Amphiphilic Beechwood Glucuronoxylan Derivatives

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ABSTRACT: Long alkyl chains (C_{12}) have been introduced onto glucuronoxylan and its sulfoethyl derivative *via O*-alkylation using 1-bromododecane in dimethylsulfoxide. The new amphiphilic xylan derivatives were characterized by chemical, elemental, and spectral analyses. They reduce the surface tension and exhibit remarkable emulsifying and protein foam-stabilizing properties. The results indicate that the development of new biopolymeric surfactants may be based on heteroxylans that are abundant in hardwoods and annual plants, and represent an unexploited renewable polysaccharide resource. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci **67:** 1523–1530, 1998

Key words: Beechwood glucuronoxylan, amphiphilic derivative, structure, surface tension, foamability, emulsifying efficiency

INTRODUCTION

Amphiphilic water-soluble polymers are receiving increasing attention due to their potential in various industrial and biotechnological applications. Most of studies in this field have been conducted on synthetic polymers, whereas polysaccharides have been selected as the hydrophilic backbone to a lesser extent. The preparation, properties, and applications of hydrophobically modified polysaccharides like *N*-saturated and *N*-unsaturated higher fatty acyl derivatives of chitosan,[1,2] stearoyl- or cholesterol-bearing pullulan,[3] cellulose alkanoates,[4] long alkyl chain ethers of cellulose,[5] hydroxyethyl cellulose,[6] sulfoethyl cellulose[7] and cellulose sulfate,[8] alkylaminated tamarind seed polysaccharide,[9] alkyl esters of pectin,[10] and alginate[11] have been published. However, no report has dealt with hydrophobically modified heteroxylans.

As a continuation of studies on heteroxylan derivatives,[12,13] this article describes the preparation and characterization of the structural, molecular, and interfacial properties of partially hydrophobized beechwood 4-*O*-methyl-D-glucurono-D-xylan (GX) and its *O*-(2-sulfoethyl) derivative. The presence of sulfoethyl groups was expected to increase solubility of the parent xylan.

EXPERIMENTAL

Materials and Chemicals

The Na^+ salt of GX was prepared in a semitechnical scale from beechwood meal.[14] It contains 13.9% of 4-*O*-methyl-D-glucuronic acid, corresponding to the xylose/uronic acid molar ratio of 10 : 1, and an average number molecular mass (M_n) of 32,500. Its solubility in water was 85%. 2-Chloroethanesulfonic acid (CHSA) and 1-bromododecane (lauryl bromide, LaB) were purchased from Aldrich Chemical Co. (Steinheim, Germany), and bovine serum albumin (BSA) was

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obtained from Sigma Chemical Co. (St. Louis, MO).

Analytical Methods

The methods used for characterization of GX and its derivatives in their salt form were described in previous articles.[15,16] The elemental analysis, performed on the elemental analyzer model 240 (Perkin-Elmer Corp., Norwalk, CT), was measured on protonated samples. For calculations of theoretical carbon and hydrogen contents, the empirical formula for GX (H^+ form of the uronic acid constituent) was $(C_{5.7}H_9O_{4.6})n$ $(151)n$. The sulfur content was determined according to Schöninger[17] and used for the calculation of the degree of substitution with sulfoethyl groups (DS_{SE}).[15] The content of carboxyl and sulfoethyl groups was determined by potentiometric titration of the protonated samples with aqueous $0.05M$ NaOH.[18]

M_n was determined by osmometry of samples dissolved in $0.1M$ NaCl or $0.2M$ NaOH using the Knauer Osmometer. High pressure gel permeation chromatography (HPGPC) of GX and its derivatives was conducted with a commercial instrument (Laboratorní Pr̂ístroje, Prague, Czechoslovakia) equipped with Separon HEMA-BIO 100 exclusion columns (Tessek Ltd., Prague, Czechoslovakia) and calibrated with pullulan standards, as previously described.[19] Fourier transform infrared spectra (FT-IR) were measured in KBr pellets (2 mg sample/200 mg KBr) using the Nicolet-Magna 750 spectrophotometer operating at 4 cm^{-1} resolution. ^{13}C-NMR spectra (75.4 MHz) were recorded with a Fourier transform-nuclear magnetic resonance spectrometer (Bruker AM-300) at 40°C in D_2O (internal methanol, δ 50.15 ppm). For the identification of CH_2 groups, the Distortionless Enhancement by Polarization Transfer (DEPT) sequence was used from the standard Bruker software library.

Preparation of Xylan Derivatives

O-(2-Sulfoethyl)xylan (SEGX)

According to the method described in a previous paper,[15] GX (1 g, 6.6 mmol) was dispersed in an emulsion prepared from isopropyl alcohol (15 mL) and 40% NaOH (13 mmol) and stirred at 10°C for 1 h. Then, 1.6 g of CHSA (11.1 mmol) was added, and the stirring was continued at 70°C for 3 h. The reaction was stopped by neutralization with acetic acid. After evaporation of the alcohol, the solution was dialyzed against distilled water and lyophilized to yield 1.37 g of the product (SEGX).

SEGX: $(C_{6.7}H_{11.5}O_{6.1}S_{0.5})n(205.5)n$

$DS_{SE} = 0.5$ Calcd. C 39.12 H 5.60 S 7.78

Found C 39.01 H 5.06 S 7.72

Sulfur content: 7.26% (Na^+ form); $DS_{SE} = 0.49$.

Alkylation of GX and SEGX with LaB

In a typical experiment, 0.5 g of the polysaccharide representing 3.3 mmol of GX and 2.3 mmol of SEGX, respectively, was suspended in dimethylsulfoxide (30 mL) at 60°C for 1 h under vigorous stirring. Two grams of LaB (0.8 mmol) and pyridine (0.6 mL) were added, and the reaction mixture was stirred at 50°C for 7 h. Then, it was poured into ethanol (200 mL), and the precipitate formed was filtered off, purified by ethanol extraction in a Soxhlet apparatus for 8–10 h, and finally dried *in vacuo* at 40°C. The products La-GX and La-SEGX were obtained in the yields of 0.50 g and 0.35 g, respectively.

La-GX: $(C_{5.8}H_{9.2}O_{4.6})n$ $(152.4)n$

$DS_{La} = 0.01$ Calcd. C 45.67 H 6.04

Found C 45.40 H 6.24

La-SEGX: $(C_{7.0}H_{12}O_{5.8}S_{0.4})n(201.4)n$

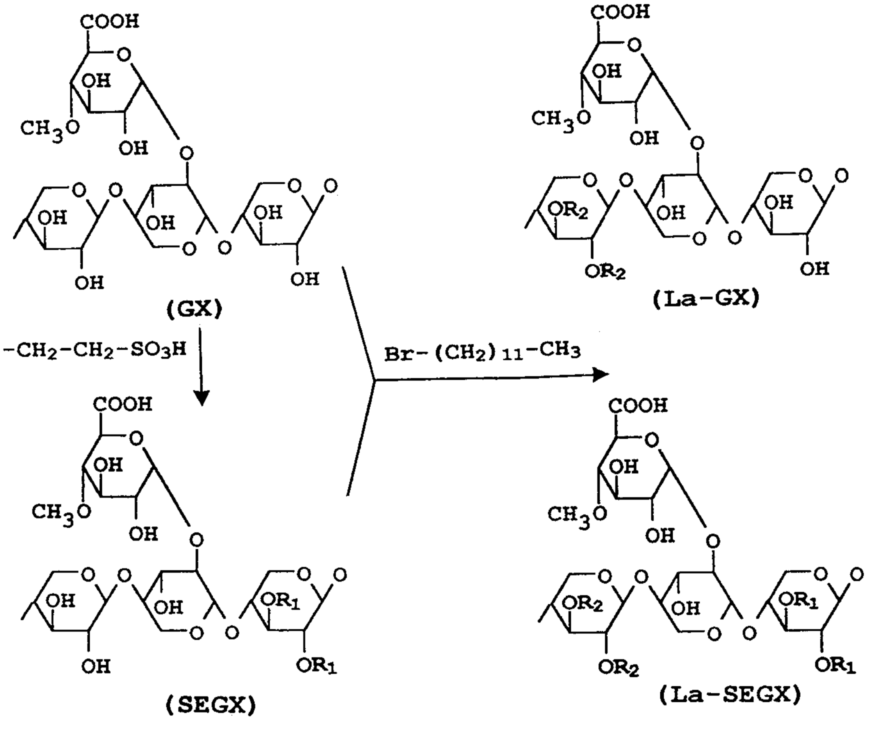
$DS_{SE} = 0.4$ Calcd. C 41.59 H 5.96 S 6.36

$DS_{LA} = 0.04$ Found C 41.33 H 5.57 S 6.78

Sulfur content: 6.07% (Na^+ form)

Preparation of the Protonated Form of the Xylan Derivatives

The protonated form was prepared[18] by treating the samples with acidified 90% ethanol containing 0.1 mol L^{-1} of HCl, followed by washing with 90% ethanol until free of Cl^- ions, and then with ethanol, acetone, and ether. The products were dried *in vacuo* at 60°C for 12 h. The residual water content was determined in nitrogen atmosphere by thermogravimetry using a Perkin-Elmer TGS-1



R_1: H or $-CH_2-CH_2-SO_3H$
R_2: H or $-CH_2-(CH_2)_{10}-CH_3$

Figure 1 Main structural features of the beechwood xylan (GX) and xylans substituted with sulfoethyl (SEGX) and/or lauryl groups (La-GX and La-SEGX);

thermobalance, and data from the elemental analyses were corrected accordingly.

Interfacial Properties

The surface tension of aqueous polysaccharide solutions was measured at 25°C using a Lecompte Du Nouy ring apparatus[20] in the concentration range of 0.01–5 g L^{-1}. The critical micelle concentration was estimated from the plot of surface tension *versus* log concentration.

Foaming activity was assessed by vigorously shaking 1.25 mL of an aqueous solution (1 g/100 mL) of the samples for 30 s and immediately measuring the foam volume.[21] Foamability was characterized by the generated foam volume and expressed as the percentage of the original liquid volume.

The protein foam stabilization effect was estimated according to Izydorczyk and colleagues[21] by adding 0.25 mL of an aqueous solution (1 g in 100 mL) of the polymer to 1 mL of the solution of BSA (2 g in 100 mL), followed by vigorously shaking for 30 s. The foam volume (mL) attained was measured immediately after the foam was generated (V_1) and after heat treatment in a water bath (95°C) for 3 min (V_2).

The emulsifying activity was assessed by mixing aqueous solutions of the sample (1 g in 90 mL H_2O) with paraffin oil (10 mL) and dyed with Sudan IV[7] by means of a laboratory mixer. The stability of emulsions was estimated in three different time intervals after the emulsions were prepared [i.e., at 5 min (h_1), 1 h (h_2), and 24 h (h_3)] and is expressed in terms of cream layer (mm) formed onto the surface.

RESULTS AND DISCUSSION

Structure and Molecular Properties of the Xylan Derivatives

The introduction of sulfoethyl groups in the xylan macromolecule yielded a derivative (SEGX; Fig.

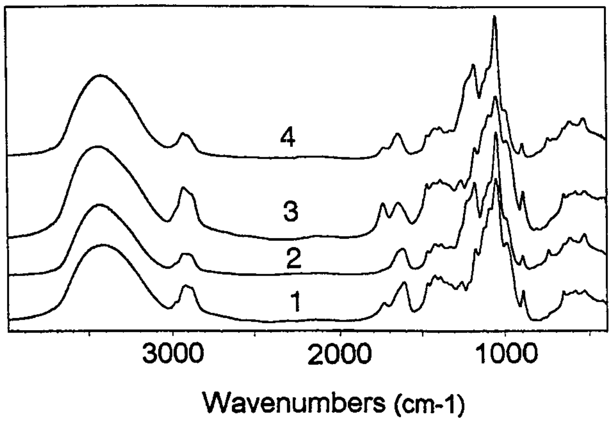


Figure 2 Fourier transform infrared spectra of GX after sulfoethylation and alkylation with LaB: (1) GX, (2) La-GX, (3) SEGX, and (4) La-SEGX.

1) fully soluble in water. The sulfur content indicates a degree of substitution[15] $DS_{SE} = 0.49$ that is comparable with that obtained by alkalimetric titration (0.52) and in accord with elemental analysis data (0.5). The hydrophobic modification of GX and SEGX was performed by *O*-alkylation with LaB (Fig. 1). From the elemental analysis data, the degree of alkylation (DS_{La}) of the derivatives were estimated to be ~ 0.01 and 0.04 for La-GX and La-SEGX, respectively. The lowered sulfur content of La-SEGX is not only due to the introduction of the sulfoethyl substituents, but also might result from losses of highly substituted xylan during the modification procedure, as indicated by the low yield of the derivative.

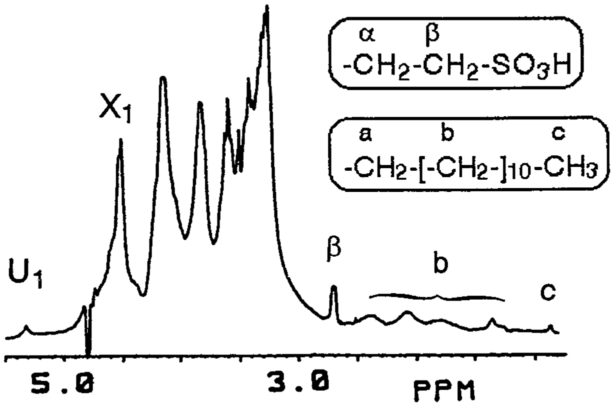


Figure 3 ^{1}H-NMR spectrum of La-SEGX (in D_2O). U, 4-*O*-methyl-α-D-glucuronic acid; X, β-D-xylopyranose.

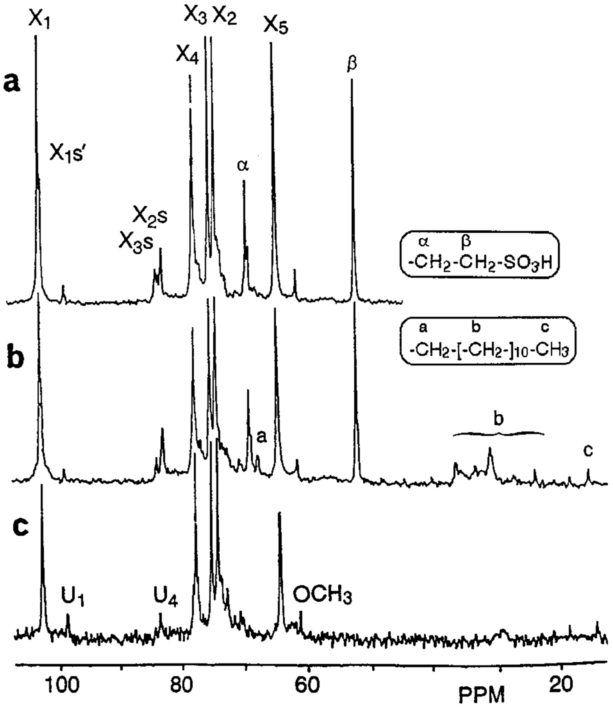


Figure 4 ^{13}C-NMR spectra (in D_2O) of (a) SEGX, (b) La-SEGX, and (c) La-GX. For U and X, see text in Figure 2; s and s′ indicate substituted and neighbor-substituted carbons, respectively of the xylose units.

As seen in Figure 2, substitution of GX with sulfoethyl and/or lauryl groups gave rise to infrared spectral changes that were assigned providing a mild Fourier Self-Deconvulation (FSD) experiment on the appropriate couples of GX and its derivatives. Thus, the vibrational modes of the sulfoethyl groups were found at 1,366 cm^{-1} ($\nu_{as}S{=}O$), 1,196 cm^{-1} ($\nu_{s}S{=}O$), and 741 cm^{-1} ($\nu C{-}S$).[22] Despite the low degree of hydrophobization, the FSD method also enabled estimation of the main vibrational modes of the lauryl groups to be at 2,927 cm^{-1} ($\nu_{as}CH_2$), 2,858 cm^{-1} ($\nu_{s}CH_2$), 2,978 cm^{-1} ($\nu_{as}CH_3$), 1,471 cm^{-1} (δCH_2), and 1,225 cm^{-1} ($\nu C{-}O{-}C$).

The ^{1}H-NMR spectrum of La-SEGX is shown in Figure 3. The signals in the anomeric region (δ, 4.48 and 5.32) correspond to H-1 resonances of the xylosyl and glucuronosyl structural units of the native GX.[23] Additional resonances were observed in the upfield region. The relatively strong, splitted signal at δ 2.88 was assigned to $CH_2{-}S$ protons of the sulfoethyl substituent. Its $O{-}CH_2$ signal expected at $\delta \sim 3.6$ is overlapped by the protons of the xylan backbone. Additional chemical shifts were observed at $\delta \sim 2.1$, 1.8, 1.3, and 0.9, which can be assigned to resonances of the methylene and methyl groups of the lauryl chains. Because they might be overlapped by sig-

Table I Molecular Properties of Xylan Derivatives SEGX, La-GX, and La-SEGX

		Molecular Mass Distribution[b]		
Compound	$M_n \times 10^{-3}$ [a]	$M_w \times 10^{-3}$	M_w/M_n	Area %
GX	32.5[c]	> 100	—	17
		35.8	2.01	83
SEGX	30.0	> 100	—	10
		39.0	1.22	90
La-GX	29.5	> 100	—	11
		38.6	1.26	89
La-SEGX	31.8	> 100	—	9
		40.0	1.21	91

[a] Determined by osmometry in 0.1 *M* NaCl.
[b] Determined by HPGPC on Separon HEMA BIO-100 columns.
[c] In 0.2 *M* NaOH.

nals of residual organic solvents, spectral data were not used for DS_{La} calculation.

The ^{13}C-NMR spectra of the xylan derivatives La-GX, SEGX, and La-SEGX are shown in Figure 4. All spectra were dominated by C-1/C-5 signals of the xylan backbone[16,24] seen at δ 102.7, 73.9, 74.9, 77.5, and 64.9. The signals for C-1/C-6 of the 4-*O*-methylglucuronic acid side chains appeared at δ 98.7, 72.5, 73.4, 83.6, 72.5, and 177.8, and that of its methoxyl group at δ 61.0. Using the DEPT experiment, the $O—CH_2$ and $CH_2—S$ signals of the sulfoethyl substituents were identified. They appeared at δ 68.7 and 51.9, respectively, as doublets reflecting different positions of the substituents. *O*-Alkylation has been shown to result in a 7–10 ppm downfield shift of attached carbons relative to the corresponding carbons of unsubstituted xylosyl units.[12,25,26] Thus, the signals at δ 82.9–83.7 correspond to substituted carbons C-2 and C-3 of the xylose units. However, this group of signals also contain a contribution of C-4 of the 4-*O*-methylglucuronic acid moieties. The signal at δ 67.4, which appeared only in the spectrum of La-SEGX, was assigned to $O—CH_2$ of the lauryl substituent. The signals of the internal CH_2 and CH_3 of the lauryl substituent were seen at $\delta \sim$ 35–18, and 14.6 in the spectra of both derivatives.

The molecular properties of GX and its derivatives are shown in Table I. The M_n values of all polysaccharide samples were similar. However, the molecular mass distribution evaluated by HPGPC showed a decrease in the proportion of the high molecular mass peak, indicating that some degradation of GX occurred during both alkylation procedures.

Interfacial Properties

The surface tension values of GX and its derivatives are given in Table II. As seen, GX and SEGX decreased the surface tension of water only moderately, and the solution was not able to form micelles. After introduction of hydrophobic substituents, the surface tension was significantly lowered, giving values of 48–58 mN m^{-1}, which are very similar to those reported for alkylaminated tamarind seed polysaccharides[9] and other polysaccharides with known surface activity (such as methylcellulose, pectin, and gum tragacanth).[27] A more pronounced decrease of surface tension ($\sim$ 38 mN m^{-1}) was reported for the lauryl derivatives of hydroxyethyl cellulose[6] and sulfoethyl cellulose.[7]

The emulsifying activity of the xylan deriva-

Table II Surface Tension (γ_{min}) and Critical Micelle Concentration of 1% Aqueous Solution of the Parent Xylan and Its Derivatives at 25°C

Compound	γ_{min} (mN m^{-1})	Critical Micelle Concentration (g L^{-1})
GX	60.5	No
SEGX	61.8	No
La-GX	57.5	0.65
La-SEGX	48.0	0.55

Table III Emulsifying Activity of the Laurylated Xylan Derivatives

Compound	Oil Layer/Cream Layer			Type of Emulsion
	h_1 (mm)	h_2 (mm)	h_3 (mm)	
La-GX	0/1	0/1	0/4	o/w[a]
La-SEGX	0/0	0/7	1/18	o/w
Tween 20	0/0	0/2	0/4	o/w

[a] o/w, oil in water.

tives was assessed for a 1% solution in a water/paraffin oil (90/10, v/v) dispersion. The initial emulsification, as well as the emulsion stability after 5 min and 24 h, was expressed in terms of the height of the formed cream layer (Table III). As expected, SEGX had no effect, similar to carboxymethyl cellulose.[9] However, both lauryl derivatives exhibited excellent emulsifying properties, comparable with those of the commercial product Tween 20 (oxyethylated monolauransorbitol).

Both viscosity and surface activity of the polysaccharides are thought to contribute to emulsion stability. However, measurement of foaming properties are more informative about the respective contributions. The foaming activity of xylan derivatives was expressed as the foam volume measured immediately after generation of foam (Table IV). As seen, there are differences in the foamability of the xylan derivatives. Again, SEGX showed, similar to carboxymethyl cellulose,[9] no effect. Hydrophobization of SEGX resulted in a small increase of foamability, whereas in the case of GX, the foamability reached a value reported for the nonylaminated tamarind polysaccharide[9] and was comparable with that of a commercial whipping protein.[9]

In contrast to the foaming properties, the surface activity of La-SEGX was higher than that of La-GX. As previously described, these xylan derivatives showed no substantial differences in their molecular properties (Table I). It seems likely that structural differences rather, than molecular properties, make important contributions to the observed interfacial properties. First, in the parent GX, the degree of substitution with carboxyl groups (from the uronic acid) is low ($DS_{UA} = 0.1$), and the degree of substitution with both carboxyl and sulfoethyl groups in SEGX is substantially higher (0.6) (i.e., on average, every second xylose unit in the polymer backbone bears one anionic group). This may influence the viscosity effects. Second, because distribution of the uronic acid side chains of GX is nonuniform,[18] the distribution of sulfoethyl groups in SEGX should be, due to the higher DS_{SE}, rather uniform. These structural features may contribute to the lower surface tension of La-SEGX in comparison with that of La-GX. Also, in the case of cellulose alkyl ethers,[28] the lowest interfacial tension values were obtained

Table IV Foaming Activity of the Xylan Derivatives

Compound	Concentration (g/100 mL)	Foam Volume (vol %)[a]
SEGX	1.0	8
La-GX	1.0	240
La-SEGX	1.0	24
BSA	2.0	520
Nonylaminated tamarind (see ref. 9)	0.5	250
Gum arabic (see ref. 9)	1.0	80
Carboxymethyl cellulose (see ref. 9)	1.0	0
D100 whipping protein (see ref. 9)	1.0	300

[a] Related to the original liquid volume.

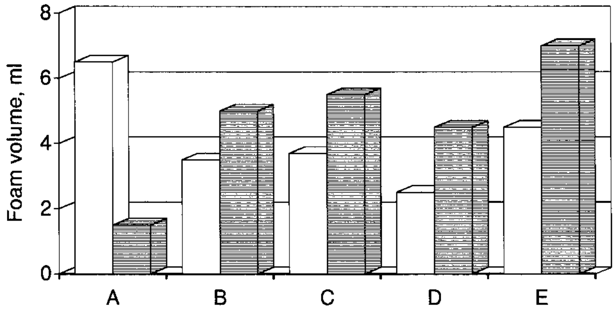


Figure 5 Effect of the GX derivatives and control on the foam volume of BSA before and after heating (90°C, 3 min). (A) BSA, (B) La-GX, (C) SEGX, (D) La-SEGX, and (E) xanthan.[21]

for derivatives that exhibited the most uniform distribution of substituents.

Because arabinoxylans were reported to stabilize protein foams during heating,[21,29] the effect of the xylan derivatives on the foam volume of a surface active protein (BSA) before and after heating was assessed. The results are demonstrated in Figure 5. The depression of the initial foam volume of BSA was the highest in the case of La-GX and lower for both SEGX and La-SEGX. However, the foam-stabilizing effect increased in the order La-GX $<$ SEGX $<$ La-SEGX. The stabilizing effect of all presented xylan derivatives was in the range reported for the wheat arabinoxylan fraction, having the highest viscosity and lowest degree of branching.[9]

The protection effect in foam stabilization is ascribed to the film-forming properties of the polysaccharide and its viscosity. However, note that, in the case of the glucuronoxylan derivatives, this effect may be also influenced by intermolecular interactions between the chains of the acidic polysaccharide and protein resulting in formation of complexes in water solutions, as recently demonstrated for pectin.[30]

CONCLUSIONS

The results suggest that both the primary and fine structure of the xylan chains play an important role in the intermolecular interactions between the molecules in solution, as well as the air–water or oil–water interface. The addition of long alkyl chains to the xylan molecules bearing ionic substituents imparts them amphiphilic characteristics and novel interesting functional properties, such as emulsifying activity and protein-foam stabilizing efficiency and exploitability in practical applications. Anionic functionality of the xylan derivatives enables them to act as sequestrants, builders, and cobuilders in detergents, the same as shown for the dodecyl derivatives of sulfoethyl cellulose[7] and cellulose sulfate.[8] Results indicate the applicability of heteroxylans from hardwoods and annual plants as renewable polysaccharide feedstocks for the production of biopolymeric surfactants.

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