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Contribution of the side branches to rheological properties of pectins

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The rheological properties of apple pectins with different degrees of side branches were investigated in terms of the zero-shear viscosity (η_0), the shear rate dependence of viscosity and the superposability of rheological data to a variety of generalization curves such as η_{sp} *versus* $c[\eta]$, η_{sp} *versus* cM_w, η/η_0 *versus* $\dot{\gamma}/\dot{\gamma}_{0.8}$ and η/η_0 *versus* $\dot{\gamma}(\eta_0-\eta_s)M_w/cRT$. Increase in branching of pectins resulted in higher η_0 and increasing shear rate dependence of viscosity. The gradients of $\eta_{sp,0}$ *versus* $c[\eta]$ in the concentrated region ($c[\eta] > c^*[\eta]$) were dependent upon the degree of branching, i.e. the higher the branching, the higher the gradients, whereas there was no significant difference in the dilute region ($c[\eta] < c^*[\eta]$) irrespective of the degree of branching. Pectins with a low degree of branching displayed better superposition in η/η_0 *versus* $\dot{\gamma}/\dot{\gamma}_{0.8}$ and η/η_0 *versus* $\dot{\gamma}(\eta_0-\eta_s)M_w/cRT$ curves when compared to the sample with more branches. Circular dichroism (CD) studies of pectins showed that the conformation of pectin molecules was not affected by the degree of branching. It is concluded that side branches of pectins can result in significant entanglements in concentrated solutions.

INTRODUCTION

Pectin molecules can be divided into two distinct regions: the homogalacturonan 'smooth region' and the rhamnogalacturonan 'hairy region' due to the covalently attached neutral side branches (DeVries *et al.*, 1982, 1983). Attached to the main backbone are the side branches consisting mainly of D-galactose, L-arabinose, D-xylose and less frequently D-mannose, L-fucose, D-glucose, and the rather rare sugars 2-*o*-methyl-D-xylose, 2-*o*-methyl-L-fucose and D-apiose, primarily through C-4 of the rhamnosyl residues, although substitution of the galacturonyl residues at either C-2 or C-3 is also found (Talmadge *et al.*, 1973; Selvendran *et al.*, 1987; Olson *et al.*, 1987; Bacic *et al.*, 1988). D-galactose and L-arabinose are frequently found as galactan, arabinan and arabinogalactan (Aspinall, 1980; McNeil *et al.*, 1984). Accordingly, heterogeneity and significant dimension are key features of side branches of pectins.

Branching significantly affects a variety of physicochemical properties and the processibility of synthetic polymers (Han, 1976; Nielsen, 1977; Rokudai, 1979; Cogswell, 1981; Graessley, 1984; Roovers, 1985). The entanglements of side branches in concentrated polymer melts have been found to be the primary mechanism influencing the unique rheological properties of branched polymers (Graessley, 1977). The effects of branching on rheology have been extensively reviewed by several authors (Small, 1975; Bywater, 1979; Graessley, 1982; Roovers, 1985; Douglas *et al.*, 1990; Hwang & Kokini, 1991).

Side branches, either natural or artificial, are also present in carbohydrate polymers. Side branches occurring naturally are found in starch (amylopectin), galactomannans, pectin, gum acacia and microbial polysaccharides such as xanthan, wellan and scleroglucan gums. Side branches can also be artificially incorporated into linear carbohydrate polymers to improve functional properties. Typical examples are a variety of modified amyloses (Wurzburg, 1986) and celluloses (Glicksman, 1986) generated by grafting. The presence of side branches significantly affects such functional properties as solubility, gelling, gelatiniz-

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ation, retrogradation, freeze-thaw stability, film formation, interaction properties and rheological properties of carbohydrate polymers (Dea, 1987; Hwang & Kokini, 1991). It is believed that the side branches affect these functionalities through the topological inhibition of intermolecular association.

The effects of branching on rheological properties have been extensively investigated in synthetic polymers, whereas studies on carbohydrate polymers are scarce (Hwang & Kokini, 1991). This may be attributed to the small size of side branches found in most carbohydrate polymers. This research aims at elucidating the branching role in the rheological properties and conformation of apple pectins. Pectins posses a high concentration of side branches consisting of neutral polysaccharides (Selvendran, 1983; John & Dey, 1986; Bacic *et al.*, 1988). It would therefore be of value to understand the significance of side branches on rheological properties.

MATERIALS AND METHODS

Preparation of pectins with different degrees of side branches

Apple pectins used in this research were purchased from Sigma Chemical Co. (St. Louis, MO). Two different metal precipitation techniques were employed to eliminate the contaminating free neutral polysaccharides and to obtain pectin samples having different degrees of the side branches. These will be referred to as pectin samples I and II.

a. Sample I

Pectin sample I was prepared by the Cu(II) precipitation and 1·0% (v/v) acid alcohol washing of Michel *et al.* (1981) with modification. One liter of 1·0% (w/v) pectin solution was mixed dropwise with 100 ml of 7·0% (w/v) $CuSO_4.5H_2O$, which was then filtered through Miracloth (Calbiochem Corporation, La Jolla, CA) and washed extensively with 10 liters of distilled, deionized water to remove the contaminating free neutral polysaccharides. Then, the complexes between Cu(II) ions and pectins were dispersed for 30 min in 60% (v/v) isopropanol containing 1·0% (v/v) HCl, which was filtered through Miracloth to remove Cu(II) ions. The acid alcohol washing was repeated four times. The remaining pectins were washed with 60% (v/v) isopropanol, until the filtrate was free of chloride ions as demonstrated through the use of silver nitrate. The resulting pectins were finally washed with 100% (v/v) isopropanol. After evaporating isopropanol for 12 h at ambient conditions, pectin samples were dissolved in 1 liter of distilled, deionized water and freeze-dried.

b. Sample II

Pectin sample II was prepared by Cu(II) precipitation and the EDTA treatment of Rombouts and Thibault (1986) with modification. The Cu(II) and pectin complexes prepared as sample I were dispersed in 500 ml of 2·5% (w/v) EDTA solution for 30 min to extract Cu(II) ions from the Cu(II) and pectin complexes. The Cu(II) and EDTA complexes formed hereby were eliminated by dialyzing against 50 mM of sodium acetate buffer (pH 4·8) and distilled, deionized water for 3 days. Then, pectins were precipitated with isopropanol. After evaporating isopropanol for 12 h at ambient conditions, the resulting pectins were dissolved in 1 liter of distilled, deionized water and freeze-dried.

Chemical characterization of pectins

The anhydrogalacturonic acid (AGA; mol. wt = 176) content of pectins was determined colorimetrically by the *m*-hydroxydiphenyl method of Blumenkrantz and Asboe-Hansen (1973). The methoxyl ($—OCH_3$; mol. wt = 31) content of pectins was measured from methanol liberated on alkaline saponification of pectins according to the method of Klavons and Bennett (1986). Alcohol oxidase (alcohol:oxygen oxidoreductase; EC 1.1.3.13.; Sigma Chemical Co., St. Louis, MO) produced from *Pichia pastoris* was employed to convert the released methanol to formaldehyde, which was then colorimetrically assayed to determine the methoxyl content of pectins. From both AGA and methoxyl content as determined above, degree of esterification (DE) was calculated by the following equation (Schultz, 1965).

$$\mathrm{DE}\,(\%) = \frac{176 \times \text{methoxyl content}\,(\%\,(\text{w/w}))}{31 \times \text{AGA content}\,(\%\,(\text{w/w}))} \times 100 \qquad (1)$$

where 176 and 31 are the molecular weights of AGA and methoxyl, respectively. The neutral sugar constituents of pectins were derivatized to alditol acetates according to the method of Blakeney *et al.* (1983), which were then identified and quantitated by gas chromatography (GC).

Determination of molecular weight of pectins

Weight average molecular weight (M_w) was measured using low-angle laser light scattering (LALLS). Specific refractive index increment or differential refractive index (dn/dc, where n is the defractive index increment and c is concentration of solute) was measured at 25°C by using the Chromatix KMX-16 laser differential refractometer (LDC/Milton Roy, Rivieva Beach, FL). For a pectin sample, five different concentrations ranging from 1·0 to 5·0 mg/ml were prepared by dissolving pectin samples in 50 mM citrate phosphate buffer (pH 4·5) for 4 h. All pectin solutions were filtered through a 0·22 μm Millipore filter (Millipore Products Division, Bedford, MA) for sample clarification. On the other hand, the Chromatix KMX-6 (LDC/Milton Roy) was used to measure the Rayleigh factor (R_θ) for solvent and samples. For a pectin sample, five different concentrations ranging from 0·1 to 0·5 mg/ml were prepared by dissolving pectin samples in 50 mM citrate phosphate buffer (pH 4·5) for 4 h. Then, the pectin solutions were ultracentrifuged at 120 000 × *g* for 4 h to remove the microgel. Prior to measurement, all pectin solutions were double-filtered through a 0·22 μm Millipore filter (Millipore Products Division, Bedford, MA) for sample clarification.

The following equations allow measurement of the weight average molecular weight:

$$\frac{Kc}{\bar{R}_\theta} = \frac{1}{M_w} + 2A_2c \tag{2}$$

$$K = \left(\frac{2\pi^2}{\lambda_0^4 N}\right)(1 + \cos^2\theta)\, n^2 \left(\frac{\mathrm{d}n}{\mathrm{d}c}\right)^2 \tag{3}$$

$$\bar{R}_\theta = R_{\theta,\,\mathrm{solution}} - R_{\theta,\,\mathrm{solvent}} \tag{4}$$

where c is the concentration, $\bar{R}_\theta$ is the excess Rayleigh factor at the scattering angle θ, λ_0 is the incident beam wavelength, K is a calibration constant obtained with sodium chloride solutions, N is the Avogadro's number, n is the refractive index of solvent, $\mathrm{d}n/\mathrm{d}c$ is the specific refractive index increment, and A_2 is the second virial coefficient. After constructing a $Kc/\bar{R}_\theta$ *versus* c curve at a few different concentrations, A_2 and M_w are obtained from half the slope and the reciprocal of the intercept, respectively.

Rheological measurements

Capillary viscosity measurements were conducted by using size 50 Cannon Fenske capillary viscometers (Induchem Lab Glass Co., Roselle, NJ). Dilute pectin solutions were prepared by dissolving the pectin samples in 50 mM citrate phosphate buffer (pH 4·5) for 1 h at room temperature. The pectin solutions were filtered through a 0·22 μm Millipore filter (Millipore Products Division) prior to viscosity measurements. Then, 10 ml of pectin solution were pipetted into the capillary viscometer for the viscosity measurements.

Specific viscosity (η_{sp}) and intrinsic viscosity ($[\eta]$) were determined as follows:

$$\eta_{sp} = (\eta - \eta_s)/\eta_s \tag{5}$$

and

$$[\eta] = \lim_{c \to 0} \eta_{sp}/C \tag{6}$$

where η is the viscosity of solution, η_s is the viscosity of solvent, and c is the concentration.

Steady shear viscosity measurements were conducted by using a Rheometrics Fluids Spectrometer (Rheometrics, Piscataway, NJ). The cone and plate geometry with cone angle of 0·0196 radian and radius of 25 mm was used for the measurements, and the gap size was 50 microns. The experiments were conducted at 25 ± 1°C. Appropriate concentrations of pectin samples were dissolved in 50 mM citrate phosphate buffer (pH 4·5) by using the magnetic stirrer for 2 h at room temperature. Preparation of pectin samples exceeding 6·0% (w/v) concentration was not successful due to solubility problems. The resulting pectin sample solutions were loaded with syringes, and the sample sizes were kept at 1·0 ml. Steady shear viscosity is reported as a function of shear rate in log–log coordinates.

Circular dichroism (CD)

All CD spectra were recorded with a Model 60DS Aviv spectropolarimeter (Aviv Associates, Lakewood, NJ) equipped with a programmable and thermoelectrically controlled cell holder. A cell of 1 mm pathlength was used, and the sample chamber was kept at 25°C. In this research CD measurements were performed by using 0·1% (w/v) pectin solution in 50 mM citrate phosphate buffer (pH 4·5), which was filtered through a 0·22 μm Millipore filter (Millipore Products Division) before taking measurements. The sample solutions were scanned from 280 to 190 nm, and the ellipticities were plotted against wavelength. The scans were conduced at every 1 nm interval with three replications, and the data were averaged automatically by the computer at the end of the run. The scan of pure solvent which was considered to be the base line was subtracted from the measured CD of the samples. The CD data were expressed in terms of mean residue ellipticity ($[\theta]$) in deg cm^2/dmole.

RESULTS AND DISCUSSION

Chemical characterization of pectins

The chemical composition of apple pectins is shown in Table 1 in terms of anhydrogalacturonic acid (AGA), methoxyl and degree of esterification (DE) and neutral sugar content. The DE of sample I was similar to that of sample II. This is consistent with the report of Michel *et al.* (1981), who described that 1% (v/v) acid alcohol washing did not significantly affect DE of commercial pectins. It has been well documented that DE is a significantly important factor to determine the conformational and rheological properties of pectins (Fishman *et al.*, 1984; Anger & Berth, 1986). Therefore, it is important to note that the possible difference in rheological properties observed in this research is not due to DE, because samples I and II exhibit the

Table 1. Chemical composition of apple pectins

Components	Samples	
	I	II
AGA (% (w/w))	74·79	71·13
Methoxyl (% (w/w))	8·09	8·06
DE (%)	61·41	64·33
Neutral sugars (% (w/w))	7·08	12·07
Rhamnose	2·71	3·10
Arabinose	0·39	0·80
Xylose	1·31	2·89
Galactose	2·37	4·68
Glucose	0·30	0·60

insignificant differences in DE. Table 1 also shows that sample I is less branched than sample II, which can be attributed to the degradation of side branches by acid alcohol washing employed during sample I preparation. Galactose and xylose are the major neutral sugar constituents present in the side branches of apple pectins, while arabinose content was relatively small.

Determination of rheological parameters of pectins

Figures 1 and 2 show $\Delta n/c$ *versus* concentration and $Kc/\bar{R}_\theta$ *versus* concentration of apple pectins, respectively. In addition, η_{sp}/c *versus* concentration of apple pectins is given in Fig. 3, in which the intercept corresponds to the intrinsic viscosity. The molecular parameters of apple pectins obtained from Figs 1, 2 and 3 are summarized in Table 2. Sample I has an M_w smaller than sample II, which is possibly due to cleavage of the glycosidic linkages of side branches by the acid alcohol washing of sample I when compared to EDTA treatment for sample II. It is also noted that the more branched sample II exhibits the smaller intrinsic viscosity and smaller radius of gyration than the relatively linear apple sample I. The results are consistent with Graessley's (1968) argument that the branching leads to smaller hydrodynamic size.

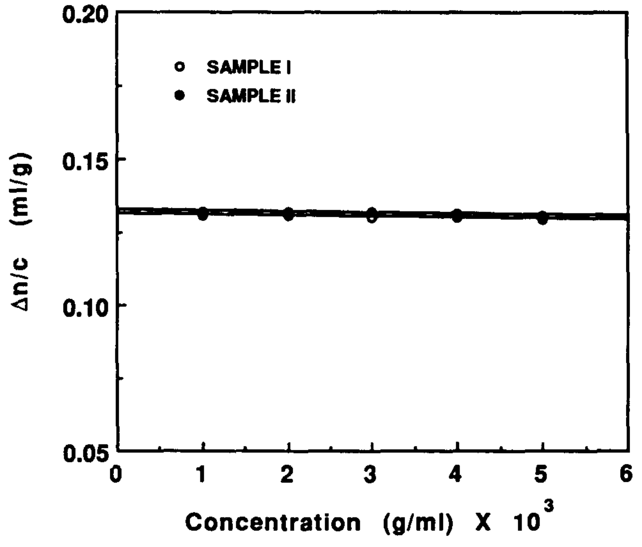


Fig. 1. $\Delta n/c$ *versus* concentration of apple pectins.

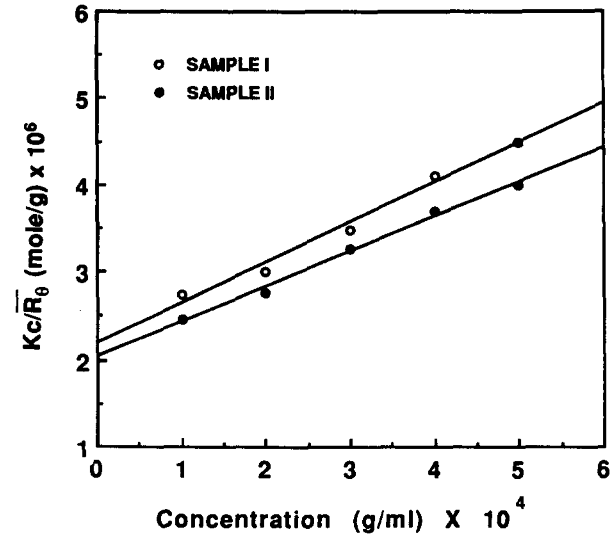


Fig. 2. $Kc/\bar{R}_\theta$ *versus* concentration of apple pectins.

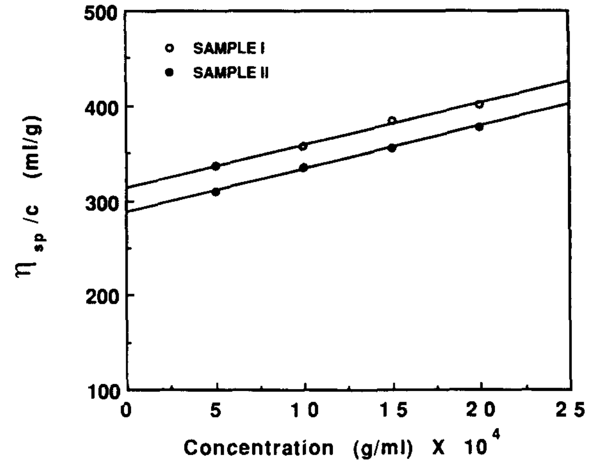


Fig. 3. Determination of intrinsic viscosities of apple pectins.

Steady shear viscosity

The concentration dependence of shear viscosity (η) for apple pectin solutions (2·0–6·0%) are given in Fig. 4 as a function of shear rate ($\dot{\gamma}$). It is found in Fig. 4 that η_0 of sample II is significantly higher than that of sample I. This trend becomes more pronounced with increasing pectin concentrations. Here it is worth noting that apple pectin sample II possesses the lower intrinsic viscosity, but higher branching than apple pectin sample I. In general, polymers that have higher intrinsic viscosities will also have higher concentrated solution viscosity, because the level of intermolecular interactions will be more significant (Mitchell, 1979). The exception to this is when the branches significantly contribute to interchain entanglements (Fujimoto

Table 2. Determination of molecular parameters of apple pectins

Samples	dn/dc (cm^3/g)	M_w (× 10^5) (g/mole)	A_2 (× 10^3) (mole cm^3/g^2)	[η] (ml/g)	S^a (× 10^6) (cm)
I	0·131	4·59	2·31	313·31	3·40
II	0·132	4·90	1·99	287·97	3·37

[a] Based on the Flory and Fox equation (Flory, 1953): $S = ([\eta]M/\Phi 6^{3/2})^{1/3}$, where $\Phi = 2{\cdot}5 \times 10^{23}$.

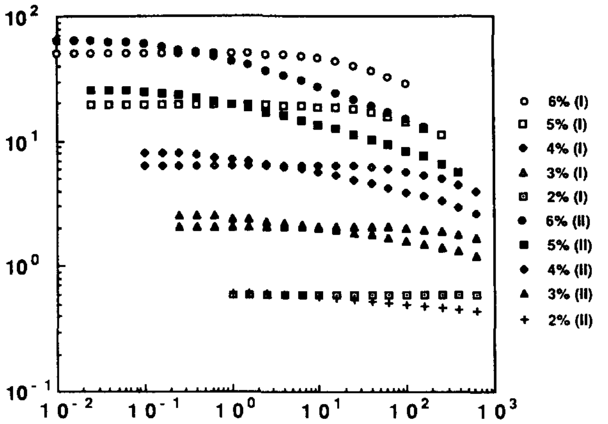


Fig. 4. Concentration dependence of shear viscosity of apple pectins.

et al., 1970; Graessley *et al.*, 1976; Graessley, 1977). In other words, branched polymers, despite the smaller hydrodynamic size, can give the higher η_0 than linear polymers in the presence of significant branching entanglements. Therefore, apple pectin sample II having the more branched structure is more entangled, resulting in higher η_0 than sample I at the same concentration.

Figure 4 also shows the shear rate dependence of viscosity. At first, increasing shear rate dependence of viscosity, i.e. more pseudoplastic, is observed with increasing pectin concentrations for both pectin samples. This can be best explained in terms of the degree of chain entanglements. As polymer concentration is increased, the freedom of movement of the individual chains becomes restricted due to the correspondingly increased number of entanglements (Graessley, 1974). This gives rise to an increase in the time required to form new entanglements to replace those disrupted by the externally imposed deformation (Graessley, 1974). Thus, the shear rate at which Newtonian behavior is lost progressively moves towards lower values with increasing polymer concentrations. This is consistent with the report of Morris *et al.* (1981). In addition, Fig. 4 reveals that at the same pectin concentration the transition shear rate of sample II from η_0 to the power law region is lower than that of sample I. In summary, sample II has a higher viscosity than sample I at low shear rates, but this trend is reversed at high shear rates. Thus, increasing the side branches of pectins leads to higher η_0 and greater shear rate dependence of viscosity. This is in good agreement with the report of Jacovic *et al.* (1979) and Graessley (1984) for synthetic polymers.

The quantitative analysis of the shear rate dependence of viscosity can be achieved by finding the characteristic shear rate at which η_0 is reduced to $0{\cdot}8\eta_0$. Shear rates ($\dot{\gamma}_{0\cdot8}$) of apple samples are given as a function of concentration in Table 3 in conjunction with the characteristic time $\lambda_{0\cdot8}$ ($= 1/\dot{\gamma}_{0\cdot8}$). Sample II with higher branching exhibited lower $\dot{\gamma}_{0\cdot8}$ than sample I at the same pectin concentration, indicating the greater rate dependence of sample II.

The shear thinning region of η *versus* $\dot{\gamma}$ curve can be expressed by the power law or the Ostward–De Waele equation, i.e. $\tau = m\dot{\gamma}^n$ or $\eta = m\dot{\gamma}^{n-1}$, where m is the consistency index, and n is the flow behavior index. The m and n values of apple pectins obtained from the power region of η *versus* $\dot{\gamma}$ are summarized in Table 4 as a function of concentration. Apple pectin sample II with higher branching gives lower n values than sample I, suggesting that sample II is more pseudoplastic.

Rheological generalization

a. η_{sp} *versus* $c[\eta]$

The zero-shear specific viscosity ($\eta_{sp,0}$) of apple pectins is plotted *versus* the coil overlap parameter ($c[\eta]$) in

Table 3. The $\dot{\gamma}_{0\cdot8}$ and $\lambda_{0\cdot8}$ values of apple pectins as a function of concentration[a]

C (% (w/v))	I		II	
	$\dot{\gamma}_{0\cdot8}$ (1/s)	$\lambda_{0\cdot8}$ (s)	$\dot{\gamma}_{0\cdot8}$ (1/s)	$\lambda_{0\cdot8}$ (s)
6·0	24·18	0·041	0·45	2·222
5·0	63·34	0·016	0·67	1·493
4·0	210·90	0·005	3·28	0·305
3·0	463·24	0·002	10·62	0·094
2·0	—	—	119·79	0·008

[a] $\lambda_{0\cdot8} = 1/\dot{\gamma}_{0\cdot8}$.

Table 4. The consistency index (m) and the flow behavior index (n) of apple pectins in the power region of η *versus* $\dot{\gamma}$ as a function of concentration[a]

C (% (w/v))	I		II	
	m	n	m	n
6·0	92·05	0·75	53·70	0·72
5·0	44·06	0·75	27·10	0·74
4·0	17·06	0·77	11·97	0·76
3·0	4·58	0·84	3·68	0·83

[a] $\eta = m\dot{\gamma}^{n-1}$.

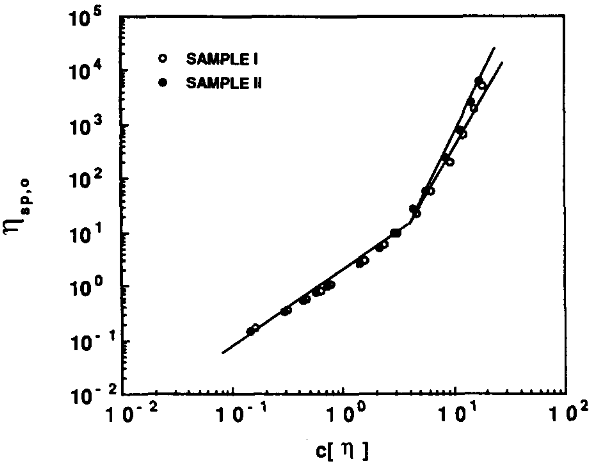


Fig. 5. $\eta_{sp,0}$ *versus* $c\,[\eta]$ of apple pectins.

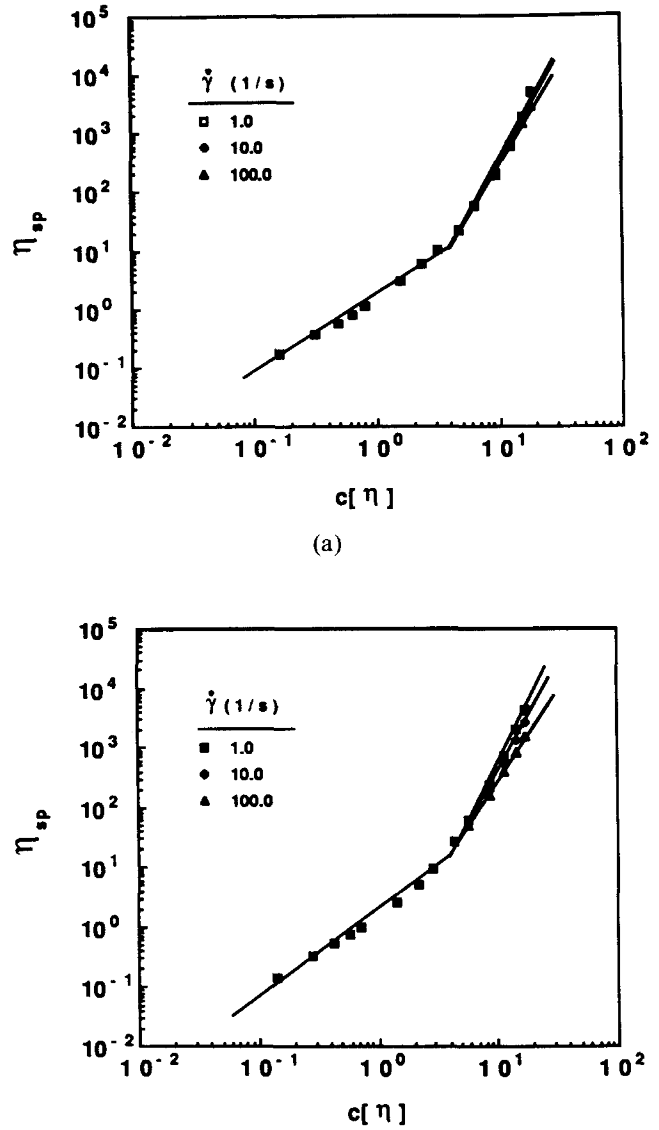
Fig. 5. Both samples exhibit a transition from the dilute to concentrated region at a $c[\eta]$ value around 4·0, irrespective of the intrinsic viscosity and the branching degree. Morris *et al.* (1981) reported that the onset of coil overlap for a variety of random-coil polysaccharides occurred when $c[\eta] \approx 4{\cdot}0$, regardless of the individual values of concentration and $[\eta]$. This indicates that the conformation of pectin remains unaffected by the degree of branching. This conformation was shown to be a Zimm-type random coil with dominant hydrodynamic interaction by Chou and Kokini (1991). Figure 5 also shows that $\eta_{sp} \approx 10$ corresponds to the intersection point for apple pectins, irrespective of the intrinsic viscosity and the amount of side branches. This is in good agreement with the report of Morris *et al.* (1981), who demonstrated that the $c^*\,[\eta] \approx 4{\cdot}0$ transition point approximately corresponded to $\eta_{sp} \approx 10$ or $\eta_0 \approx 10$ cp (mPa s) for other random-coil polysaccharides.

In the dilute region ($c[\eta] < c^*\,[\eta]$), the data for samples I and II are nearly superimposed, and their corresponding gradients are almost identical (1·31 *versus* 1·30). This indicates that the gradients in dilute solutions are almost independent of the degree of branching and the intrinsic viscosity. These results are not surprising, because the flow parameters in dilute solutions are directly related to the hydrodynamic volume of the molecule, which is taken into account by the terms $c[\eta]$. The gradients in the dilute domain are in fairly good agreement with the range of 1·1–1·4 for other polysaccharide, as reviewed by Launay *et al.* (1986). Morris *et al.* (1981) also reported that the gradient of various random-coil polysaccharides studied were proportional to $\sim c^{1\cdot 3}$ at $c[\eta] < c^*\,[\eta]$. However, in concentrated region ($c\,[\eta] > c^*\,[\eta]$), the gradients of pectin samples I and II are significantly different, i.e. 4·06 and 4·24, respectively. It is noteworthy that the gradient of sample I with the larger intrinsic viscosity is lower than the gradient of sample II with the smaller intrinsic viscosity. Clearly, entanglements are enhanced as a result of branching, and viscosity increases more sharply with concentration for sample II. Therefore, significant entanglements of side branches of sample II lead to a higher gradient for $c\,[\eta] > c^*\,[\eta]$ than sample I.

As discussed earlier, the shear viscosity of pectins is greatly influenced by shear rates at $c > c^*$. Accordingly, the slopes at $c\,[\eta] > c^*\,[\eta]$ of η_{sp} *versus* $c\,[\eta]$ also depend upon shear rate. η_{sp} *versus* $c\,[\eta]$ of apple pectins are shown for varying shear rates ($\dot{\gamma}$ = 1·0, 10·0 and 100·0 s^{-1}) in Fig. 6. The gradients obtained from the η_{sp} *versus* $c\,[\eta]$ for $c\,[\eta] > c^*\,[\eta]$ are summarized in Table 5. In general, the gradients decrease with increasing shear rate, because the polymer molecules are disentangled and more oriented by the shearing force (Krumel & Sarkar, 1975; Mitchell, 1979). At low shear rates sample II with higher branching exhibited higher gradients than sample I, but the opposite trend was observed at high shear rates. It is also noted that the gradients of sample I are less shear-rate dependent than those for sample II. These results correlated well with the observations of the shear-rate dependence of viscosity shown in Fig. 4 for apple pectins.

b. η_{sp} *versus* cM_w

A second way of characterization and superposition of viscosity data is by plotting log $\eta_{sp,0}$ *versus* log cM_w. In this case, only molecules with the same approximate shape and conformation will superimpose (Baird & Ballman, 1979; Chou & Kokini, 1987). The gradient in the concentrated solution region is also a very useful identification of the conformation of biopolymers in solution. For flexible random coil, the gradient of the η_{sp} *versus* log cM_w curve would give exponents around 3·4, while stiffer chained molecules should give gradients in the neighborhood of 8·0 (Baird & Ballman, 1979). Figure 7 shows $\eta_{sp,0}$ *versus* cM_w for apple pectin samples I and II. It can be seen from the curves that the data points are well superimposed for both samples, irrespective of the branching degrees. This is in contrast to $\eta_{sp,0}$ *versus* $c\,[\eta]$ giving a significant difference in the concentrated region ($c\,[\eta] > c^*\,[\eta]$). This clearly



(a)

(b)

Fig. 6. η_{sp} *versus* $c\,[\eta]$ of apple pectin: (a) sample I and (b) sample II as a function of shear rate.

shows that there are no significant conformational differences between the samples, and all rheological changes are due to increased entanglement density caused by side branches of sample II.

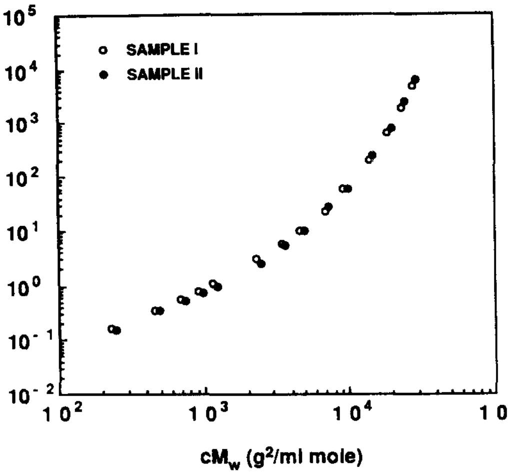


Fig. 7. $\eta_{sp,0}$ *versus* cM_w of apple pectins.

c. Superposition of steady shear data

Superposition of viscosity *versus* shear rate data obtained at different concentrations can be obtained, when the shear rate is multiplied by the time constant $\lambda_{0\cdot8}$ (Graessley, 1974). Thus, the shear-rate dependence of viscosity shown in Fig. 4 is replotted in terms of η/η_0 *versus* $\dot{\gamma}\lambda_{0\cdot8}$ or $\dot{\gamma}/\dot{\gamma}_{0\cdot8}$. In Fig. 8(a), the η/η_0 *versus* $\dot{\gamma}/\dot{\gamma}_{0\cdot8}$ curves for sample I appear to be closely superimposable for all concentrations studied. In contrast, Fig. 8(b) shows that data for sample II do not completely superimpose. This anomalous behavior of sample II may be brought about by the presence of side branches with significant dimension.

An alternative dimensionless generalized shear rate (β) can be expressed as follows (Morris & Ross-Murphy, 1981):

$$\beta = \dot{\gamma}(\eta_0 - \eta_s)M/cRT \qquad (7)$$

where η_0 is the zero-shear viscosity, η_s is the solvent viscosity, c is the concentration, R is the gas constant and T is the absolute temperature. This superposition relation uses the essence of the Rouse relaxation time (Graessley, 1974; Morris & Ross-Murphy, 1981). The results for η/η_0 *versus* $\dot{\gamma}(\eta_0 - \eta_s)M/cRT$ of apple pectins are given in Fig. 9. Sample I again exhibited

Table 5. The gradients of $\eta_{sp,0}$ *versus* $c[\eta]$ and η_{sp} *versus* $c[\eta]$ of apple pectins as a function of shear rate

Samples	$\eta_{sp,0}$ *versus* $c\,[\eta]$		η_{sp} *versus* $c\,[\eta]$ $c\,[\eta] > c^a\,[\eta]$		
	$c\,[\eta] < c^a\,[\eta]$	$c\,[\eta] > c^a\,[\eta]$	$\dot{\gamma} = 1{\cdot}0$	$\dot{\gamma} = 10{\cdot}0$	$\dot{\gamma} = 100{\cdot}0$
I	1·31 (0·996)[a]	4·06 (0·994)	4·05 (0·994)	3·98 (0·995)	3·57 (0·998)
II	1·30 (0·997)	4·24 (0·996)	3·91 (0·998)	3·54 (0·999)	3·13 (0·999)

[a] Correlation coefficients (R^2).

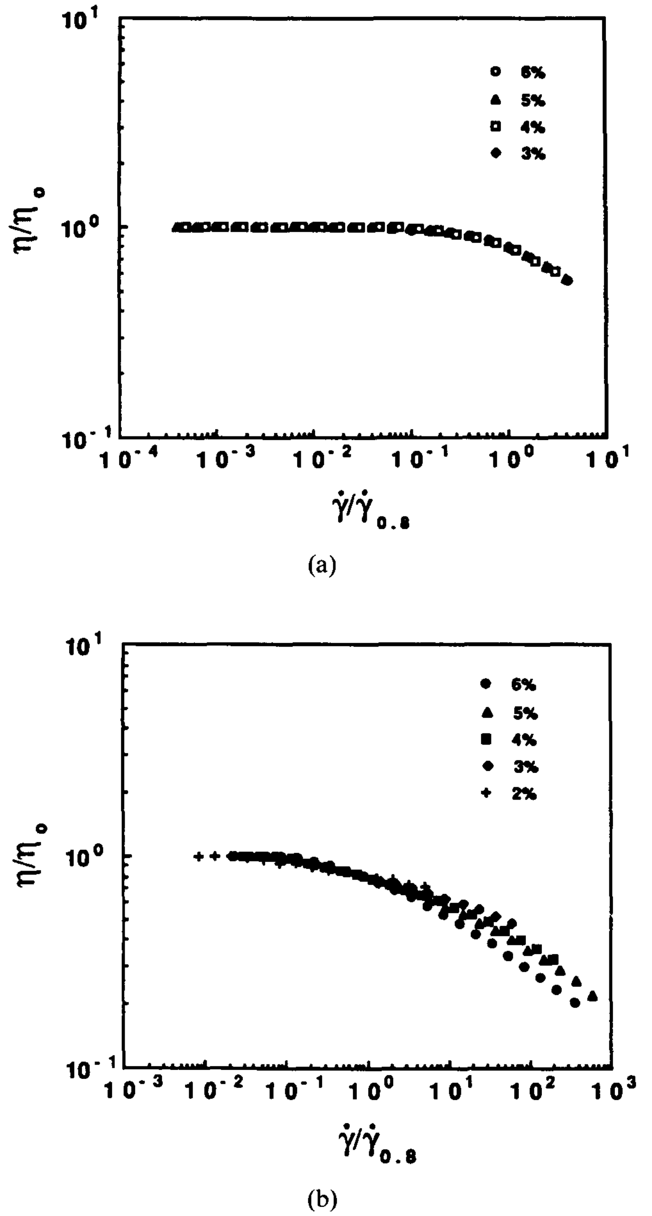


Fig. 8. η/η_0 *versus* $\dot{\gamma}/\dot{\gamma}_{0\cdot 8}$ of apple pectin: (a) sample I and (b) sample II.

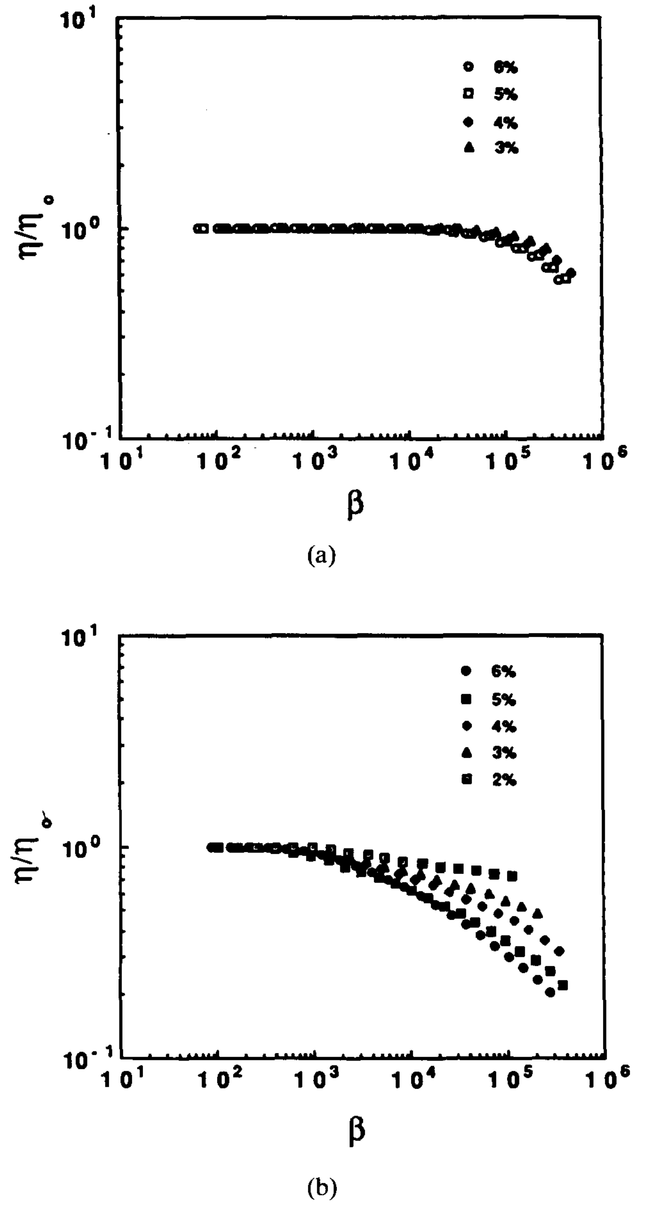


Fig. 9. η/η_0 *versus* $\dot{\gamma}\,(\eta_0 - {}_s)$ *M/cRT* of apple pectin: (a) sample I and (b) sample II.

relatively good superposition, whereas the highly branched sample II showed significant deviation.

Circular dichroism (CD)

The CD spectra of pectin samples I and II are presented in Fig. 10. Both pectin samples exhibit a strong positive CD band at 210 nm, irrespective of the degree of branching, which is due to the excitation of the carboxyl groups, i.e. the $n \rightarrow \pi^*$ transition. The same pattern of CD band has been documented elsewhere for pectins (Listowsky *et al.*, 1969, 1972; Grant *et al.*, 1973; Morris *et al.*, 1975; Gidley *et al.*, 1979; Ravanat & Rinaudo, 1980; Liang & Stevens, 1982; Thom *et al.*, 1982; Rees, 1982; Curtis Johnson, 1987). These results indicate that there is no fundamental conformational changes in terms of the branching degrees. Figure 10 also shows a linear change in $[\theta]$ between two samples. Grant *et al.* (1973) and Plaschina *et al.* (1978) reported that increasing the intermolecular association of pectin molecules resulted in the higher intensity of the CD bands. It has been well recognized that carbohydrate polymers with more linear structure can be more closely associated that the more branched ones. Therefore, sample I, having the lower degree of side branches, can be more significantly interacted with other molecules than sample II, leading to the greater magnitude in the CD spectra. The insignificant contribution of side branches to conformation of

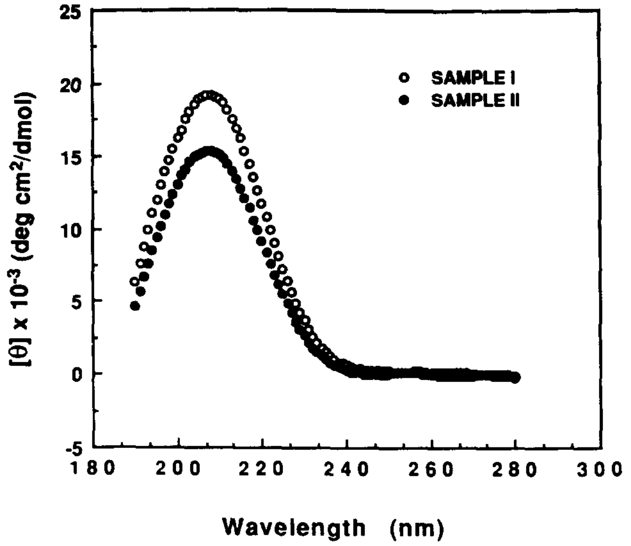


Fig. 10. The CD spectra of apple pectins.

pectins is of critical importance, because the rheological changes observed previously are not because of conformational changes, but because of the structural difference in terms of the branching degrees.

CONCLUSIONS

In this research, apple pectins with different degrees of side branches were employed to understand the role of branching in the solution properties. We clearly showed that the side branches significantly influenced the rheological properties of pectins. On the basis of the rheological theories developed for synthetic polymers and the experimental results observed in this research, it is shown that the side branches of pectins result in significant entanglements in concentrated solutions.

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