MODERN SIZE-EXCLUSION LIQUID CHROMATOGRAPHY

Practice of Gel Permeation and Gel Filtration Chromatography

SECOND EDITION

André M. Striegel
Wallace W. Yau
Joseph J. Kirkland
Donald D. Bly

WILEY
A JOHN WILEY & SONS, INC., PUBLICATION

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FOREWORD

From the very beginning, synthetic polymers were so immensely useful that their development and commercialization followed almost immediately after their invention. The same was true for size-exclusion chromatography (SEC or gel-permeation chromatography, GPC) as a method for polymer characterization. SEC yielded eminently useful information (complete molar mass distributions) much more easily and more rapidly than did previous methods. In addition, the simultaneous development of high-pressure liquid chromatography for "small" (low-molar-mass) molecules meant that SEC soon became highly precise (i.e., repeatable), robust, and automatic. SEC was — and is — embraced by industry, and the greatest experts have learned the trade there through extensive personal experience or apprenticeship. In industry, publishing the tricks of one's trade is generally discouraged, and those who do publish are often frowned upon. If we combine this with the gigantic effort it takes to write a book, the very existence of the monumental first edition of *Modern Size-Exclusion Liquid Chromatography* by Wallace Yau, Jack Kirkland, and Donald Bly may be considered a near miracle.

I am looking through my copy for the umpteenth time. I had to retrieve it from the lab. It usually finds its way onto the desk of one of the Ph.D. students — a good sign. It is decorated with a number of yellow Post-it notes marking important passages — another good sign. It is remarkable how much this 30-year old book is being used. It is also understandable and even commendable that this is the case. Reading through the book is still a humbling experience. It makes me realize how many things I don't know. It is, as the subtitle reads, a guide to *The Practice of Gel-Permeation and Gel-Filtration Chromatography*. It is also much more. It is an excellent introduction to the principles of size-exclusion chromatography and of a great number of related subjects. It reflects vast knowledge, but more importantly, it displays a thorough understanding. It is a great book.

André Striegel has accepted the daunting task of rewriting the book. I hardly think it is possible to improve the quality of the text, as this would imply producing something greater than great. Maintaining the quality of the text is already a challenging ambition. Fortunately, he has been getting the best possible help through the active involvement of the original authors.

There is, however, one aspect in which the first edition of *Modern Size-Exclusion Liquid Chromatography* can be significantly improved. We do not need something

greater than great, but we do need something more up to date than what was "modern" 30 years ago. Putting the word *modern* in the title entails the danger of a text not living up to expectations; it also provides encouragement for renewing the material. It has taken quite some time for someone to realize the latter implication, but here we are. The new edition describes twenty-first-century SEC. A large number of new developments are described and new chapters are added.

The most important question that remains is whether SEC is as important now as it was 30 years ago. Surely, measuring property distributions of polymers has become much more important, because there are many more different polymer formulations for many more applications. Moreover, both the formulations and the applications are increasingly sophisticated. We need very good tools to measure distributions. We need other liquid-chromatographic techniques to characterize other types of distributions, such as those describing the chemical composition or number and type of functional groups. In principle, we may use mass spectrometry to measure molar mass distributions and to obtain additional chemical information. However, for all but the narrowest distributions with the most homogeneous ionization profiles, SEC is still the preferred technique. In most cases this may easily remain true for the next 30 years.

We need SEC more than ever in research laboratories where polymers and materials are being investigated and applied; in material science, life science, food science, and many other fields. And perhaps most important, SEC remains an invaluable tool in industry. Chromatographers, polymer scientists, and many others should benefit from entering the era of truly *Modern Size-Exclusion Liquid Chromatography*.

Amsterdam
June 2008

PETER J. SCHOENMAKERS

PREFACE

Much has changed in size-exclusion chromatography (SEC) since publication of the first edition of this book in 1979. As a result, this second edition is an almost complete rewrite of the first, to take into account the many changes that have occurred in SEC since then. While the fundamentals of the method were well understood at the time, advances in both column and detector technology have been transformative. A half-century after its inception, the principal use of SEC remains determining the molar mass averages and distributions of natural and synthetic polymers. While this is still generally accomplished through the application of calibration curves, the popularization of robust, easy-to-use light-scattering photometers now allows users to measure these properties in absolute, calibrant-independent fashion. Similarly, the combination of multiple detection methods allows for obtaining a truly impressive variety of polymer properties. Indeed, the use of multidetector SEC has ushered in a new era of polymer analysis. A variety of chemical and physical properties of macromolecules can now be determined as a continuous function of molar mass, with many other parameters obtained from the same set of analyses.

The applicability of SEC has also extended into both smaller and more complex realms. Column advances, dictated by sample performance as well as legal requirements, have advanced the area of oligomeric SEC. Characterization and quantitation of polymers is now possible: in many cases, down to a single, monomeric repeat unit. Meanwhile, the complexity of real-world polymers and the need to understand their characteristics in order to optimize processing and end-use properties has served to further the development of polymer two-dimensional liquid chromatography (2D-LC). Because of its premier status in characterizing the molar mass distribution, SEC is virtually always one of the dimensions of separation.

In light of all of the above, we have tried to bring this book up to date on developments in multidetector, oligomeric, and two-dimensional analysis, among others. We place special emphasis on the wealth of information that can be obtained from a multidetector SEC experiment. As with the first edition, we have tried to keep this as much a "how to" book as a "why?" book. Because our main audience is the practitioner of SEC, we try to guide this scientist in designing experiments, carrying them out, and interpreting the results. No aspect of the technique is treated as a "black box," and we have tried to share with the reader as much of our (often hard-earned) practical experience as possible.

Those familiar with the first edition will see that detection techniques and structure–property relations are treated much more heavily in this second edition, as noted by the inclusion of individual chapters dealing with physical detectors (Chapter 9), chemical detectors (Chapter 10), and polymer architecture and dilute solution thermodynamics (Chapter 11). We also devote new, individual chapters to aqueous SEC (Chapter 12), to oligomeric SEC (Chapter 13), and to the role the technique plays in 2D-LC (Chapter 14). Techniques that are becoming more widespread, such as high-speed SEC, as well as niche methods such as inverse and recycle SEC, are treated in Chapter 15. Connections with rheology are explored in the final chapter (Chapter 16). This is the only chapter in the book that presupposes some familiarity by the reader with the subject matter.

The fundamental chapters dealing with retention (Chapter 2), band broadening (Chapter 3), and resolution (Chapter 4) have been updated where appropriate. The same is true of the chapters dealing with calibration methods and column technology (Chapters 8 and 6, respectively). Less emphasis is placed in this edition on column-packing techniques, for example, due to the fact that most current users employ commercially available columns. Also, the chapter on data handling in the first edition has been eliminated, due to the fact that the overwhelming majority of practitioners employ commercially available software packages for data acquisition and handling.

The original chapters on operating variables and laboratory techniques have been combined into the current chapter on experimental variables and techniques (Chapter 7). This combined chapter has also been updated with respect to a more refined understanding of analytical procedures, often due to advances in hardware. Here, the user is likely to find a good deal of practical information regarding experimental design (from selecting columns to selecting a solvent), sample preparation, execution of experiments, instrument care, and troubleshooting. For parameters that can have an adverse effect on results, we try to explain how these effects are brought about and what can be done to avoid or minimize them.

We would like to express our thanks to family, friends, and associates who have provided encouragement and support in bringing about the second edition of this book. We are particularly grateful to Professors John G. Dorsey and Peter J. Schoenmakers for their critical review of several chapters and for their insightful comments and suggestions. Any errors that remain are entirely our own fault!

Tallahassee, Florida
November 2008

A. M. STRIEGEL
W. W. YAU
J. J. KIRKLAND
D. D. BLY

1

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.

Modern Size-Exclusion Liquid Chromatography: Practice of Gel Permeation and Gel Filtration Chromatography, Second Edition
By André M. Striegel, Wallace W. Yau, Joseph J. Kirkland, and Donald D. Bly

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, semirigid, 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 μ-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 amylopectin [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,

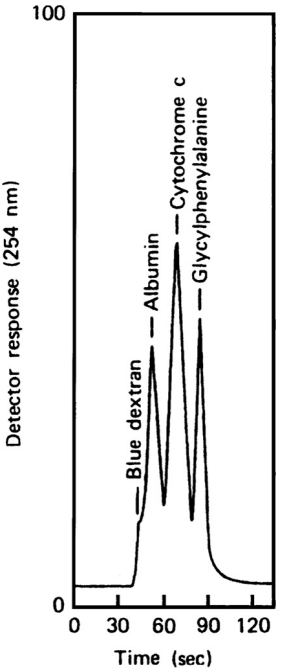

Figure 1.1 Chromatogram for size-exclusion chromatography of proteins. Column, 30 × 0.41 cm stainless steel packed with 5 to 10-μm Glycophase G/CPG, 100-Å pore diameter; temperature, 25°C; velocity, 0.7 cm/s at 2700 psi; mobile phase, 0.1 *M* KH_2PO_4 (pH 6). (Reprinted with permission from Ref. 15.)

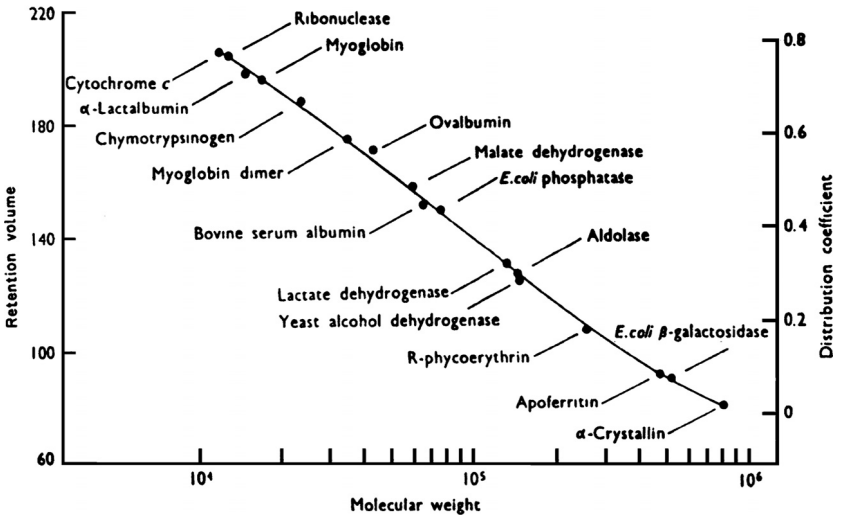

Figure 1.2 Relationship between molar mass and retention volume for certain proteins in water. (Reprinted with permission from Ref. 16.)

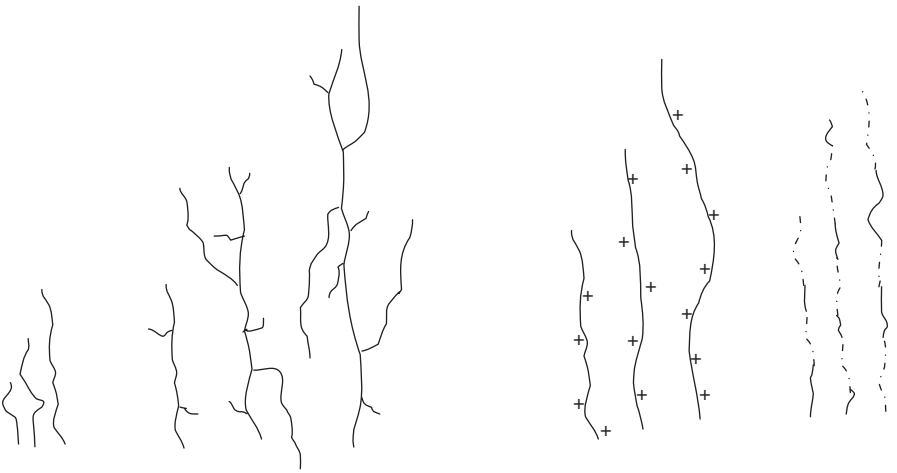

Figure 1.3 Examples of macromolecular distributions. From left: molar mass, long- and short-chain branching, polyelectrolytic charge, chemical heterogeneity.

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

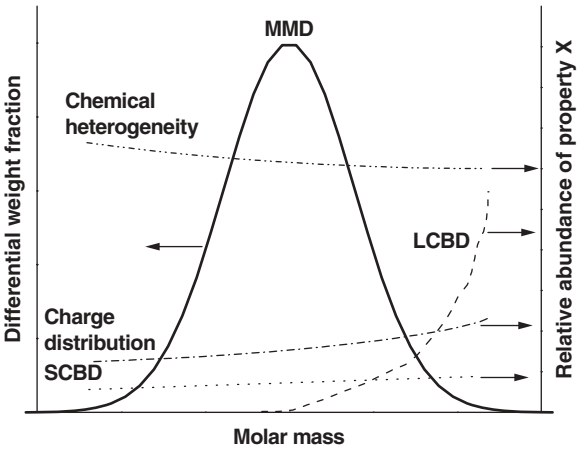

Figure 1.4 Distribution of chemical and physical properties. Property *X* refers to LCB, SCB, charge, and % co-monomer. MMD, molar mass distribution; LCBD, distribution of long-chain branches as a function of *M*; SCBD, distribution of short-chain branches as a function of *M*; charge distribution, distribution 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*.

Table 1.1 Macromolecular distributions: their measurement and end-use effects[a]

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

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; CRYSTAF, 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.

distributions that can exist or coexist in polymers, how these properties affect both processing and end use, and the types of separation methods used for measuring these distributions. As can be seen, SEC is the most widely represented technique in the table, especially when combined with a number of analytical techniques that can serve as detection methods: light scattering, viscometry, mass spectrometry, conductivity, spectroscopic methods, and so on [18,19]. The power of multidetector SEC will be a recurrent theme in this book.

Several nonseparation techniques are also listed in the last column of Table 1.1. These include enzymology, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), rheology, and sequencing. All these provide information which can, in select cases, closely complement that obtained by the separation methods. For example, the polysaccharide pullulan can possess an MMD, determined most accurately and conveniently using SEC with both a concentration-sensitive detector (e.g., a differential refractometer) and a static light-scattering detector [21]. Pullulan is composed of maltotriose units joined to each other via α-(1 $\rightarrow$ 6) linkages, but pullulan also possesses about 6.6% maltotetraose units. Whether these maltotetraose units were distributed uniformly and linearly along the pullulan backbone, were located at the chain ends, or were arranged along the backbone such as to form short-chain one- to three-glucose unit branches was not known originally. The matter was resolved using enzymatic analysis, which showed that the maltotetraose units were distributed along the pullulan backbone and were linked terminally (i.e., without resulting in short-chain branching) [22].

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 hexanedioic 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,

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

and from SEC,

$$M_n = \frac{\sum_{i=1}^{N} h_i}{\sum_{i=1}^{N} (h_i / M_i)} \tag{1.1.b}$$

where h_i is the SEC curve height at the ith volume increment and M_i is the molar mass of the species eluted at the ith 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

$$M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i} = \frac{\sum W_i M_i}{\sum W_i} \tag{1.2.a}$$

and from SEC,

$$M_w = \frac{\sum_{i=1}^{N} (h_i M_i)}{\sum_{i=1}^{N} h_i} \tag{1.2.b}$$

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[a]

	Tensile Strength	Elongation	Yield Strength	Toughness	Brittleness	Hardness	Abrasion Resistance	Softening Temperature	Melt Viscosity	Adhesion	Chemical Resistance	Solubility
Increase the molar mass	+	+	+	+	+	+	+	+	+	−	+	−
Narrow the molar mass distribution	+	−	−	+	−	−	+	+	+	−	+	0

B. Specific Correlations

Polymer	Property	Correlation
Poly(11-hydroxyundecanoic acid),[b] a polyester	Fiber and film strength, polymer solubility	Strength increases with increasing M_n while solubility decreases with increasing M_n
Polyesters from ω-hydroxydecanoic acid[c]	Fiber strength	Increases with increase in M_n
Nylon 6,6[d]	Fiber tenacity	Increases with M_n
Styrene–butadiene rubber[e]	Die swell	Increases with increase in MMD
Poly(methyl methacrylate)[f]	Sensitivity as an electron resist	Increases with higher M_n and increases with narrower MMD
Polyalkylacrylates[g]	Solution viscosity and shear stability index	Decrease with a decrease in M_w caused by shearing
Polyolefins[g]		
Polystyrenes[g]		
Polyethylene (PE)[h]	Strength, toughness	Increase with increasing M_n
	Melt fluidity, film friction	Decrease with increasing M_n
PE[h]	Strength, toughness	Increase with decreasing M_w/M_n
	Fluidity (ease of processing)	Decrease with decreasing M_w/M_n
Epoxy resins[i]	"Acceptance quality" of circuit boards	Overall SEC curve (MMD) profile
Cellulose triacetate[j]	Density (d) and shrinkage (s) of films	d increases with MMD, s decreases with MMD

Source: (A) Reprinted in part from E. A. Collins, J. Bareš, and F. W. Billmeyer, Jr., *Experiments in Polymer Science*, Wiley, New York, 1973, p. 312, with permission.

[a] Profile of performance property dependence on molecule–structure parameters for typical parameters. Key: +, property goes up; −, property goes down; 0, little change.

[b] V. V. Korshak and S. V. Vinogradovia, *Polyesters*, translated from the Russian by B. J. Hazzard, Pergamon Press, New York, 1965, p. 310.

[c] W. H. Carothers and F. J. van Natta, *J. Am. Chem. Soc.*, **55**, 4715 (1933).

[d] J. Zimmerman, *Text. Manuf.*, **101**, 19 (1974).

[e] W. Mills and F. Giurco, *Rubber Chem. Technol.*, **49**, 291 (1976).

[f] J. H. Lai and L. Shepherd, *J. Appl. Polym. Sci.*, **20**, 2367 (1976).

[g] D. E. Hillman, H. M. Lindley, J. I. Paul, and D. Pickles *Br. Polym. J.*, **7**, 397 (1975).

[h] F. W. Billmeyer, Jr., *Textbook of Polymer Science*, Wiley, New York, 1972, p. 382.

[i] *Ind. Res.*, Jan. 1977, p. C1.

[j] N. P. Zakurdaeva and T. A. Ivanova, *Plast. Massy*, **9**, 68, 1976; *Chem. Abstr.*, **85**: 193430b.

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 2×10^4 to 5×10^6 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

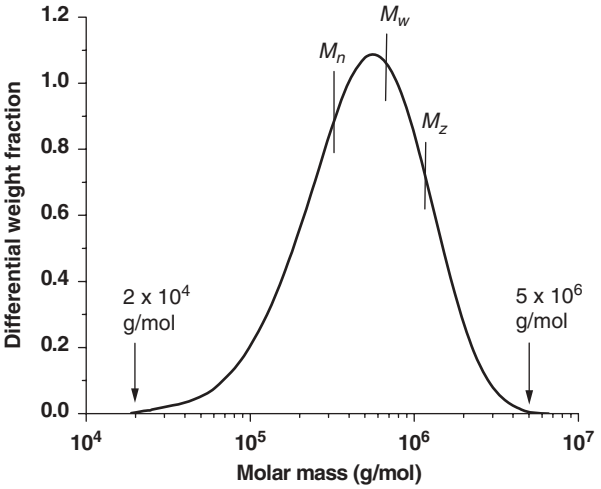

Figure 1.5 Molar mass averages and distribution of brominated polystyrene, PSBr. MMD and molar mass averages determined by SEC with differential refractive index and static multiangle light-scattering detection. Solvent, DMAc/0.5% LiCl; temperature, 35°C; flow rate, 1 mL/min; columns, three PSS GRALlinear 10-μm columns and one PSS GRAL10000 10-μm column, preceded by a guard column. $M_n = 3.26 \times 10^5$ g/mol, $M_w = 6.74 \times 10^5$ g/mol, $M_z = 1.17 \times 10^6$ g/mol, $M_w/M_n = 2.07$. (Adapted from Ref. 26.)

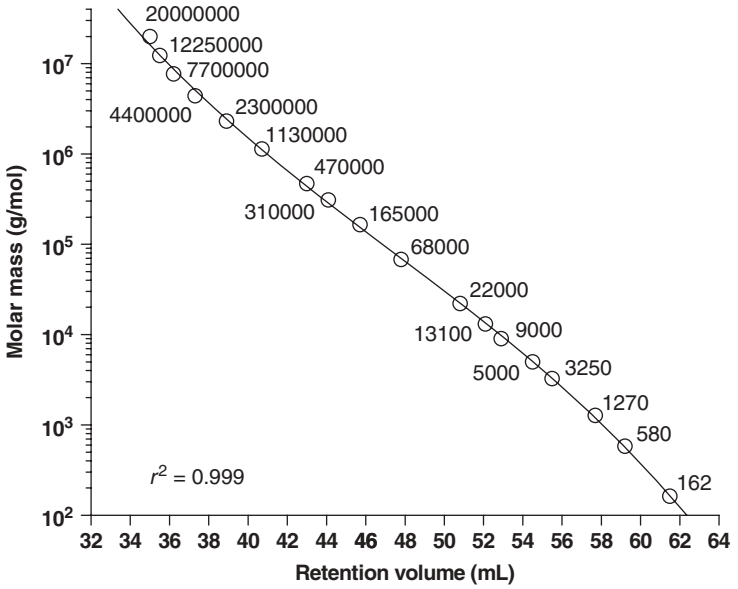

Figure 1.6 Separation range of SEC: elution of linear polystyrene standards. Circles denote average elution time of triplicate injections of each narrow polydispersity PS standard, with error bars substantially smaller than data markers and therefore not shown. Numbers next to markers denote the peak-average 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.)

mass range covered by this curve spans over five orders of magnitude, from 162 to 2×10^7 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

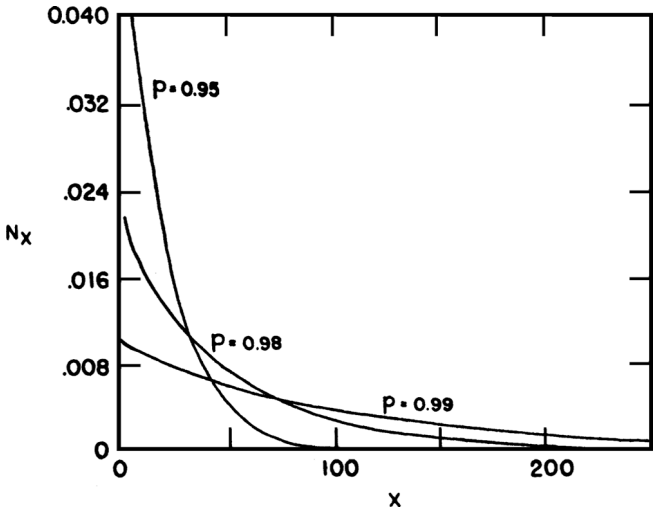

Figure 1.7 Mole fraction distribution of chain molecules in linear condensation polymers for several extents of reaction p. (Reprinted with permission from Ref. 28.)

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.

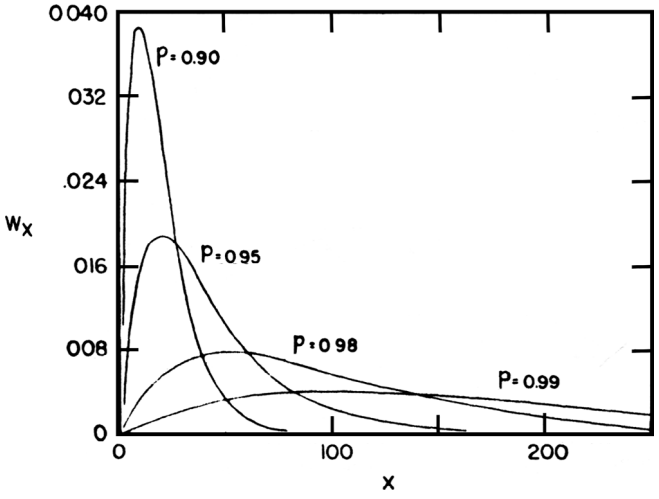

Figure 1.8 Weight fraction distributions of chain molecules in linear condensation polymers for several extents of reaction p. (Reprinted with permission from Ref. 28.)