Journal of Chromatography A, 1443 (2016) 267–271

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

A broad-standard technique for correcting for band broadening in size-exclusion chromatography

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ARTICLE INFO

Article history:
Received 6 January 2016
Received in revised form 5 March 2016
Accepted 12 March 2016
Available online 17 March 2016

Keywords:
Size exclusion chromatography (SEC)
GPC
Band broadening

ABSTRACT

Band broadening in size-exclusion chromatography (SEC) is always present to some extent. Broadening effects on averages such as the weight- and number average molecular weights ($\overline{M_W}$ and $\overline{M_n}$ respectively) are minimal with modern SEC systems. However, broadening distorts the shape of the true molecular weight distribution (MWD), which causes problems if one wants to compare the detailed form of the MWD to a model. An addition to current methods for overcoming this problem is presented. One starts with a sufficiently wide range of samples whose exact values of $\overline{M_n}$ and $\overline{M_W}$ have been measured by non-SEC methods (e.g. by fluorimetry and light scattering, respectively, of the sample without size separation). A true (unbroadened) molecular weight distribution for a sample can be obtained by deconvolution (here using a maximum-entropy algorithm) by fitting SEC data for these samples to these exact $\overline{M_n}$ and $\overline{M_W}$ values to find the values of the parameters in a sufficiently flexible assumed broadening function. This was modelled using simulated band broadening and subsequent deconvolution, with the broadening parameters least-squares fitted to the "exact" sets of values of $\overline{M_n}$ and $\overline{M_W}$. The results show that if these $\overline{M_n}$ and $\overline{M_W}$ values are for a series of broad (not narrow) standards covering a sufficient range of molecular weight, then after deconvolution, a good representation of the original molecular weight distribution used in the simulation is obtained. The method should prove useful for water-soluble polymers, for which it is often difficult to obtain narrow standards of a wide range of molecular weight, as required in a number of well-established methods for correcting for band broadening.

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1. Introduction

Size-exclusion chromatography (SEC, sometimes called GPC) separates molecules by molecular size. Band broadening (BB) is always present, causing a perfectly monodisperse sample to elute over a range of elution time (or elution volume) [1]. Effects from this artifact are relatively small with modern SEC set-ups, and do not cause very large errors in averages such as the weight- and number-*average* molecular weights, $\overline{M_W}$ and $\overline{M_n}$ [2]. However, they pose a problem with the detailed shape of the molecular weight *distribution* (MWD). For example, theory [3] indicates that the number MWD formed at any point in a free-radical polymerization should closely follow a single exponential form and thus be linear in a logarithmic plot, but BB causes curvature in such a plot [4].

There are a number of methods for correcting for BB. Methods employing various types of mass spectrometry can be very accurate (e.g. [5–7]), but these techniques cannot be applied to many polymer systems, e.g. those containing very high molecular weights. Some quantifications of this effect are as follows. The van Deemter equation [8] is used to quantify the BB effect by combining various terms for multiple paths, longitudinal diffusion and equilibration time. "Darcy's law" [9] is widely used for the quantitative analysis of flow in porous medium systems. The Tung convolution equation is the most common approach (see below), where BB effects are described by a BB function in a convenient form.

Several procedures have proposed to correct for BB, e.g. [10–17]. Some newer methods focus on describing calibration approach which involve simultaneously the use of SEC and multi-angle light scattering (MALS) techniques [13–16]. As one example, Suarez et al. [16] developed a method [16] based on diffusion effects.

For some applications, it is important to obtain an accurate shape of the MWD, which requires finding the BB function. Once this function has been obtained, various methods [18] can be

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http://dx.doi.org/10.1016/j.chroma.2016.03.030
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employed to invert the convolution equation to obtained the true size distribution of a given analyte sample.

The best method for finding this BB function is to determine it directly using ultra-narrow standards [18], but this is extremely laborious and expensive. There are a number of methods requiring moderately narrow standards (see, e.g. [19–21] for summaries of these methods and other BB effects in SEC). However, obtaining narrow standards over a wide range of M is sometimes difficult, especially in the case of water-soluble polymers where common methods, such as anionic polymerization, often cannot be employed. As pointed out elsewhere [19], this problem cannot be overcome by using multiple detection alone, because, for example, measuring absolute molecular weight using in-line light scattering detection will not help because each elution slice still contains a range of molecular weights.

Here we present a new method which should be applicable to a wide range of analytes and which uses broad (rather than narrow) standards, which are relatively easy to prepare or obtain. The principle is as follows. For a given polymer, our method uses exact values of $\overline{M}_n$ and $\overline{M}_W$ obtained without BB artifacts (i.e. without size separation by SEC), which can be done by several means. $\overline{M}_n$ can be measured by osmometry or by employing quantitative labeling and fluorimetry; quantitative labeling can be implemented by techniques appropriate to the analyte, e.g. through reaction with the chemically-unique end-group for the water-soluble α-(1 ⟶ 4)-linked linear molecules obtained by enzymatically debranching starch [22]. $\overline{M}_W$ can be measured by multiple-angle laser light scattering without size separation. We show here that if these $\overline{M}_n$ and $\overline{M}_W$ data are obtained for a sufficient number of polydisperse samples covering a sufficiently wide range of molecular weight, then the parameters in the BB function can be obtained by least-squares fitting to SEC signals for these samples. Given this function, BB can be corrected by solving the convolution equation relating the SEC signal and the actual MWD.

In this paper, simulations using realistic parameters are used to test if the technique should be able to work in the laboratory. The parameters chosen are applicable to α-(1 ⟶ 4)-linked linear glucose polymers.

If the analyte is a linear polymer, there is a one-to-one correspondence between this size and molecular weight M; for notational simplicity; the present paper only considers linear polymers, although the method is equally well applicable to branched ones, with hydrodynamic volume replacing mass throughout.

As noted by a reviewer, BB can be caused by chromatographic BB, interdetector BB, or a combination of both. The present method only uses SEC data from a single detector, the concentration-dependent differential refractive index detector, so interdetector BB will not be present. If multiple detectors were to be used, the method would be applied separately to the signal for each detector.

2. Theory/calculation

BB is quantified by the Tung convolution equation [23], which is here written in a convenient form for numerical evaluation:

$$S(V) = \int_{-\infty}^{\infty} G(\log M, V) w(\log M) d(\log M) \quad (1)$$

Here $S(V)$ is the detector signal at a given elution volume V, $w(\log M)$ is the true (unbroadened) weight SEC distribution (so named because it is the signal that would be obtained using a differential refractive index detector in an ideal SEC system without BB and with a linear calibration curve), and $G(\log M, V)$ is the BB function. It is assumed that a calibration curve $Vc(\log M)$ relating molecular weight (from relatively narrow standards) to V is available. If BB were absent, then one can obtain the SEC weight distribution from the detector signal using (e.g. [24]):

$$w(logM) = S(V)\frac{dV_c(logM)}{d(logM)}|_{V=V_{c(logM)}} \quad (2)$$

All distributions used here have arbitrary normalization unless otherwise stated.

Three types of function have some usage as broadening functions: the standard Gaussian function, the exponentially modified Gaussian (EMG), the exponentially-Gaussian hybrid (EGH) function and the rarely-used Giddings-Eyring function [25]. The first cannot adequately account for the observation that the peak shapes of ultranarrow standards in SEC are systematically skewed and the width of spreading can vary with molecular weight [17]. The other two functions can take this into account, but the EMG does not lend itself to physical interpretation [26]. For this reason, the BB function is taken to be an EGH [27] (although any appropriate function could be used):

$$G(logM, V; \sigma, \tau) = \begin{cases} \frac{1}{C}\exp\left(\frac{-(V-V_C(M))^2}{2\sigma^2+\tau(V-V_C(M))}\right), & 2\sigma^2+\tau(V-V_C(M))>0 \\ 0, & 2\sigma^2+\tau(V-V_C(M))\le 0 \end{cases} \quad (3)$$

where σ and τ are the BB parameters and C is a normalization constant.

Our simulation procedure to test the method is as follows. We assume a set of exact $I = 1, \ldots, n$ distributions $w(\log M)$, $wI(\log M)$. From these, simulated "exact" $\overline{M}_n,I$ and $\overline{M}_W,I$ values for each set (as could be obtained from quantitative labeling/fluorescence and light scattering, respectively) are calculated using:

$$\bar{M}_{n,exact} = \frac{\int_0^\infty MN(M)\,dM}{\int_0^\infty N(M)\,dM}; \bar{M}_{w,exact} = \frac{\int_0^\infty M^2N(M)\,dM}{\int_0^\infty MN(M)\,dM}; N(M) = \frac{w(logM)}{M^2} \quad (4)$$

where $N(M)$ is the number MWD.

Simulated broadened SEC distributions $wsim,I(\log M)$ are calculated from these exact $wI(\log M)$ in the present case using various values of σexact and τexact, using Eqs. (1) and (2). The least-squares fitting procedure to obtain simulated values of σ and τ is then as follows. (1) Start with assumed values of σ and τ. (2) Invert the deconvolution equation, Eq. (1), with these values to obtain first estimates of the deconvoluted values of $w(\log M)$. (3) Use these calculated $w(\log M)$ to find $\overline{M}_n$ and $\overline{M}_W$ for these values of σ and τ for each sample, $\overline{M}_n^{calc\circ I}(\sigma,\tau)$ and $\overline{M}_W^{calc\circ I}(\sigma,\tau)$. The values of σ and τ are obtained by least-squares minimizing the function:

$$\chi = \sum_{I=1\ldots n}\left(\left[\left[\overline{M}_n^{calc\circ I}(\sigma,\tau)-\overline{M}_{n,I}\right]^2-\left[\overline{M}_w^{calc\circ I}(\sigma,\tau)-\overline{M}_{w,I}\right]^2\right) \quad (5)$$

The method is illustrated in Fig. 1.

The convergence criterion used here is that the changes in the sum of $[\int|wI(\log M) - wI\text{- - }1(\log M)|\ d\ \log M]/\int|wI(\log M)|\ d\ \log M$ over all samples is less than a chosen tolerance (here 10–7). The minimization uses the Metropolis importance sampling method (see Supplementary information) [28].

The form of the assumed "exact" $wI(\log M)$ used to generate the assumed "exact" $\overline{M}_n,I$ and $\overline{M}_W,I$ and the broadened $w(\log M)$ is sums of a single-exponential $N(M)$:

$$N(M) = \sum_{i=1,3} a_i e^{-M/\alpha},\ w(logM) = M^2N(M) \quad (6)$$

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