^{13}C CP/MAS NMR Spectra of Pectins: a Peak-Fitting Analysis in the C-6 Region

Andriy SYNYTSYA[1], Jana ČOPÍKOVÁ[1] and Jiří BRUS[2]

[1]Department of Carbohydrate Chemistry and Technology, Institute of Chemical Technology, Prague, Czech Republic; [2]Institute of Macromolecular Chemistry, Czech Academy of Science, Prague, Czech Republic

Abstract

Synytsya A., Čopíková J., Brus J. (2003): **^{13}C CP/MAS NMR spectra of pectins: a peak-fitting analysis in the C-6 region**. Czech J. Food Sci., **21**: 00–00.

^{13}C CP/MAS NMR spectra of pectin samples were measured and interpreted. The influence of methylesterification and *O*-acetylation on chemical shifts of pectin carbons was studied using model pectate, pectinates, and their acetylated derivatives. The spectra were analysed also by peak fitting in C-6 region to obtain the values of methylation (DM), amidation (DAm) and acetylation (Dac), degrees. The results were in a good agreement with both convenient methods (photometry, elemental analysis) and the NMR method based on the relative areas of OCH_3 (DM) and CH_3 (DAc) resonances.

Keywords: ^{13}C CP/MAS NMR; pectins; peak fitting; degrees of methylation (DM); degrees of acetylation (DAc)

Pectins are plant polysaccharides that play an important role in cell wall structure and plant physiology. Pectins are known as gelling and stabilising agents that are widely used in food industry (BeMiller 1986; van Buren 1991). The main structural element of pectins is galacturonan a linear polymer of α(1→4) linked, partially methyl esterified D-galacturonic acid. Pectins with more than 50% methyl ester groups are classified as high-methoxyl (HM) and those with less than 50% methyl ester groups as low-methoxyl (LM). Pectins obtained from different sources reveal significant differences in their structural and technological properties. In apple and sugar beet pectins, L-rhamnose residues interrupt the galacturonic acid sequence forming rhamnogalacturonan backbones with attached neutral sugar side chains. Sugar beet pectins are also partially *O*-acetylated. NMR-based structural analysis of pectins from different sources seems to be important for the use in food technology and the characterisation of industrial scale raw materials.

The preparation of pectin solutions for NMR analysis is difficult. Although pectins are well soluble in water, their solutions are very viscous which complicates the application of NMR solution techniques for their analysis (Sullivan 1987). Solid-state ^{13}C NMR has proven to be a valuable technique in structural and conformational analysis of polysaccharides (Colquhoun & Goodfellow 1994; Jarvis 1994; Cheetham & Tao 1998; Saitô *et al*. 1990; Paric *et al*. 1999; Yu *et al*. 1999; Duarte *et al*. 2001). Partially, this NMR technique has been applied in the study of the conformation of pectin macromolecule chains in solid and gel states (Jarvis & Apperley 1995; Renard & Jarvis 1999).

In a previous work (Sinitsya *et al.* 1998), we presented ^{13}C CP/MAS NMR spectra of citrus, apple, and sugar beet pectins with different structural properties. Such characteristic values as uronic

Supported by the Ministry of Education, Youth and Sports (Project No. CEZ MSM 223300005).

acids content (UA), the degree of methylation (DM) and of acetylation (DAc) were obtained on the basis of the relative areas of C-6, COO<u>C</u>H_3 and OCO<u>C</u>H_3 resonance signals, respectively. It was shown that the C-6 region (180–160 ppm) of the spectra is very informative for the structural analysis of pectin. The chemical shift and the shape of resonances in this region are strongly influenced by the relation between different forms of uronic carboxyls in pectin.

In the solution NMR, the carbon chemical shifts of C-6 are well resolved from each other. Moreover, it has been reported that the C-6 carbon resonances are influenced by next-neighbour effects of both the preceeding and following residues, allowing the determination of the distribution of methyl ester groups in pectin (Westerlung *et al*. 1991; Catoire *et al*. 1998).

However, some problems and complications occur in the application of ^{13}C CP/MAS NMR spectroscopy for pectin structure investigation, especially in the case of amidated and highly acetylated pectins. Resonance signals of carboxylic carbons in solid--state spectra are too broad to be resolved from each other. Nevertheless, these signals could be extracted from the whole C-6 resonance mathematically using peak separation methods.

Peak-fitting analysis is widely used in chromatography and infrared spectroscopy of biopolymers including polysaccharides. This mathematical procedure permits to separate the components of the complex spectrum regions and to obtain some qualitative and quantitative information about the sample. We suggest that peak fitting could also be very useful in ^{13}C CP/MAS NMR spectroscopy of such complex polysaccharides as pectins.

In this work we present a detailed investigation of ^{13}C CP/MAS NMR spectra of pectin samples, and peak-fitting analysis of the C-6 region of the spectra.

MATERIAL AND METHODS

Sample preparation and characterisation. The samples of polygalacturonic (pectic) acid, potassium pectate, pectins and their derivatives (Table 1) were used in this work. The procedures of pectin purification and potassium pectate preparation were described in a previous work (Sinitsya *et al*. 1998).

Solid state NMR spectroscopy. ^{13}C CP/MAS NMR spectra of samples were measured using Bruker MSL 200 spectrometer operating at 50.32 MHz (2 ms contact time, recycle delay of 3 s, sweep width of 20 kHz, spinning speed of 4 kHz). All spectra were related to the carbonyl peak of glycine at 176.03 ppm.

Chemical shift calculation. The ^{13}C carbon chemical shifts were calculated by CS ChemNMR Pro 6.0 software (Upstream Solutions, Switzerland) for model structures (trigalacturonic acid and its (Cambridge Soft Corporation, USA).

Peak-fitting. Decomposition of ^{13}C CP/MAS NMR spectra in the regions of 190–165 ppm was pursued by Peak Fitting module of Microcal Origin 6.0 software (Microcal Origin, USA). The second derivative algorithm assisted peak-fitting procedure. The results of peak separation were applied to obtain the degrees of methylation (DM), amidation (DAm), and acetylation (DAc) values on the basis of the relative areas of the separated peaks.

Conventional methods. The content of galacturonic acid (GalA) in the pectin samples was measured by photometry with *m*-hydroxybiphenyl at 520 nm (Blumenkrantz & Aboe-Hansen 1973). This value expressed the total content of uronic carboxylic groups as galacturonic units. The degree of methylation (DM) was determined by photometry with chromotropic acid at 570 nm (Fillippov & Kuzminov 1971). The degree of acetylation (DAc) of samples **6**, **7**, and **15** was estimated by photometry with hydroxylamine (540 nm) (Downs & Pigman 1976). The DAc values for highly acetylated (>1) samples were obtained by solid state NMR method using the OCO<u>C</u>H_3 resonance as a marker of acetyl groups (Sinitsya *et al*. 1998). The degree of amidation (DAm) of amidated pectins was calculated from the results of organic elemental analysis (Sinitsya *et al*. 2000). The values of DM, DAm and DAc of pectins were expressed as relative contents of methoxyl (in %), amide (in %) and acetyl (in mol/mol) groups, respectively.

RESULTS AND DISCUSSION

The ^{13}C CP/MAS NMR spectra of pectin samples are presented in Figure 1(a–c). The chemical shifts of pectins' carbon resonances are summed up in Table 2. The resonances at 176–168 ppm have been assigned to C-6 carbons of galacturonic units, while O<u>C</u>OCH_3 carbons of acetyl groups in acetylated samples are also situated in this region. The resonances at ~101 ppm and ~79 ppm arise from glycosidic bond carbons C-1 and C-4, respectively. The

Table 1. Specification of the pectin samples

No	Sample	UA (%)	DM (%)	DAc (mol/mol)	DAm (%)	Source and specification
1	polygalacturonic acid, K^+-form	86	–	–	–	Koch-Light Lab., England
2	K^+ pectinate	86	22	–	–	Institute of Chemistry, Slovak Academy of Science, Slovak Republic
3	citrus pectin, K^+-form	88	49	–	–	Koch-Light Lab., England
4	HM citrus pectin, K^+-form	90	73	–	–	GENU pectin type B rapid set, Copenhagen Pectin Factory, Denmark
5	pectinic acid	90	90	–	–	Institute of Chemistry, Slovak Academy of Science, Slovak Republic
6	acetylated K^+ pectate	84	-	0.20	–	
7	acetylated K^+ pectate	50	-	0.85	–	
8	acetylated K^+ pectate	71	-	1.12*	–	
9	acetylated K^+ pectinate	25	30	1.41*	–	
10	acetylated pectinic acid	26	90	1.67*	–	
11	HM citrus pectin, H^+ form	76	68	–	–	GENU pectin type A medium rapid set
12	LM citrus pectin, H^+ form	81	13	–	–	GENU pectin type LM-1912CS
13	LMA citrus pectin, H^+ form	85	33	–	20	GENU pectin type LM-102AS
14	LM apple pectin, H^+ form	55	34	–	–	Smiřice, Danisco, Czech Republic
15	sugar beet pectin, H^+ form	83	47	0.36	–	GENU pectin type BETA, Copenhagen Pectin Factory, Denmark

Copenhagen Pectin Factory, Denmark

*obtained by NMR (Sinitsya *et al*. 1998)

peaks at 67–72 ppm come from the other carbons of pyranoid ring (Keenan *et al*. 1985; Malovíkova & Kohn 1986; Jarvis & Apperley 1995; Sinitsya *et al.* 1998; Renard & Jarvis 1999).

The NMR spectra of potassium pectate and potassium pectinates of various DM are shown in Figure 1a. An intense resonance at ~53 ppm (Table 2) represents methyl carbons of the methyl ester COO$\underline{C}H_3$ in the spectra of pectinates (Keenan *et al*. 1985; Renard & Jarvis 1999). This resonance band increases with subsequent methyl esterification and has been used for the estimation of DM values (Sullivan 1987; Sinitsya *et al.* 1998).

The NMR spectra of acetylated pectates and pectinates **6–10** in comparison with potassium pectate **1** as the non-substituted structure are demonstrated in Figure 1b. An intense resonance at ~21 ppm represents methyl carbons of the acetyl ester OCO$\underline{C}H_3$ in the spectra of acetylated samples (Keenan *et al*. 1985; Sinitsya *et al.* 1998; Renard & Jarvis 1999). This resonance band increased and slightly shifted upfield from 20.2 ppm (acetylated pectate **6**, Dac = 0.2) to 21.4 ppm (acetylated pectinic acid **10**, Dac = 1.67) with subsequent acetylation (Table 2). This band was used for the estimation of DAc values in sugar beet pectins (Sinitsya *et al.*

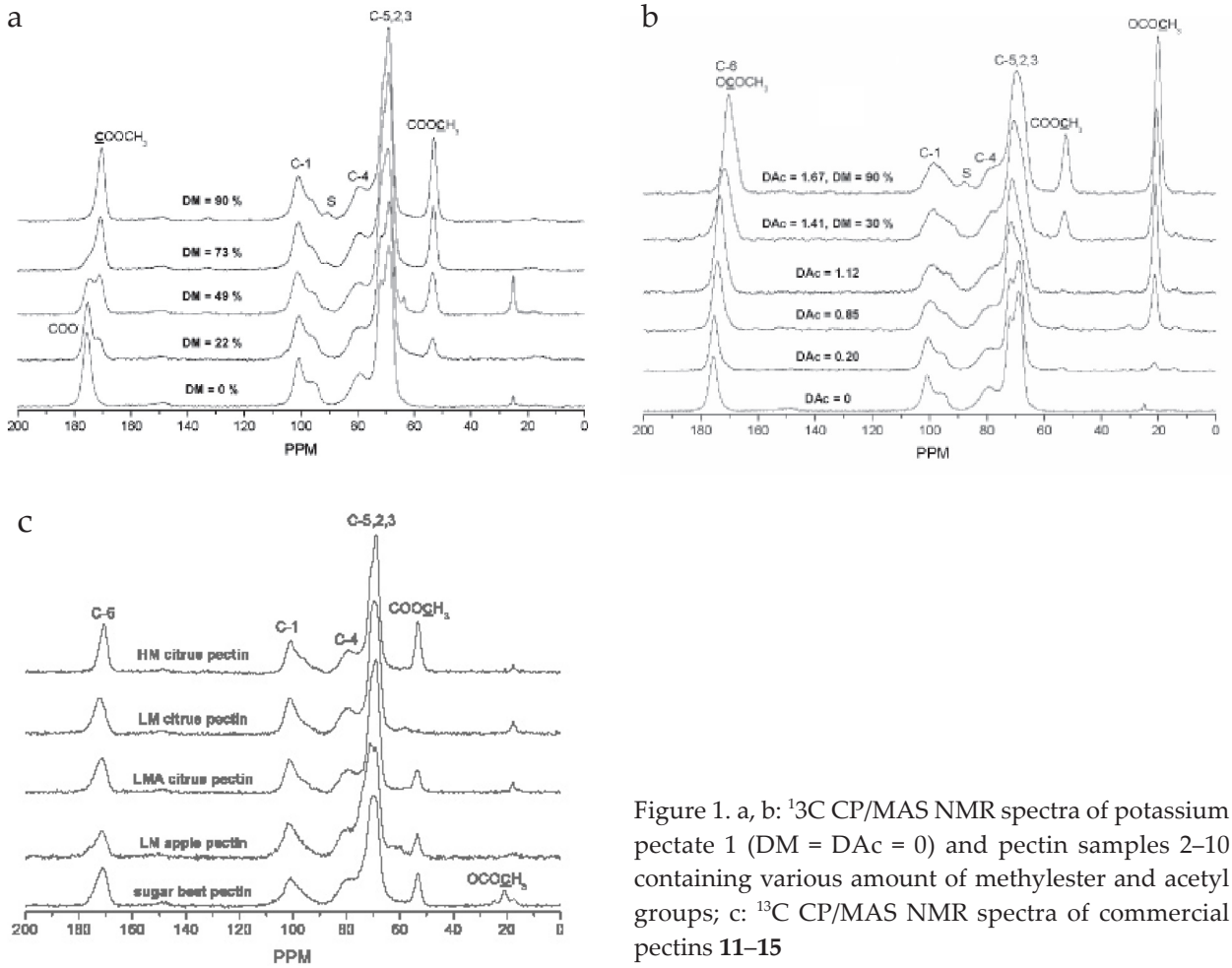

Figure 1. a, b: ^{13}C CP/MAS NMR spectra of potassium pectate 1 (DM = DAc = 0) and pectin samples 2–10 containing various amount of methylester and acetyl groups; c: ^{13}C CP/MAS NMR spectra of commercial pectins **11–15**

1998). Weak resonance at ~53 ppm was present in the spectra of acetylated pectates **6–8** indicating a small amount of methyl ester groups in these samples. In acetylated pectinates **9–10** this band was much more marked.

The resonance peak at 71.8 ppm (C-5 carbons) of potassium pectate **1** shifted upfield and became a non-resolved shoulder of a strong resonance signal at ~69 ppm (C-2, 3 carbons) in pectinates **2–4** and pectinic acid **5** with DM increasing (Figure 2a). Among all the carbons of pyranoid ring, the C-5 resonance is the most sensitive to methylation. In contrast, acetylation leads to marked changes in the resonance signals of pyranoid ring carbons (Renard & Jarvis 1999). The resonances of the glycosidic bond carbons C-1 and C-4 shifted upfield by ~2 ppm in highly acetylated samples **8–10** (Table 2). In contrast, the resonance peak at ~69 ppm (C-2, 3 carbons) of potassium pectate **1** shifted downfield and combines with the C-5 resonance into a single maximum at ~71 ppm upon acetylation (samples **7** and **8**). In acetylated pectinates **9** and **10**, this resonance signal shifted upfield to 70.6 ppm and 69.7 ppm, respectively (Figure 2b). In addition, a non-resolved upfield shoulder at ~66 ppm also occurred in highly acetylated samples. The positions of the resonance signals in the region of 85–60 ppm were estimated by second derivative algorithm (Figures 2c,d).

To explain the observed changes in the position of pyranoid ring carbon resonances, we calculated the alterations of chemical shifts in trigalacturonic acid as a result of methylation and (or) acetylation. Trigalacturonic acid and its derivatives were chosen as model compounds representing various forms of galacturonic units in pectins. In Table 3, we summed up the results of calculation for inner monosaccharide unit –D-GalA- of model trisaccharides. This residue represents internal α(1→4)-linked sugar units predominating in polysaccharide macromolecules. Previously, we observed that the substitution of both terminal GalA units had no significant influence on chemical shifts of pyranoid ring carbons in the internal –D-GalA- unit.

Table 2. ^{13}C CP/MAS NMR chemical shifts (in ppm) for the samples of pectins

Sample	Chemical shift (ppm)					
	C-1	C-2, 3, 5	C-4	C-6	$COO\underline{C}H_3$	$OCO\underline{C}H_3$
1	100.8	71.8 (C-5) 68.9 (C-2, 3)	79.3	175.6	–	–
2	100.8	68.8	78.6	175.3 (COO^-) 171.8 ($\underline{C}OOCH_3$)	53.3	–
3	100.3	69.3	79.7	174.9 (COO^-) 171.2 ($\underline{C}OOCH_3$)	53.4	–
4	100.6	69.0	79.3	170.9	53.2	–
5	101.1	69.0	79.4	170.5	53.0	–
6	100.6	71.8 (C-5) 68.8 (C-2, 3)	78.6	175.3	53.9	21.4
7	99.9	71.4	79.3	174.1	53.6	21.3
8	98.9	71.3	76.7	173.3		21.0
9	98.2	70.6	77.0	171.7	52.9	20.6
10	98.5	69.7	76.9	170.3	52.4	20.2
11	100.9	67.0	79.2	170.6	53.3	–
12	101.2	69.8	79.2	171.8	–	–
13	101.6	71.0	79.4	171.6	53.6	–
14	101.6	71.0	79.3	171.6	53.6	–
15	100.9	70.9	77.9	171.2	53.2	21.0

The results of the calculations are as follows. Firstly, the methylation at C-6 position of GalA led to upfield shift of the C-5 carbon resonance by 2.5 ppm, whereas chemical shifts of the other pyranoid ring carbons did not change significantly. Secondly, acetylation at C-2 or at C-3 position, i.e. monoacetylation, resulted in downfield shift of the resonance of the C-OH carbons (+3.4 ppm) and in upfield shift of the resonance of the carbons carrying *O*-acetyl group (–3.3 ppm). Chemical shifts of C-1 or C-4 carbons moved upfield (–3.3 ppm) after monoacetylation of –GalA- at O-2 or O-3 position, respectively. Thirdly, 2,3-diacetylation led to no significant changes of chemical shifts of C-2 and C-3 carbons, while the resonances of both C-1 and C-4 moved upfield (–3.4 ppm). And finally, the effects of methylation and acetylation mentioned above seem to be independent from each other at the first approximation.

Therefore, on the basis of the trends calculated for model compounds (Table 3) and the minima of the second derivatives of experimental NMR spectra (Table 4, Figure 2d), we may assume that, for acetylated pectin samples **6–10**, the shoulder at ~66 ppm originates from non-acetylated C-2,3 carbons of monoacetylated units, whereas the main maximum at 72–70 ppm originates from both acetylated C-2,3 carbons of monoacetylated units and all C-5 carbons. Furthermore, the residual resonance signal at ~68 ppm represents C-2,3 carbons of both non-acetylated and diacetylated units. A slight upfield shift (–1 ppm) of the C-5 carbon resonance for methylated samples **2–5** is also evident (Table 4, Figure 2c). Finally, the observed upfield shift of C-2,3,5 resonance in highly acetylated pectinates **9** and **10** may be a result of both methylation (the upfield shift of C-5 resonance) and acetylation (prevalence of diacetylated units).

Both C-1 and C-4 carbons take part in the formation of α(1→4) glycosidic bond in the pectin backbone and therefore they are more flexible than the other carbons of pyranoid ring (Jarvis & Apperley

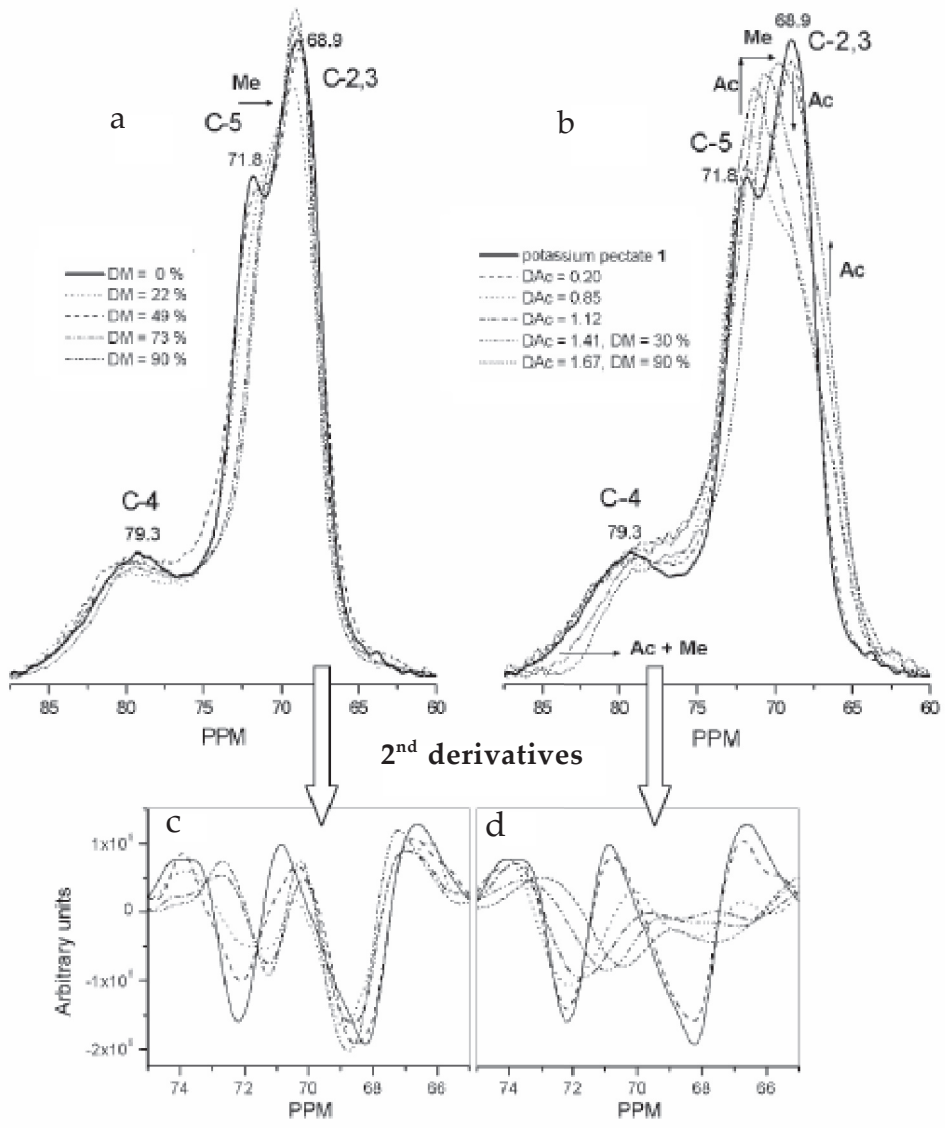

Figure 2. The ^{13}C CP/MAS NMR spectra (the region of pyranoid ring carbons C-2-5, 87.5–60 ppm) of potassium pectate **1** and pectin samples **2–10** containing various amounts of methylester and acetyl groups (a, b), and the second derivatives of these spectra at 75–65 ppm (c, d)

1995). The observed broadening of these signals can be caused by non-homogeneous broadening reflecting different conformations of glycosidic bond in regular and irregular structures as well as by homogeneous broadening resulting from destructive interference of molecular motions by the dipolar decoupling field (VanderHart *et al.* 1981). Therefore, C-1 and C-4 carbon resonances are sensitive to the conformation at glycosidic bond. In concentrated pectin gels, Jarvis and Apperley (1995) observed a C-1 resonance at ~101 ppm and the C-4 signals at 80–81 ppm for pectins forming 3_1 helices, while 21 helices had their C-4 at ~77 ppm.

For solid pectins, C-1 and C-4 carbon resonances are very broad, complex, and they overlap other signals. The upfield shift (~2 ppm) of both of them as observed for highly acetylated samples **8–10** is in agreement with the calculations and may reflect structural particularity of 2,3-*O*-diacetylated galacturonic units predominating in these samples.

The region of 190–160 ppm of ^{13}C CP/MAS NMR spectrum belongs to carboxyl C-6 carbons of galacturonic units that are present as carboxylic acid COOH, carboxylate anion COO^-, ester $\underline{C}OOCH_3$, or amide $CONH_2$ form (Sinitsya *et al.* 1998). Resonance signals of carboxyl carbons of acetyl groups

Table 3. The influence of substitution on the calculated chemical shifts (in ppm) of pyranoid ring carbons in the internal –GalA- unit of trigalacturonic acid*

Substituents**	Alteration of chemical shift (ppm)				
	C-1	C-2	C-3	C-4	C-5
6-Me	0.0	0.0	0.0	+0.3	–2.5
2-Ac	–3.3	+3.4	–3.3	–0.1	–0.3
3-Ac	–0.1	-3.3	+3.4	–3.3	+0.2
2,3-diAc	–3.4	+0.1	+0.1	–3.4	–0.1
2-Ac, 6-Me	–3.3	+3.4	–3.3	+0.2	–2.8
2-Ac, 6-Me	–0.1	–3.3	+3.4	–3.0	–2.3
2,3-diAc, 6-Me	–3.4	+0.1	+0.1	–3.1	–2.6

*The substitution of both terminal –GalA- units had no significant influence on the chemical shifts of pyranoid ring carbons in the internal –GalA– unit

**Me - methyl ester, Ac - *O*-acetyl

O<u>C</u>OCH$_3$ present in sugar beet pectins cannot be resolved due to overlapping by the intense C-6 signal. The COO$^-$ resonance (176–175 ppm) is a little shifted downfield in comparison to the other forms that have resonances at 173–170 ppm. All these resonances overlap one another because the shift difference between them is significantly less than the sum of their half widths. Therefore, in most cases, it is impossible to resolve the resonance signals of carboxylic forms. An exception is potassium pectinates **2** and **3** that have two C-6 resonances at ~175 ppm and at ~172 ppm assigned to COO$^-$ and <u>C</u>OOCH$_3$ carbons (Sullivan 1987; Sinitsya *et al.* 1998).

The spectral changes at the C-6 region of pectate and pectinates are demonstrated in Figure 3a. The resonance of COO$^-$ (~175 ppm) decreases and the resonance of <u>C</u>OOCH$_3$ (172–170 ppm) increases with methylation (Renard & Jarvis 1999). The C-6 and O<u>C</u>OCH$_3$ region of the spectra of potassium pectate **1** and acetylated samples **6–10** is shown in Figure 3b. It was observed that the main resonance signal in this region moved upfield from 175.5 ppm (potassium pectate **1**) to 170.3 ppm

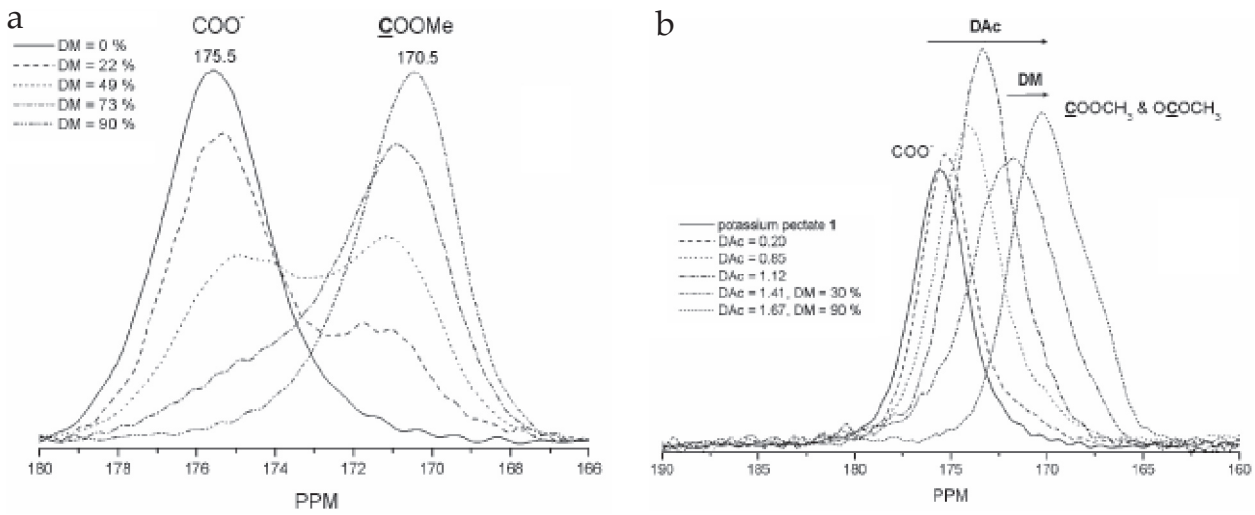

Figure 3. The C-6 & OCOCH3 region of the 13C CP/MAS NMR spectra of potassium pectate 1 and pectin samples 2-10 containing various amounts of methylester and acetyl groups (a, b)

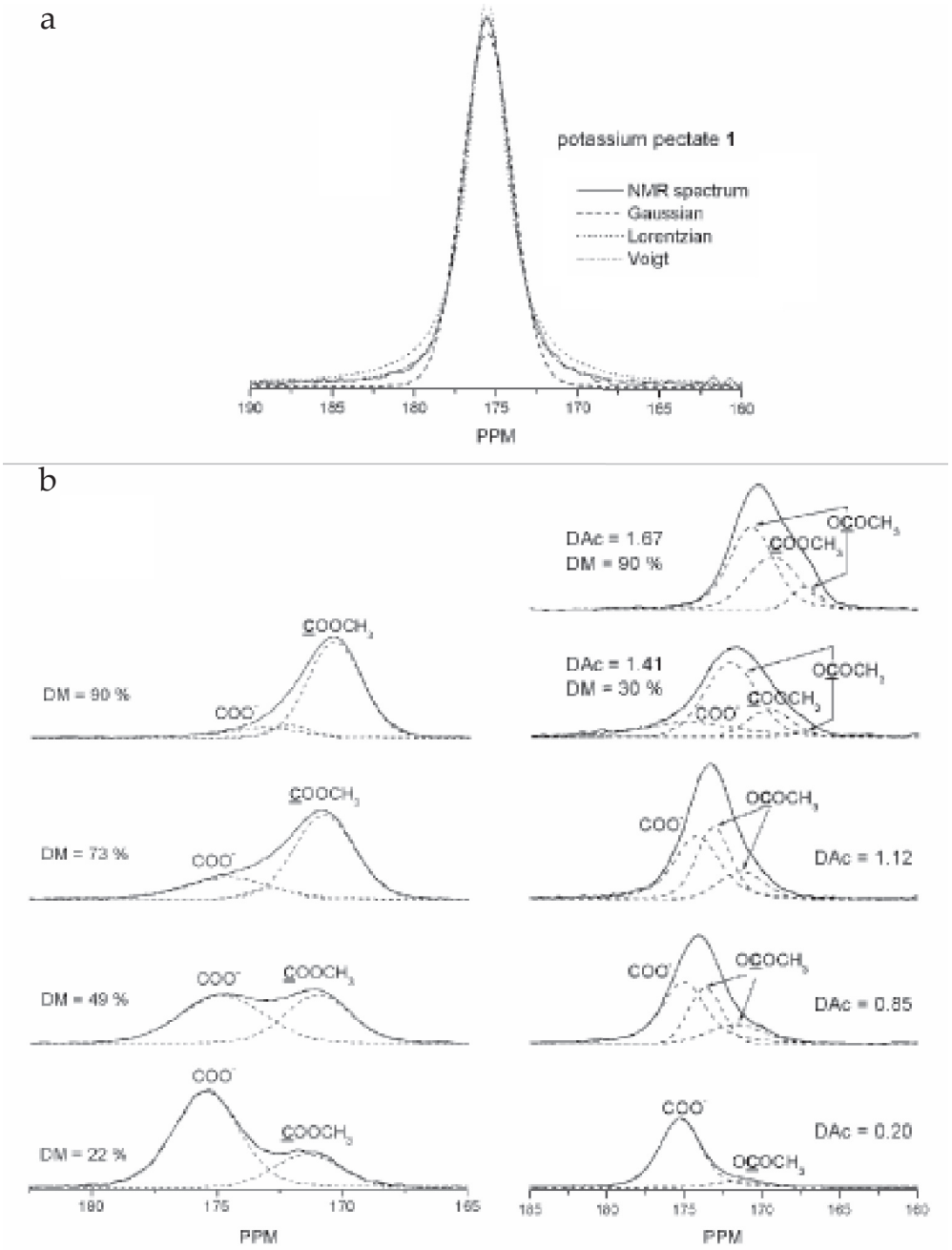

Figure 4. Modelling of the COO- carbon resonance signal of potassium pectate **1** by various fitting functions (a) and the decomposition of ^{13}C CP/MAS NMR spectra (C-6 & O<u>C</u>OCH$_3$ region, 185–160 ppm) of pectin samples **2–10** containing various amounts of methylester and acetyl groups (b)

(acetylated pectinic acid **10**). The observed shift occurred due to the contribution of ester carbons, i.e. O<u>C</u>OCH$_3$ resonances in acetylated pectates and both O<u>C</u>OCH$_3$ and <u>C</u>OOCH$_3$ resonances in acetylated pectinates.

To decompose the C-6 region of the NMR spectra, we used the multiple mixed Lorentzian-Gaussian (Voigt) functions as fitting model. The Lorentzian function is most often applied to the fitting of NMR spectra, while the Gaussian and other models has been applied, too. However, the Voigt model posseses a more accurate fitting and also provides a more accurate quantitation of NMR spectra than either Lorentzian or Gaussian model (Mierisová & Ala-Korpela 2001). Peak fittings of a single COO$^-$ carbon resonance of potassium pectate **1**

Table 4. Chemical shifts (in ppm) of C-2,3,5 carbons of samples **1**, **5–10** obtained from 2nd derivatives of ^{13}C CP/MAS NMR spectra

Sample							Assignment
1	5	6	7	8	9	10	
–	–	65.6	65.8	66.5	66.9	–	C-OH in monoacetylated units
68.2	68.7	68.3	68.1	68.7	68.2	67.6	C-2,3 in non-acetylated and diacetylated units
72.2	71.2	72.2	72.1	71.7	70.9	70.4	**C**-$OCOCH_3$ in monoacetylated units and C-5

by various models are shown in Figure 4a. It is evident that, at least for solid pectin samples, a mixed Lorentzian-Gaussian (Voigt) function is a much better model for solid state NMR than Gaussian or Lorentzian functions themselves.

Decomposition of C-6 region permits to estimate the relationship between free ionised and methyl esterified carboxyls in pectinates, between free ionised carboxyls and acetyls in acetylated pectates, and between methyl and acetyl esters in acetylated pectinates (Figure 4b). In the case of low-substituted samples **2**, **3,** and **6**, the resonance signals of each type of carbons were modelled by a single Voigt component. For highly methylated samples **4** and **5**, two Voigt components, centred at 170.4–170.8 ppm and 172.2–172.9 ppm, indicate **C**$OOCH_3$ carbons. Similarly, for highly acetylated samples **7–10**, two Voigt components at ~171.5 ppm and at ~173.5 ppm represent O**C**OCH_3 carbons of acetyls. As a rule, all ester components were shifted upfield with the increasing degree of the corresponding substitution, and the Voigt component of **C**$OOCH_3$ (169.5 ppm) was located between the acetyl components (170.7–172.1 ppm and 167.3 ppm).

The resonance signals COO^- (~176 ppm), COOH (~173 ppm) and **C**$OOCH_3$ (~170.5 ppm) carbons were obtained by decomposition of the C-6 region of NMR spectra of pectins **11–15** (Figure 5). The Voight component peak of amidated citrus pectin **13** centred at 172.4 ppm corresponds to $CONH_2$ carbons. Highly acetylated sugar beet pectin **15** has carbons **C**$OOCH_3$ and O**C**OCH_3 of very similar chemical shifts. Peak separation gives Voight

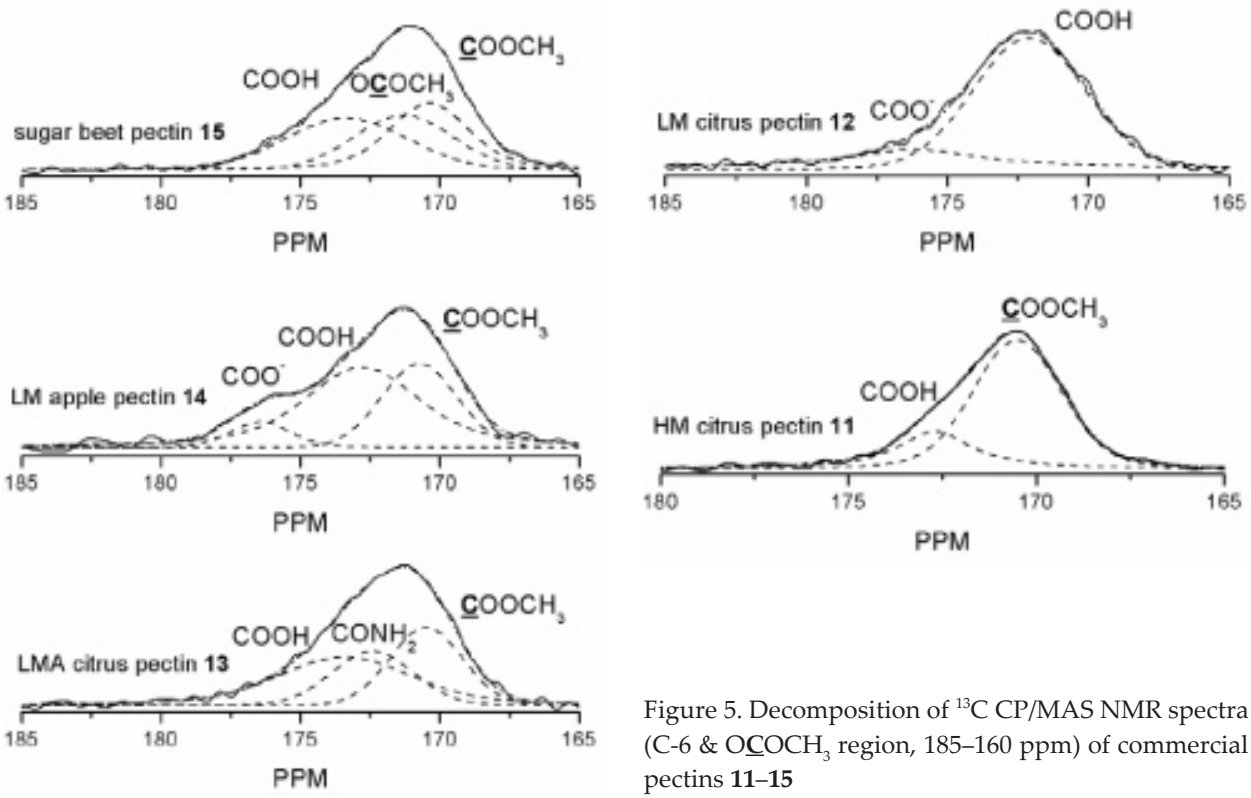

Figure 5. Decomposition of ^{13}C CP/MAS NMR spectra (C-6 & O**C**OCH_3 region, 185–160 ppm) of commercial pectins **11–15**

Table 5. Degrees of methylation (DM), amidation (DAm) and acetylation (DAc) obtained by decomposition of the C-6 region of ^{13}C CP/MAS NMR spectra*

Sample	Area, 10^7				DM (%)	DAm (%)	DAc (mol/mol)
	$A_{C\text{-}6\ tot.}$	$A_{\underline{C}OOCH_3}$	A_{CONH_2}	$A_{O\underline{C}OCH_3}$			
1	85.46	–	–	–	0	0	0
2	86.84	65.49	–	–	25	0	0
3	51.22	22.92	–	–	45	0	0
4	55.53	39.80	–	–	72	0	0
5	49.70	40.97	–	–	82	0	0
6	51.54	–	–	4.93	0	0	0.10
7	61.27	–	–	52.16	0	0	0.85
8	65.39	–	–	74.94	0	0	1.15
9	63.08	23.52	–	83.06	37	0	1.32
10	47.05	47.05	–	78.79	100	0	1.67
11	59.24	44.23	–	–	75	0	0
12	53.90	–	–	–	0	0	0
13	59.24	19.23	14.89	–	32	25	0
14	50.33	16.34	-	–	34	0	0
15	46.40	23.04	-	19.92	49	0	0.43

*DM (%) = $A_{\underline{C}OOCH_3}/A_{C\text{-}6\ tot.}\times100$; DAm (%) = $A_{CONH_2}/A_{C\text{-}6\ tot.}\times100$, DAc = $A_{O\underline{C}OCH_3}/A_{C\text{-}6\ tot.}$

component peaks at 171.3 and 170.4 ppm. These peaks represent carbonyl carbons of acetyl and methyl ester groups, respectively.

The relative contents of methyl ester, amide and acetyl groups were calculated on the basis of the areas of the corresponding Voight components (Table 5). The obtained values of DM, DAm and DAc were in good agreement with the corresponding values of the conventional methods (photometry for DM and DAc, elemental analysis for DAm) including NMR method based on the relative areas of $COO\underline{C}H_3$ and $OCO\underline{C}H_3$ resonance signals (Table 1).

CONCLUSION

^{13}C CP/MAS NMR spectroscopy is a relatively demanding but a very informative method in the analysis of pectins. In spite of the relatively low resolution and sensitivity in comparison to the solution NMR, the solid-state NMR technique may provide important information about the structure of pectins. In addition to the well-known application of OCH_3 and CH_3 resonances as effective ester and acetyl markers in the solid state NMR, the peak fitting in the C-6 spectral region seems to be a very useful tool for the estimation of the proportions of various C-6 carboxyls and *O*-acetyls in pectin.

Acknowledgements: Authors thank Dr. Anna Malovíková (Institute of Chemistry, Slovak Academy of Science, Slovak Republic) for the kind gift of the samples of potassium pectate and its methylated and acetylated derivatives, which were used in this work. Authors also thank Dr. Pavlíková (Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic) for the multiform support in the preparation of this work.

References

BeMiller J.N. (1986): An introduction to pectins: structure and properties. In: Fishman M.L., Jen J.J. (eds): Chemistry and Function of Pectins. In: ACS Symp. Ser. 310, ACS, Washington DC: 2–12.

Blumenkrantz N., Aboe-Hansen G. (1973): New method for quantitative determination of uronic acids. Anal. Biochem., **54**: 484–489.

Catoire L., Goldberg R., Pierron M., Morvan, C., du Penhoat K. H. (1998): An efficient procedure for studying pectin structure which combines limited depolymerisation and 13C NMR. Eur. Biophys. J., **27**: 127–136.

Cheetham N.W.H., Tao L. (1998): Solid state NMR studies on the structural and conformational properties of natural maize starches. Carbohyd. Polym., **36**: 285–292.

Colquhoun I.J., Goodfellow B.J. (1994): Nuclear magnetic resonance spectroscopy. In: Wilson R.H (ed.): Spectroscopic Techniques for Food Analysis. VCH, Cambridge: 87–145.

Downs F., Pigman W. (1976): Determination of *O*-acetyl groups by the Hestrin method. In: Whistler R.L., BeMiller J.N. (eds): Methods in Carbohydrate Chemistry. Academic Press, NY: 241–243.

Duarte M.L., Ferreira M.C., Marvão M.R., Rocha J. (2001): Determination of the degree of acetylation of chitin materials by ^{13}C CP/MAS NMR spectroscopy, Int. J. Biol. Macromol., **28**: 359–363.

Fillippov M.P., Kuzminov V.I. (1971): Photometric determination of methoxyl groups in pectin substances. Zh. Anal. Khim., **26**: 143–146.

Jarvis M.C. (1994): Relationship of chemical shift to glycosidic conformation in the solid-state ^{13}C NMR spectra of (1→4)-linked glucose polymers and oligomers: anomeric and related effects. Carbohyd. Res., **259**: 311–318.

Jarvis M.C., Apperley D.C. (1995): Chain conformation in concentrated pectic gels: evidence from ^{13}C NMR. Carbohydr. Res., **275**: 131.

Keenan M.H.J., Belton P.S., Mathew J.A., Howson S.J. (1985): A ^{13}C-n.m.r. study of sugar-beet pectin. Carbohyd. Res., **138**: 168–170.

Malovíkova A., Kohn R. (1986): Binding of calcium ions to 2,3-dicarboxy derivatives of pectic acid. Collect. Czech. Chem. C., **51**: 2259–2270.

Mierisová Š., Ala-Korpela M. (2001): MR spectroscopy quantitation: a review of frequency domain methods. NMR Biomed., **14**: 247–259.

Paris M., Bizot H., Emery J., Buzaré J.Y., Buléon A. (1999): Crystallinity and structuring role of water in native and recrystallized starches by ^{13}C CP/MAS NMR spectroscopy 1: Spectral decomposition. Carbohyd. Polym., **39**: 327–339.

Renard C.M.G.C., Jarvis M.C. (1999): Acetylation and methylation of homogalacturonans 2: effect on ion-binding properties and conformations. Carbohyd. Polym., **39**: 209–216.

Saitô H., Yokoi M., Yamada J. (1990): Hydration-dehydration-induced conformational changes of agarose and kappa- and iota-carrageenans as studied by high-resolution solid-state 13C-nuclear magnetic resonance spectroscopy. Carbohyd. Res., **199**: 1–10.

Sinitsya A., Čopiková J., Pavliková H. (1998): ^{13}C CP/MAS NMR spectroscopy in the analysis of pectins. J. Carbohyd. Chem., **17**: 279–292.

Sinitsya A., Čopíková J., Prutyanov V., Skoblya S., Machovič V. (2000): Amidation of highly methoxylated citrus pectin with primary amines. Carbohyd. Polym., **42**: 359–368**.**

Sullivan M. J. (1987): Industrial applications of high-resolution solid-state ^{13}C NMR techniques. Trends in Analyt. Chem., **6**: 31–36.

Van Buren J.P. (1991): Function of pectin in plant tissue structure and firmness. In: Walter R.H. (ed.): The Chemistry and Technology of Pectin. Academic Press, San Diego: 1–22.

VanderHart D.L., Earl W.L., Garroway A.N. (1981): Resolution in carbon-13 NMR of organic solids using high-power proton decoupling and magic-angle sample spinning. J. Magn. Reson., **44**: 361–401.

Westerlung E., Åman P., Andersson R.E., Andersson E. (1991): Investigation of the distribution of methyl ester groups in pectin by high-field ^{13}C NMR. Carbohyd. Polym., **14**: 179–187.

Yu G., Morin F.G., Nobes G.A.R., Marchessault R.H. (1999): Degree of acetylation of chitin and extent of grafting PHB on chitosan determined by solid state ^{15}N NMR. Macromolecules, **32**: 518–520.

Received December 9, 2002
Accepted

Souhrn

Synytsya A., Čopíková J., Brus J. (2003): **^{13}C CP/MAS NMR spektra pektinů: separace pasů v oblasti signálů C-6 uhlíků**. Czech J. Food Sci., **21**: 00–00.

Byla naměřena a interpretována ^{13}C CP/MAS NMR spektra pektinů. Vlivy esterifikace a *O*-acetylace na chemické posuny uhlíků byly sledovány na modelových vzorcích pektátu, pektinátů a jejich acetylovaných derivátů. NMR

spektra byla také analyzována pomocí separace pásů v oblasti signálů C-6 uhlíků. Na základě ploch příslušných Voigtových komponent byly vypočteny hodnoty stupňů methylace (DM), amidace (DAm) a acetylace (DAc). Výsledky výpočtu jsou v dobré shodě s konvenčními metodami (fotometrie, elementární analýza) a s NMR metodou na základě relativních ploch resonančních signálů OCH_3 (DM) a CH_3 (DAc).

Klíčová slova: ^{13}C CP/MAS NMR, pektiny, separace pasů, stupně methylace (DM); stupně acetylace (DAc)

Corresponding author:

Ing. Andriy.Synytsya, Vysoká škola chemicko-technologická, Ústav technologie a chemie sacharidů, Technická 3, 161 05 Praha 6, Česká republika
tel.: + 420 222 435 31 14, fax: + 420 231 199, e-mail: andrej.sinica@vscht.cz