Simple Methods of Gel Permeation Chromatography Calibration Developed from One or Two Polydisperse Polymer Standards

PAWEL SZEWCZYK,* *Polish Academy of Sciences, Institute of Polymer Chemistry, ul.M.Curie-Sklodowskiej 34, 41-800 Zabrze, Poland*

Synopsis

Applying the formalism of averaged elution volumes corresponding to average molecular weights or intrinsic viscosities of polydisperse polymer standards simple, single-valued equations were developed for iterative evaluation of calibration parameters of both direct and universal GPC calibration relationships. The developed evaluation procedures are simpler and converge faster than corresponding methods described in the literature.

INTRODUCTION

In order to interpret a gel permeation chromatograph (GPC) of any polymer sample in terms of its molecular weight distribution, it is necessary to establish a quantitative relationship between molecular weight M and elution volume V. It is well-known that most that are appropriate for the GPC calibration purposes are well-characterized narrow distribution samples. They are, however, difficult to prepare with the exception of polystyrene, for which good standards are readily available. The calibration established for polystyrene is not directly usable for other polymers, therefore, methods have been proposed for correlating calibrations determined for different polymers. Most of the approaches are trial and error methods in which least-squares minimization procedures are used for either linear or nonlinear relations.[1-14] The methods differ in mathematical and numerical details and in the amount of computation that must be done in order for the calibration parameters to be determined.

It seems that in the field of direct V–M calibrations for any polymer, the linear calibration method based on a single polydisperse standard, as described by Loy,[9] is simple and promising. In case of the widely used universal calibration approach of Benoit et al.,[15,16] the method proposed by Weiss and Cohn-Ginsberg[5] fulfills the requirements of accuracy and simplicity best. Another simple and generally applicable algorithm for a trial and error GPC calibration method was recently proposed,[7] and its usefulness in case of unimodal standards of any polydispersity and for polynomial model of the calibration relationship was presented.[14] In this method a concept of averaged elution volumes corresponding to either number-average $\overline{M}_n$, to weight-average $\overline{M}_w$, or to viscosity-average $\overline{M}_v$ molecular weight was introduced.

Based on the developed procedures, in this work the methods of Loy[9] and of Weiss and Cohn-Ginsberg[5] are improved as far as the simplicity and convergence

* Present address: Institute of Plastics and Paints Industry, ul. Chorzowska 50, 44-101 Gliwice, Poland.

of the iterative procedures are concerned. The functional dependence of the calibration parameters on corresponding iterative variables is shown to be monotonic. Finally, the results of application of the developed calibration methods for samples of polyvinyl chloride and polypropylene oxide are presented.

DIRECT ELUTION VOLUME—MOLECULAR WEIGHT CALIBRATION

The true V–M relationship for a given set of GPC columns is assumed to be adequately described by a polynomial of degree n of the variable $\log M$ so that

$$V = f(\log M) = \sum_{i=0}^{n} c_i (\log M)^i \tag{1}$$

where c_i is the coefficient of the polynomial.

Experimental values of the elution volumes are limited always to an interval in which the $f(\log M)$ polynomial is a monotonic function, and the function reciprocal to $f(\log M)$ exists in this interval. $\log M$ is then given by

$$\log M = f^{-1}(V) = \sum_{i=0}^{n} d_i V^i \tag{2}$$

where d_i are the coefficients of the reciprocal polynomial.

Averaged elution volumes $\overline{V}$ corresponding to average molecular weights $\overline{M}$ are defined by $\overline{V} = f(\log \overline{M})$.

Using eqs. (1) and (2), and taking advantage of the definitions of average molecular weights, the following specific forms of the defining equations of the corresponding averaged elution volumes may be derived[7]

$$\overline{V}_1 = f(\log \overline{M}_n) = f\left(-\log\left[\sum_i w_i 10^{-f^{-1}(V_i)}\right]\right) \tag{3}$$

$$\overline{V}_2 = f(\log \overline{M}_w) = f\left(\log\left[\sum_i w_i 10^{f^{-1}(V_i)}\right]\right) \tag{4}$$

$$\overline{V}_3 = f(\log \overline{M}_v) = f\left(1/a \log\left[\sum_i w_i 10^{af^{-1}(V_i)}\right]\right) \tag{5}$$

where the expressions in parentheses denote the corresponding variables of functions f, w_i are weight fractions of polymer species with elution volumes V_i, and a is the exponent in the empirical Mark–Houwink–Sakurada relationship between the limiting viscosity number $[\eta]$ and molecular weight M,

$$[\eta] = KM^a \tag{6}$$

In case of a set of columns with appropriately chosen distribution of pores the calibration curve may be described as follows[9,17]

$$V = C_1 - C_2 \log M, \qquad C_1, C_2 > 0 \tag{7}$$

Let us consider a polymer sample with a broad molecular weight distribution and measured values of $\overline{M}_n$ and $\overline{M}_w$. Assuming that, for the used set of columns, eq. (7) describes the calibration relationship well, parameter C_2 may be determined from the equation derived by Loy[9]

$$\overline{M}_w/\overline{M}_n = \left(\sum_i w_i 10^{-V_i/C_2}\right)\left(\sum_i w_i 10^{V_i/C_2}\right) \tag{8}$$

The right-hand side eq. (8) should be evaluated many times for different values of C_2 until such a C_2 is found for which the numerical difference between both sides of eq. (8) is lower than an arbitrarily chosen small number.   Finally, from an arbitrary C_1 a proper C_1 is obtained by iterating the expression

$$C_1 + C_2 \log \left(\overline{M}_w \middle/ \sum_i w_i 10^{(C_1 - V_i)/C_2} \right)$$

until

$$\left| \overline{M}_w - \sum_i w_i 10^{(C_1 - V_i)/C_2} \right| \leq 10$$

The procedure of Loy may be improved when the concept of averaged elution volumes is applied.   Assuming that the linear calibration holds, one may write the following set of linear equations

$$\begin{aligned} \overline{V}_1 &= C_1 - C_2 \log \overline{M}_n \\ \overline{V}_2 &= C_1 - C_2 \log \overline{M}_w \end{aligned} \tag{9}$$

Equations (9) may be easily solved for C_1 and C_2, namely,

$$C_2 = (\overline{V}_1 - \overline{V}_2)/\log(\overline{M}_w/\overline{M}_n) \tag{10}$$

and

$$C_1 = \overline{V}_1 + C_2 \log \overline{M}_n = \overline{V}_2 + C_2 \log \overline{M}_w \tag{11}$$

where [compare eqs. (3) and (4)]

$$\overline{V}_1 = C_2 \log \left(\sum_i w_i 10^{V_i/C_2} \right) \tag{12}$$

$$\overline{V}_2 = -C_2 \log \left(\sum_i w_i 10^{-V_i/C_2} \right) \tag{13}$$

Equation (10) can be used as a very simple iterative relation for the determination of the parameter C_2 if the functional dependence of C_2 on the difference $\overline{V}_1 - \overline{V}_2$ is monotonic.   The symbol C_2, which occurs in the defining eqs. (12) and (13), should be considered as the iterative variable.

For an assumed value of C_2, $\overline{V}_1$, and $\overline{V}_2$ are calculated from the chromatogram by eqs. (12) and (13).   Then a corrected value of C_2 is calculated by eq. (10).

In order to illustrate how the $\overline{V}_1 - \overline{V}_2$ difference behaves as a function of the iterative variable C_2, in Figure 1 a corresponding plot was made for chromatogram of a polyvinyl chloride sample that was published by Balke et al.[4]   As can be seen in Figure 1, the $\overline{V}_1 - \overline{V}_2$ vs. C_2 plot is of a hyperbolic character what proves, in this particular case, the desired monotonicity of the iterative relation, eq. (10).

Let the symbol $C_{2,t}$, marked on the abcissa axis of Figure 1, correspond to the true value of the C_2 parameter.   Starting the iterative calculations, say, with a C_2 value greater than $C_{2,t}$ a difference $\overline{V}_1 - \overline{V}_2$ will be evaluated which will be smaller than the difference $(\overline{V}_1 - \overline{V}_2)_t$, marked on the ordinate axis of Figure 1, and which corresponds to $C_{2,t}$.   Thus, in the next iteration step value of C_2 evaluated from eq. (10) will be too small.   It is obvious, that in order to speed up the convergence of the iteration procedure, in every iteration step with the exception of the initial one, the temporary value of the iterative variable C_2 should be taken as equal to, for example, the arithmetic average of the proceeding value of this variable and of value of C_2 just evaluated from eq. (10).
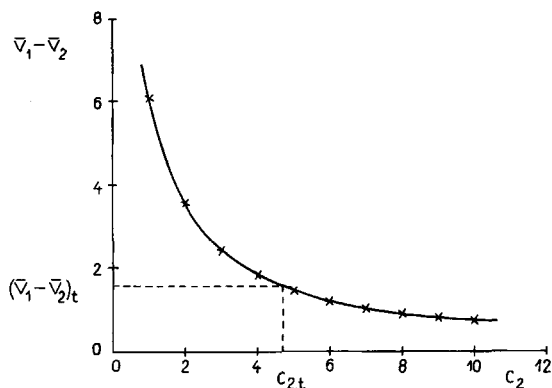
Fig. 1. Direct GPC calibration. Illustration of the functional dependence of the calibration parameter on iterative variable [compare eq. (10)]. Plot constructed for a PVC sample [data of Balke et al. (ref. 4)].

UNIVERSAL CALIBRATION

Benoit et al.[15,16] showed that the GPC calibration of many random coil polymers could be correlated by the product $[\eta]M$, which is a measure of the hydrodynamic volume of the polymeric chain in solution. Assuming that for a given set of columns the calibration is adequately described by a polynomial, one can write

$$V = g(\log[\eta]M) = \sum_{i=0}^{n} a_i(\log[\eta]M)^i \qquad (14)$$

where a_i is the coefficient and n is the degree of the polynomial g. In the typical working range of values of the product $[\eta]M$, polynomial g is a monotonic function of $\log[\eta]M$, therefore, in this range, a function reciprocal to the function g exists and can be written as follows

$$\log[\eta]M = g^{-1}(V) = \sum_{i=0}^{n} b_i V^i \qquad (15)$$

where b_i are coefficients of the reciprocal polynomial.

Using polystyrene narrow standards to determine the function $g^{-1}(V)$, the calibration relationship for any polymer may be obtained if in the GPC solvent the K and a parameters of the Mark–Houwink–Sakurada relationship, eq. (6), are known for this polymer. Namely, from eqs. (15) and (6) one can derive the following expressions

$$\log M = [g^{-1}(V) - \log K]/(a + 1) \qquad (16)$$

and

$$\log[\eta] = [ag^{-1}(V) + \log K]/(a + 1) \qquad (17)$$

When, for the analyzed polymer, the K and a parameters are not known, they may be evaluated by different procedures described in the literature.[5,8,11] Weiss and Cohn-Ginsberg[5] proposed the simplest method in which either one sample with measured values of $[\eta]$ and $\overline{M}_n$ or two samples with known values of $[\eta]$ are required. Using the notations of the present paper the corresponding Weiss et al., iterative relations for the determination of the parameter a are as follows

$$[\eta]\overline{M}_n = \sum_i w_i 10^{ag^{-1}(V_i)/(a+1)} \Big/ \sum_i w_i 10^{-g^{-1}(V_i)/(a+1)} \tag{18}$$

and

$$[\eta]_1/[\eta]_2 = \sum_i w_{1i} 10^{ag^{-1}(V_i)/(a+1)} \Big/ \sum_i w_{2i} 10^{ag^{-1}(V_i)/(a+1)} \tag{19}$$

where the subscripts 1 and 2 in eq. (19) denote quantities corresponding to samples 1 and 2, respectively.

Determination of parameter a requires the application of a trial and error method. Repetitive evaluation of the right-hand side of the corresponding equation using different values of a can be made until, for an appropriate value of a, numerical values of both sides of this equation differ less than required by the assumed accuracy of determination.

Application of averaged elution volumes simplifies the procedure of Weiss et al. Assuming that the Mark–Houwink–Sakurada relationship holds in the whole working range of molecular weights, eqs. (16) and (17) constitute a set of linear equations with two unknown parameters K and a.

After rearrangements the above mentioned equations can be written as follows

$$\begin{aligned} \log K + (a+1)\log M &= g^{-1}(V) \\ (-1/a)\log K + (a+1)/a \log[\eta] &= g^{-1}(V) \end{aligned} \tag{20}$$

Equations (20) are true for a monodisperse polymer sample, M and $[\eta]$ being its molecular weight and limiting viscosity number, respectively. According to the adopted formalism,[7,14] the quantities M, $[\eta]$, and V in eqs. (20) should be replaced by $\overline{M}$, $[\overline{\eta}]$, and $\overline{V}$, respectively, when a polydisperse polymer standard is considered. The definitions of averaged elution volumes $\overline{V}$ depend on the type of average molecular weight and average intrinsic viscosity. It was found empirically that $[\overline{\eta}]$ is, for polydisperse polymer samples, a weight-average quantity,[18] i.e.,

$$[\overline{\eta}] = \sum_i w_i[\eta]_i = [\overline{\eta}]_w \tag{21}$$

where w_i is the weight fraction of the polymer species with intrinsic viscosities equal to $[\eta]_i$.

Considering one polydisperse sample with measured values of $\overline{M}_n$ and of $[\overline{\eta}]$ = $[\overline{\eta}]_w$, the following set of linear equations is obtained

$$\begin{aligned} \log K + (a+1)\log \overline{M}_n &= g^{-1}(\overline{V}_1) \\ (-1/a)\log K + (a+1)/a \log[\overline{\eta}]_w &= g^{-1}(\overline{V}_4) \end{aligned} \tag{22}$$

where the averaged elution volumes $\overline{V}_1$ and $\overline{V}_4$ correspond to $\overline{M}_n$ and $[\overline{\eta}]_w$, respectively. Their defining formulae can be derived from eq. (14) and the definitions of $\overline{M}_n$ and $[\overline{\eta}]_w$. They are as follows

$$\overline{V}_1 = g[\log K + (a+1)\log \overline{M}_n] = g\left[-(a+1)\log\left(\sum_i w_i 10^{-g^{-1}(V_i)/(a+1)}\right)\right] \tag{23}$$

$$\overline{V}_4 = g((-1/a)\log K + (a+1)/a \log[\overline{\eta}]_w) =$$
$$= g\left[(a+1)/a \log\left(\sum_i w_i 10^{ag^{-1}(V_i)/(a+1)}\right)\right] \tag{24}$$

where the expressions in brackets denote the corresponding variables of function g.

The set of eqs. (22) can be solved for a and K giving the following simple, iterative relations for determination of the parameters

$$a = [g^{-1}(\overline{V}_1) - \log([\overline{\eta}]_w\overline{M}_n)]/[\log([\overline{\eta}]_w\overline{M}_n) - g^{-1}(\overline{V}_4)] \tag{25}$$

$$\log K = g^{-1}(\overline{V}_1) - (a + 1)\log \overline{M}_n \qquad (26)$$

Alternatively, in order to determine the parameters K and a, one may use two polydisperse samples having different intrinsic viscosities $[\overline{\eta}]_1$ and $[\overline{\eta}]_2$. Applying the averaged elution volumes the following equations (27) can be obtained

$$\begin{array}{l}
(-1/a)\log K + (a + 1)/a \log [\overline{\eta}]_1 = g^{-1}(\overline{V}_{4,1}) \\
(-1/a)\log K + (a + 1)/a \log [\overline{\eta}]_2 = g^{-1}(\overline{V}_{4,2})
\end{array} \qquad (27)$$

The corresponding iterative relations for determination of the parameters a and K, derived from eqs. (27), are as follows

$$a = \log([\eta]_1/[\eta]_2)/[g^{-1}(\overline{V}_{4,1}) - g^{-1}(\overline{V}_{4,2}) - \log([\overline{\eta}]_1/[\overline{\eta}]_2)] \qquad (28)$$

$$\log K = a \; [\log [\overline{\eta}]_1 - g^{-1}(\overline{V}_{4,1})] + \log[\overline{\eta}]_1 \qquad (29)$$

where $\overline{V}_{4,1}$ and $\overline{V}_{4,2}$ denote the averaged elution volumes $\overline{V}_4$ corresponding to $[\overline{\eta}]_1$ and $[\overline{\eta}]_2$, respectively.

Both iterative equations (25) and (28) can be applied for determination of the parameter a using a numerical procedure similar to that described earlier for determination of the parameter C_2 according to eq. (10).

COMMENTS ON MONOTONICITY OF THE DERIVED ITERATIVE RELATIONS

It was shown in Figure 1 that for a polydisperse polyvinyl chloride sample the functional dependence of the calibration parameter C_2 on the corresponding iteration variable was monotonic when a linear GPC calibration, eq. (7), was considered. Assuming that the chromatogram of a polydisperse standard sample may be approximated by a Gaussian curve, it may be generally demonstrated for linear calibration relationships that in case of all iterative relations, derived in this work, the functional dependence of the calibration parameters C_2 or a [compare eqs. (10), (25), and (28)] on the corresponding iteration variables is monotonic. The respective functional dependence may be described by a homographic function (rectangular hyperbola) (see Appendix).

Nonlinearity of a typical GPC calibration curve is limited to relatively narrow boundary regions of the range of molecular weights corresponding to low and high values of M. On the other hand, sets of GPC columns are selected in such a way that molecular weights of samples to be analyzed cover mainly central parts of the working range of elution volumes. It may be concluded thus quite generally that the iterative relations for the determination of different GPC calibration parameters, derived in this work, are monotonic. On the other hand, it is obvious that without monotonicity of the iterative relations any reproducible calibration results can not be obtained.

EXPERIMENTAL DATA AND NUMERICAL PROCEDURES

A chromatogram of a polydisperse polyvinyl chloride sample having $\overline{M}_n = 28\,200$ and $\overline{M}_w = 67\,800$ was used for determination of parameters of the direct linear calibration relation, eq. (7). Digitized data of this chromatogram could be found in the literature.[4] These data were applied in the present work in order to test the method based on the iterative equation (10).

A complete set of experimental data from studies of dilute solution properties of polypropylene oxide (PPO), made by Valles,[19] was published in a special

technical report.[20]   In this work the GPC analysis of few polydisperse PPO fractions had been made and values of $\overline{M}_n$ had been measured osmometrically. The GPC analysis had been made on a Waters model 200 instrument at 25°C, using tetrahydrofuran (THF) as the eluent and two sets of four columns each filled with styragel packings of the following average porosites: 10^6, 7×10^4, 10^4, and 3×10^3 nm (set A), and 10^4, 3×10^3, 80 and 25 nm (set B).   Tables with digitized chromatograms of the fractions had been published in the technical report.[20]   Values of $\overline{M}_n$ may be found in Table I where the original designations of Valles were cited.   Fractions numbered 1–5 had been analyzed on the first set of columns and the remaining three fractions—on the second set.   For fractions No. 1 Valles had succeeded in measuring its $\overline{M}_w$ by light scattering and the obtained result is in Table I (see footnote).   In order to test the iteration equations developed for the universal calibration, eqs. (25) and (28), $[\eta]$ values in THF at 25°C of all PPO fractions were calculated from their molecular weight distributions using the following expression

$$[\eta] = \sum_i w_i M_i^a \qquad (30)$$

where w_i was taken from the chromatograms and M_i was calculated according to the corresponding direct calibration equations (34) and (35), determined in this work for the two sets of columns.   A corresponding value of exponent a was taken from the following $[\eta]$–M relationship determined by Scholtan and Lie[21] for fractions of PPO of low molecular weights in THF at 25°C

$$[\eta] = 5.5 \times 10^{-2} M^{0.62} \text{ cm}^3/\text{g} \qquad (31)$$

The calculated values of $[\eta]$ of all PPO fractions may be found in Table I.

All calculations were made on a Wang 2200B minicomputer using BASIC as the programming language.   The difference of values of the calibration parameters being determined was regularly estimated for every two consecutive iteration steps.   The calculations were stopped when the relative change of all parameters became $\sim 10^{-3}$.

TABLE I
Data for PPO

Fraction no./symbol	$10^{-3} \times \overline{M}_n$ [a]	$[\eta]$ [b] (cm³/g). THF, 25°C
1/F-1B1A[c]	922	606.9
2/F-1B1	588	510.2
3/F-1B1B	531	411
4/F-1B2	251	289
5/F-2A	232	266.1
6/F-2B	143	152.4
7/F-2C	54.2	81.9
8/F-4	5.3	19.5

[a] Taken from ref. 19.
[b] Calculated according to eq. (30).
[c] $\overline{M}_w = 2.86 \times 10^6$, from light scattering (ref. 19).

RESULTS AND DISCUSSION

Test of the Direct GPC Calibration Procedure

In one of the earliest attempts to develop methods for direct GPC calibration based on characterized polydisperse polymer standards Balke et al.[4] had proposed a nonlinear search procedure. Applying this method to a PVC sample having $\overline{M}_n$ = 28 200 and $\overline{M}_w$ = 67 800 they had determined the following calibration relationship

$$V = 48.87 - 4.36 \log M \text{ counts (1 count = 5 cm}^3) \tag{32}$$

The chromatogram and average molecular weights of the same PVC sample were used for determination of the calibration parameters applying the iterative relation, eq. (10).

Calculations were started with different initial values of the C_2 iteration variable. Results of determination of the C_1 and C_2 parameters, rounded to four digits, may be found in Table II. In order to estimate the accuracy of the calibrations evaluated in each iterative run the percentage deviations of average molecular weights were calculated according to the formula

$$\delta \overline{M} = (\overline{M}_{\text{calc}} - \overline{M}_{\text{true}}) \, 100/\overline{M}_{\text{true}} \, (\%) \tag{33}$$

where $\overline{M}_{\text{calc}}$ is the average molecular weight calculated from the chromatogram using the corresponding calibration and $\overline{M}_{\text{true}}$ is the true (measured) value of the same average molecular weight. Values of $\delta \overline{M}_n$ and $\delta \overline{M}_w$ for the used PVC sample are in Table II and, as can be seen, they are negligible for all iterative runs. Values of the parameters C_1 and C_2, placed in Table II, are almost identical with the corresponding quantities in eq. (32), the differences being of the order of 0.1%. Thus, it was demonstrated that the developed iterative method of direct GPC calibration was accurate and it secured the determining of correct calibration relationships.

Direct Calibration for Polypropylene Oxide—Optimum Calibration Relationships

GPC analyses of the polypropylene oxide fractions had been made on two different sets of columns. Applying the general calibration method based on respective averaging of elution volumes of chromatograms of the analyzed fractions[7,14] optimum linear calibration relationships were estimated. In case of the first set of columns (set A) chromatograms of 5 PPO fractions with num-

TABLE II
Results of Determination of the Linear GPC Calibration Obtained for a Single PVC Sample ($\overline{M}_n$ = 28 200, $\overline{M}_w$ = 67 800) Using eq. (10)

C_2 initial	No. of iterations	Final results		$10^4 \times \delta \overline{M}$ (%)	
		C_1	C_2	for $\overline{M}_n$	for $\overline{M}_w$
1	6	48.84	4.353	0.5	−0.3
5	3	48.84	4.353	0.3	−0.2
10	5	48.84	4.353	3.9	−2.6
15	6	48.84	4.353	0.3	−0.2

bers 1–5 (compare Table I) and 5 values of $\overline{M}_n$ as well as 1 value of $\overline{M}_w$ were simultaneously used in the calculations. The following relationship was obtained

$$V = 49.34 - 4.09 \log M \quad \text{counts} \tag{34}$$

The corresponding optimum calibration relationship for set B of columns, evaluated for three chromatograms of PPO fractions numbered 6, 7, and 8 (compare Table I) and three values of $\overline{M}_n$, was the following

$$V = 38.06 - 3.01 \log M \quad \text{counts} \tag{35}$$

The above relationships were considered as model calibration equations for the corresponding sets of columns. In order to estimate their accuracy percentage deviations of the average molecular weights of all PPO fractions were calculated according to eq. (33), and the results may be found in Table III. Results in Table III may be regarded as the optimum results available for the analyzed set of PPO data.

It was generally assumed that corrections of the instrumental spreading effects were not needed in the calculations since all PPO fractions had broad molecular weight distributions,[22] their $\overline{M}_w/\overline{M}_n$ ratios being greater than 3 (compare Table III).

Direct Calibration Evaluated for One or Two Polydisperse PPO Fractions

In case of the first PPO fraction (No. 1, F-1B1A) both $\overline{M}_n$ and $\overline{M}_w$ had been measured (compare Table I). Using these data and the chromatogram of this fraction coefficients C_1 and C_2 were evaluated according to the iterative equation (10). For any initial value of the iterative variable C_2, taken from the interval 0.5–15, number of iteration steps never exceeded six. Evaluated values of the calibration parameters are in Table IV (first row). The accuracy of the calibration relationship was estimated by calculating the mean absolute percentage deviation of the number-average molecular weights according to the following formula

$$|\delta \overline{M}_n| = 1/n \sum_{i=1}^{n} |\delta \overline{M}_n|_i \tag{36}$$

where n is the number of analyzed fractions and $|\delta \overline{M}_n|_i$ is the absolute value of the individual percentage deviation of $\overline{M}_n$ of the ith fraction.

TABLE III
Results of GPC Analysis of PPO Fractions Using Optimum Calibrations, eqs. (34) and (35)

Fraction no.	Calibration equation	$10^{-3} \overline{M}_n$	$\delta \overline{M}_n{}^a$ (%)	$10^{-3} \overline{M}_w$	$\delta \overline{M}_w{}^a$ (%)
1	34	913	1.0	3049	7.6
2	34	543.8	7.5	2461	—
3	34	483.2	9.0	1725	—
4	34	265.2	−5.7	1098	—
5	34	247.7	−6.8	938	—
6	35	140.8	−1.5	416.8	—
7	35	54.5	0.6	157.6	—
8	35	5.25	−1.0	16.7	—

^{a} Calculated according to eq. (33).

TABLE IV

Direct Linear Calibration for PPO in THF at 25°C Evaluated According to eq. (10) for $\overline{M}_n$ and $\overline{M}_w$ and According to eq. (36) for $\overline{M}_{n1}$ and $\overline{M}_{n2}$

Set of columns	Variant	Numbers of appl. fractions	Number of variants	C_1	C_2	$|\delta\overline{M}_n|$ [a] (%)	$\delta\overline{M}_w$ [b] (%)
A	$\overline{M}_n, \overline{M}_w$	1	1	50.24	4.25	6.7	0.02
	$\overline{M}_{n1}, \overline{M}_{n2}$	1–5	10	48.78	3.57	7.8	93.4
	$\overline{M}_{n1}, \overline{M}_{n2}$	1, 3	1	52.89	4.71	10.1	−19.1
	$\overline{M}_{n1}, \overline{M}_{n2}$	3, 4	1	42.18	2.65	15.1	881
B	$\overline{M}_{n1}, \overline{M}_{n2}$	6–8	3	37.96	2.98	1.4	—
	$\overline{M}_{n1}, \overline{M}_{n2}$	7, 6	1	38.14	3.03	1.1	—
	$\overline{M}_{n1}, \overline{M}_{n2}$	6, 7	1	37.69	2.93	2.7	—

[a] Calculated according to eq. (36) for all fractions analyzed on a given set of columns.

[b] Calculated for fraction No. 1 according to eq. (33).

It is trivial to modify the iterative eq. (10) for the case of two samples whose average molecular weights are known. Assuming that $\overline{M}_n$ values of two polymer samples were measured, the respective iterative equation would be as follows

$$C_2 = (\overline{V}_{1,1} - \overline{V}_{1,2})/log(\overline{M}_{n,2}/\overline{M}_{n,1}) \tag{37}$$

where the second subscript denotes either $\overline{V}_1$ or $\overline{M}_n$ corresponding to the first or second sample, respectively.

Using five values of $\overline{M}_n$ of the PPO fractions analyzed on set A of columns and the remaining three values of $\overline{M}_n$ of fractions analyzed on set B, as well as the corresponding chromatograms, it was possible to make iterative calculations of the calibration parameters according to eq. (37) for 10 different pairs of fractions for set A and for three pairs of fractions in case of set B. The convergence of the iterative method based on eq. (37) was found to be as good as that of the previous method based on eq. (10). In Table IV the arithmetic mean values of the C_1 and C_2 parameters of all possible pairs of fractions are evaluated separately for both sets of columns, as well as the values of these parameters, which deviated to the largest extent from the mean. Finally, the corresponding absolute average percentage deviations of $\overline{M}_n$ and percentage deviations of $\overline{M}_w$ for fraction No. 1 were calculated for the cited calibration relationships. The results obtained may be found in the last two columns of Table IV.

As can be seen, the best calibration relationship was obtained for the first variant of the iteration method in which both $\overline{M}_n$ and $\overline{M}_w$ were used in the calculations. In this case value of $|\delta\overline{M}_n|$ for set A is very close to the optimum value of this quantity, the last one being equal to 6.0 (see Table III). When only $\overline{M}_n$ values were applied in evaluation of the calibration parameters for column set A, the respective percentage deviations of $\overline{M}_w$ became large or even ridiculous. Calibration parameters evaluated for any pair of PPO fractions using the iterative eq. (37) had great influence mainly on values of $\delta\overline{M}_w$, although individual $\delta\overline{M}_n$ deviations also exceeded 20%.

Consistence of the calibration parameters obtained for all pairs of fractions analyzed on set B indicate that this set had been very well chosen for the GPC analysis. Contrary to set B, the choice of columns consisting set A had been less successful.

Universal Calibration for PPO—Test of the Developed Procedures

Any set of GPC columns can be accurately calibrated with narrow polystyrene (PS) standards. Assuming that the universal calibration approach of Benoit et al. is valid for both the PS and the analyzed polymer, the calibration relationship determined for PS can be easily adopted for this polymer. This adoption resolves itself into evaluation of the K and a parameters in the $[\eta]-M$ relationship for this polymer in the GPC solvent, using any of the methods described in the literature.

In order to test the simple iterative procedures for evaluation of the parameters K and a, developed in this work [see eqs. (25) and (28)], the PPO data of Valles[19,20] were adopted. For these data the calibration relationships of both sets of columns could be adequately described by linear equations [compare eqs. (34) and (35)]. In case of linear GPC calibrations the eqs. (14) and (15) may be written as follows

$$V = g(\log[\eta]M) = A_1 - A_2 \log[\eta]M, \; A_1, A_2 > 0 \tag{14'}$$

and

$$\log[\eta]M = g^{-1}(V) = A_1/A_2 - 1/A_2 V \tag{15'}$$

where A_1 and A_2 are parameters of the universal calibration.

Using the above calibrations it is easy to derive the explicit formulae for the corresponding iterative relations and the definitions of averaged elution volumes. For example, the iterative equation (25) and the definition of $\overline{V}_4$, eq. (24), take the following simple forms

$$a = (A_1 - A_2 \log[\eta]\overline{M}_n - \overline{V}_1)/(\overline{V}_4 - A_1 + A_2 \log[\eta]\overline{M}_n) \tag{25'}$$

and

$$\overline{V}_4 = -A_2(a + 1)/a \; \log \left[\sum_i w_i 10^{-a/(a+1)V_i/A_2} \right] \tag{24'}$$

Values of the universal calibration parameters A_1 and A_2 for PPO in THF at 25°C were evaluated from the direct calibration relationships for both sets of columns, eqs. (34) and (35). Namely, it follows from eqs. (14'), (6), and (7) that

$$A_1 = C_1 + C_2 \log K/(a + 1) \tag{38}$$

$$A_2 = C_2/(a + 1) \tag{39}$$

where K and a are parameters of the $[\eta]-M$ relationship for PPO in THF at 25°C, which could be taken from eq. (31). The corresponding universal calibrations determined in this way for both sets of columns were as follows:

$$\text{Set A:} \quad V = 46.16 - 2.52 \log[\eta]M \quad \text{counts} \tag{40}$$

$$\text{Set B:} \quad V = 35.72 - 1.86 \log[\eta]M \quad \text{counts} \tag{41}$$

After completion of the above adoption of the PPO data test of the developed iteration equations (25) and (28) was made. For the variant of calibration based on two samples having different intrinsic viscosities parameter a was determined according to eq. (28) for 10 pairs of PPO fractions analyzed on set A of columns and for three pairs of fractions analyzed on set B, taking from Table I the corresponding values of $[\eta]$ in THF at 25°C. Similar calculations were made according to eq. (25) for all eight PPO fractions applying their values of $[\eta]$ and $\overline{M}_n$. Parameter K was evaluated for the two variants of iterative calibrations according to one of the corresponding eqs. (26) or (29). The tested iteration procedure converged well (number of necessary iteration steps was always less than 10).

TABLE V
Universal Calibration Iterative Procedures[a]

| | Variant $[\eta]$, $\overline{M}_n$—eq. (25) | | Variant $[\eta]_1$, $]\eta]_2$—eq. (28) | |
	Set A	Set B	Set A	Set B
No. of estimates	5	3	10	3
$\bar{a}$[b]	0.619	0.609	0.608	0.620
$s_{\bar{a}}$[c]	0.017	0.005	0.0001	0.0001
$10^2\overline{K}$[b]	6.165	6.221	6.452	5.450
$s(10^2\,\overline{K})$[c]	1.408	0.426	0.007	0.004

[a] Results of a test for PPO in THF at 25°C based on the data of Valles (ref. 19).

[b] Arithmetic average:

$$\bar{x} = \sum_{i=1}^{n} x_i/n, \; n \text{ is the number of estimates.}$$

[c] Standard error of $\bar{x}$:

$$s_{\bar{x}} = \left[\sum_{i=1}^{n} (x_i - \bar{x})^2/n(n-1) \right]^{1/2}.$$

Average results of a and K together with their standard errors calculated for all determinations and for a given variant of calibration are in Table V. The results in Table V compare well with each other and are satisfactorily consistent with the original values of a and K in eq. (31). It was thus demonstrated that the simple iterative method of evaluation of the universal GPC calibration parameters, developed in this work, converged well, and secured the determination of good results. Further analysis of the obtained results would be meaningless, however, because values of $[\eta]$ of all PPO fractions were not measured but calculated from the molecular weight distributions of the fractions.

Comparison of Calibration Methods Developed in this Work with Procedures of Loy and Weiss and Cohn-Ginsberg

Using the data of PPO fraction No. 1 (compare Table I), the iteration procedures for evaluation of calibration parameters developed by Loy[9] and by Weiss and Cohn-Ginsberg[5] were applied. It was realized that writing computer programs based on the explicit formulae developed in this work and given by eqs. (10) and (25) was a much easier task than in case of the corresponding expressions of Loy and of Weiss et al., i.e., eqs. (8) and (18), respectively. A typical example of results obtained with different procedures for the chosen data may be found in Table VI. As can be seen, convergence of the new calibration methods, proposed in this work, is better than convergence of the procedures described in the literature, while the calibration parameters obtained for corresponding relationships are the same.

CONCLUSIONS

Applying the formalism of averaged elution volumes corresponding to average molecular weights or intrinsic viscosities of polydisperse standards,[7] simple single-valued equations were developed for iterative evaluation of calibration parameters of both direct and universal GPC calibration relationships. The developed evaluation procedures are simpler and converge faster than the cor-

TABLE VI
Comparison of Calibration Procedures Developed in this Work with Procedures of Loy (ref. 9) and of Weiss et al. (ref. 5)[a]

Procedure	Direct calibration			Universal calibration		
	C_2 initial	No. of iterations	C_2 final	a initial	No. of iterations	a final
eq. (10)	4	4	4.246	—	—	—
eq. (8)—Loy	4	19	4.246	—	—	—
eq. (25)	—	—	—	0.6	3	0.5756
eq. (18)—Weiss	—	—	—	0.6	21	0.5753

[a] Results for PPO fraction 1.

responding methods of Loy[9] and of Weiss and Cohn-Ginsberg,[5] described in the literature.

Results obtained for PPO polydisperse fractions revealed that from among the simple, direct GPC calibration procedures most appropriate is the method based on one polymer standard with measured values of 2 different average molecular weights (e.g., $\overline{M}_n$ and $\overline{M}_w$). Success of the calibration procedure in which two polydisperse polymer standards with measured average molecular weights of the same type are applied (e.g., $\overline{M}_n$) strongly depends upon the choice of the GPC columns. It seems that any direct GPC calibration procedure based on one type of average molecular weights of standard, polydisperse samples secures accurate calibration relationships only in the case of the set of columns, porosities of the packing of which well-matched hydrodynamic dimensions of the analyzed macromolecules. On the other hand, results of evaluation of the calibration relationships for different pairs of polydisperse samples may serve as a simple test of the adequacy of the chosen set of GPC columns.

APPENDIX

Derivation of Analytical Forms of the Iterative Relations for Determining of the GPC Calibration Parameters

Assuming a linear GPC calibration relationship and a Gaussian chromatogram it is easy to demonstrate that in case of any of the iterative calibration equations, derived in this paper, the functional dependence of the calibration parameter on the corresponding iteration variable may be described by a homographic function. Let us consider the iteration equation of the universal calibration, eq. (25′), which may be written as follows

$$a = (\text{const} - \overline{V}_1)/(\overline{V}_4 - \text{const}) \tag{42}$$

where const contains the known universal calibration parameters and measured values of $[\eta]$ and $\overline{M}_n$.

In order to simplify the mathematical expressions let the linear universal calibration relationship have the form

$$V = a_1 - a_2 \ln[\eta]M \tag{43}$$

where ln means the natural logarithm.

Definitions of averaged retention volumes $\overline{V}_1$ and $\overline{V}_4$ may be written as follows [compare eqs. (23) and (24′)]

$$\overline{V}_1 = a_2(\alpha + 1) \ln\left(\int_0^\infty f(V)\exp[V/a_2(\alpha + 1)]dv\right) \tag{44}$$

and

$$\overline{V}_4 = -a_2(\alpha + 1)/\alpha \ln\left(\int_0^\infty f(V)\exp[-\alpha V/a_2(\alpha + 1)]dV\right) \tag{45}$$

where, for the iterative variable, the symbol α was applied in order to differentiate this variable, occurring on the right-hand side of eq. (42), from the parameter a occurring on the left-hand side of this equation. Integrals occurring in the above definitions of averaged elution volumes may be evaluated analytically using the following definite integral

$$\int_0^\infty \exp(-a^2x^2)dx = \pi^{1/2}2a, \qquad a > 0 \tag{46}$$

when a Gaussian chromatogram of the standard sample is assumed. A Gaussian chromatogram may be written in the following way

$$f(V) = h/\pi^{1/2}\exp[-h^2(V - V_p)^2] \tag{47}$$

where V_p is the peak elution volume and h is inversely proportional to the standard deviation of the Gaussian curve. Thus, for the averaged elution volumes the following results are obtained

$$\overline{V}_1 = V_p - 1/[4h^2a_2(\alpha + 1)] \tag{48}$$

and

$$\overline{V}_4 = V_p - \alpha/[4h^2a_2(\alpha + 1)] \tag{49}$$

Introducing eqs. (48) and (49) into eq. (42) and rearranging one obtains the following form of the functional dependence of calibration parameter a on the iteration variable α,

$$a = (l_1\alpha + l_2)/(m_1\alpha + m_2) \tag{50}$$

where l_1, l_2, m_1, and m_2 are constants.

Equation (50) describes the so called homographic function and plot of this function has the form of a rectangular hyperbola (compare any mathematical textbook).

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