Modern Gel Permeation Chromatography

Edward P. Otocka

Bell Laboratories, Murray Hill, New Jersey 07974

Received March 2, 1973

The determination of the molecular weight distribution (MWD) of synthetic polymers is one of the most important and persistent tasks facing the polymer chemist. The MWD of a given polymer sample is perhaps its most basic characteristic. The details of the MWD reflect the synthesis conditions and are therefore useful in determining rate constants for initiation, polymerization, chain-transfer, and termination reactions. The MWD is also a sensitive measure of degradation during processing or in service, and is therefore helpful in defining manufacturing procedure and evaluating stabilizer formulations. For polymers in more critical engineering applications, the MWD may be correlated with modulus, strength, and crack resistance, which are necessary design parameters.

All synthetic polymers are polydisperse, that is, there is a distribution of chain lengths in a given sample. This distribution is usually characterized by its principal moments: the number average molecular weight, $\bar{M}_n$, weight-average molecular weight, $\bar{M}_w$, and z-average molecular weight, $\bar{M}_z$, where n_i is

$$\bar{M}_n = \sum_{i=1}^{\infty} n_i M_i / \sum_{i=1}^{\infty} n_i \quad (1)$$

$$\bar{M}_w = \sum_{i=1}^{\infty} n_i M_i^2 / \sum_{i=1}^{\infty} n_i M_i \quad (2)$$

$$\bar{M}_z = \sum_{i=1}^{\infty} n_i M_i^3 / \sum_{i=1}^{\infty} n_i M_i^2 \quad (3)$$

the number of molecules of the ith kind and M_i is their molecular weight. Polydispersity is normally defined as the ratio, $\bar{M}_w/\bar{M}_n$. For a more specific discussion of molecular weight distributions, general references may be consulted.[1,2]

The wide variety of molecular weight distributions possible is indicated schematically in Figure 1. Condensation polymers such as polyesters achieved by reaction of a diacid chloride and diol characteristically exhibit a polydispersity of 2 or greater. Free-radical addition polymers, synthesized without chain-transfer agents and limited conversion, have a polydispersity of 1.5. However, commercial addition polymers often exhibit polydispersities of 3–10 as a result of multiple termination reactions, chain transfer, and high conversion. Finally, polymerizations where almost all polymer chains are initiated simultaneously and terminated only after complete consumption of monomer ("living" ionic polymerizations) yield polydispersities of 1.1 or less. Detailed discussions of the various polymerization kinetics are beyond the intent of this Account and may be found in general references.[1,2]

In the case of biopolymers, the situation is somewhat different. Proteins and polynucleotides in their native state are monodisperse. However, the molecular weight may often be associated with their function and is a necessary piece of information for their characterization. The biochemist requires analytical and preparative methods based on molecular weight for assay and isolation of these polymers.

Laboratory methods for the determination of these molecular weight averages individually have been available for some time. The $\bar{M}_n$ may be determined by osmotic pressure, $\bar{M}_w$ may be calculated from light-scattering data, and $\bar{M}_z$ from equilibrium sedimentation experiments. Details of these methods may be found in general polymer texts.[1,2] All these methods suffer the common drawback of considerable experimental effort expended for limited information in return.

The solubility of a given polymer decreases with increasing molecular weight. The first solution fractionation methods utilized this solubility change by precipitating successively lower molecular weight polymer by the addition of nonsolvent. After collection and isolation, the fractions were characterized, and the molecular weight distribution of the whole polymer was calculated. A column technique, the Baker-Williams method, was developed to utilize the solubility differences.[3] A column is packed with support on which the polymer had been deposited by solvent evaporation. Successively better solvents are pumped through the column to elute successively higher molecular weight functions. The methods may be used to best advantage as a preparative tool, but the technique suffers from a decreasing change in solubility at molecular weights $>2\text{–}3 \times 10^5$, and thus cannot readily provide narrow fractions above this point.

Gel Chromatography

There are two nomenclatures which have arisen from the separate developments of chromatographic methods. Gel filtration chromatography has been successfully employed in aqueous systems by biochemists for more than a decade, using soft, cross-linked dextran beads.[4] Gel permeation chromatogra-

Edward P. Otocka received a B.S. in Chemistry from Lehigh University in 1962 and a Ph.D. in Polymer Chemistry at Polytechnic Institute of Brooklyn in 1966. Since that time he has worked at Bell Laboratories, where he is currently a member of the Polymer Chemistry Research Department. His main research interests are polymer rheology, stabilization, modification, and characterization.

(1) F. W. Billmeyer, Jr., "Textbook of Polymer Science," Wiley-Interscience, New York, N. Y., Chapter 3.
(2) C. Tanford, "Physical Chemistry of Macromolecules," Wiley-Interscience, New York, N. Y., 1961, Chapters 4-6.
(3) M. J. R. Cantow, Ed., "Polymer Fractionation," Academic Press, New York, N. Y., 1967, Chapters B-1, B-2 and B-3.
(4) J. Porath and P. Flodin, *Nature (London)*, 183, 1657 (1959).

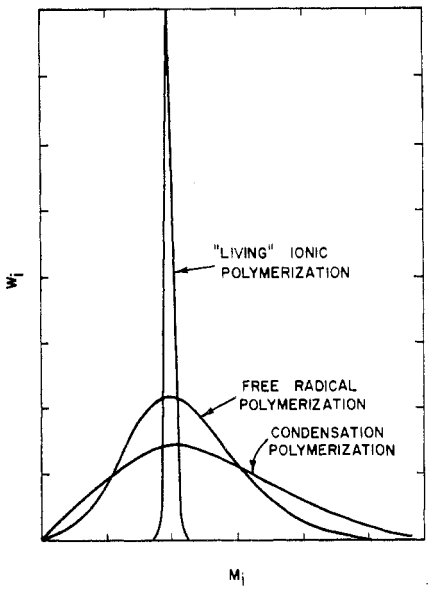

Figure 1. Typical molecular weight distributions: plot shows the weight fraction of *i*mers, W_i, *vs.* M_i for typical condensation, free-radical, and "living" ionic polymerizations.

phy (GPC) is the name given to the technique which employs the semirigid cross-linked polystyrene beads developed by Moore.[5] In either case the packing particles swell (greatly for dextran, slightly for polystyrene) in the chromatographic solvent, forming a porous gel structure, from which the method is named. The distinction between the methods is based on the degree of swelling of the packing. Subsequent developments of rigid porous packings of glass, silica, and silica gel have led to their classification as GPC packings.

The method as commonly practiced consists of employing column(s) packed with gels of varying pore sizes in a liquid chromatograph shown in a block diagram in Figure 2. Under constant flow conditions the solutes are injected onto the top of the column. They appear at the detector in order of decreasing molecular weight. The general concept of the fractionation mechanism is that the largest solute molecules cannot penetrate the pores within the gel beads, and thus elute first. Successively smaller solutes have increasing volume within the beads available to them and therefore require more time to elute. With proper flow control, calibration, injection, and detection (usually by refractive index or uv absorption) an accurate chromatographic picture of the MWD is obtained.

The advantages of the method over all previous techniques have resulted in its adoption as the method of choice for determining the MWD of synthetic polymers and one of the most useful analytical methods in the study of mixtures containing biopolymers.

In the past few years the general field of liquid chromatography has undergone a tremendous revitalization. Separations based on adsorption, partition, and ion exchange, as well as molecular size, are achieved with much greater efficiency than ever before. These practical achievements are the outgrowth

(5) J. C. Moore, *J. Polym. Sci., Part A*, **2**, 835 (1964).

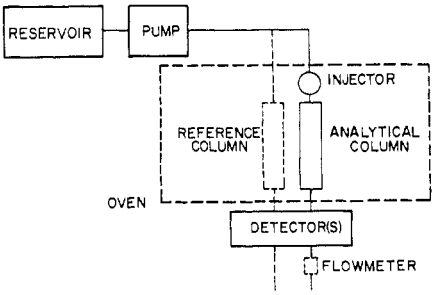

Figure 2. Block diagram of typical gel permeation chromatograph.

of a more sophisticated theoretical approach to the chromatographic process in general. The following section attempts to apply some of the general theories of GPC specifically.

General Chromatographic Theory

Chromatographic processes separate solutes by successive equilibrium distributions between the mobile (eluent) and stationary phases. The retention volume, V_r, of a given solute is simply the eluent volume between injection and appearance of the sample peak at the detector. This may be expressed as

$$V_r = V_0 + KV_s \tag{4}$$

where V_0 is the interstitial volume within the column, K is the equilibrium distribution coefficient, and V_s is the volume of the stationary phase. For GPC, the last term in eq 4 is normally written $K_{GPC}V_p$, where V_p is the pore volume of the packing in the column. This leads to eq 5, where $V_t = V_0 +$

$$K_{GPC} = (V_r - V_0)/(V_t - V_0) \tag{5}$$

V_p (measured for small solutes). In general, $V_p = 0.4\text{–}0.6V_s$ and $V_t \approx 2V_0$. Therefore, $0 \leq K_{GPC} \leq 1$ in the absence of adsorption or partition. In the other liquid chromatographic methods of adsorption, partition, and ion exchange, the eluent plays an active role in determining K. The resolution, R_s, for two solutes in a chromatogram is given by eq 6, where W_b

$$R_s \equiv 2(V_{r1} - V_{r2})/(W_{b1} + W_{b2}) \tag{6}$$

is the peak width at the base. In other liquid chromatographic methods R_s can often be increased by increasing $V_{r1} - V_{r2}$ by changing K_1 relative to K_2 simply by a judicious change in the eluent composition. In GPC, where as a first approximation the eluent is inert, this option for improving R_s is not available. Thus, for GPC more than any other liquid chromatographic technique, R_s must be increased by reducing W_b.

Models of the chromatographic process are usually based on the theoretical plate concept. The number of theoretical plates, N, in a column is given by eq 7.

$$N = (4V_r/W_b)^2 \tag{7}$$

The height equivalent to a theoretical plate is simply

$$H = N/L \tag{8}$$

where L is the length of the column. Often the reduced parameters h and v are employed in normal-

$$h = H/d_p \quad (9)$$

$$v = Ud_p/D_m \quad (10)$$

ized comparisons. Here d_p = diameter of column packing particles, U = linear velocity of eluent, and D_m is the diffusion coefficient of the solute in the eluent.

The observed W_b is the sum of individual factors according to the simple model

$$W_b^2 = \sum w_i^2 \quad (11)$$

where w_i is an individual source of dispersion.[6] Numerous individual sources which contribute to dispersion have been identified. They fall into three broad categories: mobile phase effects, interphase effects, and instrumental sources. The instrumentation itself contributes to peak broadening because of factors such as finite volume injection, detector and injector dead volume, flow cross-section changes, etc. Modern instrument design has reduced the magnitude of these contributions to ≤50 μl of peak width in a total W_b of 150–300 μl for a 1–5 μl analytical sample.

Within the column, the major dispersion sources have been identified as molecular diffusion, eddy diffusion, velocity profile, and resistance to mass transport between mobile and stationary phase and within the latter.[7-20] Modeling and reducing these phenomena have been the major activity of liquid chromatographers in the past 7 or 8 years. The use of pellicular (layered) and very small (<10 μm) packings to reduce mass transfer resistance by reducing stationary-phase dimensions has been very successful.[8,11-14] The velocity profile dispersion may be offset by the use of "infinite diameter" columns, which are defined by eq 12 where d_c is the column diame-

$$d_c \geq \sqrt{2.4Ld_p} \quad (12)$$

ter. In such a column, a point source of solute injected at the column top will not reach the disturbed flow at the column wall before it exits the column. Such columns give measurably improved performance.[7,8] Each model differs in detail, and an extensive discussion of these features is beyond the intent of this work. Specific references may be consulted.[6,9,10,15-21] Figure 3 shows the mobile phase dispersions according to Kelley and Billmeyer.[21]

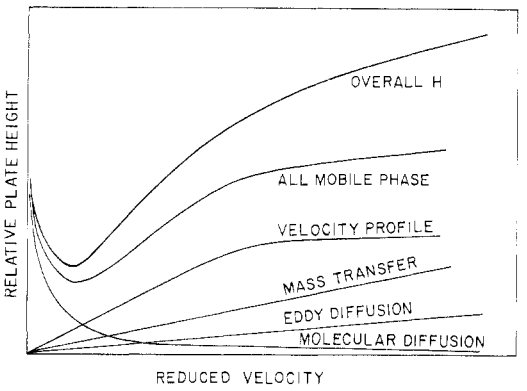

Figure 3. Schematic representation of mobile phase contributions to dispersion according to ref 21.

Rigid Packings

GPC column packings are necessarily highly porous to maximize $V_t - V_0$, so reducing mass transfer resistance by using pellicular porosity is not feasible. It has recently been demonstrated, however, that the pores in such packings do provide exclusion behavior.[22] The approach to improving GPC efficiency appears restricted to a reduction in packing particle size. In principle, there should be no fundamental barrier to using very small packing particles.

In practice, however, several factors come into play. For rigid packings, with uniform bulk distribution of pores, simply grinding to a smaller size appears feasible. To isolate a narrow distribution of particle sizes from a grinding process requires removing both fines and larger particles by elutriation. The resulting particles will not be spherical, however, and may generate sizable pressure drops in small bore columns. Recent advances in technology allow direct synthesis of small, monodisperse (5–6 μm) porous particles of silica.[8] A number of workers have found that it is necessary to pack any such particles below 20 μm in balanced density slurries under high pressure (>5000 psi) to achieve improved efficiency.[8,13]

There is an inverse correlation between particle size and efficiency in the GPC of both macromolecular and monomeric solutes using rigid packings. This is demonstrated in Figure 4. To compare efficiency for synthetic polymeric solutes is not a simple matter. The route chosen here was to take available literature data for anionically polymerized polystyrenes which have $5.1 \times 10^4 \leq M \leq 1.6 \times 10^5$ chromatographed on columns which give $K_{GPC} = 0.5 \pm 0.1$. These materials have $\bar{M}_w/\bar{M}_n = 1.06$ and are common calibration standards. An *apparent* plate height for the standard polymers, H_{poly}, was then calculated from eq 7 and 8. This normalizes the data from these diverse sources[8,13,23-27] to give curve A in Figure 4. Curve B is the efficiency of monomolecular solutes on the same columns, while curve C is the indi-

(6) J. G. Hendrickson, *J. Polym. Sci., Part A-2*, 6, 1903 (1968).
(7) J. H. Knox and J. F. Parcher, *Anal. Chem.*, 41, 1599 (1969).
(8) J. J. Kirkland, *J. Chromatogr. Sci.*, 10, 593 (1972).
(9) J. C. Giddings and K. L. Mallik, *Anal. Chem.*, 38, 997 (1966).
(10) J. C. Giddings, "Dynamics of Chromatography," Part 1, Marcel Dekker, New York, N. Y., 1965.
(11) R. E. Majors, *J. Chromatogr. Sci.*, 8, 338 (1970).
(12) J. J. Kirkland, *J. Chromatogr. Sci.*, 10, 129 (1972).
(13) R. E. Majors, *Anal. Chem.*, 44, 1722 (1972).
(14) J. N. Doane and J. H. Knox, *J. Chromatogr. Sci.*, 10, 606 (1972).
(15) J. F. K. Huber and J. A. R. Hulsman, *Anal. Chim. Acta*, 38, 305 (1967).
(16) J. J. Kirkland, Ed., "Modern Practice of Liquid Chromatography," Wiley-Interscience, New York, N. Y., 1971, Chapters 1 and 4.
(17) F. W. Billmeyer, Jr., G. W. Johnson, and R. N. Kelley, *J. Chromatogr.*, 34, 316 (1968).
(18) F. W. Billmeyer, Jr., and R. N. Kelley, *J. Chromatogr.*, 34, 322 (1968).
(19) R. N. Kelley and F. W. Billmeyer, Jr., *Anal. Chem.*, 41, 874 (1969).
(20) R. N. Kelley and F. W. Billmeyer, Jr., *Anal. Chem.*, 42, 399 (1970).
(21) R. N. Kelley and F. W. Billmeyer, Jr., *Separ. Sci.*, 5, 291 (1970).
(22) E. P. Otocka, *Anal. Chem.*, 45, 1969 (1973).
(23) E. P. Otocka, *J. Chromatogr.*, 76, 149 (1973).
(24) M. J. R. Cantow and J. Johnson, *J. Appl. Polym. Sci.*, 11, 1851 (1967).
(25) A. R. Cooper, A. R. Bruzzone, J. H. Cain, and E. M. Barall II, *J. Appl. Polym. Sci.*, 15, 571 (1971).
(26) B. J. Gudzinowicz and K. Alden, *J. Chromatogr. Sci.*, 9, 65 (1971).
(27) K. J. Bombaugh, Chapter 7 in ref 16.

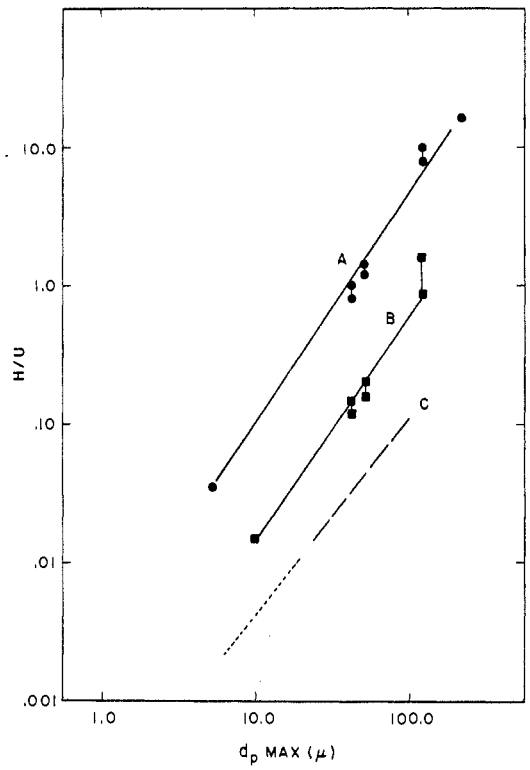

Figure 4. Plate height as a function of d_{pmax} for rigid porous packings. Curve A: H_{poly}/U for narrow polystyrenes, K_{GPC} = 0.5 ± 0.1, calculated from ref 8, 22-26. Curve B: H/U for monomeric solutes, $K_{GPC} \approx 1.0$, from ref 8, 13, 22-26. Curve C: indicated lower limit of H/U, from ref 8.

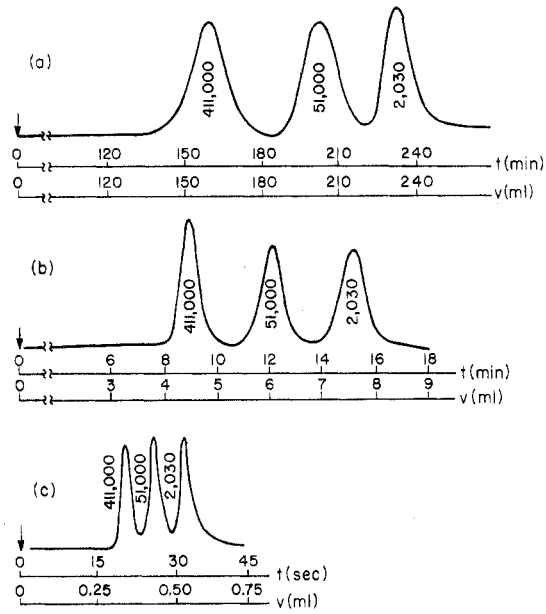

Figure 5. Chromatographic experiment time influenced by particle size: polystyrene standards. (a) Chromatogram synthesized from the data of ref 25. Conditions: d_{pmax} = 120 μ of GPC 10-2000, 10-1250, 10-700, 10-370, 10-240. Five-column series, L = 20 ft, i.d. = 0.305 in., flow rate 1.0 ml/min; toluene eluent. (b) Conditions: d_{pmax} = 44 μ; GPC 10-700, 10-350, 10-125; three columns, L = 150 cm, i.d. = 2.6 mm; F = 0.5 ml/min; tetrahydrofuran eluent.[22] (c) Conditions: d_{pmax} = 6 μ, Zorbax porous silica microspheres; one column, L = 50 cm, i.d. = 2.0 mm; flow rate 1.0 ml/min; tetrahydrofuran eluent. Redrawn from ref 8 with permission of the copyright owner.

cated efficiency limit for monodisperse solutes according to Kirkland.

There are two obvious advantages to small particle packings. The first is that the higher efficiency can be traded for increased loading or decreased experimental time at constant resolution. An example of the latter is shown in Figure 5.[8,23,26] The additional dividend in this increased efficiency is found in the capability of determining the true MWD of narrow ($\bar{M}_w/\bar{M}_n \leq 1.1$) fractions without applying laborious mathematical corrections for "instrument spreading."

Soft and Semirigid Packings

Soft and semirigid cross-linked polymer bead packings have been more extensively used and investigated than rigid packings. Despite this, a clear understanding of their chromatographic behavior has not been forthcoming.

Normally, dry bead diameters are 40-80 μm. Such materials are then slurry packed after swelling with solvent to give columns which often yield >1000 plates per foot in column diameters of 8 mm or larger. On the basis of swollen d_p, this gives reduced plate heights significantly lower than rigid packings in the same size range. Such efficiency has been attributed to differences in pore geometry, but the validity of this point of view has not been clearly established. Extensive studies of H *vs.* d_p have not been reported, and in the few cases where comparisons can be made no definitive correlation is found.[28] This unusual behavior is compounded by the fact that attempts to pack narrow bore columns (<6 mm i.d.) with soft gel packings have not been successful.[27,29] Both of these features bear closer investigation, since fundamental differences exist in relation to the aforementioned behavior of rigid packings.

The potential of trading efficiency for reduced experiment time with these packings hinges on pressure limitations. Columns packed with semirigid gels of cross-linked polystyrenes have withstood 1000 psi successfully,[28] but the softer agarose and dextran gels certainly are limited to <300 psi to avoid collapse. Such limits may be slightly higher or lower depending on the degree of cross-linking.

Separation Mechanisms

The basic mechanisms of GPC separation have received attention from widely diverse researchers. The basic chromatographic equations, (4) and (5), do not imply a mechanism, as they can be calculated from directly measurable column and packing characteristics. Three main approaches have been taken to describe the GPC separation mechanism: *equilibrium steric exclusion, flow separation,* and *restricted diffusion.* An excellent review of these approaches has been presented by Casassa,[30] and the comments here will be very limited. The equilibrium exclusion theory is the most useful in expressing K_{GPC} since it necessarily applies in the limiting case of zero eluent flow. General agreement between K_{GPC} measured on columns and in equilibrium experiments has been observed, but some exceptions have been noted.[31-33]

(28) J. N. Little, J. L. Waters, K. J. Bombaugh, and W. J. Pauplis, *J. Polym. Sci., Part A-2,* 7, 1775 (1969).

(29) W. Heitz, B. Bomer, and H. Ullner, *Makromol. Chem.,* 121, 102 (1969).

(30) E. F. Casassa, *J. Phys. Chem.,* 75, 3929 (1971).

For practical operation of columns over a range of eluent velocities, no clear understanding has been reached as to the extent of the contribution of separation by flow or nonequilibrium diffusion in determining K_{GPC}. The interested reader may find details of these approaches in a number of works.[34-41]

One of the most promising techniques for resolving these questions of separation mechanism lies in more extensive application of "vacancy permeation chromatography." Here the eluent contains a constant concentration of polymer, and pure solvent is injected. Ideally, a mirror image of the normal chromatogram is expected, but in the instance reported, this was not observed.[42] Flow rate and molecular weight dependence of the deviations from normal GPC should be very informative in revealing nonequilibrium mass transfer. If performed with both rigid and semirigid or soft packings the method may show up differences in the separation parameters displayed by different packings which were discussed previously.

Earlier, mention was made of the fact that GPC is limited in its resolving power because $0 \leq K_{GPC} \leq 1.0$. Two additional comments concerning this aspect of GPC are warranted. In certain cases it has been noted that adsorption or partition does occur between solutes and packing. In the case of peptides on dextran packings, such interactions are often advantageous, since they offer an increase in resolution. However, such behavior serves to mask the molecular weight distribution and, in the analysis of most synthetic polymers, particularly using rigid packings, efforts are made to avoid partition or adsorption. Proper deactivation of rigid packings can be achieved either by chemical treatment of the surface or by addition of a polar cosolute to the eluent.

Universal Calibration and Its Applications

The single advance of greatest practical utility in GPC has been the development of the "universal calibration" concept. Basically, the use of the intrinsic viscosity–molecular size relationship allows one to write[43,44]

$$[\eta]M = KM^{1+\alpha} = K'R^3 \qquad (13)$$

where $[\eta]$ = intrinsic viscosity, K and α are the Mark-Houwink constants for the polymer–eluent pair in question, K' is another constant, and R is a characteristic radius of the random coil polymer in solution. The choice of which radius applies to GPC has been a point of some conjecture. The equivalent hydrodynamic radius, R_c, radius of gyration, R_G, and Stokes radius, R_H, have all been suggested, usually in conjunction with a specific fractionation model (exclusion or flow). There has been considerable investigation of the universal calibration concept and some of its more subtle points,[45-51] with the consensus that with proper precautions it is generally valid. That is to say that eq 14 will adequately represent

$$\log ([\eta]M) = A + BV_r \qquad (14)$$

the elution behavior of a wide variety of polymers for a given solvent–column combination without significant variation in A and B.

The advantage of universal calibration lies in the fact that well-characterized samples of known MWD for the polymer to be studied need not be available to calibrate the column. What is needed for precise work over a broad molecular weight range are the constants K and α for the Mark-Houwink equation (eq 15) for the polymer in the GPC solvent and well-

$$[\eta] = KM^{\alpha} \qquad (15)$$

characterized standards (usually polystyrene) for which K and α are also known. In cases where less precise figures are needed, simply measuring $[\eta]$ for the polymer under study will suffice. A typical universal calibration curve for linear polyethylene and polystyrene in trichlorobenzene at 135° is shown in Figure 6.

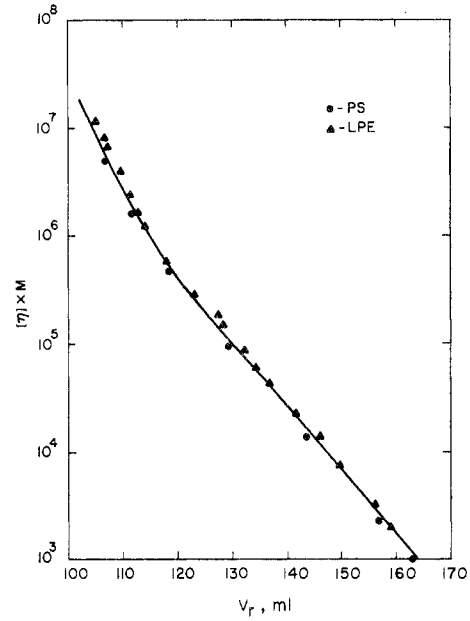

Figure 6. Universal calibration. $[\eta]M$ *vs.* V_r for linear polyethylene, LPE, and polystyrene, PS. Reprinted from ref 59 by permission of the copyright owner.

A number of workers have used the universal calibration concept combined with other analytical techniques to determine the long chain branching in

(31) G. K. Ackers, *Biochemistry*, 3, 724 (1964).
(32) W. W. Yau, C. P. Malone, and S. W. Fleming, *J. Polym. Sci., Part B*, 6, 803 (1968).
(33) W. Haller, *J. Chromatogr.*, 32, 676 (1968).
(34) E. A. DiMarzio and C. M. Guttman, *J. Polym. Sci., Part B*, 7, 267 (1969).
(35) E. A. DiMarzio and C. M. Guttman, *Macromolecules*, 3, 131 (1970).
(36) C. M. Guttman and E. A. DiMarzio, *Macromolecules*, 3, 681 (1970).
(37) J. J. Hermans, *J. Polym. Sci., Part A-2*, 6, 1217 (1968).
(38) W. W. Yau and C. P. Malone, *J. Polym. Sci., Part B*, 5, 663 (1967).
(39) W. W. Yau, *J. Polym. Sci., Part A-2*, 7, 483 (1969).
(40) J. L. White and G. W. Kingry, *J. Appl. Polym. Sci.*, 14, 2723 (1970).
(41) E. F. Casassa and Y. Tagami, *Macromolecules*, 2, 14 (1969).
(42) C. P. Malone, H. L. Sucham, and W. W. Yau, *J. Polym. Sci., Part B*, 7, 781 (1969).
(43) H. Benoit, Z. Grubisic, P. Rempp, D. Decker, and J. G. Zilliox, *J. Chem. Phys.*, 63, 1507 (1966).
(44) Z. Grubisic, P. Rempp, and H. Benoit, *J. Polym. Sci., Part B*, 5, 753 (1967).
(45) A. R. Weiss and E. Cohen-Ginsberg, *J. Polym. Sci., Part B*, 7, 379 (1969).
(46) K. A. Boni, F. A. Sliemers, and P. B. Stickney, *J. Polym. Sci., Part A-2*, 6, 1579 (1968).
(47) H. Coll and D. K. Gilding, *J. Polym. Sci., Part A-2*, 8, 89 (1970).
(48) B. L. Funt and V. Hornof, *J. Appl. Polym. Sci.*, 15, 2439 (1971).
(49) B. A. Whitehouse, *Macromolecules*, 4, 463 (1971).
(50) A. Rubin and H. L. W. Hoegy, *J. Polym. Sci., Part A-1*, 10, 217 (1972).
(51) J. V. Dawkins, *Brit. Polym. J.*, 4, 87 (1972).

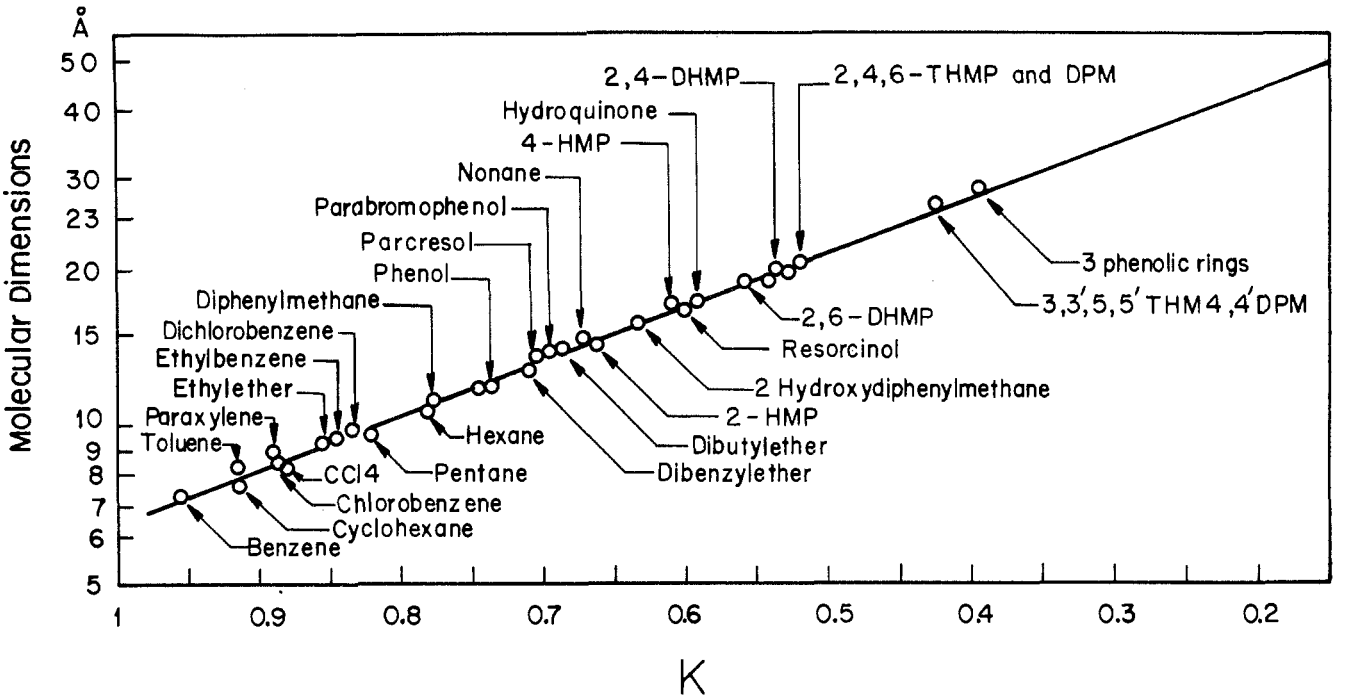

Figure 7. Solvated molecular dimensions *vs.* K_{GPC} for small molecules. Key: 2-HMP and 4-HMP, monohydroxymethylphenol isomers; 2,4-DHMP and 2,6-DHMP, dihydroxymethylphenol isomers; 2,4,6-THMP, trihydroxymonophenol; 3,3′,5,5′THM4,4′DPM, 4,4′-methylene-bis(3,5-dihydroxymethylphenol). Redrawn from ref 71 with the permission of the copyright owner.

a variety of polymers. The ratio of coil dimensions in solution for branched polymers compared to linear polymers of the same molecular weight is given by eq 16, with the Zimm–Kilb[52] relationship giving (for

$$g = \bar{R}_{G,b}{}^2/\bar{R}_{G,l}{}^2 \tag{16}$$

star-branched polymers)

$$g^{1/2} = [\eta]_b/[\eta]_l \tag{17}$$

where $\bar{R}_G{}^2$ is the root mean square radius of gyration and $[\eta]$ is the intrinsic viscosity. The subscripts b and l refer to branched and linear polymers of the same molecular weight. The values of g may be translated into branch concentrations through a variety of relationships.[53-55] To determine the distribution of branches, fractions of the polymer in question are examined to determine $[\eta]$ and V_r. This yields the $\bar{M}$. Then $[\eta]_b$–$\bar{M}_b$ plots are compared with $[\eta]_l$–$\bar{M}_l$ plots to determine $[\eta]_b/[\eta]_l$. This yields g, which may be converted to a branch concentration. The earliest results[56,57] indicated that some polymers have a random distribution of branches, as predicted by simple analysis of the polymerization reactions. More of the subsequent work[58-63] indicates that most commercial reactors yield products in which both the long and short branch concentration may vary widely across the MWD.

The availability of branch distribution data will permit a more exact correlation between the branching and physical properties on one hand and synthesis conditions on the other.

In many cases, difficult separations involve simple nonpolymeric solutes which can cover a broad range of molecular weights. Of particular interest is the generation of size calibrations in the molecular weight region below 5×10^3, where statistical methods and intrinsic viscosity correlations are not applicable. Advances in this area have been generated in the field of nonbiological organic compounds. Bombaugh, *et al.*,[64,65] have generated excellent correlations of molecular weight and size for hydrocarbons in the range C_5–C_{57}. However, it has often been found that solutes containing polar groups such as OH, NH_2, COOH do not follow a simple $\bar{M}$–K_{GPC} relationship. Furthermore, changes in the eluent have been observed to reverse the elution order of compounds. It is now recognized that the sizes of such polar compounds in solution can reflect association complexes with the eluent.[66-71] Model building has been applied, along with infrared analysis of hydrogen bond formation, to rather successfully calculate the effective molecular size of such molecules, as shown in Figure 7.[72]

(52) B. H. Zimm and R. W. Kilb, *J. Polym. Sci.*, **37**, 19 (1959).
(53) W. H. Stockmayer and M. Fixman, *Ann. N. Y. Acad. Sci.*, **57**, 334 (1953).
(54) B. H. Zimm and W. H. Stockmayer, *J. Chem. Phys.*, **17**, 1301 (1940).
(55) M. Kurata and M. Fukatsu, *J. Chem. Phys.*, **41**, 2934 (1964).
(56) L. Wild and R. Guliana, *J. Polym. Sci., Part A-2*, **5**, 1087 (1967).
(57) E. E. Drott and R. A. Mendelson, *J. Polym. Sci., Part A-2*, **8**, 1361, 1373 (1970).
(58) J. Miltz and A. Ram, *Polymer*, **12**, 685 (1971).
(59) J. A. Cote and M. Shida, *J. Polym. Sci., Part A-2*, **9**, 421 (1971).
(60) E. P. Otocka, R. J. Roe, M. Y. Hellman, and P. M. Muglia, *Macromolecules*, **4**, 507 (1971).
(61) L. Wild, R. Ranganath, and T. Ryle, *J. Polym. Sci., Part A-2*, **9**, 2137 (1971).
(62) R. Prechner, R. Panaris, and H. Benoit, *Makromol. Chem.*, **156**, 39 (1972).
(63) G. Kraus and C. J. Stacy, *J. Polym. Sci., Part A-2*, **10**, 657 (1972).
(64) K. J. Bombaugh, W. A. Dark, and R. F. Levangie, *J. Chromatogr. Sci.*, **7**, 42 (1969).
(65) K. J. Bombaugh and R. F. Levangie, *Separ. Sci.*, **5**, 6 (1970).
(66) J. Cazes and D. R. Gaskill, *Separ. Sci.*, **2**, 421 (1967).
(67) J. Cazes and D. R. Gaskill, *Separ. Sci.*, **4**, 15 (1969).
(68) A. Lambert, *J. Appl. Chem.*, **20**, 305 (1970).
(69) H. H. Oelert, *J. Chromatogr.*, **53**, 241 (1970).
(70) T. Yoshikawa, K. Kimura, and S. Fujimura, *J. Appl. Polym. Sci.*, **15**, 2513 (1971).
(71) A. Lambert, *Anal. Chem. Acta*, **53**, 63 (1971).

For analysis of monodisperse molecules of biological interest the cross-linked dextrans have enjoyed a great success across the entire molecular weight range. Commercial and laboratory applications are too numerous to mention.[73-76] As stated before, partition behavior has been observed, with compounds eluting with $V_r > V_t$. Porous glass packings are becoming more popular, and may be used in aqueous solutions up to pH 9 without ill effects. Deactivation is achieved using poly(ethylene oxide) surface treatment, and sometimes this polymer is also added to the eluent.[77-79]

(72) M. Duval, B. Bloch, and S. Kohn, *J. Appl. Polym. Sci.*, 16, 1585 (1972).
(73) H. Determan, "Gel Chromatography," Springer-Verlag, West Berlin, 1968.
(74) L. Fisher in "Laboratory Techniques in Biochemistry and Molecular Biology," T. S. Work and E. Work, Ed., North-Holland, Amsterdam, 1969.
(75) J. M. Curling, *Exp. Physiol. Biochem.*, 3, 417 (1970).
(76) "Saphadex-Gel Filtration in Theory and Practice," available from Pharmacia Fine Chemicals.
(77) H. H. Geschwinder, W. Haller, and P. H. Hofschneider, *Biochim. Biophys. Acta*, 190, 460 (1969).

Conclusion

GPC is entering its "second generation" of sophistication with the development of more advanced chromatographic theory and improved instrumentation and column packings. Already it is the most widely employed analytical technique for the laboratory MWD characterization of synthetic polymers and analysis and purification of many biological materials. The future can only bring proliferation of commercial and scientific uses of GPC in the fields of quality control, preparative-scale operation, and analytical separations.

Complete understanding of the separation mechanism and definition of differences between packing material behavior remain elusive. In this respect, GPC is one of the most fertile research fields in chromatography.

(78) W. Haller, *Virology*, 33, 740 (1967).
(79) G. L. Hawk, J. A. Cameron, and L. B. Dufault, *Prep. Biochem.*, 2, 193 (1972).

Nitrosamine Photochemistry: Reactions of Aminium Radicals

Yuan L. Chow

Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada

Received January 15, 1973

The extensive current interest in photochemical excitation of organic compounds stems largely from the synthetic utility of the reactions that ensue. The introduction of powerful techniques that permit the identification of the reactive intermediate involved in photolyses has also provided stimulus to photochemists.[1] It was such a combination that drew us to investigate the photochemical reactions of *N*-nitrosamines (1).

In the early 1960's, systematic studies by several groups, particularly Barton's, revealed the scope and synthetic utility of photolyses of nitrite esters.[2] Irradiation of these esters in neutral solutions causes homolytic dissociation to nitric oxide (NO) and alkoxy radicals; stereospecific intramolecular hydrogen atom abstractions of the latter have been utilized elegantly in syntheses of otherwise inaccessible compounds.[2] Insofar as the primary photochemical process is concerned, the behavior of nitrosamides[3] as well as *C*-nitroso compounds[4,5] parallels that of nitrite esters. The photolysis generates amido and alkyl radicals, respectively, together with NO as the counterradical. In contrast to the photolability of these nitroso compounds, dialkylnitrosamines (1) (except *N*-nitrosodibenzylamine) are stable toward uv irradiation in neutral solution.[6,7]

In 1964 we discovered that, in the presence of a dilute acid, *N*-nitrosamines rapidly undergo various photoreactions.[6] In the ensuing years, we have investigated the scope and synthetic utility of this interesting photolysis. We have employed the flash excitation technique to establish that the primary photoprocess involves generation of NO and aminium radicals ($R_2NH^{\cdot+}$).

(1) For general discussions of organic photochemistry reference is made to the monograph by N. J. Turro, "Molecular Photochemistry," W. A. Benjamin, New York, N. Y., 1965.
(2) For a review of this reaction, see M. Akhtar, *Advan. Photochem.*, 2, 263 (1964), and R. H. Hesse, *Advan. Free Radical Chem.*, 3, 83 (1969).
(3) Y. L. Chow and J. N. S. Tam, *J. Chem. Soc. C*, 1138 (1970), and references cited therein.
(4) (a) H. A. Morrison in "The Chemistry of the Nitro and Nitroso Groups," H. Feuer, Ed., Interscience, New York, N. Y., 1969, p 165; (b) J. A. Maassen, Ph.D. Dissertation, University of Amsterdam, Amsterdam, 1972.
(5) Y. L. Chow, J. N. S. Tam, and K. S. Pillay, *Can. J. Chem.*, in press.
(6) Y. L. Chow, *Tetrahedron Lett.*, 2333 (1964); *Can. J. Chem.* 45, 53 (1967).
(7) E. M. Burgess and J. M. Lavanish, *Tetrahedron Lett.*, 1227 (1964).

Y. L. (Joe) Chow received his undergraduate education in National Taiwan University, Taipei, Taiwan, and obtained his Ph.D., under H. Hary Szmant, at Duquesne University. Subsequently he studied as a postdoctoral fellow with M. L. Bender, H. Erdtman, and D. H. R. Barton, during which time he developed research interests in solution photochemistry. The nitrosamine photochemical studies were inaugurated in 1963 at the University of Alberta, Edmonton. During recent years his research interests have extended to the chemistry of "onium" radicals. He has been Professor of Chemistry at Simon Fraser University since 1969.