# Solvent Effects on the Molecular Properties of Pectins

Marshall L. Fishman,*,† Hoa K. Chau,† Frank Kolpak,‡ and James Brady‡

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, and Hercules Incorporated, Research Center, 500 Hercules Road, Wilmington, Delaware 19808

Measurements revealed that LiAc/HAc buffer, when compared with other solvents, gave relatively low values of turbidity for five commercial pectins with various apparent molar masses and degrees of methyl esterification (DE). Therefore, HPSEC with on-line light scattering and viscosity detection was employed to compare LiAc/HAc buffer against $NaNO_3$ solution, a commonly used mobile phase for measuring the molecular properties of these pectins and an additional pectin prepared by microwave extraction. Microwave-extracted pectin was included in the study for its higher molar mass and DE compared with commercial pectins. Most commercial samples were more soluble and had a higher molar mass when dissolved in $NaNO_3$ than in LiAc/HAc buffer, whereas the microwave-extracted pectin was more soluble in LiAc/HAc buffer and had about the same molar mass. Furthermore, association fragments of pectin contained in samples were more dissociated by LiAc/HAc buffer than by $NaNO_3$. For the samples studied, weight-average molar masses ranged from about 41000 to 307000, weight-average intrinsic viscosities from about 0.86 to 9.76 dL/g, z-average radii of gyration from about 13 to 45 nm, and Mark−Houwink constants from about 0.62 to 0.94.

**Keywords:** *Pectin; molar mass; viscosity; radius of gyration; aggregation; solvent effects*

## INTRODUCTION

Pectin has generated a large amount of research because of its many uses and unique functional properties (*1*−*3*). Pectin functions in processed and unprocessed foods as a gelling and texturizing agent. It is a major cell wall component in most higher plants, acting as a glue to hold the cell wall together. Furthermore, pectic fragments function as chemical signals in the development, growth, senescence, and protection of plants. Blends of pectin with starch (*4*) or with poly(vinyl alcohol) (*5*) have been shown to have potential as edible and/or biodegradable films. Moreover, pectin and/or its fragments have been reported to possess pharmacological activities that include immunostimulation, antimetastasis, hypoglycemic, and cholesterol-lowering effects (*6*).

Pectin is a group of heterogeneous polysaccharides, which are mainly blocks of 1−4-linked homogalacturonan interrupted by single 1−2-linked rhamnose units (*7*). Also located in the backbone is a much smaller percentage of regions containing rhamnogalacturonans and xylogalacturonans containing xylose side chains (*8*). The homogalacturonan region is a block copolymer of galacturonic acid and its methyl ester. Often in nature, 50% or more of the uronates are esterified. Galactose and arabinose are frequently linked to the rhamnogalacturonan portion of the backbone as side chains. The analysis of commercial pectins is further complicated in that the degree of esterification (DE) often is modified during processing to obtain desired functional properties.

* Author to whom correspondence should be addressed [telephone (215) 233-6450; fax (215) 233-6559; e-mail mfishman@arserrc.gov].

† Eastern Regional Research Center.

‡ Hercules Inc., Research Center.

Numerous studies in solution have been conducted to better understand the functional properties of pectin (*9*). In the 1970s, researchers recognized the existence of pectin aggregates in solution (*10*−*12*). Determining the existence and nature of pectin aggregates in solution under nongelling conditions is important in that these aggregates might be precursors to gelation. Studying the nature of these aggregates may provide a basis for the better understanding of the mechanism of gelation. Strong evidence for the aggregation of pectin in solution comes from visualizing pectin aggregates by electron microscopy (*13*, *14*). In addition to electron microscopy, we have investigated the aggregation of pectin by membrane osmometry and end-group analysis (*15*), by high-performance size exclusion chromatography (HPSEC) with on-line viscometry detection (*16*, *17*) and with on-line light scattering (*18*), and also by infrared spectroscopy (*19*).

Recently, we (*18*, *20*) found that flash-extracting pectin from the albedo of oranges by microwave heating in a closed vessel extracted aggregates of pectin with higher molar mass and intrinsic viscosity than were obtained by conventional extraction methods. Furthermore, lengthening the time of extraction decreased the molar mass and intrinsic viscosity of the extracted pectin aggregates. Analysis of molar mass and size distributions revealed that pectin distributions were bimodal and that with increased heating time smaller asymmetric molecules were being created at the expense of larger compact molecules. This occurred because of increased degradation as a result of longer heating times.

The complex solution behavior of pectins prompted us to undertake a study to determine how solvent effects the HPSEC characterization of several citrus pectins likely to be used in the food industry. We chose the solvent that gave the lowest turbidity for HPSEC

10.1021/jf001317l CCC: $20.00 
Published on Web 08/22/2001

characterization. We compared values of molar mass, size, and intrinsic viscosity in the low-turbidity solvent with values found for pectins dissolved in $NaNO_3$. This is a commonly used solvent for the HPSEC characterization of pectin. We also included in the HPSEC studies, for comparison with the commercial samples, a pectin that was flash extracted from orange albedo because its molecular properties were somewhat different from those of the commercial pectins produced. The results of this study are presented here.

## MATERIALS AND METHODS

**Materials.** Five citrus pectins were provided by Hercules, Inc., Wilmington, DE. The DE and "apparent" weight-average molar mass ($M_w$) as determined by their intrinsic viscosity and provided by the manufacturer were as follows: sample A, DE, 69.3%, and $M_w$, 18000; sample B, DE, 1.7%, and $M_w$, 28000; sample C, DE, 54.1%, and $M_w$, 65000; sample D, DE, 3.4%, and $M_w$, 88000; and sample E, DE, 72.5%, and $M_w$, 120000. Pectins were used as received from the manufacturer. The pectin source was a mixture of lemon and lime peels. Percentage galacturonic acid was not available from the manufacturer for this particular set of samples but is typically 85−95% depending on the DE of the sample. Generally, percentage galacturonic acid increases as DE decreases. In preliminary experiments, samples were dialyzed against water and mobile phase but gave poorer light scattering results, which indicates that neutral sugars were bound to the pectin.

One pectin sample was extracted from the albedo of early Valencia oranges (EVO). The oranges were supplied by Dr. Karel Grohmann and Fred Osick of CSPL, ARS, USDA, in Winter Haven, FL. Upon arrival, the flavedo was stripped from the skin with a potato peeler, followed by removal of the albedo with a paring knife. After the albedo had been cut into small pieces, it was stored at −20 °C in sealed polyethylene bags until extraction.

Microwave heating was performed in a CEM, model MDS-2000, microwave sample preparation system. Samples were irradiated for 2.5 min with 630 W of microwave energy at a frequency of 2450 MHZ. The oven contained a circular, 360° rotating carousel, which had the capacity to hold up to 12 microwave pressure digestion vessels. One vessel was equipped with temperature- and pressure-sensing devices, which measured and controlled the temperature and pressure within the cell. Six evenly spaced cells were placed in the carousel. After 2.5 min of irradiation, the pressure in the cells was ∼20 psi above ambient and the temperature was about 110 °C. The acid-extracting solution was adjusted to pH 2 with 0.2 M HCl. Cells were loaded with 1 g of albedo dispersed in 25 mL of acid solution. After heating, samples were cooled to room temperature, filtered through miracloth, precipitated with 70% 2-propanol (IPA), and allowed to stand for 1.5 h; the floating alcohol gel was skimmed from the mother liquor. Then, the gel was washed three times each with 70 and 100% IPA.

**Selection of "Best" Solvent by Turbidity Measurements.** Initially 0.5 wt % of the commercial pectin sample was dissolved in deionized (d.i.) water to make up the stock pectin solution. Depending on the type and concentration of electrolyte finally desired, the stock pectin solution was further diluted to 0.25 wt % with d.i. water or d.i. water in combination with 4.0 M LiCl, 4.0 M NaCl, 3.5 M KCl, 0.4 M LiAc (pH 4.75), 0.4 M KAc (pH 4.4), 0.1 M $NH_4Ac$, or 2 wt % sodium hexametaphosphate (NaHMP). Equimolar acetic acid was added to all acetate salts to yield a final pH of 4.8. When LiCl, NaCl, or KCl was added in combination with d.i. water to the stock pectin solution, the d.i. water was added first followed by the chloride salt to minimize osmotic shock. Dilutions were by percent volume with an adjustable pipet, which was calibrated at time of use with pure water for each volume metered out. Turbidity measurements were in 22 mL scintillation vials (Wheaton Glass Co., Wheaton, IL). Aliquots of the stock pectin solutions were stirred vigorously for 30 s immediately prior to turbidity measurement in order to ensure that incompletely dissolved material was thoroughly mixed. Turbidity was measured with a model 966 Orbeco-Hellige portable turbidimeter calibrated with 0 and 40 nephelometric turbidity unit (NTU) standards.

**Preparation of Pectin for Chromatography.** Ten milligrams of commercial citrus pectins was dissolved in 10 mL of either 0.05 M $NaNO_3$ or a mixture of 0.15 M LiAc and 0.15 M HAc, placed in a covered beaker, and stirred slowly for ∼3.5−4 h. After the initial dissolution period, sample B in $NaNO_3$ had to be heated for an additional 5 min at 80 °C to effect complete dissolution. The acetate buffer had a pH of 4.8.

**Chromatography.** All macromolecular solutions were passed through a 0.22 $\mu$m sterile Millex-GV filter (Millipore Corp., Bedford, MA) prior to analysis. Sample injection volume was 200 $\mu$L. The mobile phase was 0.05 M $NaNO_3$ or a mixture of 0.15 M LiAc and 0.15 M HAc (LiAc/HAc), which was filtered prior to degassing with a 0.4 $\mu$m Nucleopore (Costar Corp., Cambridge, MA) or a 0.4 $\mu$m Whatman nylon membrane filter. The nominal flow rate was 0.7 mL/min. Columns were thermoregulated at 45 °C by immersing them in a water bath.

The chromatography system consisted of a model KT-35 Shodex degasser (JM Science Inc., Grand Island, NY) connected in series to either a model 1050 or model 1100 autosampler and pump (Hewlett-Packard Corp., Wilmington, DE), an in-line 0.1 $\mu$m vv Durapore membrane filter housed in a high-pressure holder (Millipore Corp.) a 15 ft stainless steel warming coil, i.d. 0.04 in., a (10 × 3.2 mm i.d.) Synchropak cartridge guard column (Micra Scientific, Northbrook, IL), three chromatography columns, a high-pressure biocompatible 0.5 $\mu$m in-line frit filter (Upchurch Scientific, Inc., Oak Harbor, WA), a model Dawn DSP multiangle laser light scattering detector (MALLS) fitted with a helium−neon laser ($\lambda = 632.8$ nm) and a K-5 flow cell (Wyatt Tech, Santa Barbara, CA), a model 100 differential pressure viscometer detector (DPV) (Viscotek Corp., Houston, TX), and a model SE 61 Shodex differential refractive index monitor (DRI). The serially placed chromatography columns were two PL-Aquagel OH-60 and one OH-40 (Polymer Labs, Amherst, MA). The exclusion limits for these columns as specified by the manufacturer for poly(ethylene glycol) are $2 \times 10^7$ and $1 \times 10^5$ g/mol, respectively. Each column is 7.5 mm i.d. × 300 mm length.

The electronic outputs from the MALLS at 90° scattering angle and DRI and DPV detectors were sent to a Viscotek model DM 400 data manager, which in turn was interfaced to a Pentium II computer containing Viscotek Trisec 3.0 GPC software. Simultaneously, the electronic outputs from the MALLS at 16 light scattering angles and the DRI were sent to an A/D board housed in the MALLS, which in turn was interfaced to a second Pentium II PC loaded with ASTRA (v. 4.72) software (Wyatt Tech), which collected and processed the data.

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 RI response. The factor to correct the Rayleigh ratio at 90° ($R_{90}$) for instrument geometry was obtained by measuring the scattering intensity of toluene at 90° and tested with pullulan standards (*21*). The responses to scattered light intensity of the photodiodes arrayed around the scattering cell at angles other than 90° were normalized to the diode at 90° with a P-50 pullulan standard. The scattering angles in degrees available for intensity measurements were 14.4, 25.9, 34.8, 42.8, 51.5, 60.0, 69.6, 79.7, 90.0, 100.3, 110.7, 121.2, 132.2, 142.5, 152.6, and 163.3. As suggested by Jeng and Balke (*22*), molar masses and radii were extracted from data fit to Debye equations. Data were found to be best fitted by linear least squares to a first-order Debye equation. All angles between numbers 4 and 18 were used in fitting the Debye equations, but closeness of line fit to the reduced excess light scattering at any particular angle was weighted. The weighting factor was based on the standard deviation of the scattering at each angle as compared to the average standard deviation of all detectors fitted. The standard deviation of the scattering intensity at the two lowest scattering angles was greatest. Generally, for these angles the

**Table 1. Turbidity[a] of Pectin Initially Dissolved in Water**

| solvent[b] | | pectin A | pectin B | pectin C | pectin D | pectin E |
|---|---|---|---|---|---|---|
| | $M_w$[c] | 18000 | 28000 | 65000 | 88000 | 120000 |
| | DE[d] | 69.3 | 1.7 | 54.1 | 3.4 | 72.5 |
| 0.08 M NaHMP | | 63 (1)[e] | 16 (3) | 26 (1) | 12 (1) | 75 (1) |
| 0.2 M LiAc | | 72 (2) | 14 (2) | 30 (2) | 14 (2) | 79 (3) |
| 0.05 M $NH_4Ac$ | | 72 (2) | 13 (1) | 30 (2) | 14 (2) | 80 (4) |
| 0.5 M NaAc | | 74 (3) | 18 (5) | 30 (2) | 38 (3) | 79 (3) |
| 0.2 M LiCl | | 78 (4) | 14 (2) | 32 (3) | 14 (3) | 79 (3) |
| 0.2 M NaCl | | 78 (4) | ppt (6) | 32 (3) | gel (4) | 78 (2) |
| 0.2 M KCl | | 80 (5) | 17 (4) | 33 (4) | ppt (5) | 79 (3) |
| water | | 86 (6) | 18 (5) | 38 (5) | 26 (3) | 81 (5) |

[a] Nephelometric turbidity units. Triplicate analyses of single solutions. [b] 0.25 wt % pectin dissolved in final solvent concentration. pH for LiAc, $NH_4Ac$, and NaAc buffers is 4.8. [c] Nominal weight average molar mass determined by viscometry. [d] Degree of methyl esterification. [e] Number in parentheses is relative ranking of pectin solubility within column.

standard deviation decreased with increasing scattering angle. The viscometer was checked with pullulan standards to ensure that intrinsic viscosities were measured accurately. The concentration of EVO pectin was obtained from the area of its DRI chromatogram. This concentration was calculated using ASTRA software by inputting the concentration dependence of the refractive index (d*n*/d*c*). A d*n*/d*c* value of 0.146 mL/g at 670 nm in $NaNO_3$ was determined using acid-extracted lime pectin as the source. This value was within experimental error of the value 0.143 mL/g determined in LiAc/HAc buffer. The method for measuring d*n*/d*c* was described previously (*21*).

## RESULTS AND DISCUSSION

**Turbidity Measurements**. In Table 1 are the turbidity measurements, which were carried out by initially dissolving 0.5% pectin in water and then diluting it to 0.25% with an equal volume of the various prospective mobile phases, which also were at twice their final concentration. It is well-known that the turbidity of a dilute macromolecular solution on the verge of precipitation is a complex function of particle size, concentration, and solution refractive index (*23*). Nevertheless, turbidity measurements often are a useful and rapid method for qualitatively comparing the relative states of solution of identical concentrations of the same macromolecule in a series of solvents. In this application, it was assumed that the solvent which gives the lowest turbidity for the dissolved macromolecule is the "best" solvent. For purposes of comparison, precipitation or gelation is considered off the high end of the turbidity scale. The solvents chosen for comparison were ones that have been used routinely over the years to perform SEC in one of our laboratories.

If one compares samples in any one solvent for the effect of DE and molar mass on turbidity (cf. turbidities along rows in Table 1), it appears that in most solvents, turbidity increases with increasing DE.

If one compares samples at constant DE and molar mass for the effect of solvent on turbidity (cf. turbidities down columns in Table 1), with a few exceptions, pectins in water have the highest turbidity. This is probably due to the tendency of pectins to aggregate in water (*16*). Numbers in parentheses give order of ranking within columns. In the case of high DE pectin, comparison of lithium, sodium, and potassium chlorides reveals that there is no appreciable difference in turbidity among the three ions. In the case of low DE pectin it appears that $Li^+$ generally gives the lowest turbidity. In this case $Na^+$ or $K^+$ may cause precipitation or gelation. Comparison of lithium acetate (LiAc) with ammonium acetate ($NH_4Ac$) revealed that in most cases both gave about the same turbidities. Nevertheless, because the concentration of LiAc was 4 times larger than that of $NH_4Ac$, it would appear that at equal concentration, ammonium ions would produce a larger turbidity than lithium ions. Furthermore, we found that $NH_4Ac$ buffer was prone to bacterial growth, whereas the LiAc buffer was not. Comparison of LiAc with LiCl revealed that both had about the same turbidity for $Ac^-$ and $Cl^-$ with the possible exception of sample A, in which $Ac^-$ was lowest in turbidity. The lowest turbidities were given by 0.08 M NaHMP. Nevertheless, we chose LiAc buffer over NaHMP because it was found that the NaHMP interferes with the tail region of the lowest molar mass samples. Furthermore, NaHMP is a cyclic polyphosphate having a poor buffering capacity. A high buffer capacity mobile phase is useful for analyzing pectins prepared under pH extremes without further addition of acid or base. On the basis of the data in Table 1, a mixture of 0.15 M LiAc and 0.15 M HAc buffer (pH 4.8) was compared with the widely used 0.05 M $NaNO_3$ to determine which was the better mobile phase for HPSEC of pectin.

**Table 2. Effect of Solvent on Percentage Recovery of Pectin**

| sample | DE[a] (%) | LiAc buffer [b] | $NaNO_3$ (0.05 M) |
|---|---|---|---|
| A | 69.3 | 92.3 (1)[c] | 97.6 (2) |
| B[d] | 1.7 | 89.3 (0.3) | 104.0 (4) |
| C | 54.1 | 89.3 (5) | 104.0 (1) |
| D | 3.4 | 86.0 (0.6) | 97.1 (3) |
| E | 72.5 | 97.1 (1) | 102.0 (1) |
| F | 91.0 | 93.2 (1) | 89.9 (1) |

[a] Degree of methyl esterification. Galacturonic acid content for commercial samples was not available from the manufacturer but is typically 85−95%. Neutral sugar content was not determined. [b] Mixture of 0.15 M LiAc and 0.15 M HAc (pH 4.8). [c] Standard deviation of triplicate measurements. [d] Heated.

**HPSEC Measurements**. Table 2 contains the percentage recoveries based on areas of DRI chromatograms for the six pectins in LiAc/HAc buffer and $NaNO_3$. Brief heating of sample B was required to completely dissolve it in $NaNO_3$. The remaining commercial citrus pectins appear to be more soluble in 0.05 M $NaNO_3$ than in buffered 0.150 M LiAc/HAc. On the other hand, sample F, the microwave-extracted pectin, appears to be slightly more soluble in LiAc/HAc than in $NaNO_3$.

The data in Table 3 reveal that the order of $M_w$ and $R_{gz}$ values for pectins as determined by HPSEC with light scattering detection approximately followed the same order as the "apparent" $M_w$ values for pectins, which were determined by capillary viscometry (*24*) (see Table 1). Nevertheless, differences between molar masses of the samples were often quite different between HPSEC/light scattering and capillary viscometry. Not

**Table 3. Effect of Solvent on Pectins**

| | | LiAc[a]/HAc (pH 4.8) | | | | $NaNO_3$ (0.05 M) | | | |
|---|---|---|---|---|---|---|---|---|---|
| sample | DE[b] (%) | $M_w \times 10^{-3}$ | $R_{gz}$ (nm) | $[\eta]_w$ (dL/g) | $a$[c] | $M_w \times 10^{-3}$ | $R_{gz}$ (nm) | $[\eta]_w$ (dL/g) | $a$[c] |
| A | 69.3 | 41.5 (8)[d] | 13.6[e] (0.4) | 0.88 (0.01) | 0.94 (0.03) | 45.3 (4) | 13.3[e] (1) | 0.86 (0.04) | 0.90 (0.01) |
| B | 1.7 | 37.1 (3) | 13.7[e] (0.5) | 1.00 (0.01) | 1.02 (0.01) | 59.7 (2) | 15.6[e] (0.4) | 1.03 (0.04) | 0.96 (0.01) |
| C | 54.1 | 86.8 (6) | 24.8[e] (0.3) | 2.88 (0.04) | 0.86 (0.02) | 114.0 (2) | 30.0 (1) | 2.78 (0.01) | 0.84 (0.04) |
| D | 3.4 | 89.7 (2) | 29.0 (5) | 2.93 (0.03) | 0.86 (0.01) | 102.0 (8) | 28.0 (2) | 2.96 (0.02) | 0.84 (0.02) |
| E | 72.5 | 179.0 (4) | 38.0 (4) | 6.08 (0.1) | 0.67 (0.02) | 232.0 (6) | 36.0 (1) | 6.41 (0.1) | 0.65 (0.04) |
| F | 91 | 307.0 (20) | 38.0 (1) | 9.71 (0.07) | 0.62 (0.01) | 280.0 (20) | 45.0 (1) | 9.76 (0.1) | 0.72 (0.02) |

[a] Mixture of 0.15 M LiAc and 0.15 M HAc. [b] Degree of methyl esterification. [c] Mark−Houwink exponent. [d] Standard deviation of triplicate measurements. [e] Determined using LS/V method.

surprisingly, trends in $[\eta]_w$ values followed more closely the order and differences in molar mass determined by capillary viscometry than by HPSEC/light scattering. Most probably the reason is that viscometric values of $M_w$ are influenced by molecular shape and charge, unlike $M_w$ values determined by HPSEC/light scattering. In the case of samples A and B, $R_{gz}$ values were obtained by the light scattering/viscometry (LS/V) method (*18*) because they were too small to be determined by MALLS.

Comparison of values in Table 3 also revealed HPSEC values of $M_w$ were significantly higher than those determined by capillary viscometry, with the possible exception of sample D dissolved in LiAc/HAc. Furthermore, samples B−E appear to have higher values of $M_w$ in $NaNO_3$ than in LiAc/HAc, whereas samples A and F had about the same values of $M_w$ in both solvents. In Figure 1, molar mass against volume curves (calibration curves) are superimposed on DRI curves for the pectins studied. The superimposed DRI chromatograms elute in the order that would be predicted by the $M_w$ values found in Table 3. In the case of pectins dissolved in $NaNO_3$ (see Figure 1a), the high molar mass portions of the calibration curves for samples A and B appear to rise faster in molar mass than comparable portions of similar curves for samples C−F. From the greater inital slopes of the samples A and B calibration curves as compared to those for samples C−F, one may deduce that there are differences in shape. Comparison of chromatograms for samples A and B dissolved in buffered LiAc/HAc (Figure 1b) with chromatograms from pectins dissolved in $NaNO_3$ (Figure 1a) reveals high molar mass fractions missing from the samples dissolved in LiAc/HAc. The higher molar mass fraction for samples A and B in $NaNO_3$ as compared to LiAc/HAc may arise from highly associated pectin fragments, which are dissociated in LiAc/HAc but not in $NaNO_3$. Comparison of the high molar mass portions of calibration curves for samples C−F in LiAc/HAc with those in $NaNO_3$ reveals a greater spread between samples C and D with E and F in LiAc/HAc than with $NaNO_3$. These results may indicate that some aggregates still present in the LiAc/HAc solution are more expanded, possibly because they are better solvated than those found in $NaNO_3$. Recently, Corredig et al. (*25*) determined values of $M_w$ and $R_{gz}$ for a high-methoxy pectin from a similar source as sample E. Both pectins were dissolved in 0.05 M $NaNO_3$. They found values of 191000 for $M_w$ and 56 nm for $R_{gz}$, whereas we obtained values of 232000 and 36 nm for $M_w$ and $R_{gz}$, respectively. For that sample, Corredig et al. (*25*) also concluded that aggregates were present in high-methoxy pectin samples. Interestingly, unlike values of $M_w$, comparison of $R_{gz}$ and $[\eta]_w$ values in Table 3 indicates only small differences in the two solvents.

For macromolecules, it has been found that $[\eta]$ is related to $M$ by the Mark−Houwink (M−H) equation (*26*).

$$[\eta] = K'M^a \quad (1)$$

The exponent $a$ can be obtained from the slopes of logarithmic plots such as found in Figure 2. The value of $a$ is a measure of the density of monomeric residues within the macromolecular envelope. Thus, compact structures such as hard spheres and globular networks have values of $a$ below 0.5, random coils in an ideal solvent have an $a$ value of 0.5, expanded coils have an $a$ value between 0.5 and 1.8, and stiff rods have an $a$ value of 1.8.

The data in Figure 2 were for pectins studied in 0.05 M $NaNO_3$ and 0.15 M LiAc/HAc buffer (pH 4.8), respectively. These plots were obtained by the LS/V method. As shown by the data in Table 4, $M_w$ values determined by the LS/V method agree within experimental error with $M_w$ values determined by MALLS. Thus, M−H plots employing $M_w$ values obtained by either method should be comparable. The $a$ values shown in Table 3 were obtained from the average slope of the plots in Figure 2. A plot of M−H values against $M_w$ values for pectins in both solvents revealed that as $M_w$ increased, $a$ values decreased (see Figure 3). A first-order linear regression on the points gave an $r^2$ value of 0.84. From these data, we conclude that pectin becomes more compact in shape as its molar mass increases. A similar result was obtained in a previous study involving a series of pectins extracted as a function of heating time from the albedo of oranges. In that study heating time was carefully controlled by microwave heating under pressure in a closed cell (*18*). The conclusion that pectins become more compact with increasing molar mass is consistent with the concave down curvature of M−H plots for the pectins being studied here (see Figure 2). Comparison of parts a and b of Figure 2 revealed that M−H plots for commercial pectins in $NaNO_3$ showed more curvature than those in LiAc/HAc buffer. This also may indicate that commercial pectins are more dissociated in LiAc/HAc than in $NaNO_3$. For the case of high $M_w$, microwaved pectin, sample F, the M−H exponent for pectin dissolved in $NaNO_3$ is 0.72 as compared to 0.62 in LiAc/HAc buffer. Therefore, it appears that this pectin dissolved in $NaNO_3$ is less compact than when dissolved in LiAc/HAc buffer. The DE of sample F is extremely high, 91%, which may mean that $NaNO_3$ solvates highly hydrophobic pectin better than LiAc/HAc.

As indicated by the changes in slope of calibration curves in Figure 1 and by the nonlinear behavior of M−H plots in Figure 2, pectins with more than one shape are likely to be present in each sample investi-

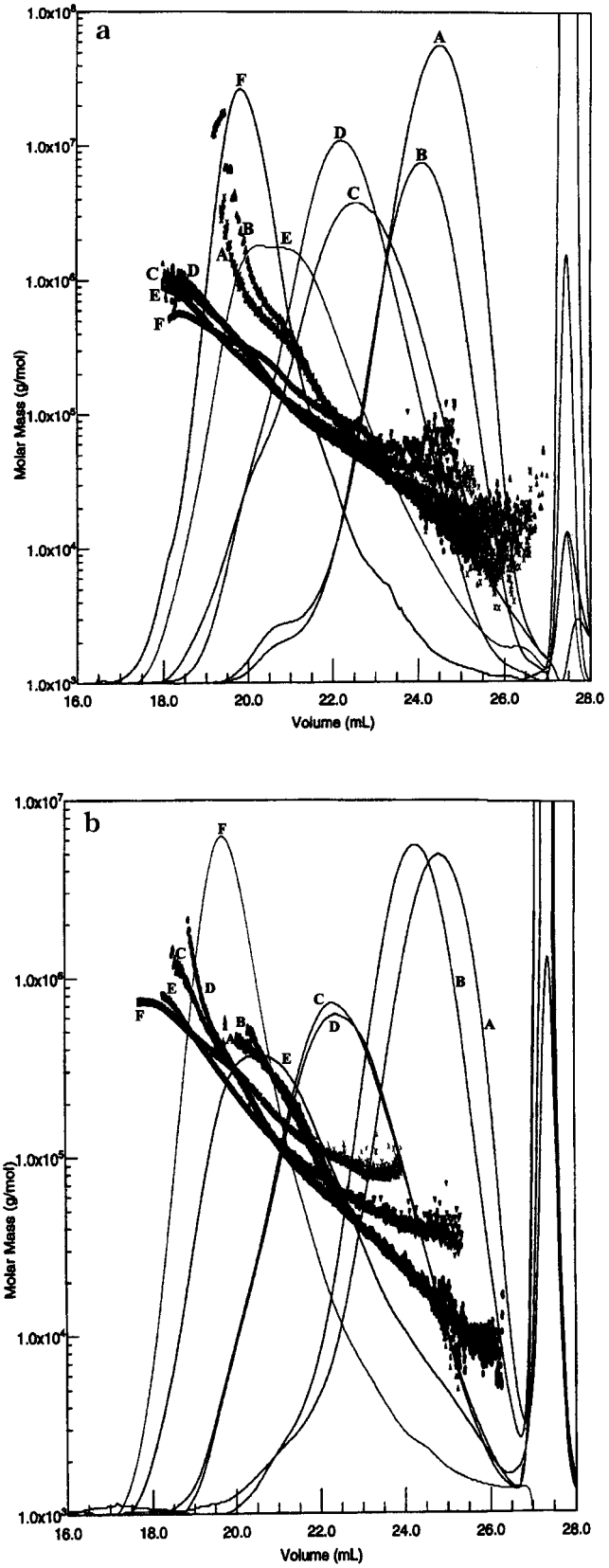


**Figure 1.** Log molar mass against elution volume (line) superimposed on DRI chromatogram (curve) for pectin samples A−F: (a) dissolved in $NaNO_3$; (b) dissolved in LiAc/HAc.

gated. This was found to be the case for pectins prepared by microwave extraction for 4 min or longer (*18*). When those samples were integrated by parts, based on bimodal SEC chromatograms detected by the intensity of light scatttered at 90°, it was found that the high

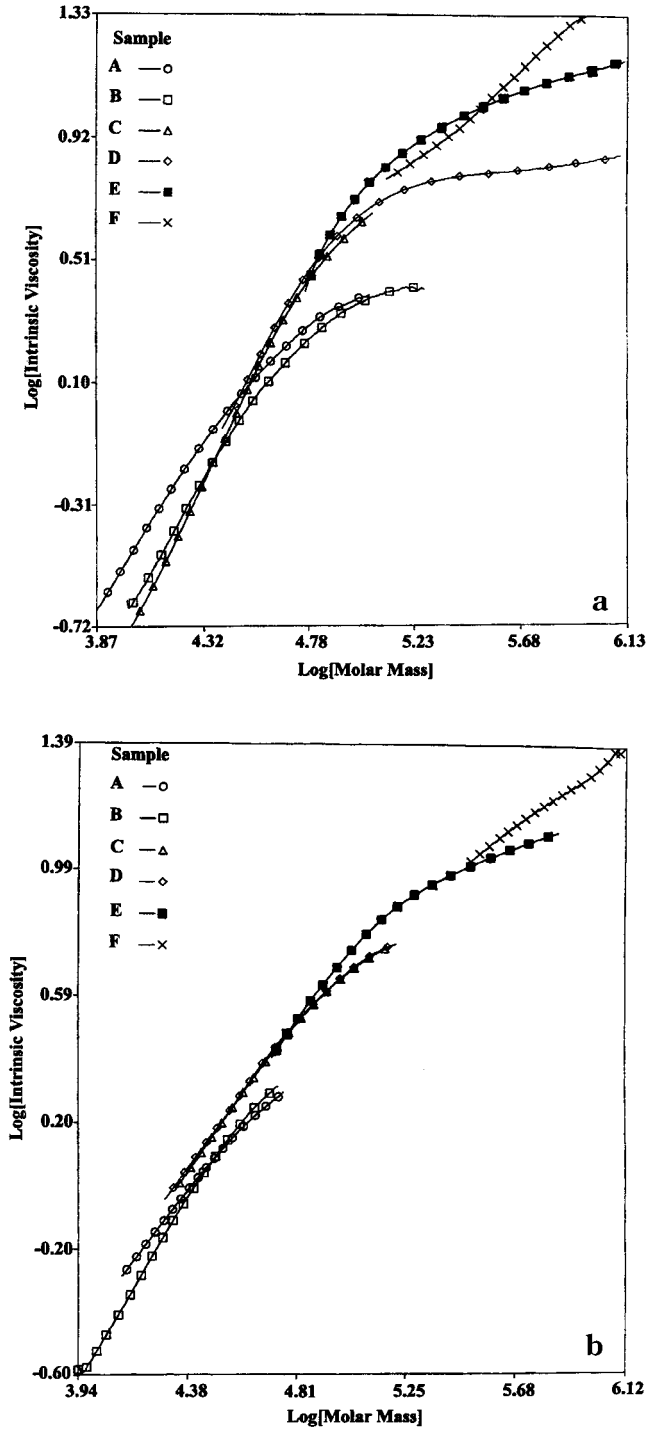


**Figure 2.** Overlaid Mark−Houwink curves for pectin samples A−F: (a) dissolved in $NaNO_3$; (b) dissolved in LiAc/HAc.

**Table 4. Comparison of Pectin Molar Masses ($M_w \times 10^{-3}$)**

| | | LiAc/HAc[a](pH 4.8) | | $NaNO_3$ (0.05 M) | |
|---|---|---|---|---|---|
| sample | DE[b] (%) | MALLS | LS/V | MALLS | LS/V |
| A | 69.3 | 41.5 (8)[c] | 40.2 (2) | 45.3 (4) | 43.1 (1) |
| B | 1.7 | 37.1 (3) | 37.6 (5) | 59.7 (2) | 55.1 (6) |
| C | 54.1 | 86.8 (6) | 83.0 (0.3) | 114.0 (2) | 104.0 (4) |
| D | 3.4 | 89.7 (2) | 85.1 (3) | 102.0 (8) | 93.8 (6) |
| E | 72.5 | 179.0 (4) | 185.0 (5) | 232.0 (6) | 222.0 (40) |
| F | 91.0 | 307.0 (20) | 341.0 (20) | 280.0 (20) | 301.0 (5) |

[a] Mixture of 0.15 M LiAc and 0.15 M HAc. [b] Degree of methyl esterification. [c] Standard deviation of triplicate measurements.

molar mass fractions had an M−H exponent of ≤0.45. The low molar mass fraction had an *a* value of ≥0.92.

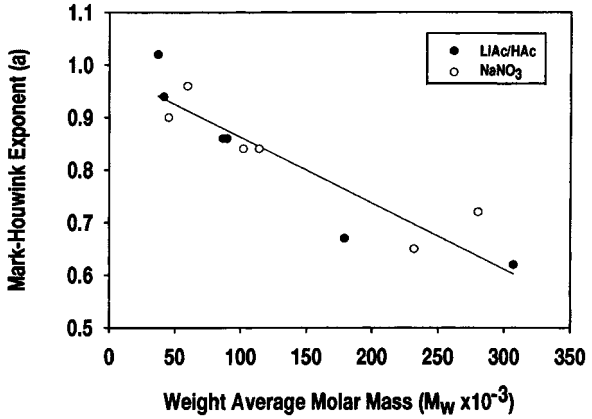


**Figure 3.** Mark−Houwink exponent plotted against weight-average molar mass for pectins dissolved in $NaNO_3$ and LiAc/HAc.

Furthermore, as $M_w$ increased, the proportion of the high molar mass fraction increased at the expense of the low molar mass fraction. The samples from this study (see Tables 5 and 6) were integrated by parts with integration limits bounded by the volumes at which the slope of the calibration curves changed.

The calibration curves of the commercial samples included in this study were approximated by three straight lines, and areas under their chromatograms were integrated by three parts. The molecular properties of these three fractions dissolved in $NaNO_3$ and LiAc/HAc are given in Tables 5 and 6, respectively. Figure 4a shows the calibration curve superimposed on the refractive index curve for sample A dissolved in $NaNO_3$, whereas Figure 4b shows how the chromatograms were integrated by parts using the information extracted from Figure 4a. In both LiAc/HAc buffer and $NaNO_3$, fraction 1 comprised ≤2 wt % of commercial samples. Fraction 1 of samples A and B dissolved in $NaNO_3$ and fraction 1 of sample A dissolved in LiAc/HAc buffer had $M_w$ values in excess of 1 million but were relatively small in $R_{gz}$ and $[\eta]_w$; $R_{gz}$ was ∼22−25 nm and $[\eta]_w$ was ∼1.8−2.8 dL/g. Fraction 1 of sample B had an $M_w$ of $\sim 5.4 \times 10^5$, an $R_{gz}$ of 22 nm, an $[\eta]_w$ of 2.3 dL/g, and an M−H exponent of 0.33. Probably, these are spherical association complexes composed of small fragments held together by weak interactions. Fraction 1 of samples C−E in both solvents had values in the ranges of $8 \times 10^5$−$11 \times 10^5$, 36−45 nm, and 7−16 dL/g for $M_w$, $R_{gz}$, and $[\eta]_w$, respectively. Sample D in $NaNO_3$ and samples C and D in LiAc/HAc had M−H exponents of 0.51, 0.50, and 0.30, respectively. Because of their larger size, and higher viscosity, these structures are probably small amounts of aggregated fragments of pectin networks. Figure 4b shows that fraction 1, although only a small portion of the wt % of sample A, contributes significantly to the light scattering signal. Furthermore, fraction 1 must be separated from the main body of the pectin if its molar mass and size are to be measured accurately. In samples A−E in both solvents, the wt % of fraction 2 increases at the expense of fraction 3 as the overall $M_w$ increases. A similar result was found by Fishman et al. (*18*) for pectins extracted by time-controlled microwave heating in a closed cell.

**Table 5. Molecular Properties of Pectin Fractions in 0.05 M $NaNO_3$**

| sample | fraction | wt % | $M_w \times 10^{-3}$ | $R_{gz}$ | $[\eta]_w$ | $a^a$ |
|---|---|---|---|---|---|---|
| A | 1 | 0.42 (0.1)[b] | 1140 (80) | 25 (1) | 2.8 (0.2) | |
| B | 1 | 0.60 (0.08) | 1940 (100) | 22 (1) | 2.4 (0.3) | |
| C | 1 | 0.91 (0.2) | 969 (70) | 41 (3) | 6.9 (0.9) | |
| D | 1 | 1.40 (0.4) | 907 (200) | 36 (3) | 6.9 (0.7) | 0.51 (0.07) |
| E | 1 | 2.00 (0.3) | 949 (50) | 45 (1) | 16.0 (2) | |
| A | 2 | 6.80 (0.4) | 274 (60) | 18 (1) | 2.3 (0.2) | |
| B | 2 | 5.20 (0.3) | 318 (20) | 20 (5) | 2.3 (0.1) | |
| C | 2 | 21.00 (1.0) | 299 (20) | 31 (1) | 5.5 (0.3) | 0.22 (0.01) |
| D | 2 | 20.40 (2.0) | 243 (30) | 30 (2) | 5.5 (0.2) | 0.17 (0.04) |
| E | 2 | 52.60 (0.5) | 347 (10) | 36 (1) | 9.4 (0.2) | 0.40 (0.04) |
| A | 3 | 92.80 (2.0) | 22 (1) | 9.63 (0.03) | 0.75 (0.03) | 1.05 (0.03) |
| B | 3 | 94.20 (1.0) | 28 (2) | 10.3 (0.1) | 0.76 (0.01) | 1.20 (0.04) |
| C | 3 | 78.10 (2.0) | 49 (2) | 16.9 (0.2) | 2.05 (0.07) | 1.29 (0.01) |
| D | 3 | 78.20 (3.0) | 51 (3) | 17.3 (0.3) | 2.21 (0.04) | 1.27 (0.6) |
| E | 3 | 45.40 (5.0) | 59 (3) | 20.6 (0.1) | 3.11 (0.1) | 1.56 (0.3) |

[a] Mark−Houwink exponent. [b] Standard deviation of triplicate measurements.

**Table 6. Molecular Properties of Pectin Fractions in LiAc/HAc Buffer[a]**

| sample | fraction | wt % | $M_w \times 10^{-3}$ | $R_{gz}$ | $[\eta]_w$ | $a^b$ |
|---|---|---|---|---|---|---|
| A | 1 | 0.54 (0.1)[c] | 1460 (200) | 23.0 (1.0) | 1.8 (0.3) | |
| B | 1 | 0.47 (0.2) | 541 (100) | 22.0 (2.0) | 2.3 (0.5) | 0.33 (0.1) |
| C | 1 | 0.69 (0.2) | 886 (400) | 38.0 (8.0) | 7.8 (3.0) | 0.50 (0.2) |
| D | 1 | 0.75 (0.1) | 1080 (100) | 37.0 (2.0) | 11.7 (1.0) | 0.30 (0.04) |
| E | 1 | 1.99 (0.2) | 809 (70) | 45.0 (1.0) | 14.6 (1.0) | |
| A | 2 | 10.2 (0.3) | 248 (10) | 22.0 (1.0) | 2.1 (0.1) | |
| B | 2 | 8.2 (0.1) | 160 (7) | 25.0 (1.0) | 2.4 (0.1) | 0.038 (0.001) |
| C | 2 | 22.5 (0.6) | 208 (10) | 29.0 (3.0) | 5.6 (0.2) | 0.23 (0.03) |
| D | 2 | 16.8 (0.1) | 237 (8) | 32.0 (2.0) | 6.1 (0.1) | 0.17 (0.02) |
| E | 2 | 45.5 (2.0) | 286 (8) | 38.0 (1.0) | 9.6 (0.2) | 0.36 (0.01) |
| A | 3 | 89.3 (1.0) | 22 (1) | 9.5 (0.5) | 0.84 (0.2) | 1.12 (0.01) |
| B | 3 | 91.3 (0.3) | 24 (1) | 10.1 (0.3) | 0.87 (0.01) | 1.21 (0.02) |
| C | 3 | 76.8 (2.0) | 44 (2) | 16.6 (0.1) | 2.2 (0.1) | 1.19 (0.04) |
| D | 3 | 82.4 (0.4) | 50 (3) | 18.5 (0.4) | 2.5 (0.1) | 1.11 (0.04) |
| E | 3 | 52.5 (2.0) | 68 (5) | 21.6 (0.4) | 3.4 (0.1) | 1.31 (0.07) |

[a] Mixture of 0.15 M LiAc and 0.15 M HAc (pH 4.8). [b] Mark−Houwink exponent. [c] Standard deviation of triplicate measurements.

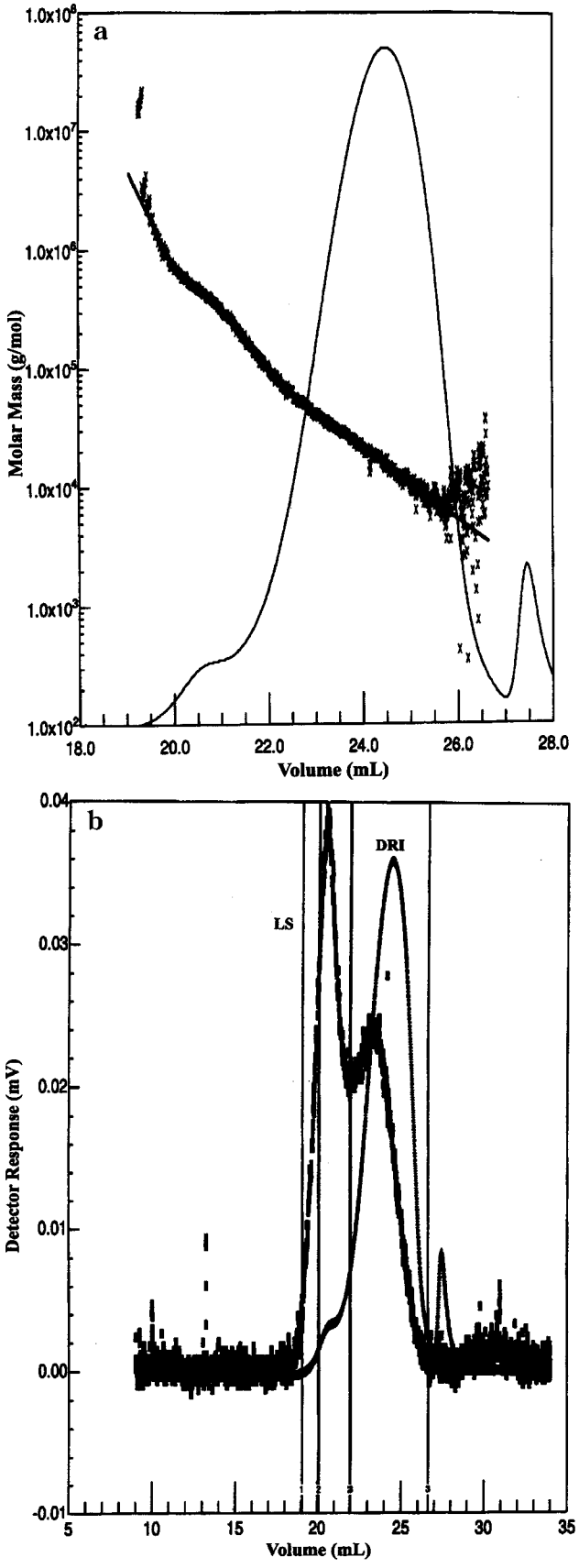


**Figure 4.** (a) Molar mass calibration curve superimposed on DRI curve for pectin sample A dissolved in 0.05 M $NaNO_3$. (b) Chromatograms for pectin sample A. Light scattering (LS) at 90 °C chromatogram superimposed on DRI chromatogram. Vertical lines indicate limits of integrations. Fraction 1 is limited by lines 1 and 2. Fraction 2 is limited by lines 2 and 3. Fraction 3 is limited by lines 3 and 3.

Comparison of global values of molecular parameters for sample F with sample E (see Table 3) revealed that sample F, the microwave-extracted sample, had higher values of $M_w$ and $[\eta]_w$ than the highest molar mass commercial sample in both solvents. It should be noted that sample F was obtained totally from orange albedo, generally considered to be an inferior source of pectin compared with the lime, lemon, or grapefruit pectin found in high-methoxy commercial pectins. Sample F in $NaNO_3$ had higher $R_g$ and $a$ values than sample F in LiAc/HAc buffer or than sample E in either solvent.

**Conclusions.** The turbidity of pectin in aqueous solution increases with DE. Turbidity measurements on a series of solvents revealed that LiAc/HAc was among those solvents which gave the lowest turbidity, whereas water generally gave the highest turbidity. Furthermore, NaCl or KCl was more likely to cause gelation or precipitation than LiCl. Comparison of the molecular properties of pectin revealed that most commercial samples were more soluble but had a higher molar mass in $NaNO_3$ than in LiAc/HAc. This result leads us to suggest that pectin is more aggregated and/or more associated in $NaNO_3$ than in LiAc/HAc and that aggregated and/or associated pectin tends to be more soluble than less aggregated and/or associated pectin. Interestingly, sample F, which had a significantly higher DE than the commercial pectins, was more soluble in LiAc/HAc than in $NaNO_3$. Perhaps this was related to smaller $Li^+$, which could better solvate the relatively small number of polar carboxyl groups than the larger $Na^+$ ions.

## LITERATURE CITED

(1) Fishman, M. L. Pectic Substances. In *Wiley Encyclopedia of Food Science and Technology*, 2nd ed.; Francis, F. L., Ed.; Wiley: New York, 2000; pp 1858−1862.

(2) Rolin, C.; Nielsen, B. U.; Glahn, P. Pectin. In *Polysaccharides: Structural Diversity and Functional Versatility*; Dumitrius, S., Ed.; Dekker: New York, 1998; pp 377−429.

(3) Voragen, A. G. J.; Pilnik, W.; Thibault, J. F.; Axelos, M. A. V.; Renard, C. M. G. C. Pectins. In *Food Polysaccharides and Their Applications*; Stephen, A. M., Ed.; Dekker: New York, 1995; pp 287−339.

(4) Fishman, M. L.; Coffin, D. R. Films fabricated from mixtures of pectin and starch. U.S. Patent 5,451,673, issued Sept 19, 1995.

(5) Coffin, D. R.; Fishman, M. L. Films fabricated from mixtures of pectin and Poly(vinyl alcohol). U.S. Patent 5,646,206, issued July 8, 1997.

(6) Yamada, H. Contribution of Pectins on Health Care. In *Pectins and Pectinases*; Visser, J., Voragen, A. G. J., Eds.; Elsevier: Amsterdam, The Netherlands, 1996; pp 173−190.

(7) BeMiller, J. N. An Introduction to Pectins: Structure and Properties. In *Chemistry and Functions of Pectins*; Fishman, M. L., Jen, J. J., Eds.; ACS Symposium Series 310; American Chemical Society: Washington, DC, 1986; pp 2−12.

(8) Schols, H. A.; Voragen, A. G. J. Complex pectins: structure elucidation using enzymes. In *Pectins and Pectinases*; Visser, J., Voragen, A. G. J., Eds.; Elsevier: Amsterdam, The Netherlands, 1996; pp 3−19.

(9) Rinaudo, M. Physicochemical properties of pectins in solution and gel states. In *Pectins and Pectinases*; Visser, J., Voragen, A. G. J., Eds.; Elsevier: Amsterdam, The Netherlands, 1996; pp 21−33.

(10) Sorochan, V. D.; Dzizenko, A. K.; Bodin, N. S.; Ovodov, Y. S. Light scattering studies on pectic substances in aqueous solution. *Carbohydr. Res.* **1971**, *20*, 243−249.

(11) Jordan, R. C.; Brant, D. A. An investigation of pectin and pectic acid in dilute aqueous solution. *Biopolymers* **1978**, *17*, 2885−2895.
(12) Davis, M. A. F.; Gidley, M. J.; Morris, E. R.; Powell, D. A.; Rees, D. A. Intermolecular association in pectin solutions. *Int. J. Macromol. Biol.* **1980**, *2*, 330−332.
(13) Hanke, D. E.; Northcote, D. H. Molecular visualization of pectin and DNA by Ruthenium Red. *Biopolymers* **1975**, *14*, 1−17.
(14) Fishman, M. L.; Cooke, P.; Hotchkiss, A.; Damert, W. Progressive dissociation of pectin. *Carbohydr. Res.* **1993**, *248*, 303−316.
(15) Fishman, M. L.; Pepper, L.; Pfeffer, P. E. Dilute solution properties of pectin. In *Water Soluble Polymers: Beauty with Performance*; Advances in Chemistry Series 213; Glass, J. E., Ed.; American Chemical Society: Washington, DC, 1986; pp 57−70.
(16) Fishman, M. L.; Gillespie, D. T.; Sondey, S. M.; Barford, R. A. Characterization of pectin by size exclusion chromatography in conjunction with viscosity detection. *J. Agric. Food Chem* **1989**, *37*, 584−591.
(17) Fishman, M. L.; Gillespie, D. T.; Sondey, S. M.; El-Atawy, Y. S. Intrinsic viscosity and molecular weight of pectin components. *Carbohydr. Res.* **1991**, *215*, 91−104.
(18) Fishman, M. L.; Chau, H. K.; Hoagland, P.; Ayyad, K. Characterization of pectin flash extracted from orange albedo by microwave heating under pressure. *Carbohydr. Res.* **2000**, *323*, 126−138.
(19) Purcell, J. M.; Fishman, M. L. Dissociation of dissolved pectin: Fourier transform infrared spectroscopy. *Carbohydr. Res.* **1987**, *159*, 185−190.
(20) Fishman, M. L.; Chau, H. K. Extraction of Pectin by Microwave Heating Under Pressure. U.S. Patent 6,143,337, issued Nov 7, 2000.
(21) Fishman, M. L.; Cescutti, P.; Fett, W. F.; Osman, S. F.; Hoagland, P. D.; Chau, H. K. Screening the physical properties of novel pseudomonas exopolysaccharides by HPSEC with multi-angle light scattering and viscosity detection. *Carbohydr. Polym.* **1997**, *32*, 213−221.
(22) Jeng, L.; Balke, S. Evaluation of light scattering detectors for size exclusion chromatography II light scattering equation selection 2. *Appl. Polym. Sci.* **1993**, *49*, 1375−1385.
(23) Hall, R. W. *The Fractionation of High Polymers in Techniques of Polymer Characterization*; Allen, P. W., Ed.; Butterworth Scientific Publications: London, U.K., 1959; pp 53−56.
(24) Christensen, P. E. Methods of grading pectin in relation to the molecular weight (intrinsic viscosity) of pectin. *Food Res.* **1954,** *19*, 163−172.
(25) Corredig, M.; Kerr, W.; Wicker, L. Molecular characterization of commercial pectins by separation with linear mix gel permeation columns in-line with multi-angle light scattering detection. *Food Hydrocolloids* **2000**, 41−47.
(26) Tanford, C. *Physical Chemistry of Macromolecules*; Wiley: New York, 1961; pp 407−411.

Received for review November 2, 2000. Revised manuscript received July 3, 2001. Accepted July 16, 2001. Mention of a brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

JF001317L