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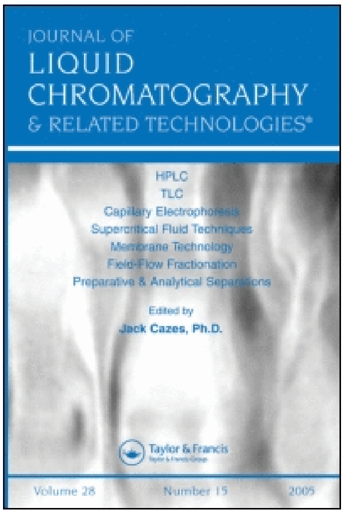

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A Calibration Technique for Size Exclusion Chromatography

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A CALIBRATION TECHNIQUE FOR SIZE EXCLUSION CHROMATOGRAPHY

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ABSTRACT

A modified Weibull distribution function is shown to be useful in calibrating the molecular size separation capabilities of both rigid and swellable gel packing materials. Two parameters are used in this function which are related to a packing material's micropore volume distribution. The calibration curves of a set of different packing materials connected in series were predicted from the Weibull calibration functions obtained for each individual packing material.

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INTRODUCTION

Quantitative size exclusion chromatography (SEC) can be accomplished only after a calibration of the system has been performed. SEC calibration relates a polymer macromolecular property, usually size or molecular weight, to the elution volume penetrated by the macromolecule. This is usually accomplished by using a calibration function which describes an SEC separation model. It is the purpose of this paper to briefly review the SEC models previously proposed and to introduce and examine a new SEC model based on a Weibull distribution function.

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SEC Calibration Models

In 1967, Grubisic, et al [1], observed that known molecular weight fractions of linear and branched polystyrene eluted at different times. The branched polymers of equal molecular weight always had a greater elution volume. From this observation, the authors concluded that the molecular hydrodynamic volume could be used as a calibration parameter.

The hydrodynamic volume of a polymer molecule in dilute solution can be obtained from the Einstein-Simha equation [2]. This equation shows that the hydrodynamic volume of a polymer molecule in dilute solution is proportional to the product of the polymer's molecular weight, M, and intrinsic viscosity $[\eta]$.

Grubisic used the logarithm of $M[\eta]$ versus elution volume for SEC calibration and also suggested that this calibration procedure was universal for any type polymer. The Grubisic calibration function is given by:

$$V_e = A + B \log (M [\eta]) \tag{1}$$

In equation (1), V_e is the elution volume of a molecule which is linearly related to the logarithm of the molecular hydrodynamic volume by the calibration constants A and B. Grubisic showed that this plot characterized SEC separation of polymers with differing structures and extents of branching [3].

The concept of hydrodynamic volume as the controlling parameter in liquid chromatography gained support in subsequent articles by Wild and Guliana [4], Coll and Gilding [5], Boni et al. [6], and Berry [7]. In all cases, a linear correlation of hydrodynamic volume with elution volume was obtained.

Dawkins [8] suggested that the molecular root mean squared, unperturbed end-to-end distance was the controlling factor in SEC. Cassassa and Tagami [9] also expressed this same view. However, there is no basis for choosing between hydrodynamic volume and end-to-end distance as the controlling size separation parameter, since both correlate data equally well.

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Giddings [10] used statistical thermodynamics to explain SEC molecular separation phenomena. Giddings modeled the packing microstructure as a network of lines. This micropore model is called a "brush pile". From this SEC model, the fraction of the micropore volume penetrated, K, by a molecule having an external length, ℓ, was related to micropore surface area, S, and volume, ν.

$$K = 1 / \mathrm{EXP}\ [\ \ell S/2\nu\ ] \qquad (2)$$

The fraction of the micropore volume penetrated by a molecule, K, is usually called the distribution coefficient. The molecular external length was compared to the hydrodynamic volume and end-to-end distance. It was found that the external length was more effective as a calibration parameter when characterizing the separation behavior. It was also concluded that the "brush pile" model was a good approximation of the micropore structure of swellable gel packing materials.

Cassassa [9] explained SEC separation phenomena by assuming that rigid spherical molecules were in equilibrium with micropores which were modeled by: (1) spherical cavities of diameter D_s, (2) long circular cylinders of diameter D_c, and (3) cavities formed by long parallel plane surfaces separated by a distance D_p. The distribution coefficient for a spherical molecule of diameter d which is in equilibrium with each cavity type is given by:

$$K = [\, 1 - (\, d/D_s \,)\,]^3 \tag{3}$$

$$K = [\, 1 - (\, d/D_c \,)\,]^2 \tag{4}$$

$$K = [\, 1 - (\, d/D_p \,)\,]^1 \tag{5}$$

The Cassassa micropore models do not describe real SEC packing micropores; however, the models do show the general effects that pore geometry will have on size exclusion.

In all three of the Cassassa micropore models, a single size pore geometry was assumed. In a more realistic approach, Dawkins [12] modeled the micropore volume by using a Schulz-Zimm exponential distribution to describe the pore volume associated with cylindrical pores. The distribution coefficient of a molecule of diameter d in this porous medium model is given by:

$$K = \int_{d}^{d_o} \left\{ \frac{d}{D^2} \Big/ \mathrm{EXP}\left[d/D \right] \right\} \partial d \tag{6a}$$

$$K = (\, 1 + d/D \,) \Big/ \mathrm{EXP}\, [\, d/D \,] \tag{6b}$$

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In equation (6), D is the average pore diameter; and the upper integration limit, d_o, represents the diameter of the smallest large molecule which will not penetrate any of the micropore volume (i.e., at K = 0). This micropore model gave a reasonable average pore diameter for real SEC packing materials.

In an SEC separation model, which is similar to that of both Giddings and Dawkins, Hester and Mitchell [13] used a simple exponential probability function to describe the distribution of the micropore volume. This model predicted that the distribution coefficient for a molecule of diameter d was a function of the mean effective pore diameter, $\overline{D}$.

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$$K = \int_{d}^{d_o} \left\{ 1 / \overline{D} \ \mathrm{EXP} \left[d / \overline{D} \right] \right\} \partial d \tag{7a}$$

$$K = 1 / \mathrm{EXP} \left[d / D \right] \tag{7b}$$

The mean effective pore diameter was shown to be a parameter which characterizes a packing material's molecular separation capabilities. Packing materials, having a larger mean effective pore diameter, have a larger range of molecular size separation. However, packing materials with a larger mean effective pore diameter are less sensitive in distinguishing between molecular sizes. It was shown that SEC calibration is more closely correlated by equation (7) than by the more commonly used Grubisic calibration function, equation (1).

It is evident from equations (6) and (7) that an SEC calibration function specifies a micropore volume distribution function.

An SEC calibration defines the fraction of the total micropore volume which is penetrated by a specific size polymer molecule. The total volume penetrated by a molecule is the volume sum of all the interstitial channels and micropores which have controlling entry sizes which are equal to or larger than the size of the molecule.

Calibration Using a Weibull Function

It will be shown that a modified Weibull distribution can be used to model the micropore volume distribution of both rigid and swellable gel SEC packing materials. The Weibull distribution function for a single packing material is given by:

$$\partial V/\partial d = (V_p n/d)\ (d/\overline{D})^n \ /\ \mathrm{EXP}\ [\ (d/\overline{D})^n] \tag{8a}$$

$$\partial V/\partial \ln d = V_p n\ (d/\overline{D})^n \ /\ \mathrm{EXP}\ [\ (d/\overline{D})^n\ ] \tag{8b}$$

In the above equation, ∂V, is the incremental change in the micropore volume associated with an incremental change in the micropore entry size, ∂d. The parameters, V_p, $\overline{D}$, and n, are material properties which characterize the packing material. They are the total micropore volume, the most dominant pore entry size, and micropore volume distribution shape factor, respectively.

The total micropore volume, V_p, can be experimentally determined. It is the volume difference between the total permeation volume, V_t, and the interstitial volume, V_o.

$$V_p = V_t - V_o \tag{9}$$

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V_t is the elution volume of a very small molecule and V_o is the elution volume of a very large molecule which only penetrates the interstitial volume.

For the distribution in equation (8b), the largest quantity of micropore volume will have a pore entry size equal to $\overline{D}$. An increase in the distribution shape factor, n, produces both a decrease in the width of the micropore volume distribution and a decrease in skewness to the smaller pore entry sizes. It will be shown that the distribution parameters, $\overline{D}$ and n, can be adjusted such that the function describes the micropore volume distribution of real packing materials.

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Equation (8) can be used to obtain an SEC calibration function. The following relationships can be obtained from the definition of the distribution coefficient.

$$K = \int_{d}^{d_o} (\partial V/\partial d)\ (\partial d/V_p) \tag{10a}$$

$$K = \int_{d}^{d_o} \left\{ (n/d)\ (d/\overline{D})^n \,/\, \mathrm{EXP}\ [\ (d/\overline{D})^n\ ] \right\} \partial d \tag{10b}$$

$$K = 1 \,/\, \mathrm{EXP}\ [\ (d/\overline{D})^n\ ] \tag{10c}$$

$$V_e = V_o + V_p \,/\, \mathrm{EXP}\ [\ (d/\overline{D})^n\ ] \tag{10d}$$

In equation (10d), V_e is the elution volume of a molecule having a diameter which is proportional to a pore entry size of d. Work by Halasz et al. [14-16] has shown that the pore entry size, as measured by capillary condensation and mercury porosimetry, is two to three times larger than the molecular hydrodynamic diameter. However, this relationship cannot be adequately tested at small pore sizes because porosimetry is not accurate if the pore entry size is less than 175 Å [20].

The hydrodynamic diameter of a polymer molecule is proportional to the cube root of the hydrodynamic volume [13].

$$d = k\,(M\,[\eta])^{1/3} \tag{11}$$

Puckett [17] has determined that for both linear or branched polymer molecules, the best value of the constant, k, is 4.8. This value for k in equation (11) will give molecular hydrodynamic diameters in Angstrom units, when the values for the intrinsic viscosity are in deciliters per gram.

Polymer standards of known molecular weight and intrinsic viscosity can be used to determine packing material properties, $\overline{D}$ and n. The best values for $\overline{D}$ and n can be obtained by fitting V_e vs d data to equation (10d). It will be shown that equation (10d) can be used as a calibration function for many different types of packing materials. In addition, it will be shown that equation (10d) can be used to calibrate a set of packing materials from a knowledge of the material properties of each individual packing material.

EXPERIMENTAL

The literature contains many papers on SEC calibration; however, most of these papers do not contain all the information needed to evaluate the utility of the modified Weibull calibration function,

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equation (10d). From the few papers which did contain sufficient SEC information, data from Dawkins [12], Squire [19], and Van Kreveld [11] were selected because they represent the major types of SEC packing materials: swellable polystyrene gels, controlled pore glass chemically bonded with hydrophilic molecules, and silica gel, respectively. For the sake of brevity, the experimental details used to obtain this data are not included in this paper.

In addition to the SEC data taken from the literature, SEC data was experimentally obtained for controlled pore glass and silica gel packing materials [19]. This SEC data will be referred to as Hester data. This data was collected using the following conditions:

Porous glass packing was obtained from Electro-Neucleonics (Fairfax, NJ). Silica gel packing material, trade named Fractosil, was obtained from E. M. Merck (Elmsford, NY).

Three sizes of controlled pore glass packing were used, CPG 75, 24, and 350. Also, three sizes of silica gel packing were used, Fractosil 200, 500, and 2500. Material specifications are given in Table I. Three columns of each size control pore glass material were packed and connected in series, then calibrated using polystyrene solutions. Only one column of each size silica gel material was packed. Each column was individually calibrated with polystyrene solutions.

Reagent grade THF or toluene were used as a mobile phase or solvent. THF was distilled (66-67^{o}C) over calcium hydride under a nitrogen atmosphere to remove an inhibitor.

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TABLE I

PACKING MATERIAL PROPERTIES

Date Source	Type Packing	Micropore Volume, ν, cm^3/g	Surface Area S, m^2/g	Ratio Volume to Surface Area, ν/S, Å	Most Dominant Micropore Size* $\bar{D}$,Å
Hester & Mitchell	CPG 350	1.39	79	176	178
	CPG 240	1.31	118	111	169
	CPG 75	0.48	207	23	48
Hester & Mitchell	Fra 2500	0.5	8	630	785
	Fra 500	0.6	35-65	90-170	118
	Fra 200	0.6	120-170	35-50	64
Van Kreveld & Van Den Hold	Porasil D	0.912	34.8	262	252
	Porasil C	0.864	71	122	119

*From fitted Weibull functions

Polystyrene standards with low polydispersity were obtained from Pressure Chemical and Waters Associates. Each standard was dissolved in THF or toluene. The concentration of each standard solution was less than the reciprocal of its measured intrinsic viscosity. This concentration level assured that a polymer solution was dilute.

A Waters UK6 sample injector equipped with a 2 ml sample loop was used for the majority of this work. However, for some experiments, a Rhoedyne (Cotati, CA) Model 7125 injector fitted with a 2 ml loop was also used.

The SEC pump used was a Waters Associates (Milford, MA) Model 6000 A. Volumetric output was monitored by a Waters siphon volumetric counter equipped with a 5 ml siphon counter. The mobile phase flow rate through the packed columns was always regulated between 0.5 and 1.0 ml/min.

A Waters Model 440 UV absorbance detector was the primary means of detecting polystyrene in the SEC eluent. The wave length used was 254 nm.

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RESULTS

The SEC data taken from the literature contained several individual data sets. However, not all of the data sets in each group were used. For example, the Dawkins data contained 23 individual sets of SEC elution data which were obtained by using combinations of two solvents, four polystyrene gel packing materials, and three different types of polymer standards. However, only four data sets were used. These four data sets were selected because they represent each size packing material and also because each data set contained a large number of data points, thereby providing the largest degree of freedom when fitting the Weibull calibration function. Similar discriminations were performed on both the Squire and Van Kreveld data.

Table II gives a summary of the data groups and the individual data sets within each group which were used to fit the Weibull calibration function. A total of 15 individual SEC data sets in five data groups were fitted to Weibull function, equation (10d), using a parametric fitting routine. The fitting routine was programmed in BASIC to run on a Hewlett-Packard HP85 microcomputer [21]. The fitting technique used a search routine to find the best fit values of the packing material parameters, $\overline{D}$ and n, which minimized the standard error between data and function. The best fit parameters for each SEC data set are listed in Table II.

Also included in Table II, is a criterion for the overall goodness of the function fit to each data set. This goodness of fit value, g, for N data points has been defined in a manner similar to that used by Samay [22]. If V_{ei} and $\hat{V}_{ei}$ are the experimental elution volume and corresponding elution volume estimated from the fitted function, respectively; then g can be expressed by:

$$g = 100 \sqrt{\frac{\sum_{i=1}^{N} (V_{ei} - \hat{V}_{ei})^2}{N - 2}} \Bigg/ \frac{\sum_{i=1}^{N} V_{ei}}{N} \qquad (12)$$

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TABLE II

DATA FOR CALIBRATION PLOTS

Data Source	Figure #	Symbol	Type Packing	Type Polymer	Type Solvent	Intrinsic or Mark Houwink Equation	V_t	V_o	Fitted Weibull Parameters		Goodness of Fit Criterion
									n	$\bar{D}$, Å	g
Dawkins & Hemming	2	x	Styragel A	PS	CL	a	10.9	9.3	1.22	500	0.59
	2	+	Styragel B	PS	CL	a	10.2	7.2	1.20	425	0.79
	2	*	Styragel C	PS	CL	a	9.4	5.6	1.08	165	1.48
	2	o	Styragel D	PS	CL	a	8.8	5.9	1.22	74	1.80
Squire	3	+	TSK Gel 4000	PEG	W	b	49.1	19.2	1.00	141	1.19
	3	o	TSK Gel 3000	PEG	W	b	47.5	18.8	1.27	59	1.07
	3	*	TSK Gel 2000	PEG	W	b	44.0	22.1	1.58	37	0.80
Van Kreveld & Van Den Hold	4	+	Porasil D	PS	THF	c	36.0	17.8	1.38	252	0.49
	4	*	Porasil C	PS	THF	c	35.3	17.0	1.40	119	0.90
Hester & Mitchell	5	+	CPG 350	PS	Tol	d	70.3	39.4	1.46	178	1.88
	5	o	CPG 240	PS	Tol	d	79.3	37.7	1.47	169	1.33
	5	*	CPG 75	PS	Tol	d	69.1	36.4	1.37	48	2.14
	7	*	CPG 75,240, 350	PS	Tol	d	218.7	113.5	-	-	2.86
Hester & Mitchell	6	*	Fractosil 2500	PS	THF	c	19.9	10.2	1.49	785	0.94
	6	o	Fractosil 500	PS	THF	c	19.9	11.4	0.95	118	0.63
	6	+	Fractosil 200	PS	THF	c	18.7	10.4	1.43	64	0.95
	8	o	Fractosil 200,500, 2500 & CPG 75	PS	THF	c	59.8	32.7	-	-	1.42

PS	polystyrene
CL	chloroform
PEG	Polyethylene glycol
W	water
Tol	toluene
THF	tetrahydrofuran

a) $[\eta] = 4.9 \times 10^{-5} M^{0.79}$

b) $[\eta] = 0.2 + 1.6 \times 10^{-4} M^{0.76}$

c) $[\eta] = 6.76 \times 10^{-5} M^{0.761}$

d) $[\eta] = 1.63 \times 10^{-4} M^{0.682}$

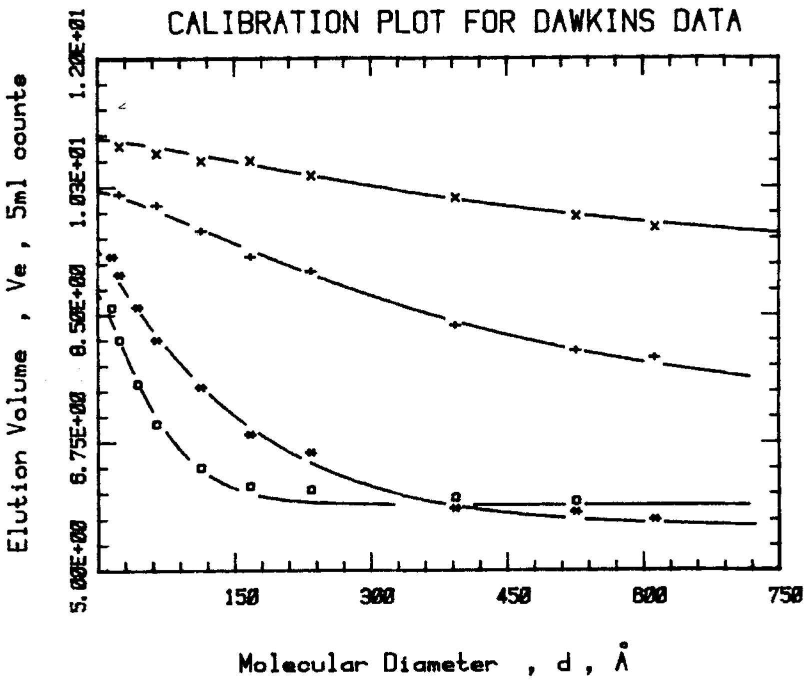

Figure 1. SEC calibration plots for styragel packing materials using polystyrene and chloroform. See Table II for details.

As shown by equation (12), the overall goodness of fit has been defined as the standard error expressed as a percentage of average experimental elution volume. A g value of zero corresponds to a perfect fit of the function to the experimental data.

The elution volume versus molecular hydrodynamic diameter has been plotted for each data set within each data group. The Dawkins, Squire, and Van Kreveld calibration plots are shown in Figures 1 through 3, respectively. The Hester data, using porous glass and silica gel, are shown in Figures 4 and 5, respectively. Also shown on the above plots are the curves representing the best fit

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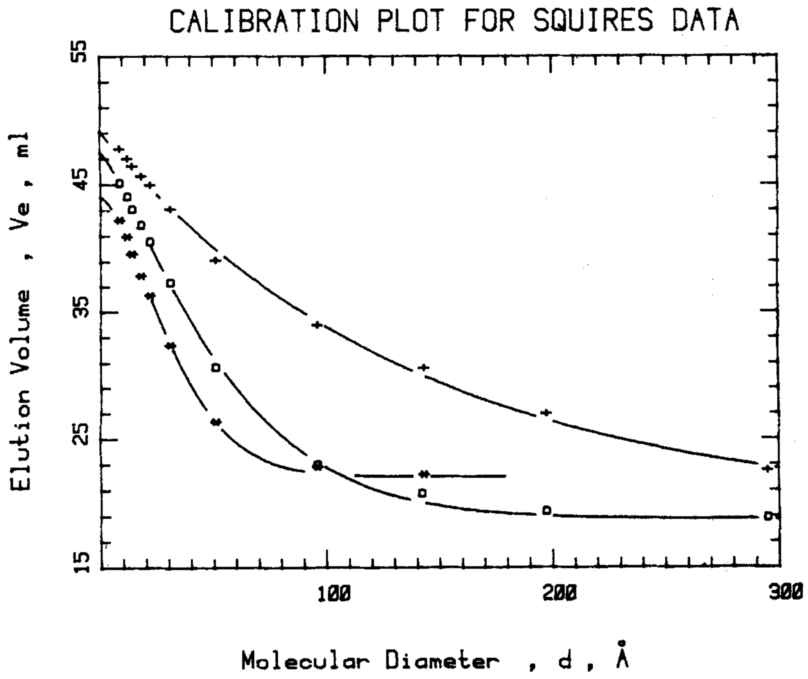

Figure 2. SEC calibration plots for TSK gel packing materials using polyethylene glycol and water. See Table II for details.

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Weibull calibration function for each data set. The molecular hydrodynamic diameters used in the calibration plots were calculated from the polymer molecular weights, the appropriate Mark Houwink equation listed in Table II and equation (11). The total permeation volume, V_t and the total interstitial volume, V_o, used in fitting the Weibull functions are also listed in Table II.

DISCUSSION

SEC Calibration of Single Packing Materials

As can be observed from the calibration curves of Figures 1 through 5 and the corresponding goodness of fit values listed in

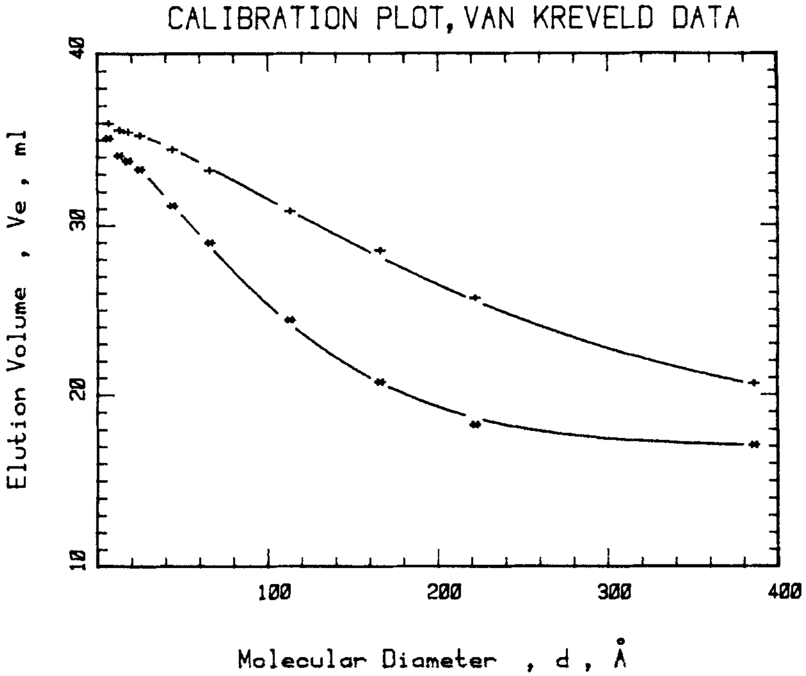

Figure 3. SEC calibration plots for Porasil packing materials using polystyrene and tetrahydrofuran. See Table II for details.

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Table II, the Weibull function developed for each data set closely models the SEC separation performance regardless of the packing material. Most of the packing materials had a shape factor, n, which ranged from 1.0 to 1.5. This indicates that the pore volume distribution of packing materials are similar. This same similarity of shape was also noted by both Scott [23] and Zhdanov [24] for silica gel packing and porous glass packing, respectively.

Most of the packing materials had a most dominant pore entry size, $\bar{D}$, which was very close to that expected from the approximate pore size specified by the manufacturers. It appears that a

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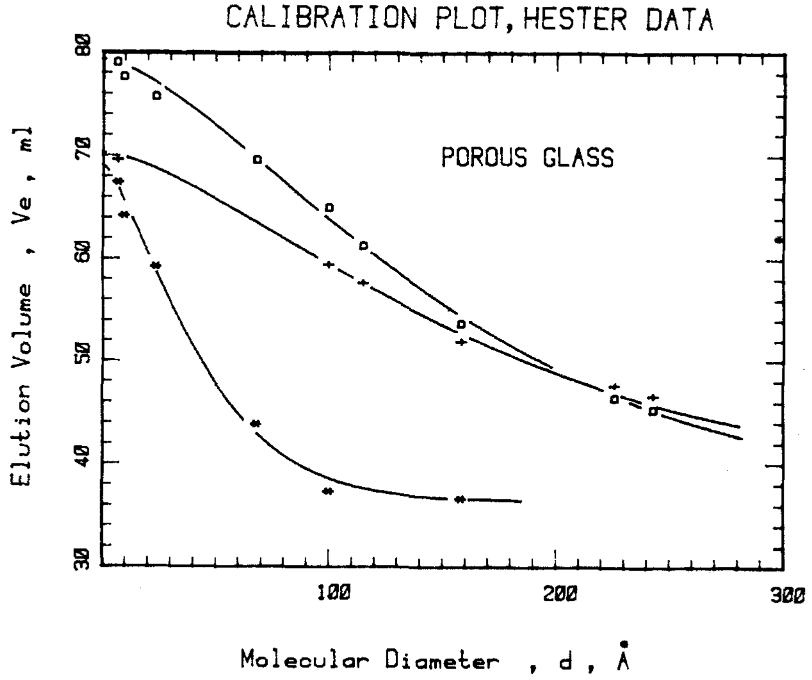

Figure 4. SEC calibration plots for Control Pore Glass packing materials using polystyrene and toluene. See Table II for details.

packing material's most dominant pore size is approximately equal to the ratio of micropore volume to surface area. This can be noted from Table I where this ratio and $\overline{D}$ can be compared. A similar observation was made by Berek [3] using polystyrene standards and silica gel packing materials. The work of Berek showed that the molecular weight, at the inflection point on an SEC elution volume versus log molecular weight calibration curve, was linearly related to the ratio of the micropore volume to surface area. It can be mathematically shown that the modified Weibull calibration function would also have an inflection point which corresponds to a molecular size equal to $\overline{D}$.

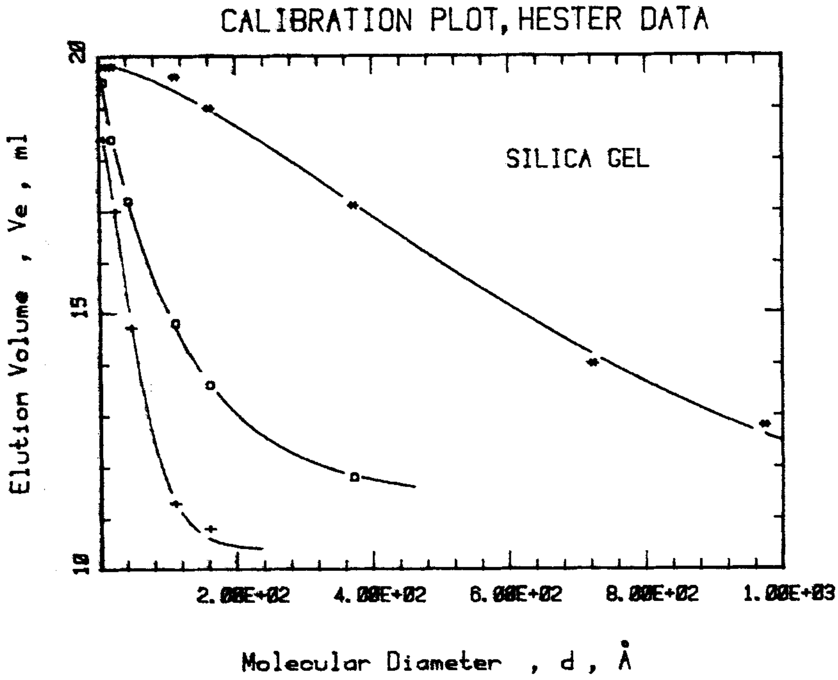

Figure 5. SEC calibration plots for Fractosil packing materials using polystyrene and tetrahydrofuran. See Table II for details.

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SEC Calibration of Several Packing Materials in Series

A definite advantage of a Weibull calibration is that the calibration of a column set of individual packing materials connected in series can be determined from the individual calibrations obtained from each single packing material. This can be shown from the following argument.

For a set of packing materials operating in true size exclusion, the elution volume of a molecule is equal to the sum of the individual packing interstitial volumes added to the sum of the individual packing micropore volumes penetrated by the molecule. Therefore, the elution volume for a set of P packing materials is given by:

$$V_e = \sum_{i=1}^{P} V_{oi} + \sum_{i=1}^{P} V_{pi} K_i \tag{13}$$

In equation (13), the subscript i refers to an individual packing material. However, equation (10c) can be used to express each individual distribution coefficient in terms of the hydrodynamic diameter of the molecule passing through the packing set. Therefore, equation (13) can be expressed as:

$$V_e = \sum_{i=1}^{P} V_{oi} + \sum_{i=1}^{P} V_{pi}/\mathrm{EXP}\ [\ (d/\overline{D}_i)^{n_i}\ ] \tag{14}$$

Equation (14) shows that, if the individual packing material parameters V_{oi}, V_{pi}, $\overline{D}_i$, and n_i are known, the calibration curve for the packing materials operating in series can be constructed.

Equation (14) was used to construct the calibration curves for two sets of packing materials. The first set was composed of three porous glass packing materials, CPG 75, 240, and 350. These packing materials have been individually calibrated and their material parameters are listed in Table II. The second set was composed of three silica gel packing materials, Fractosil 200, 500, and 2500, and a small quantity of CPG 75. The individual Fractosil materials have been individually calibrated and their material

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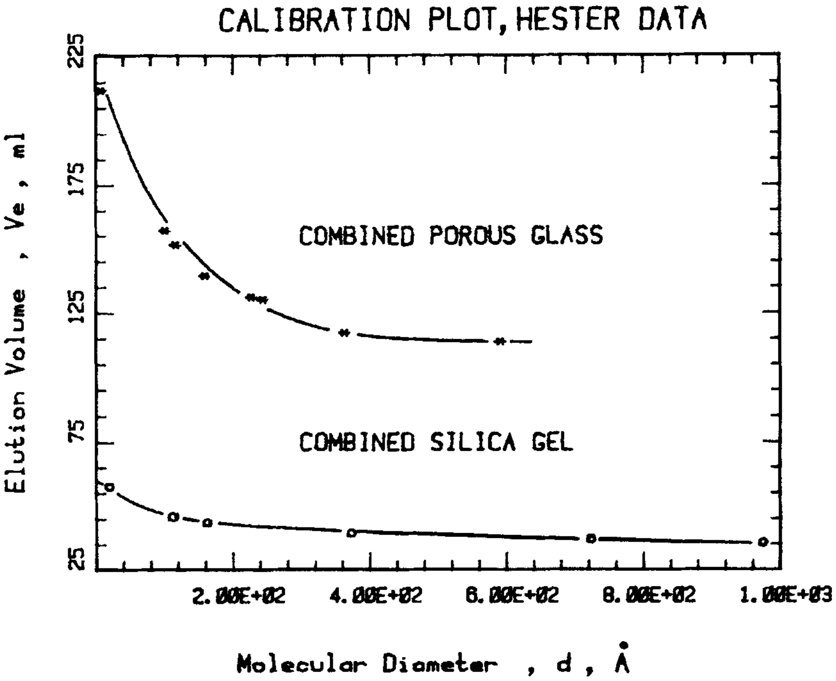

Figure 6. SEC calibration plot for the combined Control Pore Glass and combined Fractosil packing materials used in Figures 4 and 5, respectively. See Table II for details.

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parameters are listed in Table II. The CPG 75 of the second set was used in a small guard column. The guard column packing material was estimated to have a V_t and V_o of 1.3 and 0.7 ml, respectively.

Polystyrene standards were analysed on these two sets of packing material. Plots of elution volume versus molecular hydrodynamic diameter are shown in Figure 6. Equation (14) and the individual packing material parameters were used to construct the curves shown in Figure 6. The calculated calibration curves very closely predict the elution volumes experimentally obtained for the

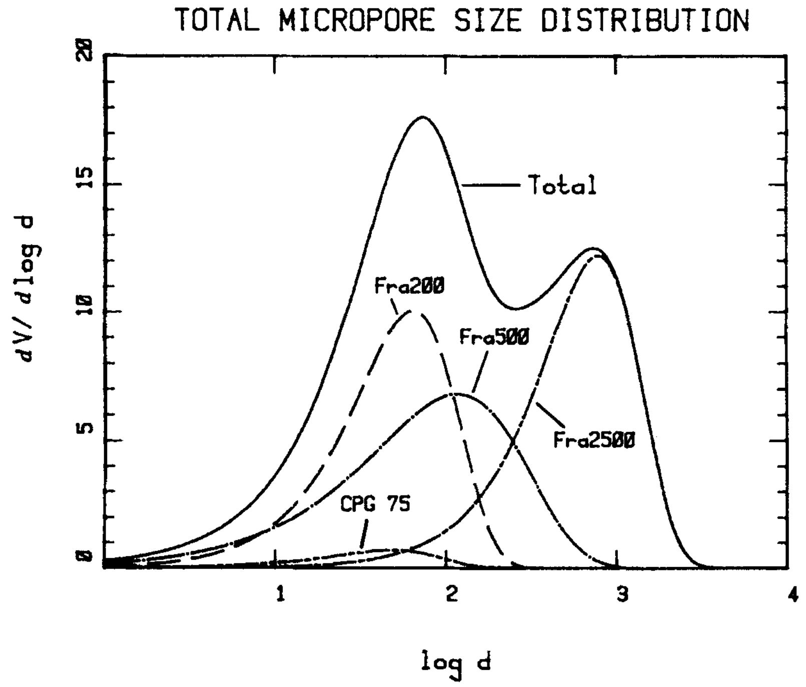

Figure 7. Combined and individual log normal size distribution curves for the Fractosil packing materials used in Figure 6.

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polystyrene molecules. Thus, the validity of equation (14) appears to be experimentally confirmed.

Equation (8) can be used to construct both a micropore volume distribution curve for individual packing materials and the total micropore volume distribution curve resulting from the combination of several individual packing materials. The same arguments used to develop equation (14) can be used to show that for P packing materials, connected in series, the total micropore volume distribution function is given by equation (15).

$$\frac{\partial V}{\partial \ln d} = \sum_{i=1}^{P} V_{pi}\, n_i \left(\frac{d}{\overline{D}_i}\right)^{n_i} \Bigg/ \text{EXP}\left[(d/D_i)^{n_i}\right] \tag{15}$$

Figure 7 shows each of the individual packing micropore volume distributions and the total micropore volume distribution for the set of four Fractosil packing materials associated with Figure 6. Figure 7 gives a visual description of how the total micropore volume is affected by adding the individual packing materials together. Together, equations (14) and (15) can be used to estimate the calibration curves and micropore volume distribution for any combination of packing materials. In addition, without extensive experimentation, both SEC separation sensitivity, $\partial V/\partial d$, and range of separation can be closely determined for any combination of packing materials.

CONCLUSION

Packing materials for size exclusion chromatography (SEC) can be calibrated for molecular size separation capabilities by using a four parameter modified Weibull distribution function. The four parameters are the interstitial volume, the total micropore volume, the most dominant pore entry size, and the micropore volume distribution shape factor. The last two parameters and the total micropore volume divided by weight of the packing material are intrinsic properties of rigid packing materials. The same parameters are not constant for swellable gel packing materials because the gel pore geometry and volume distribution vary with changes in

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solvent. However, these packing parameters should be constant for each type solvent. The interstitial volume is not an intrinsic property for any type packing material because this volume is dependent upon the efficiency used to pack material into a column.

The most dominant pore entry size appears to be approximately equal to the ratio of micropore volume to surface area. The distribution shape factor ranges between 1.0 and 1.5 and is related to the shape of the micropore volume distribution. A larger shape factor indicates a more narrow micropore size distribution. A narrow micropore size distribution reflects that a packing material has more sensitivity in separating molecules which have a hydrodynamic size close to the most dominant micropore entry size. However, a decrease in sensitivity exists at larger and smaller pore entry sizes.

If several individual columns, each composed of a single packing material, have been calibrated using the Weibull function, then an SEC calibration can be estimated, without experimentation, for any multiple column set containing any combination of the individual packing materials. The above capabilities of the calibration technique shows that it is extremely powerful and accurate.

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NOMENCLATURE

A	Constant, used in the Grubisic SEC calibration, see equation (1).
B	Constant, used in the Grubisic SEC calibration, see equation (1).
D	Average micropore diameter, used in the Dawkins SEC calibration, see equation (6).

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D	Most dominant micropore entry size, used in the Weibull SEC calibration, see equation (8).
D_c	Cylindrical micropore cavity diameter, used in the Cassassa SEC calibration, see equation (4).
D_p	Micropore cavity size defined to be the separation distance between two parallel planes, used in the Cassassa SEC calibration, see equation (5).
D_s	Spherical micropore cavity diameter, used in the Cassassa SEC calibration, see equation (3).
d	Molecular hydrodynamic diameter, see equation (11).
d_o	Smallest molecular diameter which cannot penetrate into any of the packing micropore volume, see equation (6).
g	Overall goodness of fit, defined as the standard error expressed as a percentage of the average experimental values, see equation (12).
K	Distribution coefficient, the fraction of the micropore volume that is penetrated by a molecule.
k	Proportionality constant, used in equation (11).
ℓ	Molecular external length, used in the Giddings SEC calibration, see equation (2).
M	Molecular weight.
N	Number of data points, see equation (12).
n	Micropore volume distribution shape factor, see equation (8).
P	Number of packing materials used in an SEC column set, see equation (13).
s	Micropore surface area per unit mass, see equation (2).
V_{ei}	Experimental elution volume for data point i, see equation (12).
$\hat{V}_{ei}$	Elution volume of data point i which eas estimated from a function, see equation (12).
V_o	Total interstitial volume, see equation (9).
V_p	Total micropore volume, see equation (9).
V_t	Total permeation volume, see equation (9).

$[\eta]$ Molecular intrinsic viscosity, see equation (11).

ν Micropore volume per unit mass, see equation (2).

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