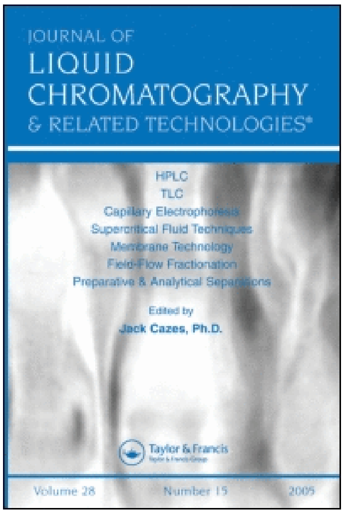

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CALIBRATION OF SIZE-EXCLUSION CHROMATOGRAPHY SYSTEMS WITH POLYDISPERSE STANDARDS

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Dedicated to Prof.O.Wichterle on the occasion of his 70th birthday

1 INTRODUCTION

Soon after its introduction to polymer science (1), size-exclusion chromatography (SEC) matured into an important tool for the determination of molecular weights and, in particular, molecular weight distributions (MWD) of diverse synthetic macromolecules, and superseded almost completely the older, tedious and less reliable fractionation methods. Different aspects of SEC have been covered in many monographs - the more recent are, e.g., (2 - 4) - and reviews including articles that deal specifically with calibration and data evaluation procedures (5 - 8).

Data evaluation via the calibration dependence molecular weight - retention volume remains an important aspect of SEC (3, 8, 9) in spite of a growing interest in dual detectors concentration/molecular weight. If the simplest method of calibration by means of narrow-distribution polymer standards is not applicable, procedures that rely on characterized polydisperse standards represent a useful alternative, as evidenced by the fact that new methods are constantly being proposed and the older recommendations are modified, improved (and sometimes reinvented).

It is the aim of the present article to summarize and critically evaluate the available information on calibration

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of SEC columns by means of characterized polymer standards having a broad molecular weight distribution.

2 BASIC CONCEPTS AND RELATIONS

It is known that under specific conditions (temperature, type of mobile phase, flowrate etc.) and on a given set of SEC columns there exists for each polymer of a given chemical and physical structure a unique relation between the molecular weight, M, and the retention volume, v, which we shall write as

$$\ln M = f(v) \tag{1}$$

and call the molecular weight calibration dependence. The retention volume is usually defined as the elution volume at the peak maximum, but it is more correct (9 - 14) to define v as the coordinate of the centroid of the peak, i.e., its first statistical moment.

The early hopes (15) that SEC with packings having known pore structure could be developed into an absolute method for measuring polymer molecular weight and MWD have not been substantiated; it is necessary to determine the shape of f(v) in Eq. (1) experimentally by means of a suitable calibration method.

As SEC separates solutes according to their size, and the size of polymer coils in solution depends on many factors which include molecular weight, chain stiffness, segment-solvent interaction, excluded-volume effects etc., the calibration dependence must be found for each polymer separately. Several attempts have been made (16 - 20) to construct a universal calibration, valid for all polymers, by plotting a suitable measure of polymer size against the retention volume. The proposal (21, 22) to use the product $[\eta] \cdot M$ as a universal calibration parameter has gained the most general acceptance; it has a sound physical basis, being proportional to the hydrodynamic volume of the solute, and is easy to apply as the intrinsic viscosity $[\eta]$ can be readily determined.

The molecular weight calibration could be found in a straightforward manner if truly monodisperse polymer standards were available. As this is not the case, however, one of the following procedures is usually used for establishing the molecular weight calibration f(v):

(i) A number of very narrow fractions ($M_w/M_n < 1.1$, where M_n and M_w are the number- and weight-average molecular weight, respectively) of the polymer in question are available, which span a sufficiently broad interval in M. These fractions are successively chromatographed and, as they are narrow, it is assumed that for the i-th standard the respective retention volume v_i corresponds to the molecular weight M_i which must be known from independent measurements by an absolute method. So far only the commercially available anionically polymerized polystyrenes fulfil the above requirements on primary calibration standards for SEC.

(ii) The system is calibrated according to the method (i) by primary standards of an auxiliary polymer (usually polystyrene) and a universal calibration is constructed as

$$\ln([\eta].M) = f_u(v) \qquad (2)$$

where the intrinsic viscosity $[\eta]$ of each standard is either directly measured (in the same solvent and at the same temperature as in the actual SEC experiment) or calculated from its molecular weight using published values of parameters K_s and α_s in the Mark-Houwink equation

$$[\eta]_s = K_s\, M_s^{\alpha_s} \qquad (3)$$

where the subscript s refers to the auxiliary standard. According to the principle of universal calibration (21,22) it holds that

$$[\eta]_p . M_p = [\eta]_s . M_s \qquad (4)$$

at the same retention volume (the index p refers to the polymer to be analysed). It is then easy to show by combining equations (3) and (4) that the sought calibration dependence f(v) can be determined from the universal function $f_u(v)$ as

$$\ln M_p = f(v) = f_u(v)/(\alpha_p+1) - (\ln K_p)/(\alpha_p+1) \qquad (5)$$

provided the Mark-Houwink parameters K_p and α_p for the polymer in question are known.

Very often one encounters a situation where the above methods of calibration are not applicable: narrow-distribution fractions of the polymer to be analysed are not at one's disposal and the universal calibration based on polystyrene primary standards cannot be used either because information on the appropriate Mark-Houwink constants K_p and α_p is lacking or the mobile phase used is a non-solvent for polystyrene. Methods that rely on secondary calibration standards (i.e. broad-distribution samples of the polymer to be analysed with one or more molecular weight characteristics known, such as M_n, M_w, $[\eta]$) must be then employed for establishing the calibration dependence; these methods will be discussed in detail below.

Separation of solutes is in real SEC systems always accompanied by processes that lead to axial dispersion (spreading) of the initially narrow bands, so that even a strictly monodisperse sample appears at the column outlet as a peak of final width. Prior to evaluating the data in terms of correct MWD and/or molecular weight averages, it is desirable to correct the chromatogram for axial spreading by solving numerically the integral equation derived by Tung (23)

$$g(V) = \int_{-\infty}^{\infty} w(v)\ G(V,v)\ dv \qquad (6)$$

where g(V) is the normalized, experimental (uncorrected) chromatogram, w(v) is the chromatogram corrected for imperfect resolution (chromatogram which would have been observed in the absence of spreading), and G(V,v) is the so-called spreading function which must be determined experimentally by calibration; G(V,v) is defined as the response of the chromatograph (as a function of elution volume V) to the injection of unit amount of a strictly monodisperse polymer having retention volume v. Calibration for spreading is then equivalent to the determination of the shape of a function of two variables G(V,v). In most instances the spreading function is assumed to be Gaussian,

$$G(V,v) = (h/\pi)^{1/2} \exp\left[-h(v - V)^2\right] \qquad (7)$$

and the spreading calibration then consists in determining the spreading factor h which is in general a function of retention volume v,

$$h = h(v) \qquad (8)$$

(More general forms of the spreading function have been proposed (24 - 27) in order to account for skew etc.)

The most general and exact method of calibration for spreading is the reverse-flow procedure proposed by Tung, Moore, and Knight (28), but this has been shown to be inapplicable (29) with the modern high-performance, high-speed SEC systems that utilize microparticulate packings in columns of relatively small volume, because of extracolumn spreading in the additional tubing and valve required for the flow reversal. Other methods that rely on manufacturer's data on M_n and M_w of primary polystyrene standards have been proposed (29 - 32); in this review some published methods of spreading calibration which employ characterized polydisperse standards will be discussed in Sections 4.1 and 4.2.

It follows from the definition of the spreading-corrected chromatogram that the product w(v) dv represents the weight fraction of the polymer eluting between volumes v and v+dv from an ideal column; accordingly, w is simply related to the molecular weight distribution F(M),

$$F(M)\, dM = -w(v)\, dv \qquad (9)$$

(The sign in Eq. (9) reflects the fact that in SEC the molecular weight decreases with increasing retention volume.) Combining Eqns (1) and (9) one arrives at

$$F(M) = -w(v)/(dM/dv) = -w(v)\exp[-f(v)]/(df/dv) \qquad (10)$$

which shows that MWD can be calculated from the corrected chromatogram, the molecular weight calibration dependence f(v) and its derivative. Once F(M) is known, the various molecular weight averages (M_n, M_w, the viscosity-average M_η etc.) can be calculated from their definitions; alternatively, they can be found directly from the corrected function w using the relationships

$$M_n = \left[\int_0^\infty \frac{F(M)\ dM}{M}\right]^{-1} = \left[\int \frac{w(v)\ dv}{\exp\,[f(v)]}\right]^{-1} \qquad (11)$$

$$M_w = \int_0^\infty M\ F(M)\ dM \quad = \int \exp\,[f(v)]w(v)\ dv \qquad (12)$$

$$M_\eta = \left[\int_0^\infty M^\alpha\ F(M)\ dM\right]^{1/\alpha} = \left[\int \exp[\alpha f(v)]\ w(v)\ \ dv\right]^{1/\alpha} \qquad (13)$$

(where α is the exponent in the Mark-Houwink equation (3) for the polymer in question). The quantity M_{GPC} calculated from the calibration dependence - Eq. (1) - for the elution volume corresponding to the peak maximum is an average of unknown type and its use for polydisperse samples is strongly discouraged.

3 MOLECULAR WEIGHT CALIBRATION

3.1 Secondary standards with known MWD

As shown in Section 2, the distribution of a sample is unequivocally connected with the corrected chromatogram and the molecular weight calibration function. In principle, it is therefore possible to determine f(v) from the chromatogram of a polydisperse polymer with known MWD, assuming that the axial spreading can be neglected. (This assumption seems to be reasonable in view of the necessity to use samples of very broad distribution).

The procedure is illustrated in Fig.1. The total area under the normalized, uncorrected chromatogram plotted in Fig.1a represents all the polymer; the fractional area A from the final elution volume V_2 at the end of chromatogram to a selected volume V_i corresponds to a weight fraction of the polymer for which $V > V_i$ (axial spreading is neglected) or, considering the integrated form of Eq. (9) with w replaced by g,

$$\int_{V_2}^{V_i} g(v)\ dv = \int_0^{M_i} F(M)\ dM \qquad (14)$$

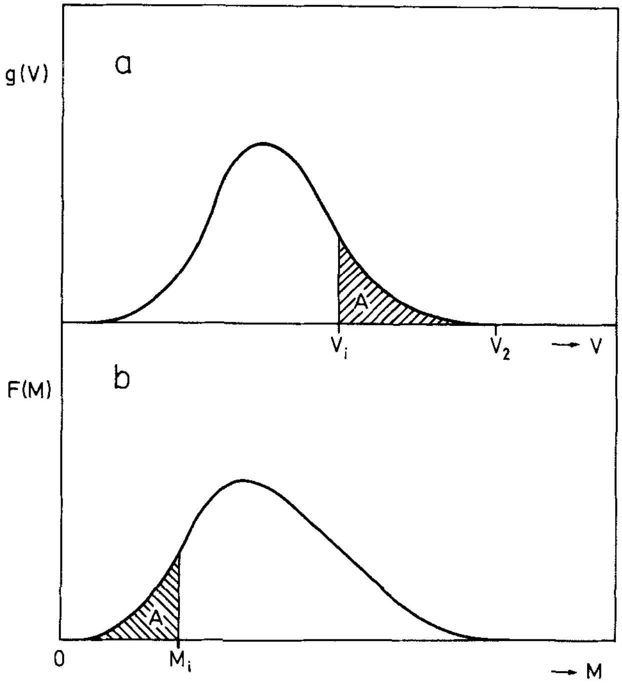

FIGURE 1

Estimation of molecular weight calibration dependence from normalized chromatogram g(V) of a polydisperse sample with known molecular weight distribution F(M). The shaded areas are equal.

for which the molecular weight is lower than M_i. One simply assigns to each V_i the corresponding abscissa M_i in Fig.1b such that the shaded areas A in both Figures are equal. In this way a series of corresponding pairs (v_i, M_i) is generated which define the calibration curve.

The method has been successfully used (33) in 1967 with broad-distribution polyisobutylenes of known MWD. The molecular weight distribution is either determined experimentally (33) (e.g., by a careful fractionation) or a commercially available sample with a known MWD is used (34 - 36). (A polyethylene sample with known MWD, SRM 1475, is available from the National Bureau of Standards, Washington; a polydisperse polystyrene standard with known MWD can be purchased from the National Physical Laboratory, Teddington, Great Britain. Dextran samples similarly characterized are available from Pharmacia Fine Chemicals, Uppsala, Sweden).

Other authors (37 - 39) assumed the distribution to be known on the basis of thoroughly studied kinetics of polymerization. It is also possible to assume the form of the distribution function in advance (e.g., the Schulz-Zimm function has been made use of by Weiss and Cohn-Ginsberg (40)) and to calculate its parameters from measured molecular weight averages.

If a suitable broad-distribution sample with known MWD is available, the procedure is very simple, but one must keep in mind the following limitations:

(i) The reliability of the resulting calibration dependence is directly given by the accuracy with which the molecular weight distribution of the standard has been determined.

(ii) The calibration curve is determined only in the interval of M spanned by the distribution (34); it is known from experience that any extrapolation of the calibration dependence is a very dubious procedure. This limitation has been overcome is some papers (35, 40) by applying the method to several samples with partially overlapping MWD's and combining the results; the coincidence of superimposed curves was surprisingly good (35, 40).

(iii) At both extreme ends of the chromatogram the error in the area A can be very large (34, 38); in addition, the difference between the corrected and uncorrected chromatogram is most pronounced in these two regions.

Some authors (37, 38) claim that because uncorrected chromatogram is used in Eq. (14), the resulting calibration dependence has the property that the calculated molecular weight averages are automatically and effectively corrected for zone broadening. This will be discussed (and mostly disproved) in the next Section in connection with the effective linear calibration.

3.2 Secondary standards with known molecular size characteristics

3.2.1 Calibration when information is scarce

If only a small number of broad-distribution secondary standards are available, one has to resort to the assumption that the sought calibration dependence can be adequately described by a linear equation of the form

$$\ln M = A - B\,v \tag{15}$$

In principle, two characteristics are then sufficient for the determination of the constants A and B, such as the number- and weight-average molecular weight of a single standard. It follows from Eqns (11), (12), and (15) that in this case

$$M_n = e^A / \int e^{Bv}\, w(v)\, dv\,, \quad M_w = e^A \cdot \int e^{-Bv}\, w(v)\, dv \tag{16}$$

Balke et al. (41) replaced in these equations the unknown corrected chromatogram w by the experimentally available chromatogram g of the secondary calibration standard in question; the equations then define not the true constants A and B, but some effective values $A^*$and B^*:

$$M_n = e^{A^*} / \int e^{B^* v} g(v)\, dv\,, \quad M_w = e^{A^*} \cdot \int e^{-B^* v}\, g(v)\, dv \tag{17}$$

Balke et al. solved equations (17) by means of a two-dimensional search algorithm for the constants $A^*$and B^* of the effective linear calibration

$$\ln M = A^* - B^*\, v \tag{18}$$

More efficient and rapid methods have been later proposed (42 - 47) for solving numerically equations (17).

It has been claimed (38, 41, 44, 48, 49) that the effective linear calibration automatically corrects the molecular weight averages calculated from the experimental chromatogram g(v) for zone broadening. This is certainly and obviously true for the standard used in establishing the effective linear calibration (see equations (17)); it is also true that for any sample the ratio M_w/M_n calculated from the uncorrected chromatogram with Eq. (18) is always lower than if the actual calibration line - Eq. (15) - established, e.g., by means of primary standards were used, because the slope B^* is always smaller (47, 49, 50) in absolute value than B. An analysis proves (51) that the ex-

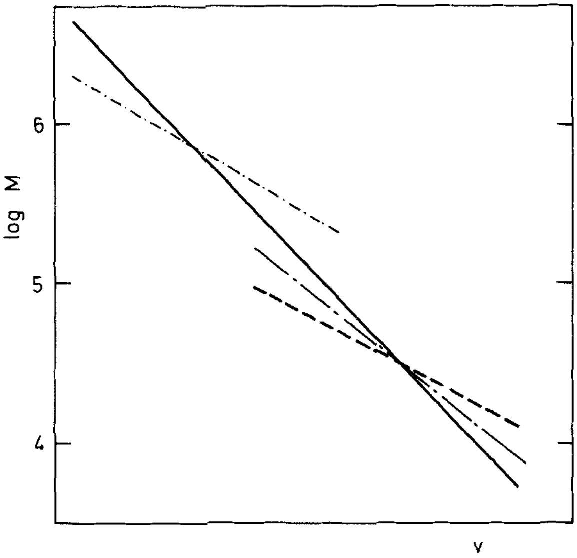

FIGURE 2

Effective linear calibrations

Full line is the true calibration. Effective linear calibration dependences from samples with

---- $M_n=5.10^4$, $M_w=6.10^4$;

— - —$M_n=4.10^4$, $M_w=7.10^4$;

-.-.-$M_n=6.10^5$, $M_w=1.10^6$.

tent of this "correction" depends both on the separation efficiency of the SEC system and on the width of the distribution of the calibration standard. The situation is illustrated in Fig.2 which shows that the difference between the effective and true calibration lines increases with decreasing width of the standard MWD and also that the effective linear calibration can lead to biassed molecular weights calculated for an unknown polymer if its mean molecular weight is sufficiently removed from that of the original calibration standard - see also (52).

Yau et al. (49) suggested an improved method which takes into account the effect of axial spreading and reduces the difference between the effective and true calibration lines.

Their procedure requires the spreading factor to be estimated beforehand; however, the method the authors suggested (49) for calculating the spreading factor from a chromatogram of a narrow-distribution standard is liable to overestimate the spreading unless the standard used is very narrow indeed (M_w/M_n < 1.005, see Knox and McLennan (53)). It was later shown by Pollock et al. (44) that from the point of view of error propagation the original method (41) and the improved version (49) are roughly equivalent.

Tsvetkovskii et al. (54) used another approach to the linear calibration which also respects peak spreading; it requires at least two secondary standards (each characterized by any combination of two values from the set M_n, M_w, $[\eta]$), but its basic simplicity is offset by the inherent assumption that the molecular weight distribution of the standards must be logarithmic-normal.

In assessing merit of the above methods it must be borne in mind that although the restriction of linearity seems to detract much from their general applicability - see, e.g., (51, 52) - with the limited amount of information one can hardly expect to do better. Moreover, with the so-called "linear" SEC columns marketed by DuPont and by Toyo Soda, one can be confident that the accuracy of a straight-line calibration dependence will be sufficient for most practical purposes. Finally, the effective linear calibration forms the starting point of some more sophisticated calibration procedures to be described below.

3.2.2 Universal calibration in combination with secondary standards

Very often the column set can be calibrated by means of polystyrene primary standards (the universal calibration dependence $f_u(v)$ is known), but this information is useless for the problem at hand because the Mark-Houwink constants for the polymer to be analysed in the given mobile phase are not known. If one or several broad-distribution samples of the polymer with at least two molecular size characteristics (such as M_n, M_w, or $[\eta]$) are at disposal, the situation can be rectified by procedures that are based on a derivation first published by Weiss and Cohn-Ginsberg (55).

Recalling equations (2), (4), and (5), we can express the molecular weight M_p by means of the known function $f_u(v)$ and the as yet unknown Mark-Houwink constants of the polymer in question:

$$M_p(v) = \exp[f_u(v)/(\alpha_p + 1)]/K_p \tag{19}$$

(As in the following derivation only the constants K_p and α_p will be required, we shall drop the subscript for simplicity). Assuming again that for the polydisperse standard(s) it is legitimate to replace the spreading-corrected chromatogram w by the experimentally available function g, we have from Eqns (11) and (12)

$$M_n = K^{-\frac{1}{\alpha+1}} \Big/ \int \exp\left[-\frac{f_u(v)}{\alpha+1}\right] g(v)\,dv \tag{20}$$

$$M_w = K^{-\frac{1}{\alpha+1}} \cdot \int \exp\left[\frac{f_u(v)}{\alpha+1}\right] g(v)\,dv \tag{21}$$

and for the intrinsic viscosity $[\eta]$ of the standard we obtain similarly

$$[\eta] = K^{\frac{1}{\alpha+1}} \cdot \int \exp\left[\alpha \cdot f_u(v)/(\alpha+1)\right] g(v)\,dv \tag{22}$$

The required Mark-Houwink constants can now be found by solving numerically two of the above equations using two experimentally determined characteristics. The solution is considerably simplified by forming groups of the molecular size characteristics such that the parameter K in the resulting equation cancels; the most useful of these are

$$[\eta] \cdot M_n = \frac{\int e^{\alpha f_u(v)/(\alpha+1)}\, g(v)\,dv}{\int e^{-f_u(v)/(\alpha+1)}\, g(v)\,dv} \tag{23}$$

$$M_w/M_n = \int e^{f_u(v)/(\alpha+1)}\, g(v)\,dv \cdot \int e^{-f_u(v)/(\alpha+1)}\, g(v)\,dv \tag{24}$$

and, if intrinsic viscosities of two polydisperse samples are known, the ratio

$$\frac{[\eta]_1}{[\eta]_2} = \frac{\int e^{\alpha f_u(v)/(\alpha+1)} g_1(v)\, dv}{\int e^{\alpha f_u(v)/(\alpha+1)} g_2(v)\, dv} \qquad (25)$$

where g_i is the uncorrected chromatogram of the i-th sample. Depending on the available characteristics, one of the last three equations is solved for α by a one-dimensional search and K is then calculated from (20), (21), or (22), as the case may be. The calibration dependence for the unknown polymer is then determined according to Eq. (5).

The method and its modifications have been extensively tested with many polymers (52, 56 - 61). The investigators agree that although the Mark-Houwink parameters obtained in this manner vary from sample to sample, a high value of K is always compensated by a low value of α so that the calculated molecular weights are in reasonable agreement with values measured by absolute methods. The possible source of error due to the uncertainty in Mark-Houwink constants determined in this manner can be diminished by combining chromatograms of several characterized samples and determining K and α by some least-squares optimization (60). Kato et al. (62) recommend to use a Θ-solvent as the mobile phase where the exponent α is known ($\alpha = 0.5$). Dobbin et al. (63) compared several of the possible combinations of molecular size characteristics and concluded that the best values of K and α are obtained when the ratio of two intrinsic viscosities - Eqn. (25) - is used.

Some proposed modifications of the method introduce additional assumptions (linearity of calibration (64, 65), Gaussian shape of chromatograms (65)) , which are unnecessary and limit the scope of the original method, but facilitate the evaluation of data.

Hamielec (66) and Taganov et al. (67) proposed generalizations of the above procedure which take into account spreading and skewing.

3.2.3 Calibration with a series of secondary standards

If a sufficient number of characterized, polydisperse standards are available, the SEC system can be calibrated without recourse to the universal calibration and also without the awkward restriction of linearity. As early as in 1968 Frank et al. (68), faced with the task of calibrating SEC columns for polyethylene, developed a somewhat cumbersome graphical-numerical procedure, where the calibration dependence was defined by a number of its enveloping curves and in the final stage had to be drawn by hand (see also Dawkins (69)).

Barlow et al. (36) extended the calibration dependence for polyethylene obtained by the method of Section 3.1 into the high-molecular-weight region (not covered by the polyethylene standard NBS 1475) by an iterative procedure that seeked to obtain coincidence between the measured intrinsic viscosities of polyethylene samples and values calculated from their chromatograms.

A number of investigators proposed calibration procedures that can be described in general terms as follows. Calibration standards with arbitrary MWD are available, characterized by N molecular weight averages $\bar{M}_\beta^{(i)}$ ($i = 1,2,..N$, $\beta = n$ or w). The molecular weight calibration dependence is described by a mathematical function $f(v;c_1,c_2,..c_n)$ with n adjustable parameters, where $n \leq N$. The values of the parameters in the model function are then determined from the requirement that the observed (i.e. calculated from SEC data) averages $M_\beta^{(i)}$ agree with the corresponding "true" values $\bar{M}_\beta^{(i)}$.

Thus, Szewczyk (70), McCrackin (71), Vrijbergen et al. (43), Chaplin and Ching (61) all used polynomials in v,

$$\ln M = \sum_{j=1}^{n} c_j v^{j-1} \tag{26}$$

for modelling the molecular weight calibration dependence. Nilsson and Nilsson (72) employed a modified polynomial function

$$\ln (M - c_5) = c_4 + c_1 v + c_2 v^2 + c_3 v^3 \qquad (27)$$

with five parameters. Cardenas and O'Driscoll (48) proposed to describe the calibration dependence by a four-parameter function

$$v = c_1 + c_2 \{\pi^{-1/2} \psi^{-1} [1 - \exp(-\psi^2)] + \operatorname{erfc}(\psi)\} \qquad (28a)$$

where

$$\psi = M^{c_4}/c_3 \qquad (28b)$$

originally derived by Yau and Malone (73) in their diffusion theory of separation in SEC (see also (12)).

All these methods, however, suffer from one deficiency, viz., the number of adjustable parameters n is set in advance. (This is obviously less important if n is sufficiently large, as in Eqns (28) or (27)). It has been repeatedly stressed (31, 48, 74, 75) that considerable errors can arise if a distinctly curvilinear calibration is forcibly fitted to a linear equation of the type (15); by the same token the number of unknowns (e.g., the degree of approximating polynomial) should be also optimized by a suitable statistical procedure. This has been recognized by Andersson (76) who tested several types of model functions and optimized also the number of parameters by minimizing the modified weighted sum of squared deviations

$$X^2 = (N - n)^{-1} \sum_{i=1}^{N} [M_\beta^{(i)} - \bar{M}_\beta^{(i)}]^2 \cdot W_i \qquad (29)$$

where the weights W_i are in the simplest case given by $W_i = [\bar{M}_\beta^{(i)}]^{-2}$ - see (18, 76) for a detailed discussion. Andersson recommends to use cubic splines for modelling the calibration dependence.

All the above authors substituted the experimental, uncorrected chromatogram into formulae that are strictly speaking valid only for the corrected function w. As a consequence, the results bear the character of effective and not true calibration dependences in the sense discussed in Section 3.2.1.

McCrackin (71) in his Method II respected the effect of axial dispersion in an elementary manner: although uncorrected chromatograms of the calibration standards were still used for calculating the average molecular weights, the known characteristics $\bar{M}_n^{(i)}$ and $\bar{M}_w^{(i)}$ were first multiplied by the appropriate correction factors derived (32) for the case of constant spreading and linear calibration dependence. In view of the latter two conditions the method is restricted to standards of moderately broad MWD, for which both $\bar{M}_n$ and $\bar{M}_w$ are known, but along with the true calibration dependence (modelled by a polynomial of a second degree) the spreading factor (assumed constant) is also determined.

The most recent approaches that respect the imperfect resolution of real SEC columns and yield the true (in general non-linear) molecular weight calibration together with the spreading calibration h(v) will be discussed separately in Section 5.

3.3 Calibration in SEC with multiple detectors

In recent years the dual detectors concentration/molecular weight find many applications in SEC. So far, viscometers (either discontinuous (77 - 85), measuring the viscosity of separate portions of the eluate, or continuous (86 - 88)) and the low-angle laser light scattering photometer - LALLSP (89 - 92), now marketed as KMX-6 by Chromatix, U.S.A, have been used in combination with a concentration detector (mostly differential refractometer). The molecular weight of the fraction leaving the column can be determined directly (LALLSP) or indirectly (automatic viscometer) by combining the data of the two detectors.

It would seem that in this case the relationship molecular weight - retention volume looses much of its importance, and that at any rate it can be readily established for any polymer simply by plotting the data obtained by chromatographing a broad-distribution sample. This is, however, not completely true. First, the calibration dependence and in particular the universal function $\ln([\eta]\cdot M) = f_u(v)$ is necessary for data evaluation when complex macromolecular systems (branched structures, chemically heterogeneous copolymers) are investigated - and here lies the true realm of SEC with dual detection. Secondly, it is now definitely established that the correction for axial spreading is necessary if meaningful estimates of the calibration curve are to be obtained (93) from the data of the LALLSP system, and this is even more important with the automatic viscometer if one considers the problem of backmixing in the tubing and in the siphon.

Zone broadening has been recognized (94) as the source of observed deviations (81, 84) between the molecular weight calibration obtained by classical methods using narrow fractions and that resulting from the uncorrected data of an automatic viscometer coupled with SEC. Park and Graessley (85) obtained correct molecular weight calibration dependence from SEC/viscometry applied to broad-distribution samples by taking into account the transport lag between the detectors, the zone spreading in the column, and post-refractometer mixing in the tubing and in siphon. The problem of finding the molecular weight calibration for the given polymer from the universal function $f_u(v)$ using the data of the SEC/viscometer system with correction for zone broadening has been investigated by Taganov (93).

Recently, Kim et al. (96) utilized the new, generalized analytical correction for imperfect resolution (93) and developed a valid method for the determination of the molecular weight calibration function (and, incidentally, the spreading factor - see Section 4.2) from a single chromatogram of a polydisperse polymer using the SEC/LALLSP detection system. In another article Hamielec (97) outlined the methodology for the determination of the molecular weight calibration, together with the Mark-Houwink constants and the elution volume dependence of spreading, from the universal curve $f_u(v)$ using the data of the dual detector (see also (98)).

4 SPREADING CALIBRATION

Calibration for spreading in SEC is seriously hindered by the fact that (with the exception of some naturally occurring macromolecules) strictly monodisperse polymers simply do not exist. As shown by Knox and McLennan (53) the contribution of polydispersity to the total peak width is considerable even for very narrow fractions. Accordingly, it is not justified to estimate the spreading directly from the variance of normal-flow (as opposed to reverse-flow) uncorrected chromatograms of primary calibration standards (25, 27, 49, 99), unless special measures (100) are adopted to ensure that the quotient M_w/M_n is lower than about 1.005.

Standards characterized as to their polydispersity are required for the spreading calibration, and it can be expected that for accurate results samples having narrow MWD will be preferable; even here, however, the scatter in the experimentally determined spreading factors is often quite high (29). Fortunately, the correction for imperfect resolution is not very sensitive to the accuracy of h, as demonstrated by the following argument. Consider the spreading correction according to Balke and Hamielec (101) to be $\exp(-B^2/4h) = 0.9$ (the corrected M_w will be then lower than the uncorrected average by some 10 %). If the value of h is now varied by ± 20 %, the correction factor changes from 0.92 to 0.88, well within the limits of accuracy of SEC results. It is therefore not surprising that the calibration for spreading with polydisperse secondary standards often yields reasonable results.

4.1 Spreading calibration with secondary standards

Taganov et al. (102) and later Berger (103) suggested "refractionation" procedures for the estimation of the shape of G(V,v) that require the chromatogram of a standard and also chromatograms of several of its fractions isolated with (and recorded on) the same set of SEC columns. The methods are tedious but require no additional information on the standard. A method of spreading calibration by means of a polymer sample with accurately determined MWD has been outlined by Berger (100) - see also (104).

Several groups (105 - 107) used recycle SEC to determine the spreading; the methods have been mostly tested with narrow-distribution samples, but there is no reason why they could not work with moderately broad polymers as well.

The method of moments (29) developed for spreading calibration with a Gaussian G(V,v) can utilize both narrow- and broad-distribution standards.

Additional methods developed recently and capable of determining the functions f(v) and h(v) simultaneously in a once-through process from the chromatograms of characterized standards with an arbitrary shape of MWD will be discussed separately in Section 5.

4.2 Spreading calibration using dual detectors

Park and Graessley (85) showed how to estimate the extent of spreading from the data of an automatic viscometer coupled with SEC: assuming that the true molecular weight calibration dependence is known, h(v) can be determined by trial and error from the chromatogram and from measured intrinsic viscosities of fractions using the Tung equation (6) for calculating the distribution of various molecular weight species among the fractions.

Berger (108) developed a systematic method for correcting the data of the dual detector for imperfect resolution by solving simultaneously the Tung equation (6) and the integral equation

$$[M^{*}(V)]^{\alpha} = \frac{\int [M(v)]^{\alpha}\, w(v)\, G(V,v)\, dv}{\int G(V,v)\, w(v)\, dv} \qquad (30)$$

where α is the Mark-Houwink exponent for the automatic viscometer and $\alpha = 1$ for the LALLSP. $M^{*}(V)$ is the uncorrected molecular weight at the elution volume V as determined from the combined signals of the detectors, M(v) is the true (spreading-corrected) molecular weight. A fairly complicated matrix algebra is involved but, provided again that the true molecular weight calibration dependence is known, the treatment yields both the corrected molecular weight M(v) of the species leaving the column at v and the spreading function (assumed to be uniform, i.e. independent of M in Berger's treatment).

This procedure has been simplified by Netopilík (109) who recognized that the two integral equations (6) and (30) can be rewritten in a form which makes them basically equivalent and amenable to be solved successively by any of the numerous methods originally developed for handling the Tung equation (6). The correct spreading factor is then found as that value of h which makes the corrected function M(v) to coincide with the molecular weight calibration established independently. Hamielec (97) outlined a procedure wherein the new analytical solution (93) of the Tung spreading equation (6) is employed and the spreading factor h(v) is determined from the data of SEC with an on-line molecular weight detector, assuming that either the molecular weight calibration f(v) or the universal function $f_u(v)$ is known.

5 SIMULTANEOUS MOLECULAR WEIGHT AND SPREADING CALIBRATION

Hamielec (97) maintains that in order to obtain accurate molecular weights, the data of SEC must be corrected for axial spreading even for broad-distribution samples chromatographed with a high-performance equipment. Several methods for evaluating normal-flow chromatograms of primary, narrow-distribution standards, as obtained in the process of molecular weight calibration, also in terms of the spreading factor and its dependence on elution volume have been proposed and tested (29, 30, 31, 101, 110). The problem of a comprehensive, once-through calibration in SEC using secondary standards having MWD of an arbitrary shape has been seriously attacked only recently. (Already in 1968 Almin (111) outlined a method for the determination of both the true molecular weight calibration (assumed to be linear) and the spreading factor as a function of retention volume, h(v), from chromatograms of polydisperse standards, but his procedure was based on rather artificial assumptions concerning the shape of experimental chromatograms.)

Andreetta and Figini (112) proposed a method for a simultaneous determination of the functions ln M = f(v) and h(v), based on the equation for the general β-average molecular weight derived by Figini (113):

$$M_\beta = \{\int M^\beta \cdot F(M)\, dM\}^{1/\beta} = \{\int g(v) \cdot [M(v)]^k\, dv\}^{1/k} \cdot L^{-1} \qquad (31)$$

where k is a function of β and of the parameters (say c_i) that define the molecular weight calibration dependence - $f(v,c_1,c_2,..)$ - as well as of the coefficients (say b_i) which enter into the elution volume dependence of spreading - $h = h(v, b_1, b_2,..)$; thus

$$k = k(\beta, c_1,c_2,\ldots;b_1,b_2,\ldots) \qquad (32)$$

Similarly, it holds for L that

$$L = L(c_1,c_2,\ldots;b_1,b_2,\ldots) \qquad (33)$$

Andreetta and Figini recognized that in many instances of practical interest the functional dependences in Eqns (32) and (33) can be explicitly derived by direct integration of the Tung's spreading equation (6). In these cases the sum of squared deviations

$$X^2 = \sum_{i=1}^{N} [M_\beta^{(i)} - \bar{M}_\beta^{(i)}]^2 \qquad (34)$$

(where $\bar{M}_\beta^{(i)}$ are again the known molecular weights of N secondary standards employed) can be minimized by Gauss iteration as the required partial derivatives can be evaluated analytically. So far only results with a constant spreading factor have been published, but the method is capable of further generalization.

Kubín (47) analysed theoretically the relationship between the true - Eqn (15) - and effective - Eqn (18) - linear calibrations for the case of Gaussian spreading and ascertained that the two straight lines intersect at the properly defined retention volume (centroid of chromatogram); as the constants A^* and B^* can be readily found by solving the equations (17) (assuming that M_n and M_w are known for each calibration standard), and the centroid of the chromatogram, μ^*_1, is easily accessible, one obtains for each standard the coordinates $(\mu^*_1, A^* - B^* \mu^*_1)$ of the intersect, i.e., of one point on the true calibration dependence. With a number of calibration standards the resulting points can be processed by some standard correlation procedure to yield the best-fit functional dependence for the true calibration f(v), e.g., in the form of a polynomial of statistically correct degree.

The slopes of Eqns (15) and (18) are related (47) through the equation

$$1 + (B^*)^2\mu_2 = \exp(-B^2/2h)\ (1 + B^2\ \mu_2) \qquad (35)$$

which also contains the spreading factor h and the variance (second statistical moment) of the respective peak,

$$\mu_2 = \int (v - \mu_1^*)^2\ g(v)\ dv \qquad (36)$$

As B^* is known and B can be taken as the slope of the true calibration dependence determined e.g. by the above method of intersects (if this is curvilinear the local slope at $v = \mu_1^*$ is assigned to B), Eqn (35) can be directly solved for the spreading factor h.

This method yields reliable molecular weight and spreading calibrations for moderately broad standards (75). For very broad polymers where the accuracy of corrected molecular weight averages calculated from the above calibration dependences is less satisfactory, an iteration loop can be initialized which improves the initial estimates of f(v) and h(v); for details see (47, 75).

6 CONCLUSION

Reliable calibration is an absolute necessity in contemporary SEC and apparently will remain so in the foreseeable future. The studies reported in this review should convince the reader that it is feasible to establish reliable molecular weight and spreading calibration dependences using normal-flow chromatograms of polydisperse, characterized polymer standards. The techniques developed differ greatly in scope and in their requirements on the extent of necessary input information; the most powerful methods involve considerable computation and require the use of a computer. It is expected that new procedures will emerge in the future in spite of the growing interest in the so-called absolute detectors in SEC.

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