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Polysaccharide Characterization by Aqueous Size Exclusion Chromatography and Low-Angle Laser Light Scattering

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Abstract

Aqueous size exclusion chromatography coupled with on-line low-angle laser light scattering (SEC/LALLS) is a valuable analytical tool for characterization of polysaccharides and other important biopolymers. This work reviews the fundamental size separation mechanism of polymers chromatographed via SEC, the development of SEC/LALLS methods for characterization of eluted polymers, and applications of this technique to determine polysaccharide physical and chemical properties. Important nonsize exclusion effects encountered in aqueous SEC of polysaccharides are discussed and attributed to intramolecular and polymer-support interactions, as well as flow-related anomalies. The necessity of absolute molecular weight detection as a direct means of calibration is presented. Low-angle laser light scattering coupled to SEC provides a simple method of direct calibration and allows determination of polymer molecular weight and molecular weight distribution. Recent applications of SEC/LALLS to determine polysaccharide branching characteristics are detailed. The combined knowledge of molecular weight distributions and branching distributions provides insight into the molecular kinetic events of polysaccharide processing operations.

INTRODUCTION

Size exclusion chromatography (SEC) is now regarded as one of the most important analytical techniques in polymer characterization. The application of this technique to water-soluble polymers, especially naturally occurring biopolymers, is of special interest. These interests

now exist as many new uses for naturally occurring polymers are envisioned. In particular, the utilization of polysaccharides as a renewable source for fuel, food, and specialty chemical production has stimulated investigators to develop specific analytical tools to characterize these important polymers. Aqueous size exclusion chromatography coupled to on-line low-angle laser light scattering (SEC/LALLS) appears to be a most promising method for measuring physical and chemical properties [e.g., average molecular weights (MW), molecular weight distribution (MWD), and branching]. Reliable determination of these properties is essential in understanding many fundamental chemical processes, most notably the kinetics of polysaccharide synthetic and degradative reactions which convert abundant low-value raw materials to high-value products.

The advances of aqueous SEC have recently been reviewed by Barth (*1*), Rollings et al. (*2*), and Dubin (*3*). Rollings et al. (*2*) pointed out that development of SEC methods for water-soluble polymers has lagged behind SEC techniques for polymers soluble in organic solvents. This exists for numerous reasons, which include the lack of readily available monodispersed calibration standards and suitable chromatographic supports of sufficient separation ranges for characterizing water-soluble polymers. In addition, SEC of water-soluble polysaccharides can be complicated by nonsize exclusion effects that result from intramolecular and polymer-support ionic interactions and flow-related anomalies (see below).

With the development of on-line low-angle laser light scattering detection in the late 1970s, molecular weights, molecular weight distributions, and polymer branching can now be rapidly determined as a function of polymer molecular size. On-line light-scattering detection provides a simple and direct calibration method. The combination of SEC molecular weight detection, and microcomputer data acquisition provides an efficient method of determining polymer physical properties. A review of SEC/LALLS and its applications in determining molecular weight and long-chain branching has recently been published by Hamielec and Meyer (*4*).

The purpose of this work is to review the fundamental size separation mechanism of polymers in aqueous solvents chromatographed via SEC, discuss SEC/LALLS methods for characterizing eluted polymers, and briefly discuss applications of this valuable technique. Important nonsize exclusion effects encountered in SEC resulting from interactions between the polymer, solvent, and support, as well as other effects (e.g., shear degradation) will be discussed briefly. Commonly used calibration techniques will be presented including the use of secondary standards,

universal calibration methods, and direct calibration from light scattering. The development of low-angle laser light-scattering detection, as it applies to SEC, will be detailed. Finally, a survey of aqueous SEC/LALLS applications for determining polysaccharide physical/chemical properties will be presented. Specifically, SEC/LALLS will be demonstrated to be a key analytical tool useful in gaining a fundamental understanding of polysaccharide synthesis and degradation processes.

SIZE EXCLUSION CHROMATOGRAPHY DEVELOPMENT

SEC Separation Mechanism

The fundamental separation mechanism in SEC is due to the partitioning of macromolecules between the flowing solvent in the interstices of the column and the nonflowing solvent within the porous matrix of the column packing (*5*, *6*). Smaller macromolecules spend a longer time in the stationary solvent inside the porous matrix compared to larger macromolecules. Larger macromolecules, therefore, elute from the SEC column before smaller macromolecules. They "see" a smaller effective column pore volume. Since the column separation is based on the solution size of the macromolecule, the polymer conformation in dilute solution is of great importance. In applying SEC to polysaccharides, polyelectrolytic interactions between the polymer, the solvent, and the support may lead to other nonsize separation effects. Macromolecular solution size and polyelectrolytic effects will be addressed in the next sections.

The retention mechanism in SEC can be best described by the following equation which relates the elution volume of a polymer, V_e, to the bed interstitial volume, V_o, and the bed pore volume, V_i:

$$V_e = V_o + K_D V_i \tag{1}$$

K_D represents an effective partition coefficient and is defined as the ratio of the polymeric solute concentration within the pores of the packing and its concentration in the bulk fluid (*1*). For a chromatographic separation solely controlled by macromolecular size, K_D ranges from 0 to 1. Figure 1 displays a typical SEC chromatogram constructed by plotting the eluting polymer's concentration (usually measured from differential refractometry) versus retention volume. For a macromolecule that is too large to

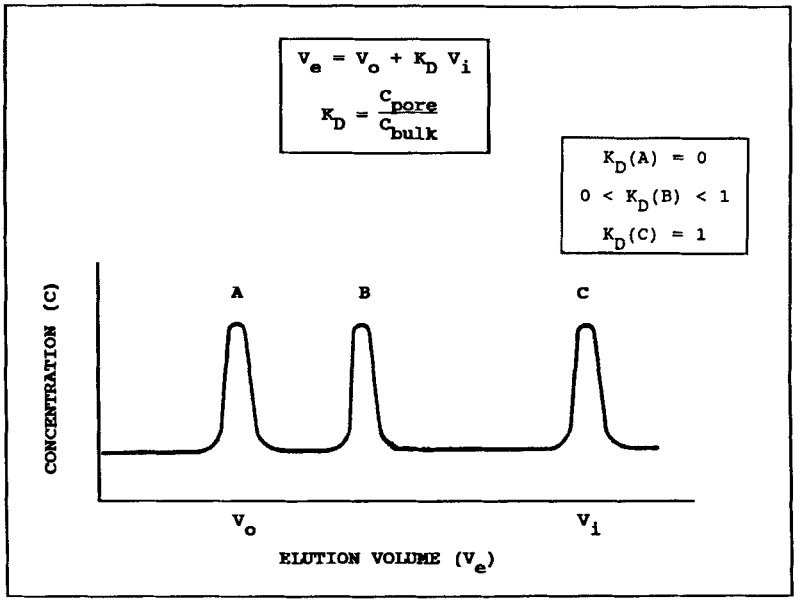


FIG. 1. SEC chromatograph depicting variation of K_D with elution volume for a polymer mixture: (A) high MW (totally excluded), (B) intermediate MW, (C) low MW (totally retained).

diffuse into the porous matrix, $K_D = 0$ and elution occurs at the interstitial bed volume; $V_e = V_o$. A value of $K_D = 1$ represents a polymer which can penetrate the entire available bed pore volume, and hence, the polymer elutes at an elution volume equal to the total volume of the bed; $V_e = V_T = V_o + V_i$. Pore size, pore shape, and pore size distribution will also influence the values of the partition coefficient (*7*). More complete reviews of theoretical SEC mechanisms are presented by Yau et al. (*5*) and Barth (*8*).

Relating the partition coefficient to elution volume can be carried out through a direct calibration procedure. Direct calibration is made by plotting known molecular weight samples versus column performance. Direct calibration can be done only with well-characterized standards of the polymer of interest. Unfortunately, primary standards are available for few water-soluble polymers. A listing of commercially available standards can be found in Barth's review (*1*). The use of secondary standards, such as sodium polystyrene sulfonate (NaPSS) and dextran, have been employed for aqueous SEC calibration (*9*). Secondary

standards which differ significantly in chemical nature (e.g., structure and ionic behavior) from the sample must be used cautiously. If polymer molecular weight information is desired and suitable calibration standards are not available, a correlation between molecular weight and solution size is needed.

Molecular Size–Molecular Weight Relationship

Fundamental separation in SEC occurs via a mechanism which is dependent upon the polymer's molecular size. As a consequence, the relationship between the macromolecule's size in solution and its molecular weight must be available to interpret SEC data correctly. Pioneering work by P. J. Flory (using statistical mechanical arguments) laid the foundation for determining the desired molecular size–molecular weight relationship. Flory's initial interests were in describing dilute solution rheological behavior of polymeric solutions. He determined that the intrinsic viscosity $[\eta]$ of a polymer solution is described by Eq. (2) (*10*):

$$[\eta] = \Phi_0 \langle r^2 \rangle^{3/2} / M = \Phi_0 \alpha^3 \langle r_0^2 \rangle^{3/2} / M \tag{2}$$

where M is the polymer molecular weight, $\langle r^2 \rangle$ is the mean-square end-to-end distance, $\langle r_0^2 \rangle$ is the unperturbed mean-square end-to-end distance, α is the expansion factor, and Φ_0 is Flory's constant (3.6×10^{21} dL/cm^3). Intrinsic viscosity is a molecular parameter obtained from linear extrapolation to zero concentration of other rheological properties. More complete discussions of these subjects can be found elsewhere (*11*). Concentrations approaching infinite dilution prevail in SEC experiments. Therefore, the applicability of Flory's equation may be useful in SEC analysis.

Grubisic, Rempp, and Benoit (*12*) employed Flory's relationship and demonstrated that molecular size separation can be correlated for various polymers differing in their specific chemical nature and configuration. They showed that plotting the product of log $[\eta]$ and MW vs volumetric throughput is applicable for many neutral polymers in nonpolar solvents. Figure 2 demonstrates that linear, comb-branched, and star-branched polymers of differing chemical make-up scale to this common separation index. This secondary calibration technique (called universal calibration) has found wide applicability in similar polymer-solvent systems. Universal calibration has not been demonstrated to be

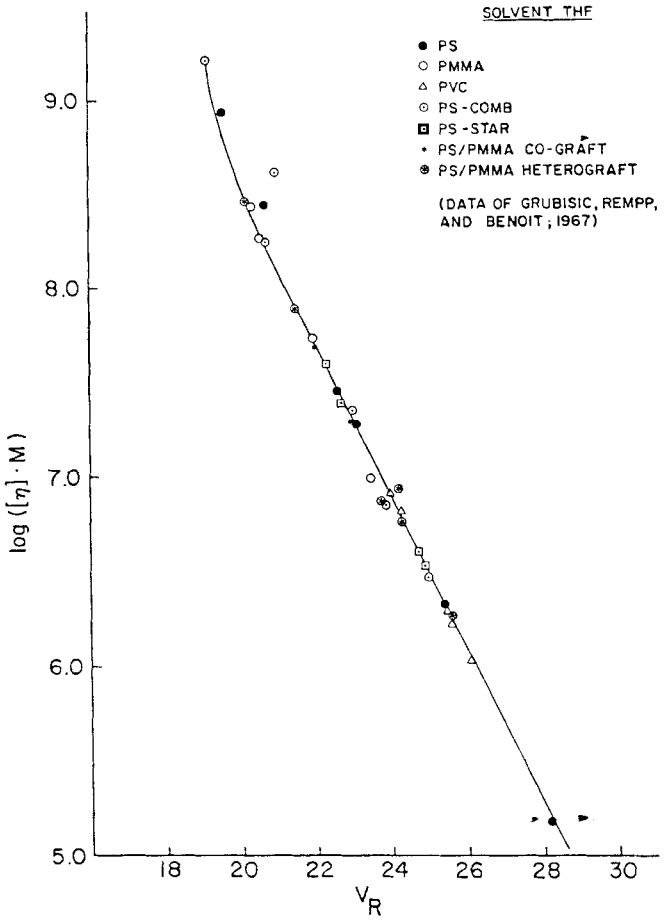


FIG. 2. Universal calibration plot of Grubisic et al. (*12*).

generally applicable for polymers soluble in polar solvents. Reasons for this failure have been presented (*13*, *14*) and suggested to result from the polymer's polyelectrolytic nature in polar solvents (e.g., water). The possibility of intramolecular polymer-polymer, polymer-solvent, and polymer-support interactions also exists. These complications must be considered in SEC analysis in order to assure that their contributions have either been accounted for or eliminated.

Polymers in dilute solution are subject to two major forms of interactions which affect their molecular size and, hence, their physical

properties: polymer-solvent interactions and intramolecular polymer-polymer interactions (*15*). In a poor solvent, intramolecular associations dominate, the polymer conforms to a tightly coiled structure, and the molecular size is small. In a good solvent, polymer-solvent interactions are greater than polymer-polymer interactions and, consequently, the polymer exists as a large, loosely coiled structure (*16*, *17*). For polyelectrolytes in solution, conformation is strongly dependent on bulk solution ionic strength (*17*, *18*). In solvents of low ionic strength, the ionizable moieties of the polyelectrolyte dissociate. The electrostatic interactions among charged groups along the polymer backbone cause the molecule to expand (*15*). These repulsive effects are suppressed in high ionic strength solvents by a process which shields the polymer's ionic groups (*18*). This leads to a contracted molecular conformation in solution. Therefore, polyelectrolyte expansion and contraction (i.e., molecular size) is strongly dependent on the solvent's ionic strength.

Coll and Prusinowski (*20*) proposed an alternative calibration scheme more suitable for highly expanded polymers. They argued that significant variation in the Flory constant exists for such systems due to solvent drainage through the coil. Coll and Prusinowski suggested a modification of the universal calibration procedure to account for this behavior. Their calibration procedure plots $[\eta]MW/f(\varepsilon)$ vs elution volume. The function $f(\varepsilon)$ is an excluded volume function which accounts for nonconstancy in the Flory parameter. Although use of the Coll-Prusinowski procedure in some systems has been shown to be applicable (*14*), these reported systems have not been exhaustively studied, and the possibility of other, unaccounted for separation phenomena may exist (*13*, *21*). Detailed work on these and other systems must be performed before concrete conclusions regarding separation phenomena are established.

Secondary Separation Phenomena in SEC

Secondary aqueous SEC separation mechanisms (nonsize) of polyelectrolytes can occur due to ionic interactions between the polymer in solution and the porous support matrix. These interactions have been reviewed by Stenlund (*22*). Ionic interactions for these systems can lead to adsorption, ion exclusion, and ion inclusion effects. This section discusses these phenomena and their potential effects on aqueous chromatography of large molecules.

Adsorption of a macromolecule to the chromatographic support has

been well documented for polymers in nonpolar solvents (e.g., polystyrene) (*23*, *24*, *26–28*). Polymer adsorption depends on numerous parameters, which include contributions due to the particular solvent used and the presence of cosolutes (*23*, *25*, *27–30*). Van der Waals forces are the primary driving forces for polymer adsorption of neutral polymers in nonpolar solvents. The mechanism is more complex for charged polymers in polar solvents undergoing separation on a support surface that also may be charged. When the chromatographic support and solute are oppositely charged, electrostatic attractions retard the movement of the polyion through the porous matrix, resulting in delayed elution. Multiple elution peaks have been reported for monodispersed polymers and attributed to adsorption (*30*, *31*). Irreversible adsorption of the polymer to the support matrix is also possible, and some authors have discussed this situation (*30*).

When the surface of the chromatographic support and the polyelectrolyte are of like charge, electrostatic repulsion or ion-exclusion occurs which prevents the polyion from freely diffusing into the pores of the matrix (*22*, *32–35*). This repulsion essentially reduces the effective pore volume available to the polymer and results in polymer elution from the column prior to that expected from a neutral polymer of the same size. This leads to an overestimation of the polymer's molecular weight. The addition of cosolutes, most often alkaline halides, has been shown to retard this exclusion effect (*22*, *35*).

In solutions containing two or more ionic solutes where one ionic species is excluded from a region of porous packing and the other species can penetrate the region, a Donnan equilibrium is established (*22*, *36–41*). This phenomenon can occur in SEC of polyelectrolytes because the size of the pore opening can exclude large polyions while allowing smaller electrolytes to completely permeate. The ionic solutes of lower permeability will force smaller ions of like charge to migrate into the pore. This ion-inclusion effect leads to further retention of low molecular weight ionic solutes. This effect can be observed with the use of on-line conductometric detection (*39*, *41*). If ion-inclusion exists, a baseline perturbation in solution conductivity is evident in the vicinity of the solvent peak. Suppression of this effect is often possible by the addition of a simple electrolyte to the eluent (*36–39*, *41*).

Other nonsize exclusion effects can be generated in SEC due to shear degradation and viscosity differences between the injected sample and the solvent. Shear degradation in SEC has recently been reviewed by Barth and Carlin (*42*) and others (*43*, *44*). High molecular weight

polymers are fairly sensitive to shear forces prevalent in SEC, which could cause mechanical chain scission. Barth and Carlin outlined the most probable sources of shear degradation in an SEC system. These sources include the injection valve, capillary tubing, column frits, and the packed column (*42*). Shear degradation is a function of polymer configuration in solution (*45*, *46*) and flow rate (*44*, *47*), as well as polymer molecular weight.

Concentration effects on the eluting chromatogram in SEC have been attributed to differences in viscosity between the eluent and the injected sample (*48–51*). As the sample concentration is increased, the elution volume of the peak increases. At even higher concentrations, the eluting peak becomes quite distorted. This effect is usually explained by two mechanisms: viscous fingering and molecular crowding (*8*). In viscous fingering, the rear boundary of the polymer sample in the column is unstable; especially if the polymer viscosity is significantly higher than the solvent. As the solvent finds the easiest pathway through the column, fingers of solute are formed. This channeling effect leads to a distortion of the elution chromatograph, most notably at the high molecular weight end of the chromatograph (larger size and viscosity) (*6*). Molecular crowding results from the compression of individual macromolecules to hydrodynamic volumes smaller than those found at infinite dilution. This reduction in size causes the elution peaks to be shifted toward higher elution volumes (*6*). Reduction in sample concentration will help to reduce both viscous fingering and molecular crowding effects. The viscosity of polyelectrolytes (and hydrodynamic volume) can be reduced by adding small amounts of electrolyte to the solvent or by increasing column temperature.

In general, aqueous SEC is more complex than SEC of neutral polymers in nonpolar solvents due to the additional phenomena enumerated above. The origins of these complexities, for the most part, arise from the polyionic nature of aqueous soluble macromolecules. Often times, these additional separation phenomena can be suppressed by an appropriate choice of system conditions. It is necessary to explore these effects in greater detail if aqueous SEC is to be routinely used. Specifically, if secondary calibration is utilized in biopolymer analysis, detailed investigation of other nonsize separation mechanisms must be studied. Alternatively, more sophisticated instrumentation which avoids these complexities and leads to direct calibration of the aqueous SEC chromatograph can be employed. The next sections detail state-of-the-art developments in this area.

DIRECT SEC CALIBRATION VIA LIGHT-SCATTERING DETECTION

The scattering of light through dilute solutions of macromolecules is a well-known phenomenon (*16*). Zimm developed the initial theoretical framework for this technique, and many reviews of this subject are available (*52*, *53*). Experimentally, the intensity of scattered light is dependent upon polymer concentration and the angle from the incident beam at which the scattered light intensity is measured. From these two independent parameters, light intensity can be mapped in a Zimm plot (see Fig. 3). These relationships were formulated mathematically by Rayleigh and are given in Eq. (3) (*52*):

$$Kc/R(\theta,c) = 1/M_w P(\theta) + 2A_2c + 3A_3c^2 + \cdots \quad (3)$$

where

$$K = (2\pi^2 n^2/\lambda^4 N)(dn/dc)^2(1 + \cos^2\theta)$$

In this equation, the weight-average molecular weight of the polymer (M_w) can be determined from the solution concentration (c), the excess Rayleigh factor for unpolarized incident radiation at the scattering angle ($R(\theta,c)$), the solution refractive index (n), the wavelength *in vacuo* (λ), Avogadro's number (N), and the second and third virial coefficients (A_2, A_3). The term $P(\theta)$ is the form factor, which is a function of the size and shape of the macromolecule in solution, and represents the modulation of light intensity due to the polymer's finite molecular size and deviation from sphericity. At the limit of the zero scattering angle, $P(\theta) = 1$. The term dn/dc is the specific refractive index increment and represents the change in solution refractive index as a function of polymer concentration. In extremely dilute solutions, terms containing c^2 can be neglected. In constructing a Zimm plot (Fig. 3), experimentally determined values of $Kc/R(\theta,c)$ are plotted vs $\sin^2\theta/2 + kc$, where k is an arbitrary constant. The resulting rectilinear grid allows extrapolation to both $c = 0$ and $\theta = 0$. The weight-average molecular weight is the inverse of the dual intercepts.

Equation (3) can be simplified if light-scattering intensity measurements are collected at low angles (approximating the zero angle extrapolation) and at dilute solution conditions (approximating the zero concentration extrapolation) to yield the LALLS working equation

$$Kc_v/R(\theta,c_v) = 1/M_v + 2A_2c_v \quad (4)$$

where the subscript v is used to denote the constant elution volume

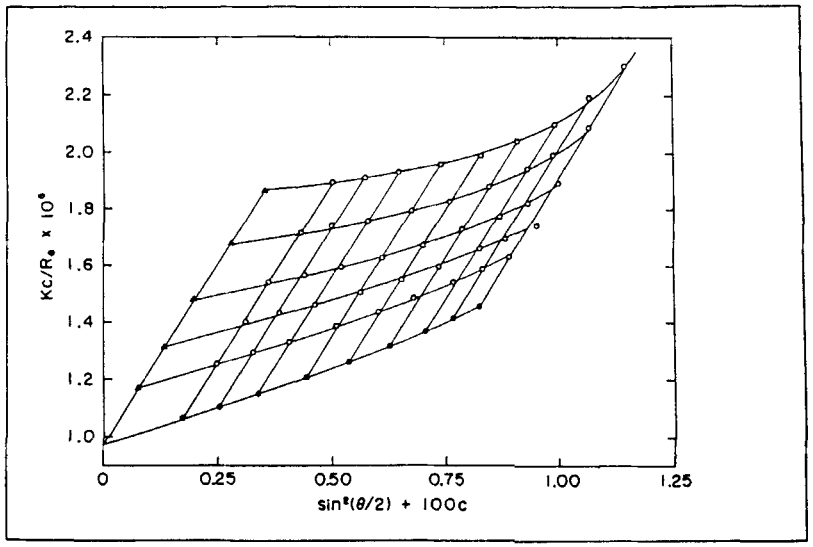


FIG. 3. Zimm plot for the light scattering of polystyrene in methyl ethyl ketone [from P. Doty and R. F. Steiner, *J. Chem. Phys., 18,* 1211 (1950)]. Filled triangles: extrapolation to $c = 0$. Filled circles: extrapolation to $\theta = 0$. Courtesy of the American Institute of Physics.

comparison. M_v represents the weight-average molecular weight of a sample within the LALLS detector cell. Thus, the molecular weight of a polymer in solution can be determined using Eq. (4) and the material's second virial coefficient.

APPLICATIONS OF SEC/LALLS IN POLYSACCHARIDE CHARACTERIZATION

Recent advances in commercial light-scattering instrumentation now provide for direct coupling to SEC. Reviews of the overall SEC/LALLS technique have been presented by Kaye (*54*), Ouano (*55*), Jordan (*56*), and Hamielic and Meyer (*4*). The enormous volume of data collected from such devices has necessitated the use of data acquisition and processing machines. Information obtained from these state-of-the-art systems provides molecular weight and molecular weight distributions of eluting polymers. Determination of polysaccharide molecular weight information via SEC/LALLS has been carried out for cellulosic materials (*57, 58*), lignins (*59*), dextran (*60*), and others (*61, 62*). The reader is referred to

these publications for a more complete discussion. Although these two fundamental molecular parameters are of primary importance, additional molecular information is desired in many systems. More recently, Yu and Rollings (*62*, *63*) (for polysaccharides) and others (*64–67*) (for polymers in organic solvents) have extended basic light-scattering theory to obtain some of this much needed information. Specifically, they have demonstrated that polymer branching as a function of column separation is obtainable with the same commercial instrumentation.

Yu and Rollings (*62*) employed SEC/LALLS to obtain data about the branching parameters, g_v and g_M, for samples of homopolymeric polysaccharides of increasing degrees of branching (amylose, amylopectin, and glycogen) and for starch (a mixture of amylose and amylopectin). The branching parameter, g_v, is obtained from molecular weight (M) comparisons of linear and branched molecules at a constant molecular size (or elution volume, v), and is defined as

$$g_v = (M_l/M_b)_v \tag{5}$$

where the subscripts l and b denote linear and branched polymers. The branching parameter, g_M, was defined by Zimm and Stockmeyer (*68*) as the ratio of the mean-square radius of gyration $\langle R^2 \rangle$ of branched and linear polymers of the same molecular weight. Yu and Rollings (*62*) showed that g_M can be obtained from the SEC/LALLS branching parameter g_v as follows:

$$g_M = (M_l/M_b)_v^{(a+1)/e} = g_v^{(a+1)/e} \tag{6}$$

where a is the Mark-Houwink coefficient and e is a polymer draining parameter which can vary between 0.5 and 1.5 depending upon the particular theoretical assumptions used in developing the model. Their branching results are shown in Fig. 4. For the three homopolymers, branching frequency (as measured by chemical means) and branching parameters are inversely related, as theoretically predicted. For starch (Amylomaize VII), a nonhomogeneous branching distribution is observed as a function of molecular weight. This observation has led to a greater understanding of starch physical and chemical properties and opened new areas of study in biopolymer kinetics.

Theoretical advancements of SEC/LALLS determined branching parameters continue. Yu and Rollings (*63*) theoretically related two measurable branching parameters $g_{v(m)} = (M_l/(M_N)_m)_v$ and $g'_{v(m)} = (M_l/(M_M)_m)_v$ of a sample mixture to the mixture's composition. Here, the

subscripts M and N denote weight-average and number-average molecular weights, respectively, and m denotes that measurements are performed on mixed polymer samples. They have determined that there exist linear relationships between $g_{v(m)}$ and $W_{b,v}$ (the mass fraction of branched component in the mixture) as well as between $g'^{-1}_{v(m)}$ and $W_{b,v}$. The latter correlation was demonstrated experimentally using SEC/LALLS and displayed excellent agreement with theoretical predictions. Representative linear plots of $g'^{-1}_{v(m)}$ vs $W_{b,v}$ for amylose/glycogen mixtures at different elution volumes are shown in Fig. 5.

Armed with the ability to efficiently determine molecular weight distribution and branching characteristics for polymer mixtures, Yu and Rollings (*63*) employed SEC/LALLS in the examination of industrially important biopolymers and, in particular, to the study of enzymatic starch hydrolysates products. Starch samples were extracted from the reaction mixture and analyzed via SEC/LALLS. As an example, the observed molecular weight distribution and branching distribution are shown in Fig. 6; (a) before hydrolysis and (b) after 2 min hydrolysis time. Here, the area under the main solid-line chromatographic trace represents the total mass of material eluted from the SEC column as a function of either elution volume or molecular weight (based on amylose hydrodynamic volume). The total branched polysaccharide material (expressed as amylopectin; narrow dashed line chromatographic region) and the mass fraction of branched material at each elution volume ($W_{b,v}$, right-hand-side axis) are also displayed. From this type of analysis, Yu and Rollings determined that starch branched components are preferentially hydrolyzed by the particular enzyme chosen (endo-acting linear depolymerase α-amylase). Combined SEC/LALLS analysis of mixed polymer systems (e.g., starch) now provides many parametric values never before obtainable. The resulting "snapshot" of this or any similar processing operation provides insight into the molecular kinetic events which occur.

CONCLUSIONS

Aqueous size exclusion chromatography coupled with on-line low-angle laser light-scattering detection is a valuable tool for characterization of polysaccharides and other polyelectrolytes. The principle separation mechanism in SEC is based on the solution size of the macromolecule. The ionic strength of the solvent influences the molecular conformation of the polyion in solution and, therefore, the elution behavior

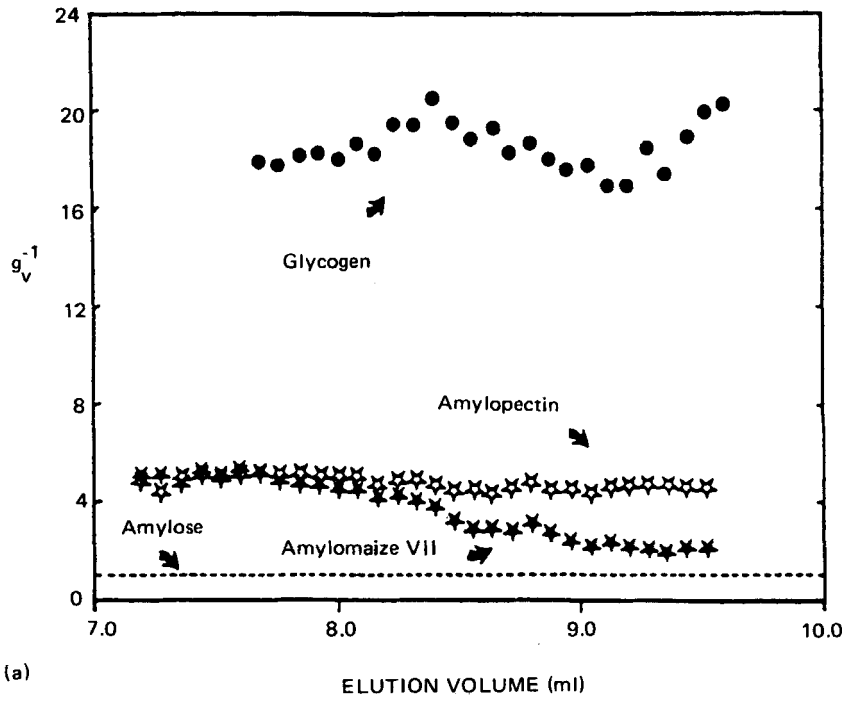
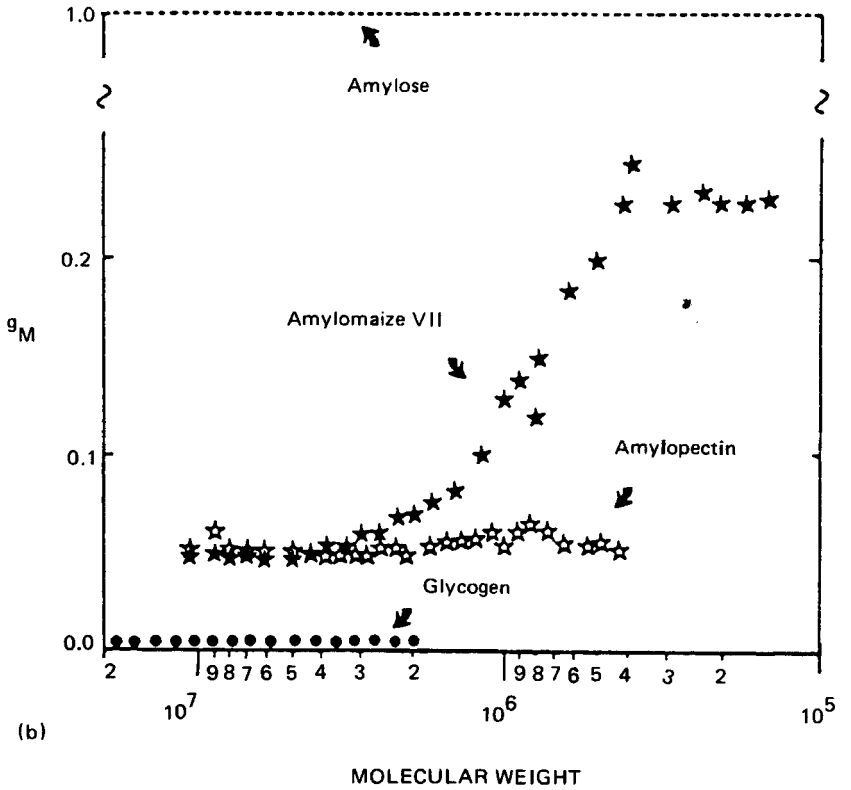


FIG. 4. Branching distribution of polysaccharides: (a) the plot of g_v^{-1} vs elution volume and (b) the plot of g_M vs molecular weight for three branched polysaccharides as compared to amylose (linear) (*62*). Courtesy of John Wiley & Sons.

in SEC. The use of polar solvents or ionic solutions in aqueous SEC requires consideration of possible polymer–support interactions such as adsorption, ion exclusion, and ion inclusion. These secondary separation phenomena can usually be suppressed by controlling solvent ionic strength. Flow-related anomalies, such as shear degradation, and viscosity differences between solvent and polymer sample may lead to other nonsize separation effects. Since primary calibration standards for most polysaccharides of interest are not available, secondary calibration techniques have been employed. These methods have met with limited success due to the inability to account for complex ionic effects present in aqueous SEC. The use of on-line light scattering as an absolute molecular weight detector has revolutionized calibration in SEC.

SEC/LALLS has been applied in determining molecular weight



information about polysaccharides. Quanitative branching measurements of both polysaccharide homopolymers and mixtures can be obtained via SEC/LALLS. The combined knowledge of molecular weight and branching distributions allows insight into the molecular kinetic events of polysaccharide processing operations. Further development of the SEC/LALLS technique, including the use of additional on- and off-line detectors, may provide the key to greater understanding of polysaccharide physical and chemical properties.

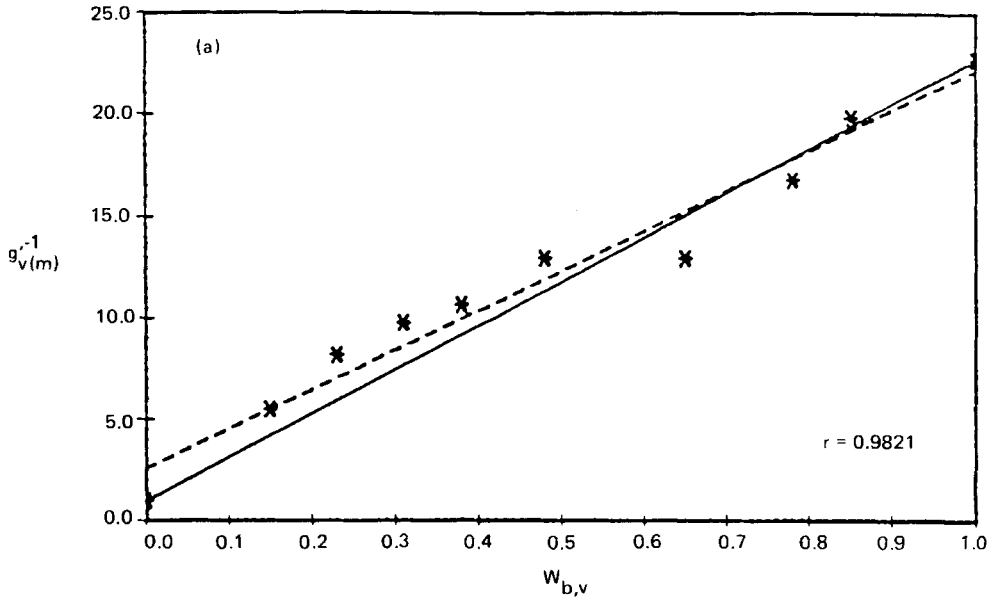
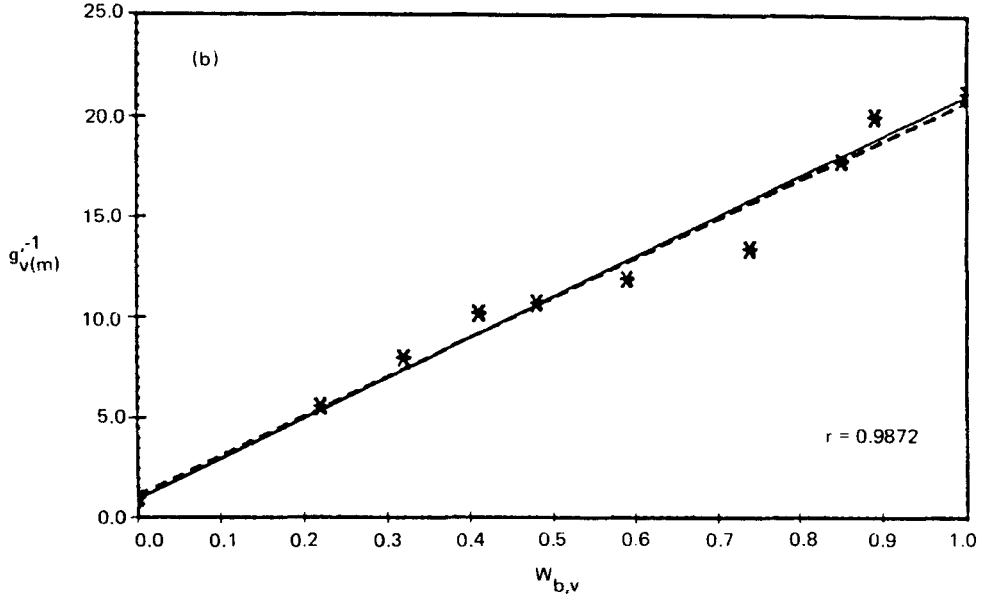
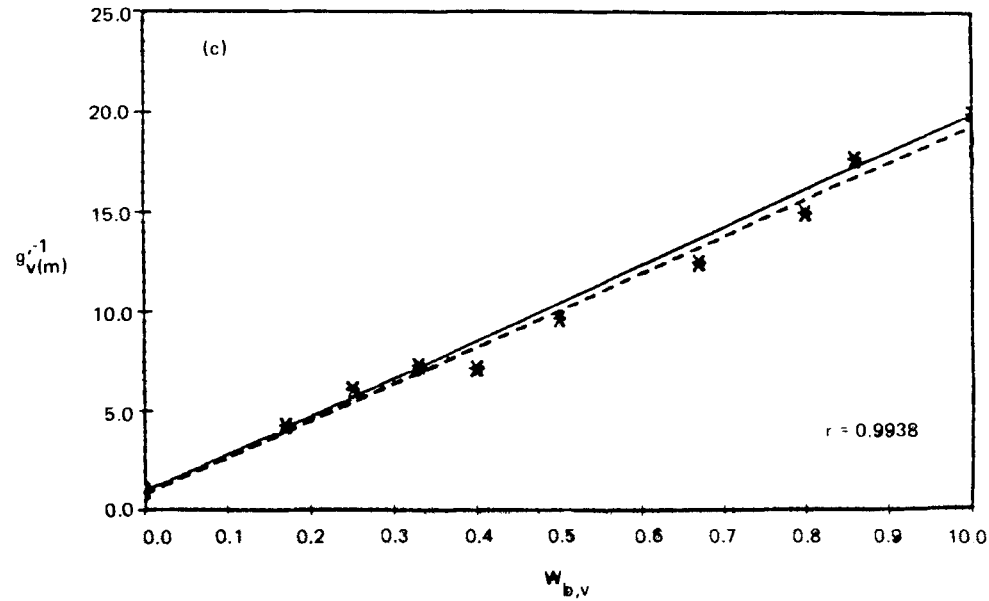


FIG. 5. The plot of $g'_v - 1$ vs weight fraction of branched material ($W_{b,v}$) for amylose/glycogen mixtures at three elution volumes (EV); (a) EV = 8.5 mL, (b) EV = 9.0 mL, (c) EV = 9.5 mL (*63*). Courtesy of John Wiley & Sons.





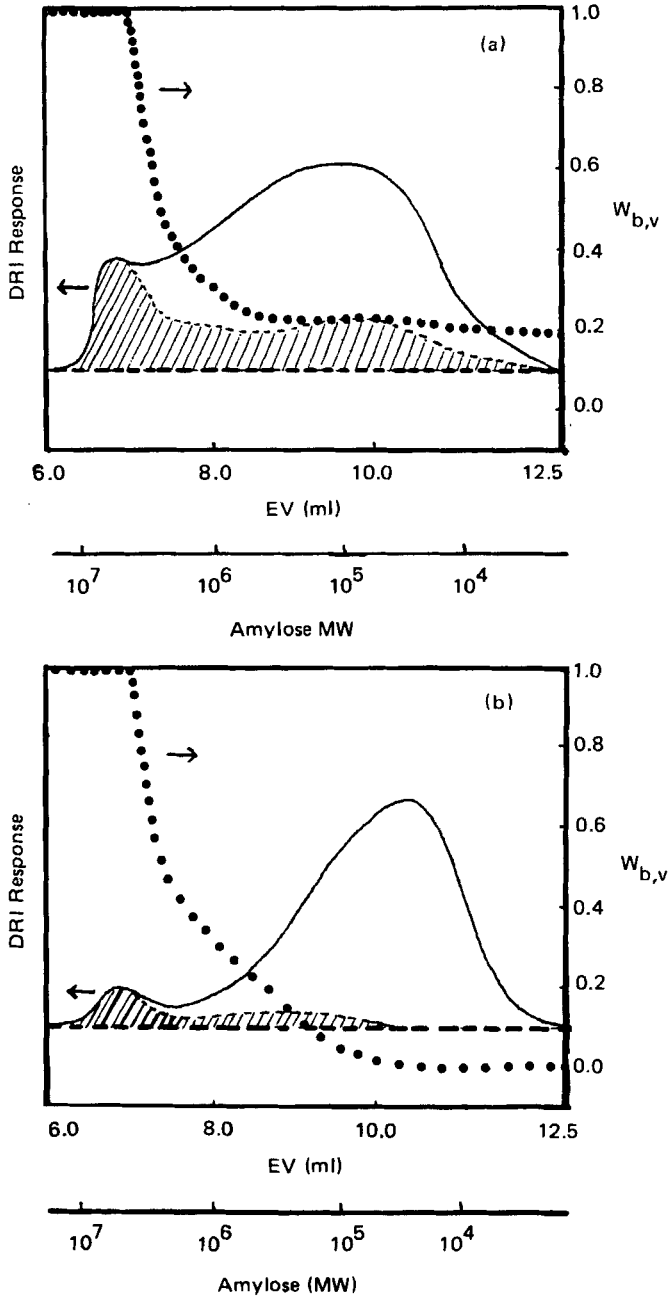


FIG. 6. Molecular weight distribution and branching distribution (dashed region) of Amylomaize VII starch: (a) before enzymatic hydrolysis and (b) after 2 min hydrolysis time (*63*). Courtesy of John Wiley & Sons.

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