution of a polymer, most common being molar mass *M* or radius *R*. *A* polymer is determined to be polydisperse when the polydispersity index (*PDI*) value is greater than one.

Polydispersity index: A measure of the distribution or heterogeneity of the molar mass *M* or radius *R* in a given polymer sample. A polymer is considered monodisperse when *PDI* = 1 and polydisperse when *PDI* > 1.

$$PDI = \frac{M_w}{M_n} \; or \; \frac{R_{x,w}}{R_{x,n}} \quad \text{where x is G, H or } \eta$$

Polyelectrolyte: A polymer in which a substantial portion of the constitutional units contain ionic or charged groups. The distribution of polyelectrolytic charge within a polymer plays a role in the flocculation, transport, and binding of metals to the polymer.

Polymer: A substance composed of molecules which have long sequences of one or more species of atoms or groups of atoms linked to each other by primary, usually covalent, bonds. A polymer is a giant molecule, or macromolecule, made up of thousands of repeating units or small parts, the monomer.

Polymerization: The process of forming giant molecules, *e.g.* polymers, from the linking together of smaller molecules through chemical reactions. The process of polymerization can be divided into two main categories: (1) condensation and addition polymerization; and (2) step-reactions and chain-reaction polymerization.

Polysaccharides: Polymeric carbohydrates (sugars) formed of repeating mono- or di-saccharide units linked together by glycosidic bonds. These structures are usually linear but may contain various degrees of branching. The structure of a polysaccharide is dictated by the monosaccharide building blocks of the polymer. The solubility of polysaccharides varies as some are soluble in both aqueous and organic solvents while others are only soluble in one or the other, for example cellulous is only soluble in organic solvents.

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Poor solvent: A polymer is dissolved in a "poor" solvent when the chemical potential between the polymer and solvent is not minimized, the molecule is not extensively solvated, and the polymer chain assumes a compact rather than extended conformation. Polymers in "poor" solvents have a negative value for the second virial coefficient.

Pore volume: The amount of stagnant solvent in the porous packing structure in a size exclusion chromatography column.

Porosity: The fraction of void space in a size exclusion chromatography column. The total column porosity in a size exclusion chromatography column incorporates both the space between the packing particles (interparticle porosity) and that within the pores of the packing material (intraparticle porosity).

Q

Quasi-elastic light scattering: A type of light scattering also referred to as dynamic light scattering or QELS, which measures the time-dependent fluctuations of scattered light. These fluctuations are a measure of the Brownian motion resulting from the dissolved particles colliding continuously with solvent molecules in a dilute solution. As the molecules undergo Brownian motion their relative positions change with time causing the intensity of scattered light reaching a photodetector to fluctuate with time about some average. The time scale of the fluctuations is characterized by the intensity of the autocorrelation function $G(\tau)$, as measured by an avalanche photodiode, which is a direct measurement of the translational diffusion coefficient of the molecule.

$$G(\tau) = \langle I(t) \rangle^2 \left(1 + \alpha e^{-2D_T \left(\frac{4\pi n_0}{\lambda_0} \sin\left(\frac{\theta}{2}\right) \right)^2 \tau} \right)$$

$\langle I(t) \rangle$: Average scattering at time t

α: An instrument constant

η_0: Refractive index of the solvent

λ_0: Vacuum wavelength of light

τ: Delay time

θ: Angle of detection with respect to the direction of the incident light

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R

Radius of gyration: The root-mean-square distance of an array of atoms from their common center of mass. The radius of gyration R_G is also known as the root-mean-square radius and is determined using multi-angle light scattering as well as other scattering techniques.
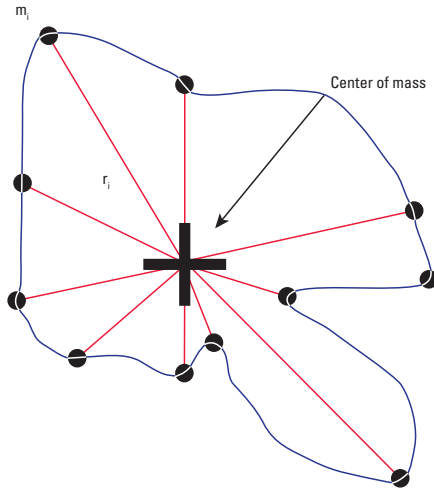

Figure 14: Radius of gyration of a polymer

$$R = \left[\left(\frac{1}{n+1} \right) \sum_i (r_i - R_{cm})^2 \right]^{1/2}$$

n: Number of bonds in the polymer or particle

r_i: Location of an individual atom or group of atoms

R_{cm}: Location of the center of mass of the particle

Random coil: The random configuration assumed by a flexible polymer in solution.

Random copolymer: A copolymer consisting of monomers distributed in a statistically random manner.

^^^A-A-A-B-A-B-A-B-A-B-B-A-A^^^

Rayleigh-Gans-Debye approximation: A mathematical generalization of light scattering theory that is applicable for particles much smaller than the wavelength of light. There are two conditions that must hold for the Rayleigh-Gans-Debye (RGD) approximation to be valid: (1) the polymer must be effectively invisible to the solvent; (2) the polymer must not disturb the phase of the laser light (*i.e.* the size of the particle must be much smaller than the wavelength of incident light).[10] The RGD approximation is also known as the basic equation of light scattering.

$$\frac{K^*c}{R(\theta)} = \frac{1}{M_w P(\theta)} + 2A_2 c + \cdots$$

$$K = \frac{4\pi^2 n_0^2 \left(\frac{\partial n}{\partial c}\right)^2}{\lambda_0^4 N_A} \qquad \frac{1}{P(\theta)} = 1 + \frac{16\pi^2}{3\lambda^2}\langle r\rangle^2 \sin^2\left(\frac{\theta}{2}\right) + \cdots$$

K^*: Optical constant

$R(\theta)$: Excess Rayleigh ratio

$P(\theta)$: Particle form factor

A_2: Second virial coefficients

N_A: Avogadro's number

c: Concentration of the solution

λ: Wavelength of light in a particular medium [$\lambda \equiv \lambda_0/n_0$]

n_0: Refractive index of the neat solvent

λ_0: Vacuum wavelength of incident radiation

M_w: Weight average molar mass

$\langle r\rangle^2$: Mean-square radius of the analyte

θ: Scattering angle

$\partial n/\partial c$: Specific refractive index increment of the solution

Refractive index: A measure of the extent in which radiation is refracted as it passes through the interface between two media.

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Relative viscosity: The ratio of the viscosity of the dilute polymer solution to the viscosity of the neat solvent. The relative viscosity η_{rel} is used in the determination of the intrinsic viscosity of a dilute polymer solution.

$$\eta_{rel} = \frac{\eta}{\eta_0}$$

η: Viscosity of the dilute polymer solution

η_0: Viscosity of the neat solvent

Resistance to mass transfer: The *C*-term in the van Deemter equation that refers to the mass transfer process. An increase in flow rate emphasizes the velocity differences between flow streams, which results in an increase in plate height.

Resolution: A measure of column efficiency related to the degree of separation of two analytes or peaks. In size exclusion chromatography resolution R_S is defined as how well the size exclusion chromatography column can distinguish between two molecules of the same polymer type but differing by a molar mass factor M_2/M_1.

$$R_S = \frac{\ln(M_2/M_1)}{2D_2(\sigma_1 + \sigma_2)} \simeq \frac{\Delta lnM}{4\sigma D_2}$$

M: Molar mass

σ: Peak standard deviation

D_2: Slope of the linear region of the calibration curve

Retention time: The amount of time required for a peak to elute from the column following sample injection. The retention time t_R is useful only for comparing peaks that have appeared in the same type of chromatography, as the value of t_R is sensitive to changes in experimental conditions, such as flow rate and the specific column used.

Retention volume: The volume of mobile phase required for a peak to elute from the column following sample injection. The retention volume V_R is the most fundamental quantity used to describe peak retention as it is independent of flow rate changes, but can still vary with differences in column size and instrument dead volume.

Reynolds number: A dimensionless ratio of the inertial and viscous forces of a moving fluid. Low Reynolds numbers *Re* correspond to slow and steady fluid motion. *Re* values greater than 4200 correspond to fully turbulent flow in an unpacked tube. In packed beds, *i.e.* columns, flow becomes turbulent for *Re* between 1 and 100.

Rho: The best known and most employed dimensionless ratio used to define polymeric architecture. The rho value ρ is the ratio of the z-averages of the radius of gyration R_G and hydrodynamic radius R_H. Values of ρ are available in the literature for a number of polymeric architectures.

$$\rho \equiv \frac{R_{G,z}}{R_{H,z}}$$

Right-angle light scattering: A type of light scattering detector that measures the intensity of light scattered at a single 90° angle. In size exclusion chromatography measurements, right-angle light scattering (RALS) is typically used in conjunction with differential viscometry and differential refractometry. This combination of detection methods is known as SEC[3].

Rigid rod: A highly extended linear structure a polymer may adapt in solution.

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S

Second virial coefficient: A measure of the excess chemical potential (*e.g.* excess Gibbs free energy of dilution) between polymer and solvent molecules in solution. The second virial coefficient A_2 describes how well solvated the polymer is at specific solvent-temperature conditions. Polymers at theta conditions are those at which $A_2=0$. Polymers that are dissolved in a thermodynamically "good" solvent at a given temperature have an $A_2>0$, while polymers that are dissolved in a thermodynamically "poor" solvent are signified by $A_2<0$.

"Good" Solvent
$A_2>0$

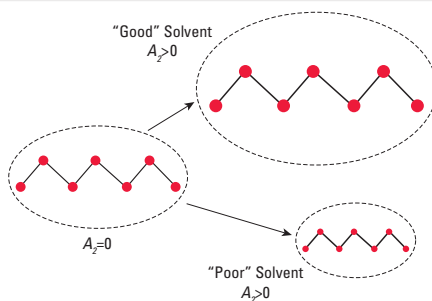$A_2=0$

"Poor" Solvent
$A_2>0$

Figure 15: Pictorial of a polymer solvated at theta conditions, in a thermodynamically "good" solvent and in a thermodynamically "poor" solvent

Selective permeation range: The range in a size exclusion chromatography column between the total exclusion and total permeation limit where the separation occurs. In the selective permeation range analytes sample a fraction of the pore volume accessible.

Short chain branching: A polymer is perceived to have short chain branching (SCB) when the branches have lengths much smaller than the length of the main macromolecular backbone. Short chain branching in a polymer can influence the haze, stress-crack resistance, and crystallinity of a polymer.

Short-term noise: A steady movement, either positive or negative, of the baseline occurring over a time constant measured in seconds or less.

GLOSSARY

Size exclusion chromatography: The general name for a solution-based separation method that operates via an inverse molecular sieving mechanism that depends on the relative size or hydrodynamic volume of a dissolved polymer with respect to the average pore size of the packing material in the column. In a size exclusion chromatography (SEC) experiment, a polymer molecule passes through a column packed with porous material sampling a portion of the available pore space. In SEC, molecules occupying a larger hydrodynamic volume will elute earlier than those occupying a smaller hydrodynamic volume due to the sampling of the pore volume of the packing material. Molecules too large to fit into any of the pores will elute together at what is called the total exclusion limit, while those that occupy a volume so small compared to the smallest pore size of the packing material will elute together, regardless of differences in size, at what is called the total permeation limit.
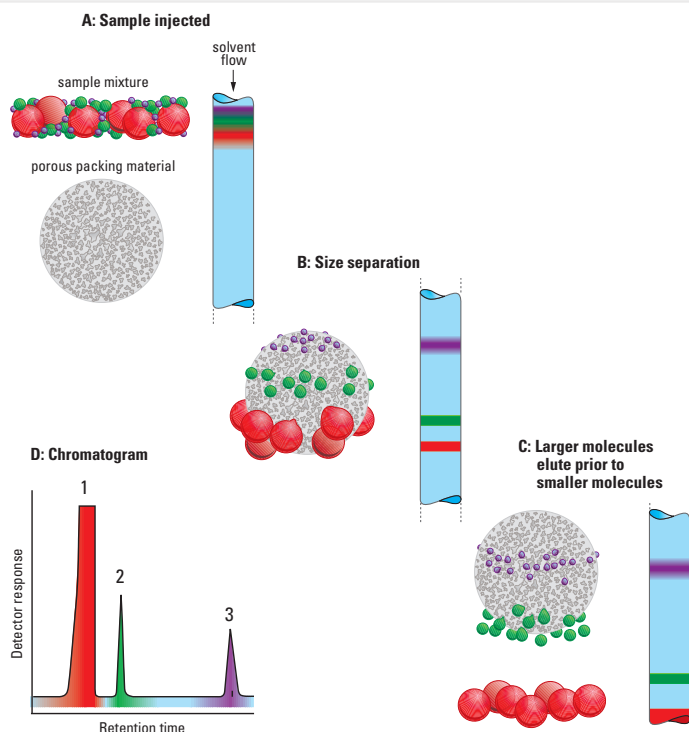

Figure 16: Separation and detection of a polymer mixture by size exclusion chromatography (SEC)

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Slalom chromatography: A separation method that typically involves ultra-high molar mass polymers that have undergone critical flow-induced extension and find themselves turning frequently around the column packing particles in their passage through the tortuous interstitial channels of the column. The repetitive and continuous turning results in longer polymers eluting later than short ones. Slalom chromatography (SC) effects can manifest themselves in size exclusion chromatography experiments involving high-molar mass polymers at high flow rates.

Snell's law: The principle employed in a deflection-type concentration-sensitive detector that describes the relation between the angles of incidence and the refraction of a wave impinging on an interface between two media with different refractive indices.

$$n_1 \sin(\theta_1) = n_2 \sin(\theta_2)$$

n: Refractive index of each medium

θ: Angle or trajectory of light in each medium

Subscripts 1 and 2: Different medium

Solubility parameter: A measure of polymer-solvent interaction. If the solubility parameter δ of the solvent is equal to that of the polymer, the two substances are mutually soluble. The greater the difference between the solubility parameters of the polymer and the solvent the less likely the two substances are soluble.

$$\delta = \left(\frac{\Delta E}{V}\right)^{1/2}$$

$\Delta E/V$: Energy of vaporization per unit volume

Solvent: The liquid used to dissolve the polymer being analyzed. The selection of a solvent for a size exclusion chromatography experiment should involve the following considerations: convenience, sample type, column packing, operating variables, safety, and purity.

Specific refractive index increment: Is the change in refractive index of a solution with respect to changes in the concentration of the solution for a given analyte at a specified wavelength and solvent-temperature conditions. The specific refractive index increment, $\partial n/\partial c$, describes how sensitive the differential refractometer (DRI) will be at measuring changes in the concentration of a particular analyte. The $\partial n/\partial c$ of a sample is determined with the DRI in batch mode (the detector uncoupled from the separation system), by measuring the detector response of a series of accurately known dilute analyte concentrations. The relationship between concentration and DRI detector response is linear, thus, the slope of a plot of the differential refractive index (the difference between detector response of the neat solvent and that of each individual dissolution) on the ordinate and the concentration of the dissolutions on the abscissa is equal to the $\partial n/\partial c$. Accurate quantification of the $\partial n/\partial c$ is essential for the characterization of particles and polymers as the signal response of both DRI and MALS detection are dependent on the $\partial n/\partial c$ value.
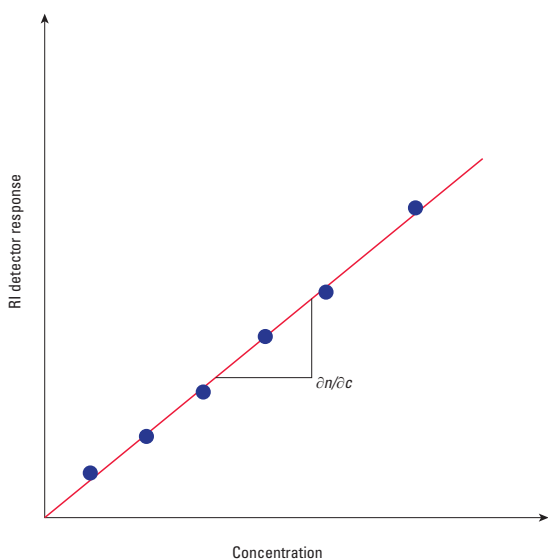

Figure 17: $\partial n/\partial c$ plot: Differential refractive index detector (DRI) signal, as a function of concentration for a series of accurately known dilute concentrations

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Specific resolution: A term used to measure resolution in size exclusion chromatography that pertains to the resolution of the elution curve as a whole and not as much on the resolution between specific pairs of eluted fractions. The specific resolution R_{sp} is not dependent on the molar mass of the sample and is only equal to the usual chromatography resolution R_s when a pair of peaks has a decade of molar mass difference.

$$R_{sp} = \frac{R_s}{\Delta log M} = \frac{0.58}{\sigma D_2}$$

M: Molar mass

σ: Peak standard deviation

D_2: Slope of the linear region of the calibration curve

Specific viscosity: A measure of the resistance to flow of a dilute polymer solution. The specific viscosity η_{sp} is usually expressed as the ratio of the absolute viscosity of the dilute polymer solution to that of the neat solvent. The specific viscosity of a polymer is determined from the flow impedances of the capillaries in a differential viscometer.

$$\eta_{sp} = \frac{\eta}{\eta_0} - 1 = \frac{4\Delta P}{IP - 2\Delta P}$$

η: Viscosity of the dilute polymer solution

η_0: Viscosity of the neat solvent

ΔP: Differential pressure

IP: Inlet pressure

Stationary phase mass transfer: A band broadening process that arises from the slow solute diffusion in and out of the pores of the packing particles. This contribution to band broadening is a result of some molecules diffusing into the pores while other molecules move with the solvent further downstream. This dispersion process is the major contributor to brand broadening in size exclusion chromatography analysis. Stationary phase mass transfer is also referred to as stagnant mobile phase mass transfer for liquid chromatography methods besides size exclusion chromatography.

Star polymer: A type of branched polymer that consists of a single branch point that gives rise to multiple linear chains or arms.

Static light scattering: A type of light scattering, also known as total-intensity light scattering, which measures the intensity of scattered light over relatively long time scales and averages the results. Static light scattering detectors have the ability to provide information about the absolute molar mass and size of a polymer, as well as dilute solution thermodynamics, long-chain branching, aggregation, conformation, and fractal dimension.

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TOSOH

T

Theoretical plate: A fundamental measure of a chromatography system's efficiency. The number of theoretical plates N can be considered a measure of how much a given solute band broadens during its time in the column and is also a descriptor of the ability of a given column to provide narrow bands and, therefore, good separations. A large number of theoretical plates correspond to a more efficient separation.

Thermal degradation: The deterioration of a polymer as a result of overheating. Thermal degradation is most commonly observed in high temperature size exclusion chromatography and can be avoided by storing the polymer solution in heat zones rather than in the run queue.

Thermodynamic radius: The radius of a hard sphere with the same excluded volume as a macromolecule. The thermodynamic radius R_T is determined by off-line, batch mode, multi-angle light scattering experiments.

$$R_T \equiv \left(\frac{3A_2 M_w^2}{16\pi N_A}\right)^{1/3}$$

A_2: Second virial coefficient

M_w: Weight-average molar mass

N_A: Avogadro's number

Theta (θ) conditions: A thermodynamically pseudo-idea state, equivalent to the Boyle temperature for gases, where the combination of solvent and temperature afford a linear polymer in dilute solution the same spatial dimensions as it would occupy in the melt.

Topology: The branching status of the macromolecule, which may be star, comb, dendritic, hyperbranched, random, or non-branched.

Total exclusion limit: The molar mass corresponding to the smallest molecule that is unable to enter the pores of the column packing material of a size exclusion chromatography column. Polymers higher in molar mass than the exclusion limit of a given column will elute in the void volume of the column and will not be separated.
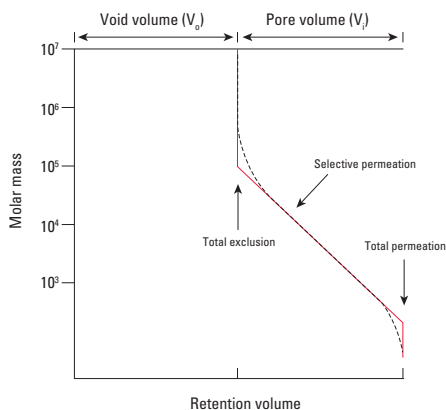

Figure 18: Separation range in size exclusion chromatography (SEC) showing the total exclusion limit, selective permeation range, and the total permeation limit (Adapted from ref 2.)

Total permeation limit: The molar mass corresponding to molecules that are small enough to access all of the pore volume of the column packing material in a size exclusion chromatography column. Polymers small enough to penetrate all of the pore volume will elute as a single chromatographic peak at the end of a size exclusion chromatography chromatogram. In traditional liquid chromatography the peak corresponding to 'total permeation' would be interpreted as the 'unretained peak'.

Turbulent flow: A type of flow that is characterized by an irregular and nearly random motion superimposed on the main motion of the fluid.

TOSOH BIOSCIENCE

U

Ultra-high molar mass polymer: A polymer with a molar mass greater than 10^6 g/mol.

Ultrasonic degradation: Polymer fracture caused by cavitational bubble collapse and associated transient elongational flow fields, resultant from irradiating a dilute polymer solution with ultrasonic frequencies.

Universal calibration: A peak position calibration curve that utilizes the concept of the hydrodynamic volume of a polymer in solution. The hydrodynamic volume of a polymer is expressed in terms of the product of the molar mass M and the intrinsic viscosity $[\eta]$ of the polymer in solution, as obtained by the viscometer. A universal calibration curve merges all polymers of different chemistries and/or architectures onto a single plot when calibration data are plotted as the log$[\eta]$M verses retention volume. This type of calibration curve allows for the determination of absolute molar masses for analytes of chemistry and/or architecture different from those of the calibrants.

UV/Visible detectors: A concentration-sensitive detection method based on ultraviolet absorption most commonly used in liquid chromatography and gel filtration chromatography. UV/Visible detectors have found limited use in gel permeation chromatography, as it is often difficult to find UV-transmitting solvents that meet the solubility requirements of polymers. UV/Visible detectors operate based on the relationship between absorbance and concentration as given by Beer's Law.

$$A = \log\frac{I_0}{I} = \varepsilon bc$$

A: Absorbance

I: Intensity of the incident

I_0: Intensity of transmitted radiation

b: Pathlength of the detector cell

ε: Molar absorptivity

c: Concentration

GLOSSARY

V

van Deemter equation: An equation that combines the three independent band broadening processes (*e.g.*, Eddy diffusion *A*, longitudinal diffusion *B*, and resistance to mass transfer *C*) to determine the effect of flow rate υ on plate height *H*.

$$H = A + \frac{B}{\upsilon} + C\upsilon$$

van Deemter plot: A graphical description of the individual band broadening processes and their sum is shown by a theoretical van Deemter plot. The sum of the band broadening processes (black line) shows a minimum plate height H_{min} which corresponds to the velocity u_{opt} at which the column has a maximum efficiency. In size exclusion chromatography the separation is controlled by the resistance to mass transfer term *C* since the longitudinal diffusion term *B* is generally insignificant and H_{min} is not observed. In practice, flow rates higher than u_{opt} are often used for reasonably fast separation.
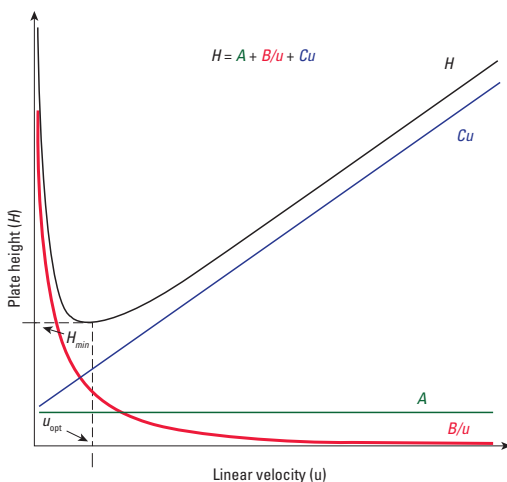

Figure 19: Theoretical van Deemter plot

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Viscometer: A physical detector that measures aberrations in the flow velocity field of a fluid or the resistance to flow of a solution according to Poiseuille's law. Viscometers can either be single capillary or differential in nature. Differential viscometers are most commonly used in conjunction with size exclusion chromatography.

Viscometric radius: The radius of a solid sphere that increases the viscosity of the fluid by the same amount as does a macromolecule or the radius of a hard sphere that feels the same force due to flow as does a macromolecule. The viscometric radius R_η is determined by using a viscometer and differential refractive index detector in combination with a molar mass-sensitive detector, such as a multi-angle light scattering.

$$R_\eta \equiv \left(\frac{3[\eta]M}{10\pi N_A} \right)^{1/3}$$

$[\eta]$: Intrinsic viscosity

M: Molar mass

N_A: Avogadro's number

Viscosity average molar mass: The only statistical moment of the molar mass distribution that is dependent on solvent-temperature conditions. The viscosity average molar mass M_v always lies between the number and weight average molar mass values and correlates with molding properties and polymer extrudability.

$$M_v = \left(\frac{\sum N_i M_i^{1+a}}{\sum N_i M_i} \right)^{1/a}$$

N_i: Number of molecules eluting in slice i

M_i: Molar mass of molecules eluting in slice i

a: Exponent in the Mark-Houwink equation

Viscosity: The measurement of the flow properties of a polymer expressed in terms of its resistance to flow.

Viscous fingering: A phenomena that occurs at the interface of two fluids of different viscosities as the fluids move through a porous bead. Viscous fingering can cause significant band broadening in size exclusion chromatography, especially when high sample concentration leads to an increased solution viscosity.

Void volume: The interstitial liquid volume between the packing particles in a size exclusion chromatography column. Polymers that are too large to penetrate the pores of the packing material will elute within the void volume, V_0, of the column.

GLOSSARY

W

Weight-average molar mass: A statistical moment of the molar mass distribution that considers the mass of the molecules. The weight-average molar mass M_w correlates with physical properties of a polymer such as tensile strength and hardness.

$$M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i}$$

N_i: Number of molecules eluting in slice i

M_i: Molar mass of molecules eluting in slice i

Weight fraction: The mass of molecules of molar mass M divided by the total mass of all the molecules present.

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Z

z-average molar mass: The highest statistical moment of the three molar mass averages typically discussed. The z-average molar mass M_z correlates to polymer processing properties that are governed by the longest chains in the molar mass distribution, such as flex life and stiffness.

$$M_Z = \frac{\sum N_i M_i^3}{\sum N_i M_i^2}$$

N_i: Number of molecules eluting in slice i

M_i: Molar mass of molecules eluting in slice i

Zimm plot: A double extrapolation plot that is used to analyze light scattering data. The weight-average molar mass M_w, z-average radius of gyration $R_{G,z}$, and second virial coefficient A_2 of a polymer can all be obtained through a Zimm plot. A Zimm plot is a plot of $K*c/R(\theta)$ versus $\sin^2(\theta/2) + kc$ where k is an arbitrary constant utilized to give a clean separation of data points. The slope of the line that results from extrapolating the angular data to concentration of zero is proportional to $R_{G,z}$. The slope of the line of concentration data extrapolated to zero angle is proportional to A_2. M_w is then the inverse of the common y-intercept of the extrapolations of the angular data and the concentration data.
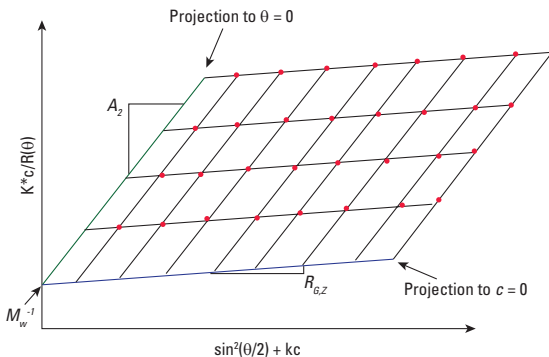

Figure 20: Zimm plot, for a series of different but known concentrations measured at many angles

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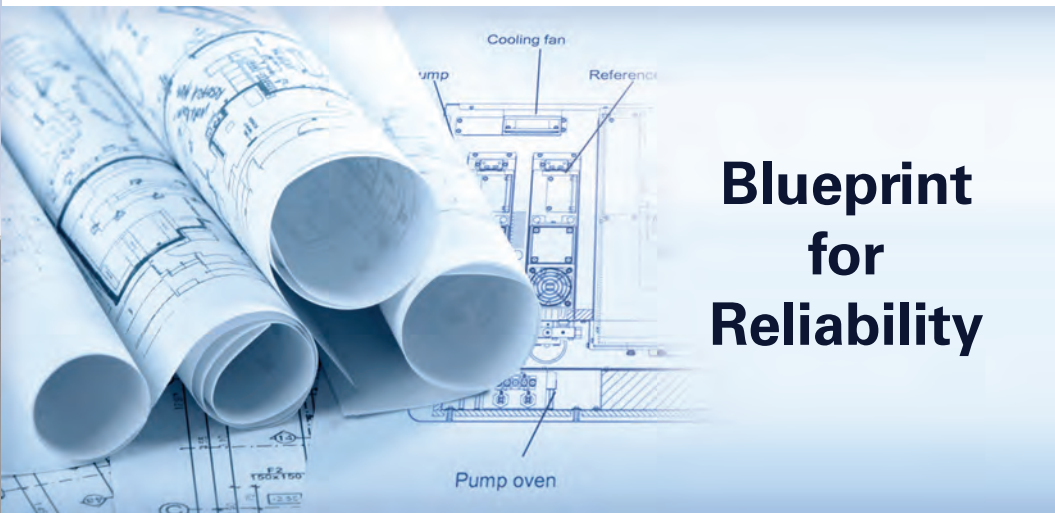
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