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The hydrodynamic characterization of waxy maize amylopectin in 90% dimethyl sulfoxide–water by analytical ultracentrifugation, dynamic, and static light scattering

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Abstract

Static light scattering of high amylopectin waxy maize starch gently dispersed in 90% dimethyl sulfoxide–water yielded a weight average molecular weight M_w and radius of gyration R_g of 560×10^6 g/mol and 342 nm, respectively. To obtain an independent hydrodynamic characterization of these solutions, we measured the sedimentation coefficient for the main component in an analytical ultracentrifuge. The value of s^0, the infinite dilution sedimentation coefficient, was 199 S. The translational diffusion coefficient D^0 in very dilute solutions was measured by dynamic light scattering at 90° and found to be 2.33×10^{-9} cm^2/s. An effective hydrodynamic radius R_h was calculated from this diffusion constant using the Stokes–Einstein equation and found to be 348 nm. The structure-related parameter $\rho = R_g/R_h$ was calculated to be 0.98. The weight average molecular weight calculated from the Svedberg equation using the values measured for s^0 and D^0 was 593×10^6 g/mol. This result is in reasonable agreement with the light scattering results. As light scattering results are subject to experimental errors due to the possibility of dust contamination, the presence of microgel or aggregates, and the questionable applicability of light scattering theory to interpret results for macromolecular sizes approaching the wave length of light used as a source for scattering, it is advisable to have corroborating hydrodynamic data when possible to further validate light scattering results in this very high molecular weight range.

Keywords: Amylopectin waxy maize; Hydrodynamic characterization; Static light scattering

1. Introduction

Amylopectin is the branched high molecular weight component of starch. The size and conformation of this macromolecule in solution have been of considerable interest. The hydrodynamic properties of amylopectin in solution, including size and shape, are dependent on the conditions used to disperse the starch granule. Early physical chemical studies on amylopectin isolated from various sources (Banks, Geddes, Greenwood & Jones, 1972) reported weight average molecular weights M_w up to 500×10^6 g/mol. The molecular size of waxy maize amylopectin varied from 10 to 400×10^6 g/mol and was found to be sensitive to the conditions of dispersal; solubilization with dimethyl sulfoxide (DMSO) under mild conditions yielded the highest values. The use of DMSO to disperse starch for various physical chemical studies was reviewed in a recent report on the effect of the conditions used to disperse starch in 90% DMSO–H_2O (Jackson, 1991). DMSO–H_2O is a good solvent in the thermodynamic sense and starch solutions in this solvent have been reported to be stable for several months.

Some recent reports of M_w values for waxy maize amylopectin vary from 53×10^6 (Bello-Perez, Paredes-Lopez, Roger & Colonna, 1996) to 76.9×10^6 (Aberle, Burchard, Vorwery & Radosta, 1994). These workers used different techniques to prepare their samples. Aberle et al. (1994) found difficulties in interpreting the light scattering data using Zimm plots (Zimm, 1948) and found it necessary to use Berry plots (Berry, 1966) to extract molecular size and shape parameters. To avoid the size limitations and possible shear affects in size exclusion chromatography, Hanselmann, Rhrat and Widmer (1995) employed sedimentation field flow fractionation combined with multiangle laser light

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[1] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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scattering to characterize the hydrodynamic properties of waxy maize starch. The molecular weights and root mean square radii of gyration of the dissolved starch species ranged from 10^6 to 10^9 g/mol and 50 to 500 nm, respectively. These authors also noted that light scattering data required Berry plots for analysis and that large errors in the extrapolation of molecular weights resulted for macromolecules of this large size.

As part of a project to characterize the hydrodynamic properties of processed waxy maize starches, we employed static light scattering to evaluate molecular size and shape changes for amylopectin starch molecules after processing (Millard, Dintzis, Willett, & Klavons, 1997; Willett, Millard & Jasberg, 1997). Light scattering results varied considerably for amylopectin gently dispersed in 90% DMSO–H_2O solution. In one study for example, the M_w varied between 250 and 750 $\times$ 10^6 g/mol and R_g varied between 50 and 350 nm depending on the processing conditions and/or dispersion techniques (Millard et al., 1997). To further substantiate our earlier results by other hydrodynamic measurements, we have measured the sedimentation coefficients of identical samples of amylopectin in 90% DMSO–H_2O by ultracentrifugation and the diffusion coefficients of these solutions by dynamic light scattering. The M_w in solution calculated from the Svedberg equation was compared with the M_w extrapolated by using Berry plots generated from static light scattering results.

2. Materials and methods

2.1. Materials

Waxy maize starch (Amioca®, Cerestar USA, Inc., Hammond, IN, USA) containing around 98% amylopectin and 11% moisture was used. DMSO 99.9% spectroscopic grade and NaN_3 were both supplied by Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Starch dispersal

Starch was gently dispersed in 90% wt/wt DMSO–H_2O containing 0.2% NaN_3. A stock dispersion of 3.0–3.5 wt.% starch sample in solvent was made up in a stoppered Erlenmeyer flask by stirring with a Teflon® stirring bar at 100 rpm for 1 h at room temperature and then diluting this mixture with solvent for hydrodynamic characterization. Starch concentrations were determined by optical rotation using a specific rotation of 190 at 589 nm (Dintzis & Tobin, 1969).

2.3. 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 prior to use. The plasma generating instrument (Plasmod, March Instruments, Inc., Concord, CA, USA) has been described elsewhere (Millard & Bartholomew, 1977). The oxygen plasma oxidizes carbonaceous material and produces an optical glass surface of exceptional clarity (Koide, Shidura, Tanaka, Yagashita & Sato, 1989).

2.4. Static light scattering

A specific refractive index increment, dn/dc, of 0.074 ml/g was used (Millard et al., 1997). Light scattering measurements were performed at room temperature with a DAWN F® multi-angle light scattering detector in the batch mode using a He–Ne laser light source operating at 632.8 nm (Wyatt Technology Corp., Santa Barbara, CA, USA). Toluene was used as the calibration standard at 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. Data were collected and processed by the use of the Wyatt version 3.00 software for Windows. All M_w and R_g values presented herein 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 dependence, and second order polynomials for the angular dependence.

Starch samples were initially diluted to approximately 10^{-3}–10^{-4} g/ml and filtered through either 5 or 1 μm polyester screen membrane filters (Poretics Corp., Livermore, CA, USA). The filtered stock solutions were then transferred to the plasma cleaned vials and diluted by weight with solvent previously filtered through a 0.1 μm polyester filter.

2.5. Dynamic light scattering

A BI-200 SM goniometer and BI-9000 AT correlator (Brookhaven Instruments, Holtsville, NY, USA) were used to measure the intensity–intensity auto correlation function for vertically polarized light (λ = 632.8 nm, 60 mW HeNe laser, Spectra-Physics, San Jose, CA, USA) scattered at 90° from dilute solutions of amylopectin starch gently dispersed in 90% DMSO–H_2O solution at room temperature. The diffusion coefficient was calculated from the first-order cumulant analysis of the time decay of the electric field correlation function (Burchard & Richtering, 1989; Burchard, 1992).

2.6. Sedimentation analyses

Sedimentation velocity measurements were made to assess the homogeneity of the amylopectin and to determine the sedimentation coefficients using a Beckman Model E

Table 1
Weight average molecular weights M_w and radii of gyration R_g calculated using the Berry plot for gently dispersed waxy maize amylopectin starch in 90% DMSO–H_2O. Each result was obtained by diluting aliquots from the same sample and measuring scattered light from each set

Set	M_w ($\times 10^6$ g/mol)	R_g (nm)
1	564 ± 20	349 ± 6
2	556 ± 20	335 ± 6

analytical ultracentrifuge (Beckman Instruments, Fullerton, CA, USA) equipped with standard phase-plate schlieren optics. Double-sector cells with a 30 mm optical path length were used. One sector was loaded with 1 ml of sample and the other with 1 ml of solvent. Alternatively, the second sector was loaded with 0.85 ml of a lower concentration of amylopectin; because of the high concentration dependence of sedimentation, the boundaries did not overlap thereby permitting measurement of two sedimentation coefficients in a single run at speeds of 8000–18 000 rpm. The sedimentation coefficients were corrected to 20°C using DMSO viscosity data of Cowie and Toporowski (1961).

3. Results

3.1. Static light scattering

The static light scattering results from two independent sets of measurements taken from aliquots of the same gently dispersed waxy maize amylopectin starch sample are given in Table 1. Molecular weights agreed well with each other and averaged 560 $\times 10^6$ g/mol. Radii of gyration likewise were in good agreement and averaged 342 nm. Berry plots were used to extract the size and shape parameters. A typical Berry plot for amylopectin in DMSO/H_2O is shown in Fig. 1.

3.2. Sedimentation velocity analysis

Sedimentation velocity measurements were made in an analytical ultracentrifuge to assess homogeneity of the amylopectin and to measure its sedimentation coefficient.

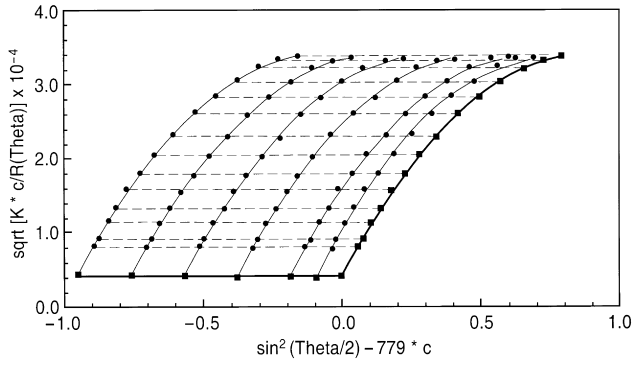

Fig. 1. Berry plot of the scattered light from amylopectin waxy maize starch in 90% DMSO–H_2O.

Sedimentation velocity schlieren diagrams for unfractionated amylopectin waxy maize starch in 90% DMSO–H_2O are shown in Fig. 2. The sedimentation pattern for a 0.35% solution (Fig. 2(a)) revealed the presence of a trace of fast sedimentating material (~ 150 S as compared to 34 S for the major component) ahead of the major boundary but which became diffuse and undetectable after sedimenting for 48 min. The major boundary existed as a hypersharp peak (Fig. 2(b)) typical of a highly concentration dependent system (Harding, Berth, Hartmann, Jumel, Cölfen & Christenson, 1996; Lelievre, Lewis & Marsden, 1986; Stacy & Foster, 1957). Only after prolonged centrifugation did the vertical schlieren line in the center of the peak disappear due to a slow broadening of the peak (Fig. 2(c)). The absence of a slower sedimenting boundary of 2–10 S indicates the absence of detectable amounts of amylose that are observed in normal maize starch (Stacy & Foster, 1957; Dintzis & Tobin, 1969) and wheat starch (Lelievre et al., 1986). The sedimenting boundaries broadened more rapidly at lower concentrations making it more difficult to locate the peaks of the boundaries as observed for maize amylopectin in water and ethylenediamine (Stacy & Foster, 1957). A plot of the sedimentation coefficients as a function of concentration confirmed a high dependence upon concentration (Fig. 3) as reported by others (Stacy & Foster, 1957; Banks et al., 1972; Lelievre et al., 1986). A value of 199 S was obtained from the plot by extrapolation to zero concentration. This value is comparable with 180 S for maize amylopectin in ethylenediamine (Stacy & Foster, 1957) and ~ 280 S in 0.2M NaCl (Banks et al., 1972) assuming a curvilinear relationship between the s values and concentration. When a straight line was fitted to the data of Banks et al., an s^0 value of ~ 205 S was obtained.

In contrast, sedimentation coefficients for wheat amylopectin tend to be somewhat lower than the value we obtained for maize amylopectin. For example, Dickinson, Lelievre, Stainsby and Waight (1984) isolated starch from wheat and dispersed it in DMSO by heating and stirring until the viscosity remained constant. The dispersion was then fractionated by ultracentrifugation and the resulting amylopectin in DMSO yielded an s^0 value of 100 S. Amylopectins isolated from a series of wheat cultivars yielded s^0 values ranging from 65 to 115 S in DMSO (Lelievre et al., 1986).

3.3. Dynamic light scattering

The translational diffusion coefficient needed to estimate the molecular weight with the Svedberg equation was measured by dynamic light scattering. In dynamic light scattering the time correlation function of the scattered electric field $g_1(t)$ is analyzed (Burchard & Richtering, 1989; Burchard, 1992; Pusey, Koppel, Schaefer, Camerini-Otero & Koenig, 1974). Scattering from polydisperse samples is

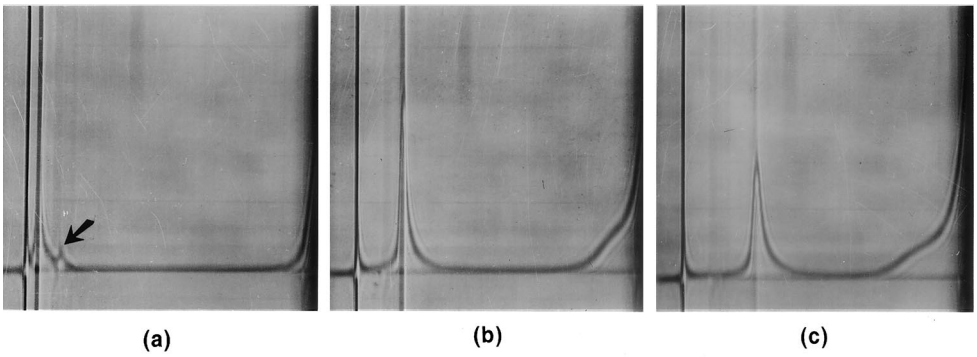

Fig. 2. Ultracentrifuge schlieren patterns for 0.35% amylomaize starch in DMSO:H_2O (90:10): (a) after sedimenting 32 min from the beginning of the run showing a small rapidly moving spikelet (arrow) ahead of the main boundary; (b) after sedimentation for 136 min exhibiting a hypersharp boundary; and (c) after sedimentation for 216 min where the boundary has widened sufficiently to eliminate the vertical schlieren line noted in (b). Rotor speed, 10 000 rpm and temperature 19.9°C.

analyzed as an exponential series in time:

$$\ln g_1(t) = -\Gamma_1 t + (\Gamma_2/2!)t^2 - (\Gamma_3/3!)t^3 + \ldots,$$

where Γ is the decay rate of the electric field fluctuations. For dilute solutions, the diffusion coefficient D_c at each concentration c, may be obtained from the extrapolation of the reduced first cummulant and the relationship $\Gamma_1 = D_c q^2$. The value of q, the magnitude of the scattering vector, is given by $q = (4\pi/\lambda_0)n_0\sin(\theta/2)$, where λ_0 is the wavelength of the light source, n_0, the refractive index, and θ, the scattering angle. At the highly dilute concentration studied and for scattering angles of 60, 90 and 120° the average calculated diffusion coefficient was 2.33×10^{-9} cm^2/s.

The diffusion coefficient was then combined with the sedimentation coefficient to calculate the M_w using the Svedberg equation (Tanford, 1961)

$$M_w = [RTs^0]/[D^0(1 - \hat{v}\rho)],$$

where R is the gas constant, T, the absolute temperature, s^0, the sedimentation coefficient at zero concentration, D^0, the diffusion coefficient, $\hat{v}$, the partial specific volume (0.60, assuming it is the same as for maize amylose in DMSO as reported by Dintzis and Tobin (1969) and Lelievre et al. (1986) obtained a value of 0.59 for wheat amylopectin in DMSO) and ρ, the density of the solvent. Substituting 199 S for s^0 and 2.33×10^{-9} cm^2/s for D^0 resulted in a value of 593×10^6 for the molecular weight. This result is in good general agreement with the static light scattering result of 560×10^6 g/mol in Table 1.

Availability of D^0 allowed calculations of the

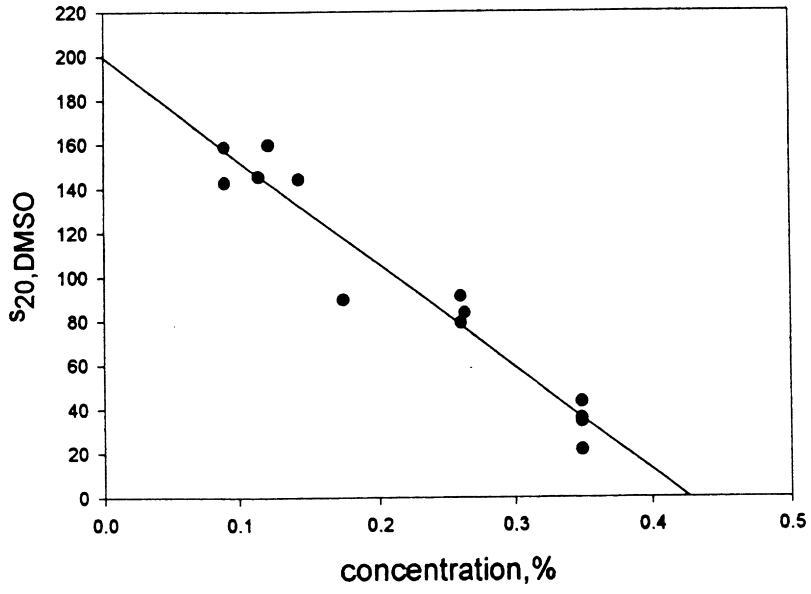

Fig. 3. Concentration dependence of the sedimentation coefficient $s_{20,DMSO}$. Line fitted to points is $s_{20,DMSO} = -466.5c + 199.4$, where c is the concentration in percent.

Table 2
Comparison of experimental values for molecular weights of waxy maize amylopectin with results of other workers for maize and wheat amylopectins

Amlyopectin source	Solvent	M_w (g/mol) $\times 10^-6$	Technique employed[a]	Reference
Maize	DMSO–H_2O	560	SLS	This work
Maize	DMSO–H_2O	593	S-D	This work
Maize	DMSO	400	SLS	Banks et al. (1972)
Maize	DMSO–H_2O	250–750	SLS	Millard et al. (1997)
Maize	H_2O	1–1000	SLS	Hanselmann et al. (1995)
Maize	DMSO–H_2O	53	SLS	Bello-Perez et al. (1996)
Maize	H_2O	76.9	SLS	Aberle et al. (1994)
Wheat	H_2O	316	SLS	Ring et al. (1985)
Wheat	DMSO	~ 10	S-D	Lelievre et al. (1986)
Wheat	DMSO	~ 1	PFGNMR	Callaghan and Lelievre (1985)

[a] SLS, static light scattering; S-D, sedimentation-diffusion; PFGNMR, pulsed-field-gradient nuclear magnetic resonance.

hydrodynamic radius R_h from the Stokes–Einstein equation (Tanford, 1961)

$$R_h = kT/6\pi\eta D^0,$$

where k is the Boltzmann constant, T, the absolute temperature, and η, the solvent viscosity (2.7 cP). The hydrodynamic radius R_h was calculated to be 348 nm. The radius of gyration R_g from static light scattering (Table 1) may be used with the hydrodynamic radius R_h from dynamic light scattering to calculate a structure parameter $\rho = R_g/R_h$ (Burchard, Schmidt & Stockmayer, 1980). This parameter is sensitive to assumed conformations of macromolecules and the relationship between ρ and conformations is given in the literature (Burchard et al., 1980; Burchard, 1992; Burchard & Richtering, 1989). Our results gave a value of 0.98 for ρ. This value is significantly lower than that for random coils in a good solvent (2.05) and is closer to values for homogeneous spheres (0.78) or regular star structures (1.08) (Burchard & Richtering, 1989). It is consistent with a dense structure for amylopectin in solution, as shown by intrinsic viscosity and light scattering results (Millard et al., 1997).

4. Discussion

Our results for the molecular weight of waxy maize amylopectin by the two techniques we employed are compared in Table 2 with results of other workers. Literature values also include data for wheat amylopectin for comparative purposes. The values for maize amylopectin vary considerably but our values are of the same order of magnitude as those reported by Banks et al. (1972) and the earlier results from this center (Millard et al., 1997). Our results also fall within the broad range of molecular weights reported by Hanselmann et al. (1995) who solubilized their samples in water at 175°C and observed a progressive decrease in molecular weight with increased time of heating. The molecular weights reported by Bello-Perez et al. (1996) and Aberle et al. (1994) are about one order of magnitude lower than our values and may reflect differences in samples and sample preparation. For example, Aberle et al. (1994) solubilized their samples in water by autoclaving whereas we solubilized our samples in DMSO–H_2O by stirring gently at ambient temperature.

As noted for maize, the literature values for the molecular weight of wheat amylopectin also vary considerably. Ring, Morris and I'Anson (1985) dispersed wheat starch granules in DMSO, removed the amylose as a butanol complex and finally transferred the amylopectin into water. Static light scattering yielded a M_w of 316×10^6 and $\langle R_g^2 \rangle$ of 6.13×10^{-15}. Ring et al. also obtained a diffusion coefficient of 1.68×10^{-8} cm^2/s by dynamic light scattering. The hydrodynamic radius R_h and radius of gyration R_g were calculated to be 131 and 101 nm, respectively. These results yield ρ values of 1.3–1.8 which are significantly greater than those measured here for waxy maize amylopectin (0.98), but still less than those expected for random coils. This difference suggests that wheat starch amylopectin has a solution structure which is less dense than that of waxy maize amylopectin.

Studies by Lelievre et al. (1986) on amylopectins isolated from various wheat cultivars yielded s^0 values ranging from 65 to 115 S. They estimated the M_w to be $\sim 10^7$ g/mol using the Svedberg equation and a diffusion coefficient measured by pulsed field gradient nuclear magnetic resonance (Callaghan & Lelievre, 1985). The diffusion coefficient of amylopectin in DMSO obtained by Callaghan and Lelievre was $3.2 \pm 0.7 \times 10^{-7}$ cm^2/s and they estimated that wheat amylopectin molecules were planar in structure with molecular weights of the order of 10^6 g/mol.

Light scattering is an absolute method for the determination of molecular weights for macromolecules in solution (Flory, 1953). In practice, light scattering measurements are difficult as solutions must be free of dust particles that can contribute as scatterers. The presence of small amounts of microgels may also complicate interpretation of light scattering measurements (Banks & Greenwood, 1975). Further, the theory used to interpret light scattering measurements is questionable when the dimensions of the scattering particles approach the wavelength of the incident light (Tanford, 1961). Difficulties in interpreting light scattering results for large amylopectin polymers have been reported in two

recent investigations using light scattering to measure the molecular size and shape of amylopectin from various sources (Aberle et al., 1994; Hanselmann et al., 1995). In their report Hanselmann et al. (1995) suggested that errors in the absolute molecular weight values for particles between 10^8 and 10^9 Da could range between 20 and 100%. During recent studies (Millard et al., 1997) on the changes in molecular size and shape of amylopectin starches after processing by extrusion, autoclaving and steam jet cooking, we also encountered this difficulty but satisfactory molecular weights resulted when Berry plots were used to interpret light scattering data.

5. Conclusion

We have used sedimentation and dynamic light scattering to determine the molecular weight and size of waxy maize starch amylopectin in DMSO–H_2O solutions. Molecular weight and R_g were determined to be 560×10^6 g/mol and 342 nm, respectively. The agreement between these results and those obtained from Berry plot analysis of static light scattering suggests that the latter method is a valid one for measuring the size of large molecules in solution. A value for the structure dependent parameter $\rho = (R_g/R_h)$ of 0.98 was determined, suggesting that waxy maize amylopectin has a dense structure in this solvent. This value of ρ is significantly lower than those obtained from wheat starch amylopectin, implying that waxy maize amylopectin has a structure which is more dense in solution than that of wheat amylopectin.

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References

Aberle, Th., Burchard, W., Vorwery, W., & Radosta, S. (1994). Conformational contributions of amylose and amylopectin to the structural properties of starches from various sources. *Starch/Stärke*, *46*, 329–335.

Banks, W., Geddes, R., Greenwood, L. T., & Jones, E. G. (1972). Physical chemical studies on starches. Part 63, The molecular size and shape of amylopectin. *Starch/Stärke*, *8*, 245–280.

Banks, W., & Greenwood, C. T. (1975). *Starch and its components*. New York: Halsted Press.

Bello-Perez, L. A., Paredes-Lopez, O., Roger, P., & Colonna, P. (1996). Molecular characterization of some amylopectins. *Cereal Chemistry*, *73*, 12–17.

Berry, G. C. (1966). Thermodynamic and conformational properties of polystyrene, I. Light-scattering studies on dilute solutions of linear polystyrenes. *Journal of Chemical Physics*, *44*, 4550–4564.

Burchard, W. (1992). Static and dynamic light scattering approaches to structure determination of biopolymers. In S. E. Harding & D. B. Sattelle & V. A. Bloomfield (Eds.), *Laser light scattering in biochemistry*, (pp. 1). Cambridge, UK: The Royal Society of Chemistry.

Burchard, W., & Richtering, W. (1989). Dynamic light scattering from polymer solutions. *Progress in Colloid and Polymer Science*, *80*, 151–163.

Burchard, W., Schmidt, M., & Stockmayer, W. H. (1980). Information on polydispersity and branching from combined quasi-elastic and integrated scattereing. *Macromolecules*, *13*, 1265–1272.

Callaghan, P. T., & Lelievre, J. (1985). The size and shape of amylopectin: a study using pulsed-field gradient nuclear magnetic resonance. *Biopolymers*, *24*, 441–460.

Cowie, J. M. G., & Toporowski, D. M. (1961). Association in the binary liquid system dimethylsulphoxide–H_2O. *Canadian Journal of Chemistry*, *39*, 2240–2243.

Dickinson, E., Lelievre, J., Stainsby, G., & Waight, S. (1984). The polymers in large and small wheat starch granules. In P. A. Williams & D. J. Wedlock & G. O. Phillips (Eds.), *Progress in food and nutrition science, gums and stabilizers for the food industry, Part II Applications of Hydrocolloids*, (pp. 277). Oxford, UK: Pergamon Press 277.

Dintzis, F. R., & Tobin, R. (1969). Optical rotation of some (-1,4-linked glucopyranosides in the system H_2O–DMSO. *Biopolymers*, *7*, 581–593.

Flory, P. J. (1953). *Principles of polymer chemistry*. Ithaca, NY: Cornell University Press.

Hanselmann, R., Rhrat, M., & Widmer, H. M. (1995). Sedimentation field flow fractionation combined with multi angle laser light scattering applied for characterization of starch polymers. *Starch/Stärke*, *46*, 345–349.

Harding, S. E., Berth, G., Hartmann, J., Jumel, K., Cölfen, H., & Christenson, B. E. (1996). Physiochemical studies on xylinan (acetan) III. Hydrodynamic characterization by analytical ultracentrifugation and dynamic light scattering. *Biopolymers*, *39*, 729–736.

Jackson, D. S. (1991). Solubility behavior of granular corn starches in methyl sulfoxide (DMSO) as measured by high performance size exclusion chromatography. *Starch/Stärke*, *43*, 422–427.

Koide, T., Shidura, T., Tanaka, K., Yagashita, A., & Sato, S. (1989). In situ d.c. oxygen discharge cleaning system for optical elements. *Review of Scientific Instruments*, *60* (7), 2034–2037 Part 2B.

Lelievre, J., Lewis, J. A., & Marsden, K. (1986). The size and shape of amylopectin: a study using analytical ultracentrifugation. *Carbohydrate Research*, *153*, 195–203.

Millard, M. M., & Bartholomew, J. C. (1977). Application of XPS to surface studies of mammalian cells grown in culture. *Analytical Chemistry*, *49*, 1290–1296.

Millard, M. M., Dintzis, F. R., Willett, J. L., & Klavons, J. A. (1997). Light-scattering molecular weights, intrinsic viscosities of processed waxy maize starches in 90% DMSO and H_2O. *Cereal Chemistry*, *74*, 687–691.

Pusey, P. N., Koppel, D. E, Schaefer, D. W., Camerini-Otero, R. D., & Koenig, S. H. (1974). Intensity fluctuation spectroscopy of laser light scattered by solutions of spherical viruses R17, Qβ, BSV, PM2, and T7. I. Light scattering technique. *Biochemistry*, *13*, 952–960.

Ring, S. G., Morris, J. J., & I'Anson, K. (1985). Characterization of the solution behaviour of starch polysaccharides. In L. Munck & R. D. Hill (Eds.), *New approaches to research on cereal carbohydrates*, (pp. 115). Amsterdam: Elsevier.

Stacy, C. J., & Foster, J. F. (1957). Molecular weight heterogeneity in starch amylopectins. *J. Polymer Science*, *25*, 39–50.

Tanford, C. (1961). Physical chemistry of macromolecules. New York: Wiley 305.

Willett, J. L., Millard, M. M., & Jasberg, B. K. (1997). Extrusion of waxy maize starch: melt rheology and molecular weight degradation of amylopectin. *Polymer*, *38*, 5983–5989.

Zimm, B. H. (1948). Apparatus and methods for measurement and interpretation of the angular variation of light scattering; preliminary results on polystyrene solutions. *Journal of Chemical Physics*, *16*, 1099–1116.