The Relation between Pore Size Distribution and Polymer Separation in Gel Permeation Chromatography

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ABSTRACT: The variation of the retention time of linear polymer in a θ solvent with the logarithm of molecular weight is studied as a function of the standard deviation of a Gaussian pore size distribution. The calculation is based on the assumption that polymer entrapment within a pore is based on the root-mean-square unperturbed end-to-end distance of the polymer and the radius of a cylindrical pore. For a mean pore size of 121 Å and a standard deviation (σ) of 0.1 Å, the linear portion of a plot of t_R *vs.* log $\langle h_0^2 \rangle^{1/2}$ covers less than one decade of molecular weight. A much broader pore size distribution of σ = 100 Å yields a linear portion which covers nearly two decades of molecular weight. This general observation will hold regardless of the shape assumed for the pores or the polymer size parameter assumed to govern entrapment. Experimental elution data on narrow fractions of polystyrene obtained by Moore and Arrington using a θ solvent agree much better with retention times calculated assuming a finite pore size distribution than with earlier calculations by this author which used a single pore size to characterize the bed.

The effect of pore size distribution on the separation of linear macromolecules in gel permeation chromatography (gpc) has not yet been considered in theoretical models. Two previous fundamental approaches to an explanation of this separation method characterized the chromatography bed solely by a single pore size.[1,2] The assumption of a Gaussian pore size distribution has, however, proven helpful in explaining the separation of inflexible biopolymers in gel filtration chromatography.[3,4]

In this work the effect of pore size distribution on the retention time of a linear random coil macromolecule is calculated by first assuming that (a) the root-mean-square unperturbed end-to-end distance ($\langle h_0^2 \rangle^{1/2}$) is the size parameter governing entrapment and (b) the chromatography bed can be represented by a Gaussian distribution of cylindrical pores. However, the same trends observed in plots of retention time *vs.* log $\langle h_0^2 \rangle^{1/2}$ with increasing breadth of pore size distribution will be observed for any reasonable assumptions used in place of a and b above.

Calculations. A. Model for a Single Pore Size. The general expression for the retention time of a monodisperse linear polymer assuming no polymer–polymer interactions (*i.e.*, injection of a dilute polymer solution into the column) was obtained earlier for a stochastic model of the chromatography process.[2]

$$t_R - t_0 = \frac{\lambda_1 t_0}{\lambda_2} = \frac{C\lambda_1' t_0}{\lambda_2} \qquad (1)$$

Equation 1 was derived assuming only one kind of site in the chromatography bed, where λ_1 is the rate constant for entrapment of a polymer molecule into the site (denoted by λ_1') multiplied by the concentration of entrapment sites per unit column length (denoted by C), and λ_2 is the rate constant for elution of the polymer molecule away from the entrapment site and into the flowing solvent carrier. When λ_1 and λ_2 are evaluated using the assumptions of a random flight polymer chain, the retention time values (denoted by t_R) thus obtained are applicable to gpc of a polymer in a θ solvent. The parameter t_0 is the minimum residence time associated with the particular column. That is, t_0 is the retention time for polymer sufficiently high in molecular weight that no entrapment into the pores can occur.

We assume that $t_R - t_0$ is proportional to λ_1'/λ_2 and that the proportionality constant is invarient with polymer molecular weight. This assumption clearly holds at equilibrium when the ratio λ_1'/λ_2 becomes a true equilibrium constant.[1,4] The assumption should also be valid at the low flow rates (approximately 1 ml/min) normally encountered in experimental gel permeation chromatography. The ratio λ_1'/λ_2 is termed the probability of entrapment and is given the symbol P_e. In the author's first paper,[2] the rate constant λ_2 was assumed to be independent of polymer molecular weight. There P_e was equated with λ_1. The present notation reflects more refined notions of the gpc process.

As before,[2] P_e is taken to be the probability that h^2 is less than the square of the radius, r^2, of a set of identical cylindrical pores. The parameter h^2 is used as the basis for obtaining numerical values of P_e for two reasons. First, the distribution for h^2 for a linear polymer in a θ solvent is rigorously Gaussian.[5] Second, a large fraction of the polymer segments lie within a hypothetical sphere obtained by locating the origin of the sphere at the polymer center of mass and using $\langle h_0^2 \rangle^{1/2}$ as its radius. The density of polymer segments lying outside this sphere is relatively low. Therefore these segments are quite easily compressed. Therefore any incremental energy gained in the entrapment process would probably make up for the small amount of energy expended by slightly compressing the polymer molecule during entrapment. Given these assumptions, P_e is easily calculated (see ref 2 for details)

(1) E. F. Casassa, *J. Polym. Sci., Part B*, **5**, 773 (1967).
(2) J. B. Carmichael, *ibid., Part A-2*, **6**, 517 (1968).
(3) G. K. Ackers, *J. Biol. Chem.*, **242**, 3237 (1967).
(4) J. B. Carmichael, *Biopolymers*, in press.

(5) In spherical coordinates, the distribution is probably better termed Maxwellian.

$$(1 - P_e) = \text{probability}(\chi^2 \geq \chi_1^2) = \int_{\chi^2=\chi_1^2}^{\chi^2=\infty} W(h)dh \quad (2)$$

where

$$\chi^2 = \frac{3h^2}{\langle h_0^2 \rangle}$$

and

$$W(h) = \left(\frac{3}{\langle h_0^2 \rangle 2\pi}\right)^3 \exp\left(\frac{-3h^2}{2\langle h_0^2 \rangle}\right)$$

Probability ($\chi^2 \geq \chi_1^2$) *vs.* χ_1^2 is termed the χ^2 distribution with three degrees of freedom. Tabulated values are available from any standard set of statistical tables.

B. Calculations Including a Pore Size Distribution. Consider a gpc bed which contains n different kinds of sites. For the numerical calculations which follow, these sites are assumed to be cylindrical pores. The only feature which distinguishes one kind of site from another is taken to be the radius of the cylinder. The n sites have associated entrapment rate constants $\lambda_{11}, \lambda_{12}, \ldots \lambda_{1n}$ and elution rate constants $\lambda_{21}, \lambda_{22}, \ldots \lambda_{2n}$. The times spent in sites 1, 2, ...n are assumed to be independent random variables. These random variables are denoted as $T_1, T_2, \ldots T_n$. The random variable, T, for the total time spent in the column is therefore given by eq 3. It follows directly that the density

$$T = T_1 + T_2 + T_3 + \ldots T_n \quad (3)$$

function describing the elution curve of a monodisperse polymer which has passed through a bed containing a distribution of n different pore sizes, $P(t)$, is calculable as the n-fold convolution of the elution curve for monodisperse polymer which has passed through a bed containing pores of a single size, $P(t_j)$;[6] *i.e.*

$$P(t) = P(t_1)*P(t_2)*\ldots P(t_n) \quad (4)$$

where the asterisks denote the convolution operation.

For simplicity in actual calculations, the time spent in the mobile phase is again considered to be constant.[2] This assumption will fail only at flow rates much higher than those attainable with commercial laboratory instruments. Under this assumption, McQuarrie has outlined the solution for $P(t)$ when the $P(t_j)$ are given by the simple one-site stochastic model.[7]

For actual computations the mean of the elution curve ($\bar{t}$) is simpler to consider than $P(t)$. Since the random variables T_j are statistically independent

$$\bar{t} = t_1 + t_2 \ldots t_n \quad (5a)$$

where $t_1, t_2, \ldots t_n$ represent, respectively, the mean times spent in entrapment sites 1, 2, ...n. Letting $t_R = \bar{t}$, where the retention time, t_R, is the time at which the maximum in the elution curve appears, we obtain

$$t_R = t_{R_1} + t_{R_2} + \ldots t_{R_n} \quad (5b)$$

Combining eq 1 and 5 for an arbitrary pore size distribution we obtain

$$t_R - t_0 = Ct_0 \sum_{j=1}^{n} a_j \frac{\lambda_{1j}'}{\lambda_{2j}} \quad (6)$$

where

$$\sum_{j=1}^{n} a_j = 1$$

The pore size distribution was taken to be Gaussian in all the calculations that follow; *i.e.*

$$a_j = \text{probability}\{r = r_j\} = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(\frac{-(r_j - r_{av})^2}{2\sigma^2}\right) \quad (7)$$

where r_{av} is the mean pore size and σ^2 is the variance of the pore size distribution.

The set of experimental retention time data by Moore and Arrington[8] was obtained using a column packed with porous glass whose pore diameter was determined to be 242 Å by Haller[9] using mercury porosimetry. The mean pore radius for all calculations in this paper was taken to be 121 Å so that the experimental retention data could be presented along with the model calculations. Moore[10] pointed out, however, that mercury penetration measurements of the pore size give the minimum neck in the pore through which the pore volume is accessible. That is, the pore structures mentioned apparently have a measured minimum diameter of 242 Å which is repeated many times throughout the macroscopic glass sample.

Moore and Arrington[8] obtained retention times for a series of narrow molecular weight polystyrenes ranging in molecular weights from 1.05×10^4 to 3.50×10^6. Experiments were run using a θ solvent and a single 122-cm column packed with the porous glass obtained from Haller.[9] For their configuration the value of t_0, the time spent in the mobile state, was found to be 26.1 min.

The value of the effective pore concentration C is calculable from eq 1 using the value of $t_R - t_0$ for benzene. This difference in retention times represents the maximum time that can be spent in the trapped state under the chromatography conditions employed. The value of $(t_R - t_0)_{max}$ was found to be 16.1 min, and for the case of benzene passing through large pores, $\lambda_1'/\lambda_2 = 1$. Using the preceding information, eq 1 yields a value of $C = 16.1/26.1 = 0.616$. Using the values of t_0 and C appropriate for the Moore and Arrington experimental configuration, eq 6 and 7 were utilized to calculate the distribution of retention times for a monodisperse polymer of given $\langle h_0^2 \rangle^{1/2}$ passing through a bed with a breadth of pore size distribution specified by σ. These data are shown in Figure 1. Along the horizontal axis are the retention times possible for each allowed pore size in the particular bed (specified by σ). The height on the vertical axis represents the probability that the particular retention time is observed (*i.e.*, it reflects the fraction of pore sizes

(6) W. Feller, "An Introduction to Probability Theory and Its Applications," Vol. II, John Wiley & Sons, Inc., New York, N. Y., 1966, Chapter 1.

(7) D. A. McQuarrie, *J. Chem. Phys.*, **38**, 437 (1963).

(8) J. C. Moore and M. C. Arrington, paper presented at the International Union of Pure and Applied Chemistry, Tokyo, Japan, Sept 1966; paper presented at the Third International Symposium on Gel Permeation Chromatography, Geneva, Switzerland, May 1966.

(9) W. Haller, *Nature*, **206**, 693 (1965).

(10) J. C. Moore, private communication.

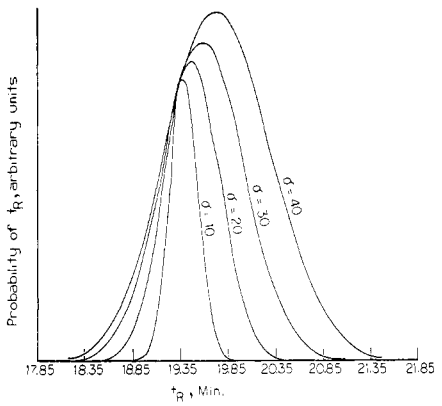

Figure 1. Distribution of mean residence times for monodisperse random coil polymer with $\langle h_0^2 \rangle^{1/2}$ = 207 Å passing through a bed of Gaussian distributed pore sizes with a mean of 121 Å and standard deviations of 10, 20, 30, and 40 Å, respectively. (Unit areas can be obtained by multiplying the areas under curves 1, 2, and 3 by factors 4, 2, and 4/3, respectively.)

that give rise to that particular t_R which are present in the bed). All of the curves shown are calculated for a polymer with $\langle h_0^2 \rangle^{1/2}$ = 207 Å (which represents, for example, a polystyrene polymer with $M = 9.8 \times 10^4$). The curves were deliberately not normalized to unit area in order to allow the set to be more easily compared in the same figure. Normalization would require multiplying the area under curve 1 by four, that under curve 2 by two, and the area under curve 3 by a factor of $^4/_3$.

As the breadth of the pore size distribution increases (shown by increasing σ), the distribution of retention times becomes broader in full accord with intuition. The maximum in the t_R values, representing the value of t_R for the elution curve $P(t)$ from eq 4, shifts to larger values as the pore size distribution becomes broader. This is because the t_R is calculated from the overlap of an asymmetric distribution (Maxwellian distribution of the h^2) with a symmetric one (Gaussian distribution of the r^2).

If a polymer coil is much larger than the average pore size, then the probability of entrapment will be minimal for a narrow pore size distribution because very few pores will be of the same order of size as the polymer coil. As the pore size distribution broadens, a greater fraction of very large pores will be present. Therefore the probability of entrapment of the large polymer will

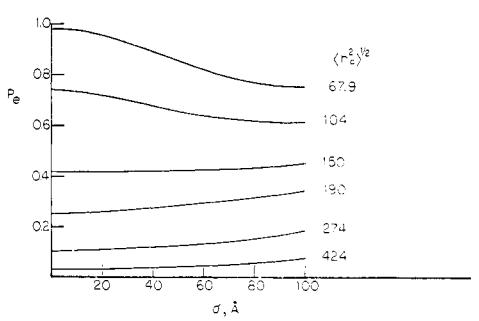

Figure 2. Probability of entrapment for monodisperse random coil polymer *vs.* the standard deviation of a Gaussian distribution of pore sizes with mean radius = 121 Å.

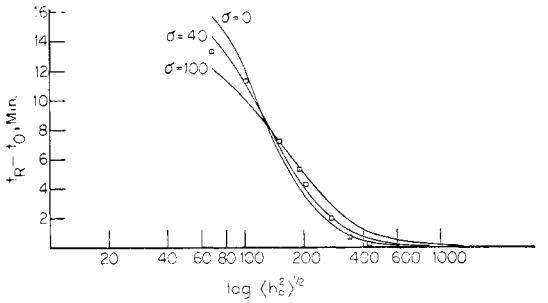

Figure 3. Effect on retention time of increasing the variance of a Gaussian distribution of pore sizes with mean radius = 121 Å: ⊡, polystyrene fractions. Experimental data are from ref 8.

increase. If the polymer coil in question is much smaller than the average pore size, then the probability of entrapment will be nearly equal to 1 for a narrow pore size distribution. If the pore size distribution were much broader, however, a substantial number of pores smaller in size than the polymer coil would be present. In this case, therefore, the entrapment probability will decrease as the pore size distribution becomes broader. This behavior is illustrated in Figure 2 for various sized polymer coils using a bed with mean pore size of 121 Å. The entrapment probability is plotted as a function of the standard deviation of the pore size distribution for the various sized polymers.

Figure 3 shows that a large increase in breadth of the pore size distribution has relatively small effect on the shape and magnitude of the $t_R - t_0$ *vs.* log $\langle h_0^2 \rangle^{1/2}$ plot. This is because the effect of increasing σ is dampened out by the broad polymer end-to-end distance distribution. That is, much more dramatic changes would be observed in such a plot for rigid biopolymers where only one polymer size is possible.

If the best straight line were drawn through the relatively flat regions of the three curves in Figure 3, one would see that for the broad pore size distribution (σ = 100) this linear plot is a good approximation to the actual curve over 1.5 decades of molecular weight. For the case of a pore size distribution which is vanishingly narrow, however, the straight line plot approximates the calculated curve only over about 0.8 decades of molecular weight. These two linear approximations roughly represent the practical range of separation that can be achieved by the column. Shifting from the narrowest pore size distribution to the broadest in Figure 3 thus roughly doubles the range of polymer molecular weights that can be effectively separated.

Discussion

Such a series of plots as shown in Figure 3 may represent an indirect way to determine some measure of pore size distribution in the swollen polystyrene resin column packing (trade name Styragel). One could measure $t_R - t_0$ for a series of well-characterized sharp fractions of linear polymer using a single column. The best fit of the experimental data to calculated values of $t_R - t_0$ would be empirically determined by using plots such as those shown in Figure 3 calculated by assuming different values of r_{av} and σ. This procedure will not yield an absolute measure of pore size distribution due to previously mentioned fallibilities in the model upon

which calculations are based. Relative measures of pore size distributions in different Styragel samples should be obtainable with quite some certainty, however.

Casassa[1] computed the equilibrium constant for the concentration of random coil polymer inside *vs.* outside the pores for a single pore size model using spheres, cylinders, and slabs as the pore shapes. He obtained no correlation with the experimental data of Moore and Arrington[8] for the spherical and cylindrical pore calculations and obtained rather good correlation with the data using the slab model.

No uniqueness is claimed in the present work by the good fit obtained in Figure 3 between the experimental retention time data from Moore and Arrington and the calculated retention times assuming a Gaussian distribution of cylindrical pore sizes and h^2 as the polymer size parameter governing entrapment. In general, if adjustment of a two-parameter model cannot produce calculated results which reasonably match experimental data, the model is obviously incorrect in principle. If a fit is obtained, however, the model may or may not be correct.

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Characterization of Sequence Distribution of Vinylidene Chloride–Vinyl Chloride Copolymers to High Conversions by Nuclear Magnetic Resonance Spectroscopy

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ABSTRACT: Dyad and tetrad sequence concentrations of vinylidene chloride–vinyl chloride copolymers were determined by nmr and successfully compared with those calculated from the copolymerization theory over wide ranges of composition and conversion. The change of the monomer sequence distribution during polymerization is discussed using the relations between the copolymer composition and the sequence concentrations as functions of conversion.

Recently[1,2] we have characterized styrene–methyl methacrylate and vinylidene chloride–vinyl acetate copolymerization systems over the wide ranges of composition and conversion. Both the copolymer composition and the sequence concentration (triad or dyad and tetrad) were determined from nmr measurement and were successfully compared with those expected from the copolymerization theory. Furthermore, we have given the relations between the copolymer composition and the particular sequence concentrations as a function of conversion,[3] by which we could characterize a monomer sequence distribution of a given copolymer sample.

Nmr spectra of vinylidene chloride–vinyl chloride copolymers were recently analyzed by the dyad and tetrad assignments by Johnsen[4] and Enomoto and Satoh.[5] Since this system is technically important, it is useful to examine the nmr spectra up to high conversions and to compare the results with other copolymerization systems in the light of the monomer sequence distribution.

(1) Y. Yamashita, K. Ito, S. Ikuma, and H. Kada, *J. Polym. Sci., Part B*, **6**, 219 (1968).
(2) Y. Yamashita and K. Ito, *J. Appl. Polym. Sci.*, in press.
(3) K. Ito and Y. Yamashita, *J. Polym. Sci., Part B*, **6**, 227 (1968).
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Experimental Section

Copolymer samples were prepared by suspension polymerization at 50° using azobisisobutyronitrile as a catalyst. Nmr spectra were measured at 80–90° in thionyl chloride using a Japan Electron Optics C-60 spectrometer working at 60 Mc/sec.

Results and Discussion

Figure 1 shows the typical CH_2 proton nmr spectra of copolymers prepared from a monomer feed of 30 mol % vinylidene chloride to 13, 38, and 92% conversions. The dyad and tetrad assignments as indicated in the figure, where A and B represent vinylidene chloride and vinyl chloride units, respectively, have been established in the literatures.[4,5] The resonances at higher magnetic fields increase in peak strengths with conversion, indicating the increasing incorporation of the less reactive vinyl chloride monomer as polymerization proceeds. Therefore, by measuring the corresponding peak areas of the normal CH_2 proton spectra, we can readily estimate the dyad concentrations $\overline{P_2\{AA\}}$, $\overline{P_2\{AB\}}$ or