Carbohydrate Polymers **16** (1991) 113–135

Solution Properties of Pectin Polysaccharides II. Conformation and Molecular Size of High Galacturonic Acid Content Isolated Pectin Chains

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(Received 30 January 1990; revised version received 20 April 1990; accepted 3 May 1990)

ABSTRACT

The solution properties of flax pectins and commercial pectins with high galacturonic acid content (AGA > 80%) were studied by low-angle laser-light scattering (LALLS), viscosity and Size Exclusion Chromatography (SEC) experiments performed in 0·2 M aq. NaCl. LALLS data were strongly affected by the presence of a small amount of high molecular weight superstructures. Molecularly dispersed pectin solutions could be obtained after complete removal of these high molecular weight particles by various procedures. The conformation of isolated pectin chains has been determined from the relationship between molecular weight, M_w, $[\eta]$ and the distribution coefficient (K_d) measured on the Sephacryl 200/0·2 M NaCl chromatographic system. Analysis of the results, combined with a set of literature data, in terms of the known theories for rods and worm-like chains, gave values of 67 Å and 6 Å for the persistence length and the hydrodynamic diameter, respectively, of pectin in 0·2 M NaCl. These results suggest that pectin with a high galacturonate content behaves in NaCl solution like an extended coil with a similar conformation to the alginate chain.

INTRODUCTION

Pectins, predominantly copolymers of $\alpha(1\rightarrow4)$-galacturonate and its methyl esters, are important heteropolysaccharides. Located in primary cell walls and middle lamellae they act as intercellular cementing material upon the cellulosic network. Experimental data from various authors, mainly from light scattering, viscosity and size exclusion

Carbohydrate Polymers 0144-8617/91/$03.50

chromatography experiments, indicate that pectin solutions generally contain a small percentage of high molecular weight particles. Their presence has a strong disturbing influence on the light-scattering behaviour, particularly the low-angle data, whereas the intrinsic viscosity, $[\eta]$, is only slightly affected. This explains the large differences between the reported Mark–Houwink plots as illustrated by literature data shown in Fig. 1. It also explains the difficulties encountered in determining the actual conformation of the pectic chain. Depending on the authors in question and the measuring techniques used, pectin molecules have been reported to behave as rigid-rod particles (Owens *et al.*, 1946; Glikman & Orlov, 1950; Vollmert, 1950; Fritzsche *et al.*, 1977) or as

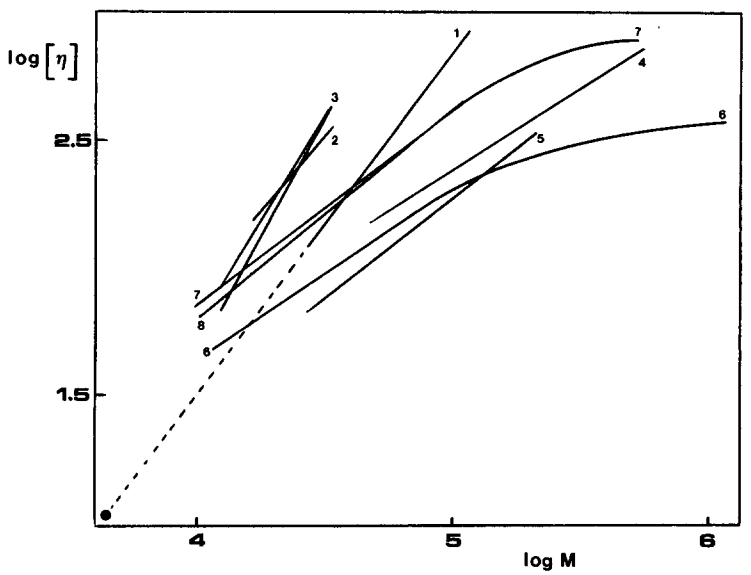

Fig. 1. Mark–Houwink relationships for pectic substances.

No.	*Authors*	*K.10³*	*a*
1.	Owens *et al.*, 1946	0·14	1·34
2.	Glikman *et al.*, 1950	1·1	1·22
3.	Fritzsche *et al.*, 1977	0·00216	1·84
		0·0113	1·68
4.	Kawabata, 1977	153	0·64
5.	Berth *et al.*, 1977	21·6	0·79
6.	Anger & Berth, 1985	85·1	0·68
7.	Anger & Berth, 1986	95·5	0·73
8.	Deckers *et al.*, 1986	49	0·79

coils of variable stiffness (Berth *et al.*, 1977; Kawabata, 1977; Anger & Berth, 1985, 1986; Deckers *et al.*, 1986; Axelos *et al.*, 1987).

Some authors have reported that the stiffness of chains could also depend on the degree of esterification (DE) (Michel *et al.*, 1982; Fishman *et al.*, 1984; Deckers *et al.*, 1986), whereas others (Owens *et al.*, 1946; Anger & Berth, 1986; Berth, 1988) have found that DE has no effect on the $[\eta]$–M relationship (M = Mw or Mn).

The neutral sugar content has been reported to affect the conformation and it has been suggested that fractions rich in neutral sugars are responsible for the high molecular weight found in some cases (Berth *et al.*, 1977; Berth, 1988).

As small quantities ($\leq 1\%$) of the microgel component have a disturbing effect on the light scattering response (Cesaro *et al.*, 1982), many different methods have been proposed for obtaining a molecularly dispersed pectin solution by removing these high molecular weight superstructures, by e.g. enzyme treatment (Smith & Stainsby, 1977), the use of hydrogen bond breakers (Robinson *et al.*, 1982), gel permeation chromatography (GPC) on Sephadex 200 (Jordan & Brant, 1978), ion-exchange chromatography (Berth *et al.*, 1977), or ultracentrifugation (Berth *et al.*, 1977; Kawabata, 1977; Plashchina *et al.*, 1985).

In a previous paper (Hourdet & Muller, 1987) the authors reported data on the physicochemical properties of flax pectins in NaCl solution. The difficulties encountered in obtaining optically clean solutions free of microgel, even after ultracentrifugation, led to more extensive examination of the problem of aggregates, in particular their effect on the solution properties of pectins, and the more effective methods for their removal.

In the present paper a set of data concerning 'microgels' in flax pectin solutions, their effect on molecular weight determination by low-angle laser light scattering (LALLS) are presented and discussed. Following complete removal of such microgels, it is shown that it is possible to measure the absolute molecular weight dispersed pectin chains and therefore to obtain reliable information on the conformation of pectin molecules with a high galacturonic acid content.

EXPERIMENTAL

Materials

The main specifications of the pectin samples investigated in this study are listed in Table 1. Flax pectins were extracted chemically (Goldberg *et*

TABLE 1
Specifications of Pectin Samples

Sample	*Origin/extraction*	*Purification*[a]	*Me*[b] *(g)*	*DE*[c]	d*n*/d*c*[d] *($cm^3\,g^{-1}$)*
F1	Flax(fibres)/water	Fract./UF.10^4D	465	38	0·136
F2	Flax(fibres)/water	Fract./UF.10^4D	405	30	—
F3	Flax(fibres)/water	Fract./UF.10^4D	395	27	0·138
F4	Flax(fibres)/water	Fract./UF.10^4D	345	24	0·139
PVw	Flax(fibres)/water	F. 8 μm	290	11	0·170
P4w	Flax(cell walls)/water	UF.10^3D	230	48	
P6w	Flax(cell walls)/water	UF.10^3D	270	49	0·156
P7w	Flax(cell walls)/water	UF.10^3D	280	47	±[g]
P8w	Flax(cell walls)/water	UF.10^3D	300	49	0·002
P12w	Flax(cell walls)/water	UF.10^3D	280	40	
P12wb	Flax(cell walls)/water	F. 8 μm	—	—	0·160
PRw	Flax(fibres)/water	EtOH/C10^4G[f]	—	—	0·152
PCALw	Flax(callus)/water	F. 8 μm	<250	—	0·151
P3ox	Flax(cell walls)/oxalate	UF.10^3D	185	13	0·149
P4ox	Flax(cell walls)/oxalate	UF.10^3D	185	13	±
P6ox	Flax(cell walls)/oxalate	UF.10^3D	190	10	0·002
P7ox	Flax(cell walls)/oxalate	UF.10^3D	220	10	0·146
P8ox	Flax(cell walls)/oxalate	UF.10^4D	210	9	±
P12ox	Flax(cell walls)/oxalate	UF.10^4D	250	7	0·002
PCALox	Flax(callus)/oxalate	UF.10^3D	<250	—	0·143
PA	Apple (Unipectine[e])	—	<250	45	0·143
PC	Citrus (Sigma[e])	—	<250	63	0·141
PGA	Orange (Sigma[e])	Na^+ form	—	0	0·142

[a]Purification: Frac = fractionated; UF = ultrafiltration; D = dalton; F = filtration; C = centrifugation; EtOH = precipitation with ethanol; Na^+ form = neutralization by NaOH.
[b]Me = Equivalent weight to an anhydrogalacturonic unit.
[c]DE = Degree of esterification.
[d]dn/dc = Refractive index increment in 0·2 M NaCl.
[e]Commercial pectins.
[f]10^4G = approx. 10 000 rpm.
[g]± = Reproductibility on the measured values.

al., 1986; Morvan *et al.*, 1989), either from cell walls or directly from flax fibres after removal of wood. Material extracted with hot water (twice, 8 h, 100°C) is termed 'pectin PW', the residue treated with a calcium ion chelating agent (ammonium oxalate 0·5% w/v, twice, 100°C) produced 'pectin POX'. After extraction, pectins PW and POX were then ultrafiltered through Pellicon membranes (Millipore 1000 D or 10 000 D) and finally freeze-dried to constant weight. Apple pectin (PA) and citrus pectin (PC) were commercial samples (Unipectine (Paris,

France) and Sigma (La Verpilliere, France), respectively) and used without further purification. Polygalacturonic acid (PGA, from Sigma) was analysed as the Na^+ form, after neutralization with NaOH.

Preparation of the solutions

All pectin samples were initially dissolved at room temperature in purified water (from Milli-ro + Milli-q system) then NaCl was added in order to obtain pectin solutions in 0.2 M NaCl. Solutions were then filtered on Millex GV 0·22 μm (Durapore membrane).

More extensive clarification of filtered solutions was obtained by ultracentrifugation using a Beckman L8-70 ultracentrifugation using a Beckman L8-70 ultracentrifuge (Rotor 70 Ti, 45 000, rpm, $\theta = 4°C$).

In all cases, pectin concentration was obtained from the dry weight of material used. All measurements were performed when the pectin in solution was in the fully dissociated form (pH $\approx$ 8·0).

Low-angle laser light scattering (LALLS)

LALLS measurements were performed on a laser photometer Chromatix KMX-6 ($\lambda = 633$ nm). At low angle of observation ($\theta = 4{\cdot}88°$) both the weight-average molecular weight (M_w) and the second virial coefficient (A_2) can be obtained from a plot of $KC/\Delta R_\theta$ versus C, where C is the polymer concentration, ΔR_θ is the measured excess Rayleigh factor between solution and solvent, and K is an optical constant and depends on geometrical factors of the apparatus, refractive index of the solvent and refractive index increment (dn/dc) of the solution.

Under the experimental conditions

$$K = 7{\cdot}2 \times 10^{-6} \left[\frac{dn}{dc}\right]^2 (\text{cm}^2\ \text{g}^{-2})$$

The values of dn/dc are given in Table 1.

Viscometry

Viscosity measurements were made at a temperature of 25°C using a modified Ubbelohde viscometer (Fica Viscomatic MS).

Size exclusion chromatography

Aliquots of pectin solution (in 0·2 M NaCl) were applied to the top of a chromatographic column (Pharmacia K26/70) wet-packed with

Pharmacia Sephacryl 200 Superfine and equilibrated with dust-free 0·2 M NaCl at a flow rate of approximately 2 $cm^3\ min^{-1}$.

Eluate was monitored at the column output using a double detection system comprising a UV-photometer (214 nm) (Pharmacia UV1/214) and a differential refractometer (Schimadzu RID-6A).

Various standards were used for the calibration of the S200/NaCl 0·2 M system:

Dextran (DX) (Pharmacia Fine Chemicals AB, Uppsala, Sweden)
Sodium polystyrene sulphonate (PSSNa) (Pressure Chemical Co., Pittsburgh, USA)
Polyethylene oxide (PEO) (Polymer Laboratories Ltd, Church Stretton, UK)
Polyethylene glycol (PEG) (Fluka Chemie AG, Buchs, Switzerland)

Blue Dextran 2000 and NaCl were used for the exclusion limits:

Blue dextran 2000 (BD 2000) molecular mass = $2 \times 10^6 \longrightarrow V_0$
Sodium chloride (NaCl) molecular mass = 58.44 $\longrightarrow V_T$

RESULTS AND DISCUSSION

Superstructures in pectin solutions

LALLS measurements were performed according to the following procedure:

(1) Preparation of pectin solutions ($C \leq 10^{-3}$ g cm^{-3}) in 0·2 M sodium chloride solution.
(2) Introduction of the solvent into the KMX cell by filtration through a Millex GV unit (0·22 μm).
(3) Recording of light-scattering intensities for injection volumes (V_i) of 5 cm^3.
(4) Operations 2 and 3 were repeated with increasing pectin concentration. (Only one filter was used for all the solutions of a pectin sample.)

The LALLS behaviour of pectins from various origins (flax, apple, citrus) exhibits evidence for the existence of non-molecularly dispersed material or large aggregates (Fig. 2). Very high values of $M_w/[\eta]$ are found, in agreement with other data reported in the literature (Smith & Stainsby, 1977; Berth *et al.*, 1977; Jordan & Brant, 1978; Anger & Berth, 1985, 1986) with $M_w > 10^5$ (Fig. 1, curves 6 and 7)). They are

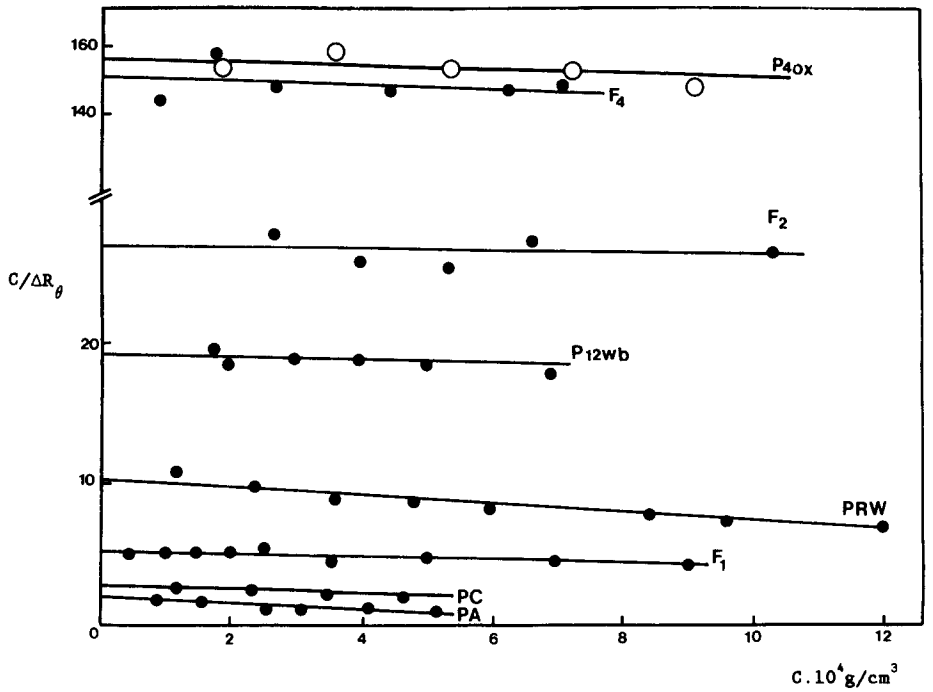

Fig. 2. $C/\Delta R_\theta$ dependence on concentration C for crude pectin solutions (0·2 M NaCl) filtered through Millex GV 0·22 μm filter.

Sample	*PA*	*PC*	*F1*	*PRw*	*P12wb*	*F2*	*F4*	*P4ox*
$[\eta]$ cm^3 g^{-1}	278	335	72·4	40·2	24·4	27·6	4·4	7
$M_w \times 10^{-5}$	46·8	27·5	14·6	6·2	2·9	2·7	0·47	0·40
$A_2 \times 10^5$ cm^3/g^2	−12·5	−6·9	−4	−16·5	−6	−2·5	−38	−28

indicative of the presence of superstructures (microgels). Such superstructures, even if present at low levels, may considerably perturb the light-scattering data, especially at low angles, without affecting the viscosity. Moreover, such superstructures can be held responsible for the abnormal negative values of A_2 observed. The same observation was reported by Smidsrød & Haug (1968) for alginates, where A_2 was used as a *clarification parameter* or *molecular dispersion parameter.*

The same experimental procedure was repeated with the addition of clarification through Millex GS 0·22 μm filters (cellulose esters) prior to sample injection. The data reported in Fig. 3 clearly show that the light-scattering behaviour strongly depends on the nature of the filter. The increase in the Rayleigh ratio R_θ with injected volume is indicative of a

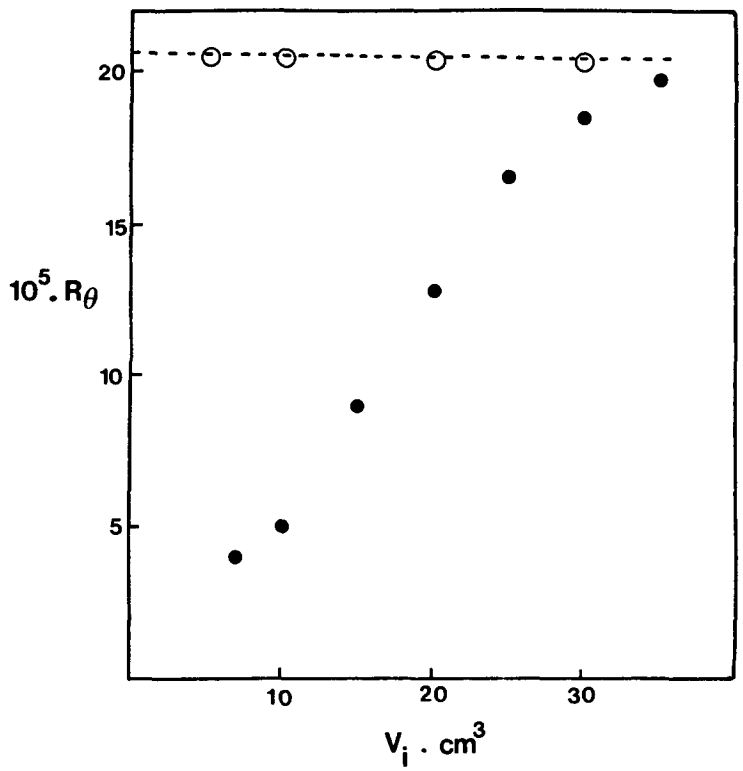

Fig. 3. Variation of the Rayleigh ratio with injected volume for pectins filtered through: ●, Millex GS 0·22 μm and ○, Millex GV 0·22 μm. (Sample PA, $C_o = 4{\cdot}06 \times 10^{-4}$ g cm^{-3} in 0·2 M NaCl.)

specific adsorption from a part of or the whole of the pectic material onto the Millipore MF membrane.

From refractometry and UV measurements (Fig. 4) it can be shown that the specific adsorption concerns only a very small fraction of the pectic material. Virtually no change in the concentration as a function of the filter is found. On the other hand, many observations seem to indicate that the superstructures are probably protein. These include:

High scattered light intensity (Fig. 3).
High UV absorption (Fig. 4).
Specific adsorption onto 'cellulosic' membranes (Millex GS and HA) (Fig. 4).
Salt-induced mechanism (no adsorption phenomenon in water).

Such observations can be compared with data of Kolodziej (1987) who showed that aggregates formation in xanthan solutions could result

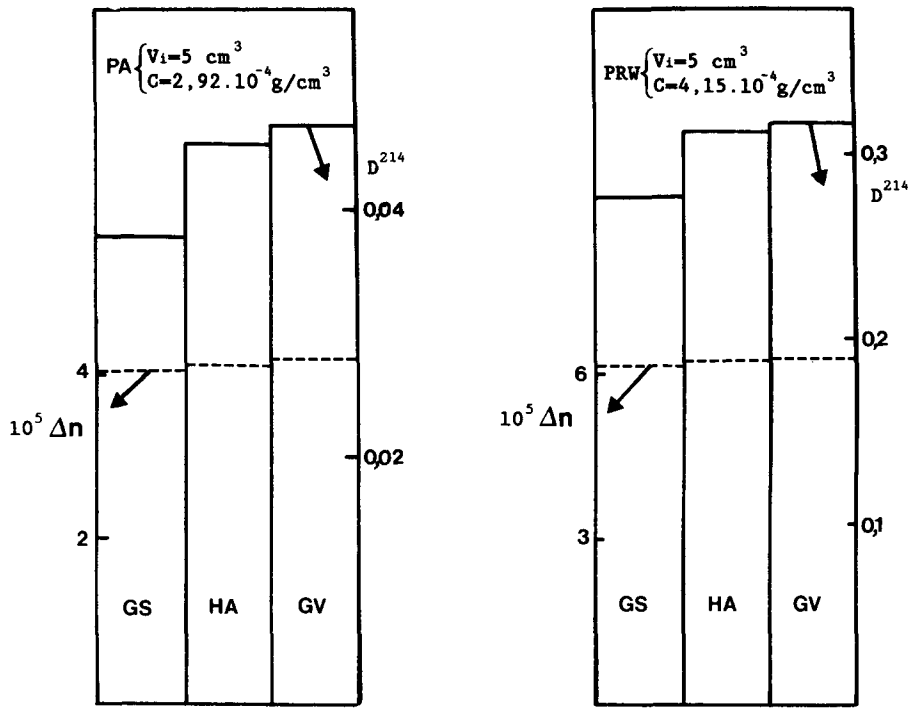

Fig. 4. Variations of pectin concentration (Δn) and absorbance at 214 nm (D^{214}) as a function of the filter used. GV, Durapore membrane (0·22 μm); GS, cellulose esters membrane (0·22 μm); HA, cellulose esters membrane (0·45 μm).

from a salt-induced interaction mechanism between denatured proteins and biopolymer molecules which establish nucleation sites for other biopolymer molecules to form a microgel aggregate. In this respect it is interesting to recall that Kolodziej (1987) used a Millipore filtration test for microgel detection based on the filterability of the polymer solution through a series of Millipore MF filters, as proposed by Kohler & Chauveteau (1981).

By taking into account this specific adsorption phenomenon of microgels onto the MF filters and more specially onto GS 0·22 μm type, the assumption can be made that for extremely dilute solutions and low injected volumes *only isolated pectin chains cross the membrane.* With this hypothesis and using the LALLS data obtained with various pectins (filter = Millex GS, injection volume = 3 cm^3) evaluation of the M_w values obtained from the experimental variation of $C/\Delta R_\theta$ against C was attempted by plotting $(C/\Delta R)^{1/2}$ versus $C^{1/2}$ (Fig. 5).

From the results reported in Table 2, a very large discrepancy exists between molecular weights measured on initial solutions (M_w^a) and the

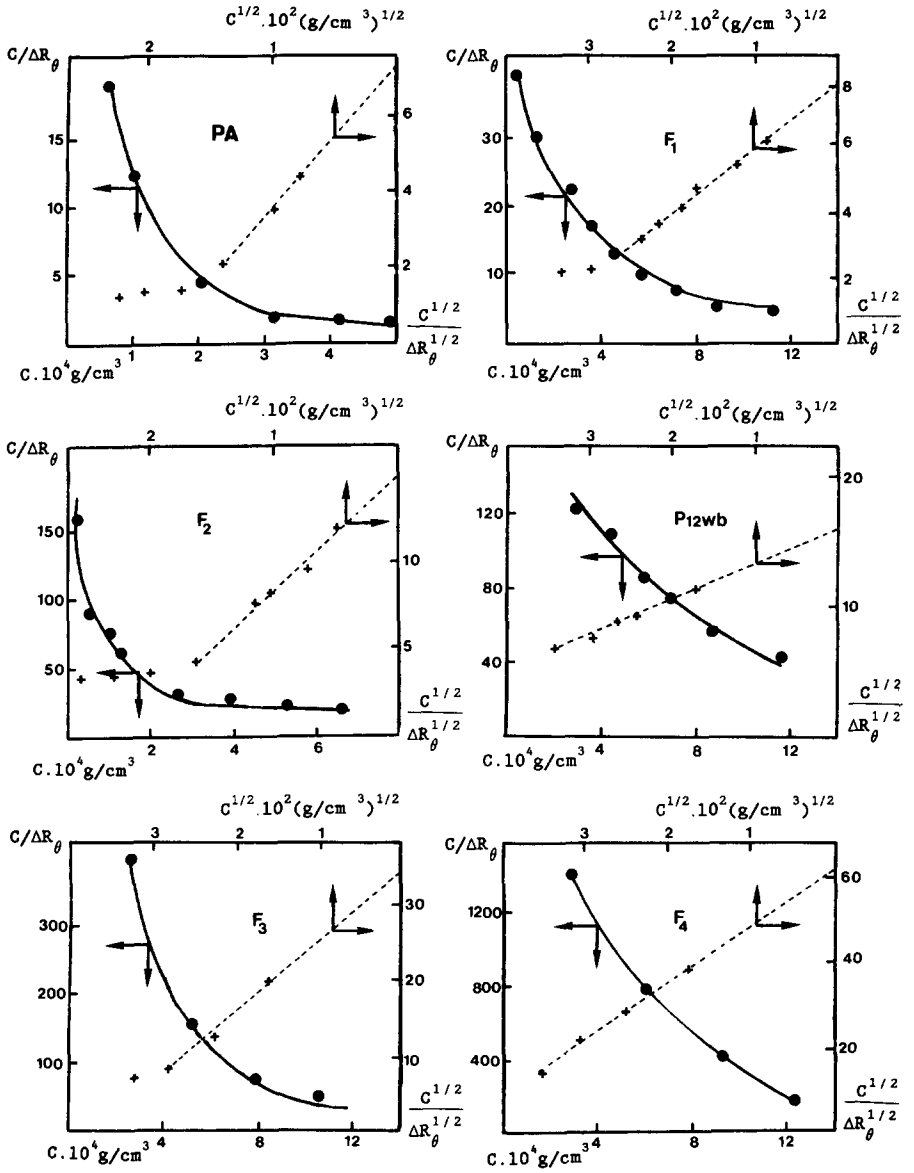

Fig. 5. Concentration dependence of $C/\Delta R_\theta$ (——) for pectin solutions clarified through Millex GS 0·22 μm. (---) Extrapolation method at $C=0$, according to $(C/\Delta R_\theta)^{1/2}$ versus $C^{1/2}$.

TABLE 2
Initial (M_w^a) and Extrapolated (M_w^b) Weight-average Molecular Weights of Pectic Substances in 0·2 M NaCl

Filter		*Sample*						
		PC	*PA*	*F1*	*P12wb*	*F2*	*F3*	*F4*
GV 0·22 μm	$[\eta]$, $cm^3\,g^{-1}$	335	278	72·4	24·7	27·6	10·7	4·4
GV 0·22 μm	$M_w^a \times 10^{-4}$	275	468	146	29	27	—	4·7
GS 0·22 μm	$M_w^b \times 10^{-4}$	14	13	12	2·1	3·1	0·63	0·2
	M_w^a/M_w^b	20	36	12	13	9	—	24

extrapolated ones (M_w^b) corresponding to molecularly dispersed pectic chains; ratios from 10 to 40 observed with different samples reflect the drastic contribution of aggregates to the measured scattered light.

Although the molecular weight of pectin samples obtained according to this empirical extrapolation procedure seem more 'consistent' with the viscosity data, these values are still dependent on both the experimental and the extrapolation method used in this work.

In an attempt to obtain molecularly dispersed pectin solution different techniques were used for achieving more extensive clarification.

Preparation of molecularly dispersed solutions

Centrifugation

In addition to the filtration techniques generally used for the preparation of polymer solutions, centrifugation and ultracentrifugation are widely carried out in order to complete the removal of extraneous material before LALLS studies.

A number of authors (Smidsrød & Haug, 1968; Berth *et al.*, 1977; Kawabata, 1977; Anger & Berth, 1986) usually clarify their polysaccharide solutions by ultracentrifugation (100000 *g*–280000 *g*). Therefore a high velocity gradient (150000 *g*, 1 h, 4°C) was applied to all the pectin solutions used in the present study.

The changes in the refractive index and viscosity following ultracentrifugation of the highest molecular weight samples (PA and PC) do not exceed 5% of the initial values. Therefore, it can be assumed that under the present conditions, ultracentrifugation does not 'affect' the initial distribution of the fully dispersed pectin chains.

In contrast to the refractometric response, which is proportional to the polymer concentration only, UV detection is a very interesting

method for determining the existence of microgels. By coupling refractometric and UV-214 detectors at the output of the Sephacryl 200/0·2 M NaCl chromatographic system, it is possible to measure the change in Δn and the extinction coefficients versus elution volume (V_e) for the fractionated pectin chains. In Fig. 6 the change in ε^{214} versus K_d for the sample P6ox before and after ultracentrifugation is reported. Following this treatment a large decrease in ε^{214} in the void volume area can be observed, which probably results from the sedimentation of microgels submitted to the high velocity gradient. Under the same conditions the extinction coefficient of galacturonic acid is $\varepsilon^{214} \simeq 350$ cm^2 g^{-1}.

LALLS measurements were performed on pectin solutions after ultracentrifugation. Two facts are worth noting (Table 3):

The second virial coefficients become slightly positive.

The weight-average molecular weights decrease in the ratio M_w^a/M_w^c from 3 to 25 according to the selected sample. (M_w^c is the molecular weight obtained for the samples clarified by ultracentrifugation.)

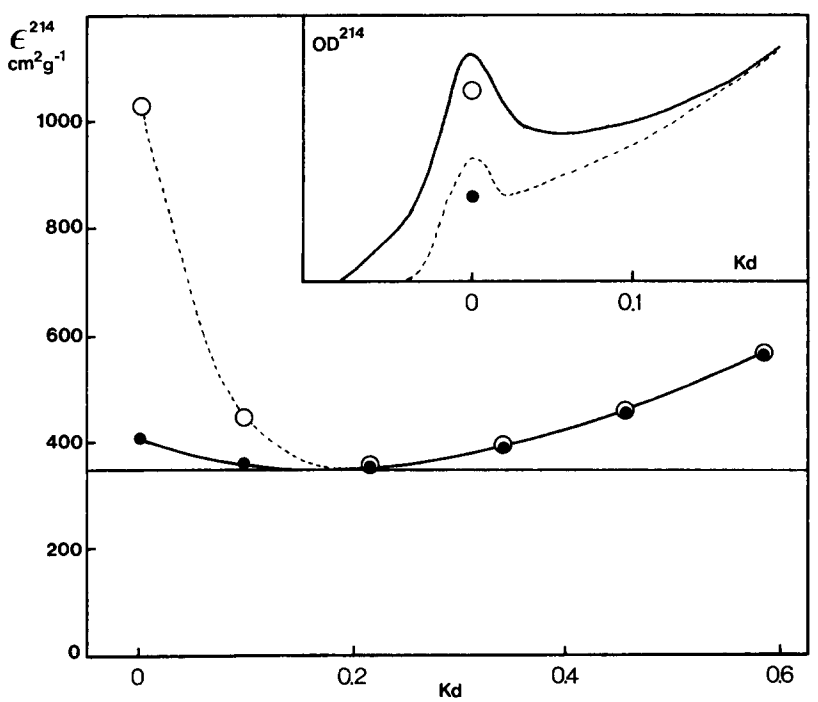

Fig. 6. Influence of ultracentrifugation on the microgel elimination. ε^{214} and optical density O.D. at 214 nm (enclosed figure) versus distribution coefficient (K_d) for P6ox sample percolated on Sephacryl 200/0·2 M NaCl. ○, Filtration through Millex GV 0·22 μm; ●, ultracentrifugation at 150 000 g and filtration through Millex GV 0·22 μm.

TABLE 3
Macromolecular Parameters of Pectic Substances in 0·2 M NaCl

Sample	$[\eta]$ $(cm^3\,g^{-1})$	$M_w^a \times 10^{-3a}$	$M_w^b \times 10^{-3b}$	$M_w^c \times 10^{-3c}$	$A_2 \times 10^4$ $(cm^3\,g^{-2})$	$M_w^a/M_w^{c\,a,c}$	Dc[e]
PC	335[f]/315[c]	2750	140	200	<1	14	98
PP	278[f]/265[c]	4680	130	187	<1	25	99
F1	72·4	1460	120	198	2	7·4	94
PRw	40·2	620	—	83[c]/69[d]	0[c]/10[d]	7·5[c]/9[d]	—
P8w	28·1	—	—	57·6	<1	—	—
F2	27·6	270	31	66	<1	4·1	85
P12wb	24·4	290	21	50	<1	5·8	89
P6w	21·8	—	—	33·0	<1	—	—
P7ox	21·8	300	—	45·8	<1	6·6	—
P12ox	20·8	—	—	46·4	<1	—	—
P7w	19·9	—	—	26	≃3	—	—
P8ox	17·2	—	—	24	<1	—	—
P3ox	11·2	120	—	27	<1	4·4	—
F3	10·7	—	6·3	35	<1	2·9	—
P4ox	7	40	—	14	<1	2·9	—
F4	4·4	47	2	17	<1	2·8	67

[a]Molecular weight measured on crude solutions (0·2 M NaCl).
[b]Molecular weight obtained by extrapolation of the specific adsorption phenomenon of aggregates onto Millex GS filters.
[c]Parameters measured on ultracentrifugated solutions (0·2 M NaCl).
[d]Parameters measured on ultracentrifugated solutions (0·01 M NaCl).
[e]Clarification degree.
[f]Parameters measured on crude solutions (0·2 M NaCl).

Nevertheless, in agreement with previously reported results (Hourdet & Muller, 1987), ultracentrifugation seems not to be completely effective in removing aggregates. There are two pieces of evidence for this.

Firstly, the second virial coefficients are still too low to admit the total absence of superstructures. This result is consistent with the work reported by Smidsrød & Haug (1968), which showed that A_2 for alginate solutions was dependent on the extent of centrifugation (time $\times$ g). Secondly, if it is accepted that the M_w values obtained previously by extrapolation of the specific adsorption phenomenon (ESAP; M_w^b) can be considered as the 'true' weight-average molecular weights of the molecularly dispersed pectin chains, it is possible to define a 'clarification degree' (D_c) by means of the relation:

$$D_c = \frac{M_w^a - M_w^c}{M_w^a - M_w^b}$$

Values of D_c for different pectins are reported in Table 3. They range from 99% to 67%, decreasing with decreasing molecular size. The low value of D_c found for the lowest molecular weight sample indicates that ultracentrifugation at 150000 g still appears inadequate to enable isolated pectin chains to be characterized 'absolutely' by low-angle laser light scattering.

The authors therefore extended the 'clarification process' or 'microgel elimination' by coupling centrifugation with complementary techniques like alkali-treatment or SEC fractionation.

NaOH treatment

Upon addition of 0·1 N NaOH (1 cm^3) to an untreated citrus pectin solution (10 cm^3), circulating inside the KMX-6 cell, a large decrease in the scattered light intensity is observed: a ratio $[\Delta G_\theta(t=0)/\Delta G_\theta(t)] \cong 15$ could be reached 45 min after NaOH injection (Fig. 7). During this experiment the light-scattering signal took a long time to stabilize.

The same treatment was applied to pectin solutions which had previously been subjected to ultracentrifugation. Very satisfactory results were then obtained with various pectins (PA, PC and PGA), as shown in Fig. 8. In particular, it was found that the second virial coefficients have positive values $[A_2 = (1.2 \times 10^{-3}$ to $3{\cdot}5 \times 10^{-3})$ cm^3 $g^{-2}]$ and that the same scattered light responses were obtained irrespective of the filter used (Millex GV or GS).

In spite of the very good results, the major drawback of this preparation method lies in the β-elimination of pectic chains that occurs during NaOH treatment. This can explain the rapid drift in the viscosity reported in Fig. 7 and the low intrinsic viscosity values found for apple and citrus pectin samples after NaOH treatment (Fig. 8), compared with those measured before treatment (Table 3).

SEC fractionation

Superstructures will be totally excluded (V_0) on the Sephacryl 200/0·2 M NaCl chromatographic system (Fig. 6). Therefore various pectin samples have been prepared and analysed by combining SEC, LALLS and viscosity measurement using the following procedure:

(1) Ultracentrifugation of the solution as previously described in order to reduce the proportion of superstructure in the void volume area.
(2) Percolation of the pectin solution through the Sephacryl 200/0·2 M NaCl column (injected weight $\cong$ 60–100 mg).
(3) Recovery of eluted substances (8–9 fractions for $0 \leq K_d \geq 1$) in known volumes (between 20 and 30 cm^3) (Fig. 9).

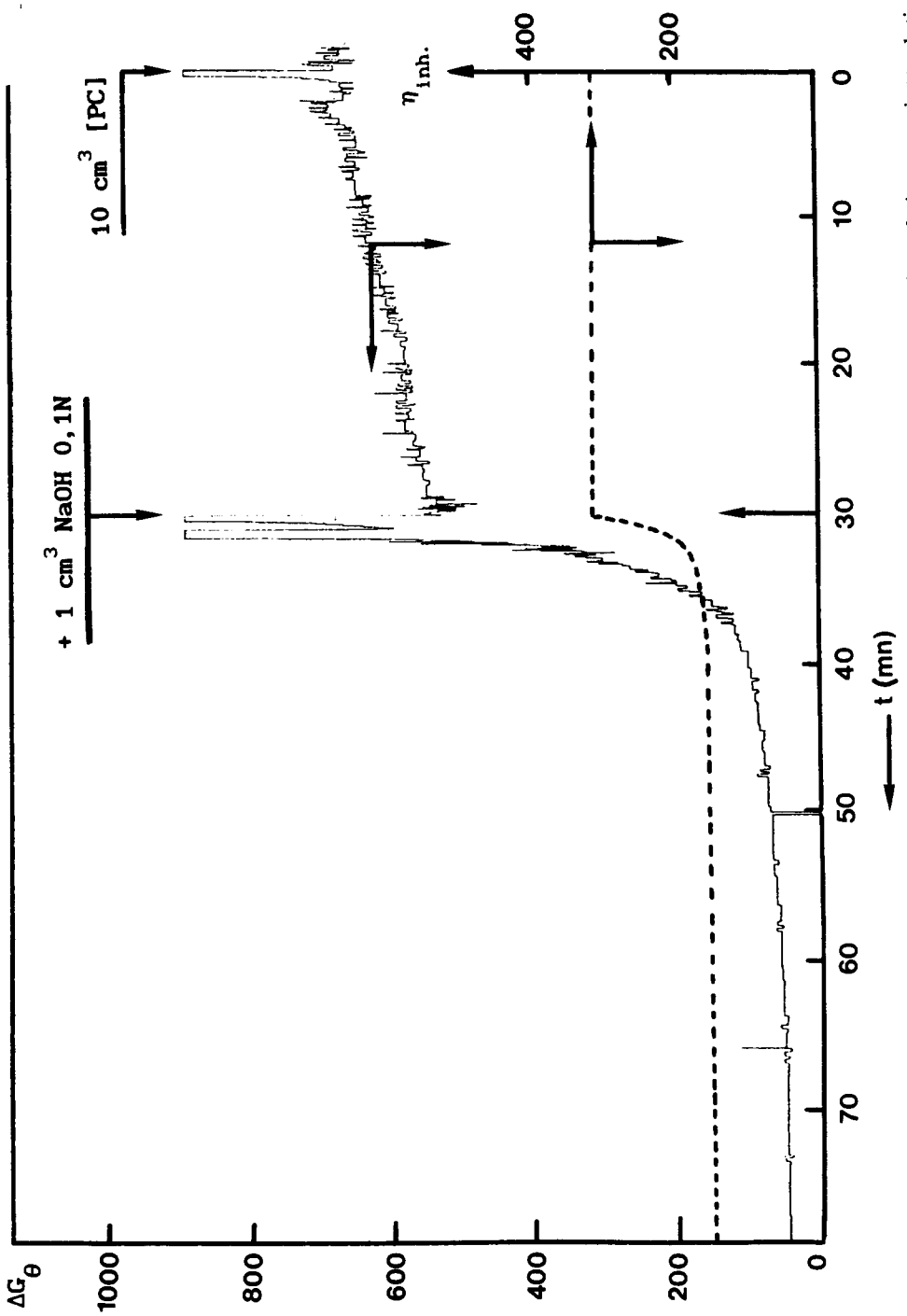

Fig. 7. μ_{inh} and scattered light signal versus time during NaOH treatment of citrus pectin. Concentration of citrus pectin × solution [PC] = 16×10^{-4} g cm^{-3}; initial solvent, 0·2 M NaCl; filter, Millex GV 0·22 μm.

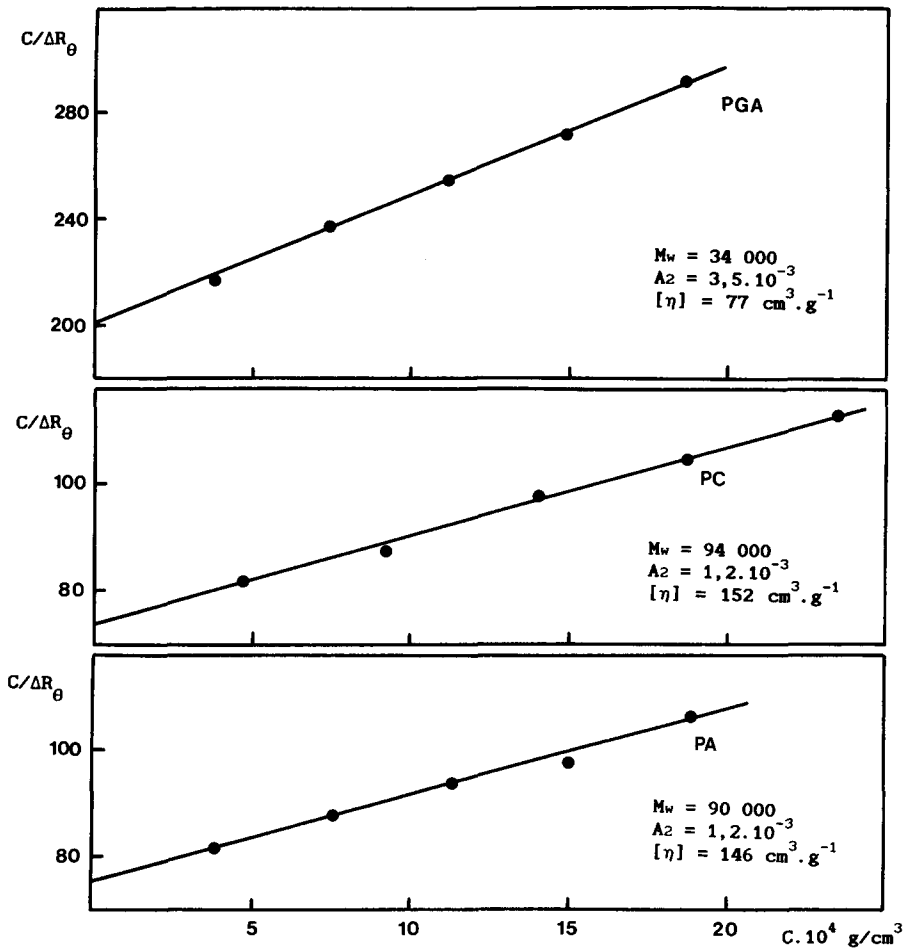

Fig. 8. $C/\Delta R_\theta$ versus concentration for pectin solutions submitted to NaOH (0·01 N) treatment and ultracentrifugation at 150 000 *g*. Initial solvent, 0·2 M NaCl; filter, Millex GV 0·22 μm.

(4) Fraction analysis as summarized below:

Refractometry $\longrightarrow C_i$

LALLS $\longrightarrow M_i \cong \dfrac{\Delta R_{\theta i}}{KC_i}$

Viscosimetry $\longrightarrow [\eta]_i \cong \eta_{inh_i} \cong \dfrac{1}{C_i} \ln \dfrac{\eta_i}{\eta_o}$

Using data obtained with four different samples, in Fig. 10 the product of $\log[\eta]M_w$ versus K_d has been plotted, according to the

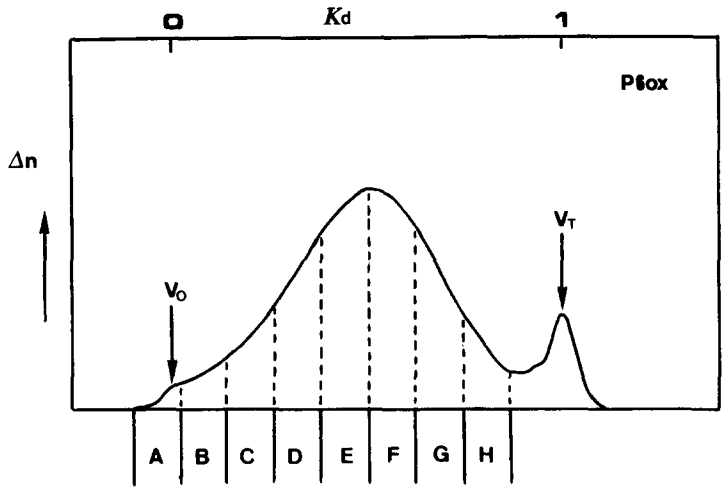

Fig. 9. Clarification of pectin samples by SEC. Fractionation of P6ox on S 200/0·2 M NaCl system before LALLS and visco analysis.

universal calibration plot of Grubisic *et al.* (1967). Two facts are worth noting:

(1) A good correlation is observed between K_d and molecular weight for the different pectic fractions over the whole steric exclusion domain ($0{\cdot}15 < K_d < 0{\cdot}8$). This shows that the reproductibility and the precision of the experiment are good in spite of some very low detection signals found for high elution volumes.

(2) The rather good correlation between pectic fractions and different standards in the selective permeation area confirms the absolute validity of the molecular analysis (LALLS, viscometry) and consequently proves the total absence of superstructure for values of $K_d > 0{\cdot}15$. Using these values as reference data for absolute molecular weights, it is thus possible to discuss more precisely the overall results and to specify the homogeneous pectic chain conformation.

For this purpose only, data obtained with pectin samples or pectin fractions characterized by a high galacturonic acid content (AGA> 80%) will be considered.

The pectic chain conformation in 0·1 M aqueous NaCl

Figure 11 shows the relationship between the intrinsic viscosity and the weight-average molecular weight M_w. The data points are fitted

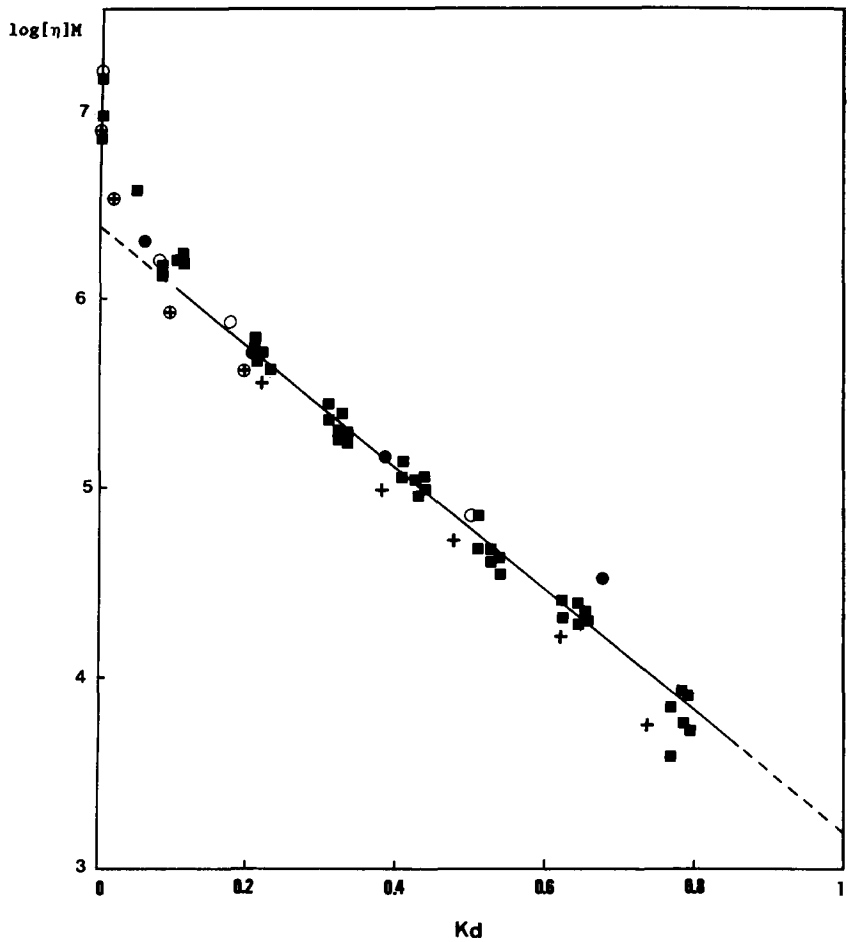

Fig. 10. Universal calibration plot of the Sephacryl 200/0·2 M NaCl system. ○, DX; ●, PSSNa; ⊕, POE; +, PEG; —■—, flax pectins.

accurately by a straight line, therefore confirming that the various clarification procedures were effective for removal of aggregates.

The following Mark–Houwink relation holds:

$$[\eta] = 0{\cdot}96 \times 10^{-3} M_w^{1{\cdot}07} \quad ([\eta] \text{ in cm}^3\ \text{g}^{-1})$$

Such a relation and particularly the value of the exponent $a(=1{\cdot}07)$ is in favour of a 'rigid' or 'semi-rigid' conformation for the 'homogalacturonan-type' chain. It is interesting to point out that the above relation (line 10 on Fig. 12) is very close to that reported by Smidsrød & Haug (1968) for alginate in 0·1 M NaCl solution (line 9 on Fig. 12). This can be

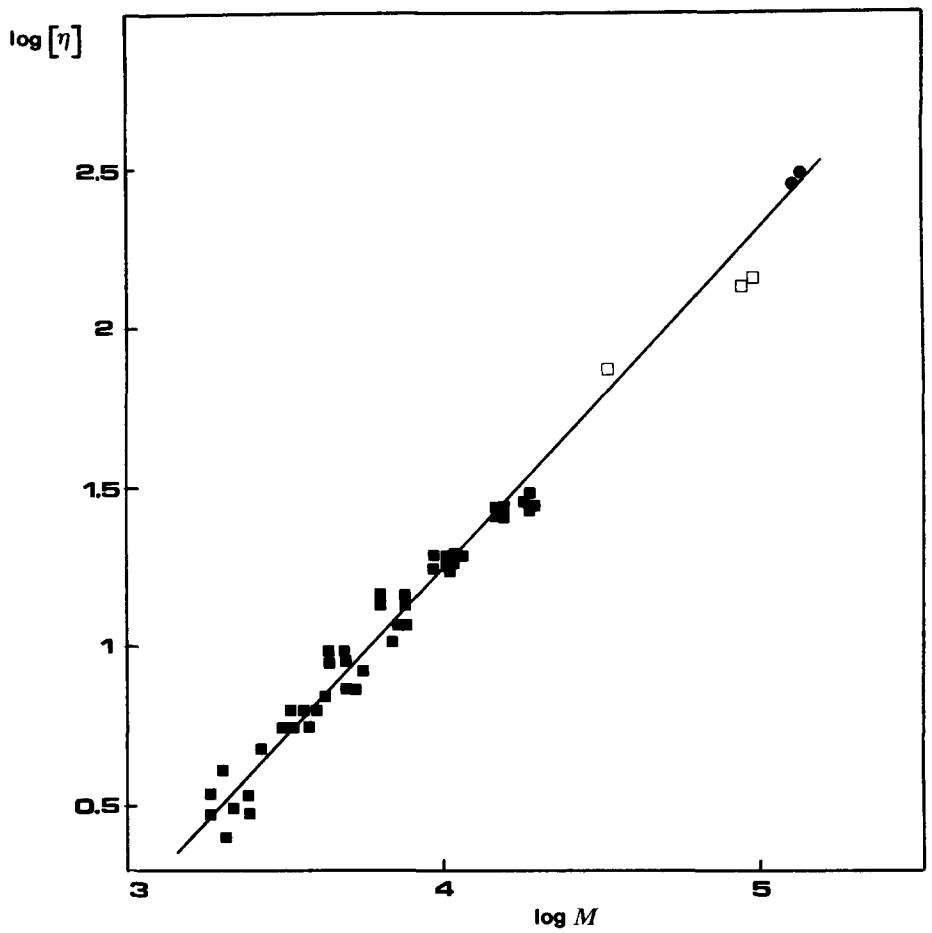

Fig. 11. $[\eta]$–M_w relationship for pectic substances in 0·2 M NaCl. M_w determined according to the three methods: ●, ESAP; □, ultracentrifugation/NaOH; ■, ultracentrifugation/SEC.

related to the similarity existing between pectins and alginates in their primary, secondary and tertiary structures (Whittington, 1971; Burton & Brant, 1983; Morris *et al.*, 1982).

For a good evaluation of the conformation of pectin chains the Kratky–Porod worm-like chain model (Kratky & Porod, 1949) was chosen. From several theories for the $[\eta]$–M_w dependence for an unperturbed worm-like cylinder the simplified version of the Yamakawa–Fujii theory (Yamakawa & Fujii, 1974) as proposed by Bohdanecky (1983) was applied:

$$[\eta]_0 = \phi_0 L_r^{3/2} (\lambda^{-1})^3 / M \quad (\text{for } L_r > 2{\cdot}28)$$

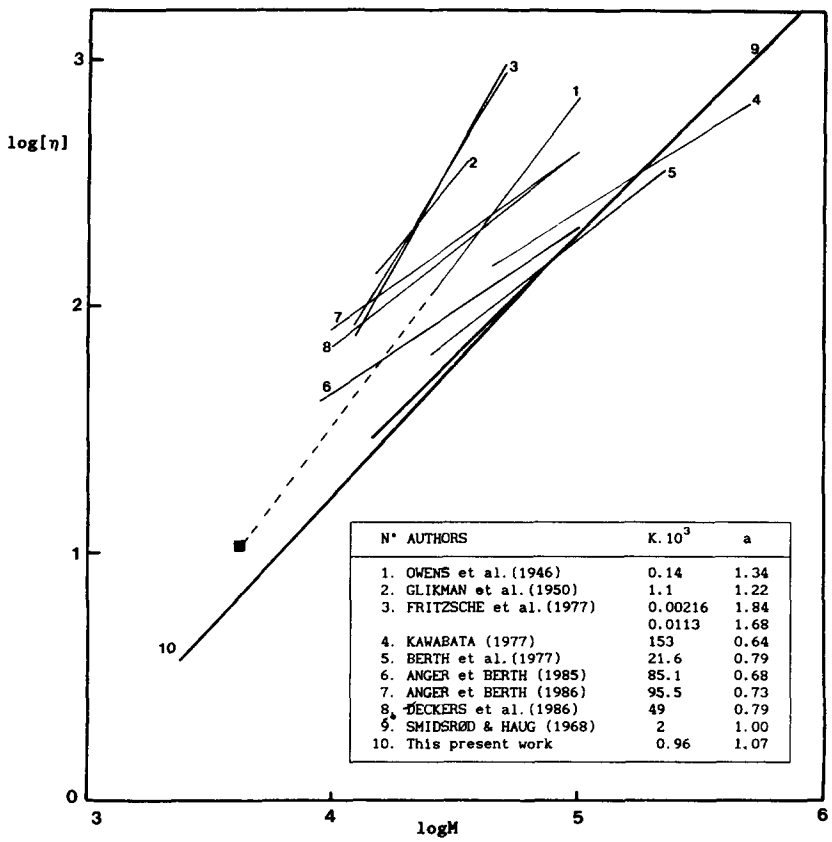

Fig. 12. Mark–Houwink relationships for pectic substances and alginates.* (* = Correlated with relation no. 9 from Smidsrød & Haug.)

No.	*Authors*	*K.10³*	*a*
1.	Owens *et al.*, 1946	0·14	1·34
2.	Glikman *et al.*, 1950	1·1	1·22
3.	Fritzsche *et al.*, 1977	0·00216	1·84
		0·0113	1·68
4.	Kawabata, 1977	153	0·64
5.	Berth *et al.*, 1977	21·6	0·79
6.	Anger & Berth, 1985	85·1	0·68
7.	Anger & Berth, 1986	95·5	0·73
8.	Deckers *et al.*, 1986	49	0·79
9.	Smidsrød & Haug, 1968	2	1·00
10.	Present work	0·96	1·07

where $L_r = \lambda M/M_L$ is the reduced contour length, M_L is the mass per unit length of the chain, $\lambda^{-1} = 2q$ is the Kuhn statistical segment length (q being the persistence length), ϕ_0 is a function of L_r and of the reduced cylinder diameter ($d_r = d/2q$) tabulated by Yamakawa & Fujii. $M_L = m_o/l_o$, was taken equal to 44·6 daltons g/Å, with $m_o = 194$ g (average mass of the repeat unit of sodium pectate chain esterified to various degrees), and $l_o = 4{\cdot}35$ Å (length of the repeat unit).

Parameters q and d_r were chosen in order to obtain the best fit for a set of experimental data including present results and those reported by Berth *et al.* (1977), Kawabata (1977), Axelos *et al.* (1987) and Panchev *et al.* (1988). As shown in Fig. 13 with this set of pectic substances, characterized as a whole by a galacturonic acid content higher than 80%

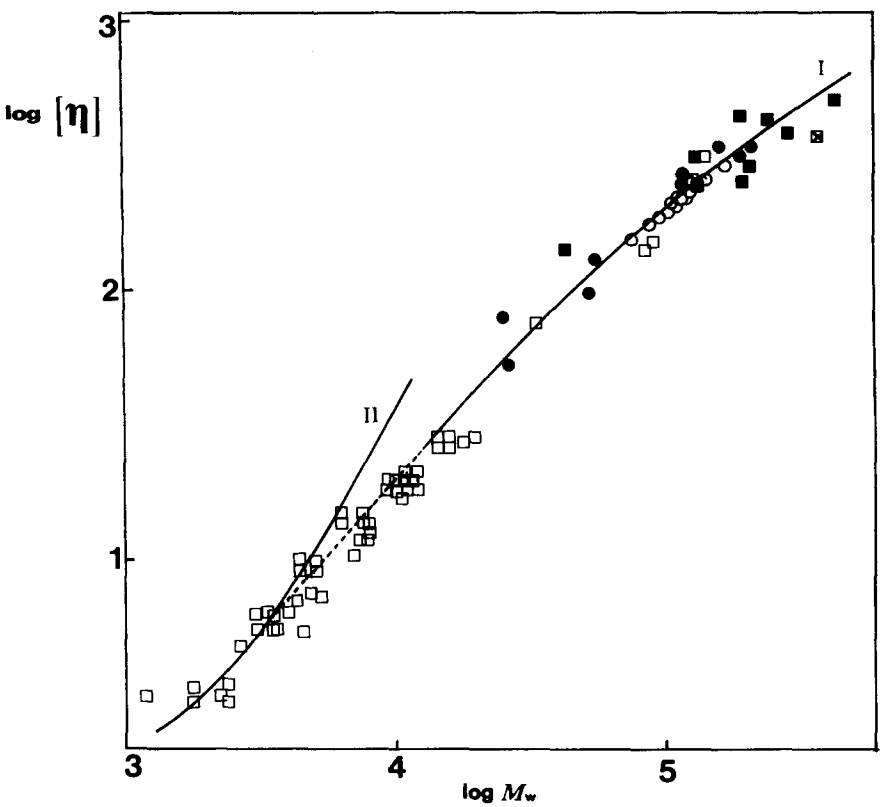

Fig. 13. Comparison between the measured [η] for Na salt pectins in 0·1–0·2 M aq. NaCl and the theoretical values calculated from Yamakawa–Fujii's theory for worm-like chains (model I) and rigid rod (model II) with $q = 67$ Å, $d = 6$ Å and $M_L = 44{\cdot}6$ daltons/Å. Experimental values: ●, Berth *et al.* (1977); ■, Kawabata (1977); ⊠, Axelos *et al.* (1987); ○, Panchev *et al.* (1988); □, this work.

and a degree of esterification $0 \leq DE \leq 80\%$, the best fit is achieved using a persistence length $q = 67$ Å and an hydrodynamic diameter $d = 6$ Å (solid line I). For the lowest molecular weight pectins, i.e. for chain lengths shorter than about 15 anhydrogalacturonic units, the worm-like chain model can be completed successfully with the rigid-rod model (solid line II) developed by Yamakawa & Fujii (1974).

The value found for the chain hydrodynamic diameter is in good agreement with the earlier reported values deduced from crystallography data or molecular models (Palmer & Hartzog, 1945; Palmer & Ballantyne, 1950; Rees & Wight, 1971).

On the other hand, the persistence length value is comparable with that reported for other similar derivatives such as alginates ($q = 65$ Å, Smidsrød & Haug, 1971) or carboxymethylcellulose (CMC) ($q = 36$–50 Å, Smidsrød & Haug, 1971; Tricot, 1984). This indicates that the homogalacturonan-type chain in NaCl solution behaves like an extended coil similar to the alginate chain. This is reasonable, considering the well-known analogies between these two polyuronides. This is, moreover, in agreement with the nearly identical values found for the empirical stiffness parameter B proposed by Smidsrød & Haug (1971) for comparing intrinsic stiffness of polyelectrolyte chains ($B \simeq 0{\cdot}04$ for alginates and pectins with $DE \leq 78\%$).

From the data shown in Fig. 13 two different Mark–Houwink relationships may be derived for high galacturonic acid content pectins independently of their degree of esterification:

$$[\eta] = 0{\cdot}8 \times 10^{-3} M_w^{1{\cdot}1} \quad \text{for } 2 \times 10^3 \leq M_w \leq 4 \times 10^4$$

$$[\eta] = 19 \times 10^{-3} M_w^{0{\cdot}8} \quad \text{for } 4 \times 10^4 \leq M_w \leq 3 \times 10^5$$

As it is expected that the shape and conformation of pectic substances should depend on their composition and principally on their neutral sugar content, the study reported here has been extended to pectins of different composition. The results will be described in a following paper.

ACKNOWLEDGEMENTS

We thank Drs C. Morvan and O. Morvan of SCUEOR (Groupe Agro-Industrie) University of Rouen, for supplying flax pectin samples.

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