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Polymer 44 (2003) 3431–3436
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Polystyrene-equivalent molecular weight versus true molecular weight in size-exclusion chromatography

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Received 7 November 2002; received in revised form 14 March 2003; accepted 18 March 2003

Abstract

The evaluation of the size-exclusion chromatography (SEC) concentration elution curves by means of a calibration dependence obtained in a given SEC set for a polymer different from the polymer to be analyzed results in an error in the determination of both molecular weight and molecular-weight distribution (MWD). The problem is analyzed assuming the validity of the universal-calibration concept. The differences between the true and apparent values of molecular weight, MWD and M_w/M_n depend on and are expressed in terms of the parameters of the Mark–Houwink–Kuhn–Sakurada equation, describing the molecular-weight dependence of intrinsic viscosity, for the polymer to be analyzed and the polymer used for calibration. The differences in molecular weight and the M_w/M_n ratio are typically tens of percent and, in extreme cases, can amount up to a factor of three for molecular weight and a factor of two for the M_w/M_n ratio.

Keywords: Size-exclusion chromatography; Polystyrene-equivalent molecular weight; Universal calibration

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1. Introduction

To determine the true molecular weight and molecular-weight distribution (MWD) of polymers by size-exclusion chromatography (SEC) using just a concentration detection, i.e. without an on-line molecular-weight sensitive detector [1], the separation system must be calibrated with a set of polymers, chemically and structurally identical with the polymers to be analyzed, the analytes. The polymers used for calibration should cover the molecular-weight range of the potential analytes and, preferably, have a narrow MWD.

In practice, frequently a suitable set of analyte polymers for calibration is not available and the separation system is calibrated with a different polymer, usually commercial polystyrene standards. Molecular weights thus obtained for polymers other than polystyrene are not their true molecular weights but values which should be called polystyrene-equivalent molecular weights. Polystyrene-equivalent molecular weight or, more generally, standard-equivalent molecular weight is the molecular weight of a standard-polymer molecule having the same hydrodynamic volume and thus eluting from the separation system at the same elution volume as a molecule of the analyte. In general, the standard-equivalent molecular weight and the true molecular weight of the analyte are not equal.

When publishing SEC data for measurements in which the analyte and the calibration polymer are not identical, it is fair to state this explicitly [2–5]. Unfortunately, many authors do not do so and often interpret the data neglecting the likely difference between the true and standard-equivalent molecular weight. Not exceptionally, this results in misinterpretation of the data.

The objective of this communication is to demonstrate how large the differences can be between the true and standard-equivalent molecular weight, between the true and apparent MWD, and between the true value of the weight-to-number-average-molecular-weight ratio and the value of this ratio obtained for calibration with a different polymer. We assume the validity of the universal-calibration concept, which postulates that the separation in SEC proceeds according to hydrodynamic volume of macromolecules or particles and the hydrodynamic volume equals the product of intrinsic viscosity and molecular weight [6].

The universal-calibration concept is operative for polymers assuming in solution conformation of flexible coils, i.e. characterized by values of the exponent in the

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doi:10.1016/S0032-3861(03)00258-1

Mark–Houwink–Kuhn–Sakurada (MHKS) equation [7] $0.5 < a < 0.8$. For such polymers and copolymers, the universal-calibration concept holds irrespective of molecular topology, i.e. the same universal-calibration dependence is valid for linear molecules as well as molecules with various types and degrees of branching [8,9]. For polymers with stiff chains, characterized by values of the MHKS exponent $a > 0.8$, the universal-calibration concept fails, the deviations increasing with increasing values of the exponent a [10,11]. Thus, the following reasoning is relevant to the most frequent case of flexible-chain polymers.

2. Theoretical

According to the universal-calibration concept in SEC separation, the product of intrinsic viscosity and molecular weight of a macromolecule is a single-valued function of elution volume, V, irrespective of chemical nature and topology of a molecule, thus [6]

$$[\eta]M = f(V) \tag{1}$$

Therefore, for two molecules of different polymers 1 and 2 eluting at the same V, the equality of the products

$$[\eta]_1 M_1 = [\eta]_2 M_2 \tag{2}$$

holds. Using the MHKS equation for the molecular-weight dependence of intrinsic viscosity

$$[\eta] = KM^a \tag{3}$$

where K and a are parameters depending on the polymer, solvent and temperature, we obtain the relation between M_1 and M_2 for molecules of polymer 1 and 2, respectively, eluting at the same elution volume

$$\log M_2 = \frac{1}{a_2+1}\log\frac{K_1}{K_2} + \frac{a_1+1}{a_2+1}\log M_1 \tag{4}$$

For a particular SEC system, the calibration dependence for polymer 1 is the dependence of molecular weight, M_1, of molecules of that polymer on elution volume, V, at which the molecules leave the separation system. Eq. (4) enables us to recalculate point by point the calibration dependence for a polymer to that dependence for another polymer, provided the parameters of the MHKS equation for both polymers are available.

In practice, the calibration dependence is often linear in semilogarithmic co-ordinates and thus

$$\log M_1 = A_1 + B_1 V \tag{5}$$

In that case, the recalculation of the calibration dependence to the dependence for another polymer is simple. Substituting Eq. (4), after cancellation of the requirement of fixed elution volume, in Eq. (5) we obtain for the calibration dependence of polymer 2

$$\log M_2 = A_2 + B_2 V \tag{6}$$

where

$$A_2 = \frac{a_1+1}{a_2+1}A_1 + \frac{1}{a_2+1}\log\frac{K_1}{K_2} \tag{7}$$

and

$$B_2 = \frac{a_1+1}{a_2+1}B_1 \tag{8}$$

From Eqs. (5)–(8) it can be seen: (i) if the calibration dependence in semilogarithmic co-ordinates is linear for polymer 1 (Eq. (5)), it is also linear for any polymer 2; (ii) if the exponents a of the MHKS equation (Eq. (3)) for polymers 1 and 2 are equal, the linear calibration dependences for both polymers have the same slope and are shifted in a parallel way.

If for a given SEC system, the calibration dependence for any polymer i is linear in semilogarithmic co-ordinates, also the universal-calibration dependence for that SEC system is linear in semilogarithmic co-ordinates, viz.

$$\log(M[\eta]) = A_\mathrm{u} + B_\mathrm{u} V \tag{9}$$

Eq. (9) can be easily proved by using the constants of the calibration dependence, and the constant and exponent of the MHKS equation (Eq. (3)) for polymer i and writing

$$\begin{aligned}\log(M_i[\eta]_i) &= A_i + B_i V + \log K_i + a_i A_i + a_i B_i V \\ &= A_\mathrm{u} + B_\mathrm{u} V\end{aligned} \tag{10}$$

where

$$A_\mathrm{u} = (1+a_i)A_i + \log K_i \tag{11}$$

and

$$B_\mathrm{u} = (1+a_i)B_i \tag{12}$$

The use of an incorrect calibration affects not only the resulting values of molecular weight, as described by Eq. (4), but also the resulting MWD. MWD obtained from an elution curve using an incorrect calibration dependence can be recalculated point by point to the true MWD, again by means of Eq. (4), for any distribution.

To get a general picture regarding the effect of using an incorrect calibration on MWD, model calculations have been made on the following assumptions: (i) the weight MWD can be described by the log–normal distribution function; (ii) there is no band broadening in the separation system; (iii) the calibration dependence can be described by Eq. (5). The weight log–normal distribution function [12, 13]

$$f(M) = \frac{1}{\beta M\sqrt{\pi}}\exp\left[-\frac{1}{\beta^2}(\ln M - \ln M_0)^2\right] \tag{13}$$

is a good approximation to real MWDs of many polymers and is amenable to an analytical solution of the problem. In Eq. (13),

$$\beta = \sqrt{2\ln(M_\mathrm{w}/M_\mathrm{n})} \tag{14}$$

where M_w and M_n are weight- and number-average molecular weight, respectively, describes the width of MWD and

$$M_0 = \sqrt{M_w \times M_n} \tag{15}$$

is the geometric-mean molecular weight.

The relation between a MWD and the corresponding concentration elution curve $W(V)$ is given by the equation [14,15]

$$W(V) = -f(M)M\frac{\mathrm{d}\ \ln M}{\mathrm{d}V} \tag{16}$$

To simplify the calculations, we express the calibration dependence for polymer i in terms of natural logarithms

$$\ln M_i = A'_i + B'_i V \tag{17}$$

From Eq. (17), we have

$$\frac{\mathrm{d}\ \ln M_i}{\mathrm{d}V} = B'_i \tag{18}$$

and

$$M_i = \exp[A'_i + B'_i V] \tag{19}$$

The experimental elution curve, $W(V)$, should be transformed to the correct MWD, $f_1(M_1)$, using the calibration dependence for polymer 1, the analyte. If this calibration is not accessible, we resort to the interpretation of $W(V)$ in terms of the available calibration dependence for standard polymer 2. This generally results in a deformed, apparent MWD, $f_2(M_2)$.

To obtain the relation between the true and apparent MWD, $f_1(M_1)$ and $f_2(M_2)$, respectively, we start from the prerequisite that both distributions must yield the same concentration elution curve (cf. Eqs. (16) and (18))

$$W(V) = f_1(M_1)M_1B'_1 = f_2(M_2)M_2B'_2 \tag{20}$$

For the apparent MWD, $f_2(M_2)$, we have

$$f_2(M_2) = \frac{M_1B'_1}{M_2B'_2}f_1(M_1) \tag{21}$$

Substitution of the explicit form of $f_1(M_1)$ (Eq. (13)) in Eq. (21) yields

$$f_2(M_2) = \frac{B'_1}{\sqrt{\pi}B'_2\beta_1M_2}\exp\left[-\frac{1}{\beta_1^2}(\ln M_1 - \ln M_{01})^2\right] \tag{22}$$

Eq. (19) for polymer 1 reads

$$M_1 = \exp[A'_1 + B'_1 V] \tag{23}$$

Using the available calibration for the standard polymer 2, the variable elution volume V can be expressed from Eq. (19) as

$$V = \frac{\ln M_2 - A'_2}{B'_2} \tag{24}$$

After substitution in Eq. (23), we have for M_1

$$M_1 = \exp\left[A'_1 + \frac{B'_1}{B'_2}\ln M_2 - \frac{B'_1}{B'_2}A'_2\right] \tag{25}$$

For the log–normal distribution, the concentration elution curve shows the maximum at an elution volume of V_0. At V_0, molecules elute that have molecular weight equal to the geometric-mean molecular weight defined by Eq. (15). Eq. (25) must be fulfilled also for M_{01} and M_{02}. Substituting Eq. (25) for $\ln M_1$ and modified Eq. (25) for $\ln M_{01}$ in Eq. (22) yields for $f_2(M_2)$

$$\begin{aligned} f_2(M_2) = {} & \frac{B'_1}{\sqrt{\pi}\beta_1B'_2M_2} \\ & \exp\left[-\frac{B'^2_1}{\beta_1^2B'^2_2}(\ln M_2 - \ln M_{02})^2\right] \\ = {} & \frac{1}{\sqrt{\pi}\beta_2M_2}\exp\left[-\frac{1}{\beta_2^2}(\ln M_2 - \ln M_{02})^2\right] \end{aligned} \tag{26}$$

This is the apparent MWD obtained when the elution curve was evaluated using the calibration dependence for polymer 2, different from the analyte, polymer 1.

According to Eq. (26), the relation between β_1 and β_2 is

$$\frac{\beta_1}{\beta_2} = \frac{B'_1}{B'_2} \tag{27}$$

As β, defined by Eq. (14), describes the width of MWD, Eq. (27) can conveniently be used to estimate the difference between the widths of the true and apparent MWD. Substitution of Eq. (14) in Eq. (27) gives

$$\ln\frac{M_{w2}}{M_{n2}} = \frac{B'^2_2}{B'^2_1}\ln\frac{M_{w1}}{M_{n1}} \tag{28}$$

Using the relation between B'_1 and B'_2 (Eq. (8), we can write

$$\frac{M_{w2}}{M_{n2}} = \left(\frac{M_{w1}}{M_{n1}}\right)^{\frac{B'^2_2}{B'^2_1}} = \left(\frac{M_{w1}}{M_{n1}}\right)^{\left(\frac{a_2+1}{a_1+1}\right)^2} \tag{29}$$

An analogous calculation has been made for MWD of the Schulz–Zimm type [16,17]. The equations are rather complex and not reproduced here. They are available from the corresponding author at request.

3. Results and discussion

The exponent a in the MHKS equation (Eq. (3)) can assume values between zero and two. The limiting values correspond to spherical and rod-like molecules or particles, respectively [18]. SEC in the simple mode with only a concentration detector is most widely and successfully used for characterization of polymers with linear flexible molecules, assuming conformation of random coils in solution. For such polymers, the value of the exponent a

Table 1
Parameters of the MHKS equation (Eq. (3)) used in calculation of calibration lines in Figs. 1 and 2; solvent tetrahydrofuran

Polymer	T (°C)	$K \times 10^3$ (ml/g)	a	Range of $M \times 10^{-4}$	Ref.	Line[a]
Polystyrene	25	13.63	0.714	2–4000	[20]	1
Poly(N–isopropylacrylamide)		6.9	0.73	2.23–130	[21]	2
	27	9.59	0.65	10–100	[22]	2′
Poly(4–*tert*–butylstyrene)	30	10.4	0.7	2.7–45.5	[23]	3
Poly(p–menthyl methacrylate)[b]	25	11.5	0.65	12–230	[24]	4
Poly(methyl methacrylate) atactic	25	7.5	0.72	1.7–1277	[6]	5
Poly(diisopropyl fumarate)	30	0.518	0.98	5.76–72.6	[11]	6
Poly(vinyl chloride)	25	19.1	0.75	1–10	[25]	7,8
	25	15	0.77	1–12	[26]	9
	25	16.3	0.766	2–30	[27]	10
	20	3.63	0.92	2–17	[28]	11
	25	49.8	0.69	4–40	[30]	12
	30	63.8	0.65	3–32	[31]	13

[a] Numbers 1 through 7 and 8 through 13 refer to lines in Figs. 1 and 2, respectively.

[b] Structures **1** and **2** in Ref. [24] are interchanged; that denoted by **1** is poly(2-*tert*-butylphenyl methacrylate), that denoted by **2** is poly(p-menthyl methacrylate).

varies between 0.5 for theta solvents and 0.8 for thermodynamically very good solvents [19]. The following discussions are limited essentially to this range of values of exponent a. The interpretation of SEC data for polymers showing values of the MHKS exponent outside this range, such as branched or stiff-chain polymers, is complex [7] and out of the scope of this study.

In Table 1, the parameters of the MHKS equation (Eq. (3)) for several flexible-chain polymer solutions are listed [29]. Substituting the parameters from Table 1 and the parameters of the calibration dependence for polystyrene ($A_2 = 11.825$ and $B_2 = -0.436\ \text{ml}^{-1}$) taken from Ref. [32] in Eqs. (7) and (8), we obtained the calibration dependences (Eq. (5)) for the individual polymers. Strictly speaking, the data in Table 1 were determined at various temperatures close to 25 °C. As small temperature differences have a negligible effect on intrinsic viscosity of polymers in a good solvent ($a \approx 0.7$) [7], this lack of rigor does not introduce any perceivable error. The calibration lines for individual polymers (Fig. 1) are dispersed above and below that for polystyrene. The difference between the highest and lowest calibration line is about $0.48 \log M$, i.e. the disparity between the lowest and highest M eluted at the same elution volume is a factor of three. The highest M is about twice higher (poly(p-menthyl methacrylate) and the lowest is 0.66 times lower (PVC) than that for polystyrene. The dependences for poly(N-isopropylacrylamide) were calculated using two sets of MHKS parameters [21,22]. The dependence calculated using the first set [21] (line 2) is practically indistinguishable from that calculated for poly(methyl methacrylate) (line 5). The dependences are relatively close to that calculated for polystyrene (line 1). This can explain a good agreement of M_w values, found for this polymer by SEC using the calibration dependence for polystyrene and values determined by other methods [5]. The dependence calculated using the second set of parameters [22] (line 2′) deviates largely from lines 2 and 5. The parameters found using polymer fractions characterized by M_n values are probably in error.

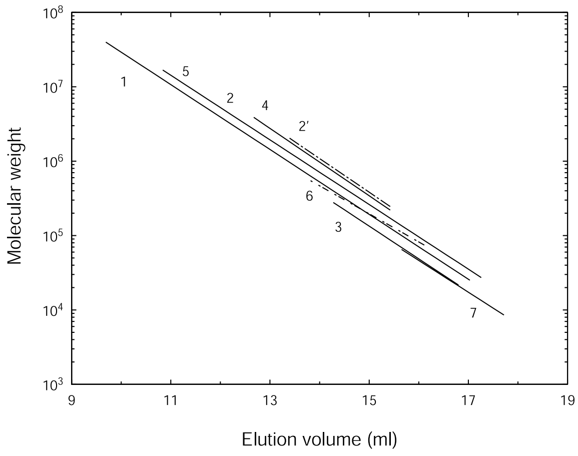

Fig. 1. Comparison of calibration lines calculated according to Eqs. (6)–(8) for parameters of Eq. (3) of polymers, denoted by numbers referring to Table 1, with that for polystyrene (line 1).

On the other hand, large differences between M_w found by SEC calibrated with polystyrene standards and light scattering (the former about one half of the latter) were reported [11] for poly(diisopropyl fumarate) where the calculated calibration dependence is very close to that for polystyrene, although differing in slope, but intersecting it just in the middle of the range of V (dashed line 6 in Fig. 1). This might be explained by a high chain stiffness and a correspondingly high value of the MHKS exponent ($a = 0.98$). It has been reported [10] that, for stiff-chain polymers such as polysaccharides and DNA in aqueous mobile phase, the universal calibration fails. For example, molecular weight of shizophyllan eluted at a particular V is considerably lower than predicted by universal calibration constructed using flexible-chain pullulan samples [10].

It is interesting to compare calibration dependences for a particular polymer, poly(vinyl chloride), calculated

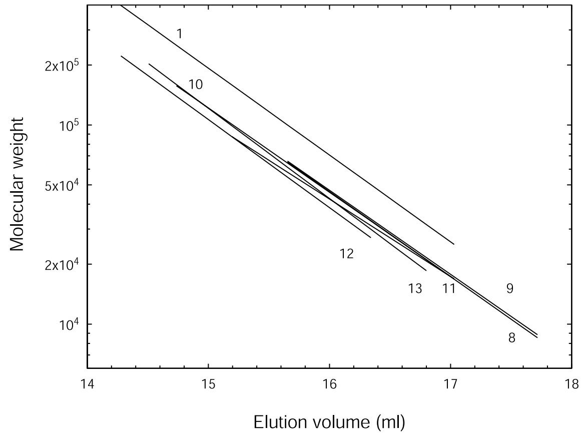

Fig. 2. Comparison of several calibration lines calculated for poly(vinyl chloride) according to Eq. (4) using various parameters of Eq. (3), denoted by numbers referring to Table 1, with that for polystyrene (line 1).

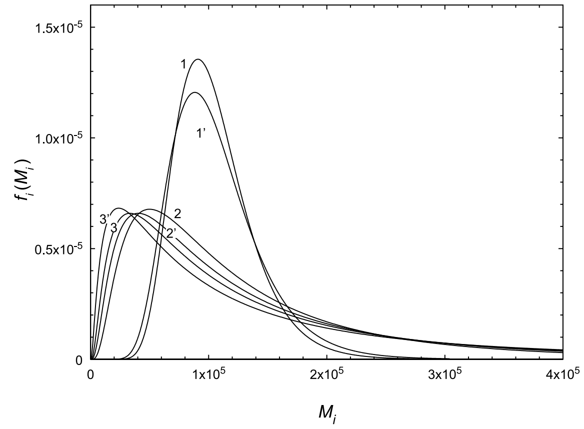

Fig. 4. Comparison of the true log–normal distributions $f_1(M_1)$ (Eq. (13) for $M_{01} = M_{02} = 10^5$, $a_1 = 0.5$ and $a_2 = 0.714$ for samples characterized by the ratios $M_{w1}/M_{n1} = 1.1$, 2 and 3 (curves 1, 2 and 3, respectively) with apparent distributions $f_2(M_2)$ characterized by $M_{w1}/M_{n1} = 1.13$, 2.47 and 4.20, respectively, calculated according to Eq. (29) (curves 1′, 2′ and 3′).

employing Eqs. (6)–(8), the calibration dependence for polystyrene and several sets of MHKS parameters from Table 1 (Fig. 2). Although the exponent varies in a large range of $0.65 \leq a_1 \leq 0.92$, the molecular weights calculated in the range where the parameters had been determined differ just by 10–15% due to partial compensation of the effects of the two MHKS parameters [33].

Another question of practical importance is what is the effect of using inappropriate calibration parameters on the estimation of the M_w/M_n ratio from a SEC concentration elution curve. The reason for doing so can be that the calibration dependence cannot be recalculated because the parameters of the MHKS equation (Eq. (3)) for the analyte are not known or are loaded with a significant error. The latter alternative occurs, e.g., if the MHKS-equation parameters were determined in a narrow or distant range of M. Fig. 3 offers an answer to that question. In the graphs, the apparent values of the M_w/M_n ratio, M_{w2}/M_{n2}, calculated for the log–normal distribution according to Eq. (29) (Fig. 3a) and the Schulz–Zimm distribution (Fig. 3b), are plotted as a function of its true value, when the evaluation was made using the calibration dependence for polystyrene, $a_2 = 0.714$, instead of the correct calibration dependence for the analyte, in the range of the MHKS exponent values $0.5 \leq a_1 \leq 0.8$. From the graphs, it can be seen that increasing a_1 causes a decrease in M_{w2}/M_{n2} and vice versa. The difference can be, with $a_2 = 0.714$ (polymer used for the calibration) and $0.5 < a_1 < 0.8$ (analyte), up to 30%. This is due to the fact that, with increasing a, $[\eta]$ and, consequently, $[\eta]M$ grows more steeply. Generally, if a_1 for the analyte is lower than a_2 for the standard polymer used for the evaluation of the concentration elution curve, the apparent ratio M_{w2}/M_{n2} is larger than the correct ratio M_{w1}/M_{n1} and vice versa. The dependence for $a_1 = 0.714$ is a straight line since for $a_1 = a_2$ the apparent and true M_w/M_n ratios are equal. The deviations of the M_w/M_n dependences from the correct line, calculated according to Eq. (29) (Fig. 3a) for the log–normal MWD, are somewhat higher than those calculated for the Schulz–Zimm MWD. This can be explained by the

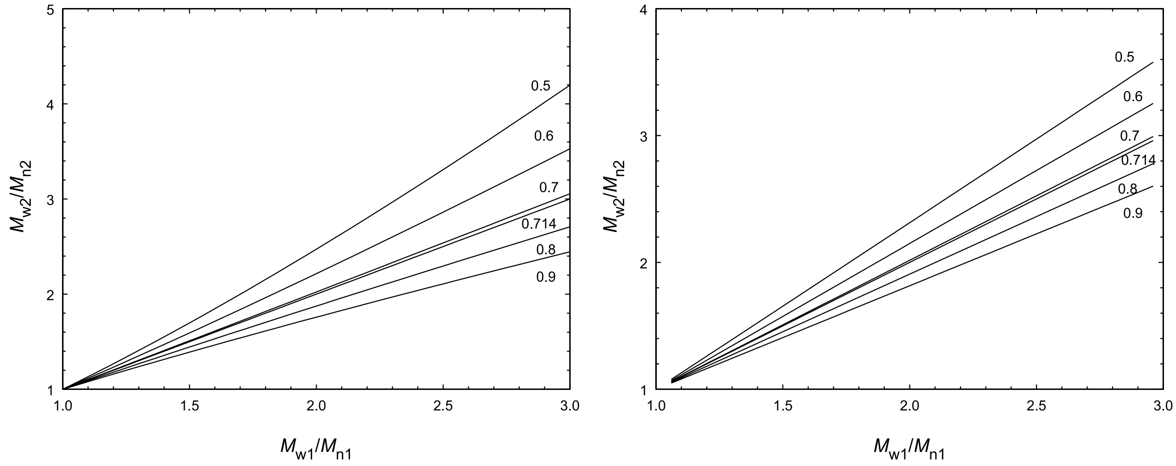

Fig. 3. Comparison of the dependence of the apparent ratio M_{w2}/M_{n2} on the true ratio M_{w1}/M_{n1} for polymers differing in a_1 (denoted on the curves) with that for polystyrene ($a_2 = 0.714$) calculated for (a) log–normal MWD according to Eq. (29). (b) Schulz–Zimm MWD.

fact that the M_w and M_n values are not equally sensitive to the change in the slope of the calibration dependence when using different types of MWD. The differences, however, are minute.

The difference between the true and apparent log-normal distributions, $f_1(M_1)$ and $f_2(M_2)$, calculated according to Eq. (13), is demonstrated in Fig. 4 for $M_{01} = M_{02} = 10^5$, $a_1 = 0.5$ and $a_2 = 0.714$ for samples characterized by the ratios $M_{w1}/M_{n1} = 1.1$, 2 and 3 ($M_{w2}/M_{n2} = 1.13$, 2.47 and 4.20, respectively, calculated according to Eq. (29). From the figure, it can be seen that the change in apparent MWD of narrow-MWD samples can be large.

4. Conclusions

Using for evaluation of SEC data a calibration dependence for a chemically different polymer can result in a considerable error in the determination of molecular weight, the M_w/M_n ratio and MWD. For equal values of the MHKS exponent of polymer 1, which is the analyte, and polymer 2, a calibration standard, i.e. $a_1 = a_2$, the calibration lines are shifted in a parallel way, the ratio M_2/M_1 is constant for any range of molecular weight, i.e. at any elution volume, and can amount up to a value of three, but the M_w/M_n ratio is not affected. An increasing difference between the values of a_1 and a_2 results, in the range of values typical of flexible-chain polymers, in a variation of the ratio M_2/M_1 with molecular weight, and in an error in the M_w/M_n ratio amounting up to a value of two.

Acknowledgements

The authors gratefully acknowledge support by the Academy of Sciences of the Czech Republic (project No. AVOZ 4050913).

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