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EFFECTS OF COLUMN COMBINATIONS ON THE MEASUREMENT OF AVERAGE MOLECULAR WEIGHTS AND THE MOLECULAR-WEIGHT DISTRIBUTION IN HIGH-PERFORMANCE SIZE-EXCLUSION CHROMATOGRAPHY

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SUMMARY

Average molecular weights and the integral molecular weight distribution curves for polystyrene NBS 706, NBS 705 and one broad polystyrene measured using different column combinations have been compared. The average molecular weights were not affected by the column combination, but the integral molecular weight distribution curves showed definite differences among several column sets. The most probable integral distribution curve will be obtained from a column packed with mixed gels of different porosity. A column combination without a gap in porosity or with many different gel porosities is preferable. The column set should have an exclusion limit of about ten times the weight-average molecular weight for the polymer sample.

INTRODUCTION

Size-exclusion chromatography (SEC) (or gel permeation chromatography, GPC) is widely used for measuring average molecular weights and the molecular-weight distribution (MWD) of polymers. In an SEC analysis the elution volume can be related to the molecular weight of the sample by calibrating the column system with polymer samples of known molecular weights followed by calculation of average molecular weights from the size exclusion chromatogram. For precise SEC measurement, the operational variables must be controlled strictly[1] and corrections for concentration effects[2] and peak broadening may be applied if necessary.

The column efficiency in high-performance liquid chromatography is expressed in terms of the number of theoretical plates per unit length, which increases on reducing the flow-rate and the injection volume of a sample solution[1]. However, the number of theoretical plates is a measure of the condition of packing the material into the column, but not the precision or accuracy of molecular weight measurements. For a comparison of column performance in polymer analysis by SEC the ratio of weight-average to number-average molecular weight[3] ($\bar{M}_w/\bar{M}_n$) for solutes with a narrow MWD, and/or the packing resolution factor[4], which is a function of the standard

deviation of the peak and the slope of the linear portion of the calibration graph for the column system, are recommended instead of the number of theoretical plates.

Column performance, operational variables and treatment of the data obtained from chromatograms have been studied in detail by several workers, but the effect of column combinations on the molecular weight and MWD has received scant attention. Ambler *et al.*[5] found that the use of sufficiently long columns and reduced flow-rates resulted in the determination of accurate molecular weights of both narrow and broad MWD samples directly from the chromatogram without the need for peak-spreading corrections and that gapped column sets were detrimental to the resolution of molecular species. Yau *et al.*[4] proposed that the relative errors between molecular weights calculated from the experimental chromatogram and those determined from the infinitely resolved or theoretical chromatogram were measures of the performance of the separating system. The relative errors are related to the term of $e^{1/2(\sigma D_2)^2}$ and increase with increase σ and/or D_2, where σ is the standard deviation of the peak caused by column dispersion and D_2 is the slope of the straight-line portion of the calibration graph. The smaller is σD_2, the higher are the resolution and accuracy of the molecular weight. It is preferable to select a column combination that has a small value of D_2 and a high value of the number of theoretical plates (small σ).

In SEC, two to four short columns, each of which has a different porosity range, are usually connected in series and it was the purpose of this work to establish the optimal combination of these columns. The effects of a continuous transition or gaps in the gel porosity were considered by linking the available GPC columns. Average molecular weights and the integral molecular weight distribution curves were determined on different column sets of the same length and correlated with the slopes or the shapes of the calibration graphs.

EXPERIMENTAL

A JASCO (Japan Spectroscopic Co., Hachioji, Tokyo, Japan) Trirotar high-performance liquid chromatograph was used with a Shodex Model SE-11 differential refractometer (Showa Denko, Minato-ku, Tokyo, Japan) and a JASCO Model VL-611 variable loop injector. Shodex A803, A804, A805 and A806 high-performance GPC columns of dimensions 500 × 8 mm I.D. were used. Polystyrene gels were packed in the columns and their nominal porosities were 10^3, 10^4, 10^5 and 10^6 Å. A Shodex A80M GPC column (500 × 8 mm I.D.) was also used, which was so-called a linear column packed with a mixture of A803–A806 polystyrene gels. The numbers of theoretical plates of these columns were found to be between 30,000 and 36,000 per metre by injection of 10 μl of a 4% benzene solution. These GPC columns were provided by courtesy of Hikari Kogyo (Chuo-ku, Tokyo, Japan) and Showa Denko. Two or four columns were combined as follows: set I, A806 + A805 + A804 + A803; set II, A80M + A80M (where M represents a mixture of gels); set III, A806 + A805; set IV, A806 + A804; set V, A806 + A803; set VI, A805 + A803; set VII, A804 + A803.

The polymers used as test samples were standard NBS 706 and NBS 705 polystyrenes and a commercial polystyrene with a broad MWD (PS C). Polymer standards for calibration were narrow MWD polystyrenes purchased from Pressure Chemical Co. (Pittsburgh, Pa., U.S.A.). The polymers were injected as 0.1% solutions

by a variable loop injector with a 0.25-ml loop. The flow-rate of tetrahydrofuran was 1.0 ml/min. The attenuation of the detector was ×8.

RESULTS

Calibration graphs for Shodex A803–A806 GPC columns are shown in Fig. 1, and those of combined columns in Figs. 2 and 3. Table I gives the slopes of the straight-line portions of the calibration graphs. The slope of a tangent was substituted for the slope of the curved portion. The values of the slopes were calculated from the equation

$$D_2 = \frac{2.3 \log(M_1/M_2)}{V_2 - V_1} \quad (1)$$

where D_2 is the slope, M the molecular weight and V the elution volume.

Average molecular weights and the polydispersity of test samples obtained from sets I to VII are listed in Table II. The relative deviations of the values were within 3%. The integral molecular weight distribution curves of NBS 706 polystyrene

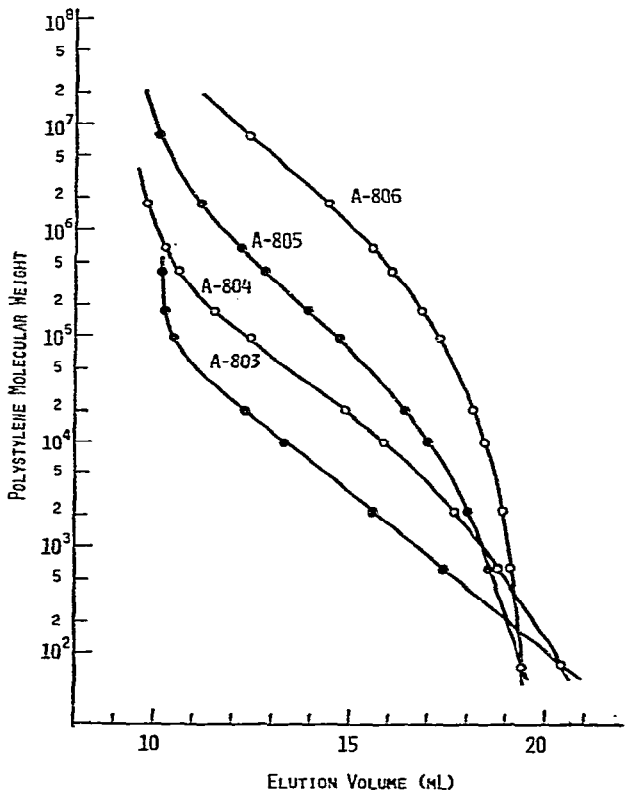

Fig. 1. Calibration graphs for Shodex GPC columns A803–A806. Column, 50 × 0.8 cm; solute, polystyrenes; mobile phase, tetrahydrofuran; flow-rate, 1.0 ml/min; sample injected, 0.25 ml; concentration, 0.1% (w/v).

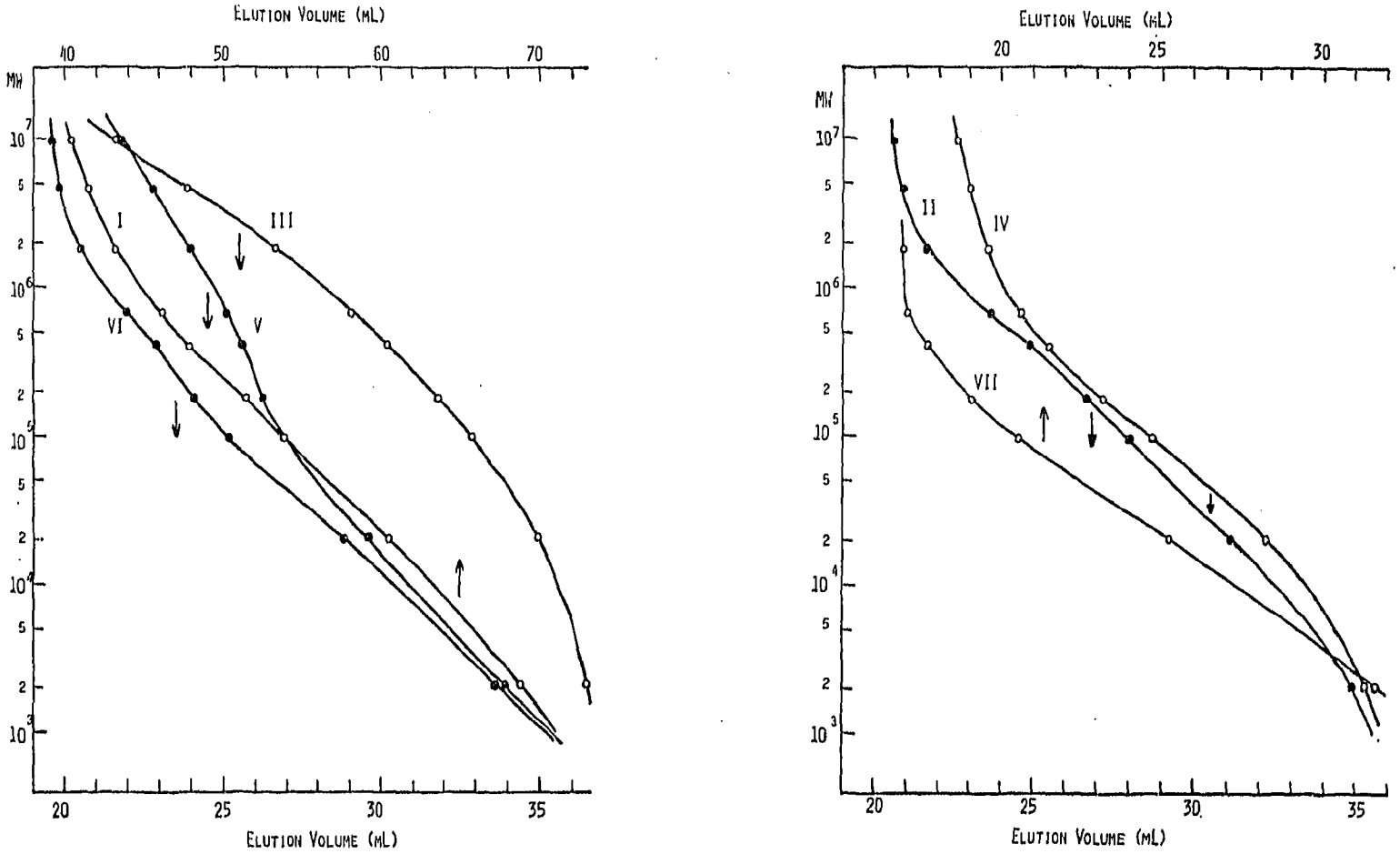

Fig. 2. Calibration graphs for several combination sets of Shodex GPC columns. Set I, A806 + A805 + A804 + A803; set III, A806 + A805; set V, A806 + A803; set VI, A805 + A803. Conditions as in Fig. 1.

Fig. 3. Calibration graphs for several combination sets of Shodex GPC columns. Set II, A80M + A80M; set IV, A806 + A804; set VII, A804 + A803. Conditions as in Fig. 1.

TABLE I

SLOPES OF THE STRAIGHT-LINE PORTIONS OF THE CALIBRATION GRAPHS

Parameter	*Column set*						
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*
Molecular weight of linear portion	$3 \cdot 10^5$ ~ $1 \cdot 10^6$	$1 \cdot 10^6$ ~ $2 \cdot 10^6$	(a) $1 \cdot 10^6$ ~ $1 \cdot 10^6$ (b) $1 \cdot 10^5$ ~ $1 \cdot 10^6$	$1 \cdot 10^6$ ~ $6 \cdot 10^5$	(a) $6 \cdot 10^5$ ~ $1 \cdot 10^5$ (b) $1 \cdot 10^5$ ~ $6 \cdot 10^5$ (c) ~ $1 \cdot 10^5$	(a) $1 \cdot 10^5$ ~ $2 \cdot 10^6$ (b) ~ $1 \cdot 10^5$	~ $2 \cdot 10^5$
Slope	−0.24	−0.48	(a) −0.34 (b) −0.49	−0.42	(a) −0.80 (b) −1.05 (c) −0.36	(a) −0.59 (b) −0.48	−0.34
σD_z*	0.135	0.170	0.221	0.185	0.135 (0.393)	0.185	0.131
Slope at curve:							
MW = $3 \cdot 10^6$	−1.05	−1.10		−1.0		−1.27	∞
$1 \cdot 10^6$				−1.0			∞
$5 \cdot 10^5$							−0.74
$5 \cdot 10^4$			−0.75				
$1 \cdot 10^4$			−1.12				
$5 \cdot 10^3$		−0.68	−1.62	−0.81			
$1 \cdot 10^3$		−0.99	−2.77	−1.54			

* σ measured with 97,200 MW polystyrene.

TABLE II

CALCULATED VALUES OF AVERAGE MOLECULAR WEIGHTS AND THE POLYDISPERSITY ($\bar{M}_w/\bar{M}_n$) OF POLYSTYRENE STANDARDS FROM THE CHROMATOGRAMS USING SEVERAL COLUMN COMBINATIONS

Set	*Parameter*	*Nominal MW*		
		20,400	*97,200*	*411,000*
Manufacturer's value	$\bar{M}_w$	20,800	97,200	394,000
	$\bar{M}_n$	20,200	96,100	392,000
	$d\ (=\bar{M}_w/\bar{M}_n)$	1.06	1.01	1.005
Set I	$\bar{M}_w$	$2.1 \cdot 10^4$	$9.32 \cdot 10^4$	$4.10 \cdot 10^5$
	$\bar{M}_n$	$2.03 \cdot 10^4$	$9.09 \cdot 10^4$	$3.98 \cdot 10^5$
	d	1.03	1.025	1.03
Set II	$\bar{M}_w$	$2.02 \cdot 10^4$	$9.70 \cdot 10^4$	$4.07 \cdot 10^5$
	$\bar{M}_n$	$1.94 \cdot 10^4$	$9.40 \cdot 10^4$	$3.93 \cdot 10^5$
	d	1.04	1.03	1.035
Set III	$\bar{M}_w$	$1.82 \cdot 10^4$	$9.26 \cdot 10^4$	$4.18 \cdot 10^5$
	$\bar{M}_n$	$1.46 \cdot 10^4$	$8.38 \cdot 10^4$	$3.89 \cdot 10^5$
	d	1.25	1.105	1.07
Set IV	$\bar{M}_w$	$2.02 \cdot 10^4$	$9.89 \cdot 10^4$	$3.91 \cdot 10^5$
	$\bar{M}_n$	$1.93 \cdot 10^4$	$9.61 \cdot 10^4$	$3.52 \cdot 10^5$
	d	1.05	1.03	1.11
Set V	$\bar{M}_w$	$2.09 \cdot 10^4$	$9.75 \cdot 10^4$	$3.69 \cdot 10^5$
	$\bar{M}_n$	$2.02 \cdot 10^4$	$9.00 \cdot 10^4$	$3.34 \cdot 10^5$
	d	1.03	1.08	1.105
Set VI	$\bar{M}_w$	$2.11 \cdot 10^4$	$9.72 \cdot 10^4$	$3.80 \cdot 10^5$
	$\bar{M}_n$	$2.02 \cdot 10^4$	$9.35 \cdot 10^4$	$3.50 \cdot 10^5$
	d	1.04	1.04	1.085
Set VII	$\bar{M}_w$	$2.02 \cdot 10^4$	$9.64 \cdot 10^4$	$3.96 \cdot 10^5$
	$\bar{M}_n$	$1.98 \cdot 10^4$	$9.47 \cdot 10^4$	$3.66 \cdot 10^5$
	d	1.02	1.02	1.08

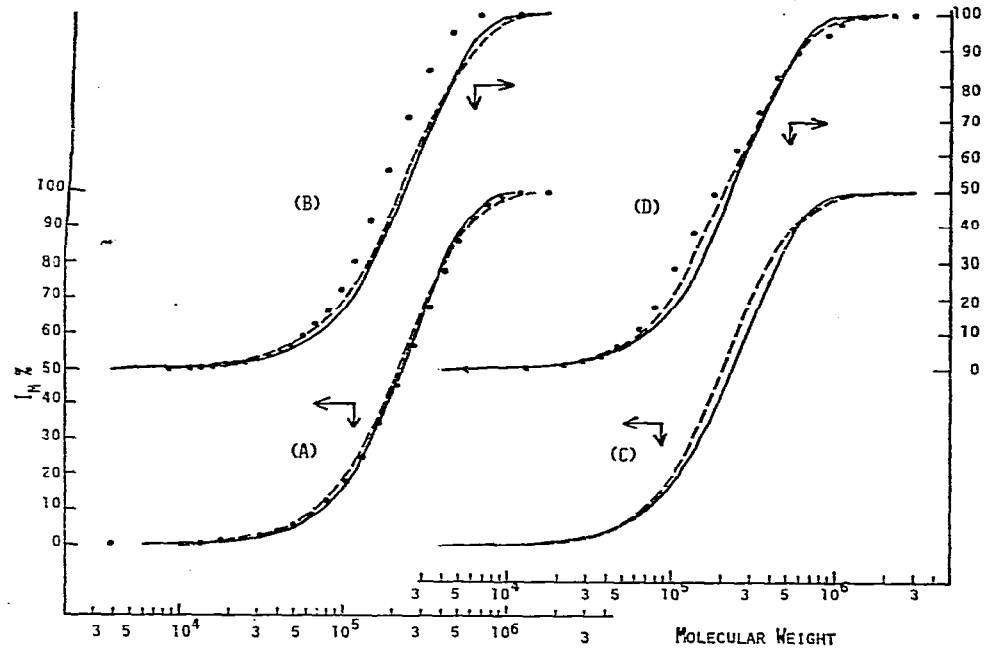

Fig. 4. Normalized integral molecular weight distribution curves for NBS 706. (A) (———) calculated from the data of Kato *et al.*[7], (— — —) set I, (. . . . .) set II; (B) (———) set II, (— — —) set III, (. . . . .) set VII· (C) (———) set II, (— — —) set IV; (D) (———) set II, (— — —) set VI, (. . . . .) set V.

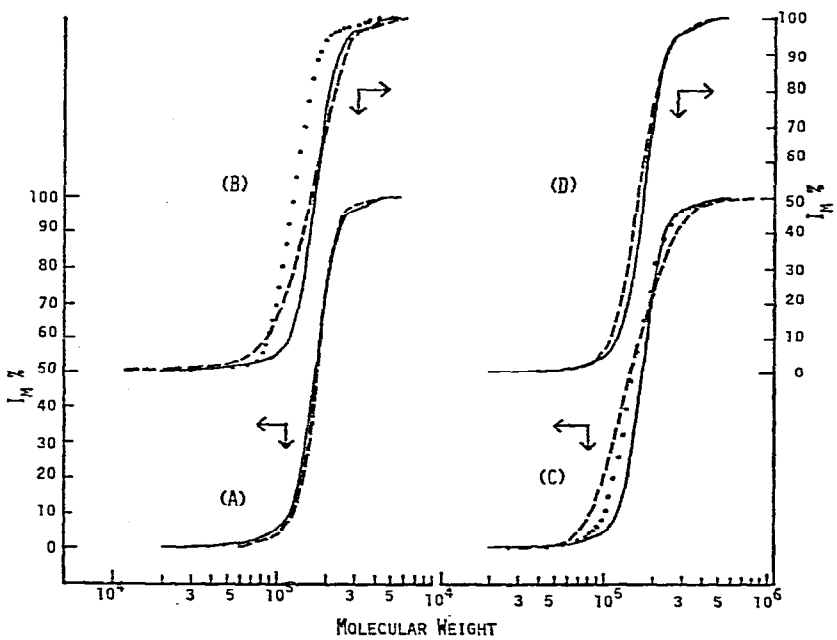

Fig. 5. Normalized integral molecular weight distribution curves for NBS 705. (A) (———) set II, (— — —) set I; (B) (———) set II, (— — —) set III, (. . . . .) set VII; (C) (———) set II, (— — —) set V, (. . . . .) set VI; (D) (———) set II, (— — —) set IV.

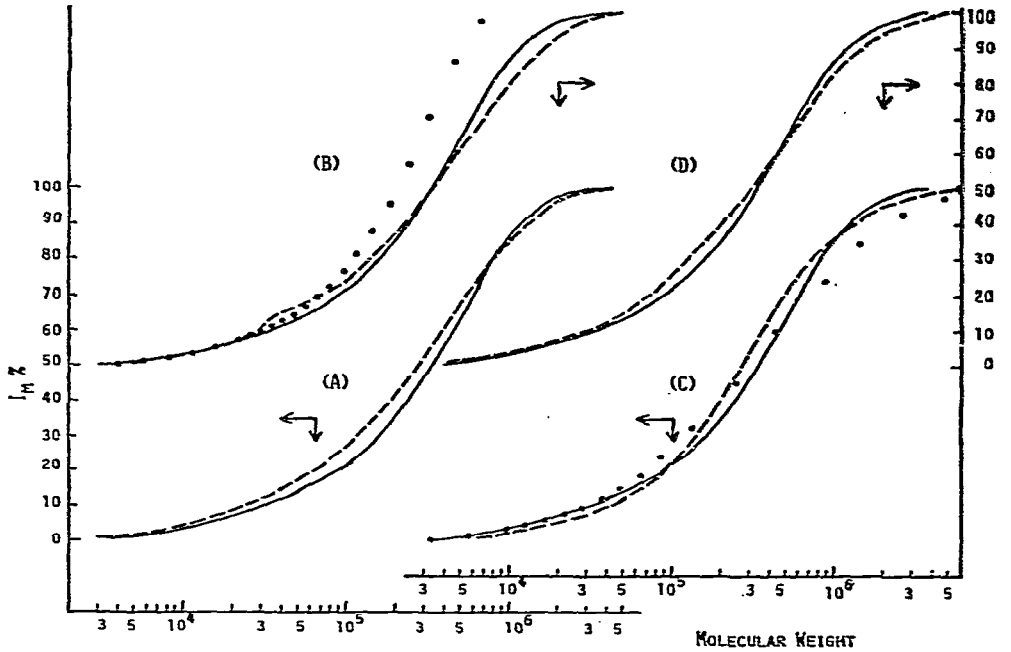

Fig. 6. Normalized integral molecular weight distribution curves for PS C. (A) (———) set II, (— — —) set I; (B) (———) set II, (— — —) set III, (. . . .) set VII; (C) (———) set II, (— — —) set IV, (. . . .) set V; (D) (———) set II, (— — —) set VI.

are shown in Fig. 4. The normalized integral molecular weight distribution curve is defined by the equation

$$I_M = \int_0^M m_M \, dM \tag{2}$$

where m_M is a mass distribution function which expresses the amount of a species having a molecular weight M in 1 g of the sample. In practice, the distribution curve is calculated from eqn. 3 and is expressed as a percentage:

$$I_{M_i} = C_{i-1} + (C_i - C_{i-1}) \cdot 1/2 \tag{3}$$

where C_i is the cumulative weight percent of the chromatogram at the point i. The chromatogram is divided into the n equal portions. C_i is larger than C_{i-1}.

The integral molecular weight distribution curves of NBS 705 and PS C are shown in Figs. 5 and 6, respectively. Table III lists the average molecular weights and the polydispersities determined from the experimental chromatograms for the three polystyrene calibration standards.

DISCUSSION

Column combinations and calibration graphs

The familiar S-shaped and linear calibration graps were obtained for the com-

TABLE III

AVERAGES MOLECULAR WEIGHTS MEASURED ON SEVERAL COLUMN COMBINATIONS

Set	*Parameter*	*NBS 706*	*NBS 705*	*PS C*
NBS values	$\bar{M}_w$	$2.58 \cdot 10^5$, $2.88 \cdot 10^5$	$1.79 \cdot 10^5$	
	$\bar{M}_n$	$1.37 \cdot 10^5$	$1.67 \cdot 10^5$	
	$d\ (=\bar{M}_w/\bar{M}_n)$	1.9, 2.1	1.07	
Set I	$\bar{M}_w$	$2.70 \cdot 10^5$	$1.80 \cdot 10^5$	$4.91 \cdot 10^5$
	$\bar{M}_n$	$1.38 \cdot 10^5$	$1.66 \cdot 10^5$	$0.61 \cdot 10^5$
	d	2.08	1.08	8.05
Set II	$\bar{M}_w$	$2.68 \cdot 10^5$	$1.77 \cdot 10^5$	$5.15 \cdot 10^5$
	$\bar{M}_n$	$1.37 \cdot 10^5$	$1.64 \cdot 10^5$	$0.69 \cdot 10^5$
	d	1.96	1.08	7.47
Set III	$\bar{M}_w$	$2.78 \cdot 10^5$	$1.71 \cdot 10^5$	$6.20 \cdot 10^5$
	$\bar{M}_n$	$1.32 \cdot 10^5$	$1.36 \cdot 10^5$	$0.65 \cdot 10^5$
	d	2.11	1.26	9.54
Set IV	$\bar{M}_w$	$2.59 \cdot 10^5$	$1.71 \cdot 10^5$	$5.26 \cdot 10^5$
	$\bar{M}_n$	$1.36 \cdot 10^5$	$1.48 \cdot 10^5$	$0.76 \cdot 10^5$
	d	1.90	1.16	6.89
Set V	$\bar{M}_w$	$2.72 \cdot 10^5$	$1.65 \cdot 10^5$	$7.98 \cdot 10^5$
	$\bar{M}_n$	$1.25 \cdot 10^5$	$1.33 \cdot 10^5$	$0.64 \cdot 10^5$
	d	2.18	1.24	12.43
Set VI	$\bar{M}_w$	$2.73 \cdot 10^5$	$1.64 \cdot 10^5$	$5.66 \cdot 10^5$
	$\bar{M}_n$	$1.30 \cdot 10^5$	$1.42 \cdot 10^5$	$0.62 \cdot 10^5$
	d	2.10	1.15	9.13
Set VII	$\bar{M}_w$	$1.98 \cdot 10^5$	$1.33 \cdot 10^5$	$2.70 \cdot 10^5$
	$\bar{M}_n$	$1.15 \cdot 10^5$	$1.20 \cdot 10^5$	$0.65 \cdot 10^5$
	d	1.72	1.11	4.18

bined column sets except for set V. A satisfactory calibration graph should have a long straight portion and a small absolute value of the slope. As D_2 is proportional to the reciprocal of the column length L, and σ to the square root of L, then D_2 and σ of set I will be 0.5 and $\sqrt{2}$, respectively, times those of set II. Then, the value of σD_2 for set I, which is a measure of the accuracy, will become $1/\sqrt{2}$ times that for set II. From the standpoint of the slope, the relative errors for sets III, IV and VII must be smaller than those for set II. However, values of the polydispersity determined from the experimental chromatograms for the polystyrene calibration standards of nominal molecular weights, 20,400, 97,200 and 411,000 indicate that the column efficiency for sets I and II is better than others (Table II). Set III has the worst values, but would have a better column efficiency for polymers with molecular weights greater than 10^6. Similarly, the set VII would have better efficiency for polymers smaller than 2×10^5 molecular weight.

It has been proposed[3] that the polydispersity for the polystyrene calibration standards is preferable to the number of theoretical plates as a measure of the column efficiency. The value of the polydispersity calculated from the chromatogram includes a factor for the range of molecular weight fractionation, which is related to the value of D_2, and is more practical than the number of theoretical plates. The results in Table II

explain the column efficiency for the three polystyrene calibration standards. For a given pair of molecular weights for polystyrene 20,400, all but set III predict the same column efficiency. For polystyrene 97,200, sets I, II, IV, VI and VII have the same efficiency. For polystyrene 411,000, only sets I and II show good efficiency. The combined use of the values in Tables I and II is advantageous for judging the column efficiency. The available fractionation range of each column set can be said to be in the portion of the calibration graph where the absolute value of D_2 is less than 1.2.

Column combinations and measured average molecular weights

The average molecular weights determined from the experimental chromatograms for NBS 706 were similar, except for those from set VII, as shown in Table III. The same results were obtained for NBS 705 and PS C. The results on set VII for these polymer samples were different from those for the calibration standards shown in Table II. The values for PS C on set VII were half and those for NBS 706 two thirds those obtained on the other sets. The fractionation range of set VII lies below molecular weight $7 \cdot 10^5$, but these polymer samples includes species with molecular weights greater than $7 \cdot 10^5$. The decrease in molecular weight for NBS 705 was 25% and that for the calibration standards was negligible. Polymers with a broader MWD require a column combination with a wider fractionation range. Empirically it can be said that the column set should have an exclusion limit about ten times the weight-average molecular weight for the polymer sample. Polymers with a narrower MWD require a more efficient column combination with a low value of σD_2. It should be emphasized that polymers with a broader MWD, such as with polydispersity 1.5 and above, need a column set with a wider fractionation range rather than an efficient column set.

Average molecular weight versus integral MWD curves

To give the true differential MWD curved, the ordinate of the chromatogram has to be changed from the weight fraction per retention volume to weight fraction per log (molecular weight) increment according to the equation[6]

$$\frac{dW}{d(\log M)} = \frac{dW}{dV}\left[\frac{dV}{d(\log M)}\right] \tag{4}$$

However, the integral molecular weight distribution (IMWD) curve can be obtained from the chromatogram without any conversion, by transposing the elution volume scale to a molecular weight scale by using the calibration graph, because the IMWD is given by the equation

$$\int \frac{dW}{d(\log M)} \cdot d(\log M) = \int \frac{dW}{dV}\left[\frac{dV}{d(\log M)}\right] d(\log M)$$

$$= \int \frac{dW}{dV} \cdot dV \tag{5}$$

The IMWD curve is obtained by dividing the chromatogram into equal portions and by calculating I_M from eqn. 3 followed by plotting I_{M_i} on the ordinate

and M_i at an elution volume V_i on the abscissa. The value of C_i is often substituted for I_{M_i}, particularly in SEC, but it should be emphasized that thus substitution is incorrect, for the following reason:

$$\sum_{i=1}^{i=j} C_i = \sum_{i=1}^{i=j} h_i / \sum_{i=1}^{i=n} h_i \tag{6}$$

$$\sum_{i=j}^{i=n} C_i = \sum_{i=j}^{i=n} h_i / \sum_{i=1}^{i=n} h_i \tag{7}$$

The value of eqn. 6 must be equal to 1 minus eqn. 7, which is expressed by eqn. 8:

$$1 - \frac{\sum_{i=j}^{i=n} h_i}{\sum_{i=1}^{i=n} h_i} = \sum_{i=1}^{i=j-1} h_i / \sum_{i=1}^{i=n} h_i \tag{8}$$

However, eqn. 6 is not equal to eqn. 8. Therefore, the IMWD curve calculated from the lowest molecular weight end is different from that calculated from the highest molecular weight end, unless n is very large.

The differences between average molecular weights calculated from the chromatograms obtained on column sets I–VI are small, but their IMWD curves differ considerably. The IMWD curves for NBS 706 polystyrene are shown in Fig. 4. The IMWD curve from the data for preparative SEC in the literature[7] (Fig. 4, A, solid line) can be regarded as the real IMWD curve, on which the IMWD curves from set I (A, broken line) and set II (dotted line) lie. This result indicates that these two sets are adequate for the fractionation of NBS 706 polystyrene. Hence, the curve from set II was employed as a criterion for the comparison of the performances of other column sets.

The IMWD curve from set III lies on the curve from set II, although the total permeation limit is in the higher molecular weight region. By comparison of the IMWD curves from other column sets, especially sets III and VII, with that from set II it can be concluded that the exclusion limit must be taken into account in preference to the total permeation. The IMWD curves from sets IV to VI differ substantially from that from set II in spite of the very small difference in the average molecular weights calculated from the chromatograms. These column sets create "gaps" in the gel porosity, that is, a continuous transition of the gel packing is not achieved by linking two columns. Closer examination of the IMWD curves from sets I and II shows that the latter is nearer than the former to the real IMWD, which indicates that columns packed with gels with a wide range of gel porosity distributed at random is more effective than a multi-column set.

The IMWD curves for NBS 705 are compared with the curve from set II and shown in Fig. 5. The IMWD from set I, which links four columns with different gel porosities, is different to that from set II, which consists of two columns with a wide porosity range, as already discussed. Most of molecular species in NBS 705 are present in the fractionation range of set VII, that is, few are above the exclusion limit and below the total permeation of set VII. However, the IMWD curve from set VII is

completely different from the others in addition to small values of measured average molecular weights. These results imply that the exclusion limit of the preferred column set should be far above the highest molecular weight species in the sample. The IMWD curves from other sets differ from that from set II and it concluded that the most satisfactory column combination for NBS 705 is set II. Polystyrene NBS 705 includes a high-molecular-weight component, and the presence of this component was clearly indicated on the chromatogram obtained by decreasing the flow-rate to 0.25 ml/min[5,8]. This bimodal distribution is indicated clearly on the chromatogram obtained for the Shodex GPC column sets except sets V and VII, even at a flow-rate of 1.0 ml/min, suggesting a high performance of these columns.

Fig. 6 shows examples for the sample PS C. Considerable differences among the IMWD curves were observed. Sets I and II have the same gel porosity range, but the former set consists of four columns with different gel porosities and the latter set two columns with a mixture of gels. Shodex GPC column A80M contains mixed gels with a wide porosity range and acts as a general-purpose column set. This column is useful for the fractionation of polymers with either a broad or a narrow MWD.

In conclusion, none of the column sets gives the same IMWD curves. The most probable IMWD curve is obtained with set II. Set I consists of four columns with different gel porosities and sets III to VII have two columns. In order to obtain a reliable IMWD curve, firstly, many columns with different gel porosities, especially columns packed with mixed gels with a wide gel porosity range, should be used. A long column is not necessary. Secondly, the average molecular weight at the exclusion limit of the column set should be about ten times the molecular weight of the measured polymer. The results are affected mostly by the exclusion limit rather than the total permeation limit.

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