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Band Broadening Function in Size Exclusion Chromatography of Polymers: Review of Some Recent Developments

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Summary: This article reviews some recent developments on the determination of the Band Broadening Function (BBF) in Size Exclusion Chromatography (SEC) of polymers. It was carried out in the frame of the IUPAC Project: ''Data Treatment in Size Exclusion Chromatography of Polymers''. The correction for band broadening (BB) is important for quantitative determinations of the molar mass distribution (MMD) of narrow-distributed (or highly multimodal) polymers, and of derived variables such as kinetic parameters. In the narrow range of a molar mass standard, the BBF is uniform and of positive skewness. In a broad chromatographic range, the BBF is non-uniform and skewed; and it can be adequately represented by an exponentially-modified Gaussian function (EMG) of 2 parameters that vary slightly with elution volume: an increasing Gaussian variance and a decreasing exponential decay. Additionally, the total BBF variance remains almost constant if not close to the total exclusion limit. The following methods for determining BBF parameters are reviewed: a) a direct method based on assuming Poisson-distributed MMDs; b) a direct method based on measuring the mass- and molar mass chromatograms of narrow standards; c) a theoretical method based on a stochastic model that is equivalent to the Gidding-s-Eyring model; and d) a theoretical method based on a deterministic model obtained through an extension of the classical van Deemter expression. Ideally, the correction for BB requires a robust numerical inversion algorithm. However, alternative simplified solutions are also possible.

Keywords: band broadening; gel permeation chromatography (GPC); molar mass distribution; size-exclusion chromatography

Introduction

This article is also the Final Report of the IUPAC project 2003-023 entitled: ''Data Treatment in SEC of Polymers'' (Coordinator: G. Meira); carried out by the authors and other participants between Jan. 2004 and Apr. 2007. The main publications[1–16] and the congress presentations[17–24] are listed in the reference section. The project was a continuation of two earlier projects entitled: ''SEC Band Broadening Correction Procedures'' (Coordinator: M. Potschka), and ''Band Broadening Correction in SEC'' (Coordinator: J. Baumgarten). An outcome of the previous project was a general review article on the problem of correction for band broadening (BB) in SEC.[25] Three formal meetings took place within the present project. First, a special morning session on Band Broadening (BB) in SEC was organized by D. Berek in the 20th Bratislava International Conference on

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Macromolecules ''Advanced Polymeric Materials'' (June 2006); that included oral presentations by M. Netopilik,[17] M. Potschka,[18] I. Schnöll-Bitai,[19] and G. Meira.[20] Second, the following contributions on SEC of polymers presented by project participants in MACRO 2006 World Polymer Congress (IUPAC), Rio de Janeiro (Brazil), July 2006: T. Chang,[21] D. Berek,[22] and J. Vega.[23] Third, G. Meira[24] presented a review of the project results in Polychar-15 World Forum on Advanced Materials, Búzios (Brazil), April 2007. There were also other presentations in international meetings.[26–32] The authors wish to dedicate this article to Prof. Klaus Lederer, on occasion of his 65th birthday.

The global project aim was to produce quantitative determinations of the MMD of narrow polymers from SEC measurements. Even though several sources of error have been investigated, the main emphasis has been the correction for BB when (narrow or multimodal-distributed) samples are analyzed. Several biases are introduced when BB is not corrected for in an otherwise ideal chromatograph. On the one hand, the total sample non-uniformity index ($\overline{M}_w/\overline{M}_n$) is overestimated when determined from a concentration chromatogram and a molar mass calibration (in turn, obtained from narrow standards). On the other hand, $\overline{M}_w/\overline{M}_n$ is underestimated when determined from ideal molar mass sensitive detectors, such as a light-scattering sensor (LS), or an intrinsic viscosity sensor (IV).[1]

When molar mass-sensitive detectors are employed, it has proven essential to properly correct for the shift introduced by the inter-detector volume (IDV). Netopilík[4] showed that small errors in the IDV may completely distort the estimated molar masses. Other articles concerning determination of the IDV and its effects in SEC data treatment were published.[5,16] The corrections for IDV and BB are interrelated. Thus, an overestimated IDV generates a clockwise rotation of the ''ad hoc'' or local calibration log$M(V)$; and this is equivalent to a (disguised and crude) correction for BB in the resulting MMD. The IDV's are overestimated when determined from the volume shift between maxima of the concentration and molar mass chromatograms of a narrow standard. Unfortunately, manufacturers of molar mass detectors do not properly distinguish between the true BB effect (that distorts all chromatogram shapes due to a deleterious process mainly occurring in the fractionation columns) from errors in the IDV (that appear when the raw molar mass chromatogram is inaccurately shifted with respect to the concentration chromatogram). Other problems with molar mass-sensitive detectors include errors at the chromatogram tails, low sensitivities toward the low molar mass species, and difficulties for analyzing copolymers.

In recent years, it was corroborated that the peak width of narrow samples is dominated by BB.[33] This effect is particularly important in fast SEC, where the so-called integrity index is a direct estimate of the width of the measured MMD.[34,35] The correction for BB is also important for accurate estimations of kinetic coefficients directly derived from SEC measurements. For example, van Berkel *et al.*[36] investigated chain-stopping and radical-loss events in seeded emulsion polymerizations of methyl methacrylate. If transfer to monomer is the dominant termination process, then the instantaneous number MMD, $N(M)$, is expected to be an exponential function when represented with a linear M axis, or a linear function when in the format ln(N) *vs.* M. The slope of this linear function provides information on rate constants; but due to BB, ln(N) *vs.* M may result nonlinear with an up-wards concavity. However, the correction for BB can be avoided by estimating the sought slope at the location of the MMD maximum.[36]

For various combinations of styrene-divinylbenzene columns differing in number, separation range, and particle diameters, a direct evidence of the effect of BB was observed when overlaying the different MMDs obtained by analyzing mixtures of polystyrene (PS) standards or multimodal PS prepared by pulsed laser polymerization

(PLP).[9,12] For quantitative results, multimodal MMDs prepared by pseudo-stationary techniques were analyzed with respect to the location of the inflection points of the individual peaks: the inflection points on the low molar mass side of each peak enabled to determine the propagation rate constant of a free radical polymerization.[37,38] Due to BB, the inflection points were systematically shifted toward lower elution volumes; and without correction, the derived rate constants can be underestimated by up to 20%.[6,7] On the basis of theoretical considerations and numerical simulations,[7] it was also demonstrated that the errors in the location of the inflection point are considerably larger than the deviations of the true average molar masses. To improve the accuracy of the rate constants determined from multimodal distributions obtained by PLP, some correction procedures have assumed the BBF as known, and have calculated the ''true'' location of the inflection points without resorting to numerical inversions.[6,7,12] These procedures were applied to polymers prepared by PLP in both homogeneous and heterogeneous media.[13,28]

Ideally, any raw chromatogram should be corrected for BB prior to performing a signal ratio or estimating the MMD. The main difficulties of this general (and in theory accurate) approach, are however: a) the BB function (BBF) is difficult to estimate; and b) the correction itself (*i.e.*, the calculation of the corrected chromatogram from the measured chromatogram and the BBF) requires an ill-conditioned numerical inversion. Several robust computer techniques have been developed for the numerical inversion of chromatograms;[10] and this important issue will not be further discussed. An important observation is that the same common BBF can be used for correcting any chromatogram, independently of the detector type. This is because BB mainly occurs in the fractionation column, and it is little affected by the injector, detection cells, and intercapillary volumes. Assuming a Gaussian and uniform BBF, the oldest methods of BB correction have aimed at obtaining unbiased estimates of the average molar masses: $\overline{M}_n$, $\overline{M}_w$, and $\overline{M}_w/\overline{M}_n$.[25,39] Clearly, the uniform assumption is only acceptable in a narrow elution volume range. Several sophisticated numerical inversion procedures have been proposed for calculating the unbiased MMD from the corrected concentration chromatogram and the molar mass calibration.[1,4,20,25] However, none of such procedures have so far been implemented in commercial software, probably due to the occasional numerical instability of the solutions. The numerical inversion becomes more complex for multimodal MMDs with narrow and overlapping peaks. The implementation of elution volume-dependent BBF parameters further adds to the problem complexity. However, such elution volume-dependence is expected to occur according to theory.[15,18,40,41]

The mathematical models that have been used for describing the BBF can be broadly classified into ''black box'' and ''grey box''. The main black box model is given by Tung's equation,[41] where the BB process is represented by a (non-causal and time-varying) linear filter. Theoretical or ''grey box'' models aim at describing the complex physico-chemical processes that take place in a SEC column. For example, the stochastic model byDondi *et al.*[42] assumes a random process where macromolecules alter between a moving zone (outside the particles) and a stagnant zone (inside the particles). The model accounts for phenomena such as the ingress/egress processes of a molecule in and out of a pore, dispersion in the moving zone, fractionation in columns with two different types of pores, and a mixed fractionation mechanism taking place both inside and outside the porous particles. Predictions from this model were experimentally validated, and a procedure was proposed for estimating the SEC partition coefficient.[43] According to this model, the BBF is asymmetrical, with its parameters being a function of the partition coefficient (and therefore of elution volume). In spite of these developments, many of the present theoretical approaches are still based on the more

classical approaches by Giddings and Eyring,[44] and van Deemter.[45]

Two-dimensional chromatography has been used for estimating the extent of BB in SEC.[46] The method consists in collecting narrow fractions in the first dimension; and on assuming that in the second dimension the peak width is only due to BB.[46] Castro *et al*.[47] developed a method for estimating the shape of a uniform BBF on the basis of analyzing broad standards with exponential number-MMDs (in turn, obtained through free-radical polymerizations with chain transfer termination). Unfortunately, such distributions can only be synthesized under perfectly-controlled conditions, and at present information is missing on whether this method will lead to reasonable results for non-uniform BBFs. Furthermore, unlike the case of narrow standards, matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF-MS) cannot be used to confirm the shape of such broad MMDs.[48]

The present article reviews some of the developments carried out within the mentioned IUPAC project for determining the BBF from the analysis of narrowly-distributed polymers. Prior to reviewing such developments, consider some of the basic models that have been used for representing the BBF.

Exponentially-Modified Gaussian Model by Busnel *et al*.[49]

The BBF would be easy to estimate if polymer standards of truly-uniform molar mass were available. Unfortunately however, this is not the case with synthetic polymers. In spite of this, Busnel *et al*.[49] aimed at determining the gross shape of the BBF by analyzing ultra-narrow samples of true non-uniformity indexes below 1.01. These samples were obtained by Chang and co-workers,[50] by fractionation of narrow anionic standards through temperature-gradient interaction chromatography. As shown in Figure 1, any of the measured concentration chromatograms can be adequately fitted with an Exponentially-Modified Gaussian function (EMG). A non-uniform EMG is obtained from the following convolution product between a Gaussian and an exponentially decaying function:[51]

$$g(V) = \frac{1}{\sqrt{2\pi}\sigma_G(V)\tau(V)} \exp\left(-\frac{[V+\tau(V)]^2}{2\sigma_G^2}\right) * \exp\left(-\frac{V}{\tau(V)}\right) \quad (1)$$

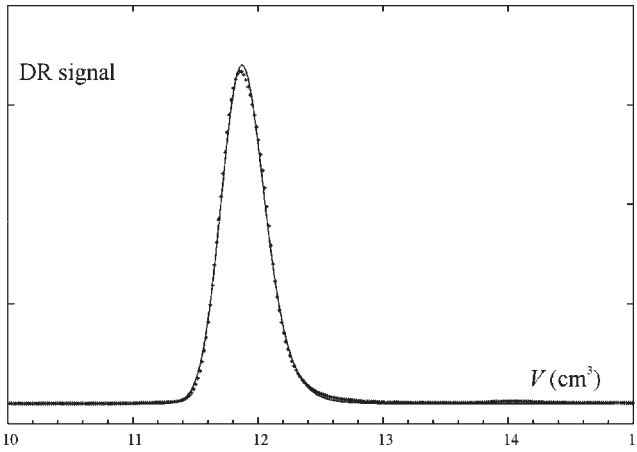

Figure 1.
Chromatogram of an ultra-narrow PS sample (continuous line) fit to an exponentially-modified Gaussian function (dotted line). The sample exhibits a molar mass of 384000 g/mol and a non-uniformity index of 1.004. The analysis was carried out using a 60 cm column from Polymer Labs. (gel mixed C). The parameters of the EMG fit resulted: $\sigma_G = 0.146$ cm^3; and $\tau = 0.124$ cm^3. After Busnel *et al*.[49]

where "*" indicates "convolution product"; *i.e.* $[f_1(V) * f_2(V) = \int_0^\infty f_1(V - \overline{V}) f_2(\overline{V}) \mathrm{d}\overline{V}]$; $\sigma_G(V)$ is the (varying) standard deviation of the Gaussian function; and $\tau(V)$ is the varying "time" constant of the exponential decay. In Equation (1), note the following: (i) the Gaussian distribution is centered at $-\tau$, and therefore the average mean volume of $g(V)$ is zero;[11,14] and (ii) the standard deviation of $g(V)$, $\sigma(V)$ is larger than $\sigma_G(V)$, since it also depends on the exponential function through:[51]

$$\sigma(V) = \sqrt{\sigma_G^2(V) + \tau^2(V)} \tag{2}$$

In addition, the skewness of $g(V)$ is given by $2(\tau/\sigma)^3$; where the ratio τ/σ_G is another measure of curve asymmetry.

For different column combinations, Figure 2 reproduces some estimated $\sigma_G(V)$ and $\tau(V)$ values.[49] Far from total exclusion, $\sigma_G(V)$ and $\tau(V)$ are relatively constant, whereas near total exclusion, $\sigma_G(V)$ decreases while $\tau(V)$ rapidly increases. The combined effects of $\sigma_G(V)$ and $\tau(V)$, determines an essentially constant (or slightly diminishing) $\sigma(V)$. Close to the exclusion limit, the variation of $\sigma(V)$ is highlighted by representing the total variance $\sigma^2(V)$. Since the values of τ, σ_G, and σ are all positive numbers, then the skewness is also positive (*i.e.*, with the tailing toward higher elution volumes). Far from total exclusion, the moderate skewness was attributed to tubing and junction zone effects; near total exclusion limit the large skewness was attributed to a reduced number of visited pores by the larger molecules.[49]

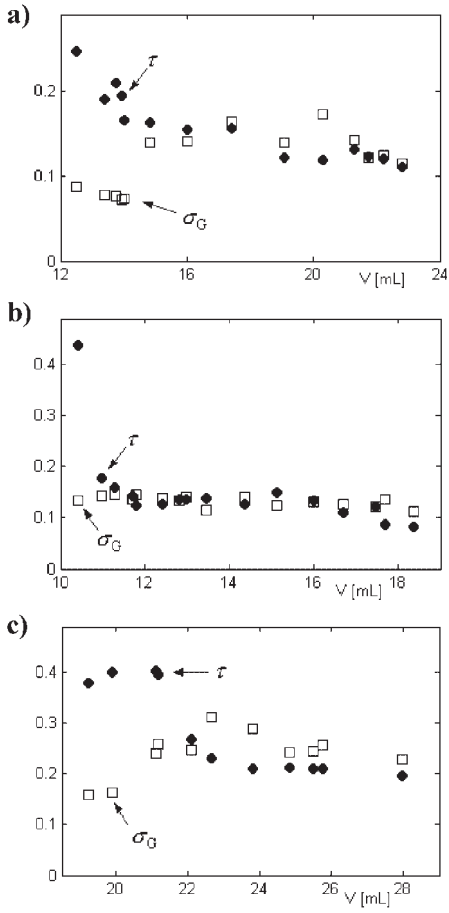

Figure 2.
EMG parameters of the BBF for the following column sets: a) Jordi gel 1000 A, 50 cm; b) PL gel mixed C, 60 cm; and c) PL gel mixed B, 2 × 60 cm. After Busnel *et al.*[49]

Black-Box Model by Tung[41]

According to Tung's equation, the concentration chromatogram obtained with a differential refractometer (DR) [represented by $s_{DR}(V)$] is a broadened version of a hypothetically true (or corrected) mass chromatogram $s_{DR}{}^c(V)$, through:

$$s_{DR}(V) = \int_0^\infty g(V, \overline{V}) s_{DR}{}^c(\overline{V}) \mathrm{d}\overline{V} \tag{3}$$

where $g(V, \overline{V})$ is the (in general, non-uniform) BBF; and $\overline{V}$ represents an average elution volume. At each $\overline{V}$, a different individual $g(V)$ function is defined. For any symmetrical $g(V)$ function, $\overline{V}$ is unambiguously assigned at its maximum (or mode). For skewed $g(V)$ functions, $\overline{V}$ can be assigned at the mode, the mean, or any other measure of central tendency. This ambiguity regarding the origin of asymmetrical BB functions, is still an unresolved matter for specifying $g(V, \overline{V})$. For uniform (or elution volume invariant) BBFs, Equation (3) reduces to a simple convolution integral. Equation (3) is applicable to any

detector type, and it constitutes the basis of correction procedures that involve numerical inversions.[10] The main advantage of Equation (3) is that no specific shape is imposed onto $g(V, \overline{V})$.

"Grey Box" Models by Giddings and Eyring[44] and by van Deemter *et al.*[45]

The stochastic model by Giddings and Eyring[44] was originally developed for liquid adsorption-desorption chromatography, but it was later extended to SEC.[52,53] According to this theory, any molecule of a uniform sample is adsorbed onto the stationary phase surface (SP) with probability k_a, and desorbed into the mobile phase (MP) with probability k_d. The probability of adsorption into MP follows a Poisson distribution, and the chromatogram of a uniform sample $g(V)$ is given by:[4,51]

$$g(V) = \frac{(k_a k_d V_0)^{1/4}}{2\sqrt{\pi}(V - V_0)^{3/4}} \times \exp\left[-\left(\sqrt{k_d(V - V_0)} - \sqrt{k_a V_0}\right)^2\right] \quad (4)$$

where k_a, k_d, are the adsorption and desorption coefficients; and V_0 is the exclusion volume. In spite of the exponential characteristics of Equation (4), in SEC measurements of synthetic polymers, the high values of k_a and k_d determine that $g(V)$ rapidly tends toward a Gaussian function of an increasing variance.

Van Deemter *et al.*[45] developed a deterministic model that relates the chromatogram variance (per unit length of fractionation column) with the linear mean velocity of the flow in the MP (u). For uniform samples, the model considers physical and kinetic phenomena such as axial and longitudinal diffusion, and mass transfer kinetics between SP and MP. From the original van Deemter expression, the following equation was derived for the total BBF variance:[40]

$$\sigma^2(V) = A + B + C = \lambda \frac{2d_p}{L} V^2 + \gamma \frac{2D_m(V)}{L} \frac{V^2}{u} + q \frac{V_0}{L} \frac{d_p^2}{D_s(V)} u(V - V_0) \quad (5.a)$$

with:

$$u = \frac{F}{\pi D_{col}^2/4} \quad (5.b)$$

where λ represents the packing quality; d_p is the diameter of the packing beads; V is the elution volume; L is the total column length; γ is a selectable weighting factor; D_m is the diffusion coefficient of the analyte in the MP; q ($\cong$1/30) is a geometrical factor;[54] V_0 is the exclusion volume; D_s is the diffusion coefficient of the analyte in the SP; F is the volumetric flow; and D_{col} is the column inner diameter. In Equation (5.a), the A-term represents the eddy diffusion (or convective mixing); and it is generally assumed u-independent in spite of some evidence in contrary.[55] The B-term accounts for axial diffusion along the column (occurring mainly in the interstitial space, but also within the pores). The C-term represents the mass transfer between MP and SP. Even though the model does not impose a specific shape for the BBFs, only its variable variance is estimated. For this reason, the derived BBF have always been considered as non-uniform Gaussians.

Recent Developments of the Estimation of the Band-Broadening Function

BBF Parameters when Assuming Poisson-Distributed MMDs

Schnöll-Bitai and co-workers estimated the BBF parameters by analyzing narrow PS standards (either commercial or home-made by PLP) with a concentration detector and assuming Poisson-distributed MMDs.[2,8,56–58] In the earlier works, the

BBF was assumed Gaussian, and only its standard deviation was estimated from the chromatograms; by assuming that the peak width obtained from the location of inflection points was twice the BBF standard deviation. The method was experimentally validated for column combinations covering a common separation range but differing in particle size ($d_p = 5$ or 10 μm).[8] The variance $\sigma^2(V)$ was adjusted through Equation (5) with $\lambda = \gamma = 1$; the diffusion coefficients ratio $D_m(V)/D_s(V)$ was assumed constant and independent of molar mass;[8,9] and the following relationship was adopted between $D_s(V)$ and the molar mass calibration $M(V)$: $D_s(V) = 1.94 \times 10^{-5}\ M(V)^{-0.564}$. At the total exclusion limit, $\sigma^2(V)$ exhibited a maximum. In agreement with the van Deemter Equation (5), the following general trends were observed: a) columns of smaller d_p's exhibit lower values of σ^2; b) σ^2 increases when increasing the number of interconnected columns; and c) the non linear dependence of $\sigma^2(V)$ was only observed for a large number of standards, while for a few standards, the strongly scattered results made it difficult to determine the real tendency.

The mentioned procedure was extended to the case when the BBF is represented by an EMG.[11] Theoretical correlations were obtained that interrelate the EMG parameters (σ_G, τ) with the peak width, the variance, and the slopes at the inflection points of the measured DR chromatograms. Based on such correlations, 3 alternative approaches were proposed for estimating σ_G and τ along V.[11] The extended method was tested by analyzing 12 narrow PS standards injected in 2 different column systems (of particle size 5 and 10 μm).[58] For the 5 μm system, σ_G slightly increased with V, while τ decreased. Also, the asymmetry τ/σ_G and the total variance $\sigma^2(V)$ decreased with V.[29,30,58] For the 10 μm columns, Figure 3.a) compares the concentration chromatograms $s_{DR}(V)$ with the derived local BBFs $g(V)$. For the total set of measurements, Figure 3.b) presents derived BBF parameters. It is seen that while σ_G remains essentially constant, τ decreases with V. Figure 3.c) compares the total variance $\sigma^2(V)$ measurements with the corresponding predictions according to Equation (2) with the model

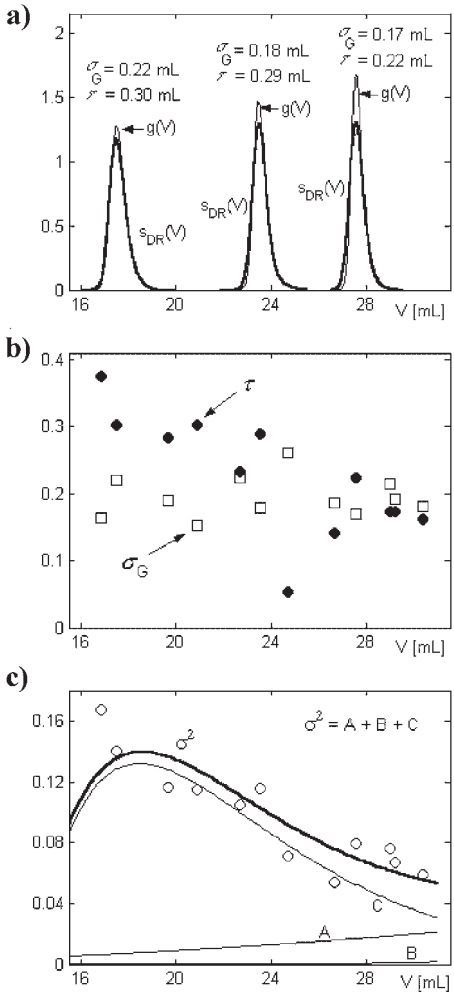

Figure 3.
Determination of the BBF parameters $\sigma_G(V)$, $\tau(V)$, and $\sigma^2(V)$, by application of the theoretical method[11] that assumes the injected PS standards as represented by Poisson-distributed MMDs. Twelve standards were analyzed in a system containing a 10 μm packing in 3 columns (0.8 cm × 30 cm each). (a) Concentration chromatograms $s_{DR}(V)$ of 3 (arbitrarily-selected) samples (in thicker curves), and corresponding EMG estimates (in thinner curves). (b) EMG parameters of the 12 samples (after Schnöll-Bitai *et al.*[58]). (c) total estimated variances (in symbols) and corresponding fit through Equation (5), with: $\lambda = 1$, $D_s/D_m = 0.057$; $D_s = 1.94 \times 10^{-5}\ M^{-0.564}$, and $\gamma = 1$ (in continuous trace).

parameters of ref.[8] As expected, the *B*-term of Equation (5) is negligible, the *C*-term dominates at low *V* but diminishes at larger *V*'s, and the *A*-term becomes significant only at the higher *V*'s.

In the context of PLP-SEC, a BB correction procedure was developed for calculating the true location of the points of inflection belonging to a peak in a MMD.[6,7,19] In addition, correction procedures have been developed for improving the accuracy of the rate constants determined by PLP from multimodal distributions.[6,7,12] These make use of an average value of σ^2 (determined explicitly beforehand), and aim at calculating the ''true'' location of the inflection points without requiring a numerical inversion of the total distribution. For polymers prepared by PLP in homogeneous and heterogeneous media, the consistency check was tested that consisted in comparing the determined points of inflection and maximum with those obtained under ideal conditions.[13,28]

Determination of the BBF when Analyzing Narrow Standards with Molar Mass-Sensitive Detectors

M. Yossen *et al.*[14] developed a technique for determining the (in general, skewed and non-uniform) BBF with the help of Tung's equation. It is based on analyzing narrow PS standards with molar mass-sensitive detectors, and it requires to know their molar mass calibration. For each standard, the BBF was assumed uniform; and such uniform BBF was estimated by comparing the DR chromatogram with its theoretical prediction assuming ideal LS or SV detection. The BBF can be assumed either arbitrary or represented by an EMG. The method employs an optimization routine that minimizes the difference between measurements and simulated chromatograms. The total non-uniform BBF is obtained by interpolation between the different uniform BBFs. The method is robust toward measurement noise and errors in the molar mass calibration, but it is highly sensitive to errors in the IDV.

Figure 4 shows a synthetic example where the analyzed PS standard is assumed to exhibit a log-normal WCLD, and the BBF is assumed to be represented by EMG[14] (see raw data in Figure 4.a). Assuming a perfect IDV correction, the mass and molar mass chromatograms [$s_{DR}(V)$ and $s_{LS}(V)$, respectively], elute in a common elution volume range [V_1–V_m]; while the elution volume range of the true or corrected chromatograms [$s_{DR}{}^c(V)$ and $s^c_{LS}(V)$] is narrower and given by [V_1^c–V_p^c]. In Figure 4.a), only the 2 limiting BBFs are

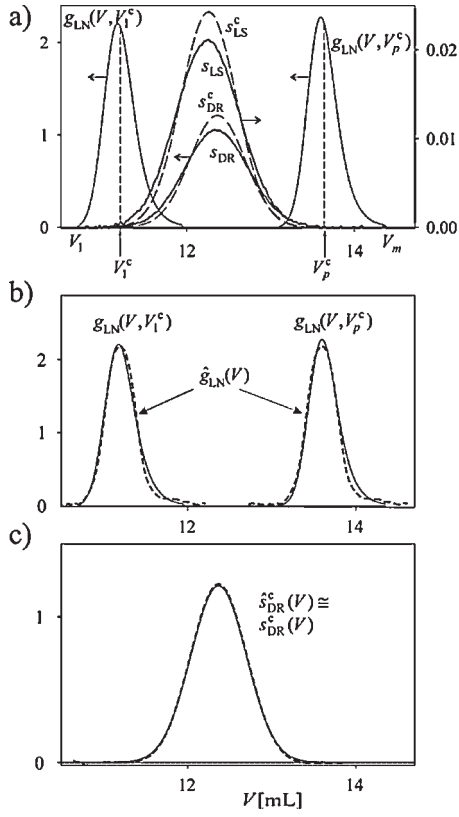

Figure 4.
Theoretical method for estimating the BBF from the analysis of a narrow PS standard with a LS detector, after Yossen *et al.*[14] (a) Corrected chromatograms [$s^c_{DR}(V)$, $s^c_{LS}(V)$]; ''Measured'' chromatograms [$s_{DR}(V)$, $s_{LS}(V)$]; and limiting BBFs [$g_{LN}(V,V_1^c)$, $g_{LN}(V,V_p^c)$]. (b) True and estimated BBFs (continuous and dashed curves, respectively). (c) True and estimated corrected DR chromatograms (continuous and dashed curves, respectively).

shown, with averages at the elution volume limits V_1^c and V_p^c. Figure 4.b) shows the final BBF estimate $\hat{g}_{LN}(V)$, as obtained from the noisy chromatograms and without imposing any specific shape onto the BBF. Figure 4.c) compares the *a priori* known corrected DR chromatogram with its estimate; obtained by numerical inversion of $s_{DR}(V)$ with $\hat{g}_{LN}(V)$.

The theoretical procedure was validated with 2 different detector configurations: a LS in series with a DR, and a SV in parallel with a DR. For the LS/DR system, the IDV is unique and it is relatively easy to estimate. For the SV/DR system, the IDV estimates were seen to depend on the analyzed molar masses.[20] In both configurations, preliminary results have shown the following tendencies: $\tau(V)$ slightly decreases; $\sigma_G(V)$ slightly increases; $\tau(V)/\sigma_G(V)$ decreases; and $\sigma^2(V)$ remains essentially constant (with a minimum somewhere in the middle of the fractionation range).

BBF Parameters Based on a Probabilistic Giddings-Type Model

For a uniform sample in dilute solution, Netopilík[3,4,59] developed a model for calculating the concentration profile along the column and the shape of the eluting chromatogram. The probabilistic approach assumes that the fractionation phenomenon is represented by two steps in series: 1) migration of a molecule between the MP and the SP until equilibrium is reached; and 2) displacement of the molecules along the MP by a small volume slice, ΔV. The concentration chromatogram is obtained by observing the time-varying concentration at the column output, and it is described by a negative binomial distribution of a moderate asymmetry and with a tailing towards the higher elution volumes. However, at the limit of an extremely long elution times (volumes), the chromatogram tends to a Gaussian distribution. This model only involves 2 molar-mass-dependent parameters: 1) the partition coefficient of the polymer molecule between MP and SP (p); and 2) the displacement interval (ΔV). For a narrow polymer, p is estimated from the ratio between the total exclusion volume and the peak volume of the mass chromatogram. Unfortunately, there is no straightforward method for estimating ΔV. In addition, the model assumes ideal intra-column flow, and neglects the effects of polymer concentration, flow tortuosity, and diffusion. The ''equilibrium model'' by Netopilík[3,4] was compared with the classical ''kinetic model'' of probabilistic-absorption by Giddings and Eyring[44] given by Equation (4); and both models result interrelated through: $p = k_d/(k_a + k_d)$. Furthermore, both models predict identical BBF statistics (mean, variance, skewness, etc.).

The construction of the BBF either from experimental data or from theoretical considerations is a complex problem. The stochastic approach[15] assumes unimolecular interactions between the analyte molecules and the solid phase, and it predicts that while near the exclusion limit, the BBF is very narrow (in contrast with the prediction of the van Deemter equation) and non-symmetrical, it quickly tends to symmetrical Gaussians for increasing V's. The theoretical model was validated with the analysis of low-molar mass substances by adsorption/desorption liquid chromatography (Figure 5). Unfortunately, the

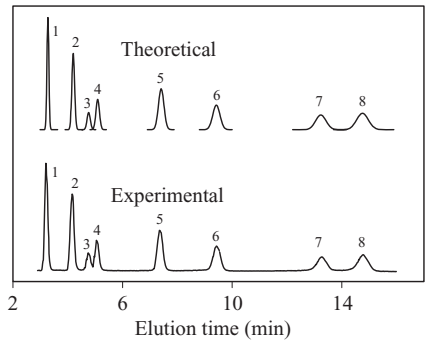

Figure 5.
Comparison of experimental elution curves for several substances with theoretical curves calculated from Equation (4). Peaks: flavone (1), 7-hydroxyflavone (2), baicalein (3), 5-hydroxyflavone (4), chrysin (5), luteolin (6), myricetin (7), quercetin (8). After Netopilík.[15]

situation turns more complex when analyzing polymers *via* SEC, because the unimolecular requirement is only valid at the limits of extremely low concentrations. The problem is particularly complicated for high-molar mass polymers near the exclusion limit, where the required ultra-dilute concentrations would produce almost no detector response.[15] On the other hand, the Gaussian shape for the BBF is an acceptable approximation for polymers eluting at sufficient distance from the exclusion limit. For more concentrated samples, the broadness of the BBF was seen to strongly depend on the concentration of the polymer samples. However, such situation is considerably reverted in the case of highly diluted samples (Figure 6). These results suggest that the separation mechanism should include deviations from the ideal unimolecular mechanism, such as viscosity effects and steric interactions. While the Gaussian approximation seems acceptable for polymers with molar masses sufficiently distant from the exclusion limit, the use of EMGs for describing the non-symmetry of the BBF (especially near the exclusion limit) seems reasonable for increasing the data-exploiting capacity of the method. The ultimate goal is the estimation of the elution-volume dependent EMG parameters.

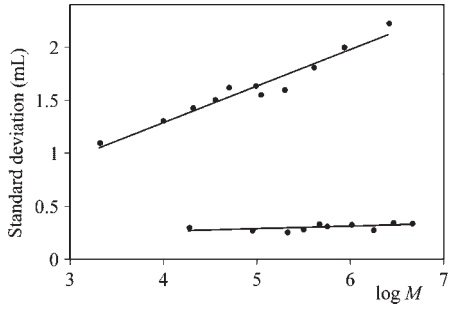

Figure 6.
Variation of the total standard deviation of the BBFs (σ) *vs.* log*M*, obtained by injecting a constant mass for each sample (upper curve), and by injecting a mass that decreases with the molar masses (lower curve). After Netopilík.[15]

BBF Parameters Based on an Extended van Deemter Model

Postchka[55] developed a model that modified the classical van Deemter Equation (5), and in particular its *C*-term. It is based on assigning an important role to the analyte convection within the pores. In effect, while in the more classical approach of dead-end pores, the mass transport is exclusively by diffusion, in the new extended model the pores behave as internally-interconnected capillaries where convection becomes important. The dispersion by convection within the pores was assumed a function of the dimensions of the column and packing beads, and it was scaled-up through the definition of the pores tortuosity (ξ_p). At each elution volume, the model predicts the peak width at the half-height of the measured chromatogram (or reduced plate height), and without specifying a BBF shape. A difficulty of this approach is the estimation of the model parameters (and in particular of ξ_p); and the fact that it requires a more comprehensive experimental validation.

Conclusions

For accurate estimations of the MMD *via* SEC of narrow or multimodal-distributed polymers, it is important to correct for the effect of BB. Unfortunately, no general and simple recommendations can be given at present on how such a correction should be implemented. One major difficulty is, as we have seen, the determination of the BBF. In the future, it is envisaged that more representative "white box" theoretical models will be developed that should provide greater insight into the complex SEC process. The required experimental effort could be in part carried out in industry by: a) SEC column manufacturers (providing better specifications of their packing material); b) polymer standards manufacturers (providing their true MMDs, perhaps determined by MALDI-TOF-MS); and c) manufacturers of liquid chromatographs and molar mass-sensitive detectors (provid-

ing appropriate software for BB correction and other biases).

In a broad range of molar masses, and in spite of some contradictory results, most experimental evidence suggests that the BBF is non-uniform and skewed (with a tailing towards the lower molar masses); and that skewness is particularly noticeable near to the total exclusion limits. Furthermore, the BBF can be conveniently adjusted by an EMG of an almost constant Gaussian standard deviation $\sigma_G(V)$, and a decreasing skewness produced by the variation of $\tau(V)$. Even if the BBF were known, there would still remain the problem of estimating the true MMD of the measured standards.

Several methods have been reviewed for estimating the BBF; and all of them exhibit both advantages and disadvantages. The methods by Schnöll-Bitai and coworkers[2,8,11,12] are experimentally simple, the developed BBF can be properly fit by non-uniform EMGs; and the average standard deviation can be interpreted in the light of the (rather inflexible) van Deemter equation. Their main limitations are the strong assumption of *a priori* imposing a Poisson-distributed MMD (while true distributions obtained by anionic mechanism are generally broader due to inevitable deactivation of ''living'' ends), and the errors involved in the determination of chromatogram characteristics such as points of inflection. So far, experimental evidence in support of the Poisson assumption has only involved the analysis of narrow polystyrene samples.[48,50]

The method by Yossen, Vega, and Meira[14] is the most general in the sense of not imposing any shape onto the BBF, but is limited to employing molar mass detectors, it requires numerical inversions, and it is highly sensitive to errors in the IDV.

The ''grey box'' approaches by Potschka[55] and Netopilik[3,4,15] have aimed at understanding and quantifying some of the physical phenomena associated with the SEC process. They are based on rather strong hypotheses such as uniform polymers at the limit of infinite dilution, and cannot easily justify the BBF skewness or the ''concentration'' effect at the high molar masses. Another major difficulty of these theoretical approaches is the adjustment of their various model parameters.

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