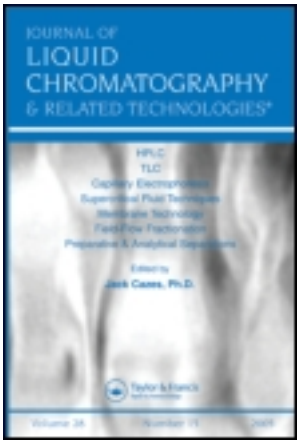

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DETERMINATION OF THE BAND BROADENING FUNCTION IN SIZE EXCLUSION CHROMATOGRAPHY WITH LIGHT-SCATTERING DETECTION

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□ *This work describes the determination of the band broadening function (BBF) in a size exclusion chromatograph fitted with 2 mixed-gel columns, a light scattering (LS) detector, and a differential refractometer (DR). The raw data were the chromatograms of 4 narrow polystyrene standards. First, the interdetector volume shift was indirectly estimated from its upper and lower limiting values. Then, for each of the standards, their "local" BBFs were estimated by application of an existing theoretical method. Each local BBF is an assumed elution-volume invariant in the narrow ranges of the analyzed standards and is represented by an exponentially-modified Gaussian of standard deviation σ_{BB} and exponential decay τ_{BB}. Finally, a "global" BBF (valid for the complete fractionation range) was interpolated from the local BBF parameters. For increasing elution volumes, the global BBF exhibits an increasing σ_{BB} and a decreasing τ_{BB}. In addition, the asymmetry factor $[\tau_{BB}/\sigma_{BB}]$ and the global variance $[\sigma_{BB}^2 + \tau_{BB}^2]$ both decrease with elution volume.*

Keywords band broadening, differential refractometer, HPLC, interdetector volume, light scattering detector, SEC

INTRODUCTION

Size exclusion chromatography (SEC) is the main analytical technique for determining the molar mass distribution (MMD) of synthetic polymers. However, ideal fractionation in SEC is according to hydrodynamic volume rather than by molar mass, in the absence of Band Broadening (BB) or any other spurious fractionation mechanism. A linear homopolymer chain exhibits a one-to-one relationship between its hydrodynamic volume and

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its corresponding molar mass. Linear homopolymers are considered to be chromatographically-simple, as perfect fractionation by hydrodynamic volume also ensures perfect fractionation by molar mass. In contrast, random-branched homopolymers, copolymers, and homopolymer blends are all chromatographically-complex, as at any given hydrodynamic volume they exhibit a whole variety of molar masses.[1,2] When due to this effect, broad instantaneous MMDs are present in the detection cells, then intolerable errors are to be expected in the estimates of the total MMD. With long-branched homopolymers, the problem arises from a hydrodynamic volume contraction of the more highly branched molecules with respect to the less branched homologues of identical molar mass.[3–5] The determination of high local dispersities in the detector cells by a combination of measurements from an on-line light scattering (LS) detector and an on-line viscometer/universal calibration does not solve for the errors in the total MMD but provides an alert for detecting such problems.[6–8] In all that follows, only linear homopolymers are analyzed, and for this reason, the instantaneous broadening caused by chromatographically-complex samples will not be further considered.

In the simplest SEC configuration, the chromatograph is fitted with a concentration detector [typically, a differential refractometer (DR)]; and the MMDs of linear homopolymers are determined from their DR chromatograms and a direct molar mass calibration [$\log M(V)$] obtained with narrow standards of the same chemical nature. When a LS detector is fitted before the DR, then the calibration with standards is in principle no longer required, and the following expression can be used for transforming elution volumes into molar masses:[2,9]

$$M_{\mathrm{w}}(V) = \mathrm{K}\frac{s_{\mathrm{LS}}(V)}{s_{\mathrm{DR}}(V+\delta)} \tag{1}$$

where V is the elution volume of the LS cell; M_{w} is the (weight-average) molar mass of the instantaneous MMD inside the LS cell; K is a constant that is estimated from the gains of the LS and DR detectors;[9] $s_{\mathrm{LS}}(V)$ is the LS chromatogram at a scattering angle of 0°; δ is the interdetector volume (IDV); and $s_{\mathrm{DR}}(V+\delta)$ is the DR chromatogram shifted by δ toward lower V's. In Eq. (1), $M_{\mathrm{w}}(V)$ can be considered as a (local or *ad-hoc*) molar mass calibration, as it depends on the analyzed MMD. The following problems are associated with Eq. (1):[2,9–11] a) the signals ratio is only accurate enough in the mid-chromatogram region; b) the LS signal is insensitive to low molar mass molecules; c) $M_{\mathrm{w}}(V)$ is highly sensitive to errors in δ; d) K depends on the specific refractive index increment of the analyzed polymer ($\partial n/\partial c$); and e) the evaluation of $s_{\mathrm{LS}}(V)$ requires an extrapolation to 0° scattering angle.

The accurate determination of δ [in Eq. (1)] is a difficult problem. For narrow standards, it has been proposed[12] to shift the DR signal until the average slope of $\log M_w(V)$ fits the average slope of $\log M(V)$ in the mid-chromatogram region. However, it can be proven that such a method provides an underestimated IDV, because $\log M(V)$ is always steeper than $\log M_w(V)$.[13] Alternatively, it has been suggested to first estimate an IDV-independent molar mass calibration from the radius of gyration obtained by LS and then to adopt δ as the shift which forces $M_w(V)$ to fit such calibration.[14] Unfortunately, this method suffers from a large propagation of errors.

The main source of BB is axial dispersion in the fractionation columns; however, other minor sources include column-end effects, finite injection volumes, finite detection cell volumes, and laminar flow profiles in the interconnection capillaries.[15–17] In addition to introducing a pure V shift, the interdetector capillary and DR cell volume also induce a (minor) extra distortion in the shape of the DR chromatogram.[18] Such distortion has been represented by an exponentially-modified Gaussian (EMG), obtained by convolution between a Gaussian function (characterized by a standard deviation σ) and a decreasing exponential function (characterized by a decay constant τ).[18] For example, an interdetector capillary of internal diameter 0.2 mm and length 50 cm induces a Gaussian component of $\sigma \approx 0.005$ mL;[15] and a 10 μL DR cell induces an exponential component of $\tau \approx 0.01$ mL.[18] As we shall see later in this work, these values are almost one order of magnitude lower than the EMG parameters of the BBF produced by our chromatographic system from the injector to the LS detector. Thus, it will be hereafter assumed that the interconnection capillary and DR cell only introduce a pure lag δ in the DR signal, but no extra shape distortion in the DR chromatogram with respect to the LS chromatogram.

Due to BB, a whole distribution of molar masses is present in the detection cells, even when linear homopolymers are analyzed. The following model has been proposed for describing the effect of BB on the LS and DR chromatograms:[19–23]

$$s_{LS}(V) = \int_0^\infty g(V, \overline{V}) s_{LS}^c(\overline{V}) d\overline{V} \tag{2a}$$

$$s_{DR}(V + \delta) = \int_0^\infty g(V, \overline{V}) s_{DR}^c(\overline{V} + \delta) d\overline{V} \tag{2b}$$

where $s_{LS}(V)$ and $s_{DR}(V)$ are the measured LS and DR chromatograms, respectively; $s_{LS}^c(V)$ is the BB-corrected LS chromatogram; $s_{DR}^c(V + \delta)$ is the IDV- and BB-corrected DR chromatogram; $g(V, \overline{V})$ is the global BBF; and $\overline{V}$ is the elution volume at which a hypothetically impulsive chromatogram would appear when analyzing a strictly uniform standard

in the absence of BB.[22,23] Note that the common $g(V, \overline{V})$ of Eqs. (2.a) and (2.b) is a consequence of having assumed that the effect of the IDV is only a pure lag in the DR signal.

The bivariate function $g(V, \overline{V})$ is here considered "global," because it describes the BBF in the complete fractionation range. In contrast, at any given $\overline{V}$, $g(V, \overline{V})$ reduces to a univariate or "local" BBF $g_{\overline{V}}(V)$. Each univariate BBF is in general unimodal, narrow, and skewed. Even though the true value of $\overline{V}$ is strictly unknown, it is typically adopted at the maximum of symmetrical $g_{\overline{V}}(V)$ functions, or at any other measure of central tendency for skewed $g_{\overline{V}}(V)$ functions. For estimating the local BBFs, $g(V, \overline{V})$ will be assumed $\overline{V}$-invariant in the narrow range of each standard. In this case, $g(V, \overline{V})$ reduces to $g_{\overline{V}}(V - \overline{V})$; that is, the different $g_{\overline{V}}(V)$ functions are simply shifted along the V-axis.

When a narrow polymer is analyzed by DR/LS, then the global weight-average molar mass ($\overline{M}_w$) directly obtained from $s_{DR}(V+\delta)$ and $M_w(V)$ [Eq. (1)] results unaffected by BB. However, the global number-average molar mass ($\overline{M}_n$) results are overestimated, and therefore the global dispersity, $\overline{M}_w/\overline{M}_n$, are underestimated.[13] For DR/LS detection, the combined effects of BB and IDV have been investigated in several publications.[24–28] When analyzing a log-normal MMD with a linear calibration $\log M(V)$ and a Gaussian and $\overline{V}$-invariant BBF, then the *ad hoc* calibration $\log M_w(V)$ [Eq. (1)] is also linear and counterclockwise-rotated with respect to $\log M(V)$.[26,27] For this reason, the (otherwise underestimated) $\overline{M}_w/\overline{M}_n$ can be indirectly corrected for BB through an appropriate underestimation of δ in Eq. (1).[28]

Chromatograms can be corrected for BB by numerical inversion of Eq. (2a) and (2b). Unfortunately however, such operations are ill-conditioned and can provide multiple solutions. Most inversion algorithms assume *a priori* knowledge of the BBF. While some methods are restricted to Gaussian BBFs,[20,21,28,29] others admit asymmetric BBFs.[22,30,31] To avoid numerical inversions, it has been proposed to indirectly correct MMDs for BB through a counterclockwise rotation of $\log M(V)$.[25,32] Unfortunately, these methods are based on the rather strong assumption that the DR chromatogram and the $\overline{V}$-invariant BBF are both Gaussian functions.

Accurate estimates of the BBF and the IDV would be simple to determine if strictly uniform standards of different molar masses were available. In effect, in this hypothetical case, any (DR or LS) chromatogram would directly provide the shape of the local BBF, and δ would be directly given by the difference in the elution volumes of the chromatogram peaks. Hatada et al.[33] described a fractionation procedure that could eventually lead to the production of uniform standards. Unfortunately however, strictly uniform standards are so far not commercially available. Instead,

narrow-distributed standards are available, with MMD dispersities that generally increase with their average molar masses. Manufacturers provide information on the average molar masses of their standards, but no information on their true MMDs. This is unfortunate, since if such information were known, then their local BBFs could be estimated by inversion of Eq. (2b). Of course, accurate determinations of the MMDs of narrow samples via SEC are impossible, due to the inevitable presence of BB.

Several theoretical models have been developed for predicting the BBF in SEC. For example, a deterministic model[34] was proposed as an extension of the classical van Deemter expression.[35] Also, stochastic models were investigated by several authors,[36–40] as an extension of the classical theory by Giddings-Eyring.[41] Other methods are based on the assumption that the analyzed polymers exhibit Poisson MMDs.[42–44] A review article on methods for estimating the BBF in SEC was coauthored by several of the participants of the IUPAC Project 2003-023-2-400: "Data Treatment in Size Exclusion Chromatography of Polymers."[45] It was concluded that local BBFs are skewed distributions that can be adequately represented by EMGs. Furthermore, it was found that the Gaussian component (of standard deviation σ_{BB}) slightly increases with $\overline{V}$, while the exponential component (of time constant τ_{BB}) slightly decreases with $\overline{V}$. In addition, the total EMG variance of the local BBFs ($\sigma^2_{EMG} = \sigma^2_{BB} + \tau^2_{BB}$) remains almost constant (or slightly decreases) with $\overline{V}$, while the asymmetry (τ_{BB}/σ_{BB}) decreases with $\overline{V}$.[44,45] Near to the total exclusion limit, the local BBF cannot be properly represented by an EMG, due to a large increase in the BBF kurtosis.[46]

The aim of this work is the determination of the BBF in SEC via DR/LS detection. An accurate estimation of the BBF is important for: 1) obtaining unbiased estimates of narrow or multimodal MMDs; 2) producing accurate mathematical models that describe the fractionation processes that take place in a column;[34–41] and 3) improving the estimates of rate coefficients of radical polymerizations obtained from the analysis of the produced MMD.[47,48]

This work applies the theoretical method proposed by Yossen et al.;[49] that is summarized in the Appendix. Several narrow polystyrene (PS) standards were analyzed by LS/DR for estimating the global BBF introduced by the chromatographic system from the sample injector to the LS detector. First, a novel technique is proposed for estimating the IDV. Then, the local BBFs of 4 polystyrene standards were calculated. Finally, the global BBF was obtained by interpolation of the local EMG parameters.

EXPERIMENTS AND DATA TREATMENT

Six PS standards (PS1-PS6) were analyzed; and their nominal average characteristics are given in the first columns of Table 1. The fractionation

TABLE 1 Nominal Characteristics of the Analyzed PS Standards, Estimates of the Lower and Upper Limits of the IDV (δ_{slope} and δ_p, Respectively), and Two Estimates of the Average Molar Masses (Obtained Without BB Correction)

	Nominal Averages			Estimates of the IDV Limits				Estimated Averages			
Standard	$\overline{M}_w$ [g/mol]	$\overline{M}_w/\overline{M}_n$ [–]	$\overline{M}_p$ [g/mol]	$V_{p,DR}$ [mL]	$V_{p,LS}$ [mL]	δ_p [mL]	δ_{slope} [mL]		$\overline{M}_w$ [g/mol]	$\overline{M}_n$ [g/mol]	$\overline{M}_w/\overline{M}_n$ [–]
PS1 (Polymer Lab.)	1,800,000	1.117	1,950,000	9.9500	9.8433	0.1067	0.0533	(a)	1,790,000	1,586,000	1.129
								(b)	1,801,000	1,779,000	1.012
PS2 (Waters)	385,400	1.082	370,500*	11.1867	11.1000	0.0867	0.0600	(a)	376,000	365,000	1.030
								(b)	424,900	415,800	1.022
PS3 (our laboratories)	113,000**	1.040**	110,800*	12.3967	12.3100	0.0867	0.0567	(a)	104,900	102,300	1.025
								(b)	113,700	111,600	1.019
PS4 (Pressure Chem.)	30,900	1.050	30,200*	13.6800	13.5933	0.0867	0.0533	(a)	30,150	29,400	1.026
								(b)	30,620	30,230	1.013
PS5 (our laboratories)	11,480**	1.048**	11,200*	14.6333	–	–	–	(a)	11,490	10,980	1.047
PS6 (Polymer Lab.)	3,250	1.086	3,100*	15.7633	–	–	–	(a)	3,054	2,754	1.109

The averages of rows (a) were calculated from $s_{DR}(V)$ and $\log M(V)$ of Figure 1. The averages of rows (b) were calculated from $s_{DR}(V+\delta)$ and $\log M_w(V)$ as obtained through Eq. (1) with $\delta = 0.0733$ mL.

*Estimated from $\sqrt{\overline{M}_n\ \overline{M}_w}$. **Characterized by MALDI-ToF.

system consisted of 2 mixed-gel columns PLgel Mixed-C (Polymer Labs), of particle diameter 5 μm and length 60 cm. The LS detector was a MiniDawn (Wyatt Tech.), and the raw measurements were the excess Rayleigh scattering $R_\theta(V)$ at $\theta = 45°$, 90°, and 135°. The concentration detector was a DR Model 2414 (Waters Corp.). The carrier solvent was tetrahydrofurane (THF) at 0.8 mL/min and 40°C. The injection loop volume was 0.1 mL, and the nominal concentration of the injection samples was 0.3 mg/mL. The signals were sampled at 300 points per mL (i.e., every $\Delta V = 0.0033$ mL). The LS signal, $s_{LS}(V) \equiv R_{0°}(V)$, was obtained through a linear extrapolation to 0° of the function $1/R_\theta(V)$ *vs.* $\sin^2(\theta/2)$. All the computer programs were coded and implemented in Matlab (MathWorks).

Figure 1 presents the measured chromatograms and the non-linear molar mass calibration $\log M(V)$. The calibration was obtained from the pairs $\{V_{p,DR}, \overline{M}_p\}$, that is, the elution volumes and molar masses at the peaks of the DR chromatograms (see values in Table 1). For the standards PS2-PS6, the molar masses at the chromatogram peaks were calculated from the $\overline{M}_n$ and $\overline{M}_w$ values through: $\overline{M}_p = (\overline{M}_n\ \overline{M}_w)^{1/2}$. This last expression implicitly assumes a Gaussian mass chromatogram and a linear calibration in the absence of BB. For the (broader and more highly skewed) chromatograms of standard PS1, the $\overline{M}_p$ value was estimated by trial-and-error, with the aim of recuperating the nominal $\overline{M}_w$ from the adjusted calibration. The final calibration (Figure 1) is represented by the following third-order polynomial: $\log M(V) = 25.9014 - 3.98227$

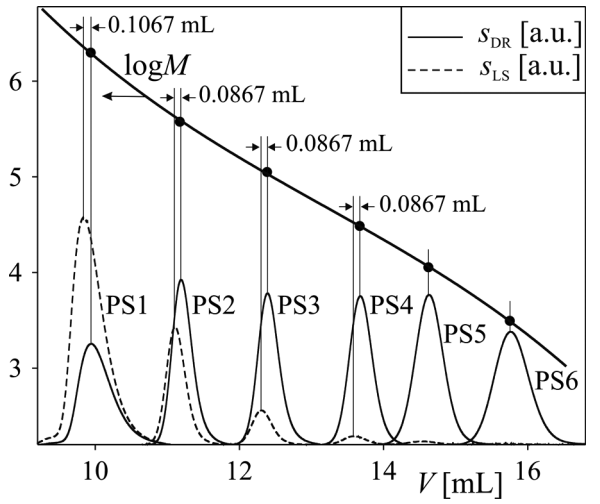

FIGURE 1 PS standards PS1-PS6: measured DR and LS chromatograms, $s_{DR}(V)$ and $s_{LS}(V)$; direct molar mass calibration based on the DR signals $[\log M(V)]$; and shifts between the chromatogram peaks of standards PS1-PS4.

$V + 0.269972\ V^2 - 0.00682263\ V^3$. Then, the average molar masses were directly calculated from $\log M(V)$ and $s_{DR}(V)$ (i.e., without correction for BB); and the results are in the last 3 columns of Table 1, rows (a). For standards PS2–PS5, the resulting global dispersion indexes are lower than the nominal values, and the opposite is observed for standards PS1 and PS6.

For the lower molar mass standards PS5 and PS6, the LS signals are low and insensitive; and for this reason, their measurements were discarded for estimating the local BBFs. The following 3-steps procedure was applied onto the chromatograms of standards PS1-PS4: 1) the IDV (δ) was estimated from limiting values indirectly obtained through the measured chromatograms; 2) the local BBF parameters (σ_{BB} and τ_{BB}) were estimated through Eqs. (A1); and 3) a global BBF was determined by interpolation of the local BBF parameters. Consider each of the mentioned steps.

Estimation of the IDV

The IDV was determined on the basis of the upper and lower IDV limits, as calculated from the chromatograms of standards PS1-PS4. The upper IDV limits (δ_p) were adopted as the difference between the peak volumes of the DR and LS chromatograms: $\delta_p \equiv V_{p,DR} - V_{p,LS}$ (see Table 1 and Figure 1). The lower IDV limits (δ_{slope}) were obtained by adjustment of δ in Eq. (1), to force the average slope of the *ad-hoc* calibrations to coincide with the average slope of $\log M(V)$. Figure 2 illustrates this adjustment for standard PS3. As expected, the resulting $\log M_w|_{\delta_{slope}}$ is parallel to $\log M(V)$

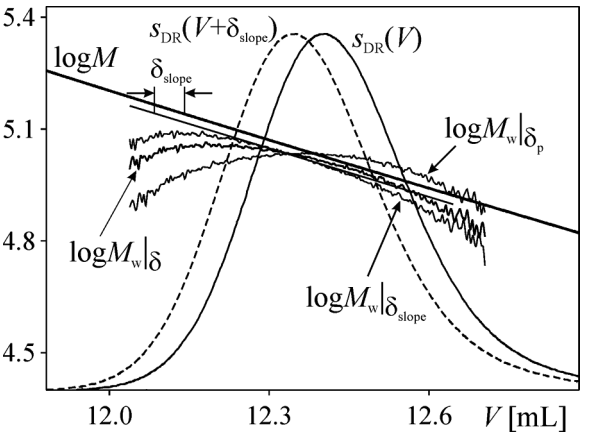

FIGURE 2 Evaluation of the IDV-limits for standard PS3. In the mid-chromatogram region, the lower limit $\delta_{slope} = 0.0567$ mL forces the average slope of $\log M_w|_{\delta_{slope}}(V)$ to coincide with the slope of $\log M(V)$, and the upper IDV limit $\delta_P = 0.0867$ mL produces the almost horizontal calibration $\log M_w|_{\delta_p}(V)$. After analyzing the four standards, the resulting average IDV ($\delta = 0.0733$ mL) produces the calibration $\log M_w|_{\delta}(V)$.

in the mid-chromatogram region (but highly oscillatory at the chromatogram tails). In contrast, note that when δ is equal to the upper limit δ_p, then $\log M_w|_{\delta_p}$ results are almost horizontal.

Table 1 presents the values of δ_{slope} for standards PS1-PS4. The final common IDV was estimated from a simple average between the highest lower IDV value ($\delta_{slope} = 0.0600$ mL) and the lowest upper IDV value ($\delta_P = 0.0867$ mL); yielding $\delta = 0.0733$ mL (Table 1). With this last value, the "true" *ad-hoc* calibration of standard PS3 is represented by $\log M_w|_\delta$ in Figure 2.

For standards PS1-PS4, Figure 3a presents the IDV-corrected DR chromatograms $s_{DR}(V+\delta)$ and the IDV-corrected calibration $\log M(V+\delta)$. Table 1, rows (b) present the average molar masses and dispersions directly estimated from $s_{DR}(V+\delta)$ and $\log M_w(V)$ [as obtained through Eq. (1)

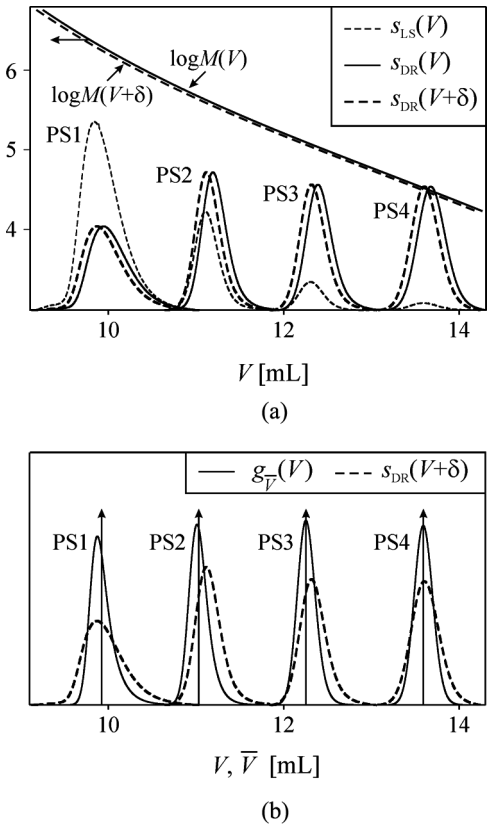

FIGURE 3 Standards PS1-PS4. a) Measured chromatograms [$s_{DR}(V)$ and $s_{LS}(V)$], IDV-corrected DR chromatograms with $\delta = 0.0733$ mL [$s_{DR}(V+\delta)$]; original molar mass calibration based on the DR signal log$M(V)$; and shifted calibration based on the LS signal log$M(V+\delta)$. b) IDV-corrected DR chromatograms [$s_{DR}(V+\delta)$] and resulting local BBFs [$g_{\overline{V}}(V)$]. The vertical arrows indicate the origins $\overline{V}$ of the local BBFs.

with $\delta = 0.0733$ mL]. As expected, the global dispersion indexes of rows (b) are in all cases lower than those of rows (a). Except for PS2, the estimates of $\overline{M}_w$ in rows (b) are close to the (independently-obtained) nominal averages of the second column of Table 1.

Estimation of the Local BBFs of Standards PS1-PS4

Four local BBFs were adjusted to EMGs; with each of such functions characterized by the parameters: σ_{BB}, τ_{BB}, and $\overline{V}$. For each standard, σ_{BB} and τ_{BB} were estimated through Eqs. (A1); while $\overline{V}$ was adopted as the elution volume of the nominal $\overline{M}_w$ according to $\log M(V+\delta)$. This criterion for the selection for $\overline{V}$ forces the (in principle accurate) estimates of $\overline{M}_w$ directly obtained from the LS/DR chromatograms [Table 1, rows (b)], to coincide with the expected estimates of $\overline{M}_w$ after correction of the chromatograms for BB. The final values of $\overline{V}$ are presented in Table 2 and are illustrated by vertical arrows in Figure 3b.

Consider the estimation of σ_{BB} and τ_{BB}. The minimal values of J were determined from plots of J *vs.* σ_{BB}, with τ_{BB} as parameter. Figure 4 presents such a plot for standard PS3, where the final optimal parameters resulted: $\sigma_{BB} = 0.09$ mL and $\tau_{BB} = 0.06$ mL. The multiple cross-points of Figure 4 are indicative of correlation between the adjusted parameters. Table 2 presents the final EMG parameters for standards PS1-PS4, together with the following derived variables: the global asymmetry (τ_{BB}/σ_{BB}) and the global standard deviation [$\sigma_{EMG} = (\sigma_{BB}^2 + \tau_{BB}^2)^{0.5}$]. Figure 3b compares the resulting local BBFs $g_{\overline{V}}(V)$ with their corresponding IDV-corrected DR chromatograms. As expected, each local BBF is skewed, with the longer tails toward the higher Vs. Note that in general, the elution volumes of $\overline{V}$ do not coincide with the elution volumes of the peaks of $g_{\overline{V}}(V)$.

Estimation of the Global BBF

A global $\overline{V}$-dependent BBF was obtained by interpolation of the values of σ_{BB} and τ_{BB} in Table 2. Those values are also represented by black dots in

TABLE 2 Local BBFs of standards PS1-PS4: the estimated EMG parameters $\overline{V}$, σ_{BB}, and τ_{BB}, and some derived parameters; the skewness (τ_{BB}/σ_{BB}) and the local standard deviation $\sigma_{EMG} = (\sigma_{BB}^2 + \tau_{BB}^2)^{0.5}$

Standard	$\overline{V}$ [mL]	τ_{BB} [mL]	σ_{BB} [mL]	τ_{BB}/σ_{BB} [-]	σ_{EMG} [mL]
PS1	9.923	0.13	0.07	1.86	0.1476
PS2	11.115	0.09	0.08	1.13	0.1204
PS3	12.259	0.06	0.09	0.67	0.1082
PS4	13.585	0.04	0.10	0.40	0.1077

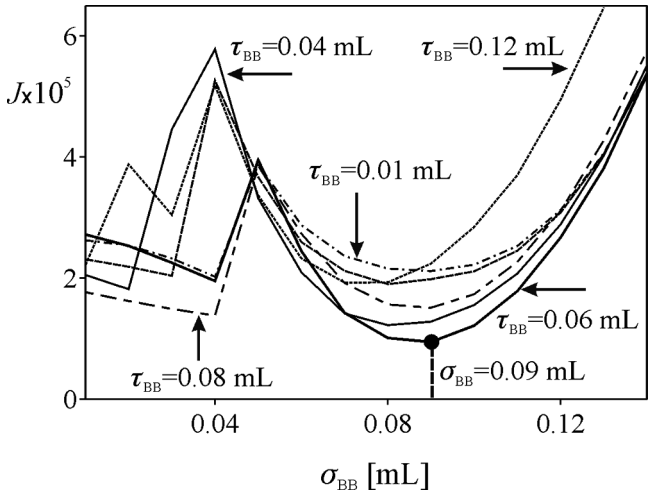

FIGURE 4 Selection of the EMG parameters for standard PS3. The optimal parameters result $\sigma_{BB} = 0.09$ mL and $\tau_{BB} = 0.06$ mL.

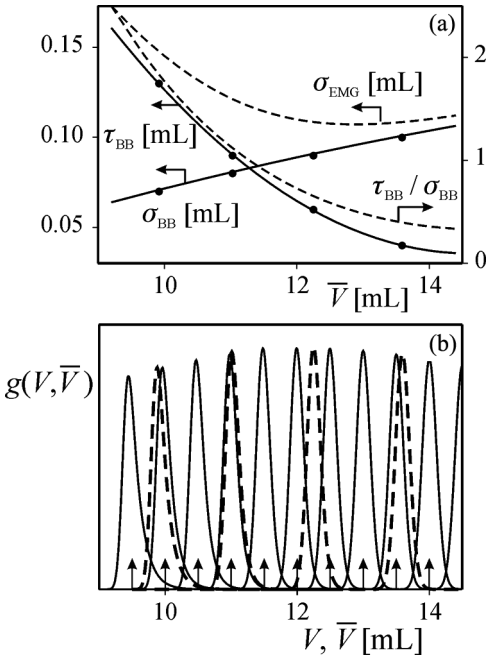

FIGURE 5 a) EMG parameters (τ_{BB} and σ_{BB}) for standards PS1-PS4 (in black dots) and interpolated polynomials (in continuous trace). Also represented are τ_{BB}/σ_{BB} and $\sigma_{EMG} = (\sigma_{BB}^2 + \tau_{BB}^2)^{0.5}$. b) The four resulting local BBFs are shown in thick dashed lines, and the global interpolated BBF is shown in solid trace. The vertical arrows indicate the $\overline{V}$ origins of the interpolated local BBFs.

Figure 5a. The following second-order polynomials were adjusted:

$$\sigma_{BB}(\overline{V}) = -0.053 + 0.0156\overline{V} - 0.000315\overline{V}^2 \quad (3a)$$

$$\tau_{BB}(\overline{V}) = 0.950 - 0.1252\overline{V} + 0.004285\overline{V}^2 \quad (3b)$$

These functions are shown in continuous trace in Figure 5a, together with the derived functions $\tau_{BB}/\sigma_{BB}(\overline{V})$ and $\sigma_{EMG}(\overline{V}) = (\sigma_{BB}^2 + \tau_{BB})^{0.5}(\overline{V})$. Figure 5b compares the local BBFs (in discontinuous trace) with the interpolated global BBF (in continuous trace). For increasing V's, σ_{BB} increases while τ_{BB} and τ_{BB}/σ_{BB} decrease. Also, the BBF variance generally decreases with V, but it remains almost constant at high V's (Figure 5a). These general tendencies are coincident with previous published results.[44–46]

CONCLUSIONS

Several narrow PS standards were analyzed by SEC with dual LS/DR detection, to estimate their (skewed and $\overline{V}$-invariant) local BBFs. The strong interdependence between the sought BBF, the IDV, and the molar mass calibration obtained from the narrow standards complicates the calculation procedure. In our case, such difficulty was overcome through the following sequential procedure. First, a molar mass calibration based on the raw DR signals was determined. Then, a common IDV was estimated from limiting values provided by the DR/LS measurements; and such common IDV was used to shift the raw DR chromatograms and their corresponding direct calibration. Finally, the local BBFs of each standard were determined, and the global BBF was obtained by interpolation of the local EMG parameters.

For each local BBF, σ_{BB} and τ_{BB} were obtained through an optimization procedure, and $\overline{V}$ was chosen as the elution volume that reproduces the nominal $\overline{M}_w$ value according to the direct calibration $\log M(V)$. This forces the (in principle, accurate) estimates of $\overline{M}_w$ directly obtained from the LS/DR chromatograms, to coincide with the same estimates obtained after BB correction. As expected,[44–46] all the local BBFs exhibited a positive skewness, and global variances and asymmetries that decreased with V. For each standard, the true unbiased global dispersion $\overline{M}_w/\overline{M}_n$ is expected to fall within the following limits: a) the overestimated value directly obtained from the DR chromatograms and the independent calibration with narrow standards [Table 1, rows (a)], and b) the underestimated value directly obtained from the LS/DR chromatograms [Table 1, rows (b)].

The numerical algorithm for estimating the BBF proved robust to unavoidable errors in the detector gains and in the concentration of the

injected samples. The reason for such robustness is that the mentioned errors are essentially cancelled off by the signals ratio of Eq. (A1b). On the negative side, the algorithm could not be applied onto the lower molar mass standards, due to the low sensitivity of their LS signals. Fortunately, however, uniform low molar mass compounds are available, and this enables a direct estimation of the BBF at the high elution volume limit.

APPENDIX: REVIEW OF THE METHOD BY YOSSEN ET AL.[49]

Consider the theoretical method proposed for determining the BBF in SEC with DR/LS detection, through the analysis of narrow PS standards.[49] For each standard, the EMG parameters $\{\sigma_{BB}, \tau_{BB}\}$ of the local BBFs $[g_{\overline{V}}(V-\overline{V})]$ are obtained from their DR and LS chromatograms, by minimizing an average squared error between the measured LS chromatogram and its estimate based on the DR chromatogram; that is:

$$\min_{\{\sigma_{BB},\tau_{BB}\}} J = \min_{\{\sigma_{BB},\tau_{BB}\}} (\tilde{\mathbf{e}}^{T}_{\mathbf{s}_{LS,DR}} \tilde{\mathbf{e}}_{\mathbf{s}_{LS,DR}}) \tag{A1a}$$

with:

$$\tilde{\mathbf{e}}_{\mathbf{s}_{LS,DR}} = \frac{\mathbf{s}_{LS}}{\|\mathbf{s}_{LS}\|_1} - \frac{\mathbf{G}[\mathbf{M}_\delta]\mathbf{G}^{[-1]}\mathbf{s}_{DR,\delta}}{\|\mathbf{G}[\mathbf{M}_\delta]\mathbf{G}^{[-1]}\mathbf{s}_{DR,\delta}\|_1} \tag{A1b}$$

where J is the scalar functional to be minimized; vector $\tilde{\mathbf{e}}_{\mathbf{s}_{LS,DR}}$ $(m \times 1)$ is the error between the normalized LS chromatogram [first term in the right hand side of Eq. (A1b)] and its estimate obtained from the DR chromatogram [last term of Eq. (A1b)]; σ_{BB} and τ_{BB} are the sought EMG parameters; vectors $\mathbf{s}_{DR,\delta}$ $(m\times1)$ and $\mathbf{s}_{LS}$ $(m\times1)$ are discrete versions of $s_{DR}(V+\delta)$ and $s_{LS}(V)$, respectively; matrix $\mathbf{G}$ $(m \times p)$ represents the local BBF $g_{\overline{V}}(V-\overline{V})$ defined by σ_{BB} and τ_{BB}; $\mathbf{G}^{[-1]}$ is a regularized pseudo-inverse of $\mathbf{G}$[22,23]; and $\mathbf{M}_\delta$ $(p\times p)$ is a diagonal matrix containing the ordinates of $M(V)$ shifted by δ towards the lower V's, that is: $M_\delta(V) \equiv M(V+\delta)$; $|\cdot|_1$ indicates the 1-norm of a vector, that is: $|\mathbf{x}|_1 = \sum_i |x_i|$. Each column of $\mathbf{G}$ contains the ordinates of $g_{\overline{V}}(V-\overline{V})$, and its elements are shifted one position downwards with respect to the previous column.[22,23,49]

Unfortunately, the sought σ_{BB} and τ_{BB} parameters are highly correlated within each other, and this complicates the resolution of Eq. (A1). To understand the reason for this correlation, note that the total variance of any s_{DR} or s_{LS} chromatogram (represented in general by s_k), is given by:[43]

$$\sigma^2_{s_k} = \sigma^2_{s_k c} + \sigma^2_{EMG} = \sigma^2_{s_k c} + \sigma^2_{BB} + \tau^2_{BB} \tag{A2}$$

where $\sigma^2_{s^c_k}$ is the variance of the BB-corrected chromatogram, and σ^2_{EMG} is the total variance of the local BBF. Clearly, many different pairs of σ_{BB} and τ_{BB} can provide similar values of σ^2_{EMG} that verify Eq. (A2).

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REFERENCES

1. Berek, D. Coupled Liquid Chromatographic Techniques for the Separation of Complex Polymers. *Prog. Polym. Sci.* **2000**, *25*, 873–908.
2. Meira, G. R.; Vega, J. R.; Yossen, M. M. Gel Permeation and Size Exclusion Chromatography. In *Ewing's Analytical Instrumentation Handbook* (3rd Ed.); Cazes, J., Ed.; Marcel Dekker, Inc.: New York, 2004, pp 825–867.
3. Hamielec, A. E.; Ouano, A. C. Generalized Universal Molecular Weight Calibration Parameter in GPC. *J. Liq. Chromatogr.* **1978**, *1* (1), 111–120.
4. Hamielec, A. Characterization of Complex Polymer Systems by Size Exclusion Chromatography – Homopolymers with Long Chain Branching and Copolymers with Composition Drift. *Pure Appl. Chem.* **1982**, *54* (2), 293–307.
5. Gaborieau, M.; Gilbert, R. G.; Gray-Weale, A.; Hernandez, J. M.; Castignolles, P. Theory of Multiple-Detection Size-Exclusion Chromatography of Complex Branched Polymers. *Macromol. Theory and Simul.* **2007**, *16*, 13–28.
6. Gaborieau, M.; Nicolas, J.; Save, M.; Charleux, B.; Vairon, J.-P.; Gilbert, R. G.; Castignolles, P. Separation of Complex Branched Polymers by Size-Exclusion Chromatography probed with Multiple Detection. *J. Chromatogr. A.* **2008**, *1190*, 215–223.
7. Castignolles, P.; Graf, R.; Parkinson, M.; Wilhelm, M.; Gaborieau, M. Detection and Quantification of Branching in Polyacrylates by Size-Exclusion Chromatography (SEC) and Melt-State ^{13}C NMR Spectroscopy. *Polymer.* **2009**, *50* (11), 2373–2383.
8. Gaborieau, M.; Castignolles, P. Size-Exclusion Chromatography (SEC) of Branched Polymers and Polysaccharides. *Anal. Bioanal. Chem.* **2011**, *399* (4), 1413–1423.
9. Baumgarten, J.; Busnel, J. P.; Meira, G. R. Band Broadening in Size Exclusion Chromatography of Polymers. State of the Art and Some Novel Solutions. *J. Liq. Chromatogr. R. T.* **2002**, *25* (13–15), 1967–2001.
10. Netopilík, M.; Podzimek, S.; Kratochvíl, P. Estimation of Width of Narrow Molecular-Weight Distributions by Size-Exclusion Chromatography with Concentration and Light Scattering Detection. *J. Chromatogr. A.* **2001**, *922*, 25–36.
11. Tackx, P.; Bosscher, F. Systematic Deviations due to Random Noise Levels in Size Exclusion Chromatography Coupled with Multi Angle Laser Light Scattering. *Anal. Commun.* **1997**, *34* (10), 295–297.
12. Balke, S. T.; Cheung, P.; Jeng, L.; Lew, R.; Mourey, T. H. Chemometrics in Quantitative Polymer Analysis: Attempts to Displace Traditional Methods. *J. Appl. Polym. Sci., Appl. Polym. Symp.* **1991**, *48*, 259–291.

13. Prougenes, P. I.; Berek, D.; Meira, G. R. Size Exclusion Chromatography of Polymers with Molar Mass Detection. Computer Simulation Study on Instrumental Broadening Biases and Proposed Correction Method. *Polymer.* **1999**, *40* (1), 117–124.
14. Netopilík, M.; Podzimek, S.; Kratochvíl, P. Determination of the Interdetector Volume by S-detection in Size-Exclusion Chromatography of Polymers with On-line Multiangle Light-scattering Detection. *J. Chromatogr. A.* **2004**, *1045* (1&2), 37–41.
15. Hupe, K. P.; Jonker, R. J.; Rozing, G. Determination of Band-Spreading Effects in High-Performance Liquid Chromatographic Instruments. *J. Chromatogr. A.* **1984**, *285*, 253–265.
16. Prüβ, A.; Kempter, C.; Gysler, J.; Jiora, T. Extracolumn Band Broadening in Capillary Liquid Chromatography. *J. Chromatogr. A.* **2003**, *1016* (2), 129–141.
17. Wyatt, P. Mean Square Radius of Molecules and Secondary Instrumental Broadening. *J. Chromatogr. A.* **1993**, *648*, 27–32.
18. Trainoff, S. P. Method for Correcting the Effects of Interdetector Band Broadening. US Patent Application Publication, US 2005/0075851 A1, April 7, 2005.
19. Tung, L. H. Method of Calculating Molecular Weight Distribution Function from Gel Permeation Chromatograms. III. Application of the Method. *J. Appl. Polym. Sci.* **1966**, *10* (9), 1271–1283.
20. Netopilík, M. Correction for Axial Dispersion in Gel Permeation Chromatography with a Detector of Molecular Masses. *Polymer Bull.* **1982**, *7*, 575–582.
21. Hamielec, A. E. Correction for Axial Dispersion. In *Steric Exclusion Liquid Chromatography of Polymers, Chromatographic Science*; Janca, J., Ed.; Marcel Dekker, Inc.: New York, 1984, Vol.25, pp 117–160.
22. Vega, J. R.; Meira, G. R. SEC of Simple Polymers with Molar Mass Detection in Presence of Instrumental Broadening. Computer Simulation Study on the Calculation of Unbiased Molecular Weight Distribution. *J. Liq. Chromatogr. R. T.* **2001**, *24*, 901–919.
23. Meira, G. R.; Vega, J. R. Band Broadening Correction Methods in GPC/SEC. In *Dekker Encyclopedia of Chromatography* (2nd Ed.); Cazes, J., Ed.; Marcel Dekker, Inc.: New York, 2005, pp 149–159.
24. Netopilík, M. Effect of Local Polydispersity in Size Exclusion Chromatography with Dual Detection. *J. Chromatogr. A.* **1998**, *793* (1), 21–30.
25. Jackson, C.; Yau, W. J. Computer Simulation Study of Size-Exclusion Chromatography with Simultaneous Viscometry and Light Scattering Measurements. *J. Chromatogr. A.* **1993**, *645* (2), 209–217.
26. Netopilík, M. Combined Effect of Interdetector Volume and Peak Spreading in Size Exclusion Chromatography with Dual Detection. *Polymer.* **1997**, *38*, 127–130.
27. Netopilík, M. Analysis of the Influence of Interdetector Volume on Local Calibration in Size Exclusion Chromatography with Dual Multiangle-Light-Scattering/Concentration Detection. *J. Chromatogr. A.* **2006**, *1113* (1&2), 162–166.
28. Jackson, C. Evaluation of the "Effective Volume Shift" Method for Axial Dispersion Corrections in Multi-Detector Size Exclusion Chromatography. *Polymer.* **1999**, *40*, 3735–3742.
29. Hamielec, A. E.; Ederer, H. J.; Ebert, K. H. Size Exclusion Chromatography of Complex Polymers. Generalized Analytical Corrections for Imperfect Resolution. *J. Liq. Chromatogr.* **1981**, *4*, 1697–1707.
30. Ishige, T.; Lee, S.; Hamielec, A. Solution of Tung's Axial Dispersion Equation by Numerical Techniques. *J. Appl. Polym. Sci.* **1971**, *15*, 1607–1622.
31. Konkolewicz, D.; Taylor, J.; Castignolles, P.; Gray-Weale, A.; Gilbert, R. Toward a More General Solution to the Band-Broadening Problem in Size Separation of Polymers. *Macromolecules* **2007**, *40*, 3477–3487.
32. Yau, W.; Stoklosa, H.; Bly, D. Calibration and Molecular Weight Calculations in GPC Using a New Practical Method for Dispersion Correction – GPCV2. *J. Appl. Polym. Sci.* **1977**, *21*, 1911–1920.
33. Hatada, K.; Kitayama, T.; Ute, K.; Nishiura, T. Synthetic Uniform Polymers and their Use for Understanding Fundamental Problems in Polymer Chemistry. *Macromol. Rapid Commun.* **2004**, *25* (16), 1447–1477.
34. Potschka, M. Mechanism of Size-Exclusion Chromatography: I. Role of Convection and Obstructed Diffusion in Size-Exclusion Chromatography. *J. Chromatogr.* **1993**, *648* (1), 41–69.
35. van Deemter, J. J.; Zuiderweg, F. J.; Klinkenberg, A. Longitudinal Diffusion and Resistance to Mass Transfer as Causes of Nonideality in Chromatography. *Chem. Eng. Sci.* **1956**, *5*, 271–289.
36. Netopilík, M. Relations between the Separation Coefficient, Longitudinal Displacement and Peak Broadening in Size Exclusion Chromatography of Macromolecules. *J. Chromatogr. A.* **2002**, *978*, 109–117.

37. Netopilík, M. Relation between the Kinetic and Equilibrium Quantities in Size-Exclusion Chromatography. *J. Chromatogr. A.* **2004**, *1038* (1&2), 67–75.
38. Netopilík, M. Using Multiple Detectors to Study Band Broadening in Size-Exclusion Chromatography. In *Multiple Detection Size-Exclusion Chromatography*; Striegel, A.M., Ed.; ACS Symp. Ser. 893: Washington, DC, 2004, pp 302–318.
39. Felinger, A.; Pasti, L.; Dondi, F.; van Hulst, M.; Schoenmakers, P.; Martin, M. Stochastic Theory of Size Exclusion Chromatography: Peak Shape Analysis on Single Columns. *Anal. Chem.* **2005**, *77*, 3138–3148.
40. Felinger, A. Molecular Dynamic Theories in Chromatography. *J. Chromatogr. A.* **2008**, *1184* (1–2), 20–41.
41. Giddings, J. C.; Eyring, H. A Molecular Dynamic Theory of Chromatography. *J. Phys. Chem.* **1955**, *59*, 416–421.
42. Schnöll-Bitai, I. The Direct Determination of Axial Dispersion in Size Exclusion Chromatography Based on Poissonian Chain Length Distributions. *Chromatographia.* **2003**, *58* (5), 375–380.
43. Vega, J. R.; Schnöll-Bitai, I. Alternative Approaches for the Estimation of the Band Broadening Parameters in Single–Detection Size Exclusion Chromatography. *J. Liq. Chromatogr. A.* **2005**, *1095* (1&2), 102–112.
44. Vega, J. R.; Schnöll-Bitai, I.; Mader, C. Estimation of the Band Broadening Parameters in Single Detection Size Exclusion Chromatography: A Comparative Study of Various Column Combinations. *Anal. Chim. Acta.* **2007**, *604* (1), 9–17.
45. Meira, G. R.; Netopilík, M.; Potschka, M.; Schnöll-Bitai, I.; Vega, J. R. Band Broadening Function in Size Exclusion Chromatography of Polymers: Review of Some Recent Developments. *Macromol. Symp.* **2007**, *258*, 186–197.
46. Busnel, J. P.; Foucault, F.; Denis, L.; Lee, W.; Chang, T. Investigation and Interpretation of Band Broadening in Size Exclusion Chromatography. *J. Chromatogr. A.* **2001**, *930* (1&2), 61–71.
47. Kornherr, A.; Olaj, O. F.; Schnöll-Bitai, I.; Zifferer, G. Masterfunctions Providing a Sound Theoretical Basis for the Extraction of Accurate k_p Data from PLP-SEC Chain Lengths Distributions Exhibiting Poissonian and Gaussian Broadening. *Macromolecules.* **2003**, *36*, 10021–10028.
48. van Berkel, K. Y.; Russell, G. T.; Gilbert, R. G. Molecular Weight Distributions and Chain-Stopping Events in the Free-Radical Polymerization of Methyl Methacrylate. *Macromolecules.* **2005**, *38*, 3214–3224.
49. Yossen, M. M.; Vega, J. R.; Meira, G. R. Estimation of Band Broadening in Size Exclusion Chromatography. I. A Method Based on Analyzing Narrow Standards with a Molar Mass-Sensitive Detector. *J. Chromatogr. A.* **2006**, *1128*, 171–180.