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Molar Masses and Sizes of Starches by High-Performance Size-Exclusion Chromatography with On-Line Multi-Angle Laser Light Scattering Detection

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Starch was characterized for analysis by high-performance size-exclusion chromatography (HPSEC) with detection by multiple-angle laser light-scattering and refractive index. Corn starches (amylopectin-to-amylose ratios of 1:0, 3:1, 1:1, and 3:7), presolubilized potato starch, and potato starch granules were analyzed. For corn starches, analysis by HPSEC revealed that weight average molar mass (M_w) and z-average root mean square radius of gyration (R_{gz}) decreased with increasing percentage of amylose. For potato starches, granules had a much higher M_w and R_{gz} than presolubilized starch. All chromatograms were polymodal in nature with at least two high M_w amylopectin components. Except for high amylose corn starch, R_{gz} values from this study were comparable with results from an earlier HPSEC/viscometry study. The R_{gz} values from microscopic studies were lower than from chromatographic studies.

Keywords: *starch; molar mass; size; HPSEC; light scattering*

INTRODUCTION

Endogenous and added starch are important components in determining the functional properties of many foods. Also, starch is used widely in industry as a coating. More recently, starch has been incorporated into plastics to render them more biodegradable (Chapman, 1994). In all of these uses, the molar mass and size of starch is important in determining its functional properties. Because starches are often high in molecular weight, difficult to dissolve, and tend to aggregate and precipitate, molar mass and size are frequently difficult to measure in neutral aqueous solution (Young, 1984). Reports in the literature indicate that amylopectin (Lelievrer et al., 1986; Aberle et al., 1994) and amylose (Roget and Colonna, 1993; Fishman and Hoagland, 1994; Aberle et al., 1994) aggregate in aqueous solution. Consequently, starch is much less frequently characterized in water. Nevertheless, characterization of starch in water is important because water is frequently the solvent of choice for its use.

Over the last few years there have been several developments in sample preparation and on-line sample detection that have facilitated the rapid characterization of starch in aqueous media. Recently, Delgado et al. (1991) developed a rapid, high-pressure, microwave procedure for solubilizing starch (often referred to as gelatinizing starch) in neutral aqueous media. This method is advantageous in that each sample can be solubilized just prior to chromatographic analysis, thus minimizing variation in analysis due to starch aggregation. We have used this solubilization method in this laboratory to characterize corn starch by high-performance size-exclusion chromatography (HPSEC) with on-line viscosity detection (Fishman and Hoagland, 1994) and electron microscopy (Fishman et al., 1995).

In this study, we solubilized four varieties of corn starch, which we studied previously by HPSEC/viscometry, by high-pressure microwave heating with multi-angle light scattering (LS) instead of viscometry to detect the dissolved starches as they eluted from the column. The starches in both studies were solubilized in a similar fashion and dissolved in aqueous media, so we were able to compare HPSEC/viscometry and HPSEC/LS as techniques for characterizing starch. Also, we included some data on potato starch for comparison with corn starch. In the course of these studies we developed a new method for testing the accuracy of the instrument constant required to obtain the correct absolute scattering intensities. Also, we tested several variations for plotting the data.

MATERIALS AND METHODS

Materials. Pullulan standards were obtained from JM Science Inc., Grand Island, NY, and dextran standards were obtained from Pharmacia LKB Biotechnology, Piscataway, NJ, and Sigma Chemical Company, St. Louis, MO. Commercial corn starches, waxy maize (~100% amylopectin), common maize (~75% amylopectin, 25% amylose), amylomaize V (~50% amylopectin, 50% amylose), and amylomaize VII (~30% amylopectin, 70% amylose) were obtained from American Maize Products Company, Hammond, IN. Potato starch granules and presolubilized potato starch were obtained from Sigma Chemical Company. Reagent grade $NaNO_3$ was obtained from J. T. Baker. HPLC grade water was obtained by passing house deionized water through a Modulab Polisher I deionizer-filtration system (Continental Water Systems Corp., San Antonio, TX).

Sample Preparation. Pullulan (12−30 mg) or dextran (18 mg) and 6 mL of 0.05 M $NaNO_3$ were placed in a capped vial and refrigerated overnight without shaking, as suggested by the manufacturer. Prior to injection, samples were stirred slowly for 15 min and equilibrated at 45 °C for 20 min.

Starch was solubilized in HPLC grade water by microwave heating (Amana 700 Watt, model R321T oven, Amana, IA) in a high-pressure vessel as described previously (Fishman and

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Hoagland, 1994), with slight modifications in heating times. Eighty-eight milligrams of starch was slurried in 20 mL of HPLC grade water, and the slurry was heated in the microwave at full power for 80 s. The microwave vessel was allowed to cool for 20 min in cold tap water, and then the sample was centrifuged for 10 min at 43500*g*. One milliliter of the supernatant was mixed with 2 mL of 0.075 M $NaNO_3$, and then a 1:1 dilution with 0.05 M $NaNO_3$ was made.

Chromatography. All macromolecular solutions were passed through a 0.40-μm Nucleopore filter (Costar Corp., Cambridge, MA) prior to analysis. Sample injection volume was 100 μL, nominal flow rate was 0.7 mL/min, and the mobile phase was 0.05 M $NaNO_3$. The mobile phase was deaerated and filtered off-line by passing it through a 0.40-μm Nucleopore filter. The chromatography system consisted of a model KT-35 Shodex deaerater (JM Science Inc., Grand Island, NY) connected in series to a model 6000A pump fitted with an M-45 pulse dampener (Waters Associates, Milford, MA), an in-line 0.1-μm vv Durapore membrane filter housed in a high-pressure holder (Millipore Corp., Milford, MA), a 15′ stainless steel warming coil (i.d., 0.04″), a model 210 injection valve (Beckman, Palo Alto, CA), a 10 × 3.2 mm i.d. Synchropak cartridge guard column (SynChrom, Inc., Lafayette, IN), three chromatography columns, a Dawn F MALLS photometer fitted with a helium-neon laser (λ = 632.8 nm) and a K-5 flow cell (Wyatt Tech., Santa Barbara, CA), and a model SE-61 Shodex differential refractive index (DRI) detector (JM Science Inc., Grand Island, NY). The serially placed chromatography columns were Shodex OH-pak SB-806, SB-805, and SB-803 (JM Science Inc.). The exclusion limits for these columns for pullulans, as specified by the manufacturer, are 4×10^7, 2×10^6, and 1×10^5 g/mol, respectively.

The electronic outputs of the DRI and the MALLS were sent to a 486 Personal Computer. The data were processed with ASTRA (Version 2.11) and EASI (Version 6.0; Wyatt Tech) software. The DRI response factor was measured by injecting a series of known NaCl concentrations directly into the detector cell with a syringe. This response factor was obtained from the slope of the linear plot between NaCl concentration and refractive index (RI) response. The factor to correct the Rayleigh ratio at 90° (R_{90}) for instrument geometry was obtained by measuring the LS intensity of toluene at 90°. The responses to LS intensity of the photodiodes arrayed around the scattering cell at angles of 22.26°, 29.11°, 36.46°, 44.72°, 54.19°, 65.02°, 77.11°, 90.00°, 102.89°, 114.98°, 125.81°, 135.28°, 143.54°, 150.89°, and 157.74° were normalized to the diode at 90° with a P-50 pullulan standard. The following values of d*n*/d*c* (change in refractive index with polymer concentration) were used in calculating polysaccharide molecular weights and radii of gyration (see *Data Treatment* section): pullulan, 0.148 (Kato et al., 1982); dextran, 0.142; and starch, 0.146 (Yu and Rollings, 1987).

Data Treatment. The Dawn F MALLS LS detector simultaneously provides up to 15 LS chromatograms, each at a different scattering angle, for a polymer solute as it emerges from the chromatography column (Wyatt, 1993). An additional chromatogram can be obtained from a concentration detector (e.g., a DRI detector) serially in line with the MALLS detector. The ASTRA software subdivides the area under the chromatograms into a number of slices determined by the operator. Each slice represents a small volume of solute eluting from the column. If the light scattered by the solute in each volume fraction and its concentration (c) is measured, then one can obtain the root mean square (RMS) radius of gyration (R_{gi}) and the absolute molecular weight (M_i) of that solute by eq 1 (Tanford, 1961):

$$(R_\Theta/K^*c)_i = M_i - 16\Pi^2/3\lambda^2 R_{gi}^2 M_i \sin^2(\Theta/2) \qquad (1)$$

In eq 1, R_Θ is the Raleigh ratio due to the excess scattering of the polymer in solution when compared with that of the solvent in the solution at the angle Θ, K^* is an optical constant, and λ is the wavelength of the scattered light. R_Θ is obtained from eq 2 (Wyatt, 1993):

Table 1. Weight-Average Molar Masses of Narrow Pullulan Standards ($\times 10^{-3}$)

sample	uncorrected	corrected	manufacturer[a]
P5	16 (2)[b]	14 (1)	5.8
P10	21 (3)	17 (1)	12.2
P20	30 (2)	25 (2)	23.7
P50	64 (9)	55 (2)	48
P100	122 (1)	95 (6)	100
P200	241 (4)	205 (5)	186
P400	477 (8)	401 (6)	380
P800	964 (3)	793 (6)	853

[a] Values supplied by manufacturer. [b] Standard deviation of triplicate determinations of molar masses (Debye second-order equation used).

$$R_\Theta = f(i_s/I_0) \qquad (2)$$

In eq 2, i_s is the excess scattering of the polymer in solution when compared with that of the solvent at the angle Θ, I_0 is the intensity of the light beam, and f is a constant dependent on the geometry of the instrument (Wyatt, 1993). The optical (K^*) constant is obtained from eq 3 (Wyatt, 1993):

$$K^* = 4\Pi^2 n_0^2 (dn/dc)^2/\lambda^4 N_A \qquad (3)$$

In eq 3, n_0 is the RI of the solvent, d*n*/d*c* is the change in RI with polymer concentration at the wavelength of the scattered light, and N_A is Avogadro's number.

According to eq 1, plotting $(R_\Theta/K^*c)_i$ against $\sin^2(\Theta/2)$ will yield values of M_i and R_{gi} from the intercept and the slope. Such a plot is called a first-order Debye plot. For molecules sufficiently large, the angular dependence may no longer be linear and an additional term in $\sin^4(\Theta/2)$ may be added to the right-hand side of eq 1, and this fitted to a second-order polynomial from which M_i and R_{gi} can be extracted. It can also be shown that plotting (K^*c/R_Θ) against $\sin^2(\Theta/2)$ will yield values of M_i and R_{gi} from the intercept and the slope. This plot is called a Zimm plot.

The weight average molar mass, M_w, and the z-average RMS radius of gyration, R_{gz}, are obtained by summing over all the slices in the chromatogram according to eqs 4 and 5, respectively (Wyatt, 1993):

$$M_w = \Sigma c_i M_i / \Sigma c_i \qquad (4)$$

$$R_{gz} = (\Sigma R_{gi}^2 c_i M_i / \Sigma c_i)^{1/2} \qquad (5)$$

RESULTS AND DISCUSSION

Pullulans. To test how well the MALLS instrument was calibrated, several pullulan standards with narrow molar mass distributions were investigated. Molar masses (M_w) were calculated by three equations: first- and second-order Debye and first-order Zimm. All three methods gave about the same results when compared with M_w values supplied by the manufacturer, which were considered to be the "true" values. Thus, the average of triplicate determinations for the three calculation methods was obtained. As shown in the second column of Table 1, these values were consistently higher than the values of M_w supplied by the manufacturer of the pullulans. One possible explanation for the consistent error in the molar masses is the existence of a consistent error in determining the geometric constant f (Dawn constant) in eq 2. If f were incorrect, it could be corrected in the following fashion. The linear least squares solution relating the uncorrected experimental molar mass values (M_{expi}, column 2, Table 1) for pullulans with those of the manufacturer supplied or "true values" (M_{ti}, column 5, Table 1) is given by eq 6:

$$cf = \Sigma M_{ti}/\Sigma M_{expi} \qquad (6)$$

Table 2. z-Average RMS Radius of Gyration (R_{gz}) for Narrow Pullulan Standards (nm)

sample	uncorrected	corrected	literature[a]
P100	5 (2)[b]	5 (9)	10.9
P200	19 (4)	23 (6)	15.6
P400	24 (1)	26 (3)	24.1
P800	33 (4)	35 (1)	38.1

[a] Fishman et al., 1987. [b] Standard deviation of triplicate determinations of RMS R_{gz} with second-order Debye equation.

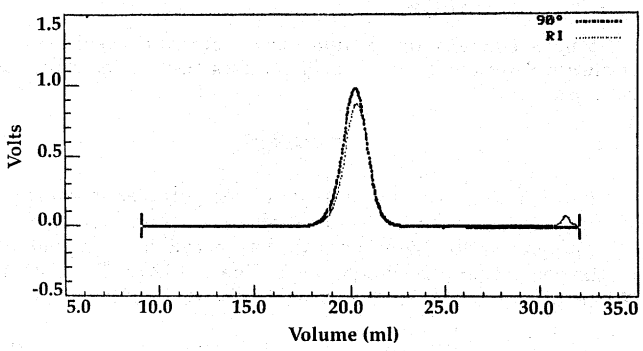

Figure 1. Superimposed chromatograms of P-800 pullulan detected by DRI and LS at an angle of 90° (sample concentration, 2 mg/mL).

Table 3. Weight-Average Molar Masses of Broad Dextran Standards ($\times 10^{-3}$)

sample	this study	manufacturer[a]
T10	10 (1)[b]	9.3
T20	27 (4)	22.3
T40	42 (3)	44.4
T70	75 (1)	70
T110	105 (3)	106
T250	254 (3)	253
T500	579 (6)	532
T2000	1551 (14)	2000
T2000	2210 (5)	2000

[a] Supplied by manufacturer; value for T2000 dextran is nominal. [b] Standard deviation of triplicate determinations of molar masses determined with second-order Debye equation.

A corrected f value, f', was then obtained by eq 7:

$$f' = f \times cf \tag{7}$$

Here, f is the value used to obtain ΣM_{exp}.

As can be seen from the results in Table 1, correcting the f value allows one to measure polysaccharide molar masses between 850 000 and 10 000 with an accuracy of ~10% or better. The data in Table 2 reveal that errors in the f value have little or no effect on R_{gz}. By inspection of standard deviations in Table 2 it appears that values of radii in column 2 do not give better agreement with the literature values than those in column 3. The results in Table 2 indicate that polysaccharides with radii of 10 nm and narrow size distributions are at the lower limit of detection. Figure 1 is the superimposed RI and 90° LS trace for P800 pullulan.

Dextrans. To demonstrate that the calibration method allowed one to measure molar masses as well as sizes absolutely, a series of broad dextran standards was measured. The results of these measurements are given in Tables 3 and 4. Again it was found that first- and second-order Debye and first-order Zimm equations gave comparable results. In this and future cases, the data were analyzed with a second-order Debye equation for consistency and to save computational time. Also, after statistical analysis comparing the various equations to extract M_w and R_{gz} from MALLS data, Balke

Table 4. z-Average RMS Radius of Gyration (R_{gz}) for Broad Dextran Standards (nm)

sample	this study	literature[a]
T110	15 (6)[b]	9.3
T250	12 (2)	13.9
T500	11 (5)	19.1
T2000	38 (2)	33.8
T2000	42 (2)	33.8

[a] Fishman et al., 1986. [b] Standard deviation of triplicate determinations made with the second-order Debye equation.

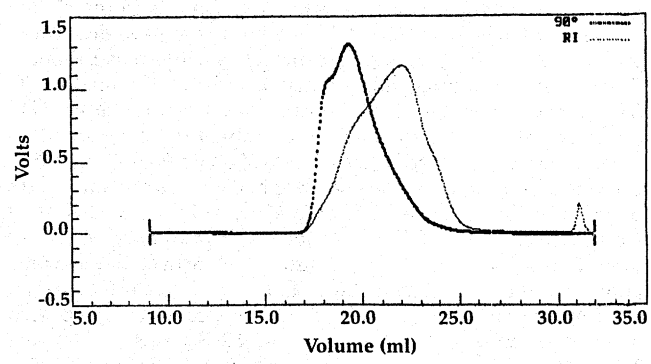

Figure 2. Superimposed chromatograms of T-2000 dextran detected by DRI and LS at an angle of 90° (sample concentration, 3 mg/mL).

(1993) concluded that using the second-order Debye equation gave the best values for polystyrene in organic solvent (Tables 3 and 4). In most cases, molar masses (Table 3) agree with manufacturers values within ~10%, whereas radii can be off by as much as 25% from the literature value. The superimposed RI and 90° LS traces for dextran T2000 are plotted in Figure 2. Comparison of Figures 1 and 2 reveal that RI and LS curves for pullulan are more nearly coincident for pullulan than for dextran. The reason for this result is that the molar mass polydispersity of pullulan as indicated by an M_w/M_n value of 1.01 is smaller than the polydispersity of dextran, which has a M_w/M_n value of 3.99.

Starches. Preliminary studies (not shown) on corn starches revealed that molar mass and R_{gz}, when plotted against solubilization time, reached a broad maximum that was somewhat dependent on the starch variety, whereas recovery of starch tended to plateau. Molar mass maxima occurred between 60 and 95 s. Corn varieties reached the time of maximum molar mass in the following order: amylomaize VII < amylomaize V = common maize < waxy maize. All samples had reached their maximum recoveries in ~80 s. Beyond 80 s, heating time recoveries reached a steady state but molar masses started to decrease. This behavior beyond 80 s of heating time could occur if the molar mass of the newly dissolving starch polymers was not sufficient to offset the loss of molar mass due to polymer degradation. Therefore, 80 s was chosen as the microwave heating time for all samples. Recoveries of the starches were calculated from the area of the RI chromatogram using the DRI response factor and the weight of starch placed in the microwave bomb. The recoveries ranged from ~59 to 81% and are given in Table 5. Recoveries in this study were ~10% higher than those found in an earlier study except for common maize, which was ~2% lower (Fishman and Hoagland, 1994). A portion of the starch remained as a gel at the bottom of the centrifuge tube. This material plugged membrane filters if we attempted to inject the sample without prior centrifugation. As determined previously, the waxy maize gave

Table 5. Percentage Recovery of Starches

sample[a]	whole[b]	MW-V[c]	RMS-V[d]	literature[e]
waxy	63 (12)[f]	24 (2)	36 (5)	49 (10)
common	65 (5)	36 (2)	34 (7)	67 (6)
amy V	72 (12)	29 (1)	15 (1)	61 (6)
amy VII	81 (4)	15 (1)	5 (1)	71 (1)
potato (IS)	59 (6)	29 (1)	26 (1)	
potato (SOL)	68 (8)	35 (4)	13 (1)	

[a] Abbreviations: waxy = waxy maize (~100% amylopectin); common = common maize (~75% amlopectin, 25% amylose); amy V (~50% amylopectin, 50% amylose); amy VII (~30% amylopectin, 70% amylose); IS = insoluble starch granules; SOL = presoluble. [b] All data points included. [c] "Well behaved" portion of molar mass plotted against elution volume; highly scattered data point at ends of plot are omitted (see Figures 4b and 6b). [d] "Well behaved" portion of RMS radius of gyration (R_{gz}) plotted against elution volume, highly scattered data at ends of plot are omitted (see Figures 4c and 6c). [e] By HPSEC/viscometry (Fishman and Hoagland, 1994). [f] Standard deviation of triplicate determinations.

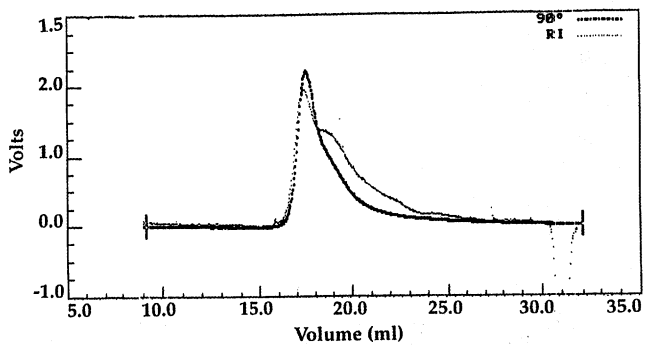

Figure 3. Superimposed chromatograms of waxy maize detected by DRI and LS at an angle of 90°.

the poorest and amylomaize VII gave the highest recovery. The improved recoveries in this study compared with the previous study may be attributed to cooling samples only 20 min rather than 30–45 min prior to removal from the microwave vessel.

The superimposed RI and 90° LS chromatograms for waxy maize are shown in Figure 3. The 90° LS trace for waxy maize, which is practically pure amylopectin, is a bimodal peak with the first peak much larger than the second one that appears as a shoulder at ~19 mL. In the case of the RI trace, the bimodality of the major peak is clear and an additional peak appears at ~25 mL. This latter peak is not visible by the LS technique. The chromatogram for common corn (maize) starch that contains ~25% amylose is shown in Figure 4a. The second peak in the bimodal LS trace increases in relation to the first peak when compared with the bimodal in Figure 3. This change is particularly evident in the RI trace. Also, the RI trace broadened more than the LS trace so that a significant portion of the RI trace appears where the LS intensity is rather small. Plots of molar mass and R_g versus volume for common starch are shown in Figures 4b and 4c, respectively. The molar mass data points decreased as expected over the elution volumes from ~17.5 to ~25.5 mL. Nevertheless, the amount of scatter in molar mass data increased considerably in the range of elution volumes between ~20.5 and 25.5 mL. The increased scatter in the data in this region of elution is probably caused by the small values of LS intensity in the tail of the chromatogram. In the case of R_g versus volume for common starch, again the data points decreased as expected in the range of elution volumes between 16.5 and 19.5 mL. This smaller range of reliable data compared with molar mass data is due to the lower sensitivity in measuring the slope of eq 1

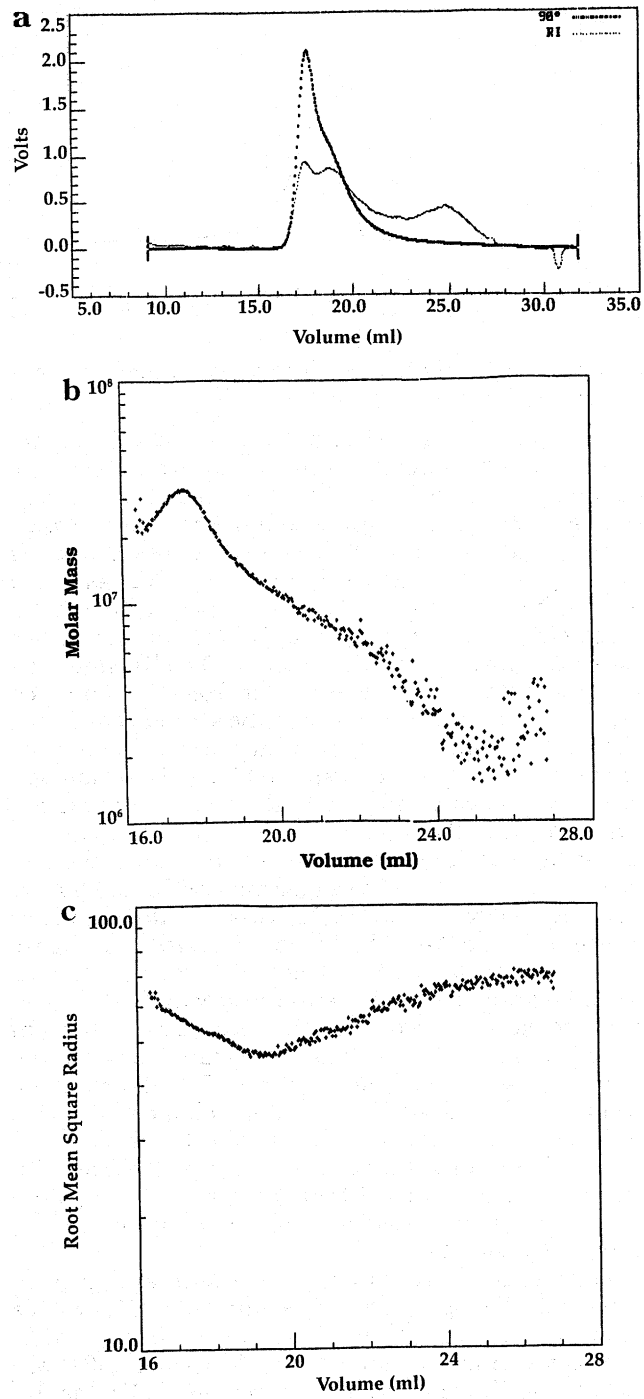

Figure 4. (a) Superimposed chromatograms of common maize detected by DRI and LS at an angle of 90°; (b) dependence of molar mass on elution volume for common maize; and (c) dependence of RMS radius of gyration (R_{gz}) on elution volume for common maize.

than in measuring its intercept. The trend toward a larger proportion of the chromatogram area occurring at higher elution volumes continues for amylomaize V (Figure 5) and amylomaize VII (Figure 6). This trend coincides with increases in the proportion of amylose to amylopectin. Molar mass and R_g are plotted versus elution volume for amylomaize VII in Figures 6b and 6c, respectively. The molar mass data are "well behaved" over the elution volume range of 17 to 23 mL, whereas the R_g data are "well behaved" over the elution volume range of 17 to 20 mL. The useful data range for amylomaize VII is roughly comparable with that obtained for common starch.

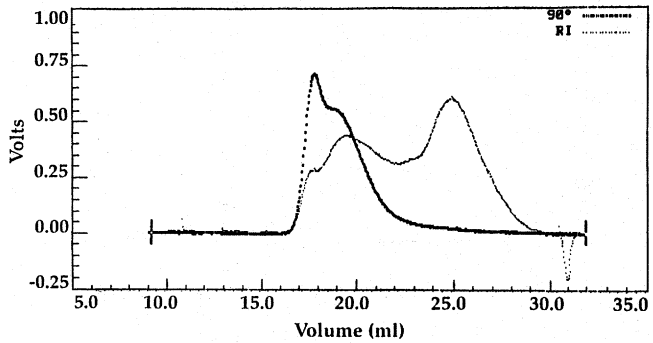

Figure 5. Superimposed chromatograms of amylomaize V detected by DRI and LS at an angle of 90°.

The superimposed RI and 90° LS traces for potato starch granules solubilized by microwave heating are shown in Figure 7a. The 90° LS chromatogram is very similar to the chromatogram obtained for common corn starch granules also solubilized by microwave heating (see Figure 4a). This similarity may be expected because the amylose:amylopectin ratio does not differ greatly (Shannon and Garwood, 1984) for common corn starch compared with potato starch. The RI chromatogram for potato starch is different from that obtained from common corn starch in that the second peak in the bimodal distribution is somewhat lower in relation to the first peak in the potato starch bimodal distribution. In the case of common starch, the first two peaks are almost equal. Also, there appears to be more low molecular weight fractions in potato starch than in common corn starch. The superimposed RI and 90° LS traces for microwaved presolubilized potato starch are shown in Figure 7b. The LS chromatogram in Figure 7b is monomodal with a long tail, whereas the RI chromatogram is bimodal with the second peak almost as large as the first. The first LS peak maximum shifted from ~17.2 to 19.6 mL compared with the microwave-solubilized potato starch. The RI chromatogram also shifted to higher elution volumes compared with the RI trace for the microwave-solubilized potato starch. Thus, qualitatively, comparison of RI and LS chromatograms revealed that the resolubilized potato starch is lower in molecular weight than the microwave-solubilized potato starch.

Molar mass averages for the starches are given in Table 6.. For purposes of comparison, M_w values obtained for corn starches in our earlier HPSEC/viscometry study (Fishman and Hoagland, 1994) are included in Table 6. The first number under the M_w column determined by HPSEC/viscometry is the global value. The first number after the standard deviation is the weight-average molar mass of the components identified as amylopectin, whereas the second number is the weight-average molar mass of the components identified as amylose. In the case of waxy maize, the second number represents low molecular weight amylopectin. In the HPSEC/viscometry study, sample preparation was similar to the method used here in that component analysis by curve fitting and universal calibration were used to obtain values of molar mass and RMS radius of gyration in addition to intrinsic viscosity. The molar masses obtained by HPSEC/LS for the corn starches gave M_w values that were somewhat higher than those obtained by viscometry. The reason for this difference is the higher sensitivity of viscosity over LS for lower molecular weight species. Because of the higher sensitivity of viscosity compared with LS towards amylose, the starch M_n is lower and the

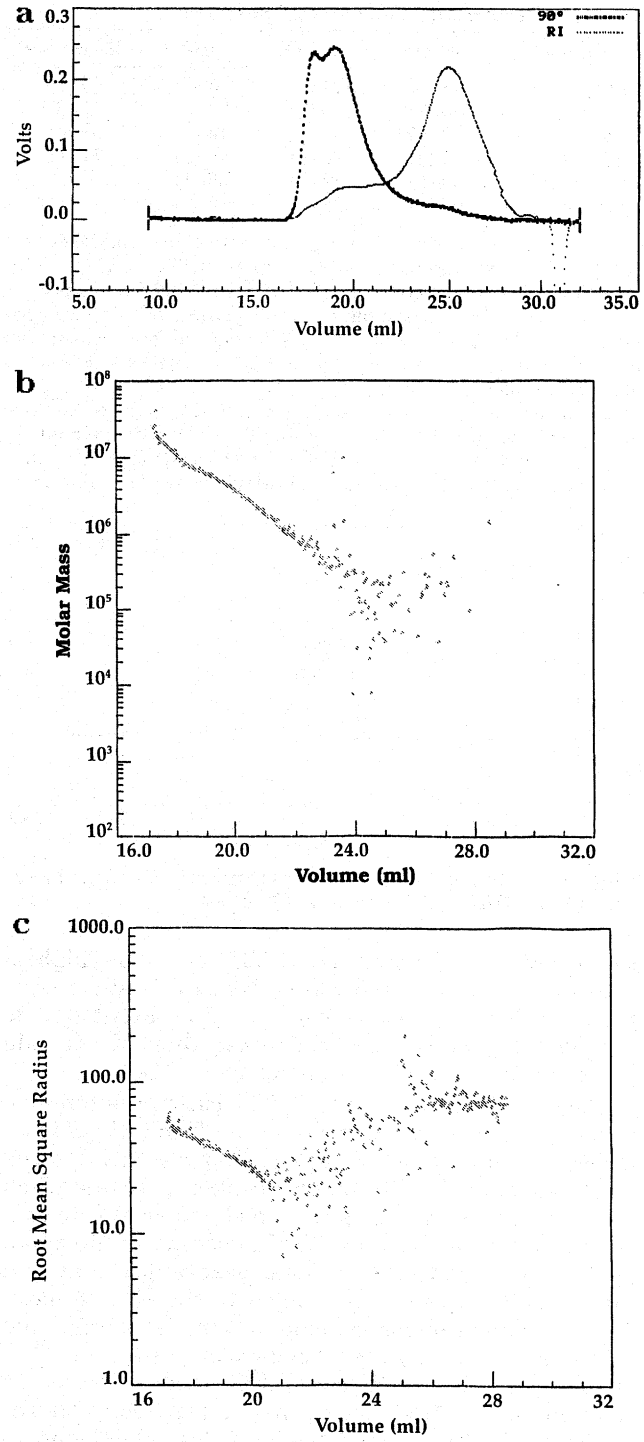

Figure 6. (a) Superimposed chromatograms of amylomaize VII detected by DRI and LS at an angle of 90°; (b) dependence of molar mass on elution volume for amylomaize VII; and (c) dependence of RMS radius of gyration (R_{gz}) on elution volume for amylomaize VII.

polydispersity, M_w/M_n, is higher as determined by viscosity than by LS. It is noteworthy that both studies indicate a decrease in M_w with increasing amylose content in the starch. Component analysis of the viscosity data reveals that in those starches containing amylose the decrease in M_w is due to a decrease in the molar mass of amylopectin as well as an increase in the percentage of the lower molar mass amylose. Because LS predominantly measures amylopectin when both amylopectin and amylose are present (Aberle et al., 1994; also see Figures 4a, 5, and 6a), LS measurements also demonstrate that the molar mass of amylopectin

Table 6. Number-Average (M_n) and Weight-Average (M_w) Molar Masses of Starch ($\times 10^{-6}$)

sample[a]	M_n	M_w	M_w/M_n	M_n[b]	M_w[b]	M_w/M_n[b]	M_w[c]
waxy	24 (5)[d]	26 (4)	1.1 (0.1)	2.6 (0.5)	20 (1), 27, 0.99	7.7	21.8
common	12 (4)	15 (4)	1.3 (0.2)	2.8 (0.6)	13 (2) 22, 0.13	4.6	14.5
amy V	5.9 (1)	9.9 (2)	1.7 (0.1)	2.0 (0.3)	8.0 (.4) 17, 0.13	4.0	5.75
amy VII	2.7 (0.4)	6.3 (0.9)	2.3 (0.1)	1.3 (0.3)	3.2 (.8) 12, 0.18	2.5	3.96
potato (IS)	13 (3)	17 (3)	1.3 (0.1)				
potato (SOL)	1.2 (0.2)	2.0 (0.4)	1.6 (0.1)				

[a] Abbreviations: waxy = waxy maize (~100% amylopectin); common = common maize (~75% amylopectin, 25% amylose); amy V = amylomaize V (~50% amylopectin, 50% amylose); amy VII = amylomaize VII (~30% amylopectin, 70% amylose); IS = insoluble starch granules; SOL = presolublized. [b] By HPSEC/viscometry (Fishman and Hoagland, 1994). [c] Data taken from Young, 1984. [d] Standard deviation of triplicate determinations with second-order Debye equation.

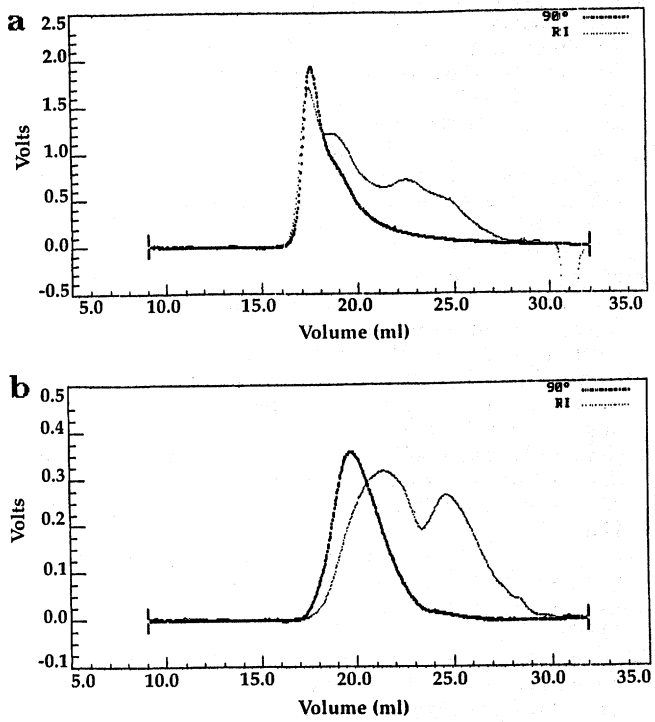

Figure 7. (a) Superimposed chromatograms of microwaved potato starch granules detected by DRI and LS at an angle of 90°; and (b) superimposed chromatograms of microwaved presolubilized potato starch detected by DRI and LS at an angle of 90°.

decreases with the increase in the percentage of amylose in corn starch under the conditions of extraction.

In the Introduction we indicated that method of extraction and solvent employed greatly affect the molar mass of the starch solubilized. Nevertheless, Young (1984) reported M_w values of 21.8, 14.5, 5.75, and 3.96 $\times 10^6$ for waxy maize, common corn starch, amylomaize V, and amylomaize VII, respectively. In that study, starches were extracted with DMSO at 150 °C for 1 h, analysis of the starches was by low-performance size-exclusion chromatography on glass beads, and the column was calibrated with dextran standards. The M_w values obtained by Young (1984) are comparable to the M_w values were obtained by HPSEC/LS and HPSEC/viscometry (see Table 6). Comparison of M_w of microwaved potato starch granules and microwaved presolubilized potato starch reveals that resolubilization in the microwave occurred with a significant decrease in the molar mass of amylopectin.

The R_{gz} values from this study in addition to R_{gz} values determined by HPSEC/viscometry (Fishman and Hoagland, 1994) and by electron microscopy (Fishman et al., 1995) are shown in Table 7. Waxy, common, and amylomaize V all gave values that were within or almost within the precision of the measurements if one

Table 7. *z*-Average RMS Radius of Gyration for Starch (nm)

sample[a]	R_{gz}	R_{gz}[b]	R_{gz}[c]
waxy	76 (3)[d]	81 (2), 85, 18	64
common	68 (1)	72 (4), 79, 16	
amy V	63 (3)	61 (3), 72, 17	
amy VII	62 (6)	41 (4), 59, 11	31 (2)
potato (IS)	73 (3)		
potato (SOL)	39 (3)		

[a] Abbreviations: waxy = waxy maize (~100% amylopectin); common = common maize (~75% amylopectin, 25% amylose); amy V = amylomaize V (~50% amylopectin, 50% amylose); amy VII = amylomaize VII (~30% amylopectin, 70% amylose); IS = insoluble starch granules; SOL = presolublized. [b] By HPSEC (viscometry, from Fishman and Hoagland (1994). [c] By microscope, from Fishman, Cooke, White, and Damert (1995). [d] Standard deviation of triplicate determinations of RMS radius of gyration by second-order Debye equation.

compares global values from LS with those from viscometry (values in second column preceding the standard deviation). The R_{gz} value for amylomaize VII determined by universal calibration in R_g (Fishman et al., 1986) was significantly below the value obtained by LS. As is the case with universal calibration in hydrodynamic volume (intrinsic viscosity $\times M$), R_g universal calibration measures the smaller sizes more sensitively than LS. In the case of amylomaize VII, the sensitivities of the two methods are sufficiently different to be reflected by a large disparity in the global averages of R_{gz} because of the large proportion of amylose in amylomaize VII. Similarly, comparison of M_w values by LS and viscometry in Table 6 reveals that a greater difference exists in values from the two methods for amylomaize VII than for any of the other corn varieties. The two values following the parentheses in column two of Table 7 are the R_{gz} values identified for amylopectin and amylose, respectively. These results indicate that the size of amylopectin decreases with decreasing amylopectin composition as indicated by LS and viscometry measurements, whereas viscometry indicates a decrease in amylose as well. Global R_{gz} values from microscopy (Fishman et al., 1995) of amylopectin and amylose are smaller than global R_{gz} values from either LS or viscometry. These differences may be explained by differences in sample preparation for chromatography as compared with microscopy in addition to differences in the sensitivity of the techniques toward the various sized molecules present in the distribution. Comparison of RMS R_{gz} values for insoluble and soluble potato starch parallels the molar mass results. Namely, that the size of insoluble starch is significantly larger than the resolubilized starch.

CONCLUSIONS

Visual inspection of superimposed chromatograms obtained by LS at 90° and DRI indicated that only a

portion of the eluted starch was detected by LS. Comparison with previously published chromatograms obtained by HPSEC/viscometry revealed that a portion of the amylose was not being measured by LS. The M_w values from viscometry were lower than M_w values from LS because of the greater sensitivity of viscometry for lower molar mass species. Further evidence of the higher sensitivity of viscometry over LS for smaller-sized species is the lower values of M_n for the various corn varieties as determined by the two methods. It should be noted that whereas values of M_w are systematically higher for LS than viscometry, the difference is within or almost within the precision of the measurements in our laboratory. The exception is amylomaize VII, for which the M_w value determined by LS is significantly higher than that determined by viscometry.

Comparison of the corn variety R_{gz} values for LS and viscometry revealed that the R_{gz} value from viscometry is significantly lower than the LS value only in the case of amylomaize VII, which contains ~70% amylose. Because *Z*-average values are more heavily weighted toward large molecules than lower weighted average values, only relatively high concentrations of amylose, such as is found in amylomaize VII, will greatly affect R_{gz}. A comparable effect appears to explain the lower global M_w values obtained for amylomaize VII from viscometry as compared with LS methods.

It is clear that LS compared with viscosity detection is relatively insensitive to amylose in the presence of amylopectin because the molar mass of amylopectin is 30−100 times greater for amylopectin than amylose and the R_g of amylopectin is about 4−5 times greater than that of amylose. Nevertheless, LS is an absolute method for molar mass and size measurement, whereas viscosity determination of these parameters is relative and requires column calibration. At present, there are no well-characterized water-soluble standards with molar masses in excess of 800 000 and R_g values in excess of 34 nm; therefore, linear extrapolation of calibration curves at the high molar mass and size end is required to obtain these quantities for many starches when determined by HPSEC/viscometry. Comparison of M_w and R_{gz} values between LS and viscometry detection reveals that these values agree within experimental error for waxy maize, common maize, and amylomaize V. Thus, for these starches, amylose has little impact on M_w and R_{gz} values. Furthermore, agreement between LS and viscometry lends credibility to the extrapolation procedure required to obtain M_w and R_{gz} by HPSEC/viscometry. In the case of amylomaize VII, in which the concentration of amylose is sufficiently high to affect M_w and R_{gz}, HPSEC/viscometry is the method of choice because it can accurately measure amylopectin in the size and molar mass range found in these starches.

In general, HPSEC/viscometry and HPSEC/LS are complementary techniques in that the former is useful for broad distributions of molecules and the latter should be employed in cases where an absolute method of measurement is required and provided the distribution is not too broad. In cases in which the distribution is broad and it is desired to characterize the distribution by LS, the sample should be fractionated prior to column chromatography. The smaller-sized species, in this case amylose, should be concentrated and chromatographed.

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