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N-octadecylpectinamide, a hydrophobic sorbent based on modification of highly methoxylated citrus pectin

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Abstract

N-octadecylpectinamides with degrees of substitution (DA) of 5–32% were prepared by heterogeneous amino-de-alkoxylation of HM citrus pectin (DM = 73%) with *n*-octadecylamine. FT-Raman, FT-IR, FT-NIR and ^{13}C CP/MAS NMR spectroscopic studies indicated both secondary amide and *n*-octadecyl groups in the modified pectin samples. Comparing laser diffraction, image analysis and electronic microscopy of the initial HM citrus pectin and the final product of aminolysis confirmed that the size distribution and the shape of solid pectin particles significantly changed on substitution. The sorption properties of the water-insoluble modified pectin (DA = 32%) were tested by reverse phase (RP) chromatography using this product as the stationary phase. According to RP chromatography, *N*-octadecylpectinamide is able to absorb selectively non-polar molecules and polar molecules with non-polar parts. However, it still has some residual hydrophilicity due to the presence of polar groups in the pectin framework.

Keywords: *N*-octadecylpectinamide; HM citrus pectin; *n*-Octadecylamine; Aminolysis; FT-IR; FT-Raman; FT-NIR; ^{13}C CP/MAS NMR; Laser diffraction; Particle size distribution; Image analysis; Reverse phase chromatography

1. Introduction

Preparation of various *N*-alkylpectinamides by heterogeneous amino-de-alkoxylation (aminolysis) of highly methoxylated (HM) citrus pectin has been described in our previous article (Sinitsya, Čopíková, Prutyanov, Skoblya, & Machovič, 2000). This method has been shown to give a good reaction yield for non-branched aliphatic amines. Among the derivatives obtained, *N*-octadecylpectinamide is interesting due to its marked amphiphilic properties that make it acceptable for the preparation of new materials.

Amphiphilic polymers contain both polar and non-polar groups (Tribet, 1998). *N*-octadecylpectinamide is an example of an amphiphilic polymer with a polar backbone (D-galacturonic units of pectin) and non-polar side chains (*n*-octadecyls). The hydrophilicity–lipophilicity relationship of such polymers depends on the degree of substitution, i.e. the content of non-polar substituents attached to the original polar macromolecule. At small degrees of substitution, the polymer is soluble in water like the hydrophilic precursor. Slightly substituted polymers have surface-active

Abbreviations: AGA, anhydrogalacturonic acid; CP/MAS, cross polarisation and magic angle spinning; DA, degree of amidation; DM, degree of methylation (methoxylation, esterification); FT, Fourier transformed; HM, highly methylated (methoxylated, esterified); IR, iInfrared; NIR, near infrared; NMR, nuclear magnetic resonance; RP, reverse phase; SD, second derivative.

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properties and can interact with lipid bilayers and globular proteins (Tribet, 1998). Higher level of substitution with non-polar groups leads to total insolubility in water. A polymer carrying a large amount of hydrophobic groups is able to absorb non-polar molecules from aqueous solutions.

Hydrophobically modified pectins have been considered to be good emulsion stabilisers and bile acid sorbents. It has been reported that alkyl esters of pectin and pectic acid can form complexes with such natural compounds as bile acids and isolated soy protein (Klavons & Bennet, 1995). Ethyl esterified apple pectin at concentration of 1% in water conferred good emulsion stability in oil-in-water emulsions containing 17–60% oil (Krachanov, Stamov, Popova, & Pancheva, 1982). Müllner, Wiesner, and Ahlers (1993) has prepared partially depolymerised (degree of polymerisation is = 10–500) hydrophobically modified pectins including some alkylamides. These pectin derivatives have been tested as potential sorbents of bile acids.

N-octadecylpectinamide could be of interest as a cheap bioavailable surfactant or hydrophobic sorbent. This pectin derivative could also be applied to the stabilisation of oil-in-water emulsions or the removal of non-polar pollutants from water. In addition, due to its non-polar groups, *N*-octadecylpectinamide may have a stronger affinity to biologically important molecules containing non-polar residues, such as bile acids, fats or cholesterol, than natural HM pectin or other food polysaccharides. To determine applications, the structural, physical and chemical properties of *N*-octadecylpectinamide, particularly its water solubility and its affinity to various organic compounds, needs to be studied.

In this work we describe the preparation of *N*-octadecylpectinamides of various degrees of substitution and their characterisation by spectroscopic and other methods.

2. Experimental

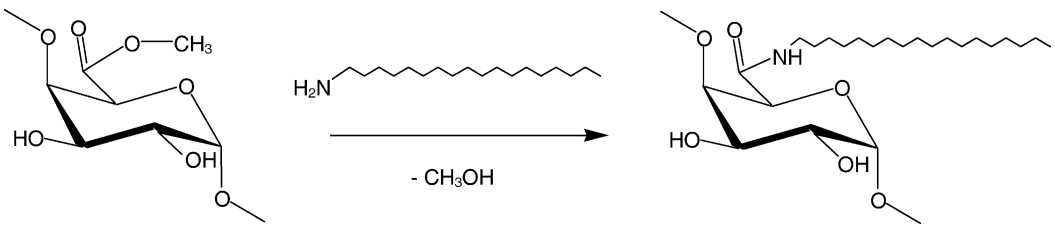
2.1. Initial HM citrus pectin

High methoxyl citrus pectin **1** (Genu, HM type B, Denmark) with a degree of methylation (DM) of 73% and anhydrogalacturonic acid (AGA) content of 89.7% was used for the preparation of the *N*-octadecylamide derivatives. The commercial pectin was purified and converted into H-form by washing with 0.1 mol l^{-1} of HCl dissolved in ethanol-water mixture (1:1 v/v). Then pectin was washed several times with the ethanol-water mixture and then with 96% ethanol, filtered repeatable through a paper filter until the chloride reaction was negative, and finally dried in laboratory oven at 60 °C for 6 h.

The content of AGA, which expresses the total content of uronic carboxyls, was estimated as *m*-hydroxybiphenyl complex with products of uronic acid thermal decomposition by photometry at 520 nm (Blumenkrantz & Asboe-Hansen, 1973). The degree of methylation (DM) was determined as formaldehyde-chromotropic acid complex by photometry at 570 nm (Filippov & Kuzminov, 1971). Formaldehyde was obtained by oxidation of methanol liberated from the samples by alkali hydrolysis of methyl ester groups. Both these photometric measurements were made in the medium of concentrated sulphuric acid.

2.2. Preparation of N-octadecylpectinamides

The reaction of HM citrus pectin with N-octadecylamine was carried out in a heterogeneous system in methanol according to Sinitsya et al. (2000):



Pectin powder (29 g) was weighted into a 200-ml flask and suspended in a small volume of methanol. *n*-octadecylamine (42 g) (Fluka) was dissolved in 150 ml of methanol. The solution was gradually added to the flask under stirring. The reaction was carried out in covered flasks at 25 °C for 460 h under continuous mixing. Small volumes of reaction mixture (about 1 ml), which contained 100–150 mg of dried product, were collected at different time intervals during the reaction. After the solids settled, the liquid phase was decanted. Product was washed with chloroform to remove free amine and then washed with 0.1 M HCl in ethanol–water (1:1 v/v) mixture to convert free carboxylic groups into the protonated form. Finally, the product was washed by 80% aqueous ethanol, filtered and finally dried in a laboratory oven at 60 °C for 6 h. The mass of final product was 34 g and the total mass of intermediate products was near 1 g.

The water content in the powder samples was determined by drying at 100 °C to constant mass and by volumetric titration on a Karl Fischer titrator AF8 (ThermoOrion).

According to both these methods, the samples of *N*-alkylpectinamides had 87–89% of dry matter that is similar to dry matter content of the initial HM pectin (89.4%).

The degrees of amidation (DA), mass and molar yields of reaction (Y_m and Y_n, respectively) were calculated from results of organic elemental analysis according to the following equations (Sinitsya et al., 2000):

$$DA = \frac{M_N}{M_C}\left[6 + \frac{73}{100} + (18-1)\frac{M_N}{14}\right]100$$

$$Y_m = \frac{M_N M_A}{14}$$

$$Y_n = \frac{DA}{73}100$$

where DA is the degree of amidation (%), Y_m the mass yield of reaction, i.e. the relative mass of bound amine (%) in the reaction product, Y_a the molar yield of the reaction, i.e. the relative content of ester groups substituted by amine (%), M_N the nitrogen content (%), M_C the carbon content (%), M_A the molar mass of *n*-octadecylamine (g mol^{-1}), 14 the nitrogen atomic mass (g mol^{-1}), 6 the number of carbons in the galacturonic unit, 18 the number of carbons in the amine molecule, 73 the DM of the original pectin (%).

2.3. Spectroscopic methods

Fourier transformed diffusion reflectance infrared (FT-IR) and near-infrared (FT-NIR) spectra were measured on a Nicolet 740 and Nicolet AVATAR 360 (Nicolet Analytical Instruments, USA) spectrometers, respectively. FT-Raman spectra were recorded using a Bruker FT-Raman (FRA 106/S, Equinox 55/S) spectrometer (Bruker Optics Inc., USA). The positions of the overlapping bands were determined using minima of smoothed second derivatives of the spectra.

High-resolution ^{13}C CP/MAS spectra (Pines, Gibby, & Waugh, 1973) were measured by using NMR spectrometer BRUKER DSX 200 in 7 mm ZrO_2 rotors at a frequency of 50.33 MHz (Bruker Instruments Inc., USA). Number of data point was 2k, magic angle spinning (MAS) frequency 4 kHz, strength of B1 field was 50.0 kHz. The number of scans for accumulation of NMR spectra was 1200–22000 depending on the signal-to-noise ratio, spectral width was 25 kHz, repetition delay and spin lock pulse length were 3 s and 2 ms, respectively. The ^{13}C scale was calibrated using the external standard glycine (176.03 ppm—low field carbonyl signal). The areas of resonance signals at 171, 53 and 14 ppm were used for the calculation of DA and DM values.

FT-IR, FT-Raman and FT-NIR (in second derivative (SD) form) spectra were applied to the estimation of the substitution degrees of the samples. FT-IR and FT-Raman spectra have been analysed by the normalised least-squares curve fitting procedure (Microcal Origin 6.0 software) using multiply Voigt curves and linear baselines. Spectra were decomposed in the regions of 1820–1490 cm^{-1} (FT-IR) and 1470–1415 cm^{-1} (Raman). The areas of the separate peaks were used for the quantification. The SD algorithm assisted the curve fitting procedure.

The quantification was also made using the relative height H_1/H_0, where H_0 is the height at standard wavenumber (2957 cm^{-1} for Raman and 4224 cm^{-1} for SD of NIR) and H_1 is the height at analytical wavenumber (2852 cm^{-1} for Raman and 4254 cm^{-1} for SD of NIR). The values of H_0 and H_1 were measured from linear baselines: 3090–2760 cm^{-1} for Raman and $y = 3.5 \times 10^{-5}$ a.u. for SD of NIR.

2.4. Particle size distribution and image analysis

Particle size distribution of initial HM pectin **1** and the final *N*-octadecylpectinamide **10** was studied by laser diffraction, laser-particle-sizer 'Analysette 22' (Fritsch GmbH, Idar-Oberstein, Germany), measuring range 0.2–500 μm, resolution 124 channels, measurement duration 20 scans. Dry powder samples (1 g) were suspended in 96% ethanol using ultrasonic cleaning bath, mechanical stirrer and feed pump, which are the components of standard liquid dispersing unit of 'Analysette 22' system. Results were presented as cumulative and frequency curves.

The samples **1** and **10** were also analysed by image analysis. The samples were spread on the sample glass through the special steel tube and put under objective 4 of an optical scanning microscope Nicon SM2-2T (Nicon, Japan) equipped with Intralux 4000-1 lamp, colour camera and digitizer Micro-Movies. Images were focused, digitised and decomposed into pixels using a 256-dot scale. The image processing was made using a LUCIA system.

2.5. Scanning electron microscopy

Samples **1** and **10** were deposited on carbon tape, sprayed with carbon and put under the objective of cold field emission scanning electron microscope Hitachi S-4700 (Hitachi, Japan) at working distance 12 mm, integrated electron detector, resolution 2.1 nm at an acceleration voltage of 1 kV.

2.6. Solubility assay

An aliquot (20 mg) of the pectin derivatives was weighted in a small beaker and wetted with a drop of ethanol. Then 10 ml of distilled water or aqueous 0.02 mol l^{-1} NaOH was added into the beaker. The mixture was stirred and heated on a magnetic stirrer for 10–15 min, cooled to 25 °C and filtered on paper filters. The filters with residual solids were dried and weighted for the estimation of non-soluble solids. The samples were defined as well soluble (no solids), partially soluble (markedly lower

amount of solids) and insoluble (insignificant decline of solids, i.e. less than 5%).

2.7. Reverse phase chromatography

A liquid chromatography system HPP 4001 (Lab. Equipment, Prague, Czech Republic) in combination with manual sampler (250 μl), glass column (295 × 52 mm^2) packed with *N*-octadecylpectinamide (**10**, 60–100 μm), refractive index detector RIDK 101 and computer integrator Spectra Physics 1000 was used for the analysis of sorbent selectivity. The column was operated at 25 °C and a flow rate 0.5 ml min^{-1} with methanol as the eluent. The capacity factors of tested compounds (k') were calculated according to the formula:

$$k' = (t_R - t_0)/t_0$$

where t_R is the retention time and t_0 the death time.

3. Results and discussion

3.1. Molecular structure and the substitution degrees

The samples of HM citrus pectin **1** and *N*-octadecylpectinamides obtained at various reaction times (**2**–**10**) as well as reaction conditions are characterised in Table 1. Initial pectin **1** contains only a small amount of nitrogen (0.25%) originating probably from integrated cell wall proteins. Consequent increase in the content of nitrogen in samples **2**–**10** confirms the process of substitution. According to elemental analysis, the molar reaction yield for final product was 43% and its degree of amidation (DA) was 32%. Graphical representation for the time-dependencies of DA and DM values is shown in Fig. 1. These empirical curves permit the preparation of *N*-octadecypectinamides of predicted DA by varying the reaction time under the above conditions.

Table 1
Reaction conditions and characterisation of initial HM pectin **1** and obtained *N*-octadecylpectinamides **2**–**10** according to elemental analysis

Sample	t (h)	M_N (%)	M_C (%)	DA (%)	DM (%)	Y_n (%)	Y_m (%)
1	–	0.24	41.38	–	73	–	–
2	1	0.32	39.80	5	67	6.2	7
3	4	0.45	39.74	7	61	8.7	10
4	22	0.68	42.42	10	58	13.1	14
5	47	0.94	44.47	14	53	18.1	20
6	71	1.12	48.02	16	49	21.6	22
7	105	1.28	49.11	18	45	24.6	25
8	167	1.42	47.94	21	40	27.3	29
9	285	1.73	49.63	26	33	33.3	36
10	460	2.14	54.18	32	28	41.2	43

t: the reaction time, M_N : the nitrogen content, M_C : the carbon content, DA: the degree of amidation, DM: the degree of methylation, Y_m : the mass yield of reaction, Y_n : the molar yield of reaction.

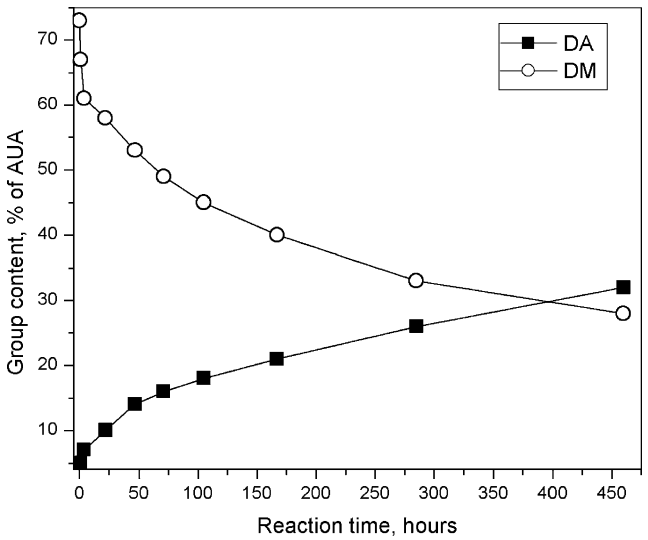


Fig. 1. Time dependence of DA and DM values (in %) during amino-de-alkoxylation of HM citrus pectin **1** with *n*-octadecylamine.

FT-Raman, FT-IR and FT-NIR spectra of HM pectin **1** and *N*-octadecylpectinamides **2**–**10** are presented in Fig. 2(a)–(c). In all cases, the spectral differences between the initial pectin and the derivatives were explained by the subsequent addition of *N*-octadecylamide substituents.

The vibration band assignment, which is presented in Table 2, was made according to the literature (Filippov, 1978, 1972; Engelsen & Nørgaard, 1996; Kacuráková et al., 1999; Silverstein, Basssler, & Morrill, 1997). Two strong infrared and Raman bands of *N*-octadecylpectinamides in the region of 2920–2850 cm^{-1} were assigned to asymmetric and symmetric stretching of -CH_2- groups of *n*-octadecyl substituents ($\nu_{as}(CH_2)$ and $\nu_s(CH_2)$). The weak Raman band at 2730 cm^{-1} appeared upon amidation was assigned to combination mode of these groups. Methylene units have also the following vibration bands: at 1460–1440 cm^{-1} (scissoring vibration $\delta_s(CH_2)$), at ~1300 cm^{-1} (wagging and twisting vibrations $\omega,\tau(CH_2)$, strong in Raman) and at 720 cm^{-1} (rocking vibration $\rho(CH_2)$, strong in IR). Two strong vibration bands at 1660 and at 1550 cm^{-1} were assigned to amide I and amide II modes, respectively. The bending of water at 1630 cm^{-1} overlaps the former band. All these bands gradually increased in the direction of *N*-octadecylpectinamides **2**–**10**.

Usually, the interpretation of NIR spectra is very difficult due to the complex origin of vibration bands in the near-infrared region. However, the NIR band assignment can be successful for the analysis of structurally similar compounds (Murray, 1987). In the case of HM pectin and *N*-octadecylpectinamides, which differ from each other only by several structural characteristics such as DM and DA, it is possible to assign the NIR bands changing in the row of samples **1**–**10** directly to methyl esters or *N*-octadecylamide substituents. FT-NIR spectra of *N*-octadecylpectinamides **2**–**10** have several characteristic bands absent in the spectrum of HM pectin **1** (Fig. 2(c)). These bands were

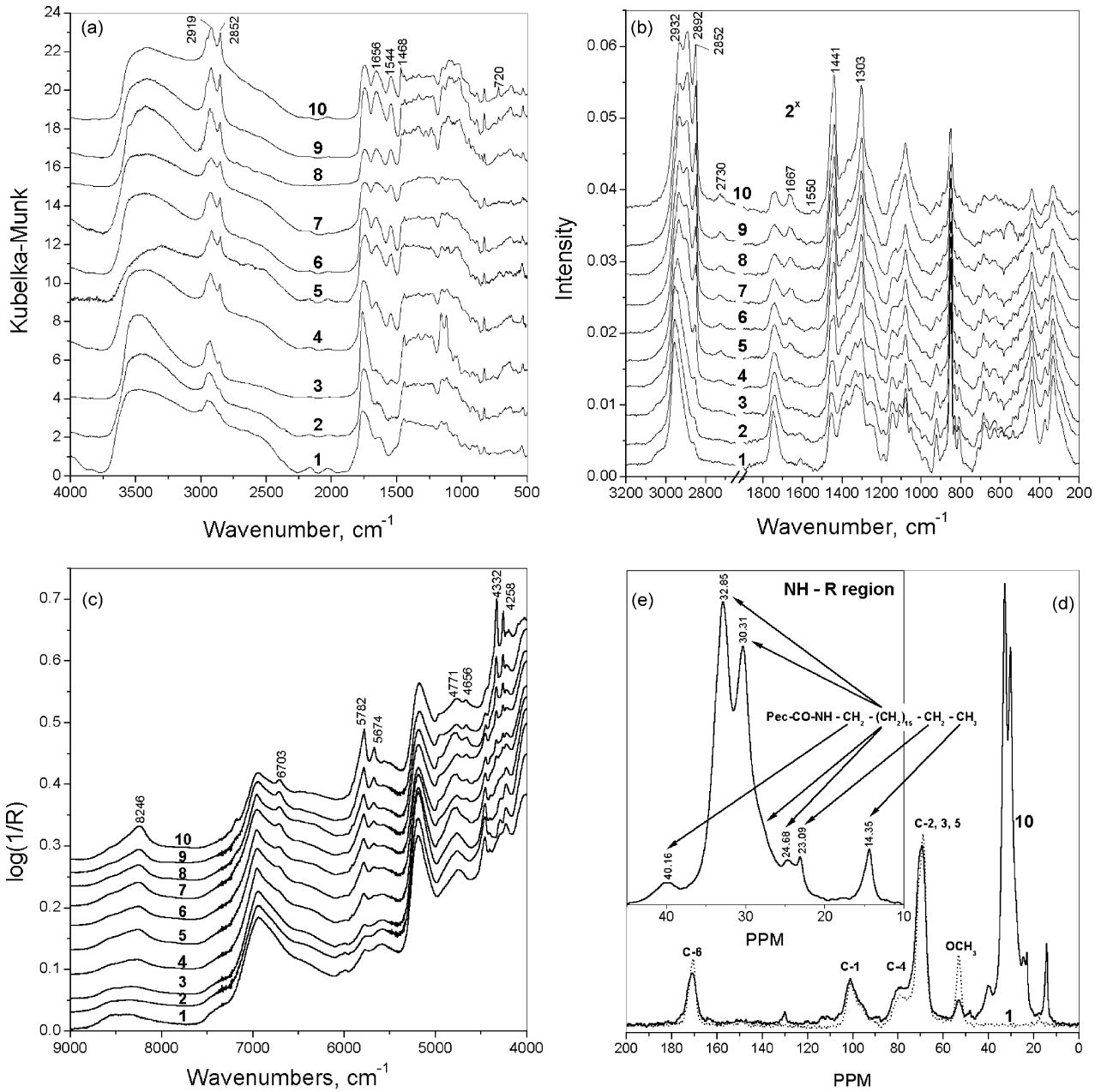


Fig. 2. FT-IR (a), FT-Raman (b) and FT-NIR (c) spectra of HM citrus pectin **1** and *N*-octadecylpectinamides **2**–**10**; ^{13}C CP/MAS NMR spectra (d,e) of *N*-octadecylpectinamide **10** (—) and HM citrus pectin **1** (– – –).

assigned to combinations and overtones of the vibration modes of amide bonds and *n*-octadecyls (Table 3). All these NIR bands increase with increasing of DA values. Among the bands of *N*-octadecylamide substituents observed, the most intense were combination modes and overtones of CH_2 vibrations at 4254, 4326, 5670 and 5782 cm^{-1}. Most of the other NIR bands, which were similar for both HM pectin and its derivatives, were assigned to the pectin backbone.

The ^{13}C CP/MAS NMR spectra of samples **1** and **10** are shown in Fig. 2(d,e). The carbon chemical shifts and their assignments based on literature (Jarvis & Apperley, 1995; Sinitsya, Čopíková, & Pavlíková, 1998) are presented in Table 4. The uronic C-6 and pyranoid ring carbons of *N*-octadecylpectinamide have similar chemical shifts as corresponding carbons of HM citrus pectin. The resonance signal at 53 ppm assigned to OCH_3 carbons of methyl esters significantly decreased after amino-de-alkoxylation. The group of strong resonance signals in the region of 45–15 ppm appeared in the spectrum of *N*-octadecylpectinamide. These signals belong to the alkyl carbons of *n*-octadecyls. Some of these resonances were assigned to definite carbons: $-NH-CH_2-$ (40 ppm), $-CH_2-CH_3$ (23 ppm) and $-CH_3$ (14 ppm). The other chemical shifts inside the region of 45–15 ppm belong to inner methylene $-CH_2-CH_2-CH_2-$ carbons (Fig. 2(e)).

Table 2
FT-Raman and FT-IR band positions (in cm^{-1}) and assignments for initial HM pectin **1** and *N*-octadecylpectinamides **2–10**

1		**2–10**		Assignments	Structure
FT-Raman	FT-IR	FT-Raman	FT-IR		
	3475		3386	ν(OH)	Water, ROH
2957	2958	2957sh	2956	$\nu_{as}(CH_3)$	$COOCH_3$
		2932	2939sh	$\nu_{as}(CH_3)$	Octadecyls
			2919	$\nu_{as}(CH_2)$	Octadecyls
		2892		$\nu_s(CH_3)$	Octadecyls
2864	2858			$\nu_s(CH_3)$	$COOCH_3$
		2852	2852	$\nu_s(CH_2)$	Octadecyls
1749	1763	1739	1748	ν(C=O)	COOH, $COOCH_3$
		1667	1656	Amide I	CONHR
		1550	1544	Amide II	CONHR
			1468	$\delta(CH_2)$	Octadecyls
		1441	1442	$\delta(CH_2)$, $\delta_{as}(CH_3)$	Octadecyls
1456	1444	1456sh		$\delta_{as}(CH_3)$	$COOCH_3$, Octadecyls
1396	1402	1396	1396	ν,δ(COH)	COOH
1379	1383	1368	1378	$\delta_s(CH_3)$	$COOCH_3$, Octadecyls
1338	1340	1329	1332	δ(CH)	Pyranoid ring
		1303		$\omega,\tau(CH_2)$	Octadecyls
1270	1277	1275	1272	ν(COC)	$COOCH_3$
1261		1257	1254	δ(CH)	Pyranoid ring
1238	1230		1236	ν(COC)	$COOCH_3$
1224		1227	1224	ν(OH)	COOH
1147	1160	1154	1148	ν(COC)	Glycosidic bond
		1131		ν(CC)	Octadecyls
1110	1123		1098	ν(CC)(CO)	Pyranoid ring
1081	1083	1080	1079	ν,δ(COH)	Pyranoid ring
1053	1055		1048	ν(CC)(CO)	Pyranoid ring
		1034sh	1027	ν(CC)	Octadecyls
	981		985	γ(OH)	ROH
923	920	925	925	$\rho(CH_3)$	$COOCH_3$
883	887	891	887	γ(OH)	ROH
852		852		Skeletal	Pyranoid ring
833	833	828	830	γ(OH)	ROH
772	758	773	753	Ring breathing	Pyranoid ring
			720	$\rho(CH_2)$	Octadecyls
684–537		676–538		ν(CCO)	Pyranoid ring
442		442		τ(COC) def.	Pyranoid ring
332		332		τ(COC) def.	Pyranoid ring

ν: stretching, δ: in-plan bending, γ: out-off-plan bending, ρ: rocking, as: antysymmetric, s: symmetric, sh: shoulder.

An integration of the ^{13}C CP/MAS NMR resonance signals at 171 ppm (C-6 carbons), 53 ppm (OCH_3 carbons of carboxylate esters) and 14 ppm (CH_3 carbons of *n*-octadecyls) permits to estimate the values of DA and DM as the ratios of peak areas:

$$DA = A_{14}/A_{171}$$

$$DM = A_{53}/A_{171}$$

where A_i is the area of resonance signal centred at i ppm. Calculated value of DA was 38% for sample **10** and calculated values of DM were 71 and 22% for samples **1** and **10**, respectively. All these values are comparable with those obtained by elemental analysis (DA) and spectroscopicaly determined (DM) (Table 1).

Table 3
FT-NIR band positions (in cm^{-1}) and band assignments for initial HM pectin **1** and *N*-octadecylpectinamides **2–10**

1	**2–10**	Assignments	Structure
	4254	$\nu_s(CH_2) + \delta(CH_2)$	Octadecyls
4297	4293	ν(CH) + δ(CH)	Pyranoid rings
	4326	$\nu_{as}(CH_2) + \delta(CH_2)$	Octadecyls
4405	4401	ν(OH) + ν(CC)	ROH
4455	4455	ν(OH) + δ(OH)	ROH
	4656	2 × amide I + amide III	CONHR
	4717	ν_s(NH) + amide III	CONHR
4764	4760	2 × δ(OH) + 2 × ν(CO)	ROH
	4964	ν_s(NH) + amide II	CONHR
5196	5196	$\nu(H_2O) + \delta(H_2O)$	Water
	5670	$\nu_s(CH_2)$ 1st Ov.	Octadecyls
	5782	$\nu_{as}(CH_2)$ 1st Ov.	Octadecyls
	6703	ν(NH) 1st Ov.	CONHR
6943	6939	2 × ν(CH) + δ(CH)	Pyranoid rings
	7186	2 × $\nu_{as}(CH_2) + \delta(CH_2)$	Octadecyls
	8246	$\nu_{as}(CH_2)$ 2nd Ov.	Octadecyls

ν: stretching, δ: in-plan bending, as: antysymmetric, s: symmetric.

The vibration spectroscopic methods mentioned above were also used for the estimation of the degrees of substitution. Fig. 3 shows the examples of quantification based on FT-IR (Fig. 3(a)), FT-Raman (Fig. 3(c) and (d)) and FT-NIR (Fig. 3(g)) spectra. Quantification was made using areas of separated peaks obtained by spectra decomposition (Fig. 3(a) and (d)) or using relative heights (Fig. 3(c) and (g)).

The FT-IR spectra of all the samples were decomposed into six Voight (mix Gaussian–Lorentzian) components in the region of 1825–1490 cm^{-1} (Fig. 3(a)). The peak of C=O stretching vibrations was decomposed into three Voight components centred at 1770–1760 cm^{-1} (carboxylic monomers), 1755–1735 cm^{-1} (methyl esters) and 1720–1710 cm^{-1} (carboxylic dimers). The Voight

Table 4
^{13}C CP/MAS NMR chemical shifts (in ppm) for initial HM pectin **1** and *N*-octadecylpectinamide **10**

1	**10**	Assignments
170.57	171.09	C-6
100.98	101.09	C-1
84sh	84sh	C-4
69.17	69.30	C-2,3,5
52.85	53.27	OCH_3
	40.16	$NHCH_2$
	32.85	CH_2
	30.31	CH_2
	24.68	CH_2
	23.09	CH_2CH_3
	14.35	CH_3

sh: shoulder.

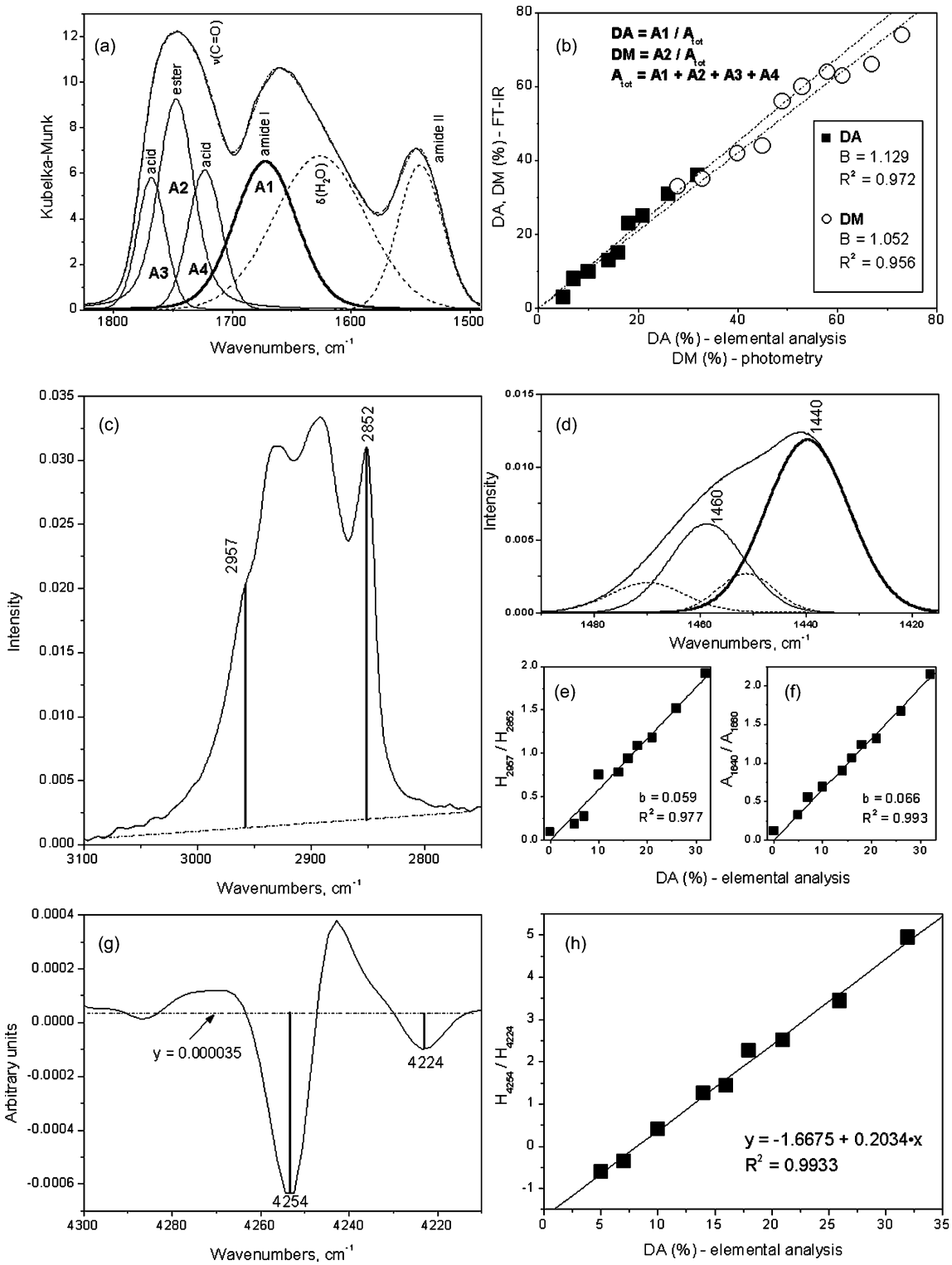


Fig. 3. (a) Curve-fitting decomposition of FT-IR spectrum of sample **10** in the region of 1825–1490 cm^{-1}; (b) Correlation between experimental DA and DM values of the samples and corresponding values calculated from FT-IR data; (c) Quantification on the basis of FT-Raman spectrum of sample **10** in the region of 3100–2775 cm^{-1}; (d) Curve-fitting decomposition of FT-Raman spectrum of sample **10** in the region of 1490–1410 cm^{-1}; (e) Correlation between experimental DA values of the samples and H_{2957}/H_{2852} values calculated from FT-Raman data; (f) Correlation between experimental DA values of the samples and A_{1440}/A_{1460} values calculated from FT-Raman data; (g) Quantification on the basis of second derivative of FT-NIR spectrum of sample **10**; (h) Correlation between experimental DA values of the samples and H_{4254}/H_{4224} values calculated from FT-NIR data.

components centred at 1675–1660 and 1570–1560 cm^{-1} were indicated as amide I and amide II vibrations, respectively. The Voight component centred at near 1630 cm^{-1} was assigned as the in-plane deformation mode of bound water.

The ν(C=O) ester and amide I components were used for determination of DM and DA, respectively. These values were calculated as the ratio of the corresponding component area to the sum of the areas of amide I and all C=O components (Sinitsya et al., 2000). The values of DA and DM obtained from FT-IR spectra decomposition correlated well with corresponding values obtained from elementary analysis and photometry (Fig. 3(b)).

Two regions of FT-Raman spectra, i.e. those of 3100–2775 cm^{-1} and of 1490–1410 cm^{-1}, were applied to quantification. In the C–H stretching region, two adduced to baseline intensities were measured at 2852 cm^{-1} (H_1, analytical height) and 2957 cm^{-1} (H_0, standard height) (Fig. 3(c)). The second region was decomposed into four Voight component peaks and the areas of two components centred at 1460 cm^{-1} (A_0, standard area) and 1440 cm^{-1} (A_1, analytical area) were calculated (Fig. 3(d)). Both H_1/H_0 and A_1/A_0 ratios showed a good correlation with the values of DA obtained by elemental analysis (Fig. 3(e) and (f)).

Second derivatives of FT-NIR spectra of *N*-octadecylpectinamides were also used for quantification of DA based on the relative heights. The negative band heights from linear baseline $y = 3.5 \times 10^{-5}$ were measured at 4254 cm^{-1} (H_1, analytical height) and 4224 cm^{-1} (H_0, standard height) (Fig. 3(g)). Calculated values of H_1/H_0 correlated well with the experimental data (Fig. 3(h)).

3.2. Particle size distribution, particle morphology and solubility

Amidation of HM citrus pectin with *n*-octadecylamine is a heterogeneous process, so it is very important to analyse the structural changes of solid polysaccharide phase via substitution. The powder particles of HM citrus pectin and *N*-octadecylpectinamide were studied by laser diffraction and image analysis.

The particle distribution of the samples is ascribed in Fig. 4, whereas the microscopic images of particles are shown in Fig. 5. According to results obtained, the structural parameters of pectin particles significantly changed during the reaction. Substitution with *n*-octadecylamine led to decreasing of particle size that can be seen from the results of particle size distribution (Fig. 4). The arithmetic mean diameter of particles decreased from 175.5 to 80.9 μm, whereas their specular surface area increased from 0.10 to 0.28 m^2/cc.

Furthermore, the shape of particles also significantly changes in the process substitution (Fig. 5(a) and (b)). The pectin powder consists of compact, roundish or sub-angular, and near isodiametral particles. The particles of final product **10** are friable, oblong or shapeless, and mainly non-isodiametral. The surface of the pectin particles was also changed upon amino-de-alkoxylation. Particles of HM pectin **1** are relatively smooth or slightly uneven, whereas *N*-octadecylpectinamide particles are highly rough and have protrusions (Fig. 5(c) and (d)).

All these structural changes can be rationalised in terms of the interaction of *n*-octadecylamine reagent with the solid particles of pectin. The particles consist of complex and compactly intertwined polymeric strains. Interaction of the reagent molecules with the surface of solid particles leads firstly to the substitution of methyl ester groups locating on the phase boundary, and, secondly, to partial untangling of polysaccharide strains. Then *n*-octadecylamine molecules gradually penetrate inside the particle reacting with internal methyl ester groups. In this process some breaks of strains occur leading to fragmentation of initial particles. As a result, the heterogeneous reaction leads to a drastic change of the physical structure of pectin particles, especially in their surface layer.

In addition to mechanical changes of solid pectin particles mentioned above, the substitution caused a gradual loss of its hydrophilic properties, first of all its solubility in water (Table 5). *N*-octadecylpectinamides **4**–**10** are completely insoluble in water, while less substituted samples **2** and **3** are noticeable soluble. In alkali conditions (0.02 mol l^{-1} NaOH) the solubility of the derivatives markedly increased owing to ionisation of free carboxyls

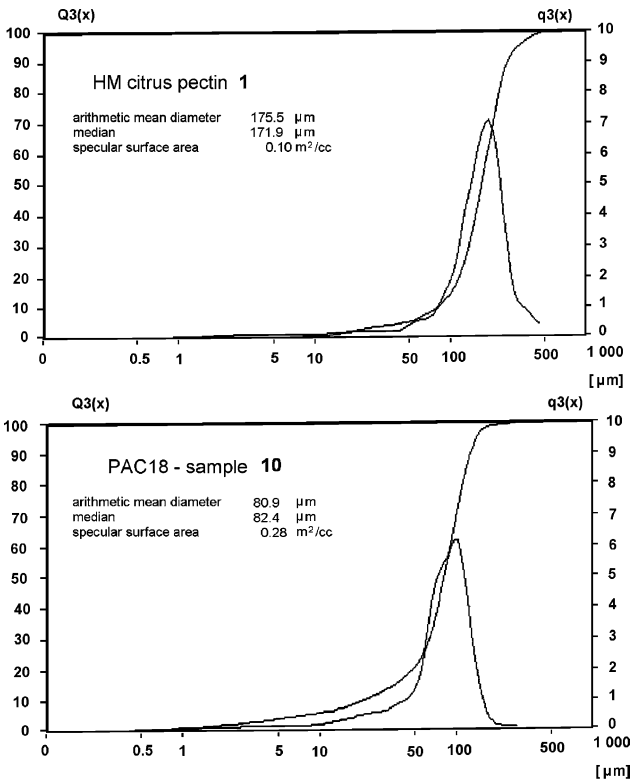


Fig. 4. Particle distribution of HM citrus pectin **1** and *N*-octadecylpectinamide **10**.

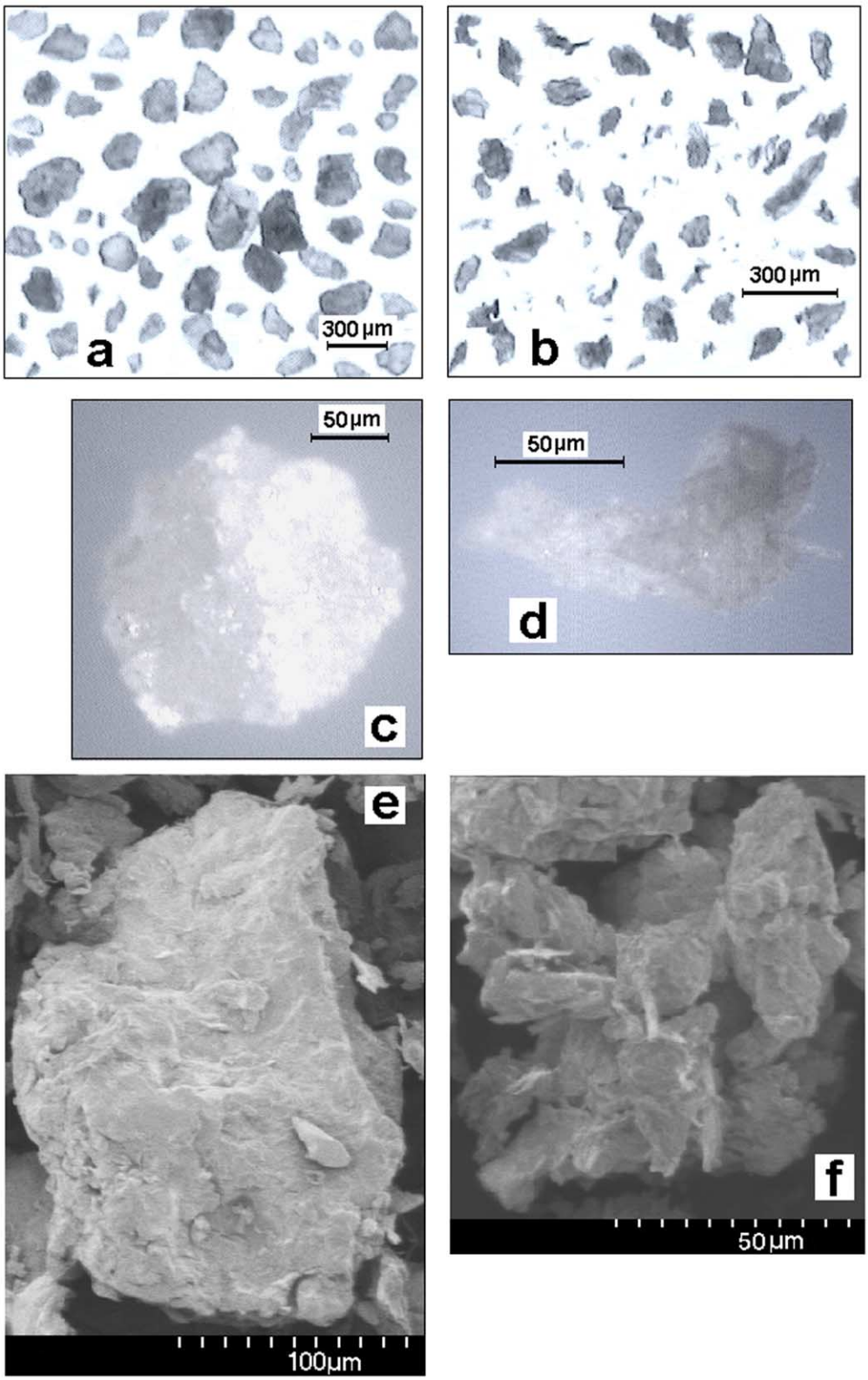


Fig. 5. Images of the particle ensembles (a, b) and single particles (c–f) of HM citrus pectin **1** (a, c, e) and *N*-octadecylpectinamide **10** (b, d, f) obtained by light scanning microscope (a–d) and by electronic microscope (e, f).

Table 5
Solubility of *N*-octadecylpectinamides **2**–**10** in water and in aqueous 0.02 mol l^{-1} NaOH

Sample (20 mg)	Water (10 ml)	0.02 mol l^{-1} NaOH (10 ml)
2	++	++
3	+	++
4	−	++
5	−	++
6	−	+
7	−	+
8	−	−
9	−	−
10	−	−

++: well soluble, +: partially soluble, −: insoluble.

and some degradation of polysaccharide molecules via β-elimination.

3.3. Affinity to polar and non-polar molecules

We suppose that the introduction of non-polar *n*-octadecyl groups into pectin matrix has to cause the increasing of its affinity to hydrophobic molecules. The reverse phase (RP) chromatography is able to determine quickly and simply the affinity and selectivity of the sorbent used as stationary phase. RP chromatography also permits to compare sorbent affinity to small molecules of various homologous series. This method was chosen for testing of *N*-octadecylpectinamide sorption properties.

Methanol was chosen as the mobile phase because it has the highest elution power for hydrophobic compounds in conditions of RP chromatography. This property of methanol permits to eluate rapidly non-polar molecules. In comparison to water, in methanol the retention time of non-polar compounds significantly decreases, while the selectivity is similar. In the case of aqueous elution, non-polar compounds hold on column for a long time and, therefore, cannot be detected exactly. In addition, some compounds are insoluble in water.

Chosen compounds, i.e. *n*-alkanes, *n*- and branched alcohols, polyols, alkylbenzenes and polyaromates, were injected on glass column packed by *N*-octadecylpectinamide as stationary phase and eluted by methanol. The range of compounds tested was limited by their solubility in methanol.

The dependence between carbon number in molecule and log(k'), where k' is capacity factor, is presented in Fig. 6. This data confirm that *N*-octadecylpectinamide is able to bind selectively non-polar molecules and (or) non-polar residues of polar molecules. High retention of non-polar compounds by this pectin-based sorbent is possible mainly due to the presence of *n*-octadecyl substituents. In the series of *n*-alkanes, alkylbenzenes, *n*-alcohols and branched alcohols, the values of log(k') increase with increasing of methylene group number in alkyls. Similarly, in the row of

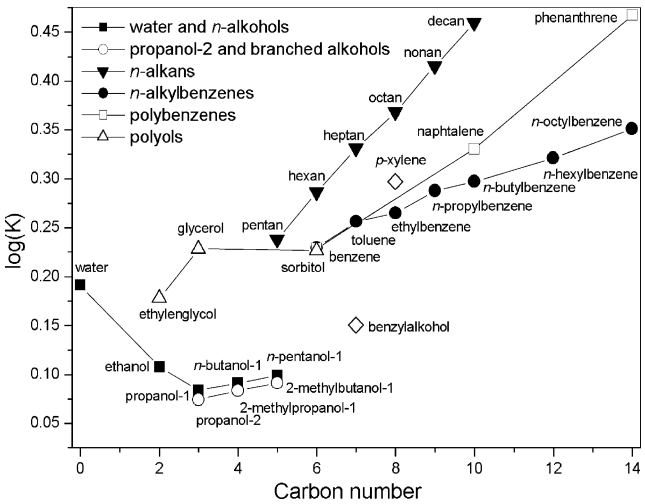


Fig. 6. Carbon number—log(k') dependence plot for selected compounds on the basis of reverse phase chromatography on *N*-octadecylpectinamide **10** as stationary phase.

polyaromates, the values of log(k') increase with the number of condensed rings. Branched alcohols had a somewhat weaker retention than their unbranched analogues due to steric hindrances.

Nevertheless, *N*-octadecylpectinamide still has residual hydrophilicity due to hydroxyls and free carboxyls of polysaccharide framework. These polar and charged groups of the sorbent can interact with water and small polar molecules. As a result, water and polyols have relatively strong retention (Fig. 6). Ethyleneglycol (2 carbons, 2 hydroxyls) and glycerol (3 carbons, 3 hydroxyls) had higher log(k') values than corresponding monohydroxylic alcohols, i.e. ethanol and *n*-propanol. Glucitol (6 carbons, 6 hydroxyls), in turn, showed nearly the same retention to that of glycerol but significantly weaker than that of hexane.

4. Conclusions

N-octadecylpectinamide is an example of hydrophobically modified pectin that could be used in various applications. An introduction of *n*-octadecyl groups into pectin macromolecules has led to significant changes of physical and chemical properties of pectin, first of all to increasing of its hydrophobicity. We suppose that this pectin derivative could be used as cheap, bioavailable and regeneratable technological sorbent for removing of non-polar compounds, such as oil pollution, from water or aqueous solutions for the purpose of using of solvent in following technological cycles.

The sorption ability of *N*-octadecylpectinamide may be improved by increasing of substitution degree and (or) by blocking of hydrophilic sites (OH and COOH groups). The first approach is to get optimal conditions of pectin

substitution. However, the reaction yield of *N*-alkylamidation is still restricted by steric hindrance. As the second approach we may propose previous methylation of hydroxylic and (or) carboxylic groups in HM pectin. The methylation of carboxyls also possesses additional sites for subsequent *N*-alkylamidation.

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