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SEC OF SIMPLE POLYMERS WITH MOLAR MASS DETECTION IN PRESENCE OF INSTRUMENTAL BROADENING. COMPUTER SIMULATION STUDY ON THE CALCULATION OF UNBIASED MOLECULAR WEIGHT DISTRIBUTIONS

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**SEC OF SIMPLE POLYMERS WITH MOLAR
MASS DETECTION IN PRESENCE OF
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CALCULATION OF UNBIASED
MOLECULAR WEIGHT DISTRIBUTIONS**

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ABSTRACT

This theoretical work presents a correction procedure for calculating an unbiased MWD when analyzing a linear homopolymer by size exclusion chromatography with molar mass detection. The fractionation and the measurements are ideal, but the chromatograms are distorted by a nonuniform and skewed instrumental broadening (IB), and are contaminated with a zero-mean random noise. The main assumption is that the molecular weight calibration is linear in the chromatogram range.

The following phenomenological procedure was proposed: (i) independently correct the mass- and molar mass chromatograms for IB through a robust inverse filtering technique; (ii) estimate the unbiased calibration from the ratio of the corrected chromatograms; (iii) adjust a straight line to the mid-values of that cal-

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ibration; and (iv) estimate the unbiased MWD from the linear calibration and the corrected mass chromatogram.

For the chromatogram inversions, a minimum sized broadening matrix was selected and a singular value decomposition technique was applied. The following effects were evaluated: of an increased measurement noise, of uncertainties in the range of the measured chromatograms, and of systematic errors in the IB function. The method fails for very narrow MWDs.

INTRODUCTION

Size exclusion chromatography (SEC) is the main analytical technique for measuring the molecular weight distribution (MWD) of a polymer. When a chromatographically-simple polymer (i.e., a linear homopolymer) is analyzed by ideal SEC, then a perfect fractionation according to both hydrodynamic volume and molecular weight is produced. In contrast, chromatographically-complex polymers (i.e., branched homopolymers, copolymers, and polymer blends) determine that (even under perfect resolution) a one-to-one relationship between elution volume and molecular weights can no longer be established; and some bias is introduced in the estimated MWDs.^{1,2}

Perfect SEC fractionation is impossible due to secondary fractionations and to instrumental broadening (IB). Secondary fractionations result from physico-chemical interactions between the polymer, the solvent, and the polymer packing;³ and will not be further discussed. IB is mainly due to axial dispersion in the columns, while other minor IB sources include column end-fitting effects, finite injection volume, finite detection cell volume, and flow profiles in the capillaries.^{4,5} In multidetection SEC, two or more detectors can be installed in series. This introduces the interdetector volume problem, by which the downstream signals may be shifted and distorted with respect to the upstream signals.⁵⁻¹⁰

This work is a continuation of the theoretical publication by Prougenes *et al.*,¹¹ where the SEC analysis of a simple polymer was simulated with the following assumptions: perfect mass- and molar mass detection, a uniform and Gaussian IB, and a linear molecular weight calibration. With ideal $M_w(V)$ -sensitive detectors, only the global \bar{M}_w results unbiased while the other averages are biased. Similarly, $M_n(V)$ -sensitive detectors only produce unbiased \bar{M}_n 's. In both cases, the global polydispersity \bar{M}_w/\bar{M}_n is underestimated, and a method was proposed for correcting these biases on the basis of estimating the instantaneous polydispersity.¹¹

The presence of noise and the recuperation of an unbiased MWD were not considered in Prougenes *et al.*¹¹ For ideal $M_w(V)$ -sensitive detectors, the global

polydispersity may be either underestimated (when the exact Mark-Houwink constants are used) or overestimated (when the universal calibration is used).^{7,12}

For a mass chromatogram $w(V)$, the IB process is modeled by Tung's equation:¹³

$$w(V) = \int_{V_1^c}^{V_2^c} g(V, \tilde{V}) w^c(\tilde{V}) d\tilde{V} \quad (1)$$

where $g(V, \tilde{V})$ is the (in general nonuniform) broadening function; \tilde{V} is a dummy integration variable that represents the mean elution volume of each individual $g(V)$ function; $w^c(V)$ is the IB-corrected chromatogram; and $[V_1^c - V_2^c]$ is the elution volume range of $w^c(V)$. Note that $[V_1^c - V_2^c]$ is narrower than the range of $w(V)$, that we shall indicate by $[V_1 - V_2]$. For symmetrical $g(V)$ functions, \tilde{V} is unambiguously assigned at the maximum of g . For skewed $g(V)$ functions, \tilde{V} can be assigned at the mode, the mean, or the median of $g(V)$. This introduces an uncertainty in the definition of $g(V, \tilde{V})$ even when truly monodisperse standards are available.

By neglecting the interdetector volume problem, Eq. (1) was extended to any generic molar mass chromatogram $s_k(V)$ ($k = w, v, n$), as follows:^{14,15}

$$s_k(V) = \int_{V_1^c}^{V_2^c} g(V, \tilde{V}) s_k^c(\tilde{V}) d\tilde{V}; \quad (k = w, v, n) \quad (2)$$

where $g(V, \tilde{V})$ coincides with the IB function of Eq. (1), and $s_k^c(V)$ is the IB-corrected molar mass chromatogram. The instantaneous average molecular weight $M_k(V)$ is related to the measured chromatograms through:^{14,15}

$$s_k(V) \propto [M_k(V)]^a w(V); \quad (k = w, v, n) \quad (3)$$

where $k = w$, $a = 1$ for a light-scattering sensor; $k = v$, $0.5 < a < 0.8$ for a viscosity sensor; and $k = n$, $a = -1$ for an osmotic pressure sensor.¹⁶⁻¹⁸ Equation (3) yields:

$$M_w(V) \propto s_w(V)/w(V); \quad M_v(V) \propto [s_v(V)/w(V)]^{1/a}; \quad M_n(V) \propto w(V)/s_n(V). \quad (4)$$

Note that a signals ratio is required to calculate any of the instantaneous average molecular weights, and therefore, large errors are to be expected at the chromatogram tails where the signal-to-noise ratios are low. Since $M_w(V)$, $M_v(V)$, and $M_n(V)$ all depend on the analyzed MWD, then these functions can be considered a set of biased (or *ad hoc*) molecular weight calibrations.

Some publications have investigated the propagation of errors in Eqs. (3) and (4).^{19,20} Procházka and Kratochvíl¹⁹ theoretically evaluated a light scattering detector. They determined that acceptable molecular weight estimates are only feasible in the central chromatogram region; recommending to use an independently-determined calibration for estimating the molecular weights at the chromatogram tails.

Lew *et al.*²⁰ investigated on-line viscometers. They proposed to simultaneously estimate the MWD and the Mark-Houwink constants by combining the universal calibration with the intrinsic viscosity calibration. This last calibration was adjusted to a fourth-order polynomial, weighing more strongly the mid-chromatogram measurements than the measurements at the chromatogram tails.

In ideal SEC without IB, the instantaneous MWD is monodisperse. In this case, any molar mass sensor type would provide the same instantaneous molecular weight, and therefore:

$$M(V) \propto [s_k^c(V)/w^c(V)]^{1/a}; (k = w, v, n) \quad (5)$$

where $M(V)$ [and $\log M(V)$] represent an unbiased molecular weight calibration. This calibration is expected to coincide with that obtained in a real chromatograph with IB, from monodisperse standards of the analyzed polymer. Note that an unbiased MWD is produced from $w^c(V)$ and $\log M(V)$.

For broad and smooth MWDs, the correction for IB is generally unnecessary. In contrast, the correction is important when the MWD is narrow and/or when 'sharp' details of a MWD are required. To apply Eq. (5), $w^c(V)$ and $s_k^c(V)$ must be first independently estimated by inverting Eqs. (1) and (2) from the knowledge of $w(V)$, $s_k(V)$, and $g(V, \tilde{V})$. This procedure has been suggested in several publications,^{10,14,15} but independent inversions of the measured chromatograms were apparently never implemented. The main reason for disregarding this more direct or "phenomenological" approach is the ill-conditioned nature of the inversion.^{21,22} Another difficulty is the ill-defined nature of the IB function $g(V, \tilde{V})$; by which even gross definitions, such as if the IB is symmetrical or skewed, uniform or nonuniform, are still a matter of controversy.^{23,24}

The IB correction is considerably simplified when the following (rather strong) hypotheses are adopted: (a) the MWD is log-normal; (b) the IB function is uniform and Gaussian; and (c) the molecular weight calibration $\log M(V)$ is linear. In this case, the *ad hoc* calibrations $\log M_n(V)$, $\log M_w(V)$, and $\log M_z(V)$ are also linear and rotated counterclockwise with respect to $\log M(V)$. Therefore, $\log M(V)$ can be estimated by simply rotating any of the *ad hoc* calibrations clockwise around its corresponding average retention volume.^{12,25} Using these concepts, Billiani *et al.*²⁶ proposed to simultaneously determine the slope of $\log M(V)$ and the standard deviation of the IB function from the central values of $w(V)$ and $s_w(V)$.

To this effect, theoretical expressions for the measured chromatograms were applied, and the central region was specified by requiring both signals to be above 10% of their maximum values.²⁶ By assuming that the interdetector volume only introduces a pure shift with no signal distortion, then the interdetector volume effect is equivalent to a clockwise rotation of the linear calibration.⁶⁻⁸ Thus, one can in principle simultaneously correct for the IB and the interdetector volume by simply shifting one of the chromatograms with an appropriate "effective

volume shift". Even though the method is strictly applicable to log-normal MWDs with Gaussian IBs, the technique was applied to a Schulz-Flory MWD, to evaluate the resulting deviations.¹⁰ By assuming that the interdetector volume also distorts the downstream peak,⁹ then the rotation technique is no longer applicable, even with log-normal MWDs and Gaussian IBs.

In this work, the analysis of a simple polymer by SEC with molar mass detection is simulated, with the aim of obtaining an unbiased (or IB-corrected) MWD. No assumptions on the shapes of the MWD or the IB function are imposed. The main assumption is that the unbiased molecular weight calibration is linear. This condition is in general verified, especially if the MWD is narrow and it does not contain ultra high molecular weight material.

THE NUMERICAL EXAMPLE

"Synthetic" or simulated examples are ideal for investigating alternative data treatment procedures. This is because the solutions are *a priori* known, and each of the intervening effects can be individually evaluated. In contrast, in a real SEC analysis, the "true" MWD is never exactly known, and it is difficult to quantify all possible cause-effect interrelationships.

The simulations that follow emulate the analysis of a linear homopolymer *via* SEC. The main assumptions are: (a) the molecular weight calibration is linear; (b) the fractionation and the detection are ideal, but the chromatograms are distorted by a nonuniform and skewed IB, and are corrupted with an additive zero-mean noise; (c) both measured and corrected chromatograms are discrete, with values sampled at a fixed elution volume interval ΔV ; (d) the MWD contains only a (relatively low) number of molecular species as defined by the molecular weight calibration and the points of the corrected mass chromatogram; and (e) the interdetector volume effects are neglected.

The reason for choosing a common ΔV for both the measured and the corrected chromatograms, is in order to simplify the data treatment. The selection of ΔV is in general a compromise between the number of points of the resulting MWD and the difficulty of the numerical inversion. (For example, when reducing ΔV a more detailed MWD is produced, but at a higher computational cost and with potentially deteriorated solutions.) In all calculations, the discrete equivalents of Eqs. (1–5) were used. For example, Eqs. (1, 2) yield:

$$\mathbf{w} = \mathbf{G} \mathbf{w}^c \quad (6a)$$

$$\mathbf{s}_k = \mathbf{G} \mathbf{s}_k^c; \quad (k = w, v, n) \quad (6b)$$

where \mathbf{w} and \mathbf{s}_k are $(m \times 1)$ column vectors containing the ordinates of $w(V)$ and $s_k(V)$, respectively; \mathbf{w}^c and \mathbf{s}_k^c are $(p \times 1)$ column vectors containing the ordinates of $w^c(V)$ and $s_k^c(V)$, respectively; and \mathbf{G} is a $(m \times p)$ matrix that represents $g(V, \tilde{V})$.

The selection of \mathbf{G} is important for an adequate inversion of Eqs. (6). First, it is recommended to develop an analytical expression for $g(V, \tilde{V})$. This allows the calculation of the discrete values of a $g(V)$ function with \tilde{V} at any elution volume in the columns operational range. The continuous functions that typically represent $g(V, \tilde{V})$ never strictly drop to zero, thus, producing "full" \mathbf{G} matrixes with only nonzero elements. The numerical solutions were seen to considerably improve if all the very small elements of \mathbf{G} (e.g., smaller than 1% of the maximum) are set to 0. Also, it was recommendable to choose \mathbf{G} rectangular with $m > p$, and of minimal dimensions. This means that one must include in \mathbf{G} only the individual $g(V)$ functions of \tilde{V} values in the range of the corrected chromatograms $[V_1^c - V_2^c]$. Thus, this range must be estimated before performing the chromatogram inversions.

Assume first that $g(V, \tilde{V})$ is skewed but uniform. In this case, all individual $g(V)$ functions are identical and defined by $(c + 1 + d)$ nonzero points, with c points before the maximum and d points after the maximum. Thus, the number of points of the corrected chromatograms p is simply: $p = m - c - d$. This expression can be extended to nonuniform and skewed IB functions, but in this case, c must be defined as the number of points before the maximum of the limiting $g(V)$ that contributes to the first point of the measured chromatograms, and d as the number of points after the maximum of the limiting $g(V)$ that contributes to the last point of the measured chromatograms. The limits of the corrected chromatograms can be obtained from the range of the measured chromatograms and the limiting $g(V)$ functions, as follows: $V_1^c = V_1 + c \Delta V$, and $V_2^c = V_2 - d \Delta V$.

From p and $[V_1^c - V_2^c]$, \mathbf{G} is obtained by discretizing $g(V, \tilde{V})$ as follows:

$$\mathbf{G} = \begin{bmatrix} g(1,1) & \cdots & 0 & \cdots & 0 \\ \vdots & \ddots & 0 & & \vdots \\ g(c+1,1) & & g(j,j) & & \\ \vdots & \ddots & \vdots & \ddots & 0 \\ g(c+d+1,1) & & g(c+j,j) & & g(p,p) \\ 0 & \ddots & \vdots & \ddots & \vdots \\ \vdots & & g(c+d+j,j) & & g(c+p,p) \\ & & 0 & \ddots & \vdots \\ 0 & \cdots & 0 & \cdots & g(m,p) \end{bmatrix}; (m > p) \quad (6c)$$

where each j -th column of \mathbf{G} contains $c + 1 + d$ nonzero elements that define the discrete $g(V)$ function of $\tilde{V} = V_1^c + (j-1) \Delta V$. Also, the following is verified: (a) \tilde{V} ranges from $\tilde{V} = V_1^c$ in the first column to $\tilde{V} = V_2^c$ in the last column; and (b) if

\tilde{V} is specified at the mode of $g(V)$, then the largest elements of \mathbf{G} appear c rows below the corresponding (j,j) diagonal element.

The direct inversion of Eq. (6a) through for example $\hat{\mathbf{w}}^c = [\mathbf{G}^T \mathbf{G}]^{-1} \mathbf{G}^T \mathbf{w}$ (where “ $\hat{}$ ” indicates estimated value) is not recommendable because $\mathbf{G}^T \mathbf{G}$ is normally bad-conditioned; and this can produce a highly oscillatory $w^c(V)$ with negative peaks. The difficulty of the inversion operation is measured by: (a) the condition number of $\mathbf{G}^T \mathbf{G}$ (defined as the ratio between the largest and the smallest eigenvalues of $\mathbf{G}^T \mathbf{G}$); and (b) the noise contents of $w(V)$.

To invert Eq. (6a), the following singular value decomposition expression was applied.²⁷

$$\hat{\mathbf{w}}^c = \sum_{i=1}^r \frac{\mathbf{u}_i^T \mathbf{w}}{\sigma_i} \mathbf{v}_i \quad (r \leq p); \quad (\sigma_1 \geq \sigma_2 \geq \dots \geq \sigma_r \geq \sigma_{r+1} \geq \dots \geq \sigma_p \geq 0) \quad (7)$$

where \mathbf{u}_i and \mathbf{v}_i are the i -th eigenvectors of $\mathbf{G}\mathbf{G}^T$ and $\mathbf{G}^T\mathbf{G}$, respectively; and σ_i with $(1 \leq i \leq p)$ is a singular value of \mathbf{G} , obtained from the square root of the i -th eigenvalue of $\mathbf{G}^T\mathbf{G}$. The number of “effective” terms in the summation of Eq. (7) is limited to r for producing smooth and nonnegative estimates of \mathbf{w}^c . Thus, r can be seen as the adjustment parameter of the presented algorithm. To invert Eq. (6b), a similar expression to Eq. (7) was used.

The Base Example and the Standard Data Treatment

In Figure 1, a noise-free example is presented which aims at illustrating the deterministic effects of IB. The basic raw data are given by: (a) the “true” or corrected mass chromatogram $w^c(V)$ of Figure 1a); (b) the molecular weight calibration $\log M(V)$ of Figure 1c); and (c) the nonuniform and skewed IB function $g(V, \tilde{V})$ of Figure 1a). All of these functions are discrete, with points defined every $\Delta V = 0.1$ mL. Even though all calculations were developed for a light scattering detector, the case of an $M_n(V)$ -sensitive detector is also included in Figure 1 for comparison reasons.

The corrected mass chromatogram is specified by the sum of two Gaussians as follows: $w^c(V) = 0.2667 \exp[-(V - 41.5)^2/0.72] + 0.24 \exp[-(V - 43.5)^2/2]$; and it consists of $p = 70$ nonzero points in the range $[V_1^c - V_1^c] = 39.6 \text{ mL} - 46.5 \text{ mL}$ [Figure 1a)]. The total sample mass is equal to 10 arbitrary units, and it is equal to the sum of all chromatogram heights. The molecular weight calibration is given by: $\log M(V) = 13.0076 - 0.179941 V$ [Figure 1c)]. The “true” or corrected molar mass chromatograms that would be obtained in the absence of IB are defined by: $s_w^c(V) = 0.02 [M(V) w^c(V)]$ and $s_n^c(V) = 5 \times 10^8 [w^c(V)/M(V)]$; with $M(V)$ as determined from the linear calibration.

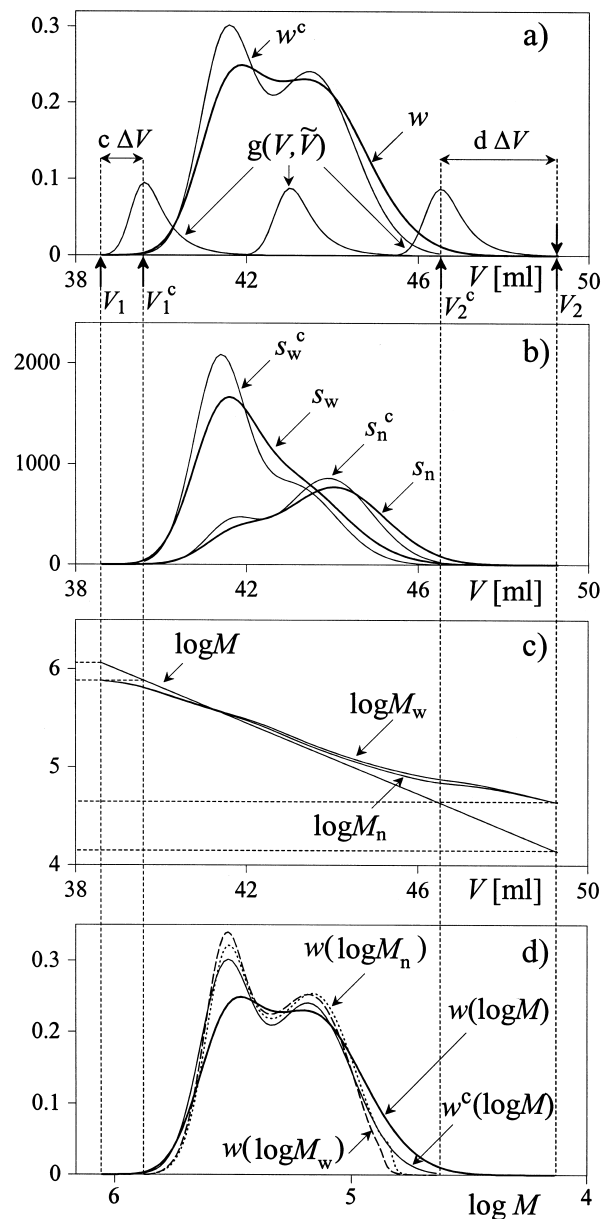


Figure 1. The effects of IB in the noise-free Base Example. a) “True” mass chromatogram, $w^c(V)$; three samples of the nonuniform and skewed spreading function, $g(V, \tilde{V})$; and resulting “measured” mass chromatogram, $w(V)$. b) “True” M_n - and M_w -sensitive molar mass chromatograms, $s_n^c(V)$ and $s_w^c(V)$, respectively; and resulting “measured” chromatograms $s_n(V)$ and $s_w(V)$. c) Unbiased linear calibration $\log M(V)$; and “true” *ad hoc* calibrations $\log M_n(V)$ and $\log M_w(V)$. d) “True” MWD $w^c(\log M)$; MWD estimate $w(\log M)$ obtained from $w(V)$ and $\log M(V)$; MWD estimate $w(\log M_n)$ obtained from $w(V)$ and $\log M_n(V)$; and MWD estimate $w(\log M_w)$ obtained from $w(V)$ and $\log M_w(V)$.

Table 1. The Numerical Example. Average Molecular Weights of the “True” Base MWD and of Various Biased and Unbiased MWD Estimates

MWD	\bar{M}_n	\bar{M}_w	\bar{M}_w/\bar{M}_n
^a $w^c(\log M)$ of Fig. 1d	182000	242000	1.33
^b $w(\log M)$ of Fig. 1d	160000	224000	1.40
^b $w(\log M_n)$ of Fig. 1d	182000	233000	1.28
^b $w(\log M_w)$ of Fig. 1d	191000	242000	1.26
^c $\hat{w}^c(\log \hat{M}_{in})$ of Fig. 3d	185000	243000	1.32
^c $\hat{w}^c(\log \hat{M}_{in}^c)$ of Fig. 5d	188000	243000	1.30
^c $\hat{w}^c(\log \hat{M}_{in}^c)$ of Fig. 6d	183000	241000	1.32

^a “True” Base MWD^b MWD estimate without IB correction.^c Unbiased MWD estimate.

From $w^c(V)$ and $\log M(V)$, the “true” MWD $w^c(\log M)$ of Figure 1d) is obtained. The molar mass range results 43700 g/mol – 762000 g/mol, and the average molecular weights are given in the first row of Table 1. Throughout this work, the MWD abscissas are represented by an inverted $\log M$ axis. This has the advantage of not requiring corrections in the MWD heights with respect to $w^c(V)$ when the molecular weight calibration is linear; and only minor corrections when the calibration is nonlinear. Also, the $\log M$ values of Figure 1d) vertically correspond (through the linear calibration) to the V values of Figures 1a-c).

The IB function $g(V, \tilde{V})$ is an exponentially-modified Gaussian of a constant skewness and a variable standard deviation $\sigma_g = 0.3 - 0.0015 (\tilde{V} - 45)^2$. It is defined by the convolution of $(\sqrt{2\pi} \sigma_g)^{-1} \exp[-(V - \tilde{V} + 0.2)^2 / 2\sigma_g^2]$ with $\exp[-2V]$. Each individual $g(V)$ function exhibits 39 nonzero points, and is normalized in the sense that the sum of all its heights is equal to 1. Since the average retention volume \tilde{V} is adopted at the peak of each $g(V)$; then there are $c = 10$ nonzero points before the maximum of $g(V)$ and $d = 28$ nonzero points after the maximum. In Figure 1a), only the two limiting $g(V)$ functions and one intermediate $g(V)$ are shown. The limiting $g(V)$ functions correspond to $\tilde{V} = V_1^c$ and $\tilde{V} = V_2^c$. Note that $g(V, V_1^c)$ is slightly narrower than the other two $g(V)$ functions illustrated in Figure 1a).

By application of Eqs. (1) and (2), the following “measured” chromatograms were calculated: $w(V)$ of Figure 1a), and $s_w(V)$ and $s_n(V)$ of Figure 1b). All of these chromatograms are defined by $m = 70 + 39 - 1 = 108$ nonzero points in the range $[V_1 - V_2] = 38.6 \text{ mL} - 49.3 \text{ mL}$ [Figure 1a)]. Note that $[V_1^c - V_2^c]$ is narrower than $[V_1 - V_2]$ by $c\Delta V$ at the left hand side and by $d\Delta V$ at the right hand side (Figure 1a).

From the mass chromatogram and the linear calibration, the (broadened) MWD $w(\log M)$ of Figure 1d) is obtained. The average molecular weights are pre-

sented in Table 1. Both averages are underestimated as a consequence of the IB skewness. The global polydispersity is (as expected) overestimated.

At each retention volume of $w(V)$, the instantaneous MWD is calculated by considering the contributions (toward that V) of all the molecular species of $w^c(V)$. The instantaneous distributions are not shown for reasons of space. From such distributions, $M_n(V)$ and $M_w(V)$ were calculated, and the corresponding *ad hoc* calibrations $\log M_n(V)$ and $\log M_w(V)$ are represented in Figure 1c). As expected, these calibrations are nonlinear and in general less steep than $\log M(V)$. Also, $\log M_w(V)$ is above $\log M_n(V)$ at all points except at the limits of the measured chromatograms where strictly monodisperse species are to be expected.¹¹

From $w(V)$ and $\log M_n(V)$, the MWD estimate $w(\log M_n)$ of Figure 1d) was obtained. Similarly, $w(\log M_w)$ of Figure 1d) was obtained from $w(V)$ and $\log M_w(V)$. The deviations of $w(\log M_n)$ and $w(\log M_w)$ from $w^c(\log M)$ are only caused by the IB. The average molecular weights are presented in Table 1. As expected, $w(\log M_n)$ accurately estimates \bar{M}_n but underestimates \bar{M}_w , while $w(\log M_w)$ accurately estimates \bar{M}_w but estimates \bar{M}_n . Thus, in both cases the global polydispersity is underestimated.

In Figure 2, the Base Example of Figure 1 is reconsidered, but disregarding the M_n -sensitive detector and adding a random noise into the noise-free measurements. The additive noises correspond to zero-mean Gaussian distributions. The noise of $w(V)$ in Figure 2a) exhibits a standard deviation of 0.0016. The noise of $s_w(V)$ in Figure 2b) presents a standard deviation of 18.3.

In the standard data treatment, the estimate of $M_w(V)$ is directly obtained from $\hat{M}_w(V) = s_w(V) / [0.02 w(V)]$. Due to the measurement noise, the resulting $\log \hat{M}_w(V)$ of Figure 2c) is highly oscillatory at the chromatogram tails. For comparison, note that the “true” $\log M_w(V)$ is smooth and it varies monotonically with V . The oscillatory nature of $\log \hat{M}_w(V)$ makes it impossible to recuperate a MWD. In effect, the estimate $w(\log \hat{M}_w)$ of Figure 2c) is not a function at the distribution tails, and for this reason the average molecular weights were not calculated.

The Correction Method

The following method is proposed for producing an unbiased MWD: (i) estimate $w^c(V)$ and $s_k^c(V)$ by inversion of Eqs. (1)–(2); (ii) estimate the unbiased molecular weight calibration $\log \hat{M}(V)$ through Eq. (5), and use its mid-region for adjusting an unbiased linear calibration $\log \hat{M}_{in}(V)$; and (iii) recuperate an unbiased MWD $\hat{w}^c(\log \hat{M}_{in})$ from $\hat{w}^c(V)$ and $\log \hat{M}_{in}(V)$. Note that the linear calibration requirement can be independently verified through a normal SEC calibration with narrow standards. Also note that these standards must not necessarily be of the same chemical nature of the analyzed polymer. (If the fractionation is by hydrodynamic volume and the molecular weight calibration for a given set of

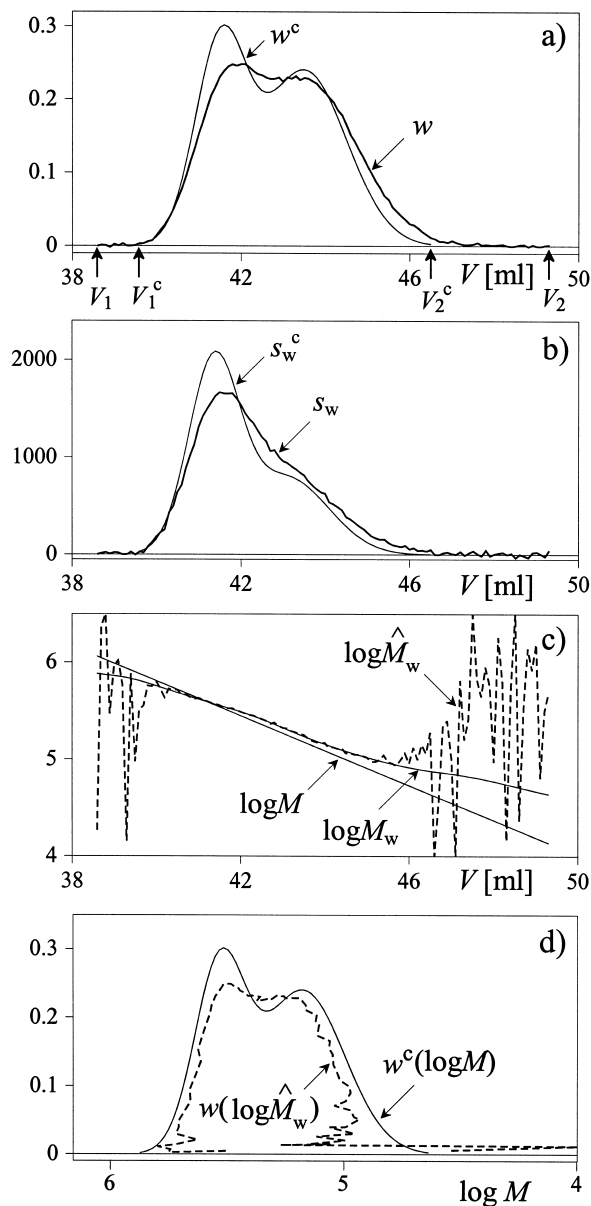


Figure 2. Base Example and standard data treatment. a) “True” mass chromatogram $w^c(V)$; and measured (noisy) mass chromatogram $w(V)$. b) “True” molar mass chromatogram $s_w^c(V)$; and measured (noisy) molar mass chromatogram $s_w(V)$. c) Unbiased linear calibration, $\log M(V)$; “true” *ad hoc* calibration $\log M_w(V)$; and estimated *ad hoc* calibration $\log \hat{M}_w(V)$. d) “True” MWD $w^c(\log M)$; and (unacceptable) MWD estimate $w(\log \hat{M}_w)$, obtained from $w(V)$ and $\log \hat{M}_w(V)$.

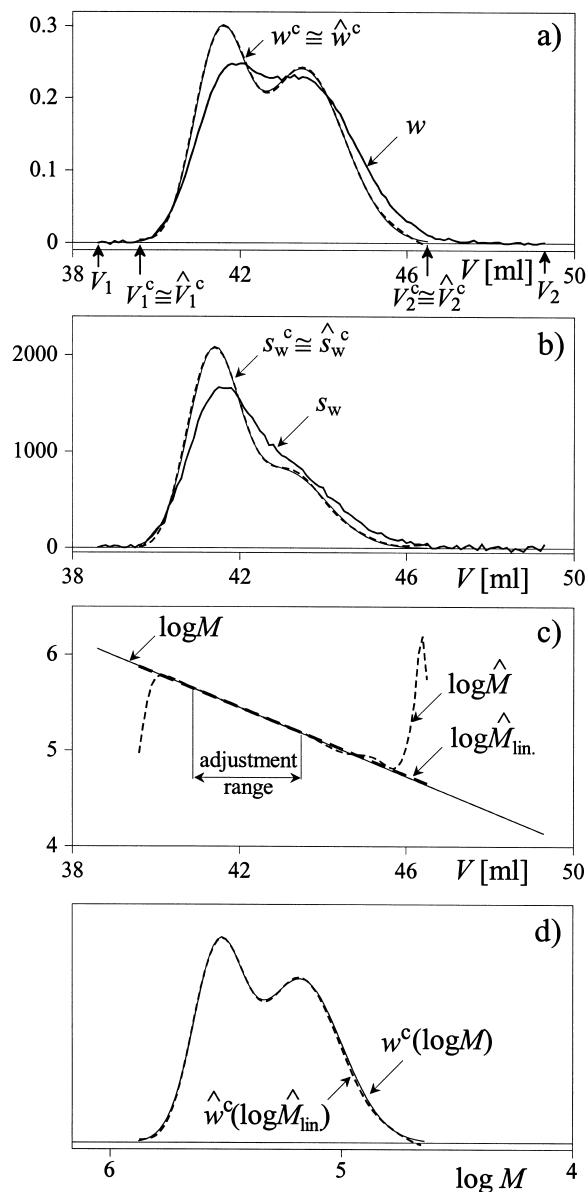


Figure 3. Base Example and proposed correction method assuming the exact chromatogram range. a) "True" mass chromatogram $w(V)$; measured mass chromatogram $w^c(V)$; and estimated true chromatogram $\hat{w}^c(V)$. b) "True" molar mass chromatogram $s_w(V)$; measured molar mass chromatogram $s_w^c(V)$; and estimated true molar mass chromatogram $\hat{s}_w^c(V)$. c) Unbiased linear calibration $\log M(V)$; estimated unbiased calibration $\log \hat{M}(V)$, and estimated linear unbiased calibration $\log \hat{M}_{lin}(V)$. d) "True" MWD $w^c(\log M)$ and MWD estimate $\hat{w}^c(\log \hat{M}_{lin})$, obtained from $w^c(V)$ and $\log \hat{M}_{lin}(V)$.

standards is linear, then it will also remain linear for standards of a different nature.)

The procedure was applied to the noisy chromatograms $w(V)$ and $s_w(V)$ of Figures 2a, b), that are reproduced in Figures 3a, b). The dimensions of \mathbf{G} are $(m \times p) = (108 \times 70)$. For the mass chromatogram inversion, Eq. (7) was adjusted to $r = 14$ nonzero terms (from a maximum of 70 terms). The adjustment was carried out as if the true corrected chromatograms were unknown, and with the criterion of producing smooth solutions with minimal negative peaks. In the molar mass chromatogram inversion, the solving expression was adjusted to 12 nonzero terms.

The final estimates are $\hat{w}^c(V)$ and $\hat{s}_w^c(V)$ of Figures 3a, b). These functions are smooth and close to the true $w^c(V)$ and $s_w^c(V)$. In both inversions, the range of the measured chromatograms was assumed known. For this reason, the range of the estimated corrected chromatograms $[\hat{V}_1^c - \hat{V}_2^c]$ exactly coincides with the true range $[V_1^c - V_2^c]$ [Figure 3a)].

The unbiased calibration estimate was calculated from $\log \hat{M}(V) = \log\{\hat{s}_w^c(V)/[0.02 \hat{w}^c(V)]\}$ [Figure 3c)]. This function almost coincides with the “true” $\log M(V)$ in the mid-chromatogram region, while it diverges at the tails. The linear calibration $\log \hat{M}_{in}(V)$ was adjusted from the points of $\log \hat{M}(V)$ contained in the “adjustment range” that is indicated in Figure 3c). Note that $\log \hat{M}_{in}(V)$ needs to be calculated only in $[V_1^c - V_2^c]$.

Finally, the unbiased distribution $\hat{w}^c(\log \hat{M}_{in})$ of Figure 3d) was obtained from $\hat{w}^c(V)$ and $\log \hat{M}_{in}(V)$. The MWD estimate is smooth and close to the “true” $w_c(\log M)$. Also, the estimated average molecular weights are close to the real values (Table 1).

Some Checks on the Method's Robustness

The proposed procedure requires a good estimation of the corrected chromatograms. Following are discussions of some of the problems associated to the chromatogram inversions.

In the Base Example of Figure 3, the true range of the measured chromatograms was assumed known for the inversion operations. In real practice, it may be difficult to determine this range due to detector inaccuracies, the presence of a baseline noise and drift, and the possible contamination with nonpolymeric low molecular weight material. In Figure 4, the Base Example of Figure 3 is reconsidered, but adopting an underestimated range of the measured chromatograms given by $[\hat{V}_1 - \hat{V}_2] = 39.0 \text{ mL} - 47.0 \text{ mL}$ (Figure 4).

This range was obtained by admitting only positive values in the measured chromatograms. Then, the range of the corrected chromatograms was estimated from the limiting $g(V)$ functions presented in Figure 4a), and is indicated by $[\hat{V}_1^c -$

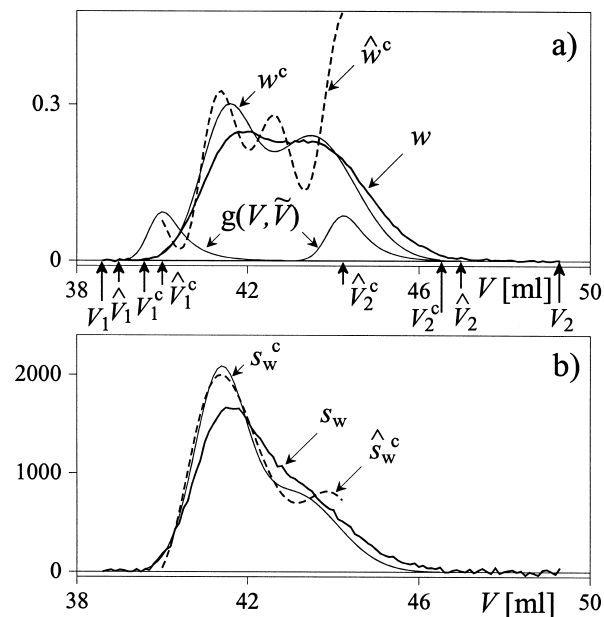


Figure 4. Base Example and proposed correction method assuming a reduced chromatogram range. a) “True” mass chromatogram $w^c(V)$; measured mass chromatogram $w(V)$; estimation of the true mass chromatogram $\hat{w}^c(V)$; and two samples of $g(V, \tilde{V})$ at the limits of the estimated corrected chromatogram range. b) “True” molar mass chromatogram $s_w^c(V)$; measured molar mass chromatogram $s_w(V)$; and estimation of the true molar mass chromatogram $\hat{s}_w^c(V)$.

\hat{V}_2^c] in Figure 4a). As expected, this range is narrower than the true $[V_1-V_2]$; and the dimensions of the \mathbf{G} matrix are now (81×43) . In this case, it is impossible to produce reasonable estimates of the corrected chromatograms [see $\hat{w}^c(V)$ and $\hat{s}_w^c(V)$ of Figures 4a,b)]. Thus, it is impossible to recuperate the unbiased molecular weights, and the method fails.

In Figure 5, the adopted chromatogram range was extended with respect to the real $[V_1-V_2]$. To this effect, 10 zeroes were added before V_1 and 10 zeroes were added after V_2 . The resulting range $[\hat{V}_1-\hat{V}_2] = 37.6 \text{ mL} - 50.3 \text{ mL}$ falls outside the scale of Figures 5a-c); and the dimensions of \mathbf{G} are increased to (128×90) . In this case, the recuperated corrected chromatograms are quite close to the real functions in spite of the fact that the new estimated range $[\hat{V}_1^c-\hat{V}_2^c]$ is broader than $[V_1^c-V_2^c]$ [Figures 5a,b)]. The final MWD estimate is quite acceptable, except perhaps for the negative peak observed at the low molecular weight end [Figure 5d)]. The averages are also acceptable as estimated (Table 1).

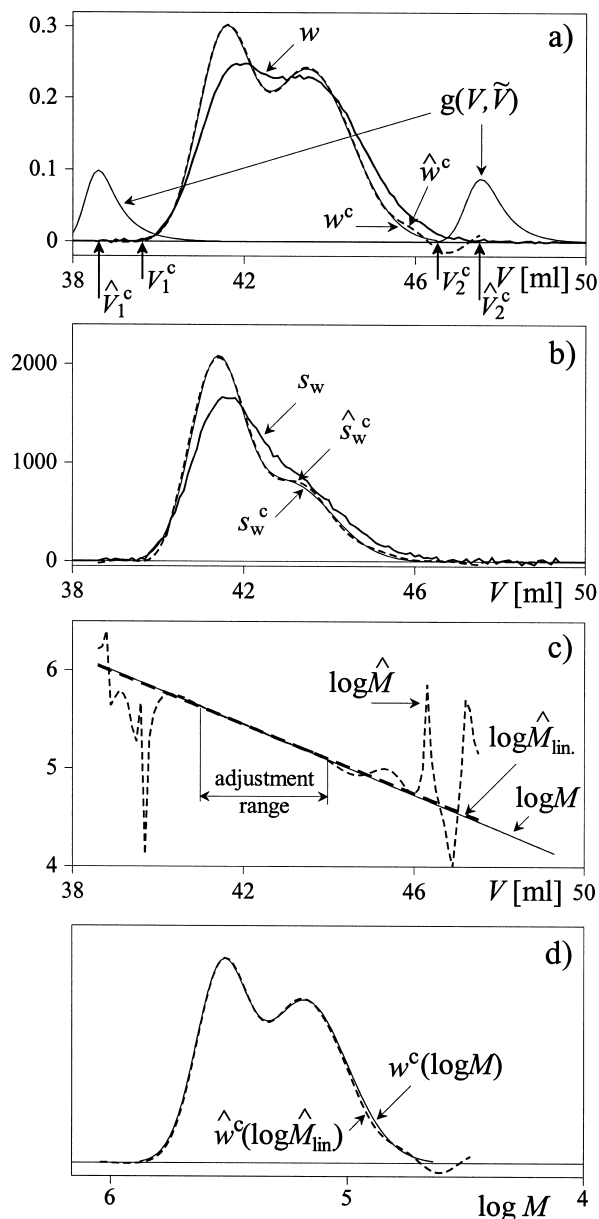


Figure 5. Base Example and proposed correction method assuming an extended chromatogram range. a) “True” mass chromatogram $w^c(V)$; measured mass chromatogram $w(V)$; estimated true mass chromatogram $\hat{w}^c(V)$; and two samples of $g(V, \tilde{V})$ at the limits of the estimated corrected chromatogram range. b) “True” molar mass chromatogram $s_w^c(V)$; measured molar mass chromatogram $s_w(V)$; and estimation of the true molar mass chromatogram $\hat{s}_w^c(V)$. c) Unbiased linear calibration $\log M(V)$; estimated unbiased calibration $\log \hat{M}(V)$; and estimated linear unbiased calibration $\log \hat{M}_{\text{lin}}(V)$. d) “True” MWD $w^c(\log M)$ and estimated MWD $\hat{w}^c(\log \hat{M}_{\text{lin}}(V))$, obtained from $w^c(V)$ and $\log \hat{M}_{\text{lin}}(V)$.

From the results of Figures 4 and 5, it is concluded that when the limits of the measured chromatograms are uncertain, then it is preferable to overestimate the chromatogram range rather than to underestimate it.

In most SEC experiments, the true IB function is only scarcely known. To simulate this situation, the case of an ill-defined IB function is presented in Figure 6. Like in Figures 1 – 5, the “true” $g(V, \tilde{V})$ is nonuniform and skewed [Figure 6a)]. Also, the measured chromatograms of Figures 2 – 5 are reproduced in Figures 6a, b). However, for the chromatogram inversions, we shall adopt a uniform and symmetrical IB function. It is defined by: $\hat{g}(V, \tilde{V}) = (\sqrt{2\pi} \cdot 4)^{-1} \exp[-(V - \tilde{V})^2/0.32]$, and each individual $\hat{g}(V)$ consists of 27 nonzero points [Figure 6a)].

For the inversion operations, the extended range 37.6 mL–50.3 mL previously considered in Figure 5 is readopted. The corrected chromatogram estimates are $\hat{w}^c(V)$ and $\hat{s}_w^c(V)$ of Figures 6a, b). Relatively large deviations are observed in $\hat{w}^c(V)$ and $\hat{s}_w^c(V)$. The estimated range $[\hat{V}_1^c - \hat{V}_2^c] = 38.9 \text{ mL} - 49.0 \text{ mL}$ is, as before, broader than $[V_1^c - V_2^c]$. The molecular weight calibration estimate $\log \hat{M}(V)$ exhibits a bias toward the higher molecular weights, and this is also observed in $\log \hat{M}_{in}(V)$ [Figure 6c)]. The deviations in the corrected chromatograms are partly compensated by the deviations in the linear calibration estimate. For this reason, the final MWD estimate $\hat{w}^c(\log \hat{M}_{in})$ of Figure 6d) presents only moderate deviations with respect to the true function, and also the errors in the average molecular weight estimates are relatively small (Table 1).

Even though no numerical results will be shown for reasons of space, the following tests were also carried out on the basis of the Base Example of Figure 3. First, the standard deviation of the measurement noises were duplicated. In this case, an almost identical final MWD estimate was obtained, thus, verifying the method robustness in front of (a rather high) measurement noise.

Second, the effect of the discredited interval was evaluated, by halving and doubling ΔV with respect to the original value. Again, practically identical final results were obtained. Finally, the whole MWD shape was narrowed while maintaining the same IB function. In this case, the method fails when the polydispersity becomes lower than around 1.1. This is to be expected for two reasons: (i) the inversion operations become worse conditioned; and (ii) the linear range of the estimated calibration is narrowed, which is the reason why the calibration slope becomes increasingly difficult to estimate.

CONCLUSIONS

The correction for IB is important when quantitative MWDs must be obtained from SEC with molar mass detection. A phenomenological correction procedure was proposed, and therefore, no restrictions on the shapes of the MWD

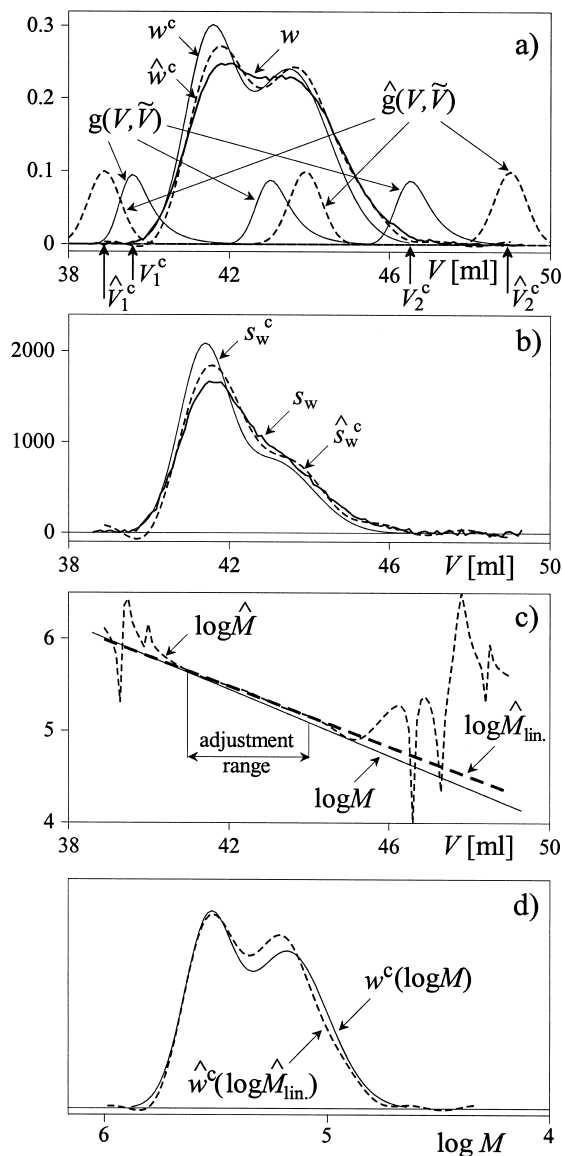


Figure 6. Base Example and proposed correction method assuming an erroneous IB function. a) "True" mass chromatogram $w^c(V)$; measured mass chromatogram $w(V)$; estimate of the true mass chromatogram $\hat{w}^c(V)$; "true" IB function $g(V, \tilde{V})$; and IB function adopted for the inversion operation $\hat{g}(V, \tilde{V})$. b) "True" molar mass chromatogram $s_w^c(V)$; measured molar mass chromatogram $s_w(V)$; and estimate of the corrected molar mass chromatogram $\hat{s}_w^c(V)$. c) Unbiased linear calibration $\log M(V)$; estimated unbiased calibration $\log \hat{M}_{lin}(V)$, and estimated unbiased linear calibration $\log \hat{M}_{lin}(V)$. d) "True" MWD $w^c(\log M)$ and estimated MWD $\hat{w}^c(\log \hat{M}_{lin})$, obtained from $w^c(V)$ and $\log \hat{M}_{lin}(V)$.

or the IB function are imposed. In the simulated example, the procedure was almost exactly able to recuperate the true MWD.

The main limitation of the technique is the potential instability of the chromatogram inversions. To improve the inversions, it was recommended to minimize the dimensions of \mathbf{G} for the given discredited interval, and to make zero all the elements of \mathbf{G} lower than 1% of the maximum value. The singular value decomposition technique is able to provide good inversion solutions; it is simple to implement, and it is simple to adjust. When the range of the measured chromatograms is uncertain, then it is preferable to adopt an extended range by simply adding zeroes before and after the chromatograms.

The technique seems ideal for moderately narrow MWDs or for broader MWDs that are multimodal and/or "rich" in high frequency contents. For very narrow MWDs, the method fails since it becomes incapable of recuperating the linear calibration.

Even though light scattering detectors were mainly investigated, the method is also applicable to viscosity sensors. This will be the subject of a future communication.

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