Carbohydrate Polymers 116 (2015) 207–214

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Cholesterol and fat lowering with hydrophobic polysaccharide derivatives

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ARTICLE INFO

Article history:
Received 30 December 2013
Received in revised form 5 May 2014
Accepted 6 May 2014
Available online 13 May 2014

Keywords:
Hydrophobically modified polysaccharides
Structure
Thermal analysis
Cholate and cholesterol sorption

ABSTRACT

Hydrophobic derivatives of highly methylated citrus pectin, chitosan and cellulose were prepared and tested as potential cholesterol lowering agents. Elemental analysis and spectroscopic methods confirmed high substitution degree for all of them. Substitution with long alkyl/acyl groups led to significant changes in physical and thermal properties of modified polysaccharides. Sorption of cholate and cholesterol by these polysaccharide-based sorbents was estimated in comparison with the synthetic drug cholestyramine. It was found that modified polysaccharides have high affinity to cholesterol. By contrast, cholestyramine was effective only in cholate sorption.

1. Introduction

High cholesterol level in low density lipoproteins (LDL) improves the risk of cardiovascular diseases. Cholestyramine is an example of effective cholesterol lowering agents (Léonard, Clas, & Brown, 1993; Zhu, Brizard, Piché, Yim, & Brown, 2000). Administration of cholestyramine reduces cholesterol absorption by sequestering bile acid conjugates (McNamara, Davidson, Samuel, & Ahrens, 1980). Thus this polymeric resin interrupts the enterohepatic circulation of bile acids, increases their faecal excretion and supports compensatory oxidation of cholesterol in the liver. Cholestyramine also binds anionic drugs, vitamins, and salts (Johansson, Adamsson, Stierner, & Lindsten, 1978; Harmon & Seifert, 1991), and the competition for its binding sites in the digestive tract decreases the binding capacity for bile acids (Stedronsky, 1994). As a result, large doses are required for the effective sorption that may cause serious side effects for some patients. Another way of cholesterol level lowering is the consumption of dietary fibres that can also remove bile acids from the digestive tract (Spiller, 1994). The dietary fibres consist mainly of water soluble and insoluble polysaccharides, which are not hydrolysed by the enzymes of the small intestine (Ylitalo, Lehtinen, Wuolijoki, Ylitalo, & Lehtimäki, 2002). The combination of cholestyramine with such well-tolerated polysaccharides has been shown to be effective in the treatment of patients with familial hypercholesterolemia (Schwandt, Richter, Weisweiler, & Neureuther, 1982). Chemical modifications may increase cholesterol lowering effect of polysaccharides. Hydrophobically modified polysaccharides have a potential value as drug carriers and sorbents for non-polar compounds. For example, amino-de-alkoxylation of highly methylated (HM) citrus pectin with *n*-alkylamines leads to *N*-alkylpectinamides (Sinitsya, Čopíková, Prutyanov, Skoblya, & Machovič, 2000; Synytsya et al., 2003), and water insoluble *N*-octadecylpectinamide is interesting due to its marked amphiphilic properties, i.e. its ability to absorb non-polar and polar molecules (Synytsya et al., 2004). *N*- and *O*-acylated derivatives of chitosan showed more hydrophobic properties, than the original chitosan (An et al., 2010; Kim et al., 2010; Le Tien, Lacroix, Ispas-Szabo, & Mateescu, 2003) and thus can be applied for the preparation of hydrophobic matrices for controlled drug release (Tůma, Marounek, Dušková, Čopíková, & Synytsya, 2011). Among cellulose derivatives, monocarboxy cellulose (MCC) and carboxymethyl cellulose (CMC) are suitable for hydrophobic modification via amino-de-methoxylation (Taubner, Čopíková, Havelka, & Synytsya, 2013).

This work is devoted to further characterisation of hydrophobic polysaccharide derivatives as potential cholesterol lowering agents by the use of spectroscopic and thermal analyses and by

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http://dx.doi.org/10.1016/j.carbpol.2014.05.009
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in vitro sorption experiments on model molecules (sodium cholate, cholesterol).

2. Experimental

2.1. Materials and reagents

Chitosan (free base) with the degree of deacetylation (DD) 0.86, palmitoyl chloride (purum ≥ 97%), *n*-octadecylamine (90%) and cholesterol were purchased from Fluka (Germany). Highly methylated (HM) citrus pectin (HMP) with the degree of methylation (DM) 73 mol.% 0.86 was purchased from Danisco Cultor Bohemia (Smiřice, Czech Republic). Oxidised monocarboxycellulose (MCC) in the acidic form with carboxylic groups of 18% m/m was obtained from VUOS Plc., Pardubice, Czech Republic. Sodium salt of carboxymethylcellulose (CMC) containing 0.65–0.85 carboxymethyl (CM) groups per anhydroglucose unit, sodium cholate, methyl iodide (purum ≥ 99%), anhydrous pyridine (99.8%), cholestyramine resin and acetonitryl (NAc) were purchased from Sigma Aldrich Chemie GmbH, Steinheim, Germany. CMC was converted into the acidic form by treatment with 0.2 mol l^{-1} HCl in 80% aqueous ethanol. Dibasic and monobasic sodium phosphates (puriss.) were purchased from Riedel-de-Haën, Germany. Organic solvents, i.e. chloroform, methanol, ethanol, acetone, tetrahydrofuran (THF) and *N*,*N*-dimethylformamide (DMF), were obtained from Lach-Ner, Czech Republic; hydrochloric acid (35%) and monobasic potassium carbonate (p.a.) from Penta, Czech Republic.

2.2. Preparation of polysaccharide derivatives

N,*O*-Palmitoylchitosan (PCh) was prepared by the reaction of chitosan with palmitoyl chloride (Choi, Kim, Pak, Yoo, & Chung, 2007; Hirano, Ohe, & Ono, 1976; Hirano, Yamaguschi, & Kamiya, 2002). The chitosan powder (10 g) was suspended in 200 ml of dry pyridine and boiled under reflux for 12 h. Palmitoyl chloride (100 g) was dissolved in 80 mL of DMF at 50 °C, and the solution was poured into the suspension under continuous mixing. Reaction was carried out at 60 °C for 3 days. Then the mixture was filtered and the solids were subsequently washed with chloroform, acetone and ethanol. The final product (26 g) was dried in oven at 50 °C.

N-Octadecylpectinamide (HMP-C18) was prepared by heterogeneous amino-dealkoxylation of the HM pectin with *n*-octadecylamide in DMF medium (Sinitsya et al., 2000; Synytsya et al., 2000, 2004). Raw polysaccharide (29 g) was weighted into a 200-ml flask and suspended in a small volume of DMF. *n*-Octadecylamine (42 g) was dissolved in 150 ml of DMF with heating up to 40 °C. The solution was gradually added to the flask under stirring. The reaction was carried out in covered flasks at 40 °C for 5 days under continuous mixing. Then the mixing was stopped and, after the solids settled, the liquid phase was decanted. The product was washed with petroleum ether to remove free amine and then washed with 0.1 M HCl in ethanol–water (1:1 v/v) mixture to convert alkylammonium carboxylates into protonated carboxyls. Finally, the product (~31 g) was washed by 80% aqueous ethanol, filtered and finally dried in a laboratory oven at 50 °C for 6 h.

N-Octadecylamides of MCC and CMC (MCC-C18 and CMC-C18, respectively) were prepared in two steps: (a) esterification with methanol and (b) amino-de-alkoxylation of methyl ester with *n*-octadecylamine (Taubner et al., 2013). In the first step, raw polysaccharides (each 150 g) were suspended in 2 l of methanol containing 12 ml of sulphuric acid. The mixture was heated (65 °C) in a reflux condenser and intensively stirred for 72 h. Then, the suspension was filtered, and the residue was washed twice with ethanol. The resulting esters (~133 g for MCC, ~107 g for CMC) were dried in an oven at 50 °C and then stored at room temperature. In the second step, *n*-octadecylamine (1 kg) was melted and dissolved in 2 l of DMF and tempered at 55 °C for 30 min. The methyl esters (100 g) were suspended in the solvent, and the reagent solution was subsequently added while stirring. The reaction continued at 60 °C in a reflux condenser and intensively stirred for the next 5 days. Then, the suspension was decanted, and the excess medium was removed. Concentrated products were filtered, and the residues were washed twice with ethanol, petroleum ether, 0.2 M HCl in 80% aqueous ethanol, and finally with ethanol. Final *N*-octadecylamides MCC-C18 (117 g) and CMC-C18 (110 g) were dried in an oven at 50 °C and then stored at room temperature. Structure of cholestyramine and hydrophobically modified polysaccharides is shown in Fig. 1.

2.3. Organic elemental analysis

The C, H and N contents (% m/m) in the native and derived polysaccharides were determined on Elementar vario EL III (Elementar, Germany) equipment. The degree of substitution (DS, mol mol^{-1}) of the final products was calculated based on the carbon (C) and nitrogen (N) contents (% m/m) according to the formulae:

$$DS = \left(\frac{C \cdot 14}{N \cdot 12} - 6 - 2 \cdot DAc\right) \times \frac{1}{16} \qquad \text{(PCh)}$$

$$DS = \frac{N \cdot 12 \cdot 6}{C \cdot 14 - N \cdot 18 \cdot 12} \qquad \text{(HMP-C18, MCC-C18)}$$

$$DA = \frac{N \cdot 12 \cdot (6 + 2 \cdot DCM)}{C \cdot 14 - N \cdot 18 \cdot 12} \qquad \text{(CMC-C18)}$$

where DAc (mol mol^{-1}) is degree of acetylation, DCM (mol mol^{-1}) is degree of carboxymethylation.

2.4. FTIR spectroscopy

FTIR spectra of the samples in KBr discs were measured on FTIR spectrometer Nicolet 6700 (Thermo Scientific, USA), the spectral range of 4000–400 cm^{-1}, 64 scans, spectral resolution 2.0 cm^{-1}. All the obtained spectra were smoothed and baselines corrected by the Origin 6.0 (Microcal Origin, USA) and Omnic 8.0 (Nicolet Analytical Instruments, USA) software, respectively. The second derivative algorithm was applied for determination of overlapped band positions.

2.5. Solid state ^{13}C CP-MAS NMR

High-resolution solid state ^{13}C CP-MAS NMR spectra were recorded using a Bruker Avance 500 spectrometer in 4 mm ZrO_2 rotors at the frequency 125.8 MHz with contact time 2 ms, repetition delay 4 s and spinning frequency 11 kHz. The number of scans was 1800–5400. Chemical shifts were referred to the carbonyl line of glycine with a signal at 176.0 ppm from TMS by sample replacement. Obtained NMR data were processed with the MestReNova (Mestrelab research, Spain) and Microcal Origin 6.0 (Microcal software, USA) software. The substitution degrees (DS, mol mol^{-1}) were calculated based on the ratio of integrating areas of carbon NMR signals assigned to the substituents (δ 20–40 ppm for PCh, δ 10–45 ppm for *N*-octadecylamides) and the polysaccharides (δ 50–120 ppm for PCh, δ 55–110 ppm for *N*-octadecylamides).

2.6. Thermal analyses

DSC measurements were performed in the DSC 131 modulus from SETARAM, France. The DSC modulus was calibrated by an indium standard. Samples of polysaccharides (10 mg) were placed into closed platinum cells and heated under nitrogen atmosphere

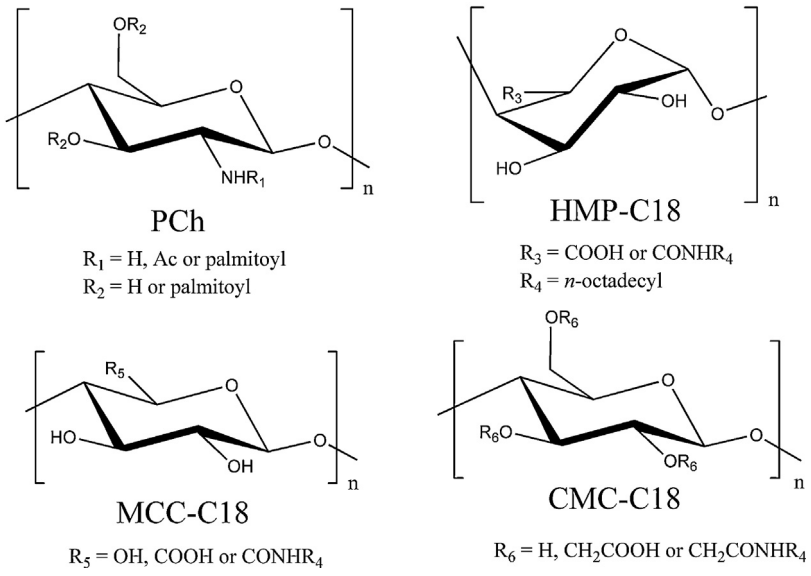

Fig. 1. Structure of cholestyramine and hydrophobically modified polysaccharides.

from −25 to 500 °C at the heating rate of 5 °C min^{-1}. A TGA–FTIR instrument (Perkin-Elmer, USA) including ultramicrobalance Pyris 1 (sensitivity 0.1 mg) and FTIR spectrometer Spectrum 100 was used to analyse the weight loss of the polysaccharide samples and the nature of the species released from the heated samples simultaneously. Samples of polysaccharides (8–10 mg) were analysed with temperature programme: heat from 45 °C to 600 °C at 10 °C min^{-1} in nitrogen atmosphere with a purge rate of 20 ml min^{-1}.

2.7. Sorption experiments

Sorption properties of polysaccharide derivatives were compared with those of cholestyramine, an effective synthetic sorbent of bile acids. Sodium cholate and cholesterol were used as model compounds in sorption experiments. Preliminary kinetic study confirmed that 60 min is sufficient to reach equilibrium in the sorption of both cholate and cholesterol by the sorbents used in this work. The sodium cholate sorption experiments were carried out in sodium phosphate buffer (pH 7). The dry sorbent (0.02 g) was suspended in the buffer solution (40 ml) with a given initial concentration of sodium cholate, covering the range of 0.1–20 mmol l^{-1}. The mixture was rigorously stirred for 60 min at 37 °C. Then the suspension was filtered through paper filter and the filtrate was analysed by HPLC. The cholesterol sorption experiments were carried out in THF:water (2:3) mixture. The dry sorbent (0.25 g) was suspended in the medium (20 ml) with a given initial concentration of cholesterol (0.1–5 mmol l^{-1}). The mixture was rigorously stirred for 60 min at 37 °C. Then the suspension was filtered through paper filter and the filtrate was analysed by HPLC. All these procedures were repeated three times for every cholate and cholesterol concentration. The function of Langmuir–Freundlich (Sips) isotherm (Marczewski & Jaroniec, 1983; Sohn & Kim, 2005) describing the process of monolayer single-solute adsorption from a dilute solution onto an energetically heterogeneous solid was applied to fit the experimental data:

$$Q_e = Q_m \cdot \frac{(b \cdot C_e)^n}{1 + (b \cdot C_e)^n}$$

where Q_e (mmol g^{-1}) is amount of bound sorbate at equilibrium, C_e is concentration of free sorbate at equilibrium, Q_m (mmol g^{-1}) is maximal sorption capacity, b (l mmol^{-1}) is the average value of binding energy characterising the sorbent affinity (the lower b, the higher the affinity), and n is the surface heterogeneity factor ($n = 1$ for homogeneous sorbent–Langmuir isotherm). This isotherm corresponds to symmetrical quasi-Gaussian energy distribution of binding sites, reduces to Freundlich equation for low adsorption values, and does not show Henry (linear) behaviour for low concentrations (Marczewski & Jaroniec, 1983). Fitting of the sorption experimental data was made using Origin 6.7 software (Microcal Origin, USA).

2.8. HPLC analysis

The HPLC system (Shimadzu, Japan) containing liquid chromatograph with low pressure gradient unit, high pressure pump LC-20 AD (0.0001–10 ml min^{-1}), autosampler SIL-20AC, column oven CTO-20AC, degasser DGU-20A5, communications bus module CBM-20A, and low temperature evaporative light scattering detector (ELSD–LT) was used for the determination of sodium cholate and cholesterol in the corresponding media (Roda et al., 1992). Steel column Nova-Pak C-18, 3.9 × 300 mm, particle size 4 μm was used in analysis of sodium cholate; detector 80 °C, 272 kPa; mobile phase 75% methanol (v/v) and 15 mmol l^{-1} ammonium acetate (pH 5.40 ± 0.1); flow 0.9 ml min^{-1}, working temperature 37 °C. Column Xterra RP8, 150 × 4.6 mm, particle size 3.5 μm was used in analysis of cholesterol; detector 60 °C, 152 kPa; mobile phase AcN, flow 1 ml min^{-1}, working temperature 35 °C. GC solution 2.3 (Shimadzu, Japan) and Origin 6.0 (Microcal Origin, USA) software were used in the data processing and preparation of the graphs.

3. Results and discussion

3.1. Elemental analysis

Results of organic elemental analysis for hydrophobically modified and parent polysaccharides are summarised in Table 1. Chemical modifications led to significant decrease of nitrogen content for PCh in comparison with parent chitosan. By contrast, nitrogen contents increased for all the other derivatives. HM citrus pectin contained very low amount of nitrogen; parent MCC and CMC contained no nitrogen. According to the nitrogen to carbon ratios, the DS were found to be 1.23 mol mol^{-1} for PCh, 0.61 mol mol^{-1} for HMP-C18, 0.30 mol mol^{-1} for MCC-C18 and 0.63 mol mol^{-1} for CMC-C18.

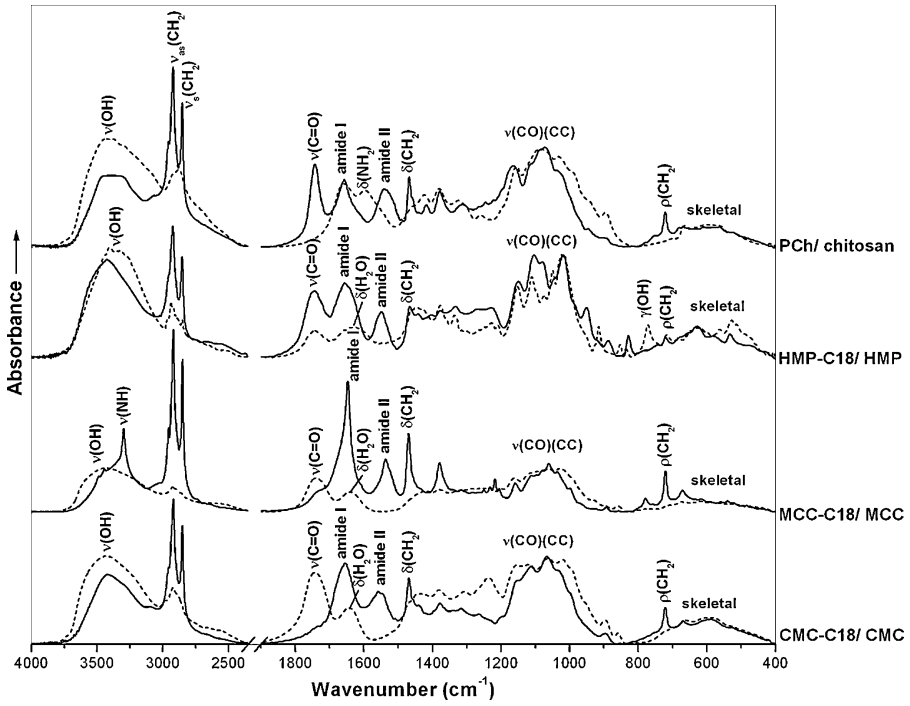

Fig. 2. FTIR spectra of hydrophobically modified (solid) and parent (dash) polysaccharides.

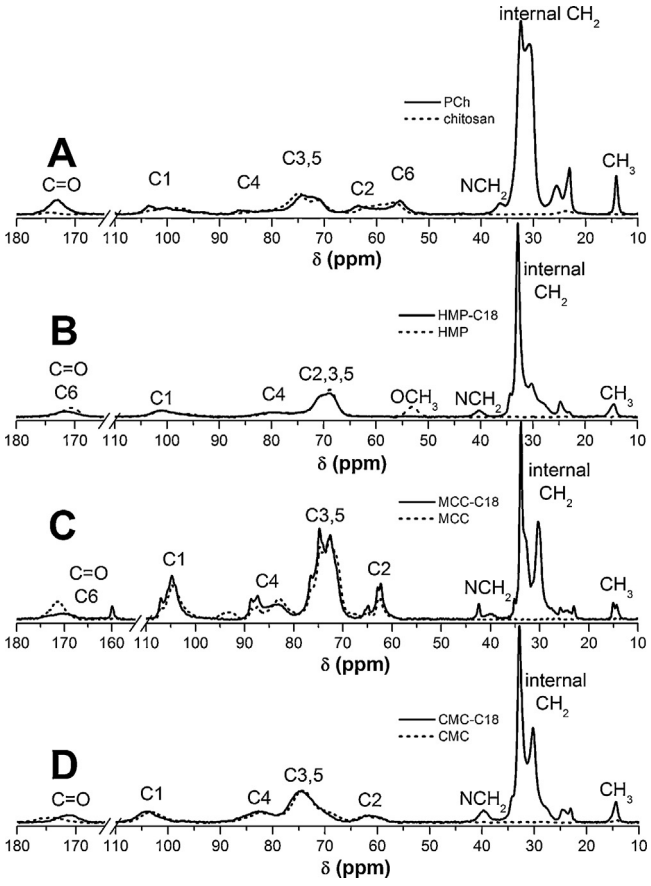

Fig. 3. ^{13}C CP-MAS NMR spectra of hydrophobically modified (solid) and parent (dash) polysaccharides including chitin and cellulose.

3.2. FTIR spectra

FTIR spectra of the hydrophobically modified and initial polysaccharides are shown in Fig. 2. Three bands of PCh at 1744 cm^{-1} (C=O stretching), 1657 cm^{-1} (amide I) and 1546 cm^{-1} (amide II) indicate the presence of ester and amide groups (Choi et al., 2007; Le Tien et al., 2003). FTIR spectra of HMP-C18, MCC-C18 and CMC-C18 also had two intense bands of amide vibrations at 1647–1655 cm^{-1} (amide I) and 1537–1558 cm^{-1} (amide II), which are absent in the spectra of parent polysaccharides. The presence of these two intense IR bands confirmed that the substituents are bound to HMP, MCC or CMC as an amide (Sinitsya et al., 2000; Synytsya et al., 2003; Taubner et al., 2013; Tůma et al., 2011). Several bands near 2920, 2850, 1465 and 722 cm^{-1} were found in the spectra of all the derivatives. These bands were assigned to vibrations of methylene groups in the alkyl or acyl substituents. All these IR features confirmed significant substitution (amidation) with *n*-octadecylamine for HMP-C18, MCC-C18 and CMC-C18, while in PCh palmitoyl groups are bound to chitosan as esters and amides (*N*,*O*-palmitoyl substitution).

Table 1
Organic elemental analysis of polysaccharide derivatives and calculated values of substitution degrees (DS) (Tůma et al., 2011).

Sample	Contents of elements (%m/m)			DS (mol mol^{-1})	Substituent
	N	C	H		
Chitosan	7.58	41.08	7.24		
HMP	0.22	43.40	5.23		
MCC	0	37.09	4.99		
CMC	0	36.67	5.56		
PCh	2.85	63.10	10.75	1.24	*N*,*O*-Palmitoyl
HMP-C18	2.16	51.62	8.99	0.61	*N*-Octadecylamine
MCC-C18	1.63	53.63	8.56	0.30	*N*-Octadecylamine
CMC-C18	2.38	60.74	10.08	0.63	*N*-Octadecylamine

Table 2
Parameters of Langmuir–Freundlich (Sips) model for cholestyramine, chitosan and polysaccharide derivatives.

Sorbent/sorbate	Cholate			Cholesterol		
	Q_m	b	n	Q_m	b	n
Cholestyramine	5.82	0.24	1.14	ND	ND	ND
Chitosan	0.47	0.37	1.89	ND	ND	ND
PCh	0.55	0.13	0.69	0.12	0.43	1.69
HMP-C18	1.34	0.08	0.79	0.08	0.35	2.18
MCC-C18	1.03	0.15	1.04	0.10	0.18	1.61
CMC-C18	1.01	0.04	0.79	0.12	0.33	1.59

3.3. ^{13}C CP-MAS NMR spectra

Solid state ^{13}C CP-MAS NMR spectra of the derivatives and corresponding initial polysaccharides are shown in Fig. 3. The signals of CH_3 in the end of *N*,*O*-palmitoyl or *N*-octadecyl substituents were found at 14–18 ppm. An envelope of very intense highly overlapped signals at 20–45 ppm was assigned to the CH_2 groups of these moieties (Synytsya et al., 2003, 2004; Tůma et al., 2011). These signals confirmed the substitution. Resonance signals in the region of 55–105 ppm were assigned to polysaccharide carbons. The OCH_2 signals of carboxymethyls (CMC-C18) and *O*-palmitoyls (PCh) should be situated in this region, but are overlapped by sugar carbon signals. Resonance signals of C=O carbons in esters, amides, uronic carboxyls and carboxymethyls were found at 165–180 ppm. According to NMR data, the DS were found to be 1.18 mol mol^{-1} for PCh, 0.58 mol mol^{-1} for HMP-C18, 0.21 mol mol^{-1} for MCC-C18 and 0.59 mol mol^{-1} for CMC-C18. These values are in agreement with those obtained from elemental analysis.

3.4. Thermal analysis

The DSC thermograms of the initial and hydrophobically modified polysaccharides are demonstrated in Fig. 4a–d. For all the initial polysaccharides, first broad endothermic peak of the curves at ~71–75 °C was assigned to water evaporation. The DSC thermograms of all modified polysaccharides have several narrower endothermic peaks in the region of 56–126 °C that could be assigned to melting of crystalline structures from the long aliphatic side chains. The water evaporation endotherm slightly contributes to this region because the derivatives retain much less amounts of water than corresponding parent polysaccharides. The DSC curve of chitosan has a strong exothermic peak at 298 °C. This peak was attributed to thermal degradation of this polysaccharide by cross linking reactions of free amino groups (Pawlak & Mucha, 2003; Tang, Wang, & Chen, 2005). Similarly, exothermic peaks observed for the other initial polysaccharides at 243 °C (HMP), 141–336 °C (MCC) and 292 °C (CMC) could be assigned to the process of thermal degradation (Einhorn-Stoll, Kunzek, & Dongowski, 2007). The DSC thermogram of PCh has two endothermic peaks at 236 °C and 256 °C that may be attributed to elimination of *O*- and *N*-palmitoyl moieties (Tůma et al., 2011). For the other hydrophobically modified polysaccharides, a number of overlapped exo- and endothermic peaks were found in the region above 200 °C. These peaks may indicate various processes including elimination of the functional groups, cross-linking reactions and degradation of the polysaccharide chain.

The TG and the corresponding DTG curves of the initial and hydrophobically modified polysaccharides are shown in Fig. 4e–h. The first thermal event is endothermic and occurs in the temperatures up to 130 °C. Corresponding mass loss was in the range from 2–5% (hydrophobically modified polysaccharides) to 8–15% (initial polysaccharides). This process was attributed to the water evaporation, and the mass loss decreased for the derivatives due to the presence of hydrophobic substituents (Choi et al., 2007). The second thermal event occurs in the temperature range 170–575 °C. It was attributed to the thermal destruction of the polysaccharides. Negative DTG peak of chitosan at 319 °C indicate elimination of acetamide and other volatile products of degradation (Pawlak & Mucha, 2003; Tang et al., 2005). Similar DTG peak of HMP was found at 208 °C; modified celluloses demonstrated DTG peaks at 182–296 °C (MCC) and 324 °C (CMC). Degradation of these polysaccharides could be connected with decarboxylation. For the hydrophobic polysaccharide derivatives, the DTG peaks were observed at 307 °C (PCh, with two shoulders at both sides), 240–282 °C (HMP-C18), 395 °C (CMC-C18), 280 °C and 408 °C (MCC-C18). These features were attributed to polysaccharide destruction. Further degradation of the chart involving deep depolymerisation and pyrolysis continued at higher temperatures. The mass losses at 600 °C were of 34.5% for chitosan, 29.1% for HMP, 22.4% for MCC-C18 and 38.2% for CMC-C18. The corresponding values for hydrophobically modified polysaccharides were significantly lower: 11.8% for PCh, 20.2% for HMP-C18, 12.2% for MCC-C18 and 9.4% for CMC-C18. More completed degradation was achieved because of the protection of functional groups (–OH, –NH_2 and/or –COOH) by alkyl/acyl substitution that inhibited cross-linking reactions.

Fig. 5 shows FTIR spectra of volatile compounds released during thermal decomposition of HMP-C18, MCC-C18 and CMC-C18 that were heated from 45 °C to 600 °C at 10 °C min^{-1} in nitrogen atmosphere. Weak bands of H_2O stretching and bending vibrations were found in the regions of water vapour absorption, i.e. at 3000–4000 and 1300–1800 cm^{-1}, respectively. It means that water evaporation was not pronounced for all the polysaccharide derivatives. By contrast, the increasing absorbance of CO_2 was observed in the region of 2200–2200 cm^{-1} and below 730 cm^{-1} in all temperature range. The exception was the temperature 148 °C when the strong decomposition occurred for HMP-C18. In contrast to pyrolysis of cellulose (Wang, Liu, Luo, Wen, & Cen, 2007), the CO bands centred near 2110 and 2180 cm^{-1} were not found in all the cases. The formation of CO_2 may occur as a result of decarboxylation, i.e. elimination of free uronic or carboxymethyl carboxyls. Therefore, the pyrolytic degradation of HMP-C18, MCC-C18 and CMC-C18 was different from that of cellulose probably because of the presence of carboxyls. In addition, several bands near 2965, 1273, 1095, 1025, 970, 930 and 830 cm^{-1} subsequently increased during heating. These bands were assigned to several CH, CC and CCO vibrations in alcohols and other non-defined organic compounds. The carbonyl bands at 1400–1800 cm^{-1} were non-detectable owing to overlapping with water bands.

3.5. Sorption isotherms

Sorption experiments are described in Section 2.7. Based on the experimental data the sorption isotherms were calculated. Sorption isotherms for cholate and cholesterol uptake by cholestyramine and polysaccharide derivatives are shown in Fig. 6a and b. The Langmuir–Freundlich model parameters calculated for cholate and cholesterol sorption are summarised in Table 2.

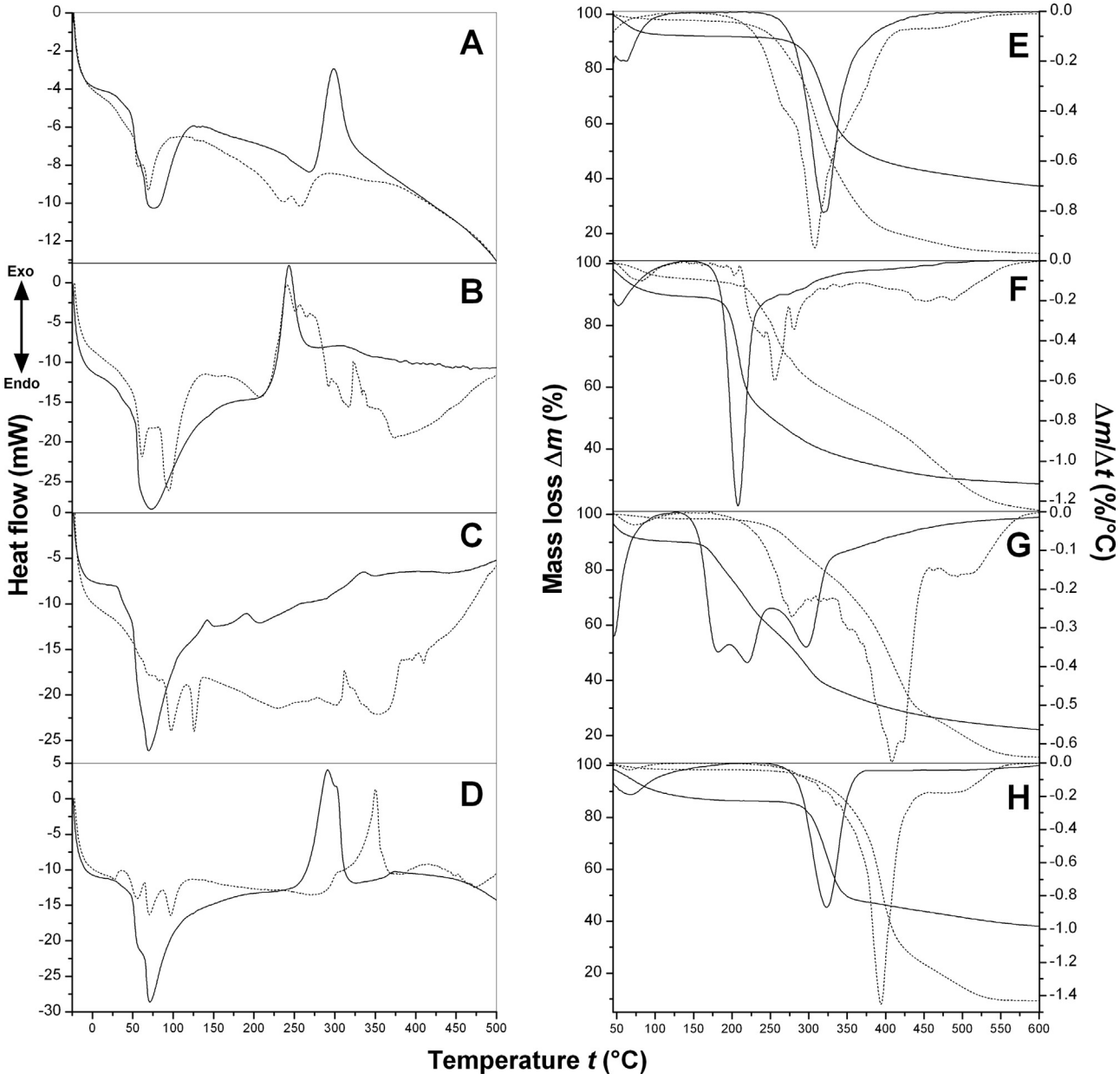

Fig. 4. DSC (a–d), TG and DTG (e–h) thermograms of initial (solid) and hydrophobically modified (dash) polysaccharides: chitosan/PCh (a, e), HMP/HMP-C18 (b, f), MCC/MCC-C18 (c, g) and CMC/CMC-C18 (d, h).

Generally, high performance sorbents should have both high uptake capacity Q_m and high affinity to sorbate indicated by low values of b (Volesky, 2003). The comparison of sorption performance should be made on whole sorption isotherm plots. It is evident from Fig. 6a that cholestyramine demonstrates the best sorption of cholate. According to the Langmuir–Freundlich model, this synthetic sorbent is able to absorb up to 5.82 mmol g^{-1} of cholate (Tůma et al., 2011). By contrast, chitosan and PCh showed much weaker sorption capacities, which were an order lower than that of cholestyramine. A little higher sorption capacities (1.01–1.34) were obtained for the derivatives of cellulose and HM pectin. The value of b was maximal for chitosan (0.37) and significantly decreased for polysaccharide derivatives (0.04–0.15), which were lower than the corresponding value of cholestyramine (0.24). Thus the substitution of chitosan with *N*,*O*-palmitoyls supports the cholate uptake. The third parameter n of the Langmuir–Freundlich model has been used to characterise sorption cooperativity: $n = 1$ for purely independent non-interacting sites, $n > 1$ for positive cooperativity, and $0 < n < 1$ for negative cooperativity (Sharma & Agarwal, 2001). Chitosan showed pronounced positive cooperativity ($n = 1.89$), while cholestyramine demonstrated lower positive cooperativity ($n = 1.14$). By contrast, MCC-C18 had nearly independent sites ($n = 1.04$) and other polysaccharide derivatives showed negative cooperativity ($n = 0.69$–0.79). Obtained results confirmed that alkyl/acyl substitution may support or inhibit cholate sorption depending on substitution degree and sorbate concentration in the medium.

Sorption of cholate is mainly based on electrostatic interactions involving charged amino groups, while hydrophobic interactions are less important. In contrast to this, effective sorption of cholesterol needs an introduction of hydrophobic groups. Indeed, all hydrophobically modified polysaccharides showed significant sorption of cholesterol that increased with substitution degree (Fig. 6b, Table 2). This process is characterised by positive

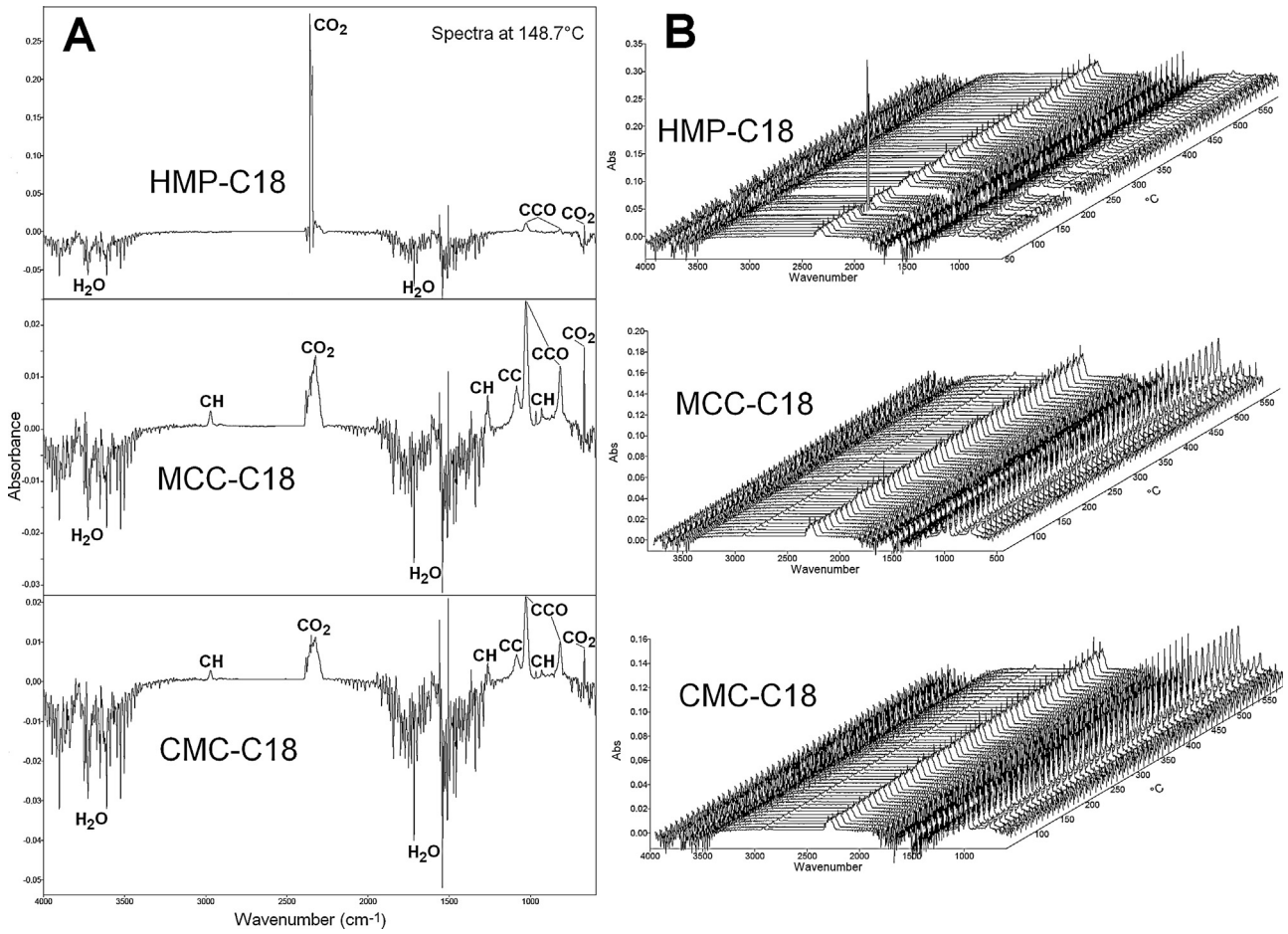

Fig. 5. FTIR spectra of volatile compounds released during thermal decomposition of HMP-C18, MCC-C18 and CMC-C18 measured at fixed temperature of 148.7 °C (a) and during the whole heating process (b).

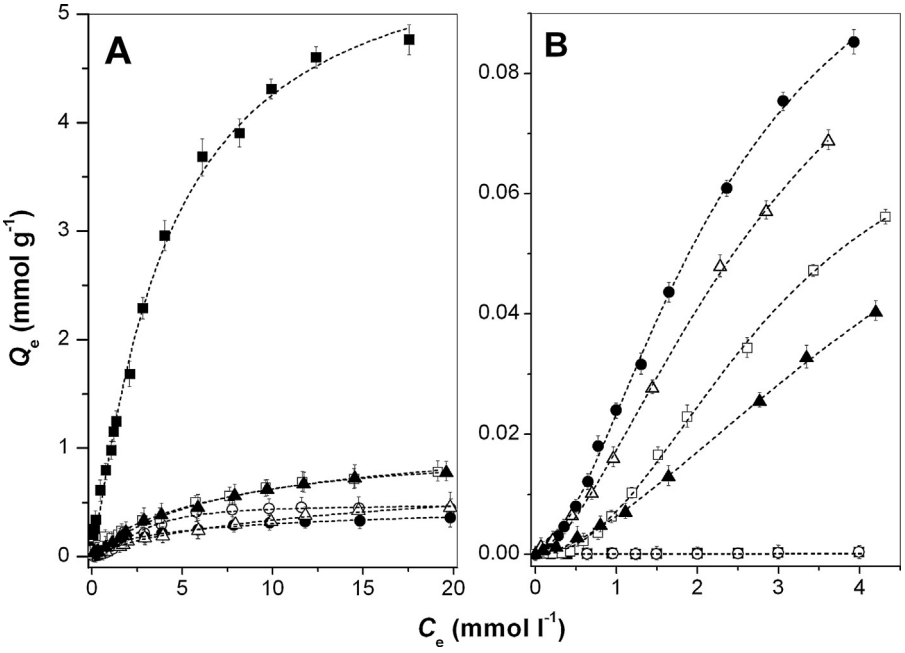

Fig. 6. Sorption isotherms for cholate (a) and cholesterol (b) uptake by cholestyramine (■), chitosan (○) and hydrophobically modified polysaccharides: PCh (●), HMP-C18 (□), MCC-C18 (▲) and CMC-C18 (△).

cooperativity (n = 1.59–2.18) because subsequent sorption of cholesterol enhance hydrophobic interactions involving alkyl/acyl substituents and thus support further uptake. By contrast, cholestyramine and chitosan showed negligible sorption of cholesterol.

4. Conclusions

Hydrophobic derivatives of HM citrus pectin, chitosan and cellulose were prepared and tested as potential cholesterol and fat agents for intestinal sorption of cholesterol, fats and bile acids. Elemental analysis and spectroscopic methods (FTIR, ^{13}C CP-MAS NMR) confirmed high substitution degrees for all of modified polysaccharides. According to thermal analyses (DSC, TG/DTG, TGA–FTIR), substitution with long alkyl/acyl groups led to significant changes in physical and thermal properties of modified polysaccharides. All the derivatives are able to adsorb cholesterol, but they demonstrated lower sorption of cholate than those of cholestyramine. Among hydrophobically modified polysaccharides of this study, *N*-octadecylamidated derivatives of HM pectin were confirmed as effective cholesterol-lowering agents and *in vivo* sorbents of dietary fat (Marounek et al., 2007; Marounek, Volek, Skřivanová, & Tůma, 2010; Marounek, Volek, Skrivanova, Tůma, & Duskova, 2010; Marounek, Volek, Dušková, Tůma, & Taubner, 2013); corresponding data for *N*-octadecylamidated celluloses are prepared for publication.

Acknowledgement

This work was supported by the Czech Science Foundation (Project No. 503/11/2479).

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