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# Estimation of the band broadening parameters in single detection size-exclusion chromatography: A comparative study of various column combinations

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ABSTRACT

Alternative approaches for the determination of band broadening in size-exclusion chromatography based on the use of exponentially modified Gaussian (EMG) functions were used to experimentally investigate the performance of two different column sets. In both cases, the columns were combined in order to cover the complete fractionation range (from $10^3$ to $5 \times 10^6$ g mol$^{-1}$), which is of interest in many applications. When analyzing experimental chromatograms the question of proper data treatment (especially the necessary smoothing routines) became obvious and is discussed accordingly. First results indicate that the exponential decay time of the EMG decreases and the standard deviation of its Gaussian component slightly increases (or remains almost constant) with increasing retention volumes. As a consequence, the total variance and the asymmetry of the EMG both decrease with the retention volume. A favorable agreement with independent experimental results (obtained by other researchers on the basis of analyzing ultra narrow standards) was found. Additionally, the skew was also investigated as a function of the retention volume and the trend was found to be in concordance with the predictions of theoretical models. The comparison with theoretical models is also discussed.



## 1. Introduction

In recent publications [1–4], the influence of band broadening (BB) on the accuracy of data deduced from chromatograms measured by size-exclusion chromatography (SEC) was discussed. For example, BB can strongly affect the width and the shape of the molar mass distribution (MMD) corresponding to narrow polymers and numerical data treatment must be applied to obtain the correct MMD [5,6]. It was also demonstrated that particularly the location of the inflection points of a narrow peak is seriously influenced by the phenomenon of BB and in some cases can be shifted by about 20%. Thus, for higher accuracy of some kinetic data deduced from inflection points of narrow polymeric samples [7–10], it is essential to know how strong BB is, and to be able to apply the appropriate correction. An IUPAC project [11] comprised the objectives of a concise survey and critical evaluation of existing methods for the determination of the extent of BB, the development and implementation of new techniques, information about the proper data treatment as well as the assignment of experimental results with theory.

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Many publications dealing with the determination of BB [12–21] in SEC implicitly use Gaussian functions as approximate substitutes of the BB function and sometimes the true MMD (expressed in a logarithmic molar mass axis). In such cases, and assuming a linear molar mass calibration, the resulting mass chromatogram peak is a Gaussian too; and consequently its standard deviation, $\sigma$, can be deduced from the chromatogram width measured either at: (a) the half height (i.e. width = $2.354\sigma$), (b) the intercepts of the tangent at the inflection points with the abscissa axis (i.e. width = $4\sigma$), or (c) the points of inflection (i.e. width = $2\sigma$). From a simple inspection of peak shapes, it becomes immediately obvious that this approximation is a rather crude one in most cases. Furthermore, experimental results [22] and theory [23–26] also indicate that peak shapes should be asymmetric, thus making it necessary to use more elaborate functions instead of the simple Gaussian approximation.

Asymmetry of the chromatogram peak can be attributed to the combined effect of: (i) an asymmetric MMD of the sample; (ii) an asymmetric BB function; (iii) a nonlinear detector response; and (iv) a nonlinear molar mass calibration. The last effect can normally be neglected when a narrow polymer is analyzed. Light-scattering sensors and viscometers are molar mass-sensitive detectors that provide signals proportional to the molar mass, and therefore introduce asymmetries in the chromatograms inducing tails towards the higher retention volumes, V. In contrast, the more classical differential refractometers (DR) or the UV sensors are mass-sensitive detectors that provide signals proportional to the weight chain length distribution (WCLD) when a homopolymer is analyzed [27]. Asymmetries originated by the effects (i) and (ii) cannot be avoided. In fact, the narrow DR chromatograms corresponding to polymers obtained by ideal anionic polymerizations are often assumed to follow Poisson distributions. Chang and coworkers [28] justified this assumption for polystyrene (PS) with a molar mass above 20,000 g mol$^{-1}$.

At a given retention volume $\bar{V}$, an asymmetric BB function, $g_{\bar{V}}(V)$, can be modeled through an EMG [22,24,29–31]; i.e. the convolution of a Gaussian of variance $\sigma_{BB}$, and an exponentially decaying function of time constant $\tau_{BB}$, as follows [31]:

$$g_{\bar{V}}(V) = \frac{1}{\sqrt{2\pi}\sigma_{BB}(\bar{V})\tau_{BB}(\bar{V})} \exp\left[-\frac{[V+\tau_{BB}(\bar{V})]^2}{2[\sigma_{BB}(\bar{V})]^2}\right] * \exp\left(-\frac{V}{\tau_{BB}(\bar{V})}\right) \quad (1)$$

where the symbol '*' stands for "convolution product" [24]. Therefore, the determination of the BB function for a complete column set consists in estimating the variation of the parameters $\sigma_{BB}$ and $\tau_{BB}$ with V.

In this work, two different column sets are investigated with the aim of determining the BB extent and its dependence on the retention volume (or the molar mass). The BB is modeled through EMG functions, and their parameters are estimated by means of a previously developed method [31], which is briefly summarized in what follows.

## 2. Correlations for the estimation of the BB parameters

The new method for estimating the BB extent [31] is based on two basic assumptions: (i) the number chain length distribution (NCLD) of a narrow polymer standard can be regarded as being a Poisson distribution [32] and (ii) the BB function is represented by an EMG. Although an asymmetric Poisson distribution tends to a symmetric Gauss distribution for high values of the average chain length, $\lambda$ that characterize the location of the peak maximum, the transformation of the NCLD to chromatographic dimensions converts this distribution to an asymmetric one. The reason is that not only the linear chain length axis is changed to a logarithmic molar mass one by making use of a molar mass calibration $\log M(V)$, but also the ordinate values are multiplied by their corresponding $M^2$ values (when a DR detector is used). The resulting signal is called the BB-corrected mass chromatogram, $s^c_{DR}(V)$, and represents the chromatogram that one would obtain in an ideal BB-free chromatographic system.

As an approximation, $s^c_{DR}(V)$ corresponding to a Poisson NCLD can be adjusted by an EMG of parameters $\{\sigma_p, \tau_p\}$. With the aid of simulations, the following empirical correlations were derived for a PS sample [31]:

$$\sigma_P(\lambda, b) \cong \frac{\sqrt{-(1.256/\lambda^2)+(0.155/\lambda)+(2.38\times10^{-5})}}{b} \quad (2a)$$

$$\tau_P(\lambda, b) \cong \frac{-(27.70/\lambda^2)+(1.933/\lambda)+(4.342\times10^{-3})}{b} \quad (2b)$$

where $b$ is the slope of the linear molar mass calibration expressed as $\log M(V) = a - b\ V$. Both $\sigma_P$ and $\tau_P$ decrease with increasing $\lambda$ (or with the respective decreasing retention volume). In Eqs. (2a) and (2a,b), the essential parameter $\lambda$ represents the characteristic chain length corresponding to the Poisson NCLD, and can directly be derived from the retention volume $V_{max}$ corresponding to the maximum of $s^c_{DR}(V)$, as follows [31]:

$$\lambda \cong \frac{10^{a-bV_{max}}}{M_0} - 2.5 \quad (3)$$

where $M_0$ (=104.15 g mol$^{-1}$ for PS) is the molar mass of the repetitive unit. In practice, Eq. (3) can only be applied on the measured or broadened chromatogram, $s_{DR}(V)$, and therefore the parameter $\lambda$ is overestimated by around 2%.

The analysis of simulated broadened chromatograms [31] leads to the essential relations for the experimental peak width $\Delta[s_{DR}(V)]$, the peak variance, $\mathrm{Var}[s_{DR}(V)]$, and a newly introduced quantity, namely the so-called asymmetry factor, $r$. The peak width is defined via the inflection points of the DR chromatogram as: $\Delta[s_{DR}(V)] = V_{high} - V_{low}$, where $V_{low}$ and $V_{high}$ are the locations of inflection points as deduced from the location of the corresponding maximum and minimum of the first derivative of $s_{DR}(V)$. The calculation of $\Delta[s_{DR}(V)]$ is related

to the broadening parameters by [31]:

$$(V_{high} - V_{low})^2 \cong \left(\frac{1}{b}\log\left[\frac{\lambda+\sqrt{\lambda}}{\lambda-\sqrt{\lambda}}\right]\right)^2 + 4\sigma_{BB}^2 + 2\tau_{BB}^2 \quad (4)$$

The first summand on the r.h.s. of Eq. (4) is the theoretical peak width of a Poisson NCLD transformed to chromatographic dimensions [33–35]; while the last two terms represent the contribution of BB to the peak width.

From the DR chromatogram, the variance of a single peak can be determined, and it is related to the broadening parameters by [31]:

$$\mathrm{Var}[s_{DR}(V)] \cong \frac{\lambda^{-1} + (11/4)\lambda^{-2} + (137/12)\lambda^{-3}}{b^2 \ln^2 10} + \sigma_{BB}^2 + \tau_{BB}^2 \quad (5)$$

The first summand on the r.h.s. of Eq. (5) is the theoretical variance of a Poisson NCLD transformed to chromatographic dimensions, and was deduced [31] from an expression published by Knox and McLennan [36], and by making use of the appropriate polydispersity index for Poisson distributions. The last two terms represent the variance added by the EMG that is used for modeling the BB.

The asymmetry factor, $r$, is defined as the ratio of the slopes at the points of inflection of $s_{DR}(V)$, and is in practice calculated as the ratio of the ordinate values of the maximum and minimum of the first derivative, $h(V)$; i.e. [31]:

$$r = \frac{h(V_{low})}{h(V_{high})} \cong \frac{1/(\sqrt{2\pi}\sqrt{\sigma_{BB}^2+\sigma_P^2})\,\exp -((V_{low} - V_{max} + \tau_{BB})^2/(2(\sigma_{BB}^2+\sigma_P^2))) - \tilde{s}_{DR}(V_{low})}{1/(\sqrt{2\pi}\sqrt{\sigma_{BB}^2+\sigma_P^2})\,\exp -((V_{high} - V_{max} + \tau_{BB})^2/(2(\sigma_{BB}^2+\sigma_P^2))) - \tilde{s}_{DR}(V_{high})} \quad (6)$$

where $\tilde{s}_{DR}(V)$ is the measured DR chromatogram normalized to unit area.

### 2.1. *Compilation of strategies [31]*

In general, to estimate the BB parameters the following information is required: (1) the molar mass calibration constants $\{a, b\}$; (2) the volume corresponding to the maximum of $s_{DR}(V)$, $V_{max}$; (3) the volumes corresponding to the inflection points of $s_{DR}(V)$, $\{V_{low}, V_{high}\}$; (4) the variance of $s_{DR}(V)$, $\mathrm{Var}[s_{DR}(V)]$; (5) the ratio of the slopes at the inflection points of $s_{DR}(V)$, $r$; and (6) the normalized DR chromatogram, $\tilde{s}_{DR}(V)$.

The first step consists in calculating $\lambda$ from Eq. (3). Then, three different methods can be implemented:

*Method 1*: estimate $\{\sigma_{BB}, \tau_{BB}\}$ from Eqs. (2a), (4) and (6).
*Method 2*: estimate $\{\sigma_{BB}, \tau_{BB}\}$ from Eqs. (2a), (5) and (6).
*Method 3*: estimate $\{\sigma_{BB}, \tau_{BB}\}$ from Eqs. (4) and (5).

*Methods 2* and *3* can only be applied when chromatograms are perfectly baseline separated. In such cases, the variance can be calculated through any standard numerical procedure. Usually, the inflection points and their slopes are calculated from the first derivative of $s_{DR}(V)$. Since the DR chromatogram is always contaminated by noise, a smoothened version of $s_{DR}(V)$ is required previously to evaluate its derivative to avoid unacceptable noise amplification. Several smoothing methods can be applied (e.g. the classical Savitzky–Golay algorithm or some Fourier transform-based filter [24]). Any smoothing algorithm will perturb the original chromatogram $s_{DR}(V)$. Therefore, some errors in the derived $V_{low}$, $V_{high}$, and $r$ parameters must be expected, which will consequently propagate through Eqs. (2)–(6) originating erroneously estimated BB parameters. Unfortunately, there is no known theoretical expression to accurately compensate for such errors when the above-mentioned filters are applied. As a practical alternative, a smoothing procedure is here proposed (see Appendix). The method basically consists in convoluting $s_{DR}(V)$ with a narrow Gaussian (characterized by its variance, $\sigma_s$), and therefore it only involves $\sigma_s$ as unique adjustable parameter. The method is simple to be implemented, does not affect the estimated $\tau_{BB}$, and provides a theoretical expression to correct the error propagated on $\sigma_{BB}$.

## 3. Experiments

The polystyrene standards (from Polymer Standard Service, Mainz, Germany and Scientific Products, Ontario, New York, USA) already measured by SEC in the context of BB [1,37] were analyzed according to the Methods 1–3 as outlined before. For an experimental test of the methods, two combinations of three SDV (30 cm × 0.8 cm) columns ($10^6$, $10^4$, $10^3$ A) from Polymer Standard Service (Mainz, Germany) covering the total separation range from $10^3$ to $5 \times 10^6$ g mol$^{-1}$ but with different particle sizes (5 and 10 μ) were investigated. The PS samples were dissolved in THF and injected in the chromatographic system, with THF as carrier solvent (at 30 °C, and 1.0 mL min$^{-1}$). In all experiments, the sampling rate of the DR signal was 45 samples per minute.

Unfortunately, experimental SEC data has two shortcomings in comparison to simulated ones: (1) the density of the data points is limited by the total number of points accepted by the commercial software (and also by the range of retention volumes per chromatogram) and (2) the data is contaminated by noise. According to the acquired data, it was necessary to employ a smoothing routine, not only for the determination of the peak maximum but also for the calculation of the first derivative by numerical differentiation. Consequently, the measured chromatograms were exported to ASCII files, and the smoothing (as described in the Appendix) was carried out with a homemade computer routine. Then, the numerical differentiation and the estimation methods were applied through already available homemade routines [31].

## 4. Results and discussion

Most theories on SEC fractionation assume that all the pores are identical and concentrate exclusively on the contribution of the columns to the extent of BB; i.e. all other disturbing contributions (like from the injector, connecting tubes or detector) must be treated separately or the experimental results must be corrected beforehand if a considerable extra column con-

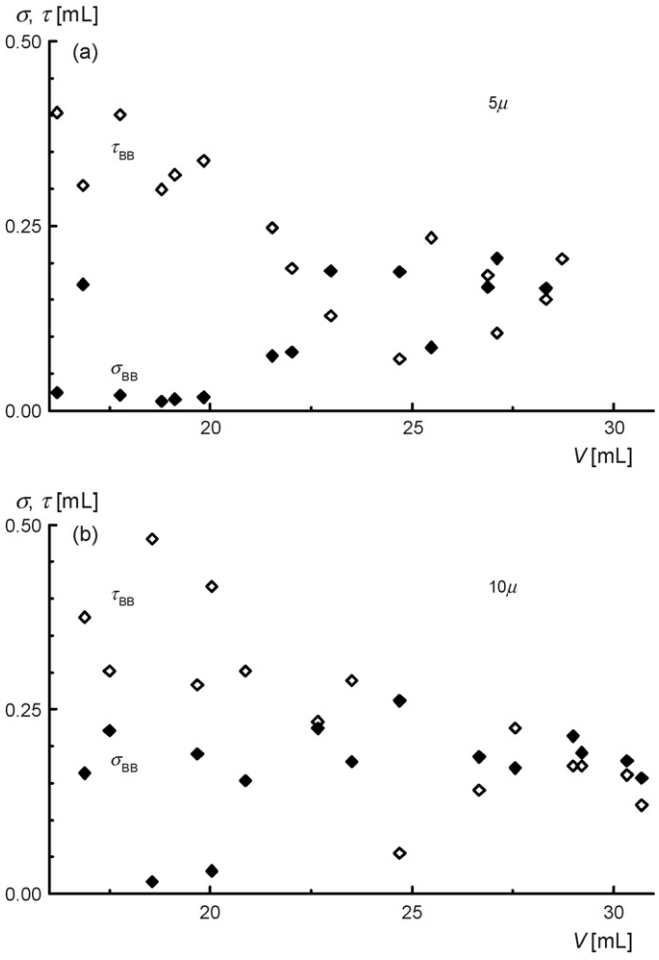


**Fig. 1 – The BB parameters $\sigma_{BB}$ and $\tau_{BB}$ (as a function of V) derived from DR chromatograms of PS standards, for two different column sets (as indicated in the diagrams). The parameters were determined based on the assumptions that: (a) the narrow standards follow a Poisson NCLD, and (b) the BB function can approximately be described by an EMG function.**

tribution might occur. In order to be able to compare the experimental results with theory, it was therefore assumed that the determined extent of BB is dominated by the column performance alone and all other effects are negligible. This approximation was also supported by Pasti et al. [23], who demonstrated for their system that the contribution of extra column effects amounted to approximately 1% of $\sigma_{BB}$ in the most unfavorable case of low retention volumes (or high molar masses) close to the exclusion limit; whereas this contribution reduced to approximately 0.1% for high retention volumes.

The first experimental results for $\sigma_{BB}$ and $\tau_{BB}$ are summarized in Fig. 1 and Tables 1 and 2. Methods (1) and (2) did not always yield data whereas with Method (3) $\sigma_{BB}$ was found to be zero in some cases. From the averaged values it can be seen that with increasing retention volume, $\sigma_{BB}$ increases for the 5 μ columns, but remains almost constant for 10 μ columns. In contrast, $\tau_{BB}$ decreases for both types of columns. These general trends were used to rate the data. For instance, when the results obtained with Method (1) are regarded, it becomes obvious that the $\sigma_{BB}$ value for the sample $\lambda = 549$ (see Table 1) does not conform to the general trend obtained with Methods (2) and (3). Moreover, the rare cases where $\tau_{BB} = 0$ were found suggest that this might be due to a lack of convergence in the numerical method, probably due to excessive experimental noise. Similar considerations can be applied to the samples with λ values 21610, 251, and 176 in Table 1, and 22524, 4509, and 548 in Table 2.

The comparison with the results from Busnel et al. [22] can be carried out in the most direct way as $\sigma_{BB}$ and $\tau_{BB}$ were deduced based also on an assumed EMG function for BB and are published as a function of the retention volume. The following agreements in the general trends are observed: (i) for the 10 μ columns (from PSS and Polymer Laboratories), a decreasing $\tau_{BB}$ and an almost constant $\sigma_{BB}$ with increasing V; (ii) the limiting values of $\sigma_{BB}$ and $\tau_{BB}$ are similar (although the columns have different lengths); (iii) for the 5 μ columns, changes in $\sigma_{BB}$ and $\tau_{BB}$ seem to be more pronounced. Furthermore, when the variances of the EMGs ($\sigma^2_{EMG} = \sigma^2_{BB} + \tau^2_{BB}$) as a function of V (c.f. Fig. 2) are compared, a decrease with increasing retention volume can be observed, which is predicted by a somewhat more elaborate van Deemter equation and was also found for other column combinations [1,12,17,37–39].

The monopore Giddings–Eyring–Carmichael (GEC) model predicts an increase in $\sigma_{GEC}$ with increasing values of the SEC partition coefficient, $K_{SEC}$, according to [23]:

$$\sigma^2_{GEC} = AK^{1+\alpha}_{SEC} \quad (7)$$

where A is a constant and $\alpha$ ($0 \le \alpha \le 1$) is the relative contribution originated by the pore-egress process to the overall size-exclusion effect. As $K_{SEC}$ varies continuously from 0 to 1 with V, then $\sigma_{GEC}$ must increase with V. This model predicts that the variance is zero at the exclusion limit which is not in agreement with experimental results. When the moving zone dispersion (MZD) [23] is also taken into account, the theory predicts a somewhat stronger increase of the variance with $K_{SEC}$:

$$\sigma^2_{MZD} = AK^{1+\alpha}_{SEC} + \frac{t^2}{B} = \sigma^2_{GEC} + \frac{t^2}{B} \quad (8)$$

where t is the retention time (or retention volume), and B is a dimensionless constant. Even if more than one type of pores are present in the column the partition coefficient should increase accordingly and thus should also $\sigma_{GEC}$ or $\sigma_{MZD}$. Furthermore, according to the equilibrium–displacement (ED) model [25], the predicted standard deviation $\sigma_{ED}$ should either remain constant or increase with V. Thus, all these models predict a continuous increase of the theoretical variance, $\sigma^2_{theory}$, with V, which is in disagreement with the results: (i) presented in this contribution as well as in previous publications [1,37], (ii) published by Busnel et al. [22], and (iii) predicted by the van Deemter equation upgraded by the concept of obstructed diffusion [12,17]. This obvious discrepancy leaves us with the question of how to compare theoretical and experimental results.

The stochastic model and the equilibrium–displacement model predict an increase in $\sigma^2_{theory}$ with increasing V. The experimental results presented by Busnel et al. [22] and those

**Table 1 – Compilation of the determined parameters $\sigma_{BB}$ and $\tau_{BB}$ obtained with the Methods (1)–(3) for the combination of three 5 μ columns; the averaged values and the skew calculated with Eq. (11) are also included**

| $\overline{V_P}$ | λ | -r | $\sigma_s$ | Method 1 | | Method 2 | | Method 3 | | Averaged | | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $\sigma_{BB}$ | $\tau_{BB}$ | $\sigma_{BB}$ | $\tau_{BB}$ | $\sigma_{BB}$ | $\tau_{BB}$ | $\sigma_{BB}$ | $\tau_{BB}$ | |
| 16.197 | 36430 | 1.914 | 0.080 | 0.048 | 0.365 | | | 0 | 0.024 | 0.024 | 0.404 | 1.989 |
| 16.831 | 21610 | 1.550 | 0.070 | 0.067 | 0.407 | 0.202 | 0.272 | 0.244 | 0.235 | 0.171 | 0.305 | 1.921(1) |
| 17.763 | 10880 | 1.673 | 0.050 | 0.041 | 0.272 | | | 0 | 0.530 | 0.021 | 0.401 | 1.992 |
| 18.788 | 5365 | 1.316 | 0.040 | 0.024 | 0.259 | | | 0 | 0.340 | 0.012 | 0.299 | 1.995 |
| 19.119 | 4443 | 1.264 | 0.050 | 0.030 | 0.288 | | | 0 | 0.350 | 0.015 | 0.319 | 1.993 |
| 19.831 | 2815 | 1.169 | 0.080 | 0.035 | 0.301 | | | 0 | 0.375 | 0.018 | 0.338 | 1.991 |
| 21.533 | 1222 | 1.265 | 0.045 | | | 0.148 | 0.225 | 0 | 0.269 | 0.074 | 0.247 | 1.759 |
| 22.019 | 874 | 1.219 | 0.050 | | | 0.158 | 0.262 | 0 | 0.306 | 0.079 | 0.192 | 1.585 |
| 22.985 | 549 | 1.237 | 0.045 | 0.264 | 0 | 0.169 | 0.179 | 0.134 | 0.206 | 0.189 | 0.128 | 0.742(2),(3) |
| 24.697 | 251 | 1.138 | 0.050 | | | 0.225 | 0 | 0.151 | 0.138 | 0.188 | 0.070 | 0.617(3) |
| 25.485 | 176 | 1.224 | 0.050 | | | 0.171 | 0.203 | 0 | 0.265 | 0.085 | 0.234 | 0.897(2) |
| 26.885 | 92.8 | 1.187 | 0.045 | 0.171 | 0.170 | 0.176 | 0.180 | 0.155 | 0.198 | 0.167 | 0.183 | 0.804 |
| 27.116 | 83.1 | 1.152 | 0.080 | 0.210 | 0.063 | 0.228 | 0.087 | 0.180 | 0.164 | 0.206 | 0.104 | 0.547(3) |
| 28.325 | 45.5 | 1.151 | 0.050 | 0.166 | 0.213 | 0.109 | 0.200 | 0.221 | 0.056 | 0.165 | 0.151 | 0.611 |
| 28.729 | 36.2 | 1.171 | 0.060 | 0.166 | 0.218 | 0.150 | 0.214 | 0.184 | 0.185 | 0.167 | 0.206 | 0.939 |

In some cases the skew was calculated based on the parameters obtained with the method as indicated by the superscript.

presented here exhibit the same trend in the Gaussian component $\sigma^2_{BB}$. However, such a comparison is invalid because both models claim to take into account asymmetric contributions, and therefore $\sigma^2_{theory}$ is the total variance of the BB function. Thus, we have a strong disagreement between the trends of the total variance predicted by the models and that deduced from experiments.

Busnel et al. [22] tried to justify their results by theoretical considerations based on the assumption that a polymer molecule visits $N$ pores, where $N$ is a random variable that follows a Poisson distribution of mean $n$. They calculated the relative standard deviation and the Fisher asymmetry coefficient and found that both are inversely proportional to the square root of $n$, i.e. $n^{-1/2}$. Thus, the simulations revealed that the distributions become narrower and more symmetric the higher $n$. This means that the peak width becomes smaller with decreasing molar mass (or increasing retention volume). With their simulation they were also able to explain why peak asymmetry becomes extremely important near the total exclusion volume when $n$ becomes small. As they made use of the relative standard deviation it is necessary to divide the standard deviations by the average number of pore visits. When we assume that the number of theoretical plates [23] is also a measure for the average number of visits we can make use of the following equation:

$$n = CK_{SEC}^{1-\alpha} \tag{9}$$

where $C$ is a constant. By combining Eqs. (7) and (9), it results:

$$\frac{\sigma^2_{GEC}}{n^2} = \frac{A}{C^2} K_{SEC}^{3\alpha-1} \tag{10}$$

**Table 2 – Compilation of the determined parameters $\sigma_{BB}$ and $\tau_{BB}$ as obtained with the different methods for the combination of three 10 μ columns; the averaged values and the skew calculated with Eq. (11) are also included**

| $\overline{V_P}$ | λ | -r | $\sigma_s$ | Method 1 | | Method 2 | | Method 3 | | Averaged | | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $\sigma_{BB}$ | $\tau_{BB}$ | $\sigma_{BB}$ | $\tau_{BB}$ | $\sigma_{BB}$ | $\tau_{BB}$ | $\sigma_{BB}$ | $\tau_{BB}$ | |
| 16.875 | 36355 | 1.617 | 0.080 | 0.073 | 0.437 | 0.219 | 0.338 | 0.198 | 0.350 | 0.163 | 0.375 | 1.920(1) |
| 17.494 | 22524 | 1.469 | 0.060 | 0.224 | 0.295 | 0.223 | 0.302 | 0.215 | 0.308 | 0.221 | 0.302 | (1.05) |
| 18.550 | 10617 | 1.672 | 0.050 | 0.033 | 0.336 | | | 0 | 0.625 | 0.016 | 0.481 | 1.972 |
| 19.675 | 5298 | 1.426 | 0.060 | 0.208 | 0.248 | 0.205 | 0.286 | 0.157 | 0.315 | 0.190 | 0.283 | 1.436 |
| 20.035 | 4509 | 1.365 | 0.050 | 0.062 | 0.391 | | | 0 | 0.442 | 0.031 | 0.416 | 1.926(1) |
| 20.867 | 2812 | 1.214 | 0.050 | 0.040 | 0.379 | 0.242 | 0.237 | 0.178 | 0.289 | 0.153 | 0.302 | 1.968(1) |
| 22.663 | 1234 | 1.244 | 0.040 | 0.243 | 0.184 | 0.233 | 0.243 | 0.197 | 0.273 | 0.224 | 0.233 | 1.070(3) |
| 23.506 | 873 | 1.175 | 0.050 | | | 0.239 | 0.252 | 0.119 | 0.326 | 0.179 | 0.289 | 1.231 |
| 24.697 | 548 | 1.114 | 0.050 | 0.296 | 0 | 0.283 | 0 | 0.206 | 0.166 | 0.262 | 0.053 | 0.493(3) |
| 26.670 | 253 | 1.128 | 0.050 | 0.188 | 0.129 | 0.189 | 0.141 | 0.179 | 0.152 | 0.186 | 0.141 | 0.428 |
| 27.563 | 175 | 1.218 | 0.060 | | | 0.204 | 0.199 | 0.136 | 0.250 | 0.170 | 0.224 | 1.011 |
| 28.994 | 92.2 | 1.167 | 0.040 | 0.211 | 0.186 | 0.207 | 0.178 | 0.223 | 0.157 | 0.214 | 0.173 | 0.501 |
| 29.216 | 82.7 | 1.149 | 0.050 | 0.192 | 0.170 | 0.193 | 0.173 | 0.189 | 0.177 | 0.191 | 0.173 | 0.606 |
| 30.338 | 45.2 | 1.129 | 0.050 | 0.179 | 0.195 | 0.147 | 0.186 | 0.214 | 0.102 | 0.180 | 0.161 | 0.592 |

In some cases the skew was calculated based on the parameters obtained with the method as indicated by the superscript.

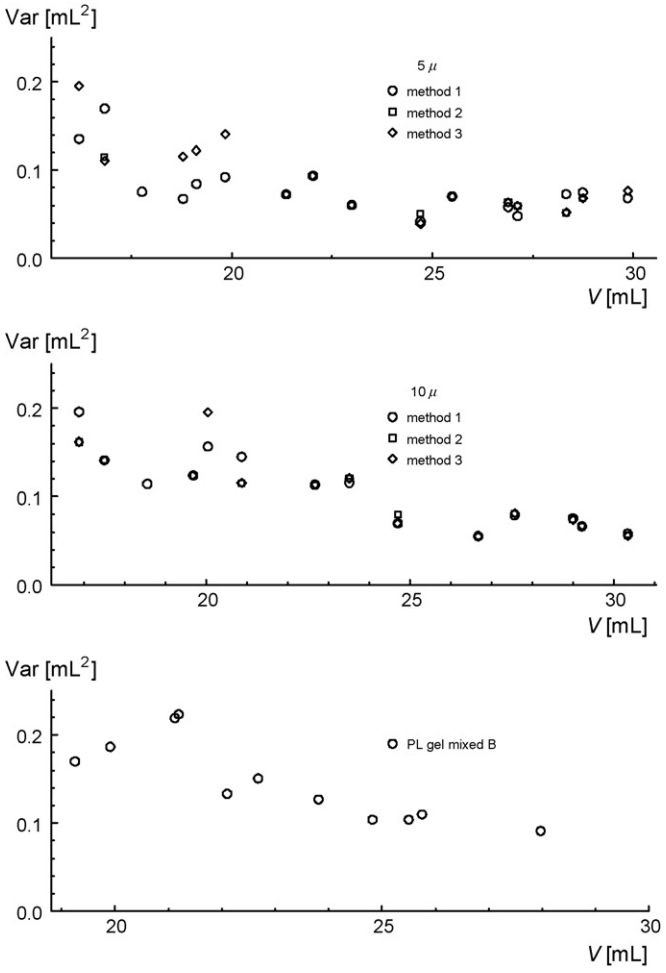


**Fig. 2 – The total variance $\sigma_{BB}^2 + \tau_{BB}^2$ of the EMG describing BB of the two column combinations as a function of the retention volume, *V*. For comparison, the results from Busnel et al. [22] are included.**

This means that $\sigma_{GEC}^2/n^2$ should decrease with *V* whenever $\alpha$ is smaller than 1/3, whereas it will certainly increase whenever $\alpha$ is bigger than 1/3. The decrease will be more pronounced close to the exclusion limit where $\alpha$ approaches zero [23]; and in this case the size-exclusion effect is completely dominated by the pore-ingress. For $\alpha < 1/3$, there is a qualitative agreement of the trends of $\sigma_{GEC}^2/n^2$ and the experimentally observed total variance of the EMGs. Furthermore, by comparison of Eqs. (7) and (9), it becomes obvious that the variance depends on *n* and thus implicitly also on the number of theoretical plates and consequently also on the length of the columns:

$$\sigma_{GEC}^2 = \frac{A}{C} n K_{SEC}^{2\alpha} \tag{7a}$$

The chromatogram skew, S, (usually calculated from the chromatogram moments) is also used in the context of the theoretical models to describe the column performance and the extent of BB. The skew of an EMG can be expressed as [24]:

$$S = \frac{2\tau_{BB}^3}{(\sigma_{BB}^2 + \tau_{BB}^2)^{3/2}} \quad (0 \le S \le 2) \tag{11}$$

According to Eq. (11), an EMG exhibits a lower limit of S = 0 for symmetrical distributions ($\tau_{BB} \to 0$), and an upper limit of S = 2 for $\tau_{BB} \gg \sigma_{BB}$. The data shown in Fig. 3 were calculated with the averaged values as indicated in the Tables 1 and 2.

Fig. 4 summarizes the skew calculated according to Eq. (11) of the data by Busnel et al. [22]. The qualitative agreement between the trends for the different data sets is obvious. This constitutes an interesting result, since the data by Busnel et al. [22] were obtained from SEC experiments of PS samples fractionated by temperature gradient interaction chromatography (and therefore assumed to be uniform); whereas the results presented in this work were obtained by direct analysis of commercial PS standards. Again there is agreement of the results obtained via an EMG function for BB, but how does this compare to the theories?

The monopore GEC model [23] predicts a continuously decreasing value of the skew with increasing $K_{SEC}$, according to:

$$S = B(K_{SEC})^{(\alpha-1)/2} \tag{12}$$

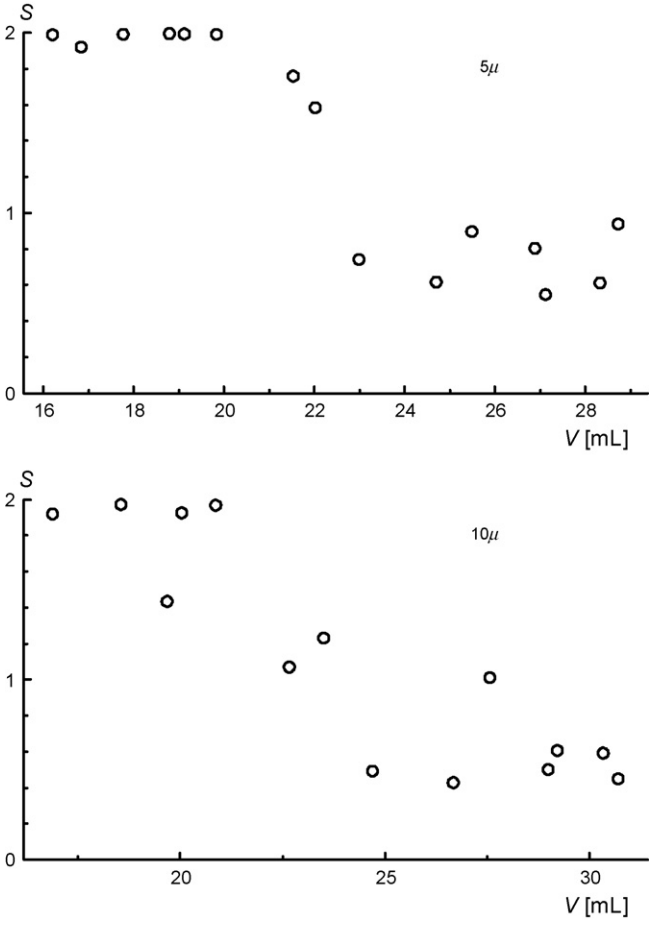


**Fig. 3 – The skew S as a function of V calculated with Eq. (11) with the estimated (and averaged) $\sigma_{BB}$ and $\tau_{BB}$ values.**

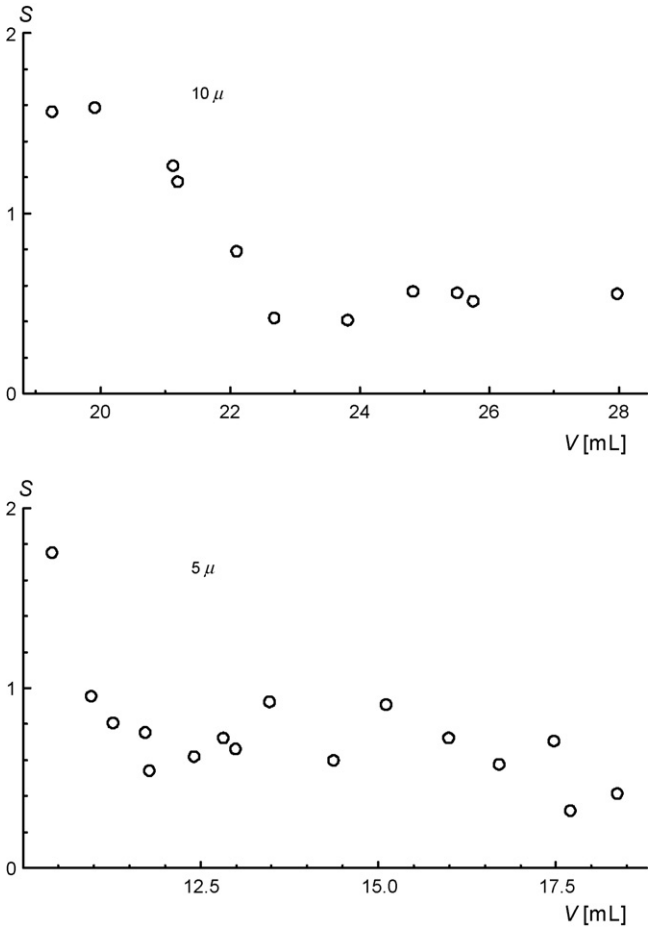


**Fig. 4 – The skew S as a function of V calculated with Eq. (11) from the $\sigma_{BB}$ and $\tau_{BB}$ data published by Busnel et al. [22]. Upper diagram: PL gel mixed B (2 × 60 cm, 10 μ), lower diagram: PL gel mixed C (60 cm, 5 μ).**

According to Eq. (12), $S > 2$ for small values of $K_{SEC}$ (i.e. for low V); whereas theoretically $S \to \infty$ when $K_{SEC}$ approaches zero (total exclusion zone). The skew determined by Pasti et al. [23] was almost 2 for $K_{SEC}$ close to zero. Whether this is just a mere coincidence or an independently found evidence for the appropriateness of the EMG cannot be decided at the moment, as any information is missing of how to take into account a possible contribution of the polymer polydispersity when the skew is calculated via the moments of the chromatogram.

In our case, the skew [calculated with Eq. (11)] decreases with increasing V, which is in agreement with the above given models [Eq. (12), with $\alpha < 1$]. For both column combinations, a plateau close to the limiting value of $S = 2$ is observed at low V, and the values start to diminish around a molar mass of $1.5 \times 10^5\ g\,mol^{-1}$. An explanation for the plateau is not provided by the theory, but might stem from the fact that an EMG is used to describe BB, or that column combinations were used with different pore sizes. The equilibrium–displacement model [25] also predicts a reduction of S with increasing V.

Neither the publication by Pasti et al. [23] nor the one by Netopilik [25] contain any information about $\tau_{BB}$. Therefore, a rearrangement of Eq. (11) was carried out in order to get information about $\tau_{BB}$:

$$\left(\frac{\sigma_{BB}}{\tau_{BB}}\right)^2 = \left(\frac{2}{S}\right)^{2/3} - 1 \tag{13}$$

From Eq. (13), it is obvious that $\sigma_{BB}/\tau_{BB} \to 0$, for $S = 2$; which leads to the expectation that $\sigma_{BB} = 0$ at the exclusion limit. So far, all experimental results give evidence of a nonvanishing variance at the exclusion limit, and therefore in the case of an EMG, the contribution of the exponential decay term is dominant; with decreasing skew the ratio becomes larger. Thus, the trends observed for $\sigma_{BB}$ and $\tau_{BB}$ are in qualitative agreement with the commonly accepted decrease of the asymmetry with decreasing S (increasing V).

In order to find out what conditions must be fulfilled for the variance of an EMG to decrease, we combine $\sigma^2_{EMG}$ with S, as follows:

$$S^2_{EMG}\sigma^2_{EMG} = \left(\frac{2\tau^3_{BB}}{\sigma^2_{EMG}}\right)^2 \tag{14}$$

If we assume that besides the variance also the skew is independent of the function used to describe BB, then the product on the l.h.s. of Eq. (14) can be replaced by the same product obtained with any model, i.e. $S^2_{EMG}\sigma^2_{EMG} = S^2_{model}\sigma^2_{model}$. Therefore, with the aid of the skew and variance determined for the different models together with the knowledge that $\tau_{BB}$ decreases monotonically, the dependence of $\sigma^2_{EMG}$ on V can be calculated via a rearranged Eq. (14):

$$\sigma^2_{EMG} = \frac{2\tau^3_{BB}}{S_{model}\sigma_{model}} \tag{14a}$$

The model by Busnel et al. [22] predicts that $S^2\,\sigma^2$ has a constant value independent of the number of visited pores, which can be associated with the size of the molecules; and therefore Eq. (14a) predicts that $\sigma^2_{EMG}$ must decrease accordingly. In the equilibrium–displacement model by Netopilik [25], $S^2\sigma^2 = \Delta t^2$, where $\Delta t$ is a time increment which is constant only in the case of liquid chromatography, and therefore the same argument is valid. In the case of SEC, however, an experimental investigation [40] revealed that $\Delta t$ decreases with increasing V. This leads to a ratio where both the numerator and the denominator of Eq. (14a) decrease, and in principle three possibilities for $\sigma^2_{EMG}$ can occur, namely decreasing, constant, and increasing. Therefore, the results of Netopilik [40] might be only occasionally conform to the results presented by Pasti et al. [23] as in their case one can derive that $S^2\sigma^2$ is increasing proportional to $K^{2\alpha}$ (even for $\alpha = 0$) which will lead again to a decreasing variance of the EMG.

Thus, according to the reasoning above it is possible to demonstrate that the decrease in the variance of an EMG is qualitatively in agreement with the theoretical models which do not make any assumption with respect to the shape of the BB function. In this context immediately two questions emerge: (i) what would happen when functions other than an EMG [41] are used for the description of the BB processes? (This was certainly not the scope of this contribution but is currently investigated and will be presented in due course) and (ii) how

must a proper comparison of theory and experimental results be carried out? For instance, it is clear (and a common practice) that the variance must be corrected for the contribution of the polymer polydispersity, but so far no information was found with respect to the skew [41]. Furthermore, as in some cases not only the skew is determined but also the excess, *E*, [24,26] an analogous information is missing. On the other hand, the excess of an EMG can be calculated through [24]:

$$E = \frac{\tau_{BB}^4}{\left(\sigma_{BB}^2 + \tau_{BB}^2\right)^2} \tag{15}$$

In the case of an EMG, the excess does not offer additional information as $E = (S/2)^{4/3}$ and consequently *E* decreases with *V* (not shown here).

## 5. Conclusion

This work presents the first experimental tests of the previously developed method [31] to estimate the BB extent in SEC. The EMG parameters $\sigma_{BB}$ and $\tau_{BB}$ were determined on the basis of several commercial narrow PS standards. In general, both $\sigma_{BB}$ and $\tau_{BB}$ exhibited changes with the retention volume, thus indicating a nonuniformity of the BB function. The plausibility of the data was controlled by comparing the obtained results with (i) already published data obtained with almost uniform PS samples and (ii) the predictions derived from theoretical fractionation models. The general trends of the BB parameters and (particularly) of the skew are in qualitative agreement with the expectations of the different models. Our results also indicate that BB is not constant over the total separation range. It also turned out that for the correct comparison of different models and experimental results it is essential to know what quantities should be taken into consideration and a concise investigation of this topic will follow in a forthcoming contribution [41].

The results of this investigation are of interest for researchers who try to adjust theoretical models to experimental results. In this context the question was discussed of how to compare theory and experiment. Additionally, it turned out that it would be necessary to develop theories that take into account more than one pore size in the fractionation columns, as this is of interest for many practitioners who usually employ column combinations with a broad separation range. The results are also of interest for those who are devoted to the delicate task of estimating the true polymer MMD from experimental chromatograms [42,43], since the knowledge of the BB function in the whole fractionation range is an unavoidable prerequisite. Besides the determination of the complete true distribution, a point wise correction can be carried out by making use of the BB parameters [44,3].

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## Appendix A

### A.1. A method to smooth chromatograms

This Appendix presents a method to smooth chromatograms obtained by SEC. The procedure is intended to deal with the narrow DR chromatograms, $s_{DR}(V)$, that are used to determine the extent of BB through the method proposed in [31].

In a narrow volume range, a uniform (but asymmetric) BB function, $g(V)$, can be modeled by a first-order EMG. In [31], $g(V)$ is represented by a zero-mean EMG, obtained by convoluting a Gaussian of mean volume $(-\tau_{BB})$ and standard deviation $\sigma_{BB}$, $N_{-\tau_{BB},\sigma_{BB}}(V)$, with an exponential function of decaying volume $\tau_{BB}$; i.e. [see also Eq. (1)]:

$$g(V) = N_{-\tau_{BB},\sigma_{BB}}(V) * \frac{\exp(-V/\tau_{BB})}{\tau_{BB}} \tag{A.1}$$

Then, the DR chromatogram $s_{DR}(V)$ corresponding to a polymer exhibiting a narrow MMD is represented by [31]:

$$s_{DR}(V) = g(V) * s_{DR}^{c}(V) = \left[N_{-\tau_{BB},\sigma_{BB}}(V) * \frac{\exp(-V/\tau_{BB})}{\tau_{BB}}\right] * s_{DR}^{c}(V) \tag{A.2}$$

where $s_{DR}^{c}(V)$ is the DR chromatogram in absence of BB. Since $g(V)$ is a zero-mean function, then the mean volume of $s_{DR}(V)$ coincides with that of $s_{DR}^{c}(V)$.

To successfully estimate $\{\sigma_{BB}, \tau_{BB}\}$ through Eqs. (2)–(5), the first derivative of $s_{DR}(V)$, $h(V)$, must be smoothened. The main goal of this Appendix is to propose an efficient procedure that allows an acceptable estimation of $h(V)$, and a further correction of the artificial biases affecting the estimated BB parameters, introduced by the smoothening operation. In practice, a smoothened DR chromatogram, $s_{DR,s}(V)$, can be obtained by convoluting $s_{DR}(V)$ with any smoothing function, $g_s(V)$. Assume that $g_s(V)$ is selected as a zero-mean Gaussian of standard deviation $\sigma_s$; i.e. $g_s(V) = N_{0,\sigma_s}(V)$. Bearing in mind that: (i) the convolution is a commutative operation and (ii) the convolution of 2 Gaussians $N_{\mu_1,\sigma_1}$ and $N_{\mu_2,\sigma_2}$ is a new Gaussian $N_{\mu_1+\mu_2,\sqrt{\sigma_1{}^2+\sigma_2{}^2}}$, then:

$$\begin{aligned} s_{DR,s}(V) &= g_s(V) * s_{DR}(V) = g_s(V) * g(V) * s_{DR}^{c}(V) \\ &= \left[N_{-\tau_{BB},\sqrt{\sigma_{BB}^2+\sigma_s^2}}(V) * \frac{\exp(-V/\tau_{BB})}{\tau_{BB}}\right] * s_{DR}^{c}(V) \end{aligned} \tag{A.3}$$

Eq. (A.3) is a new EMG convoluted with $s_{DR}^{c}(V)$; and therefore any of the described *Methods* 1–3 [Eqs. (2)–(5)] can directly be applied on the smoothened chromatogram to simultaneously estimate $\sigma_{BB}^2 + \sigma_s^2$ and $\tau_{BB}$. Then, the (known) variance $\sigma_s^2$ introduced during the smoothening should be discounted to obtain the true $\sigma_{BB}$ value.

In principle, $\sigma_s$ should be selected as small as possible, to minimize the error propagation. However, a low $\sigma_s$ will only produce a poor smoothening of $s_{DR}(V)$, and therefore ambiguous estimations of $V_{low}$, $V_{high}$, and *r*. In practice, $\sigma_s$ can be selected as the smaller value that produce an acceptably soft shape of the first derivative of $s_{DR}(V)$.

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