Theoretical and Practical Problems in Characterizing Polymers with Extremely Narrow Molecular-weight Distribution by SEC with Dual Detection

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Theoretical and Practical Problems in Characterizing Polymers with Extremely Narrow Molecular-weight Distribution by SEC with Dual Detection*

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Peak broadening in size exclusion chromatography of polymers is closely related to local polydispersity. Under simplifying conditions, the log-normal molecular-weight distribution and Gaussian spreading function, both phenomena can analytically be described. Local polydispersity is constant throughout the whole span of peak on the elution-volume axis depending only on the sample polydispersity and the extent of peak broadening. Due to peak broadening, the dependence of molecular weight *vs.* elution volume obtained from the dual-detection data is rotated with respect to that obtained in a broad range of elution volumes from analyses of several samples with narrow molecular-weight distribution. The extent of peak broadening and local polydispersity can be found from the concentration/light scattering dual-detection SEC analysis data. The relations between the concentration and light-scattering elution curves, with respect to peak broadening, are discussed.

INTRODUCTION

Soon after its introduction,[1–4] size exclusion chromatography (SEC) was employed in the dual-detection mode: the concentration

detector was supplemented by a viscometric[5-9] and, later on, by a light-scattering detector.[10-14] In order to obtain molecular-weight distribution (MWD), the former combination of detectors requires calibration of the separation system: either a known dependence of molecular weight, $\log M$, or $\log[\eta]M$, ($[\eta]$ is intrinsic viscosity) on elution volume V. The dependence of $\log M$ on V obtained using a series of standards of narrow MWD and known molecular-weight averages, is sometimes called 'conventional calibration'. The latter combination makes possible the so called absolute detection, i.e., direct determination of molecular weight, M, in the eluent.

However, it has soon been found that although the combination of light scattering and concentration detectors yields a theoretical possibility of finding MWD of a polymer sample, the experimental results are plagued by large errors, especially in the determination of number-average molecular weight, M_n. There are several causes of errors, among them the existence of peak broadening[15-39] (axial dispersion) and related local polydispersity[37-39] (which is especially complicated in dual detection[26-45]), by uncertainty in the determination of interdetector volume[40-45] and decreasing signal-to-noise ratio in the marginal parts of the elution curves.[47]

Thanks to progress in instrumentation, the sources of errors have been largely eliminated except for samples of extremely narrow MWD.[13] Experimental characteristics of such samples, obtained from slopes of $\log M_d$ vs. elution volume V obtained in one-sample analysis are aggravated by errors to the extent that they are quite unrealistic as can be seen by comparing them with the slope of the 'conventional calibration'. Samples with narrow MWD are important and interesting because they are used as calibration standards. Knowledge of their weight-to-number average ratio, M_w/M_n, is of crucial importance enhanced by the fact that there is no reliable absolute method for M_n determination of high-molecular-weight samples. Hence, peak broadening and related phenomena, such as the uncertainty in the determination of the interdetector volume, are still important in the analyses of MWD of polymers; in the following they will be treated in detail. The phenomena will be treated with respect to the light-scattering detection. However, all concepts can be extended to viscometric detection.[39]

Single- and Dual-detection SEC

If conventional calibration is employed and only the concentration detector is used, it is not necessary to know the exact value of concentration. Differential refractometer is conveniently used even though the signal-to-concentration ratio, for a given polymer species and solvent, depends not only on the refractive index increment, dn/dc, but is influenced by variations of intensity of the light source, sensor sensitivity, *etc.*

In the dual-detection SEC mode, the differential refractometer is not reliable because concentration has to be determined absolutely for the determination of molecular weight which requires either frequent calibration[48] or, more advisably, the use of an interference refractometer.[12]

Molecular weight is determined by light scattering. The angle and concentration dependence of the intensity of the scattered light is given by[49]

$$\frac{Kc}{R(\theta, c)} = \frac{1}{M_w P(\theta)} + 2A_2 c + \cdots \tag{1}$$

where K is an optical constant (which includes dn/dc), $R(\theta, c)$ is the Rayleigh ratio of the intensity of light scattered at the angle θ from a solution of polymer of concentration c to that of the incident light, $P(\theta)$ is the normalized intensity distribution function and A_2 is the second virial coefficient.

In the off-line measurements, experimental data are extrapolated according to Eq. (1) to zero angle and concentration.[50]

For light-scattering data evaluation in SEC, the product of molecular weight and concentration in the limit of zero angle and concentration is necessary.[34] If the value of A_2 is known, this product can conveniently be obtained from Eq. (1) by neglecting higher terms, dividing the equation by c and solving for $M_w c$

$$M_w c = \frac{1}{P(\theta)} \left(\frac{K}{R(\theta, c)} - 2A_2 \right)^{-1} \tag{2}$$

At low angle $\theta \to 0$, $P(\theta) \to 1$, we have according to Eq. (1)

$$\frac{Kc}{R(0, 0)} = \frac{1}{M_w} \tag{3}$$

By combining Eqs. (2) and (3), the Rayleigh ratio extrapolated to zero angle and concentration can be expressed as

$$R(0,0) = \frac{K}{P(\theta)} \frac{R(\theta, c)}{K - 2A_2 R(\theta, c)} \qquad (4)$$

The function $P(\theta)$ depends on the shape and dimensions of the macromolecules [12−14, 49] and usually is unknown before the analysis. For this reason, the measurements have to be performed so that the value of the Rayleigh ratio corresponding to a low angle is obtained $(P(\theta) \to 1)$

$$R(0,0) = \frac{KR(0, c)}{K - 2A_2 R(0, c)} \qquad (5)$$

On the basis of Eq. (5), the light scattering elution curve can be defined, as the light scattering signal extrapolated to zero angle and concentration (although the local value of concentration is not necessarily known), and treated with respect to the peak broadening parallely with the concentration elution curve.

The frequent practice in SEC is the measurement at one angle [43] (e.g., at 90°, 'right angle light scattering', RALS), at several angles [43] or at one angle close to zero ('low angle light scattering', LALS). However, the ultimate solution is the measurement using an array of detectors at different angles ('multiangle light scattering', MALS) which makes the extrapolation procedure reliable. [12−14]

The dependence of concentration c on elution volume V is called the concentration elution curve in SEC denoted by $F(V)$. This symbol often means a normalized curve (the area between the curve and baseline is equal to unity). The product of concentration and molecular weight determined from the detector data, is called the light-scattering elution curve, $F(V)M_d(V)$. The subscript 'd' means that the molecular weight is determined from the detector data. If light-scattering detection is employed, M_d is the local weight-average molecular weight [39] as can be seen from Eq. (2). The curves $F(V)$ and $F(V)M_d(V)$ do not correspond exactly to MWD of the polymer because of experimental error, including peak broadening. The theoretical counterparts of the (experimental) concentration and light-scattering elution curves are defined as $W(V)$ and $W(V)M(V)$

respectively in the following theoretical elution curves. In the next section, the relation of peak broadening and local polydispersity will be discussed.

Peak Broadening and Local Polydispersity

The usual cause of local polydispersity is peak broadening. In addition, the source of local polydispersity can also be the nonuniformity in molecular topology of the polymer analyzed. For instance, with nonuniform branched polymers, molecules with different combinations of molecular weight, degree of branching and topology may have the same hydrodynamic volume and elute at the same elution volume.[37] Both sources of local polydispersity have to be distinguished and treated separately, although they may contribute at the same time. In the following, under the term 'local polydispersity', peak broadening is the cause.

Theory of peak broadening is based on the well-known Tung equation [15]

$$F(V) = \int W(y)G(V,y)dy \qquad (6)$$

where $F(V)$ is the uncorrected elution curve, $W(y)$ is the corrected curve and the kernel $G(V,y)$ is called the spreading function. The limits of integration are within the limits of the elution curves. For the elution volume, two equivalent variables, V and y are used. Equation (6) says that the contribution of fraction $W(y)$ of polymer eluted theoretically at elution volume y to the fraction of polymer eluted is *really* at elution volume V given by $W(y)G(V,y)$. This is the source of local polydispersity caused by peak broadening.

Only $F(V)$ in Eq. (6) is obtained directly by experiment. Because the process of peak broadening is complicated and cannot be understood in all details,[46] it is necessary to accept simplifying approximations. The most common and important of them is the approximation of $G(V,y)$ by the well-known Gauss normal distribution, centered[15] at y, with a mean deviation σ^2. (Sometimes the spreading factor defined[15] $h = 1/\sigma^2$ is used.)

Local polydispersity is difficult to prove experimentally because it is below the resolution power of the particular columns; it is, however,

essential for understanding of peak broadening namely in dual detection SEC.

In general, mathematical treatment of peak broadening in connection with dual detection is complex. Some important details will be discussed in the last section. In a special case of a polymer with MWD which can be approximated by a log-normal model MWD, *i.e.*, for a polymer with elution curve approximated by the Gaussian curve, the problem of peak broadening and local polydispersity can be described analytically. This makes it possible to demonstrate graphically basic principles of peak broadening and local polydispersity. It is assumed that the ('conventional') calibration dependence is given by

$$\ln M = A + BV \tag{7}$$

where A and B are constants. In this case, the ratio of the sample polydispersity, expressed as the M_w/M_n ratio, to its local value, $(M_w/M_n)_l$, is constant throughout the whole range of elution volume and given by [38]

$$(M_w/M_n)_l = (M_w/M_n)^{1-\Sigma} \tag{8}$$

where

$$\Sigma = \frac{\beta^2}{(2\sigma^2 B^2 + \beta^2)} \tag{9}$$

where $\beta^2 = \ln M_w/M_n$ and σ^2 is the variance of the Gaussian spreading function $G(V, y)$ in Eq. (6).

According to Eq. (8), with increasing resolution of the separation system, *i.e.*, for $\sigma \to 0$, local polydispersity decreases, *i.e.*, $\Sigma \to 1$ and $(M_w/M_n)_l \to 1$. For decreasing column resolution, *i.e.*, for high σ^2, $\Sigma \to 0$, local polydispersity approaches its upper limit, *i.e.*, the sample polydispersity $(M_w/M_n)_l \to M_w/M_n$. This is shown in Figure 1 for several polymers of M_w/M_n ranging between 1.2 and 1.005 analyzed in a separation system with $B = 0.2$. It is obvious that, at low values of σ for narrow-MWD polymers $(M_w/M_n)_l$ reaches values comparable with that of the whole polymer, M_w/M_n, and does not change with increasing σ whereas for a sample with relatively broad MWD $(M_w/M_n = 1.2)$, $(M_w/M_n)_l$ increases throughout the whole range of σ. This demonstrates the fact that for a polymer with narrow MWD,
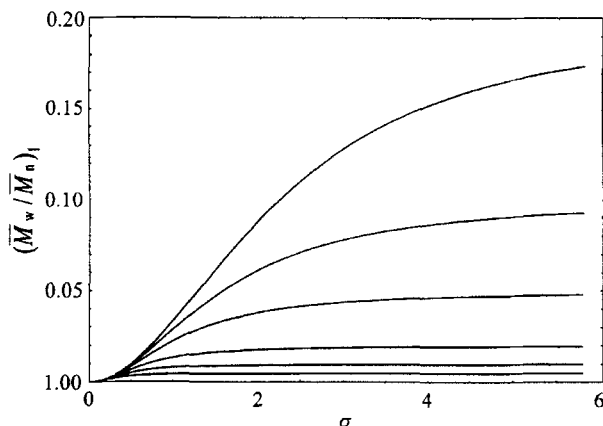
FIGURE 1 Dependency of local polydispersity expressed as the (M_w/M_n) ratio calculated dependent on the standard deviation σ of the spreading function $G(V, y)$ in Eq. (15) for polymers of (curves from the top) $M_w/M_n = 1.2$, 1.1, 1.05, 1.02, 1.01 and 1.005.

peak broadening is more important than for the broad-MWD ones, even though local polydispersity is lower. One can compare the situation of narrow-MWD samples with that of broad-MWD in the early stages of SEC when the resolution power was low. In next sections, this will be demonstrated also for other, experimentally accessible polymer characteristics, e.g., for dependences of $\ln M_d$ vs. V.

Local polydispersity is not directly detectable and it is independent of polymer detection. However, the detector response depends on the sample MWD, column characteristics and resulting local polydispersity as well as on the configuration of detectors. This will be discussed in the next section.

Peak Broadening in Single- and Dual-detection SEC

Peak broadening leads to broadening of MWD obtained from experimental data if ('conventional') calibration, i.e., Eq. (7), is used for calculation of molecular weight. The situation is more complex if the dual detection is employed and the values of M_d determined from the data of detectors are used for the MWD determination. The reason is the decrease in the slope of the calibration, i.e., of the dependence of $\ln M$ vs. V calculated from the detector data, compared with the slope

of the 'conventional' calibration given by Eq. (7) determined from analyses of a series of narrow-MWD samples (standards). For log-normal MWD, the calibration is linear and can be found analytically. Only its slope is important for determination of the M_w/M_n ratio; it is given by [35, 38, 39]

$$B_d = B\Sigma \qquad (10)$$

For illustration, dependences of B_d calculated from Eq. (10) on the mean deviation of the spreading function for polymers of the same M_w/M_n ratios, respectively from top to bottom, in Figure 1, and $B = 0.2$ are plotted in Figure 2. With increasing σ, the values of B_d decrease. With decreasing M_w/M_n ratio, the decrease is more pronounced.

For different polymer MWD's, the calibration may be curved and the model and correction calculations have to be performed numerically even if MWD is given by an analytical function. The situation is graphically demonstrated for a polymer with $M_w/M_n = 1.2$ and MWD given by the Schulz-Zimm function in Figure 3. The conversion between the elution curves and MWDs, $w(\log M)$, is given, in the first approximation, by perpendiculars to the elution volume and $\log M$ axes intersecting at the dependence of $\log M$ on elution
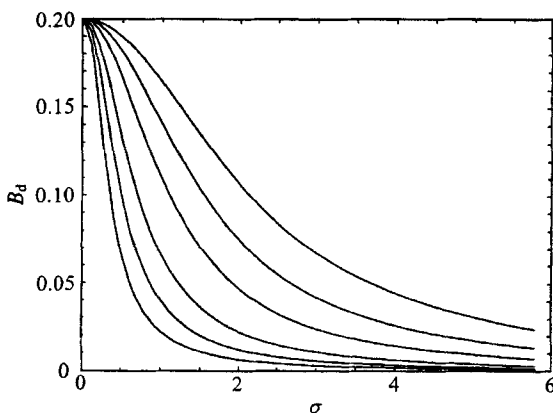

FIGURE 2 Comparison of dependences of calibration slope (Eq. (7)) affected by peak broadening, B_d, calculated from Eq. (10), on the mean deviation of the spreading function, σ, for polymers of the same M_w/M_n ratios, respectively from top to bottom, as in Figure 1.
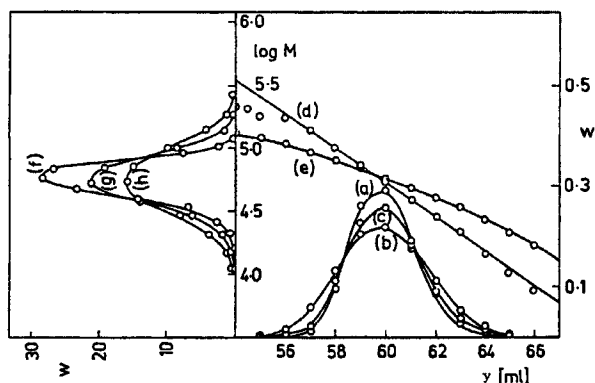
FIGURE 3   Results of a model calculation for a polymer with $M_w/M_n = 1.2$: theoretical elution curve (a), broadened elution curve (b), numerically corrected broadened elution curve (c), theoretical calibration (d), calibration deformed by peak broadening (e), MWD corresponding to detector data (f), theoretical MWD (g) and MWD corresponding to broadened elution curve and theoretical calibration (h) (Reproduced with permission from Ref. [35]).

volume used for the calculation of MWD. The influence of the slope B_d on the resulting MWD can be seen from the Figure 3. If the slope is decreased, the corresponding MWD is narrower (and higher in its maximum), compared with the theoretical one, even if the elution curve is broadened (elution curves and MWDs in the Fig. 3 are normalized). From Figure 3, one can also see that the theoretical curve can be, to a large degree, restored by a numerical procedure.

For the log-normal MWD, the weight-to-number average ratio from the dual-detection data $(M_w/M_n)_d$, is given by [39]

$$(M_w/M_n)_d = (M_w/M_n)^{\Sigma} \tag{11}$$

However, the M_w/M_n ratio, calculated from the broadened elution curves and conventional calibration is broader and is given by[20, 24]

$$(M_w/M_n)_c = (M_w/M_n) \exp[\sigma^2 B^2] \tag{12}$$

Equation (12) has an alternative form[35, 38] (see also note in Ref. [33]).

$$(M_w/M_n)_c = (M_w/M_n)^{1/\Sigma} \tag{13}$$

The dependences of $(M_w/M_n)_d$ and $(M_w/M_n)_c$ vs. σ calculated from Eqs. (11) and (12) or (13) are plotted in Figure 4. It can be seen that the relative error in determination of the M_w/M_n ratio increases with decreasing sample polydispersity. For $(M_w/M_n)_c$, the error rises without limit, for $(M_w/M_n)_d$, the error is lower; in dual detection, the error is lower compared with the 'conventional' calibration.

From Eqs. (11) and (12) and from Figure 4, one can see that the true M_w/M_n ratio is between $(M_w/M_n)_c$ and $(M_w/M_n)_d$ and can be found from them by correcting them to the extent that they meet. This is shown in Figure 5 for a sample of polystyrene of $M_w = 247 \times 10^3$ and $M_w/M_n = 2.445$, analyzed [57] on a separation system with $B = 0.415\,\mathrm{mL}^{-1}$ (columns Styragel HR 5E $300 \times 7.8\,\mathrm{mm}$, particle size $7-10\,\mu\mathrm{m}$) equipped with miniDAWN scattering photometer (Wyatt Technology Corp.). One can see from the figure that the true value of $M_w/M_n = 2.445$ is obtained by correcting both $(M_w/M_n)_c$ and $(M_w/M_n)_d$ with the same value [57] of $\sigma = 0.797\,\mathrm{mL}$.
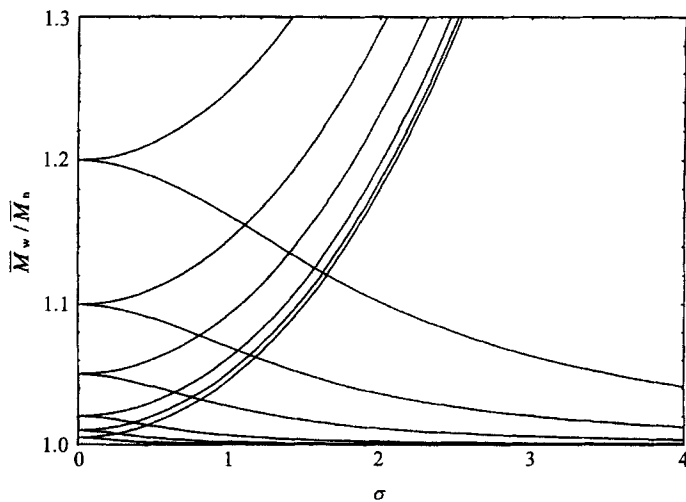

FIGURE 4   Comparison of the dependences of the $(M_w/M_n)_d$ (descending curves) and $(M_w/M_n)_c$ (rising curves) ratios, calculated according to Eqs. (11) and (12) (or (13)), respectively, on the mean deviation of the spreading function, σ, for polymers of the same M_w/M_n ratios, respectively from top to bottom (at the beginnings of the curves), in Figure 1.
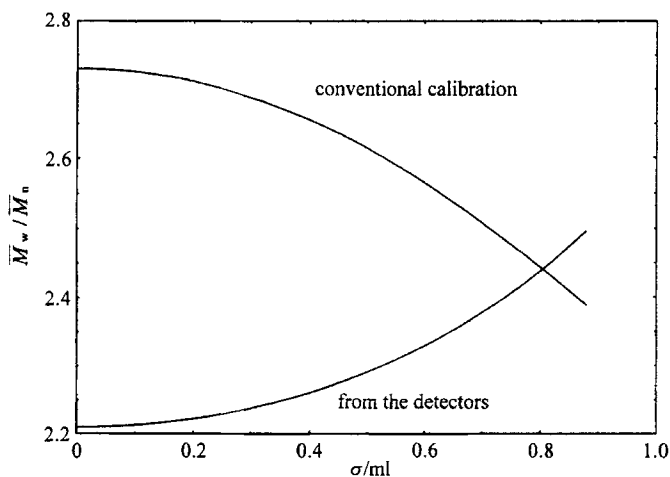
FIGURE 5 The dependences of M_w/M_n from conventional calibration and detector data (denoted with the curves) on the mean deviation of the spreading function, σ, used for the correction, for a sample of polystyrene with $M_w/M_n = 2.445$.

Solution to the Peak-broadening Problem
in Dual-detection SEC

The theoretical molecular weight, M, which is identical with that obtained from dual detection analysis corrected for peak broadening and which can be practically obtained from the 'conventional' calibration using a series of narrow-MWD samples, is related to molecular weight influenced by peak broadening, M_d, by [29]

$$M_d(V)F(V) = \int M(V)W(y)G(V,y)dy \qquad (14)$$

where the variables V and y have the same meaning as in Eq. (6). Equation (14) says that the contribution of fraction $W(y)$ of polymer eluted theoretically at elution volume y to the light-scattering elution curve of polymer eluted is *really* at elution volume V is given by $M(y)W(y)G(V,y)$.

At first, attempts were made[26, 29] to solve Eq. (14) for $M(y)$. These were unsuccessful because Eq. (14) presents the so called 'ill-posed' problem which means that small changes in the input

functions cause large changes in the output results. Progress was achieved by realizing that the underlined functions in Eq. (14), $M_d(V)F(V)$ and $M(V)W(y)$ are, from the point of view of solving the peak-broadening problem, identical with $F(V)$ and $W(y)$, respectively, in Eq. (6). This makes it possible to apply both numerical and analytical methods developed for the correction of $F(V)$ also on the $F(V)M_d(V)$ function, i.e., to the light scattering elution curve. The use of the numerical correction method of Pierce and Armonas[19] was demonstrated for a polymer with MWD approximated by the Schulz-Zimm MWD.[52] The result is in Figure 2. The relations between all curves have been discussed in the previous section. Here it will be only pointed out that the curvature of the function $\log M_d$ is due to the asymmetry of the elution curves of the polymer with MWD approximated by the Schulz-Zimm MWD. This curvature is restored as can be seen from the experimental points on the linear theoretical $\log M$ dependence, even though the elution curves are not completely restored (only $F(V)$ elution curve (c) is shown).

The numerical solution procedures are sensitive to the smallest errors in the numerical input of values of individual experimental points. This is done away by the strategy based on the approximation the narrow elution curves by the Gaussian curve[15, 16] (which follows from the approximation of MWD by the log-normal function [55, 56]) and by simultaneous correcting (Fig. 5) the upper limit (based on the combination of concentration elution curve with broad-range calibration) and the lower one (combination of concentration elution curve with the local calibration), as expressed by Eqs. (11) and (13). This strategy ensures that the resulting polydispersity and the calculated variance of the spreading function cannot exceed natural limits given by the experiment. However, an error in determination of the global input parameters, i.e., the elution-curve width and the slope of the local calibration is not excluded. Especially in the determination of the latter one has to be aware of the fact that its experimental value depends on the precision of the interdetector volume determination. The analytical solution of all effects is complex and by including various input parameters, as the variance of the spreading function, M_w/M_n, the error in the interdetector volume, various effects can be studied. Nevertheless, there is a general strategy in the elaboration of analytical solutions[38-45] of individual effects which can be

expressed in the following outline:

(1) The $W(V)$ elution curve is constructed from MWD.[53, 54] If the log-normal function[55, 56] is used, a Gaussian elution curve results.[15]

(2) The $M(V)W(V)$ elution curve is constructed using calibration (7) and elution curve $W(V)$. It is also a Gaussian curve, shifted along the elution curve axis.[35, 38, 40, 42, 43]

(3) Peak broadening is modeled by multiplying both $W(V)$ and $M(V)W(V)$ elution curves by the spreading function $G(V,y)$ and integrating according to Eqs. (15) and (14). The extent of broadening is given by the mean deviation, σ, of the spreading function $G(V,y)$. By a change of the shift factor between the elution curves, the error, caused by the error in the interdetector volume determination, can also be estimated.[39]

(4) The $\ln M_d$ vs. V dependence is derived by dividing the broadened elution curves and taking the logarithm.

(5) The molecular-weight averages corresponding to broadened elution curves are conveniently computed[35, 38, 39] from the definition sums of the number-, weight- and z-averages where the weight fraction of polymer eluted between V and $V+dV$ is expressed as elution curve $F(V)$ and is multiplied by molecular weight (in the power given by the definition of the average) expressed as fraction of elution curves $M_dF(V)$ and $F(V)$.

(6) The above described procedures can be extended also to viscosity[39] detection, detection of end-groups[38] or based on the detection of the radius of gyration.[38, 57] In the case of viscometric detection, it is necessary either to know the Mark–Houwink–Sakurada coefficients or to rely on the universal calibration. In the latter case, the resulting dependences $\ln M_d$ vs. V behave in a different way.[41]

The above described procedures can be utilized for finding the true value of M_w/M_n by an iterative method based on the following procedure. From experimental data, the M_w/M_n ratios are calculated in two ways: one using the concentration and light-scattering elution curve for the polymer measured, and the other based on the concentration elution curve and calibration line for a wide range of molecular weights. An iteration method has been developed making

the two M_w/M_n ratios converge. The method has been successfully applied to a series of narrow-MWD polystyrene standards.[57]

CONCLUSIONS

(1) Peak broadening and local polydispersity are closely interconnected.
(2) For a polymer with MWD obeying the log-normal function, peak broadening dependence of $\ln M_d$ on V and local polydispersity can be found as functions of variance σ^2 of the spreading function $G(V,y)$.
(3) The extent of peak broadening can be found from the dual light-scattering/concentration SEC analysis.
(4) All procedures described for the light-scattering detection can be used, with some restrictions, also for viscometric detection.

Acknowledgments

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