

Determination of the interdetector volume by s-detection in size-exclusion chromatography of polymers with on-line multiangle light-scattering detection

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Received 25 January 2002; received in revised form 8 March 2004; accepted 4 June 2004

Abstract

The determination of the interdetector volume (IDV) in size-exclusion chromatography (SEC) of polymers with dual multiangle light-scattering/concentration detection based on the s-detection is demonstrated. By s-detection the determination is meant of local molecular mass from a known relation between molecular mass and radius of gyration, obtained from the angle dependence of the intensity of scattered light. IDV is found basing on the stipulation of equal slope of local calibrations found by the dual light-scattering/concentration detection, referred to as the w- and s-detection. As the s-detection uses just the light-scattering detector and, therefore, is independent of the value of IDV, this quantity can be found by searching for its value that gives the local calibration dependence obtained by the dual detection closest to that determined independently by the s-detection.

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Keywords: Detection, LC; Multiangle light scattering detection; Interdetector volume; Radius of gyration; Peak broadening; Polymers

1. Introduction

In the evaluation of multiple-detector size-exclusion chromatography (SEC) data, the accurate value of the interdetector volume (IDV) is of prime importance, particularly so in analyses of samples with narrow molecular-weight distribution (MWD) [1]. For two subsequent detectors, IDV is the volume of tubing between both measuring cells. For parallel arranged detectors, IDV is the difference between volumes of tubing connecting the separation system with individual measuring cells. The geometric determination of IDV leads to unsatisfactory results [2] and the most frequently used way for its determination is finding it as the difference between the elution volumes of apexes of narrow elution curves recorded by two successive detectors [3]. The polystyrene reference standards, usually used for the calibration as well as for finding the interdetector volume, although of very narrow MWD, are not perfectly uniform in

molecular weight, M . Their non-uniformity, expressed as the weight-to-number-average molecular-weight ratio, \bar{M}_w/\bar{M}_n , is larger than one. As a consequence, the maxima of the elution curves, corrected for IDV, are shifted apart and IDV determined from the difference between the elution volumes of the maxima of the two elution curves is charged with an error. This error is often neglected. Precise calculations for a SEC set with concentration and light-scattering detection showed that, if extremely narrow-MWD samples of $1.001 < \bar{M}_w/\bar{M}_n < 1.005$ are used for the determination of the interdetector volume, the expected error for a sample of $\bar{M}_w/\bar{M}_n = 1.05$ is between 5% and 20% in terms of $(\bar{M}_w/\bar{M}_n) - 1$ and decreases with increasing \bar{M}_w/\bar{M}_n ratio of the sample [4].

Various other procedures have been suggested for indirect assessment of IDV, such as estimation from the difference in peak onset of concentration and light-scattering elution curves [5], replacing the SEC columns by a piece of empty tubing and measuring peak onsets or peak maxima at reduced flow rate [6]. Several authors recommend [5,7,8] for the IDV determination a method in which the local calibration dependences are employed, i.e., dependences of M

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on elution volume obtained by SEC analysis of one sample with a concentration and a molecular-weight-sensitive detector. The slope of the local calibration dependence of broad-MWD samples is much less affected by band broadening than the slope of that dependence for narrow-MWD samples, the latter slope being decreased more by band broadening. IDV is determined by searching for its value that gives the best fit between the slope of the local calibration dependence for a broad-MWD sample and the slope of the calibration dependence obtained by measuring the elution volumes for a series of narrow-MWD samples covering a broad range of M . The method was tested with low-angle light-scattering [5] and viscometric detection [8]. When using a viscometric detector, calibration dependences can be expressed in terms of intrinsic viscosities or in terms of molecular weights [8], when parameters of the Mark–Houwink–Kuhn–Sakurada (MHKS) equation, describing the molecular-weight dependence of intrinsic viscosity, are known.

Each method of IDV determination faces specific problems [5]. Clearly, there is a need for an accurate method of IDV determination independent of sample parameters, potential experimentalist's bias, and not requiring computer simulations [9]. The complexity of the problem consists in the fact that the experimentally found local calibration is a result of superposition of the effects of sample MWD, IDV and peak broadening, the extent of the latter being preferably found from the local calibration slope [10].

A powerful and frequently used array of detectors in present-day SEC is the combination of a multiangle light-scattering (MALS) photometer with a concentration detector. The MALS photometer enables us, for sufficiently large macromolecules, to determine at all elution volumes not only the local molecular weight but also the local value of the root-mean-square radius of gyration, $\langle s^2 \rangle^{1/2}$ [11]. (For the sake of simplicity, the term "radius of gyration" and the symbol s are used for $\langle s^2 \rangle^{1/2}$ hereinafter.) For linear chains, radius of gyration is a single-valued function of M and its value can thus be used for an alternative molecular-weight determination, provided that the $s - M$ relation is known. Such M value results from MALS data solely and does not require knowledge of IDV, unlike the calculation of molecular weight from MALS and concentration data.

SEC with MALS and concentration detectors has the potential of yielding two local calibrations, viz., that obtained from the signals of both detectors and that resulting from the MALS signal only. In the following, the former mode of detection is called the w-detection and the latter one the s-detection.

Both local calibrations are affected by band broadening. The local calibration obtained by the w-detection depends, in addition to band broadening, also very sensitively on the IDV value used for the data processing, whereas the local calibration resulting from the s-detection is invariant to IDV.

This offers a unique possibility for assessing a reasonably accurate value of IDV for SEC sets with MALS and

concentration detectors. To get a good approximation to the correct IDV value, we calculate the local calibration from the w-detection data for varying IDV values and the IDV value that makes the local calibrations calculated from the w- and s-detection data coincide is the correct, or nearly correct, value of IDV.

This procedure is satisfying for most practical purposes. When, however, polymers with narrow MWD are analyzed, an accurate value of IDV is absolutely essential [4]. And here, a delicate question present itself. In SEC analyses the peak-broadening effect is always operative and results in local non-uniformity. The local molecular weight found by the w-detection is the weight-average value, \bar{M}_w . The s-detection yields a z -average molecular weight, \bar{M}_z , for theta conditions and a complex average, \bar{M}_s , depending on the extent of the excluded volume effect in thermodynamically good solvents [12]. Thus, generally, the two molecular-weight averages are not identical and one worries how much this non-identity might affect the accuracy of IDV determination. From our model calculations [13] it follows that, for efficient SEC systems, the differences between the local molecular-weight averages yielded by the w- and s-detection are far below the effect of experimental error and do not devalue the suggested method of IDV determination.

2. Theory

The dependence on the angle of observation of the intensity of light scattered from an infinitely dilute solution of a non-uniform polymer can be written in a form convenient for data evaluation as [14]:

$$\frac{K(\text{dn/dc})^2 c}{R(\theta)} = \frac{1}{\bar{M}_w} \left(1 + \frac{16\pi^2}{3\lambda^2} \sin^2 \frac{\theta}{2} \frac{1}{\bar{M}_w} \sum_i w_i M_i s_i^2 \right) \quad (1)$$

where $R(\theta)$ is the intensity of light scattered at the angle θ expressed in terms of the excess Rayleigh ratio, dn/dc is the refractive index increment, K is an optical constant, c is the mass concentration of the polymer, w_i is the weight fraction of polymer molecules with radius of gyration s_i and molecular weight M_i , and λ is the wavelength of the incident light in the medium.

The expression $\bar{M}_w^{-1} \sum_i w_i M_i s_i^2$ defines an average value of the radius of gyration that can be obtained from the measured angle dependence of scattering intensity. This average is usually referred to as the z -average radius of gyration. Thus [12],

$$\bar{s}_z^2 = \bar{M}_w^{-1} \sum_i w_i M_i s_i^2 \quad (2)$$

The square of the radius of gyration for uniform coil molecules is proportional to M^α :

$$s^2 = kM^\alpha \quad (3)$$

the value of α depending on excluded-volume effect. $\alpha = 1$ for theta conditions and increases with improving thermodynamic quality of solvent. Introducing Eq. (3) into Eq. (2), we obtain an average molecular weight, \bar{M}_s , the value of which is equal to the molecular weight of molecules having the radius of gyration equal to the measured z -average value of s :

$$\bar{M}_s = \left[\bar{M}_w^{-1} \sum_i w_i M_i^{1+\alpha} \right]^{1/\alpha} \quad (4)$$

For theta conditions, $\bar{M}_s = \bar{M}_z$. In thermodynamically good solvents, polymer coils expand, $\alpha > 1$ and $\bar{M}_s > \bar{M}_z$ [12,15]. To avoid potential confusion, a note on symbols and terminology is pertinent. The average radius of gyration obtained by light scattering and defined by Eq. (2) is always called the z -average, irrespective of the value of α . On the other hand, the average molecular weight calculated from the z -average radius of gyration depends on α (Eq. (4)) and we call it the s -average molecular weight, \bar{M}_s .

The combination of Eqs. (1)–(4) enables us to obtain \bar{M}_s from the angle dependence of scattered light at any elution volume, provided the parameters of Eq. (3) are known.

3. Experimental

SEC measurements with dual light-scattering/concentration detection were performed using a Pump Deltachrom (Watrex company), autosampler Midas, two columns with PL gel Mixed, particle size 10 μm , separating according to the producer in the range of molecular weights approximately 400– 10^7 , and a differential refractometer Showdex. The set was connected to a light-scattering photometer DAWN, measuring at 18 angles of observation (Wyatt Technology Corp.). The photometer was placed as the first detector, i.e., between the columns and the refractometer. Mobile phase was tetrahydrofuran at ambient temperature. The flow rate was 0.5 ml min^{-1} . The data were accumulated and processed using the ASTRA Software [16]; some calculations were performed using home-modified software. The separation system was calibrated using polystyrene reference standards. The calibration dependence was fitted using linear regression:

$$\log M = 13.56 - 0.605V \quad (5)$$

The results for the standards are summarized in Table 1.

4. Results and discussion

As an example of the determination of s from the angle dependence of scattering intensity, Fig. 1 shows the dependence of $Kc/R(\theta)$ on $\sin^2(\theta/2)$ according to Eq. (1). As s is found from the initial slope of this plot, low scatter of points is critical in the s -detection. For narrow-MWD stan-

Table 1

Characteristics of the high-molecular-weight polystyrene standards used for the s -detection: molecular weight given by the producer (named in the footnote), \bar{M}_p , determined by SEC with light-scattering/concentration detection, \bar{M}_w , the weight-to-number-average molecular weight ratio given by the producer, $(\bar{M}_w/\bar{M}_n)_p$, its value determined by the method described in [4], \bar{M}_w/\bar{M}_n , and the variance of the spreading function, σ

$\bar{M}_p \times 10^{-6}$	$\bar{M}_w \times 10^{-6}$	$(\bar{M}_w/\bar{M}_n)_p$	\bar{M}_w/\bar{M}_n	σ (ml)
0.400 ^a	0.393	1.06	1.008	0.222
0.575 ^a	0.628	1.06	1.012	0.227
0.900 ^a	1.11	1.10	1.026	0.250
1.600 ^a	1.61	1.12	1.080	0.143
3.00 ^a	2.70	–	1.449	0.209
3.84 ^b	3.82	1.04	1.241	0.169
	2.25 ^c	–	1.582	0.200
20 ^b	19.7	–	–	–

^a Pressure chemical.

^b Toyo soda.

^c Laboratory prepared sample.

dards, a reliable determination of this slope was possible for molecular weights $M > 5 \times 10^5$.

The dependence of s on M for the polystyrene standards measured (Fig. 2) can be described the relation:

$$\log s = -1.84 + 0.59 \log M \quad (6)$$

The slope 0.59 is virtually equal to the asymptotic limit 0.588 for thermodynamically good solvents [17].

As for narrow MWD standards and efficient separation system the difference between the local molecular-weight averages obtained by the w - and s -detection is negligible [13], the local molecular weight can be calculated from the local value of the radius of gyration as:

$$\log M = 1.69 \log s - 3.12 \quad (7)$$

In this way, a local calibration independent of IDV is obtained.

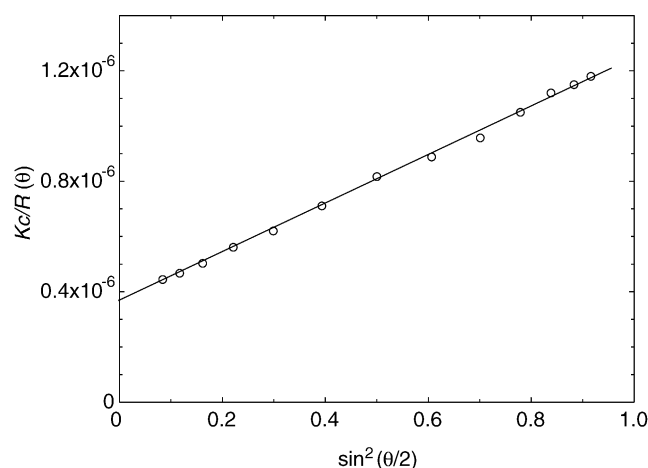


Fig. 1. The angular dependence of intensity of the scattered light obtained for a polystyrene standard of molecular weight $M = 3 \times 10^6$ at elution volume 12.05 ml, concentration $c = 4.56 \times 10^{-6}$ g/ml. K and $R(\theta)$ are, respectively, an optical constant and the excess Rayleigh ratio in Eq. (1).

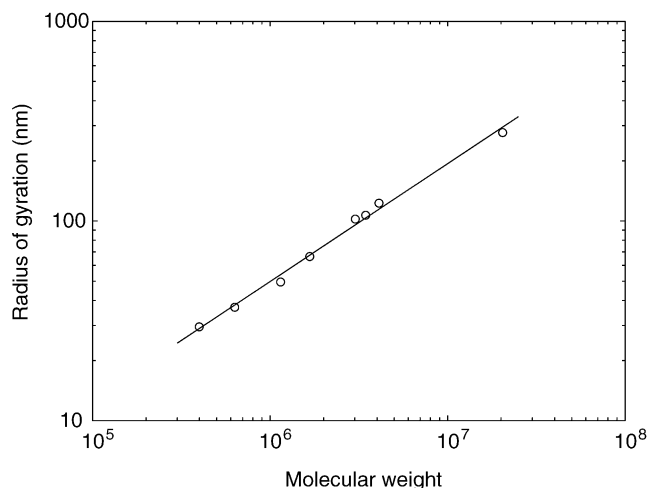


Fig. 2. The bi-logarithmic dependence of radius of gyration, on molecular weight with a line obtained by linear regression of experimental points.

The sensitivity of the local calibration found by the w-detection to the value of IDV is demonstrated for three polystyrene standards with $M \times 10^{-6} = 0.47, 1.02$ and 2.85 in Fig. 3. The diagrams contain also local calibrations obtained by the s-detection. In the area of elution volumes close to the peak apex, the local calibration dependences obtained by s-detection nearly coincide with those found by w-detection for a value of IDV = 0.148 ml, determined by a procedure described in the following section. In the following, this IDV is referred to as correct IDV.

A visual comparison of the local calibrations is difficult and may be biased. To make the determination of IDV sample- and operator-independent, a graphical method is suggested, requiring only the determination of slopes of local calibrations in the region of the concentration-elution-peak apex. In that region of elution volumes, the local calibrations can, as a rule, be satisfactorily approximated by straight lines.

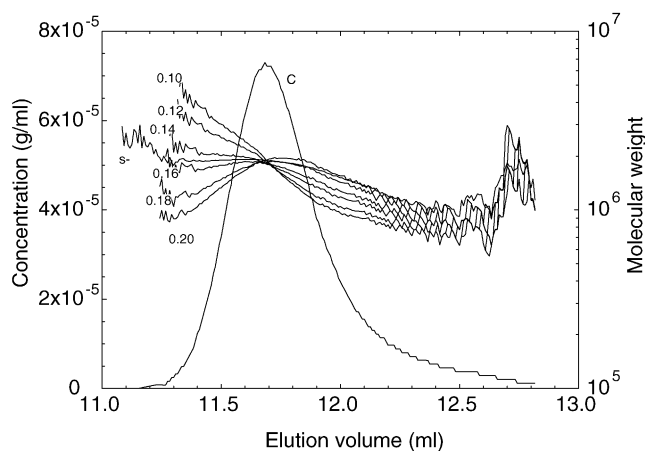


Fig. 3. Comparison of local calibration dependences obtained by the s-detection with those obtained by the w-detection for particular values of IDV, denoted with the curves, for polystyrene standard $M = 1.6 \times 10^6$. 'C' is the concentration elution curve.

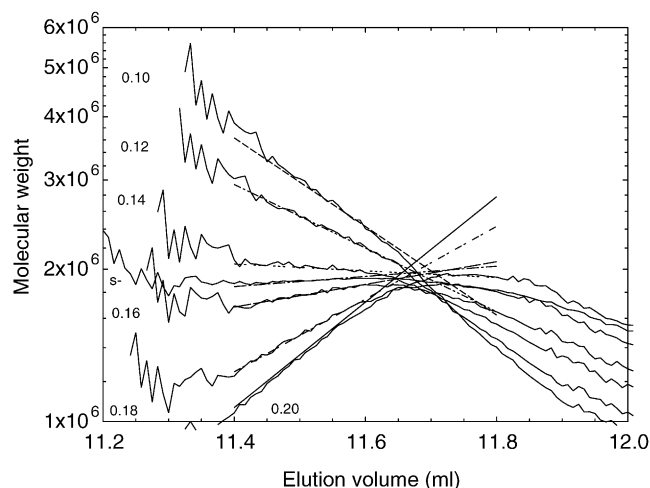


Fig. 4. Blow-up of the central sections of local calibration dependences obtained by the s- and w-detections for particular IDV values, denoted with the curves, of polystyrene standard $M = 1.60 \times 10^6$. Dashed straight lines are tangents of local calibrations.

The local calibration obtained by the s-detection and its slope do not depend on the IDV, unlike the local calibration resulting from the w-detection. Thus, we must search for an IDV value yielding a slope of the w-detection local calibration identical with that found by the s-detection. A trial-and-error procedure is feasible yet awkward. The Astra Software [16] is a suitable tool for that calculation.

To determine with a good accuracy the slopes of the individual local calibrations obtained by the w-detection for particular values of IDV and the slope of the local calibration obtained by the s-detection, the local calibrations in the area of elution volumes close to the apex of the elution curve are blown up, straight lines are plotted through the central sections of calibrations (Fig. 4) and their slopes are determined. Then, the difference between the slopes of the local

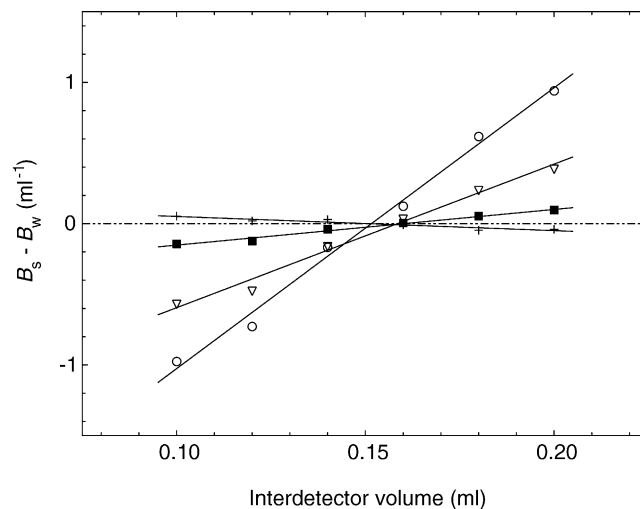


Fig. 5. Difference between the slopes of local calibrations obtained by the s-detection, B_s , and that found by the w-detection, B_w , as a function of the value of interdetector volume used for the calculations, for polystyrene standards $M \times 10^{-6} = 1.6$ (\circ), 2.25 ($+$), 2.70 (\blacksquare), and 3.82 (∇).

calibrations for the s-detection and w-detections for particular IDV values, $B_s - B_w$, is plotted as a function of IDV (Fig. 5). All dependences thus obtained are linear and intersect in nearly one point with an ordinate of $B_s - B_w \approx 0$. The abscissa of that point is the best approximation to the correct value of IDV. Using this procedure, the value of $IDV = 0.148 \pm 0.004$ ml was determined.

5. Conclusions

1. The local calibration can be constructed by the s-detection from the local dependence of radius of gyration, s , on elution volume and an independently determined relation between s and M .
2. The slope of the local calibration found by the w-detection is very sensitive to the interdetector-volume error, whereas the slope obtained by the s-detection, using data from the light-scattering detector only, does not require knowledge of the interdetector volume. The correct IDV value yields a local calibration found by the w-detection with the same slope as the local calibration determined by the s-detection. In this way, a sufficiently accurate value of IDV can be found.

Acknowledgements

The authors gratefully acknowledge the loan of the DAWN laser photometer from the Wyatt Technologies Ltd.

as well as support by the Academy of Sciences of the Czech Republic (project No. AVOZ 4050913).

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