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EFFECT OF COLUMN PERFORMANCE ON THE ACCURACY OF MOLECULAR WEIGHTS OBTAINED FROM SIZE EXCLUSION CHROMATOGRAPHY (GEL PERMEATION CHROMATOGRAPHY)

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SUMMARY

This paper presents a functional molecular weight accuracy criterion for gel permeation chromatography column performance that connects the traditional concepts of column resolution with molecular weight accuracy in gel permeation chromatographic measurements.

The criterion predicts accuracy of molecular weights directly from column parameters; conversely it can be used to specify needed column parameters to obtain a selected level of gel permeation chromatography-molecular weight accuracy.

According to this accuracy criterion, the performance of the columns can be quantitatively rated in terms of the product of two fundamental column parameters: σ, the standard deviation of the peak (related to the column plate count for a monodispersed polymer), and D_2, related to the slope of the linear portion of the chromatographic calibration curve. Use of this σD_2 criterion is illustrated with data obtained on different types of column packings and various sets of columns. The effects of pore geometry and column operating variables on the column resolution are discussed in terms of separation and dispersion theories.

A quantitative theory for interpreting the effect of packing pore size distribution on gel permeation chromatographic performance was developed. The theory indicates that the effect on D_2 diminishes at narrow pore size distribution.

INTRODUCTION

Traditionally chromatographic column performance has been expressed in terms of number of theoretical plates (N), plate height (H) or, in the separation of mixtures, by column resolution, R_s[1,2]. The relationships describing these performance parameters are:

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

$$R_s = \frac{2(V_{R_2} - V_{R_1})}{(W_1 + W_2)} \quad (2)$$

where V_R is the peak retention volume, and W the chromatogram peak width formed by intersection of the tangents to the curve inflection points with the baseline in units of retention volume. The subscripts refer to solutes 1 and 2, respectively. The plate height H (or HETP, height equivalent to a theoretical plate) $= L/N$, where L is the column length. The R_s and N values obtained in this way are dependent on the columns used, the manner in which they are operated, and on the probe solutes chosen to test the performance.

These equations describing column performance also are used in size exclusion or gel permeation chromatography (GPC). However, in GPC polymer analysis an opportunity exists to eliminate the dependence of the resolution expression on the test or polymeric probe samples. It has been shown that in GPC[3] for the linear region of the calibration curve (log MW *versus* V_R), R_s can be normalized as expressed in eqn. 3.

$$R_{sp} = \left[\frac{2\,(V_{R_2} - V_{R_1})}{(W_1 + W_2)}\right] \cdot \left[\frac{1}{\log_{10}(MW_1/MW_2)}\right] \tag{3}$$

In this performance expression, the specific resolution factor R_{sp} is independent of the sample. The symbols in this expression are the same as above with the addition that MW is the known molecular weight for a polymer standard.

Since the width of the chromatographic peak W is affected by the sample molecular weight distribution (MWD), the specific resolution described in eqn. 3 only is sample independent if the probe samples have very narrow MWD. Another derivation and form of the specific resolution equation is given in this paper which promotes the usefulness and the understanding of the resolution concept in GPC.

Column plate count often is the only value reported by many workers or vendors for GPC to indicate expected or observed performance. When obtained for monomeric species such as toluene, this value is inappropriate[4], and as the present work shows, can lead to erroneous conclusions. We suspect that the continued use of plate count as a measure of GPC column performance is based on tradition and the ease with which the values can be obtained. This paper develops an easily applied criterion for column performance which is recommended for general use in GPC. A new form for the specific resolution factor R_{sp} and a new column performance parameter, the molecular weight accuracy $\bar{M}^*$, are given below. A theoretical treatment of the effect of packing pore geometry on GPC resolution is presented later in the paper.

Resolution

Eqn. 2 also may be written as,

$$R_s = \frac{V_{R_2} - V_{R_1}}{2\,(\sigma_1 + \sigma_2)} \approx \frac{\Delta V_R}{4\sigma} \tag{4}$$

where σ is the peak standard deviation (in ml or counts) caused by column dispersion. To a first approximation, $\sigma_1 = \sigma_2$ and is measured as the experimental σ of a very narrow MWD polystyrene standard (see Experimental). Now, the calibration relationship between molecular weight as a function of retention volume can be expressed by

$$M_T(V_R) = D_1\, e^{-D_2 V_R} \tag{5}$$

where D_1 is related to the projected intercept, and D_2 the slope, of the straight line portion of the calibration[5,6]. For this and succeeding equations the numerical value of D_2 is a positive quantity for all GPC experiments. By solving the calibration equation for V_R and substituting in the above eqn. 4, one obtains the usual chromatographic resolution R_s,

$$R_s = \frac{1}{2\,D_2\,(\sigma_1 + \sigma_2)} \ln\left(\frac{M_1}{M_2}\right) = \frac{\Delta \ln M}{4\,D_2\,\sigma} \tag{6}$$

This equation describes how well the GPC column distinguishes between two molecules of the same polymer differing by a molecular weight factor MW_1/MW_2 (or M_1/M_2). Dividing eqn. 6 by log (M_1/M_2) leads directly to the expression for specific resolution:

$$R_{sp} = \frac{\Delta \ln M}{4\,D_2\,\sigma\,\Delta \log M} = \frac{2.303\,\Delta \log M}{4\,\sigma\,D_2\,\Delta \log M} = \frac{0.576}{D_2\,\sigma} \tag{7}$$

The parameter R_{sp} in eqn. 7 is functionally the same as in eqn. 3 but easier to use and visualize. Specifically, eqn. 7 states that the resolution factor R_{sp} in the linear region of calibration is the usual chromatographic resolution R_s for a pair of peaks having a decade of MW difference. In actual practice any two samples of differing MW can be used in eqn. 7 to obtain R_{sp}.

To provide a resolution factor for comparison of column packings the expression must be compensated for the length of the columns. Since D_2 is proportional to the reciprocal of the column length L, and σ is proportional to $\sqrt{L}$, eqn. 7 can be expanded to give R_{sp} normalized for column length. The packing resolution factor R_{sp}^* is equivalent to R_{sp} for a 1-cm column as shown by,

$$R_{sp}^* = \frac{0.576}{\sigma\,D_2\,\sqrt{L}} \tag{8}$$

This R_{sp}^* value has been used to compare different packing materials for high-performance GPC[7].

Eqn. 7 shows that columns of different σ and D_2 can work equally well as long as they have the same σD_2 vlaue. This concept is consistent with Hamielec's discussion of GPC column resolution[8] except that the parameter R_{sp} here relates more directly to the usual sense of chromatographic resolution.

Molecular weight accuracy

In another study we developed a new calibration method which includes a molecular weight correction for column dispersion in GPC[5]. We developed eqns. 9 and 10,

$$\bar{M}_n = e^{\frac{1}{2}(D_2\sigma)^2} \Big/ \Sigma_{V_R} \frac{F(V_R)}{D_1\,e^{-D_2 V_R}} \tag{9}$$

$$\bar{M}_w = e^{-\frac{1}{2}(D_2\sigma)^2} \cdot \Sigma_{V_R}\,[F(V_R)\,D_1\,e^{-D_2 V_R}] \tag{10}$$

which also have been derived by Balke and Hamielec[6,8] and extended by Provder and Rosen[9], using a different mathematical approach. In eqns. 9 and 10, $F(V_R)$ represents the normalized experimental chromatogram of the sample ($\Sigma_{V_R} F(V_R) = 1$)

and $D_1 e^{-D_2 V_R}$ the linear portion of the molecular weight calibration curve and σ is the peak standard derivation measured to a first approximation as the experimental value for a very narrow MWD polystyrene standard. Since σ has a small dependency on GPC retention volume[10], it would be most accurate to account for this variation. However, this dependency is system dependent, and corrections are small relative to the magnitude of σ. Therefore, this minor correction is not used in this work. It is expected though, that σ is dependent on solvent viscosity, but essentially independent of polymer type. Of course, at infinite resolution $\sigma = 0$, there is no column dispersion, and $F(V_R)$ becomes the true or infinitely resolved curve $W(V_R)$.

We propose that the relative errors ($\bar{M}_w^*$ and $\bar{M}_n^*$) between the molecular weights calculated from the real or experimental $F(V_R)$ curve, $(\bar{M})_{exp}$, and the molecular weights determined from the infinitely resolved or theoretical $W(V_R)$ curve, $(\bar{M})_{true}$, are important measures of the performance of the separating system. From eqns. 9 and 10 this performance may be stated as

$$\bar{M}_w^* = \frac{(\bar{M}_w)_{exp} - (\bar{M}_w)_{true}}{(\bar{M}_w)_{true}} = (e^{\frac{1}{2}(\sigma D_2)^2} - 1) \tag{11}$$

$$\bar{M}_n^* = \frac{(\bar{M}_n)_{exp} - (\bar{M}_n)_{true}}{(\bar{M}_n)_{true}} = (e^{-\frac{1}{2}(\sigma D_2)^2} - 1) \tag{12}$$

Note that $\bar{M}_n^*$ and $\bar{M}_w^*$ are errors caused by column band broadening only. The product σD_2 is a dimensionless quantity and eqns. 11 and 12 are valid regardless of whether retention volume, time, or syphon counts are used in defining the GPC calibration and elution curves. One should seek systems with small σD_2 values to achieve high resolution and high molecular weight accuracy. These equations provide criteria for specifying MW error in terms of column performance and these criteria provide guidance for column packing syntheses and/or selection.

EXPERIMENTAL

The polystyrene standards used as probes and test materials in this study were commercially available, characterized standards (Pressure Chemical Co., Pittsburgh, Pa., U.S.A.). The apparatus and general technique have been described previously[5,11,12,16]. Styragel (122 × 0.95 cm O.D.), Porasil (122 × 0.95 cm O.D.) and μ-Styragel columns (30 × 0.76 cm I.D.) (Waters Assoc., Milford, Mass., U.S.A.), and Vit-X columns (50 × 0.46 cm I.D.; Perkin-Elmer, Norwalk, Conn., U.S.A.), were obtained prepacked. The porous silica microsphere (PSM) packings developed in this laboratory have been described previously for use as column packing materials[7,13–15]. LiChrospher column packings were obtained from the E. M. Labs. (Elmsford, N.Y., U.S.A.). To eliminate alkaline impurities, this material was heated in concentrated nitric acid on a steam bath for 2 h and washed to neutrality with distilled water. Large "aggregates and fines" were removed by sedimentation in 0.001 *M* ammonium hydroxide. The resulting material was again washed to neutrality with distilled water, filtered and dried at 150° for 2 h. The PSM and heated LiChrospher packing materials were made into highly efficient chromatographic columns (PSM: 15 or 10 × 0.78 cm I.D.; LiChrospher: 25 × 0.62 cm) using the high-pressure slurry

packing technique[16]. The pore size distribution of PSM and LiChrospher packings were obtained by mercury intrusion.

With the column packings described above, no solutes were observed to adsorb during the experiments conducted; however, no detailed study of this effect was made.

R_{sp} was calculated from eqn. 7. The value, D_2 was calculated from,

$$D_2 = \frac{\ln (M_1/M_2)}{V_{R2} - V_{R1}} \tag{13}$$

Sigma values (σ) were computer-calculated according to the procedure of James and Martin[17] using the relationship $\sigma = (A/h\sqrt{2\pi})$ where h is peak height and A is peak area. The predicted MW accuracy $\bar{M}^*$ (average of $\bar{M}_w^*$ and $\bar{M}_n^*$), or the $\bar{M}_n$ and $\bar{M}_w$ error curves used in Figs. 2–4, were calculated from eqns. 11 and 12 using the experimental D_2 and σ values. The MW values of the narrow MWD polystyrene standards as reported by the manufacturers were used as $(\bar{M}_w)_{true}$ and $(\bar{M}_n)_{true}$ in the calculations.

RESULTS AND CONCLUSIONS

$\bar{M}^*$ is uniquely defined by the σD_2 value regardless of the separate values of σ and D_2 which can be coupled to give the same σD_2 product. D_2 is varied by connecting columns of different column packing pore sizes to broaden the molecular weight range through which the calibration curve is linear. In actual practice, the D_2 value of an assembled column set remains essentially constant. The various column sets discussed in this work were arranged to provide linear D_2 values for polystyrene over a fairly wide range of MW, approximately 10^3–10^6. For specific column sets, the MW accuracy criterion can be described by a $\bar{M}^*$ *versus* σ plot for the specific D_2 value. Since the expressions for R_{sp} and $\bar{M}^*$ do not show sample MW or MWD dependence, the R_{sp} and the $\bar{M}^*$ values describe the intrinsic characteristics of the GPC columns (applicable to all solutes in the linear separation range regardless of their MW or MWD differences).

To test eqns. 11 and 12 on a specific column set, it is necessary to vary σ and to compare the theoretical and experimental values of $\bar{M}^*$ (the molecular weight accuracy) as a function of σ. The value of σ can easily be changed by varying the flow-rate of the carrier solvent. Results for σ (Polystyrene 4a of 97,200 MW) for various particle size packings and flow-rates are shown in Fig. 1.

Experimental $\bar{M}^*$ values closely correspond to theoretical $\bar{M}^*$ values predicted by σ and D_2 as shown in Fig. 2. The dashed plots are the predicted values and the dots are the experimental MW results for 97,200 MW polystyrene (4a) calculated directly from the elution curves at different flow-rates (not from σ and D_2 values). A Vit-X column set was chosen arbitrarily for illustration, and similar results were obtained for the other packing materials and column sets in Table I.

In Fig. 2 the data points at the largest σ value were obtained at a flow-rate corresponding to a 4-min GPC analysis. The *ca.* 35% MW error at this flow-rate reduces the utility of these Vit-X columns for accurate high-speed GPC analyses. However, PSM columns are very satisfactory for fast GPC owing to the small σ dependence of the columns on flow-rate as illustrated by the PSM plot in Fig. 1. Fig. 3 shows the separation obtained with a 40-cm set of PSM columns in 90 sec. In this

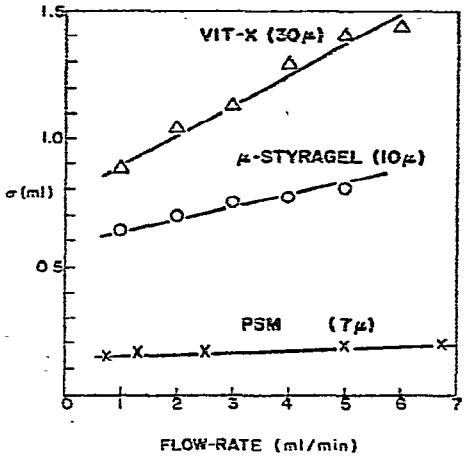

Fig. 1. Dependence of σ on flow-rate.

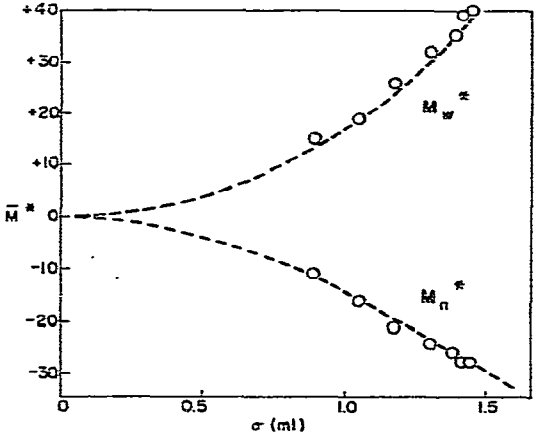

Fig. 2. Effect of column dispersion on MW accuracy.

TABLE I

PERFORMANCE COMPARISON OF SEVERAL COLUMN SETS USING VARIOUS GPC COLUMN PACKING MATERIALS

Column packing	*No. of columns*	*Total length (cm)*	*Flow-rate (ml/min)*	*Sample analysis time (min)*	*Particle size (μm)*	*Linear calibration range (MW)*	*Plate count (N) toluene*	σD_2*	R_{sp}	$\bar{M}$** *(ave.) (%)* **
Styragel	4	488	1	180	50	10^3–10^6	7,500	0.45	1.27	11
Porasil	4	488	1	180	75–150	$2\cdot10^4$–10^6	2,700	0.37	1.56	7
Vit-X	4	200	2	15	30	$5\cdot10^3$–10^6	3,500	0.59	0.97	18
μ-Styragel	4	120	2	15	10	$2\cdot10^3$–10^6	13,000	0.50	1.14	13
LiChrospher	4	100	1.5	15	10	$5\cdot10^3$–10^6	5,800	0.23	2.50	3
PSM	5	60	1.25	15	7	10^3–$2\cdot10^6$	24,500	0.21	2.72	2

* σ measured with 97,200 MW polystyrene.

** $\bar{M}$* is obtained from eqns. 11 and 12 using the measured σ and D_2 values.

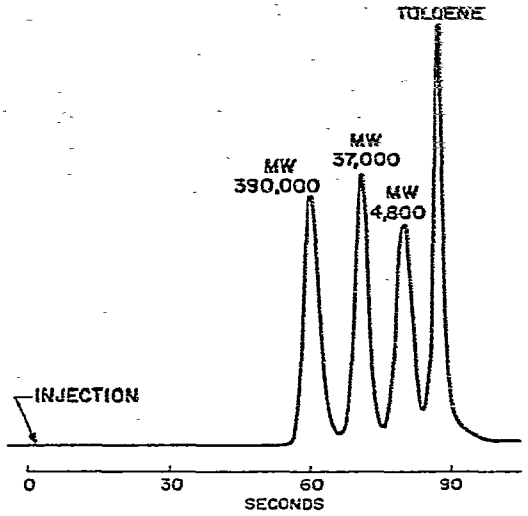

Fig. 3. Rapid GPC: a 90-sec separation of polystyrenes on PSM columns.

90-sec separation, baseline resolution of each decade of MW of polystyrene up to 400,000 was obtained with an average $\bar{M}^*$ of 9%. The data in Table I also show that the PSM column set exhibits an excellent MW accuracy level of 2% for a 15-min analyses. Particle size is the most significant factor which differentiates the 15-min "high-performance" GPC from the 3-h conventional GPC analysis.

Table I also verifies that plate count (N) alone is a poor indicator of column performance in terms of resolution or molecular weight accuracy. For example, for the 2,700-plate Porasil column set, the molecular weight error ($\bar{M}^*$) caused by column dispersion was 7% compared with 11% for a Styragel column set of $N = 7{,}500$. The better MW accuracy of the Porasil column set in this case is partly due to its narrower linear calibration range as compared with Styragel. These data support the contention that column plate count measured from a monomer peak does not accurately reflect the capability of the GPC system for polymer MW analyses. We propose that the R_{sp} and $\bar{M}^*$ accuracy values are useful quantitative criteria for GPC column performance. The effects of certain chromatographic variables on GPC column performance are discussed by Kirkland in a companion paper[7].

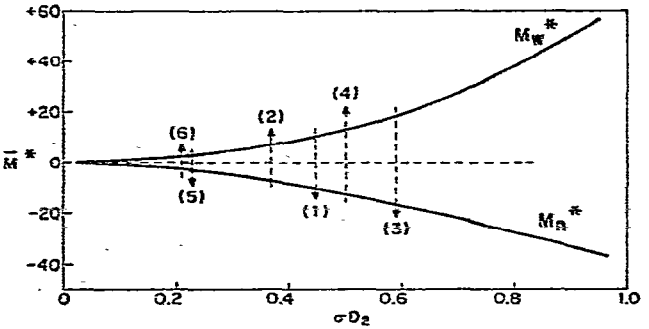

Fig. 4. Comparison of column sets using the molecular weight criterion $\bar{M}^*$. 1 = Styragel; 2 = Porasil; 3 = Vit-X; 4 = μ-Styragel; 5 = LiChrospher; 6 = PSM.

The performance of the column sets of Table I is compared directly in Fig. 4, a master plot of $\bar{M}^*$ *versus* σD_2. It should be emphasized again that $\bar{M}^*$ is the error caused by column dispersion alone and does not include errors in values of MW assigned to standards, errors due to flow-rate variation[11], operator errors, etc. In actual polymer sample analyses, we correct for $\bar{M}^*$ through eqns. 9 and 10 using the GPC calibration and MW calculation method previously described[5]. Developments are continuing in our laboratory to eliminate small residual errors in the analysis since these equations do not permit use of a variable σ in the GPC chromatogram nor do they include correction for peak skewing.

Effect of pore geometry

The theoretical treatment developed in the following section interprets the effect of pore geometry on GPC separation capacity (D_2). The effect of the pore geometry on column dispersion (σ) was not studied because theoretical analysis is relatively difficult and the effect will probably be small.

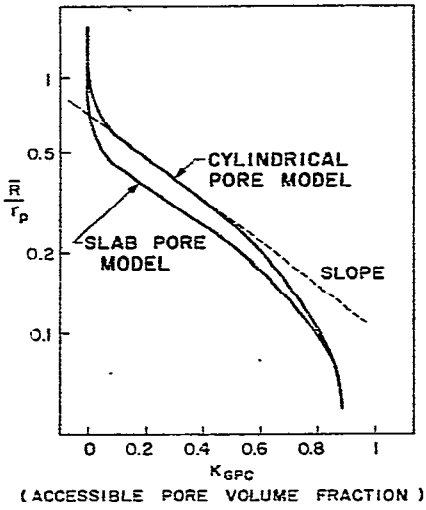

Fig. 5. Theoretical GPC calibration curves for two pore shaped models.

The effect of packing porosity, pore shape and size on D_2 can be understood by analyzing the basic GPC separation theory[18,19]. The theoretical GPC calibration curves for two pore shape models are reproduced in Fig. 5, where r_p is the equivalent hydraulic radius of the pore, $\bar{R}$ is the root-mean-square radius of gyration of the solute polymer molecule, and K_{GPC} is the GPC distribution coefficient (which equals the accessible pore volume fraction). The slope of these curves represents the theoretical limit of GPC separation capacity normalized by the internal pore volume of the packing particle. The particle porosity (γ) is inversely proportional to D_2 so that separation capacity increases with γ,

$$\left(D_2 \propto \frac{\text{slope}}{\gamma} \propto \frac{1}{R}\right) \tag{14}$$

The effect of pore shape on D_2 is relatively small. In fact, the large difference in restricted-cylindrical and open-slab pores causes only a 20% difference in the molecular weight calibration curve slope or D_2 (see Fig. 5). Particles with pores having only size differences have identical values. Because the ordinate $\bar{R}/r_p$ is plotted on the log scale, variation in pore radius (r_p) only linearly shifts the curves on the ordinate without change in slope.

There is no adequate theory for the effect of packing pore size distribution (PSD) on GPC separation, and it has been generally assumed that GPC separations can be improved indefinitely by narrowing the PSD. The assumption made in this classical thinking is that the slope of the GPC calibration curve is directly related to the PSD of the packing[20,21]. However because there is a finite limit to GPC separation capacity, this model is incorrect.

Because of the basic separation mechanism, the GPC calibration curve approaches a limiting, not horizontal, slope (see Fig. 5), even at infinitely narrow PSD. In fact, the slope of the theoretical GPC calibration curve actually may be quite different from the PSD curve. To illustrate this point, in Fig. 6 we reversed the axes of the theoretical GPC calibration curve for the cylindrical pore model to compare directly with the experimental PSD curve of the PSM 300 packing. The average pore radius is designated $\bar{r}_p$ while all other symbols are as defined earlier. The large difference of the slope for the two curves in Fig. 6 indicates that PSD alone is not sufficient to explain GPC separation.

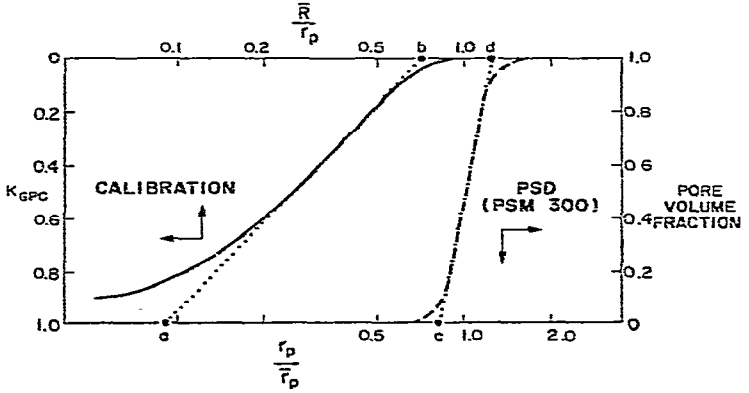

Fig. 6. Comparison of single size cylindrical pore model calibration curve with experimental cumulative pore size distribution curve.

We consider the overall slope of the GPC calibration curve (D_2) to be the result of the convolution between the slopes of the theoretical calibration limit (W_{cal}) and the PSD curve (W_{psd}), that is,

$$D_2 = k\sqrt{W_{cal}^2 + W_{psd}^2} \tag{15}$$

where k is a proportionality constant. The manual calculation of W_{cal} and W_{psd} is illustrated by the dotted lines (Fig. 6). In this analysis the tangents ab and cd are constructed. The desired values are then calculated by:

$$W_{cal} = \log\sqrt{b/a} = 0.477 \text{ and } W_{psd} = \log\sqrt{d/c}$$

From eqn. 15 as W_{psd} approaches zero, D_2 approaches the limiting value of D_2^0:

$$D_2^0 = k\, W_{cal} \tag{16}$$

In Fig. 7, we have plotted the ratio D_2/D_2^0 from eqns. 15 and 16 as a function of W_{psd} shown as curve B. The line A in Fig. 7 calculated by,

$$D_2 = k\, W_{psd} \tag{17}$$

is the classical (linear) interpretation. Since R_{sp} is inversely proportional to D_2, the ratio of R_{sp} to its limiting value R_{sp}^0 is also indicated along the D_2/D_2^0 axis. The difference between curves A and B increases at small W_{psd} values. According to line A or the classical interpretation, GPC resolution is always very sensitive to the PSD of the packing; while the correct interpretation (curve B) indicates that *the effect of PSD on R_{sp} diminishes at small W_{psd}.*

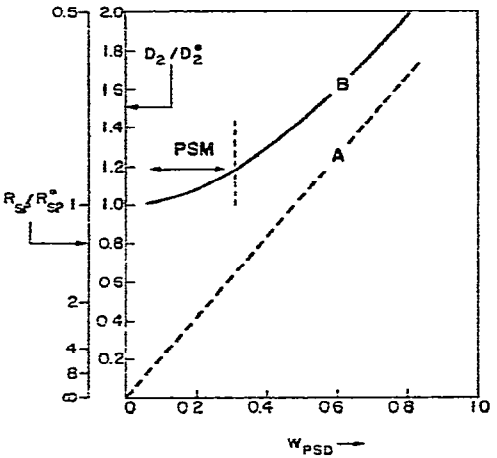

Fig. 7. Effect of pore size distribution on GPC separation.

The W_{psd} values of the PSM and the LiChrospher packings of different pore sizes are listed in Table II along with the R_{sp}/R_{sp}^0 and D_2/D_2^0 values calculated according to our theory. On the average, the PSD of the PSM packings appears to be narrower than that of the LiChrospher packings. Most of the PSM packings fall within 20% of the theoretical limit as shown in both Fig. 7 and Table II. This suggests that further reducing the PSD of the PSM packing, except for the large pore size ones, would not result in substantial gain in column performance. This theory suggests that porosity is a more important factor affecting the PSM column performance than the PSD and the pore shape. The theory also provides a means to evaluate quantitatively the effect of pore structure on the performance of GPC column packings.

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TABLE II

PACKING PSD AND GPC PERFORMANCE PARAMETERS

$W_{crt} \approx \log 3 = 0.477$, theoretical limits of D_2/D_2^0 and $R_{sp}/R_{sp}^0 = 1$.

Packing	*Pore size (μm)*		W_{psd}	D_2/D_2^0	R_{sp}/R_{sp}^0
	d	*c*			
PSM					
4000	1.0	0.095	0.51	1.47	0.68
1500	0.17	0.03	0.38	1.27	0.79
800	0.034	0.026	0.06	1.0	0.99
600	0.023	0.017	0.07	1.17	0.99
500	0.050	0.013	0.29	1.01	0.85
300	0.0014	0.01	0.07	1.07	0.99
150	0.0073	0.0056	0.06	1.01	0.99
50	0.0069	0.0043	0.10	1.05	0.96
Average			0.19	1.13	0.91
LiChrospher					
4000	0.8	0.13	0.14	1.30	0.77
1000	0.14	0.07	0.15	1.05	0.95
500	0.059	0.024	0.020	1.08	0.92
100	0.060	0.01	0.39	1.29	0.78
Average			0.28	1.18	0.86

LIST OF SYMBOLS

Presented in the order of appearance in the text.

N	Number of theoretical plates or column plate numbers
V_R	Solute (peak) retention volume
W	Solute (peak) width
R_s	Resolution function
R_{sp}	Specific resolution function
H (or HETP)	Height equivalent of a theoretical plate
MW (or M)	Molecular weight
$\bar{M}_n$	Number average molecular weight
$\bar{M}_w$	Weight average molecular weight
σ	Standard deviation (dispersion)
D_1	A constant related to the intercept of the GPC calibration line
D_2	A constant related to the slope of the GPC calibration line
$\bar{M}_n^*$	Relative number average molecular weight error
$\bar{M}_w^*$	Relative weight average molecular weight error
$\bar{M}^*$	Average of $\bar{M}_w^*$ and $\bar{M}_n^*$
$F(V_R)$	Experimental chromatographic height at every V_R
PSM	Porous silica microspheres
r_p	Equivalent hydraulic radius of a (packing) pore
$\bar{R}$	Root mean square radius of gyration of a solute polymer molecule
K_{GPC}	Solute distribution coefficient or accessible pore volume fraction
γ	Particle porosity

W_{cal}	Theoretical limit on slope of GPC calibration
W_{psd}	Slope of the cumulative pore size distribution curve from mercury intrusion
D_2^0	Limit value of D_2
R_{sp}^0	Limit value of R_{sp}

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