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Gel Permeation Chromatography. VIII. A Study of the Effect of Column Arrangement of Resolution at Normal and Overloading Concentrations

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Synopsis

In the determination of molecular weight distributions by GPC, the traditional column arrangement is such that the fractionation process proceeds from a high-permeability limit to a low-permeability limit column. We report computer comparisons of data obtained from columns in their normal ordering (high- to low-permeability limit), reverse ordering, and random ordering. The columns had a permeability limit range from 1×10^6 Å down to 1×10^3 Å, and the polymers had a molecular weight range of 1.8×10^6 down to 2.1×10^3. The concentrations used varied from 0.05% up to 0.5%. The data show significantly different results, with the random arrangement the preferred ordering. A qualitative model for the separation mechanism is presented to account for the improvement in resolution. Additional data are presented which show that serious errors (as high as 45%, depending on concentration) will be encountered in GPC studies, unless the calibration curve is obtained at the same relative concentration as the samples, with definite overloading effects occurring at the higher concentrations. A new method of curve fitting was used in the higher molecular weight region to give meaningful calibration curves.

INTRODUCTION

The advent of gel permeation chromatography (GPC) has been of great assistance to polymer chemists for the rapid determination of the molecular weight distributions, various molecular weight averages ($\bar{M}_w$, $\bar{M}_n$, $\bar{M}_z$, and $\bar{M}_v$), and polydispersity indices ($\bar{M}_w/\bar{M}_n$). Moore[1] introduced this technique for the determination of molecular weight in 1964, and other authors[2,3,4] give a history of GPC and its relative usefulness when compared with other methods of measuring molecular weight distributions. Lambert[5] has written an excellent review and has qualitatively addressed the inherent problems in the technique. Two of the problems addressed in this work are (1) the nonlinearity effects (coupling of the different molecular species in the solute) on the fractionation process, and (2) the variation in resolution, both as a function of the ordering of the permeability limits of separating columns and of the concentrations.

The term permeability limit, as related to GPC columns, connotes a parameter (pore size) of the gel beads whereby molecules bigger than a

given size are excluded from diffusing into the pores of the beads. Each of the columns is packed with beads of a different pore size permitting a broad range of molecular species to be separated in a single run. The columns are connected in series. The traditional column arrangement is from a high-permeability limit column down to a low-permeability limit column, i.e., 1×10^6 Å to 1×10^3 Å. This range of permeabilities gives effective distributions from a molecular weight of more than 1.5 million down to less than 2000.

Since the separation process is not well understood, questions have arisen as to the effect on the fractionation process of purposely altering the molecular environment in the immediate vicinity of the molecules undergoing fractionation. This can be accomplished by changing the ordering of the columns such that the molecules "see" the gel pores in variant disposition.

In the determination of some molecular weight distributions, it may be necessary to increase the solute concentration considerably beyond what is desired. This is true, for instance, when the refractive index of the solute is very near that of the solvent (silicone oils in tetrahydrofuran), such that the sensitivity of the differential refractometer is reduced. One must be careful when increasing the concentration, not only of the overloading of the columns, but also to ensure that the calibration curve is constituted at the same relative concentration as the sample. Rather gross errors can be incorporated into the data if "low" concentration calibration curves are used with "high" concentration samples. Ouano[6] has recently developed an absolute molecular weight detector which will obviate this problem as it does not require a calibration curve.

Ouano,[7] Moore,[8] and Lambert[9] have addressed the overloading problem in GPC with the columns in their normal arrangement. Ouano has shown that definite loss of sensitivity and resolution occurs as the solute concentration is increased beyond optimum levels. This phenomenon is also considered here for the reversed and random arrangements.

EXPERIMENTAL

In order to study the effect of ordering on the GPC separation process, a series of seven polystyrene standards (Pressure Chemical Company, Pittsburgh, Pennsylvania) was selected covering a molecular weight range of 1.8×10^6 down to 2100 (Table I).

The concentrations used were 0.05%, 0.25%, and 0.50% polymer in tetrahydrofuran (THF). The seven component mixtures were made from the standards such that the sample loading of each individual species was kept constant with a total sample loading of 7 mg for the 0.05%, 35 mg for the 0.25%, and 70 mg for the 0.50% concentration.

The instrument used was a Waters Associates Model 200 gel permeation chromatograph equipped with an automatic six-sample injection system. The automatic sample injector allows unattended overnight operation

TABLE I
Polystyrene Standards

Standard no.	$\bar{M}_w$	$\bar{M}_w/\bar{M}_n$
1	1.8×10^6	<1.20
2	4.1×10^5	<1.06
3	1.6×10^5	<1.06
4	5.1×10^4	<1.06
1	2.0×10^4	<1.06
6	1.0×10^4	<1.06
7	2.1×10^3	<1.10

which was imperative for these types of studies. Five 4 ft × 3/8 in. columns were connected in series with the effluent being fed directly to a differential refractometer. The column arrangements and permeability limits are given in Table II. The availability of only five columns poses a problem of obtaining a truly random arrangement in column ordering. Hence, in Table II, the "random" ordering appears to be a combination of normal and reverse arrangements rather than a true random configuration. Rearranging the columns further can only result in an alternating-type arrangement. An ideal random arrangement can be obtained either by using a large number of very short columns or by mixing beads of different permeability limits i.e., Waters linear columns.

The normal ordering is conventional and was the criterion by which the other systems were judged. The column packings were the standard polystyrene–divinylbenzene beads (Styragel), and the flow rate of THF was 1 ml/min.

Data collection was accomplished through the use of an automated laboratory system developed in this laboratory as discussed by Gladney.[10] The GPC arrangement and modifications were described by Gregges, Dowden, Barrall, and Horikawa.[11] The signal from the differential refractometer was fed to an IBM 1800 computer by a shielded twisted-pair cable. The data were stored on disk files until needed by the data reduction programs executed at the termination of the runs. The computer calculates the number-average molecular weight ($\bar{M}_n$), the weight-average molecular weight ($\bar{M}_w$), the polydispersity index ($\bar{M}_w/\bar{M}_n$), and the viscosity-average molecular weight ($\bar{M}_v$) when the proper Mark-Houwink constants are supplied.

TABLE II
Column Arrangements and Permeability Limits

Column no.	Normal	Reverse	Random
1	1×10^6 Å	1×10^3 Å	1×10^4 Å
2	1×10^5 Å	1×10^4 Å	5×10^4 Å
3	5×10^4 Å	5×10^4 Å	1×10^6 Å
4	1×10^4 Å	1×10^5 Å	1×10^5 Å
5	1×10^3 Å	1×10^6 Å	1×10^3 Å

The most important data reduction program employed in this study uses the punch card inputs from the programs described above.[7] This program utilizes the individual chromatograms of each component to construct the envelope of a mixture by linear superposition. The computer compares the synthesized chromatogram to the normalized chromatogram of the actual mixture with any differences being ascribed to nonlinearity of the fractionation process. These differences are indicated in both analog and digital form by the computer generated plots and printout.

DISCUSSION OF RESULTS

One of the most interesting results of this study is that the random ordering used gives better resolution and less error due to nonlinearity than either the normal or the reverse ordering. This is seen by a careful comparison of Figures 1–9, which are the overlay plots. In each, the synthesized chromatogram and the actual chromatogram are shown in their normalized form. The curve along the bottom of each figure is a plot of the absolute magnitude of the differences between the curves. It should be noted that the areas under the two chromatograms are *equal* since the algebraic sum of the difference is zero (see Fig. 1). The summation of the higher magnitudes of peaks 1, 3, and 7 (left to right) of the synthesized envelope is exactly equal to the higher magnitudes of peaks 2, 4, and 5 of the chromatogram of the mixture (albeit one is negative and one is positive). Figures 1–3 are similar to those previously obtained by Ouano[7] and show the constancy of the present GPC system over a long period of time.

As can be seen by comparing Figures 2, 5, and 8, the *resolution* in the 20,000–60,000 range is particularly enhanced by the "random" ordering

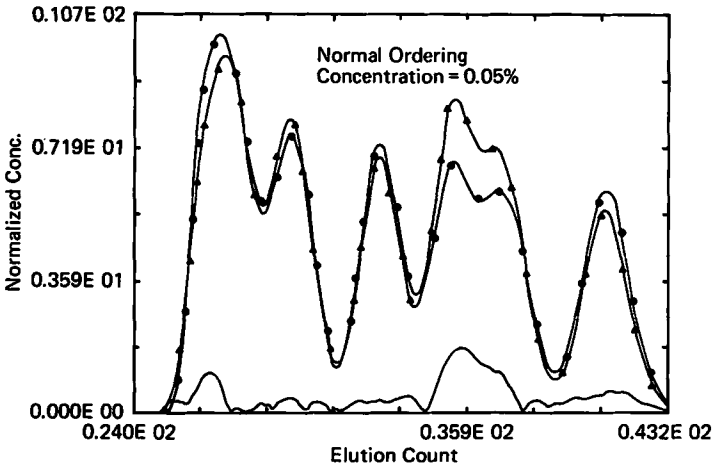

Fig. 1. Overlay plots of the normalized chromatograms of a solution of equal mixtures of the standards in Table I (▲) and the computer-synthesized envelope (●) from the seven individual chromatograms: normal ordering, 0.05 g/100 ml (elution count = 5 ml/count, normalized concentration in per cent).

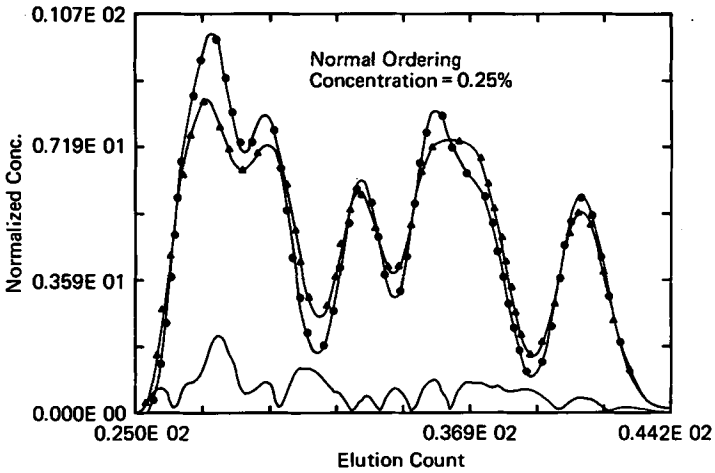

Fig. 2. Overlay plots of the normalized chromatograms of a solution of equal mixtures of the standards in Table 1 (▲) and the computer-synthesized envelope (●) from the seven individual chromatograms: normal ordering, 0.25 g/100 ml (elution count = 5 ml/count, normalized concentration in per cent).

and no doubt is due, in part, to the fact that this is a very linear area of the calibration curve. This is shown in Table III, which summarizes the plotted data for the 0.25 g/100 ml concentrations and shows the relative degree of disagreement between the different orderings at this concentration.

Less overloading effects were experienced in the random ordering than either the normal or reverse (Figs. 3, 6, and 9). Table IV gives the molecular weight averages for the seven component mixtures at the three con-

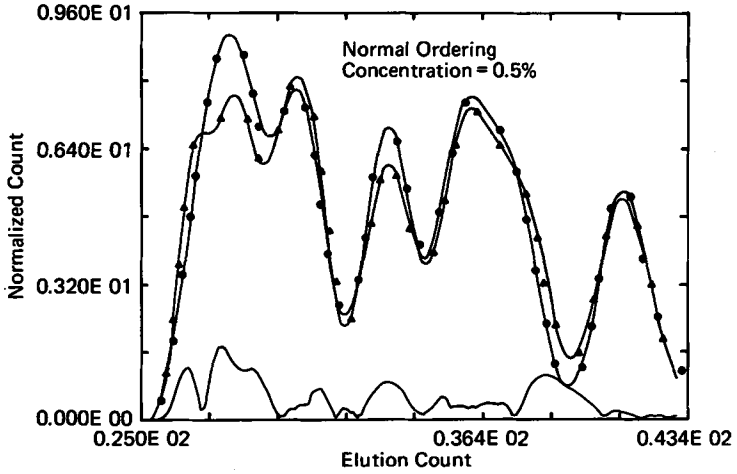

Fig. 3. Overlay plots of the normalized chromatograms of a solution of equal mixtures of the standards in Table I (▲) and the computer-synthesized envelope (●) from the seven individual chromatograms: normal ordering, 0.50 g/100 ml (elution count = 5 ml/count, normalized concentration in per cent).

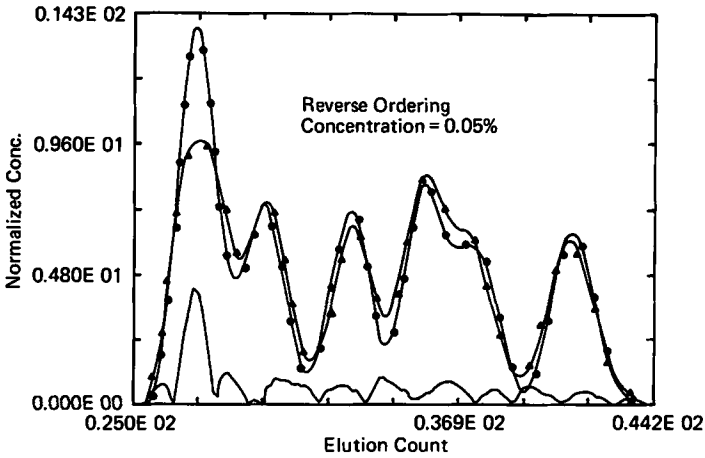

Fig. 4. Overlay plots of the normalized chromatograms of a solution of equal mixtures of the standards in Table I (▲) and the computer-synthesized envelope (●) from the seven individual chromatograms: reverse ordering, 0.05 g/100 ml (elution count = 5 ml/count, normalized concentration in per cent).

centrations for the three orderings. The per cent variance shows that the random ordering is a factor of 5 times less sensitive to overloading than the normal arrangement.

A qualitative model has been developed to explain this improvement. If the separation process is an entrapment phenomenon, then the partitioning of the molecules between the eluent stream and the pores of the gels is directly dependent on the effective molecular hydrodynamic volume. In the usual high- to low-permeability ordering of the columns, the molecules are separated as shown in Figure 10.

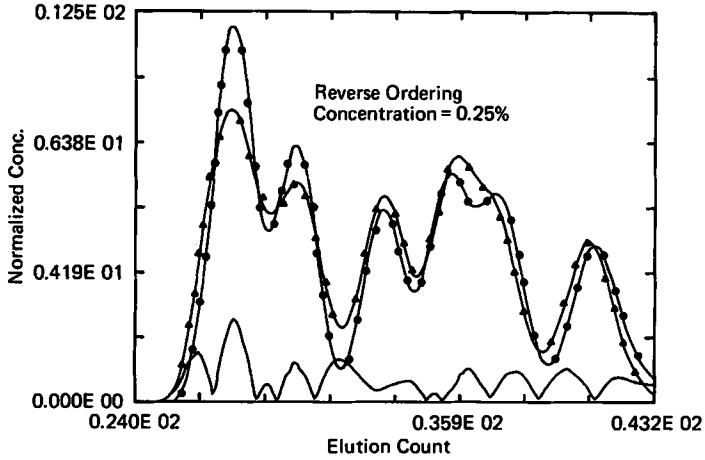

Fig. 5. Overlay plots of the normalized chromatograms of a solution of equal mixtures of the standards in Table I (▲) and the computer-synthesized envelope (●) from the seven individual chromatograms: reverse ordering, 0.25 g/100 ml (elution count = 5 ml/count, normalized concentration in per cent).

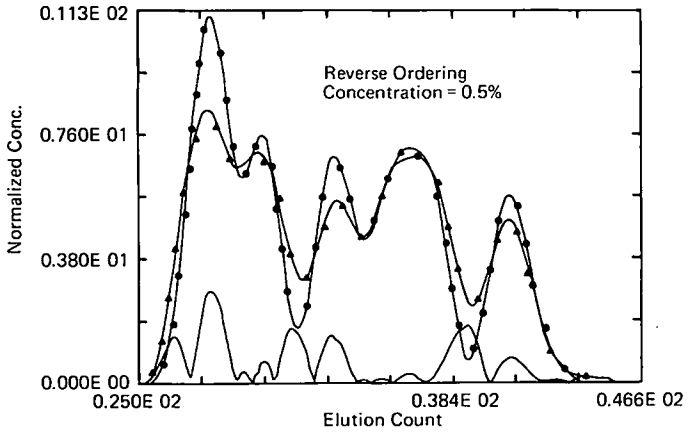

Fig. 6. Overlay plots of the normalized chromatograms of a solution of equal mixtures of the standards in Table I (▲) and the computer-synthesized envelope (●) from the seven individual chromatograms: reverse ordering, 0.50 g/100 ml (elution count = ml/count, normalized concentration in per cent).

It is seen that the intermolecular distances between the larger molecules are greater than those between the smaller molecules. This allows a greater interaction time between the smaller molecules.

If the ordering is now reversed, the separation process is as given in Figure 11.

The relative interaction times of the low and high molecular weight species have now been drastically altered. The larger molecules now have a much longer time in which their effective hydrodynamic volume is affected by the smaller molecules.

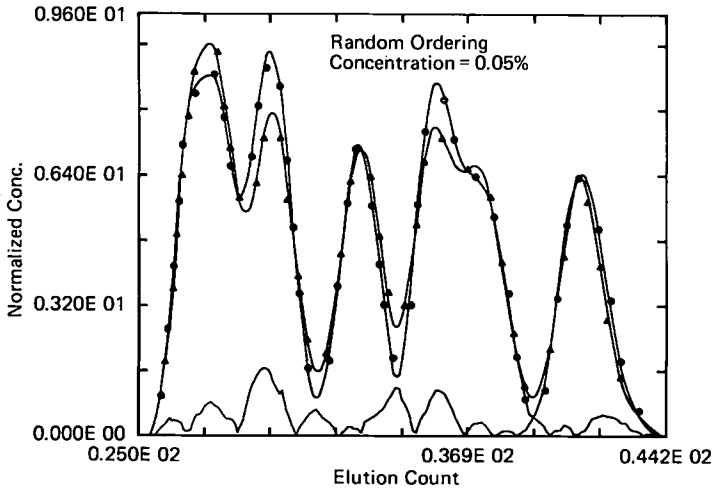

Fig. 7. Overlay plots of the normalized chromatograms of a solution of equal mixtures of the standards in Table I (▲) and the computer-synthesized envelope (●) from the seven individual chromatograms: random ordering, 0.05 g/100 ml (elution count = 5 ml/count, normalized concentration in per cent).

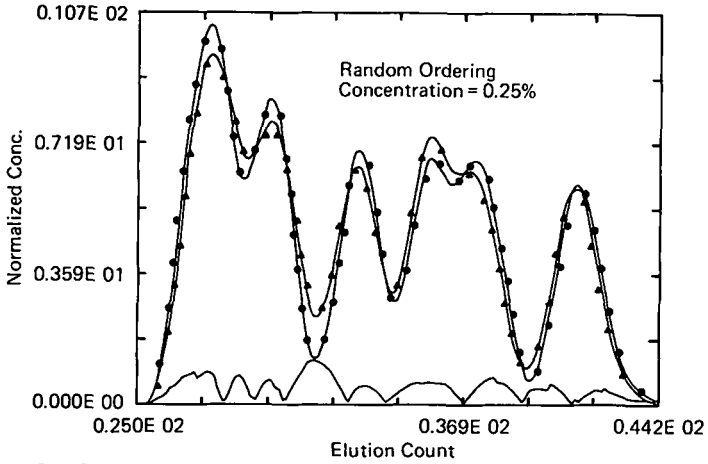

Fig. 8. Overlay plots of the normalized chromatograms of a solution of equal mixtures of the standards in Table I (▲) and the computer-synthesized envelope (●) from the seven individual chromatograms: random ordering, 0.25 g/100 ml (elution count = 5 ml/count, normalized concentration in per cent).

By randomizing the column arrangement, the solute plug is essentially "segregated" as is shown in Figure 12.

This segregation essentially serves to control the environment of the molecular species during the separation process and reduces fluctuations in the effective hydrodynamic radii. This improves the efficiency of the operative utilization of the column beads and leads to better separations.

Additional support for this model can perhaps be drawn by a careful evaluation of the data in Table IV. The use of $\bar{M}_w$ values alone for com-

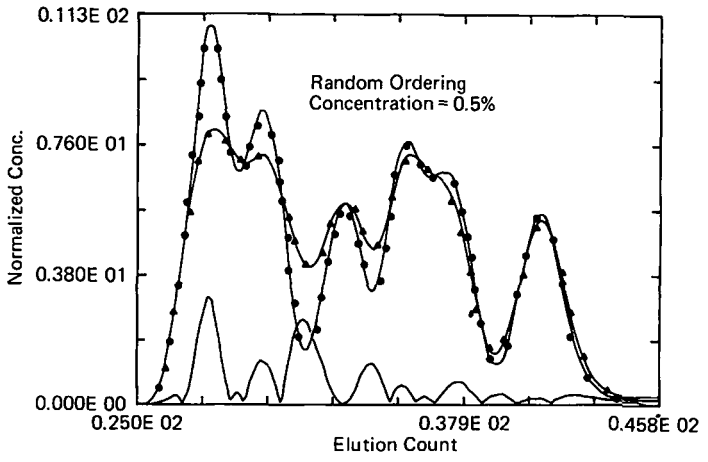

Fig. 9. Overlay plots of the normalized chromatograms of a solution of equal mixtures of the standards in Table I (▲) and the computer-synthesized envelope (●) from the seven individual chromatograms: random ordering, 0.50 g/100 ml (elution count = 5 ml/count, normalized concentration in per cent).

TABLE III
Degree of Nonlinearity Between Synthesized Plot and Actual Mixture[a]

Sample concentration, %	Ordering	Degree of disagreement[b]
0.25	normal	82.5
0.25	reverse	106.3
0.25	random	65.3

[a] Calibration concentration = 0.25 g/100 ml.

[b] Degree of disagreement is the computer-determined digital summation of the difference between the two curves (see Figs 2, 5, and 8).

paring chromatograms is normally not a valid approach, since a Gaussian curve can have the same $\bar{M}_w$ as a highly skewed curve. However, a direct comparison based on $\bar{M}_w$ values *is* valid where the shapes of the chromatograms are similar. The data in Table IV show the $\bar{M}_w$ values for the random arrangement to be between the normal and reverse column orderings. This is an indication that band spreading dominates in the high end of the distribution in the normal arrangement (Fig. 10), while occlusion predominates in the reverse arrangement (Fig. 11). This phenomenon would result in an overestimation and underestimation of the $\bar{M}_w$ values, respectively. This observation agrees well with the proposed qualitative model.

The use of an accurate calibration curve is a necessary adjunct to GPC determinations. However, the use of calibration values measured at concentrations other than the sample concentrations may lead to gross errors. Table V shows the relative error as a function of both concentration and column ordering.

TABLE IV
Percent Variances Between $\bar{M}_w$ at Various Loadings and Column Arrangements

Ordering	Calibration concentration, g/100 ml	Sample concentration, g/100 ml	$\bar{M}_w \times 10^{-5}$	Per cent variance[a] A	Per cent variance[a] B
	0.05	0.05	2.92		
Normal	0.25	0.25	2.42	17.1	25.7
	0.50	0.50	2.17		
	0.05	0.05	2.03		
Reverse	0.25	0.25	2.28	12.3	3.9
	0.50	0.50	2.11		
	0.05	0.05	2.22		
Random	0.25	0.25	2.36	6.3	4.7
	0.50	0.50	2.12		

[a] A = Variance between 0.05 g/100 ml and 0.25 g/100 ml; B = variance between 0.05 g/100 ml and 0.50 g/100 ml.

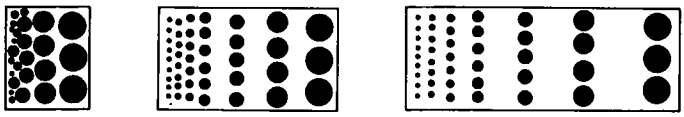

Fig. 10. Accordion effect on molecular species. High-permeability to low-permeability columns. Normal ordering.

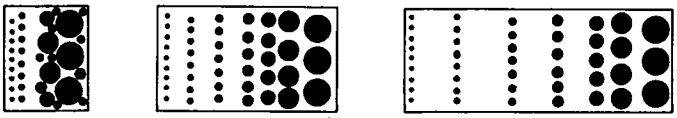

Fig. 11. Accordion effect on molecular species. Low-permeability to high-permeability columns. Reverse ordering.

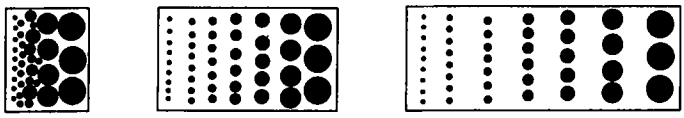

Fig. 12. Isochronal effect as a result of the randomizing of the permeability limits.

Note that the calibration error at the 0.50 g/100 ml concentration is reduced by a factor of 2 by using the random ordering.

An auxiliary problem involved with the selection of the proper calibration curve is the changing slope above a molecular weight of 500,000. The relationship between the molecular size (M) and the elution volume (V) is given by

$$\alpha = -\frac{d \log (M)}{d (V)}$$

where α is the slope of the calibration curve. For best operating efficiency, it is desirable to stay on the straight line portion of the curve (i.e., where α is independent of molecular weight). This usually ranges between 1,000 and 500,000.

Inasmuch as many calibration curves were required in this work (different orderings, different concentrations, etc.), a new method was used to correct for the error in the high molecular weight ranges. GPC envelopes were obtained for each of the seven standards and these data were used to construct the best fit for calibration. The data reduction programs were then utilized to calculate the molecular weights of the higher molecular weight standards. These values were compared to the actual weight-average molecular weights (Table I), and the curve fit was then changed by a trial-and-error method to give the correct computer-calculated values. The slopes at the high molecular weight ends were used to construct a family of curves at the other orderings and concentrations. The molecular weight moments were then calculated. This entire technique is predicated on the standards being accurate, low-dispersity polymers.

TABLE V
Relative Errors Caused by Misuse of Calibration Curves

Configuration	Sample concentration, g/100 ml	$\bar{M}_w \times 10^{-5}$	Relative error between 0.05% and 0.25%	Relative error between 0.05% and 0.50%
Normal ordering	0.05	2.92		
Calib. standards	0.25	2.22	24.0	46.0
Concentration = 0.05 g/100 ml	0.50	2.00		
Reverse ordering	0.05	2.03		
Calib. standards	0.25	1.72	15.3	30.5
Concentration = 0.05 g/100 ml	0.50	1.41		
Random ordering	0.05	2.22		
Calib. ordering	0.25	2.09	5.9	23.4
Concentration = 0.05 g/100 ml	0.50	1.70		

Subsequent discussions and considerations of the voluminous amount of data generated in this study have engendered several interesting items. If, indeed, one is interested in optimizing the segregation in the solute plug as it proceeds to the refractometer, then the idealized situation would be to alternate the columns; e.g., 10^3 Å, 10^6 Å, 10^4 Å, 10^5 Å, 5×10^4 Å. This would provide maximum separation and allow minimum interaction time between the different molecular species. In effect, this exerts maximum control on the molecular environment.

Another sidelight of this study involves the consideration of the interaction time of each molecular species as a function of the molecular weight distribution. If the separation model presented above is correct, then the peak of the retention volume distribution for the standards should occur slightly later in the reverse ordering than in the normal ordering. This was true *in every case* (usually by a small amount; $\approx$0.5 cc) and is attributed to the greater interaction times for the large and small molecules in the reverse ordering (larger effective hydrodynamic volume).

In summary, this study has shown that a randomizing of the permeability of the limits of the columns (or more probably an alternating arrangement) will give improved resolution and linearity to the fractionation process. Also, this type of system will be less sensitive to overloading effects and to errors caused by misuse of calibration curves. This study indicates that new so-called linear columns recently available may provide better performance than standard (narrow pore size distribution) columns.

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