

# HPSEC with Component Analysis of Citrus and Apple Pectins After Hollow Fiber Ultrafiltration

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## ABSTRACT

Component analysis of high performance size-exclusion chromatograms was applied to  $3.3 \times$  concentration of citrus and apple pectins in dilute solution by ultrafiltration with a hollow fiber module fitted with a 100K membrane. Viscosities of the pectins in retained solutions were unchanged and for citrus pectin there was a 10% reduction of weight fraction of the largest-sized component at the last stage of concentration. A small proportion of apple pectin (radius of gyration = 7 nm) was recovered from one permeate by freeze-drying, and, when redissolved in water, produced large aggregates (z-average diam > 100 nm, measured by dynamic light scattering). Results can provide guidelines for use of the method during industrial pectin processing.

Key Words: citrus pectin, apple, hollow fiber, ultrafiltration, size-exclusion

## INTRODUCTION

COMPONENT ANALYSIS of size-exclusion chromatograms (HPSEC) has been used to characterize size and viscosity distributions of pectin, starch, and other complex polysaccharides (Fishman, et al., 1991a, b; 1993b, c; Hoagland et al., 1993a; Fishman and Hoagland, 1994). Changes in distributions of size and viscosity after plate module ultrafiltration have also been investigated with component analysis of HPSEC (Hoagland et al., 1993b). Pectin was isolated by dialysis and freeze-drying before chromatography. To avoid possible complications due to effects of dialysis and/or freeze-drying we investigated the ultrafiltration of pectin with hollow fiber membranes using low concentrations of pectin in order to permit direct injection of retentate and permeate. Our objective was to evaluate the effectiveness of component analysis to detect changes in distributions of sizes and viscosities during ultrafiltration of dilute pectin solutions. Such laboratory evaluation could then provide guidelines for the application of this analytical method under conditions of industrial pectin processing.

## MATERIALS & METHODS

### Materials

Two nonstandardized pectins for research evaluation were obtained from commercial suppliers: a citrus pectin, rapid set, 70% DE (degree of esterification) and an apple pectin, 70% DE. All chemicals were reagent grade and ultrapurified water was used for HPSEC (Hoagland et al., 1993a). Pullulan standards for universal calibration were obtained from Polymer Laboratories and dextran T-110 was a product of Pharmacia.

### Filtration

Laboratory scale hollow fiber modules (Romicon) type PM 100 and PM 500 were used with fixed starting volumes of 5L (batch operation). Operating conditions were inlet 1.8 bar; outlet 1.0 bar; and solution temperature 20°C. During filtration the solution temperature rose 5°C. Initial 5L solution concentrations were 0.025% (1250 mg) pectin in wa-

ter, in 0.01% citric acid, or 0.01% sodium nitrate. Samples (30 mL) for analysis were taken from the retentate after each 0.5L of permeate was collected.

### Size-exclusion chromatography

HPSEC was performed with the system previously described (Hoagland et al., 1993a, b). The column set consisted of a Waters u-Bondagel E-HighM, E1000, and SynChrom GPC-100 connected in series. Degassed mobile phase, 0.05M NaNO<sub>3</sub>, was pumped, with pulse dampening, through the column set at  $35 \pm 0.1^\circ\text{C}$  at a flowrate of  $0.473 \pm 0.003$  mL/min. The eluate passed through a differential pressure (DP) detector (Viscotek, Model 100) and then through a differential refractive index (RI) detector (Erma Optical Works, Model 7510). The RI detector was calibrated with dextran standards (7% moisture) (Hoagland et al., 1993a). The DP detector was tested with pullulan standards to insure that accurate specific viscosities were measured (Hoagland et al., 1993a). Pullulan standards were used to obtain universal ( $[\eta]$ Mw) and  $R_{pw}$  calibration curves;  $R_{pw}$  values for pullulan were taken from Hoagland et al. (1993a).

### Component analysis

A unique set of Gaussian curves was fitted to the combined RI and offset DP response curves (Hoagland et al., 1993a, b). In effect, the curve fitted to an observed chromatogram duplicated that chromatogram with appropriate weight fractions of six uniformly size-distributed monodisperse molecules or aggregates. These components had associated properties of size, viscosity, and molecular weight that were characteristic of the population of molecules or aggregates in the injected sample. Bandspreading precluded use of a larger number of components. Any mathematical software that implements the well established numerical procedures of Press et al. (1988) could be used. Curve fitting was done with Igor Pro by WaveMetrics on a Macintosh platform. The sigma, which determines the width at half height, for each Gaussian curve was constrained by its median (peak elution time) through an experimentally determined relationship from the set of pullulan standards. This sigma effectively represents the maximum resolution limited by bandspreading, which was a characteristic property for each column. The weight fraction of each component was obtained by dividing its RI area by the total RI areas for all components. The  $R_{pw}$  for pectin represented by each component was obtained from the pullulan calibration curve using the Gaussian median of the component to calculate its  $K_{AV}$ . The intrinsic viscosity ( $[\eta]$ ) of the pectin corresponding to each Gaussian component was calculated by dividing the specific viscosity, from the DP component, by its concentration, from the area of its associated RI component (Hoagland et al., 1993a, b). The molecular weight (Mw) of the pectin corresponding to each Gaussian component was obtained from its  $[\eta]$  and  $K_{AV}$  from the universal calibration curve of pullulan standards (Hoagland et al., 1993a, b).

### Dynamic light scattering

Pectin aggregate sizes were measured by dynamic light scattering (DLS) at 488 Å (Spectra-Physics Model 2020 argon laser) with a Malvern 4700c Sub-Micron Particle Analyzer. The data were analyzed with Malvern Automeasure software, version 4.12. Solutions of apple pectin (0.25%, 2.5 mg/mL), recovered by freeze-drying of retentate and permeate, were prepared with HPLC grade purified water and filtered through a 0.45 μm Nucleopore filter. A cylindrical quartz sample cell was used after steam cleaning with ultrapurified water. The z-average particle size for dextran T-110 was determined to provide a value for the cut-off size for a polysaccharide known to be just retained by the 100K membrane used for hollow fiber ultrafiltration.

## RESULTS & DISCUSSION

NOTE THAT A GIVEN PECTIN in solution probably exists as a collection of aggregates and molecules. The size distributions of such aggregates depend upon temperature, ionic strength, and presence of H-bond competing molecules other than water (i.e., citric acid). During HPSEC of pectin, temperature and ionic strength were held constant so that pectins subjected to different treatments before analysis could be compared. We wanted to determine how ultrafiltration of pectin under specified conditions may affect its size distribution as measured in 0.05M NaNO<sub>3</sub> by HPSEC.

Both citrus and apple pectins in dilute solution were concentrated (3.3x) by the hollow fiber ultrafiltration. During ultrafiltration of the first 3L of solution only traces of pectin were found in the permeate. The levels were well below useful signal-to-noise ratios and were estimated to be <<1% of original pectin and no precipitates were found. Based on the areas of the RI concentration chromatograms at 5L and 1.5L, 97% to 98% of original pectin was present in the final retentates. A common set of 6 RI components and 5 DP components resulted from analyses of over 100 concentration-viscosity chromatograms (Fig. 1). Concentration component 6 had an intrinsic viscosity below the practical level of detection. The radius of gyration ( $R_{gw}$ ) for the pectin represented by each component was determined from the partition coefficient and the  $R_{gw}$  calibration curve. Throughout the ultrafiltration of citrus pectin, the  $R_{gw}$  associated with each component was unchanged from starting solution. Average values for  $R_{gw}$ , including starting solutions, for all components were compared (Table 1).

The intrinsic viscosity,  $[\eta]$ , did not change for the pectin associated with each component before or during ultrafiltration (Table 2). The moderately high standard deviations for  $[\eta]$  were attributed to fluctuations in room temperature. Global values for the weight average radius of gyration ( $R_{gw}$ ) and  $[\eta]$  were obtained by summing the products of weight fractions (Tables 3, 4 and 5) and respective values from Tables 1 and 2. For citrus pectin the weight fractions for all components remained fairly uniform, and were similar to starting solution, until the last stage of ultrafiltration from 2.0 to 1.5L, at which point there was a small (~10%) decrease in weight fraction of the largest size component 1 (Table 1). A global  $R_{gw}$  of 18 nm, global  $[\eta]$  of 2.9-3.1 dL/g, and a global Mw of 79-111 kilodaltons were found for citrus pectin. The molecular weights of pectins associated with the Gaussian components were calculated using universal calibration (Table 6). For apple pectin the weight fractions for all components remained fairly uniform from start to finish of ultrafiltration (Table 5). A global  $R_{gw}$  of 14 nm, a global  $[\eta]$  of 1.5-1.6 dL/g, and global Mw of 96-99 kilodaltons were found for apple pectin.

### Citrus pectin

When concentrated with a 500K membrane, the final 0.5L permeate from citrus pectin contained 1.6% (20 mg) of the pectin that was in the 2L retentate (1235 mg) at the last stage of concentration. This permeate pectin had a much reduced global radius of gyration of 7.6 nm (retentate pectin 18.8 nm) with an  $[\eta]$  below the level of detection at this low concentration (Fig. 2). Of particular note was the 10% decrease in weight fraction of component 1 that was associated with the passage of smaller sized pectin through the membrane (Table 3). An increase in weight fraction of the largest sized component (1) might reasonably be expected.

When citrus pectin in water was concentrated with a 100K membrane the pectin in the last 0.5L permeate had a global  $R_{gw}$  of 4 nm and was 1.1% (14 mg) of the pectin in the 2L retentate (1240 mg). Clearly the 100K membrane allowed reduced passage of smaller sized pectin than did the 500K membrane above. Also there was a 12% reduction in weight fraction of large sized

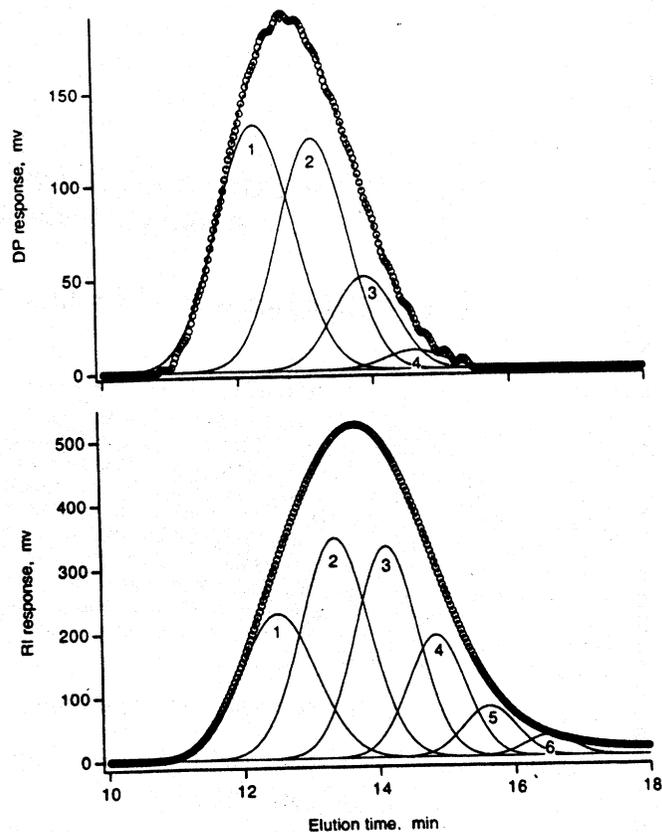


Fig. 1—HPSEC chromatogram of citrus pectin fraction in retentate after hollow fiber ultrafiltration with 100K membrane. Concentration (RI) and viscosity (excess pressure, DP) data points are circles. Gaussian components are solid lines and fitted curve, the sum of gaussian components is heavy dashed line. RI components 5 and 6 did not have an associated detectable DP response.

pectin associated with component 1 as smaller sized pectin passed through the membrane (Table 3).

When citrus pectin in 0.01% citric acid was concentrated (100K membrane) pectin in the last 0.5L permeate had a global  $R_{gw}$  of 5 nm and was 0.9% (11 mg) of that in the 2L retentate (1235 mg). Ultrafiltration in 0.01% citric acid produced the highest global  $[\eta]$ , lowest  $R_{gw}$ , and, therefore, the lowest Mw for that fraction of citrus pectin in the retentate (Tables 1, 2 and 6). There was 11% reduction in the weight fraction of large sized pectin associated with component 1 (Table 4) as smaller aggregates or molecules passed the membrane.

Ultrafiltration was also carried out with dilute pectin in 0.01% NaNO<sub>3</sub> in order to simulate closely the conditions for the injected pectin in the mobile phase. The citrus pectin retained during concentration by HFUF with a 100K membrane was not different from that retained by the 500K membrane in water. The  $R_{gw}$ ,  $[\eta]$ , and Mw of the pectin represented by each Gaussian component were essentially the same. The decrease in weight fraction of component 1 was 9% (Table 4). The permeate citrus pectin had a 9 nm component, midway between retentate components 3 and 4, that was not found in 100K-permeate pectin in water, but was found in 500K-permeate pectin in water. The two 100K-permeate pectin concentration chromatograms, with components, were compared (Fig. 3).

### Apple pectin

When apple pectin in water was concentrated with a 100K membrane the pectin in the last 0.5L permeate had a global  $R_{gw}$  of 4.6 nm (Table 1) and was 0.5% (6 mg) of the pectin in the 2L retentate (1240 mg). No apparent change in weight fraction

**Table 1**—Average  $R_{gw}$  of HPSEC components of citrus and apple pectin fractions before and after ultrafiltration

Membrane (K): Pectin: Solvent:	500 citrus water	100 citrus water	100 apple water	100 citrus 0.01% citric acid	100 citrus 0.01% NaNO <sub>3</sub>	100 apple 0.01% NaNO <sub>3</sub>
Retentate fraction Component	$R_{gw}$ , nm					
1	34.7 ± 0.8 <sup>a</sup>	35.7 ± 1.0	34.5 ± 0.4	32.3 ± 1.5	34.4 ± 1.2	33.8 ± 0.8
2	18.6 ± 0.6	19.3 ± 0.5	18.0 ± 0.1	18.4 ± 0.6	18.6 ± 0.6	17.8 ± 0.3
3	11.4 ± 0.3	11.7 ± 0.3	11.1 ± 0.0	11.3 ± 0.4	11.3 ± 0.3	11.1 ± 0.2
4	7.5 ± 0.3	7.5 ± 0.2	7.3 ± 0.0	7.3 ± 0.2	7.4 ± 0.2	7.3 ± 0.1
5	5.0 ± 0.1	5.0 ± 0.0	4.9 ± 0.0	4.9 ± 0.1	4.9 ± 0.1	4.8 ± 0.1
global	18.8 ± 1.12 <sup>a</sup>	18.9 ± 1.0	14.4 ± 0.4	17.3 ± 1.1	18.4 ± 0.7	14.4 ± 0.9
Last permeate fraction Component						
3	9.0	6.9	8.1	8.3	9.4	—
4	5.8	4.8	5.6	5.6	6.5	6.5
5	3.7	3.2	3.8	3.8	3.1	4.4
6	—	2.2	2.5	2.6	2.2	3.0
global	7.6	4.4	5.7	5.0	4.8	4.6

<sup>a</sup> Standard deviation of 16 determinations.

**Table 2**—Average intrinsic viscosities of HPSEC components of citrus and apple pectin retentate fractions before and after ultrafiltration

Membrane (K): Pectin: Solvent:	500 citrus water	100 citrus water	100 apple water	100 citrus 0.01% citric acid	100 citrus 0.01% NaNO <sub>3</sub>	100 apple 0.01% NaNO <sub>3</sub>
Component	dL/g					
1	6.40 ± 0.52 <sup>a</sup>	6.05 ± 0.38	3.95 ± 0.05	6.38 ± 0.39	6.26 ± 0.16	3.51 ± 0.36
2	2.95 ± 0.26	3.11 ± 0.30	2.41 ± 0.12	3.24 ± 0.34	3.01 ± 0.18	3.51 ± 0.36
3	1.28 ± 0.22	1.24 ± 0.24	1.09 ± 0.14	1.74 ± 0.24	1.37 ± 0.23	1.07 ± 0.15
4	0.67 ± 0.24	0.73 ± 0.28	0.59 ± 0.12	0.96 ± 0.19	0.68 ± 0.26	0.51 ± 0.20
global	2.99 ± 0.14	2.87 ± 0.13	1.64 ± 0.05	3.06 ± 0.22	2.93 ± 0.13	1.53 ± 0.13

<sup>a</sup> Standard deviation of 16 determinations

**Table 3**—Average weight fractions of components separated by concentration chromatography of retained citrus pectin fractions in water before and after final 0.5L batch and of pectin fraction in final permeate

Component	Citrus pectin, 500K <sup>a</sup> , water		Citrus pectin, 100K, water	
	Before final retentate	Final retentate	Before final retentate	Final retentate
	5 - 2L	1.5L	5 - 2L	1.5L
1	0.268 ± 0.015	0.241 ± 0.003	0.257 ± 0.015	0.225 ± 0.003
2	0.316 ± 0.014	0.322 ± 0.005	0.309 ± 0.009	0.306 ± 0.004
3	0.221 ± 0.012	0.251 ± 0.007	0.235 ± 0.007	0.260 ± 0.005
4	0.113 ± 0.010	0.119 ± 0.002	0.123 ± 0.008	0.137 ± 0.003
5	0.054 ± 0.012	0.047 ± 0.005	0.053 ± 0.007	0.053 ± 0.003
		final permeate		final permeate
3		0.407		0.218
4		0.445		0.351
5		0.147		0.233
6		—		0.197

<sup>a</sup> Size of hollow fiber membrane.

**Table 4**—Average weight fractions of components separated by concentration chromatography of retained citrus pectin in 0.01% citric acid or NaNO<sub>3</sub> before and after final 0.5 L batch and of pectin fraction in final permeate

Component	Citrus pectin, 100K <sup>a</sup> , 0.01% citric acid		Citrus pectin, 100K, 0.01% NaNO <sub>3</sub>	
	Before final retentate	Final retentate	Before final retentate	Final retentate
	5-2L	1.5L	5-2L	1.5L
1	0.243 ± 0.017	0.217 ± 0.005	0.258 ± 0.008	0.236 ± 0.006
2	0.299 ± 0.013	0.305 ± 0.004	0.310 ± 0.008	0.316 ± 0.006
3	0.236 ± 0.007	0.263 ± 0.003	0.232 ± 0.007	0.255 ± 0.002
4	0.132 ± 0.010	0.138 ± 0.004	0.121 ± 0.004	0.124 ± 0.006
5	0.062 ± 0.010	0.057 ± 0.002	0.054 ± 0.005	0.049 ± 0.004
		Final permeate		Final permeate
3		0.159		0.169
4		0.372		0.329
5		0.270		0.241
6		0.197		0.147

<sup>a</sup> Size of hollow fiber membrane.

**Table 5**—Average weight fractions of components separated by concentration chromatography of retained apple pectin in water or 0.01% NaNO<sub>3</sub> before and after final 0.5 L batch and of pectin fraction in final permeate

Component	Apple pectin, 100K <sup>a</sup> , water		Apple pectin, 100K, 0.05 M NaNO <sub>3</sub>	
	Before final retentate	Final retentate	Before final retentate	Final retentate
	5-2L	1.5L	5-2L	1.5L
1	0.138 ± 0.008	0.139 ± 0.001	0.141 ± 0.003	0.137 ± 0.002
2	0.259 ± 0.017	0.265 ± 0.006	0.252 ± 0.007	0.251 ± 0.002
3	0.305 ± 0.012	0.332 ± 0.001	0.291 ± 0.007	0.305 ± 0.001
4	0.180 ± 0.013	0.173 ± 0.006	0.186 ± 0.004	0.193 ± 0.001
5	0.082 ± 0.013	0.065 ± 0.001	0.091 ± 0.008	0.082 ± 0.001
		Final permeate		Final permeate
3		0.166		—
4		0.373		0.281
5		0.290		0.434
6		0.170		0.281

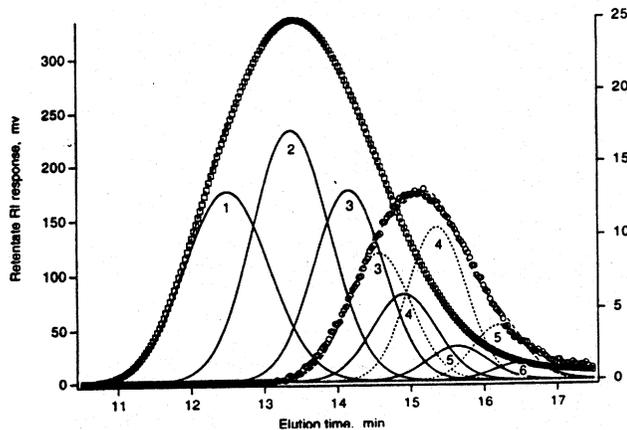
<sup>a</sup> Size of hollow fiber membrane.

**Table 6**—Calculated average molecular weights of pectin associated with components separated by concentration chromatography of retained pectin

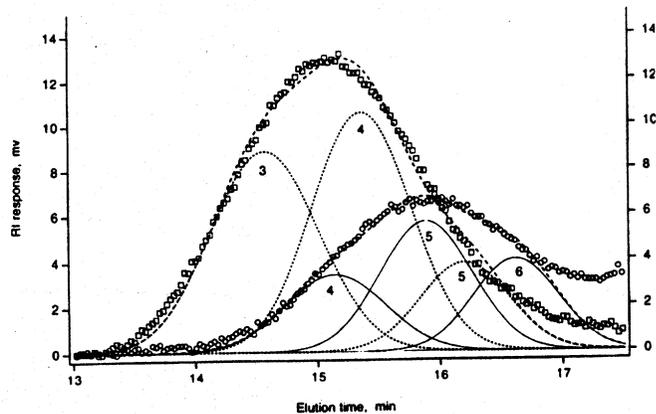
Pectin	component MW, Kdaltons					Global
	1	2	3	4	5	
Citrus, water <sup>b</sup>	245 ± 16	82.9 ± 7.3	42.5 ± 3.5	22.3 ± 2.2	1.49 ± 0.10	103.0 ± 7.7
Water	281 ± 23	86.9 ± 7.2	47.5 ± 3.9	21.1 ± 1.7	1.53 ± 0.07	111.3 ± 9.2
Citric acid	198 ± 26	72.3 ± 7.1	30.7 ± 3.1	14.6 ± 1.4	1.36 ± 0.08	78.7 ± 9.2
NaNO <sub>3</sub>	242 ± 24	80.2 ± 7.6	38.9 ± 3.4	21.0 ± 1.6	1.43 ± 0.09	98.6 ± 9.4
Apple, water	411 ± 29	89.0 ± 4.8	46.8 ± 2.3	27.3 ± 1.2	2.63 ± 0.06	99.3 ± 6.2
NaNO <sub>3</sub>	388 ± 12	91.7 ± 1.7	46.8 ± 0.7	23.6 ± 0.4	2.51 ± 0.04	95.7 ± 2.4

<sup>a</sup>Values obtained from universal calibration.

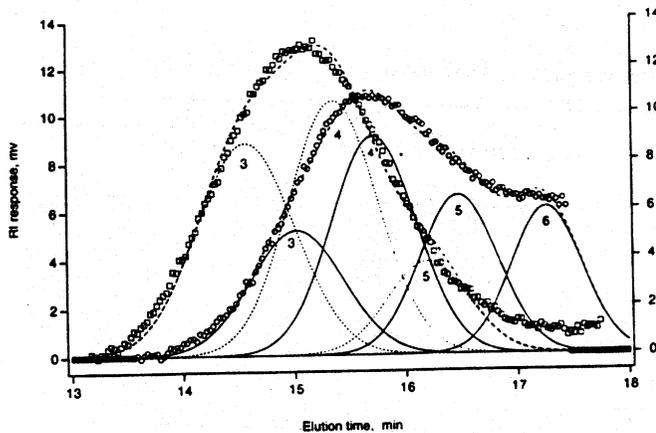
<sup>b</sup>500K membrane.



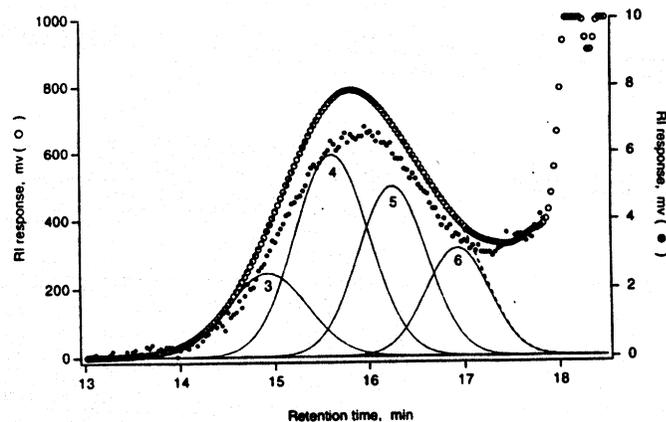
**Fig. 2**—HPSE concentration chromatograms of citrus pectin fraction after hollow fiber ultrafiltration in water with a 500K membrane. Concentration data points: retentate, squares; permeate, circles. Gaussian components: retentate, shaded; permeate, dashed lines. Fitted curve, the sum of gaussian components, is the heavy dashed line. Component numbers key to physical properties in Tables 1,2,3, and 6.



**Fig. 4**—HPSE concentration chromatograms of permeate pectin fraction in 0.01% NaNO<sub>3</sub> after HFUF with 100K membrane. Data points: citrus, squares; apple, circles. Gaussian components: citrus, dashed lines; apple, solid lines. Fitted curve, the sum of gaussian components, is heavy dashed line. Analysis cut off at 17 minutes.



**Fig. 3**—HPSE Concentration chromatogram of permeate citrus pectin fractions in water. Data Points: 500K membrane, squares; 100K membrane, circles. Gaussian components: 500K, dashed lines; 100K, solid lines. Fitted curve, the sum of gaussian components, is heavy dashed line. Component numbers key to physical properties in Tables 1,2,3, and 6.



**Fig. 5**—HPSE concentration chromatograms of apple pectin fraction in final water permeate (solid circles) and recovered from freeze-dried permeate (open circles) after HFUF. Components (3 to 6) for recovered pectin are dashed lines and fitted curve, the sum of gaussian components, is heavy dashed line through open circles.

of component 1 pectin occurred (Table 5). For apple pectin in 0.01% NaNO<sub>3</sub>, a global  $R_{gw}$  of 6 nm was obtained for the permeate pectin fraction that was 2.7% (33 mg) of the pectin in the 2L of retentate (1235 mg). Concentration chromatograms, with components, for final permeate apple and citrus pectins in dilute NaNO<sub>3</sub> were compared (Fig. 4). The permeate citrus pectin fraction was present at higher concentration and larger size than the permeate apple pectin fraction.

Apple pectin was recovered from the water permeate by freeze-drying. Its concentration chromatogram in 0.05M NaNO<sub>3</sub>,

with components (Fig. 5) was compared with a chromatogram of original permeate. The pectin concentration in the permeate chromatogram after direct injection was about 100 times more dilute than the solution (2.5 mg/mL) prepared with the pectin fraction recovered from the permeate. The similarity of the two chromatograms and associated Gaussian components suggests that freeze-drying did not alter size ( $R_{gw}$ ) or  $[\eta]$  of this small sized pectin fraction. This also suggests that the earlier study of pectin behavior during plate module ultrafiltration was not altered by freeze-drying (Hoagland et al., 1993b). However, when this freeze-dried fraction was dissolved in pure water, dynamic light scattering (DLS) revealed large aggregates that scattered readily detectable amounts of light.

In addition, apple pectin was recovered from the final water retentate by freeze-drying and was also examined, in pure water, by DLS. Water solutions of dextran T-110 were used as non-aggregating polysaccharide controls (Table 7). The z-average particle diameter for dextran T-110, ca 16 nm, was about two-fold greater than its  $R_{gw}$ , ca 8.5 nm. The z-average aggregate diameters for both retentate and permeate recovered apple pectins in water were  $\approx 10$ -20 times larger than corresponding  $R_{gw}$  for the same fractions in 0.05M NaNO<sub>3</sub>. Furthermore, the marked dependence of aggregate size on angle of scattered light (Table 6) is characteristic of large, possibly asymmetric structures.

### Aggregation

Many studies support the hypothesis that pectin in aqueous, low-ionic strength solution exists as aggregates that resist dialysis, ultrafiltration, and size exclusion chromatography (Fishman et al., 1984; Mort et al., 1991; Hoagland et al., 1993b). Results from hollow fiber ultrafiltration of citrus and apple pectins can be explained in terms of such an aggregating system. First, there was high recovery (>95%) of citrus pectin concentrated in water with a 500K membrane. The membrane allows passage of  $R_{gw}$  material <20 nm. This indicates that pectin associated with components 3, 4, and 5 ( $R_{gw}$ s >20 nm in 0.05M NaNO<sub>3</sub>) in water was retained because of aggregation. Second, aggregation in water was supported by the fact that the largest component of permeate pectins in 0.05M NaNO<sub>3</sub> had a much lower  $R_{gw}$  (9 nm) than the ca 20 nm retention limit of the 500K membrane. If pectin were present only as monomolecular species, passage of smaller sized pectin into the permeate would increase the weight fraction of component 1.

With citrus pectin, in each of the four ultrafiltrations, from 5L to 2L of retentate, the weight fraction of component 1 was unchanged. Thereafter, from 2L to 1.5L the weight fraction of this largest component decreased by ca 10%. Furthermore, an increase in global  $[\eta]$  should have been measured, but none was found. Similar behavior was observed during plate module ultrafiltration of citrus and lime pectins (Hoagland et al., 1993a). This could be explained if large aggregates of pectin in water were destabilized during ultrafiltration and some small sized (i.e.,  $R_{gw}$  <20 nm) pectin species were released and passed through the membrane. Removal of such smaller species of pectin would not improve the functional bulk properties in solution because they were involved in stabilization of the largest aggregates of pectin. The low 0.01% levels of citric acid or NaNO<sub>3</sub> used for three of the hollow fiber ultrafiltrations did not affect the recovery or bulk properties of the retained citrus or apple pectins. Extensive dissociation of pectin aggregates (Fishman et al., 1992, 1993a) appears to require higher levels of salt than ca 0.4%. However, citric acid appeared to have small effects on component  $R_{gw}$ ,  $[\eta]$ , and Mw. The small diminishment of these properties of pectin corresponding to Gaussian components in Tables 1, 2, and 6 may have resulted from some disruption of H-bonds and/or chelation of traces of polyvalent cations (such as Ca<sup>++</sup>) that may be present in commercial pectins.

The marked difference between apple and citrus pectin was the global  $[\eta]$  of 1.5 and 3.0 dL/g, respectively. This difference was largely due to the  $[\eta]$  of component 1 pectin, which for apple was nearly 50% the 6 dL/g value for citrus. The weight fractions of components 1 and 2 were also lower than those for citrus pectin. However, since the  $R_{gw}$  of the pectin associated with component 1 for apple pectin and citrus pectin was the same, the apparent molecular weight of this fraction of apple pectin ( $\approx 400K$ ) had to be much larger than that of citrus pectin ( $\approx 250K$ , Table 6).

The basic secondary structure for pectin is probably that of a segmented rod (Rees and Wight, 1971; Chapman et al., 1987; Fishman et al., 1992, 1993a). Each segment is a region of methyl esterified homogalacturonan, joined by single rhamnopyranoside units, which may have an attached, short neutral sugar side

Table 7—Dynamic light scattering aggregate sizes for apple pectin fractions recovered from final permeate and retentate and dissolved in water,  $R_{gw}$  values for respective pectin-fractions from HPSEC, and corresponding values for dextran T-100, which did not aggregate in water

Angle	z-Average diameter, nm		
	Retentate <sup>a</sup>	Permeate <sup>a</sup>	Dextran T-110 <sup>b</sup>
150	246 ± 4	118 ± 1	15.8
120	235 ± 11	122 ± 11	16.1
90	283 ± 5	194 ± 25	15.8 ± 0.2
60	303 ± 18	266 ± 31	14.9
	----- $R_{gw}$ <sup>c</sup> , nm -----		
HPSEC	14.4 ± 0.4	4.6	8.5 ± 0.2

<sup>a</sup> Freeze-dried apple pectin redissolved in water 0.25 mg/mL.

<sup>b</sup> Dextran T-110, 2.7 mg/mL 0.05M NaNO<sub>3</sub>.

<sup>c</sup> 0.05M NaNO<sub>3</sub>.

chain. The average length of homogalacturonan segment appears to vary with both plant source and region of plant cell wall, and ranges from 25 to 100 units. Longer homogalacturonan segments would imply a lower content of rhamnose.

Aggregation of pectin is hypothesized to involve segment-segment H-bonds (Rees, 1982) and methyl-methyl hydrophobic interactions (Oakenfull and Scott, 1984). Intermolecular segment-segment interactions have been called junction zones (Rees and Wight, 1971) and they confer on pectin aggregates the characteristic extended, kinked structures observed in electron micrographs (Fishman et al., 1992, 1993a; McCann et al., 1992). The major difference between component 1 pectin for apple and citrus may be that the apple pectin had shorter segments, with correspondingly more kinks, than did the citrus pectin. The global weight average molecular weights for citrus and apple pectins were nearly the same ( $\approx 100K$ ) as found by Anger and Berth (1986) for citrus pectin by light scattering and by Harding (1995) using sedimentation analysis. Fishman et al. (1991a, b) showed that those pectins with >90% galacturonic acid (pomegranate, grapefruit, and garlic skin) had a global intrinsic viscosity and radius of gyration inversely related to rhamnose content. This relationship further suggests that the physical properties of pectin aggregates may relate highly to the average length of homogalacturonan regions involved in junction zones. Low levels of rhamnagalacturonan, associated with 'hairy' regions probably affect the stability of some aggregates (Renard et al., 1995).

During extraction of pectin from any plant cell wall with calcium ion chelators or mild base some disaggregation of pectin probably is induced. Further purification and fractionation that occurs in commercial processing of pectin would result in further disaggregation. The pectins in our permeates were unable to form aggregates as large as those pectins represented by component 1 or 2 in the retentate. This suggests that some large molecular weight species of pectin probably with long, extended homogalacturonan segments, are required to form stable aggregates with smaller sized pectin species. However, aggregation of small sized apple pectin recovered from the final permeate of hollow fiber ultrafiltration occurred in water as determined by DLS (Table 7). The observed z-average aggregate diameters (Table 7) could only be accounted for by aggregation of apple pectin recovered from retentate or permeate. In this instance, the larger aggregates of the permeate pectin fraction water were still smaller than those for the corresponding retentate fraction.

### CONCLUSIONS

COMPONENT ANALYSIS of HPSEC can be used to follow changes in distributions of size and viscosity of pectin in dilute solution during hollow fiber ultrafiltration. This method, which can use direct injection of a solution, has potential for measuring changes in distributions of size and viscosity of pectin during processing. Concentration of citrus pectin resulted in about 10% reduction of the weight fraction of the largest component. Apparently, the small sized pectin fraction that passed through the

membrane during concentration had an effect on the stabilization of large aggregates. The fraction of small-sized apple pectin recovered from the permeate could form large aggregates in water with z-average diameters >100 nm.

## REFERENCES

- Anger, H. and Berth, G. 1986. Gel permeation chromatography and the Mark-Houwink relation for pectins with different degrees of esterification. *Carbohydr. Polym.* 6: 193-202.
- Chapman, H.D., Morris, V.J., Selvendran, R.R., and O'Neill, M.A. 1987. Static and dynamic light-scattering studies of pectic polysaccharides from the middle lamellae and primary cell walls of cider apples. *Carbohydr. Res.* 165: 53-68.
- Fishman, M.L., Pfeffer, P.E., Barford, R.A., and Doner, L.W. 1984. Studies of pectin solution properties by high performance size exclusion chromatography. *J. Agric. Food Chem.* 32: 372-378.
- Fishman, M.L., El-Atawy, Y.S., Sondey, S.M., Gillespie, D.T., and Hicks, K.B. 1991a. Component and global average radii of gyration of pectins from various sources. *Carbohydr. Polym.* 15: 89-104.
- Fishman, M.L., Gillespie, D.T., Sondey, S.M., and El-Atawy, Y.S. 1991b. Intrinsic viscosity and molecular weight of pectin components. *Carbohydr. Res.* 215: 91-104.
- Fishman, M.L., Cooke, P., Levaj, B., Gillespie, D.T., Sondey, S.M., and Scorza, R. 1992. Pectin microgels and their subunit structure. *Arch. Biochem. Biophys.* 294: 253-260.
- Fishman, M.L., Cooke, P., Hotchkiss, A., and Damert, W. 1993a. Progressive dissociation of pectin. *Carbohydr. Res.* 248: 303-316.
- Fishman, M.L., Gillespie, D.T., and Levaj, B. 1993b. Structural analysis of aggregated polysaccharides by HPSEC/viscometry. In *Chromatography of Polymers: Characterization By SEC and FFF*, T. Provder (Ed.), ACS Symposium Series No. 521, p. 314-325. American Chemical Society, Washington, DC.
- Fishman, M.L., Levaj, B., Gillespie, D.T., and Scorza, R. 1993c. Changes in the physico-chemical properties of peach fruit pectin during on-tree ripening and storage. *J. Amer. Soc. Hort. Sci.* 118: 343-349.
- Fishman, M.L. and Hoagland, P.D. 1994. Characterization of starches dissolved in water by microwave heating in a high pressure vessel. *Carbohydr. Polym.* 23: 175-183.
- Harding, S.E. 1995. Some recent developments in the size and shape analysis of industrial polysaccharides in solution using sedimentation analysis in the analytical ultracentrifuge. *Carbohydr. Polym.* 28: 227-237.
- Hoagland, P.D., Fishman, M.L., Konja, G., and Clauss, E. 1993a. Size exclusion chromatography with viscosity detection of complex polysaccharide component analysis. *J. Agric. Food Chem.* 41: 1274-1281.
- Hoagland, P.D., Konja, G., and Fishman, M.L. 1993b. Component analysis of disaggregation of pectin during plate module ultrafiltration. *J. Food Sci.* 58: 680-687.
- McCann, M.C., Wells, B., and Roberts, K. 1992. Complexity in the spatial localization and length distribution of plant cell-wall matrix polysaccharides. *J. Microsc.* 166: 123-126.
- Mort, A.J., Moerschbacher, B.M., Pierce, M.L., and Maness, N.O. 1991. Problems encountered during the extraction, purification, and chromatography of pectin fragments, and some solutions to them. *Carbohydr. Res.* 215: 219-227.
- Oakenfull, D. and Scott, A. 1984. Hydrophobic interaction in the gelation of high methoxyl pectins. *J. Food Sci.* 49: 1093-1098.
- Press, W.H., Flannery, B.P., Teukolsky, S.A., and Vetterlin, W.T. 1988. *Numerical Recipes in C*. Cambridge University Press, New York.
- Rees, D.A. 1982. Polysaccharide conformation in solutions and gels—Recent results on pectins. *Carbohydr. Polym.* 2: 254-263.
- Rees, D.A. and Wight, A.W. 1971. Polysaccharide conformation. Part VI. Model building computations for  $\alpha$ -1,4-galacturonan and the kinking function of L-rhamnose residues in pectic substances. *J. Chem. Soc.* 1971: 1366-1372.
- Renard, C.M.G.C., Crépeau, M.-J., and Thibault, J.-F. 1995. Structure of the repeating units in the rhamnogalacturonic backbone of apple, beet and citrus pectins. *Carbohydr. Res.* 275: 155-165.

Ms received 3/10/96; revised 8/2/96; accepted 8/13/96.