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Influence of Chemical Heterogeneity of Copolymers on the Separation Process in Size-Exclusion Chromatography

M. Netopilík,* M. Bohdanecký, and P. Kratochvíl

Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, 162 06 Prague 6, Czech Republic

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ABSTRACT: The effect of the chemical-composition heterogeneity of flexible-chain binary copolymers on their separation in size-exclusion chromatography (SEC) was theoretically examined. The two-parameter theory of polymer solutions was engaged to estimate the coil size. The variation in molecular weight is shown for copolymer molecules which differ in the ratio of the two constituent monomeric units but have the same coil size and, therefore, are eluted at the same elution volume. The result was applied to a theoretical case of analysis of copolymers, the composition and molecular-weight distributions of which are given by theoretical functions. If the refractive index increments of homopolymers whose units constitute the copolymer are equal or close to each other, the influence of the chemical heterogeneity on the experimental data is below the experimentally detectable limit. A significant error in the determination of the molecular-weight averages can occur only if the difference in the refractive index increments exceeds physically reasonable limits.

Introduction

The combination of size-exclusion chromatography (SEC) with an absolute method for the molecular-weight determination, e.g., light scattering (LS), brings new possibilities to polymer analysis. On the other hand, theoretical problems arise in the evaluation of experimental data. This holds true especially in SEC of copolymers, where questions of two kinds arise: first, how a copolymer is separated and, second, how it is detected and what kind of information may be drawn from the raw detector data.

There is no unambiguous relation between the coil structure and the elution volume of a particular polymer species. It is believed that the separation process is governed by the size-exclusion radius of the molecule, R_{SEC}, but there is no general agreement whether R_{SEC} corresponds to the theoretically founded mean projection on a line, or to the viscosity radius or radius of gyration.[1,2] On the other hand, as pointed out by Dubin et al.,[3] if the SEC studies are confined to a single structural type, virtually any dimensional parameter will prove successful. Hence, the well-known universal calibration concept[4] based on the product of molecular weight and intrinsic viscosity, $[\eta]M$, holds among polymers of similar chain characteristics.

Our study is focused on the flexible-chain copolymers of two comonomers, A and B, and the hydrodynamic volume $[\eta]M$ will be taken as a measure of the molecular size.

With copolymers, the chain dimensions depend not only on the molecular weight or chain length but also on the composition, i.e., on the weight fraction w_{A} (in the following, the subscript will be dropped) of monomeric units A in the copolymer AB. It is obvious that the two quantities may compensate each other in some sense, i.e., that some molecules of different composition and molecular weight may have the same coil dimensions and, consequently, be eluted at the same elution volume V. Due to this, polydispersity of fractions and deteriorated separation according to molecular weight (in addition to the well-known axial dispersion effect[5]) have to be expected. In consequence of that, there is uncertainty connected with the interpretation of experimental data,[6−12] which is a serious obstacle, e.g., to the confirmation of theories that forecast the statistics of copolymerization. Therefore, the effect of chemical composition on the size separation should be analyzed in order to estimate its influence on the precision of the experimental data.

Generally, light scattering from copolymer solutions yields only an apparent (weight-average) molecular weight $\bar{M}_{\mathrm{H}}$*, rather than its true value.[13−15] Another complication appears if LS is used in the dual-detection arrangement: the correct value of concentration cannot be computed from the differential refractometer (DR) data unless w at every elution volume is known.

Based on the assumptions listed above, a SEC analysis of a copolymer with the distribution of molecular weight $W(M)$ and chemical composition $W'(w)$ described by theoretical functions was modeled. Our aim was to find whether differences in the structure and properties of comonomers influence the separation process and whether, and how, the information provided by the single DR detector or by the dual DR/LS detector combination corresponds to the true distribution characteristics of a particular copolymer.

Theory

Coil Dimensions. According to the two-parameter theory of polymer solutions, the hydrodynamic volume $[\eta]M$ can be calculated from the intrinsic viscosity by means of the equation[16,17]

$$[\eta] = \Phi_{0,\infty}(\langle R^2\rangle_0/M)_\infty^{\ 3/2}M^{1/2}\alpha_\eta^{\ 3} \qquad (1)$$

where $\Phi_{0,\infty}$ is the limiting value of the Flory viscosity function for nondraining random coils and $(\langle R^2\rangle_0/M)_\infty$ is the random-coil value of the ratio of the unperturbed mean-square end-to-end distance $\langle R^2\rangle_0$ and molecular weight. This study is confined to copolymers of vinyl monomers, i.e., with both monomeric units having identical length; the value $l = 1.5 \times 10^{-8}$ cm has been

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used throughout the calculations. It is convenient to write

$$(\langle R^2\rangle_0/M)_\infty = \frac{2l^2}{m_0}C_\infty \tag{2}$$

where m_0 is the molecular weight of the monomeric unit, l is the length of the main-chain bond, and C_∞ is the characteristic ratio

$$C_\infty = (\langle R^2\rangle_0/nl^2)_\infty \tag{3}$$

where n is the number of main-chain bonds in the chain.

The viscosity expansion factor, defined as[16,17]

$$\alpha_\eta{}^3 = [\eta]/[\eta]_0 \tag{4}$$

where $[\eta]_0$ is the intrinsic viscosity corresponding to the unperturbed state, can be expressed as a function of the excluded-volume parameter z[18,19]

$$\alpha_\eta{}^3 = (1 + 1.9z)^{3/5} \tag{5}$$

where[16]

$$z = \left(\frac{3}{2\pi}\right)^{3/2}\left(\frac{\langle R^2\rangle_0}{M}\right)_\infty^{-3/2} BM^{1/2} \tag{6}$$

and the interaction parameter, B, is a function of the binary cluster integral.

With copolymers, similar relations are valid, but the chemical composition impacts on the chain dimensions.

The characteristic ratio of the copolymer molecule can be written as[20]

$$C_{\infty,\mathrm{C}} = xC_{\infty,\mathrm{A}} + (1 - x)C_{\infty,\mathrm{B}} + \delta C_{\infty,\mathrm{C}} \tag{7}$$

where x is the molar fraction of A units in the copolymer molecule and where the term $\delta C_{\infty,\mathrm{C}}$ corrects for the population of unlike diads (AB and BA) of the monomeric units.[20] For block copolymers and for copolymers where $C_{\infty,\mathrm{A}}$ is close to $C_{\infty,\mathrm{B}}$, the equation obeys the approximation proposed previously by Stockmayer,[13] i.e., $\delta C_{\infty,\mathrm{C}} = 0$.

Similarly for the interaction parameter of a copolymer, we have[9]

$$B_\mathrm{C} = xB_\mathrm{A} + (1 - x)B_\mathrm{B} + 2x(1 - x)B_\mathrm{AB} \tag{8}$$

where B_AB is the term expressing the interaction of monomeric units A and B.

Chemical-Composition Distributions. The distribution of chemical composition and molecular weight of a copolymer is characterized by a two-dimensional distribution function, which gives the probability density of the presence of a molecule of a given composition (e.g., molar fraction x or weight fraction w of component A in the copolymer molecule AB) and molecular weight M in the sample. For the sake of simplicity, it is usually assumed that this function may be expressed as a product of two marginal distribution functions, that of the chemical composition, $W'(w)$, and the other of molecular weight, $W(M)$.[21−27] In other words, it is assumed that $W'(w)$ is the same for molecules with any specific value of molecular weight, and $W(M)$ is the same for molecules with any specific composition. This assumption is generally not fulfilled. Its potential effect on the results of SEC analysis of copolymers is being investigated by the authors.

Three types of $W'(w)$ are employed in the calculations: $W'(w)$ due to the conversion heterogeneity (which is generated by the change of ratio of monomer concentrations during the polymerization), $W'(w)$ due to the statistical heterogeneity (caused by the statistical nature of the chain-growth reaction), and, as a case of an extremely broad $W'(w)$, the rectangular distribution where the probability density is uniform throughout the whole span of w, i.e., from unity to zero.

Results and Discussion

In accordance with the principle of universal calibration, the molecules eluted at a fixed elution volume V should have identical hydrodynamic volumes, i.e., the values of the product $[\eta]M$.[4] As a starting point to the modeling of SEC experiments, the variation in molecular weight and composition of copolymer molecules at a constant $[\eta]M$ will be discussed.

Molecular Weight and Composition at Constant $[\eta]M$. Combining eqs 1−6, one gets

$$[\eta]M/\Phi_{0,\infty} = G_1M^{3/2}(1 + G_2M^{1/2})^{3/5} \tag{9}$$

where

$$G_1 = (2l^2/\bar{m}_0)^{3/2}C_{\infty,\mathrm{C}}{}^{3/2} \tag{10}$$

$$G_2 = 1.9\left(\frac{3\bar{m}_0}{4\pi lC_{\infty,\mathrm{C}}}\right)^{3/2}B_\mathrm{C} \tag{11}$$

and the average molecular weight per monomeric unit $\bar{m}_0$ is

$$\bar{m}_0 = xm_{0,\mathrm{A}} + (1 - x)m_{0,\mathrm{B}} \tag{12}$$

It follows from eq 9 that the molecular weight of copolymer molecules eluted at a fixed V, denoted hereinafter by $\bar{M}_V$, depends on several variables specific to the system: characteristic ratios, interaction parameters, copolymer composition, and molecular weight of monomeric units. In practice, the effects of these factors overlap and may be cooperative or antagonistic. (The molecular weight M_V and the corresponding degree of polymerization P_V should not be confused with the viscosity-average values.)

In order to evaluate the weight- and number-average molecular weights, $\bar{M}_{\mathrm{w},V}$ and $\bar{M}_{\mathrm{n},V}$, of molecules eluted at a fixed elution volume V, it is necessary to take into consideration all possible chemical compositions ($0 < x < 1$) and to calculate, according to the dual molecular weight-composition distribution, the fraction of the particular polymer species characterized by x and M. To illustrate the role of the characteristic ratio, reflecting the chain stiffness, we calculate the M_V values for molecules of different compositions and the $\bar{M}_{\mathrm{w},V}/\bar{M}_{\mathrm{n},V}$ ratio assuming a vinyl-type copolymer and $m_{0,\mathrm{A}} = m_{0,\mathrm{B}}$, $B_\mathrm{A} = B_\mathrm{B} = B_\mathrm{AB} = 0$ (i.e., $G_2 = 0$ and $\alpha_\eta{}^3 = 1$). It follows from eqs 1, 2, and 7 for $\delta C_{\infty,\mathrm{C}} = 0$ that, in this case,

$$\langle R^2\rangle_{0,\mathrm{C}} = 2C_{\infty,\mathrm{A}}l^2Pq \tag{13}$$

where

$$q = p - (p - 1)x \tag{14}$$

$$p = C_{\infty,\mathrm{B}}/C_{\infty,\mathrm{A}} \tag{15}$$

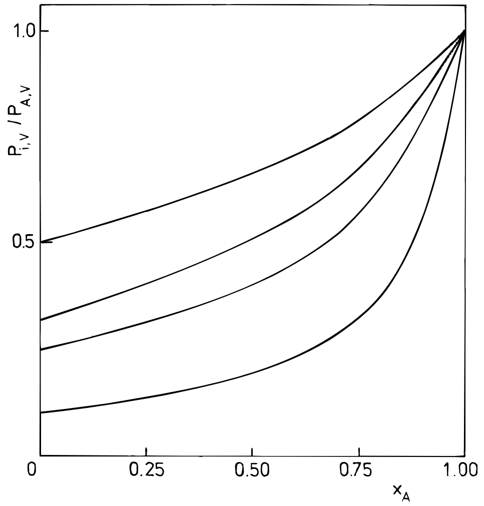

Figure 1. Dependence of the ratio of the degree of polymerization of the copolymer to that of the homopolymer A, $P_{i,V}/P_{A,V}$, eluted at fixed elution volume, V, on the composition, x_B, calculated according to eq 16 for $p \equiv C_{\infty,A}/C_{\infty,B} = 2, 3, 5$, and 10 (curves from top to bottom).

and P is the degree of polymerization ($P = n/2$). To fulfill the condition of equal hydrodynamic volumes of molecules eluted at a constant V, the $P_{i,V}$ values must satisfy the relation

$$P_{i,V}q_i = \text{const} \tag{16}$$

where subscript i refers to copolymer molecules characterized by the molar fraction x of monomeric units A. The constant in eq 16 is obtained by choosing homopolymer A ($x = 1$) as a reference composition, where $P_{i,V} = P_{A,V}$ and $q_i = 1$. Figure 1 presents the dependences of $P_{i,V}/P_{A,V}$ on x for several values of p. The increasing trend of the dependences is a consequence of $p > 1$ ($C_{\infty,A} < C_{\infty,B}$).

The differences in M (or P) of the polymer molecules eluted at a fixed elution volume, V, lead to the polymolecularity of fractions, $\bar{P}_{w,V}/\bar{P}_{n,V}$, where the weight- and number-average degrees of polymerization are defined

$$\bar{P}_{n,V} = [\int_0^1 \tilde{W}'(x)/P_V(x)\,dx]^{-1} \tag{17}$$

$$\bar{P}_{w,V} = \int_0^1 \tilde{W}'(x)P_V(x)\,dx \tag{18}$$

$\tilde{W}'(x)$ is the distribution of chemical composition at the elution volume V. The $\bar{P}_{w,V}/\bar{P}_{n,V}$ ratio will now be assessed. Assuming further, for the sake of simplicity, the rectangular frequency distribution of chemical composition (each composition with the same frequency, $n_{i,V}$ = const) and assuming $m_{0,A} = m_{0,B}$, the weight fraction of molecules of composition x may be expressed as

$$\tilde{W}'_i = P_{i,V}/\sum_i P_{i,V} \tag{19}$$

Substituting for $P_{i,V}$ from eq 16 and replacing sums by integrals (in limits $0 \leq x_i \leq 1$), we obtain

$$\tilde{W}'(x) = (p-1)[p-(p-1)x]^{-1}/\ln p \tag{20}$$

By substituting into eqs 17 and 18 for $P_V(x)$ from eq 16 and for $\tilde{W}'(x)$ from eq 20, we obtain

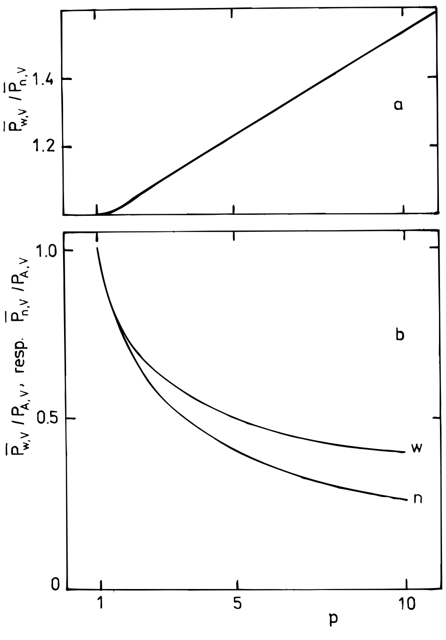

Figure 2. Dependence on the ratio $p \equiv C_{\infty,B}/C_{\infty,A}$ for the copolymer AB eluted at a fixed elution volume, V, of the $\bar{P}_{w,V}/\bar{P}_{n,V}$ ratio (a) and the ratios $\bar{P}_{w,V}/P_{A,V}$ (upper curve) and $\bar{P}_{n,V}/P_{A,V}$ (lower curve) (b).

$$\bar{P}_{w,V} = (p-1)/p \ln p \tag{21}$$

$$\bar{P}_{n,V} = \ln p/(p-1) \tag{22}$$

The results of these calculations for $1 \leq p \leq 10$ are presented in Figure 2. The $\bar{P}_{w,V}/\bar{P}_{n,V}$ ratio of fractions increases with increasing p (Figure 2a), which is due to the fact that $\bar{P}_{n,V}/P_{A,V}$ decreases more rapidly than $\bar{P}_{w,V}/P_{A,V}$ (Figure 2b). The plot of $\bar{P}_{w,V}/\bar{P}_{n,V}$ vs p is almost linear, particularly at $p > 2$.

While, under ideal conditions, the polymolecularity index of fractions of homopolymers eluted in an SEC experiment should be equal to unity, it may be significantly increased above this value with copolymers, if the characteristic ratios $C_{\infty,A}$ and $C_{\infty,B}$ are different.

The range of p values considered in the present calculations is broad, whereas the C_∞ values of most vinyl-type polymers are between 5 and 25. For the range $1 \leq p \leq 5$, the values of $\bar{P}_{w,V}/P_{A,V}$ and $\bar{P}_{n,V}/P_{A,V}$ decrease from 1 to 0.5 and from 1 to 0.4, respectively. Hence the $\bar{P}_{w,V}/\bar{P}_{n,V}$ ratio increases from unity to 1.25.

Unlike the effect of the characteristic ratio, the effect of the values of the interaction parameters B cannot be expressed in general terms, and eq 9 has to be solved for M numerically; the Newton method of tangents was used. To show this influence, we present an example, assuming the rectangular distribution of chemical composition, letting $C_{\infty,A} = C_{\infty,B} = 10$, $B_A = 0.1 \times 10^{-26}$, $B_{AB} = 0$ and varying B_B in the range $0.1 \leq B_B \times 10^{-26} \leq 3$; $m_{0,A} = m_{0,B} = 100$ was assumed in these calculations. The dependences on B_B of the $\bar{P}_{w,V}/\bar{P}_{n,V}$ ratio calculated for $[\eta]M/\Phi_{0,\infty} \times 10^{-15} = 18$, 2, and 0.8 (corresponding to $M \times 10^{-6} = 5.6$, 1.3, and 0.7 and $[\eta]/(\text{mL/g}) = 827$, 398, and 110, respectively) are depicted in Figure 3. It can be seen that the polymolecularity of copolymer fractions eluted at a fixed elution volume becomes pronounced only at very high, rather improbable differences in the interaction parameters of homopolymers. The role of

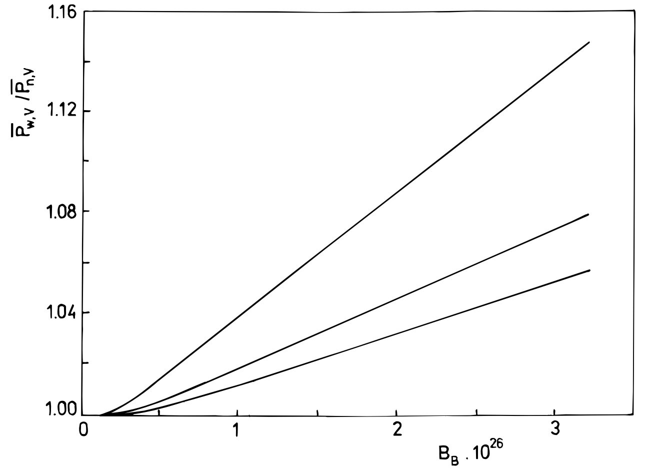

Figure 3. Dependence on the interaction parameter B_B of the calculated $\bar{P}_{w,V}/\bar{P}_{n,V}$ ratio of the copolymer eluted at elution volume V. Calculation with $B_A = 1 \times 10^{-27}$, $B_{AB} = 0$, $C_{\infty,A} = C_{\infty,B} = 10$, and $m_{0,A} = m_{0,B} = 100$ for $[\eta]M/\Phi_{0,\infty} \times 10^{-3} = 50$, 10, and 5 (curves from top to bottom).

Table 1. Values of the Interaction Parameter (*B*), Partial Specific Volume ($\bar{v}$), Molar Volume of the Solvent (V_S), and Characteristic Ratio (C_∞), Taken from Ref 16 for the Model Calculation (cf. Figure 4) for Polystyrene (PS) and Poly(methyl methacrylate) (PMMA) in Various Solvents

			PS ($C_\infty = 9.85$)		PMMA ($C_\infty = 6.7$)	
solvent	temp (°C)	V_S (mL/g)	$\bar{v}$ (mL/g)	$10^{29}B$ (cm^3)	$\bar{v}$ (mL/g)	$10^{29}B$ (cm^3)
THF[a]	25.05	82.3	0.9102	6.15	0.8085	4.21
toluene	30.0	107.4	0.917	6.25	0.807	2.38
MEK[b]	30.0	90.8	0.9078	1.07	0.7993	2.83

[a] Tetrahydrofuran. [b] 2-Butanone.

the polymer–solvent interaction turns out to be less important than that of the chain stiffness.

To illustrate these considerations by a typical pair of comonomers, viz., methyl methacrylate and styrene, we repeat the calculations using data (cf. Table 1) taken from the literature and setting $[\eta]M/\Phi_{0,\infty} = 3 \times 10^{-18}$, a value giving M in a reasonable range. The result obtained for several solvents by numerically solving eq 9 for M is depicted in Figure 4 as a function of the weight fraction of methacrylate units. The hypothetical case where $B_A = B_B = B_{AB} = 0$ is also included. The difference in molecular weights M_V eluted under the given conditions may amount to as much as 30%, but the $\bar{M}_{w,V}/\bar{M}_{n,V}$ ratio is still less than 1.01. This is probably due to a low value of 1.47 of the parameter p.

Molecular-Weight-Distribution Analysis of Copolymers by SEC. The question arises to what extent the facts demonstrated in the preceding part are reflected in the results of the molecular-weight-distribution analyses carried out by SEC with copolymer samples which are polydisperse in molecular weight and heterogeneous in composition. To answer this question, the weight- and number-average molecular weights obtained by model calculation ("experimental values") will be confronted with the "true" ones. To reflect only the polydispersity in chemical composition and molecular weight and to exclude trivial effects such as changes of molecular weight with the variation of $m_{0,A}$ or $m_{0,B}$, the results of modeled analyses of two species will be compared: that of a copolymer of given $\bar{M}_n$, $\bar{M}_w$, etc., with a homopolymer of identical average molecular weights and molecular characteristics, i.e., monomer molecular weight (eq 12), characteristic ratio (eq 7), and parameter of interaction (eq 8).

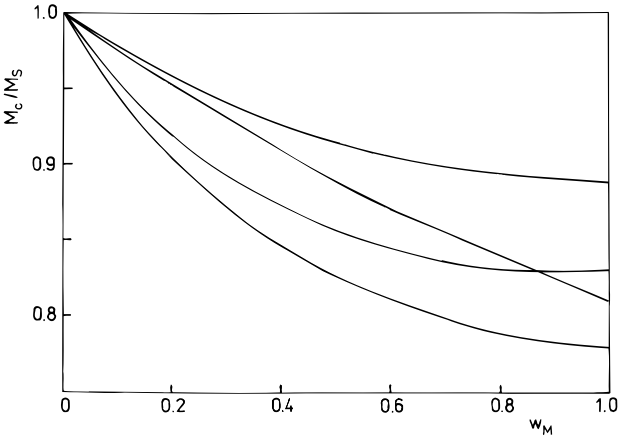

Figure 4. Dependence on the copolymer composition w_H (weight fraction of methyl methacrylate units) of the ratio of molecular weight of poly(styrene-*co*-methyl methacrylate), M_C, to molecular weight of styrene homopolymer, M_S, eluted at constant elution volume, V, in 2-butanone, hypothetical Θ solvent, tetrahydrofuran, and toluene (curves 1 to 4, respectively from top to bottom). Parameter values from Table 1 were used for computation.

The discussion of the problem is divided into two parts, depending on the conditions of detection in the SEC experiment. In the former, identity of the refractive index increments of the parent homopolymers, $\nu_A = \nu_B$, and thus true concentration detection will be assumed. In the latter, a real situation will be modeled where $\nu_A \neq \nu_B$.

True Concentration Detection. We now calculate the normalized chromatogram heights $F(V)$ at elution volume V for copolymers. We assume that the concentration detector is capable of detecting the mass of polymer in solvent regardless of the refractive index increment of its constituents. Let the contribution of the copolymer species with composition w to the chromatogram at elution volume V be $f(w,V)$. The integrals both of the normalized chromatogram over the composition and elution volume and of the normalized dual-composition molecular-weight distribution should be unity. This condition may be written in the differential form

$$f(w,V)\,\mathrm{d}w\,\mathrm{d}V = W'(w)W(M_V(w))\,\mathrm{d}w\,\mathrm{d}M_V(w) \qquad (23)$$

(where the subscript V indicates M at a fixed V) or

$$f(w,V) = W'(w)W(M_V(w))\frac{\mathrm{d}M_V(w)}{\mathrm{d}V} \qquad (24)$$

The differential term can be expressed as

$$\frac{\mathrm{d}M_V(w)}{\mathrm{d}V} = M_V\frac{\mathrm{d}\log M_V(w)}{\mathrm{d}\log M_{H,V}}\frac{\mathrm{d}\log M_{H,V}}{\mathrm{d}V} \qquad (25)$$

where $M_{H,V}$ is the molecular weight of homopolymer eluted at V. The last differential can be evaluated from the well-known calibration relation for homopolymers

$$\log M_{H,V} = D_1 - D_2V \qquad (26)$$

as

$$\frac{\mathrm{d}\log M_{H,V}}{\mathrm{d}V} = -D_2 \qquad (27)$$

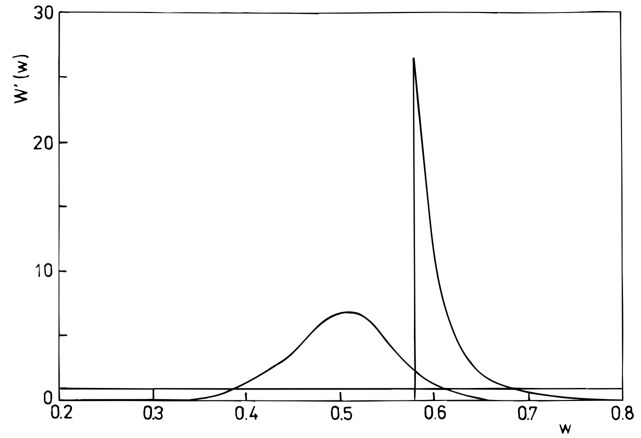

Figure 5. Example of chemical-composition distribution functions $W'(w)$ used in the model calculations: (a) rectangular; (b) conversional with $\bar{w} = 0.75$ ($r_A = 0.48$, $r_B = 0.46$; the polymer composition was $w = 0.68$ and $w = 0.88$ at the beginning and at the end of the copolymerization; degree of conversion was $\varphi = 0.91$); (c) statistical ($r_A r_B = 0.25$ and $\bar{M}_w = 10^5$).

The result is

$$\frac{\mathrm{d}M_V(w)}{\mathrm{d}V} = -M_V(w)D_2\frac{\mathrm{d}\log M_V(w)}{\mathrm{d}\log M_{\mathrm{H},V}} \tag{28}$$

From eqs 25 and 28 we obtain

$$f(w,V) = -M_V(w)D_2\frac{\mathrm{d}\log M_V(w)}{\mathrm{d}\log M_{\mathrm{H},V}}W'(w)W(M_V(w)) \tag{29}$$

For the chromatogram height we have

$$F(V) = \int_0^1 f(w,V)\,\mathrm{d}w \tag{30}$$

If M is independent of w (i.e., $M_V(w) \equiv M_V$), which also is the case for homopolymers, combining eqs 29 and 30 leads to the well-known equation for the chromatogram heights[31]

$$F(V) = -M_V D_2 W(M) \tag{31}$$

The normalized chromatogram of the hypothetical homopolymer is generated using eqs 30 and 26 with the values of the calibration constants ($D_1 = 10.00$ and $D_2 = 0.20$) corresponding to real systems. The Schulz–Zimm model distribution function[30] of molecular weight in the form

$$W(M) = \frac{a^{b+1}M^b}{\Gamma(b+1)}\mathrm{e}^{-aM} \tag{32}$$

where Γ is the gamma function, and

$$a = \frac{b}{\bar{M}_n} \qquad b = \frac{\bar{M}_n}{\bar{M}_w - \bar{M}_n} \tag{33}$$

was used in the calculations. The calculation for the copolymers was performed using eq 29 for three model chemical-composition distributions, $W'(w)$, which are depicted in Figure 5. The differential factor d log $M_V(w)$/d log $M_{\mathrm{H},V}$ is evaluated numerically as the ratio of differences log $M_{V+\Delta V}(w)$ − log $M_V(w)$ and log $M_{\mathrm{H},V+\Delta V}$ − log $M_{\mathrm{H},V}$, where ΔV is the increment of the elution volume in each step of the computation (usually $\Delta V =$ 1). An example of this factor as a function of the composition for a sample of the average composition $\bar{w} = 0.5$ is shown in Figure 6.

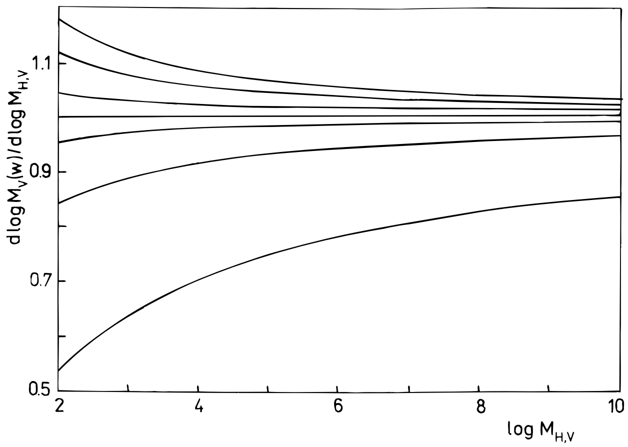

Figure 6. Example of the differential factor d log $M_V(w)$/d log $M_{\mathrm{H},V}$ as a function of log $M_{\mathrm{H},V}$, calculated according to eq 26 for a sample with average composition $\bar{w}_A = 0.5$, $C_{\infty,A} = 5$, $C_{\infty,B} = 10$, $m_{0,A} = 100$, $m_{0,B} = 50$; curves (from top to bottom) for $w_A = 0$, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.

The molecular-weight averages of fractions eluted at constant elution volume (cross-sections of the chromatogram) as well as the total apparent average molecular weights of the copolymer are calculated in the standard way. The molecular weight at a given elution volume is assumed to be either determined by a molecular-weight-sensitive detector, such as the light-scattering detector, or read from the calibration for a homopolymer. The latter procedure is methodically wrong but is often used in practice, because calibrations for copolymers with different compositions are not available. The results summarized in Table 2 are divided into three parts according to the function $W'(w)$ used. We first discuss the most unfavorable case, the broad rectangular distribution function. It is seen that, using the light-scattering detector, the differences between the “true” and “experimental” values of the average molecular weights are within the experimental error (LS^a, LS^c) unless the ratio $p = C_{\infty,B}/C_{\infty,A}$ is large (LS^b). On the contrary, large differences in the interaction parameter (LS^c) have a minor effect. The values obtained according to the calibration curve (C) for the homopolymer are more influenced than the values obtained with the light-scattering detector; this is obviously due to the discordance between the molecular weight of the copolymer and that of the homopolymer for which the calibration holds. The data in Table 2, in particular the nonrigorous evaluation of the copolymer chromatogram according to the calibration for the homopolymer (case C), may deserve a few more comments. When the input parameters have common values (case C^a), the deviations from the true molecular weights are negligible. When, however, the difference in unperturbed dimensions of the constituents are larger (case C^b), the apparent polydispersity may change severely but only in the most inconvenient case of the rectangular chemical-composition distribution function. The effect can also be seen in the light-scattering data (case LS^b). The broadening of the apparent molecular-weight distribution calculated according to the given calibration and simultaneous narrowing of the distribution calculated from the dual-detector data were generally demonstrated to be the consequence of deteriorated column separation.[32] Here it is obviously due to mixing of fractions of polymer of

Table 2. Results of the Modeled SEC Analyses Evaluating the Dual DR/LS Record (LS) and the DR Record and the Calibration Dependence (C) Assuming Ideal Detection ($\nu_A = \nu_B$)

computed as	$\bar{M}_n \times 10^{-6}$	$\bar{M}_w \times 10^{-6}$	$\bar{M}_z \times 10^{-6}$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_z/\bar{M}_n$
True Values					
	5.00	10.00	15.00	2.00	1.50
Rectangular Chemical-Composition Distribution					
LS[a]	4.96	10.13	15.51	2.04	1.53
LS[b]	6.67	9.99	12.88	1.50	1.28
LS[c]	4.94	9.91	14.89	2.01	1.50
C[a]	4.98	10.10	15.35	2.03	1.52
C[b]	6.47	18.76	35.58	2.90	1.89
C[c]	11.00	22.01	33.00	2.00	1.50
Conversion Chemical-Composition Distribution					
LS[a]	4.99	10.00	15.02	2.00	1.50
LS[b]	5.04	10.00	14.83	1.98	1.48
LS[c]	4.99	9.98	14.98	2.00	1.50
C[a]	4.99	10.00	15.01	2.00	1.50
C[b]	9.69	19.58	29.72	2.02	1.52
C[c]	11.11	22.18	33.22	1.99	1.50
Statistical Chemical-Composition Distribution					
LS[a]	4.99	9.98	14.98	2.00	1.50
LS[b]	5.00	9.99	14.99	1.99	1.50
LS[c]	4.93	9.91	14.89	2.01	1.50
C[a]	4.99	9.98	14.98	2.00	1.50
C[b]	8.65	17.32	25.99	2.00	1.50
C[c]	11.00	22.00	32.99	2.00	1.50

[a] $C_{\infty,A} = 9.85$, $C_{\infty,B} = 6.7$, $m_{0,A} = 104$, $m_{0,B} = 100$, $B_{AA} = 6 \times 10^{-29}$ cm², $B_{BB} = 4 \times 10^{-29}$ cm². [b] $C_{\infty,A} = 1$, $C_{\infty,B} = 5$, $m_{0,A} = 100$, $m_{0,B} = 100$, $B_{AA} = 10 \times 10^{-29}$ cm², $B_{BB} = 0$ cm². [c] $C_{\infty,A} = 5$, $C_{\infty,B} = 5$, $m_{0,A} = 100$, $m_{0,B} = 100$, $B_{AA} = 6 \times 10^{-29}$ cm², $B_{BB} = 0$ cm².

the same dimensions but different molecular weight. On the other hand, with large differences in polymer–solvent interaction parameters (case C), the molecular weights change without notable change in polydispersity ($\bar{M}_w/\bar{M}_n$ or $\bar{M}_z/\bar{M}_w$). This may be interpreted as a shift of the chromatogram along the elution volume axis without radically changing its broadness. As expected, the molecular weights calculated from the light-scattering data are not influenced in this case at all.

With realistic $W'(w)$ functions (conversional and statistical composition distribution), there are no observable differences between the chromatograms of a chemically heterogeneous copolymer and a parent, chemically homogeneous homopolymer even in the case of high p values (LS[b]). Thus the influence of the chemical heterogeneity may be neglected in practice, provided the conditions of true concentration and molecular-weight detection are fulfilled.

Copolymers of Nonisorefractive Components. The excess Rayleigh ratio (normalized ratio of the contribution of the polymer solute to the intensity of the scattered light to the intensity of the incident beam, measured on a copolymer eluent and extrapolated to zero angle of observation) is[13,14]

$$R_\Theta = K\sum c_i M_i \nu_i^2 \tag{34}$$

where c_i is the concentration of species i,

$$K = \frac{2\pi^2 \tilde{n}_0^{\,2}}{N\lambda_0^{\,4}} \tag{35}$$

is the optical constant where $\tilde{n}_0$ is the refractive index of the solvent, λ_0 is the wavelength of the light in vacuo, and N is the Avogadro constant, and

$$\nu_i = w_i\nu_A + (1 - w_i)\nu_B \tag{36}$$

is the refractive index increment of a copolymer species i with weight fraction w_i of monomeric units of type A. Its value is not known and therefore the excess Rayleigh ratio (cf. eq 3 in ref 14) can be written in terms of the instant apparent molecular weight M_i^* of species i as

$$R_\Theta = K\nu^2\sum c_i M_i^* \tag{37}$$

where

$$\nu = \bar{w}\nu_A + (1 - \bar{w})\nu_B \tag{38}$$

is the macroscopic refractive index increment measured on the unfractioned sample with average weight fraction $\bar{w}$ of monomeric units of type A, and the apparent molecular weight of species i, M_i^*, is defined as

$$M_i^* = M_i(\nu_i/\nu)^2 \tag{39}$$

M_i^* is related to the well-known apparent weight-average molecular weight[14] as

$$M_w^* = \frac{\sum c_i M_i^*}{\sum c_i} \tag{40}$$

Equations 34–40 apply only if the concentration-detector signal is proportional to the mass of the polymer in the eluent and, consequently, the concentration is determined correctly. For nonisorefractive components of the copolymer, i.e., $\nu_A \neq \nu_B$, this can be achieved, e.g., by the combination of the ultraviolet (UV) and DR detection. For instance, in the case of copolymers of styrene and methyl methacrylate, the UV detector is sensitive to the concentration of styrene units and the DR detector to those of both. This enables us to determine the concentration and composition of the copolymer eluted.

If only the DR detection is employed, the apparent concentration

$$c_i^* = c_i(\nu_i/\nu) \tag{41}$$

rather than the true concentration is recorded because the contribution of species i to the DR signal is proportional to $c_i\nu_i$.

The apparent concentration detected by the DR detector is the sum of the contributions c_i^* of individual species i. Then, the excess Rayleigh ratio measured can be expressed as

$$R_\Theta = K\nu^2\sum c_i^* M_i^{**} \tag{42}$$

The sums in eqs 34, 37, and 42 are identical because the contribution of the individual copolymer species to the experimental R_Θ does not depend on the way of determination of concentration. Comparing eqs 37 and 42, the apparent molecular weight of the polymer species i in the SEC experiment with the on-line LS/DR detection is obtained as

$$M_i^{**} = M_i(\nu_i/\nu) \tag{43}$$

Similarly to M_i^*, M_i^{**} also is related to the apparent weight-average molecular weight as

$$\bar{M}_w^* = \frac{\sum c_i^* M_i^{**}}{\sum c_i^*} \qquad (44)$$

According to eqs 40 and 44, the apparent SEC-LS weight-average molecular weight of a sample does not depend on whether the polymer concentration is determined by DR or mass-sensitive detection. (Clearly, the mass of the polymer is introduced into the calculation in both cases with the concentration of the injected sample. The concentration at a given elution volume may, however, differ from the true one in the case of the DR detection.)

Now the results of the numerical simulations of the SEC analysis for $\nu_A \neq \nu_B$ will be discussed. The $\bar{M}_w/\bar{M}_n = 1.2$ ratio was chosen rather low to make the effect of chemical heterogeneity pronounced. The results presented in Figure 7 refer to a copolymer with the molecular-weight distribution $W(M)$ given by the Schulz–Zimm model distribution function (eq 32) and three types of the compositional distribution $W'(w)$. The values of C_∞, m_0, and B used correspond to those for poly(styrene-*co*-methyl methacrylate) but the results are not sensitive to the values of these parameters.

The broad rectangular distribution function, $W'(w) \equiv 1$, was chosen as the most unfavorable one (Figure 7). In this case, the impact of the ν_A/ν_B ratio on the experimentally obtained $W(M)$ is most pronounced. In spite of that, the M averages deviate from the true ones only if ν_A and ν_B differ considerably. In the realistic range of $0.05 < \nu/(\text{mL/g}) < 0.2$, the molecular weights determined are essentially correct.

The calculation was also performed assuming the correct (mass-sensitive) detection of concentration (cf. discussion of eq 37). As shown in Figure 7, the $\bar{M}_w^*$ values thus obtained are identical with those obtained for the DR detection whereas the $\bar{M}_n^*$ values are slightly different.

Calculations with narrow $W'(w)$ distributions due to statistical as well as conversional heterogeneity (Figure 7) showed an even smaller effect. The same can be said about broader molecular-weight distribution. The apparent molecular weights deviated from the true ones only when the refractive index increments had opposite signs, $\nu_A \neq -\nu_B$.

The impact of the difference in the refractive index increments of the components on the experimentally obtained dependences of log M^* vs V is demonstrated in Figure 8. The curvature as well as the change of the slope in the region of the peak maximum also appears only for large differences in the refractive index increments.

To the extent that the conversional heterogeneity, which is the main source of the heterogeneity of statistical copolymers, is independent of the average molecular weight, similar results may be expected throughout the whole span of molecular weights. Only a negligible change of the ratio $\bar{M}_k^*/\bar{M}_k$ due to the increase in the viscosity expansion factor (cf. eqs 5 and 6) was observed. The statistical heterogeneity is known to be less pronounced as compared to the conversional one and practically disappears with increasing M. Considering this, the effect of statistical heterogeneity on M^* may be neglected totally.

Conclusions

(i) The molecular weight of a chemically heterogeneous copolymer excluded at an elution volume V may vary with the chemical composition (weight fraction of component A in copolymer AB) to a considerable extent, e.g., for poly(styrene-*co*-methyl methacrylate) by about 30%.

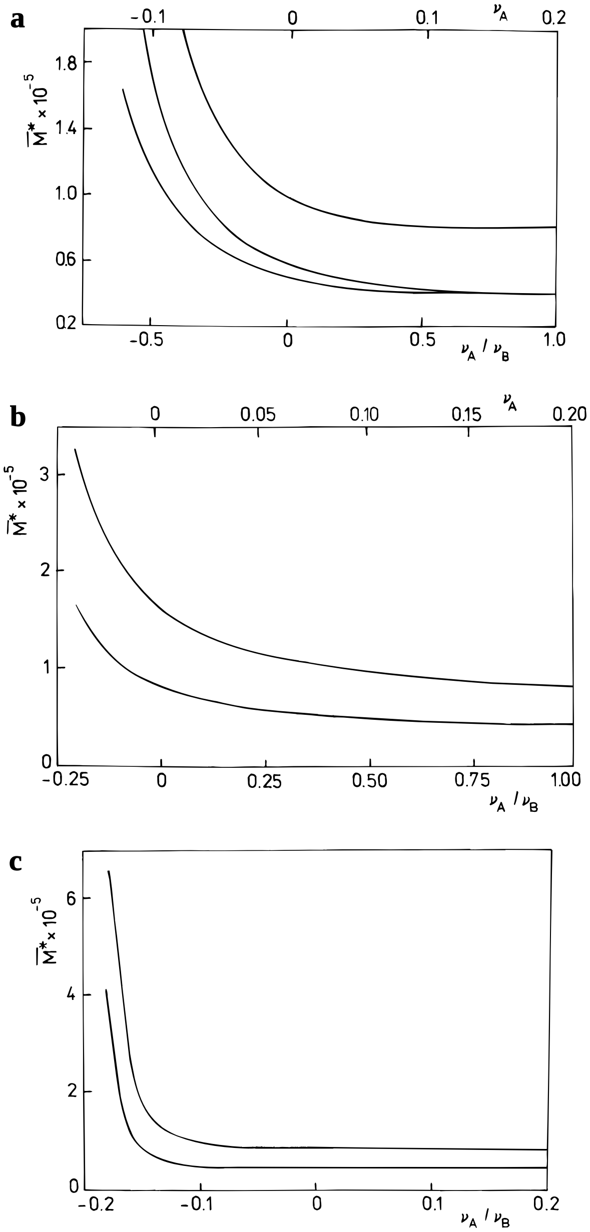

Figure 7. Apparent number- and weight-average molecular weights, $\bar{M}_n^*$ and $\bar{M}_w^*$, vs the ratio of the refractive index increments of the components, ν_A/ν_B (lower scale), or vs ν_A (upper scale) with fixed $\nu_B = 0.2$. Result of the model SEC analysis assuming the mass-sensitive detection of concentration with $\bar{M}_n = 8 \times 10^4$, $\bar{M}_w = 1 \times 10^5$, $C_{\infty,A} = 9.85$, $C_{\infty,B} = 6.7$, $m_{0,A} = 104$, $m_{0,B} = 100$, $B_{AA} = 6 \times 10^{-29}$, and $B_{BB} = 4 \times 10^{-29}$ (parameters corresponding to poly(styrene-*co*-methyl methacrylate)) for various composition distribution functions $W'(w)$: (a) rectangular distribution function and both mass-sensitive (M) and DR detections (for $\bar{M}_w$ the curves are identical); (b) due to conversion heterogeneity, $r_A = r_B = 0.5$, $\bar{w} = 0.38$; (c) due to statistical heterogeneity, $r_A r_B = 1.00$, $\bar{w} = 0.5$.

(ii) In the case of correct detection, i.e., with DR detectors for equal refractive index increments of both constituents, the error due to chemical heterogeneity in the experimentally obtained molecular-weight averages is within the experimental error and may be neglected. A larger error appears at highly different values of the characteristic ratio of the constituents or of the interaction parameters.

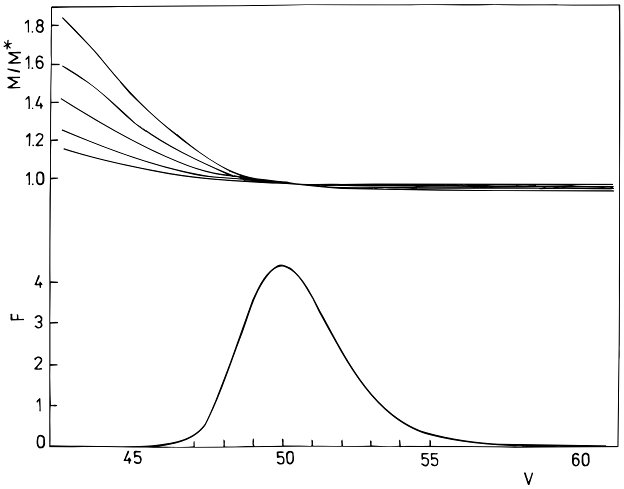

Figure 8. Comparison of the normalized experimental chromatogram *F* (lower part) and the ratio *M*/*M** calculated with $C_{\infty,A} = 10$, $C_{\infty,B} = 5$, $m_{0,A} = 100$, $m_{0,B} = 105$, and $\bar{w} = 0.5$ for a copolymer differing in the ratio of the refractive index increments of the constituent homolopymers, $\nu_A/\nu_B = -0.75$, -0.5, -0.25, 0.05, 0.5, and 1 (true detection) (curves in the upper part from top to bottom, respectively).

(iii) Significant deviations of experimental molecular-weight averages from the true ones due to the effect of composition heterogeneity may be expected for largely different refractive index increments of both constituents ($\nu_A \to -\nu_B$).

(iv) It is necessary to investigate the separation of copolymer in the case where the distributions of molecular weight and composition are not independent.

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