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Gel Permeation Chromatography: The Effect of Pore Size Distribution on Separation Efficiency*

ANTHONY R. COOPER† and JULIAN F. JOHNSON,‡ *Chevron Research Company, Richmond, California, 94802*

Synopsis

Columns packed with Bio-Glas (porous glass manufactured by Bio-Rad Laboratories, Richmond, California) of varying porosities were made which had the same cumulative pore volume. The pore radii were equal to or larger than the mean end-to-end distance of the 160,000 molecular weight polystyrene molecule in carbon tetrachloride. The efficiency of these columns was studied for the elution of polystyrene $\bar{M}_w$ = 160,000 and two sharp polyisobutene fractions having $\bar{M}_w$ = 114,700 and 145,700. Elution volume was found to be independent of flow rate below a flow rate of 1 ml/min for all columns studied. Two pairs of columns with the differing cumulative pore volumes were compared, and only small differences were found in their efficiencies, as measured by the number of theoretical plates per foot. The pore size distribution is important in that it determines elution volumes and the effective separating range of the column, but it does not appear to materially affect the efficiency of the separation process.

INTRODUCTION

The efficiency of the gel permeation chromatography (GPC) separation process with respect to pore size and its distribution does not appear to have been investigated thoroughly. Moore[1] showed that for polystyrene and polypropylene glycol samples of similar molecular weight distribution (MWD), using three polystyrene gel columns in series, the base widths of the peaks increased with increasing molecular weight. This effect was also observed by Hess and Kratz[2] using polystyrene samples with $\bar{M}_w/\bar{M}_n < 1.1$ and polystyrene gel columns. Haller,[3] however, observed, with virus species eluting from a single porous glass column, that the experimental base widths decreased with increasing molecular weight. DeVries[4] considered that pore size distribution was extremely important for selectivity in GPC separations. Later, DeVries et al.[5] showed that plate height was independent of column length and pore size distribution for porous silica beads and narrow-MWD polystyrene solutes. The plate height in GPC has been related to the diffusion coefficient of the solute[6,7] for various small molecules

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† Present address: Department of Pure and Applied Chemistry, University of Strathclyde, Glascow, Scotland.

‡ Present address: Department of Chemistry and Institute of Materials Science, University of Connecticut, Storrs, Connecticut 06268.

eluting from a given column and also for polymeric solutes.[8] An excellent review of various aspects of peak broadening has been given by Kelley and Billmeyer.[18]

Theoretical predictions by Carmichael[9] suggested that, for unperturbed random coils, the experimental base widths should decrease with increasing molecular weight for a given column for samples of similar $\bar{M}_w/\bar{M}_n$. Later,[10] the passage of a monodisperse random coil polymer through a bed of Gaussian-distributed pore sizes having the same mean, but different variances, was considered. The chromatographic dispersion was predicted to increase as the pore size variance increased. Giddings[11] has suggested that pore sizes should be small and their total volume large. For spherical and rigid rod molecules, various pore geometries were considered and found to be important for maximum efficiency in the separation of monodisperse and polydisperse solutes.

In this stydy, the 160,000-molecular-weight polystyrene standard and two polyisobutene fractions of similar mean end-to-end distance, one slightly larger and one slightly smaller, were chromatographed from columns having the same cumulative pore volume between pore radii of 66,968 and 383 Å. The columns were of different lengths, and the conclusions from this study are dependent upon the plate height per unit length being independent of column length.

EXPERIMENTAL

The solutes were chromatographed from the columns listed in Table I. The porous glass packings were treated with hexamethyldisilazane as

TABLE I
Column Characteristics

Column designation	Column packing	Column length, in.	Weight of packing, g	Cumulative pore volume (radius 66,968 → 383 Å), ml
A	Bio-Glas 1000, 100–200 mesh, disilazaned	50.7	41.2	13.20
B	Bio-Glas, experimental, broad pore distribution, 100–200 mesh, disilazaned	64.5	55.37	13.32
C	Bio-Glas 500, 100–200 mesh, disilazaned	96	75.6	4.59
D	Bio-Glas 1000, 100–200 mesh, disilazaned	17.7	14.53	4.65

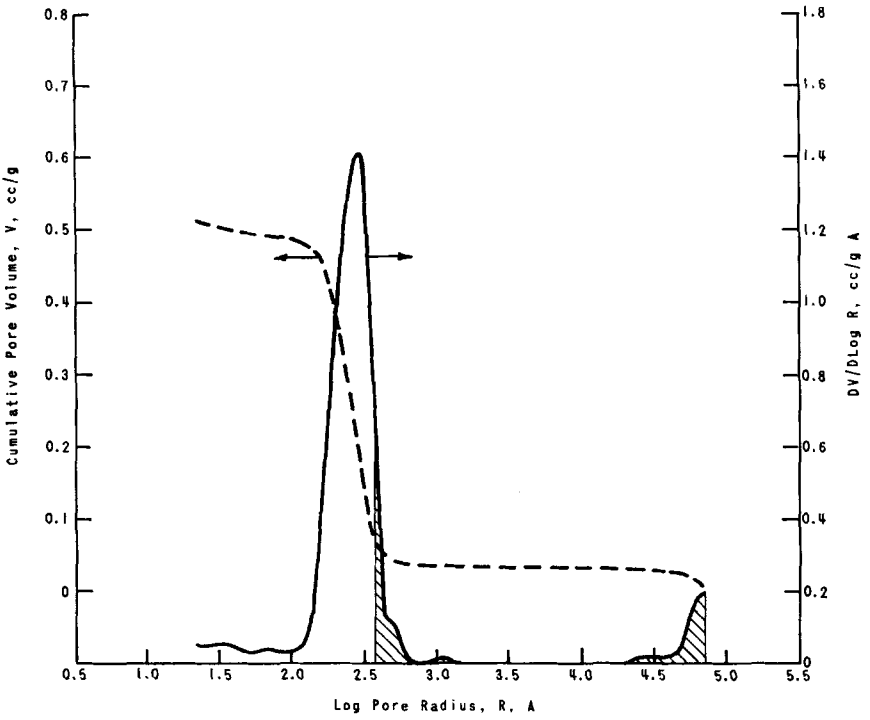

Fig. 1. Characterization of porous glass 500, 100–200 mesh, disilazaned, by mercury porosimetry.

previously described.[12] The eluting solvent was carbon tetrachloride, and the detection system was by infrared absorption monitoring the carbon–hydrogen stretching vibration at 2940 cm^{-1}. Pulseless flow was maintained by a Waters pulseless pump, and injection volumes were 0.56 cc.

Sample sizes were sufficiently small so that there were no overloading effects. The porous glasses (Bio-Rad Laboratories) were characterized by mercury porosimetry, and the pore size distributions are shown in Figures 1 to 3, respectively, for Bio-Glas 500, Bio-Glas 1000, and an experimental broad pore-size-distribution glass, all having identical particle sizes, mesh range 100–200.

The mean end-to-end distance of 160,000-molecular-weight polystyrene in carbon tetrachloride at 25°C was calculated from the equation of Ptitsyn and Eizner[13]:

$$(\bar{h}^2)^{3/2} = \frac{M[\eta]}{\phi(\epsilon)}$$

where $\bar{h}^2$ is the mean square distance between chain ends, M is the molecular weight, η is the intrinsic viscosity in cm^3/g, and $\phi(\epsilon) = 2.86 \times 10^{23}(1 - 2.63\epsilon + 2.86\epsilon^2)$ where $\epsilon = (2a - 1)/3$ and a is the exponent of the Mark-Houwink relationship $[\eta] = kM^a$.

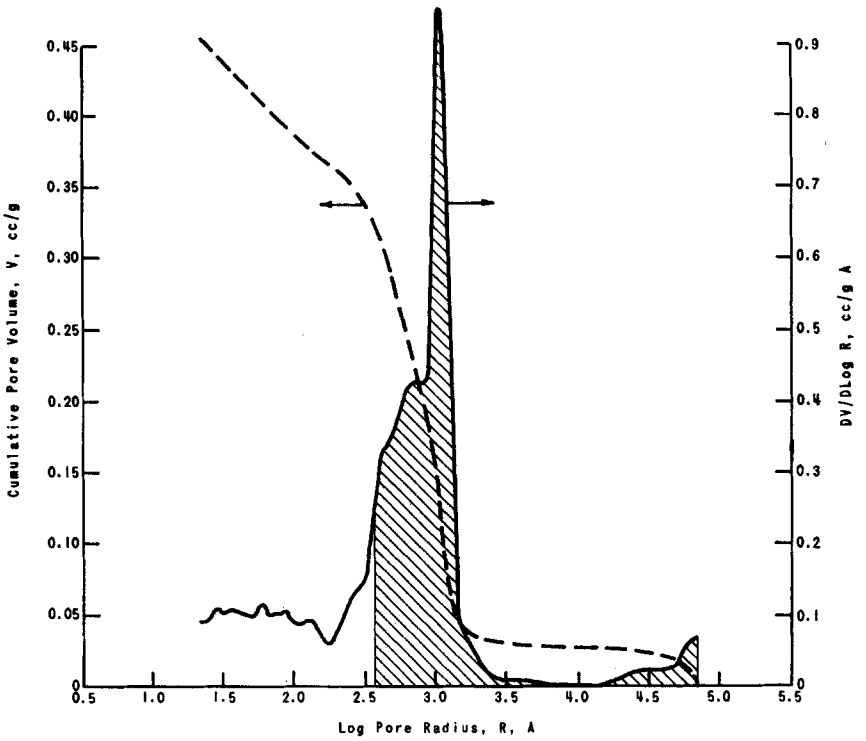

Fig. 2. Characterization of porous glass 1000, 100–200 mesh, disilazaned, by mercury porosimetry.

The plot of η_{sp}/c versus η_{sp}, where η_{sp} is the specific viscosity and c is the concentration in g/100 cc, is shown in Figure 4 for the 160,000- and 97,200-molecular-weight polystyrene standards in carbon tetrachloride at 25°C. From the determination of $[\eta]$ for these two molecular weights only, the Mark-Houwink exponent, a, was found to be 0.75, and $k = 8.06\times10^{-5}$ dl/g. Thus, $\epsilon = 0.167$ and $\phi(\epsilon) = 1.834\times10^{23}$. Hence, the value of $\bar{h}$ for 160,000-molecular-weight polystyrene was calculated to be 383 Å.

The molecular weight of a polyisobutene molecule having the same value of $\bar{h}$ as the 160,000-polystyrene molecule was calculated using the viscosity data of Fox and Flory,[14] $k = 29.0 \times 10^{-5}$, $a = 0.68$, and was found to be 132,430. The fractions available having approximately this molecular weight and a narrow molecular weight distribution had $\bar{M}_v$ values of 114,700 and 145,700.

Previous work[15] had shown that the pore diameter of the column packing had to be approximately twice the coil size for appreciable penetration to occur. Thus, columns C and D were constructed so that they contained the same pore volume, $\simeq$4.6 ml, of pores having radii of 383 Å and larger determined by mercury porosimetry. The shaded portions of the differential pore size distribution curvex, Figures 1 and 2, indicate the pore vol-

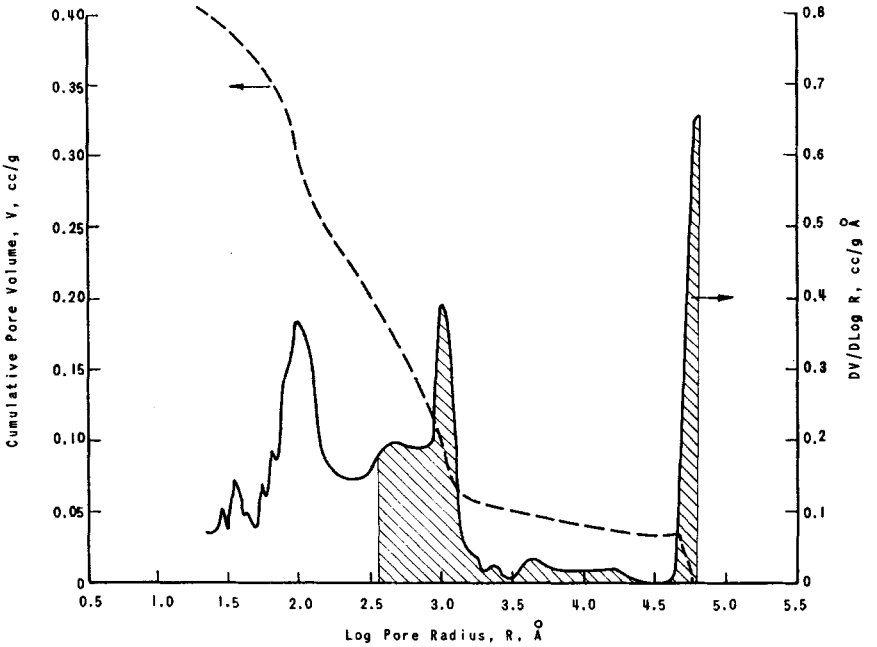

Fig. 3. Characterization of porous glass, broad pore-size distribution, 100–200 mesh, disilazaned, by mercury porosimetry.

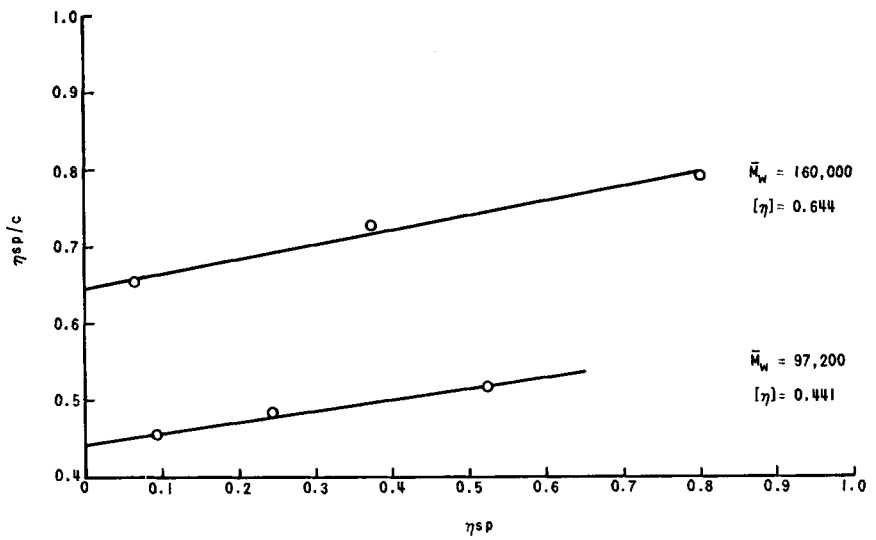

Fig. 4. Determination of intrinsic viscosity of polystyrene of molecular weight 160,000 and 97,200 in carbon tetrachloride at 25°C.

ume available to the 160,000-polystyrene molecule based on this simple model. When comparing columns C and D, it is clear that the chromatographic process takes place on column C, Bio-Glas 500, in a much narrower pore size range than Bio-Glas 1000, column D.

Similarly, columns A and B were constructed to have the same pore volume, $\simeq$13.2 ml, of pores having radii of 383 Å or larger. The shaded portions of differential pore size distribution, Figures 2 and 3, represent the pore volume available to the 160,000-polystyrene molecule. In this case, the pore size range available for the separation is smaller for Bio-Glas 1000, column B, than the board pore size distribution, column A. The results of the comparison of columns A and B are collected in Table II where the elution volumes, V_e, peak width at the base, W_b, and the number of theoretical plates per foot, n_b, are given for the polystyrene having $\bar{M}_w$ = 160,000 and the polyisobutenes having molecular weights of 145,700 and 114,700. The subscript b denotes the width of the peak base between the tangents drawn to the points of inflection of the curve. The relevant equation is

$$n_b = \frac{1}{L}\left(\frac{V_e}{W_b/4}\right)^2.$$

TABLE II
Column Efficiency with Respect to Pore Size Distribution
at a Flow rate of 1 ml/min

Sample (weight injected)	Column[a]	Elution volume V_e, ml	Peak width at base W_b, ml	No. of theoretical plates/ft (n_b)
Toluene	A	42.19	14.00	34.4
(2.71 mg)	B	51.05	8.39	101.2
Polystyrene	A	40.13	15.68	24.8
standard,	B	48.73	10.61	62.8
$\bar{M}_w$ = 900				
(5.6 mg)				
Polystyrene	A	32.93	15.5	17.2
standard,	B	36.59	11.02	32.8
$\bar{M}_w$ = 160,000				
(4.7 mg)				
Polystyrene	A	28.79	14.3	15.3
standard,	B	34.20	13.16	20.2
$\bar{M}_w$ = 860,000				
(4.7 mg)				
Polyisobutene	A	33.8	16.59	15.1
fraction,	B	36.30	12.61	25.1
$\bar{M}_v$ = 114,700				
(3.58 mg)				
Polyisobutene	A	32.4	17.28	13.4
fraction,	B	36.30	12.61	24.6
$\bar{M}_v$ = 145,700				
(3.34 mg)				

[a] See Table I for details.

Also included in this table are similar results for toluene and a low molecular weight polystyrene, $\bar{M}_w$ = 900, to indicate approximately the total liquid volume V_t and the polystyrene standard having $\bar{M}_w$ = 860,000 to indicate the approximate void volume V_0. These latter results show that the molecules of interest are being eluted in the effective separating range of the columns. It is clear that column B is more efficient than column A for the GPC separation of the polymers with $\bar{h}$ = 383 Å.

TABLE III
Column Efficiency with Respect to Pore Size Distribution at a Flow Rate of 1 ml/min

Sample (weight injected)	Column[a]	Elution volume V_e, ml	Peak width at base W_b, ml	No. of theoretical plates/ft (n_b),
Toluene (2.71 mg)	C	80.6	15.63	53.2
	D	16.26	7.00	58.6
Polystyrene standard, $\bar{M}_w$ = 900 (5.6 mg)	C	79.2	18.28	37.6
	D	15.65	8.23	39.2
Polystyrene standard, $\bar{M}_w$ = 160,000 (4.7 mg)	C	57.2	18.21	19.8
	D	12.87	8.44	25.2
Polystyrene standard, $\bar{M}_w$ = 860,000 (4.7 mg)	C	49.4	13.17	28.1
	D	12.56	9.06	20.8
Polyisobutene fraction, $\bar{M}_v$ = 114,700 (3.58 mg)	C	59.1	28.24	8.8
	D	13.69	9.57	22.2
Polyisobutene fraction, $\bar{M}_v$ = 145,700 (3.34 mg)	C	57.0	28.68	7.9
	D	12.87	9.57	19.6

[a] See Table I for details.

This is also true for the other solutes studied. Similar comparisons of the results obtained from columns C and D show a slightly higher plate count for column D with the broader pore size distribution for the GPC of the 160,000-molecular-weight polystyrene.

In order to separate the effects contributing to the peak width in GPC, several approximations can be made for the systems studied here. Since the slopes of the calibration curves are similar for Bio-Glas columns,[16] the peak width, W_{MWD}, due to the discreet molecular weight range in the samples studied (160,000-molecular-weight polystyrene and the two poly-

TABLE IV
Elution Volume and Number of Theoretical Plates for 160,000 Molecular-Weight Polystyrene Eluted from Treated Bio-Glas Columns at Various Flow Rates[a]

Column	Flow rate, ml/min	Elution volume V_e, ml	No. of theoretical plates/ft, (n_b)
Bio-Glas 1000			
Column A	1.02	34.68	28.5
	0.42	34.71	36.8
Broad Pore-Size Distribution			
Column B	1.06	39.52	51.0
	0.39	39.50	66.4

[a] The elution volumes and number of theoretical plates are slightly different from those in Table II because these were determined in a different instrument.

siobutenes) is constant for each compound. The broadening due to the chromatographic process, W_{GPC}, is given by

$$W^2_{TOTAL} = W^2_{MWD} + W^2_{GPC} + W^2_{INST}$$

where W_{INST} is the broadening due to the injection system, connecting tubing and the detector system. Thus, W^2_{TOTAL} for columns A and B or C and D reflects the value of W^2_{GPC} for these solutes under investigation.

If it is assumed that the broadening per foot due to flow through the interstitial volume is the same for all columns (in agreement with data in Tables II and III), then the number of plates per foot observed shows that in comparing columns A and B it is not the column with the largest pore volume per foot (column A) which is the most efficient. Comparing columns C and D, the opposite effect is observed. The differences between the number of theoretical plates per foot of these columns are not very large. In the case of columns C and D, the difference may be due to packing geometry differences. The difference between columns A and B is greater; column B, with the broader pore size distribution, is the more efficient column. Table IV shows the results obtained by slowing the flow rate from approximately 1.0 ml/min to 0.4 ml/min. The increase in the number of theoretical plates increases by about 30% for both columns. This is somewhat less than the increase, $\simeq$50%, found[17] for a similar reduction in flow rate for the elution of 160,000-molecular-weight polystyrene from a polystyrene gel column.

The elution volumes (Table IV) for the 160,000-molecular-weight polystyrene solute are independent of flow rate, for each column, at 1 ml/min and less. This is also true for the Bio-Glas 500 column where there is very little pore volume, with the pore radius equal to or larger than the mean end-to-end distance.

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