

Size Exclusion Chromatography Molecular Weight Separation and Column Dispersion

Simultaneous Calibration with Characterized Polymer Standards

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With the aid of the theoretical relationship between the calibration relation of a SEC column for the monodisperse polymer species under ideal working conditions and the effective relations between the molecular weight and the elution volume for characterized polymer samples, a computational procedure for simultaneous calibration of molecular weight separation and column dispersion is proposed. From the experimental chromatograms of narrow MWD polystyrene standards and broad MWD 1,2-polybutadiene fractions the spreading factors of a SEC column was deduced by the proposed method. The variation of the spreading factor with the elution volume is independent upon the polymer sample used.

A number of computer searching methods for estimating the molecular weight calibration curve of SEC with characterized polydisperse polymer standards had been proposed (1-9). Recently it has been shown that the calibration curve for a SEC column and the calculated effective relation or experimental relation between the molecular weight and the elution volume for a sample are quite different (10,11) and it is possible to estimate the molecular weight calibration curve and the spreading factor simultaneously by coupling SEC with LALLS (12). In this paper, a simple digital searching method is proposed for calibrating the molecular weight separation and column dispersion of a SEC column simultaneously with characterized polymer samples.

Theory

The molecular weight calibration function $M(V_R)$ of a SEC column may be defined as the relationship between the molecular weights of the monodisperse polymer species and their retention volume V_R under ideal working condition, i.e. in the absence of instrumental spreading effect. It is unique for a given column and the true weight and number average molecular weight of any polydis-

0097-6156/84/0245-0125\$06.00/0
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perse polymer sample may be calculated by definition as

$$\langle M \rangle_w = \int W(V_R) M(V_R) dV_R \quad (1)$$

$$\langle M \rangle_n = 1 / \int (W(V_R) / M(V_R)) dV_R \quad (2)$$

where $W(V_R)$ is the true chromatogram of the sample. In a real SEC column, the experimental chromatogram $F(V)$ of a sample is broadened by the instrumental spreading effect and the molecular weights calculated by Equation 1 and 2 using $F(V)$ instead of $W(V_R)$ differ from the true values. We may define an effective relation between the molecular weight and elution volume $M^*(V)$ so that the true average molecular weights also satisfy the following relations:

$$\langle M \rangle_w = \int F(V) M^*(V) dV \quad (3)$$

$$\langle M \rangle_n = 1 / \int (F(V) / M^*(V)) dV \quad (4)$$

The effective relation $M^*(V)$ is not unique to a given SEC column but varies with samples and also differs from the calibration relation $M(V_R)$.

For a linear SEC column, the monodisperse calibration relation and the effective relations may be represented by

$$M(V_R) : \quad \ln M = A_m - B_m V_R \quad (5)$$

$$M^*(V) : \quad \ln M = A_m^* - B_m^* V \quad (6)$$

respectively. By using the results of the moment analysis of Tung's integral equation of instrumental spreading (13), the effective relation of a polydisperse sample may be written as (10,11)

$$M^*(V) : \quad \ln M = (A_m - (1 - \xi) B_m \bar{V}) - \xi B_m V \quad (7)$$

where \bar{V} is the mean elution volume of $F(V)$ and ξ is a parameter defined as

$$\xi^2 = (\sigma_F^2 - \langle \sigma_0^2 \rangle) / \sigma_F^2 \quad (8)$$

in which σ_F^2 is the variance of $F(V)$ and $\langle \sigma_0^2 \rangle$ is the average spreading factor of the polydisperse sample exerted on the column as expressed by

$$\langle \sigma_0^2 \rangle = \int W(V_R) \sigma_0^2(V_R) dV_R$$

The spreading factor σ_0^2 is the variance of the chromatograms of the monodisperse polymer species, i.e. of the instrumental spreading function $G(V, V_R)$. If σ_0^2 varies linearly with the retention volume of the monodisperse polymer, then $\langle \sigma_0^2 \rangle$ is numerically equal to the interpolated value $\sigma_0^2(\bar{V})$ of the function $\sigma_0^2(V_R)$ for the polydisperse sample at its mean elution volume.

It can be seen from Equation 5 and 7 that the effective rela-

tion $M^*(V)$ of a sample crosses with the unique calibration relation of the column at the mean elution volume \bar{V} of that sample. After the effective relations of several samples have been deduced, the molecular weight of each sample at its crosspoint may be calculated by Equation 6 and the line connecting all the crosspoints is just the calibration relation $M(V_R)$ of the column. The calibration relation may be linear or otherwise nonlinear. For the latter case the coordinates of the crosspoints may be fitted by a polynomial and then Equation 5 should be regarded as the tangent line of the polynomial which varies with the mean elution volume of the sample.

Comparing the coefficients of Equation 6 with that of Equation 7, we get

$$A_m^* = A_m - (1 - \xi) B_m \bar{V} \quad (9)$$

$$B_m^* = \xi B_m \quad (10)$$

The parameter ξ of a sample could be deduced from the slope or intercept of the effective relation and the calibration relation or its tangent and thereafter the spreading factor $\langle \sigma_0^2 \rangle$ could be determined from ξ by Equation 8.

With the procedure outlined above, simple programs of programmable calculator (TI 59) and microprocessor (Z80) for finding $M(V_R)$ and $\sigma_0^2(V_R)$ were written. The mean elution volume and total variance of the experimental chromatograms of well characterized polymer samples are first calculated according to

$$\bar{V} = \sum H_i V_i / \sum H_i \quad (11)$$

$$\sigma_T^2 = \sum H_i V_i^2 / \sum H_i - \bar{V}^2 \quad (12)$$

where H_i is the height of the chromatogram at elution volume V_i .

Next the coefficients of the effective relation of each sample with known weight and number average molecular weight are evaluated by iteration. Combining Equation 3, 4 and 6, the average molecular weights and inhomogeneity index may be expressed as

$$\langle M \rangle_w = \text{Exp}(A_m^*) \sum H_i \text{Exp}(-B_m^* V_i) / \sum H_i \quad (13)$$

$$\langle M \rangle_n = \text{Exp}(A_m^*) \sum H_i / \sum H_i \text{Exp}(B_m^* V_i) \quad (14)$$

$$\begin{aligned} D &= \langle M \rangle_w / \langle M \rangle_n \\ &= (\sum H_i \text{Exp}(B_m^* V_i)) (\sum H_i \text{Exp}(-B_m^* V_i)) / (\sum H_i)^2 \end{aligned} \quad (15)$$

Putting

$$f(B_m^*) = (\sum H_i \text{Exp}(B_m^* V_i)) (\sum H_i \text{Exp}(-B_m^* V_i)) - D (\sum H_i)^2 \quad (16)$$

and taking the first derivative

$$f'(B_m^*) = \left(\sum H_i V_i \exp(B_m^* V_i) \right) \left(\sum H_i \exp(-B_m^* V_i) \right) - \left(\sum H_i \exp(B_m^* V_i) \right) \left(\sum H_i V_i \exp(-B_m^* V_i) \right) \quad (17)$$

the coefficient B_m^* could be evaluated by the Newtonian iteration formula

$$B_m^*(k+1) = B_m^*(k) - f(B_m^*)/f'(B_m^*) \quad (18)$$

with the known inhomogeneity index and the experimental chromatogram of the sample using

$$\mathcal{E} \geq B_m^*(k+1) - B_m^*(k) \quad (19)$$

as the objective function. Coefficients A_m^* are then evaluated by substituting B_m^* into Equation 13 or 14.^m

The third step of computation is to find $M(V_R)$ by linear regression or polynomial fitting after the crosspoint coordinates of all of the samples have been evaluated.

The fourth step is to estimate the parameter ξ and the spreading factor by Equation 9, 10 and 8, taking $\sigma_0^2(V)$ as an approximation of function $\sigma_0^2(V_R)$.

Experimental

Six commercial narrow MWD polystyrene standards (Applied Research Laboratories Limited, England) and five broad MWD 1,2-polybutadiene fractions were used to calibrate an ARL 950 GPC instrument with silica bead packed columns. The ARL polystyrene standards were also used to calibrate a number of home-made SEC column units packed with silica beads or styrene-divinylbenzene copolymer beads. The average molecular weights of these samples are listed in Table I. Tetrahydrofuran or toluene was used as eluents for these columns.

Table I. The Average Molecular Weight and SEC Data of Polystyrene and 1,2-Polybutadiene

Polymer		$\langle M \rangle_w 10^{-5}$	$\langle M \rangle_n 10^{-5}$	\bar{V}	σ_1^2
Polystyrene	A1	0.04	0.037	166.4	17.8
	A2	0.10	0.093	157.9	18.5
	A3	0.204	0.198	151.5	17.9
	A4	1.1	1.09	140.4	15.8
	A5	3.9	3.85	132.2	17.0
	A6	28.6	24.7	115.0	42.5
Polybutadiene	S1A	2.33	1.60	133.9	46.4
	S1	6.46	3.10	127.3	79.2
	S2	10.7	6.41	120.4	61.0
	S3	13.4	8.69	117.9	55.5
	S4	21.2	15.1	114.8	47.3

Results and Discussion

The mean elution volume and total variance calculated from the experimental chromatograms of polystyrene and 1,2-polybutadiene on the ARL 950 GPC instrument are also listed in Table I. The coefficients of the effective relation, coordinates of the cross-point, parameter ξ and spreading factor were computed by the scheme outlined above. The results obtained are listed in Table II and III. The effective relations and calibration

Table II. The Calculated Coefficients of the Effective Relation

Polymer		A_m^*	B_m^*	$M^*(\bar{V})10^{-5}$
Polystyrene	A1	19.19	0.0657	0.038
	A2	19.42	0.0649	0.096
	A3	16.08	0.0407	0.201
	A4	14.98	0.0241	1.10
	A5	16.51	0.0276	3.88
	A6	21.61	0.0593	26.6
Polybutadiene	S1A	24.29	0.0906	1.92
	S1	25.53	0.0984	4.49
	S2	24.76	0.0925	8.30
	S3	24.41	0.0892	10.8
	S4	24.18	0.0853	17.8

Table III. The Calculated Results of the Parameter ξ and the Spreading Factor

Polymer		ξ		σ^2	
		S	I	S	I
Polystyrene	A1	0.500	0.505	13.4	13.3
	A2	0.494	0.489	14.0	14.1
	A3	0.310	0.300	16.2	16.3
	A4	0.183	0.185	15.3	15.3
	A5	0.210	0.233	16.3	16.2
	A6	0.450	0.444	33.9	34.1
Polybutadiene	S1A	0.816	0.814	15.4	15.5
	S1	0.886	0.893	17.0	16.1
	S2	0.833	0.829	18.6	19.1
	S3	0.804	0.799	19.6	20.1
	S4	0.769	0.775	19.3	18.9

S: from slope, I: from intercept.

relation for polystyrene and 1,2-polybutadiene are shown in Figure 1 and 2 respectively. The coefficients of the calibration relation obtained by linear regression are $A_m = 30.30$, 27.05 and $B_m = 0.1316$, 0.1110 for polystyrene and 1,2-polybutadiene respectively. The variation of spreading factor with elution volume derived from polystyrene and 1,2-polybutadiene are coincident and in accord with that obtained by the method of coupling GPC with LALLS for the same column (12) as shown in Figure 3.

The uncertainty of the calculated spreading factor $\Delta(\sigma_o^2)$ depends upon the accuracy of the inhomogeneity index of the sample and that of the total variance of experimental chromatogram. It may be expressed as

$$\Delta(\sigma_o^2) = (\partial \sigma_o^2 / \partial \sigma_T^2) \Delta(\sigma_T^2) - (\partial \sigma_o^2 / \partial D) \Delta D. \quad (20)$$

If the experimental chromatogram is Gaussian, the spreading factor could be represented by

$$\sigma_o^2 = \sigma_T^2 - \ln D / B_m^2 \quad (21)$$

Substituting its partial derivatives into Equation 20 we have

$$\Delta(\sigma_o^2) = \Delta(\sigma_T^2) - (1 / B_m^2) (\Delta D / D) \quad (22)$$

Thus the absolute error of the calculated spreading factor depends upon the absolute uncertainty of total variance, the slope of the calibration curve and the relative uncertainty of the inhomogeneity index $\Delta D/D$ of the sample. Equation 22 was verified by arbitrarily changing the inhomogeneity index of polystyrene standards, recalculating the spreading factor with the same computing program and plotting the deviation $\Delta(\sigma_o^2)$ versus $\Delta D/D$ as shown in Figure 4. Therefore, if the standard sample was well characterized, the error of the calculated spreading factor is mainly caused by the uncertainty of the total variance. It is shown in Figure 3 that the spreading factor derived from polystyrene sample A6 is much larger than others'. It is probably caused by the larger total variance due to the greater extent of adsorption of high molecular weight polystyrene on the porous silica gel.

It is interesting to examine the effect of the molecular weight of polymer and the role of the packing material on the spreading factor. The ARL polystyrene standards were used to determine the spreading factor of a number of SEC units packed with styrene-divinylbenzene copolymer gel (SDG) or silica gel (SG) beads using tetrahydrofuran or toluene as eluent by the present method. The data cannot be compared directly because the volumes of the siphon tubes and columns of these SEC units are different. But if the relative value of spreading factor i.e. the ratio of the spreading factor of the standards to that with lowest molecular weight (A1) is considered, some interesting features could be realized as shown in Figure 5 in which the relative spreading factor is plotted as a function of molecular weight. The molecular weight dependency of the spreading factor, in other words the restricted diffusion of the macromolecule in the pore is much pronounced for styrene-divinylbenzene copolymer gel.

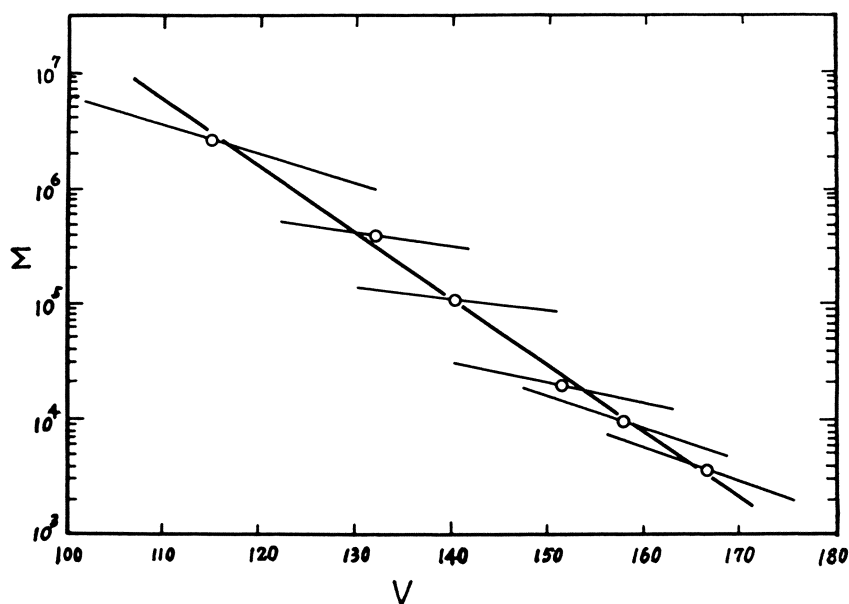


Figure 1. The calibration relation $M(V_R)$ and effective relations $M^*(V)$ of narrow MWD polystyrene standards.

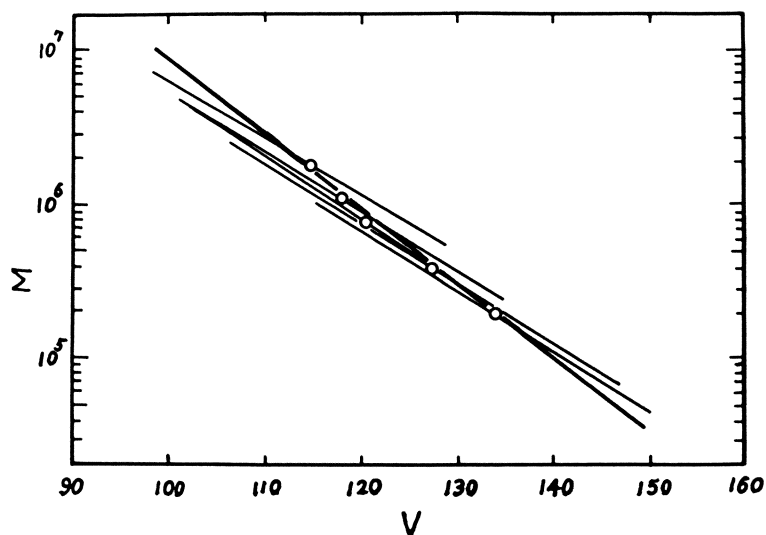


Figure 2. The calibration relation $M(V_R)$ and effective relations $M^*(V)$ of broad MWD 1,2-polybutadiene fractions.

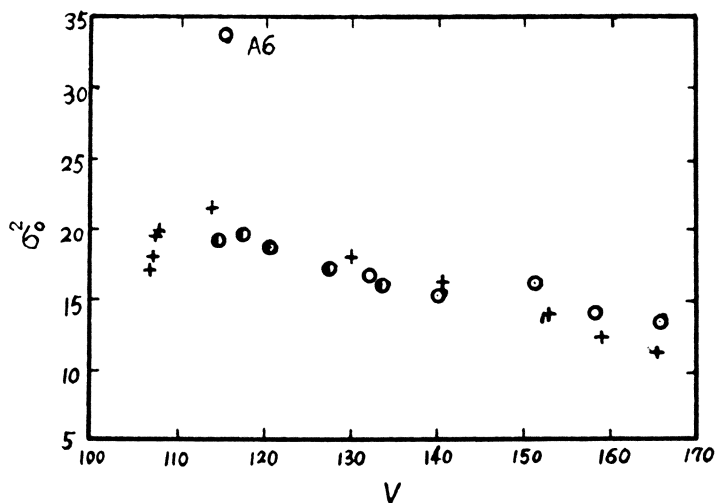


Figure 3. Variation of the spreading factor with the elution volume. Key: \circ , PS; \bullet , PB, $+$, GPC-LALLS, PS.

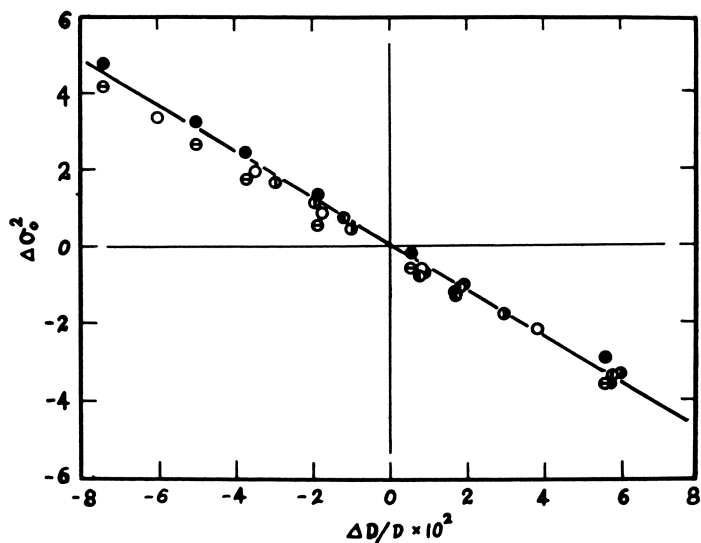


Figure 4. Dependency of the uncertainty of spreading factor on the relative error of inhomogeneity index. Key: \circ , A1; \bullet , A2; \circ , A3; \bullet , A4; \circ , A5; and \bullet , A6.

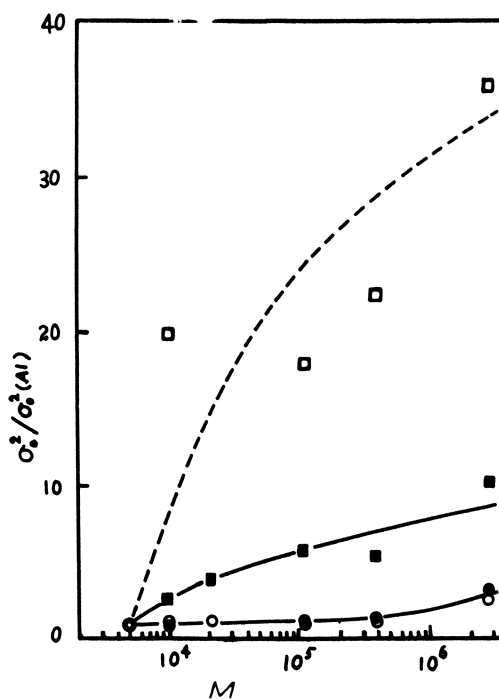


Figure 5. Variation of relative spreading factor with the molecular weight of polystyrene standards. Key: \circ \bullet SG, SEC unit 1 and 2; \square \blacksquare SDG, SEC unit 3 and 4.

It is believed that the surface structure of the porous packing material plays an important role. The presence of the free chain ends of styrene-divinylbenzene copolymer may prevent the movement of the macromolecules in the pore.

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RECEIVED September 12, 1983