Calibration of Size-Exclusion Chromatography Columns with Polydisperse Polymer Standards. I. Theory

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Synopsis

Relationships have been derived between the spreading factor and the respective slopes of actual and effective linear calibrations in size-exclusion chromatography. On this basis a new calibration procedure has been developed for determining simultaneously the dependences of sample molecular weight and of the spreading factor on elution volume from chromatograms of polydisperse polymer samples with known values of number- and weight-average molecular weight. No assumptions about the shape of sample molecular weight distribution are required. If necessary, the calibration dependences may be further improved by an iteration procedure described.

INTRODUCTION

Prior to evaluating the data of size-exclusion chromatography (SEC) in terms of correct molecular weight distribution (MWD) and/or average molecular weights, it is necessary to determine, for the given combination of columns, the relationship between the molecular weight of the polymer and its retention volume (molecular weight calibration), and the shape of the spreading function $G(v,y)$ in the integral (Tung) equation

$$f(v) = \int_{-\infty}^{\infty} G(v,y)w(y)\,dy \tag{1}$$

(calibration for axial spreading), where f is the normalized uncorrected chromatogram and w is the chromatogram corrected for zone spreading. Molecular weight calibration in SEC with characterized, polydisperse polymer samples is a useful alternative to the simplest method, which employs standards with narrow molecular weight distribution, in particular when narrow fractions of the polymer to be analyzed are not at one's disposal and the universal calibration based on commercially available "monodisperse" polystyrene (PS) standards cannot be applied either because information on the appropriate Mark–Houwink constants is lacking or the mobile phase used is a nonsolvent for PS.

Many attempts have been undertaken in this area with varying degree of success. Some of them[1-6] start from the knowledge of cumulative MWD; the others require one or more molecular weight averages to be known for all calibration standards.[7-11] Frank et al.[7] proposed a rather cumbersome graphical procedure. Balke et al.[8] developed a method for determining the so-called effective linear calibration; several alternatives and improvements of their procedure have been published.[6,12-15] Other investigators attempted to circumvent the awkward restriction of linearity by employing more general forms of the

log (molecular weight) vs. retention volume dependence.[9–11,16] These authors, however, substituted the experimentally available, uncorrected chromatogram f into formulae that are, strictly speaking, valid only for the ideal, spreading-corrected function w. In testing the polynomial methods of Vrijbergen et al.[11] and Szewczyk[9] on computer-generated data, we confirmed the previous finding[12] that neglecting the axial spreading resulted in a distorted molecular weight calibration (see also Ref. 11). Correction for axial spreading has been incorporated into the methods developed by Yau et al.[12] and by McCrackin,[10] but their results are limited to a special form of the dependence $\log M = g(v)$ (linear in Ref. 12; quadratic polynomial in Ref. 10) and/or to calibration standards with relatively narrow molecular weight distributions.[10]

In this article (Paper I) a general method is developed for calibrating SEC columns by polymer standards having MWD of an arbitrary shape with known number- and weight-average molecular weights, M_n and M_w. The method yields both the dependence of molecular weight on elution volume as a polynomial of statistically correct degree and the dependence of the spreading factor h on elution volume, to be used in subsequent corrections for axial spreading of chromatograms of unknown samples. Applications of the new procedure are described in Paper II.[17]

THEORETICAL

Yau et al.[12] observed that with increasing spreading the effective linear calibration dependence derived from the chromatogram of a polydisperse polymer sample according to Balke et al.[8] tends to rotate counterclockwise around an anchor point located near the average elution volume (see also Ref. 18). We shall first put this observation on a quantitative basis by proving that the abscissa of the intercept between the true and effective calibration lines lies very close to the first statistical moment (centroid) of the uncorrected chromatogram, and derive the relationship between the respective slopes of the actual and effective linear calibrations and the spreading factor. On this basis it will be shown how to construct an approximate polynomial molecular weight calibration and to determine the dependence of the spreading factor on elution volume from uncorrected chromatograms of several polydisperse polymer standards with known M_n and M_w. The chromatograms can be then corrected for axial spreading and subsequently used in an iteration procedure in order to improve the initial estimates of both the molecular weight and spreading calibrations. Similarly to the previous methods,[8–11] the proposed procedure involves considerable calculation and requires the use of a computer.

Intercept of Actual and Effective Linear Calibrations

Let $f(v)$ and $w(v)$ be the respective uncorrected and corrected chromatograms of a polydisperse polymer standard characterized by known values of M_n and M_w. At first let us assume that the actual (as yet unknown) calibration dependence of the given combination of SEC columns is linear:

$$\ln M = A^* + B^* v \tag{2}$$

It is known that in this case it holds for the averages M_n and M_w.

$$M_n = e^{A^*} / \int e^{-B^* v} w(v) \, dv \tag{3a}$$

$$M_w = e^{A*} \cdot \int e^{B*v} w(v)\, dv \qquad (3b)$$

Following Balke et al.,[8] we define the constants A and B of effective linear calibration

$$\ln M = A + Bv \qquad (4)$$

such that

$$M_n = e^A / \int e^{-Bv} f(v)\, dv \qquad (5a)$$

$$M_w = e^A \cdot \int e^{Bv} f(v)\, dv \qquad (5b)$$

(B and B^* are both negative).

As M_n, M_w, and $f(v)$ are known, eqs. (5) can be always solved for the constants A and B; a very rapid iteration procedure is described in Appendix A. Accordingly, throughout the following development we shall consider A and B as known.

Assuming for simplicity that the kernel $G(v,y)$ in Tung integral equation (1) is Gaussian,

$$G(v,y) = (h/\pi)^{1/2} \exp[-h(v - y)^2] \qquad (6)$$

(where h is the spreading factor), we can evaluate the integrals $\int e^{\pm Bv} f(v)\, dv$ in eqs. (5) by substituting for $f(v)$ from eq. (1), using eq. (6). We have

$$\int_{-\infty}^{\infty} e^{\pm Bv} f(v)\, dv = \int_{-\infty}^{\infty} e^{\pm Bv}\, dv \int_{-\infty}^{\infty} \sqrt{h/\pi} \exp[-h(v - y)^2] w(y)\, dy$$

$$= \sqrt{h/\pi} \int_{-\infty}^{\infty} w\, dy \int_{-\infty}^{\infty} \exp[\pm Bv - h(v - y)^2]\, dv$$

The integral over v can be evaluated analytically, and the result is

$$\int_{-\infty}^{\infty} e^{\pm Bv} f(v)\, dv = \exp(B^2/4h) \int_{-\infty}^{\infty} e^{\pm By} w(y)\, dy \qquad (7)$$

Substitution into eqs. (5) gives for the number and weight averages

$$M_n = \frac{\exp(A - B^2/4h)}{\int \exp(-By) w(y)\, dy} = \frac{\exp(A^*)}{\int \exp(-B^*y) w(y)\, dy} \qquad (8a)$$

$$M_w = \exp(A + B^2/4h) \int \exp(By) w\, dy$$

$$= e^{A*} \int e^{B*y} w\, dy \qquad (8b)$$

where the integrals contain the as-yet unknown function w. However, in a completely analogous manner it can be shown that

$$\int_{-\infty}^{\infty} e^{\pm B*v} f(v)\, dv = \exp(B^{*2}/4h) \int_{-\infty}^{\infty} e^{\pm B*y} w(y)\, dy \qquad (9)$$

Using (7) and (9) it is now possible to write, instead of eqs. (8),

$$M_n = \frac{e^A}{\int \exp(-Bv) f\, dv} = \frac{\exp(A^* + B^{*2}/4h)}{\int \exp(-B^*v) f\, dv} \qquad (10a)$$

and

$$M_w = e^A \int e^{Bv} f\, dv = \exp(A^* - B^{*2}/4h) \int e^{B*v} f\, dv \qquad (10b)$$

where the integrals now contain the experimentally available uncorrected chromatogram.

The spreading factor can be eliminated from eqs. (10), and we may write

$$e^{(A*-A)} \frac{\int e^{-Bv}f dv}{\int e^{-B*v}f dv} = e^{(A-A*)} \frac{\int e^{Bv}f dv}{\int e^{B*v}f dv} \tag{11}$$

Now, A and B will be certainly close to their starred counterparts, so that it is possible to expand both sides of eq. (11) into a Taylor series in variables A and B around the point $(A = A*, B = B*)$ and retain only the leading terms. The result can be written in the form

$$\frac{A - A*}{B* - B} = \frac{1}{2}\left[\frac{\int v e^{-B*v}f dv}{\int e^{-B*v}f dv} + \frac{\int v e^{B*v}f dv}{\int e^{B*v}f dv}\right] \tag{12}$$

Notice, however, that for the abscissa (say $\bar{v}$) of the intercept between the actual—eq. (2)—and effective—eq. (4)—calibration lines we have $A + B\bar{v} = A* + B*\bar{v}$ or

$$(A - A*)/(B* - B) = \bar{v} \tag{13}$$

A comparison of the last two equations shows that

$$\bar{v} = \frac{1}{2}\left[\frac{\int v e^{-B*v}f dv}{\int e^{-B*v}f dv} + \frac{\int v e^{B*v}f dv}{\int e^{B*v}f dv}\right] \tag{14}$$

This result can be given a more lucid meaning by expansion of the exponentials into McLaurin series followed by term-by-term integration; each integral is then transformed into an infinite series containing statistical moments about zero of the uncorrected chromatogram,

$$\mu'_k = \int v^k f dv \tag{15}$$

After an awkward but straightforward algebra that relies on the connection[18] between moments about zero and central statistical moments,

$$\mu_k = \int (v - \mu'_1)^k f dv \tag{16}$$

the result reduces to the form

$$\bar{v} = \mu'_1 \frac{1 + B*^2(\mu_2 + \mu_3/2\mu'_1) + O(B*^4)}{1 + B*^2\mu_2 + O(B*^4)}$$

Neglecting the higher terms, we have finally

$$\bar{v} = \mu'_1\left[1 + \frac{B*^2\mu_3}{2\mu'_1(1 + B*^2\mu_2)}\right] \cong \mu'_1 \tag{17}$$

When actual values of $B*$ and the μ's from experimental chromatograms recorded on different column combinations (both in classical and high-speed SEC) are inserted into eq. (17), the second term in square brackets always turns out to be much smaller than unity; thus, it has been proved that the intercept between the actual and effective linear calibrations is very close to the centroid μ'_1 of the uncorrected chromatogram.

Now, if chromatograms for a series $(i = 1, 2, \ldots, n_s)$ of polymer samples with broad MWD are recorded on a combination of SEC columns, the parameters A_i and B_i of the effective calibration lines can be determined for each chromatogram

by solving eqs. (5), using the method of Appendix A, and the first statistical moments $\mu'_{1,i}$ can be calculated by numerical integration according to eq. (15). Thus, for each sample a single point $(\mu'_{1,i}, \ln M_i)$ of the actual calibration dependence sought can be determined from

$$\ln M_i = A + B\mu'_{1,i} \qquad (18)$$

These points can be then processed by some standard statistical procedure to yield the best-fit functional dependence for the actual calibration, e.g., in the form of a polynomial. Henceforth, this procedure will be referred to as the method of intercepts.

Although the derivation of the underlying eq. (17) rests on the assumptions that the actual calibration is also linear and that the spreading function is Gaussian, it is reasonable to expect that slight deviations from linearity and from the assumed shape of $G(v,y)$ will not invalidate the proposed procedure. This expectation has been fully confirmed (see Paper II). At any rate, the calibration obtained by the above method of intercepts will serve only as the first approximation and can be further improved, as shown below.

Determination of the Spreading Factor

When eq. (10b) is divided by eq. (10a), we see that

$$\int e^{Bv} f \, dv \cdot \int e^{-Bv} f \, dv = \exp(-B^{*2}/2h) \int e^{B^*v} f \, dv \cdot \int e^{-B^*v} f \, dv$$

where A and A^* have been eliminated. Expanding again the exponentials under the integral sign and integrating term by term, we obtain after some manipulation

$$1 + B^2(\mu'_2 - \mu'^2_1) + (B^4/12)(\mu'_4 - 4\mu'_1\mu'_3 + 3\mu''^2_2) + \cdots$$
$$= \exp(-B^{*2}/2h) \left[1 + B^{*2}(\mu'_2 - \mu'^2_1) + (B^{*4}/12)(\mu'_4 - 4\mu'_1\mu'_3 + 3\mu''^2_2) + \cdots\right]$$

which can be written (see Ref. 19) as

$$1 + B^2\mu_2 + (B^4/12)(\mu_4 + 3\mu^2_2)$$
$$= \exp(-B^{*2}/2h)[1 + B^{*2}\mu_2 + (B^{*4}/12)(\mu_4 + 3\mu^2_2)]$$

When terms containing higher powers of B and B^* are neglected, the result reduces to

$$1 + B^2\mu_2 = \exp(-B^{*2}/2h)(1 + B^{*2}\mu_2) \qquad (19)$$

It is now possible to calculate the spreading factor: B^* is taken as the local slope of the calibration dependence $\ln M = g(v)$ (at $v = \mu'_1$), determined by the method of intercepts, and eq. (19) is solved directly for h; the process is repeated for all chromatograms and the values $h_i = h(\mu'_{1,i})$ for $i = 1, 2, \ldots, n_s$ are then processed statistically to yield a smoothed dependence $h(v)$ in a suitable analytical form.

Methods for Improving the Initial Calibrations

For relatively narrow calibration standards the curvature of the actual calibration as well as the elution volume dependence of spreading can be safely neglected within the volume interval spanned by the sample; in this case the pro-

posed method of intercepts will yield a reliable molecular weight calibration, and, accordingly, also the spreading factor determined from eq. (19) using the slope B^* of this function will be correct. However, when only standards with broad MWD are available, the inherent assumptions cast some doubt on the accuracy of the method of intercepts. Moreover, the number of points on the calibration is equal to the number of calibration standards employed (n_s), and this can lead to a polynomial of unnecessarily low degree to be accepted by the statistical test, owing to a low number of degrees of freedom. In this section, methods are discussed that can be used for improving the initial estimates of molecular weight and spreading calibrations.

As soon as the dependence $h(v)$ is known (see the previous section), all chromatograms can be corrected for imperfect resolution by solving the Tung equation (1) by means of some rapid numerical procedure (e.g., Refs. 20 and 21). Second central moments ν_2 of the corrected chromatograms are then calculated,

$$\nu_2 = \int (v - \mu_1')^2 w(v) \, dv \tag{20}$$

and improved values of h are determined from the equation

$$\nu_2 = \mu_2 - 1/2h \tag{21}$$

derived in Ref. 22.

We may then assume that, within the interval of elution volume spanned by two adjacent chromatograms, the calibration dependence can be adequately represented by a second-degree polynomial

$$\ln M = a + bv + cv^2 \tag{22}$$

The three coefficients a, b, c are found by solving numerically the appropriate equations derived from eq. (22) for the number and weight averages, M_n and M_w, of the two calibration standards in question:

$$
\begin{aligned}
M_{n,i} &= \frac{e^a}{\int w_i(v) e^{-bv - cv^2} \, dv} \\
M_{w,i} &= e^a \cdot \int w_i e^{bv + cv^2} \, dv \\
M_{n,i+1} &= \frac{e^a}{\int w_{i+1} e^{-bv - cv^2} \, dv} \\
M_{w,i+1} &= e^a \cdot \int w_{i+1} e^{bv + cv^2} \, dv
\end{aligned}
\tag{23}
$$

where the indices i and $i +1$ refer to two adjacent chromatograms of calibration standards, ordered according to increasing values of average M. A rapid iteration procedure for solving these equations for a, b, and c is described in Appendix B.

A number of points ($v_j, \ln M_j$) are then calculated from eq. (22) with a set of elution volumes v_j selected from the interval covered by significant portions of the two chromatograms [excluding their tails, where eq. (22) can deviate from the true course] and stored for further use. The process is repeated with the next pair of adjacent chromatograms until all ($n_s - 1$) pairs have been exhausted. All points thus obtained are then used together for calculating the coefficients of a best-fit polynomial of statistically significant degree, as the number of points is no longer restricted to the number of chromatograms and can be as high as convenient.
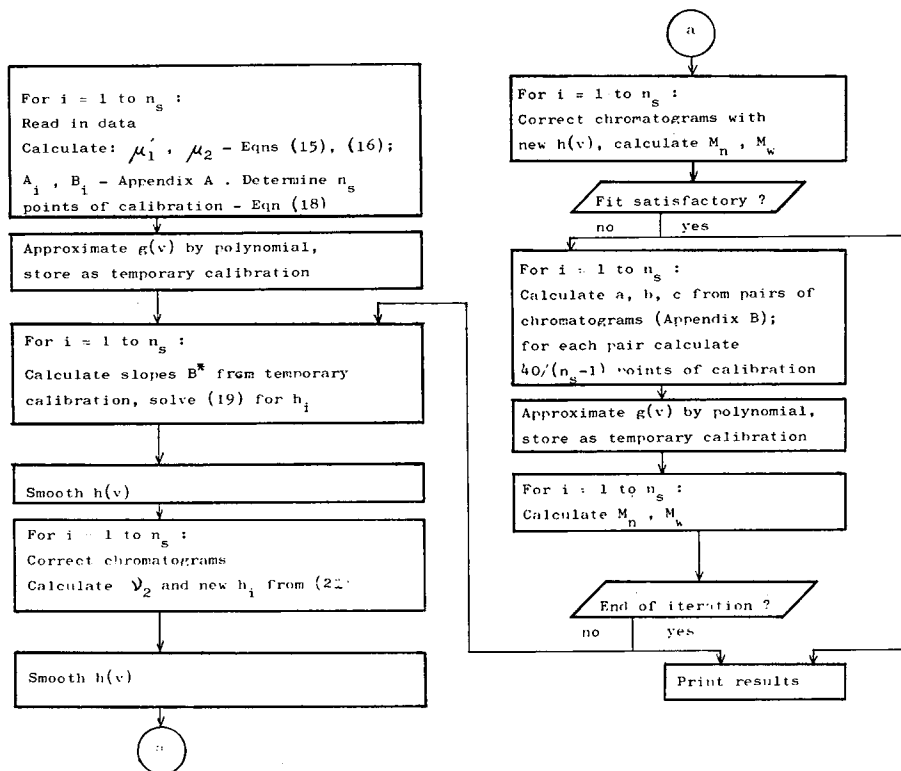
Fig. 1. Flow chart of the program (schematical).

By using the new polynomial calibration thus obtained, the number and weight average molecular weights are calculated for each sample and compared with the known values.   If the fit is not satisfactory, an iteration loop can be initiated wherein the slope $B*$ calculated from the improved polynomial at the centroid of each chromatogram is inserted into eq. (19) together with the corresponding variance, new values of the spreading factor are determined, etc.   This method will be referred to in Paper II as iteration procedure. A flow chart of the computer program devised to handle these calculations is shown in Figure 1.

The procedures described in this paper could be easily modified for the case when other two averages, e.g., M_n and the viscosity average M_η, are known for some or all calibration standards, but they would then require the knowledge of the appropriate Mark–Houwink constants for the polymer in the mobile phase employed.

No assumption about the shape of sample MWD has been introduced in the development of the calibration methods described; accordingly, both should work equally well with standards having unimodal and multimodal distributions. Their application to artificial (computer-generated) and experimental chromatograms is described in Paper II.

APPENDIX A

An efficient iteration process devised for solving eqs. (5) can be described as follows.   First divide (5b) by (5a) to eliminate A:

$$(M_w/M_n) = \int e^{Bv} f(v)\, dv \cdot \int e^{-Bv} f(v)\, dv \qquad (24)$$

This equation is valid for the correct value of B.   With B variable we may write

$$\lambda(B) = P(B) \cdot Q(B)$$

where

$$P(B) = \int e^{Bv} f \, dv \qquad (25)$$

and

$$Q(B) = \int e^{-Bv} f \, dv \qquad (26)$$

It is readily seen that

$$\Delta\lambda = [Q(dP/dB) + P(dQ/dB)]\Delta B$$

If B_k represents the value of B at the kth iteration step, we may write

$$\frac{M_w}{M_n} - P(B_k) \cdot Q(B_k) = \left[Q(B_k) \left| \frac{dP}{dB} \right|_{B=B_k} + P(B_k) \left| \frac{dQ}{dB} \right|_{B=B_k} \right] (B_{k+1} - B_k) \qquad (27)$$

Solving for B_{k+1}, we obtain the iteration formula

$$B_{k+1} = B_k + \frac{M_w/M_n - P(B_k)Q(B_k)}{Q(B_k)P_1 + P(B_k)Q_1} \qquad (28)$$

where we have defined for simplicity

$$P_1 = \left| \frac{dP}{dB} \right|_{B=B_k} = \int e^{B_k v} v f(v) \, dv \qquad (29)$$

and

$$Q_1 = \left| \frac{dQ}{dB} \right|_{B=B_k} = -\int v e^{-B_k v} f(v) \, dv \qquad (30)$$

The quantities P, Q, P_1, and Q_1 can be easily calculated by numerical integration from eqs. (25), (26), (29), and (30). As soon as B is determined with sufficient precision (convergence of the product $P \cdot Q$ to the known ratio M_w/M_n is a suitable stopping criterion), A is calculated as the mean of two values obtained by inserting the final B into eqs. (5a) and (5b). The initial value B_0 for the first chromatogram is estimated as the slope of the straight line joining the points $[\mu_1', \ln (M_n M_w)^{1/2}]$ corresponding to the first two calibration standards; for subsequent samples the final value of B arrived at with the previous chromatogram is used as the initial estimate.

The method proved to be almost 10 times as rapid as the iteration procedure described in Ref. 11: three to four steps have been sufficient in most cases.

APPENDIX B

Equations (23) can be solved iteratively by a generalization of the procedure described in Appendix A. Let $(M_w/M_n)_i$ be the ratio of known averages for the ith sample and w_i the corresponding corrected chromatogram. From eq. (23) we have

$$(M_w/M_n)_i = \int e^{bv + cv^2} w_i(v) \, dv \cdot \int e^{-bv - cv^2} w_i(v) \, dv$$

and

$$(M_w/M_n)_{i+1} = \int e^{bv + cv^2} w_{i+1} \, dv \cdot \int e^{-bv - cv^2} w_{i+1} \, dv$$

and the parameter a has been eliminated. Similarly as in eq. (27), we write

$$(M_w/M_n)_i - P_i M_i = (R_i M_i + L_i P_i)(b_{k+1} - b_k) + (S_i M_i + K_i P_i)(c_{k+1} - c_k) \qquad (31)$$

$$(M_w/M_n)_{i+1} - P_{i+1} M_{i+1} = (R_{i+1} M_{i+1} + L_{i+1} P_{i+1})(b_{k+1} - b_k) \\ + (S_{i+1} M_{i+1} + K_{i+1} P_{i+1})(c_{k+1} - c_k) \qquad (32)$$

where the quantities in eqs. (31) and (32) are defined by the integrals

$$P_i = \int \exp(b_k v + c_k v^2) w_i \, dv$$
$$M_i = \int \exp(-b_k v - c_k v^2) w_i \, dv$$
$$R_i = \left| \frac{\partial P_i}{\partial b} \right|_{\substack{b=b_k \\ c=c_k}} = \int v \exp(b_k v + c_k v^2) w_i \, dv$$

$$S_i = \left|\frac{\partial P_i}{\partial c}\right|_{b_k,c_k} = \int v^2 \exp(b_k v + c_k v^2) w_i \, dv$$

$$L_i = \left|\frac{\partial M_i}{\partial b}\right|_{b_k,c_k} = -\int v \exp(-b_k v - c_k v^2) w_i \, dv$$

$$K_i = \left|\frac{\partial M_i}{\partial c}\right|_{b_k,c_k} = -\int v^2 \exp(-b_k v - c_k v^2) w_i \, dv$$

and similarly for $(i + 1)$; b_k and c_k are the values of b and c at the kth iteration step.

Solving eqs. (31) and (32) for b_{k+1} and c_{k+1}, we arrive at two iteration formulae in the form

$$b_{k+1} = b_k + \frac{x_2 z_1 - x_1 z_2}{d}, \quad c_{k+1} = c_k + \frac{x_1 y_2 - x_2 y_1}{d} \tag{33}$$

with

$$x_1 = (M_w/M_n)_i - M_i P_i, \quad x_2 = (M_w/M_n)_{i+1} - M_{i+1} P_{i+1}$$
$$y_1 = M_i R_i + P_i L_i, \quad y_2 = M_{i+1} R_{i+1} + P_{i+1} L_{i+1}$$
$$z_1 = M_i S_i + P_i K_i, \quad z_2 = M_{i+1} S_{i+1} + P_{i+1} K_{i+1}$$
$$d = z_1 y_2 - z_2 y_1$$

Twelve integrals must be evaluated numerically, but the process described by formulae (33) converges very rapidly, and no more than three iterations are normally needed to attain an agreement between the calculated and known values of both ratios M_w/M_n within 0.005. The coefficient a is again determined as the mean of four values obtained by inserting the final values of b and c into eqs. (23).

References

1. M. J. R. Cantow, R. S. Porter, and J. F. Johnson, *J. Polym. Sci. A-1*, **5**, 1391 (1967).
2. L. Wild, R. Ranganath, and T. Ryle, *J. Polym. Sci. A-2*, **9**, 2137 (1971).
3. T. D. Swartz, D. D. Bly, and A. S. Edwards, *J. Appl. Polym. Sci.*, **16**, 3353 (1972).
4. A. H. Abdel-Alim and A. E. Hamielec, *J. Appl. Polym. Sci.*, **18**, 297 (1974).
5. A. R. Weiss and E. Cohn-Ginsberg, *J. Polym. Sci. A-2*, **8**, 148 (1970).
6. J. A. P. P. Van Dijk, W. C. M. Henkens, and J. A. M. Smit, *J. Polym. Sci., Polym. Phys. Ed.*, **14**, 1485 (1976).
7. F. C. Frank, I. M. Ward, and T. Williams, *J. Polym. Sci. A-2*, **6**, 1357 (1968).
8. S. T. Balke, A. E. Hamielec, B. P. LeClair, and S. L. Pearce, *Ind. Eng. Chem., Prod. Res. Dev.*, **8**, 54 (1969).
9. P. Szewczyk, *Polymer*, **17**, 90 (1976).
10. F. L. McCrackin, *J. Appl. Polym. Sci.*, **21**, 191 (1977).
11. R. R. Vrijbergen, A. A. Soeteman, and J. A. M. Smit, *J. Appl. Polym. Sci.*, **22**, 1267 (1978).
12. W. W. Yau, H. J. Stoklosa, and D. D. Bly, *J. Appl. Polym. Sci.*, **21**, 1911 (1977).
13. M. J. Pollock, J. F. MacGregor, and A. E. Hamielec, *J. Liq. Chromatogr.*, **2**, 895 (1979).
14. E. G. Malawer and A. J. Montana, *J. Polym. Sci., Polym. Phys. Ed.*, **18**, 2303 (1980).
15. J. V. Dawkins, *Eur. Polym. J.*, **6**, 831 (1970).
16. G. Nilsson and K. Nilsson, *J. Chromatogr.*, **101**, 137 (1974).
17. M. Kubín, *J. Appl. Polym. Sci.*, **27**, 2943 (1982).
18. T. Kotaka, *J. Appl. Polym. Sci.*, **21**, 501 (1977).
19. C. A. Bennett and N. L. Franklin, *Statistical Analysis in Chemistry and the Chemical Industry*, Wiley, New York, 1954, p. 77.
20. P. E. Pierce and J. E. Armonas, *J. Polym. Sci., C*, **21**, 23 (1968).
21. S. Vozka and M. Kubín, *J. Chromatogr.*, **139**, 225 (1977).
22. S. Vozka, M. Kubín, and G. Samay, *J. Polym. Sci., Polym. Symp.*, **68**, 199 (1980).