Quantitative Branching of Linear and Branched Polysaccharide Mixtures by Size Exclusion Chromatography and On-Line Low-Angle Laser Light Scattering Detection

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Synopsis

The quantitative branching characterization of polysaccharide via size exclusion chromatography (SEC) and on-line low-angle laser light scattering (LALLS) detection is presented from both theoretical arguments as well as direct experimental evidence. The two measurable branching parameters $g_{v(m)}$ [$= (M_l/(\bar{M}_n)_m)_v$] and $g'_{v(m)}$ [$= (M_l/(\bar{M}_w)_m)_v$] of a sample mixture have been related theoretically to the mixture's composition. There exists linear relationships between $g_{v(m)}$ and $W_{b,v}$ (the mass fraction of branched component in mixture) as well as between $g'^{-1}_{v(m)}$ and $W_{b,v}$. The latter correlation has been demonstrated experimentally employing a combined SEC/LALLS technique, and displays excellent agreement with the theoretical predictions. Furthermore, this polymer branching characterization method has been applied to study enzymatic starch hydrolysates products. The molecular weight distribution and branching distribution have been obtained.

INTRODUCTION

Polymer samples which are composed of mixtures of linear and branched polymers occur often in many applications. These mixtures may result as a consequence of synthetic (or degradative) chemical kinetic process or from formulation processes. In either case, it is known that such polymer mixtures may possess unique physical properties such as bulk rheology, yield strength, electrical properties, and many others. Fundamentally, these properties will (to a large extent) depend on the polymer mixtures' average molecular weights, the distribution of these molecular weights, and conformational states. The analysis of such polymer mixtures is difficult, at best, by traditional chemical and thermodynamic measurements.[1-4] It is therefore desirable to develop methods whereby routine analytical tools can be employed to obtain the polymer mixture's fundamental molecular information. We recently reported on direct measurements of polymer system branching characteristics where qualitative differences can be determined.[5]

It is generally recognized that linear and branched polymers exhibit much different physical properties such as their intrinsic viscosity–molecular weight relationships, conformational structures in bulk and dilute solutions, and many others.[11] Of specific interest in our laboratory are those physical properties of naturally occurring polymers (biopolymers). In many biotechnology applications, biopolymer mixtures are encountered; most specifically are

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the cases seen for polysaccharides (e.g., starches). In this paper, we report quantitative branching characterization of polymer mixtures via size exclusion chromatography and coupled on-line detectors presented from both theoretical arguments as well as direct experimental evidence. The experimental studies employ polysaccharide materials as an example, but the methods are generally applicable to any polymer mixture.

BACKGROUND

Size exclusion chromatography (SEC) is a powerful tool for determining fundamental molecular information of polymers. The relatively fast and convenient method is capable of determining average molecular weights and molecular weight distributions.[6] SEC can also be coupled to an on-line low angle laser light scattering (LALLS) detector and be used to obtain qualitative polymer branching information.[5,7] Quantitative branching information of polymer samples can be established if the polymer is commonly branched throughout its measured molecular weight distribution.[5] Additional complications are present when the polymer sample is not commonly branched throughout its entire molecular weight distribution or if the analysis is done on polymer mixtures. These complications arise as the fundamental separation mechanism in SEC is caused by the size of soluble polymer molecules as a function of chromatographic elution volume.

THEORETICAL DEVELOPMENT

Zimm and Stockmeyer[8] defined the branching parameter g_M as the ratio of mean square radius of gyration of branched and linear polymer molecules of the same molecular weight

$$g_M = \left(\langle R^2\rangle_b / \langle R^2\rangle_l\right)_M \tag{1}$$

where the subscripts b and l denote branched and linear polymers, respectively. The calculation of g_M is usually done employing measured intrinsic viscosity data. One general expression (5) is

$$\left([\eta]_b/[\eta]_l\right)_M = g_M^e \tag{2}$$

where the exponent can assume values of 0.5–1.5 depending on the particular theoretical assumptions used in the model development of intrinsic viscosity.[9–11]

Branched polymer samples that are commonly branched as a function of their molecular weight distribution can be analyzed quantitatively by SEC provided sample dispersion throughout the SEC system can be assumed negligible. According to the universal calibration concept of Grubisic et al.,[12] at any given elution volume, linear and branched polymer molecule separation can be related by

$$\left([\eta]_b \cdot M_b\right)_v = \left([\eta]_l \cdot M_l\right)_v \tag{3}$$

where $[\eta]_t$ is the intrinsic viscosity of polymer type t, M_t is the molecular

weight of polymer type t, $t = l, b$ denotes linear or branched polymer, respectively, and v denotes common elution volume. Rearrangement of eq. (3) defines the SEC branching parameter g_v:

$$g_v = ([\eta]_b/[\eta]_l)_v = (M_l/M_b)_v \tag{4}$$

The two branching indices can be related[13] by eq. (5) as

$$g_M^e = g_v^{a+1}$$

or

$$g_M = (M_l/M_b)_v^{(a+1)/e} \tag{5}$$

where a is the Mark–Houwink exponent of the linear polymer. Thus, with the knowledge of a and e, the branching parameter g_M can be calculated by comparing the molecular weight of the eluting polymer to that of the corresponding linear homolog at each elution volume.

Mixtures of branched and linear polymer samples can be analyzed by combined SEC/LALLS if (a) the mixtures contain only two polymer components (e.g., one linear polymer and one branched polymer) and (b) sample dispersion is negligible in the SEC system. When mixed polymer samples elute from the SEC column, the contents may contain a mixture of the two polymer types (linear and branched) having the same hydrodynamic volume. With assumption (b), these two materials can each be considered monodisperse. Hamielec and Ouano[14] have derived the relationship shown in eq. (6), applicable for such cases:

$$\left([\bar{\eta}]_m(\bar{M}_n)_m\right)_v = ([\eta]_l M_l)_v \tag{6}$$

where $[\bar{\eta}]_m$ is the weighted intrinsic viscosity of the polymer mixture and $(\bar{M}_n)_m$ is the number-average molecular weight of the polymer mixture. All other nomenclature is as defined above. The weighted intrinsic viscosity of the polymer mixture is assumed to follow the relationship

$$[\bar{\eta}]_m = \sum w_i[\eta]_i \tag{7}$$

where i denotes the contributions to the intrinsic viscosity of the individual components of the polymer mixture, in agreement with the arguments of Rodriguez[15] and Pannell.[16] The weighting parameter w_i is the mass fraction of the polymer types (linear, l, and branched, b). Equation (6) can be rearranged to define the branching parameter of a polymer mixture $g_{v(m)}$ as

$$g_{v(m)} = \left(M_l/(\bar{M}_n)_m\right)_v = ([\bar{\eta}]_m/[\eta]_l)_v \tag{8}$$

The use of light scattering as a detector in SEC analysis presents a problem in that direct measurement of polymer mixture *number*-average molecular weight is not available. This method directly determines polymer mixture *weight*-average molecular weight. Consequently, this method is capable of

directly measuring the branching parameter $g'_{v(m)}$

$$g'_{v(m)} = \left(M_l/(\bar{M}_w)_m\right)_v \tag{9}$$

where the prime denotes parametric determination based on weight averaged quantities. The relationship between $g'_{v(m)}$ and $g_{v(m)}$ is

$$g_{v(m)} = g'_{v(m)}\left[(\bar{M}_w)_m/(\bar{M}_n)_m\right]_v \tag{10}$$

where the major bracketed term on the right-hand side is simply the polydispersity of the polymer mixture in the sample detector cell at a given retention volume v.

Our purposes for the use SEC/LALLS are quite practical, in that we wish to use these measurements in the subsequent analysis of kinetic processes. For such applications (i.e., quantitative compositional data determinations of linear and branched polymer component mixtures), it is necessary to determine the relationship between the amount of branched material present in a sample cell and the two measurable (or determinable) branching parameters $g_{v(m)}$ and $g'_{v(m)}$ of a sample mixture. In other words, if polymer branching determination is required for some applications, then branching must be determinable from the parameters $g_{v(m)}$ and $g'_{v(m)}$. Furthermore, these parameters must be related to the relative amounts of the branched components for polymer mixtures. We consider two subcases below here (I) the branching parameter $g_{v(m)}$ related to mixed polymer blends compositional correlation and (II) the branching parameter $g'_{v(m)}$ related to mixed polymer blends compositional correlations:

Subcase I: $g_{v(m)}$ vs. Branch / Linear Compositional Polymer Blends

Intrinsic viscosity measurements are the key to relating $g_{v(m)}$ to branching compositional characteristics. Equation (7) can be extended to include the contributions of both linear and branched eluting material components. Thus,

$$[\bar{\eta}]_{v(m)} = \left[W_l[\eta]_l + W_b[\eta]_b\right]_v \tag{11}$$

where $W_{l,v}$ and $W_{b,v}$ are the mass fractions of linear and branched components, respectively, in the eluted fraction and $[\eta]_{l,v}$ and $[\eta]_{b,v}$ are the corresponding intrinsic viscosities. By definition,

$$W_{l,v} + W_{b,v} = 1.0 \tag{12}$$

and

$$g_{v(m)} = \left([\bar{\eta}]_m/[\eta]_l\right)_v \tag{13}$$

Recombining eqs. (11)–(13) results in

$$g_{v(m)} = \left[(W_l[\eta]_l + W_{b,v}[\eta]_b)/[\eta]_l\right]_v \tag{14}$$

$$= 1 + W_{b,v}\left[([\eta]_b/[\eta]_l)_v - 1\right] \tag{15}$$

$$= 1 + W_{b,v}(g_v - 1) \tag{16}$$

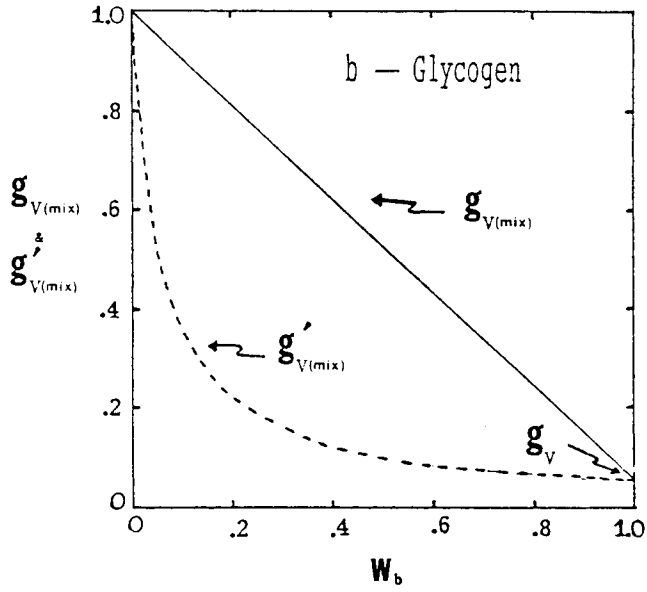

Fig. 1. Theoretical prediction of the relationship between branching parameters and weight fraction of branched polymers in a polymer mixture. Example shown is that of amylose/glycogen.

For quantitative determinations, g_v can be obtained from the analysis of the pure component branched polymer samples (5). The salient result here is that there exists a linear relationship between $g_{v(m)}$ and the mass fraction $W_{b,v}$ of the branched component in the polymer sample mixture. This result is depicted for a particular polymer mixture situation (employing glycogen and amylose) in Figure 1 (solid line). For polymer samples that are purely linear, $g_{v(m)}$ has a value of unity and the value of $g_{v(m)}$ assumes the value of the pure component branched polymer when $W_{b,v}$ is unity. Thus, with knowledge of these two extremes, and with the direct measurement of $g_{v(m)}$, the mass fraction of branched material in an eluting sample can be determined. The most suitable tool available today for direct $g_{v(m)}$ measurements is by employing an on-line intrinsic viscometer coupled to the SEC system. Unfortunately, this equipment is not available in our laboratory at this time.

Subcase II: $g'_{v(m)}$ vs. Branch / Linear Compositional Blends

LALLS measurements can be employed to establish the relationship between the mass fraction of polymer mixture branched components $W_{b,v}$. Equation (9) serves as the starting point in this theoretical development. The basic difference, here, compared to subcase I is that LALLS measurements provide weight-average molecular weight values instead of number-average molecular weight values. The number and weight average molecular weights are calculated from

$$\overline{M}_n = \left[\sum (W_i/M_i)\right]^{-1} \tag{17}$$

$$\overline{M}_w = \sum W_i M_i \tag{18}$$

where M_i denotes the monodisperse molecular weights of the polymer sample with mass fraction W_i. The same basic relationship can be extended to include polymer mixtures composed of linear and branched material eluted from an SEC system as

$$(\bar{M}_n)_{m,v} = [W_l/M_l + W_b/M_b]_v^{-1} \tag{19}$$

$$(\bar{M}_w)_{m,v} = [W_l M_l + W_b M_b]_v \tag{20}$$

where, here, the subscripts l and b are used to denote the individual monodisperse fractions of linear and branched polymer materials. Assuming negligible band broadening, the summation signs are eliminated in eqs. (19) and (20). The detector cells will contain two monodispersed polymer species of the same hydrodynamic volume, one linear and one branched. The mass fraction of the linear species in the cell is $W_{l,v}$ with molecular weight $M_{l,v}$ and the corresponding parameters of the branched species are $W_{b,v}$ and $M_{b,v}$. Polydispersity (PD) of the eluting polymers in the detector cell at any given elution volume can be calculated from

$$\text{PD} = (\bar{M}_w)_{m,v}/(\bar{M}_n)_{m,v} \tag{21}$$

and after rearrangement:

$$\text{PD} = [1 + W_{b,v}((M_b/M_l)_v - 1)] \times [1 + W_{b,v}((M_l/M_b)_v - 1)] \tag{22}$$

By definition, $g_v = (M_l/M_b)_v$ and therefore

$$\text{PD} = [1 + W_{b,v}(g_v^{-1} - 1)] \times [1 + W_{b,v}(g_v - 1)] \tag{23}$$

From eq. (10), $g'_{v(m)}$ of a polymer mixture is the product of $g_{v(m)}$ and $(\text{PD})^{-1}$. Hence, substituting eqs. (16) and (23) into eq. (10) results in

$$g'_{v(m)} = [1 + W_{b,v}(g_v^{-1} - 1)]^{-1} \tag{24}$$

Figure 1 displays $g'_{v(m)}$ as a function of $W_{b,v}$ (dashed line) for a particular branched/linear polymer system (glycogen/amylose). If the reciprocal of $g'_{v(m)}$ had been plotted vs. mass fraction of branched material, a straight line would have been obtained. This demonstrates that with SEC/LALLS it is possible to predict branched polymer compositions of polymer mixtures with $g'_{v(m)}$ measurements. The applicability of employing eq. (24) as our working model is further demonstrated experimentally in this paper. Moreover, the potential application of SEC combined with multiple on-line detectors (LALLS and intrinsic viscosity) offers even greater opportunities. For example, combining eqs. (16) and (24) results in

$$W_{b,v} = [g_{v(m)}g'_{v(m)} - g_{v(m)} - g'_{v(m)} + 1]/[g_{v(m)}g'_{v(m)} - 2g'_{v(m)} + 1] \tag{25}$$

In principle, the branching parameters $g_{v(m)}$ and $g'_{v(m)}$ can be measured, respectively, by on-line intrinsic viscometry and LALLS simultaneously. With this method, the weight fraction of branched polymer in a mixture can be obtained directly without independent g_v measurement (i.e., knowledge of pure component branching parameter's value). We are currently working on such developments.

EXPERIMENTAL

Instruments

The SEC/LALLS system used in this study consisted of a Waters Associates (Milford, MA) pump (M6000), injector (U6K) and differential refractive index (DRI) (R401), LDC/Milton Roy (Riviera Beach, FL) KMX-6 LALLS detector, and our self-packed TSK (40S and 65F) Fractogel HW columns.[17] The combined column system exhibited a plate count of 3900 ppm (plates/meter) relative to a theoretical plate count of 4300 ppm according to the procedure of Yau et al.[6] The experiments were run with flow rate 0.10 mL/min at ambient temperature.

The LALLS photometer with a flowthrough sample cell was serially connected with the DRI detector. Scattering intensity data were collected at the 6–7° annulus with 6328 Å wavelength, He–Ne laser. The mobile phase was filtered through an on-line 0.2-μm Fluoropore filter (Millipore Corp. Bedford, MA) just before the LALLS cell. The value of dn/dc determined independently for polysaccharides is 0.146 mL/g. Using the software package, MOLWT3 (LDC/Milton-Roy), the analog data from both DRI and LALLS detectors were collected and digitized through a CMX10A A/D converter during a sample run on the SEC/LALLS system. Collected data were processed to give the molecular weight at each elution volume.

Materials and Sample Preparation

The polysaccharides used in these experiments are all regarded as α-(1,4)-D-glucan linkages. Amylose, a purely linear α-(1,4) polysaccharide, purchased from Hayashibara Biochemical Lab. Inc. (Japan) (Lot No. 71063012) and is reported by the manufacturer as having a nominal molecular weight 1.8×10^4. Two branched polymers (amylopectin and glycogen) were obtained from Sigma Chemical Co. (St. Louis, MO). Because the purchased amylopectin possessed an extremely high molecular weight, it was necessary to partially hydrolyze the amylopectin sample before SEC/LALLS analysis. In addition, a high amylose corn starch referred to as amylomaize VII supplied by American Maize-Products Co. (Chicago, IL.) was hydrolyzed by enzyme and examined via SEC/LALLS.

For the hydrolysis experiments, 50 mL of 10 wt % substrate solution was enzymatically digested at 90°C with the α-amylase from Bacillus licheniformis (Sigma, batch No. A3403). The substrate solution was then heated at 100°C for 10 min before the addition of enzyme. Reaction was stopped by adding a few drops of 1N NaOH, bringing the pH beyond where the enzyme exhibits hydrolytic activity.

All solutions for analysis were prepared in degassed 0.5N NaOH, the same solvent used as the SEC eluent. Polymer solutions were prepared by dissolving known mass quantities of the polysaccharide materials and diluted to volume with the caustic solvent. For the blends of linear and branched polymer studies, several artificial mixtures of linear and branched polysaccharides solutions were prepared. These samples were amylose/amylopectin mixtures and amylose/glycogen mixtures.

RESULTS AND DISCUSSION

Through the combined use of SEC and direct weight average molecular weight measurements (via LALLS), the molecular characteristics of a sample can be plotted as a function of chromatographic separation. Figure 2 is an example of such a graphical representation for the data collected with our analytical system. The solid lines are the linearly regressed elution profiles of three polysaccharides (amylose, amylopectin, and glycogen), labeled as such, and reported earlier[5] and are used here as reference lines. These lines are not parallel, but rather would intersect at some low molecular weight as theoretically predicted.[8] Artificially mixed samples of these linear and branched polymers (i.e., mixtures of amylose and glycogen and amylose and amylopectin) are also presented in Figure 2 for two representative polymer mixtures (1 : 2 ratio of amylose: amylopectin and 4 : 1 ratio of amylose: glycogen, where the ratios are the bulk mass fractions of the two polysaccharides). For these polymer mixture samples, two general trends in the data representation are apparent. For the polymer mixture of amylose and amylopectin, the data points map out a curve that lies between the amylose and amylopectin reference lines and is nearly parallel to these two lines.

In contrast, the data for the amylose–glycogen mixtures exhibit extreme curvature but are still bounded by the corresponding reference lines (i.e., amylose and glycogen). This situation exists, as the individual polymer samples used in the artificial mixtures are not guaranteed to have the same molecular size distribution. The eluted material detected at each elution volume varies compositionally in the relative amounts of linear and branched polysaccharide. Only if the mixed polymer sample's individual components possessed the same molecular size distributions would the data be exactly linear and lie proportionately equal distance between the two reference lines. This situation is represented in Figure 3(a).

In cases where molecular size distributions of the two component polymers in the sample mixture chromatographed are not exactly the same (as is most often the case), curvature of the data, as represented in Figure 2, will result. This situation is represented in Figure 3(b). In either case, however, the information of Figure 2 can be employed to calculate $g'_{v(m)}$ data at each elution volume by use of eq. (9). Such data are tabulated for various artificial polymer mixtures at several elution volumes and presented in Table I.

In order to calculate the mass fraction of branched material at each elution volume for the various mixed polymer samples chromatographed, the normalized DRI detector response was calculated via

$$NX_v = X_v / \sum X_v \tag{26}$$

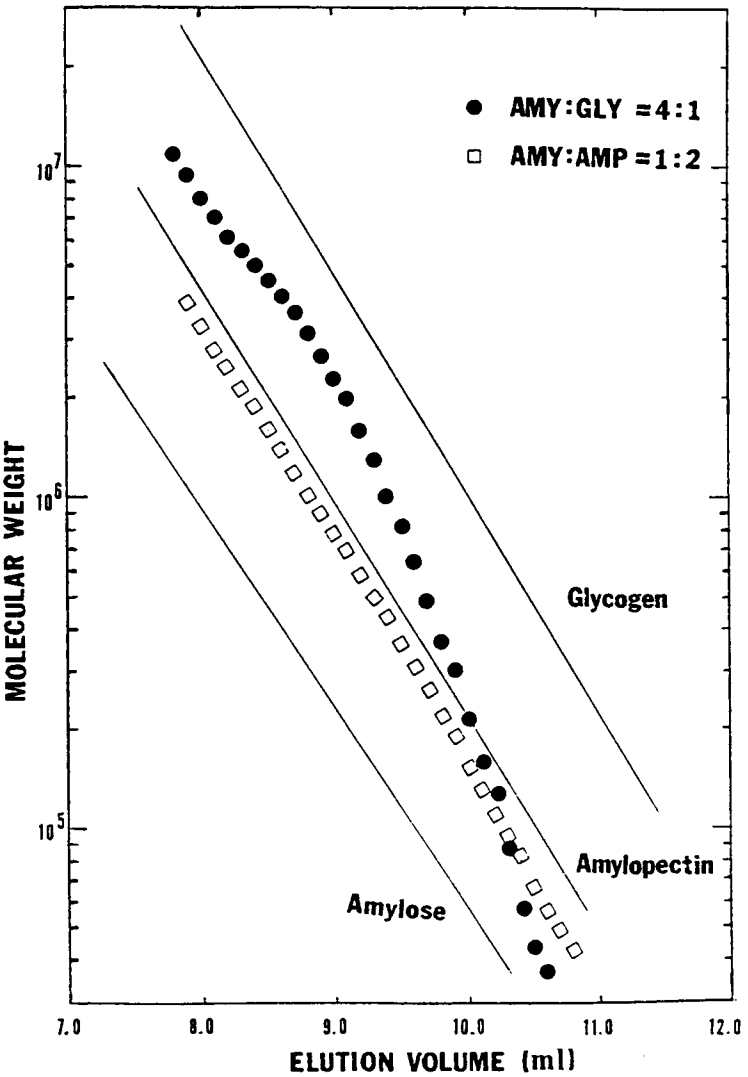

Fig. 2. The plot of molecular weight vs. elution volume for polysaccharide mixtures by SEC: (●) for 4 : 1 mass ratio of amylose : glycogen mixture; (□) for 1 : 2 mass ratio of amylose : amylopectin mixture.

where NX_v is the normalized response and X_v is the DRI response at a given elution volume. The mass fraction of branched material at each elution volume was then calculated via

$$W_{b,v} = (NX_v)_b / [\gamma_{lb} \cdot (NX_v)_l + (NX_v)_b] \tag{27}$$

where γ_{lb} is the total mass ratio of linear and branched components in the mixture.

The $W_{b,v}$ values so determined are presented in Tables II and III for each polymer mixture tested at each indicated elution volume. Table II gives the

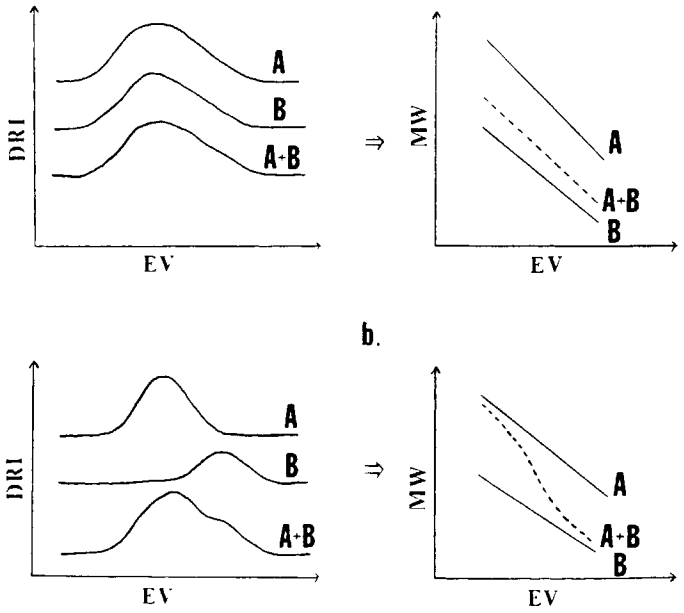

Fig. 3. Schematic chromatograph and corresponded calibration curves for two types of polymer mixtures: (a) for the case of mixture's individual components with the same molecular size distribution; and (b) for the case of mixture's individual components with different molecular size distributions. *EV* is the elution volume.

TABLE I
$g'_{v(m)}{}^{-1}$ for Polysaccharide Mixtures

Samples	Elution volume (mL) 8.5	9.0	9.5	10.0
Amylose/glycogen				
AMY : GLY = 0 : 1	22.7	21.1	20.0	18.0
AMY : GLY = 1 : 3	19.8	20.0	17.7	15.7
AMY : GLY = 1 : 2	16.8	17.8	15.0	12.1
AMY : GLY = 1 : 1	13.0	13.4	12.5	9.4
AMY : GLY = 2 : 1	13.0	11.9	9.7	6.2
AMY : GLY = 3 : 1	10.7	10.7	7.2	3.4
AMY : GLY = 4 : 1	9.8	10.2	7.3	3.8
AMY : GLY = 6 : 1	8.2	8.0	6.1	3.8
AMY : GLY = 10 : 1	5.5	5.6	4.3	2.0
Amylose/amylopectin				
AMY : AMP = 0 : 1	4.5	4.2	3.95	3.7
AMY : AMP = 1 : 4	4.5	4.1	3.7	3.0
AMY : AMP = 1 : 3	4.0	3.75	3.5	3.0
AMY : AMP = 2 : 5	3.55	3.4	2.8	2.45
AMY : AMP = 1 : 2	3.6	3.5	3.2	2.7
AMY : AMP = 1 : 1	3.0	3.2	3.0	2.9
AMY : AMP = 2 : 1	2.2	2.3	2.3	2.1
AMY : AMP = 3 : 1	1.9	1.7	1.6	1.6
AMY : AMP = 4 : 1	1.8	2.0	1.85	1.7
AMY : AMP = 6 : 1	1.6	1.7	1.5	1.5

TABLE II
$W_{b,c}$ Values for Amylose/Glycogen Mixtures[a]

γ_{lb}	EV = 8.5 mL, NX_l/NX_b = 0.55	EV = 9.0 mL, NX_l/NX_b = 0.355	EV = 9.5 mL, NX_l/NX_b = 0.5	EV = 10.0 mL, NX_l/NX_b = 1.25
1/3	0.845	0.894	0.857	0.706
1/2	0.784	0.849	0.80	0.615
1/1	0.645	0.738	0.667	0.444
2/1	0.476	0.585	0.5	0.286
3/1	0.377	0.484	0.40	0.21
4/1	0.312	0.413	0.333	0.167
6/1	0.233	0.319	0.25	0.188
10/1	0.154	0.220	0.167	0.074

[a] γ_{lb} = total mass ratio of linear and branched components in the mixture. NX = normalized DRI response.

data for various amylose/glycogen mixtures, and Table III the values for amylose/amylopectin mixtures. These experimental data (in Tables I–III) can be used to test the theoretical predictions discussed above.

Figures 4 and 5 compare experimental data to theoretical predictions. Equation (24) predicts that $g'_{v(m)}{}^{-1}$ should vary linearly with branched polymer mass fraction. This linear relationship is shown to be valid for each of the elution volume data represented in Figures 4(a)–(c) for the amylose/glycogen mixtures and in Figures 5(a)–(c) for the amylose/amylopectin mixtures examined. In these figures the solid line is the theoretical prediction and the dashed line is the line resulting from the linear regression of the collected data. The correlation coefficients (r) of each data set are included in each figure. Moreover, it is clear that as elution volume increases, the intercept values of $g'_{v(m)}{}^{-1}$ (i.e., at $W_{b,v} = 1.0$) decreases for both polymer systems tested. Zimm and Stockmeyer[8] theoretically predicted that g_M should decrease with increasing relative molecular weight, and our data are consistant with this prediction. We cannot, at this time, directly compare the quantitative predictions[8] to our experimental data. Three reasons exist for this

TABLE III
$W_{b,v}$ Values for Amylose/Amylopectin Mixtures[a]

γ_{lb}	EV = 8.5 mL, NX_l/NX_b = 0.85	EV = 9.0 mL, NX_l/NX_b = 0.82	EV = 9.5 mL, NX_l/NX_b = 0.83	EV = 10.0 mL, NX_l/NX_b = 0.76
1/4	0.825	0.830	0.828	0.84
1/3	0.779	0.785	0.783	0.80
2/5	0.746	0.753	0.751	0.767
1/2	0.70	0.709	0.707	0.725
1/1	0.54	0.55	0.55	0.57
2/1	0.37	0.379	0.376	0.40
3/1	0.28	0.289	0.286	0.305
4/1	0.23	0.23	0.23	0.25
6/1	0.164	0.169	0.167	0.18

[a] γ_{lb} = total mass ratio of linear and branched components in the mixture. NX = normalized DRI response.

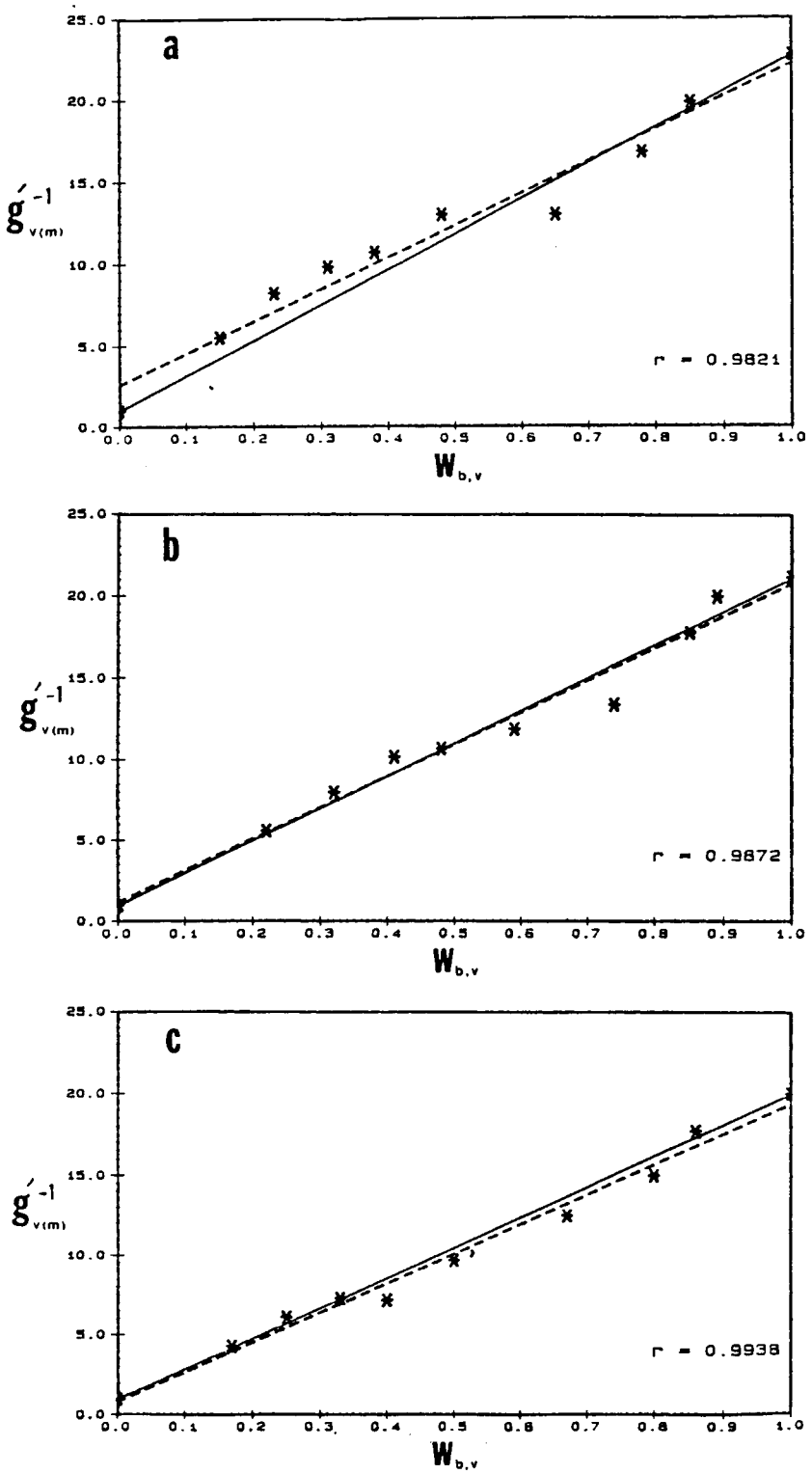

Fig. 4. The plot of $g'^{-1}_{v(m)}$ vs. weight fraction of branched material ($W_{b,v}$) for amylose/glycogen mixtures at three different elution volumes (EV); (a) 8.5 mL; (b) 9.0 mL; (c) 9.5 mL.

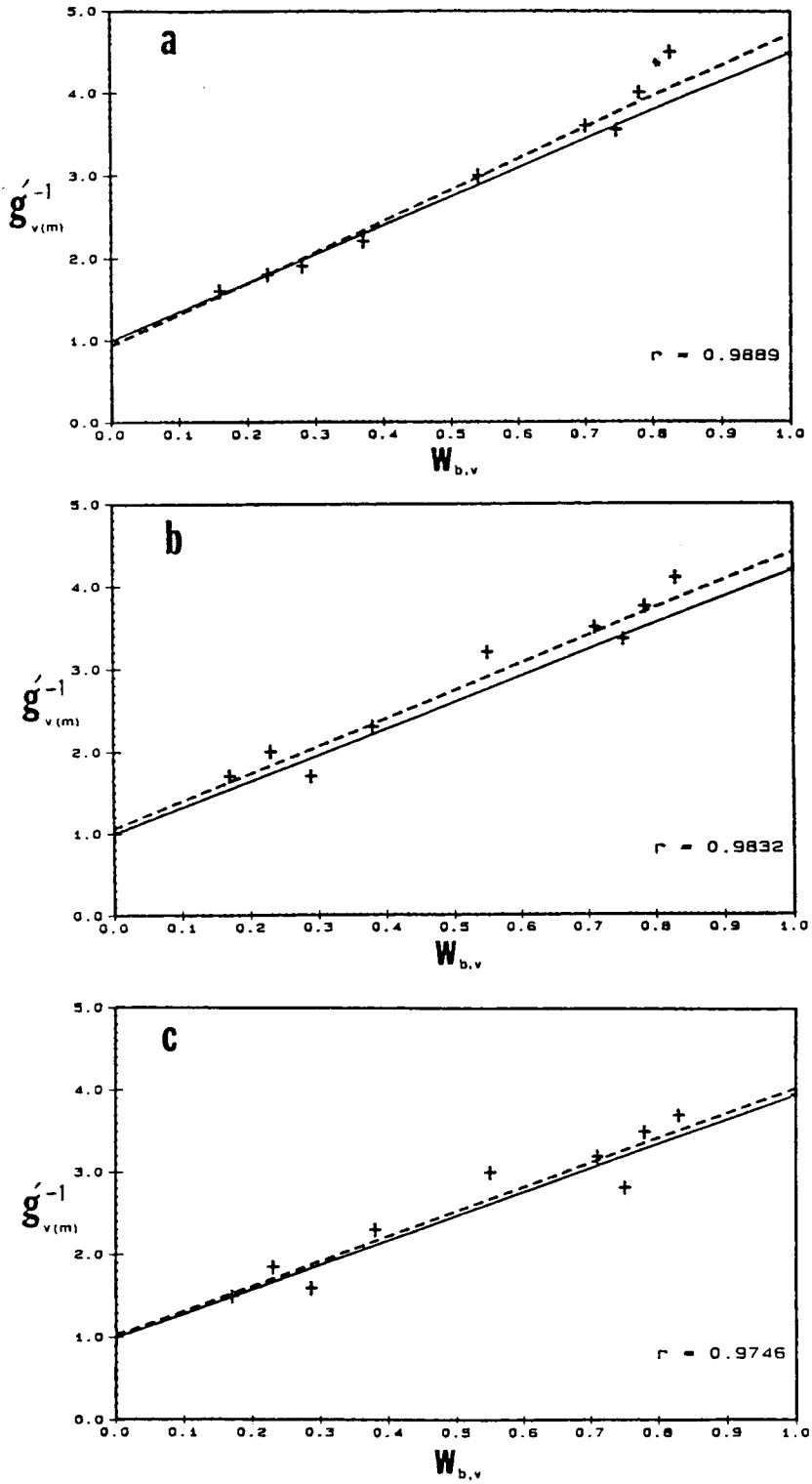

Fig. 5. The plot of $g'^{-1}_{v(m)}$ vs. weight fraction of branched material ($W_{b,v}$) for amylose/amylopectin mixtures at three different elution volumes (EV); (a) 8.5 mL; (b) 9.0 mL; (c) 9.5 mL.

situation: (1) "effective" polymer branching functionality is unknown at this time,[18-20] (2) polymer drainage characteristics are unknown, and (3) polysaccharide bond lengths (i.e., allowable rotations) vary with bonding type and hence the polysaccharides may, in this manner, be considered copolymeric. We are working on these problems at this time and anticipate reporting our findings at a later date.

Our purpose in developing this branching analysis procedure is in the examination of industrially important biopolymers. One specific application of this analysis is in starch processing. Starch is an abundant natural polysaccharide that is used in numerous industries.[21] Two polymer molecular types are present in natural starch: amylose and amylopectin. Amylose is linear composed exclusively of α-1,4 glycosidic linkages. Amylopectin is branched whose composition is 96% linear α-1,4 and 4% α-1,6 branched glycosidic linkages. In some applications, starch is modified prior to use. One such modification is enzymatic- or acid-catalyzed depolymerization in which our interest lie.[22]

The method described above can be applied to characterize starch hydrolysate materials. The information contained in Figure 5 can serve as a calibration curve, whereby SEC/LALLS measurements of $g'_{v(m)}$ at various elution volumes can be employed to determine the mass fraction of branched material (expressed as amylopectin). For such studies, one can consider $g'_{v(m)}$ as the independent variable and $W_{l,b}$ as the dependent variable. Starch samples extracted from some process can be then analyzed via SEC/LALLS. An example of this approach is shown in Figures 6 & 7. Combined SEC/LALLS analysis of a mixed polymer system (in this case starch; i.e., amylose and amylopectin) provides many parametric values. First of all, a molecular weight distribution is provided. Secondly, differentiation of the branched and linear components of a starch sample as a function of molecular size are obtained. Thirdly, the resulting molecular weight distribution profile "snapshots" of any processing operation will provide insight into the molecular kinetic events which occurred. As an example, Figure 6 displays chromatographic profiles at zero reaction time for both the total mass distribution (the solid line is the DRI chromatographic trace) of material eluted as a function of either elution volume or molecular weight (based on amylose hydrodynamic volume) and the mass distribution of branched polysaccharide material (expressed as amylopectin, narrow-line shaded chromatographic trace) against the same separation parameters. This data confirms independent chemical information that this particular starch (amylomaize VII) is composed of approximately 70% amylose and 30% amylopectin.

Figure 6 also displays the mass fraction of branched material at each elution volume $W_{b,v}$ (dotted line), calculated from eq. (24), on the right-hand axis of the graph. The curve indicates that the high molecular weight region ($MW > 5 \times 10^6$) is dominated by the branched material; and that branching decreases with decreasing molecular weight. The dotted region of the chromatograph blocks the so-called "dissolving gap" region of amylose. This molecular weight region of amylose is known to be insoluble in neutral aqueous solvents.[23] Approximately 60% of the linear polysaccharide material of the unreacted starch sample is within this dissolving gap region.

Figure 7 displays similar data for the same starch after 2 min hydrolysis time with the endo-acting enzyme, α-amylase. The relative amount of branched

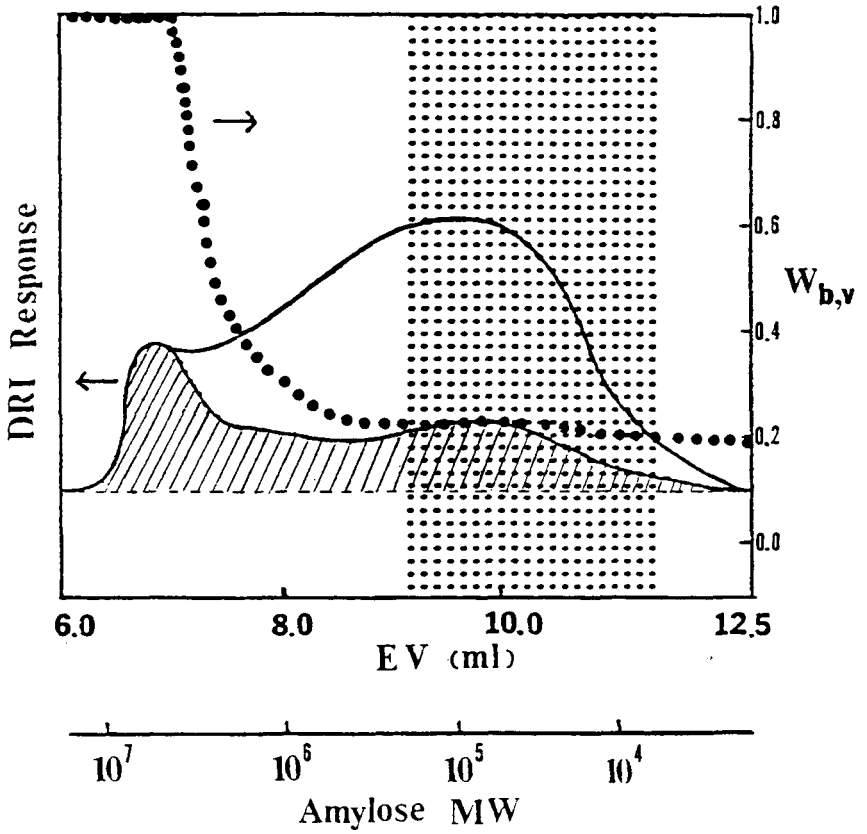

Fig. 6. Molecular weight distribution (solid line) and branching distribution (shaded curve) of amylomaize VII starch.

material above amylose molecular weight 5×10^3 (DP $\approx$ 31) is drastically reduced to approximately 11% compared to the original 30% value. This indicates that the starch branched component material is preferentially hydrolyzed by the endo-acting linear depolymerase α-amylase and is present as low molecular weight oligosaccharide fractions, too small to be detected by LALLS. This most likely results from selective hydrolysis of the α-1,4 bonds of starch components in the vicinity of branching points. Clearly, the majority of branched material must exist at the lower end of the molecular weight distribution in order to close the material balance. Our recent NMR data confirm this speculation.

The proton ^{1}H-NMR spectra of polysaccharide samples in deuterated dimethyl sulfoxide (DMSO-d_6) have been studied. A dramatic difference exists in the ^{1}H-NMR spectra of α-1,4-linked and α-1,6-linked glucose monomeric units within the polymer. This information can be employed to independently test starch hydrolysate fractions' molecular bonding patterns; i.e., α-1,4 and α-1,6 linkages for different molecular size starch components can be assayed. Qualitatively, and specifically for this study, a relatively high

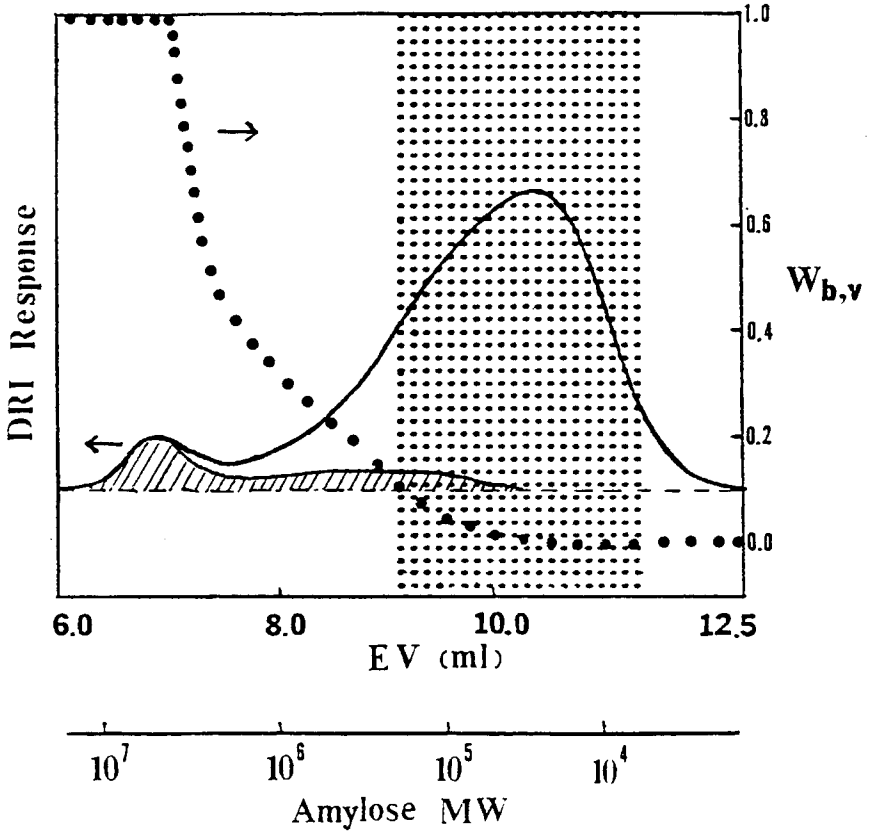

Fig. 7. Molecular weight distribution (solid line) and branching distribution (shaded curve) of amylomaize VII starch hydrolysate after 2 min enzymatic hydrolysis.

quantity of α-1,6 branching is evident in the low molecular weight starch hydrolysate residue as seen by the ^{1}H-NMR spectra. Moreover, the ^{1}H-NMR spectra of the precipitated starch hydrolysate fraction indicates a nearly pure α-1,4-linked material. This independently confirms our SEC/LALLS data displayed in Figure 7.

CONCLUSION

This work has demonstrated that quantitative information on polymer branching as a function of polymer molecular size can be obtained from size exclusion chromatography combined with on-line low-angle laser light scattering detectors (or other physical measurements). The basic technique has been demonstrated to be applicable for polysaccharide analysis (starches in this case) and has been shown useful in understanding some interesting features of the kinetics associated with starch depolymerization via industrially important enzymes. The same technique should be useful in various studies of a broad range of polymeric materials.

APPENDIX: NOMENCLATURE

a	Mark–Houwink exponent
dn/dc	refractive index increment
DRI	differential refractometer
e	exponent used in eq. (2), $0.5 \leq e \leq 1.5$
EV	elution volume (mL)
g	branching parameter
g'	branching parameter determined by SEC/LALLS
K	Mark–Houwink parameter
LALLS	low angle laser light scattering
M	molecular weight of monodisperse polymer
$\overline{M}_n$	number average molecular weight
$\overline{M}_w$	weight average molecular weight
NX	normalized DRI response
PD	polydispersity
r	correlation coefficient
$\langle R^2 \rangle$	mean square radius of gyration
SEC	size exclusion chromatography
W	weight fraction
X	DRI response
γ_{lb}	total mass ratio of linear and branched components in the mixture
$[\eta]$	intrinsic viscosity

Subscripts:

b	branched polymer
l	linear polymer
M	common molecular weight
m or (m)	mixture polymer
v	common elution volume

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