BACKGROUND

1.1 INTRODUCTION

This book is about modern size-exclusion chromatography (SEC). Size-exclusion chromatography is a liquid column chromatographic technique that sorts molecules according to their size in solution. The sample solution is introduced onto the column, which is filled with a rigid-structure, porous-particle column packing, and is carried by solvent (mobile phase) through the column. The size sorting takes place by repeated exchange of the solute molecules between the bulk solvent of the mobile phase and the stagnant liquid phase within the pores of the packing. The pore size of the packing particles determines the molecular size range within which separation occurs.

Throughout the book we use the term size-exclusion chromatography, which is meant to include the techniques originally (and sometimes still) referred to as gel permeation chromatography (GPC) and gel filtration chromatography (GFC). The term GPC was traditionally used when referring to analyses employing organic solvents and mobile phases for the separation. When aqueous solvents and mobile phases were used, the term GFC was used. Nowadays, gels are not always used as column packing materials. Also, one might employ aqueous solvents for separation one week and organic solvents the next, while the separation mechanism remains the same. Hence, the more general, all-inclusive term size-exclusion chromatography is preferred.
BACKGROUND

1.2 HISTORY

Size-exclusion chromatography has its roots in conventional liquid chromatography (LC). Ettre’s interesting paper, “The Development of Chromatography” [1], describes how David Talbot Day demonstrated in 1897 that crude oil fractions could be separated through pulverized fuller’s earth. Unfortunately, Day did not properly interpret the phenomenon that was occurring and, because of this, the original founding of chromatography is often ascribed to Michael S. Tswett. In 1903–1906, Tswett clearly described the chromatographic separation of colored vegetable pigments in petroleum ether on calcium carbonate and recognized the method as a general process. From Tswett’s early beginning, a large number of workers have continued to develop liquid chromatography into its present high-performance capabilities. Today, high-performance liquid chromatography is used widely in various forms within many scientific disciplines [2].

The origin of gel filtration chromatography is generally attributed to J. Porath and P. Flodin [3]. In 1959, these workers of the Institute of Biochemistry of the University of Uppsala (Porath) and of the Pharmacia Research Laboratories (Flodin), in Sweden, demonstrated that columns packed with cross-linked polydextran gels, swollen in aqueous media, could be used to size-separate various water-soluble macromolecules. The gels for this technique were made commercially available and have been used extensively for biomolecule separations in low-pressure systems. The technique has been reviewed by Porath [4] and, more recently, by Flodin [5].

In 1964, J. C. Moore of the Dow Chemical Company disclosed the use of cross-linked polystyrene “gels” for separating synthetic polymers soluble in organic solvents [6] and, with this event, conventional gel permeation chromatography (GPC) was born. It was recognized immediately that with proper calibration, gel permeation chromatography was capable of providing molar mass (M) and molar mass distribution (MMD) information for synthetic polymers. Because this information was difficult to obtain by other methods, gel permeation chromatography came rapidly into extensive use. The inception of GPC was reviewed some years later by Moore himself [7], while the background and applications of conventional early gel permeation chromatography have been reviewed by Bly [8].

The column packing materials used by Porath and Flodin for gel filtration and by Moore for gel permeation were particles of lightly cross-linked, porous, semi-rigid, organic-polymer networks. As such, they could be packed into columns and used with various mobile phases only at relatively low flow rates and pressures, less than 17 bar or 250 psi. At high pressures and flow rates, these packings collapse, and separations cannot be made. Because of these limitations, both conventional gel filtration chromatography and gel permeation chromatography are relatively slow techniques.

Modern, high-performance size-exclusion chromatography is a result of the development of small, more rigid porous particles for column packings. The first small particles introduced commercially for SEC were ε-Styragel (a trade name for microparticle cross-linked polystyrene gel) by Waters Associates, Milford, Massachusetts. Packed into efficient columns, these semirigid 10-μm particles
withstand relatively high pressure (e.g., 2000 to 3000 psi) and provide performance approximately 10 times better than that of the macroparticle cross-linked polystyrene (e.g., 70 to 150 µm Styragel) widely used previously. Subsequent to the introduction of µ-Styragel, completely rigid inorganic-based particle packings were developed (Chapter 6). Unger et al. [9,10] and Kirkland [11,12] have described porous silica particles, and Sato et al. [13] have discussed porous alumina for SEC.

1.3 UTILITY OF SEC

For water-soluble macromolecules of biochemical origin, separation by size-exclusion chromatography is normally desired for one or more of the following reasons:

1. To prepare molecular fractions for characterization or further use
2. To serve as a method for desalting or buffer exchange (i.e., to act as a substitute for dialysis)
3. To estimate molar mass using calibration standards or an absolute method (e.g., light scattering)
4. To estimate molecular association constants:
   a. Complexes of small molecules with macromolecules
   b. Macromolecular aggregation

Many examples of these uses are presented throughout this book, especially in Chapter 12.

The utility of aqueous size-exclusion chromatography is illustrated in Figure 1.1, where the separation of a number of protein molecules is made in a matter of minutes. Traditionally, this analysis takes several hours to perform. A calibration relating the molar mass of carbohydrate-free globular proteins in water to their retention volume is shown in Figure 1.2. This calibration plot, which was obtained in a few hours, would have taken much longer to obtain by large-particle-based conventional gel filtration techniques. Reference 14 provides a good review of the size-exclusion chromatography separation of proteins in both denaturing and nondenaturing solvents.

It is well known that many macromolecules, both natural and synthetic, are polydisperse with respect to molar mass. This is the case for biopolymers such as cellulose and the starch fractions amylose and amylpectin [17] and for all synthetic polymers, which can range from being narrowly to broadly polydisperse. As seen in Figure 1.3, in addition to an MMD, macromolecules can possess distributions in a variety of chemical and physical properties, including branching (long- and short-chain), chemical heterogeneity, and polyelectrolytic charge. A generic example of how the distribution of several of these properties as a function of $M$ may overlay the MMD of a polymer is shown in Figure 1.4.

The applications of polymers are often determined by the distributions of the chemical and physical properties present. The breadth of the MMD, for example,
BACKGROUND

Figure 1.1 Chromatogram for size-exclusion chromatography of proteins. Column, 30 × 0.41 cm stainless steel packed with 5 to 10-µm Glycophase G/CPG, 100-Å pore diameter; temperature, 25°C; velocity, 0.7 cm/s at 2700 psi; mobile phase, 0.1 M KH₂PO₄ (pH 6). (Reprinted with permission from Ref. 15.)

Figure 1.2 Relationship between molar mass and retention volume for certain proteins in water. (Reprinted with permission from Ref. 16.)
1.3 UTILITY OF SEC

Figure 1.3 Examples of macromolecular distributions. From left: molar mass, long- and short-chain branching, polyelectrolytic charge, chemical heterogeneity.

can affect the elongation and tensile strength of the macromolecule and adhesive properties of the final product; long-chain branching has a profound impact on such rheological properties as the viscosity of melts and solutions and the shear strength of formed products; chemical heterogeneity can affect the toughness, brittleness, and biodegradability of plastics. Table 1.1 lists the types of macromolecular property

<table>
<thead>
<tr>
<th>Differential weight fraction</th>
<th>Molar mass</th>
<th>Relative abundance of property X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical heterogeneity</td>
<td>MMD</td>
<td>Molar mass distribution</td>
</tr>
<tr>
<td>Charge distribution</td>
<td>SCBD</td>
<td>Distribution of long-chain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>branches as a function of $M$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Distribution of short-chain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>branches as a function of $M$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Charge distribution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Distribution of polyelectrolytic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>charge as a function of $M$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chemical heterogeneity,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>distribution of the percentage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>of one component of a copolymer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>as a function of copolymer $M$</td>
</tr>
</tbody>
</table>

Figure 1.4 Distribution of chemical and physical properties. Property X refers to LCB, SCB, charge, and % co-monomer. MMD, molar mass distribution; LCBD, distribution of long-chain branches as a function of $M$; SCBD, distribution of short-chain branches as a function of $M$; charge distribution, distribution of polyelectrolytic charge as a function of $M$; chemical heterogeneity, distribution of the percentage of one component of a copolymer as a function of copolymer $M$. 
### Background

#### Table 1.1 Macromolecular distributions: their measurement and end-use effects

| Macromolecular Property | Representative End-Use Properties Affected | Separation Method Used for Determination$^a$
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Molar mass</td>
<td>Elongation, tensile strength, adhesion</td>
<td>SEC, FFF, HDC, TGIC, CEC, SFC, MALDI-MS, rheology</td>
</tr>
<tr>
<td>Long-chain branching</td>
<td>Shear strength, tack, peel, crystallinity</td>
<td>SEC-MALS, SEC-VISC, rheology enzymeology</td>
</tr>
<tr>
<td>Short-chain branching</td>
<td>Haze, stress-crack resistance, crystallinity</td>
<td>SEC-IR, SEC-NMR, TREF,$^c$ enzymology</td>
</tr>
<tr>
<td>Cross-linking</td>
<td>Gelation, vulcanization, surface roughness</td>
<td>SEC-MALS, SEC-VIS, rheology</td>
</tr>
<tr>
<td>Architecture</td>
<td>Flow modification, diffusion, encapsulation</td>
<td>SEC-MALS-QELS-VISC</td>
</tr>
<tr>
<td>Tacticity</td>
<td>Crystallinity, anisotropy, solubility</td>
<td>SEC-NMR, TGIC, LCCC,</td>
</tr>
<tr>
<td>Chemical composition</td>
<td>Morphology, miscibility, solubility</td>
<td>GPEC, TGIC</td>
</tr>
<tr>
<td>Chemical heterogeneity</td>
<td>Toughness, brittleness, biodegradability</td>
<td>SEC-spectroscopy/ spectrometry, LCCC, PFC</td>
</tr>
<tr>
<td>Chemical composition vs. molar mass</td>
<td>Mechanical properties, blending, plasticization</td>
<td>2D-LC (e.g., SEC-GPEC)</td>
</tr>
<tr>
<td>Block sequence</td>
<td>Dielectric properties, reactivity, miscibility</td>
<td>SEC-spectroscopy, 2D-LC (e.g., PFC-SEC)</td>
</tr>
<tr>
<td>Base-pair sequence</td>
<td>Genetic code, heredity, miscibility</td>
<td>Automated DNA sequencing, MALDI-MS</td>
</tr>
<tr>
<td>Polyelectrolytic charge</td>
<td>Flocculation, transport, binding of metals</td>
<td>SEC-conductivity</td>
</tr>
<tr>
<td>Particle size</td>
<td>Packing, drag, friction, mixing</td>
<td>FFF, HDC, PSDA, sieving</td>
</tr>
</tbody>
</table>

Source: Ref. 20.

$^a$Many techniques require a concentration-sensitive detector (e.g., a differential refractometer), not included here for simplicity.

$^b$SEC, size-exclusion chromatography; FFF, field-flow fractionation; HDC, hydrodynamic chromatography; TGIC, temperature-gradient interaction chromatography; CEC, capillary electrokinetic chromatography; SFC, supercritical fluid chromatography; MALDI-MS, matrix-assisted laser desorption/ionization mass spectrometry; MALS, multiangle light scattering; VISC, viscometry; IR, infrared spectroscopy; NMR, nuclear magnetic resonance spectroscopy; TREF, temperature-rising elution fractionation; CRYSTALF, crystallization fractionation; QELS, quasielastic (dynamic) light scattering; LCCC, liquid chromatography at the critical condition; GPEC, gradient polymer elution chromatography; PFC, phase fluctuation chromatography; 2D-LC, two-dimensional liquid chromatography; PSDA, particle-size distribution analyzer.

$^c$For crystalline polymers only.
1.4 MOLAR MASS AVERAGES AND MOLAR MASS DISTRIBUTION

Size-exclusion chromatography normally is used as an analytical procedure for separating molecules by their difference in size and to obtain molar mass averages \(M_n\), \(M_w\), \(M_z\) or information on the molar mass distribution (MMD) of polymers. At times, however, it is also used for preparing various molar mass fractions for further use (Chapter 15). The raw-data SEC curve is a molecular size-distribution curve. If a concentration-sensitive detector is used, the SEC curve is really a size distribution curve in weight concentration. With calibration (Chapter 8) or static light-scattering detection (Chapter 9), the raw data are converted to a molar mass distribution curve and the respective molar mass averages can be calculated. Because determining molar mass averages and distributions remains the principal use of SEC, we present here a short overview for polymers of the meaning of molar mass distribution and molar mass averages \(M_n\), \(M_w\), and \(M_z\).

Various reaction mechanisms are employed for the synthesis of high polymers. Examples are the addition reaction to form polyethylene from ethylene, and the condensation polymerization of hexanedioc acid and hexamethylenediamine to form the polyamide (nylon). During the course of a polymerization reaction, a large quantity of polymer chains are initiated, grow, and then are terminated (i.e., stop growing). The number and length (or weight) of the polymeric chains formed during the reaction vary with the reaction mechanism and the reaction conditions employed. At
times, the distribution of these chains is accurately predictable from statistical considerations; at other times (nonequilibrium processes), a priori predictions are not accurate. In either case SEC can be used to determine experimentally the distributions and the molar mass averages of the polymer formed.

One convenient way of measuring the “average” chain length in a polymer sample provides a quantity known as $M_n$, the number-average molar mass. $M_n$ is historically significant because for many years it has been a characterizing value obtained directly in the laboratory by colligative property methods. $M_n$ also has been correlated with a number of polymer physical properties (Table 1.2) and is defined as the mass of the sample in grams $\sum W_i$, or $\sum N_i M_i$, divided by the total number of chains present, $N$, which is $\sum N_i$. Here $W_i$ and $N_i$ are the weight and number of molecules of molar mass $M_i$, respectively, and $i$ is an incrementing index over all molar mass present. Thus,

\[ M_n = \frac{\sum N_i M_i}{\sum N_i} = \frac{\sum W_i}{\sum (W_i/M_i)} \] (1.1.a)

and from SEC,

\[ M_n = \frac{\sum_{i=1}^{N_i} h_i}{\sum_{i=1}^{N_i} (h_i/M_i)} \] (1.1.b)

where $h_i$ is the SEC curve height at the $i$th volume increment and $M_i$ is the molar mass of the species eluted at the $i$th retention volume. The equation assumes that $h_i$ is proportional to solute concentration and $M_i$ is sampled in equal volume increments.

Another molar mass average that can be correlated with physical properties is the weight-average molar mass, $M_w$, which is determined in the laboratory from static light scattering (Section 9.3) and ultracentrifugation measurements as well as from SEC. It is defined as

\[ M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i} = \frac{\sum W_i M_i}{\sum W_i} \] (1.2.a)

and from SEC,

\[ M_w = \frac{\sum_{i=1}^{N_i} (h_i M_i)}{\sum_{i=1}^{N_i} h_i} \] (1.2.b)

Some observations about the relative properties of $M_n$ and $M_w$ have been made [15]. The value of $M_w$ is always larger than $M_n$, except that the values are identical for a monodisperse system. The ratio $M_w/M_n$, termed the molar mass polydispersity or, more simply, the polydispersity, is a measure of the breadth of the polymer molar mass distribution. $M_w/M_n$, is equal to unity for monodisperse systems, has a value of 2 for a Flory most probable distribution, and is exceedingly large for a
Table 1.2 Examples of effect of molar mass or molar mass distribution on various polymer properties

### A. General Correlations

<table>
<thead>
<tr>
<th>Polymer Property</th>
<th>Tensile Strength</th>
<th>Elongation Strength</th>
<th>Yield Strength</th>
<th>Toughness</th>
<th>Brittleness</th>
<th>Hardness</th>
<th>Abrasion Resistance</th>
<th>Softening Temperature</th>
<th>Melt Viscosity</th>
<th>Adhesion</th>
<th>Chemical Resistance</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase the molar mass</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Narrow the molar mass distribution</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

### B. Specific Correlations

<table>
<thead>
<tr>
<th>Polymer Property</th>
<th>Polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber and film strength, polymer solubility</td>
<td>Poly(11-hydroxyundecanoic acid), a polyester</td>
</tr>
<tr>
<td>Fiber strength</td>
<td>Polyesters from ω-hydroxydecanoic acid, b</td>
</tr>
<tr>
<td>Fiber tenacity</td>
<td>Nylon 6.6, c</td>
</tr>
<tr>
<td>Die swell</td>
<td>Styrene–butadiene rubber, c</td>
</tr>
<tr>
<td>Sensitivity as an electron resist</td>
<td>Poly(methyl methacrylate), d</td>
</tr>
<tr>
<td>Solution viscosity and shear stability index</td>
<td>Polyalkylacrylates, e</td>
</tr>
<tr>
<td>Die swell</td>
<td>Polyolefins, f</td>
</tr>
<tr>
<td>“Acceptance quality” of circuit boards</td>
<td>Polyethylene (PE), g</td>
</tr>
<tr>
<td>Strength, toughness</td>
<td>Polystyrenes, g</td>
</tr>
<tr>
<td>Melt fluidity, film friction</td>
<td>Polystyrenes, g</td>
</tr>
<tr>
<td>Strength, toughness</td>
<td>Polyethylene (PE), h</td>
</tr>
<tr>
<td>Fluidity (ease of processing)</td>
<td>Epoxy resins, i</td>
</tr>
<tr>
<td>Density (d) and shrinkage (s) of films</td>
<td>Cellulose triacetate, j</td>
</tr>
</tbody>
</table>

Correlation:
- Increase with increasing M<sub>n</sub> while solubility decreases with increasing M<sub>n</sub>.
- Increases with increase in M<sub>n</sub>.
- Increases with MMD.
- Increases with higher M<sub>n</sub> and increases with narrower MMD.
- Decrease with a decrease in M<sub>w</sub> caused by shearing.
- Increase with increasing M<sub>n</sub>.
- Decrease with increasing M<sub>n</sub>.
- Overall SEC curve (MMD) profile.
- d increases with MMD, s decreases with MMD.

- Profile of performance property dependence on molecule–structure parameters for typical parameters. Key: +, property goes up; −, property goes down; 0, little change.
cross-linked polymer. High-molar-mass species particularly influence the value of $M_w$, whereas the value obtained for $M_n$ is influenced more by species at the lower end of the molar mass distribution. If equal weights of molecules with $M = 10,000$ and $M = 1,000,000$ are mixed, $M_w = 55,000$ and $M_n = 18,200$; if equal numbers of each kind of molecule are mixed, $M_w = 92,000$ and $M_n = 55,000$ [23].

The molar mass distribution (MMD) can be expressed graphically in integral form as the cumulative weight fraction or cumulative number fraction versus molar mass ($M$) (or $X$, the number of repeat units in the chain). The MMD may also be in the differential form as the weight fraction or number fraction versus $M$ (or $X$). As used here, $M$ is a generic term for the molar mass, which is obtained by multiplying the repeat unit $M$ by the number of repeat units $X$. The true MMD can be deduced from the SEC curve only via careful application of calibration curves or by the use of static light-scattering detection. Figure 1.5 shows the differential MMD of a sample of brominated polystyrene, PSBr, as determined by SEC with both differential refractive index and static multiangle light-scattering detection (both detection methods are described in Chapter 9) [24–26]. Marked on the curve are the number-, weight-, and $z$-averages of the molar mass ($M_z$ is described below). It is worth noting the broad molar mass range covered by this sample’s MMD, extending from $2 \times 10^4$ to $5 \times 10^6$ g/mol.

By proper selection of columns and other experimental conditions, the molar mass range accessible by SEC can be very large. Figure 1.6 shows a calibration curve based on narrow polydispersity linear polystyrene (PS) standards. The molar mass range accessible by SEC can be very large. Figure 1.6 shows a calibration curve based on narrow polydispersity linear polystyrene (PS) standards.
1.4 MOLAR MASS AVERAGES AND MOLAR MASS DISTRIBUTION

![Graph showing molar mass distribution](image)

**Figure 1.6** Separation range of SEC: elution of linear polystyrene standards. Circles denote average elution time of triplicate injections of each narrow polydispersity PS standard, with error bars substantially smaller than data markers and therefore not shown. Numbers next to markers denote the peak-average molar mass, $M_p$, of each standard in g/mol. Solid line is a third-order fit to the data, with $r^2 = 0.999$. Solvent, 1,2,4-trichlorobenzene (with 1.5 mg/mL Santonox); temperature, 135°C; columns, PLgel Mixed A; flow rate, 0.1 mL/min; detector, DRI. (Reprinted with permission from Ref. 27.)

The mass range covered by this curve spans over five orders of magnitude, from 162 to $2 \times 10^7$ g/mol!

Historically, before SEC became available, the MMD curves were very difficult to obtain. Examples of some of the various $M$ and MMD parameters are shown in Figures 1.7 to 1.9, which represent theoretical plots for condensation polymers (e.g., nylon) and other distribution functions. In the figures, the extent of reaction $p$ is defined as the mole fraction (of all functional groups available for polymerization both in monomer and in growing polymer chains) that has reacted at various times. The great utility of $M_n$, $M_w$, and the MMD is shown in Table 1.2, where various correlations with physical properties for synthetic polymers are compiled. Calculations of $M_n$, $M_w$, $M_z$, and MMD are performed routinely by most commercial SEC software.

It is not always necessary to calculate the molar mass averages or MMD to obtain useful information about a sample from the SEC curve. Simple inspection of chromatograms often reveals important information. For example, Figure 1.10 shows raw-data chromatograms of two batches of supposedly the same epoxy resin. Inspection indicates immediately, however, that batch 1443 is missing a significant amount of material on the low-molar-mass side of the main peak. This absence of certain material could account for differences in sample properties. There also might be...
differences in $M_n$ or $M_w$ between these lots, but the values obtained would not indicate where differences occur in the overall MMD.

As mentioned above, values of $M_w/M_n$ have often been used traditionally to express the breadth of the molar mass distribution. Figure 1.11 shows, however, that three different distribution curves can provide identical values of $M_n$, $M_w$, and $M_z$.  

![Figure 1.7](image1.png)

**Figure 1.7** Mole fraction distribution of chain molecules in linear condensation polymers for several extents of reaction $p$. (Reprinted with permission from Ref. 28.)

![Figure 1.8](image2.png)

**Figure 1.8** Weight fraction distributions of chain molecules in linear condensation polymers for several extents of reaction $p$. (Reprinted with permission from Ref. 28.)
1.4 MOLAR MASS AVERAGES AND MOLAR MASS DISTRIBUTION

Figure 1.9  Theoretical size-exclusion chromatograms for three values of \( \frac{X_w}{X_n} \) according to various distribution function formulations. Dashed-dotted curves, logarithmic normal; dashed curves, Schulz–Zimm; solid curves, modified Stockmayer; \( X_w \), weight-average chain length; \( X_n \), number-average chain length. (Reprinted with permission from Ref. 29.)

The parameter \( M_z \) is related to a higher moment of the distribution defined by

\[
M_z = \frac{\sum N_i M_i^3}{\sum N_i M_i^2} \tag{1.3}
\]

At times, \( M_z \) is correlated to polymer processing properties, in particular to properties such as flex life and stiffness that are governed by the longest chains in the MMD. If molar mass values were obtained for these three distributions by light scattering, osmometry, or centrifugation, all the polymers would have identical \( M_n \) or \( M_w \) or \( M_z \) values and identical polydispersity \( M_w / M_n \). Yet, clearly, the distributions are not alike, and physical properties of materials fabricated from these polymers
BACKGROUND

Figure 1.10 Comparison of two lots of SU-8 resin by SEC showing batch variations. (Reprinted with permission from Ref. 30.)

Figure 1.11 Three differential weight distribution curves corresponding to identical values of $M_n$, $M_w$, and $M_z$. Curve 1 is a logarithmic normal function; curves 2 and 3 are sums of two exponential functions. (Reprinted with permission from Ref. 31.)
could be different. This information illustrates the utility of the entire MMD profile as provided by SEC.

Two other molar mass averages are used in this book and will be encountered in the literature and in daily use. These are the peak-average molar mass, $M_p$, and the viscosity-average molar mass, $M_v$ or $M_\eta$. The peak-average molar mass is simply the molar mass of the slice eluting at the peak apex in an SEC chromatogram. It is used primarily in assigning molar masses when constructing peak-position calibration curves based on narrow MMD standards (see Section 8.2).

The viscosity-average molar mass is defined as

$$M_v = M_\eta = \left[ \frac{\sum_{i=1}^{N} h_i (M_i)^\alpha}{\sum h_i} \right]^{1/\alpha}$$  \hspace{1cm} (1.4)

The term $\alpha$ corresponds to the exponent in the Mark–Houwink equation (Equation 8.2). Molding properties and polymer extrudability have often been found to correlate with $M_v$. The viscosity-average molar mass is unlike $M_n$, $M_w$, and $M_z$. The latter three averages are “absolute” in the sense that, properly measured, their values are independent of the solvent–temperature conditions of analysis. $M_v$, however, will depend on experimental conditions; the latter, as we will see in Chapters 8 and 9, enter the equation through the $\alpha$ term.

1.5 STRUCTURE OF THE BOOK

The next three chapters (Chapters 2 to 4) serve to introduce the reader to the fundamental chromatographic aspects of size-exclusion chromatography: retention, band broadening, and resolution. The treatment of these topics is rather detailed in the hopes of establishing a strong foundation on which to design and optimize separations. In Chapter 5 we describe the various components of an analytical SEC system, concentrating on the hardware that precedes the column. The latter is the focus of Chapter 6, where we describe the types of columns and column packing materials available and how packing materials are synthesized and columns packed. Chapter 7 provides a lengthy discussion of experimental variables, an extremely practical discussion about most of the considerations that an actual SEC practitioner must take into account to obtain reliable, reproducible data in a safe manner, all the while ensuring that the equipment is taken care of.

In the chapter on calibration techniques, Chapter 8, we differentiate between the various types of calibration effected using narrow polydispersity standards, giving the relative advantages and disadvantages of each. We also discuss calibration methods based on broad MMD standards, the accuracy and linear ranges of the various calibrations, and recent developments regarding band-broadening corrections for certain types of calibration methods.

Chapters 9 and 10 deal with physical and chemical detection methods, respectively. The discussion in Chapter 9 revolves mostly around the methods themselves.
BACKGROUND

In Chapter 10 we also describe the type of information obtained from the chemical detection methods, as these methods are likely to be more familiar to the reader than the physical methods from Chapter 9. Because of this, we devote Chapter 11 to the architectural and thermodynamic information obtainable when a multiplicity of physical detection methods is used. Indeed, the use of multiple detection methods in SEC has transformed the technique over the last two decades [18,19,32].

Because of the types of analytes that are water-soluble (e.g., proteins and peptides) and the types of effects that can be encountered when using water as a solvent and chromatographic mobile phase, we have dedicated one chapter (Chapter 12) to aqueous SEC. As discussed, not only is aqueous SEC used for proteins and peptides but also for analyzing a variety of polysaccharides and synthetic polymers, including dendrimers and polyelectrolytes.

Like the use of multiple detection methods, another area where SEC has experienced tremendous growth in the last decade is in the analysis of oligomers [32]. This is due to the great advances in column technology for oligomeric analysis, driven in many ways by regulatory requirements. Oligomeric SEC is the subject of Chapter 13.

Two current areas of growth for SEC are two-dimensional (2D) chromatography and high-speed analysis [32]. Understanding the physicochemical composition of complex polymers is not always straightforward, but is vital to optimizing the processing and end use of materials. This “deformulation” of a material is best done using more than one separation dimension. The capability of SEC to separate analytes based on size (which can then be related to molar mass) affords it a preeminent role in 2D-LC macromolecular analysis, the subject of Chapter 14.

High-speed SEC analysis, vital for high-throughput screening, for combinatorial research, and to meet the increasing quality assurance and quality control (QA/QC) demands of industrial production, is treated in Chapter 15. We also discuss a number of other “special techniques,” niche methods such as recycle, inverse, vacancy, and differential SEC, as well as more widespread applications such as preparative SEC and size-exclusion electrochromatography.

In the final chapter, Chapter 16, we look at high-temperature SEC (used primarily, although not exclusively, in the study of polyolefins) and at connections between SEC and rheology. This chapter distinguishes itself from the others in that some familiarity by the reader with rheological methods and terminology is assumed. The particular connections we explore are the rheological determination of the MMD of polymers, which is a primary application of SEC; how to obtain rheological properties of polymers from SEC measurements; and how SEC and rheology combine in the study of dilute oligomer solutions. New theories, based on a generalized $M$-averaging concept, are developed to help to close the gap between SEC and rheology measurements.

REFERENCES

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