Carbohydrate Polymers **16** (1991) 409–432

Solution Properties of Pectin Polysaccharides — III: Molecular Size of Heterogeneous Pectin Chains. Calibration and Application of SEC to Pectin Analysis

Dominique Hourdet & Guy Muller

URA 500 du CNRS 'Polymères, Biopolymères, Membranes', Faculté des Sciences de Rouen, 76130 Mont Saint Aignan, France

(Received 2 October 1990; revised version received 10 December 1990; accepted 18 December 1990)

ABSTRACT

Flax pectins, with a low anhydrogalacturonic acid content (AGA ≤ 50%), have been studied by viscometry, light scattering and size exclusion chromatography. After removal of interfering superstructures, intrinsic viscosities were correlated with weight average molecular weights to give a Mark-Houwink coefficient $a = 0{\cdot}69$. The persistence length of these heterogeneous chains (PI: $q = 20$–25 Å), compared with that of homogalacturonan ones previously studied (PII–PIII: $q = 67$ Å), shows a higher segment density (branched conformation). Using a wide range of experimental data ((η), M_i, K_d) obtained on narrow pectic fractions of known composition, specific calibration curves of the Sephacryl 200/0·2 M NaCl/ Flax pectins (PI or PII–PIII) system were established. Applied to the indirect characterization of flax pectins, this size exclusion chromatography (SEC) system constitutes a very convenient method for analysing the molecular and macromolecular parameters ((η), M_w, M_n, I_p, ε^{UV}) or for controlling the molecular weight distribution of pectins in flax cell walls during the physiological steps of growth and retting.

INTRODUCTION

Pectic substances are complex structural polysaccharides located in primary plant cell walls and middle lamellae where they play an important role as hydrating agents and cementing material for the

Carbohydrate Polymers 0144-8617/91/$03.50

cellulosic network. Mostly studied for their thickening and gelling properties which are used in the food industry (Rees, 1972; Kohn & Luknar, 1977; Morris, 1986), they are also of importance in other activity fields such as the textile industry. In fibre plants like flax, jute or hemp, the enzymic degradation of pectic cement, which occurs during the technological step of retting, takes place as the major part in the release of the cellulosic bundles, which become textile fibres (Rosemberg & De Franca, 1967; Morvan *et al.*, 1985; Morvan *et al.*, 1988).

If the basic backbone of these polysaccharides is constituted by (1→4)-linked α-D-galacturopyranosyl residues, either free or in ester form, these homogalacturonan sequences may be 'kinked' at intervals (Rees & Wight, 1971) with β-L-rhamnopyranosyl residues carrying the major amount of neutral sugar side-chains, mainly arabinans, galactans or arabinogalactans (Aspinall, 1980; Darvill *et al.*, 1980). Many authors have actually focused on the microstructure of pectic substances by analysing residual fragments after enzymic or chemical degradation of the carbohydrate backbone (Knee *et al.*, 1975; Darvill *et al.*, 1978; McNeil *et al.*, 1980; De Vries *et al.*, 1982; Thibault, 1983; Saulnier & Brillouet, 1988; Kiyohara & Yamada, 1989). From the literature data, an interesting model of the primary structure of pectins (Fig. 1) has been proposed by Jarvis (1984). It consists of a blockwise distribution of the main sequences, namely a smooth free or esterified homogalacturonan-type region and a 'hairy' rhamnogalacturonan-type region, the latter being rich in neutral side-chains.

In the physicochemical analysis of pectins, the characterization of both their size and shape 'has always been a challenge' (Brigand *et al.*, 1990) and many techniques have been applied. Initially, osmometry measurements were used for the determination of the number average molecular weight (M_n) of fruit pectins (Owens *et al.*, 1946; Glikman & Orlov, 1950; Vollmert, 1950; Fritzsche *et al.*, 1977; Fishman *et al.*, 1986), but in the last two decades, wide and low angle laser light scattering (LALLS) measurements have prevailed (Berth *et al.*, 1977; Kawabata, 1977; Anger & Berth, 1985, 1986; Axelos *et al.*, 1987; Panchev *et al.*, 1988). As pointed out by Harding (1987), low speed sedimentation equilibrium, although less frequently used because of the very few centres of expertise in this technique left, would be the most accurate method. The reported literature data only show a poor reliability of the $(\eta) - M$ relationship for pectins (Fig. 2), mainly originating from:

- the heterogeneity of the primary structure which can be dependent on both the origin and the extraction conditions of pectins. Many

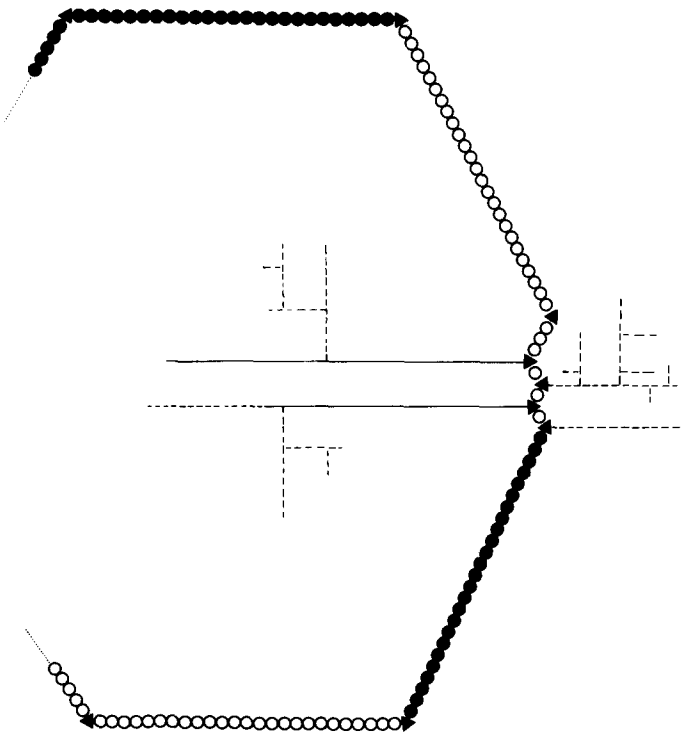


Fig. 1. Block structure of pectins (from Jarvis (1984)): ○, methyl-esterified galacturonan block; ●, non-esterified galacturonan block; ▶, rhamnose; ——, galactan side-chain; - - -, arabinan side-chain.

authors have reported variation of neutral sugar composition in the molecular weight distribution of pectin samples (Berth *et al.*, 1977; Hourdet & Muller, 1987; Berth, 1988; Brigand *et al.*, 1990),

- the presence of aggregates which strongly affect the light scattering behaviour, particularly at low angles, whereas the viscosity is only slightly modified (Kawabata, 1977; Plashchina *et al.*, 1985; Anger & Berth, 1985, 1986; Hourdet & Muller, 1987, 1991).

To overcome such problems, attention has recently been focused on the indirect characterization of pectins by the use of (high performance) size exclusion chromatography (SEC) (Fishman *et al.*, 1984; Deckers *et al.*, 1986; Fishman *et al.*, 1989; Brigand *et al.*, 1990). However, the use of extraneous standards, according to the universal calibration method proposed by Benoit and coworkers (Grubisic *et al.*, 1967), gives only partial, relative and sometimes unrealistic information on the actual size and conformation of pectic chains. Such a problem may be avoided by

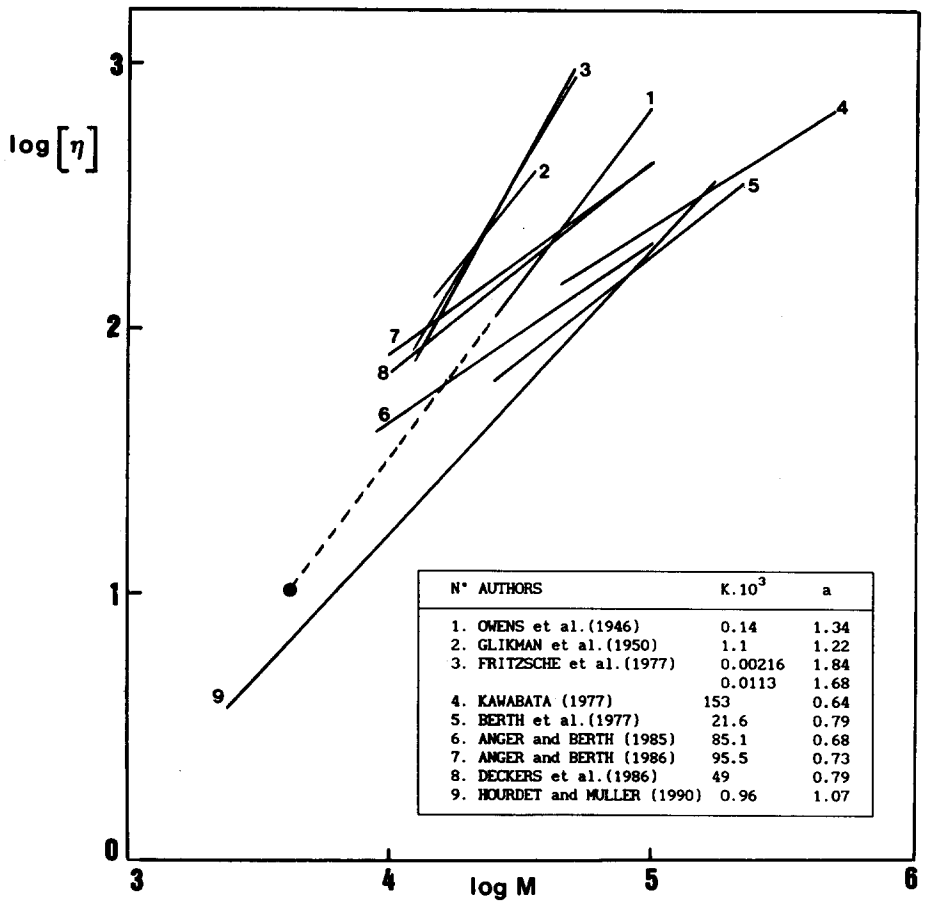


Fig. 2. Mark-Houwink relationships for pectic substances.

using coupled techniques such as SEC/LALLS or SEC/LALLS/ Viscometry.

In a previous report (Hourdet & Muller, 1991) the authors gave evidence that the true molecular weight of pectins could be determined by LALLS after complete removal of interfering superstructures by suitable procedures. It was also reported that the pectic chain with a high anhydrogalacturonic acid content behaves in NaCl solution as an extended coil with a persistence length ($q = 67$ Å) similar to alginates (Smidsrød & Haug, 1968, 1971; Wedlock *et al.*, 1986).

This paper concerns the macromolecular characterization of the low anhydrogalacturonic acid content polysaccharide which is extracted with

hot water from flax cell walls. The data reported here and previously are used to achieve the specific calibration of a chromatographic system (Sephacryl 200/0·2 M NaCl) which will be applied to the indirect characterization of flax pectins during the physiological steps of growth and retting.

EXPERIMENTAL

Samples and solutions

The main specifications of the pectins have been given previously (Hourdet & Muller, 1991) and are listed in Table 1. The same procedure for preparing solutions was used as previously. Pectin solutions were filtered on Millex GV 0·22 μm (Millipore) and then centrifuged at 150 000 g for 1 h in a Beckman L8-70 ultracentrifuge (rotor 70Ti). Concentrations were estimated on a dry weight basis.

All flax pectin samples were kindly supplied by Drs C. Morvan and O. Morvan of SCUEOR (Groupe agro-Industrie), University of Rouen. The degree of esterification (DE) and anhydrogalacturonic acid content (AGA) were determined titrimetrically in the same institute.

Size exclusion chromatography (SEC)

As described previously (Hourdet & Muller, 1987, 1991), the gel permeation system consists of a Pharmacia-P3 pump delivering dust-free solvent, at a flow rate of approximately 2 $cm^3\ min^{-1}$ to a K26-70 Pharmacia column wet-packed with Sephacryl 200 Superfine (Pharmacia) equilibrated with 0·2 M NaCl at room temperature. Eluate is monitored at the column output using RI (Schimadzu RID-6A) and UV (Pharmacia UV1/214) detectors. Exclusion limits of the S200/0·2 M NaCl system were defined with Blue Dextran 2000 ($M_w = 2 \times 10^6$ — void volume (V_0): $K_d = 0$) and NaCl (total permeation volume (V_t): $K_d = 1$).

Viscometry

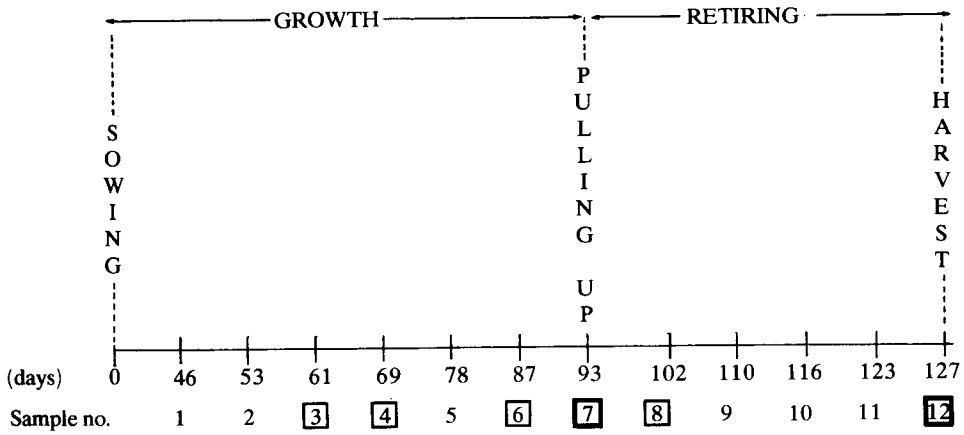
Viscosity measurements were made using a modified Ubbelohde capillary viscometer (FICA VISCOMATIC MS) thermostated at 25°C. Except for very dilute solutions and very low viscosity solutions for which inherent viscosity ($\eta_{inh} \simeq (\eta)$) was used, intrinsic viscosity was

TABLE 1
Specifications of Pectin Samples

Sample	*Origin/extraction*	*Purification*	M_e *(g)*	*DE*	d*n*/d*c* *(cm³/g)*
F_1	FLAX fib./water	Fract./UF× 10^4 D	465	38	0·136
F_2	FLAX fib./water	Fract./UF× 10^4 D	405	30	—
F_3	FLAX fib./water	Fract./UF× 10^4 D	395	27	0·138
F_4	FLAX fib./water	Fract./UF× 10^4 D	345	24	0·139
P_{VW}	FLAX fib./water	F. 8 μm	290	11	0·170
P_{4W}	FLAX c.w./water	UF× 10^3 D	230	48	
P_{6W}	FLAX c.w./water	UF× 10^3 D	270	49	
P_{7W}	FLAX c.w./water	UF× 10^3 D	280	47	0·156 ± 0·002
P_{8W}	FLAX c.w./water	UF× 10^3 D	300	49	
P_{12W}	FLAX c.w./water	UF× 10^3 D	280	40	
P_{12WB}	FLAX c.w./water	F. 8 μm	—	—	0·160
P_{RW}	FLAX fib./water	EtOH/C 10^4 G	—	—	0·152
P_{CALW}	FLAX cal./water	F. 8 μm	<250	—	0·151
P_{3OX}	FLAX c.w./oxalate	UF× 10^3 D	185	13	
P_{4OX}	FLAX c.w./oxalate	UF× 10^3 D	185	13	0·149 ± 0·002
P_{6OX}	FLAX c.w./oxalate	UF× 10^3 D	190	10	
P_{7OX}	FLAX c.w./oxalate	UF× 10^3 D	220	10	
P_{8OX}	FLAX c.w./oxalate	UF× 10^4 D	210	09	0·146 ± 0·002
P_{12OX}	FLAX c.w./oxalate	UF× 10^4 D	250	07	
P_{CALOX}	FLAX cal./oxalate	UF× 10^3 D	<250	—	0·143
PA	APPLE/UNIPECTINE©	—	<250	45	0·143
PC	CITRUS/SIGMA©	—	<250	63	0·141
PGA	ORANGE/SIGMA©	Na^+ form	—	0	0·142

Flax pectins are extracted from:

- fib. ▶ fibres
- cal. ▶ callus
- c.w. ▶ cell walls during:



- ©: Commercial pectins

Purification:
- UF Ultrafiltration
- F Filtration
- C Centrifugation
- Fract. Fractionation
- EtOH Precipitation with ethanol
- Na^+ form Neutralization by NaOH

Me: Equivalent weight to an anhydrogalacturonic unit
DE: Degree of esterification
dn/dc: Refractive index increment in 0·2 molar NaCl

generally extrapolated to $C = 0$ according to the equation:

$$[\eta] = \lim_{C \to 0} \eta_{inh} = \lim_{C \to 0} \left[\frac{\ln t/t_0}{C} \right] \quad (1)$$

where t and t_0 are respectively the flow times for the solvent (0·2 M NaCl) and the pectin solution at a concentration C.

Low angle laser light scattering (LALLS)

Measurements of the excess Rayleigh factor $\overline{R}_0$ at scattering angle of $\theta = 4{\cdot}88°$ were performed with a Chromatix KMX-6 (LDC Milton Roy) low angle laser light scattering photometer. As LALLS is very sensitive to superstructures the same procedure as described previously (Hourdet & Muller, 1991) was used here, namely:

(1) specific adsorption of aggregates onto the MF filters *or*
(2) microgel removal by NaOH treatment *or*
(3) fractionation of the flax pectin samples onto the S200/0·2 M NaCl system.

As clearly indicated in Fig. 3, the good correlation between pectic fractions and isomolecular standards shows that this third procedure presents a dual advantage. First, the superstructures are isolated in the void volume of the S200/0·2 M NaCl system and second, it allows an accurate characterization of narrow pectic fractions by LALLS and viscometry.

RESULTS AND DISCUSSION

Main components in flax pectin samples

From experimental data on both the partial and the overall composition of pectin samples (Hourdet & Muller, 1987; Morvan *et al.*, 1989*a*, *b*) and their molecular weight distribution, three main pectic components were obtained as a result of both hot water and ammonium oxalate extraction. They are presented in a schematic way in Fig. 4 by using the chromatograms of various representative samples.

Pectins I (PI)
Pectins I are esterified to a moderate extent (DE ≤ 50%) and have a low anhydrogalacturonic acid content (AGA ≤ 50%). They assume a ramified

structure with neutral sugar (NS) side-chains comparable to the Rhamnogalacturonan I (RGI) described by McNeil *et al.* (1980) as can be deduced from the ratio AGA/NS (Hourdet, 1989) and from the neutral sugar composition (Morvan *et al.*, 1989*a*) determined by NMR studies (Davis *et al.*, 1990). Residually present in the oxalate extracted pectins P_{Ox} (cf. P_{6OX}, P_{7OX} and P_{8OX} on Fig. 3), Pectins I are largely extracted with hot water (samples P_W) and are distributed mainly in the void volume area of S200/0·2 M NaCl system with a peak broadening towards higher distribution coefficients.

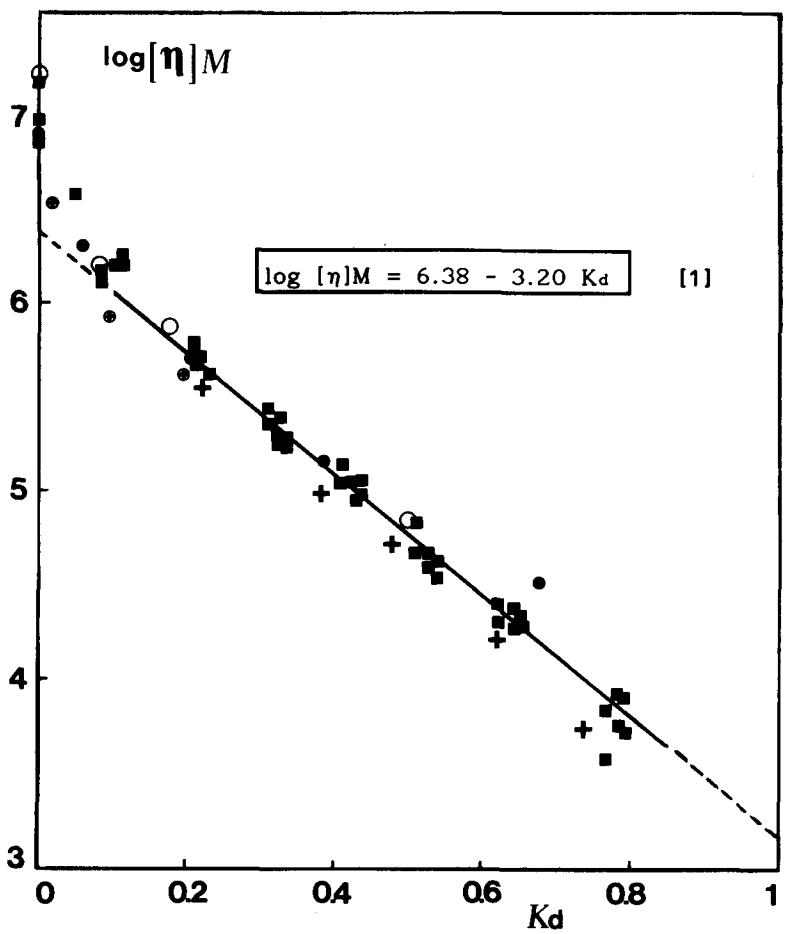


Fig. 3. Universal calibration plot of the S200/0·2 M NaCl system: ○, DEX; ●, PSSNa; ⊕, POE; +, PEG; ——■——, flax pectins.

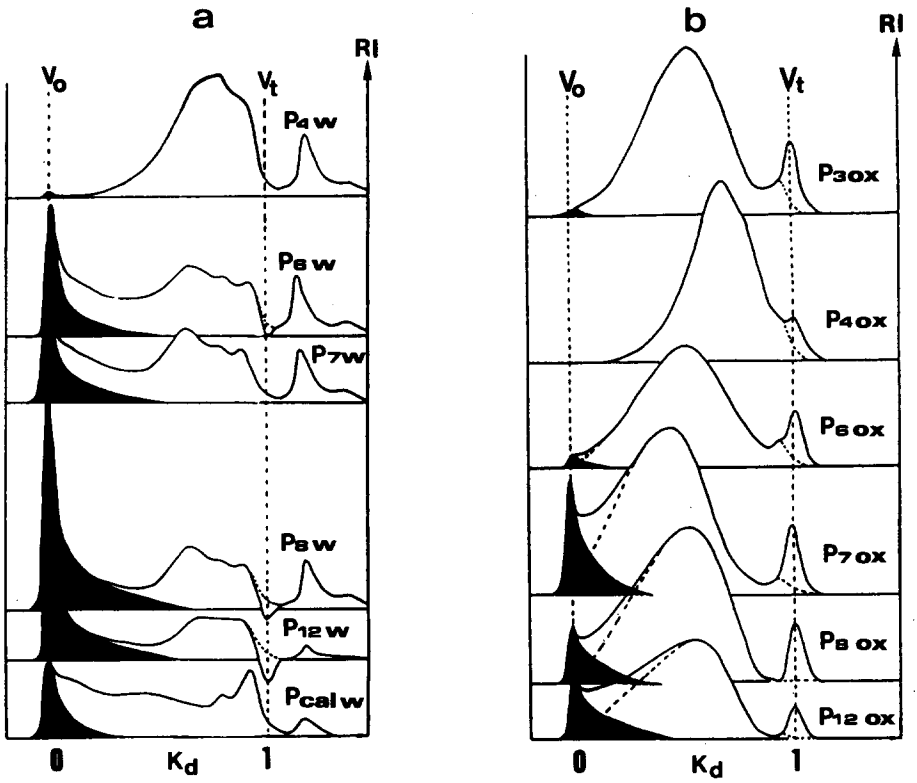


Fig. 4. Schematic distribution of pectic components in flax pectin samples. (a) Hot water extracted (P_w), ■ PI, □ PIII; (b) oxalate extracted (P_{OX}), ■ PI, □ PII.

Pectins II (PII)

Pectins II consist essentially of weakly esterified anhydrogalacturonic acids (DE ≤ 15%). These low polydispersity homogalacturonan chains, extracted with oxalate, are specific to middle lamellae where they play an important part in the intercellular cohesiveness. Their elimination is closely correlated to the degree of retting (Morvan *et al.*, 1985; Hourdet, 1989).

Pectins III (PIII)

Pectins III have a low neutral sugar content (AGA ≥ 80%) and are esterified to a moderate extent (DE ≤ 50%). They are easily solubilized in hot water (samples P_W on Fig. 3) and widely distributed in the overall selective permeation area but principally in the area $K_d \geq 0{\cdot}5$. Mainly present in P_W samples extracted from flax calli (P_{CALW}) or cell walls

during growth (P_{4W}, P_{6W}, P_{7W}) their elimination occurs just after uprooting. This explains the difference observed in the molecular weight distribution between growth (P_{6W}, P_{7W}) and retting (P_{8W}, P_{12W}).

Size and conformation of Pectins I (AGA ≤ 50%; DE ≤ 50%)

The $[\eta]$, M_w parameters of pectin fractions obtained both from the P_{7W} and P_{8W} samples, according to the combination of SEC/LALLS/Viscosity (Hourdet & Muller, in press), and from the P_{VW} sample, after fractionation ($F_1 \rightarrow F_4$) (Hourdet & Muller, 1987) and extrapolation of the specific absorption phenomenon of aggregates onto the MF filters (Hourdet & Muller, in press), are shown on Fig. 5 together with the relationship previously established for Pectins PII–PIII (solid line):

$$[\eta] = 0{\cdot}96 \times 10^{-3} M_w^{1{\cdot}07} \tag{2}$$

By taking into account only fractions rich in Pectins I, namely F_1, F_2, F_3, F_4 and P_{8W}s (with the exception of P_{8WA} and P_{8WB} which are eluted in the void volume area and therefore contain residual superstructures), the following relationship for Pectins I in the range $4 \times 10^3 \leq M_w \leq 120 \times 10^3$ holds:

$$[\eta] = 21 \times 10^{-3} M_w^{0{\cdot}69} \tag{3}$$

As shown in Fig. 5, the $[\eta]$–M_w relationships for Pectins PI and Pectins PII–PIII are convergent in the low M_w range. This behaviour is indicative of change in conformation (linear or branched chains) occurring within a same family of macromolecules.

A good estimation of the conformational characteristics of pectins can be derived from their intrinsic viscosity behaviour by using the simplified form of the Yamakawa–Fujii equation (Yamakawa & Fujii, 1974 as proposed by Bohdanecky, 1983):

$$[M^2/[\eta]_0]^{1/3} = A_\eta + B_\eta M^{1/2}$$

where

A_η:	$A_0 M_L \phi_0^{-1/3}$
B_η:	$B_0 \phi_0^{-1/3}$ $\quad (\langle R_0 \rangle^2/M)^{-1/2}$
M_w:	the weight average molecular weight,
$[\eta]_0$:	the intrinsic viscosity ($cm^3\ g^{-1}$),
$\langle R_0 \rangle^2$:	the mean square end to end distance of the chain,
M_L:	the mass per unit length of the chain,
$\phi_0 = 2{\cdot}86 \times 10^{23}$:	the theoretical Flory constant for infinitely large molecular weights,
A_0 and B_0:	functions of the reduced cylinder diameter.

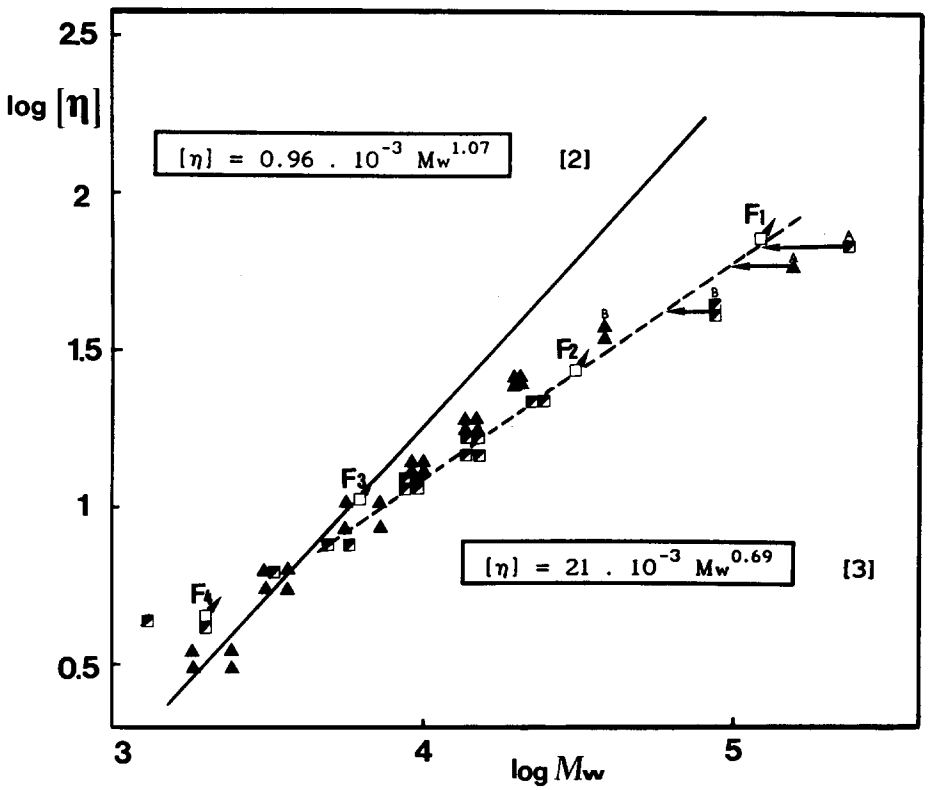


Fig. 5. (η)–M_w relationships for pectic substances in 0·2 M NaCl: ---, pectins PI; ——, pectins PII–PIII. Flax pectin samples: ▲ P_{7W}, ◪ P_{8W}, □ P_{VW} (F_1, F_2, F_3, F_4).

This allows the estimation of $(\langle R_0\rangle^2/M_w)$ and hence the persistence length q from the slope B_η and the intercept A_η of the plot of $[M_w^2/[\eta]_0]^{1/3}$ versus $M_w^{1/2}$. Such plots for Pectins PI and PII–PIII are represented in Fig. 6. From the random coil value $(\langle R_0\rangle^2/M_w)$ the following values were obtained for the persistence lengths q:

$$q = 67\ \text{Å (PII–PIII)} \qquad q = 20\text{–}25\ \text{Å (PI)}$$

As previously reported, the conformation of the homogalacturonan chain is comparable to that of other similar derivatives such as alginates or CMC. Therefore the lower persistence length found for Pectins PI ($q = 20$–25 Å) is probably the consequence of a higher density (branched conformation) and not of a lower intrinsic stiffness.

As a consequence of their heterogeneous character, pectic substances assume various kinds of chain conformations ranging from an extended

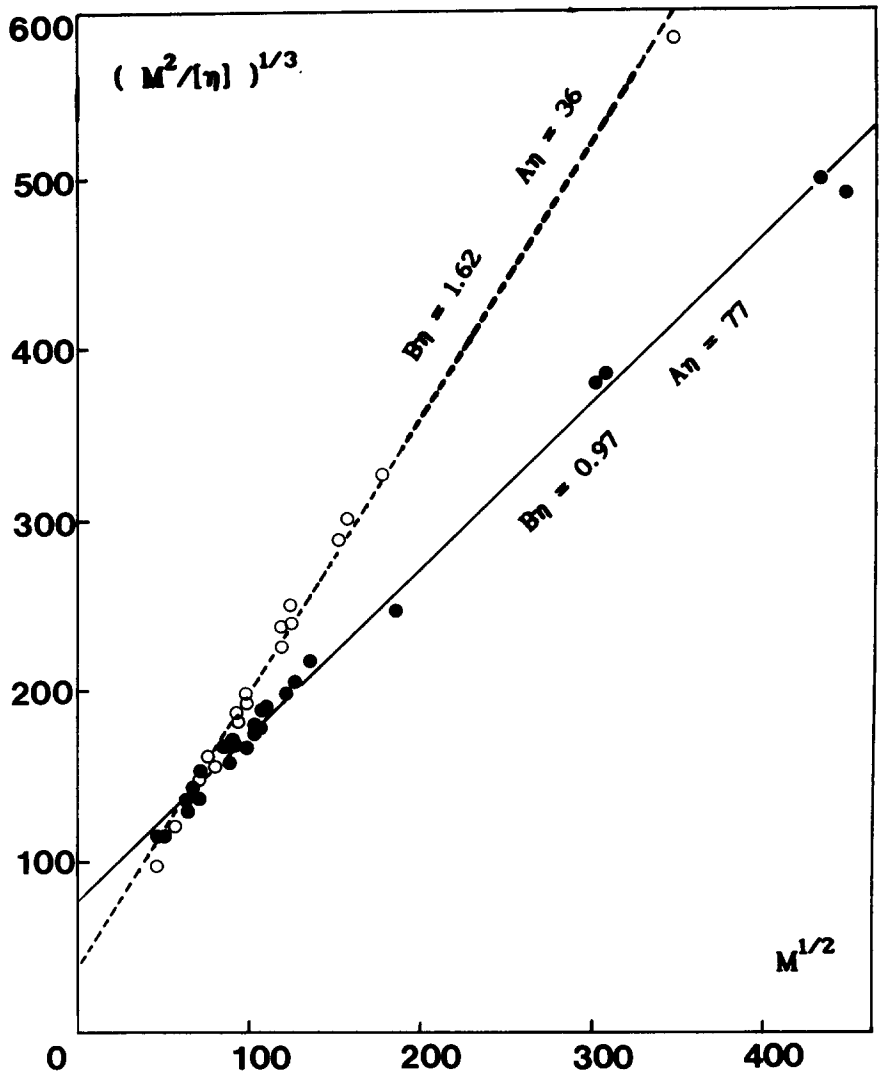


Fig. 6. Determination of the wormlike-chain parameters of pectin chains according to Bohdanecky's method: $(M^2/[\eta])^{1/3} = f(M^{1/2})$. ○, *Pectins PI*: $q = 20$–25 Å. ●, *Pectins PII–PIII*: $q = 67$ Å.

coil (homogalacturonan chain) to a more compact one (Pectin PI). Therefore their distribution both in conformation and in composition must be taken into account in order to consistently correlate the macromolecular parameters. The same holds for most of the pectin samples, e.g. apple and citrus pectins analysed by Anger and Berth (1985, 1986) and Berth (1988) (Fig. 7) or flax pectins (this work: samples

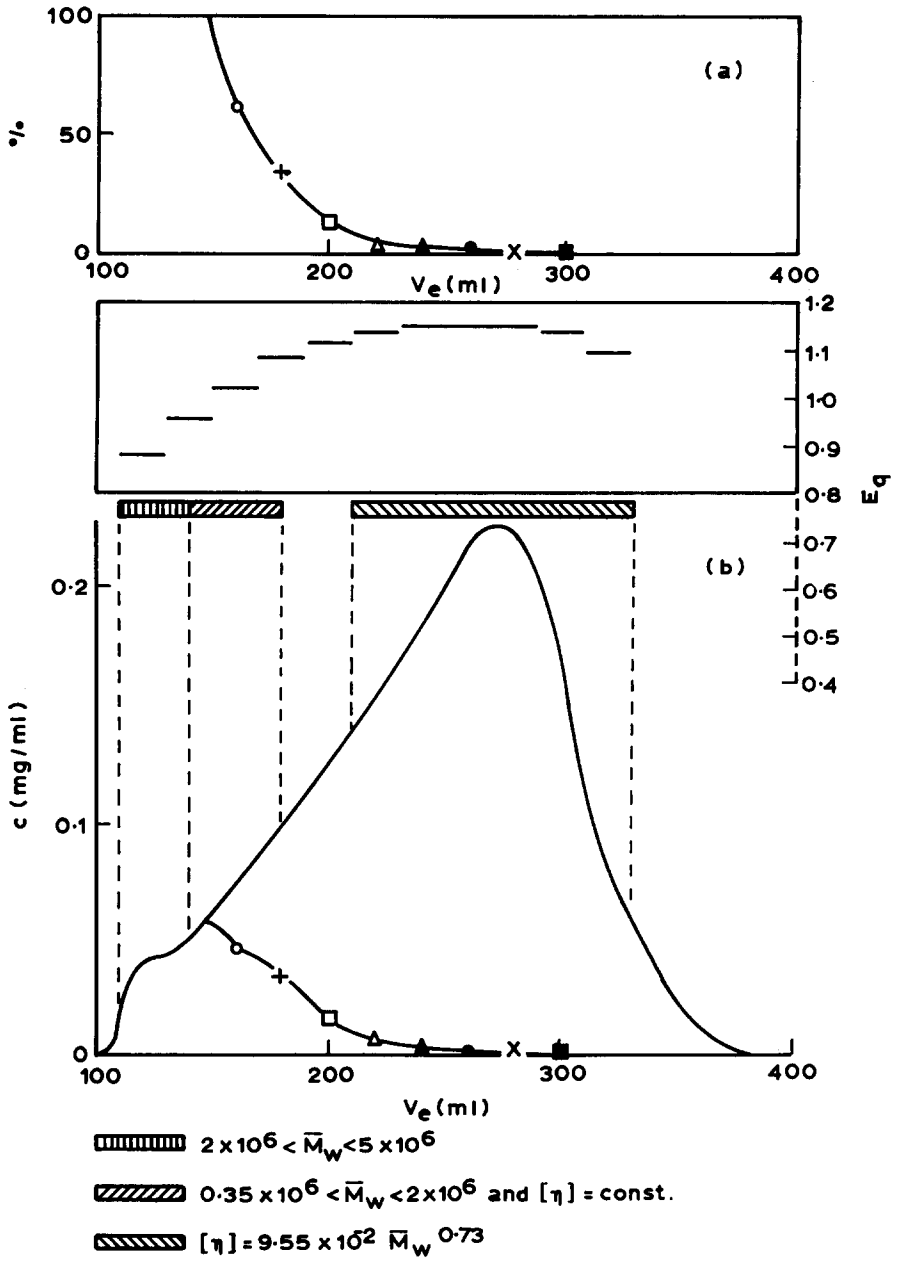


Fig. 7. Percentages of the high molecular weight 'impurity' of samples against the elution volume section used for their preparation (A) and its distribution within the eluate of citrus pectin (B) including the E_q value as a measure for the neutral sugar/galacturonic acid ratio. From Berth (1988).

P_w, Fig. 4(b)). In both cases the heteropolymolecularity, heterogeneity both in size and chemical composition, as initially applied to Acacia Senegal gum (Anderson *et al.*, 1967), is the consequence of the presence of a high M_w fraction rich in neutral sugar. The two Mark-Houwink relations shown on Fig. 5 may be applied to characterize homogeneous samples or fractions from PI-type or PII–PIII-type respectively. The intermediate data obtained from P_{7W} (Fig. 5) are consistent with the heterogeneity of its fractions eluted in the range $0{\cdot}1 \leq K_d \leq 0{\cdot}5$ (Fig. 4: mixed composition PI/PIII). For fractions with a high PI content (P_{7WA}, P_{8WA}, P_{8WB}) which are eluted in the void volume area, it is possible to evaluate the true M_w of molecularly dispersed chains from the values of (η) by referring to the Mark-Houwink relation for Pectins PI (relation (C); Fig. 5). Doing this, a more realistic average M_w is found for P_{8WA} ($M_w = 125\,000$) whereas initially $M_w = 236\,000$ was measured.

Specific calibration of the S200/0·2 M NaCl/flax pectins system

From the universal calibration plot (Fig. 2) and Mark-Houwink relations (Fig. 5) it becomes possible to correlate the intrinsic viscosity (η) or the molecular weight $\bar{M}_w$ with the elution volume V_e or the distribution coefficient K_d in order to establish the specific calibration curves of the S200/0·2 M NaCl/flax pectins system. From eqns (1), (Fig. 3), (2) and (3); two relations between M_i and K_d can be deduced for pectins PI (relation (4)) and pectins PII–PIII (relation (5)) respectively:

Pectins PI $(0{\cdot}1 \leq K_d \leq 0{\cdot}6)$*:* $\log M_i = 4{\cdot}76 - 1{\cdot}89\, K_d$ (4)

Pectins PII–PIII $(0{\cdot}1 \leq K_d \leq 0{\cdot}8)$*:* $\log M_i = 4{\cdot}54 - 1{\cdot}55\, K_d$ (5)

As shown in Fig. 8, the above equations fit the experimental data fairly well with account being taken of the AGA content (PI and PII–PIII). From this calibration, indirect characterization of flax pectins within the range $M_w = 2000$–$24\,000$ for pectins PII–PII, $M_w = 2000$–$38\,000$ for PI, is possible in the selective permeation area $(0{\cdot}1 \leq K_d \leq 0{\cdot}8)$. In the same way, a second calibration plot $\log[\eta] = f(K_d)$ (Fig. 9) could be established for Pectins PI (relation (6)) and Pectins PII–PIII (relation (7)):

Pectins PI $(0{\cdot}1 \leq K_d \leq 0{\cdot}6)$*:* $\log[\eta]_i = 1{\cdot}62 - 1{\cdot}31\, K_d$ (6)

Pectins PII–PIII $(0{\cdot}1 \leq K_d \leq 0{\cdot}8)$*:* $\log[\eta]_i = 1{\cdot}84 - 1{\cdot}65\, K_d$ (7)

These four relations may be used for the characterization of well-defined pectins such as PI or PII–PIII. For heterogeneous samples it

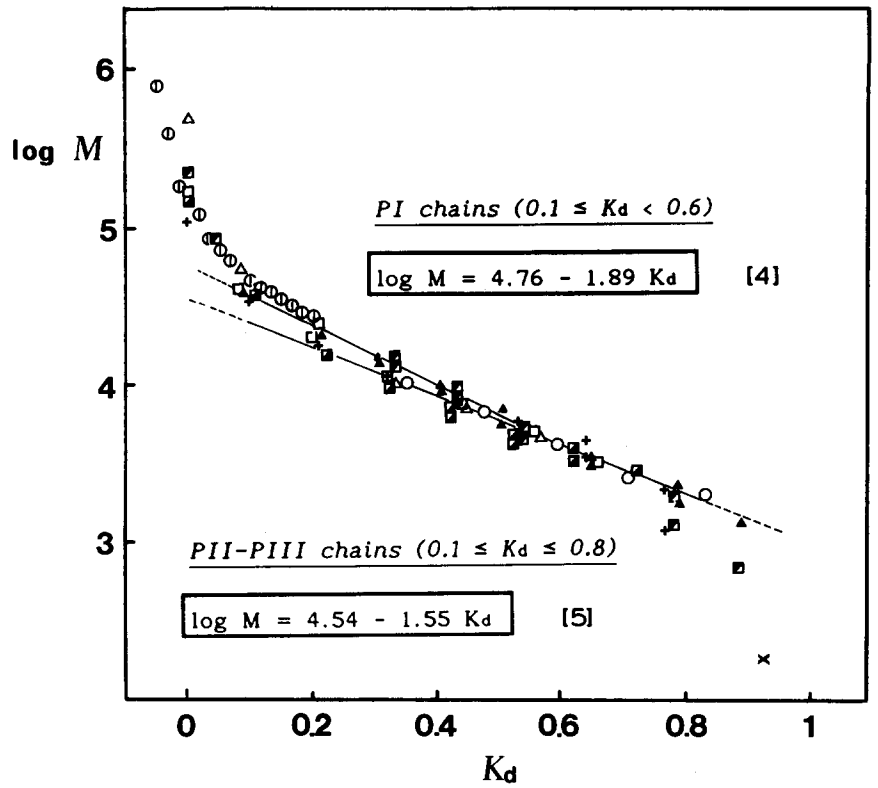


Fig. 8. Specific calibration plot $\log M = f(K_d)$ of the S200/0·2 M NaCl/flax pectins system: ○, P_{4OX}; □, P_{6OX}; △, P_{7OX}; ▣, P_{8OX}; ×, galacturonic acid; +, P_{CALW}; ▲, P_{7W}; ▣, P_{8W}; ⊕, P_{RW}.

should be better to apply intermediate equations which in the worst case give maximal deviation of only 20% on $[\eta]_i$ and M_i.

The S200/0·2 M NaCl column, once calibrated, constitutes a very convenient system for rationalizing the molecular and macromolecular analysis of flax pectins. Amongst different applications of this column worth mentioning are:

- the preparative and analytical fractionation of pectin samples,
- the macromolecular characterization ($\langle\eta\rangle$, $\bar{M}_w$, $\bar{M}_n$, I_p) of a whole sample, a fraction or a specific component, and
- the control of enzymic or chemical degradation or other phenomena leading to changes in the initial MWD.

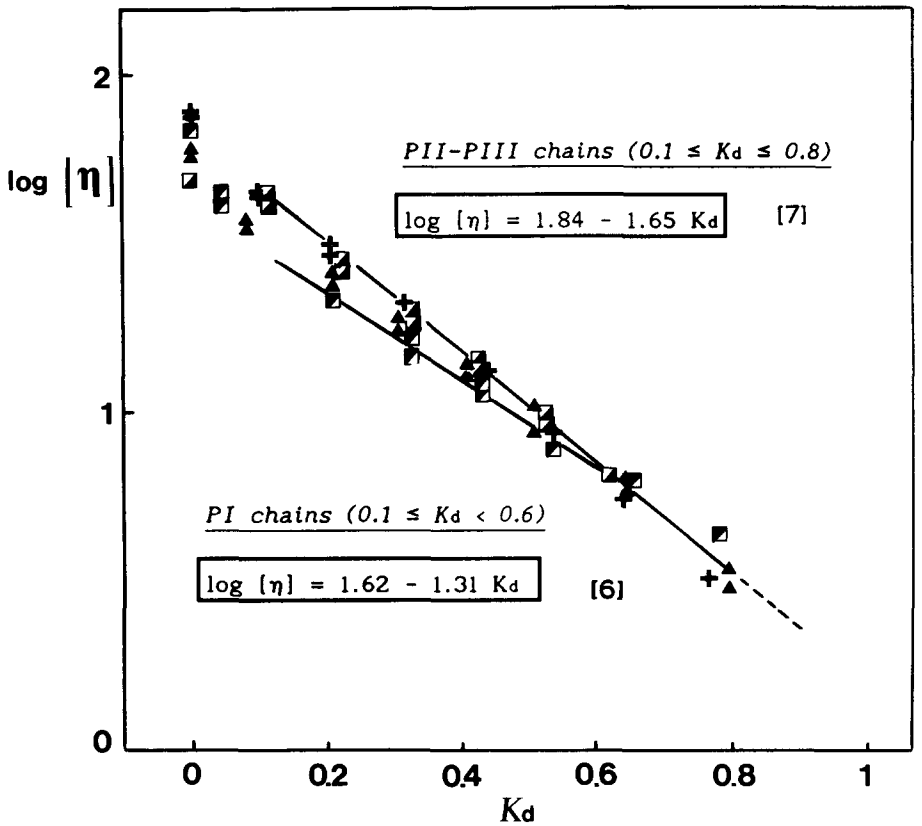
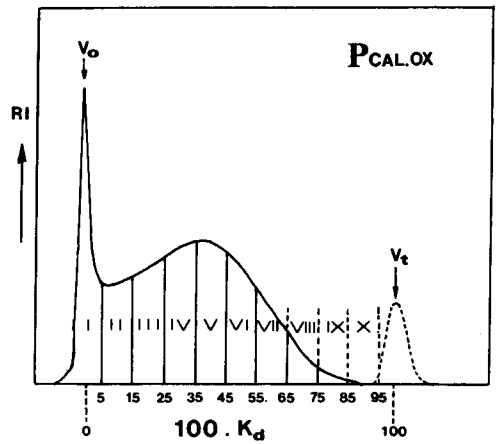


Fig. 9. Specific calibration plot $\log(\eta) = f(K_d)$ of the S200/0·2 M NaCl/flax pectins system: ◪, P_{8OX}; +, P_{CALW}; ▲, P_{7W}; ◪, P_{8W}.

Application of SEC to flax pectins analysis

The macromolecular parameters of pectin samples were evaluated from chromatograms which have been split up into ten equal fractions (I–X) as shown in Fig. 10. For each of them, $[\eta]_i$ and M_i were calculated by considering the main composition of the pectin fractions (Table 2). This whole set of data leads to the macromolecular parameters of pectin samples reported in Table 3. Good agreement between experimental and calculated intrinsic viscosities is found. This is particularly true for pectins of the two first series for which excluded components ($K_d < 0{\cdot}1$) have been characterized by viscometry (SEC/LALLS/viscosity coupling) but also for lower fractions (F_3, F_4) mainly excluded in the selective



	Sample	P_{3OX}	P_{4OX}	P_{6OX}	P_{7OX}	P_{8OX}	P_{12OX}	P_{CALOX}	P_{4W}	P_{6W}	P_{7W}	P_{8W}	P_{12W}	P_{CALW}	*PRW*	F_1	F_3	F_4
F	$10^2.K_d$	FRACTIONAL PERCENTAGE($\%_I$)																
I	<5	0·6	0·08	1·7	8·6	4·7	7·9	16·4	0·4	14	14·1	27	26·1	11·9	27·9	70	1·8	1
II	5–15	2	0·02	3·7	9·3	6·5	9	11·7	0·3	10·8	10·8	12	11·9	12·7	18·4	26	1·1	0·1
III	15–25	4·6	0·43	7	11·6	9·2	10·3	13·7	0·3	8·6	8·4	6·9	7·2	10·6	10·3	4	2·3	—
IV	25–35	9·8	2	11·8	15	12·8	12·2	16·2	1·9	7·3	6·9	5·2	5·5	9·5	7	—	4·5	—
V	35–45	16·6	5·6	17	17·9	16·4	14·7	16·3	4·3	7·2	6·3	4·5	4·5	9·4	5·3	—	14·1	0·5
VI	45–55	22	12·5	19·9	17·4	18·7	16·5	13	8·7	8·5	8·2	5·8	4·6	9·1	4·4	—	25·6	2·5
VII	55–65	21·2	22·7	17·7	11·7	17·4	15·6	8·2	17·1	11·9	13·1	10·6	8·2	8·3	5·8	—	32·7	13·9
VIII	65–75	14·4	27·7	11·6	5·3	10·9	10·1	3·3	23·5	12	12·4	11·3	11·6	7·3	4·9	—	13·6	29·8
IX	75–85	7·1	19·7	5·8	1·9	3	3·2	1	24	10·2	10·6	9·3	11·3	8·7	6·2	—	3	39·8
X	85–95	2	9·2	3·7	1·2	0·3	0·7	0·1	19·3	9·4	9·3	7·5	9·2	12·3	9·8	—	1·2	12·3

Fig. 10. Schematic partition and molecular weight distribution of flax pectin samples percolated on the S200/0·2 M NaCl system.

TABLE 2
Macromolecular Parameters of Flax Pectin Fractions According to Their Main Composition

		I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*
SP. A	$(\eta)_i$	50[d]	45[d]	32·5[a]	22·2[a]	15·2[a]	10·4[a]	7·1[a]	4·8[a]	3·3[a]	2[g]
	$M_i \times 10^3$	78[e]	23[f]	16·9[a]	11·8[a]	8·3[a]	5·8[a]	4·1[a]	2·9[a]	2[a]	1[g]
SP. B	$(\eta)_i$	60[d]	38[d]	27·2[b]	19·3[b]	13·7[b]	9·8[b]	6·9[b]	4·8[a]	3·3[a]	2[g]
	$M_i \times 10^3$	100[e]	53[e]	20·2[b]	13·6[b]	9·1[b]	6·2[b]	4·1[b]	2·9[a]	2[a]	1[g]
SP. C	$(\eta)_i$	69[d]	45[d]	22·7[c]	16·8[c]	12·4[c]	9·2[c]	6·8[c]	4·8[a]	3·3[a]	2[g]
	$M_i \times 10^3$	125[e]	67·2[e]	24·2[c]	15·7[c]	10·1[c]	6·5[c]	4·2[c]	2·9[a]	2[a]	1[g]

SP. A: P_{3OX}, P_{4OX}, P_{6OX}, P_{7OX}, P_{8OX}, P_{12OX}, P_{CALOX}, P_{4W} and P_{CALW} for P_{CALW}, $(\eta)_I^d = 76$ $cm^3\,g^{-1}$ and $M_I^e = 145 \times 10^3$.
SP. B: P_{6W}, P_{7W}.
SP. C: P_{8W}, P_{12W}, *PRW*, F_1, F_3, F_4.
[a]Macromolecular parameters calculated from eqns (5) and (7).
[b]Macromolecular parameters calculated from eqns (4) and (6).
[c]Macromolecular parameters calculated from intermediate equations.
[d]Intrinsic viscosities measured during coupling SEC/LALLS/Viscosity.
[e]Average molecular weights calculated from eqn (3).
[f]Average molecular weights calculated from eqn (2).
[g]Arbitrary parameters estimated for fraction X.

permeation area of the chromatographic system. For higher fractions (F_1 and P_{RW}), which are eluted with a broad peak in the void volume, use of mean values to characterize the excluded components leads to a higher discrepancy of $\Delta(\eta)/(\eta) \simeq 17\%$. Although no reference may be used to compare average molecular weights, it can nevertheless be estimated that the calculated values of M_w and M_n can be used for defining the size of percolated samples with the same accuracy as the intrinsic viscosities.

These data show that the calibration curves give reliable information in the whole selective permeation area. However, the indirect characterization of pectin samples appears to be restricted owing to the fact that some components are excluded on this chromatographic system. Although this major drawback may be circumvented by analysing the substances eluted in the void volume by SEC/LALLS/viscosity coupling, it would be better, in the future, to use columns of higher porosities (S300/S400) in addition to the Sephacryl 200.

An interesting comparison between the different samples can now be drawn from the SEC analysis. Pectins extracted with hot water from cell walls or flax callus are characterized during the growth (P_{6W}, P_{7W} and

TABLE 3
Macromolecular Parameters of Flax Pectin Samples

Sample	$(\eta)_{exp.}$	$(\eta)_{cal.}$[a]	$M_w \times 10^{3}$[b]	$M_n \times 10^{3}$[c]	I[d]
P_{3OX}	11·2	12·1	7	4·4	1·6
P_{4OX}	7	6·6	3·8	2·7	1·4
P_{6OX}	14·1	14·1	8·5	4·5	1·9
P_{7OX}	21·8	21·3	15·8	6·9	2·3
P_{8OX}	17·2	17·4	11·8	5·9	2
P_{12OX}	20·8	19·7	14·6	6·1	2·4
P_{CALOX}	—	26·2	22·3	9·2	2·4
P_{4W}	5·8	6·1	3·6	2·2	1·6
P_{6W}	21·8	20	24·8	4	6·2
P_{7W}	19·9	19·9	24·7	3·9	6·3
P_{8W}	28·1	29·3	46·1	4·8	9·6
P_{12W}	27·1	28·6	44·9	4·3	10·4
P_{CALW}	—	24·2	24·6	3·8	6·5
P_{RW}	40·2	33·2	52	5·4	9·6
F_1	72·4	60·6	106	90	1·2
F_3	10·7	10·1	9·1	4·8	1·9
F_4	4·4	4·9	3·9	2·2	1·8

[a] $(\eta) = \dfrac{\Sigma(\eta)_i . \%_i}{\Sigma \%_i}$

[b] $M_w = \dfrac{\Sigma M_i . \%_i}{\Sigma \%_i}$

[c] $M_n = \dfrac{\Sigma \%_i}{\Sigma \%_i / M_i}$

[d] $I = M_w / M_n$.

P_{CALW}) by an average moleular weight $M_w = 25\,000$ and a high polydispersity ($I_p = 6{\cdot}5$). Just after up rooting, the rapid elimination of pectins PIII (Fig. 11) leads to an increase both in M_w ($M_w = 45\,000$) and polydispersity ($I_p \simeq 10$). These values are quite similar to those reported with P_{RW} extracted from retted flax fibres. With the exception of P_{4W}, a sample extracted from cell walls which were accidently degraded by fungi, Fig. 11 shows that little or no evolution of pectins PI occurs until pulling up. Thereafter a decrease of 20–30% at the void volume occurs during dew-retting ($P_{8W} \rightarrow P_{12W}$). This elimination of pectins PI during

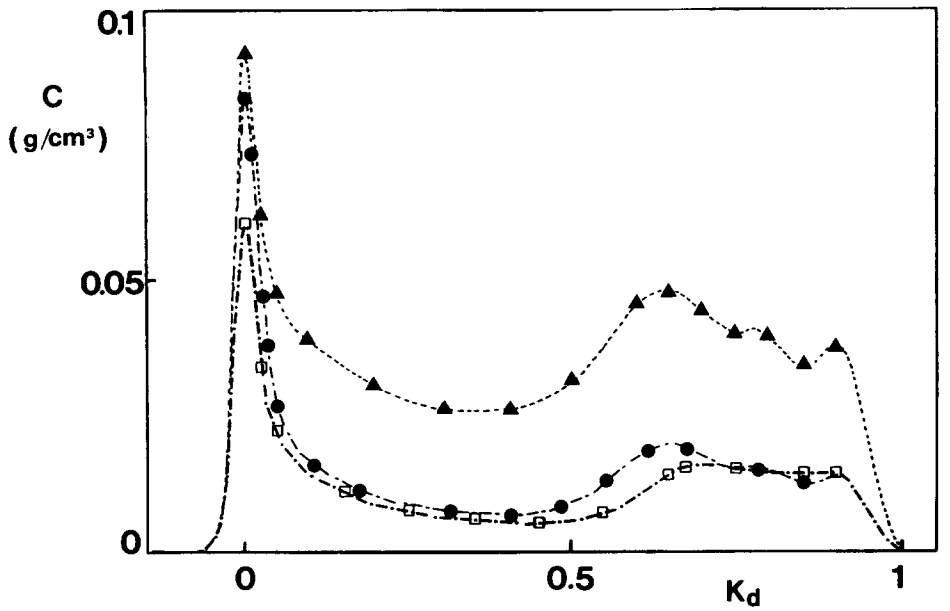


Fig. 11. Size distribution, on the S200/0·2 M NaCl system, of pectins P_w extracted with hot water from 100 g of flax cell walls: ▲, P_{6W}/P_{7W}, end of growth; ●, P_{8W}, start of the retting process; □, P_{12W}, end of retting.

the retting process may also be related to the variation of extinction coefficient at 214 nm. Figure 12 displays the variation of ε^{214} versus the distribution coefficient K_d. A difference clearly appears between unretted (curve I) and retted (curve II) samples. The higher coefficients obtained for both P_{12W} and P_{RW} may be partly ascribed to a β-elimination of pectic chains by lyase.

Data reported in Table 3 indicate that pectins extracted with oxalate (P_{OX}) are more homogeneous both in size and composition. Owing to ultrafiltration conditions applied for oxalate removal, only samples P_{3OX}, P_{4OX}, P_{6OX} and P_{CALOX} (ultrafiltered with a molecular cut-off 1000 D) can be considered to describe the actual size of these chains. Such pectins P_{OX} are characterized by a $\bar{M}_w$ in the range 4000–22 000 and a low polydispersity ($1{\cdot}5 \leq I_p \leq 2{\cdot}5$). By considering only the pectin component PII, a constant $M_w = 6500$–7000 is found during the entire step of growth ($P_{3OX} \rightarrow P_{6OX}$) for samples extracted from cell walls whereas $M_w = 11\,000$ is obtained for those extracted from callus indifferentiated cells (P_{CALOX}). If no value may be used to characterize pectins PII during and at the end of dew-retting, we nevertheless mention $M_w = 3700$ for P_{4OX}PII extracted from degraded cell walls. These data relating to

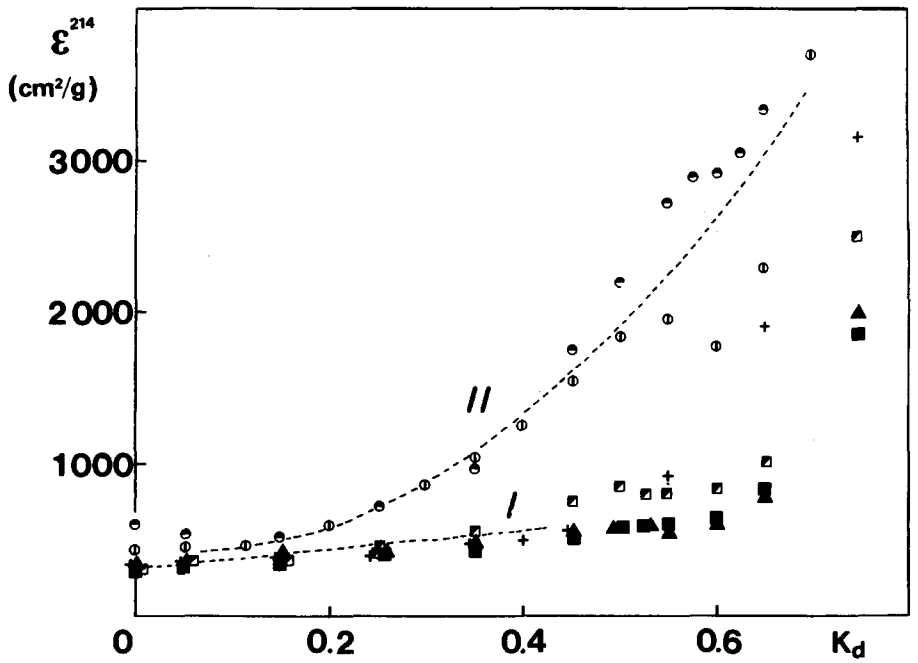


Fig. 12. Extinction coefficients at $\lambda = 214$ nm versus K_d for pectins extracted with hot water: during growth (I) — ■, P_{6W}; ▲, P_{7W}; +, P_{CALW}; and retting (II) — ◪, P_{8W}; ⊖, P_{12W}; ⊕, P_{RW}.

pectins PII may be compared favourably with those recorded by Schneider and Bock (1937). Those authors, working on nitrated pectins extracted from flax, reported viscosity average molecular weights of $M_\eta = 11\,000$–$16\,000$ for flax straw and $M_\eta = 3000$–$10\,000$ for retted flax.

In spite of scarcity of data and references which prohibits any sound conclusion about the general evolution of flax pectins during growth and retting, the authors nevertheless maintain that SEC is a very convenient tool for characterizing pectic cements. It provides important information about the size, the molecular weight distribution and the composition of flax pectins, which form part of a whole set of parameters necessary to understand better the wall organization and the physiological mechanisms that intervene in the primary cell walls and middle lamellae during growth and retting. This is the aim with which this work will continue.

REFERENCES

Anderson, D. M. W., Hirst, E. L., Rahman, S. & Stainsby, G. (1967). *Carbohyd. Res.*, **3**, 308–17.
Anger, H. & Berth, G. (1985). *Carbohyd. Polymers*, **5**, 241–50.
Anger, H. & Berth, G. (1986). *Carbohyd. Polymers*, **6**, 193–202.
Aspinall, G. O. (1980). *The Biochemistry of Plants*, Vol. 3, ed. J. Preiss, p. 473.
Axelos, M. A. V., Lefebvre, J. & Thibault, J. F. (1987). *Food Hydrocolloid*, **1**, 569–70.
Berth, G., Anger, H. & Linow, F. (1977). *Die Nahrung*, **21**, 939–50.
Berth, G. (1988). *Carbohyd. Polymers*, **8**, 105–17.
Bohdanecky, M. (1983). *Macromolecules*, **16**, 1483–92.
Brigand, G., Denis, A., Grall, M. & Lecacheux, D. (1990). *Carbohyd. Polymers*, **12**, 61–77.
Darvill, A. G., McNeil, M. & Albersheim, P. (1978). *Plant Physiol.*, **62**, 418–22.
Darvill, A. G., McNeil, M., Albersheim, P. & Delmer, D. P. (1980). *The Biochemistry of Plants*, Vol. 1, ed. N. E. Tolbert, pp. 91–162.
Davis, E. A., Derouet, C., Herve du Penhoat, C. & Morvan, C. (1990). *Carbohyd. Res.*, **197**, 205–15.
Deckers, H. A., Olieman, C., Rombouts, F. M. & Pilnik, W. (1986). *Carbohyd. Polymers*, **6**, 361–73.
De Vries, J. A., Rombouts, F. M., Voragen, A. G. J. & Pilnik, W. (1982). *Carbohyd. Polymers*, **2**, 25–33.
Fishman, M. L., Pfeffer, P. E., Barford, R. A. & Doner, L. W. (1984). *J. Agric. Food Chem.*, **32**, 372–8.
Fishman, M. L., Pepper, L. & Pfeffer, P. E. (1986). *Adv. Chem. Ser.*, **213**, 57–70.
Fishman, M. L., Gross, K. C., Gillespie, D. T. & Sondey, S. M. (1989). *Archives of Biochemistry and Biophysics*, **1**, 179–91.
Fritzsche, P., Lehmann, I., Dongowski, G. & Bock, W. (1977). *Fazerforschung und Textiltechnik*, **28**, 543–5.
Glikman, S. A. & Orlov, S. I. (1950). *Dokl. Akad. Nauk. SSSR*, **71**, 895–98.
Grubisic, Z., Rempp, P. & Benoit, H. (1967). *J. Polym. Sci.*, **B5**, 753–9.
Harding, S. E. (1987). *Gums and Stabilisers in the Food Industry*, Vol. 4, ed. G. O. Phillips, D. J. Wedlock & P. A. Williams, p. 15.
Hourdet, D. & Muller, G. (1987). *Carbohyd. Polymers*, **7**, 301–12.
Hourdet, D. & Muller, G. (1990). *Carbohyd. Polymers*, **16**, 113.
Hourdet, D. (1989). Thèse de Doctorat de l'Université Paris 6.
Jarvis, M. C. (1984). *Plant, Cell and Environment*, **7**, 153–64.
Kawabata, A. (1977). *Memoirs of the Tokyo University of Agriculture*, **XIX**, 115–201.
Kiyohara, H. & Yamada, H. (1989). *Carb. Res.*, **187**, 117–29.
Knee, M., Fielding, A. H., Archer, S. A. & Laborda, F. (1975). *Phytochem.*, **14**, 2213–22.
Kohn, R. & Luknar, O. (1977). *Coll. Czech. Chem. Commun.*, **42**, 731–44.
McNeil, M., Darvill, A. G. & Albersheim, P. (1980). *Plant Physiol.*, **66**, 1128–34.
Morris, V. J. (1986). *Functional Properties of Food Macromolecules*, Vol. 3, ed. J. R. Mitchell & D. A. Leward. Elsevier Applied Science Publishers, p. 121.

Morvan, C., Morvan, O., Jauneau, A. & Demarty, M. (1985). *C. R. Acad. Agri.*, **71**, 837–41.
Morvan, O., Jauneau, A., Morvan, C., Demarty, M. & Ripoll, C. (1988). *Ann. Appl. Biol.*, **112**, 107–18.
Morvan, C., Abdul-Hafez, A., Morvan, O. & Jauneau, A. (1989*a*). *Plant Physiol. Biochem.*, **27**, 451–9.
Morvan, O., Jauneau, A., Morvan, C., Voreux, H. & Demarty, M. (1989*b*). *Can. J. Bot.*, **67**, 135–9.
Owens, H. S., Lotzkar, H., Schultz, T. H. & Maclay, W. D. (1946). *J. Amer. Chem. Soc.*, **68**, 1628–32.
Panchev, I. N., Kirtchev, N. A., Kratchanov, C. R. & Proichev, T. (1988). *Carbohyd. Polymers*, **8**, 257–69.
Plashchina, I. G., Semenova, M. G., Braudo, E. E. & Tolstoguzov, V. B. (1985). *Carbohyd. Polymers*, **5**, 159–79.
Rees, D. A. (1972). *Biochem. J.*, **126**, 257–69.
Rees, D. A. & Wight, A. W. (1971). *J. Chem. Soc.*, **B**, 1366–72.
Rosemberg, J. A. & De Franca, F. P. (1967). *Appl. Microbiol.*, **15**, 484–6.
Saulnier, L. & Brillouet, J. M. (1988). *Carbohyd. Res.*, **182**, 63–78.
Schneider, G. G. & Bock, H. (1937). *Ber.*, **70B**, 1617–30.
Smidsrød, O. & Haug, A. (1968). *Acta Chem. Sc.*, **22**, 797–810.
Smidsrød, O. & Haug, A. (1971). *Biopolymers*, **10**, 1213–27.
Thibault, J.-F. (1983). *Carbohyd. Polymers*, **3**, 259–72.
Vollmert, B. (1950). *Makromol. Chemie*, **5**, 128–38.
Wedlock, D. J., Fasihuddin, B. A. & Phillips, G. O. (1986). *Gums and Stabilisers for the Food Industry*, Vol. 3, ed. G. O. Phillips, D. J. Wedlock & P. A. Williams, p. 47.
Yamakawa, N. & Fujii, M. (1974). *Macromolecules*, **7**, 128–35.