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Cationisation of galactomannan and xylan hemicelluloses

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ABSTRACT

In this study, the cationisation of galactomannan and xylan hemicellulose types was investigated with 2,3-epoxypropyltrimethylammonium chloride (ETA) as reagent, under alkaline conditions. The degree of substitution was accurately determined by ^{1}H NMR spectroscopy after hydrolysis in a DCl/D_2O mixture and confirmed by elemental analyses. The influence of reactional parameters on yield and DS was studied systematically and the reactivity of both polysaccharides was compared. DS values from 0.1 to 1.3 were obtained by varying reactional parameters, the most influential being the hemicellulose concentration and the epoxide amount. The corresponding mass yields varied from 40 to 80% of recovered hemicelluloses and were strongly correlated to DS values. The cationisation of xylan was studied more particularly with a D-optimal experimental design in order to identify interactions between parameters. The optimization of reactional parameters as a function of a desired DS allowed obtaining a maximal grafting rate of 48% with a DS value of 1.3 and 64% of mass yield.

1. Introduction

Hemicellulose is the second most abundant natural polymer in the vegetal world after cellulose, representing 15–35% of plants and wood. Contrary to cellulose which has a unique structure of glucose polymer, hemicelluloses refer to a multitude of heteropolymers composed of different sugar units (D-xylose, L-arabinose, D-galactose, D-glucuronic acid, etc.) depending on the vegetal species (Ebringerová, Hromádková, & Heinze, 2005). Ramifications are variable in terms of frequency and regularity on the backbone, conferring a higher level of complexity to these polysaccharides.

Hemicelluloses are found in large amounts in forest and agricultural residues. In the last decade, there has been a regain of interest to explore potential applications for this available resource but the main difficulty is to extract the hemicelluloses without deteriorating the polymer chain. Therefore, advances have been done in this area, offering new perspectives of utilization (Goksu, Karamanlioglu, Bakir, Yilmaz, & Yilmazer, 2007). However, today, few studies report on chemical modifications of hemicelluloses, compared to other polysaccharides (Ebringerová et al., 2005). We are interested here in their cationisation by grafting a cationic reagent through an etherification reaction.

Cationic polysaccharides have been known since the 70s in several applications. Derivatives of starch, cellulose (Rodríguez, Alvarez-Lorenzo, & Concheiro, 2001; Song, Sun, Zhang, Zhou, & Zhang, 2008) and galactomannan with low degrees of substitution (DS up to 0.2) are now important commercial products. As far as hemicelluloses are concerned, cationic derivatives of galactomannans from plant seed (guar or xanthan gums) are commercially available with DS lower than 0.1 (Dasgupta, 1994). Higher DS (0.2–1) have been obtained in isopropyl alcohol/water media (Cottrell, Martino, & Fewkes, 2001) or in pure aqueous media (Pal, 2007) for potential use in cosmetic and food industries.

More recently, some reports described the cationisation of non-commercial extracted hemicelluloses. The common procedure of cationisation consists in the reaction of the polymer with a cationic reagent by etherification of hydroxyl functions in alkaline medium. Ebringerova et al. have investigated cationisation of xylans extracted from different plant sources (Ebringerova, Hromadkova, Kacurakova, & Antal, 1994) in aqueous media with 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHMAC) as etherifying agent, giving DS from 0.1 to 0.5. The same group also reported the heterogeneous cationisation of commercial birchwood xylan (Schwikal, Heinze, Ebringerová, & Petzold, 2005) in alkaline water-dimethoxyethane slurry, with 2,3-epoxypropyltrimethylammonium chloride (ETA) as etherifying

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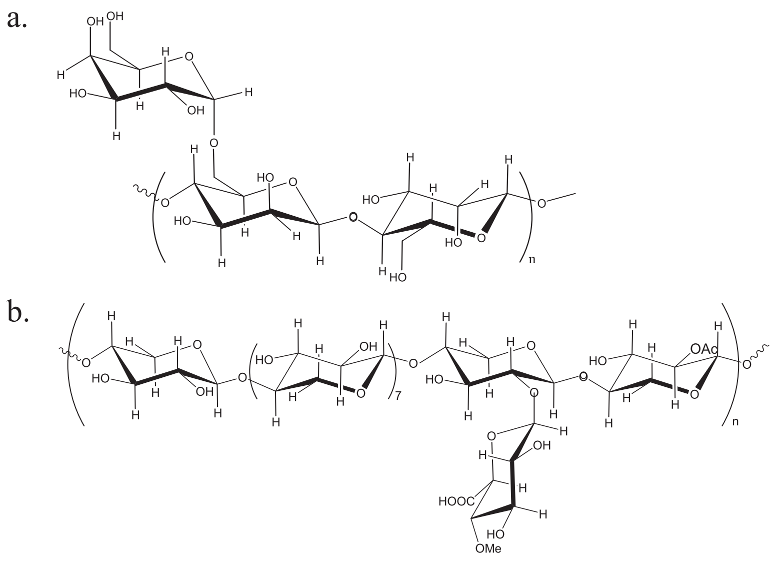


c.

	Guar gum	Birchwood xylan
Main sugar	Mannose, Galactose	Xylose
Branching	High (Man:Gal 2:1)	Low (10%)
DP	6000	200
OH-groups/sugar (DS_{max})	3	2

Fig. 1. (a) Guar gum structure, (b) birchwood xylan structure and (c) summary of their characteristics.

agent and DS from 0.2 to 1.6 were obtained. Ren et al. have investigated the cationisation of hemicelluloses extracted from sugarcane bagasse (xylose as main sugar) with the two etherifying agents CHMAC and ETA (Ren, Sun, Liu, Chao, & Luo, 2006), in different reaction media. The reaction was more efficient in homogeneous phase, preferentially in aqueous phase (Ren, Liu, Sun, She, & Liu, 2007). A maximum substitution degree of 0.54 has been attained by using 1 equiv./OH of epoxide and 0.5 equiv./OH of NaOH.

In this study, we focused on the preparation of cationic hemicelluloses with various degrees of substitution. The reactivity of two commercial model hemicellulose types representative of the main families (mannan and xylan) was compared. These hemicelluloses differ in source, structure, and degree of polymerization. Guar gum was chosen as galactomannan type. Its structure consists in a backbone of β-1,4-D-mannopyranosyl units, branched with α-1,6-D-galactopyranosyl unit every two units (Fig. 1), yielding a ratio mannose/galactose of 2:1. This hemicellulose is characterized by an important degree of polymerization (about 6000) (Tuinier, ten Grotenhuis, & de Kruif, 2000) and a high level of regular ramifications. This polysaccharide forms a very viscous gel in water even at low concentration. Considering the predominant presence of hexose units, the average number of OH functions per sugar unit of this substrate is 3 (Fig. 1a). The second polysaccharide is a xylan type extracted from birch wood. This is a more linear polymer consisting in β-1,4-D-xylopyranosyl units as backbone, with 10% of acetylated hydroxyl groups and containing 10% of 4-*O*-methyl-D-glucuronic acid (Fig. 1b). Its degree of polymerization is relatively low: about 200 (Teleman, Larsson, & Iversen, 2001). The xylan forms a suspension in water and is soluble in hot alkaline water. The average number of OH functions per sugar unit is 2 as it is formed mainly of pentose units (Fig. 1).

The cationisation of the two polysaccharides was achieved through an etherification reaction involving the cationic reagent ETA. The influence of reactional parameters on the degree of substitution and the mass yield was studied. The case of xylan was detailed with a D-optimal experimental design in order to identify the most influent parameters and their interaction and to optimize the reaction conditions depending on expected degrees of substitution.

2. Experimental

2.1. Materials

Guar gum (Ref G4129), birchwood xylan (Ref X0502) and 2,3-epoxypropyltrimethylammonium chloride (ETA, Ref 50053, >90%) were purchased from Sigma–Aldrich. Water content was determined by drying both hemicelluloses in an oven at reduced pressure for 16 h at 80 °C: 8% for guar gum, 4% for xylan. Absolute ethanol (99%) was purchased from Carlo Erba.

2.2. Preparation of cationic hemicelluloses by etherification

2.2.1. Etherification of guar gum hemicellulose

In a typical synthesis, 0.36 g (2 mmol sugar/6 mmol hydroxyl groups) of guar gum was dispersed in 13 mL of distilled water and the suspension was heated at 60 °C for 20 min to form a gel. A fixed amount of sodium hydroxide dissolved in 2 mL of distilled water was added to activate the substrate for 20 min at 60 °C. Then glycidyl trimethylammonium chloride (ETA) was added and the mixture was stirred for 5 h at 60 °C. After cooling to room temperature, 50 mL of ethanol were added dropwise under vigorous stirring to precipitate the cationised guar gum. After filtration and washing with ethanol, the crude product was recovered and dried for 16 h at 80 °C under reduced pressure. The dry product was characterized by ^{1}H NMR spectroscopy to determine its degree of substitution (DS) and the yield was calculated from the mass.

2.2.2. *Etherification of xylan birchwood hemicellulose*

In a typical synthesis, 1 g (7.6 mmol or 15.2 mmol hydroxyl functionality) of xylan was dispersed in 22 mL of distilled water and the suspension was heated at 60 °C for 20 min. A fixed amount of sodium hydroxide dissolved in 3 mL of distilled water was then added to activate the substrate for 20 min at 60 °C. Then a specific quantity of glycidyl trimethylammonium chloride was added and the mixture was stirred for 5 h at 60 °C. After cooling to room temperature and neutralization to pH 5, 50 mL of ethanol were added dropwise under stirring to precipitate the cationised hemicelluloses. After filtration and washing with ethanol, the crude product was collected and dried in a reduced pressure oven for 16 h at 80 °C. After a first analysis by ^{1}H NMR spectroscopy, the crude product was purified by solubilisation in 8 mL of water at ambient temperature and precipitation in ethanol. After filtration and washing with ethanol, the purified product was collected and dried in a reduced pressure oven for 16 h at 80 °C. The dry product was characterized by ^{1}H NMR spectroscopy to determine its degree of substitution (DS) and the yield was calculated from the mass.

2.3. *Characterization of native and modified hemicelluloses*

2.3.1. *^{1}H and ^{13}C NMR spectroscopy*

The liquid-state ^{1}H and ^{13}C NMR spectra were obtained on a 250 MHz Bruker spectrometer at 25 °C. The ^{1}H NMR spectra of both hemicelluloses were recorded from 10 mg of sample dissolved in 1 mL of D_2O (192 scans), or after hydrolysis of 10 mg dissolved in a DCl/D_2O mixture (32 scans). The procedure of hydrolysis was as follow: a solution of 10 mg of product in 0.2 mL of 35% DCl/D_2O was heated at 100 °C for 1 min. The yellow solution was then diluted with 0.7 mL of 99% deuterium oxide and the spectrum was recorded directly from this mixture, at ambient temperature. The ^{1}H NMR analysis after hydrolysis in DCl/D_2O was preferred to determine degrees of substitution with a better accuracy than in D_2O. The ^{13}C NMR spectra were recorded after hydrolysis of 40 mg of sample in a DCl/D_2O mixture by the same procedure (3000 scans).

2.3.2. *Infrared spectroscopy*

FT-IR spectra were obtained on a Fourier transform infrared (FT-IR) spectrophotometer (Bruker Vector 22) using a KBr disc containing 1% (w/w) of solid product. All the spectra were obtained by accumulation of 16 scans, with a resolution of 4 cm^{-1}, at 400–4000 cm^{-1}.

2.3.3. *Elemental analyses*

Nitrogen contents of the products (N %) were measured at the Service Central d'Analyse (CNRS, Solaize). The degree of substitution determined by nitrogen contents according to Eq. (1) could be compared with results from ^{1}H NMR spectroscopy:

Table 1
Level limits of the five variables studied in D-optimal design.

	Variable	Unit	Lower level (−1)	Higher level (+1)
A	Xylan Concentration	g/L	40	200
B	Epoxide	equiv./OH	1	5
C	NaOH	equiv./OH	0.1	1.2
D	Time	h	1	5
E	Temperature	°C	30	80

$$\mathrm{DS} = \frac{M_S \times N\%}{14 - 151.5 \times N\%} \qquad (1)$$

M_S: molar mass of sugar by taking into account the water content.

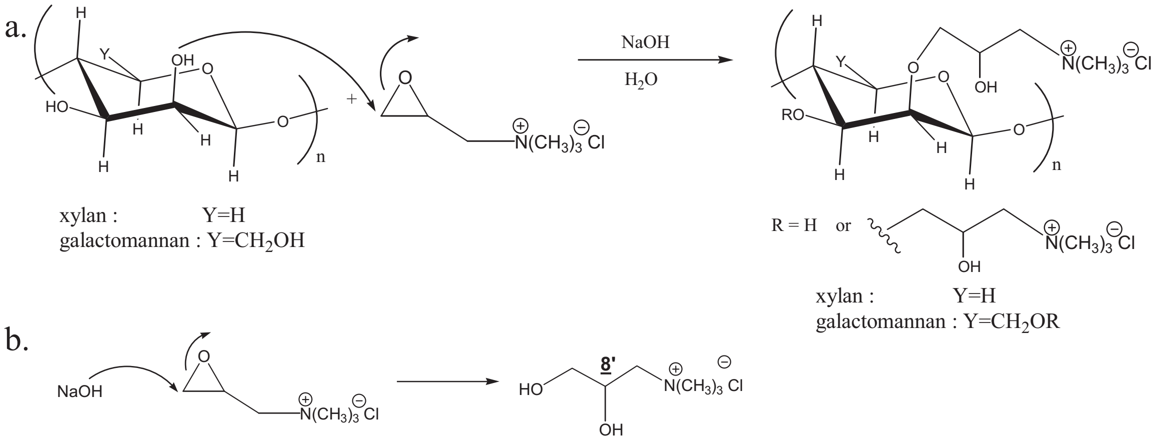
2.4. *Experimental design*

A D-optimal experimental design was carried out on xylan cationisation in order to identify interactions between reactional parameters and optimize the conditions. Five variables were considered: xylan concentration (A), epoxide (B) and sodium hydroxide (C) amounts, time (D) and temperature of reaction (E). Their levels are listed in Table 1. The two responses to study were DS value and mass yield. Data were analyzed through multi-linear regression. A quadratic model with interactions was fitted to the data, for each response. The statistical significance of the coefficients of the model was determined by analysis of variance (ANOVA). Models were built by a backward elimination technique, with a significance level to exit of 0.05. Optimum reaction conditions for chosen DS values were identified through numerical optimization. All calculations were performed using the Design-Expert for Windows software, version 7 (Stat-Ease).

3. Results and discussion

The reactivity of the two polysaccharides towards cationisation by etherification was compared according to Scheme 1a.

The reaction consists in grafting a cationic group on the hemicellulose backbone, by etherification of the hydroxyl functions. The reaction efficiency is characterized by the degree of substitution (DS) which corresponds to the number of cationic group grafted per sugar unit. The maximal degree of substitution (DS_{max}), achieved when all hydroxyl groups are etherified, is different for the two hemicelluloses since they do not have the same number of OH groups available on their backbone (Fig. 1).



Scheme 1. (a) Cationisation of the hemicelluloses and (b) concurrent reaction forming the by-product.

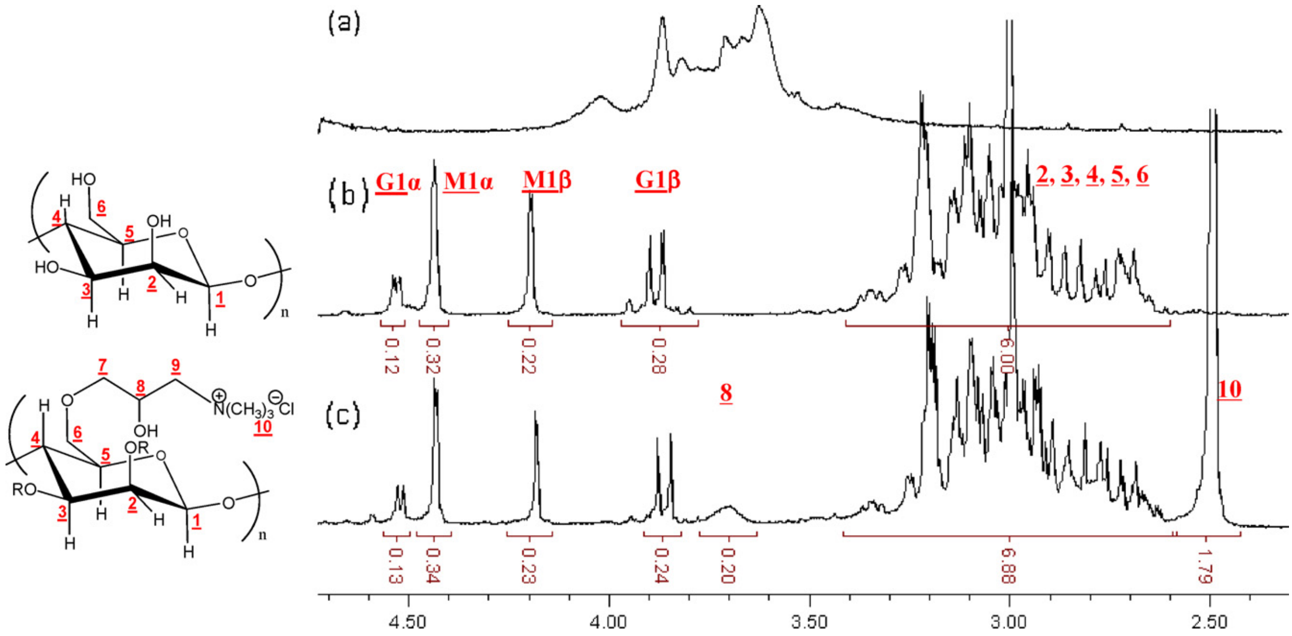


Fig. 2. ^{1}H NMR spectra of native guar gum (a) in D_2O, (b) in DCl/D_2O, and (c) cationic guar gum (DS = 0.2) in DCl/D_2O.

3.1. *Characterizations*

3.1.1. *^{1}H NMR spectroscopy*

^{1}H NMR spectroscopy was used to characterize native and cationic hemicelluloses and to determine the degree of substitution. When the analysis was done in D_2O (Figs. 2a and 3a), we noticed that the low resolution due to the polymeric structure prevented us to determine the DS with good accuracy and repeatability. To overcome this uncertainty, the NMR experiments were performed in DCl/D_2O in order to hydrolyze in situ the hemicelluloses into monosaccharides and to achieve therefore a better spectrum resolution.

In resolved spectra obtained from hydrolyzed native and cationic hemicelluloses in DCl/D_2O (Figs. 2 and 3), signals of anomeric protons from monosaccharides are observable from 3.8 to 4.6 ppm, indicating the complete hydrolysis of hemicellulose. In the galactomannan spectra, 4 signals are identified in the anomeric zone, corresponding to α and β conformations of galactose and

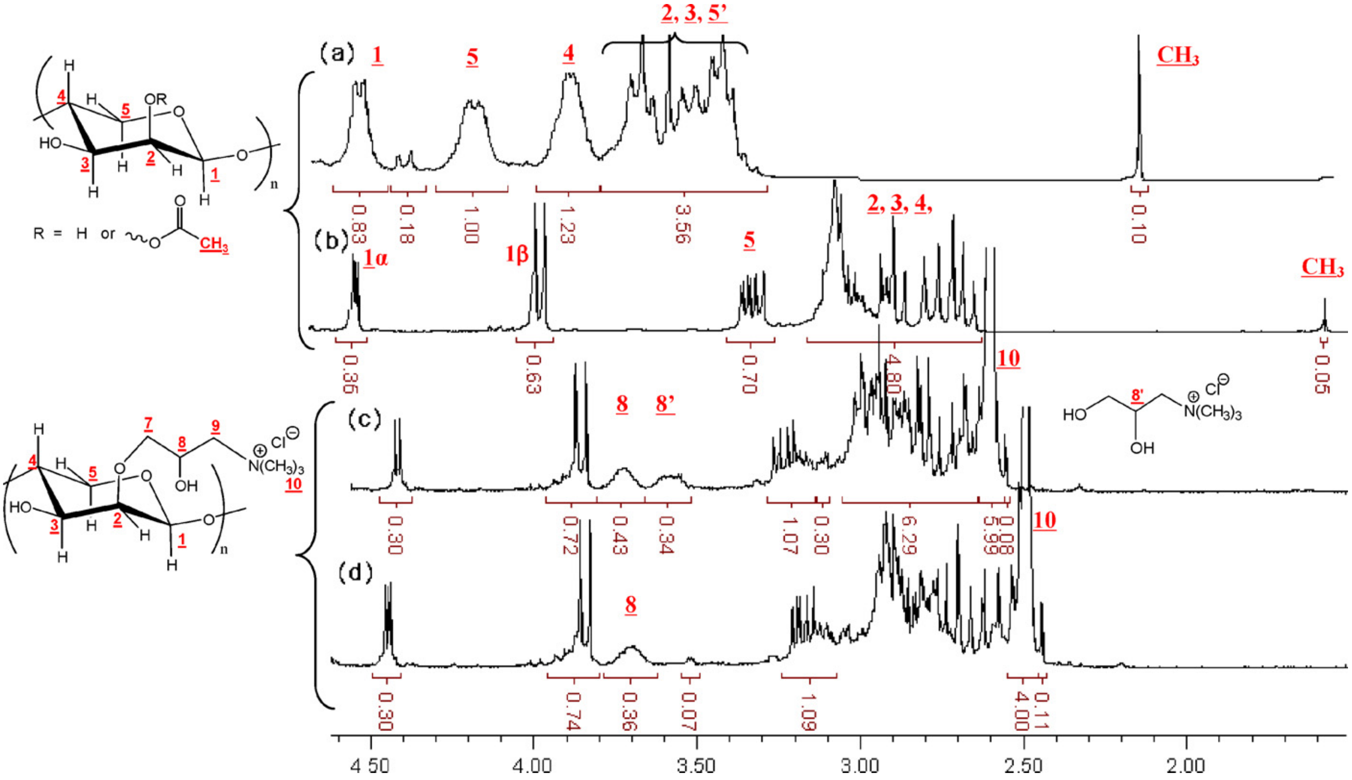


Fig. 3. ^{1}H NMR spectra of native xylan (a) in D_2O, (b) in DCl/D_2O, (c) cationic xylan before purification, and (d) after purification.

Table 2
Comparison of DS values obtained by elemental analyses and ^{1}H NMR spectroscopy.

	Water content (%)	M_S[a] (g/mol)	%N[b]	$DS_{\%N}$	DS_{NMR}
Cationic guar gum	8	180	1.83	0.29	0.25
Cationic xylan	4	140	2.37	0.32	0.35

[a] Molar mass of sugar by taking into account the water content.
[b] Nitrogen content of the product obtained by elemental analyses.

mannose (Fig. 3b). The ratios G_α/G_β (30/70) and M_α/M_β (60/40) calculated from relative integrations of the 4 signals correspond to the percentage compositions of mannose and galactose sugars in aqueous solutions at equilibrium (Collins & Ferrier, 1995). In hydrolyzed xylan spectra, the two anomeric protons from α and β xylose are displayed in Fig. 3b with the expected ratio X_α/X_β: 65/35. The singlet at 2 ppm corresponding to the methyl group of acetyl functions shows that 10% of hydroxyl groups are acetylated.

The spectra of both cationic hemicelluloses show additional signals corresponding to the grafted chain. A singlet corresponding to the protons **10** of the methyl groups of the ammonium is observed at 2.5 ppm (relative integration 9) and a broad signal appears at 3.75 ppm corresponding to the central proton **8** of the lateral chain (relative integration 1). The degree of substitution can be directly determined from the relative integration of protons **8** or **10** to the integration of the anomeric protons.

In the case of cationic xylan spectrum, a supplementary signal appeared at 3.54 ppm (Fig. 3c), that we attributed to proton **8**′ of the by-product formed during the reaction (Scheme 1b).

Unlike the cationic guar gum which precipitated as a slightly yellow powder with ethanol addition, cationic xylan precipitated as viscous aggregate. Then, some by-product could be trapped with aggregate and co-precipitated with the product. The purification of the cationic xylan was carried out by solubilisation of the residue in water followed by selective reprecipitation of the polymer in ethanol. With this procedure, one step of washing was enough to completely purify the product, as shown in spectrum d (Fig. 3d) with the disappearance of the additional signal.

DS values calculated from elemental analyses (%N) are in accordance with ^{1}H NMR spectroscopy, as reported in Table 2.

By comparing FT-IR spectra of native and cationic polysaccharides (data not shown), the introduction of the cationic pattern was confirmed by the peak at 1470 cm^{-1} in the cationic spectrum corresponding to the H–C–H bending mode of the methyl groups of the ammonium (Yu, Huang, Ying, & Xiao, 2007).

The ^{13}C NMR spectra of cationic products clearly showed the appearance of news signals characteristic of the cationic chain. More specifically, the methyl groups of the ammonium exhibited a strong signal at 53.9 ppm. Smaller additional signals corresponding to the three carbons of the chain were observed at δ 67.8 (C8), 68.5 (C9) and 70.5 (C7) (Ebringerova et al., 1994; Heinze, Haack, & Rensing, 2004).

3.2. *Systematic study*

Cationisation of both hemicelluloses was carried out in alkaline aqueous media, according to the procedure described by Ren et al. for bagasse hemicellulose (mainly xylan type) (Ren, Sun, & Liu, 2007). ETA was chosen as etherifying agent since the reaction is more efficient by introducing directly the reactive epoxide than forming it in situ from CHMAC (Ren et al., 2006). Hydroxyl functions were firstly activated at 60 °C during 20 min under basic conditions, before reaction with the epoxide at 60 °C for 5 h to form the cationic polymer. Water was chosen as solvent to perform the reaction in an environmentally friendly homogeneous medium. In the case of guar gum, the reaction medium consisted of a high viscous gel and all experiments were performed at 24 g/L of guar, which correspond to the maximal concentration for the gel to be homogeneously stirred. On the other hand, xylan formed a suspension in water, and was soluble in hot alkaline aqueous medium up to 200 g/L.

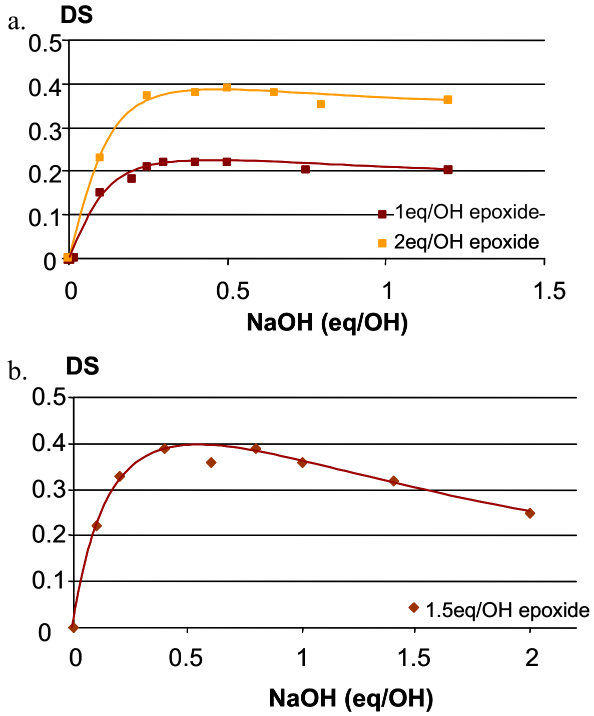


Fig. 4. (a) DS values for cationic guar gum in function of NaOH using 1 and 2 equiv./OH epoxide (60 °C, 5 h, 24 g/L) and (b) DS values for cationic xylan in function of NaOH using 1.5 equiv./OH epoxide (60 °C, 5 h, 40 g/L).

The same procedure of cationisation was used for the two polysaccharides but a supplementary purification step was necessary for xylan as discussed above. The influence of sodium hydroxide and epoxide amounts on yield and DS was studied for both hemicelluloses. For a better understanding, NaOH and ETA amounts are expressed in equivalent number per OH function of hemicellulose (equiv./OH).

3.2.1. *Influence of NaOH concentration*

3.2.1.1. Guar gum. The influence of sodium hydroxide amount was studied for two epoxide amounts: 1 and 2 equiv./OH corresponding to 3 and 6 equiv./mannose unit. Firstly, we clearly observed that no reaction occurred in the absence of NaOH, which confirms the absolute necessity to activate the hydroxyl functions of the saccharide. Whatever the epoxide amount, the DS evolution followed the same profile: from 0 to 0.25 equiv./OH of sodium hydroxide, the DS increased linearly with NaOH concentration to reach a plateau (Fig. 4a). A further increase of NaOH ratio did not allow increasing the DS. At the plateau value, the yield based on epoxide was almost the same in both cases: 7.3% (DS = 0.22) and 6.5% (DS = 0.39) for 1 and 2 equiv./OH of epoxide, respectively.

To explain this behavior it must be considered that sodium hydroxide catalyses two competitive reactions: the activation of hydroxyl functions into nucleophilic alkoxide and the epoxide ring opening into the corresponding diol as seen above (Scheme 1b). The optimal amount of sodium hydroxide corresponds to an optimal activation of hydroxyl functions with the minimization of the by-product formation. The best compromise was reached at 0.25–0.3 equiv./OH for the guar gum. Up to this value, the reaction was limited by the activation of hydroxyl functions. When the amount of NaOH increased above 0.25 equiv./OH, the DS did not increase anymore: the degradation of the epoxide became predominant and was in competition with the etherification of the less reactive hydroxyl groups. It is expected that the substitution occurs both in primary and secondary hydroxyl functions (Wu et al., 2010) but unfortunately, we could not determine which hydroxyl functions were etherified preferentially.

3.2.1.2. Xylan. Due to its lower molecular weight, the xylan was soluble in water under basic conditions and preliminary experiments were performed at 40 g/L. The influence of sodium hydroxide was studied with 1.5 equiv./OH of epoxide corresponding to 3 equiv./xylose unit. Fig. 4b shows the DS evolution profile: as for guar gum, from 0 to 0.4 eq of NaOH, a uniform increase of the DS was observed, the optimum being reached at 0.4 eq. With a further increase of NaOH amount, the DS decreased slowly from 0.4 to 0.25 in the presence of 2 eq of NaOH. These results are in accordance with those published by Schwikal et al. (2005) in heterogeneous etherification of birchwood xylan where the maximal DS value was observed at 0.3 equiv./OH of NaOH. The reaction efficiency was greater for xylan than for guar gum as the DS value of 0.4 is attained with a lower excess of epoxide, despite the presence of only secondary OH functions. This could be attributed to the higher concentration and the easier diffusion of the reagent in xylan solution compared to the gel phase of the galactomannan.

At high NaOH concentration, the direct opening of epoxide is faster than etherification of OH functions, driving to lower DS. That means that the kinetic profiles of the reactions are different for the galactomannan and the xylan. Probably, the etherification is slower with xylan because the 2 secondary OH functions are less accessible than primary OH of the galactomannan. Moreover, the side reaction is favoured in xylan solution as diffusion of reagents is easier than in the gel phase of galactomannan.

Then, the optimal amount of sodium hydroxide was similar for guar gum and xylan (0.3–0.4 equiv./OH) in the conditions used but the kinetic of etherification and side reaction are different for the two polysaccharides.

3.2.2. Influence of epoxide amount

The influence of epoxide amount was studied for both polysaccharides in the same conditions (60 °C, 5 h) with an excess of NaOH in order not to be limited by hydroxyl activation. In addition to DS values, the mass yield corresponding to the yield of recovered hemicelluloses after reaction was reported. This yield takes into account the DS value in the molecular weight calculation of the modified polymer (Fig. 5a). Moreover, the grafting percentage was calculated considering the amount of grafted cationic chains over the initial amount of epoxide (Fig. 5b).

As shown in Fig. 5, DS values increase linearly with epoxide concentration, up to 0.85 by using 10 equiv. of epoxide. Nevertheless, the mass yield decreased when DS values increased. This is probably ascribed to the higher solubility in water of products with high DS. Then, their recovery was limited by precipitation upon ethanol addition, resulting in lower yields. In addition, the grafting percentage decreased as the initial epoxide amount increased (5% with 3 eq. epoxide; 2.8% with 10 equiv./OH epoxide), indicating a lower efficiency of the reaction compared to those in stoichiometric amount (7% of grafting). The DS increase was limited compared to the involved amounts of epoxide, which confirmed the limited accessibility to hydroxyl functions. The highest DS (0.85) was achieved using 10 eq. of epoxide, so the grafting rate reached only 2.8%.

In the case of xylan (Fig. 6), the DS increase was similar to guar gum by increasing epoxide amount, with a greater slope which is due to the higher medium concentration and easier diffusion of the reagent (as for sodium hydroxide). A maximum DS value of 1 was obtained by using 6 equiv. of epoxide. A similar phenomenon of

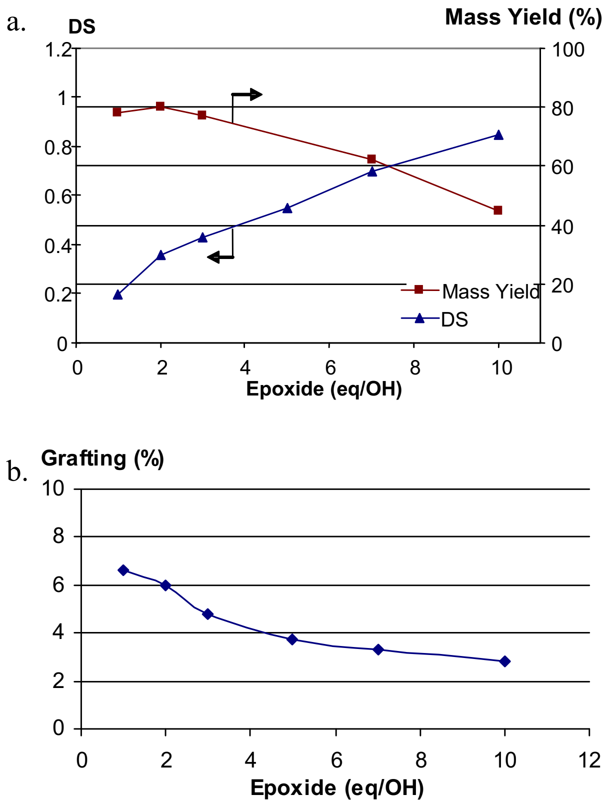


Fig. 5. (a) Yield and DS of cationic guar gum and (b) grafting rate in function of epoxide amount. Conditions: 24 g/L; 1.2 equiv./OH NaOH

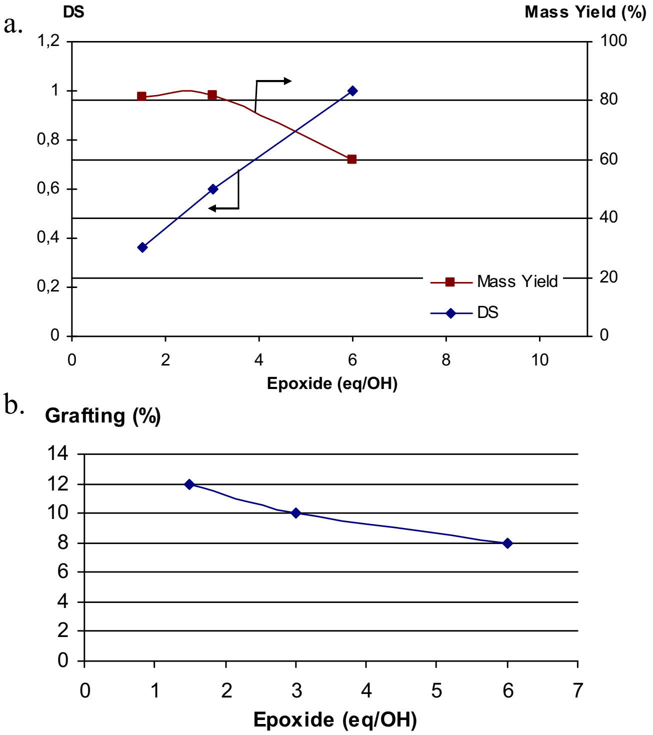


Fig. 6. (a) Yield and DS of cationic xylan and (b) grafting rate in function of epoxide amount. Conditions: 40 g/L; 0.6 equiv./OH NaOH.

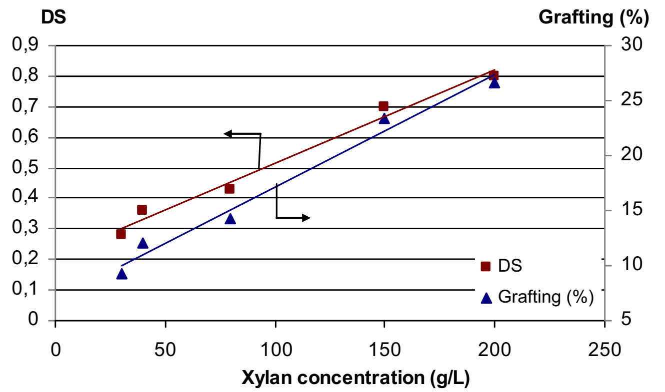


Fig. 7. DS vs xylan concentration. Conditions: 5 h, 60 °C, 1.5 eq epoxide, 0.6 eq NaOH.

Table 3
Yield and DS values of cationic xylans by varying reaction time and temperature. Conditions: 40 g/L, 1.5 eq epoxide, 0.6 eq NaOH.

Time (h)	Temp (°C)	DS	Mass yield (%)
3	60	0.36	92
5	60	0.36	82
15	60	0.33	80
24	60	0.32	70
5	40	0.3	–
5	80	0.32	75

yield decrease was observed by increasing the DS, but the decrease of grafting was less significant than for guar gum: from 12 to 8%. These values are in accordance with those obtained with the xylan of bagasse (Ren, Sun, et al., 2007).

To summarize, high DS derivatives could be prepared by increasing the epoxide concentration but at the expense of yield and reaction efficiency.

3.2.3. Influence of the medium concentration

For guar gum hemicellulose, the medium concentration could not be investigated because of the gel viscosity (maximum concentration of 24 g/L). In the case of xylan, the concentration could be increased up to 200 g/L for which one the medium is highly viscous. The concentration of the medium was done by reducing the solvent volume, thus the concentration of all species was increased. As shown in Fig. 7, the DS increased linearly with the xylan concentration (other parameters being kept constant, including epoxide amount), with corresponding grafting rates running from 9 to 27%. Then, by working at high concentration, grafting rates can be improved and epoxide amounts can be reduced for a given DS value.

3.2.4. Influence of reaction time and temperature for xylan

The influence of reaction time and temperature on the DS and mass yield was then studied.

As shown in Table 3, nor the reaction time neither the temperature affected significantly the DS in the used conditions, with values from 0.32 to 0.36. However, the increase of reaction time to 15 h and 24 h resulted in lower yields (80 and 70%, respectively). The same phenomenon was observed with an increased temperature of 80 °C, with 75% yield. It was supposed that an extended reaction time or a high temperature in alkaline medium could result in partial depolymerization of xylan into shorter chains, as observed in preliminary SEC analyses. The precipitation of these smaller polymers upon ethanol addition was not complete and therefore the mass yield decreased.

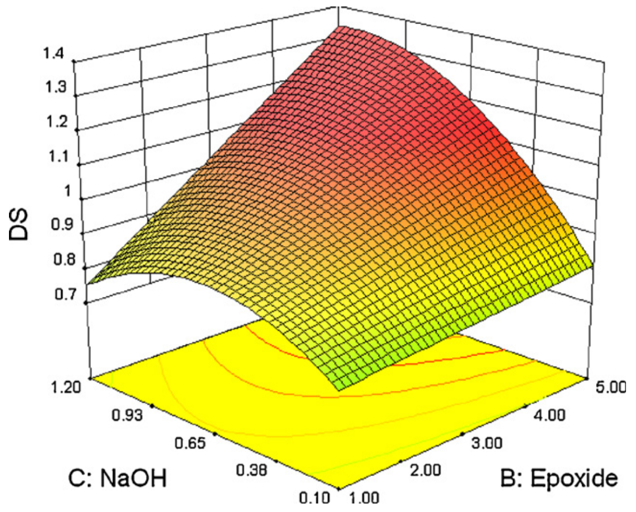


Fig. 8. Surface plot of DS as a function of Epoxide and NaOH amounts. Conditions: A: 120 g/L; D: 3 h; E: 55 °C.

3.3. Experimental design

From these observations, we decided to study the cationisation of xylan more specifically with an experimental design in order to identify possible interactions between parameters and to detect new effects. Previous experiments allowed us to select 5 parameters to study and their levels (Table 1). The two responses to be considered were the DS value and the mass yield for each experiment. The Design-Expert software generated a 31 runs experimental design and their responses are presented in Table 4.

Limits of each parameter were chosen according to previous experiments. The concentration was limited from 40 g/L to 200 g/L to have a homogeneous stirring of the mixture. Concerning the epoxide, its amount was increased up to 10 equivalents in the systematic study but this value is actually too high in term of reaction efficiency. Thus we decided to set the higher level of epoxide to 5 equivalents in the experimental design, to cover a wide range of experimental conditions, knowing that the lower amount of epoxide would be desirable. The NaOH amount was limited to 1.2 equivalents since we observed that DS values decreased when a higher amount of sodium hydroxide was used (Fig. 4b). Time was fixed from 1 to 5 h and temperature from 30 to 80 °C.

3.3.1. Analysis of DS model

The model for the DS response was significant ($F = 27.45$), with an Adj-R^2 value of 0.92 and a reasonably close value of Pred-R^2 of 0.84. For this model, only runs 5 and 29 were ignored since the pertinence of the model is lower when they are included.

Coefficients of selected significant effects (remaining after Backward elimination) are presented in Fig. 9a. As expected, the 5 parameters influenced the substitution degree. The epoxide amount (B) had a linear influence (no quadratic term in the model) which is consistent with the systematic study. Moreover, four interactions were identified: The two more significant being the interactions epoxide–NaOH (BC), already mentioned in the systematic study, and epoxide–temperature (BE) (Fig. 11).

The effect of epoxide and NaOH amounts on DS is represented by a surface plot in Fig. 8. The interaction between the two parameters is obvious as the optimal amount of NaOH varied by increasing the epoxide concentration. Then, with a low epoxide amount, there was no significant evolution of DS by varying sodium hydroxide, which was in accordance with previous experiments (Fig. 4b). Indeed, the

Table 4
Testing matrix and responses of 31 runs D-optimal design.

Run	Factor 1 A: xylan concentration (g/L)	Factor 2 B: epoxide (equiv./OH)	Factor 3 C: NaOH (equiv./OH)	Factor 4 D: time (h)	Factor 5 E: temperature (°C)	Response 1 DS value	Response 2 Mass yield (%)
1	40	1	1.2	1	30	0.27	90
2	120	5	1.2	1	30	0.8	72
3	200	5	0.1	5	30	0.05	100
4	120	1	0.1	1	80	0.42	75
5	200	5	1.2	5	30	1.5	37
6	40	1	0.1	5	80	0.2	80
7	40	5	0.1	1	55	0.26	81
8	200	3	1.2	5	80	0.7	63
9	200	1	0.1	5	30	0.29	71
10	200	1	0.1	3	80	0.56	64
11	200	5	0.1	1	80	0.58	63
12	40	3	1.2	1	80	0.20	80
13	200	1	0.65	5	80	0.61	67
14	40	1	0.1	5	80	0.2	84
15	40	5	0.1	5	30	0.15	80
16	40	5	0.65	5	80	0.8	67
17	40	5	1.2	5	55	0.85	67
18	40	1	1.2	3	80	0.28	84
19	200	5	1.2	1	80	1.14	60
20	40	1	0.1	1	30	0	97
21	80	2	0.375	4	55	0.85	68
22	200	1	1.2	5	30	0.38	57
23	200	1	1.2	1	55	0.35	70
24	120	1	0.1	1	80	0.32	67
25	200	1	0.65	5	80	0.46	74
26	200	1	0.1	1	30	0.15	82
27	40	1	1.2	5	30	0.34	73
28	200	5	0.1	5	80	0.73	69
29	200	5	0.1	1	30	0.18	82
30	80	2	0.375	4	55	0.73	72
31	40	1	1.2	3	80	0.20	82

amount of epoxide was insufficient but above all, the increase of NaOH resulted in direct opening of the epoxide, faster than etherification (at 1.2eq NaOH, NaOH/ETA = 1.2). However, in the presence of a large excess of epoxide (5 equiv.), the competitive reaction stays negligible by increasing NaOH from 0.1 to 1.2 (at 1.2eq NaOH, NaOH/ETA = 0.24), allowing a greater activation of OH functions and a significant increase of DS. Then, there is an optimal ratio NaOH/ETA for each epoxide amount, corresponding to a sufficient activation of OH functions and a negligible opening of epoxide into the corresponding diol.

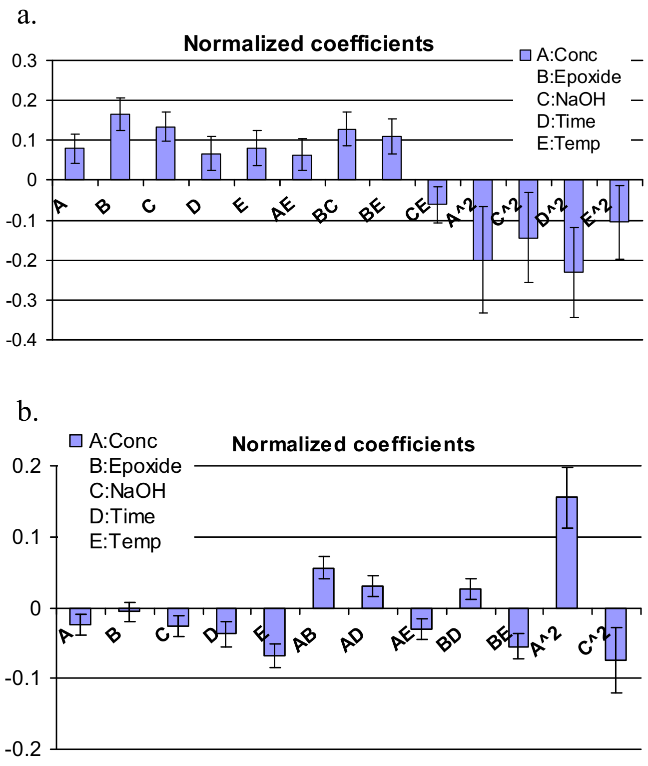


Fig. 9. (a) Normalized coefficients of the DS model and (b) the mass yield model with 95% confidence intervals.

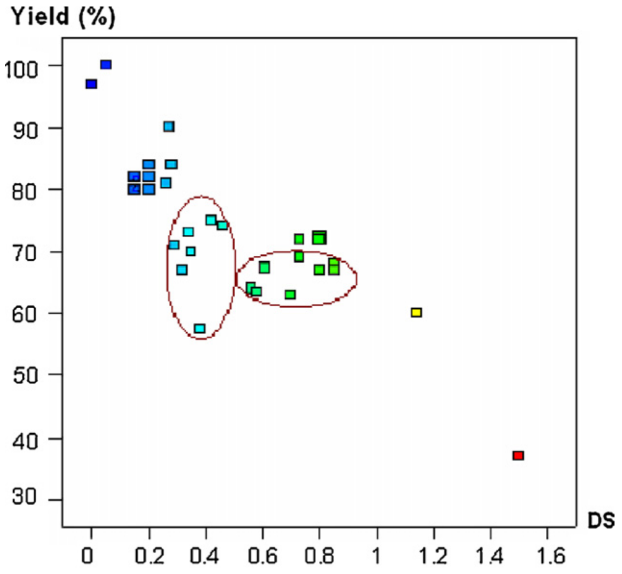


Fig. 10. Correlation between yield and DS for the 31 experiments. Vertical Circle is dispersion in yield for a same DS and horizontal circle is dispersion of DS for a same yield.

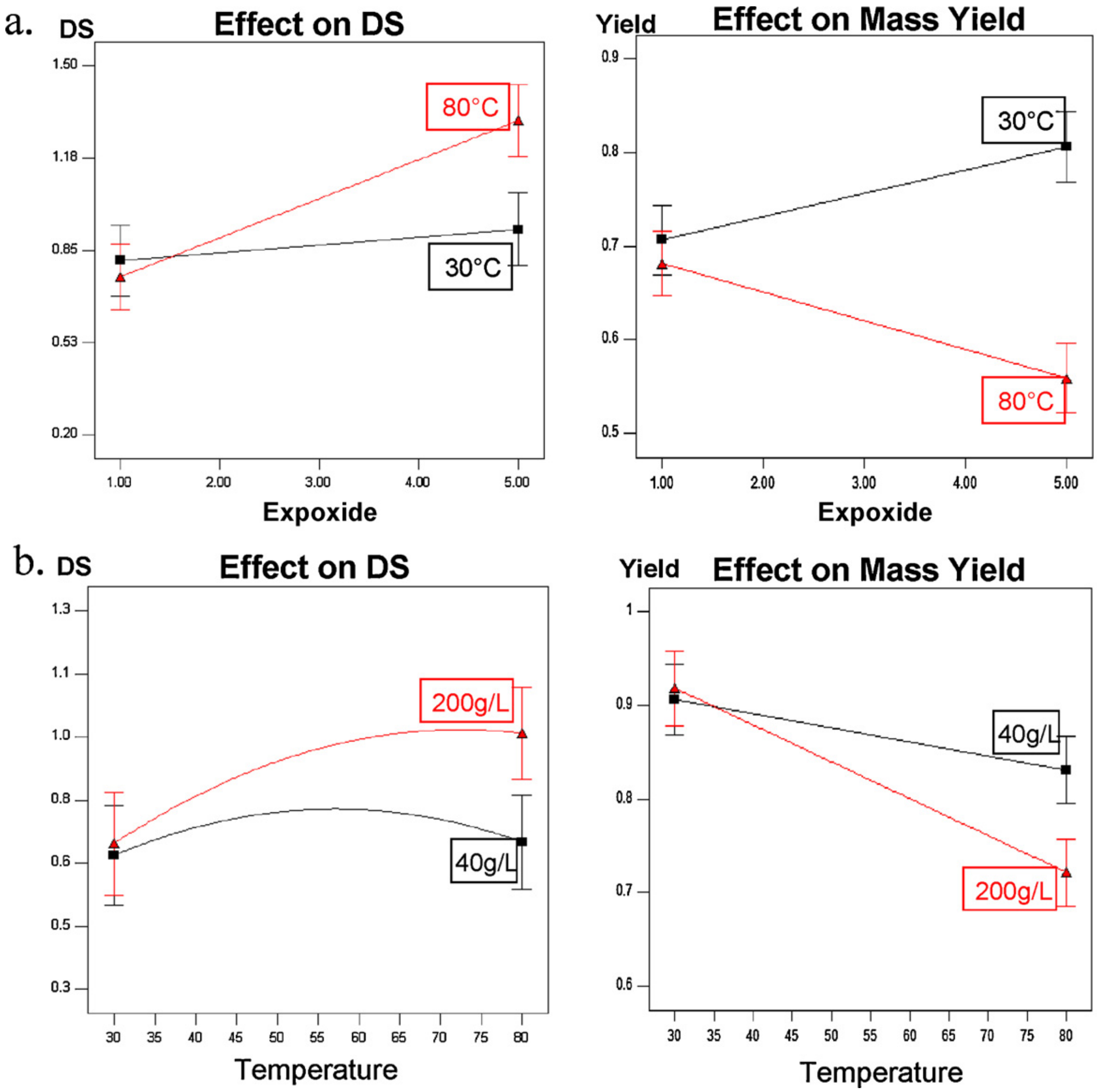


Fig. 11. (a) Effect of the temperature–epoxide interaction on DS and mass yield and (b) effect of the temperature–concentration interaction on DS and mass yield.

3.4. Analysis of mass yield model

The model for the yield response was significant ($F = 24.83$), with an Adj-R^2 value of 0.91 and a reasonably close value of Pred-R^2 of 0.85. For this model, runs 5, 29 and 22 were ignored since the pertinence of the model is lower when they are included.

Selected significant linear effects identified are four parameters A, C, D, E (the epoxide (B) was forced back to enter the model, to maintain model hierarchy, as it is involved in interactions terms only). Five interactions were detected (Fig. 9b). To analyse the mass yield response, the correlation between DS and yield, already observed in previous experiments (Fig. 6a), must be taken into account.

Fig. 10 shows a clear negative correlation between yield and DS (Pearson's $R = -0.83$); the higher the DS, the lower the yield. Then, from the effects detected to influence the mass yield, some of them are indirect or combined effects which mean that an interaction can influence indirectly the yield by its influence on DS. However, the DS is not the only factor to affect the yield as different yields are obtained for a same DS value, and different DS values are obtained for a similar yield (red circles), showing opportunities for process optimization. These observations are in accordance with the normalized coefficients of the 5 individual parameters (Fig. 9) which are positive for the DS model, but negative for the yield model. It is clear that time and temperature parameters are not strongly influential in the case of DS, while they appear to most significantly affect the mass yield, individually or in interaction with others parameters. This could correspond to the supplementary effect, independent of the indirect effect of DS, observed in the correlation graph (Fig. 10). Note that the influence of time and temperature on yield was already observed in the systematic study.

3.4.1. Other interactions

From all identified interactions, two of them were recovered in both DS and mass yield models. That is the case for the interactions temperature–epoxide and temperature–concentration. As far as the interaction epoxide–temperature was concerned (Fig. 11a), it appeared that epoxide was more efficient to increase the DS at high temperature. The inverse effect is observed for the mass yield (more pronounced negative effect of epoxide on yield at high temperature). Similar effects were observed for the interaction xylan concentration–temperature (Fig. 11b) in which a high concentration combined with high temperature yielded a higher DS and lower yield. In both cases, the effect on mass yield was closely correlated to the effect on DS. These inverse effects on DS and mass yield are confirmed with the normalized coefficients, which were

Table 5
Optimized conditions for two DS ranges.

Parameter/response	Goal (importance degree /5)	Optimized conditions	
		DS0.35–0.40	DS0.70–0.75
Concentration	max (3)	200	200
Epoxide	min (5)	1.08	1.35
NaOH	min (3)	0.30	0.56
Time	min (3)	1.5	2.6
Temperature	min (3)	30	41
Pred (exp) DS		0.35 (0.37)	0.7 (1.3)
Pred (exp) yield	max (5)	82 (78)	82 (64)

positive for both interactions in the DS model, and negative for the mass yield model. Nevertheless, it is not excluded that an additional effect, independent of the DS, contributed to the decrease of yield in these two interactions, high temperatures favouring the depolymerization of xylan in basic conditions.

Three other interactions involving xylan concentration, epoxide amount and time are identified to positively influence the mass yield.

This experimental design confirmed the tendencies identified with the systematic study and brings new informations. The interaction NaOH–epoxide is significant and new interactions having an influence on DS were detected such as the interaction temperature–epoxide. Concerning the mass yield, it depends mainly on the DS value but additional effects of time and temperature could also play a role in the decrease of yield. From these observations, it appears that optimized cationisation conditions may be achieved by maximising the concentration, minimising the time and temperature and using an appropriate epoxide–NaOH ratio.

3.4.2. Optimization

Optimization of reactional parameters as a function of a desired DS was performed with the Numerical Optimization function of the Design-Expert software. The goal was to maximise the reaction yield for a given DS. This was achieved by trying to minimise reagents consumption (especially for epoxide since it is the most expensive), time and temperature, while working at the highest possible xylan concentration (to lower overall chemicals cost and heating energy).

Two scenarios were submitted to aim for a low DS (0.35–0.40) and moderate DS (0.70–0.75). Optimized conditions found by the software are listed in Table 5. In both cases, the concentration should be maximised, while epoxide, time and temperature parameters were kept near to their lowest level. New experiments were then carried out in these optimized conditions and corresponding experimental DS and yield values are reported in Table 5. For DS in range 0.3–0.4, a good agreement between predicted and experimental values was obtained with an experimental DS of 0.37 and a yield of 78%. The corresponding grafting rate is 18%. With conditions optimized for a DS of about 0.7, a higher DS of 1.3 was obtained, corresponding to 48% of grafting epoxide. This value was superior to the ones obtained in the systematic study (27% maximum at 200 g/L with DS 0.8 by using 1.5eq epoxide) and to the ones reported in the literature (Schwikal et al., 2005; Ren et al., 2006). A yield of 64% could be maintained despite the high DS value by using mild conditions (41 °C, 2.6 h). For comparison, a yield of 60% was obtained for a DS value of 1 by working at 60 °C for 5 h (systematic study).

The difference between predicted and experimental values is more pronounced. Then the model was reliable for low DS but it underestimated moderate DS values.

From this data, it should be interesting to carry out a new experimental design by restricting the parameters limits, and preferentially to study epoxide amounts lower than 1 equivalent.

4. Conclusions

Two families of cationic hemicelluloses have been synthesized from a galactomannan and a xylan type. DS values from 0.1 to 1.3 have been obtained and accurately determined using a new ^{1}H NMR spectroscopy method. The reactivity of both polysaccharides was compared in a systematic study. The most influential parameters on DS are the concentration of the medium and the epoxide amount, the kinetics of reaction being different for the two polysaccharides. An experimental design on xylan confirmed these tendencies with two reliable models for DS and mass yield, and constituted a good complement to the systematic study. The optimization of reaction parameters could be achieved for both high and low DS. Xylan is a good hemicellulose candidate for this cationisation reaction since it can be used at high concentration which allows decreasing the epoxide amount and thus increasing the reaction efficiency.

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