

Simultaneous Calibration of Separation and Axial Dispersion in Size Exclusion Chromatography Coupled with Light Scattering

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Synopsis

An improved method for simultaneous calibration of separation and axial dispersion in SEC/LALLS is presented which partially overcomes restriction imposed in the procedure of He et al. (1982). Both methods are comparatively tested by evaluation of SEC/LALLS experiments with samples from commercial grades of polyisobutylene. On the basis of these findings, the range of applicability and the advantage of the novel procedure are discussed.

INTRODUCTION

The interpretation of data from size exclusion chromatography (SEC) requires calibration of separation and corrections for axial dispersion as has recently been reviewed by Dawkins¹ and Hamielec.² In the case of SEC coupled with low-angle laser light scattering (LALLS) as a detector of molecular mass, special methods of data evaluation permit a simultaneous calibration of separation and axial dispersion. He et al.³ have recently described such a method which can be applied under the following conditions: (i) the calibration function has the form $\ln M(v) = A - Bv$, (ii) the axial dispersion is described by a Gaussian, and (iii) the chromatogram (response of a mass concentration detector as a function of retention volume) is also Gaussian. In conjunction with conditions (i) and (ii), condition (iii) restricts this method to polymer samples with logarithmic normal distribution of molecular mass.

In this paper, a modified method for simultaneous calibration of separation and axial dispersion is presented, which is based on assumptions (i) and (ii) being valid in the limited range of molecular mass covered by each polymer sample used for calibration, but is not restricted to samples with logarithmic normal distribution of molecular mass. This method is tested by evaluation of SEC/LALLS experiments with samples from commercial grades of polyisobutylene.

THEORETICAL

Tung's equation⁴ for the effect of axial dispersion on the chromatogram $e(v)$

$$e(v) = \int_0^{\infty} w(v_0) \cdot D(v - v_0) \cdot dv_0 \quad (1)$$

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with $e(v)$ (ml^{-1}) = response of mass concentration detector as a function of retention volume, normalized to $\int_0^\infty e(v) \cdot dv = 1$, $w(v_0)$ (ml^{-1}) = mass distribution of retention volume (v_0 = retention volume of a certain component of the solute), normalized to $\int_0^\infty w(v_0) \cdot dv_0 = 1$, and $D(v)$ (ml^{-1}) = dispersion function, normalized to

$$\int_{-\infty}^{\infty} D(v) dv = 1,$$

can be extended to work with a molecular mass detector, e.g., a LALLS detector, by the equation⁵

$$E(v) = \int_0^\infty w(v_0) \cdot D(v - v_0) \cdot M(v_0) \cdot dv_0 \quad (2)$$

where $E(v)$ ($\text{g mol}^{-1} \text{ml}^{-1}$) = response of LALLS detector, extrapolated to scattering angle $\theta = 0$ and mass concentration $c = 0$, as a function of retention volume, normalized, so that $E(v)/e(v) = \bar{M}_w(v)$, where $\bar{M}_w(v)$ is the weight-average molecular mass within an infinitesimal volume fraction at retention volume v [cf. eq. (15)], and $M(v_0)$ = calibration function (molecular mass as a function of v_0).

Assuming the dispersion function to be of the form

$$D(v) = \frac{1}{\sigma \cdot \sqrt{2\pi}} \cdot \exp\left(-\frac{v^2}{2\sigma^2}\right), \quad (3)$$

a Gaussian function with variance σ^2 , and the calibration function to be of the form

$$\ln M(v) = A - B \cdot v, \quad (4)$$

Yau et al.⁶ derived the relationship

$$\bar{M}_w(v) = \frac{e(v - B \cdot \sigma^2)}{e(v)} \cdot \exp\left(\frac{B^2 \cdot \sigma^2}{2}\right) \cdot M(v) \quad (5)$$

With eq. (4), eq. (5) is modified to

$$\bar{M}_w(v) = \frac{e(v - B\sigma^2)}{e(v)} \cdot \exp\left(\frac{B^2 \cdot \sigma^2}{2}\right) \cdot \exp(A - B \cdot v) \quad (5')$$

Assuming the chromatogram $e(v)$ to be Gaussian,

$$e(v) = \frac{1}{\sigma_v \sqrt{2\pi}} \cdot \exp\left(-\frac{(v - \bar{v})^2}{2\sigma_v^2}\right) \quad (6)$$

with \bar{v} = mean retention volume and σ_v^2 = variance, and introducing a param-

eter ξ defined by

$$\xi^2 = \frac{\sigma_v^2 - \sigma^2}{\sigma_v^2} \quad (7)$$

He et al.³ derived from eqs. (5') and (6) that

$$\ln \bar{M}_w(v) = A_w - B_w \cdot v \quad (8)$$

where

$$A_w = A - (1 - \xi^2) B \left(\bar{v} - \frac{1}{2} \cdot B \cdot \xi^2 \cdot \sigma_v^2 \right), \quad (8a)$$

and

$$B_w = \xi^2 \cdot B \quad (8b)$$

with A and B as defined in eq. (4).

The coordinates of the cross point (v^* , $\ln M^*$) of the straight lines (in a plot of $\ln M$ vs. v) defined by $\ln \bar{M}_w(v)$ [cf. eq. (8)] and $\ln M(v)$ [cf. eq. (4)], respectively, are in this case defined by

$$v^* = \bar{v} - \frac{1}{2} \cdot B_w \cdot \xi^2 \cdot \sigma_v^2 \quad (9a)$$

and

$$\ln M^* = A_w - B_w \cdot v^* \quad (9b)$$

These cross points for a series of samples with different molecular mass [but each with logarithmic normal distribution; cf. assumption (iii) given above] can be connected by a straight line which is just the calibration function $\ln M(v)$. From the slope of both straight lines describing $\ln \bar{M}_w(v)$ and $\ln M(v)$ the variance σ^2 of the dispersion function [eq. (3)] can be evaluated, using eqs. (7) and (8b):

$$\sigma^2 = \left(1 - \frac{B_w^2}{B^2} \right) \cdot \sigma_v^2 \quad (10)$$

In our attempt to overcome the restriction given by assumption (iii) in the method of He et al., eq. (5') was written in the form:

$$E(v) = e(v - B\sigma^2) \cdot \exp\left(\frac{B^2 \cdot \sigma^2}{2}\right) \cdot \exp(A - Bv) \quad (11)$$

Assuming a certain value of σ , the function $E(v)$ was approximated by the least-squares method (with respect to E) using eq. (11); this numerical procedure leads to a pair of values for A and B in eq. (4) corresponding to the value of σ chosen. Varying σ within a certain range results in a bundle of straight lines $M(v, \sigma)$ in the plot of $\log M$ vs. v (cf. Fig. 1) which cross the function $\bar{M}_w(v)$ nearly at one point taking into account the precision of measurement for $\bar{M}_w(v)$ (cf. Experimental).

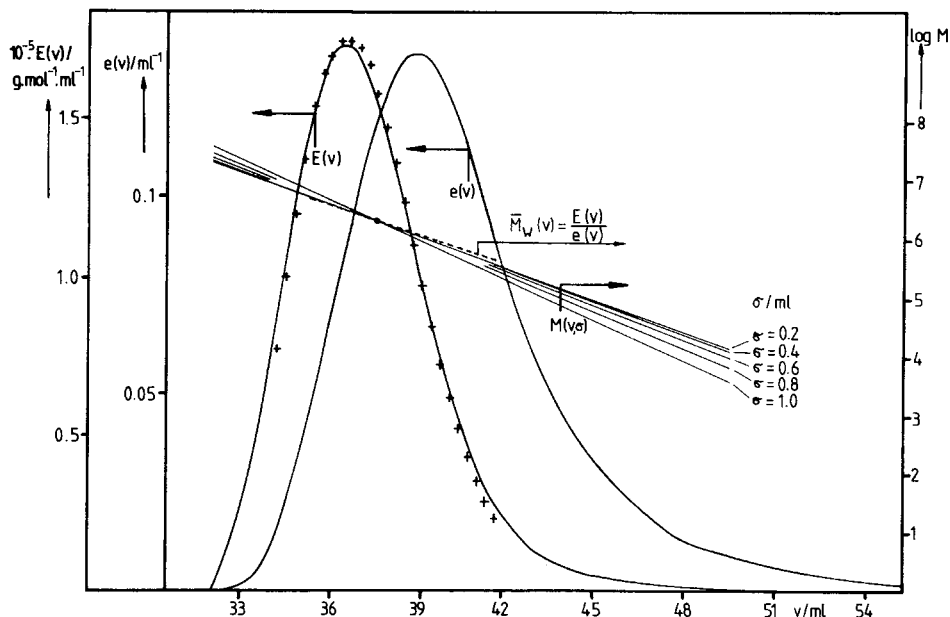


Fig. 1. Test of the numerical procedure to approximate the function $E(v)$ with a given function $e(v)$ and σ [cf. eqs. (1), (2), and (11)]; (+ + + +) closest fit obtained for $\sigma = 0.3$; $M(v, \sigma)$ = calibration functions found by variation of σ .

TEST OF THE NUMERICAL PROCEDURE

As can be seen from Figure 1, a rather close fit of $E(v)$ is reached by this procedure [cf. $E(v)$ and the fit of $E(v)$ obtained with $\sigma = 0.3$ ml given in Fig. 1 by the points marked with small crosses]. The efficiency of the numerical method was further tested by simulating the effect of axial dispersion given by a Gaussian dispersion function with $\sigma = 0.6$ ml and $\sigma = 2.1$ ml, respectively, as described by eqs. (1) and (2) and assuming an arbitrary mass distribution of retention volume $w(v)$ and an arbitrary calibration function [$\log M(v) = 12.38 - 0.170v$], as shown in Figure 2. Applying this numerical procedure to the simulated functions $e(v)$ and $E(v)$, both in the case of $\sigma = 0.6$ ml and $\sigma = 2.1$ ml, the resulting straight lines for the calibration function $\log M(v, \sigma)$ cannot be distinguished within the graphical resolution given in Figure 2 from the calibration function chosen for simulation.

EXPERIMENTAL

To test the applicability of this numerical procedure to actual data from SEC/LALLS experiments, three samples of polyisobutylene taken from commercial preparations (Oppanol 10, Oppanol 50, Oppanol 100; BASF, Ludwigshafen/West Germany) were investigated. Average values of molecular mass of these samples are summarized in Table I. THF (reagent grade, Merck, Darmstadt) was used as the solvent. Prior to all measurements, sample solutions were slowly rotated at room temperature for several days to assure their homogeneity.

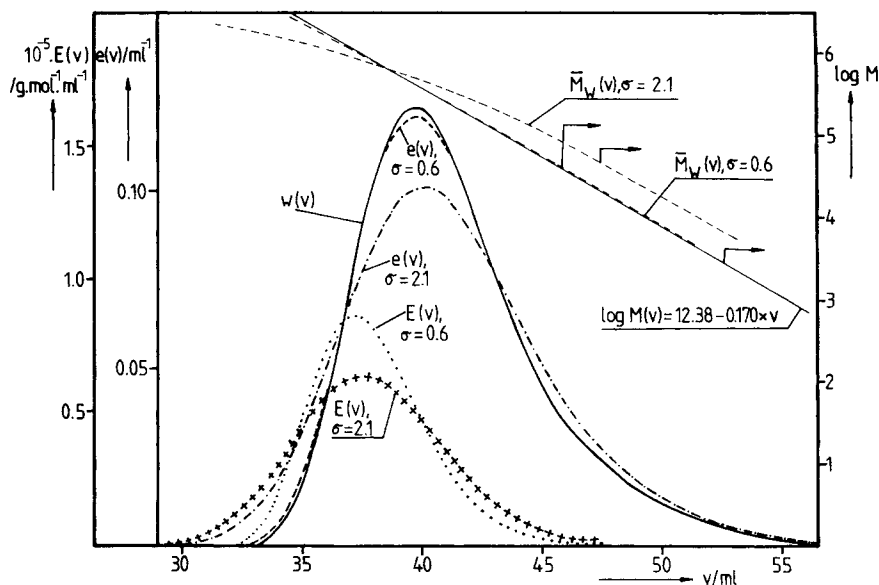


Fig. 2. Test of the numerical procedure; $e(v)$ and $E(v)$ simulated according to eqs. (1) and (2) with Gaussian dispersion functions ($\sigma = 0.6$ and 2.1) assuming $w(v)$ as given and $\log M(v) = 12.38 - 0.170v$. The resulting straight lines for $\log M(v, \sigma = 0.6)$ and $\log M(v, \sigma = 2.1)$, respectively, and indiscernible from $\log M(v)$ assumed for the simulation.

SEC was carried out in the conventional way at 25°C with THF as eluant using six columns filled with polystyrene/divinylbenzene-gel in series (inner diameter 0.78 cm, each of 30 cm length, specified range of particle size about $10\ \mu\text{m}$ in diameter) with exclusion limits of 10^6 , 10^5 , 10^4 , 10^3 , and $10^3\ \text{\AA}$ at a flow rate of 1 ml/min. The concentration of sample solutions varied from 10 to 15 mg/ml. Injection was carried out via a 0.180 ml sample loop.

A differential refractometer (Knauer, Berlin) was used for detection of concentration. The signal $S(v)$ from this detector is considered to be proportional to the concentration and can be normalized to obtain the function $e(v)$

TABLE I
Average Values of Molecular Mass of Polyisobutylene
Samples Investigated in SEC/LALLS Experiments

Sample	\bar{M}_n	$\bar{M}_w^a \pm \bar{M}_w$	Number of SEC/LALLS experiments	\bar{M}_w/\bar{M}_n
PIB 10	6500 ^b	40,500 \pm 1540	5	6,4
PIB 50	60,000 ^b	325,000 \pm 1610	8	5,4
PIB 100	300,000 ^c	1010,000 \pm 15,000	15	3,4

^a Measured by SEC/LALLS; average value and standard deviation determined in a series of experiments (cf. next column).

^b Estimated from SEC/LALLS.

^c Measured by membrane osmometry.⁷

as defined in eq. (1):

$$e(v) = \frac{S(v)}{\int_0^\infty S(v) \cdot dv} \quad (12)$$

The weight average molecular mass \bar{M}_w of the whole sample and of the fraction eluated at v , $\bar{M}_w(v)$, respectively, was determined by SEC/LALLS coupling using the low-angle laser photometer KMX-6 (wavelength $\lambda = 632.8$ nm) (Chromatix, Inc., Sunnyvale, CA) on filtered solutions (0.2 or 0.5 μm Fluoropore filter, Millipore, Bedford, MA) at 25°C using the flow-through-sample-cell-accessory of Chromatix (4.93 mm). The measurement were carried out at an angle of 6–7° with field stop 0.15. The refractive index increment was measured at 25°C and 632.8 nm using a Brice Phoenix differential refractometer with an interference filter.

In the KMX-6, the reduced scattering intensity (Rayleigh factor) is determined as

$$R_\theta(v) = \frac{D \cdot (\sigma' l)^{-1}}{G_0} \cdot [G_{\theta, \text{solution}}(v) - G_{\theta, \text{solvent}}] \quad (13)$$

where G_θ is the photomultiplier signal for the scattered beam, G_0 is the signal for the incident beam; D and $(\sigma' l)^{-1}$ are apparative constants. $E(v)$ as defined in eq. (2) is given by the following expression:

$$E(v) = \frac{R_\theta(v)}{K \cdot m} \cdot C(v) \quad (14)$$

where

$$K = \frac{4\pi^2 \cdot n^2 \cdot (dn/dc)^2}{A \cdot \lambda^4}$$

with $A = \text{Avogadro's number}$, $n = \text{refractive index} = 1.4037$,⁷ $dn/dc = \text{refractive index increment} = 0.125 \text{ ml/g}$,⁷ $\lambda = \text{wave length}$, $m = \text{mass injected in SEC}$, and $C(v) = [1 + 2A_2 \cdot mE(v)] [1 + 16\pi^2 R^2 \sin^2(\theta/2)/3\lambda^2]$. $C(v)$ is a factor for correction of the concentration dependence and angular dependence of light scattering ($A_2 = \text{second virial coefficient}$, $R = \text{radius of gyration}$, $\theta = \text{scattering angle}$); the angular dependence of light scattering can be neglected under the given experimental conditions. A_2 was measured on fractions of PIB 100 in the range of $M = 240,000$ – $1,050,000$,⁷ and was assumed to be constant ($A_2 = 3.5 \times 10^{-4} \text{ ml mol g}^{-2}$) in the whole range of molecular weight covered in this study.

The shift in the retention volume between the measuring cells of both detectors was determined by running a monodisperse sample with $M \approx 10^3$. The weight-average molecular mass of the detector cell content eluated with retention volume v is given by the quotient [cf. eq. (2)]:

$$\bar{M}_w(v) = E(v)/e(v) \quad (15)$$

The weight average molecular mass of the total solute eluted in one SEC experiment is given by the integral

$$\bar{M}_w = \int_{v=0}^{\infty} E(v) \cdot dv \quad (16)$$

RESULTS AND DISCUSSION

The function $e(v)$ and $E(v)$ measured under the conditions described above are shown in Figure 3 for typical SEC/LALLS runs with the samples PIB 100, PIB 50, and PIB 10. The values of \bar{M}_w determined from $E(v)$ by eq. (16) are given in Table I and agree very well with the values of \bar{M}_w measured on these samples with the LALLS instrument in the off-line mode. The reproducibility of the values for \bar{M}_w determined from $E(v)$ appears to be even higher than the reproducibility when measuring in the off-line mode which might be due to the high reproducibility of sample preparation given by passage through the SEC system.

Only in the case of partial clogging at the inlet-frits of the SEC columns which results in a marked increase of the operating pressure, shear degradation was observed in the case of PIB 100 with a drop of \bar{M}_w calculated via $E(v)$ from $\bar{M}_w = 1,010,000$ to $\bar{M}_w = 750,000$ as was reported in a previous study by Huber and one of us (K. L.).⁷

As can be seen from Figure 3, the functions $M(v)$ calculated by the numerical procedure described above for various values of σ [straight lines $M(v, \sigma = \dots)$ in the plot of $\log M$ vs. v] cross one another within a small domain which lies close to the measured function $M_w(v) = E(v)/e(v)$. The scatter of the values for the coordinate v^* of the cross points determined from different pairs of the $M(v, \sigma)$ -lines shown in Figure 3 is about $\pm 1\%$ in the case of PIB 10 and even smaller in the case of PIB 50 and PIB 100 ($< \pm 0.5\%$). A considerable larger spreading is, however, observed with the values of the coordinate M^* of the respective cross points which is about $\pm 10\%$ in the case of PIB 10, about $\pm 2\%$ in the case of PIB 50 and about $\pm 4\%$ in the case of PIB 100. Considering the limited reproducibility encountered in these measurements of the values for the retention volume ($\approx \pm 0.5\%$) and for the function $\bar{M}_w(v)$ ($> 2\%$, depending on the range of M), it does appear to be justified to represent this small domain covered by the respective cross points (v^* , M^*) by the small ellipsoids shown in Figure 4. These ellipsoidal domains can be considered as a measure for the precision attained in these experiments for the determination of the calibration function.

Given the function $M(v)$ as drawn in Figure 4, it is possible to estimate the value of the Gaussian parameter σ of the function governing the axial dispersion [eq. (3)] by drawing a tangent to $M(v)$ within the cross point domain. Comparison of the slope of this tangent with the slope of the calculated functions $M(v, \sigma = \dots)$ in Figure 3 leads to the result that the value of the parameter σ for the axial dispersion in these experiments is about 1.3 ml in the range of molecular mass covered by PIB 100, and about 1.2 ml with PIB 50 and PIB 10, respectively. In both cases the precision for the estimate of σ appears to be restricted to ± 0.1 ml.

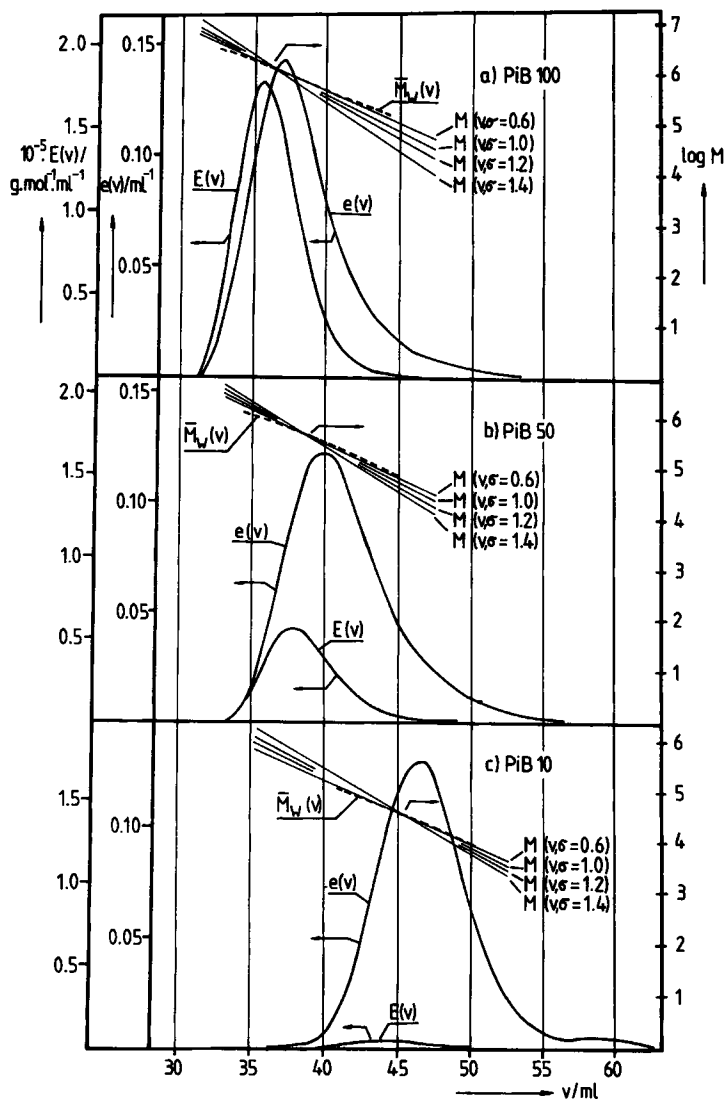


Fig. 3. Typical results from SEC/LALLS experiments with samples from commercial grades of polyisobutylene: (a) PIB 100; (b) PIB 50; (c) PIB 10. $e(v)$ = normalized response of refractive index detector as a function of retention volume v [cf. eq. (1)], $E(v)$ = normalized response of LALLS detector [cf. eq. (2)], $\bar{M}_w(v)$ = weight-average molecular mass of solute in the detector cell at retention volume v [cf. eq. (15)], and $M(v, \sigma)$ = calibration function $M(v) = \exp(A - Bv)$ found with numerical procedure for a certain value of σ (/ml).

The applicability of the method of simultaneous determination of $M(v)$ and σ demonstrated above is restricted to a limited range of the ratio between the breadth of the chromatogram and the variance of the dispersion function. Approximating the chromatogram by a Gaussian function with the same v coordinates of the inflection points as the main peak of the chromatogram, the breadth of the chromatogram may be roughly described by the variance of this approximating Gaussian, σ_{app}^2 . From the experience gained in the present

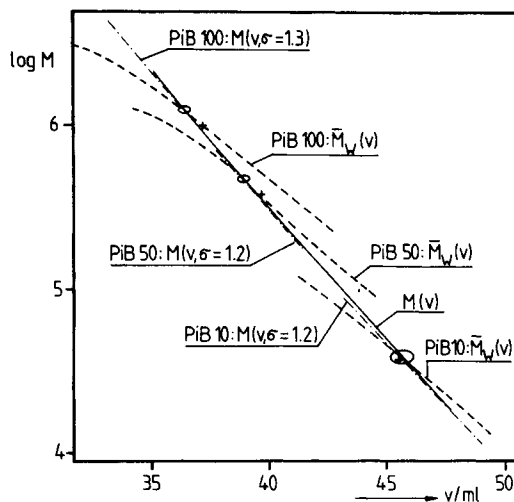


Fig. 4. Calibration function $M(v)$ obtained from the data in Figure 3: (o) domains of cross points between $M(v, \sigma)$ functions found for different values of σ ; (---) best fit of tangent to $M(v)$ by $M(v, \sigma = \dots)$; (---) measured functions $\bar{M}_w(v) = E(v)/e(v)$ from Figure 3; (+) points of the calibration function $M(v)$ from the data for PIB 100, PIB 50, and PIB 10 evaluated following He et al.³ (cf. last two paragraphs in the text).

study, simultaneous determination of $M(v)$ and σ appears to be possible in the range of $\sigma_{\text{app}}/\sigma \leq 4$. In the range $\sigma_{\text{app}}/\sigma \geq 10$, $M(v)$ and $M_w(v)$ become indiscernible, i.e., identical within the precision of measurement attained in this investigation. Measuring the function of $M_w(v)$ on samples with $\sigma_{\text{app}}/\sigma > 10$ can therefore be recommended for direct determination of the calibration function, as was already pointed out by Kim et al.⁸ On the other hand, SEC/LALLS data from samples with $\sigma_{\text{app}}/\sigma \rightarrow 1$ appear to be best suited for the determination of σ , when leaving aside the problems arising in this case from the lack of precision in the determination of the volume shift between the detector of mass concentration and molecular mass, respectively.⁹ The method presented and demonstrated above will be especially advantageous in the case of polymers of which standards with narrow distribution of molecular mass are not readily available.

We also carried out an evaluation of the data shown in Figure 3 by approximating the chromatograms $e(v)$ with Gaussian functions having the same v coordinates of the inflection points as the main peaks of $e(v)$ and using the method of He et al.³ This procedure leads to pairs of values for v^* and M^* [cf. eqs. (9a) and (9b)] shown in Figure 4 as small crosses which deviate considerably from the cross point domain of $M(v, \sigma)$ for PIB 100 and PIB 50, but not for PIB 10; the connecting line of these points (M^*, v^*), i.e., the calibration function $M(v)$ determined by this procedure, does not deviate drastically from $M(v)$ as determined by the method newly presented in this paper. Applying the procedure of He et al. to the data shown in Figure 3 leads, however, to considerably higher values of σ [cf. eq. (10)], namely, $\sigma = 1.42$ for PIB 10, $\sigma = 1.68$ for PIB 50, and $\sigma = 1.47$ for PIB 100.

These findings demonstrate the value of our improved method for simultaneous calibration of separation and axial dispersion in those cases where the

chromatogram $e(v)$ deviates considerably from a Gaussian distribution as in the case of PIB 50. The fact, that both procedures of evaluation give nearly the same results in the case of PIB 10 can be explained by the symmetrical shape of the main peak of $e(v)$ for this sample and the negligible influence of its low molecular mass side peak with respect to $E(v)$ and $M_w(v)$.

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