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Retention Mechanism of Branched Macromolecules in Size Exclusion Chromatography

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ABSTRACT: The theory of Stockmayer in the modifications of Thurmond and Zimm has been used for the description of the size exclusion chromatography separation of randomly branched molecules with tetrafunctional branch points. It is assumed that free chain ends, created by the branching process, cause the molecules to be entrapped in the pores of the column packing with the time of their release given by the exponential law characteristic for the monomolecular reactions. Using this assumption, the anomalous elution behavior of such molecules can be modeled. With increasing elution volume, the average values of radius of gyration and, to a lesser degree, of molecular weight decrease and, after passing a minimum, again increase in the low-molecular weight region.

"Conformation plot"

experiment
γ = 1
γ = 0.75
γ = 0.5
γ = 0.25
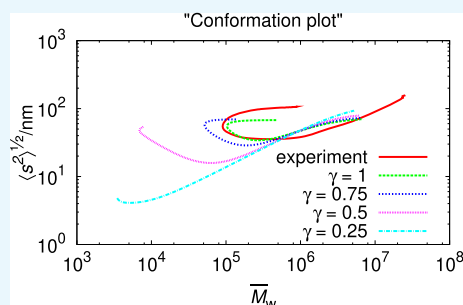
1. INTRODUCTION

It is generally assumed that polymer molecules are separated in size exclusion chromatography (SEC) according to their hydrodynamic volume. However, the elution behavior of branched[1] and brush[2] polymers deviates strongly from that of linear-chain polymers. The usual decrease in molecular weight and radius of gyration with increasing elution volume, V, is frequently followed by an abrupt increase in both measures of the molecular size in the tail of the elution curve (EC),[3] where a decrease is expected in the analogy with the linear-chain molecules. Percec and Johann et al. suggested that "the extremely high molar mass fraction of the sample molecules interact with the column material (i.e., they get entrapped) and elute at higher elution volumes than expected by a separation mechanism based on pure size exclusion".[4,5] The mechanism of the entrapment is not clear but it can be assumed that it is connected with chain ends, occurring in both branched and brush polymers in increased quantities compared with the linear-chain molecules.

The Stockmayer theory of branching, in particular tetrafunctional,[6,7] is the only theoretical treatment comprising fully this phenomenon. Although Stockmayer himself admits that the copolymer poly(styrene-divinylbenzene) is not an ideal representative of this family of polymers, because the reactivity of the vinyl groups on the two monomers differs,[6] no other system conforming better to this theory and no superior theory describing this system have been presented so far. The modification of Thurmond and Zimm[8] by using the limiting formula for the base of exponent close to unity made the theory treatable for computers and proper for model calculations.[9]

The capture and release of entrapped molecules with multiple chain ends is a complicated topological problem. No reference to this subject was found in the literature. Although the topology of the pore formed by a cross-linked polymer packing is complex, it is usually modeled by a cylinder with smooth walls and the mechanical interaction of the free-chain ends with the structure of the cylinder-wall can be taken into consideration.[10] This explanation of the delay is supported by the fact that the effect of the delayed elution occurs only in SEC of branched polymers but in the separation by the field flow fractionation,[1] using a different separation principle, including no porous column filling, it has not been observed at all.[11]

As some of the molecules may be entrapped for the whole time of the experiment or even for the lifetime of the column, their release can be considered a monomolecular reaction. The estimation of the timing of the release from topological considerations appears impossible at this stage. On the other hand, modeling of the SEC experiment so that its results agree with the measured data may help this estimation.

The SEC is considered the best method for the study of branching, especially if the apparatus is equipped with a multiangle light-scattering (MALS) photometer additionally to the usual concentration detector. The MALS photometer enables us, for sufficiently large macromolecules, to find at all

elution volumes not only the local molecular weight[3,12] (weight average), $\bar{M}_w$, but also the local value of the root-mean-square radius of gyration (z-average), $\langle s^2 \rangle^{1/2}$, hereinafter called "radius of gyration".

The goal of this paper is to present a model of the separation of poly(styrene-divinylbenzene) sample on the basis of theory of Stockmayer[6,7] in the modification of Thurmond and Zimm[8] and from comparison with experiments to estimate the conditions of the release of the entrapped molecules.

1.1. Distribution of Degrees of Polymerization and Unbroadened ECs.

The distribution of the relative degree of polymerization (DP) x with m branch points is given by a recursion formula derived by Thurmond and Zimm[8,9]

$$w_{m,x} = \frac{\gamma x^3}{2m(2m+1)(2m+2)} w_{m-1,x} \tag{1}$$

with the first term given by

$$w_{0,x} = x \exp[-x(1 + \gamma/2)] \tag{2}$$

where $W_{m,x}$ is the mass fraction of molecules with relative DP x comprising m tetrafunctional branch points, that is, cross-links.

The branching parameter $\gamma = \varrho \bar{y}_w$ is a product of fraction tetrafunctional (vinyl) groups reacted ϱ and the weight-average DP of the primary chains $\bar{y}_w$ (that would exist if all cross-links were severed). At the beginning of the reaction $\gamma = 0$ and at the gel point $\gamma = 1$. For $\varrho \ll 1$ and $\bar{y}_w \gg 1$, the average DP of the cross-linked material is given by[7]

$$\bar{x}_w \approx \bar{y}_w / (1 - \gamma) \tag{3}$$

The molecules in fractions with average number m of branch-points per molecule are contracted with respect to linear-chain molecules of the same M as described by the branching index defined by

$$g_m = \langle r^2 \rangle_b / \langle r^2 \rangle_l \tag{4}$$

which is for the random tetrafunctional branching well approximated by[13]

$$g_m = \left[\left(1 + \frac{m}{6}\right)^{1/2} + \left(\frac{4m}{3\pi}\right) \right]^{-1/2} \tag{5}$$

For modeling the SEC separation, the calibration dependence is given by

$$\ln M = A_{cal} + B_{cal} V_0 \tag{6}$$

where V_0 is the elution volume of linear molecules.

The theoretical (unbroadened) EC of the polymer fraction with n branch points, $W_n(V)$, is then given by[14]

$$W_m(V) = \frac{M}{B_{cal}} w_{m,\ln M} \tag{7}$$

where $M = f_M x$ where f_M is a constant of molecular weight per unit x of the chain and $w_{m,\ln M}$ is the function defined by eqs 1 and 2 calculated for $M = f_M x$ in logarithmic scale.

With the gyration radius given by

$$\langle s^2 \rangle^{1/2} = K_s \times M^\alpha \tag{8}$$

where $K = 2.09 \times 10^{-2}$ nm and $\alpha = 0.56$ for linear-chain polystyrene in tetrahydrofuran (THF) at 30 °C[15] and by expressing the elution volume in terms of $\langle s^2 \rangle^{1/2}$ and taking derivative according to $\ln\langle s^2 \rangle^{1/2}$, the shift ΔV_m of the EC

$W_m(V)$ of the fraction with m branch points is expressed independently of their molecular weight by a combination of eqs 4, 6, and 8 by

$$\Delta V_m = \frac{\Delta \ln g_m}{2\alpha B_{cal}} \tag{9}$$

as a shift of the distribution of molecular sizes with respect to the distribution of the pore diameters.[16]

Although there is a discussion which molecular parameter is deciding for the SEC separation, the difference between the use of hydrodynamic volume and gyration radius for the description of the separation is expected for linear and less-branched polymers as self-similar objects (but not for very densely branched structures), to result only in a slight shift with respect to the elution volume axis.[17] Here, $\langle s^2 \rangle^{1/2}$ was chosen for its simple relation with the molecular size.

1.2. Delayed Release of the Stacked Molecules and Band Broadening.

Let f be the number of functionalities of the branch monomer unit. As the formation of closed structures in randomly branched structures is not considered, each of $m > 1$ branch-points brings into the molecule $f - 2$ new ends, giving thus the total number of $f + (m - 1)(f - 2)$ ends. Thus, each branch-point contributes to probability of the entrapment by a new chain end pointing out of the structure. The quantification of this contribution is a complicated topological problem, which reflects properties of polymer molecules and, probably to some extent, also of the stationary phase (SP). No reference to this problem was found in the literature. This justifies the use of a trial model with a criterion of an agreement of calculated functions with the experiment.

We assume that release of the entrapped molecule with m branch-points at any site of the column packing or time of the analysis is the first-order reaction with the probability-density λ_m and can be described by

$$P_m(V) = \exp[-\lambda_m V] \tag{10}$$

where the numerical values of the constant λ_m will be discussed later. This assumption is in accord with the scaling concept, proposing a similar behavior of branched molecules of various chain-length with m branch point in pores of corresponding size of the chromatography column packing.[10]

Band-broadening function, BBF, that is, the EC of a single polymer species (uniform in chain-length and chemical composition), of linear-chain molecules can be described by the symmetric Gauss distribution[18,19]

$$G_0(V) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left[\frac{V^2}{2\sigma^2}\right] \tag{11}$$

under the condition that maximum of EC is sufficiently distant from the exclusion limit. As branching decreases the size of molecules, this condition is fulfilled even for high-molecular-weight highly branched structures. The function defined by eq 11 will be used as BBF for linear-chain fractions (subscript $m = 0$). For $m > 0$, the resulting BBF is obtained by the convolution of the functions defined by eqs 10 and 11

$$G_m(V) = \int G_0(V - y) P_m(V) dV = \int G_0(V) P_m(V - y) dy \tag{12}$$

which gives the well-known exponentially modified Gaussian (EMG)[20,21]

$$G_m(V) = \frac{\lambda_m}{2} \exp\left[\frac{\lambda_m}{2}(2\mu + \lambda_m\sigma^2 - 2V)\right]$$
$$\text{erfc}\left[\frac{\mu + \lambda_m\sigma^2 - V}{\sigma\sqrt{2}}\right] \tag{13}$$

with parameters σ, μ, and λ_m, where erfc is the complementary error function defined as

$$\text{erfc}(x) = 1 - \text{erf}(x) = \frac{2}{\sqrt{\pi}} \int_x^\infty \exp -t^2 \, dt \tag{14}$$

where erf is the error function.[22]

The mean μ_{EMG} of EMG is given by

$$\mu_{\text{EMG}} = \mu + 1/\lambda_m \tag{15}$$

the peak variance is given by[20]

$$\sigma_{\text{EMG}}^2 = \sigma^2 + 1/\lambda_m^2 \tag{16}$$

and the skew (nonsymmetry) β is given by[20]

$$\beta = \frac{2}{\sigma^3 \lambda_m^3}\left(1 + \frac{1}{\sigma^2\lambda_m^2}\right)^{-3/2} \tag{17}$$

The theoretically constructed ECs undergo the band broadening, which is a part of the separation process. It is described by the well-known Tung equation, which relates the theoretical EC $W_m(V)$ and the calculated (broadened) "experimental" EC $F_m(V)$ by a convolution

$$F_m(V) = \int G_m(V)W_m(V-y)dy = \int G_m(V-y)W_m(V)dy \tag{18}$$

with the BBF $G_m(V)$, which is the EC of an analyte uniform in molecular weight and chemical composition specific for the polymer-chain with m branch-points, where V and y are two variables denoting the elution volume.

With theoretical EC given in N points, W_i, and BBF, G_i, given in $2N + 1$ points with its center in the Nth point, the "experimental" EC in the i-th point is numerically simply calculated by convolution of these functions by

$$F_i = \sum_{j=1}^{N} W_j G_{N+i-j} \tag{19}$$

The local molecular-weight averages (i-th point) are calculated by

$$\bar{M}_{i,k} = \frac{\sum_{j=1}^{N} W_j G_{N+i-j}(f_M x_j)^k}{\sum_{j=1}^{N} W_j G_{N+i-j}(f_M x_j)^{k-1}} \tag{20}$$

for $k = 0, 1, 2$ giving $\bar{M}_n$, $\bar{M}_w$, and $\bar{M}_z$, and the local gyration radius (z-average) is, based on its definition[23]

$$\langle s^2 \rangle = \frac{\sum_{j=1}^{N} W_j M_j s_j^2}{\sum_{j=1}^{N} W_j M_j} \tag{21}$$

expressed by

$$\langle s^2 \rangle_i = \frac{\sum_{j=1}^{N} W_j G_{N+i-j} K_s^2 g_{m,j}(f_M x_j)^{1+2\alpha}}{\sum_{j=1}^{N} W_j G_{N+i-j} f_M x_j} \tag{22}$$

2. RESULTS AND DISCUSSION

LS and refractive index (RI) ECs of sample PS show some undulation or local bumps occurring usually in highly branched samples which may be explained by irregularities in forming molecules differing in the number of branch-points, $m = 0, 1, 2$, coming, for example, from the unequal reactivity of vinyl groups on styrene and divinylbenzene,[6] or irregularity of the polymerization in late stages[24] (Figure 1).
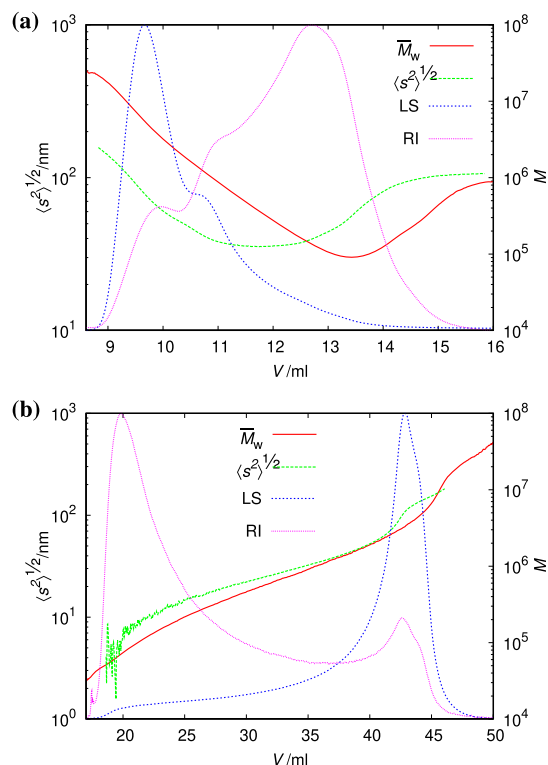

Figure 1. Comparison of the concentration (RI) and light scattering (LS, 90°) ECs (arbitrary units) of the sample PS with dependences of root-mean-square radius $\langle s^2 \rangle^{1/2}$ and local molecular weight $\bar{M}_w$ on elution volume V, separated by SEC (a) and FFF (b).

The parameter γ was estimated by a method suggested by Thurmond and Zimm.[8] With $\bar{M}_w = 1.24 \times 10^6$ ($\bar{M}_w/\bar{M}_n = 5.86$), obtained by SEC with LS detection, we have $\bar{x}_w = 1.188 \times 10^4$. Assuming all divinylbenzene and styrene molecules reacted in the ratio of the reaction mixture (cf. Experimental Section), from the number fraction branch-points (divinyl benzene units) $N_{BP} = 81$ per molecule, that is, $N_B = N_{BP}(f-1) = 244$ branches per molecule, where $f = 4$ is functionality of the branch points, were estimated which gives $\bar{y}_w \approx 49$. The value of $\gamma \approx 0.996$ was then found from eq 3, in accord with that the system was on the verge of gelation. The assumption of the equal reactivity was already questioned by Stockmayer.[6] It turns out that divinyl benzene, as a molecule, is converted somewhat faster than styrene because it has two vinyl groups.[25,26] The conversion of the pendent vinyl group is quite different, which is a very complex phenomenon and depends on the combination of the monomer and the divinyl monomer.[27] On the other hand, there is no other system better fitting the assumptions than the currently investigated one.

The dependences of $\langle s^2 \rangle^{1/2}$ and M obtained by SEC show a decrease with increasing V, expected in the separation according to the hydrodynamic volume of the molecules,

followed by an increase in the low-molecular-weight region in both $\overline{M}_w$ and $\langle s^2 \rangle^{1/2}$ (local values, Figure 1a). This can be explained by the assumption of the capture and delay of highly branched macromolecules eluted in the late stages of the analysis. This assumption is corroborated by an absence of this phenomenon when using FFF for the separation of this sample. As this method does not include an SP containing pores but separates strictly on the principle of the hydrodynamic volume,[11] the capture and delay of the sample is not present when employing it. In this case, the dependences of both $\langle s^2 \rangle^{1/2}$ and M are continually increasing with V in accord with the FFF mechanism,[11] except for irregularities in the low-molecular-weight region (Figure 1b).

For modeling the separation process, the choice of the time-constant λ_m of the delayed elution in eq 10 m is critical. There is no theoretical clue for its increase and therefore it had to be estimated tentatively. We used the dependence $\lambda_m = \lambda_1 / m^\varepsilon$ where the exponent ε was chosen tentatively together with λ_1. The consequence of the increase in m is the increase in the nonsymmetry of BBF, shown in Figure 2 for $\sigma = 0.18$ mL, a
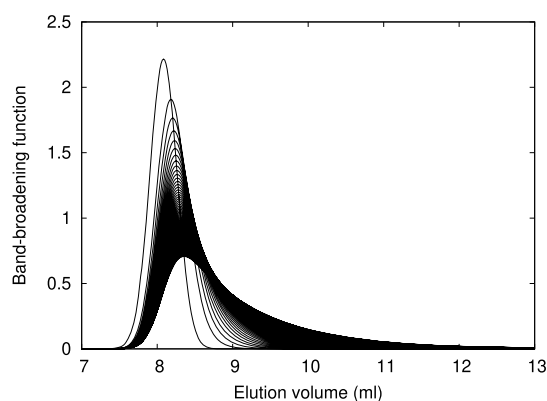

Figure 2. BBFs for increasing number of the branch-points $0 \leq m \leq$ 100: symmetrical curve, $m = 0$, curves with increased tailing to the left, $m > 0$.

typical value of the BBF standard deviation,[28] $\lambda_1 = 8$ and $\varepsilon = 0.45$ obtained as values giving the best agreement of the calculated dependences of local $\langle s^2 \rangle^{1/2}$ and $\overline{M}_w$ on V (see below).

For a comparison with the experiment, we used calibration constants (cf. eq 6) for a linear-chain polymer, $A = 14.5$ and $B_{cal} = -0.016$ mL^{-1}, fitting best the experimental data.

The calculated dependences of $\langle s^2 \rangle^{1/2}$ and of molecular-weight averages versus V are curved upward in the low-molecular-weight region (Figure 3) in dependence on the branching parameter γ and the power of M which they depend on (cf. eqs 20 and 22). In the lower degree of branching, that is, with $\gamma = 0.25$ (Figure 3a) only small curvatures upwards appear at the end of the dependences of $\overline{M}_z$ and $\langle s^2 \rangle^{1/2}$, the others appear almost linear. For $\gamma = 0.5$ (Figure 3b) the minima are pronounced and the dependence of $\langle s^2 \rangle^{1/2}$ is turned strongly upward in the low-molecular-weight region, which can be explained by an increasing number of strongly delayed high-molecular-weight fractions. For $\gamma = 1$ the values of $\langle s^2 \rangle^{1/2}$ in the central part are higher than for $\gamma = 0.5$ and the tail becomes flat because of the presence of the high-molecular-weight fractions even in the central parts of the ECs. The dependence of $\overline{M}_n$ versus V shows no curvature even for $\gamma = 1$ (Figure 3c). For the appraisal of the influence of the maximum
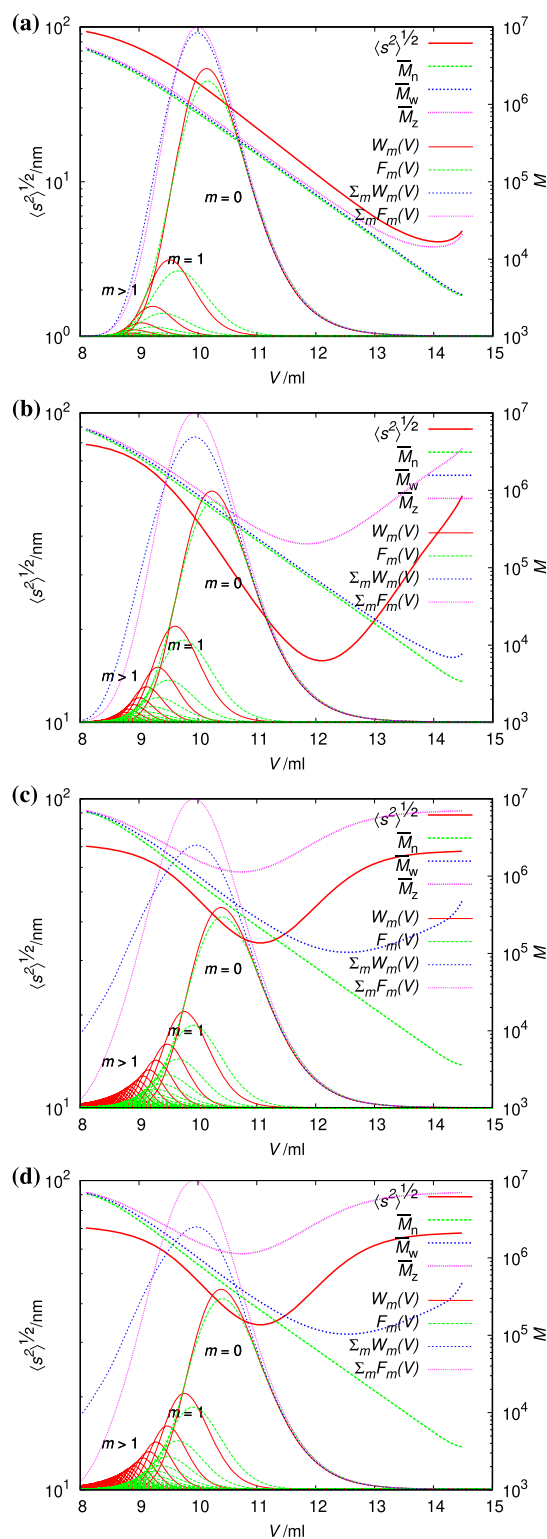

Figure 3. Comparison of the theoretical ECs (arbitrary units) $W_m(V)$ calculated for the fraction of the polymer differing in the number of the branch points m from eq 1 through 6 and broadened ECs $F_m(V)$ calculated from eq 19 and their sums $\sum_m W_m(V)$ and $\sum_m F_m(V)$ with dependences of molecular weight averages and $\langle s^2 \rangle^{1/2}$ calculated from eqs 20 and 22, respectively, for $0 \leq m \leq 100$ and $\gamma = 0.25$ (a), $\gamma = 0.5$ (b) and $\gamma = 1$ (c) and for $0 \leq m \leq 200$ and $\gamma = 1$ (d).

number of the branch-points, m, taken into the calculation, we compared the results for $\gamma = 1$ and $0 \leq m \leq 100$ (Figure 3c) and for $0 \leq m \leq 200$ (Figure 3d). The figures are virtually

identical. Taking a strong decrease in the weight fraction with increasing m into consideration,[9] the range $0 \leq m \leq 100$ is highly sufficient for the description of the separation process. Thus, in addition to the parameters describing the separation system, as constants of eq 6, the delay of the molecules and their separation are described by the above-discussed constant of the release $\lambda_0 = 8$ and the exponent $\varepsilon = 0.45$ of its increase with the number of the branch-points m. The dependence of these two constants on the properties of SP, its porosity, structure of pores, and so forth is an open question requiring more experimental material and potentially topological studies.

The diverse dependences of local molecular weights may contribute to a solution of one interesting problem: the local dispersity in M calculated on the basis of a separation of branched molecules according to the hydrodynamic volume is expected in the high-molecular-weight region only and to a negligible degree[29] ($\bar{M}_w/\bar{M}_n \rightarrow 1$). On the other hand, its indirect determination based on combination of viscometry with the universal calibration,[30] yielding[31,32] $\bar{M}_n$, and light scattering, yielding $\bar{M}_w$, detected a large increase in local dispersity[33] in the low-M region in accord with the diverting dependences of the averages calculated in the low-M region. The calculated dispersities $\bar{M}_w/\bar{M}_n$ (Figure 4a) and $\bar{M}_z/\bar{M}_w$
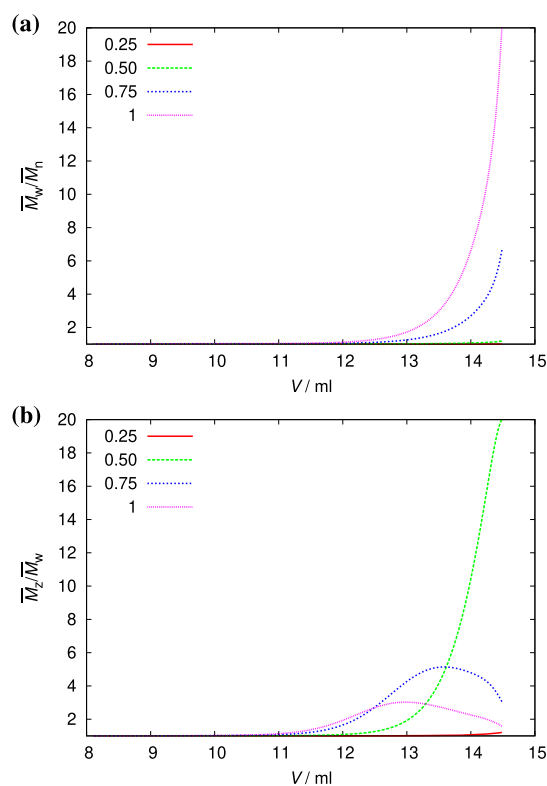
(a)

(b)

Figure 4. Dependences of local dispersities $\bar{M}_w/\bar{M}_n$ (a) and $\bar{M}_z/\bar{M}_w$ (b) for γ given with the curves.

(Figure 4b) rise with γ in the low-molecular-weight region but they may even go down when the high-molecular-weight moieties prevail (Figure 4b), that is, for $\gamma \rightarrow 1$ in the region where the dependence of $\langle s^2 \rangle^{1/2}$ becomes flat (cf. Figure 3c). This is also supported by the fact that a coelution of branched molecules together with linear ones has been observed.[34] Although the mechanism of polymerization of acrylates using laser pulses may be different from random polymerization with the tetrafunctional branch points, high values of local dispersity

found for such samples[33] may account for the effect of the delayed elution of highly branched fractions.

The average values of the contraction factor $\langle g \rangle$, calculated as weighted fractions of g_m given by eq 4, show maxima in the middle of the dependences (Figure 5) as expected from the U-
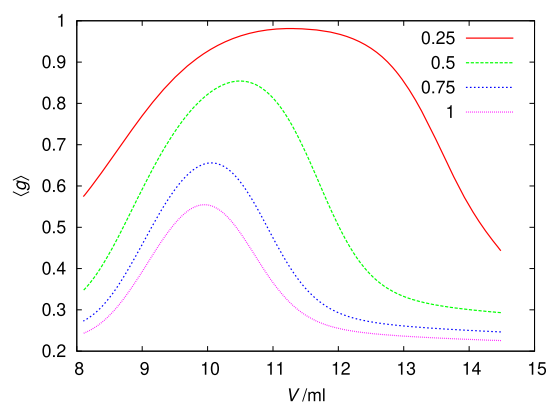
Figure 5. Average contraction factor $\langle g \rangle$ calculated for values of the branching parameter γ denoted with the curves in dependence on elution volume V.

shaped dependences of $\langle s^2 \rangle^{1/2}$. The fronting part of the dependence decreasing with decreasing elution volume is similar to that obtained experimentally by an on-line viscometer as the $g' = [\eta]_b/[\eta]_l$ ratio of the intrinsic viscosities of branched ($[\eta]_b$) and linear-chain ($[\eta]_l$) polymer (polystyrene).[29] The decreasing parts in the low-molecular-weight region are not easily experimentally accessible and there is no experimental evidence of them. A comprehensive study including a combination of preparative fractionation with viscometric characterization[34,35] is desirable to clarify the question.

The dependence $\langle s^2 \rangle^{1/2}$ versus $\bar{M}_w$, constructed from the dual-detection on-line data is for well-separated linear-chain polymers sometimes called the "conformation plot". For the branched polymers, its curvature is a sign of the branching and delayed elution[3] as a consequence of the curvature of the $\langle s^2 \rangle^{1/2}$ versus V dependence. Figure 6 shows a comparison of this plot calculated for several values of γ with the experimentally obtained dependence. The curve for $\gamma = 1$ fits the experimental curve best.

We used the accordance of the theoretical dependences with the experimental ones as a criterion for the choice of
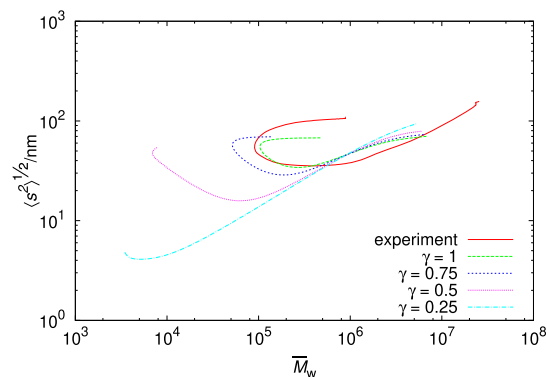
Figure 6. Comparison of the plot $\langle s^2 \rangle^{1/2}$ vs $\bar{M}_w$ ("conformation" plot) for sample a with the curves calculated for γ denoted with the curves.

parameters of interaction of molecules with SP, using the Stockmayer theory of branching. Thus, the theoretical insight helps to design simulations and to rationalize the available macroscopic information. On the other hand, simulations provide the necessary independent evidence for the justification of phenomenological models or the relevance and they can supply benchmark results for the comparison of theory with experimental data. On the other hand, the applicability of the Stockmayer theory on the experiment, just using several simple assumptions on input parameters, is amazing.

3. CONCLUSIONS

1. The assumption of the delay in release is sufficient to reproduce the results of the SEC analyses by use of the Stockmayer theory of branching process in the modification of Thurmond and Zimm, expressed as endless sums. However, the experiment can be depicted using a constrained number of terms in the sums.

2. The dependence of $\langle s^2 \rangle^{1/2}$ and $\bar{M}_w$ versus V are consistent with the delayed elution of the highly branched molecules.

3. Strongly branched macromolecules are delayed in elution from the SEC column proportionally to their number of branch-points, that is, number of chain ends. This delay manifests itself by the increase in average molecular weight and radius of gyration in the low-molecular-weight region.

4. EXPERIMENTAL SECTION

The sample PS of randomly branched polystyrene−divinyl-benzene used in this study was prepared by radical solution polymerization of 50% solution of styrene/divinylbenzene mixture in toluene. The styrene/divinylbenzene ratio was 99.15/0.85 by weight. The polymerization was carried out in a glass sealed vial using azobisisobutyronitrile (0.1% by weight to monomers) as an initiator at 80 °C. The reaction time was 8 h. The polymer was precipitated with petroleum ether with the yield of 28%.

The experimental SEC setup consisted of an Agilent 1100 pump, a Waters 717 autosampler, two Agilent PLgel Mixed-C columns 300 × 7.5 mm, a DAWN HELEOS MALS photometer from Wyatt Technology, and a Waters RI detector 2410. THF was used as the mobile phase at a flow rate of 1 mL·min^{-1}. The sample was dissolved in THF at the concentration of 2 mg·mL^{-1}, filtered with a 0.45 μm filter, and injected in the volume of 100 μL.

The measurement by asymmetric flow field flow fractiona-tion (AF4) was carried out using Wyatt Technology AF4 System Eclipse 3+ connected to the same system of detectors as used for SEC−MALS. THF was used as the carrier at a detector flow rate of 1 mL·min^{-1} and cross flow gradient from 3 to 0.1 mL·min^{-1} within 20 min, with a 5 min isocratic section before the gradient and a 10 min isocratic section after the gradient. The focus time was 13 min. The separation was achieved by means of a Wyatt Technology long channel with a 350 μm spacer and 5 kDa Nadir regenerated cellulose membrane. The concentration and injected volume were identical as those used for SEC−MALS. The mass recovery was 96%, which means only small fraction of oligomeric species permeated through the semipermeable membrane.

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Notes
The authors declare no competing financial interest.

■ REFERENCES

(1) Podzimek, S.; Vlček, T.; Johann, C. Chromatography of Branched Polymers by Size Exclusion Chromatography Coupled with Multiangle Light Scattering Detector. I. Size Exclusion Chromatography Elution Behaviour of Branched Polymers. *J. Appl. Polym. Sci.* **2001**, *81*, 1588−1594.

(2) Gerle, M.; Fischer, K.; Roos, S.; Müller, A. H. E.; Schmidt, M.; Sheiko, S. S.; Prokhorova, S.; Möller, M. Main Chain Conformation and Anomalous Elution Behavior of Cylindrical Brushes as Revealed by GPC/MALLS, Light Scattering, and SFM. *Macromolecules* **1999**, *32*, 2629−2637.

(3) Wyatt, P. J. Light Scattering and the Absolute Characterization of Macromolecules. *Anal. Chim. Acta* **1993**, *272*, 1−40.

(4) Johann, C.; Kilz, P. Utilization of Size-Exclusion Chromatog-raphy/Multiangle Laser Light Scattering for the Analysis of Structured Polymers. *J. Appl. Polym. Sci.: Appl. Polym. Symp.* **1991**, *48*, 111−122.

(5) Percec, V.; Ahn, C.-H.; Cho, W.-D.; Jamieson, A. M.; Kim, J.; Leman, T.; Schmidt, M.; Gerle, M.; Möller, M.; Prokhorova, S. A.; Sheiko, S. S.; Cheng, S. Z. D.; Zhang, A.; Ungar, G.; Yeardley, D. J. P. Visualizable Cylindrical Macromolecules with Controlled Stiffness from Backbones Containing Libraries of Self-Assembling Dendritic Side Groups. *J. Am. Chem. Soc.* **1998**, *120*, 8619−8631.

(6) Stockmayer, W. H. Theory of Molecular Size Distribution and Gel Formation in Branched-Chain Polymers. *J. Chem. Phys.* **1943**, *11*, 45−55.

(7) Stockmayer, W. H. Theory of Molecular Size Distribution and Gel Formation in Branched Polymers. *J. Chem. Phys.* **1944**, *12*, 125−131.

(8) Thurmond, C. D.; Zimm, B. H. Size and shape of the Molecules in Artificially Branched Polystyrene. *J. Polym. Sci.* **1952**, *8*, 477−494.

(9) Netopilík, M.; Kratochvíl, P. Distribution of Degrees of Polymerization in Statistically Branched Polymers with Tetrafunc-tional Branch Points: Model Calculations. *Polym. Int.* **2006**, *55*, 196−203.

(10) Dimitrov, D. I.; Milchev, A.; Binder, K. Polymer Brushes in Cylindrical Pores: Simulation versus Scaling Theory. *J. Chem. Phys.* **2006**, *125*, 034905.

(11) Podzimek, S. *Light Scattering, Size Exclusion Chromatography and Asymetric Field Flow Fractionation*; J. Wiley & Sons, Inc., 2011.

(12) Podzimek, S. Truths and Myths about the Determination of Molar Mass Distribution of Synthetic and Natural Polymers by Size Exclusion Chromatography. *J. Appl. Polym. Sci.* **2014**, *131*, 40111.

(13) Zimm, B. H.; Stockmayer, W. H. The Dimensions of Chain Molecules Containing Branches and Rings. *J. Chem. Phys.* **1949**, *17*, 1301−1314.

(14) Shortt, D. W. Differential Molecular-Weight Distributions in High Performance Size Exclusion Chromatography. *J. Liq. Chromatogr.* **1993**, *16*, 3371–3391.

(15) Venkataswamy, K.; Jamieson, A. M.; Petschek, R. G. Static and Dynamic Properties of Polystyrene in Good Solvents: Ethylbenzene and Tetrahydrofuran. *Macromolecules* **1986**, *19*, 124–133.

(16) Lastoskie, C.; Gubbins, K. E.; Quirke, N. Pore Size Distriution Analysis of Microporous Carbons: A Density Functional Theory Approach. *J. Phys. Chem.* **1993**, *97*, 4786–4796.

(17) Farmer, B. S.; Terao, K.; Mays, J. W. Characterization of Model Branched Polymers by Multi-detector SEC in good and Theta Solvents. *Int. J. Polym. Anal. Charact.* **2006**, *11*, 3–19.

(18) Giddings, J. C.; Eyring, H. A molecular Dynamic Theory of Chromatography. *J. Phys. Chem.* **1955**, *59*, 416–421.

(19) Tung, L. H. Method of Calculating Molecular Weight Distribution Function From Gel Permeation Chromatogram. *J. Appl. Polym. Sci.* **1966**, *10*, 375–385.

(20) Grushka, E. Characterization of Exponentially Modified Gaussian Peaks in Chromatography. *Anal. Chem.* **1972**, *44*, 1733–1738.

(21) Hanggi, D.; Carr, P. W. Errors in Exponentially Modified Gaussian Equations in the Literature. *Anal. Chem.* **1985**, *57*, 2394–2395.

(22) *Handbook of Mathematical Functions with Formulas, Graphs, and Mathematical Tables*; Abramowitz, M., Stegun, I., Eds.; Applied Mathematics Series 55; National Bureau of Standards, tenth printing, 1972.

(23) Kratochvíl, P.; Huglin, M. B. *Light Scattering from Polymer Solutions*; Academic Press: London and New York, 1972; p 345.

(24) Chekal, B. P. *Understanding the Roles of Chemically-Controlled and Diffusion-Limited Processes in Determining the Severity of Autoacceleration Behavior in Free Radical Polymerization*; Northwestern University, 2002.

(25) Bannister, I.; Billingham, N. C.; Armes, S. P.; Rannard, S. P.; Findlay, P. Development of Branching in Living Radical Copolymerization of Vinyl and Divinyl Monomers. *Macromolecules* **2006**, *39*, 7483–7492.

(26) Yang, H.-J.; Jiang, B.-B.; Huang, W.-Y.; Zhang, D.-L.; Kong, L.-Z.; Chen, J.-H.; Liu, C.-L.; Gong, F.-H.; Yu, Q.; Yang, Y. Development of Branching in Atom Transfer Radical Copolymerization of Styrene with Triethylene Glycol Dimethacrylate. *Macromolecules* **2009**, *42*, 5976–5982.

(27) Huang, W.; Yang, H.; Xue, X.; Jiang, B.; Chen, J.; Yang, Y.; Pu, H.; Liu, Y.; Zhang, D.; Kong, L.; Zhai, G. Polymerization Behaviors and Polymer Branching Structures in ATRP of Monovinyl and Divinyl Monomers. *Polym. Chem.* **2013**, *4*, 3204–3211.

(28) Netopilík, M. Towards Ideal Separation by Size-Exclusion Chromatography. *J. Chromatogr. A* **2017**, *1487*, 139–146.

(29) Netopilík, M. Size-exclusion-chromatography Separation of Randomly Branched Polymers with Tetrafunctional Branch Points and Local Dispersity. *J. Chromatogr. A* **2012**, *1260*, 97–101.

(30) Grubisic, Z.; Rempp, P.; Benoit, H. A Universal Calibration for Gel Permeation Chromatography. *J. Polym. Sci., Part B: Polym. Lett.* **1967**, *5*, 753–759.

(31) Goldwasser, J. M. Absolute $\bar{M}_n$ Determination by Gel Permeation Chromatography-Differential Viscometry. In *Chromatography of Polymers, Characterization by SEC and FFF*; Provder, T., Ed.; ACS Symposium Series 521; American Chemical Society: Washington, DC, 1993; p 243.

(32) Netopilík, M.; Podešva, J.; Lokaj, J.; Kratochvíl, P. Number-average Molecular Weight of Branched Polymers from SEC with Viscosity Detection and Universal Calibration. *Polym. Int.* **2008**, *57*, 1152–1158.

(33) Castignolles, P. Transfer to Polymer and Long-chain Branching in PLP-SEC of Acrylates. *Macromol. Rapid Commun.* **2009**, *30*, 1995–2001.

(34) Edam, R.; Meunier, D. M.; Mes, E. P. C.; Van Damme, F. A.; Schoenmakers, P. J. Branched Polymer Separations Using Compre-hensive Two-Dimensional Molecular-Topology Fractionation × Size-exclusion Chromatography. *J. Chromatogr. A* **2008**, *1201*, 208–214.

(35) Bungu, P. S. E.; Pflug, K.; Pasch, H. Combination of preparative and two-dimensional chromatographic fractionation with thermal analysis for the branching analysis of polyethylene. *Polym. Chem.* **2018**, *9*, 3142–3157.