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Estimation of band broadening in size-exclusion chromatography. I. A method based on analyzing narrow standards with a molar mass-sensitive detector

Mariana M. Yossen, Jorge R. Vega, Gregorio R. Meira *

INTEC (Universidad Nacional del Litoral and CONICET), Güemes 3450, 3000 Santa Fe, Argentina

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Abstract

A method is proposed for estimating the (asymmetrical and non-uniform) band broadening function (BBF) in size-exclusion chromatography (SEC). The following data are required: the molar mass calibration and the concentration- and molar mass chromatograms of a set of narrow standards. In the narrow range of each standard, the BBF is uniform but skewed. Each uniform BBF is estimated through a nonlinear optimization procedure that compares one (of the two) measured chromatograms with its theoretical prediction based on the other chromatogram. The method is validated with numerical examples that simulate the analyses of narrow standards exhibiting log-normal and Poisson weight chain length distributions. The BBF can be assumed of arbitrary shape, or represented by an exponentially-modified Gaussian (EMG). From the uniform BBF estimate, the true polydispersity of the standard can be determined. The global non-uniform BBF is obtained by interpolation between a set of uniform BBFs covering a wide range of elution volumes.

Keywords: SEC; Band broadening; Molar mass detectors; Exponentially-modified gaussian; Molar mass distribution

1. Introduction

Size-exclusion chromatography (SEC) is the main analytical technique for measuring the molar mass distribution (MMD) of a polymer [1]. In ideal SEC, molecules are fractionated according to their hydrodynamic volume. Unfortunately, perfect fractionation is impossible, due to secondary fractionation mechanisms and band broadening (BB). Secondary fractionations result from physicochemical interactions between the analyzed polymer, the solvent, and the column packing [2], and will not be further discussed. BB is mainly due to axial dispersion in the fractionation columns, while other minor sources include column-end effects, finite injection volumes, finite detection cell volumes, and laminar flow profiles in the capillaries [3,4]. Due to BB, an instantaneous MMD is present in the detector cell, even when analyzing chromatographically simple polymers [5,6].

Assume that the MMD is directly determined from the concentration (or mass) chromatogram and an independent molar mass calibration. In this case, the effect of BB is to underestimate the global number-average molar mass ($\bar{M}_n$), overestimate the global weight-average molar mass ($\bar{M}_w$), and therefore overestimate the global polydispersity, $\bar{M}_w/\bar{M}_n$. In contrast, with molar mass sensitive detectors, the global polydispersity can be either over- or underestimated. Thus, ideal light-scattering detectors produce unbiased $\bar{M}_w$ values, but overestimated $\bar{M}_n$'s, and therefore the polydispersities are underestimated [7]. Also, ideal specific viscometers underestimate $\bar{M}_w/\bar{M}_n$ when the molar masses are calculated with the exact Mark–Houwink (M–H) constants of the analyzed polymer, or overestimate $\bar{M}_w/\bar{M}_n$ when employing an exact universal calibration [8–10]. An important point of molar mass sensitive detectors, is to properly correct for the inter-detector volume (IDV) shift [9–12].

Several mathematical models have been developed that describe the complex physicochemical processes that take place in a SEC analysis. Their aim is to simulate the chromatograms from *a priori* knowledge of the MMD, the polymer-solvent-matrix interactions, the column characteristics, and the flow

* Corresponding author. Fax: +54 342 451 0348.
E-mail address: gmeira@ceride.gov.ar (G.R. Meira).

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conditions [13–16]. Unfortunately, these models involve a large number of unknown physicochemical parameters, and as far as the authors are aware, they have not yet been applied for BB corrections. Alternatively, in the phenomenological (or "black box") approach by Tung [17], the concentration chromatogram $w(V)$ is considered a broadened (or filtered) version of a hypothetically true (or corrected) chromatogram $w^c(V)$, as follows:

$$w(V) = \int_0^\infty g(V, \bar{V}) w^c(\bar{V})\, d\bar{V} \quad (1)$$

where $g(V, \bar{V})$ is the (in general, non-uniform) BB function (BBF); and $\bar{V}$ are the average retention volumes. At each $\bar{V}$, a different individual $g(V)$ function is defined. For symmetrical $g(V)$ functions, then $\bar{V}$ is unambiguously assigned at the maximum (or mode). For skewed $g(V)$ functions, then $\bar{V}$ can be assigned at the mode, the mean, or any other measure of central tendency. This ambiguity regarding asymmetrical BBFs is a still unresolved problem in SEC data treatment, but it will not be further discussed. For uniform (or elution volume invariant) BBFs, Eq. (1) reduces to a simple convolution integral.

When neglecting the extra broadenings produced outside the fractionation columns (in the injector, detector cells, and interdetector capillaries), then any generic k-chromatogram is distorted by the same (common) BBF, as follows [18,19]:

$$s_k(V) = \int_0^\infty g(V, \bar{V}) s_k^c(\bar{V})\, d\bar{V}, \quad (k = \mathrm{DR, LS, SV, OS}) \quad (2)$$

where $s_k(V)$ is any measured chromatogram; $s_k^c(V)$ is its corresponding BB-corrected chromatogram; and DR, LS, SV, and OS indicate a differential refractometer, a light-scattering detector, a specific viscosity detector, or a (still commercially unavailable) colligative-property osmometer.

When linear homopolymers are analyzed with perfect or ideal sensors, then the following expression interrelates the BB-corrected concentration chromatogram $s_{DR}^c(V)$ with any BB-corrected molar mass chromatogram $s_k^c(V)$ (k = LS, SV, OS) [18–20]:

$$s_k^c(V) = K_k [M^c(V)]^{a_k} s_{DR}^c(V),$$
$$(a_{DR} = 0;\ a_{LS} = 1;\ a_{SV} = \alpha;\ a_{OS} = -1) \quad (3)$$

where K_k is a detector gain; $M^c(V)$ is the molar mass calibration; and $a_{SV} = \alpha$ is the M–H exponent for the given polymer-solvent-temperature combination. In $M^c(V)$, the superscript 'c' is introduced to highlight the assumption that a molar mass calibration obtained from narrow standards is unaffected by BB.

The correction for BB is important when the chromatograms are narrow, or are broad but multimodal with sharp elbows and/or narrow peaks. In the most general approach, the correction for BB involves the three following difficulties: (1) estimation of the BBF; (2) calculation of the corrected chromatograms from the measured chromatograms and the BBF; and (3) estimation of the corrected MMD. The numerical algorithms associated with the last two items have been extensively treated in the literature [19–25], and will not be further discussed.

If strictly monodisperse (or uniform) standards were available, then the BBF would be simple to determine, since any $s_k(V)$ chromatogram would directly provide the shape of the individual BBFs; and the nonuniform BBF would be obtained by interpolation between a set of such chromatograms. Unfortunately, uniform standards are only available for low molar mass materials (e.g., a pure solvent), and for some water-soluble biopolymers. Almost-uniform synthetic standards have been obtained by fractionation of narrow PS standards in temperature-gradient interaction chromatography; and their chromatograms have been adequately represented by exponentially-modified Gaussian (EMG) functions [26]. Inside the linear calibration range, these functions were only moderately non-uniform but skewed, with the exponential decay or tailing toward the higher elution volumes. Near to the limit of total exclusion, the BB function became narrower and more skewed, and could not be properly approximated by an EMG [26]. In general, the BB effects are particularly serious near to the limit of total exclusion.

Arbitrary-shaped BBFs can be estimated through a technique that is based on recycling narrow standards [27]. However, this method requires of two ill-conditioned deconvolutions, to compensate for the large BB introduced by the pump and recycle tubing (not present in a normal fractionation run).

When the shape of a narrow MMD is known *a priori* (e.g., it is represented by a Poisson distribution on a linear molar mass axis), then a uniform and Gaussian BBF can be estimated by comparing the DR chromatogram with the theoretical chromatogram that would be obtained in the absence of BB [28]. More recently, the method was extended to BBFs represented by asymmetrical EMG functions [29]. For samples exhibiting a Poisson or a log-normal weight-chain length distribution (WCLD), theoretical correlations have been developed for determining the EMG parameters on the basis of the peak width, the variance, and the inflection points of the DR chromatogram [29]. These methods do not require of any ill-conditioned numerical inversion, but are restricted to imposing a shape to the analyzed MMD.

Several publications have employed molar mass chromatograms for estimating uniform and Gaussian BBFs. Thus, a method has been proposed [30,31] for simultaneously estimating the standard deviation of the Gaussian BBF and the molar mass calibration coefficients from the DR and LS chromatograms and a linear molar mass calibration. In addition, an iterative procedure has been proposed [18] for simultaneously estimating the true MMD and the standard deviation of a uniform Gaussian BBF from the DR and LS chromatograms of a narrow standard and a linear calibration. The procedure is as follows: (i) guess a standard deviation for the BBF; (ii) estimate $s_{DR}^c(V)$ and $s_{LS}^c(V)$ by inversion of Eq. (2); (iii) estimate $M^c(V)$ through Eq. (3); and (iv) iterate until the slope of $\log M^c(V)$ coincides with that of the (independently determined) molar mass calibration. The method was evaluated by adopting a narrow Schulz–Zimm MMD, with the following average molar masses and polydispersity: $\bar{M}_n = 50,000$ g/mol, $\bar{M}_w = 60,000$ g/mol, and $\bar{M}_w/\bar{M}_n = 1.20$. In this case, the original MMD was well recuperated, but large errors were observed in the standard deviation estimate [18].

This work presents a new method for estimating the BBF in SEC. The raw data are the (concentration and molar mass) chromatograms of narrow standards and the molar mass calibration. The BBF can be assumed either arbitrary or represented by an EMG. At present, two of the authors participate of the IUPAC Project entitled: "Data Treatment in the Size-Exclusion Chromatography of Polymers"; and one of the project objectives is the evaluation and standardization of the available BB correction techniques. Thus, the present article is a contribution toward such goal.

2. Theoretical considerations

Consider a discrete SEC model that has been previously described in Refs [20] and [24]. The model assumptions are as follows: (i) the mass and molar mass detectors are ideal in the sense that they respond to Eqs. (2) and (3), except for the fact that their signals are contaminated with additive zero-mean random noises; (ii) the IDV has been accurately corrected for; (iii) the mass and molar mass chromatograms elute in a common elution volume range $[V_1–V_m]$, and are sampled at regular elution volume intervals ΔV; and (iv) the molar mass calibration $\log M^c(V)$ is known, and can be in general "nonlinear".

Rewritten in discrete form, Eqs (2) and (3) yield:

$$\mathbf{s}_k = \mathbf{G}\mathbf{s}_k^c; \quad (k = \mathrm{DR, LS, SV, OS}) \tag{4a}$$

$$\mathbf{s}_k^c = K_k[\mathbf{M}^c]^{a_k}\mathbf{s}_{\mathrm{DR}}^c; \quad (k = \mathrm{LS, SV, OS}) \tag{4b}$$

where $\mathbf{s}_k$ is a $(m \times 1)$-column vector containing the heights of $s_k(V)$ in the range $[V_1–V_m]$; $\mathbf{s}_k^c$ is a $(p \times 1)$-column vector containing the heights of $s_k^c(V)$ in the (narrower) range $[V_1^c - V_p^c]$; $\mathbf{M}^c$ is a $(p \times p)$ diagonal matrix containing the ordinates of $M^c(V)$ in the range $[V_1^c - V_p^c]$; and $\mathbf{G}$ is a $(m \times p)$ rectangular matrix representing $g(V, \bar{V})$, with V in $[V_1–V_m]$, and $\bar{V}$ in $[V_1^c - V_p^c]$. The sampling interval ΔV is selected from a compromise between small ΔVs that produce highly-resolved corrected chromatograms, and large ΔVs that improve the numerical inversion of $\mathbf{G}$. Typical ΔV values are in the range 0.01–0.03 mL.

Consider building the (true and nonuniform) $\mathbf{G}$ matrix from a (known and continuous) analytical expression of $g(V, \bar{V})$. At each discrete elution volume, the heights of all the individual $g(V)$ functions with average retention volumes in the range $[V_1^c - V_p^c]$ must be calculated. Most analytical functions (e.g., a Gaussian distribution) never strictly drop to zero, and this would produce "full" $\mathbf{G}$ matrixes, with most of their elements close to zero. This increases the correlation between columns of $\mathbf{G}$; and therefore it increases the ill-conditioned nature of its numerical inversion. To improve the numerical inversion of $\mathbf{G}$, we propose to set to zero all the smaller components of $\mathbf{G}$ (e.g., lower than 1% of the maximum). Furthermore, we fix the total number of nonzero points of any individual $g(V)$ function to $n(=c+d+1)$, where c and d are respectively the number of nonzero points before and after $\bar{V}$.

The ideal $\mathbf{G}$ is of minimal dimensions, with p columns strictly covering the corrected chromatogram range $[V_1^c - V_p^c]$, and m rows strictly covering the measured chromatogram range $[V_1–V_m]$. For measured chromatograms containing m points, the corrected chromatograms will contain $p = m - c - d$ points, and the minimum sized $(m \times p)$ matrix $\mathbf{G}$ is:

$$\mathbf{G} = \begin{bmatrix} g(V_1, V_1^c) & \cdots & 0 & \cdots & 0 \\ \vdots & \ddots & 0 & & \vdots \\ g(V_{c+1}, V_1^c) & & g(V_j, V_j^c) & & \\ \vdots & \ddots & \vdots & \ddots & 0 \\ g(V_{c+1+d}, V_1^c) & & g(V_{c+j}, V_j^c) & & g(V_p, V_p^c) \\ 0 & \ddots & \vdots & \ddots & \vdots \\ \vdots & & g(V_{c+j+d}, V_j^c) & & g(V_{c+p}, V_p^c) \\ & & 0 & \ddots & \vdots \\ 0 & \cdots & 0 & \cdots & g(V_m, V_p^c) \end{bmatrix}; \quad (m > p) \tag{5}$$

In Eq. (5), any generic jth column contains the n nonzero heights of $g(V, \bar{V})$, at $\bar{V} = V_j^c$.

In a wide range of elution volumes, the BBF and its corresponding $\mathbf{G}$ matrix are both non-uniform. In contrast, in the narrow range of a calibration standard, the BBF is uniform and represented by $\mathbf{g} = [g_1, g_2, \ldots, g_n]^T$, where the superscript 'T' indicates transpose. Its corresponding $\mathbf{G}$ matrix is also uniform, and is totally specified by only the n nonzero elements of any $g(V)$ function. Furthermore, any two consecutive columns jth and $(j+1)$th of $\mathbf{G}$ are identical, except for the fact that the $(j+1)$th column contains all its nonzero elements shifted one position downwards.

An estimate of any corrected k-chromatogram is obtained by inversion of its corresponding $\mathbf{G}$ matrix through [Eq. (4a)]:

$$\hat{\mathbf{s}}_k^c = \mathbf{G}^{[-1]}\mathbf{s}_k; \quad (k = \mathrm{DR, LS, SV, OS}) \tag{6}$$

where the $\mathbf{G}^{[-1]}$ is $(p \times m)$-matrix that represents a regularized pseudo-inverse of $\mathbf{G}$; and the symbol "$\wedge$" indicates "estimate of". Several numerical procedures have been developed for calculating $\mathbf{G}^{[-1]}\mathbf{s}_k$ [20,22]. In this work, the following singular

value decomposition expression [32] is employed:

$$\hat{\mathbf{s}}_k^{\mathrm{c}} = \mathbf{G}^{[-1]}\mathbf{s}_k = \sum_{j=1}^{r} \frac{\mathbf{u}_j^{\mathrm{T}}\mathbf{s}_k}{\sigma_j}\mathbf{v}_j; \quad (r \le p);$$

$$(\sigma_1 \ge \sigma_2 \ge \ldots \ge \sigma_r \ge \ldots \ge \sigma_p \ge 0) \tag{7}$$

where $\boldsymbol{u}_j$ and $\boldsymbol{v}_j$ are the eigenvectors of $\mathbf{G}\mathbf{G}^{\mathrm{T}}$ and $\mathbf{G}^{\mathrm{T}}\mathbf{G}$, respectively; and the σ_js are the singular values of $\mathbf{G}$ (or square roots of the eigenvalues of $\mathbf{G}^{\mathrm{T}}\mathbf{G}$). In the summation of Eq. (7), the number of "effective" terms is limited to r, to avoid amplifying the measurement noise. The parameter r is selected from a trade-off between a highly oscillatory and an excessively smoothened solution.

2.1. Estimation algorithms

The uniform $g(V)$ function can be assumed either arbitrary or represented by an EMG. Consider first an arbitrary $g(V)$ given by $\mathbf{g} = [g_1, g_2, \ldots, g_n]^{\mathrm{T}}$. The raw data are the measured mass chromatogram $\boldsymbol{s}_{\mathrm{DR}}$, the measured molar mass chromatogram $\boldsymbol{s}_k$ (with k either LS or SV), and the molar mass calibration $\mathbf{M}^{\mathrm{c}}$.

Call $\hat{\mathbf{s}}_{k,\mathrm{DR}}$ (k = LS or SV) an estimate of the molar mass chromatogram, calculated from the measured DR chromatogram. Such estimate is obtained by replacing Eq. (6) into Eqs. (4a) and (4b), yielding:

$$\hat{\mathbf{s}}_{k,\mathrm{DR}} = K_k \mathbf{G}[\mathbf{M}^{\mathrm{c}}]^{a_k}\mathbf{G}^{[-1]}\mathbf{s}_{\mathrm{DR}}; \quad (k = \mathrm{LS}, \mathrm{SV}) \tag{8}$$

We define the following estimation error vector:

$$\tilde{\mathbf{e}}_{\mathbf{s}_{k,\mathrm{DR}}} = \mathbf{s}_k - \hat{\mathbf{s}}_{k,\mathrm{DR}} = \mathbf{s}_k - K_k\mathbf{G}[\mathbf{M}^{\mathrm{c}}]^{a_k}\mathbf{G}^{[-1]}\mathbf{s}_{\mathrm{DR}};$$

$$(k = \mathrm{LS}, \mathrm{SV}) \tag{9}$$

Ideally, $\tilde{\mathbf{e}}_{\mathbf{s}_{k,\mathrm{DR}}} \cong [0, \ldots, 0]^{\mathrm{T}}$. Eq. (9) represents m algebraic equations in n ($<m$) unknowns, $g_1, g_2, \ldots, g_n$. We avoid for possible errors in the detector gain, K_k, by normalizing Eq. (9) as follows:

$$\mathbf{e}_{\mathbf{s}_{k,\mathrm{DR}}} = \frac{\mathbf{s}_k}{||\mathbf{s}_k||_1} - \frac{\mathbf{G}[\mathbf{M}^{\mathrm{c}}]^{a_k}\mathbf{G}^{[-1]}\,\mathbf{s}_{\mathrm{DR}}}{||\mathbf{G}[\mathbf{M}^{\mathrm{c}}]^{a_k}\mathbf{G}^{[-1]}\,\mathbf{s}_{\mathrm{DR}}||_1}; \quad (k = \mathrm{LS}, \mathrm{SV}) \tag{10}$$

where $\mathbf{e}_{\mathbf{s}_{k,\mathrm{DR}}}$ is the normalized estimation error vector; and $||^{\bullet}||_1$ is the 1-norm of a vector, i.e.: $||x||_1 = \Sigma_i|x_i|$.

Finally, $\mathbf{g}$ is obtained from the following minimization process:

$$\min_{\mathbf{g}}(e_{\mathbf{s}_{k,\mathrm{DR}}}) = \min_{\mathbf{g}}(\mathbf{e}^{\mathbf{T}}_{\mathbf{s}_{k,\mathrm{DR}}}\mathbf{e}_{\mathbf{s}_{k,\mathrm{DR}}} + \phi_g^2); \quad (k = \mathrm{LS}, \mathrm{SV}) \tag{11a}$$

with

$$\phi_g^2 = \beta\sum_{i=1}^{n}[2g_i - g_{i+1} - g_{i-1}]^2, \quad \text{and} \quad g_0 = g_{n+1} = 0 \tag{11b}$$

where $\mathbf{e}_{\mathbf{s}_{k,\mathrm{DR}}}$ is the scalar functional or mean square error of $\mathbf{e}_{\mathbf{s}_{k,\mathrm{DR}}}$; and β ($\ge$0) is a weighting factor. In Eq. (11b), ϕ_g^2 is a scalar filtering function that is introduced to impose some correlation between any three consecutive points of $\mathbf{g}$. The square bracket of Eq. (11b) is an estimate of the second derivative of $g(V)$, and therefore the effect of ϕ_g^2 is to reduce the high frequency oscillations in the estimates of $g(V)$.

In essence, the proposed procedure is based on estimating one of the chromatograms from the knowledge of the other. Eqs. (11) can be generalized to any pair of generic detectors $\{k_1, k_2\}$, as follows:

$$\min_{\mathbf{g}}(e_{\mathbf{s}_{k_1,k_2}}) = \min_{\mathbf{g}}(\mathbf{e}^{\mathrm{T}}_{\mathbf{s}_{k_1,k_2}}\mathbf{e}_{\mathbf{s}_{k_1,k_2}} + \phi_g^2) \tag{12a}$$

with

$$\mathbf{e}_{\mathbf{s}_{k_1,k_2}} = \frac{\mathbf{s}_{k_1}}{||\mathbf{s}_{k_1}||_1} - \frac{\mathbf{G}[\mathbf{M}^{\mathrm{c}}]^{(a_{k_1}-a_{k_2})}\mathbf{G}^{[-1]}\mathbf{s}_{k_2}}{||\mathbf{G}[\mathbf{M}^{\mathrm{c}}]^{(a_{k_1}-a_{k_2})}\mathbf{G}^{[-1]}\mathbf{s}_{k_2}||_1};$$

$$(k_1, k_2 = \mathrm{DR}, \mathrm{LS}, \mathrm{SV}) \tag{12b}$$

with ϕ_g^2 as in Eq. (11b). For all the possible combinations of DR, LS, and SV sensors, Table 1 presents the resulting expressions of $\mathbf{G}[\mathbf{M}^{\mathrm{c}}]^{(a_{k_1}-a_{k_2})}\mathbf{G}^{[-1]}$ in Eq. (12b). Note that for SV sensors, the M–H slope α is also required (see Table 1). Also, note that the product $\mathbf{G}^{[-1]}\mathbf{s}_{k_2}$ in Eq. (12b) is an estimate of $\mathbf{s}^{\mathrm{c}}_{k_2}$.

Table 1
Final Expressions for the numerator of the second term of Eq. (12b), when the k_1-chromatogram is estimated from the k_2-chromatogram

k_1	k_2	$\mathbf{G}[M^{\mathrm{c}}]^{(a_{k_1}-a_{k_2})}\mathbf{G}^{[-1]}$
LS	DR	$\mathbf{G}\,[\mathbf{M}^{\mathrm{c}}]\,\mathbf{G}^{[-1]}$
SV	DR	$\mathbf{G}\,[\mathbf{M}^{\mathrm{c}}]^{\alpha}\,\mathbf{G}^{[-1]}$
LS	SV	$\mathbf{G}\,[\mathbf{M}^{\mathrm{c}}]^{(1-\alpha)}\,\mathbf{G}^{[-1]}$
DR	LS	$\mathbf{G}\,[\mathbf{M}^{\mathrm{c}}]^{-1}\,\mathbf{G}^{[-1]}$
DR	SV	$\mathbf{G}\,[\mathbf{M}^{\mathrm{c}}]^{-\alpha}\,\mathbf{G}^{[-1]}$
SV	LS	$\mathbf{G}\,[\mathbf{M}^{\mathrm{c}}]^{(\alpha-1)}\,\mathbf{G}^{[-1]}$

Assume now that $g(V)$ is represented by a first-order EMG. A convolution product between a Gaussian and an exponentially decaying function defines the EMG [29,33–35]:

$$g(V) = \frac{1}{\sqrt{2\pi}\sigma_{\mathrm{BB}}\tau_{\mathrm{BB}}}\exp\left(-\frac{(V-\bar{V}_{\mathrm{G}})^2}{2\sigma_{\mathrm{BB}}^2}\right) * \exp\left(-\frac{V}{\tau_{\mathrm{BB}}}\right) \tag{13}$$

where '*' indicates "convolution product"; i.e. $[f_1(V) * f_2(V) = \int_0^{\infty} f_1(V-\bar{V})f_2(\bar{V})\,\mathrm{d}\bar{V}]$; $\bar{V}_{\mathrm{G}}$ and σ_{BB} are respectively the mean and standard deviation of the Gaussian function; and τ_{BB} is the "time" constant of the exponential decay. Note that an EMG defined as in Eq. (13): (i) involves three parameters: $\bar{V}_{\mathrm{G}}$, σ_{BB}, and τ_{BB}; and (ii) is a smooth function, because it results from the convolution of two smooth functions.

In Eq. (13), $g(V)$ is normalized in the sense that $\int_0^{\infty} g(V)\,\mathrm{d}V = 1$. Also, the mean volume of $g(V)$ results: $\bar{V} = \int_0^{\infty} Vg(V)\,\mathrm{d}V = \bar{V}_{\mathrm{G}} + \tau_{\mathrm{BB}}$. If $\bar{V} = 0$ is imposed onto all individual $g(V)$ functions, then the mean retention volume of the corrected chromatograms will coincide with the mean retention volume of the measured chromatogram. This condition is automatically ensured by adopting $\bar{V}_{\mathrm{G}} = -\tau_{\mathrm{BB}}$; and in this case, Eq. (13) reduces to the following two-parameter expression:

$$g(V) = \frac{1}{\sqrt{2\pi}\sigma_{\mathrm{BB}}\tau_{\mathrm{BB}}}\exp\left(-\frac{(V+\tau_{\mathrm{BB}})^2}{2\sigma_{\mathrm{BB}}^2}\right) * \exp\left(-\frac{V}{\tau_{\mathrm{BB}}}\right) \tag{14}$$

Since the EMG of Eq. (14) is a smooth function, then the filtering factor of Eq. (11b) is no longer required. Thus, the minimization problem of Eq. (12a) reduces to the following two-parameters search:

$$\min_{\{\sigma_{BB},\tau_{BB}\}}(e_{\mathbf{s}_{k_1,k_2}}) = \min_{\{\sigma_{BB},\tau_{BB}\}}(\mathbf{e}^{T}_{\mathbf{s}_{k_1,k_2}}\mathbf{e}_{\mathbf{s}_{k_1,k_2}}) \quad (15)$$

with $\mathbf{e}_{\mathbf{s}_{k_1,k_2}}$ as in Eq. (12b).

Initial guesses are required on the sought uniform BBF (or equivalently on vector $\boldsymbol{g}$ and on the uniform matrix $\mathbf{G}$), to solve Eqs. (12) and (15). For arbitrary BBFs, we propose to obtain an initial guess of $\mathbf{g}$ by simply "contracting" the DR chromatogram into a reduced number of n points. For BBFs represented by an EMG, we propose to select an initial pair of parameters σ_{BB} and τ_{BB} that grossly approximates the DR chromatogram.

Finally, consider a criterion for the initial selection of dimensions n and p of $\mathbf{G}$. The measured chromatograms contain m nonzero points in $[V_1–V_m]$. Since $m=p+n-1$, many combinations of n and p satisfy such expression. We propose the following two-steps procedure. In *Step I*, we adopt $\hat{n}_I = \hat{p}_I \approx m$; and to this effect we must extend the original chromatograms with leading and lagging zeroes, such their original m points are transformed into $\hat{m}_I = 2m - 1$ points. Then, the optimization algorithm is applied, and the intermediate estimates of $g(V)$ and $s^c_{k_2}(V)$ are obtained, that we shall call $\hat{g}_I(V)$ and $\hat{s}^c_{k_2,I}(V)$, respectively. The overestimated range of $\hat{s}^c_{k_2,I}(V)$ generates spurious oscillations in the corrected chromatogram tails. In *Step II*, such spurious oscillations are eliminated by simply reducing the system dimensions into $\hat{n} < \hat{n}_I$ and $\hat{p} < \hat{p}_I$. Then, the optimization algorithm is applied for the second time, to produce the final estimates $\hat{g}(V)$ and $\hat{s}^c_{k_2}(V)$.

To evaluate the quality of alternative BBF estimates $\hat{\mathbf{g}}$, the following (scalar) mean square error is defined:

$$e_g = \frac{(\hat{\mathbf{g}}-\mathbf{g})^T(\hat{\mathbf{g}}-\mathbf{g})}{\mathbf{g}^T\mathbf{g}} \quad (16)$$

where $\mathbf{g}$ is the true *a priori* known solution. Clearly, Eq. (16) is inapplicable in a real experiment.

3. Simulation examples

3.1. Raw data

"Synthetic" or simulated examples are useful for evaluating numerical procedures, because the sought solutions are known *a priori*. Except for the MMDs, we simulated the experimental conditions and BBF that were determined by Busnel et al. [26]. The simulations involved ambient temperature, tetrahydrofurane (THF) as carrier solvent, and a mixed-gel column from Polymer Laboratories (5 μm, 60 cm length) [26]. The molar mass calibration was assumed linear (Fig. 1a), and given by [26]:

$$\log M^c(V) = 10.562 - 0.4223\,V \quad (17)$$

The continuous BBF is represented by a non-uniform EMG (see Fig. 1a). Its theoretical expression is in Eq. (14), and its parameters are slightly reduced along the elution volume according to [26]:

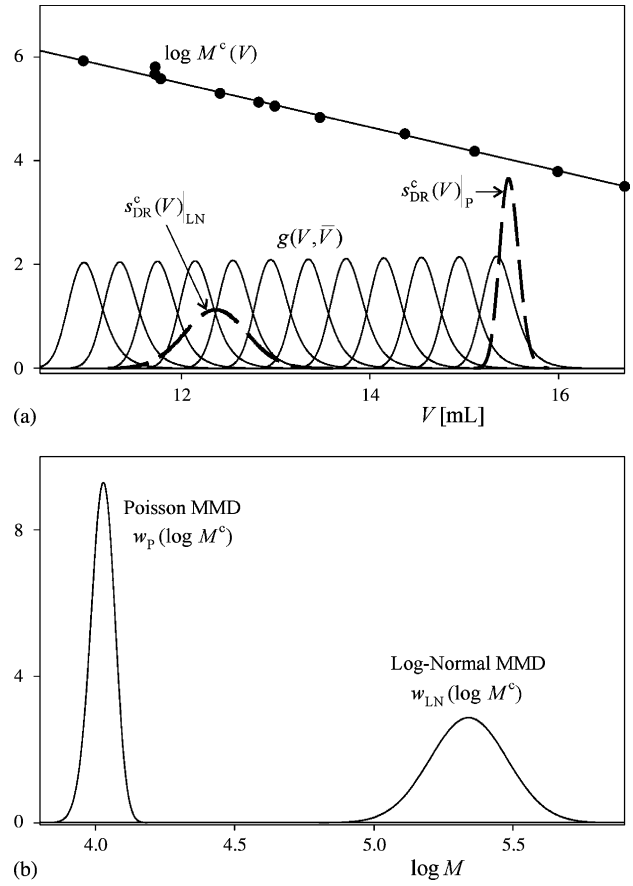

Fig. 1. Simulated examples: raw data. (a) Molar mass calibration, $\log M^c(V)$, non-uniform BBF, $g(V,\bar{V})$, and corrected DR chromatograms, $s^c_{DR}(V)|_{LN}$ and $s^c_{DR}(V)|_P$. (b) Proposed MMDs: log-normal distribution, $w_{LN}(\log M^c)$, and Poisson distribution, $w_P(\log M^c)$.

$$\sigma_{BB}(V) = 0.165 - 0.002\,V \quad (18a)$$

$$\tau_{BB}(V) = 0.160 - 0.0015\,V \quad (18b)$$

We simulated the analyses of two narrow PS standards of known WCLDs. Their analytical expressions are in Table 2, where i is the number of repetitive units. They correspond to a lower molar mass Poisson distribution $w_P(i)$ with $\bar{M}_w/\bar{M}_n = 1.010$; and to a higher molar mass log-normal distribution $w_{LN}(i)$ with $\bar{M}_w/\bar{M}_n = 1.107$. These distributions are shown in Fig. 1b with a common logarithmic molar mass axis. A Poisson WCLD is the narrowest possible distribution that is obtainable through a synthetic polymerization process. Note that the molar masses of the Poisson distribution are in the limit of low sensitivity of a LS or SV detector. However, this problem is not considered here, since we are assuming perfect ideal sensors. The log-normal WCLD was chosen to emulate the typical polydispersities of standards with molar masses around 200,000 g/mol. (If a Poisson WCLD with $\bar{M}_w \cong 200,000$ g/mol had been chosen, then its polydispersity of around 1.0005 would be unrealistic in practice.)

For each simulated WCLD, the true (or corrected) mass chromatograms were obtained from the MMDs and the linear

Table 2
Simulated examples: raw data[a]

	Log-normal WCLD	Poisson WCLD
Analytical expressions and averages		
	$w_{LN}(i) = \frac{1}{\sqrt{2\pi}\sigma_{LN} i} \exp\left[-\frac{[\ln(i/\bar{i})]^2}{2\sigma_{LN}^2}\right]$ (with $\bar{i} = 2100$ and $\sigma_{LN}^2 = 0.32$)	$w_P(i) = \frac{i}{\lambda+1} \frac{e^{-\lambda}\lambda^{i-1}}{(i-1)!}$ (with $\lambda = 100$)
$\bar{M}_n$ (g/mol)[b]	207780	10519
$\bar{M}_w$ (g/mol)[b]	230100	10623
$\bar{M}_w/\bar{M}_n$	1.107	1.010
Corrected chromatograms: number of points and elution volume range		
p	122	41
$[V_1^c - V_p^c]$ (mL)	[11.22–13.64]	[15.10–15.90]
BBF: number of points and limiting parameter values		
$n(=c+d+1)$	63(=26 + 36 + 1)	63 (=26 + 36 + 1)
σ_{BB} (mL)	[0.1377–0.1426]	[0.1332–0.1348]
τ_{BB} (mL)	[0.1395–0.1432]	[0.1361–0.1374]
Measured chromatograms: number of points and elution volume range		
$m = p + n - 1$	184	103
$[V_1 – V_m]$ (mL)	[10.70–14.36]	[14.58–16.62]

[a] For the discretizations, the elution volume interval was $\Delta V = 0.02$ mL.
[b] Styrene molar mass: $M_{St} = 104.15$ g/mol.

calibration, yielding the dashed curves $s^c_{DR}(V)|_{LN}$ and $s^c_{DR}(V)|_P$ of Fig. 1a. All the chromatograms are discrete, with values at regular elution volume intervals $\Delta V = 0.02$ mL. The true (or corrected) LS chromatograms, $s^c_{LS}(V)|_{LN}$ and $s^c_{LS}(V)|_P$, were obtained from the DR chromatograms through Eq. (3), and adopting $K_{LS} = 0.02$. Table 2 presents the total number of points (p) and elution volume ranges $[V_1^c - V_p^c]$ of the corrected chromatograms. Fig. 2 presents the corrected DR and LS chromatograms (in dashed trace). Also, Fig. 2 shows the first and last "effective" BBFs, placed at the first and last nonzero points of the corrected chromatograms. Each individual $g(V)$ function exhibits $n = 63$ nonzero points, with $c = 26$ leading points, and $d = 36$ lagging points (Table 2). Note that the average volumes of the individual $g(V)$ functions are not placed at their maxima.

For each analyzed WCLD, two (true and non-uniform) **G** matrixes were built with Eqs. (5), (14) and (18), for strictly covering their elution volume ranges. At any elution volume V_j, the jth column of **G** contains the ordinates of the particular $g(V)$ function with $\bar{V} = V_j$. The columns of the non-uniform **G** matrixes were obtained as follows: (i) at each V_j, calculate the true σ_{BB} and τ_{BB} parameters, with Eqs. (18a) and (18b); (ii) with $\bar{V} = V_j$, calculate the n heights of the individual $g(V)$ function with Eq. (14); (iii) set to zero all the column elements smaller than 1% of the maximum value; and (iv) normalize the column elements, such that their sum is equal to 1. For the log-normal distribution, a (184 × 122)-matrix **G** was defined, that covered the elution volume range 10.70–14.36 mL. For the Poisson distribution, a (103 × 41)-matrix **G** was defined, that covered the elution volume range 14.58–16.62 mL (Table 2).

The noise-free "measured" chromatograms were calculated with Eqs. (4a) and (4b). Then, zero-mean Gaussian sequences were added onto the noise-free chromatograms to produce the final measured chromatograms $s_{DR}(V)$ and $s_{LS}(V)$ of Fig. 2. In all cases, the variances of the additive noises were equal to 0.25% of the chromatogram maxima. For the measured chromatograms, Table 2 presents the total number points (m) and the volume

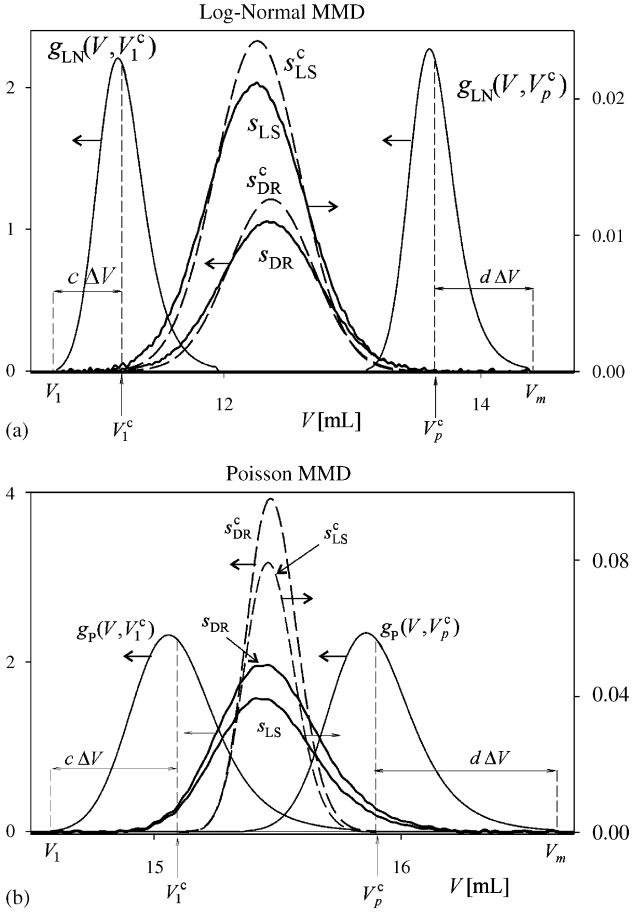

Fig. 2. Simulated examples: raw data. (a) Log-normal distribution. (b) Poisson distribution. Limiting BBFs [$g_{LN}(V, V_1^c)$, $g_{LN}(V, V_p^c)$, $g_P(V, V_1^c)$, $g_P(V, V_p^c)$], "measured" chromatograms [$s_{DR}(V)$, $s_{LS}(V)$], and corrected chromatograms [$s^c_{DR}(V)$, $s^c_{LS}(V)$].

Table 3
Simulated examples: intermediate adjustments and Final global results

	Log-normal WCLD[a]	Poisson WCLD[b]
Estimated dimensions and ranges of the BBF and the measured chromatograms		
Step I:		
$\hat{n}_I = \hat{p}_I = m$	184	103
$\hat{m}_I = 2m - 1$	367	205
$[V_1–V_m]_I$ (mL)	[8.88–16.20]	[13.56–17.64]
Step II:		
$\hat{m}(= \hat{n} + \hat{p} - 1)$	244 (= 95 + 150 − 1)	163 (= 95 + 69 − 1)
$[V_1–V_m]$ (mL)	[10.10–14.96]	[13.98–17.22]
BBF estimates and performance indexes		
Arbitrary BBF:	$\hat{g}_{LN}(V)$ of Fig. 3a	$\hat{g}_P(V)$ of Fig. 3d
e_g ($\times 10^3$)	0.43	0.57
BBF as EMG:	$\hat{g}_{LN}(V)$ of Fig. 4a	$\hat{g}_P(V)$ of Fig. 4b
$\hat{\sigma}_{BB}$ (mL)	0.143	0.137
$\hat{\tau}_{BB}$ (mL)	0.136	0.133
e_g ($\times 10^3$)	0.19	0.13
MMD average estimates		
Arbitrary BBF:		
$\hat{\bar{M}}_n$ (g/mol)	210120	10540
$\hat{\bar{M}}_w$ (g/mol)	230050	10610
$\hat{\bar{M}}_w/\hat{\bar{M}}_n$	1.095	1.007
BBF as EMG:		
$\hat{\bar{M}}_n$ (g/mol)	209020	10510
$\hat{\bar{M}}_w$ (g/mol)	230990	10620
$\hat{\bar{M}}_w/\hat{\bar{M}}_n$	1.105	1.010

[a] See Fig. 3a–c.
[b] See Fig. 3d–f.

ranges $[V_1–V_m]$. Note that the measured chromatograms are only slightly wider than any individual BBF.

3.2. BBF estimates

For each of the two simulated examples, consider estimating their uniform BBFs. The raw data are the measured chromatograms and the molar mass calibration. First, consider applying the proposed algorithm of Eqs. (11a) and (11), for the case of arbitrary shaped BBF estimates. For both WCLDs, the following common parameters were adopted: (a) $r = 10$ for the deconvolution operations; and (b) $\beta = 500$ for the smoothing term of Eq. (11b). These values were obtained from a compromise between smooth BBF estimates with high objective functionals $e_{\mathbf{s}_{LS,DR}}$, and oscillatory BBF estimates with low values of $e_{\mathbf{s}_{LS,DR}}$.

The final solutions are in Table 3 and in Fig. 3. Table 3 shows the intermediate and final vector dimensions and elution volume intervals. For the log-normal WCLD, the intermediate and final BBF estimates ($\hat{g}_{I,LN}(V)$ and $\hat{g}_{LN}(V)$, respectively) are in Fig. 3a. For the Poisson WCLD, the intermediate and final BBF estimates $\hat{g}_{I,P}(V)$ and $\hat{g}_P(V)$ are in Fig. 3d. Their corresponding corrected chromatograms are in Fig. 3b,e. In both examples, spurious oscillations are observed in the intermediate estimates. Such oscillations were eliminated in *Step II*, when reducing the dimensions of $\hat{n}_I$ and $\hat{p}_I$ (see Table 3). In both examples, the final BBF estimates are slightly oscillatory and exhibit $\hat{n} = 95$ non-zero points (i.e., they are somewhat broader than the real $n = 63$). However, the final estimates are quite acceptable, with the (broader) log-normal distribution presenting slightly better results. The true BBF is only slightly non-uniform in the narrow ranges of the chromatograms. For this reason, $\hat{g}_{LN}(V)$ is close to the true initial and last individual broadening functions $g_{LN}(V, V_1^c)$ and $g_{LN}(V, V_p^c)$ (Fig. 3a); and similarly, $\hat{g}_P(V)$ is close to $g_P(V, V_1^c)$ and $g_P(V, V_p^c)$ (Fig. 3d).

Finally, consider recuperating the original MMDs from the estimated uniform arbitrary BBFs, the concentration chromatograms, and the molar mass calibration. The procedure was as follows: (i) calculate the corrected mass chromatograms through Eqs. (6) and (7), by deconvolution of the measured mass chromatograms; and (ii) combine the corrected mass chromatograms and the molar mass calibration to obtain the MMD estimates $\hat{w}_{LN}(\log M^c)$ and $\hat{w}_P(\log M^c)$. The results are in Fig. 3c,f. For the broader log-normal MMD, the estimate is quite acceptable. For the narrower Poisson distribution, the MMD estimate is more oscillatory, due to the worse posed nature of its deconvolution operation. Table 3 presents the estimated average molar masses and polydispersities.

Now, let us apply the proposed algorithm of Eq. (15), for the case of uniform BBFs represented by EMGs. Better results are to be expected in this case because: (a) the true BBF was originally defined as a (slightly non-uniform) EMG; and (b) in each numerical example, the algorithm needs to estimate only two constant parameters: σ_{BB} and τ_{BB} (rather than $n = 95$ unknowns). For the intermediate and final estimates of the elution volume intervals, the $\hat{n}$, $\hat{p}$, and $\hat{m}$ dimensions, and the r parameter, the same values previously described for the arbitrary BBF cases were readopted (upper section of Table 3).

The BBF estimates are in Fig. 4, and the global results are in the second section of Table 3. As expected, the results are better than before, for which reason the average errors e_g are somewhat lower than for the arbitrary BBF cases. Even though not shown, the intermediate chromatogram estimates of *Steps I* and *II* were also improved versions of the arbitrary BBF case. Compared with the true EMG parameters of Table 2, the estimated EMG parameters are in the upper or lower limits of their true values. Also, the estimated parameters σ_{BB} and τ_{BB} are slightly reduced when changing from higher molar masses of the log-normal distribution to the lower molar masses of the Poisson distribution. The lower section of Table 3 presents the estimated average molar masses and polydispersities. In general, all estimates are in good agreement with their true values presented in Table 2. Again, the averages were slightly better estimated when the BBFs were assumed EMG functions. This is reasonable, since the MMDs were estimated from the BBF estimates, and the best BBF estimates corresponded to the EMG functions.

3.3. Checks of robustness

The presented examples were simulated with varying conditions and detector combinations. The results are summarized in Table 4. In Case I, the variances of the additive noise that contaminate the measured chromatograms were increased from

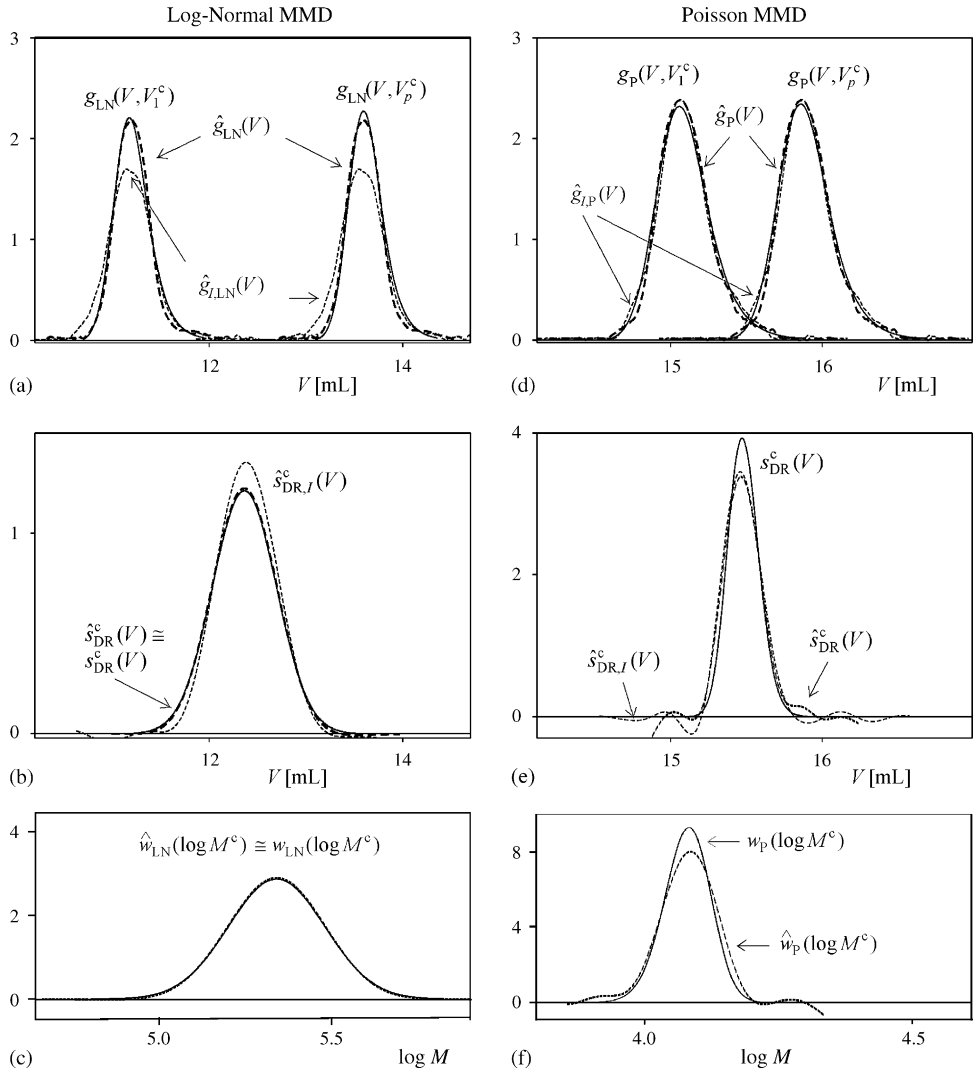

Fig. 3. Simulated examples: estimates when assuming arbitrary BBFs: (a–c) Log-normal MMD; (d–f) Poisson MMD; (a, d) BBF estimates after *Step I* (thin dashed curves), and after *Step II* (thick dashed curves). The limiting "true" BBFs are shown in continuous trace: (b, e) True and estimated corrected chromatograms after *Steps I* and *II*; (c, f) True and estimated MMDs.

0.25% to 2.5% of the chromatogram maxima. In spite of this change, the BBF estimates were only slightly deteriorated, as it can be seen from the resulting e_g values and from the estimated EMG parameters.

In Case II of Table 4, ±5% errors were introduced into the slope of the calibration $\log M^c(V)$. Again, negligible deviations were observed in the BBF estimates. But while clockwise rotations of $\log M^c(V)$ produce broader-than-real estimates on

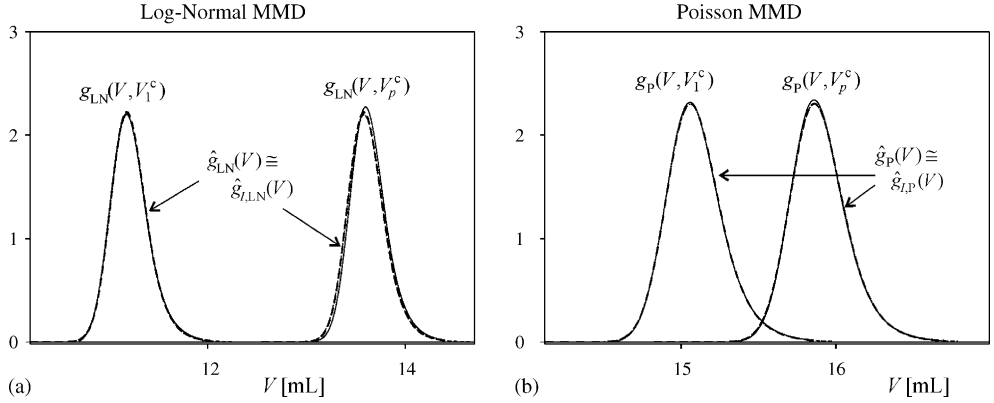

Fig. 4. Simulated examples: estimates when assuming the BBFs represented by EMGs. (a) Log-normal MMD. (b) Poisson MMD. The BBF estimates after *Step I* (in thin dashed curves), and after *Step II* (in thick dashed curves). The limiting "true" BBFs are in continuous trace.

Table 4
Simulated examples: checks of robustness

Case no.	Log-normal WCLD		Poisson WCLD	
	Arbitrary BBF	EMG	Arbitrary BBF	EMG
I) Measurement noise variances: 2.5% of the chromatograms maxima				
e_g ($\times 10^3$)	6.2	0.87	2.8	1.1
$\hat{\sigma}_{BB}$ (mL)	–	0.140	–	0.143
$\hat{\tau}_{BB}$ (mL)	–	0.132	–	0.127
II) Changes of ±5% in the molar mass calibration slope				
e_g ($\times 10^3$)[a]	6.5/5.6	4.2/7.5	1.5/0.68	0.32/0.15
$\hat{\sigma}_{BB}$ (mL)[a]	–	0.156/0.128	–	0.139/0.136
$\hat{\tau}_{BB}$ (mL)[a]	–	0.143/0.128	–	0.132/0.132
III) SV sensor with changes of ±5% in the M–H slope α				
e_g ($\times 10^3$)[a]	3.9/3.9	4.2/7.8	3.5/8.0	0.47/0.53
$\hat{\sigma}_{BB}$ (mL)[a]	–	0.156/0.133	–	0.140/0.138
$\hat{\tau}_{BB}$ (mL)[a]	–	0.143/0.121	–	0.131/0.128
IV) Changes of ±0.02 mL in the IDV				
e_g ($\times 10^3$)[a]	117/62	190/43	365/71	422/49
$\hat{\sigma}_{BB}$ (mL)[a]	–	0.091/0.191	–	0.041/0.195
$\hat{\tau}_{BB}$ (mL)[a]	–	0.089/0.163	–	0.118/0.140

[a] The first value corresponds to positive changes, and the second value to negative changes.

both the BBF and the MMD, the opposite occurs for counter clockwise rotations.

In Case III of Table 4, the LS detector was changed to an ideal SV detector with the M–H slope $\alpha = 0.712$ (corresponding to PS in THF at 25 °C) [36]. In this case, ±5% variations in the value of α produced negligible deviations in the BBF estimates. While positive variations produced broader than real BBF estimates, the opposite occurred for negative variations. Thus, errors in α are qualitatively equivalent to errors in the linear calibration slope.

In Case IV of Table 4, errors in the IDVs were simulated. In this case, a very high parameter sensitivity was observed. Thus, when shifting the LS chromatograms towards lower elution volumes by only 0.02 mL (i.e., the adopted discretization interval), then unacceptably large errors were produced (Table 4). Also, the BBF estimates were narrower than real when the LS chromatograms were shifted towards lower elution volumes; and wider than real when the shift was in the opposite direction.

Finally, both wider and narrower WCLDs were simulated. A (rather wide) Schulz–Flory WCLD with $\bar{M}_n = 42,800$ g/mol and $\bar{M}_w/\bar{M}_n \cong 1.995$, produced high errors in the BBF estimates, with $e_g = 34.2 \times 10^{-3}$ for arbitrary-shaped BBFs, and with $e_g = 19.6 \times 10^{-3}$ for EMGs. The reason is that the BB negligibly affects a broad MMD, and therefore little information on the BBF can be recuperated from the corresponding chromatograms. Similarly, the algorithm did not provide good results for a narrow Poisson WCLD with $\lambda = 500$ and $\bar{M}_w/\bar{M}_n \cong 1.002$. In this case, the small differences between the DR and LS chromatograms were hidden by the measurement noise. After a series of simulations involving log-normal and Poisson WCLDs of different averages and noises, the procedure proved effective for samples with polydispersities in the range 1.005–1.50.

4. Conclusions

A method was proposed for estimating a uniform (but skewed) BBF, based on analyzing narrow standards with a molar mass sensitive detector. The simulations involved a Poisson distribution of a relatively low molar mass, and a log-normal distribution of a higher molar mass. In both cases, quite reasonable BBF estimates were observed. The BBF estimates were best and faster when adopting EMG functions. In addition, the EMG parameter estimates showed a correct variation with the molar mass; making it possible to estimate the overall non-uniform BBF by simple interpolation of the obtained EMG parameters.

The numerical algorithm is free from errors in the detectors gains, and it has proven immune to different shapes of the WCLD, to high-frequency noises, to errors in the calibration slope, and to errors in the M–H parameter α (when SV sensors are applied). However, the method has proven to be very sensitive to errors in the IDV. Assuming no errors in the IDV, very reasonable BBF estimates were recuperated for standards with true polydispersities in the range 1.005–1.50. For extremely low polydispersities, the procedure fails because the molar mass chromatogram almost coincides with the concentration chromatogram. In such cases, however, the mass chromatogram could be considered as a good approximation to the sought BBF. For high polydispersities, the procedure fails because the BB has a negligible effect of on the measured chromatograms.

Molar mass calibrations are in general determined from the concentration chromatograms that are also required in the proposed BBF estimation technique. The errors in the molar mass calibrations are in part due to an incorrect assignment of the average elution volumes to the average molar masses. Perhaps, better estimates could be obtained if (rather than using the mass chromatograms of narrow standards), the corresponding BB-corrected chromatograms were used.

In triple detection, a second (independent) molar mass-sensitive sensor is incorporated, and this could introduce additional consistency tests between the measured chromatograms, the molar mass calibration, and the BBF estimates. For triple detection, Eqs. (12a) and (12) could be extended and a similar iterative procedure to that of Netopilík [18] could be applied to simultaneously estimate the uniform BBF and the molar mass calibration.

Applications of the proposed technique onto LS–DR and SV–DR measurements will be presented in a future work. In the first case, the detectors were interconnected in series, and the IDV could be unambiguously determined. In the second case, the detectors were interconnected in parallel, and important uncertainties arose in relation with the sign and magnitude of the IDV.

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