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Food Hydrocolloids 14 (2000) 227–235

FOOD HYDROCOLLOIDS

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The effect of the degree of esterification on the hydrodynamic properties of citrus pectin

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Received 2 September 1999; accepted 13 January 2000

Abstract

Estimation of the influence of the degree of esterification on the hydrodynamic properties of citrus pectins provides a simple demonstration of how chemical variation can influence structural properties of polysaccharides. Five citrus pectins with average degree of esterification 77.8, 65.0, 53.9, 37.8 and 27.9%, respectively were studied using capillary viscometry, sedimentation velocity, sedimentation equilibrium and size exclusion chromatography coupled to multi-angle laser light scattering (SEC-MALLS). Molecular weights (weight average) for all five pectin samples were within the range 190 000 ± 30 000 g/mol as confirmed by the *independent* techniques of sedimentation equilibrium and SEC-MALLS. Estimates for the conformation dependent Wales–van Holde ($k_s/[\eta]$) and frictional (f/f_0) ratios from the hydrodynamic data clearly indicates increasing chain stiffness with decreasing degree of esterification. It appears that both steric and electrostatic interactions are important in these conformational changes.

Keywords: Citrus pectin; Degree of esterification; Hydrodynamic properties

1. Introduction

Pectin is the family of complex polysaccharides that constitute approximately one third of the dry weight of higher plant cell walls (Tombs & Harding, 1998; Walter, 1991). Pectins are particularly prevalent in fruit cell walls, especially citrus fruits and apple pommace. The main pectin chain is composed of α(1 → 4) linked D-galacturonic acid residues.

···→ 4)α-DGal*p*A-(1 → ···

Many of the galacturonic acid residues have been esterified to form methyl esters. Theoretically the degree of esterification (DE) can range from 0 to 100%. Pectins with a degree of esterification DE > 50% are known as high methoxyl (HM) pectins and consequently low methoxyl (LM) pectins have a DE < 50% (Walter, 1991). Rhamnose residues are incorporated into the main chain at random intervals, which results in a kink in the otherwise linear chain. Side chains of arabinoses and galactans, are also present, either randomly dispersed or in localised "hairy" regions. The degree of esterification and therefore the charge on a pectin molecule is important to the functional properties in the plant cell wall. It also significantly affects their commercial use as gelling and thickening agents. HM pectins form gels at low pH (<4.0) or in the presence of a low amount of soluble solids, usually sucrose (>55%). HM pectin gels are stabilised by hydrophobic interactions (Lapasin & Pricl, 1995; Tombs & Harding, 1998). Conversely, LM pectins form electrostatically stabilised gel networks with divalent metal cations, usually calcium. It would be therefore interesting to see if these differences are also borne out in the hydrodynamic properties of these substances: this is the subject of the current study.

2. Materials and methods

2.1. Materials

Pectin samples 7000–7004 were obtained from Copenhagen Pectin, (Copenhagen, Denmark). The parent pectin 7000 DE 77.8% was systematically de-esterified to 27.9% pectin 7004. All samples were prepared and solubilised for approximately 24 h in standard pH 6.8, $I = 0.1$ "Paley" buffer, of the following composition $Na_2HPO_4 \cdot 12H_2O$, 4.595 g; KH_2PO_4, 1.561 g and NaCl, 2.923 g all made up to 1 l (Green, 1933).

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0268-005X/00/$ - see front matter
PII: S0268-005X(00)00007-2

2.2. Viscometry

Solutions and reference solvents were analysed using a 2 ml automatic Schott-Geräte Oswald viscometer, under precise temperature control (25.04 ± 0.01°C). The relative, η_{rel} and specific, η_{sp} viscosities were calculated from:

$$\eta_{rel} = (t/t_0) \times (\rho/\rho_0) \quad (1)$$

where t is the flow time for the pectin solution, t_0 is the flow time for the solvent (82.63 ± 0.01 s). Because of the low concentration used (ρ/ρ_0) was taken as unity, and

$$\eta_{sp} = \eta_{rel} - 1 \quad (2)$$

A common method for measuring intrinsic viscosity is to calculate the relative and specific viscosity at one concentration (2.5 mg/ml) and employ the Solomon/Ciuta approximation (see e.g. Harding, 1997).

$$[\eta] \approx [2\eta_{sp} - 2 \ln \eta_{rel}]^{1/2}/c \quad (3)$$

2.3. Sedimentation velocity in the analytical ultracentrifuge

The Optima XLI (Beckman Instruments, Palo Alto, CA) equipped with Rayleigh interference optics was used to determine the sedimentation behaviour of the pectin samples. Rotor speeds of 40 000 rpm and a 4 mm column length in 12 mm optical path length double sector cells were used together with an accurately controlled temperature of 20.0°C. A weighted average partial specific volume, $\bar{v}$ of 0.630 ± 0.005 ml/g was calculated from density measurements following the procedure of Kratky, Leopold and Stabinger (1973). The so-called $g(s^*)$ (sedimentation time derivative) method (Stafford, 1992) was used to determine apparent sedimentation coefficients at each concentration. As the sedimenting boundary moves towards the cell base the change in concentration (of the sedimenting species) over time (dc/dt) is calculated from the subtraction of multiple pairs of scans (maximum 20 pairs): an apparent distribution $g(s^*)$ of sedimentation coefficients can in this way be produced (Stafford, 1992), where the " * " indicates "apparent" in the sense of not being corrected for diffusion effects nor non-ideality. The apparent *weight average* sedimentation coefficient, s^* can then be calculated (Stafford, 1992), corresponding to particular buffer conditions and temperature (T). From this, normalisation to $s_{20,w}$ values (i.e. to the density and viscosity of water at 20.0°C) can then be performed, according to the standard equation (see e.g. Ralston, 1993):

$$s_{20,w} = s^*[(1 - \bar{v}\rho_{20,w})\eta_{T,b}]/[(1 - \bar{v}\rho_{T,b})\eta_{20,w}] \quad (4)$$

where $\eta_{T,b}$, $\eta_{20,w}$ are, respectively, the viscosities of the solvent at temperature T and water at 20.0°C, and $\rho_{T,b}$ and $\rho_{20,w}$, are the corresponding solvent densities.

The $s_{20,w}$ values were evaluated at various concentrations from 0.5 to 2.5 mg/ml and extrapolated to zero concentration (to remove the effects of non-ideality) using the standard equation (see e.g. Ralston, 1993).

$$s_{20,w} = s^0_{20,w}(1 - k_s c) \quad (5)$$

where the Gralen parameter (Gralen, 1944), k_s is a measure of concentration dependence.

2.4. Sedimentation equilibrium in the analytical ultracentrifuge

The Beckman Optima XLI ultracentrifuge was also used also to determinate the weight average molecular weight, M_w using low speed sedimentation equilibrium. A rotor speed of 10 000 rpm and a 1 mm solution column length in 12 mm path length double sector cells were employed at a running temperature of 20.0°C. Equilibrium was reached after approximately 24 h. Rayleigh interference optics were used to record the solute distributions at sedimentation equilibrium and data subsequently analysed using the QUICKBASIC algorithm MSTARI (Cölfen & Harding, 1997).

This program estimates:

1. Molecular weight for the whole distribution of solute in the ultracentrifuge cell (from meniscus to cell base) using the operational point average, the "star average" M^*, and the identity M^* (cell base) = $M_{w,app}$ (the apparent weight average molecular weight).
2. The apparent point average weight average molecular weight as a function of radial position, $M_{w,app(r)}$. These values are obtained by the sliding strip procedure as described in Teller (1973).

Since interference optical records are of concentration relative to the meniscus, the meniscus concentration needs to be calculated before absolute concentrations can be obtained and (1) and (2) subsequently performed. For this, the "intercept over slope" method as described in Creeth and Harding (1982) was employed in the initial stage of running MSTARI.

2.5. SEC-MALLS

SEC-MALLS (size exclusion chromatography coupled to multi-angle laser light scattering) allows on-line light scattering of a heterogeneous solute fractionated by size exclusion chromatography, permitting the extraction of absolute molecular weights and molecular weight distributions (van Holde, 1985; Jumel, 1994; Jumel, Browne & Kennedy, 1992). The Wyatt Technology (Santa Barbara, CA) Dawn F multi-angle laser light scattering photometer (Wyatt, 1992) was coupled to TSK Gel 4000, TSK Gel 5000 and TSK Gel 6000 columns protected by a similarly packed guard column (purchased from Anachem Ltd., Luton, UK). The eluent was the standard pH 6.8 $I = 0.1$ "Paley" buffer as described above and the injection volume was 100 μl. A value for the refractive index increment of 0.146 ml/g was used (Chapman, Morris, Selvendran & O'Neill, 1987).

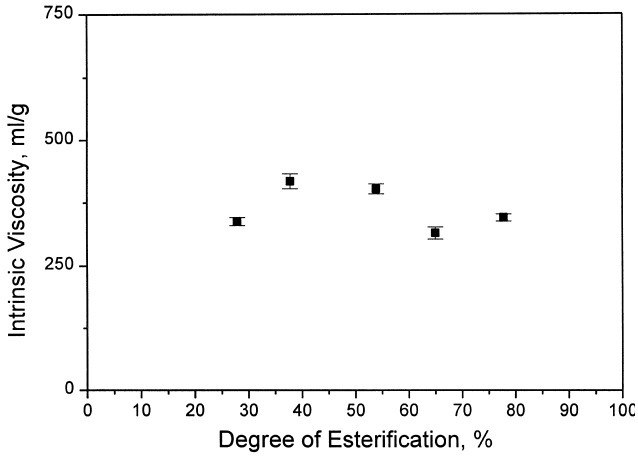


Fig. 1. Intrinsic viscosity vs. DE for Pectin 7000 Series.

3. Results and discussion

3.1. Viscometry

It appears that there is a general decrease in $[\eta]$ as DE increases (Fig. 1) and that there is a distinct relationship between the intrinsic viscosity and the degree of esterification. Intrinsic viscosities in the range 350–450 ml/g are lower than pervious results for a citrus pectin (Harding, Berth, Ball, Mitchell & Garcia de la Torre, 1991) which would indicate either a lower molecular weight and/or a less rigid molecule. The change in $[\eta]$ with DE would indicate that there is a general decrease in rigidity with increased DE (7003 most rigid, 7001 least rigid)—(see e.g. Tanford, 1961).

3.2. Sedimentation velocity

The $g(s^*)$ plots for each pectin sample appear to show that the samples are pure, with only one peak (Fig. 2). There appears to be a general increase in $s^0_{20,w}$ with increasing DE: the same is true for k_s (Figs. 3a–e and 4; Table 1). The increase in $s^0_{20,w}$ with increased DE is in agreement with the viscosity measurements indicating a decrease in rigidity with increase in DE. This trend is also borne out with an increase in the Wales–van Holde ratio, $k_s/[\eta]$, with increase in DE.

3.3. Sedimentation equilibrium

Apparent molecular weights were calculated over the concentration range 0.5–2.5 mg/ml and extrapolated to zero concentration to obtain "ideal" molecular weights (Fig. 5a–e). It is seen that there is very little difference between the pectins.

3.4. SEC-MALLS

All material appeared to be eluted in the void/ total volume limits. A single, wide peak at the elution volume ~16–30 ml is evident for all samples. Again, there appears to be very little difference in molecular weights and molecular weight distributions for each pectin sample (Figs. 6a–e and 7) which agree with the results of sedimentation equilibrium, based on completely independent principles.

4. Discussion

It would appear from intrinsic viscosity and sedimentation velocity data that there are conformation differences between pectins with different degrees of esterification.

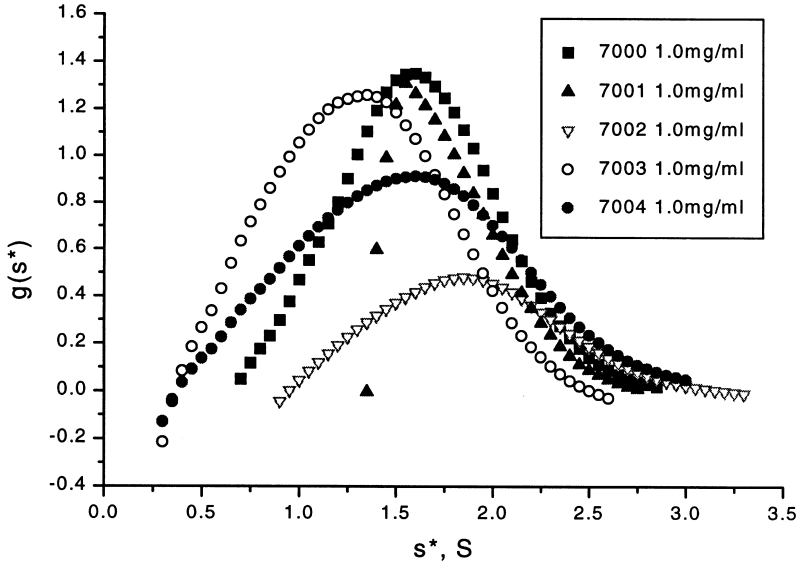


Fig. 2. $g(s^*)$ profiles for pectin samples.

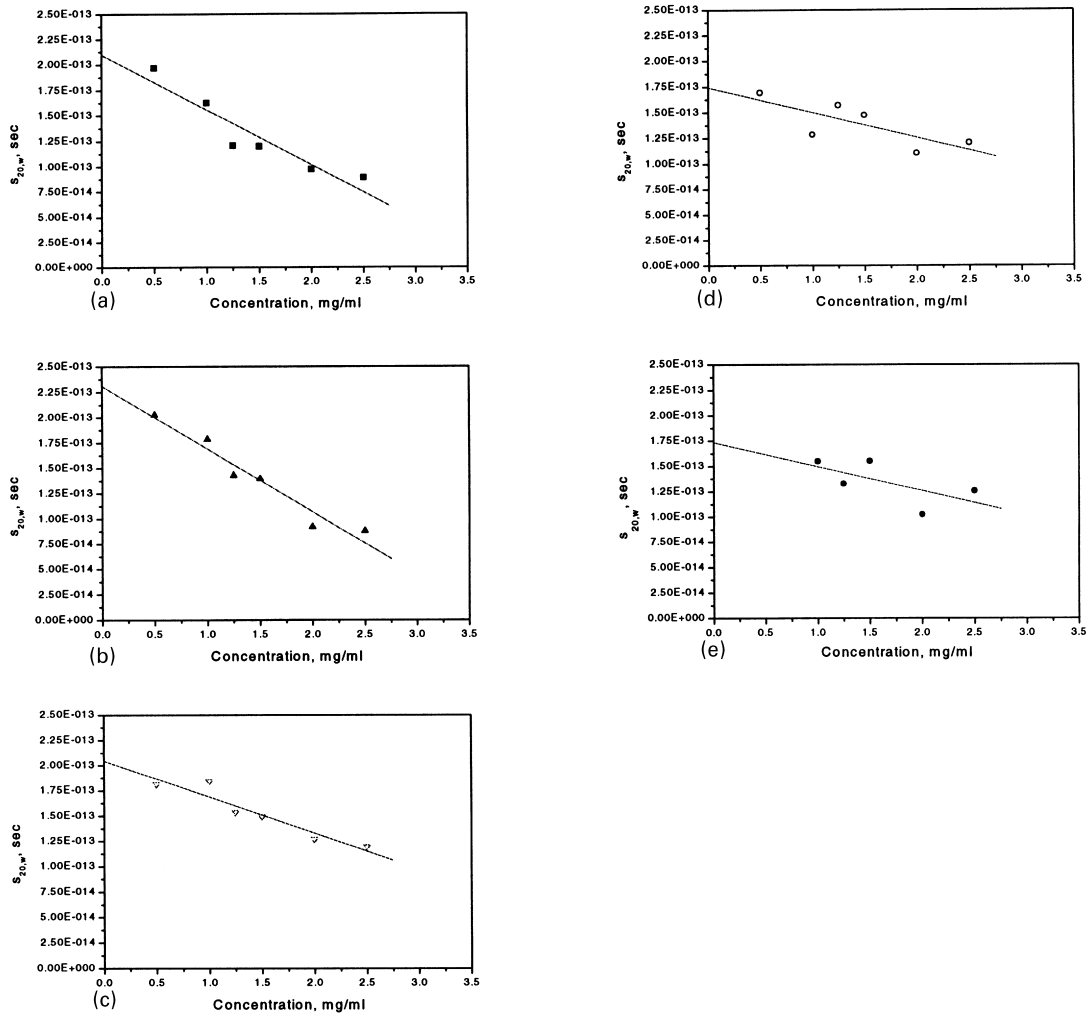


Fig. 3. Sedimentation coefficient vs. concentration: (a) pectin 7000; (b) pectin 7001; (c) pectin 7002; (d) pectin 7003; (e) pectin 7004.

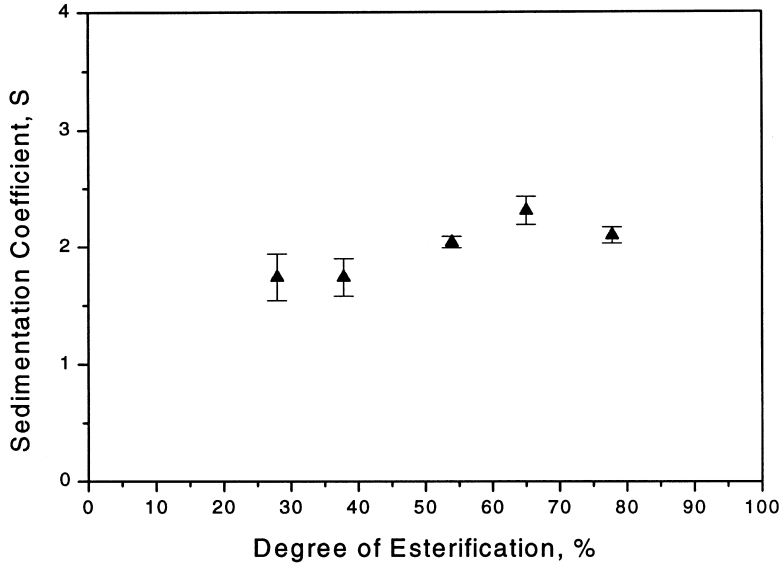


Fig. 4. Sedimentation coefficient vs. DE for pectin 7000 series.

Table 1
Hydrodynamic data for pectin 7000 series

	Pectin				
	7000	7001	7002	7003	7004
DE (%)	77.8 ± 0.2	65.0 ± 0.2	53.9 ± 0.2	37.8 ± 0.2	27.9 ± 0.2
$[\eta]$ (ml/g)	345 ± 7	315 ± 12	402 ± 10	417 ± 15	338 ± 8
$s^0_{20,w}$ (S)	2.10 ± 0.07	2.31 ± 0.12	2.04 ± 0.05	1.74 ± 0.16	1.74 ± 0.20
k_s (ml/g)	257 ± 20	270 ± 30	175 ± 30	140 ± 30	137 ± 20
$10^{-5} \times M_w$ [a] (g/mol)	1.76 ± 0.20	1.99 ± 0.09	2.17 ± 0.02	1.65 ± 0.17	1.65 ± 0.20
$10^{-5} \times M_w$ [b] (g/mol)	1.86 ± 0.09	2.11 ± 0.10	2.11 ± 0.10	1.88 ± 0.09	1.87 ± 0.09
$k_s/[\eta]$	0.75 ± 0.07	0.85 ± 0.10	0.44 ± 0.08	0.34 ± 0.08	0.41 ± 0.07
f/f_0	7.9 ± 0.4	7.8 ± 0.4	8.9 ± 0.4	9.6 ± 0.5	9.6 ± 0.5

[a] Sedimentation equilibrium.
[b] SEC-MALLS.

Both data sets suggest that pectin 7001 (DE 65.0%) is the most flexible (highest sedimentation coefficient, lowest intrinsic viscosity) and that pectin 7003 (DE 37.8%) is least flexible (lowest sedimentation coefficient, highest intrinsic viscosity). The more asymmetric a structure is then the higher generally is the intrinsic viscosity and the lower the sedimentation coefficient (see e.g. Tanford, 1961). We can however be more quantitative. For example, the

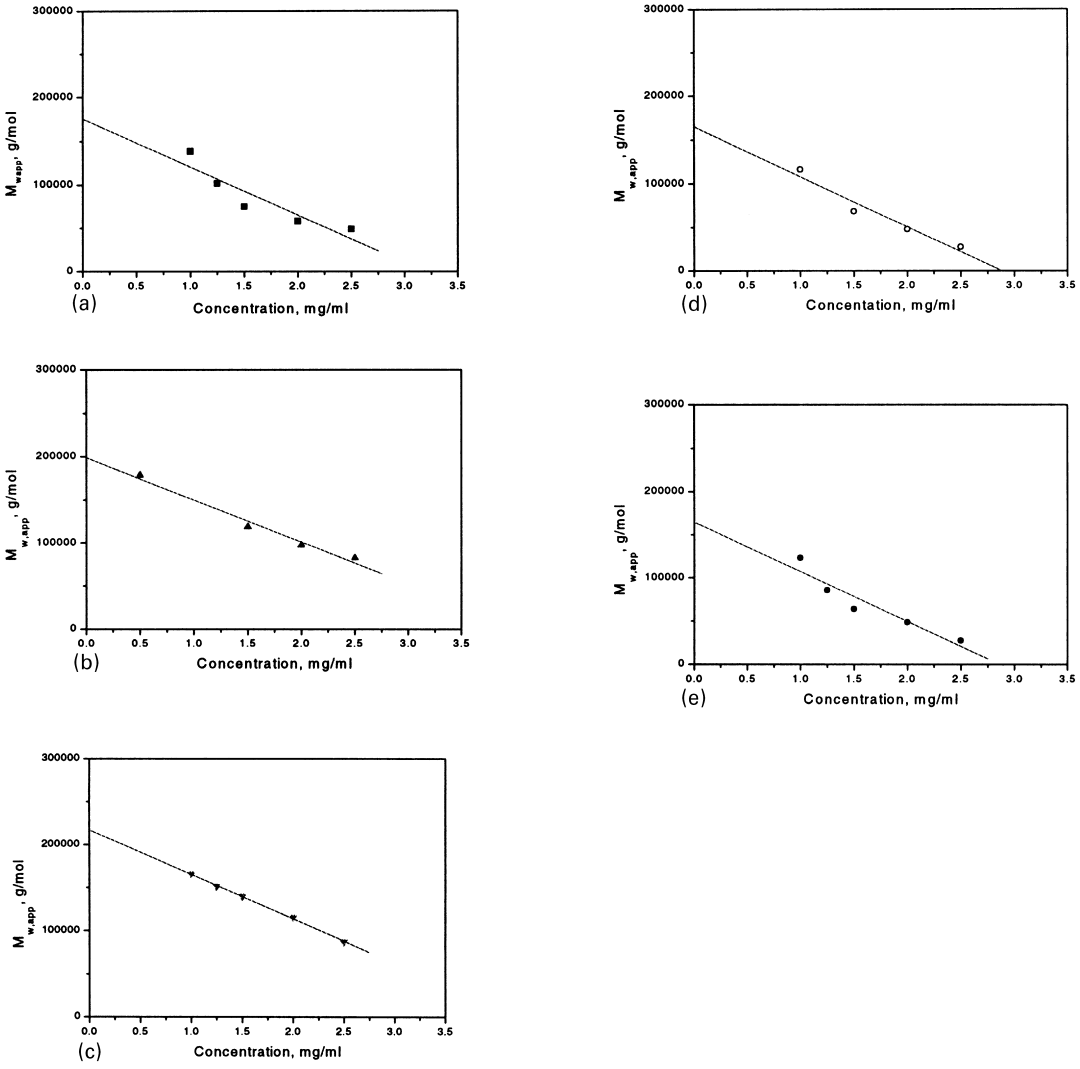


Fig. 5. Apparent weight average molecular weight vs. concentration: (a) pectin 7000; (b) pectin 7001; (c) pectin 7002; (d) pectin 7003; (e) pectin 7004.

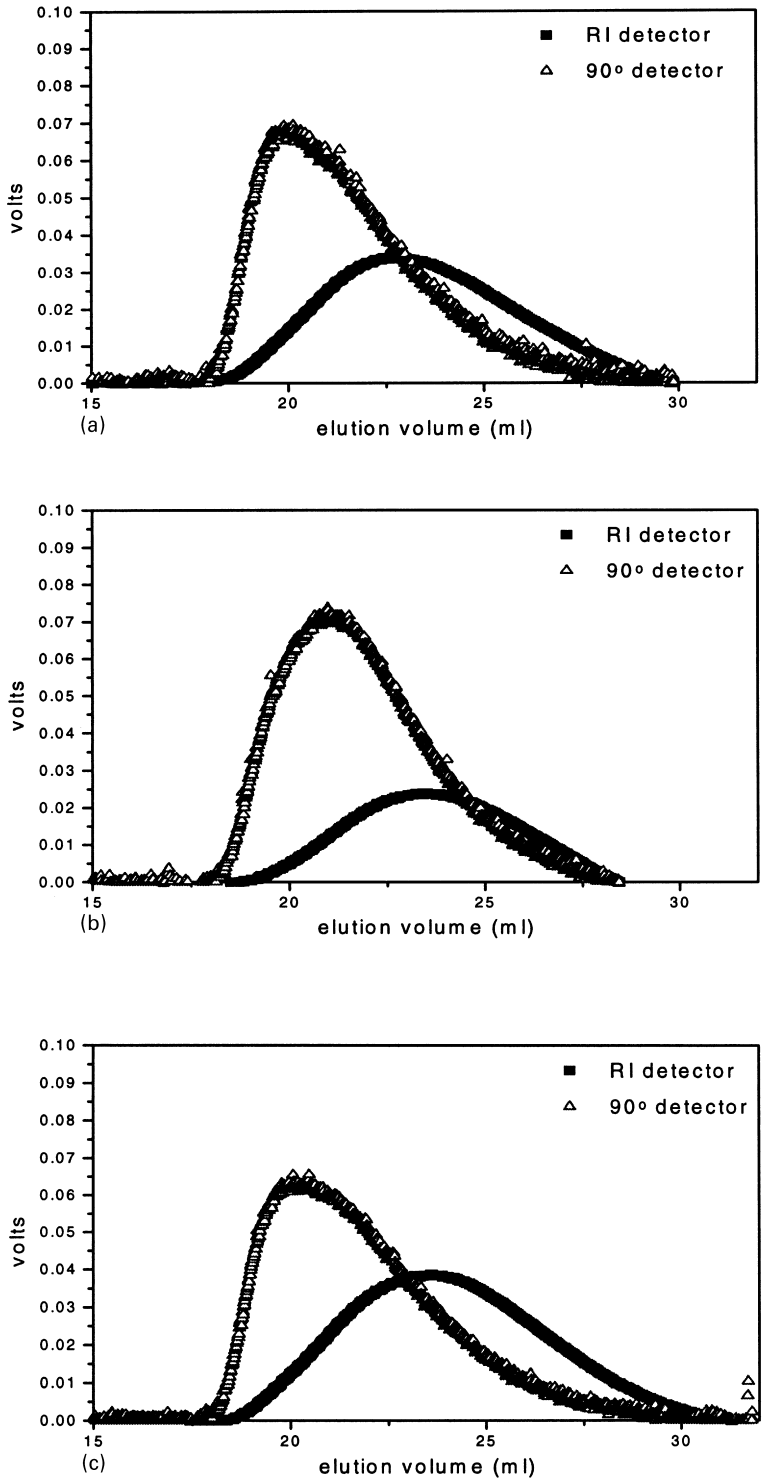
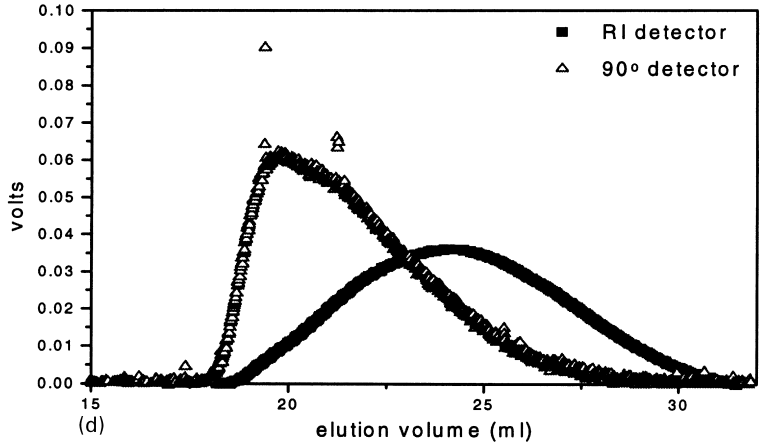


Fig. 6. Elution profile: (a) pectin 7000; (b) pectin 7001; (c) pectin 7002; (d) pectin 7003; (e) pectin 7004.

Wales–van Holde ratio, $R = k_s/[\eta]$ is a good indication of chain flexibility (see e.g. Lavrenko, Linow & Gornitz, 1992), having a value of ~1.6 for spheres and random coils and <1.6 for asymmetric structures, approaching a lower limit of ~0.15 for a rigid rod (Rowe, 1992). A Wales–van Holde ratio of 0.15 has already been quoted for pectin (Harding et al., 1991) It can thus be seen from Table 1 and Fig. 8 that pectin 7001 is the most flexible ($R = 0.85 \pm 0.10$) and 7003 the least ($R = 0.34 \pm 0.08$). A further manifestation of the effect of DE on chain flexibility is the translational frictional ratio, f/f_0 (Tanford, 1961) a parameter which depends on conformation and



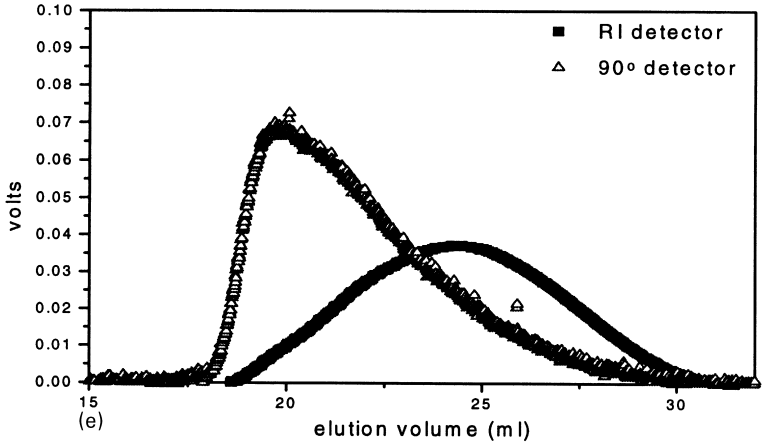


Fig. 6. (*continued*)

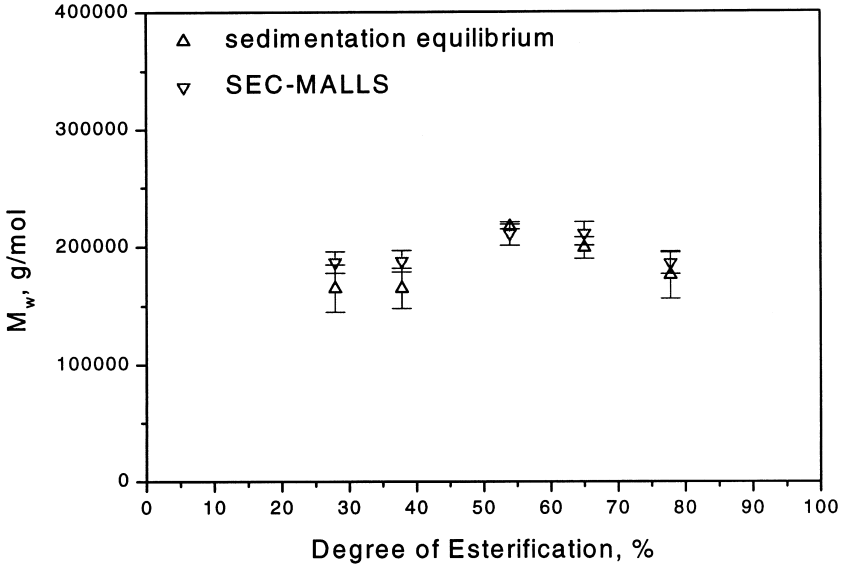


Fig. 7. Molecular weight vs. DE for pectin 7000 series.

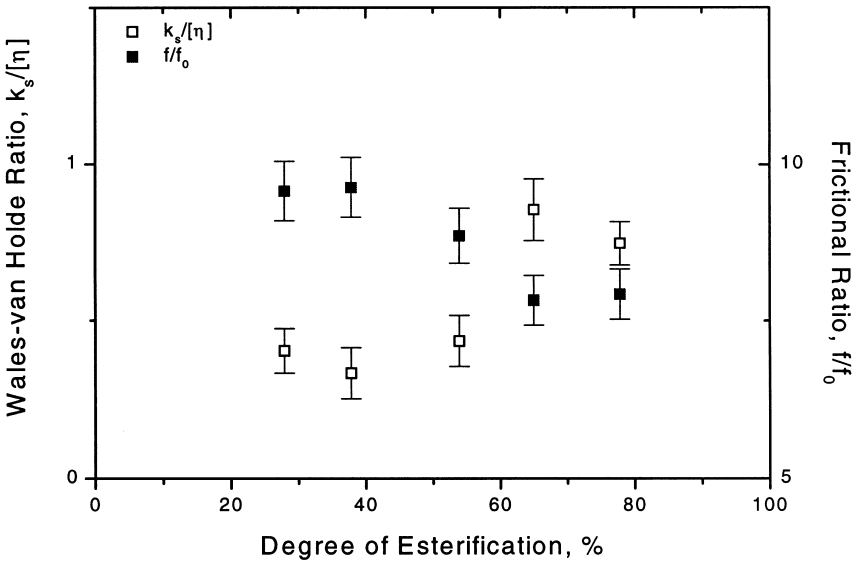


Fig. 8. Wales–van Holde and frictional ratios vs. DE for the pectin 7000 series.

molecular hydration

$$f/f_0 = M_w(1 - \bar{v}\rho_0)/(N_A 6\pi\eta_0 s^0_{20,w})(4\pi N_A/3\bar{v}M_w)^{1/3} \quad (6)$$

where N_A is Avogadro's number, η_0 and ρ_0 are the viscosity and density of water at 20.0°C, respectively. It is clear that the DE of the pectin molecule does influence significantly the hydrodynamic properties. These changes in rigidity of the pectin with change in DE cannot be explained in terms of change in molecular weight increase, since the weight average molecular weight of all five pectin samples is approximately constant in the range 190 000 ± 30 000 g/mol, and confirmed by two independent absolute techniques.

We can though take the analysis one step further based on rigid particle hydrodynamics to estimate relative molecular hydration using the premise that R is a hydration independent function (Creeth & Knight, 1965; Lavrenko et al., 1992; Pavlov, 1997; Rowe, 1977) whereas f/f_0 depends on hydration *and* conformation (Table 2). The Wales–van Holde ratio allows us to calculate the apparent axial ratio (Harding & Cölfen, 1995), using the ELLIPS1 program (Harding, Horten & Cölfen, 1997b). The Perrin function (frictional ratio due to shape), P and hydration, δ can then be estimated from Eqs. (6) and (7) (Harding, Day, Dhami & Lowe, 1997a).

$$P = (f/f_0)[\bar{v}/v_s]^{1/3} \quad (7)$$

where v_s is the swollen specific volume (volume of hydrated molecule per unit anhydrous mass), and

$$\delta = (v_s - \bar{v})\rho_0 \quad (8)$$

It should be stressed that this type of analysis is applicable to quasi-rigid types of macromolecule (e.g. proteins and highly charged or double/triple helical types of polysaccharide). However trends can be discerned and we can see that the molecule becomes less extended and more coiled as DE increases, with a large hydration volume ($\delta \sim 50$ g/g for the low DE species).

Table 2
Calculated parameters for pectin 7000 series

	Pectin				
	7000	7001	7002	7003	7004
DE (%)	77.8	65.0	53.9	37.8	27.9
$k_s/[\eta]$	0.75	0.85	0.44	0.34	0.41
a/b	8.6	6.9	21.0	37.0	23.8
P	1.5	1.4	2.0	2.6	2.1
V_s (ml/g)	99	117	52	33	58
δ	99	117	52	33	58

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