

Modification of pectin with UV-absorbing substituents and its effect on the structural and hydrodynamic properties of the water-soluble derivatives

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Abstract

Citrus pectin with a low degree of methyl esterification (LMP) and its deesterified form, potassium pectate (KP), were modified with a low amount of UV-absorbing substituents. For this purpose, two different substitution reactions were used (a) alkylation of hydroxyl groups with *p*-carboxybenzyl bromide in aqueous alkali and (b) alkylation of the carboxylate group with benzyl bromide in the DMSO/TBAI/catalyst system. Chemical and spectroscopic methods reveal a low degree of substitution ($DS < 0.1$) for the derivatives. The hydrodynamic properties were assessed by analytical ultracentrifugation, viscometry, and HPGPC. The results indicate that the introduction of small amounts of *p*-carboxybenzyl ether groups practically had no effect on the hydrodynamic properties in the case of KP, whereas, it was accompanied with a decrease of the molecular mass for LMP. The degradation was more pronounced during the benzyl esterification of LMP. The results confirmed that LMP is susceptible to chain cleavage due to β -elimination during both modification reactions. However, KP seems to be more tolerant of the reaction conditions. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Targeted chemical modification of pectins can give new products of industrial importance. Partial hydrophobisation of pectin has been reported to yield water-soluble alkyl esters which are able to bind to bile acids and soy protein (Klavons & Bennet, 1995). Recently, the hydrophobic modification of pectin was described by Crescenzi and Callegaro (1993) and reports the preparation of various water-insoluble alkyl and aryl esters with high degrees of substitution (DS). In our previous studies (Ebringerová, Novotná, Kačuráková & Machová, 1996; Ebringerová, Alföldi, Hromádková, Pavlov & Harding, 2000), the partial hydrophobisation of a series of polysaccharides, including pectin, by etherification with *p*-carboxybenzyl bromide was reported to yield water-soluble, tensioactive *p*-carboxybenzyl

(CB) derivatives. The advantage of this modification is the UV-tagging of molecular chains and, thus, the applicability of UV-based analyses to characterise the structural and molecular properties of the derivatives. In the case of the CB-derivative prepared from corn cob xylan it was shown (Morris, Ebringerová, Harding, & Hromádková, 1999) that the structural integrity and hydrodynamic properties of the xylan, evaluated by the analytical ultracentrifuge with UV-absorption optics, were unaffected by the modification.

The aim of our present study was to compare the effect of UV-tagging with aromatic substituents on the hydrodynamic properties of two pectin samples, low methoxy pectin and potassium pectate. Two reaction methods were applied for this purpose, i.e. etherification of the hydroxyl groups of the pectin samples by *p*-carboxybenzyl bromide and of the carboxylate group by benzyl bromide.

2. Experimental

2.1. Materials and methods

The low methylesterified pectin (LMP) was a gift from

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Pektinfabrik (Copenhagen, Denmark). Potassium pectate (KP) was prepared from the purified GENU pectin, Medium Rapid set — type A (Pektinfabrik Copenhagen, Denmark) by alkaline deesterification with 0.5 M KOH in suspension of 60% ethanol. *p*-Carboxybenzyl bromide (CBB) was prepared according to Daub and Castle (1954). Benzyl bromide (BzB) was supplied from Merck KGaA (Darmstadt, Germany).

FT-IR spectra were measured in KBr pellets (2 mg sample/200 mg KBr) using the Nicolet-Magna 750 spectrophotometer operating at resolution 4 cm⁻¹. The NMR spectra were recorded at 25°C on a Bruker DPX AVANCE-300 spectrometer equipped with a selective excitation unit and gradient enhanced spectroscopy kit (GRASP) for generation of Z-gradients operating at 300 and 75.46 MHz for ¹H- and ¹³C NMR, respectively. Acetone was used as internal standard ($\delta = 2.225$ ppm for ¹H and 31.07 ppm for ¹³C). The samples were dissolved in D₂O (99.99 atom%) and measured in 5 mm tubes. For the identification of CH₂ groups, the DEPT sequence was used from the standard Bruker software library. The following pulse programs were used: in the heteronuclear single quantum correlation (HSQC) experiments the signal from water was suppressed using pulse sequence with pulse field gradients (Schleucher, Schwendiger, Sattler, Smidt, Schedletzky, Glaser et al., 1994). The heteronuclear multiple bond correlation (HMBC) pulse program was applied with low-pass J-filter to suppress single bond correlations (Bax & Summers, 1986). For HSQC and HMBC experiments a multiplication with a squared sine function was applied. All processing was performed using Bruker software XWIN-NMR version 1.3 on a Silicon Graphics INDY computer system. For the identification of CH₂ groups, the DEPT sequence was used from the standard Bruker software library. UV-Vis spectra of the samples in water ($c = 1$ mg ml⁻¹) were recorded on the UV-Visible Spectrophotometer UVmini-1240 (Shimadzu cooperation, Japan).

2.2. Modification methods

Etherification with CBB of LMP and KP in dilute aqueous alkali at ambient temperature was performed as described earlier (Ebringerová et al., 1996). The reaction time was 24 h. The derivative was isolated by precipitation with four volumes of 96% ethanol and purified by re-precipitation from an aqueous solution with 80% ethanol. Then the precipitate was separated by filtration, dialysed against distilled water and lyophilised to yield the carboxybenzylated derivatives CB-LMP and CB-KP.

Alkylation of the carboxylate groups of LMP was performed by a slight modification of the published method (Della Valle & Romeo, 1988), in presence of a catalyst. LMP (1 g) was suspended in dry DMSO (75 ml) and stirred at room temperature overnight. Then, 1.5 g of tetrabutylammonium iodide (TBAI), 0.5 ml of BzB and a small amount of a catalyst were added. The suspension was heated to 30°C

for 6 h and agitated for 24 h at ambient temperature. The reaction mixture was poured into 500 ml of ethyl acetate, and the precipitate was filtered, washed with ethyl acetate to remove residual BzB and degradation products, then dissolved in water. After adjusting the pH to 7 the solution was dialysed against distilled water. The non-dialysable portion after lyophilisation yielded the benzyl ester Bz-LMP.

2.3. Physicochemical methods

2.3.1. Capillary viscometry

Solutions and reference solvents were analysed using a 2 ml automatic Schott-Geräte Oswald viscometer, under precise temperature control (25.33 ± 0.01)°C. The relative, η_{rel} , specific, η_{sp} and intrinsic viscosities were calculated from Eq. 1–3, respectively.

$$\frac{\eta}{\eta_0} = \left(\frac{t}{t_0}\right)\left(\frac{\rho}{\rho_0}\right) = \eta_{rel} \quad (1)$$

where t is the flow time for the macromolecular solution, t_0 is the flow time for the solvent in seconds. Because of the low concentration used (ρ/ρ_0) can be, to a reasonable approximation, taken as unity (see e.g. Harding, 1997). The specific viscosity η_{sp} is obtained as follows

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

A common method for measuring intrinsic viscosity is to calculate the relative and specific viscosity at one concentration (in this case 2.5 mg ml⁻¹) and employ the Solomon-Ciuta approximation (Eq. 3) (see e.g. Abel-Azim, Atta, Farahat & Boutros, 1998; Harding, 1997; Kravtchenko & Pilnik, 1990; Morris, Foster & Harding, 2000). According to Kravtchenko and Pilnik, 1990, the intrinsic viscosity can be accurately estimated (error 1%) by a single measurement: these workers reported good agreement between single point measurements and traditional multi-point (Kravtchenko & Pilnik, 1990) for pectin solutions up to 5 mg ml⁻¹.

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

2.3.2. Sedimentation velocity in the analytical ultracentrifuge

The Optima XLI (Beckman Instruments, Palo Alto, USA) equipped with Rayleigh interference optics was used to determine the sedimentation behaviour of the pectin samples. Rotor speeds of 50 000 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} (0.630 ± 0.01) ml g⁻¹ was assumed for both native and substituted pectins (Morris, 2001). The $g^*(s)$ (sedimentation time derivative) method 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 sedimentation coefficient distribution $g^*(s)$ can in this way be produced (Laue & Stafford, 1999; Stafford, 1992a,b). The apparent weight average sedimentation coefficient, s^* is then calculated. $s_{20,w}$ values can then be generated according to the standard equation (see e.g. Pavlov, 1997; Ralston, 1993) (Eq. 4). Apparent sedimentation coefficients, $s_{20,w}$ were calculated at various concentrations from 0.5 to 2.5 mg ml⁻¹ and extrapolated to zero concentration using the standard Eq. 5 (Ralston, 1993). In addition the concentration of the water-soluble fraction was estimated from the areas under the $g^*(s^*)$ curve.

$$s_{20,w} = s_{T,b} \left[\frac{(1 - \bar{v}\rho_{20,w})\eta_{T,b}}{(1 - \bar{v}\rho_{T,b})\eta_{20,w}} \right] \quad (4)$$

where $\eta_{T,b}$, $\eta_{20,w}$ are the viscosities of the solvent at temperature T and water at 20.0°C, respectively and $\rho_{T,b}$ and $\rho_{20,w}$, are the corresponding solvent densities. This correction can be done before (convention) or after the concentration extrapolation of Eq. 5.

$$s = s^0(1 - k_s c) \quad (5)$$

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

2.3.3. SEC-MALLS

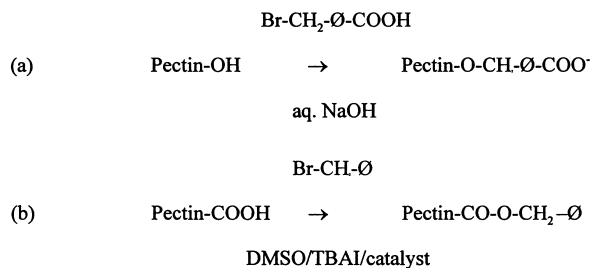
SEC-MALLS (size exclusion chromatography coupled to multi-angle laser light scattering) allows on-line light scattering of a heterogeneous solute fractionated by size exclusion chromatography, permitting the extraction of absolute molecular weights and molecular weight distributions (see e.g. van Holde, 1985; Jumel, 1994; Jumel, Browne, & Kennedy, 1992). The Wyatt Technology (Santa Barbara, USA) Dawn F multi-angle laser light scattering photometer was coupled to TSK Gel 4000, TSK Gel 5000 and TSK Gel 6000 columns protected by a similarly packed guard column (Anachem Ltd., Luton, UK). The eluent was the standard pH 6.8 $I=0.1$ 'Paley' buffer and the injection volume was 100 μ l. A value for the refractive index increment of 0.146 ml g⁻¹ was used (Chapman, Morris, Selvendran & O'Neill, 1987). The area under the refractive index vs. elution volume curve gave an additional estimation of concentration.

3. Results and discussion

3.1. Modification and compositional analysis

For the modification of the pectin samples LMP and KP, two different reaction protocols were used: (a) alkylation of hydroxyl groups with CBB in dilute aqueous alkali, and (b)

alkylation of the carboxylate groups of LMP, transformed into their tetrabutylammonium (TBA) salt, with BzB in dimethyl sulphoxide in the presence of a catalyst.



where \emptyset represents a phenyl group.

The presence of the aromatic substituents in the pectin derivatives was hardly detectable from FT-IR spectra because of the low DS achieved. In spectra of CB-KP the shoulder at 1545 cm⁻¹ corresponds to the $\nu(\text{COO}^-)$ vibrations of the CB substituent. In CB-LMP, the two bands at ~ 1730 and 1710 cm⁻¹ originate from the protonated carboxyl group of the GalA and the CB substituent, respectively (Ebringerová et al., 1996). Bz-LMP seems to have a lower degree of esterification than the parent pectin, as suggested from the lower intensity of the ester band at 1745 cm⁻¹ for Bz-LMP. However, at least a part of the non-methylesterified carboxyl groups of the parent LMP were present in the protonated form, which causes overlapping of the ester band by the $\nu(\text{COOH})$ vibration at 1730 cm⁻¹. The vibration of the aromatic ring expected at 1505–1510 cm⁻¹ is not distinguishable in the spectrum.

The UV-Vis spectra of the derivatives shown in Fig. 1 are more informative. In addition to the absorption of the carboxyl groups at 198–210 nm, the CB-KP and CB-LMP derivatives exhibit new maxima at ~ 240 nm (Ebringerová et al., 1996) and at ~ 280 nm for Bz-LMP. The last two maxima can be used for quantification of the DS. However, β -elimination of the methylesterified galacturonic acid residues is most probably taking place during modification of LMP. The unsaturated 4-deoxy-D-hex-4-enopyranuronic acid residues formed during β -elimination may contribute to the absorption band at 240 nm (Johansson & Samuelson, 1977), thus, exaggerating CB-substitution. However, the presence of the UV-absorbing groups is confirmed by the HPGPC (results not shown). The elution volume profiles recorded by UV absorption at 254 nm follow the same distribution as the molar mass distribution (using the RI-detector — not shown).

To confirm the modification of the pectin chains, ¹H- and ¹³C NMR analyses are the most convenient. The ¹³C NMR spectrum of CB-KP (Fig. 2) is dominated by the signals of the α -(1,4)-D-galacturonan chains at δ 99.9 (C-1), 69.0 (C-2), 69.5 (C-3), 78.9 (C-4) 72.1 (C-5), and 176.3 (C-6) which is in accord with reported data (see for example Ebringerová, Banzragch, Malvovíková & Kačuráková, 1993; Keenan, Belton, Matthew, & Howson, 1985; Odonmazhig, Badgaa, Ebringerová & Alföldi, 1992). The spectrum also contains

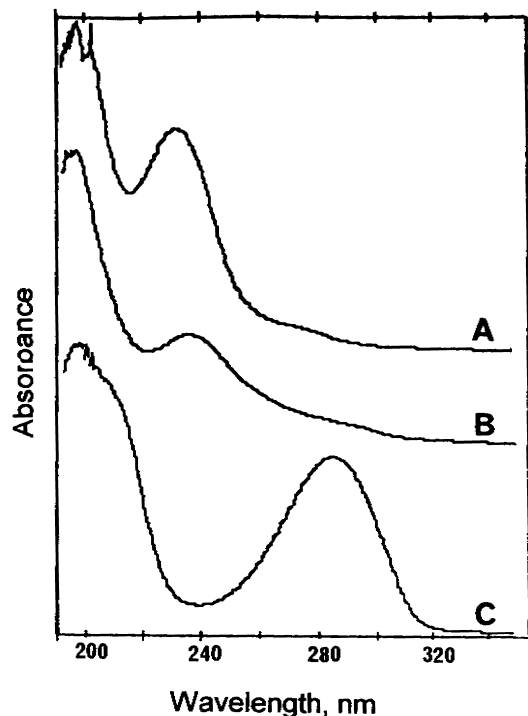


Fig. 1. UV-Vis spectra in H₂O ($c = 1 \text{ mg ml}^{-1}$) of (A) CB-KP, (B) CB-LMP and (C) Bz-LMP.

weak but distinguishable signals of the aromatic carbons (b, c, e, f) of the CB substituent at δ 129 and 130. In the case of CB-LMP (Fig. 3), the ^{13}C NMR spectrum contains, in addition to the resonances of the galacturonan chain and the aromatic carbons, a group of signals at δ 108.4 and 105.2 are attributed to the anomeric carbons of α -arabinofuranosyl and β -galactopyranosyl residues, respectively, which are indicative of arabinan and galactan polymers known to form neutral side chains in the 'hairy' regions of pectins (Thomas, Darvill & Albersheim, 1989). By the HSQC NMR technique, the C/H cross-peaks indicate the presence of β -(1,4)-D-galactan and α -(1,5)-L-arabinan branched at positions O-2 and O-3 (Table 2) assigned in accord with

earlier reported NMR data (Colquhoun, de Ruiter, Schols, & Voragen, 1990; Ryden, Colquhoun & Selvendran, 1988; Schols, Posthumus & Voragen, 1990). The presence of CB groups was deduced from the C/H chemical shifts of the benzylic CH₂ at δ 72.0/4.95, and confirmed by the DEPT experiment (not shown). The data is in accord with the earlier reported shifts for the CB-xylan derivative (Ebringerová et al., 2000). Due to the alkaline reaction conditions, CB-LMP lost most of the methoxyl groups as indicated by the lack of C/H chemical shifts at δ 53.8/3.80. From the ^{13}C NMR spectra of CB-KP and CB-LMP, DS values can be roughly estimated by calculating the ratio of ^{13}C signal areas of the aromatic carbons and C-1 of GalA (δ 99.8–100.3). As shown in Table 1, the obtained DS values of both carboxybenzyl ethers were very low.

In the HSQC spectrum of Bz-LMP (Fig. 4A), the weak C/H cross-peak at δ 68.9/5.28 can be assigned to the benzylic CH₂ group (Table 2). Values 66.4 and 5.16 ppm (in DMSO-*d*₆) have been reported (Kwam, Atzori, Toffanin, Paoletti, & Biviano, 1992) for the benzyl ester of hyaluronic acid. Also the DEPT experiment (not shown) confirmed this assignment, as it allowed us to distinguish this cross-peak from that of the C-5 atoms of esterified GalA residues resonating at δ 71.8/5.10–5.16 (Schols, Bakx, Schipper & Voragen, 1995). The main C/H cross-peak at δ 171.7/3.80, seen in the subsection of the heteronuclear multiple bond correlation (HMBC) experiment (Fig. 4B), corresponds to correlation of C-6 of the methyl-esterified GalA units with protons of the methyl ester group. The further correlations of the C-6 carbon with two H-5 protons at δ 5.18 and 5.10 are indicative of different local environments of these units. The cross-peak at δ 175.6/4.76 corresponds to C-6/H-5 correlations of the non-esterified GalA residues. However, the intensity of the benzylic CH₂ resonance was too low to give a cross-peak in the HMBC spectrum.

From the ^{13}C NMR spectrum of Bz-LMP, the DS calculated from the ^{13}C signal areas of the aromatic protons (b, c, d, e, f) at δ ~129.8 and C-1 of GalA, was as low as that of the CB-LMP derivative. However, the total degree of esterification, calculated from the ratio of the ^{13}C signal areas of

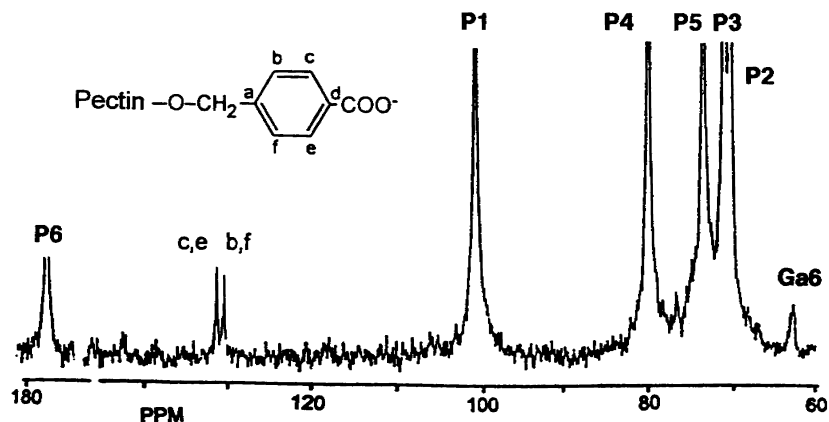


Fig. 2. ^{13}C NMR spectrum of CB-KP. P: 4-linked α -D-GalpA.

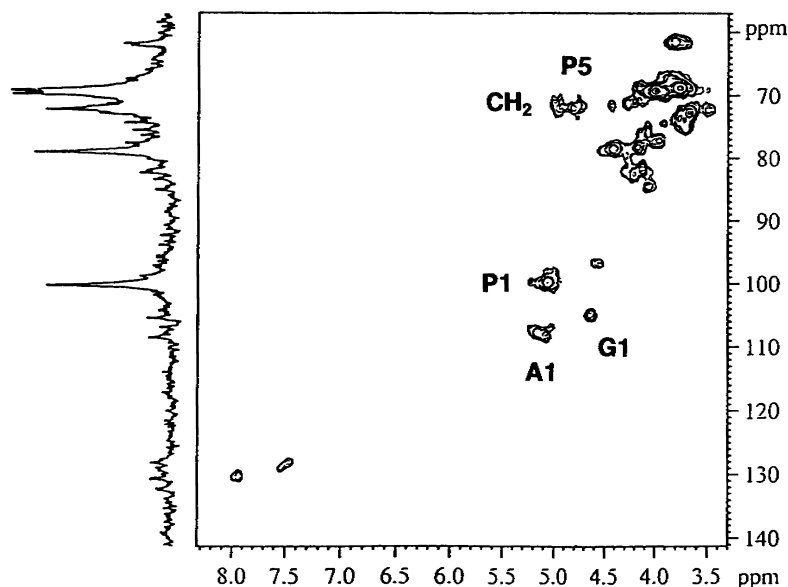


Fig. 3. $^1\text{H}/^{13}\text{C}$ NMR HSQC spectrum of CB-LMP. P: 4-linked α -D-GalpA; A: α -L-Araf, G: 4-linked β -D-Galp.

C-6 atoms of the nonesterified (δ 175.6) and esterified GalA units (δ 170.1) was shown to be about 18%. This value is substantially lower than that claimed for the parent LMP to be 27.9% (Table 1). The β -eliminative cleavage of the macromolecular chains of LMP during the modification was indicated by the small but distinct signal at δ 5.78 in the ^1H NMR spectrum of Bz-LMP. In accord with published data (Alföldi, Palovčík, Peciar, Hirsch & Kováč, 1975; Tjan, Voragen & Pilnik, 1974), it was assigned to the vinylic (H-4) proton of the formed 4-deoxyhex-4-enopyranosyluronic acid non-reducing end

unit which has the double bond located between C-4 and C-5. In the case of Bz-LMP it represents about 0.8% of the signal area at δ 5.08 corresponding to the H-1 resonance of GalA residues, whereas in CB-LMP, only traces are distinguishable. This suggests that low molecular weight degradation products rich in methyl ester groups, formed by the β -eliminative cleavage of the chains, were lost during isolation and dialysis of the derivative. This also means that the methoxyl groups were non-uniformly distributed in the parent pectin chains, otherwise, no changes in the DS would occur.

Table 1
Analytical characteristics of the parent pectins and pectin derivatives.

Sample	GalA (%)	DS (%) ^a	Sugar composition (mol%) ^b		
			Ara	Gal	GalA
KP	89 ^c	0	1	11	88
CB-KP	–	5 ^d	1	10	89
LMP	91 ^c	27.9 ^c	–	–	–
CB-LMP	–	4.0 ^d	5	6	89
Bz-LMP	–	18.0 ^f , 7.0 ^g	6	11	84

^a DS, degree of substitution expressed by the moles of the substituent related to 100 moles of galacturonic acid (GalA).

^b Calculated from the ^{13}C signal areas of the corresponding anomeric carbon atoms of galactose (Gal), arabinose (Ara) and galacturonic acid (GalA).

^c Determined by alkalimetry (Ebringerová et al., 1996).

^d DS was calculated from the ^{13}C signal areas of the aromatic protons (b, c, e, f) of the CB-substituent and C-1 of GalA.

^e T.J. Foster, personal communication.

^f DS represents the sum of methoxyl and benzyl ester groups, calculated from the ^{13}C signal areas of the C-6 carbon of GalA in its carboxylate and ester form.

^g DS was calculated from the ^{13}C signal areas of the aromatic protons (b, c, d, e, f) of the benzyl ester group and C-1 of GalA.

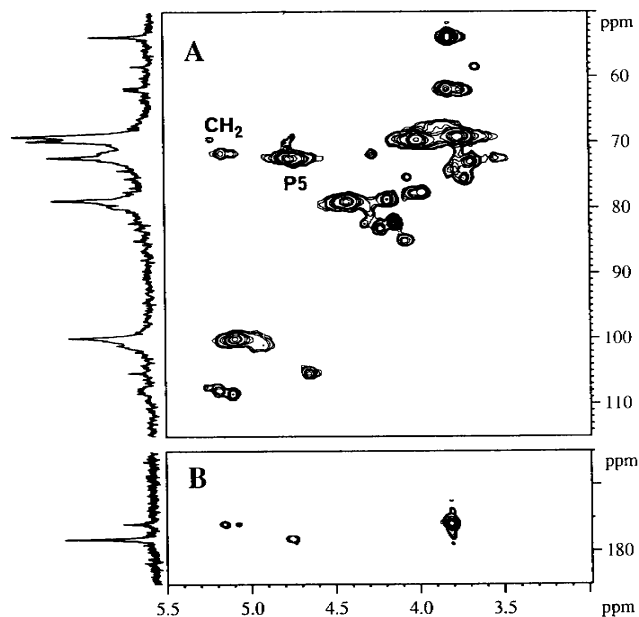


Fig. 4. (A) $^1\text{H}/^{13}\text{C}$ NMR HSQC spectrum of Bz-LMP. (B) partial $^1\text{H}/^{13}\text{C}$ NMR HMBC spectrum of Bz-LMP.

Table 2

¹³C and ¹H chemical shifts (ppm) and assignments for the monomeric units of galacturonic acid, galactose and arabinose found in the CB-LMP and Bz-LMP derivatives

Atom no.	GalpA ^a		Galp ^b		Araf ^c	
	C	H	C	H	C	H
1	99.9–100.1, 101.1 ^c	5.06–5.16, 4.95 ^c	105.3	4.65	108.4 ^{d,e} , 107.9 ^f , 107.4 ^g	5.10 ^{d,e} , 5.18 ^f , 5.25 ^g
2	69.0	3.74	7.31	3.69	81.9 ^{d,e} , 86.0 ^g	4.14 ^{d,e,f}
3	69.5	4.00	7.42	3.78	77.7 ^{d,e} , 83.1 ^f , 76.2 ^g	3.98 ^d , 4.05 ^h , 4.22 ^f , 4.07 ^g
4	78.9	4.41	79.0	4.19	85.0 ^d , 83.1 ^{e,g} , 82.2 ^f	4.08 ^d , 4.22 ^{e,g} , 4.32 ^f
5	72.0, 71.8 ^c	4.76, 5.10 ^c , 5.16 ^c	76.0	3.72	62.0 ^d , 67.8 ^{e,f,g}	– ^h
6	175.6, 171.7 ^c	5.28 ⁱ	61.6	– ^k		
CH ₃	53.9 ^l	3.80 ^l				
CH ₂	68.9 ^l , 72.0 ^l	5.28 ^l , 4.95 ^j				

^a GalpA, non-esterified 4-linked α -D-galactopyranosyl uronic acid residue.

^b Galp, 4-linked β -D-galactopyranosyl residue.

^c GalpA, esterified 4-linked α -D-galactopyranosyl uronic acid residue.

^d Non-reducing terminal end unit α -L-arabinofuranosyl residue.

^e Araf, 5-linked α -L-arabinofuranosyl residue.

^f Araf, 3,5-linked α -L-arabinofuranosyl residue.

^g Araf, 2,5-linked α -L-arabinofuranosyl residue.

^h Indistinguishable signals.

ⁱ Benzylic CH₂ of the carboxybenzyl group.

^j Benzylic CH₂ of the benzyl ester group.

^k The chemical shifts of H-6 of the galactopyranosyl residues could not be distinguished from that of H-5 of the arabinofuranosyl residues.

^l Methylene group.

3.2. Physicochemical characterisation of the parent and modified pectins

3.2.1. SEC-MALLS: molecular weight

The weight average molecular weight for KP is significantly lower than that of LMP, this is consistent with both viscosity and sedimentation data (Table 3). Again it can be seen that the derivatives CB-LMP and Bz-LMP (Fig. 5) are of significantly lower molecular weight than the native pectin LMP and that there is little or no difference between the molecular weights for KP and CB-KP: this is again consistent with other techniques (Table 3).

One can again see the loss in soluble material for the derivitised samples; this is especially noticeable for CB-KP (curve 5).

3.2.2. Capillary viscometry

There are significant differences in the intrinsic viscosities of the two native pectins KP and LMP, 89 and

283 ml g⁻¹, respectively. However in the case of KP there is little or no difference between the intrinsic viscosities of the native and derivatised samples. This is very different for the LMP sample, for which there is approximately a 30% decrease in viscosity during alkaline derivatisation and a 65% decrease in viscosity during (neutral) DMSO modification (Table 3).

3.2.3. Sedimentation velocity in the analytical ultracentrifuge

It is clear from Table 3 that a large amount of material remains insoluble after both alkaline and neutral derivitisation. This can be seen by the decrease in area (Fig. 6) of the $g^*(s^*)$ curve, which is a manifestation of (weight) concentration of the solute (Philo, 1997).

After the sedimentation coefficients have been extrapolated to infinite dilution ($s_{20,w}^0$) we see a trend similar to that of intrinsic viscosity, i.e. there is a significant difference

Table 3

Some physico-chemical data of the parent and modified pectin samples

Sample	Soluble fraction (%)	$[\eta]^a$ (ml g ⁻¹)	$10^{13} \times s_{20,w}^0$ (sec)	$10^{-3} \times M_w^b$ (g mol ⁻¹)	$k_s/[\eta]$	ff_0^c
KP	~100	89	1.91	59	–	4.1
CB-KP	~52	90	1.97	61	–	4.1
LMP	~100	283	2.31	160	0.5	6.6
CB-LMP	~80	201	2.17	116	0.6	5.7
Bz-LMP	~80	106	2.06	90	0.9	5.1

^a Calculated from the Solomon–Ciuta approximation (see Eq. 3).

^b From SEC-MALLS.

^c From Eq. 6.

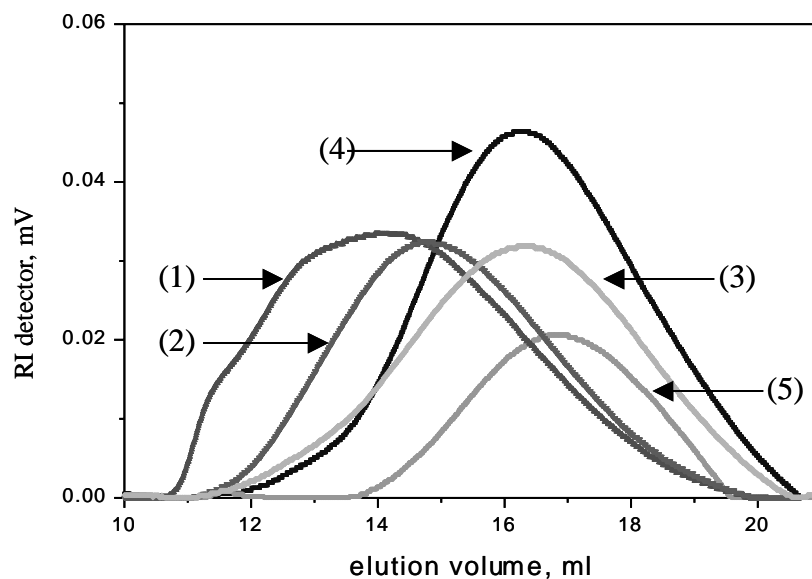


Fig. 5. SEC-MALLS elution profiles for parent and substituted pectins. (1) LMP, (2) Bz-LMP, (3) CB-LMP, (4) KP and (5) CB-KP.

between the sedimentation coefficients of the native pectins (Table 3), and in the case of LMP there is a decrease in sedimentation coefficient upon derivatisation (Table 3). These data can be further interpreted in terms of a decrease in translational frictional ratio defined by (Tanford, 1961)

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

where N_A is Avogadro's number. This together with a decrease in the so-called Wales–van Holde ratio ($R = k_s/[\eta]$) both suggest a decrease in particle asymmetry.

4. Conclusions

Modification of KP and LMP under mild reaction conditions yielded water-soluble derivatives exhibiting low DS. During the modification of LMP in alkaline medium (carboxybenzylation of hydroxyl groups) as well as in neutral reaction medium (benzylation of the carboxylate groups), cleavage of the pectin chains occurred due to the susceptibility of the esterified galacturonic acid units towards β -elimination. However, it was less pronounced in the first case due to the faster alkaline hydrolysis of the methyl ester groups.

The introduction of small amounts of CB ether groups practically had no effect on the hydrodynamic properties

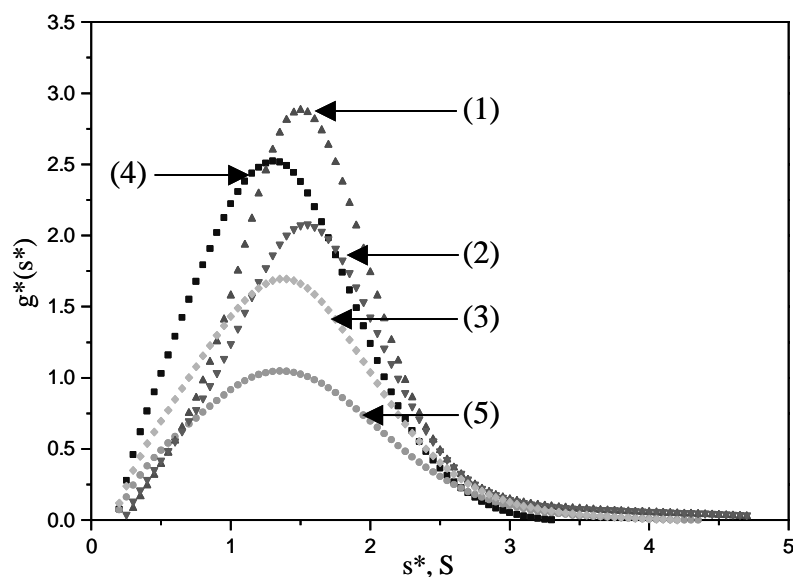


Fig. 6. Sedimentation velocity $g^*(s^*)$ profiles for parent and substituted pectins. (1) LMP, (2) Bz-LMP, (3) CB-LMP, (4) KP and (5) CB-KP.

only in the case of KP. The soluble fraction of CB-KP has similar hydrodynamic properties to those of the parent KP. The etherified pectin CB-LMP as well as the ester Bz-LMP show significantly reduced molecular mass (M_w), sedimentation coefficient ($s_{20,w}^0$) and intrinsic viscosity ($[\eta]$), and increased Wales–van Holde parameter ($k_s/[\eta]$) all symptomatic of a decrease in asymmetry compared to the native LMP.

We suggest that the decrease in molecular weight is due to chain cleavage from β -elimination and that the increased reaction time for the neutral (benzylation of the carboxylate groups in DMSO) protocol results in increased cleavage. KP appears to more tolerant to reaction conditions; this is perhaps due to the fact that a large amount of the native methyl esters had been removed prior to derivitisation, as the methyl esters are the primary driving force in β -elimination reactions this should at least partially explain the increased tolerance and the low molecular weight of the native pectin, KP.

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