JOURNAL OF APPLIED POLYMER SCIENCE VOL. 21, 191–198 (1977)

Calibration of Gel Permeation Chromatography Columns Using Polydisperse Polymer Standards

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Synopsis

Two methods of calibrating gel permeation chromatography columns are given. The first method uses polymer standards that may have broad molecular weight distributions. Either the weight-, number-, or viscosity-average molecular weight of each standard must be known. This method neglects column peak spreading. The second method requires polymer standards of moderately narrow molecular weight distributions for which both the weight- and number-average molecular weights are known. However, the second method determines both the column peak spreading and calibration of the column. The second method is applied to calibration of a column using polystyrene standards. The column peak spreading is found to be small and independent of molecular weight for this column.

INTRODUCTION

A column used for gel permeation chromatography (GPC) must be calibrated by determining the relationship between the molecular weight of a polymer and its retention volume, v, when it is passed through the column. The common method of calibrating a column requires passing a series of very narrow fractions of known molecular weight through the column. The calibration curve of the column is then obtained by plotting the log of the weight-average molecular weight of each fraction versus the retention volume at the peak of the chromatogram of the fraction. This calibration method requires very narrow fractions. Also, even for narrow fractions, the peak of the chromatogram need not occur exactly at its weight-average molecular weight, so this calibration method is approximate even when narrow fractions are used. In addition, this method neglects the effects of column peak spreading.

A method of calibrating GPC columns using polydisperse polymer samples was developed by Blake et al.,[1] but was applied only to linear calibration curves. Another calibration method was developed by Weiss et al.,[2] but requires the molecular weight distribution of the polymer samples used for calibration to be of a particular shape, so it is not generally applicable.

Two calibration methods are given in this paper. The first method utilizes polydisperse calibrating samples for which any molecular weight averages are known, but neglects column peak spreading. The second method determines both the calibration and column spreading for the GPC column, but requires calibrating samples with fairly narrow molecular weight distributions for which both the weight- and number-average molecular weights must be known. These

methods are more mathematically involved than the common calibration method and require considerable calculation. However, the calculations have been programmed for a computer so these methods may be applied without difficulty.

CALIBRATION METHOD I: USE OF POLYDISPERSE SAMPLES

Let the chromatogram of a polymer sample be given by its height $H(v)$ as a function of retention volume v. Let $H(v)$ be normalized so that

$$\int_0^\infty H(v)dv = 1 \tag{1}$$

The calibration of the column is expressed by the function $M(v)$ of the molecular weight M versus the retention volume v. The weight-, number-, and viscosity-average molecular weights of a polymer sample may be computed from the chromatogram $H(v)$ of the sample and the calibration $M(v)$ of the column by the formulas

$$M_w^c = \int_0^\infty H(v)M(v)dv \tag{2}$$

$$M_n^c = 1 \Big/ \int_0^\infty H(v)M^{-1}(v)dv \tag{3}$$

and

$$M_v^c = \left[\int_0^\infty H(v)M^a(v)dv \right]^{1/a} \tag{4}$$

where the superscript c indicates calculated molecular weight averages. For eq. (4), the polymer is assumed to obey the Mark-Houwink relationship

$$[\eta] = kM^a \tag{5}$$

If the function $M(v)$ is given, the molecular weight averages of polymer samples may be computed by eqs. (2) – (4) from their chromatograms. We wish to solve the inverse problem, i.e., to find the function $M(v)$, given the chromatograms $H(v)$ of polymer samples for which some of the molecular weight averages are known.

Let m molecular weight averages, M_i, of polymers samples be measured by an absolute method, such as light scattering, osmometry, or viscometry, where M_i may be a weight-, number-, or viscosity-average molecular weight. For a given calibrating function $M(v)$, the deviation of the given molecular weight and the molecular weight calculated by eqs. (2), (3), or (4) is $M_i - M_i^c$, so one may consider finding the function $M(v)$ that will minimize

$$E = \sum_{i=1}^{m} w_i(M_i - M_i^c)^2 \tag{6}$$

where the weights w_i are inversely proportional to the variance (square of the standard error) of the measurements M_i. If the values of M_i were all of the same accuracy, weights $w_i = 1$ would be used. However, the values of M_i will differ by several orders of magnitude and will have errors approximately proportional to their magnitudes, so we use

$$w_i = 1/M_i^2 \tag{7}$$

The calculation is simplified by use of an empirical equation for the function $M(v)$. The calibration curve of a column given as a plot of log M versus v is generally a smooth curve with only slight curvature in the molecular weight range for which the column will be applied. Therefore, the calibration curve may be adequately represented by the empirical formula

$$\log M = A + Bv + Cv^2 \tag{8}$$

and the constants A, B, and C are to be evaluated.

Substituting eq. (8) in eqs. (2), (3), and (4) gives

$$M_i^c = S_i e^A \tag{9}$$

where

$$S_i = \int_0^\infty H(v) \exp(Bv + Cv^2)dv \tag{10}$$

for a weight-average molecular weight, or

$$S_i = 1 \Big/ \int_0^\infty H(v) \exp(-Bv - Cv^2)dv \tag{11}$$

for a number-average molecular weight, or

$$S_i = \left[\int_0^\infty H(v) \exp(aBv + aCv^2)dv \right]^{1/a} \tag{12}$$

for a viscosity-average molecular weight. Substituting eqs. (7) and (9) in eq. (6) gives

$$E = \sum_{i=1}^{m} \left[1 - S_i e^A / M_i \right]^2 \tag{13}$$

For E to be a minimum,

$$\frac{\partial E}{\partial A} = \frac{\partial E}{\partial B} = \frac{\partial E}{\partial C} = 0 \tag{14}$$

where E depends on B and C through the terms S_i. The first condition gives

$$\sum_{i=1}^{m} \left[1 - e^A S_i / M_i \right] (S_i / M_i) = 0 \tag{15}$$

The other two conditions give two more equations in A and S_i. Because S_i depends on B and C, these three equations could be solved for A, B, and C calibrate the column. However, because the equations are complex and difficult to solve, a trial-and-error method of minimizing E was developed. Equation (15) is solved for e^A to give

$$e^A = \frac{\sum S_i / M_i}{\sum S_i^2 / M_i^2} \tag{16}$$

A GPC column may now be calibrated from the chromatographs of a number of polymer samples, which may have broad molecular weight distributions, for which molecular weight-averages have been measured by absolute means. Trial

values of B and C are assumed, the value of A is calculated by eq. (16), the molecular weight averages calculated by eqs. (9)–(12), and the error function E is then calculated by eq. (6). The calculations are repeated with other values of B and C until E is minimized. These calculations were performed by a nonlinear regression subroutine called NREG written by Ryshpan and Henkel.[3]

A GPC column could also be calibrated by directly minimizing E given by either eq. (6) or (13) by nonlinear regression with respect to variables A, B, and C. However, by calculating A by eq. (16), nonlinear regression need be performed for only two variables, so less calculation is required.

CALIBRATION METHOD 2: CALIBRATION WITH COLUMN SPREADING

The above calibration method does not take column spreading into account. With column spreading, the measured chromatogram $H(v)$ is given in terms of the true chromatogram $W(v)$ that would be obtained with no column spreading by Tung's equation[4]

$$H(v) = \int_0^\infty K(v,y)W(y)dy \tag{17}$$

The spreading function, $K(v,y)$, may be approximated by a Gaussian function, so eq. (17) may be approximated as

$$H(v) = (h/\pi)^{1/2} \int_0^\infty W(y)e^{-h(v-y)^2}dy \tag{18}$$

where h is a slowly varying function of v that measures the column spreading and y is a dummy variable of integration.

We now restrict the polymer samples used for calibration to moderately narrow fractions so that over the molecular weights in each fraction, h will be approximately constant and the calibration equation, eq. (8), may be approximated by a linear calibration

$$\log M = D_1 - D_2 v \tag{19}$$

for the narrow range of molecular weights in the sample.

For these conditions, Hamielec and Ray[5] have shown that the true molecular weight averages M_w, M_n, and M_v are given in terms of the molecular weight averages M_w^c, M_n^c, and M_v^c calculated from GPC chromatograms uncorrected for column spreading, i.e., calculated by eqs. (2)–(4), by

$$M_w = PM_w^c \tag{20}$$

$$M_n = P^{-1}M_n^c \tag{21}$$

$$M_v = P^a M_v^c \tag{22}$$

where the molecular weight correction factor P is

$$P = \exp(-D_2^2/4h) \tag{23}$$

Let both M_w and M_n of a moderately narrow fraction be known. Then, by eqs. (20) and (21),

$$M_w M_n = M_w^c M_n^c \tag{24}$$

so that the product of M_w and M_n is independent of column spreading. By eqs. (9) and (24),

$$M_w M_n = e^{2A} S_w S_n \tag{25}$$

where S_w and S_n are given by eqs. (10) and (11).

By a method similar to the above, we may calibrate a column by minimizing the sum of squares of relative deviations of $M_w M_n$ determined by absolute measurements and calculated by eq. (25) from the chromatograms for a series of fractions. That is, minimizing,

$$T = \sum \left[\frac{M_{w,i} M_{n,i} - e^{2A} S_{w,i} S_{n,i}}{M_{w,i} M_{n,i},} \right]^2 \tag{26}$$

where $M_{w,i}$ and $M_{n,i}$ are the weight- and number-averages of the ith fraction determined by an absolute method. The summation is, of course, over the fractions used by the calibration.

Applying the condition

$$\frac{\partial T}{\partial A} = 0 \tag{27}$$

and solving for e^A gives

$$e^{2A} = \frac{\sum \dfrac{S_{w,i} S_{n,i}}{M_{w,i} M_{n,i}}}{\sum \dfrac{S_{w,i}^2 S_{n,i}^2}{M_{w,i}^2 M_{n,i}^2}} \tag{28}$$

We proceed as above by assuming values for B and C, calculating e^{2A} by eq. (28) and then T by eq. (26). The calculation is repeated for other values of B and C until T is minimized and A, B, and C are determined. This was performed by the nonlinear regression subroutine written by Ryshpan and Henkel.[3] After the column is calibrated, the value of P for each fraction is computed by eq. (20) or (21). P may then be plotted versus the value of v at the peaks of the chromatogram. Thus, both the calibration of the column, given by A, B, and C, and the column spreading, given by values of P, are determined from the chromatograms of polymer samples of known weight- and number-average molecular weights.

This calibration method with column spreading taken into account was applied to a series of chromatograms supplied by Lewis Fetters of the polystyrene fractions shown in Table I. The chromatograms were run on a commercial chromatograph with seven Styragel columns. Tetrahydrofuran was the carrier solvent at a temperature of 25°. Solution concentrations were 0.25% (w/v), and the low flow rate of ¼ ml/min was used in order to reduce column spreading. More experimental details are given in reference 5. The values of P obtained from this calibration procedure are also shown in Table I. They are seen to be close to 1 for all the samples, so the amount of column spreading is small. No trend in the value of P with molecular weight is evident in Table I, so column spreading does not vary appreciably with molecular weight, and an average value of $P = 0.99$ may be used. The calibration is given by

$$\log_{10} M = 8.084 - 0.02193v - 0.00081v^2 \tag{29}$$

TABLE I
Polystyrene Samples Used for Calibration[a]

Sample no.	Source	$M_n \times 10^{-3}$	$M_w \times 10^{-3}$	P
25169	Waters Assoc.	3.7[b]	4.0	1.01
LJF-A	Univ. of Akron	5.3	5.4	.99
25171	Waters Assoc.	9.7	10.0	.98
25168	Waters Assoc.	20.0	20.8	1.00
LJF-7	Univ. of Akron	39.	40.	.99
41995	Waters Assoc.	111.	111.	.98
SRM 705	NBS	171	179	.98
SMD-3500	Union Carbide Corp.	78.4	236	.98
SRM 706	NBS	136	257.8	.97
3b	Pressure Chem.	355	390	1.01

[a] The molecular weight averages are those given by the source, except where otherwise noted.

[b] This average was measured by the University of Akron.

The molecular weight averages M_w and M_n calculated by eqs. (29), (9), (20), and (21) with $P = 0.99$ agreed with their measured values within a relative standard deviation of 5%. This calibration method was also applied to chromatograms of ten fractions obtained by Fetters at a flow rate of 1 ml/min. The calibration

$$\log_{10}M = 8.7756 - 0.047v - 0.00060v^2 \tag{30}$$

and a value of $P = 0.98$ was obtained. The molecular weight averages M_w and M_n calculated by eqs. (29), (9), (20), and (21) agreed with their measured values within a relative standard deviation of 5%.

Smith[7] described another method of calculating the molecular weight correction factor P. (The molecular weight correction factor $P = 1/\bar{G}$ (1) in Smith's terminology.) He determined that $P = 0.93$, 0.93, and 0.91 for three combinations of standard commercial Styragel columns operated with a flow rate of 2 ml/min, and $P = 0.85$ for a special high-speed Styragel column operated with a flow rate of 6 ml/min. He also found that P was approximately constant over a broad molecular weight range. The molecular weight correction factors for the columns measured by Smith are seen to be much less than the factor of the column measured by Fetters, indicating more spreading. This is believed to be partly due to the higher flow rates used by Smith.

Two methods of calibration gel permeation chromatography columns have been presented. Although these methods involve considerable calculations, the calculations may be easily performed on a computer.

The first method may be used with polymer samples with broad molecular weight distributions for which any molecular weight average (M_w, M_n, or M_v) is known, but neglects column spreading. The effect of neglecting column spreading is now investigated.

Let the calibration of a column be given by eq. (8). The weight-average molecular weight calculated from a chromatograph of a polymer sample neglecting column spreading by eqs. (8) and (2) is then given by

$$M_w^c = \int_0^\infty H(v) \exp(A + Bv + Cv^2)dv \tag{31}$$

Substituting in eq. (20) gives

$$M_w = \int_0^\infty H(v) \exp(A + \log P + Bv + Cv^2)dv \tag{32}$$

However, eq. (32), which includes the effect of column spreading, is the formula for computing the weight-average molecular weight neglecting column spreading using the column calibration

$$\log M = A + \log P + Bv + Cv^2 \tag{33}$$

Therefore, if the column is calibrated by method I using samples with known weight-average molecular weights, the calibration given by eq. (33) will be obtained. Also, if the weight-average molecular weight of any polymer sample is computed from its chromatogram neglecting column spreading and using the calibration given by eq. (33), the correct value will be obtained. However, if the number-average molecular weight of a polymer sample is calculated using eqs. (3) and (33) and neglecting column spreading, the result will be

$$M_n^c = 1 \Big/ \int_0^\infty H(v) \exp(-A - \log P - Bv - Cv^2)dv \tag{34}$$

By eqs. (3), (8), and (21), the true number-average molecular weight of the sample may be written

$$M_u = P^{-1} \Big/ \int_0^\infty H(v) \exp(-A - Bv - Cv^2)dv \tag{35}$$

Comparing eqs. (34) and (35),

$$M_n^c = P^2 M_n \tag{36}$$

so that the calculated weight average molecular weight will be P^2 times the true value.

It can similarly be shown that if column spreading is neglected and a column is calibrated by method I using only number-average molecular weights of samples, then the number-average molecular weight of a sample calculated from its chromatogram will be correct while its calculated weight-average molecular weight will be $1/P^2$ times its true value. For values of P close to 1, the errors due to neglecting column spreading may be allowable for many practical investigations.

The second method determines both the molecular weight correction factor and calibration of the column, but requires samples of moderately narrow molecular weight distributions for which both the weight- and number-average molecular weight averages (M_w and M_n) are known. However, if the molecular weight correction factor P is substantially independent of molecular weight, as for the columns of Smith and Fetters, or even if it is slightly dependent on molecular weight, the polymer samples used for the second calibration method may have fairly broad molecular weight distributions. The second method is to be preferred if the required calibrating samples are available, otherwise the first method should be used. It can use polydisperse polymer samples and takes the molecular weight distribution of the samples properly into account, in comparison with previously used calibration methods that require sample of narrow molecular weight distributions and also neglect column spreading.

A third calibration method may be possible. If future measurements show that the molecular weight correction value P is constant for a particular type of GPC column although their calibrations differ, P may be considered to be known before a column of this type is to be calibrated. Then, by eqs. (20)–(22), the uncorrected molecular weight averages (M_w^c, M_n^c, or M_v^c) may be calculated for the series of polymer samples used to calibrate the column. Using these uncorrected molecular weight averages with the first calibration method will then take column spreading into account.

The author wishes to thank Lewis Fetters and Peter Verdier for many helpful discussions and Lewis Fetters for providing the data and chromatograms used.

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Received February 5, 1975
Revised January 21, 1976