

RELIABILITY OF THE LINEAR EFFECTIVE CALIBRATION METHOD IN GEL PERMEATION CHROMATOGRAPHY

Miroslav KUBÍN

*Institute of Macromolecular Chemistry,
Czechoslovak Academy of Sciences, 16206 Prague 6*

Received October 17th, 1983

The validity of the effective linear calibration (ELC) method in gel permeation chromatography of polymers has been checked by means of computer generated chromatograms. The ratio M_w/M_n calculated using ELC from an uncorrected chromatogram is always smaller than that determined by means of the true calibration dependence, but the extent of this "correction for longitudinal spreading" depends on the polydispersity of both the original calibration standard and the analyzed polymer, and also on the separation efficiency of the given column. If the respective molecular weight averages of the analyzed sample and of the calibration standard differ considerably, the effective linear calibration method leads to serious systematic errors in the calculated average molar mass values.

Evaluation of gel permeation chromatography (GPC) data of polymers by using an experimental calibration dependence between the logarithm of relative molecular weight M and retention volume v is not losing importance, although lately there has been an increased interest in the so-called dual detectors in GPC which make possible a simultaneous determination of the concentration and relative molecular weight of components in the eluate leaving the column. If primary calibration standards having a very narrow molar mass distribution are not available for the analyzed polymer and the universal calibration based on polystyrene standards cannot be used, either because the necessary values of the Mark-Houwink constants are not known, or because polystyrene does not dissolve in the mobile phase employed, the given system of columns may be calibrated by means of characterized polymer samples with a broad distribution curve — *cf.* the review¹. If only a small number (1 or 2) of such secondary calibration standards are at disposal, one must resort to an assumption that the sought calibration dependence is linear, *i.e.*,

$$\ln M = A^* - B^*v. \quad (1)$$

It is then in principle sufficient to know two characteristics (*e.g.*, the number — M_n — and weight — M_w — average molecular weight) of a single polydisperse sample in order to determine the constants A^* and B^* .

As early as in 1969, Balke and coworkers² suggested for this case the so-called effective linear calibration method (ELC): if equation (1) is valid, the number- and mass-average M may be calculated from a chromatogram corrected for longitudinal spreading, $w(v)$, by means of the relations

$$M_n = \exp(A^*) / \int \exp(B^* \cdot v') w(v') dv', \quad (2)$$

$$M_w = \exp(A^*) \cdot \int \exp(-B^* v') w(v') dv'; \quad (3)$$

(the integration limits cover the whole range of elution volume of the given system of columns). If the corrected chromatogram w in Eqs (2) and (3) is replaced by the experimentally available uncorrected chromatogram g of the secondary standard in question, the equations

$$\bar{M}_n = \exp(A) / \int \exp(B \cdot v') g(v') dv', \quad (4)$$

$$\bar{M}_w = \exp(A) \cdot \int \exp(-B \cdot v') g(v') dv' \quad (5)$$

(in which \bar{M}_n, \bar{M}_w are the known characteristics of the calibration standard) no longer define the actual constants (A^* and B^*) but relate to some effective values A, B . Balke and coworkers² solved Eqs (4) and (5) for the sought constants A, B of the so-called effective linear calibration

$$\ln M = A - Bv. \quad (6)$$

A view has been forwarded in several papers²⁻⁶ that, if ELC thus determined is used in the calculation of molar mass averages from the uncorrected chromatogram of an unknown sample, the resulting values are automatically corrected for longitudinal spreading. This is of course true for the standard used in the determination of ELC — *cf.* equations (4) and (5); it is also true that for any other polymer the value of M_w/M_n calculated from the uncorrected chromatogram by using equation (6) is always smaller than that determined from the true calibration dependence (1), since the slope B is always smaller than B^* (*cf.*⁶⁻⁸).

Lately, some manufacturers have supplied so-called “linear” columns for GPC where, with a suitable combination of porous packings, the calibration dependence is made to satisfy adequately Eq. (1); thus, the simple effective linear calibration method has once again become topical. This study has as its objective a critical

evaluation of the ELC method with respect to reliability of the calculated molecular weight averages and a verification of the supposed automatic data correction. The effect of a slight curvature of the real calibration dependence was also investigated.

RESULTS AND DISCUSSION

To be able to verify the method proper, irrespective of uncertainty in the molecular weights of calibration standards and of the possible errors due to the apparatus and data evaluation procedure, artificial, computer-generated chromatograms of "polymeric standards" with known (Schulz-Zimm) distribution and known \bar{M}_n , \bar{M}_w values were used in the testing. The procedure of calculating uncorrected chromatograms from model distributions has been described in detail earlier^{9,10}. A number of chromatograms were generated for polymers having distributions differing greatly in their position on the M axis, width (characterized by \bar{M}_w/\bar{M}_n) and shape (uni- or bimodal); the actual calibration dependence was assumed either linear according to equation (1) or slightly curved. The dependence of the spreading factor h on the elution volume (with a Gaussian spreading function in Tung's integral

TABLE I

Characteristics of model polymers with the Schulz-Zimm molecular weight distribution used in the computation of artificial chromatograms and constants of the resulting effective linear calibration dependences

Sample	$\bar{M}_n \cdot 10^{-4}$	\bar{M}_w/\bar{M}_n	ELC constants	
			<i>A</i>	<i>B</i>
Actual calibration linear, $\log M = 12.80259 - 0.1345v$				
A	9.1	1.2	10.6816	0.09797
B	8.2	1.5	11.6062	0.11395
C	7.1	2.0	12.0570	0.12180
D	50	2.0	11.7667	0.11477
Actual calibration quadratic, $\log M = 21.64 - 0.43933v + 0.002533v^2$				
E	9.1	1.2	10.6566	0.10121
F	8.2	1.5	11.7615	0.12092
G	50	2.0	12.6869	0.13454
H ^a	7.8	1.76	12.2045	0.12872

^a Molecular weight distribution bimodal, obtained as a superposition of two Schulz-Zimm curves.

equation) was chosen as: $h(v) = -0.93102 + 0.02541v - 7.3 \cdot 10^{-5}v^2$. From uncorrected chromatograms, the constants A , B of the effective linear calibration were then calculated by employing the iterative method described in ⁷. The basic characteristics of model polymers are summarized in Table I.

Fig. 1 shows the straight lines of effective linear calibration calculated for samples A, B, D along with the straight line of the true calibration dependence. It can be easily seen that with decreasing polydispersity of the secondary standard (samples A, B) the straight lines of ELC deviate more and more from the true calibration dependence. This behaviour has already been observed for both real⁶ and model (computer generated) chromatograms⁸. In our earlier paper⁷ it has been demonstrated, on the basis of theoretical analysis, that for a Gaussian spreading function the slopes of the actual (B^*) and effective (B) linear calibration lines are related by the equation

$$1 + B^2\mu_2 = \exp(-B^{*2}/2h)(1 + B^{*2}\mu_2), \quad (7)$$

which contains the second central moment μ_2 (variance) of the uncorrected chromatogram of the respective calibration standard used in the construction of ELC and the spreading factor h characterizing the separation efficiency of the given system of columns ($1/2h = \sigma_G^2$, where σ_G^2 is the variance of the spreading function $G(v, y)$). Since for real GPC systems with separation efficiency sufficiently high for practical pur-

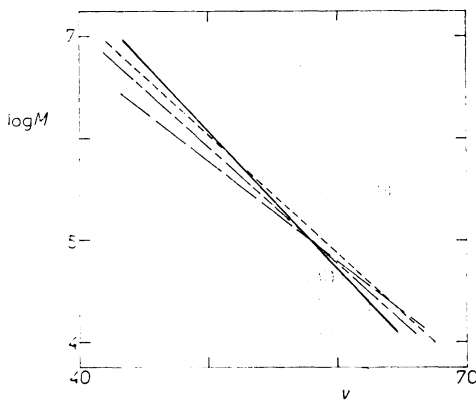


FIG. 1

Actual, straight line calibration dependence (full line) and straight lines representing effective linear calibrations calculated for samples A (— — —), B (---), and D (-----)

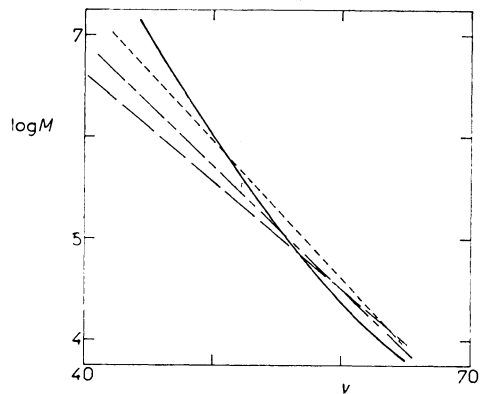


FIG. 2

Actual, curved calibration dependence (full line) and straight lines representing effective linear calibrations calculated for samples E (— — —), F (---), and G (-----)

poses the ratio $B^{*2}/2h$ is always sufficiently low, the exponential in Eq. (7) can be expanded in an infinite series with sufficient accuracy, and terms containing higher powers of B^* can be neglected. The approximate relation

$$-B^2 \cdot \mu_2 \approx B^{*2}(1/2h - \mu_2) \quad (8)$$

is obtained as a result. Since μ_2 reflects both the polydispersity of the given calibration standard and the separation efficiency of the system of columns under consideration, we shall rearrange Eq. (8) to a more lucid form by substituting for μ_2 from the equation

$$\mu_2 = v_2 + 1/2h \quad (9)$$

derived in ref.¹¹, in which v_2 is the variance of the corrected chromatogram $w(v)$ which reflects only the polydispersity of the calibration standard. This gives the resulting approximate relation

$$B^{*2}/B^2 \approx 1 + \sigma_G^2/v_2 \quad (10)$$

which indicates that the ratio of the respective slopes of the real calibration straight line and ELC is the greater, the smaller the separation efficiency of the column (*i.e.* the higher σ_G^2) and the narrower the distribution of the secondary standard used.

It can also be seen in Fig. 1 that due to this difference in the slopes the deviation of the ELC line from the actual calibration dependence becomes unacceptably large, if we move along the v -axis from the point of their intersection (according to the theoretical analysis, the point of intersection corresponds to the elution volume at the centroid of the chromatogram of the calibration standard⁷). This means that if the given ELC is used in the evaluation of chromatograms of polymers whose average relative molecular weight differs greatly from that of the secondary standard used in establishing the ELC line, a considerable systematic error is committed (*cf.* also the difference between the ELC's for samples A and D).

A similar situation for a nonlinear actual calibration dependence is shown in Fig 2: in this case the systematic deviation of ELC's from the actual calibration curve is still more pronounced, in particular in the range of large M .

From each generated uncorrected chromatogram, the M_n and M_w values were calculated (a) on the basis of ELC's obtained from chromatograms of the other samples (thus considered as secondary calibration standards) and (b) by using the true calibration dependence (as determined, *e.g.*, by means of primary "monodisperse" standards) either without correction for longitudinal spreading or by correcting the data using the simplest correction method according to Pierce and Armonas¹². Deviations of the number and mass averages calculated by the above procedures

from the correct M_n and M_w values are summarized in Table II for the case of the linear and in Table III for the curved actual calibration dependence.

Data in the Tables unambiguously confirm that the effective linear calibration may be used in the calculation of M_n and M_w , if both the analyzed sample and the secondary standard used in the construction of ELC have a similar molecular weight distribution. In such case errors of the calculated M_n and M_w values are of little importance and are also mostly smaller than errors of the average values determined

TABLE II

Error of molecular weight averages calculated by means of effective linear calibration dependences obtained from various samples. (Actual calibration dependence linear)

ELC from sample	$\Delta M_n, \%$	$\Delta M_w, \%$
	Sample A, $\bar{M}_n = 9.1 \cdot 10^4$,	$\bar{M}_w = 10.9 \cdot 10^4$
B	- 3.8	+ 2.7
C	- 6.4	+ 3.3
D	+ 24.7	+ 33.5
-	- $2.0^a(-7.5)^b$	+ $2.5(+8.9)^b$
	Sample B, $\bar{M}_n = 8.2 \cdot 10^4$,	$\bar{M}_w = 12.3 \cdot 10^4$
A	+ 6.2	- 9.2
C	- 4.0	+ 1.8
D	+ 29.4	+ 30.2
-	- $2.0^a(-7.2)^b$	+ $2.5^a(+9.1)^b$
	Sample C, $\bar{M}_n = 7.1 \cdot 10^4$,	$\bar{M}_w = 14.2 \cdot 10^4$
A	+ 16.4	- 9.5
B	- 6.2	- 3.0
D	+ 37.1	+ 26.5
-	- $1.9^a(-6.8)^b$	+ $2.3^a(+9.4)^b$
	Sample D, $\bar{M}_n = 5.0 \cdot 10^4$,	$\bar{M}_w = 10.0 \cdot 10^4$
A	- 33.0	- 44.8
B	- 23.5	- 24.2
C	- 19.5	- 11.8
-	- $3.8^a(-10.2)^b$	+ $4.4^a(+17.7)^b$

^a Errors of the molecular weight averages calculated using the actual calibration dependence by means of the correction procedure according to Pierce and Armonas¹² ($\Delta M = (M_x - \bar{M}_x) \cdot 100/\bar{M}_x$). ^b Errors of the molecular weight averages calculated by means of the actual calibration dependence without correction.

from the actual calibration dependence without correction for longitudinal spreading. If, however, this condition is not satisfied, and particularly if both distributions differ considerably in their position on the M axis, evaluation of the chromatograms by means of ELC leads to serious systematic errors in the calculated averages. It also follows that even the simplest procedures of correction for longitudinal spreading in connection with the actual calibration dependence of the given system of columns (as determined by means of primary calibration standards or by correct methods

TABLE III

Error of the molecular weight averages calculated by means of effective linear calibration dependences obtained from various samples. (Actual calibration dependence curved)

ELC from sample	$\Delta M_n, \%$	$\Delta M_w, \%$
	Sample E, $\bar{M}_n = 9.1 \cdot 10^4$,	$\bar{M}_w = 10.9 \cdot 10^4$
F	- 3.3	+ 4.8
G	+ 36.8	+ 57.6
H	- 3.4	+ 8.4
-	- $2.3^a(-9.8^b)$	+ $2.7^a(+16.1^b)$
	Sample F, $\bar{M}_n = 9.2 \cdot 10^4$,	$\bar{M}_w = 12.3 \cdot 10^4$
E	+ 6.0	- 6.2
G	+ 38.3	+ 52.7
H	- 1.3	+ 4.3
-	- $2.2^a(-9.0^b)$	+ $2.5^a(+17.4^b)$
	Sample G, $\bar{M}_n = 50 \cdot 10^4$,	$\bar{M}_w = 100 \cdot 10^4$
E	- 45.6	- 60.1
F	- 37.0	- 45.1
H	- 32.7	- 36.7
-	- $6.9^a(-16.5^b)$	+ $9.0^a(+22.9^b)$
	Sample H, $\bar{M}_n = 7.83 \cdot 10^4$,	$\bar{M}_w = 13.8 \cdot 10^4$
E	+ 8.3	- 12.5
F	+ 1.6	- 4.8
G	+ 40.0	+ 47.5
-	- $3.7^a(-8.9^b)$	+ $4.2^a(+19.0^b)$

^a Errors of the number and mass average values of M calculated using the actual calibration dependence by means of the correction procedure according to Pierce and Armonas¹². ^b Errors of the molecular weight averages calculated by means of the actual calibration dependence without correction.

reported in the literature for characterized secondary standards¹) lead to accurate M_n and M_w values; the latter procedure of data evaluation (if applicable) should always be given preference.

REFERENCES

1. Kubín M.: *J. Liquid Chromatogr.* 7 (Suppl. 1), 41 (1984).
2. Balke S. T., Hamielec A. E., LeClair B. P., Pearce S. L.: *Ind. Eng. Chem., Prod. Res. Develop.* 8, 54 (1969).
3. Swartz T. D., Bly D. D., Edwards A. S.: *J. Appl. Polym. Sci.* 16, 3353 (1972).
4. Pollock M. J., MacGregor J. F., Hamielec A. E.: *J. Liquid Chromatogr.* 2, 895 (1979).
5. Cardenas J. N., O'Driscoll K. F.: *J. Polym. Sci. (Polym. Letters Ed.)* 13, 657 (1975).
6. Yau W. W., Stoklasa H. J., Bly D. D.: *J. Appl. Polym. Sci.* 21, 1911 (1977).
7. Kubín M.: *J. Appl. Polym. Sci.* 27, 2933 (1982).
8. Kotaka T.: *J. Appl. Polym. Sci.* 21, 501 (1977).
9. Vozka S., Kubín M.: *J. Chromatogr.* 139, 225 (1977).
10. Kubín M.: *J. Appl. Polym. Sci.* 27, 2943 (1982).
11. Vozka S., Kubín M., Samay G.: *J. Polym. Sci. (Polym. Symposia)* 68, 199 (1980).
12. Pierce P. E., Armonas J. E.: *J. Polym. Sci. C* 21, 23 (1968).

Translated by L. Kopecká.