

Light-Scattering Molecular Weights and Intrinsic Viscosities of Processed Waxy Maize Starches in 90% Dimethyl Sulfoxide and H₂O

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ABSTRACT

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Waxy maize starch was treated by a variety of gentle and severe methods: direct dispersion-solubilization into 90% dimethyl sulfoxide (DMSO) and H₂O solvent, extrusion followed by dispersion-solubilization of the ground exudate into solvent, or jet-cooking or stirred autoclaving of an aqueous starch slurry followed by transfer into solvent. Intrinsic viscosities $[\eta]$ and multiangle light-scattering measurements were made in 90% DMSO-H₂O. A Mark-Houwink relation, $[\eta] = (0.28-1.2) M_w^{0.29 \pm 0.04}$, was obtained over a molecular weight range of $\approx 30-700$

million. However, there was a large amount of scatter in the data when $[\eta]$ were >140 mL/g. The power law relationship $R_g \propto M_w^{0.41 \pm 0.04}$ was noted between radii of gyration and molecular weights. We infer from our data that over the entire range of M_w distributions, the amylopectin existed in solution as relatively compact molecules or aggregates and that in the higher molecular weight region, the size and possibly the shape of the “dissolved” amylopectin was highly sensitive to the method of dispersal and treatment.

The purposes of this study were to: 1) characterize the solution state of amylopectin from processed waxy maize starches by obtaining relationships between weight-average molecular weights (M_w), intrinsic viscosities ($[\eta]$), and radii of gyration (R_g) in the solvent 90% dimethyl sulfoxide (DMSO) and H₂O (w/w); and 2) consider effects of processing methods on the molecular parameters of waxy maize amylopectin. A commercial multiangle laser light-scattering detector (MALLS) was used in a batch mode to examine these very high molecular weight biopolymers. Amylopectins are the high molecular weight, highly branched polysaccharide components of starches. The sensitivity of amylopectins to high or moderate shear degradation has been deduced from $[\eta]$ and light-scattering measurements (Whitnauer et al 1955, Banks et al 1972). In this study, we showed that dispersal techniques involving low shear, time, and temperature also strongly influence the solution state of the dispersed amylopectin.

In a study of the effects of thermomechanical processing on the viscosity behavior of corn starches (Dintzis and Bagley 1995), a lack of a unique correlation was noted between the intrinsic viscosity ($[\eta]$) and the shear viscosity (η) of the dispersed starch. In addition, there was greater variation in values of these parameters when waxy maize starch was prepared by gentle procedures rather than by severe treatments. These results, which we interpreted as evidence for nonuniform degradation and dissociation of the amylopectin when waxy maize starch granules were gently dispersed and solubilized, led to the present study.

The M_w for amylopectins obtained by light-scattering before 1975 had a range of $\approx (10-450) \times 10^6$. There was concern in that era, that is valid today, that “microgel” particles might make significant contributions to high molecular weight values (Sitaramaiah and Goring 1962, Banks and Greenwood 1975). Recent reports of amylopectin molecular weights determined by MALLS instruments (Aberle et al 1994; Hanselmann et al 1995,

1996) verify such high M_w values. Using sedimentation field flow fractionation combined with MALLS, Hanselmann et al (1995) reported apparent average molecular weights for waxy maize amylopectin molecules or aggregates that, depending on sample heating time at 175°C in an autoclave, had a range in water of $\approx 37-360 \times 10^6$ and a corresponding range of R_g of 120–320 nm. This article will provide evidence from MALLS and $[\eta]$ data that there exists a region of polysaccharide breakdown in which initial disruption of the waxy maize starch granule yields species of molecules or aggregates that vary significantly in molecular weight, R_g , and $[\eta]$.

MATERIALS AND METHODS

Starches

Two industrial waxy maize starches were used in this study: Staley 7300, Waxy No. 1 Starch was donated by A.E. Staley (Decatur, IL); Amioca starch was purchased from American Maize-Products Co. (Hammond IN). These starches were $\approx 98\%$ amylopectin, contained $\approx 11\%$ moisture, and were used as is.

Starch Dispersal

The starches were dispersed-solubilized by a variety of methods intended to include conditions from very gentle to severe treatments. The solvent was 90% DMSO-H₂O (w/w) containing 0.2% NaN₃ prepared from 99.9 % spectroscopic grade DMSO, and NaN₃ (Sigma Chemical Co., St. Louis MO) and distilled water. Some samples were directly dispersed into the solvent. A gentle dispersal-solubilization method was used to prepare 50 mL of a 3.0–3.5 wt% starch sample in solvent in a 125-mL glass-stoppered Erlenmeyer flask by stirring with a Teflon-coated stirring bar at 100 rpm for 1–2 hr at room temperature. One starch sample was cooked at pH 3.8 as an aqueous slurry of 10 wt% starch in a stirred autoclave. Other samples were cooked as 10 wt% starch slurries in a steam jet-cooker at steam pressure settings of 70/65 (gentle jet-cook), 70/40 (standard jet-cook), or 120/40 (severe jet-cook), where the ratio is defined as inlet steam pressure/steam pressure in the Venturi chamber of the cooker (Dintzis and Fanta 1996). The cooked aqueous samples were then transferred into DMSO to form a solution of 1.0 wt% starch in 90% DMSO-H₂O containing 0.2% NaN₃. Another treatment was to extrude starch through a cylindrical 2.5-mm die by use of a 19-mm diameter single-screw extruder (C.W. Brabender) at 120°C with 23% moisture content. The extrudate then was cryogenically ground in a high-speed mill (Fritsch) to pass through a 20-mesh screen. This

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ground extrudate was then added to 90% DMSO solvent and dispersed by gentle swirling or moderate heating and stirring until a clear 0.2–0.3 wt% solution was obtained. Starch concentrations in the 90% DMSO solvent were determined by optical rotation using a specific rotation of 190 at 589 nm (Dintzis and Tobin 1969). A few gravimetric determinations of processed starch lyophilized from aqueous solutions or precipitated as the ethanol-starch complex from DMSO solvent verified that at least 98% of the starting material was recovered.

Intrinsic Viscosity

Flow times in solvent were obtained at $25 \pm 0.1^\circ\text{C}$ in a shear dilution viscometer (CUSDC-11 series, size 150, Cannon-Ubbelohde) at the lowest available shear rates ($\approx 60/\text{sec}$). Intrinsic viscosities were obtained by the usual method of plotting reduced viscosity versus concentration. Data were extrapolated to zero concentration by linear regression of concentrations (C) generally: $C < 25 \times 10^{-4}$ g/mL. Values of $[\eta]$ generally were reproducible within a range of 5%.

Plasma Cleaning of Light-Scattering Glassware

All flasks and scintillation vials used for light-scattering measurements were cleaned by exposure to a low temperature oxygen plasma for 30 min immediately before use. The plasma generating instrument (Plasmod, March Instruments, Concord, CA) has been described elsewhere (Millard and Bartholomew 1977). The oxygen plasma oxidizes carbonaceous material and produces an optical glass surface of exceptional optical clarity (Koide et al 1989).

Light-Scattering Measurements

All scattering measurements were done in 90% DMSO-H₂O (w/w). A specific refractive index increment (dn/dc) of 0.074 mL/g was used in this study and verified by measurements with a Chromatix KMX-16 laser differential refractometer at 25°C with a 632.8 nm light source (Chromatix Inc., Sunnyvale, CA). Light-scattering measurements were performed at room temperature with a DAWN F multiangle light-scattering detector in the batch mode using a He-Ne laser light source operating at 632.8 nm

(Wyatt Technology, Santa Barbara, CA). Toluene was used as the calibration standard at the 90° scattering angle. Light intensity at the other angles was calibrated using the isotropic “magic glass” cylinder supplied by the manufacturer. Triplicate light-scattering measurements were made on a few samples to obtain estimates of experimental error. Some samples were prepared in replicate to test the reproducibility of solubilizing the starch under conditions of gentle treatment. Data were collected and processed by use of the Wyatt version 3.00 software for Windows. All M_w and R_g values presented here were calculated from Berry plots of the scattered light. The software calculations were done in a manner to minimize the error in M_w . It was necessary to specify the polynomial degree for the best least-squares fit of the extrapolation to zero scattering angle and zero concentration. Calculated values with the least error generally were achieved using zero or first degree polynomials for concentration and second or third order polynomials for the angular dependence. Typical examples of Berry plots obtained from amylopectin in the higher molecular weight and lower molecular weight ranges are presented in Fig. 1A and B.

Starch samples were initially diluted to ≈ 0.1 – 0.25% and filtered through either 5- or 1- μm polyester screen membrane filters (Poretics Corp., Livermore, CA). The filtered stock solution was then transferred to the plasma-cleaned vials and diluted by weight with solvent previously filtered through a 0.1- μm polyester filter.

RESULTS

All error values presented are standard deviations (SD). The error bars (+) in the figures are the errors calculated by the light-scattering software for a single measurement. The arrows designate experimental error obtained from three measurements on a processed sample.

At the highest molecular weights, the errors in our measurements were of the order: $M_w > R_g > [\eta]$. Our measurements on the processed starches revealed no significant differences between the two batches of industrial waxy maize starches used in this study.

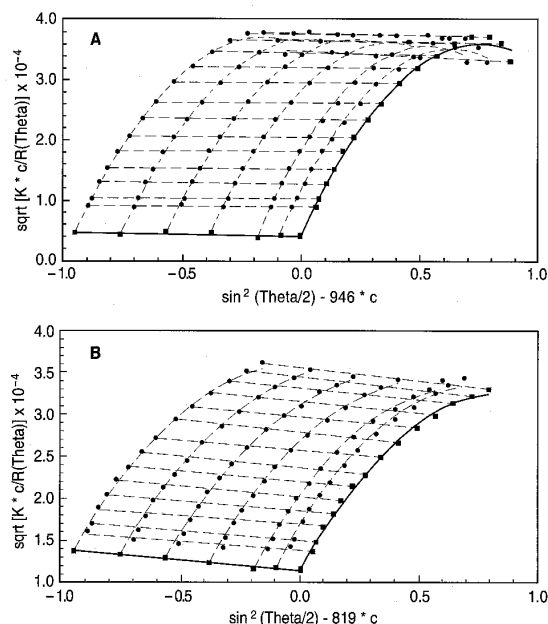


Fig. 1. Berry plots of two processed amylopectin samples in 90% dimethyl sulfoxide (DMSO) and H₂O. Weight-average molecular weights (M_w), radii of gyration (R_g), and intrinsic viscosities ($[\eta]$). **A**, Cooked in water in stirred autoclave at 130°C for 45 min at pH 7.0. $M_w = 586 \times 10^6$, $R_g = 376$ nm, $[\eta] = 172$ mL/g. **B**, Cooked in water in stirred autoclave at 130°C for 10 min at pH 3.8. $M_w = 65 \times 10^6$, $R_g = 148$ nm, $[\eta] = 95$ mL/g.

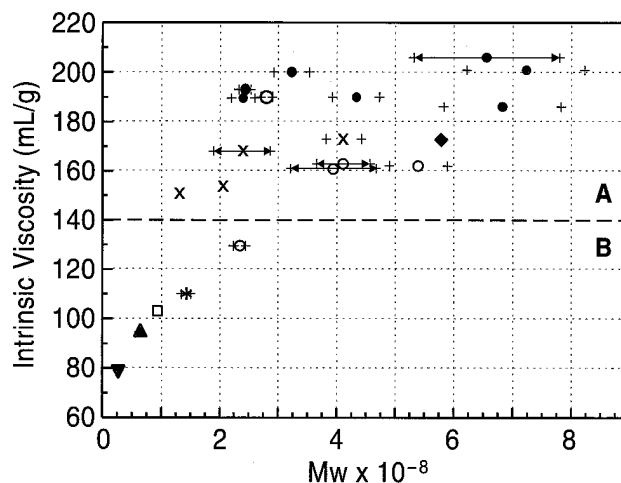


Fig. 2. Intrinsic viscosity vs. molecular weight behavior of processed waxy maize starch. ● = Sample gently stirred in 90% dimethyl sulfoxide (DMSO) and H₂O at room temperature for various times. →← = Sample stirred in 90% DMSO-H₂O on hot plate at 160°C for 12 hr. ○ = Aqueous sample jet-cooked at 70/65 steam conditions. □ = Aqueous sample jet-cooked under 70/40 steam conditions. ▼ = Aqueous sample jet-cooked under 120/40 steam conditions. × = Sample extruded and dispersed with gentle stirring in 90% DMSO-H₂O. ▲ = Aqueous sample cooked in stirred autoclave at 130°C for 10 min at pH 3.8. ◆ = Aqueous sample cooked in stirred autoclave at 130°C for 45 min at pH 7.0. In region A $[\eta] > 140$ mL/g. Error bars (+) are standard deviation calculated by the light-scattering software for a single measurement. Arrows designate experimental error obtained from three measurements on a processed sample.

The relationship between M_w and $[\eta]$ of our processed waxy maize starch samples is shown in Fig. 2. In this figure, region A (above the dashed line) corresponds to those starch fluids that had $[\eta] > 140$ mL/g. Region B represents starch fluids that exhibited lower $[\eta]$. The methods of processing the samples are indicated by different symbols in the plot.

When the M_w and $[\eta]$ data were plotted on a log-log scale as in Fig. 3 and fitted to a straight line by regression, a Mark-Houwink relation was obtained:

$$[\eta] = (0.59) \times M_w^{0.29 \pm 0.04} \quad (1)$$

The standard deviation of ± 0.040 in the exponent was calculated from an analysis of variance (ANOVA) of the regression line. This analysis also provided an intercept value of -0.229 ± 0.319 , which means the coefficient (0.59) has an estimated range of $+0.28$ to 1.2 .

Since both R_g and $[\eta]$ of polymers are considered to be proportional to the volumes occupied by the molecules in solution, we have compared the two parameters in Fig. 4. The linear regression line fitted to the data has a slope of 1.8 ± 0.3 , thus indicating there is not a one-to-one correspondence between the parameters. The intercept -21.9 has a large error of ± 49.6 .

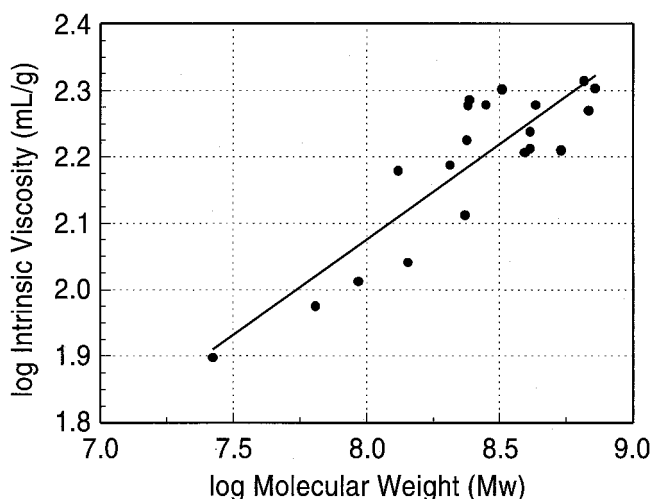


Fig. 3. Data in Fig. 2 plotted on log-log scale. Regression fit shown by solid line.

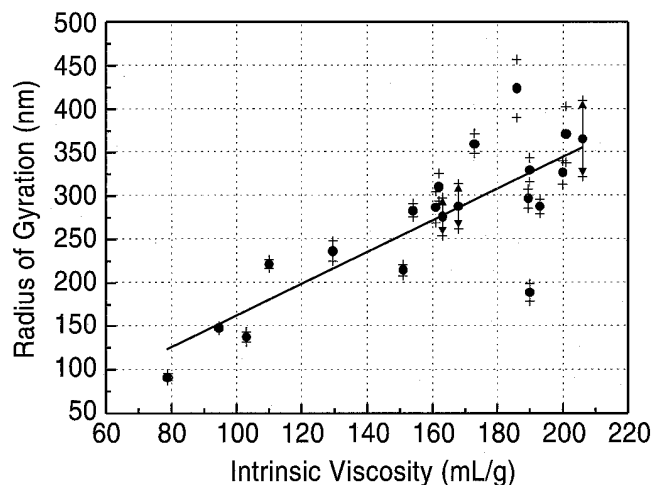


Fig. 4. Radius of gyration vs. intrinsic viscosity behavior of processed waxy maize starch. Error bars (+) are standard deviation calculated by the light-scattering software for a single measurement. Arrows designate experimental error obtained from three measurements on a processed sample.

When the data for R_g vs. M_w are plotted on a log-log scale and fitted by regression (Fig. 5), we obtain the relationship:

$$R_g = 7.3 \times 10^{-2} M_w^{0.422 \pm 0.04} \quad (2)$$

ANOVA provided an intercept value of -1.14 ± 0.34 , which means the coefficient of 7.3×10^{-2} has an estimated range of $(0.34-1.6) \times 10^{-1}$.

DISCUSSION

The viscosity values were the most reproducible of the data presented in this work. Stock samples in 90% DMSO-H₂O stored frozen, or with 0.2% NaN₃ added for microbial protection at room temperature storage, were stable with respect to $[\eta]$ over long periods (greater than six months). The stability of starches stored in 90% DMSO-H₂O was also observed by Bradbury and Bello (1993) and the high solubility of starches in this solvent has been examined by Jackson (1991). The advantage of using plasma-cleaned glassware was evident by inspection of the plots of scattered light intensity which had noticeably less fluctuation when compared to plots obtained from samples exposed to glassware that was not plasma-cleaned.

The high M_w values we report are compatible with values reported recently for waxy maize starch (Aberle et al 1994, Hanselmann et al 1995). The issue of large errors associated with light-scattering determinations of high molecular weights has been discussed by Hanselmann et al (1995), who employed sedimentation field flow fractionation to separate distributions of cooked starch and commented that "absolute molecular weight values of particles larger than 10^8 D are determined with an error greater than 20%," and that for M_w values of 10^9 D, the error could be $>100\%$. For samples of $1 \times 10^8 < M_w < 8 \times 10^8$, our software indicated sample (random) standard deviations of $\approx 4-17\%$ for a given measurement. Standard deviations of $\approx 3-9\%$ were obtained for R_g values. We found, as did Aberle et al (1994) and Hanselmann et al (1995), that for very high molecular weights ($250 \times 10^6 < M_w < 750 \times 10^6$), Zimm plots could yield negative numbers or physically unreasonable results, and that Berry plots would lead to apparently reasonable M_w and R_g . Therefore, all of our light-scattering results are from Berry plots.

In region A of Fig. 2, we observed a M_w range of $250-750 \times 10^6$ while the $[\eta]$ varied between 190 and 205 mL/g. This is presumably due to the fact that, because of internal branching, the volumes occupied by the molecules no longer increased in direct proportion to M_w . The larger errors in M_w in this region are also pre-

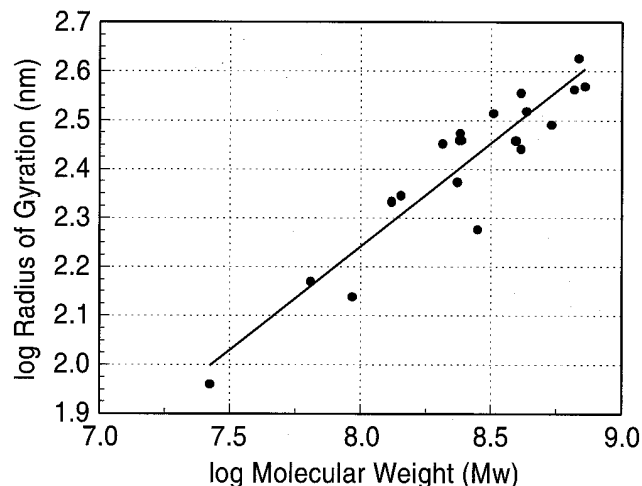


Fig. 5. Radius of gyration vs. molecular weight of processed waxy maize starch plotted on log-log scale. Regression fit shown by solid line.

sumably due to the insensitivity of the extrapolation of the data by the Berry plot of those high molecular weights. In addition, we speculate that a significant amount of scatter, particularly in region A, is caused by the confounding influences of different dispersion methods, which in combination with a possible inherent nonuniform initial disruption of granule contents, results in high molecular weight species of different hydrodynamic characteristics and M_w values. The fact that it was difficult to replicate pairs of M_w and $[\eta]$ values for these gently dispersed samples supports this speculation.

The five jet-cooked samples with $M_w > 125 \times 10^6$ were prepared by the same gentle jet-cooking conditions (70/65 steam conditions) and transferred into DMSO (Dintzis and Fanta 1996). High molecular weight samples ($M_w > 225 \times 10^6$) prepared by gentle dispersion into 90% DMSO-H₂O, scatter significantly to a degree that also may not be explained fully by the uncertainties in determinations of M_w or $[\eta]$. We interpret these data as evidence that the initial gentle dispersion of the waxy maize starch granule into a solvated state produces species that have size and shape characteristics dependent to a significant degree on the dispersal method and subsequent treatment. This postulated dependence on these two factors could partially explain the lower M_w values (53×10^6) recently obtained for waxy maize starch in 10% (v/v) DMSO-H₂O by Bello-Perez et al (1996) and by Fishman et al (1996), who obtained M_w values of 26×10^6 for partially recovered ($63 \pm 12\%$) waxy maize starch cooked in water by microwave digestion and examined in water by size-exclusion chromatography.

If one were to plot in Fig. 3 only the data for which $\log [\eta] < 2.2$ ($27 \times 10^6 < M_w < 142 \times 10^6$), the scatter would be much less and $[\eta]$ would be proportional to $M_w^{0.22}$. If one were to plot only the data for which $\log M_w < 8.55$, $[\eta]$ would be proportional to $M_w^{0.39}$. However, we have no basis on which to omit any of the data. The influence of a small amount of very high molecular weight particles to skew a distribution to high values is well known, and we consider it likely that such an effect, possibly caused by incompletely solubilized particles or aggregates, is a major factor in the scatter of our highest molecular weight values. When flasks containing the 3.0–3.5% stock starch samples dispersed in 90% DMSO-H₂O at room temperature by gentle stirring were tilted and rotated so that a film of starch fluid flowed to the bottom of the flask, refractive index inhomogeneities (fish eyes) were observed in the fluid. Samples stirred for longer times showed fewer inhomogeneities. The filled circles of region A in Fig. 2 represent data obtained from these gently stirred samples after they had been diluted further and filtered for light scattering and viscosity measurements. Consequently, some of the data in region A represent greatly diluted samples that still may contain undefined species, perhaps small particles of gel or aggregates that easily deform and are able to pass through a 1- μ m filter. These observations are compatible with Jackson's (1991) comments that waxy starches could only be fully solubilized by exposure to shear.

The concentration dependence of the viscosity of our processed waxy maize starch samples may be compared with other polymer systems by use of the Huggins relationship:

$$\eta_{sp}/C = [\eta](1 + k_H[\eta]C) \quad (3)$$

where η_{sp} is the specific viscosity and k_H is the Huggins constant (Stickler and Sütterlin 1989). We find that $k_H = 1.0 \pm 0.31$ with a 95% confidence interval of 0.84–1.23 for a sample size of $n = 16$. There was no correlation between values of the Huggins constant and molecular weights. Values of $k_H \geq 1$ are considered to represent aggregates, while k_H values of 0.3–0.7 are considered to represent perfect solution, thus we consider our Huggins constants to be evidence of compact molecular entities in solution over the entire molecular weight range examined.

The low value of 0.29 for the exponent in the Mark-Houwink relation (Eq. 1) also is indicative of a compact hydrodynamic shape in solution. Hence, gently processed waxy maize amylopectin can maintain a compact shape in 90% DMSO-H₂O to M_w of the order of 7×10^8 . This evidence of a compact shape is consistent with diffusion and sedimentation measurements on wheat amylopectin in DMSO that indicated the hydrodynamic configuration was that of a disk-like, oblate ellipsoid (Callaghan and Lelievre 1985, Lelievre et al 1986). Intrinsic viscosity measurements (in 0.2N KOH, 25°C) on the amylopectin fraction of wheat starches that had been cooked by extrusion and drum dried (Colonna et al 1984) led to the relationship:

$$[\eta] = 0.414 M_w^{0.306} \quad (4)$$

These workers commented that the $[\eta]$ of all of their modified starches were reduced compared to the native starch. The low value of the exponent (0.306) has been attributed to the branched structure of amylopectin (Quivoron 1972).

Our value of 0.29 for the exponent in the Mark-Houwink relation (Eq. 1), while in agreement with the value of 0.306 obtained by Colonna et al (1984), is different from values reported by Salemis and Rinaudo (1984) from their investigations using fractions of acid-hydrolyzed waxy maize starch in pure DMSO. They reported that for values of M_w from ≈ 6.5 to 20 million $[\eta] \propto M_w^{0.67}$; at lower molecular weights, from ≈ 0.51 to 6.5 million, their exponent value was 0.36.

Our experimental results have yielded Eq. 1, which may be transformed to the relationship $[\eta]^{3.4} \sim M_w$. If this is so, one may ask (as did a referee) why the intrinsic viscosity did not vary more extensively as M_w changed. A partial answer to this aspect of Eq. 1 is to note that it was obtained from a regression in which $\log M_w$ was the independent variable, that is, one presumed the M_w values were correct. If one does the regression such that $\log [\eta]$ is the independent variable, one obtains the relationship: $M_w \sim [\eta]^{2.65}$. Thus, perhaps the scatter in our data combined with the properties of the regression technique contribute to the low value of the exponent in Eq. 1.

Figure 4 is presented to show another aspect of the scatter in the data and to demonstrate that the data can be reasonably fitted by a straight line.

Our exponent value of 0.42 ± 0.04 for the processed amylopectin samples in 90% DMSO-H₂O (Fig. 5, Eq. 2) may be compared to a value of 0.41 ± 0.01 obtained in water at room temperature for waxy corn starch dispersed or dissolved by heating in an autoclave to 175°C for 20, 40, and 60 min (Hanselmann et al 1995). From their exponent value of 0.41, Hanselmann et al (1996) conclude, with the use of fractal theory, that over their entire M_w range of $(36.5\text{--}360) \times 10^6$, the waxy maize starch exists as self-similar structures with internal structure between "a hard sphere and a fully swollen randomly branched macromolecule in a thermodynamically good solvent."

In a theoretical study using cascade branching theory, Thurn and Buchard (1985) calculated the angular dependence of scattered light, R_g , M_w , and number-average molecular weight (M_n) values for amylopectin and its β -limit dextrin. These calculations led to the conclusions that amylopectin is heterogeneously branched with densely branched clusters connected by longer chains that are preferentially B-chains of 22 units in length and carry on the average 1.4 clusters. Determination of wheat amylopectin diffusion coefficients by pulsed-field nuclear magnetic resonance (Callaghan and Lelievre 1985) and of sedimentation coefficients (Lelievre et al 1986) has provided important hydrodynamic characteristics of this material. The benefits of combining static with dynamic light-scattering experiments to obtain both a root mean square average R_g and a hydrodynamic radius (R_n) for more complete structure determinations have been discussed by Burchard (1992). Further studies, including dynamic light-scattering

tering and analytical ultracentrifugation are needed to better characterize the hydrodynamic properties of gently processed waxy maize starch. Thus, while major experimental and theoretical efforts have partly elucidated the molecular structures and properties of amylopectin in solution, there is only limited understanding of how amylopectin in the solid state may be altered when a waxy maize starch granule is processed by various methods to initially form amylopectin in dilute solution.

CONCLUSIONS

Light-scattering measurements indicate that very gently dispersed waxy maize starch can have a broad range of molecular weights in 90% DMSO-H₂O of $(30-70) \times 10^7$. Intrinsic viscosity and light-scattering measurements show that suitably degraded waxy maize amylopectin maintains a relatively compact configuration in this solvent over a molecular weight range of $\approx(3-70) \times 10^7$. Although our light-scattering measurements at the higher molecular weight ranges are subject to significant uncertainty, we conclude, particularly from the scatter of data of samples gently dispersed or dissolved in 90% DMSO-H₂O, that the size and possibly the shape of waxy maize starch in solution is dependent on the method of dispersal. The data at the higher molecular weights could represent aggregates of incompletely solubilized amylopectin.

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