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

# Peak Resolution and Separation Power In Gel Permeation Chromatography

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**To cite this Article** Harmon, Dale J.(1970) 'Peak Resolution and Separation Power In Gel Permeation Chromatography', Separation Science and Technology, 5: 3, 283 — 289
**To link to this Article: DOI:** 10.1080/01496397008080033
**URL:** http://dx.doi.org/10.1080/01496397008080033

SEPARATION SCIENCE, 5(3), pp. 283–289, June, 1970

# Peak Resolution and Separation Power In Gel Permeation Chromatography

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**Summary**

Plate count alone cannot predict the separation ability of permeation columns. Separation is a function of the size range of the molecules in the sample, the size range of the pores in the packing, and how well a column is packed. Ideally one should select columns which have a pore size range exactly covering the size distribution of the sample and which do not spread the peaks. In practice, one usually settles for columns which at least cover the size range of the sample and have some pores of every size present. The lower the slope of the calibration plot (log, $A$, $M$ or $[n]M$ vs $V_R$), the better the resolution. The more nearly the plot approaches a straight line the less the distortion and the simplier the treatment of the data.

Since the objective of GPC is to separate species by their molecular size in solution, it is desirable to have some means of describing how well a column will do this job. Not only is the information of value to the practitioner to guide him in selecting the proper column or columns to successfully carry out a specific analysis, but such information is necessary for comparison of data between laboratories. Any published data should also contain descriptions of the columns used and of their resolution capability.

The separation efficiency of columns has usually been compared by measuring the number of theoretical plates using equations originally developed for liquid-liquid chromatography (*1*)

$$N = 16\left(\frac{V_R}{W}\right)^2 \tag{1}$$

where $V_R$ equals retention volume and $W$ equals peak base width. As pointed out by Cazes (*2*) "The theoretical plate concept is borrowed from that area of chemical engineering involving fractional distillation. A theoretical plate, in the case of distillation, refers to a discreet distillation stage constituting a simple distillation in which complete equilibrium is established between the liquid and vapor phases. In the case of gel permeation chromatography where the two phases are in constant motion, i.e., the solvent in the interstitial volume and the solvent within the gel pores, equilibrium is probably never achieved. The true significance of the theoretical plate is lost. It must be realized, moreover, that the calculated theoretical plate in a chromatographic column represents smaller separating ability than the theoretical plate in a distillation column by a factor of twenty-five to fifty." Quite often plate count, which is the number of theoretical plates per foot,

$$PC = \frac{N}{L} = \left(\frac{16}{L}\right)\left(\frac{V_R}{W}\right)^2 \tag{2}$$

with $L$ = total column length in feet, or its reciprocal, the "height equivalent to a theoretical plate"

$$H = \frac{1}{PC} = \left(\frac{L}{16}\right)\left(\frac{W}{V_R}\right)^2 \tag{3}$$

are used instead of theoretical plates since these terms take into consideration the total column length.

As pointed out by Giddings (*3*), gel permeation is unique in that there is a well-defined limit to peak capacity. The reason is that the peaks are confined to a definite retention volume range determined by the interstitial volume at one end ($V_0$) and the maximum solvent volume (both in and out of the pores) at the other end ($V$). The largest molecules with no penetration will elute at the first limit and the smallest molecules at the second. In most other forms of chromatography, while there is a similar lower limit to retention volume, the upper limit is indefinite and may be 10 or 100 times larger than the lower limit.

Assuming a fixed number of plates, $N$, equal for each solute and a minimum base line separation, Giddings (*3*) developed the following highly simplified expression for peak capacity of a permeation column.

$$n \cong 1 + 0.2N^{1/2} \tag{4}$$

where $n$ is the number of peaks resolvable. Table 1 compares the

TABLE 1

Comparative Peak Capacity of Gel Filtration and Other Columns for Given Numbers of Theoretical Plates[a]

| Theoretical plates ($N$) | Peak capacities ($n$) | | |
|---|---|---|---|
| | Gel chromatography | Gas chromatography | Liquid chromatography |
| 100 | 3 | 11 | 7 |
| 400 | 5 | 21 | 13 |
| 1,000 | 7 | 33 | 20 |
| 2,500 | 11 | 51 | 31 |
| 10,000 | 21 | 101 | 61 |

[a] From Ref. 3.



number of peaks resolvable at various theoretical plate levels, using Eq. (4), for gas, liquid, and gel permeation chromatography. Gel columns generally have around 1000 theoretical plates and therefore may be expected to separate a maximum of about seven peaks.

Plate counts determined by Eq. (2) are more a measure of how well a column is packed, that is, how much peak spreading it will cause, than how well it will resolve. This is clearly illustrated in Fig. 1. Two GPC traces for a polystyrene are shown. The traces were obtained under identical conditions except in one case (A) four Porasil columns

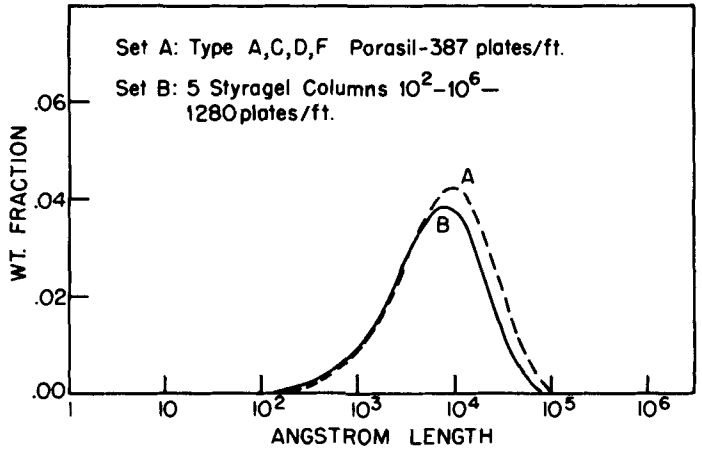


FIG. 1. Chromatograms of polystyrene. Conditions identical except for columns.

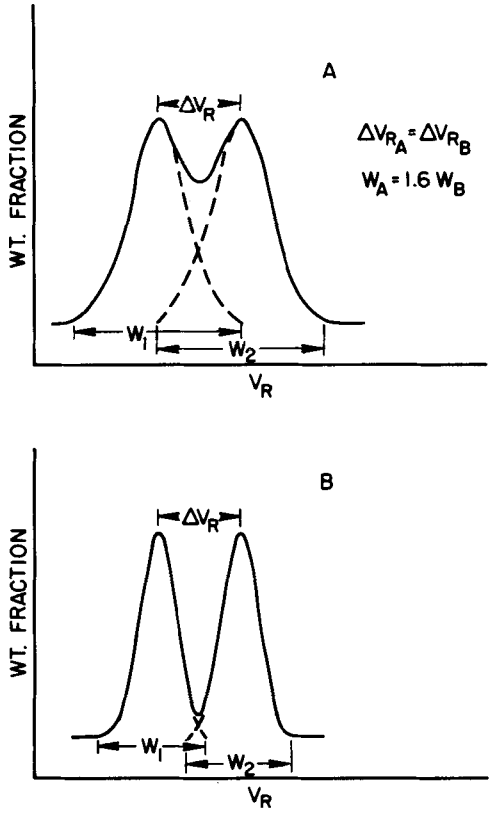


FIG. 2. Effect of band spreading on peak resolution.

were employed and in the other (B) five Styragel columns. The plate counts were 387 and 1280/ft, respectively, yet the two curves show nearly equal resolution. No corrections were applied to the data.

The effectiveness of the GPC separation is determined by the distance separating the centers of the peaks of two species and the width of the two peaks. This is illustrated in Fig. 2. The conventional definition of resolution ($R$) in chromatography is

$$R = \frac{2(V_{R_2} - V_{R_1})}{W_1 + W_2} \tag{5}$$

where $V_{R_1}$ and $V_{R_2}$ are the retention volumes of species 1 and 2, respectively, measured from injection to the peak maximum and $W_1$ and $W_2$ are the base widths measured between two tangents drawn on the

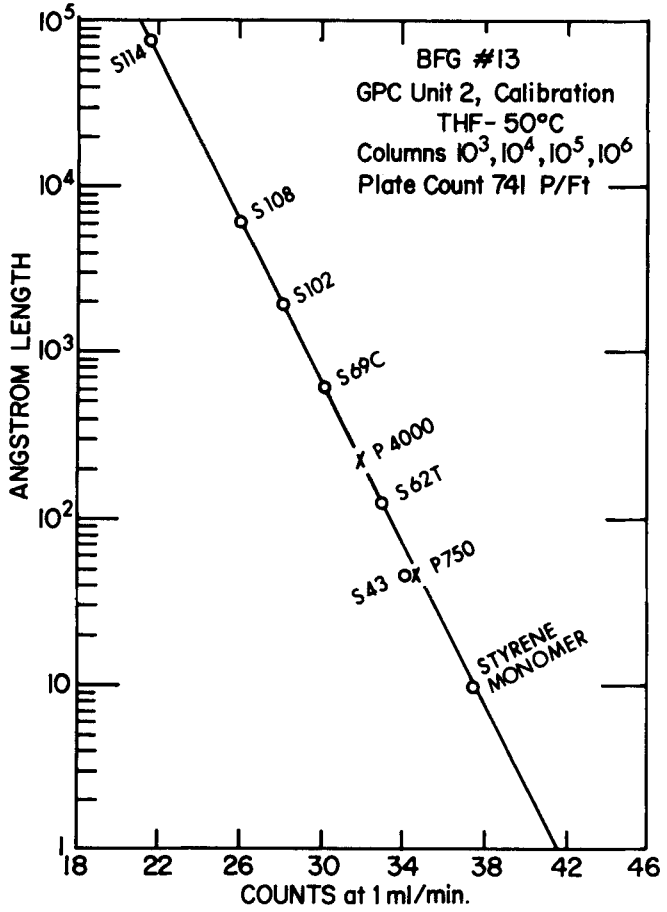


FIG. 3. Typical calibration curve for Styragel columns.

points of inflection of the curve and extended to the base line (4). For complete separation $R$ must be equal to or greater than 1.

For high molecular weight, polydispersed materials resolution decreases with increasing molecular weight. This is readily apparent if one looks at a calibration curve such as shown in Fig. 3 where Angstrom size or molecular weight is plotted against elution volume for a 20-ft Styragel column system. The reason resolution decreases with increasing molecular weight is that fewer pores are available to the large molecules than are available to the small molecules. A realistic approach, then, is to calculate some sort of resolution index

TABLE 2

Resolution Indices for GPC Columns

| Column | Pore size (A°) | Plates/ft. | Solvent | Flow rate (ml/min) | Resolution index $2 \times 10^5$ | Resolution index $8.6 \times 10^5$ |
|---|---|---|---|---|---|---|
| $A_1$ | $10^6$ | 450 | THF | 1 | 0.097 | 0.10 |
| $A_2$ | $10^6$ | 990 | THF | 1 | 0.21 | 0.24 |
| B | $7 \times 10^5$ | 1370 | THF | 1 | 0.062 | 0.11 |
| C | $4 \times 10^5$ | 243 | THF | 1 | 0.15 | 0.23 |
| D | $3 \times 10^4$ | 103 | THF | 1 | 0.19 | 0.17 |
| E | $10^4$ | 800 | THF | 1 | 0.28 | 0.03 |

| Combination of columns | | | | Measured | Calc. | Measured | Calc. |
|---|---|---|---|---|---|---|---|
| $A_2 + C$ | | THF | 1 | 0.32 | 0.29 | 0.38 | 0.36 |
| $A_2 + C + E$ | | THF | 1 | 0.43 | 0.41 | 0.38 | 0.35 |
| $A_1 + C + D + E$ | | THF | 1 | 0.39 | 0.42 | 0.36 | 0.37 |
| $A_1 + C + D + E$ | | THF | 1 | 0.39 | | 0.40 | |

[a] Data taken from Ref. *6*.



that takes into consideration molecular weight and base width. Bly (*5*) defines a specific resolution ($R_s$) based on the assumption that the two polymer species used to determine $R_s$ have the same molecular weight distribution and that

$$V_e = A - B \log M \tag{6}$$

which usually holds over a fairly wide range of molecular weight. Bly's equation is

$$R_s = \frac{2(V_2 - V_1)}{(W_1 + W_2)(\log \bar{M}_{w_1} - \log \bar{M}_{w_2})} \tag{7}$$

where $V$ and $W$ are as before and $\bar{M}_{w_1}$ and $\bar{M}_{w_2}$ are the molecular weights of the two species used to determine $R_s$.

Feldman and Smith (*6*) have proposed a similar sort of resolution

$$RI = \left(\frac{M_1}{M_2}\right)^{W_{12}/(P_1 - P_2)} \tag{8}$$

index where $W_{12}$ is the average width of the two species and $P$ is the peak position or retention volume. If one assumes that the relative displacements of two polymer peaks are additive in a series combi-

nation of columns and that the square of the peak width for the series is equal to the sum of the squares of the individual peak widths, i.e., $W^2 = \Sigma_i W_i^2$, the resolution index ($RI$) of a series combination of columns is given by

$$\frac{1}{\log (RI)} = \Sigma_i \left[\frac{1}{\log (RK)_i}\right]\left[\frac{W_i}{(\Sigma_j W_j^2)^{1/2}}\right] \tag{9}$$

Table 2 illustrates how plate count may be misleading as an indicator of separation ability and how RI values calculated using Eq. (9) agree with measured values.

**Acknowledgments**

The author wishes to acknowledge the courtesy of the following editors and publishers for permission to reproduce the designated material: The American Chemical Society, publishers of *Analytical Chemistry*, Table 1; Interscience Publishers, a division of John Wiley and Sons, Inc., publishers of *Journal of Polymer Science*, Table 2.



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*Received by editor October 30, 1969*