

Combined effect of interdetector volume and peak spreading in size exclusion chromatography with dual detection

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The relation between the error in interdetector volume determination and peak spreading was studied for a polymer sample whose molecular weight distribution obeys the log-normal function. A compensation of these quantities was shown, i.e. their values were found for which the ratio of weight- to number-average molecular weight, \bar{M}_w/\bar{M}_n , was constant. The influence of non-uniformity in the molecular weight of the sample on the compensation is demonstrated. Copyright © 1996 Elsevier Science Ltd.

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INTRODUCTION

The peak broadening (spreading) and interdetector volume are two factors of decisive importance in size exclusion chromatography (s.e.c.) with dual detection, e.g. with a differential refractometer (DR) as concentration detector and a light scattering¹ (LS) or viscometric^{2–6} (VIS) detector. The effect of these factors on the chromatograms can be eliminated if the variance σ^2 of the spreading function (or the resolution factor $h = 1/\sigma^2$) and the interdetector volume are known. Since the corrections may be inaccurate, it is legitimate to deal theoretically with the impact of this inaccuracy on the result of the s.e.c. experiment (i.e. the correlation of the molecular weight M and elution volume V ; the average molecular weights and the molecular weight distribution; the parameters of the Mark–Houwink–Kuhn–Sakurada relationship between the molecular weight and the intrinsic viscosity $[\eta] = KM^a$, etc.).

Both effects of peak broadening and error in the interdetector volume, δ , have been studied^{3,7,8*}†, assuming that σ^2 and δ are not related. In fact, they are interrelated. Moreover, the impact of σ^2 and δ on the dependence of $M(V)$ and $[\eta](M)$ depends on the nonuniformity of the sample. It is obvious that a detailed study of the effect of the instrument parameters on the results of the s.e.c. analysis is desirable. Therefore, we decided to extend the theoretical studies^{7*}† of s.e.c. of polymers with log-normal molecular weight distribution.

RESULTS AND DISCUSSION

The dependence of molecular weight M on elution volume V is currently described by the equation

$$\ln M = A + BV \quad (1)$$

where the constants A and B are obtained by calibration with standard samples of high uniformity, i.e. of low values of \bar{M}_w/\bar{M}_n (weight- to number-average molecular weight) ratio. It will be shown in this paper that a set of different values of the instrument parameters, σ^2 and δ , may be obtained if the s.e.c. analysis with dual detection is applied to a nonuniform sample. That may have an impact on the results of the analysis.

We simulated a s.e.c. experiment with a sample with the molecular-weight distribution described by the log-normal distribution function⁹

$$w(M) = \frac{1}{\beta\sqrt{\pi M}} \exp\left(-\frac{1}{\beta^2} \ln^2 \frac{M}{M_0}\right) \quad (2)$$

where $\beta = \sqrt{2 \ln \bar{M}_w/\bar{M}_n}$ and $M_0 = \sqrt{\bar{M}_w/\bar{M}_n}$. The parameters σ^2 and δ were properly taken into account and the 'experimental' values of the average molecular weights (\bar{M}_w , \bar{M}_n), their ratio \bar{M}_w/\bar{M}_n , and the values of the MHKS parameters (\bar{K} , \bar{a}) were evaluated and compared with the 'correct' ones (\bar{M}_w , \bar{M}_n , K , a).

The s.e.c. experiment with dual detection yields two records: the DR chromatogram and the LS chromatogram. The former is a normalized plot of signal of the differential refractometer influenced by the peak broadening vs V and can be described by the equation^{10,11}

$$F(V) = \frac{1}{\sqrt{\pi(2\sigma^2 + \beta^2/B^2)}} \exp\left(-\frac{(V - V_0)^2}{2\sigma^2 + \beta^2/B^2}\right) \quad (3)$$

The theoretical LS chromatogram (chromatographic peak height) can be expressed, e.g. as a product of the

* Equation (5) of ref. 7 should read exactly as equation (2) of this paper
† Equation (19) in ref. 9 should correctly read

$$(\bar{M}_w/\bar{M}_n)_d = (\bar{M}_w/\bar{M}_n)_c^{\Lambda^2} \quad (26)$$

(A^2 instead of A) as can be easily proved by combining equations (17) and (18) in ref. 9

theoretical DR (with $\sigma^2 = 0$) and molecular weight given by equation (1); by 'spreading' it according to the same law as the DR chromatogram (equation (3)), the 'experimental' LS chromatogram is obtained¹². The latter may be expressed as a product of two experimental functions: the 'experimental' light scattering molecular weight of the fraction obtained at the elution volume V , $\tilde{M}(V)$, and the (DR) chromatogram, $F(V)$ ⁸,

$$\tilde{M}(V)F(V) = \frac{M_0 \exp(\beta^2/4)}{\sqrt{\pi(2\sigma^2 + \beta^2/B^2)}} \times \exp\left(-\frac{[V - (V_0 + \beta^2/2B + \delta)]^2}{2\sigma^2 + \beta^2/B^2}\right) \quad (4)$$

where $\ln M_0 = A + BV_0$, $F(V)$ is given by equation (3) and δ is the error in determination of the interdetector volume. It is seen from these equations that both records are broadened and, additionally, the LS chromatogram is shifted with respect to the DR chromatogram due to an error in determination of the interdetector volume.

The dependence $\tilde{M}(V)$ will be expressed as

$$\ln \tilde{M} = \tilde{A} + \tilde{B}V \quad (5)$$

where the parameters \tilde{A} and \tilde{B} differ from A and B in equation (1). They are found by dividing equation (4) by equation (3) and taking the logarithm:

$$A = \frac{2(\sigma^2 + \delta/B) \ln M_0 + \sigma^2 \beta^2/2 + \beta^2 A/B^2 + \delta(2A/B - \delta \beta^2/B)}{2\sigma^2 + \beta^2/B^2} \quad (6)$$

and

$$\tilde{B} = \frac{\beta^2/B^2 + 2\delta/B}{2\sigma^2 + \beta^2/B^2} B \quad (7)$$

The elution volumes corresponding to weight- and number-average molecular weights are, respectively,

$$V_w = V_0 - \frac{\beta^2}{4B} \quad (8)$$

and

$$V_n = V_0 + \frac{\beta^2}{4B} \quad (9)$$

The situation is sketched in Figure 1. For comparison and later discussion, the (experimental) effective linear calibration (ELC) is also depicted (by use of which true \bar{M}_w and \bar{M}_n are calculated from the 'experimental' chromatogram) with constants^{7*†}

$$\tilde{A} = \ln M_0 + \frac{\beta}{B} \frac{A - \ln M_0}{\sqrt{2\sigma^2 + \beta^2/B^2}} \quad (10)$$

and

$$\tilde{B} = \frac{\beta}{\sqrt{2\sigma^2 + \beta^2/B^2}} \quad (11)$$

For $\sigma^2 = 0 = \delta = 0$, all the experimental dependences of $\ln \tilde{M}(V)$ (equations (6)–(11)) as well as chromatograms (equations (3) and (4)) turn into theoretical ones and equation (5) into (1).

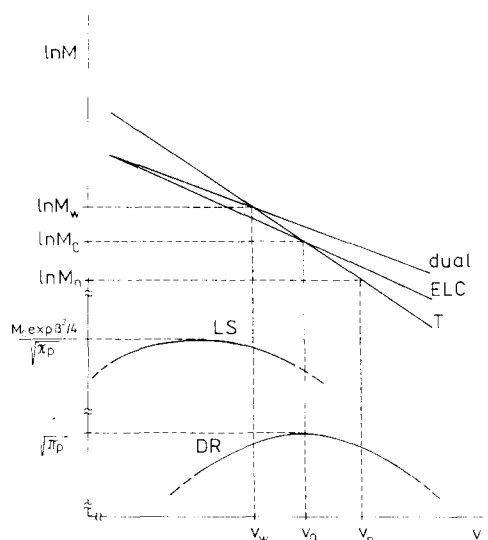


Figure 1 Comparison of experimental concentration record (DR), light-scattering record (LS), experimental calibration calculated from the dual DR/LS record, effective linear record (ELC) and 'traditional' record (T) for a polymer sample of MWD defined by equation (2)

The 'experimental' values of the weight- and number-average molecular weights, \tilde{M}_w and \tilde{M}_n , can be calculated by defining

$$\nu_i = \int_{-\infty}^{\infty} \tilde{M}^i(V)F(V) dV \quad (12)$$

The calculation is easy to perform by use of the integral

$$I_n = \int_{-\infty}^{\infty} \left(\frac{\exp[-k(x-x_1)^2]}{\exp[-k(x-x_0)^2]} \right)^n \exp[-k(x-x_0)^2] dx \quad (13)$$

which is

$$I_n = \sqrt{\frac{\pi}{k}} \exp[kn(n-1)(x_0-x_1)^2] \quad (14)$$

We get $\nu_0 = 1$, and for the weight-average molecular weight we obtain

$$\nu_1 = \tilde{M}_w = M_0 \exp \frac{\beta^2}{4} = \bar{M}_w \quad (15)$$

This result is expected because the determination of \tilde{M}_w depends on the correct determination of scattered light (LS area) and overall concentration rather than on the shapes of the records. For the number- and z-average molecular weights, $\tilde{M}_n = (\nu_{-1})^{-1}$ and $\tilde{M}_z = \nu_2/\nu_1$, respectively, we have

$$\tilde{M}_n = M_w (\bar{M}_w/\bar{M}_n)^{-(\Sigma+\Delta)} \exp\left(\frac{-2\delta^2}{\beta^2 + 2\sigma^2 B^2}\right) \quad (16)$$

and

$$\tilde{M}_z = M_w (\bar{M}_w/\bar{M}_n)^{(\Sigma+\Delta)} \exp\left(\frac{2\delta^2}{\beta^2 + 2\sigma^2 B^2}\right) \quad (17)$$

where

$$\Sigma = \frac{\beta^2}{\beta^2 + 2\sigma^2 B^2} \quad (18)$$

and

$$\Delta = \frac{4\delta/B}{\beta^2 + 2\sigma^2 B^2} \quad (19)$$

By combining equations (15), (16) and (17) it is obvious that in the limit of the 'ideal' experimental conditions ($\sigma^2 \rightarrow 0$; $\delta \rightarrow 0$)

$$\tilde{M}_z/\tilde{M}_w \rightarrow \tilde{M}_w/\tilde{M}_n \rightarrow \bar{M}_w/\bar{M}_n \quad (20)$$

This is a well known property of the log-normal MWD.

The nonuniformity index calculated from the detector data, $(\tilde{M}_w/\tilde{M}_n)_d$, is

$$(\tilde{M}_w/\tilde{M}_n)_d = (\bar{M}_w/\bar{M}_n)^{\Sigma+\Delta} \exp\left(\frac{2\delta^2}{2\sigma^2 + \beta^2/B^2}\right) \quad (21)$$

If the DR chromatogram is evaluated by means of equation (1), the nonuniformity index $(\tilde{M}_w/\tilde{M}_n)_c$ is obtained. According to refs 7 and 10,

$$(\tilde{M}_w/\tilde{M}_n)_c = (\bar{M}_w/\bar{M}_n)^{1/\Sigma} \quad (22)$$

and we can write

$$(\tilde{M}_w/\tilde{M}_n)_d = (\tilde{M}_w/\tilde{M}_n)_c^{\Sigma(\Sigma+\Delta)} \exp\left(\frac{2\delta^2}{2\sigma^2 + \beta^2/B^2}\right) \quad (23)$$

The subscript c denotes here that the nonuniformity ratio is calculated using the calibration dependence (1). For $\delta \rightarrow 0$, this equation turns in equation (19) of ref. 8†.

It is notable that $\tilde{M}_w = \bar{M}_w$ for all values of σ^2 and δ , whereas $\tilde{M}_n \rightarrow \bar{M}_n$ only for $\delta \rightarrow 0$ and $\sigma^2 \rightarrow 0$. On the other hand, $\tilde{M}_n = \bar{M}_w$ for $\sigma^2 \rightarrow \infty$.

It follows from equations (15)–(23) that the instrument parameters σ^2 and δ may compensate each other. This is demonstrated by converting equation (21) into the form

$$\sigma^2 = \frac{(\beta^2/B^2 + 4\delta/B) \ln \bar{M}_w/\bar{M}_n + 2\delta^2 - (\beta^2/B^2) \ln \tilde{M}_w/\tilde{M}_n}{2 \ln \tilde{M}_w/\tilde{M}_n} \quad (24)$$

The results are presented in Figures 2 and 3. The former shows that different combinations of σ^2 and δ may result in the same value of \tilde{M}_w/\tilde{M}_n . Figure 3 presents the combinations of σ^2 and δ leading to the correct nonuniformity index (i.e. $\tilde{M}_w/\tilde{M}_n = \bar{M}_w/\bar{M}_n$). The curves start at $\sigma^2 = \delta = 0$. Except for very low nonuniformity, the curves deviate from each other only at relatively high values of instrument parameters. This means that, in analyses of polymers with $\bar{M}_w/\bar{M}_n > 1.5$, some error in the instrument parameters is admissible provided that the condition of correct determination of the molecular weight is fulfilled. On the other hand, the instrument parameter obtained by analysing polymers with broad MWD should not be used with samples with narrow MWD.

† Equation (19) in ref. 9 should correctly read

$$(\tilde{M}_w/\tilde{M}_n)_d = (\tilde{M}_w/\tilde{M}_n)_c^{A^2} \quad (26)$$

(A^2 instead of A) as can be easily proved by combining equations (17) and (18) in ref. 9

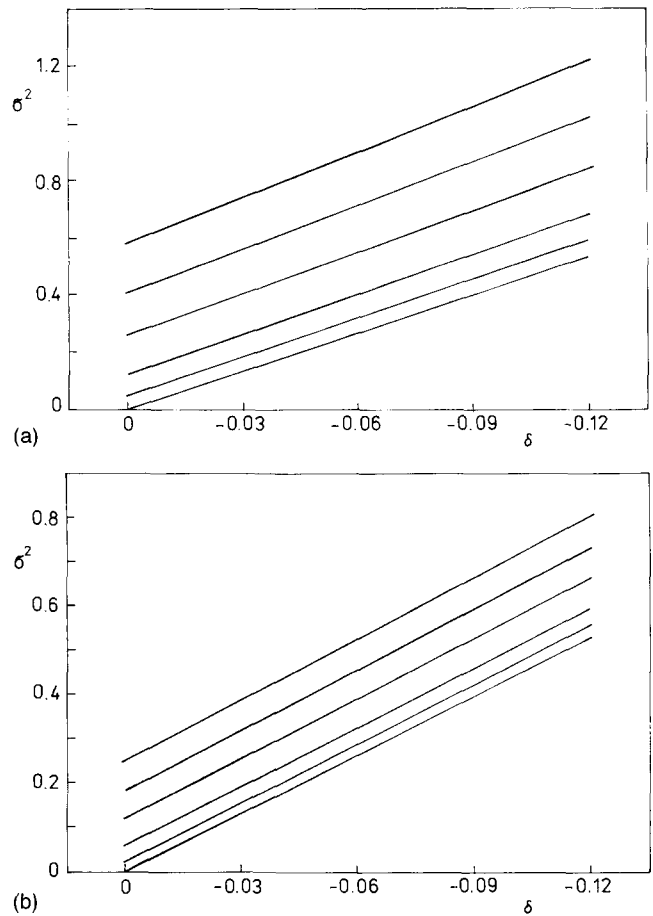


Figure 2 Comparison of the peak spreading variance, σ^2 , and error in determination of the interdetector volume, δ , calculated according to equation (24) for sample with $\bar{M}_w/\bar{M}_n = 2$ and experimental polymolecularity $\tilde{M}_w/\tilde{M}_n = 1.8, 1.85, 1.9, 1.95$ and 2 (from top to bottom) (a), and for sample with $\bar{M}_w/\bar{M}_n = 4$ and experimental polymolecularity $\tilde{M}_w/\tilde{M}_n = 3.8, 3.85, 3.9, 3.98$ and 4 (from top to bottom) (b)

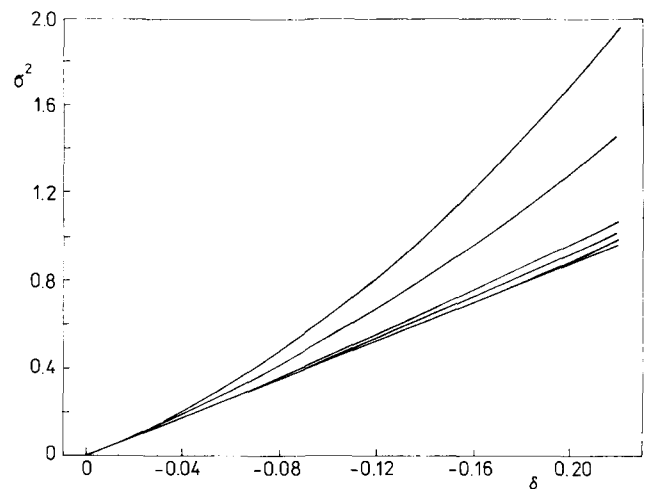


Figure 3 Comparison of the peak spreading variance, σ^2 , and error in determination of the interdetector volume, δ , calculated according to equation (24) under the assumption that experimental and theoretical polymolecularities are equal, $\bar{M}_w/\bar{M}_n = \tilde{M}_w/\tilde{M}_n = 1.05, 1.1, 1.5, 2, 5,$ and 10 (curves from top to bottom; the last two cannot be distinguished)

Table 1 Experimental values of KMHS parameters

Method	\tilde{a}	$\ln(\tilde{K}/K)$
Primary + UC	$(a+1)\frac{B}{\tilde{B}} - 1$	$(a+1)A - (\tilde{a}+1)\tilde{A}$
Secondary + UC	$\frac{a\tilde{B}}{a(B-\tilde{B})+B}$	$(\tilde{a}+1)a\tilde{A} - (a+1)\tilde{a}A$
Primary + VIS ^a	$\frac{\tilde{B}_V}{\tilde{B}_P} a$	$a\left(\tilde{A}_V - \frac{\tilde{B}_V}{\tilde{B}_P} A_P\right)$

^a Subscripts P and V, respectively, are used to distinguish experimental constants of the primary calibrations and the secondary calibration calculated from the viscosity record and from the universal calibration

The MHKS parameters

Based on the above equations, the relationship between the theoretical or 'correct' (K and a) and 'experimental' (\tilde{K} and \tilde{a}) MHKS parameters will now be discussed. We distinguish two types of correlations depending on the procedure used to obtain the dependence $\tilde{M}(V)$.

- If the molecular weight \tilde{M} is estimated directly by light scattering or calculated from a viscometric record with known MHKS parameters, the correlation is referred to as the 'primary' one.
- If the MHKS parameters are unknown, the dependence $\tilde{M}(V)$ can be computed from a viscometric record and the universal calibration⁶ (i.e. from the dependence of $\ln[\eta]M$ vs V determined in a broad range of molecular weights). This type of correlation is called 'secondary'. Supposing, in that case, that $[\eta]M = [\tilde{\eta}]\tilde{M}$ the $\tilde{M}(V)$ function can be expressed by

$$\ln \tilde{M} = A + a(A - \tilde{A}) - (B + a(B - \tilde{B}))V \quad (25)$$

The parameters \tilde{A} and \tilde{B} are found by combining the experimental correlations (primary or secondary) with the universal calibration. In *Table 1* they are expressed in terms of correct values (a and K) and the parameters A , \tilde{A} , B , and \tilde{B} .

It is notable that primary calibrations become less steep due to the increasing peak spreading (decreasing value of δ , i.e. a shift of the LS to the DR record has the same effect), contrary to the secondary one, which becomes steeper. This is because the product $[\eta]M$ is constant; if, e.g. $[\eta]$ is higher than it should be, M is lower and vice versa.

CONCLUSIONS

It was demonstrated using the example of a polymer with log-normal MWD that the peak-spreading variance, σ^2 , and the error in determination of the interdetector volume, δ , may lead either to correct or incorrect results in determination of MWD, in particular \tilde{M}_n , \tilde{M}_w is determined correctly independently of the values of the instrument parameters.

With increasing \tilde{M}_w/\tilde{M}_n ratio, the error as well as the dependence of the experimental \tilde{M}_w/\tilde{M}_n ratio on the instrument parameters diminishes.

A criterion for the correctness of the values of interdetector volume and σ^2 is that the $\ln \tilde{M}(V)$ dependence determined in analysis of one nonuniform sample is parallel to the correct one (determined in the broad range of elution volumes). The value of instrument parameters obtained with nonuniform samples should not be used in the evaluation of analyses of samples with narrower MWD.

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