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Modification of polygalacturonic acid hydroxyls with trimethylammonium- and/or sulfonate-2-hydroxypropyl group

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ABSTRACT

Polygalacturonic acid (PGA) was quaternized with 2,3-glycidyltrimethylammonium chloride (GTMAC) and/or sulfonated with 3-chloro-2-hydroxy-1-propanesulfonate (CHPS) under vacuum or at ambient pressure in the absence or presence of NaOH. The aim was to find out what optimal ratio of reactants are required to obtain the highest possible degree of substitution (DS), yield and molar masses. The highest DS of PGA obtained (calculated on a fully substituted derivate) for quaternized sample was around one according to elemental analyses. At ambient pressure the DS was lower than under a vacuum treatment. Lower DS and yield (36%) of PGA were obtained when PGA was sulfonated. At ambient pressure the sulfonation has not taken place. Repeated quaternization of PGA has not increased the DSs of PGA, while sulfonation was beneficial for increase of DS. When PGA was modified simultaneously with both alkylating agents, the yield of PGA was further improved (up to 52%). Further, the DS of the ammonium substituent was higher than that of the sulfonate group, and the yield of molar mass of the modified PGA was the highest from all tested experiments. At ambient pressure the DS of PGA treated with both reagents was lower than under vacuum. The treatment of PGA with NaOH at the absence of GTMAC or CHPS caused M_w decrease to 11,150 g/mol, while when quaternized in the absence of NaOH the values were between 20,940 and 32,540 g/mol. The repeated quaternization caused even more dramatic decrease of molar mass (to 6400 g/mol). According to NMR both C-2 and C-3 positions of PGA were substituted equally due to a use of the alkylating reagent or a certain charge.

1. Introduction

Pectin is a family of complex polysaccharides present in all plant primary cell walls (Ridley, O'Neill, & Mohnen, 2001). Pectic polysaccharides are important components of food products (Savary, Hotchkiss, Fishman, Cameron, & Shatters, 2003; Thakur, Singh, & Handa, 1997). Their solubility in water depends uppon the degree of esterification, acetyl content, pH, etc. In our previous work we have prepared water-insoluble PGA derivatives with ion-exchanging properties (Šimkovic, 1997; Šimkovic, Hricovíni, & Sasinková, 2002). The derivatives were suitable as stationary phases for purification of glycanases (Ondrášová, Omelková, Schubertová, & Šimkovic, 2002). In the present work we have focused on the preparation of water-soluble PGA derivatives using monofunctional quaternizing or sulfonating reagents. We have employed repeated quaternizing as well as applied sulfonating procedures, and have further used combinations of these modification procedures. The substrate itself contains carboxyl groups in a salt form and is not soluble in water without a pretreatment with base. Due to the fact that, in water/NaOH environment the alkylating agents are hydrolyzed to correspoding alcohols, which are innactive reaction side-products, we developed a modification procedure under vacuum to minimize the hydrolysis of the agent. The products were characterized by NMR and light scattering techniques and complemented by elemental analyses. The aim of the work was to prepare water-soluble polysaccharides in highest possible yields and molar masses. We believe that they could find uses in contolled drug delivery, film or suspension preparations and other composite applications (Liu, Fishman, & Hicks, 2007; Sinitsya, Čopíková, Prutzanov, Skoblza, & Machvič, 2000).

2. Experimental

2.1. Materials

PGA sodium salt (Sigma, batch #: 053K3780; C, 32.16; H, 5.13), GTMAC (Fluka) and CHPS (Aldrich) were used as a substrate or ionization reagents. All other chemicals used were without further purification (Merck).

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2.2. Analysis

The elemental analysis were run on a Fisons EA-1108 instrument, while NMR measurements were performed on INOVA-600 VARIAN spectrometer at 25 °C in D_2O with 3-(trimethylsilyl)-propionic acid (TSP) as an internal standard at a relaxation delay of 1 s. For HSQC the mixing time was 0.5 s; acquisition time 0.23 s; width 6000.6 Hz; 2D width 35987.4 Hz; 256 repetitions; 2 × 128 increments; power 34 dB, on during acquisition, off during delay. For HMBC the acquisition time was 0.175 s with adiabatic pulses. The size exclusion chromatography–multiple angle laser light scattering (SEC–MALLS) experimental conditions were: Dawn DSP multi-angle laser light scattering photometer from Wyatt (Santa Barbara, CA, USA) on line to a Alliance 2690 size exclusion chromatography system from Waters (Milford, MA, USA) by using two Waters Ultrahydrogel columns (1000–250 Å) and 0.05 M acetic buffer at pH 4.0 or 0.1 M $NaNO_3$ at pH 6.0 or 0.2 M NaCl + 0.1 M Tris buffer at pH 8.0; 35 °C as elution systems with 0.8 mL/min flow rate; dn/dc = 0.160 g/mL and KMX-16 differential refractometer from Milton Roy (Riviera Beach, FL, USA). To eliminate the aggregation effect of samples the experiments were repeated at pH 10.0 (0.1 M carbonate buffer as a mobile phase) using two TSK PW (G4000–G3000) columns setting.

Table 1
Quaternization/sulfonation of PGA

Sample #	Molar ratios of reaction components				Yield[a] (%)	X[b] (%)
	PGA	Y[c]	NaOH	H_2O		
1	1	4[d]	0	0–1000[e]	39	1.94[f]
2	1	10[d]	0	0–200[e]	43	3.02[f]
3	1	20[d]	0	0–200[e]	45	3.41[f]
4	1[g]	10[d]	0	0–200[e]	17	0.98[f]
5	0.5[h]	1[d]	0	0–1000[e]	30	2.86[f]
6	1	4[d]	4	0–1000[e]	40	3.36[f]
6*	1	4[d]	4	1000	43	2.10[f]
7	1	4[d]	10	0–1000[e]	32	2.28[f]
8	1	0	10	0–1000[e]	78	0
9	1	5[i]	10	0–1000[e]	34	1.83[j]
10	0.3[k]	1[i]	10	0–1000[e]	36	3.10[j]
11	1	10[i]	20	0–1000[e]	35	1.45[j]
11*	1	5[i]	10	1000	16	0[j]
12	1	5[d]/5[i]	10	0–1000[e]	52	3.27[f]/1.39[j]
13	1	10[d]/10[i]	20	0–1000[e]	40	3.13[f]/1.92[j]
13*	1	10[d]/10[i]	20	1000	37	1.81[f]/0.25[j]

[a] Water-soluble part was calculated on a fully substituted product.
[b] Nitrogen or sulfur content.
[c] Ionizing agent.
[d] GTMAC.
[e] See Section 2.
[f] Nitrogen content.
[g] Repeated quaternization of sample 2.
[h] Repeated quaternization of sample 3.
[i] CHPS.
[j] Sulfur content.
[k] Repeated sulfonation of sample 9.
* Experiments performed under ambient pressure.

2.3. Derivatization

The molar ratios of reagents are listed in Table 1. The PGA was mixed with water/NaOH solution, treated on vacum rotary evaporator for 1 h at 60 °C and subsequently the alkylating agent was added and the mixture was further modified on vacum rotary evaporator at 60 °C for 3 h under vacuum (3.3–4 kPa) or at ambient pressure. The reaction was stopped by dilution with water, neuralized to pH 7 when necessary, dialyzed (1 kDa MWCO; Spectra/Por®), filtered through 0.2 μm filter (PALL Gelman Laboratory) and lyophilized.

3. Results and discussion

3.1. Modification of PGA with glycidyltrimethylammonium chloride

The first quaternization experiments were performed without NaOH to find out how the reaction would proceed. The degree of substitution could be increased by changing the PGA/GTMAC mo-

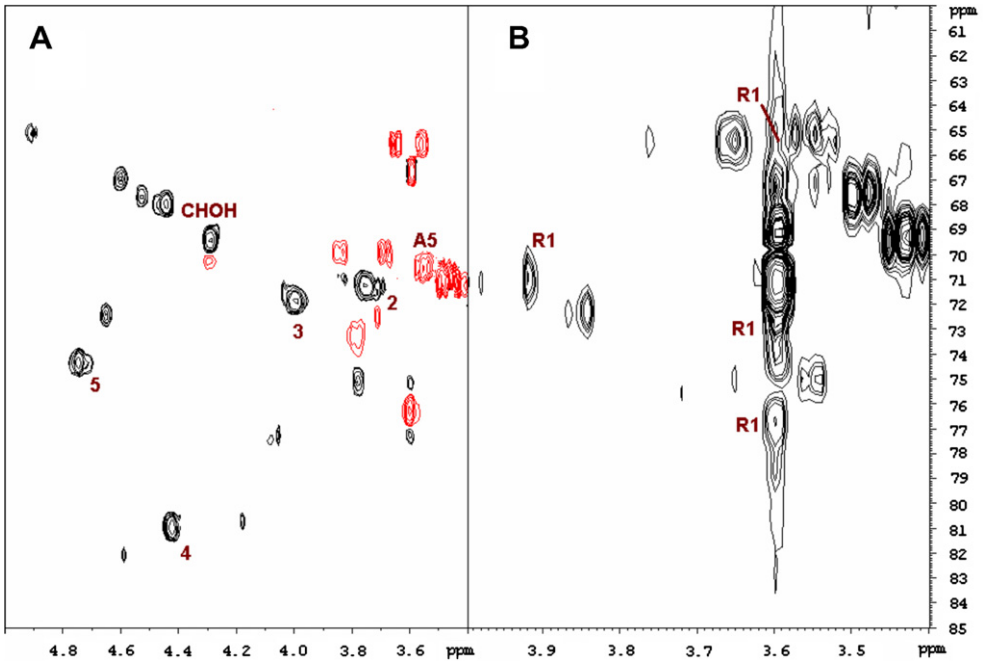

Fig. 1. The HMQC-DEPT (A) and HMBC (B) spectra of sample 3. The numbers assigned to peaks indicate C/H atoms in galacturonic acid residues; A5 is C/H-5 signal of arabinose; CHOH are signals of the TMAHP-substituent and R1 are HMBC multiplets confirming the correlation of the substitunet with C/H-2 and 3 signals of HSQC.

lar ratio from 1/4 to 1/20 (Table 1, samples 1–3). Sample 3 (DS 1.13; 45% yield) gave at the PGA:GTMAC = 1:20 ratio the highest nitrogen content (3.41%) and M_w = 20,940 g/mol (M_w/M_n = 1.8, recovered mass 64%). The molar mass of this sample has decreased in comparison to sample 2 (PGA:GTMAC = 1:10; M_w = 32,540 g/mol, M_w/M_n = 2.0, recovered mass 67%). By a repeated quaternization of sample 2 in the absence of NaOH under the reduced pressure lower yield, nitrogen content and molar mass were obtained (sample 4; M_w = 6400 g/mol). The quaternization of sample 3 (PGA:GTMAC = 1:2) resulted in decreased yield of PGA, although nitrogen content has increased (sample 5). In the presence of NaOH much smaller amount of GTMAC was needed to reach the high DS but a lower yield of product was obtained (cf. samples 6 and 3). When quaternized at ambient pressure the yield of PGA was slightly higher than in the case of sample 6, but lower than in the case of sample 3 (sample 6*; 43%), while lower DS was always present. At an excess of NaOH both the yield and DS kept decreasing (sample 7; PGA:GTMAC:NaOH = 1:4:10). The PGA polysaccharide could be recovered after pretreatment with NaOH and without the use of GTMAC (sample 8; PGA:NaOH = 1:10; 78%

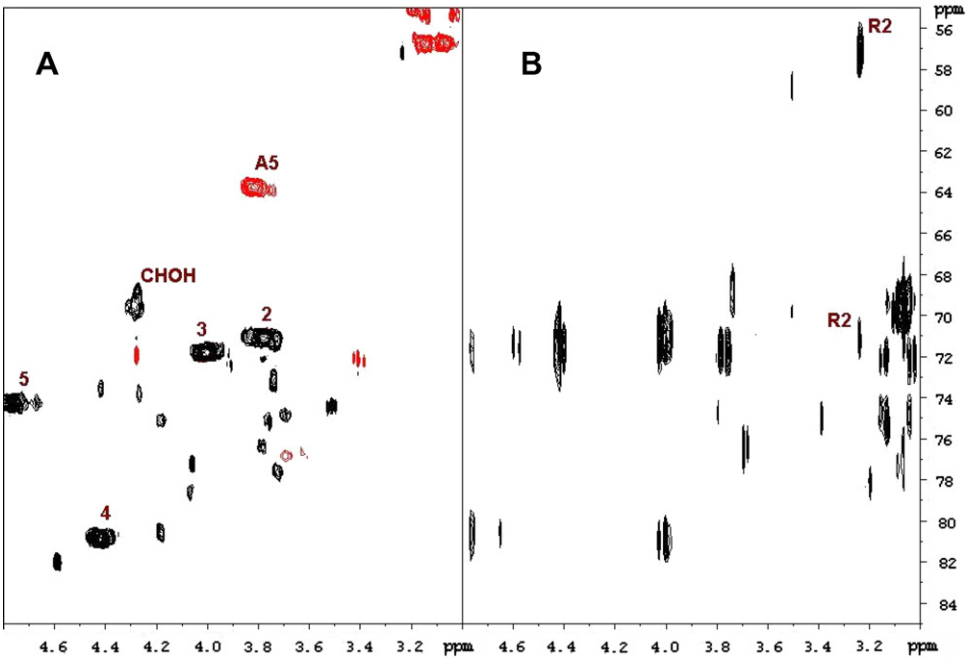

Fig. 2. The HMQC-DEPT (A) and HMBC (B) spectra of sample 11. The symbols used are identical to those in Fig. 1, except where R2 indicates multiplets confirming that the HPS-group correlate with C/H-2 and 3 signals.

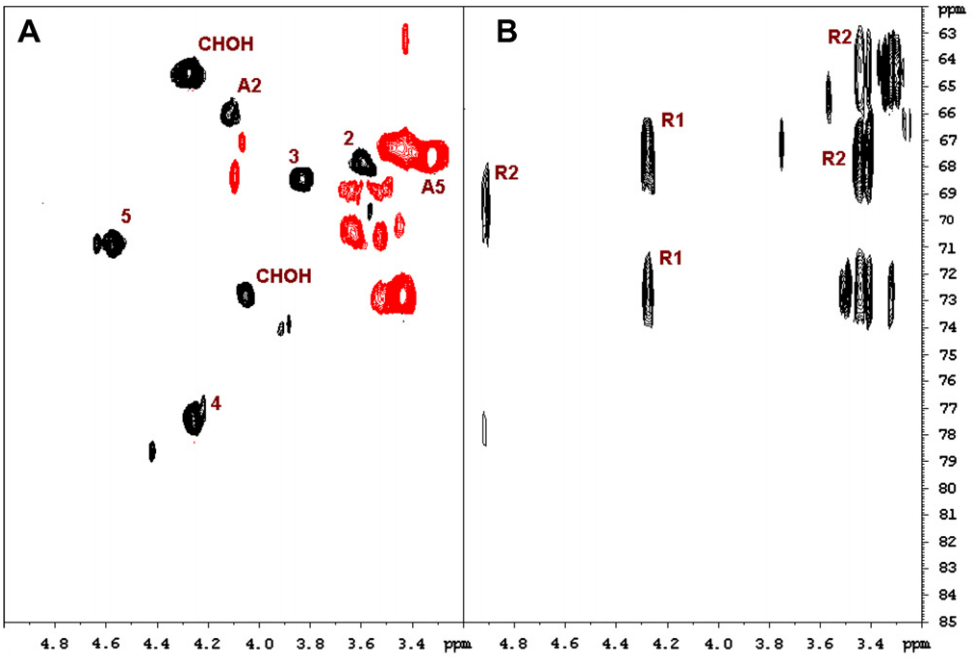

Fig. 3. The HMQC-DEPT (A) and HMBC (B) spectra of sample 13. The symbols used are identical to those in Figs. 1 and 2.

yield). This material had decreased molar mass (M_w = 11,150 g/mol), although the elemental composition was similar to the starting material (C, 31.76; H, 4.89). This comparison indicates that in the absence of alkylating reagent the polysaccharide is degraded to a higher extent than in its presence, when NaOH is neutralized with protons dissociated from PGA hydroxyl groups that have been affected by the reagent. The yields were higher in comparison to experiments with 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHMAC) at ambient temperature and presure (Šimkovic, 1997). It is due to higher efficiency of GTMAC and higher ratio of GTMAC/PGA used in comparison to experiments with CHMAC.

According to the NMR specroscopy (HSQC, Fig. 1A; sample 3; COSY data not shown) an anomeric signal at 101.82/5.08 (C/H) ppm was present. The other related signals were at 71.40/3.75 (C/H-2), 72.00/4.00 (C/H-3), 80.90/4.42 (C/H-4) and 74.20/4.74 (C/H-5) ppm. These data has also occurred with unsubstituted sample 8 and are close to data measured on oligogalacturonides (Ló, Hahn, & van Halbeek, 1994). Additionally, a signal at 70.70/3.55 ppm was present, which also occurred in unsubstituted sample 8, and according to COSY we ascribe it to the primary hydroxyls of the linked arabinosyl units as also confirmed by DEPT (the signal in red).[1] The CH_2 signals of the substituent are occurring in 76.40/3.60, 73.30/3.78, 69.90/3.84 and 3.67, 66.60/3.58 regions as well as in 65.60/3.64 and 3.56 ppm regions. The other new signals were ascribed to CHOH groups of the substituent with chemical shifts at 69.40/4.28, 67.90/4.45, 67.60/4.74 and 66.80/4.60 ppm. The methyl group signals of the substituent linked to quaternary nitrogen, which are not included in HSQC spectrum due to artificial signals, are present at 57.12/3.13 ppm. In the HMBC (Fig. 1B) the spectrum represents a multiplet at 3.60 ppm, which is correlated with C-2 and C-3 signals as well as with CH_2 groups at 65.60–66.60, 73.30 and 76.40 ppm. This confirms the presence of linkages of substituents to both secondary hydroxyl groups. That was the reason, why we assumed that both C-2 and C-3 were substituted equally with trimethylammonium-2-hydroxyprophyl (TMAHP) groups. Because the additional CH_2 groups were present in the spectrum, we assumed that arabinosyl C-5 groups have also been substituted. This was confirmed by the multiplet at 3.92 ppm in the HMBC spectrum. Only one signal for carboxyl carbon was present in ^{13}C NMR spectrum (178.20 ppm). This finding led us to believe that the interactions between the polygalacturonic carboxyls and quaternary groups were not occurring.

3.2. Modification of PGA with 3-chloro-2-hydroxy-1-propanesulfonate

Sulfonation is a less effective reaction than quaternization. For sample 9 the calculated DS was 0.29 according to sulfur content (Table 1; PGA:CHPS:NaOH = 1:5:10; S = 1.83%; 34% yield). By repeating alkylation of sample 9 the DS could be increased to 0.50 (sample 10; S = 3.10%; 36% yield). At higher concentrations of CHPS and NaOH, the sulfur content has been decreasing (sample 11; S = 1.45%; 35% yield). Under ambient pressure the reaction has not taken place (sample 11*, S = 0%). The molar masses of these samples were at 10,100 (sample 10) or 30,900 g/mol (sample 11). When sample 9 was analyzed by SEC–MALLS at four different concentrations of H^+, the amounts of recovered samples (80% at pH 4.5; 70% at pH 6; 76% at pH 8.6 and 45% at pH 10) have increased with decreasing pH, which indicates that the samples have increasing solubility when pH is lowered.

According to NMR spectroscopy (HSQC; sample 11; Fig. 2A; COSY not shown) the shifts of C/H-2 and C/H-3 are at 71.20/3.78 and 71.70/4.01 ppm. The CH_2 groups (in red)[1] are according to DEPT at 63.70/3.83, 56.70/3.16 and 3.07 as well as at 72.10/3.43 ppm. The strong signal at 63.70/3.83 ppm (in red)[1] belongs according to COSY and DEPT to C/H-5 of arabinosyl unit. The peak occurring at 56.70/3.16 and 3.07 ppm (in red)[1] is ascribed to CH_2 groups of the substituent. Additional signal at 69.25/4.25 ppm in the HSQC spectrum belongs to the CHOH of the substituent, and was not present in the spectrum of the starting material. In the HMBC spectrum (Fig. 2B), the cross-peaks at 71.20–71.70/3.24 ppm indicate that the correlation of signals at C-2 and C-3 with the protons of the substituents of CH_2 groups were at 56.70/3.24 ppm. On the basis of this finding we assume that the substituent is linked to both C-2 and C-3 positions of PGA through an ether bond.

3.3. Preparation of mixed ethers

When alkylated simultaneously with both agents (sample 12; 52% yield; PGA:GTMAC:CHPS:NaOH = 1:5:5:10 molar ratio) the product was found to contain more nitrogen (DS 1.4) than sulfur (DS 0.2). The yield of this sample was higher than in all previous

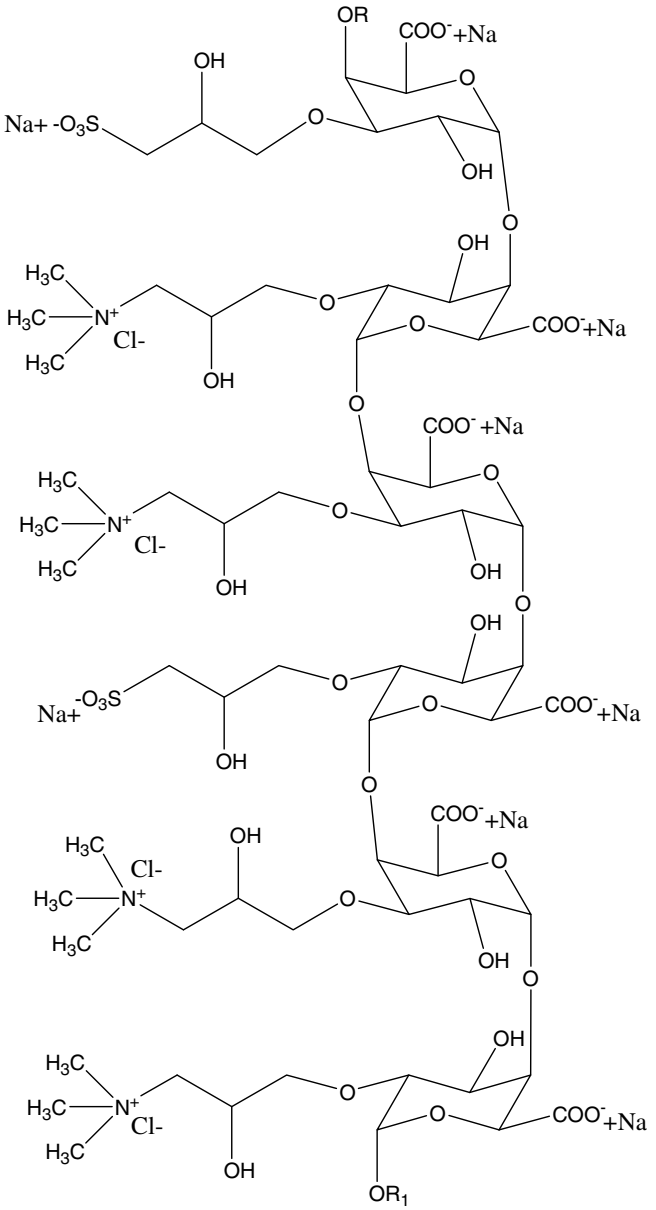

Fig. 4. Hypothetical structure of samples 12 or 13 (R and R_1 are the macromolecular residues).

[1] For interpretation of the references to color in this figure, the reader is referred to the web version of this paper.

Table 2
Molar masses [g/mol] and polydispersity values for selected samples

Sample	2	3	4	8	10	11	12	13
M_w	32,540	20,940	6400	11,150	10,100	30,900	48,400	28,000
D	2.0	1.8	–	1.5	–	2.0	1.7	1.9

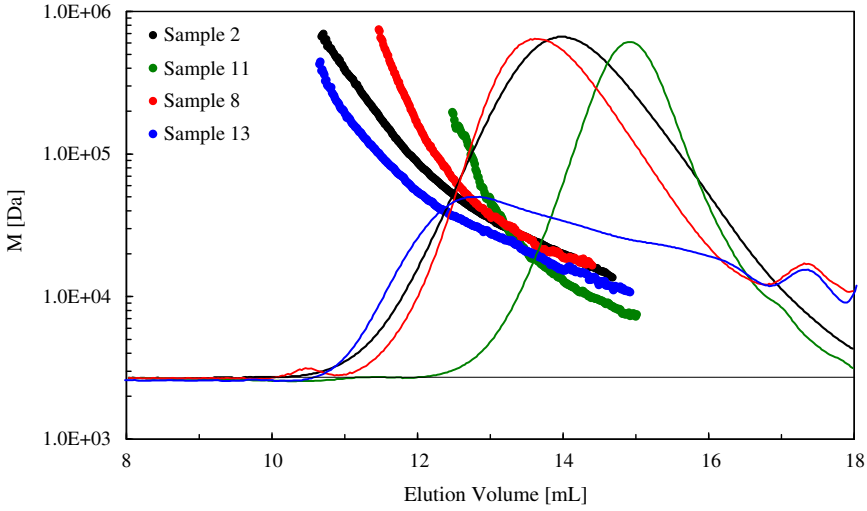

Fig. 5. Comparison of $M = f(V)$ experimental functions (broad symbols) or refractometer signals of size exclusion chromatography curves (thin lines) of samples 2, 11, 8 and 13.

experiments. With the increased amounts of reagents and NaOH (sample 13), the DS (0.3) of 2-hydroxypropylsulfonate group could be increased at the expence of a lower yield (40%). This observation indicates that the conversion of GTMAC is higher in comparison to the sulfonating agent. The DS values have not improved by using treatments under ambient pressure (sample 13*), although the yields of product were similar.

According to NMR (HSQC; sample 13; COSY data not shown; Fig. 3A), the signals for C/H-2 and C/H-3 PGA positions were at 67.90/3.62 and 68.50/3.86 ppm. The CH_2 group signals (in red)[1] are at 63.20/3.46, 67.10/4.07, 67.40/3.52, 67.60/3.44, 67.70/3.35, 68.50/4.10, 68.9/3.64, 69.00/3.51 and 3.53, 70.20/3.48, 70.40/3.56, 70.60/3.67, 73.10/3.46 and 73.30/3.53 ppm. The CHOH and CH_3 of quaternary groups are at 64.50/4.30 as well as 72.80/4.08 and 53.70/3.09 (CH_3, not shown) ppm. According to COSY additional signals of arabinosyl residue were present at 66.10/4.14 (C/H-2) and 67.7/3.35 (C/H-5, CH_2 in red)[1]. Due to the lower intensities of signals at 63.20/3.46, 70.30/3.46, 70.60/3.53 and 70.60/3.64 ppm, these signals were assigned to CH_2 groups. The signal at 66.25/4.15 ppm were assigned to CHOH group of the hydroxypropylsulfonate (HPS) substituent. The more intense signals belonging to the CH_2 groups in the HSQC window showed correlation cross-peaks with the C-2 and C-3 in the HMBC experiment (Fig. 3B), with multiplets at 4.28 ppm due to the presence of TMAHP-group (R1). Further, the HMBC spectrum showed that less intense CH_2 signals at 70.30/3.46, 70.60/3.53 and 70.60/3.64 ppm correlated with multiplet at 4.92 ppm due to C/H-2 and 3 linkage of HPS-group (R2) in the HSQC spectrum. Another less intense correlation is between the CH_2 signal at 63.2/3.46 ppm of the HSQC spectrum and the HMBC signal at 3.46 ppm, which also correlates with C/H-2 and 3 signals in the HSQC spectrum. We assume that this phenomenon is due to the linkage of the HPS-group. On the basis of these results we assume that PGA modified with both reagents is predominantly substituted with quaternary groups as described in Fig. 4.

As listed in Table 2, the SEC–MALLS analysis showed lower polydispersity of sample 12 ($D = 1.7$; $M_w = 48{,}400$ g/mol, 46% recovered) than of sample 13 ($D = 1.9$; $M_w = 28{,}000$ g/mol, 74% recovered). When we compared the molar masses of products, using the light scattering curves, the sulfonated sample 11 ($D = 2.0$; $M_w = 30{,}900$) was located partially above the quaternized sample 2 ($D = 2.0$), while the quternized/sufonated sample 13 was mostly bellow the previous two samples (Fig. 5). The alkalized sample 8 ($M_w = 11{,}150$ g/mol, $M_{peak} = 7420$, $D = 1.5$, 60% recoverd) was found to have the lowest polydispersity, although a part of the MALLS curve was located above the curves of the substituted samples.

4. Conclusions

PGA could be quaternized or sulfonated with GTMAC or CHPS under vacuum, whereby conversion of PGA with GTMAC is higher than with CHPS. While PGA could be quaternized also in the absence of NaOH, the excess of CHPS and NaOH is needed for sulfonation. The yields of product were the highest when combined quaternization and sulfonation procedure was applied in one step. The treatment under ambient pressure has not improved the DS of PGA. According to NMR the substituents were introduced in C-2 and C-3 positions of galacturonate residues. According to the elemental analysis, when the modification proceeded with both reagents in one step, a randomly substituted derivative could be obtained with predominance of quaternary groups. The molar mass of PGA using this procedure, was the highest from all the tested experiments.

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