Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

The Sizes of Polymer Molecules and the GPC Separation

Fred W. Billmeyer Jr.[a]; K. H. Altgelt[b]
[a] DEPARTMENT OF CHEMISTRY, RENSSELAER POLYTECHNIC INSTITUTE, TROY, NEW YORK [b] CHEVRON RESEARCH COMPANY, RICHMOND, CALIFORNIA

To cite this Article Billmeyer Jr., Fred W. and Altgelt, K. H.(1970) 'The Sizes of Polymer Molecules and the GPC Separation', Separation Science and Technology, 5: 4, 393 — 402
To link to this Article: DOI: 10.1080/00372367008068438
URL: http://dx.doi.org/10.1080/00372367008068438

SEPARATION SCIENCE, 5(4), pp. 393–402, August, 1970

The Sizes of Polymer Molecules and the GPC Separation*

FRED W. BILLMEYER, JR.
DEPARTMENT OF CHEMISTRY
RENSSELAER POLYTECHNIC INSTITUTE
TROY, NEW YORK 12181

and K. H. ALTGELT
CHEVRON RESEARCH COMPANY
RICHMOND, CALIFORNIA 94802

Summary

This introductory review explains in simplest terms the separation mechanism in GPC and the concept of size as its discriminant. Sample molecules permeate the gel to different degrees depending on their size and are kept out of the solvent stream in the interstices in correspondingly different time ratios. For rigid molecules the size is determined either by the volume or by the most prominent linear dimension. A better approximation seems to be Giddings's "mean external length."

With polymers the decisive size parameter is the hydrodynamic volume. Its calculation from molecular weight must take into account the coiling of the polymer, its flexibility, and its interaction with the solvent. Another important consideration is the statistical nature of polymer properties which results in average values for molecular weight and size. Chain statistics yield polymer sizes that are compatible with pore dimensions of appropriate gels.

Gel permeation chromatography (GPC) is a method to separate molecules by size. Basically, any soluble molecules can be separated by GPC, small ones of less than 100 molecular weight (MW) as well as large ones of several millions MW.

* Presented at the ACS Symposium on Gel Permeation Chromatography, sponsored by the Division of Petroleum Chemistry at the 159th National Meeting of the American Chemical Society, Houston, Texas, February, 1970.

The separation is usually carried out on columns that are tightly packed with a gel or some other porous material and completely filled with solvent. The same solvent is used to dissolve the sample before introducing it into the column and also for elution. Small sample molecules can diffuse into the pores of the gel, large ones are excluded, others of intermediate size can penetrate some of the larger pores. The molecules are constantly diffusing back and forth between the pores and the interstices. Solvent pumped through the column flows only in the interstices, sweeping along all sample molecules present there. The molecules in the pores stay behind until they diffuse back out. The large molecules which are always or mostly excluded from the pores are, therefore, eluted first; the small ones which are mostly inside the pores come out last.

A species is eluted at a volume exactly equal to the volume available to it in the column. For large completely excluded molecules, the elution volume V_e is equal to the interstitial volume V_0; for small molecules which can completely penetrate all pores of the gel it is equal to the total liquid volume of the column, i.e., equal to the sum of V_0 and the internal (pore) volume V_i. For molecules of intermediate size, the elution volume is:

$$V_e = V_0 + K_d V_i \tag{1}$$

where K_d, the partition coefficient, is equal to the ratio of accessible pore volume to total pore volume:

$$K_d = \frac{V_{i,\text{acc}}}{V_i} \tag{2}$$

Figure 1 illustrates the statement made in Eq. (1). The center shows a schematic of a gel column with the interstitial volume as the core and the internal volume toward the walls. The small column on the right has a volume equal to the interstitial column volume; the large column on the left has the size of the whole gel column. Visualize both model columns, left and right, filled with solvent and an immiscible lighter sample put on top of both. If we drain both columns, the samples will come out at exactly the column volumes, i.e., at V_0 in the case of the small one and at $V_0 + V_i$ in the case of the large one. If a real sample can only penetrate part of the internal volume, its imaginary column volume in the sense of Fig. 1 is $V_0 + V_{i,\text{acc}}$, or $V_0 + K_d V_i$ as stated in Eq. (1). This equation then holds for all cases including those of complete exclusion or complete permeation with

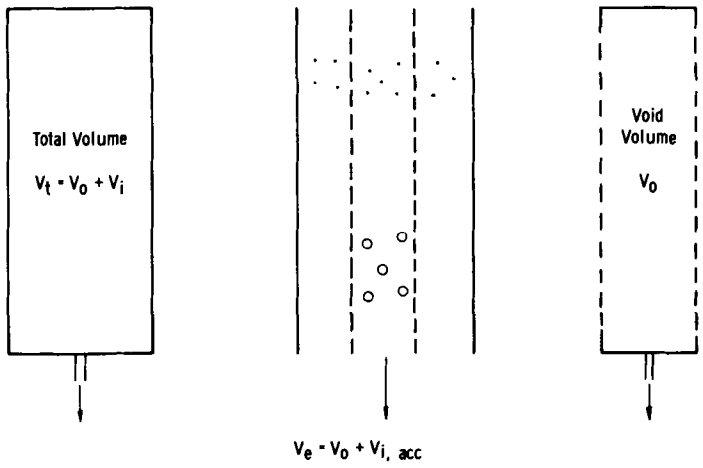

FIG. 1. Elution volume and accessible column volume. Illustration of the equation $V_e = V_0 + V_{i,acc}$ as explained in the text.

$K_d = 0$ and $K_d = 1$, respectively. GPC may thus be considered a special case of partition chromatography where partition occurs between like solvents but different locations, viz., between the spaces outside and inside the gel particles (mobile and stationary phases).

It is generally assumed that under ordinary GPC run conditions, a molecule can diffuse in and out of the gel pores several times before the sample zone has passed by a gel particle. The system is then considered in equilibrium. Other papers given in this Symposium discuss equilibrium conditions and deviations in greater detail. Here it may suffice to point out that complete or incomplete attainment of equilibrium affects primarily the peak width and only slightly the elution volume (*1*, *2*).

Various workers have tried to predict V_e in terms of molecular size parameters. All of the rather diverse models gave good agreement with experimental results, even in cases where the models were obviously unsuitable (*3*). The reason for such apparent agreement was a rather insensitive square or cube root relation between elution volume and molecular size on one hand and an insufficient range of molecular sizes in the experiments on the other hand.

Today the emphasis is not so much on a detailed theoretical model of GPC as on either a fundamental understanding of the separation process or on a universal calibration method. On both aspects we will hear more in later papers in this Symposium. For those who have not

worked with GPC yet, some basic considerations on calibration in GPC may be of value.

Linear relation was found empirically between the elution volume of a species and the logarithm of its molecular weight. The upper and the lower ends of these calibration curves bend to 90° slopes at the limits of resolution. The total curve is S-shaped. The broader the pore size distribution of the gel or the more types of gels used in a GPC system, the longer is the linear part of the curve. Gels with very narrow pore ranges give S-shaped curves with little or no linearity. Better than plotting the logarithm of molecular weight is plotting the logarithm of molecular size versus elution time. In this way different shapes, flexibilities, and degrees of swelling do not affect the calibration curves. Since molecular size is the primary selective parameter in GPC, such a plot yields a universal curve which seems to hold for all kinds of molecules (*4*, *5*).

What exactly is molecular size in our context? Is it the length, the cross section, or the volume of a molecule? In the case of spherical molecules, any of these parameters could be used equally well. For rodlike molecules, either the length or the volume is suitable; the same would hold for prolate ellipsoids. For oblate ellipsoids it would be cross section or volume. It is always the most prominent dimension or the volume that determines size for GPC. Giddings et al. (*6*) proposed a "mean external length" $\bar{L}$ which is a mean length of projection of the molecule along an infinite number of axes. For rigid molecules, this length $\bar{L}$ seems to correlate better than other size parameters with V_e (*6*).

In contrast to rigid molecules, in the case of polymers we must distinguish between the size of a molecule, i.e., the amount of space it takes up in solution, and its mass. We shall have to develop the relationships, if any, between these two independent quantities.

With the aid of a model, we can demonstrate the long-chain nature of a typical polymer, the basic flexibility of the molecular chain, and its randomly coiling nature when it is surrounded by small solvent molecules. Let us consider for the moment a single polymer molecule with a fixed chain length, say 2000 carbon-carbon bond repeat units, each 1.54 Å long. We can calculate the size of this chain if we make enough simplifying assumptions.

If we assume that there are absolutely no restrictions on the positions of successive atoms of the chain except that they be 1.54 Å apart, the calculation is the very old one developed by Lord Rayleigh around

1870 and known as the random flight calculation. The answer has to be a statistical one, for the chain can take up a vast number of arrangements or conformations at different times, and it is appropriate only to ask about its *average* size. If we take, as the particular average quantity to calculate, the root-mean-square distance between the ends of the chain, $\sqrt{r^2}$, the answer for the random-flight calculation is $\sqrt{r_f^2} = l\sqrt{n}$. Putting $n = 2000$ and $l = 1.54$ Å, we obtain an end-to-end distance of about 69 Å.

This calculation disregards short-range interactions restricting the arrangement of successive atoms in the chain. We know that in real polymer chains the carbon-carbon bond angle must be preserved and that even in a very simple polymer such as polyethylene there are restrictions to free rotation about the carbon-carbon bonds, including those which rule out conformations putting near-neighbor atoms (say, the first and fifth in a sequence) on top of one another. In recent years, polymer chemists have been able to calculate the results of these restrictions with good accuracy; the contributions of Flory and his colleagues (*7*) have been outstanding in this way. He has shown, for example, that the root-mean-square end-to-end distance for polyethylene is increased by a factor of about 2.6 by all these restrictions. For our model chain, the resulting value, known as the unperturbed dimension, is $\sqrt{r_0^2} = 178$ Å.

Flory's calculation is in good agreement with the experimental value for the unperturbed dimensions of polyethylene, as computed from their hydrodynamic volume at the theta temperature. In good solvents, the chains are further expanded, of course, because of favorable polymer-solvent interactions and long-range interactions. We will come back to this later.

At this stage, we can draw the following conclusions:

1. For linear chains, the relation between size and the square root of the number of atoms (and this means the square root of molecular weight) is preserved, though the proportionality constant depends on polymer type, solvent, and temperature, and very slightly on molecular weight. This implies that for a given linear system there is a unique relation between size and mass, justifying the assumption that almost all GPC workers take for granted.

2. Even though we can talk only about the average behavior of a polymer chain, this is enough to explain its behavior in the GPC experiment. We can think of the average either as an instantaneous one

over many polymer chains of the same length or as a time average over the many conformations taken up by a single chain. During the time of a GPC experiment, a given molecule will take up so many conformations that its behavior is very well approximated by assuming it has its average size all the time.

3. The randomly coiling nature of the polymer chain also implies something about its shape. It can be shown that, as long as the chain has more than about ten segments, the Gaussian statistics required by the random-flight calculation hold. They require that the average shape for all conformations of the freely coiling chain, without any outside force fields or restraints, is spherical. Only if restraints are introduced do deviations from spherical symmetry occur. Thus, the average shape of all conformations in which both ends of the chain are at fixed locations is a prolate ellipsoid of revolution; the average when the center segment is fixed also is described as "bean-shaped" and so on. There is no reason to expect that in the GPC experiment the chain will be constrained to assume only these special conformations so that its shape can be considered spherical for our purposes.

To get some feel for the relation between the sizes of polymer molecules and GPC gel structures, we can make the following calculations. Consider a monodisperse polystyrene dissolved in tetrahydrofuran at room temperature for the usual GPC experiment. For any given molecular weight, we can calculate the random-flight end-to-end distance of this chain by the usual methods. Next, taking Flory's result (*7*) that the effect of short-range interactions in polystyrene is to expand the chain by a factor of about 3.2, we can calculate the unperturbed dimensions of the molecule.

The easiest way to get the effect of long-range interactions on the size of the chain is to compare its viscosity in the good solvent and in a θ-solvent. We can do this using Benoit's relationship (*8*) between viscosity and molecular weight in THF and that of Cantow (*9*) for a θ-solvent. The ratio of the viscosities gives the cube of the factor by which the chain dimensions are expanded in THF over their unperturbed dimensions. For MW = 100,000 the expansion factor is about 1.24. (Both this number and the factor due to short-range interactions are slightly molecular-weight dependent, but we have neglected this.)

We can further ask, what is the amount of space effectively oc-

cupied by a polymer with a certain end-to-end distance? Roughly speaking, it is that of a sphere whose diameter is about five times the end-to-end distance; all the segments of the polymer chain ought to be inside a sphere of this diameter about 95% of the time.

Putting all this together, we estimate the effective diameter of a polystyrene molecule in THF whose molecular weight is 100,000 to be just under 1000 Å; and that of a molecule with MW = 1,000,000 to be about 3000 Å. In Figs. 2 and 3 "random coils" of these sizes are sketched very crudely onto electron photomicrographs (*10*) of Styragel.

Figure 2 shows the sketch at MW = 100,000 in relation to a 10^4 Å Styragel. It seems to fit comfortably in the larger openings of the gel structure, which is reassuring since the exclusion limit for this material is about 400,000 molecular weight.

In Fig. 3, both this sketch and that corresponding to MW = 1,000,000

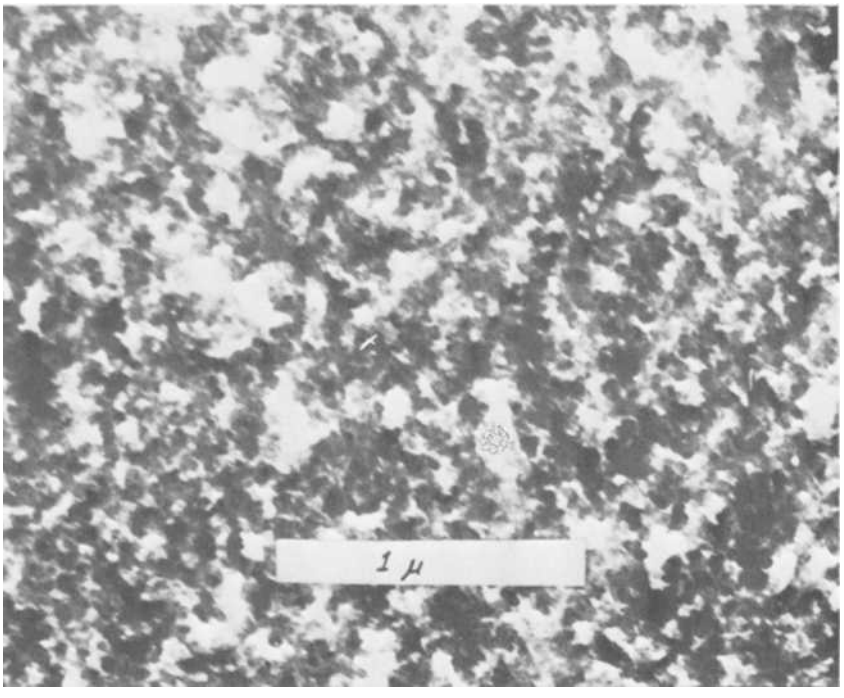

FIG. 2. Sketch of the effective size of a polystyrene molecule in THF at MW = 100,000, on an electron photomicrograph of 10^4 Å Styragel.

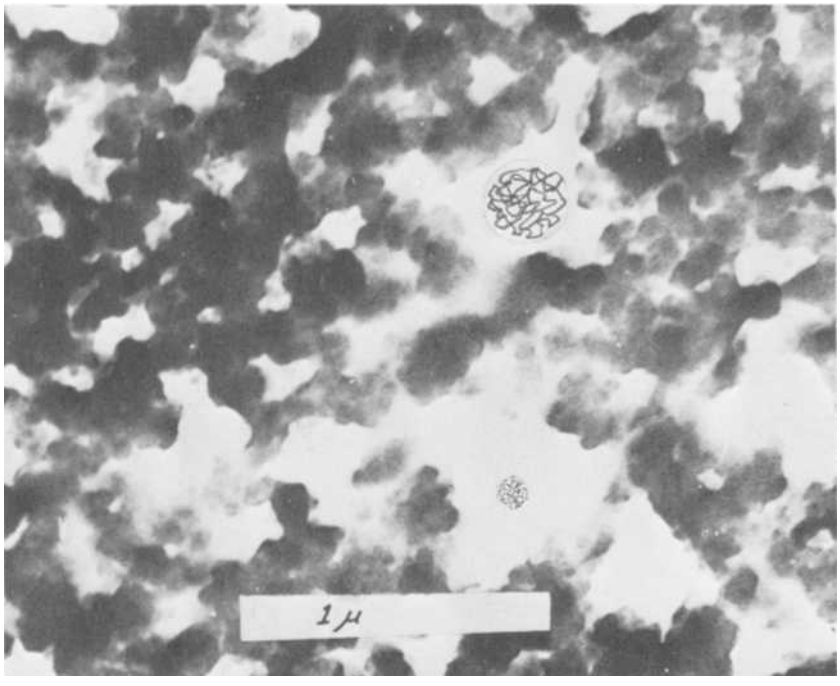

FIG. 3. Sketch of the effective sizes of polystyrene molecules in THF at MW = 100,000 and MW = 1,000,000 on an electron photomicrograph of a 10^6 (old designation) Styragel.

are shown on a 10^6 Styragel (old designation; now designated 10^5) where the exclusion limit is about MW = 4,000,000. Again, things seem to look about reasonable.

Now let us turn to the more usual type of polymer sample where we are always faced with a distribution of molecular weights and corresponding chain lengths. Again assuming that a fixed relation exists between over-all chain length and end-to-end distance, it follows that a distribution of end-to-end distances exists as a result of the various molecular species present in the sample. It would seem that two distributions exist simultaneously, the one just mentioned and that considered previously, the distribution of end-to-end distances resulting from the various conformations of a single molecular species. How can GPC separate the effects of these two?

The answer lies in the averaging nature of the many repeated steps of permeation in the GPC process. During the experiment the polymer

chains assume many conformations so that the average behavior of any single species corresponds to a fixed size parameter, such as the root-mean-square end-to-end distance, uniquely related to total chain length or molecular weight. The different molecular species display average behavior characteristic of their position in the distribution of such species present, and the separation occurs on the basis of this latter distribution.

What is the result? In the usual experiment, it is a plot of some measure of the amount of material existing in the column as a function of elution volume. One of the major efforts in the development of GPC has been to provide methods allowing the correlation of elution volume with molecular size or molecular weight. Details of these studies will be reported in later papers in this Symposium; here we shall make only one point:

The GPC experiment alone does not provide any information on either average molecular weights or molecular weight distribution. It is solely a separation technique. All the rest of the information must come from the calibration step. Ultimately, this requires the use of absolute methods for determining the average molecular weights of polymers. These methods have been reviewed in the literature from time to time (*11*) and will not be discussed further here.

Acknowledgments

One of us (FWB) would like to acknowledge support of his research on GPC by the Texas Division, Dow Chemical Co., and Waters Associates, Inc. The research is carried out in Rensselaer's Materials Research Center, a facility supported by the National Aeronautics and Space Administration.

REFERENCES

1. T. C. Laurent and E. P. Laurent, *J. Chromatogr.*, **16**, 89 (1962).
2. H. Vink, *J. Chromatogr.*, **25**, 71 (1966).
3. K. H. Altgelt, *Advances in Chromatography*, Vol. 7 (J. C. Giddings and R. Keller, eds.), Dekker, New York, 1968.
4. Z. Grubisic, P. Rempp, and H. Benoit, *J. Polym. Sci., Part B*, **5**, 753 (1967).
5. J. Cazes and D. R. Gaskill, *Separ. Sci.*, **2**, 421 (1967).
6. J. C. Giddings, E. Kucera, C. P. Russell, and M. N. Myers, *J. Phys. Chem.*, **72**, 4397 (1968).
7. P. J. Flory, *Statistical Mechanics of Chain Molecules*, Wiley, New York, 1969.

8. H. Benoit, Z. Grubisic, P. Rempp, D. Decker, and J. G. Zilliox, *J. Chim. Phys.* **63,** 1507 (1966).
9. H. J. Cantow, *Makromol. Chem.*, **30,** 169 (1959).
10. Electron micrographs kindly supplied by J. C. Moore.
11. F. W. Billmeyer, Jr., *J. Polym. Sci., Part C,* **8,** 161 (1965); *Polym. Eng. Sci.,* **6,** 359 (1966); *Appl. Polym. Symp.,* **10,** 1 (1969).

Received by editor March 2, 1970