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Influence of peak-broadening and interdetector volume error on size-exclusive chromatographic analysis with dual viscometric–concentration detection using the universal calibration method

Miloš Netopilík*

Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, 162 06 Prague 6, Czech Republic

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Abstract

The effect of peak-broadening and error in interdetector volume on the local calibration curve and experimental molecular-mass averages obtained by size-exclusion chromatography (SEC) with dual concentration/viscosity detection, and determination of molecular mass using the universal calibration (UC) method, is theoretically examined using a polymer sample with a molecular-mass distribution (MMD) approximated by the log-normal function. Although peak-broadening is often neglected, its effect on the slope of the local calibration curve and, consequently, on the experimentally obtained values of the weight-to-number average ratio is large. To obtain the right values of these parameters, a numerical correction is usually recommended. While using the UC method, the relationships between the extent of peak broadening, calibration slopes and interdetector volume are complex and can contribute to the occurrence of undiscovered errors. For this reason, an understanding of this problem, using a model, is necessary. The results of the UC method are compared with those obtained using dual-detection with known Mark–Houwink–Kuhn–Sakurada parameters (MHKS method), light-scattering (LS)/concentration detection as well as with the results obtained using conventional calibration. Due to peak-broadening, the slope of a local calibration curve and the weight-to-number average ratio, $(\overline{M}_w/\overline{M}_n)^n$, obtained using the UC method, increase compared to the theoretical values, whereas they decrease using the MHKS or LS methods. The increase when using the UC method is even larger compared to evaluation using conventional calibration. The effect of the error in interdetector volume on the slopes of local calibrations and the weight-to-number average ratios is opposite in the UC method to that found using the MHKS and LS methods. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Peak-broadening; Interdetector volume; Viscometry; Light scattering; Dual detection; Detection, LC

1. Introduction

Dual, light-scattering [1–6] or viscosity/concentration [7–12] detection methods are efficient ways of obtaining the maximum amount of information

[13–25] about a polymer analyzed by size-exclusion chromatography (SEC). Light scattering is the only absolute method for the determination of the molecular mass of the whole polymer [26], as well as of its local values [1–6]. Nevertheless, viscosity/concentration detection is also frequently used because of its relative simplicity, low requirement for sample preparation, sensitivity to chain branching [27], etc. This detection is a relative method because the local

*Tel.: +420-220-403-296; fax: +420-235-357-981.

E-mail address: netopilik@imc.cas.cz (M. Netopilík).

molecular mass is estimated from local values of intrinsic viscosity, $[\eta]$.

Calculation of the molecular mass, M , from $[\eta]$ can be done, in principle, in two ways. If the Mark–Houwink–Kuhn–Sakurada (MHKS) parameters are known, the calculation is straightforward using the well-known MHKS equation (hereafter termed the MHKS method). If the values are not known, it is possible to use the universal calibration method [28–32], which is based on the quantity $[\eta]M$, which is proportional to hydrodynamic volume not only for chemically identical polymers but also, to a large degree, for chemically different polymers of a similar architecture. (For coils, this law holds well [28], although deviations are observed [32] when comparing rods with coils of low M .) Thus, polymers with unknown MHKS parameters can be analyzed if universal calibration is determined for a particular separation system (hereafter called the UC method). The MHKS parameters can be determined using the UC method. However, their values are subject to a large error, especially when examining polymer samples with narrow molecular-mass distributions. This was demonstrated by computer simulations of the process [16].

All dual-detection experiments are aggravated by errors of various origin: peak broadening [33–43] (axial dispersion), decreasing signal-to-noise ratio at marginal parts of elution curves [44], etc. Some of the errors of dual detection are specific to concentration/viscosity detection, e.g., wrong local values of $[\eta]$ due to flow-rate fluctuations caused by the passage of polymer through to the detection system [45–48], interdetector peak-broadening [23], i.e., the difference between peak-broadening of the concentration and viscosity elution curves caused by the non-negligible inner volume of a viscometric detector in the bridge connection [49]. The LS and MHKS methods have been thoroughly examined from the point of view of peak-broadening [13–25] and interdetector volume [22,23,50,51] for polymers with molecular-mass distributions (MMDs) that can be approximated by the log-normal distribution function [52–54]. This is a restriction because this model function can be used only for samples with symmetrical (Gaussian) elution curves, typically of standards with narrow molecular-mass distributions. On the other hand, such characterized standards are

widely accessible and, therefore, the results of an analysis can be evaluated by both MHKS and UC methods and the results compared. From theoretical results [14,16–25] as well as from experimental results [5], it follows that the slope of the local calibration curve, i.e., of the dependence of $\log M$ vs. V obtained from analysis of a single sample by dual detection and evaluation, either by the MHKS or LS method, as well as the experimental value of the $\overline{M}_w/\overline{M}_n$ ratio, decreases compared with the theoretical (correct) values. However, the UC method has not yet been analyzed from this viewpoint. This is the scope of the present paper.

2. Theory

2.1. Local calibration curve

The theoretical (true) dependence of molecular mass of the analyzed polymer on elution volume, which can be obtained by calibration with several, narrow-MMD standards, is given by:

$$\ln M = A + BV \quad (1)$$

The local calibration curve obtained by the MHKS or LS ($\alpha = 1$) method, which can be affected by peak-broadening and errors in the interdetector volume, is given by [22,23]:

$$\ln M' = A' + B'V \quad (2)$$

where:

$$\begin{aligned} A' = & \left[\frac{1 + \alpha}{2}(1 - \Sigma) - \frac{1 + 2\alpha}{2} \frac{\Delta}{2\alpha} \right] \ln \overline{M}_w \\ & + \left[\frac{1 - \alpha}{2}(1 - \Sigma) - \frac{1 - 2\alpha}{2} \frac{\Delta}{2\alpha} \right] \ln \overline{M}_n \\ & + \left(\Sigma + \frac{\Delta}{2\alpha} \right) - \frac{Z}{2\alpha} \end{aligned} \quad (3)$$

and:

$$B' = \left(\Sigma + \frac{\Delta}{2\alpha} \right) B \quad (4)$$

where:

$$\Sigma = (\beta^2/B^2)p \quad (5)$$

where:

$$\beta^2 = 2 \ln \overline{M}_w / \overline{M}_n \quad (6)$$

$$p = (2\sigma^2 + \beta^2/B^2)^{-1} \quad (7)$$

where σ is the standard deviation of the spreading function (common to viscosity and concentration elution curves):

$$\Delta = (4\delta/B)p \quad (8)$$

where δ is the error in the interdetector volume, defined [22] as the relative shift of the light-scattering or viscometric elution curve with respect to the concentration curve, and:

$$Z = (2\delta^2)p \quad (9)$$

The universal calibration method [28] is based on the dependence of $\ln [\eta]M$ vs. elution volume, V , determined over a broad range of V . In the UC method, the local experimental value of molecular mass, M'' , is determined from the universal calibration value and the local (experimental) value of intrinsic viscosity, $[\eta]'$. This can be written in the logarithmic form as:

$$\ln M'' = \ln [\eta]M - \ln [\eta]' \quad (10)$$

Using the parameters of the MHKS equation [55], written for the experimentally determined (local) intrinsic viscosity:

$$[\eta]' = KM'^{\alpha} \quad (11)$$

Eq. (10) is expressed in terms of molecular mass:

$$\ln M'' = \ln [\eta]M - \ln K - \alpha \ln M' \quad (12)$$

where M' is the molecular mass calculated from $[\eta]'$ according to Eq. (11). For the parameters of the obtained local calibration curve:

$$\ln M'' = A'' + B''V \quad (13)$$

we get [20], by combining Eqs. (1), (2) and (12):

$$A'' = A + \alpha(A - A') \quad (14)$$

and:

$$B'' = B + \alpha(B - B') \quad (15)$$

Eq. (13) is a local calibration curve determined from dual concentration/viscosity elution curves (influenced by peak-broadening and the error in the interdetector volume) and the universal calibration.

It might be of interest to know the relation of constants A'' and B'' to the constants A_U and B_U of the universal calibration method:

$$\ln [\eta]M = A_U + B_U V \quad (16)$$

This can be shown as follows. Using Eq. (11), we have, from Eq. (16):

$$A_U = (\alpha + 1)A + \ln K \quad (17)$$

and:

$$B_U = (\alpha + 1)B \quad (18)$$

Introducing A and B from Eqs. (17) and (18) into Eqs. (14) and (15), for the local calibration curve constants obtained using the UC method, we have:

$$A'' = (A_U - \ln K) \left(1 - \frac{\alpha}{\alpha + 1} \frac{A'}{A} \right) \quad (19)$$

and:

$$B'' = B_U \left(1 - \frac{\alpha}{\alpha + 1} \frac{B'}{B} \right) \quad (20)$$

The effect of interdetector peak-broadening, i.e., of the difference between the standard deviation of the spreading function of the concentration record, σ , and that of the viscometric record, σ_η , on the local calibration curve obtained by the UC method, can be demonstrated as follows: due to interdetector peak-broadening, the calibration [Eq. (2)] converts into Eq. (23):

$$\ln M' = (1/2\alpha) \ln(q/p) + \frac{p-q}{\alpha B^2} \times (\ln M_0 - A - BV)^2 + A'' + B''V \quad (21)$$

where:

$$M_0 = \sqrt{\overline{M}_w \overline{M}_n} \quad (22)$$

and:

$$q = (2\sigma_\eta^2 + \beta^2/B^2)^{-1} \quad (23)$$

where σ_η is the standard deviation of the spreading function of the viscosity elution curve. In this case, the constants of Eq. (13) cannot be found because the local calibrations are not linear; the local calibration curve found by the UC method is described by Eq. (12), where M is calculated from Eq. (1), $[\eta]$ from Eq. (11) and M' from Eq. (21).

2.2. Molecular mass averages

The experimental MMD and molecular mass averages are associated with the experimental elution curve determined using the particular calibration used for the data evaluation.

The averages corresponding to the UC method are calculated using the calibration [Eq. (13)] and the experimental concentration elution curve that is given by Eqs. (22) and (23):

$$F(V) = \frac{1}{\sqrt{\pi} \sqrt{2\sigma^2 + \beta^2/B^2}} \exp \left[-\frac{(V_0 - V)^2}{2\sigma^2 + \beta^2/B^2} \right] \quad (24)$$

where:

$$V_0 = (\ln M_0 - A)/B \quad (25)$$

In the MHKS method, with α being the MHKS exponent (in the LS method, $\alpha = 1$), the experimental values of molecular mass averages are given by [23]:

$$\bar{M}'_n = P \left(\frac{\bar{M}_w}{\bar{M}_n} \right)^{-\frac{\alpha+1}{2\alpha}(\Sigma+\Delta)} \exp \left[-\frac{\alpha+1}{2\alpha^2} Z \right] \quad (26)$$

where:

$$P = \frac{\bar{M}_w^{\alpha+1} \bar{M}_n^{\alpha-1}}{\bar{M}_w^2 \bar{M}_n^{2\alpha}} \quad (27)$$

$$\bar{M}'_w = P \left(\frac{\bar{M}_w}{\bar{M}_n} \right)^{\frac{1-\alpha}{2}(\Sigma+\Delta)} \exp \left[\frac{1-\alpha}{2\alpha^2} Z \right] \quad (28)$$

which gives for the $(\bar{M}_w/\bar{M}_n)'$ ratio [23]:

$$\left(\frac{\bar{M}_w}{\bar{M}_n} \right)' = \left(\frac{\bar{M}_w}{\bar{M}_n} \right)^{-(\Sigma+\frac{\Delta}{\alpha})} \exp[-Z/\alpha^2] \quad (29)$$

The uncorrected molecular mass averages of the whole polymer obtained using of the UC method are distinguished in the following by double primes. For the uncorrected $(\bar{M}_w/\bar{M}_n)''$ ratio, according to Eq. (6), we have:

$$\beta''^2 = 2 \ln(\bar{M}_w/\bar{M}_n)'' \quad (30)$$

From Eqs. (15), (24), (6) and (30) we have:

$$2\sigma^2 + \beta^2/B^2 = \frac{\beta''^2}{(B + \alpha(B - B'))^2} \quad (31)$$

which, for the weight-to-number average ratio determined by the UC method, gives:

$$(\bar{M}_w/\bar{M}_n)'' = (\bar{M}_w/\bar{M}_n)^D \exp[B^2\sigma^2] \quad (32)$$

where:

$$D^{1/2} = \frac{B + \alpha(B - B')}{B} \quad (33)$$

Eq. (32) gives the uncorrected $(\bar{M}_w/\bar{M}_n)''$ ratio obtained from the broadened concentration elution curve and local calibration curve, available from broadened concentration and viscosity elution curves and the universal calibration. The meaning of individual terms in Eq. (32) is obvious: the term D corresponds to the change in the local calibration curve slope [cf. Eq. (15)]. The exponential term corresponds to the broadening of the concentration elution curve. If the calibration according to Eq. (1) is used, $D = 1$ and Eq. (32) reduces to the well-known equation derived by Hamielec [43], which holds irrespective of the type of sample MMD:

$$(\bar{M}_w/\bar{M}_n)_c = (\bar{M}_w/\bar{M}_n) \exp[B^2\sigma^2] \quad (34)$$

(subscript 'c' denotes the use of conventional calibration.)

The formulae for \bar{M}''_w and \bar{M}''_n will now be derived. For the median (abscissa of the maximum) on the elution volume axis of the concentration elution curve, which is not changed by peak-broadening, we have:

$$V_0 = (\ln M''_0 - A'')/B'' \quad (35)$$

where:

$$M''_0 = \sqrt{\bar{M}''_w \times \bar{M}''_n} \quad (36)$$

By combining Eqs. (25), (22), (35) and (36), we get:

$$\bar{M}''_w \bar{M}''_n = \exp \left[2A'' + 2B'' \left(\frac{\ln M_0 - A}{B} \right) \right] \quad (37)$$

Solving the system of Eqs. (32) and (37) for \bar{M}''_n and \bar{M}''_w , we get:

$$\bar{M}''_n = (\bar{M}_w/\bar{M}_n)^{-D/2} \exp \left[-\frac{B^2\sigma^2}{2} + A'' + B'' \frac{0.5 \ln \bar{M}_w \bar{M}_n - A}{B} \right] \quad (38)$$

and:

$$\overline{M}_w'' = (\overline{M}_w/\overline{M}_n)^{D/2} \exp \left[\frac{B^2 \sigma^2}{2} + A'' + B'' \frac{0.5 \ln \overline{M}_w \overline{M}_n - A}{B} \right] \quad (39)$$

Using equation:

$$\overline{M}_z/\overline{M}_w = \overline{M}_w/\overline{M}_n \quad (40)$$

which is valid for the log-normal MMD (i.e., for a Gaussian concentration elution curve and a linear

calibration), we get:

$$\overline{M}_z'' = (\overline{M}_w/\overline{M}_n)^{3D/2} \exp \left[\frac{3B^2 \sigma^2}{2} + A'' + B'' \frac{0.5 \ln \overline{M}_w \overline{M}_n - A}{B} \right] \quad (41)$$

From Eqs. (38), (39) and (41), the experimental molecular-mass averages, corresponding to the calculation of M from local calibration curve (13) using concentration elution (24), can be computed.

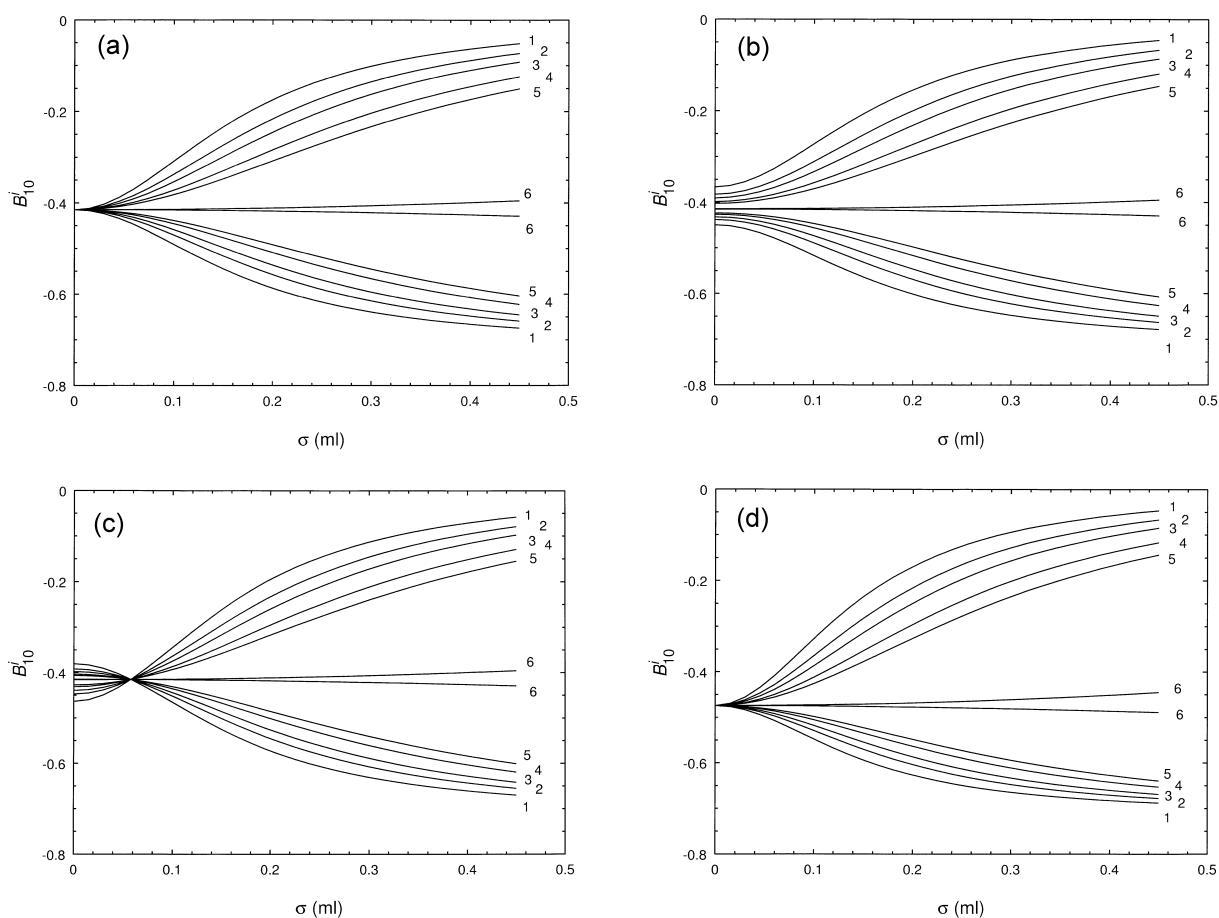


Fig. 1. Dependence on σ of B'_{10} (rising curves) and B''_{10} (descending curves) calculated according to Eqs. (4) and (15), respectively, for polymer samples of $\overline{M}_w/\overline{M}_n = 1.005, 1.0075, 1.01, 1.015, 1.02$ and 2 (curves 1 through 6, respectively), for (a) $\delta = 0$, $\alpha = 0.714$ and $B_{10} = -0.415$ ml⁻¹; (b) $\delta = 0.001$ ml, $\alpha = 0.714$ and $B_{10} = -0.415$ ml⁻¹; (c) $\delta = -0.001$ ml, $\alpha = 0.714$ and $B_{10} = 0.415$ ml⁻¹; (d) $\delta = 0$, $\alpha = 0.5$ and $B_{10} = -0.474$ ml⁻¹.

3. Results and discussion

A separation system is characterized by the slope of the calibration curve (1) and the standard deviation, σ , of the spreading function for a particular polymer. Calibration:

$$\log M = A_{10} + B_{10}V \quad (42)$$

of a real system [24], obtained using polystyrene standards (MHKS parameters are $K = 13.63 \times 10^{-3}$ (g/ml) and $\alpha_i = 0.714$ [56]), where $A_{10} = 12.2$ and $B_{10} = -0.415 \text{ ml}^{-1}$ and $\sigma \approx 0.3 \text{ ml}$, will be used for further discussions. Subscript '10' denotes the constant of the calibrations using decadic logarithms, which is usual in practical applications. In the following, the parameters of local calibrations will be expressed and distinguished in this way, if necessary. Further discussion includes the influence of the MHKS exponent α on the separation and detection in the UC method. In the discussion concerning a particular separation system, the term:

$$\frac{d \ln[\eta]M}{dV} = (\alpha + 1)B \quad (43)$$

which is the physical meaning of the slope, B_U , of universal calibration [Eq. (16)], given by Eq. (18), has to be kept constant. If the MHKS exponent is changed from α_i to α_j , the ratio of the corresponding slopes of the calibration Eq. (1) is then:

$$\frac{B_j}{B_i} = \frac{\alpha_i + 1}{\alpha_j + 1} \quad (44)$$

Note that this effect is independent of the method of detection and therefore is also present with LS detection, even though $\alpha = 1$ in Eqs. (3) and (4).

Fig. 1 shows the dependences on σ of experimental slopes B'_{10} and B''_{10} , calculated according to Eqs. (4) and (15) for polymer samples with $\overline{M}_w/\overline{M}_n = 1.005, 1.0075, 1.01, 1.015, 1.02$ and 2 (curves 1 through 6, respectively) for $\delta = 0$ and $\pm 0.001 \text{ ml}$, $\alpha = 0.714$ ($B_{10} = -0.415 \text{ ml}^{-1}$) and for $\alpha = 0.5$ ($B_{10} = -0.474 \text{ ml}^{-1}$). The values of B''_{10} decrease monotonically (absolute values decrease) with increasing σ (decreasing resolution power of the separation system) whereas the dependences of B' increase (absolute values decrease). The difference from the true value of $B_{10} = -0.415 \text{ ml}^{-1}$ at realistic values of the standard deviation of the

spreading function ($\sigma \approx 0.3 \text{ ml}$ for a separation system with comparable B_{10}) is large for all samples except that with the broadest MMD ($\overline{M}_w/\overline{M}_n = 2$). The effect of the error, δ , in the interdetector volume increases with increasing resolution power ($\sigma \rightarrow 0$), which is expected due to narrowing of the peak with $\sigma \rightarrow 0$. If the error is positive (the shift of the viscosity record to higher values of V (Fig. 1b), B' values increase (absolute values decrease) and the B' value is not reached even for $\sigma \rightarrow 0$. If the error is negative, the B'_{10} values decrease (absolute values increase) (Fig. 1c). As the slope B'_{10} does not change only due to the effect of δ but also due to peak broadening, the question arises as to what degree the effects on B'_{10} of the two phenomena may compensate. From Fig. 1c, one can see that, at approximately $\sigma \approx 0.1 \text{ ml}$, all dependences intersect regardless of the sample $\overline{M}_w/\overline{M}_n$. At first, this may seem surprising but can be understood on the basis of Eq. (4), which can be rearranged to give an equation:

$$2\sigma^2 B' B + \beta^2 \left(\frac{B'}{B} - 1 \right) = 2\delta B / \alpha \quad (45)$$

relating σ and δ at a constant ratio of B'/B . For $B' = B$, the term containing β drops from Eq. (45). This means that σ and δ may compensate regardless of the sample's $\overline{M}_w/\overline{M}_n$ ratio so that a correct value of the calibration slope, B , is found. On the other hand, determination of the correct slope of the local calibration curve is not a criterion that guarantees the correct determination of the interdetector volume. If extremely narrow MMD samples are to be characterized, the slope of the local calibration curve as well as the interdetector volume have to be characterized by an independent method. This can be accomplished using an independent method in the multi-angle LS detection system [24].

The errors in Fig. 1 appear to be nearly mirror images of each other for the two methods evaluated. This can be used for the first estimate of the true slope B_{10} from uncorrected data, especially for a uncalibrated SEC system, or for detection of the error if data are not properly corrected. However, the consequence of the decreased values of the MHKS exponent, $\alpha = 0.5$, is a higher decrease in B'_{10} and increase in B''_{10} with σ compared to the dependences calculated for $\alpha = 0.714$. This can be explained by a higher value of $B_{10} = -0.472 \text{ ml}^{-1}$, calculated

according to Eq. (44) (Fig. 1d), which means that, at a given value of σ , there will be a lower resolution power of the system characterized by the given σ .

The difference in peak broadening of the concentration and viscosity elution curves manifests itself, in accord with computer simulations [16], in the curvature of the dependences of $\log M''$ on V , calculated from Eq. (12), for calibration given by Eq. (42), for samples with $\overline{M}_w/\overline{M}_n = 1.005, 1.01, 1.015, 1.02$ and 2 . The number-average molecular mass $\overline{M}_n = 7 \times 10^4$ was chosen for sample with $\overline{M}_w/\overline{M}_n = 2$ and $\overline{M}_n = 10^5$ was chosen for other samples so that the maxima of the elution curves are approximately the same (Fig. 2). The difference between the standard deviations of the spreading functions, $\sigma = 0.1$ ml and $\sigma_\eta = 0.11$ ml, causes the dependences of $\log M''$ on V to be curved downwards. Even though the shape of the calibration is very sensitive to the difference between σ and σ_η , this type of error is easily detected and corrected for by a proper choice of these parameters. On the other hand, taking the interdetector peak-broadening into consideration makes the results complicated and unsuitable for practical applications. For this reason, interdetector peak-broadening will be neglected in the following.

The consequences of peak-broadening and the error in the interdetector volume on the determi-

nation of the molecular mass averages using the MHKS and UC methods will be examined now. Figs. 3 and 4 present a comparison of dependences on σ of $(\overline{M}_w/\overline{M}_n)''$ calculated according to Eq. (32) and $(\overline{M}_w/\overline{M}_n)'$ calculated according to Eq. (29) for $\delta = 0$ and ± 0.001 ml, $\alpha = 0.714$ ($B_{10} = -0.415$ ml⁻¹) and $\alpha = 0.5$ ($B_{10} = -0.474$ ml⁻¹) for several samples differing in the $\overline{M}_w/\overline{M}_n$ ratio, as denoted in the legend to Fig. 1. All dependences of $(\overline{M}_w/\overline{M}_n)''$ increase with increasing σ , whereas the $(\overline{M}_w/\overline{M}_n)'$ decreases. The increase in $(\overline{M}_w/\overline{M}_n)''$ is much higher than that found when using conventional calibration, i.e., than the rise of dependences of $(\overline{M}_w/\overline{M}_n)_c$ (Fig. 5) calculated according to Eq. (34). The dependences also reflect the value of δ . If $\delta > 0$, the values of $(\overline{M}_w/\overline{M}_n)''$ increase for $\sigma = 0$, (Fig. 3b), whereas the values of $(\overline{M}_w/\overline{M}_n)'$ decrease (Fig. 4b), as expected from the decreased values of the slope of the local calibration curve (Fig. 1b). On the other hand, for $\delta < 0$, the values $(\overline{M}_w/\overline{M}_n)''$ decrease (Fig. 3c) whereas the values of $(\overline{M}_w/\overline{M}_n)'$ increase (Fig. 4c), as expected from the increased values of the slope of the local calibration curve (Fig. 1c).

Higher sensitivities and steeper dependences of ratios $(\overline{M}_w/\overline{M}_n)'$ and $(\overline{M}_w/\overline{M}_n)''$ vs. σ for $\alpha = 0.5$, compared to the case of $\alpha = 0.714$ (Figs. 3d and 3a), is in accord with higher sensitivity of the slopes of local calibrations discussed above and can also be explained by the less favourable ratio of the actual value of σ to the calibration slope.

The results of this paper can be summarized as follows:

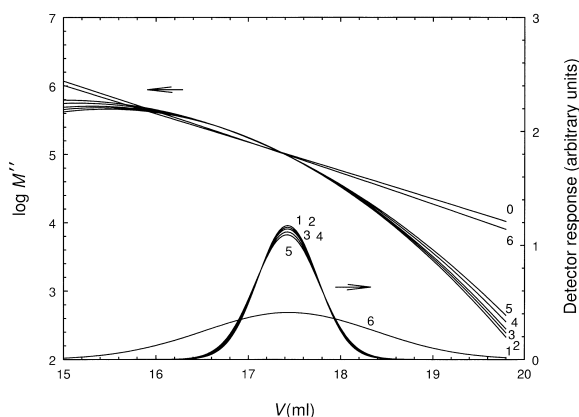


Fig. 2. Comparison of (concentration) elution curves calculated according to Eq. (24) and the dependences of $\log M''$ vs. V calculated according to Eq. (21) for polymer samples with the $\overline{M}_w/\overline{M}_n$ (in the legend to Fig. 1) for $\sigma = 0.1$ ml and $\sigma_\eta = 0.11$ ml [curve '0' is the correct calibration ($\sigma = \sigma_\eta = 0$) according to Eq. (1)]. Details concerning the choice of \overline{M}_w and \overline{M}_n for the calculation are given in the text.

4. Conclusions

1. The absolute value of slope of the local calibration curve, B'' , and the $(\overline{M}_w/\overline{M}_n)''$ ratio determined from the dual concentration/viscometric SEC elution curve and universal calibration (UC) method increase due to peak-broadening, in contrast to the slope B' of the local calibration curve and the $(\overline{M}_w/\overline{M}_n)'$ ratio determined from dual concentration/viscosity elution curves and MHKS parameters (MHKS method), or from dual concentration/light-scattering elution curves.

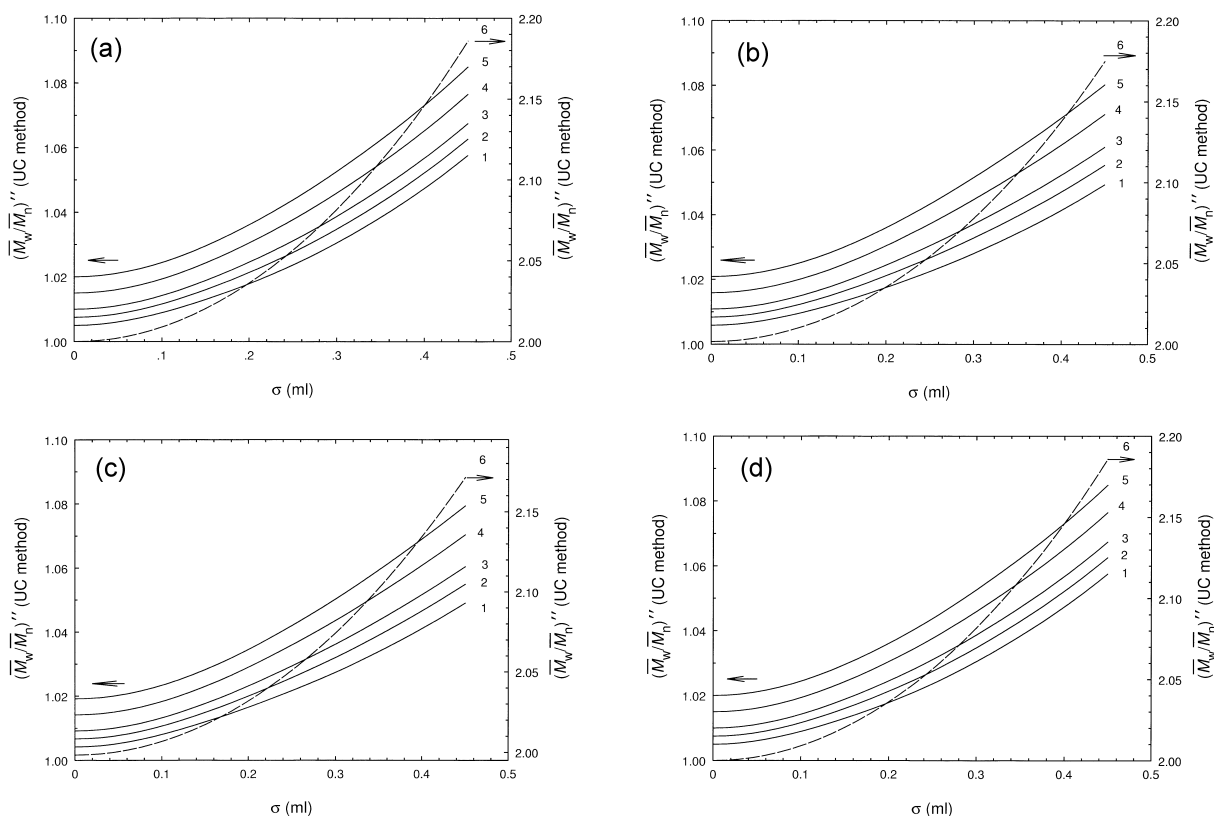


Fig. 3. Comparison of the dependences of $(\overline{M}_w/\overline{M}_n)''$, determined by the UC method, on σ , calculated according to Eq. (32), for samples with the $\overline{M}_w/\overline{M}_n$ given in the legend to Fig. 1 (the left scale is for samples 1 through 5 and the right scale is for sample 6, as denoted by the dashed line and arrows) for (a) $\delta = 0$, $\alpha = 0.714$ and $B_{10} = -0.415 \text{ ml}^{-1}$; (b) $\delta = 0.001 \text{ ml}$, $\alpha = 0.714$ and $B_{10} = -0.415 \text{ ml}^{-1}$; (c) $\delta = -0.001 \text{ ml}$, $\alpha = 0.714$ and $B_{10} = -0.415 \text{ ml}^{-1}$; (d) $\delta = 0$, $\alpha = 0.5$ and $B_{10} = -0.474 \text{ ml}^{-1}$.

- The increase in the $(\overline{M}_w/\overline{M}_n)''$ ratio obtained using the UC method greatly exceeds that of the $(\overline{M}_w/\overline{M}_n)_c$ ratio obtained evaluating the uncorrected concentration elution curve from conventional calibration.
- The increase in the slopes of the local calibration curve obtained using the UC methods as well as the decrease in the calibration curves obtained using the MHKS and LS methods increase with decreasing values of the the MHKS exponent, α .
- Interdetector peak-broadening is present if detectors with non-negligible inner volumes (e.g. a viscometric one) are used. As a consequence of different peak broadening, the mean deviation of the spreading function of the viscometric elution curve is higher than that of the concentration elution curve, $\sigma_\eta < \sigma$. If uncorrected, this results in the local calibrations determined using the UC

- method bending upwards whereas the calibrations determined using the MHKS bend downwards. If $\sigma_\eta > \sigma$ (e.g. a single-capillary viscometer), curvatures in the opposite direction can be expected.
- The slopes of the local calibrations, determined both by UC and MHKS methods, are associated not only with peak broadening but also with the error, δ , in the interdetector volume determination. There exists a value of δ for which the slopes are correctly determined irrespective of the $\overline{M}_w/\overline{M}_n$ ratio of the sample (provided that the sample MMD can be approximated with log-normal distribution). Hence, the determination of the correct slope of the local calibration curve (i.e., identical with that determined using several narrow-MMD samples), determined either by the UC or MHKS method, is not a reliable proof that the interdetector volume has been determined

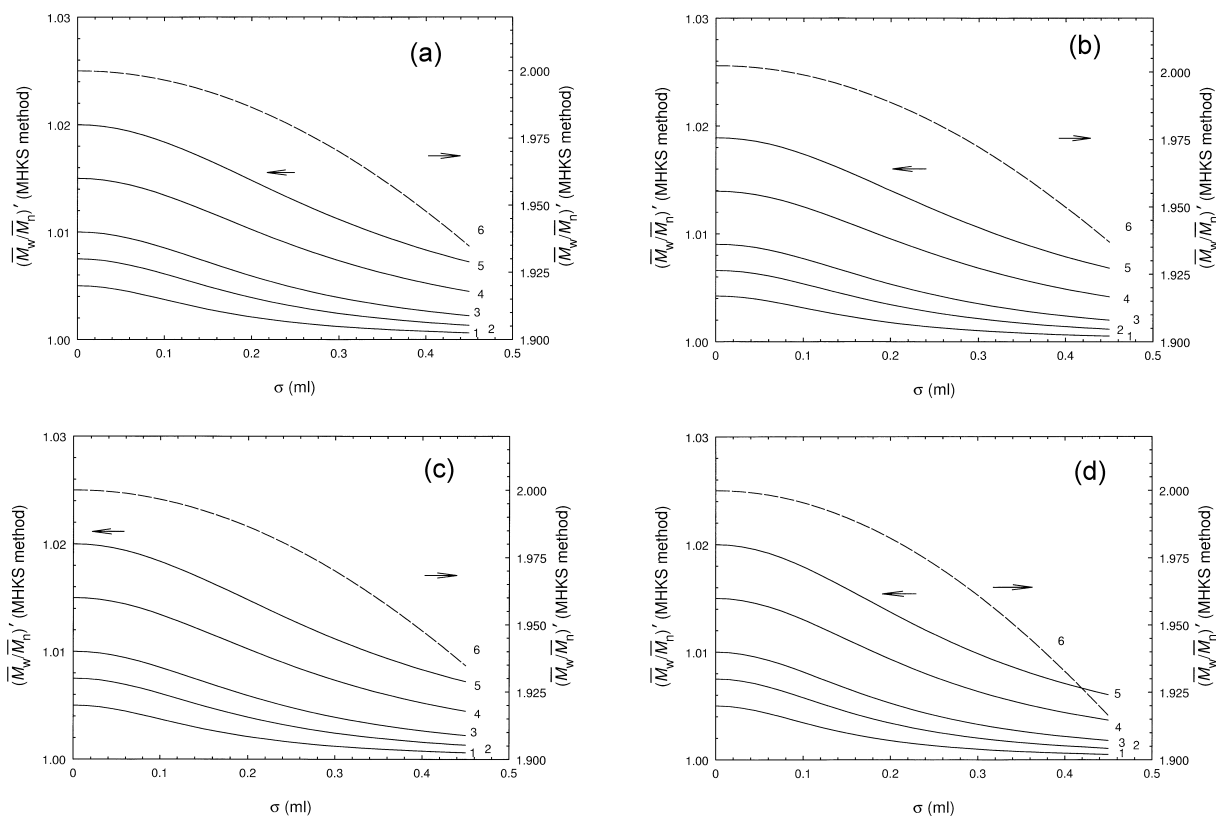


Fig. 4. Comparison of the dependences of $(\overline{M}_w/\overline{M}_n)'$, determined by the MHKS method, on σ , calculated according to Eq. (29), for samples with the $\overline{M}_w/\overline{M}_n$ given in the legend to Fig. 1 (the left scale is for samples 1 through 5 and the right scale is for sample 6, as denoted by the dashed line and arrows) for (a) $\delta = 0$, $\alpha = 0.714$ and $B_{10} = -0.415 \text{ ml}^{-1}$; (b) $\delta = 0.001 \text{ ml}$, $\alpha = 0.714$ and $B_{10} = -0.415 \text{ ml}^{-1}$; (c) $\delta = -0.001 \text{ ml}$, $\alpha = 0.714$ and $B_{10} = -0.415 \text{ ml}^{-1}$; (d) $\delta = 0$, $\alpha = 0.5$ and $B_{10} = -0.474 \text{ ml}^{-1}$.

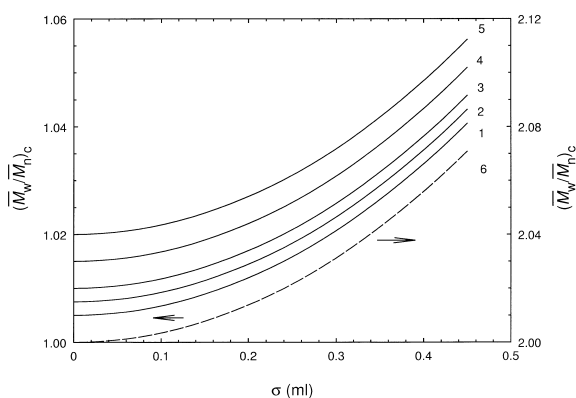


Fig. 5. Comparison of the dependences of the $(\overline{M}_w/\overline{M}_n)_c$ ratio determined using the conventional calibration, on σ calculated according to Eq. (34) for samples with the $\overline{M}_w/\overline{M}_n$ denoted in the legend to Fig. 1 (the left scale is for samples 1 through 5 and the right one is for sample 6, as denoted by the dashed line and arrows).

correctly. Its effective value can be used for the evaluation of SEC analyses of samples with a $\overline{M}_w/\overline{M}_n$ ratio exceeding that of the sample used for its determination.

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