

Characterization of Crosslinked Starch Materials with Spectroscopic Techniques

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ABSTRACT: The structure, mobility, and properties of crosslinked starch materials of various compositions were investigated with FTIR and NMR spectroscopy, and relaxation time measurements were taken with cross-polarization/magic-angle-spinning (CP-MAS) and magic-angle-spinning (MAS) spectroscopy. Characterization by Fourier transform infrared spectroscopy confirmed the crosslinking reaction. The CP-MAS and MAS spectra allowed the assignment of the principal ¹³C signals. The molecular mobility of these polysaccharides was analyzed in terms of the cross-relaxation time between the protons and carbons, the ¹H spin-lattice relaxation time in the rotating frame, and the ¹H

longitudinal spin-lattice relaxation time. Relaxation studies showed that increasing the crosslinking degree increased the amorphous content, and the material became rigid as an increasing number of covalent bonds in the polymer network reduced mobility. The values of the spin-lattice relaxation in the rotating frame reflected the homogeneous nature of the materials. The correlation between the crosslinking degree, structure, and mobility and the sorption properties of these sorbents was examined. © 2004 Wiley Periodicals, Inc. *J Appl Polym Sci* 93: 2650–2663, 2004

Key words: crosslinking; NMR; relaxation

INTRODUCTION

Starches, naturally occurring polysaccharides, are among the most widely used carbohydrate polymers because of their safety and low cost. Starches possess several other advantages and characteristics that make them excellent materials for industrial use. For example, starches are unique raw materials in that they are very abundant natural polymers, widely available in many countries and renewable. They also have biological and chemical properties such as nontoxicity, polyfunctionality, and high chemical reactivity. However, despite these properties, some problems can occur. The hydrophilic nature of starches is a major constraint that seriously limits the development of starch-based materials. Chemical derivatization has been proposed as a way of solving this problem and of producing water-resistant materials. For many applications, starches need to be modified to prevent deg-

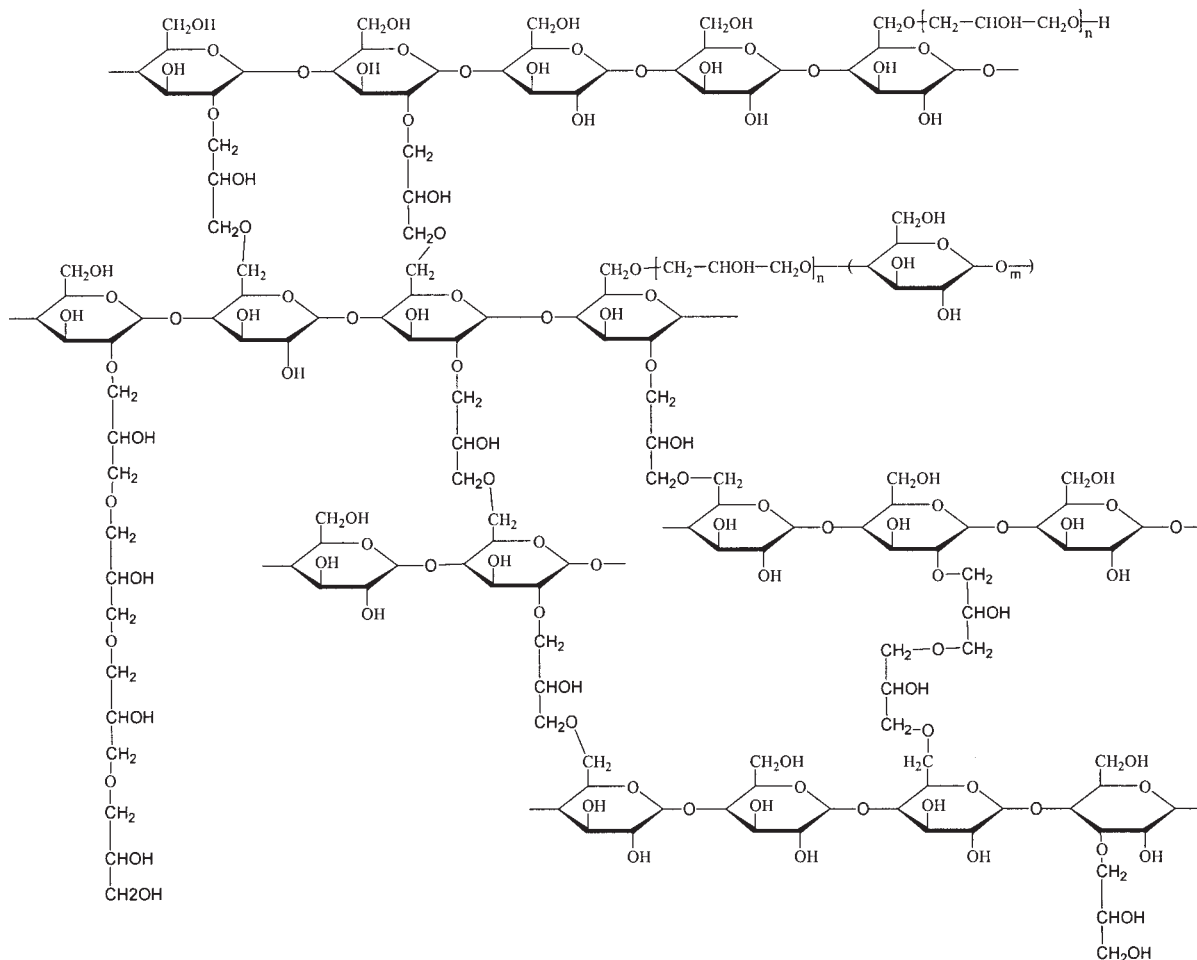
radation and to improve certain chemical and physical properties.

Crosslinking starches is the most common method used in polysaccharide chemistry. Crosslinking occurs when a crosslinking agent introduces intermolecular bridges and/or crosslinks between polysaccharide macromolecules. Epichlorohydrin (EPI) is the most popular crosslinking agent. Crosslinking starches with EPI is a well-documented reaction and a relatively easy method of preparing polysaccharide-based derivatives. Abundant information on this reaction can be found in the literature. However, although the crosslinking reaction between EPI and polysaccharides has been known for 80 years,¹ some basic questions concerning this reaction continue yet to interest the scientific community.

The industrial uses of chemically modified starches are numerous. In particular, crosslinked starch materials (gels, microbeads, polymers, films, composites, and hydrophilic networks) are widely used in biomedical and pharmaceutical research^{2,3} (e.g., for the transport of molecules, excipients, and hydrogels), in the food industry⁴ (as additives, thickeners, and aroma retainers), and in other fields such as wastewater treatment^{5–7} (as sorbents for decolorization, chelation of pollutants, or extraction of metals). Several

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Scheme 1 Possible structure for a crosslinked starch-enriched flour polymer.

polymeric insoluble materials are commercial products, such as Contramid.

In the literature, there are numerous studies^{8–17} on the synthesis and characterization of crosslinked materials with EPI, and in these polymers, two kinds of structures exist: starch crosslinked by EPI due to the crosslinking reaction between the glucose units and epoxide and polymerized EPI due to the homopolymerization reaction of EPI with itself. However, because they contain only glucose units and hydroxyl groups, the structures of these polymers are complicated: many glucose units are interconnected, and a three-dimensional network is formed (Scheme 1).

In our previous studies,^{18–20} we focused our attention on the determination of the sorption properties of crosslinked starch derivatives. We presented the syntheses of several new materials containing starch and their applications as sorbents to wastewater treatment. We chemically modified starch-enriched flour to valorize an industrial agro alimentary byproduct. These polymers were prepared by a crosslinking reaction of flour with EPI as a crosslinking agent and afterward were used for the recovery of various organic pollut-

ants (e.g., phenol derivatives and dyes) from aqueous solutions. The sorption experiments showed that they had high sorption capacities. We demonstrated that the crosslinking agent plays an important role in the mechanism of sorption. The sorption is more important for polysaccharides that have chemical structures compatible with the aromatic compound. Now, our aim is to determine the relationship between their retention behavior and molecular structure to obtain detailed knowledge of the chemical structure and molecular mobility of different crosslinked starch derivatives.

Under the experimental conditions, this polymer network is highly hydrophilic, swells strongly in aqueous solutions, and is insoluble in organic solvents. Polymers and related gel structures have been characterized by their water-retention, density, chemical and mechanical stability, and thermal transitions with several methods, such as Fourier transform infrared (FTIR),^{1,11} X-ray diffraction analysis,^{1,14} differential scanning calorimetry,⁸ fluorescence,²¹ and enzymatic degradation.¹⁵ High-resolution solid-state NMR is also known to be a rich source of information about

TABLE I
Determination of the Soluble Part and Glucose Percentage Present in the Polymers

	EPI ^a	EPI/glucose ^b	Soluble part ^c	Glucose ^d
Flour	0	0	100	90
Polymer 1	10	5.2	100	58
Polymer 2	20	10.4	100	43.2
Polymer 3	35	18.2	80	33.2
Polymer 4	50	26	73.5	32
Polymer 5	60	31.2	62.9	17.7
Polymer 6	70	36.4	40	15.5

^a EPI (mL) incorporated during the synthesis.

^b Molar ratio in the synthesis.

^c Percentage after the hydrolysis step.

^d Percentage in the soluble part.

the polymeric materials themselves, particularly when their structures and molecular mobilities are concerned.^{22–30} Hartmann–Hahn cross-polarization (CP), combined with magic-angle spinning (MAS) and dipolar decoupling (DD), has been widely applied in NMR studies of solids, including crosslinked polymers and gels. This useful technique not only permits us to identify the structure and to characterize the different kinds of polymers but also is sensitive to different molecular mobilities. Moreover, individual relaxation times give information on the local mobility.

In this study, the method introduced by Dubois et al.^{31,32} was used to quantify the amount of glucose in the products. The industrial flour and the crosslinked starch derivatives were investigated with FTIR spectroscopy and ¹³C-NMR spectroscopy techniques such as cross-polarization/magic-angle spinning (CP-MAS) with DD and MAS. The FTIR and NMR spectra were examined. The homogeneity and molecular mobility of these polysaccharides was also analyzed in terms of relaxation parameters, such as the ¹H spin-lattice relaxation in the rotating frame ($T_{1\rho}$, ¹H), the cross-relaxation time between the protons and carbons

(T_{CH}), and the ¹H longitudinal spin–lattice relaxation time (T_1^1H). The aim of this work was to characterize a new crosslinked starch-enriched flour and to determine the precise role of the crosslinking reaction.

EXPERIMENTAL

Materials

Starch-enriched flour was kindly provided by Sauvin S.A. (Patornay, France). This product was industrial agro alimentary garbage. Other compounds were purchased from various suppliers and used as received.

Polymer synthesis

The crosslinked polymers were prepared in one step via the crosslinking of starch-enriched flour with EPI as a bifunctional crosslinking agent, according to the procedure introduced by Morcellet and Crini.^{33–35} The synthesis of the starch derivatives has been described in detail elsewhere.¹⁹ EPI was reacted with glucose units of starch molecules (crosslinking step) and/or itself (polymerization step). A number of chains were interconnected by the formation of new interchain linkages, and the resulting polymers formed a network containing glucose units joined by repeating glyceryl linkers. A three-dimensional network was then formed (see Scheme 1). If the degree of reticulation was sufficiently high, the matrix of the polymers became insoluble in water and in organic solvents. It was possible to obtain a large range of materials with different crosslinking degrees with different amounts of EPI.

Sample characterization

The method used for the determination of the amount of glucose in the polymer was the measurement of the reducing sugars with phenol after acidic hydrolysis, as described by Dubois et al.^{31,32} This method is a sensitive colorimetric technique specific to carbohydrates

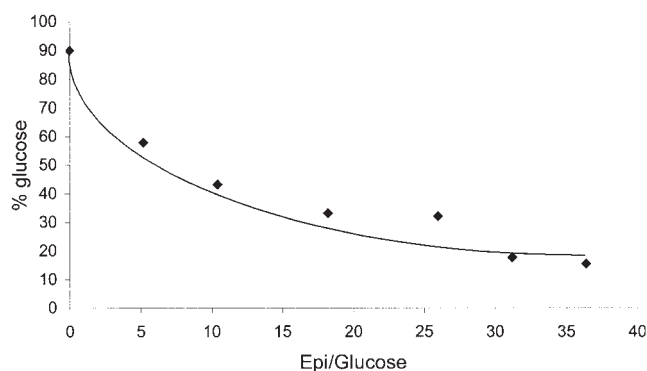


Figure 1 Influence of the amount of EPI added to the synthesis on the amount of glucose present in the soluble parts of the materials.

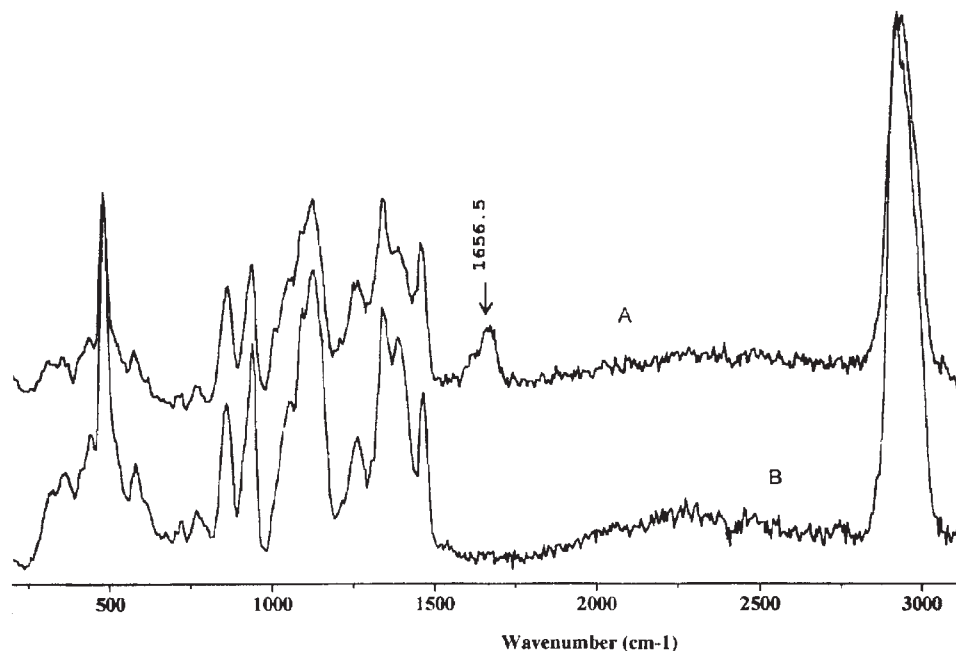


Figure 2 Raman spectra of (A) starch-enriched flour and (B) crosslinked polymer 1.

and widely applicable. A typical experiment was carried out as follows. A sample (5.5 mg) was hydrolyzed with 10 mL of 2M trifluoroacetic acid for 8 h at 120°C. After filtration, the solution was neutralized by several washings with water with a rotavapor until the solution became neutral. Once again, the soluble part was washed, and exactly 10 mL of distilled water was added. Several fractions of the aqueous solution containing the sugar were placed into tubes. In some cases, an insoluble part was obtained and weighed. A 5% phenol solution (0.5 mL) and 5 mL of 96% sulfuric acid were added to each tube. The sulfuric acid induced convection currents at the surface of the liquid, leading to good mixing and even heat distribution. Each tube was then agitated with a vortex. After 10 min, the tubes were reshaken and placed in a water bath at 25–30°C for 20 min. A yellow-orange color appeared that was stable for several hours. The adsorbance was measured with a Biotek Instrument Uvikon XL spectrophotometer (UVK-Lab, Trappes, France) at 480 nm, which corresponded to the characteristic wavelength of the colored complex. The amount of sugar was determined with a standard curve as a reference previously prepared for the particular sugar assayed.

The Fourier transform infrared (FTIR) spectroscopy studies were carried out with a Nexus Nicolet spectrometer (Thermo Electron, Montigny Le Bretonneux, France) between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1} . The samples were ground with KBr in an agate mortar: 500 mg of KBr was mixed with each sample (2% w/w). All the powder samples were pressed into KBr pellets for the FTIR transmission measurements. Raman spectra were recorded with a

Kaiser optical spectrometer, which was provided by Bruno Lenain (Ecully, France).

The NMR solution spectra were recorded with a Bruker AC 200 spectrometer and a Bruker Avance 400 spectrometer (Bruker, Wissembourg, France) at room temperature. The samples were suspended in a D_2O solution in a 5- or 10-mm tube. Solid-state ^{13}C -NMR data were collected with a Bruker ASX wide-bore system equipped with a 7-mm CP-MAS probe head with a field strength equal to a ^1H Larmor frequency of 300.13 MHz. The samples were finely powdered, packed into a zircon rotor, and sealed with Kel-F caps. The rotor held 220 mg of each sample. The spectra were referenced to external ME_4Si via the low-field resonance of adamantane at 38.6 ppm. Data were obtained at an MAS speed of 4 kHz. The $\pi/2$ pulse widths were equal to 3.7 μs , the contact time for the CP experiment was 1.2 ms, and the relaxation delay was 6 s. Spectra were obtained with 1024 data points in the time domain, 512 scans being performed for each experiment. Baseline correction was manually set when necessary after Fourier transform and phasing. A series of experiments was performed as a function of the contact time. The intensity of the resonance was reported against the contact time; from these data, T_{CH} and $T_{1\rho}(^1\text{H})$ were obtained. $T_1(^1\text{H})$ was determined with the classical inversion-recovery method.²⁸

RESULTS AND DISCUSSION

Determination of the amount of glucose

The results (Table I and Fig. 1) show that for the flour, the whole sample fraction was soluble after acid hy-

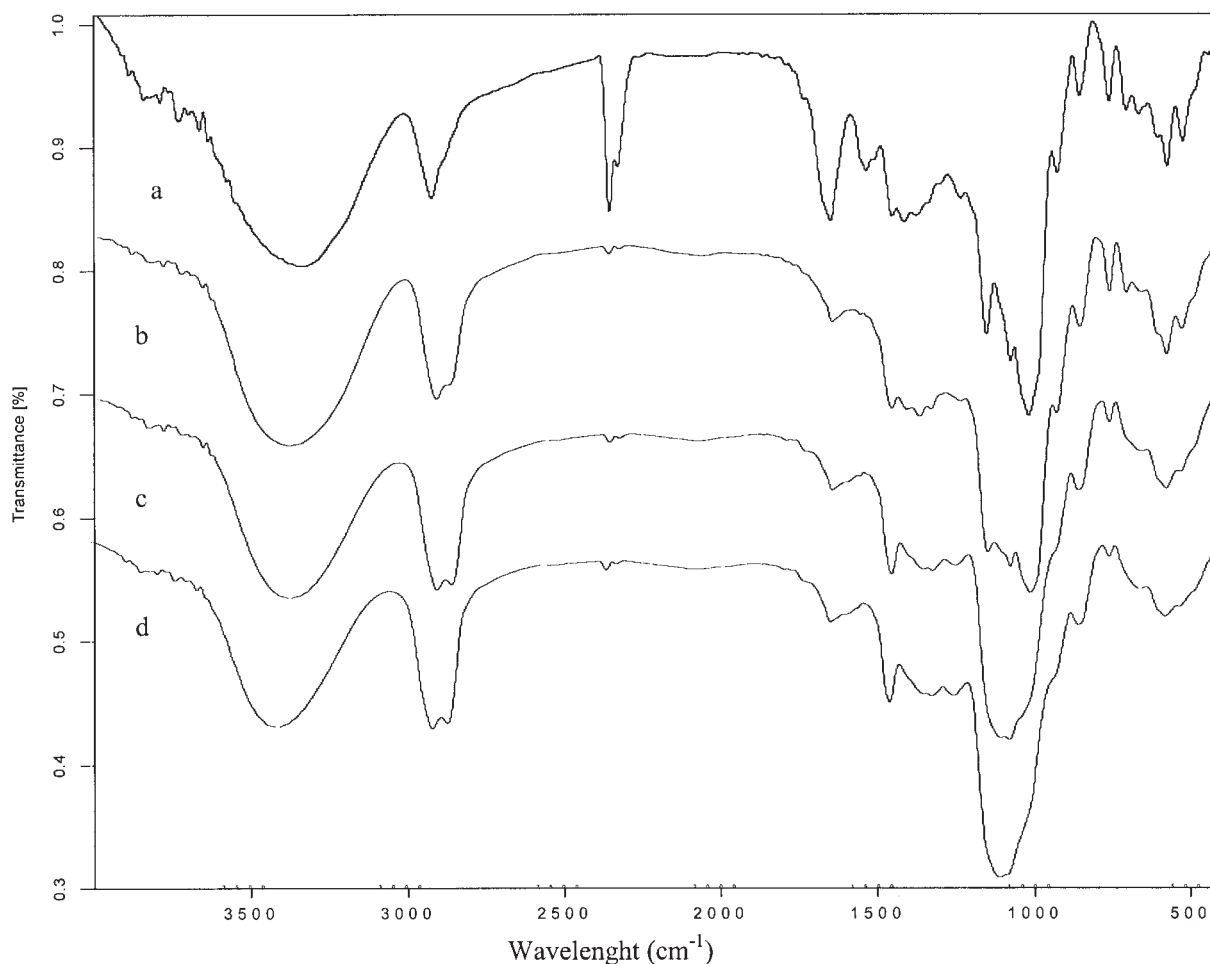


Figure 3 Typical FTIR spectra (transmission-mode) of (a) starch-enriched flour and (b–d) crosslinked polymers 1, 3, and 6, respectively.

drolysis. Therefore, this step could be used to evaluate the fraction impurities of the industrial flours, which were estimated to be 10%. This value allowed us to calculate the EPI/glucose ratio for each synthesis. For polymers 1 and 2, which were synthesized with low amounts of EPI, the entire fraction was also soluble after acid hydrolysis. This indicates a low degree of crosslinking for the two polymers. Moreover, we observed a reduction of the glucose with an increase in the amount of the crosslinking agent used during the synthesis. Indeed, EPI could react with the glucose molecules (crosslinked step) and/or itself (polymerization step), and this led to a decreasing amount of glucose in polymer 2. The other polymers, synthesized with larger amounts of EPI, had insoluble parts, which became larger with the amount of EPI. As expected, the overall trend was an increase in the substitution reactions as the amount of EPI increased. Thus, a more important crosslinking step inducing a rigid structure with a higher degree of crosslinking could be expected. This could induce a more difficult hydrolysis of the polymer and also lead to a less accessible net-

work for the acid. For polymers 3–6, the glucose determination was performed on the soluble part. The results showed that the amount of glucose decreased, probably because of more extensive homopolymerization for these polymers. The previous conclusions were confirmed by NMR experiments.

Raman and FTIR studies

The Raman spectra of the industrial flour and polymer 1 are presented in Figure 2. The two spectra are similar. There is a characteristic peak at 1656.5 cm^{-1} in the flour spectrum that disappears in the polymer 1 spectrum. This peak might belong to a carbonyl of an amide function due to the residual proteins present in this industrial flour. The Soxhlet washing performed in the last step of the synthesis allowed us to purify the polymer and was responsible for the absence of this protein peak. The Raman spectra show that the polymer was purified, but they cannot provide any information about the crosslinking reaction. We ob-

TABLE II
Assignment of the Main Bands of the IR Spectra of the Industrial Flour and Starch Polymers

Industrial flour		Polymers	
Wavelength (cm ⁻¹)	Assignment	Wavelength (cm ⁻¹)	Assignment
3345	OH stretching (H-bonded)	3400–3385	OH stretching (H-bonded) ^a
2920	CH stretching	2920	CH stretching
1645	OH bending (water)	2880	CH stretching ^{b,c}
1530	Impurity (amide band)	1645	OH bending (water)
1450	CH bending (CH ₂)	1455	CH bending (CH ₂) ^c
1410	OH bending	1407	OH bending
1370		1360	
1330	CH bending	1330	CH bending ^d
1300		1300	
1260–1210	OH bending	1260–1240	OH bending ^d
1200	OH bending	1215	OH bending
1150	CO stretching glycosidic bond, CO stretching/OH bending	1150–1100	CO stretching/OH bending of COH group/CO stretching glycosidic bond
1070	CO/CC stretching	1080–1060	CO/CC stretching ^b
1020	CO/CC stretching	1030	CO/CC stretching
940	Pyranose ring vibrations	950	CO/CC stretching ^b
860		940	Pyranose ring vibrations
750–700	Pyranose ring vibrations	860	Anomeric CH deformation
650	Anomeric CH deformation	750–700	Pyranose ring vibrations
600–520	Pyranose ring vibrations	650	OH bending (out of plane)
		600–520	Pyranose ring vibrations

^a Shift to higher frequency.

^b Absent in flour.

^c Intensity increase with crosslinking.

^d Peak broadening.

^e Intensity decrease with crosslinking.

tained the same kind of Raman spectra for the other polymers.

Figure 3 shows the FTIR spectra obtained for the industrial flour and the polymers. Table II shows the origins of the peaks. The industrial flour spectrum displays the typical profile of a polysaccharide in the 1200–920-cm⁻¹ range (characteristic peaks attributed to C—O bond stretching). The peaks at 1020 and 1070 cm⁻¹ are characteristic of the anhydroglucose ring. The peak at 1645 cm⁻¹ is due to the water present in the starch. The band at 2920 cm⁻¹ is characteristic of C—H stretching associated with the ring methine hydrogen atoms. The broad band at 3345 cm⁻¹ is due to hydrogen-bonded hydroxyl groups (O—H). Moreover, another broad band at 1450 cm⁻¹ suggests the presence of other kinds of compounds that could be impurities, such as proteins. The main IR bands and their assignments are summarized in Table II.^{36–38}

Comparing the IR spectra of the industrial flour and polymers **1**, **4**, and **8**, we noted the following: the characteristic ring vibrations and the anomeric C—H deformations were still present in the polymers. At 950, 1060–1080, and 1100–1150 cm⁻¹ the spectra of the polymers showed broad peaks suggesting additional C—O and C—C bonds present in the glyceryl bridge. The intensity of the coupled C—OH group vibration and C—O—C stretching mode of the glycosidic bond

was lowered. The lowering of the relative intensity of the 1150-cm⁻¹ did not disagree with the presence of one bridge decreasing the number of OH groups by one. The intensity of the CH₂ peak (1450 cm⁻¹) increased with the crosslinking reaction according to the introduction of the glyceryl bridges. A characteristic C—H stretching gradually appeared at 2880 cm⁻¹ from polymer **1** to polymer **6**. This peak was characteristic of the CH groups in the glyceryl bridges. The hydrogen-bonded O—H stretching at 3400 cm⁻¹ shifted to higher frequencies, indicating a loosening of hydrogen bonds. Indeed, the introduction of the glyceryl bridges may have caused this effect.³⁸

The IR spectra showed that the crosslinking reaction occurred but could not provide any information about the location of the bridges and tails to the pyranose rings or on the molecular mobility of the different polymers. This was the reason that we thought it necessary to use another technique, such as NMR spectroscopy.

Solution NMR study

Before the study of the polymers, we investigated starch-enriched flour. Figure 4(a) shows the solution carbon spectrum of starch-enriched flour suspended in dimethyl sulfoxide-*d*₆ (DMSO-*d*₆). This spectrum

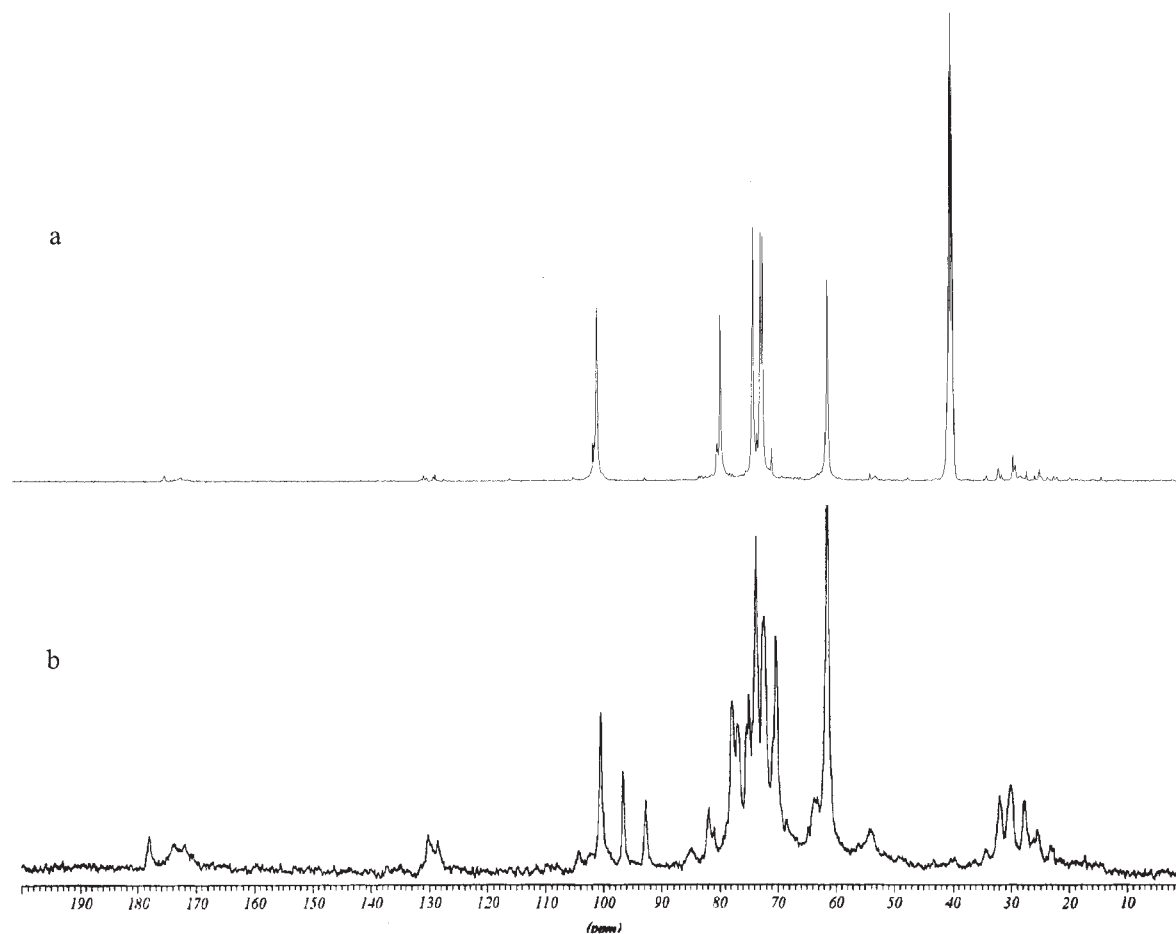


Figure 4 ^{13}C -NMR spectra of starch-enriched flour in (a) D_2O and (b) $\text{DMSO}-d_6$.

resembles a classical starch spectrum; there are six important signals at 100, 79, 74, 72, 71 and 61.5 ppm due to the C-1, C-4, C-3, C-2, C-5, and C-6 atoms of the glucopyranose unit, respectively. The resonances at 79 and 70 ppm are due to C-4 involved in the α -(1,4)-linkages and C-4 of the nonreducing terminal units, respectively. As the spectrum is resolved, a quantitative analysis can provide an estimation of the proportion of branched molecules present, that is, the ratio of (1,4)- and (1,6)-linkages.³⁹ The degree of branched chains was estimated to be 5.1%. This result could also be obtained from the ratio of C-6 involved in the α -(1,6)-linkages to C-6 of the nonreducing terminal units, but for these carbons (in the range 67–68 ppm), a broad signal can be observed. We also note low-intensity signals, at 30, 130, and 180 ppm, out of this characteristic polysaccharide region. These signals, occurring in aliphatic, carbonyl, and olefin carbon regions, could be attributed to impurities present in this industrial byproduct. Figure 4(b) shows the ^{13}C spectrum of a sample in D_2O . It emphasizes the presence of maltose and maltotriose derivatives (at 100–90 ppm) and impurities such as proteins (at 30, 130, and 180 ppm). In this case, the peaks are more evident than in

the spectrum of flour suspended in dimethyl sulfoxide. In fact, when the sample was suspended in D_2O , the solubility of the impurities and the swelling behavior of the starch were more important; this explained the better resolution in the NMR signals.

Figure 5 shows the liquid ^{13}C -NMR spectra of crosslinked starch derivatives with different amounts of EPI. These experiments were performed with partially hydrated polymers in D_2O (polymers 1–3 swelled in water) in a 5-mm-diameter tube at room temperature and required 3 days of accumulation for a signal of sufficient resolution to be obtained. These spectra were recorded on a 200-MHz spectrometer. Under these conditions, a typical profile of a crosslinked polysaccharide was observed. The spectra show the peaks of a disordered starch in the range of 50–110 ppm. The resonance at 101 ppm is a well-defined peak and is due to anomeric C-1. The resonances at 61.5 and 63.5 ppm are attributable either to the hydroxymethyl group at C-6 in the glucose unit of starch or to the hydroxymethyl group of the EPI terminal residue, according to literature data.^{35,40} There is a large degree of signal overlap in the range of 66–85 ppm due to the crosslinking step, which in-

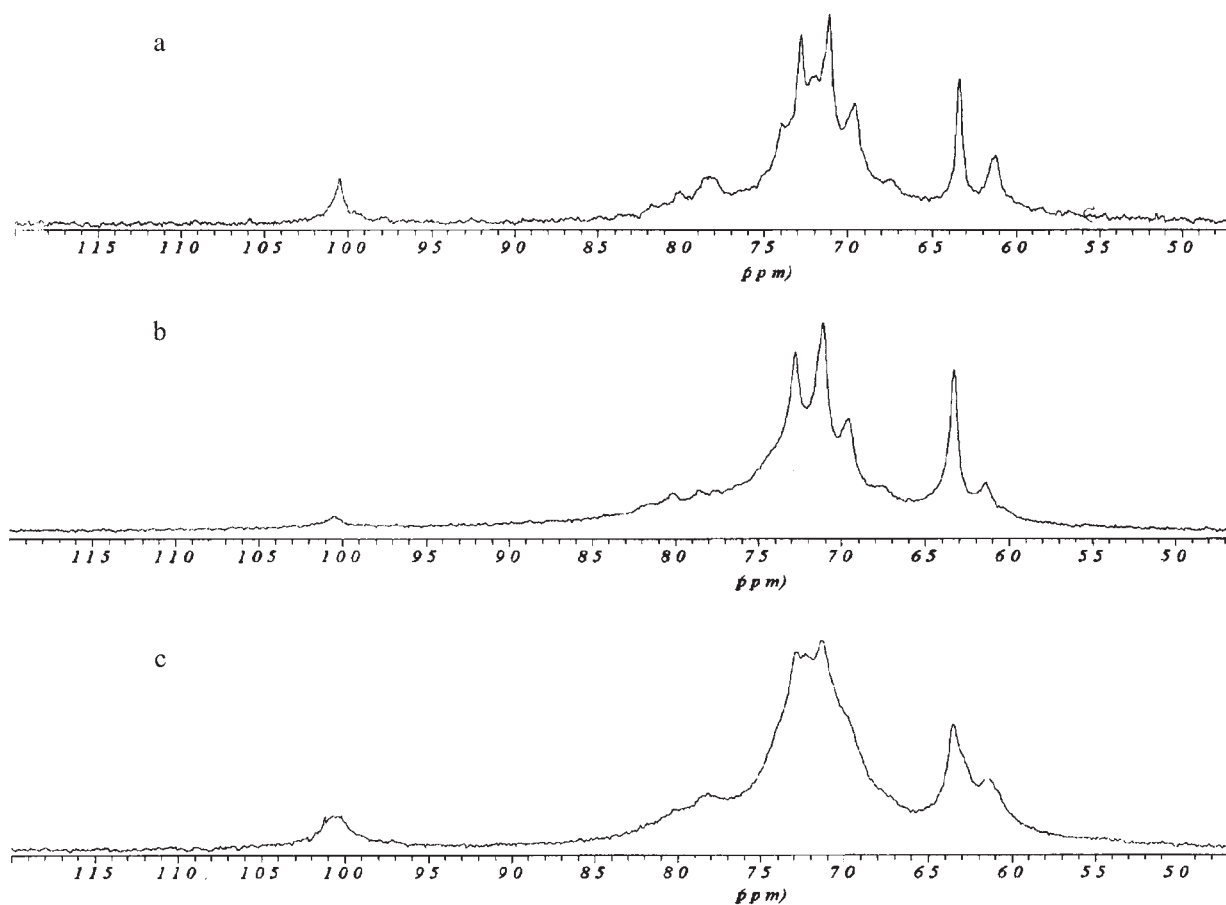


Figure 5 ^{13}C NMR solution spectra of crosslinked starch derivatives in D_2O of (a) polymer 1, (b) polymer 2, and (c) polymer 3.

duces the formation of a polymer network. We suppose that the signals of EPI are completely hidden by the C-2, C-3, and C-5 starch peaks. These spectra also show that the different amounts of EPI used during the synthesis changed the interactions of the solvent with the polymers and, as a result, the width of the signals as the C-1/C-6 ratio resonance changed. For polymer 3 [Fig. 5(c)], there is a very broad band typical of a structure that does not interact with the solvent. This result can be interpreted as a resolution reduction when the amount of the crosslinking agent increased, probably due to a decrease in the swelling capability induced by a higher crosslinking degree. It is well known that polysaccharides with a low crosslinking rate have higher swelling behavior.

With a good understanding of the polymer hydration, we hydrated polymers 3 and 6 with an excess of water. The spectra were recorded with a 400-MHz spectrometer at 313°K with a 10-mm tube (Fig. 6). Under these conditions, a well-defined resolution can be observed in the 65–85 ppm range for polymer 3 [Fig. 6(a)], probably because of more important homopolymerized chain–solvent interactions. For polymer 6, which was synthesized with a larger amount of EPI, the same resonances can be observed, except for

C-1, which disappears completely [Fig. 6(b)]. The presence of the C-6 resonance and the determination of the amount of glucose involve the presence of glucose units in this polymer, but this disappearance can be explained by the increase in the rigidity due to more extensive crosslinking. In this case, only the mobile part of the polymer (homopolymerized EPI chains) could be investigated because of the better interaction with the solvent. This effect limited the investigation of the reticulated polymers by the NMR solution technique. Thus, solid-state NMR spectroscopy such as ^{13}C DD/CP–MAS and ^{13}C DD/MAS techniques were necessary to characterize the structure and molecular mobility of the different components.

Solid-state NMR studies

Generally, the use of the carbon–proton dipolar interaction in a ^{13}C DD/CP–MAS experiment is a very efficient tool for the analysis of rigid structures as it is even able to investigate the mobile components in solid polymers. The ^{13}C DD/MAS method principally shows signals arising from the mobile component. MAS spectra are taken with different values of D_1 (i.e., the time between each acquisition). This time, which is

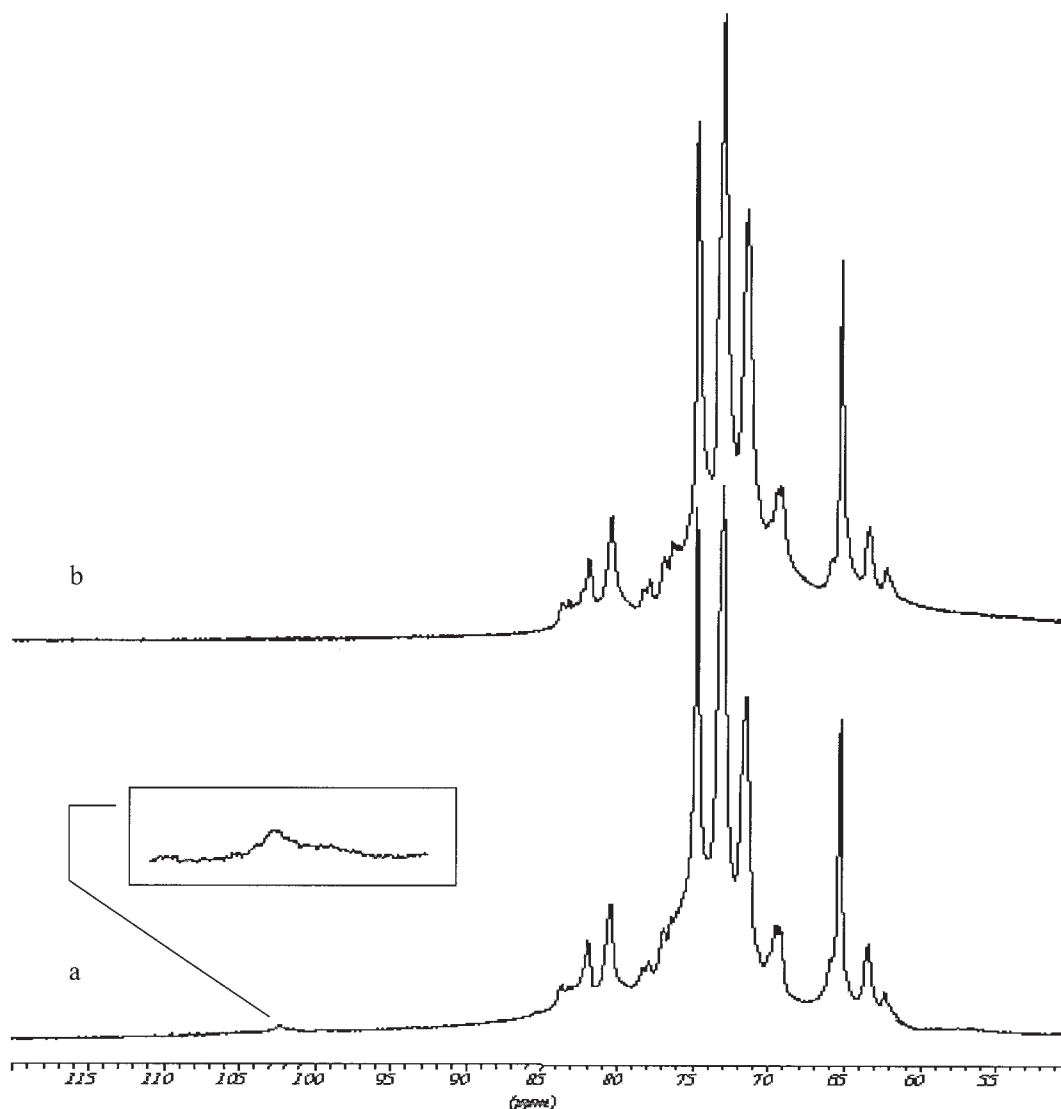


Figure 6 ^{13}C NMR solution spectra of crosslinked starch derivatives in D_2O of (a) polymer 3 and (b) polymer 6.

correlated to relaxation time T_1 , provides an idea of the polymer rigidity. If the D_1 value used in the sequence is short, carbons with rigid structures have no time to return to the zero of magnetization and thus provide less important and resolute signals. In this way, it is possible to obtain well-defined peaks for highly mobile components of materials.

Figure 7 shows the ^{13}C DD/CP-MAS spectrum and ^{13}C DD/MAS spectra of starch-enriched flour with two different repetition times (4 and 20 s). The MAS spectra, with and without CP, show the classical peaks of corn starch.^{41,42} Figure 7(a) seems to have better resolution; in this case, the CP-MAS technique allowed us to obtain some more important signals. The flour showed different allomorph states as functions of their origins, as revealed by the number of resonances, particularly for the carbon C-1 signal (99–103 ppm). As expected, these signals were marked for

corn starch with allomorph A and V states and also an amorphous structure.^{41,43} In fact, for $D_1 = 4$ s, corresponding to a short time for the carbohydrate MAS spectrum, a different ratio between the areas of the signals was observed. The peak due to C-6 became more intense because of the high mobility of this group. As expected, a higher contribution to the total signal intensity of the most mobile carbons could be observed under these conditions. For $D_1 = 20$ s, the spectrum was close to the one obtained with ^{13}C DD/CP-MAS. Indeed, a larger D_1 value allowed the less mobile group to have time to return to the magnetic ground state and thus to give a stronger signal with a better resolution. Therefore, the spectra were close to those obtained with the ^{13}C DD/CP-MAS experiments.

Figure 8 shows ^{13}C DD/CP-MAS spectra of the flour and polymers 1–3 and 6 taken at the appropriate

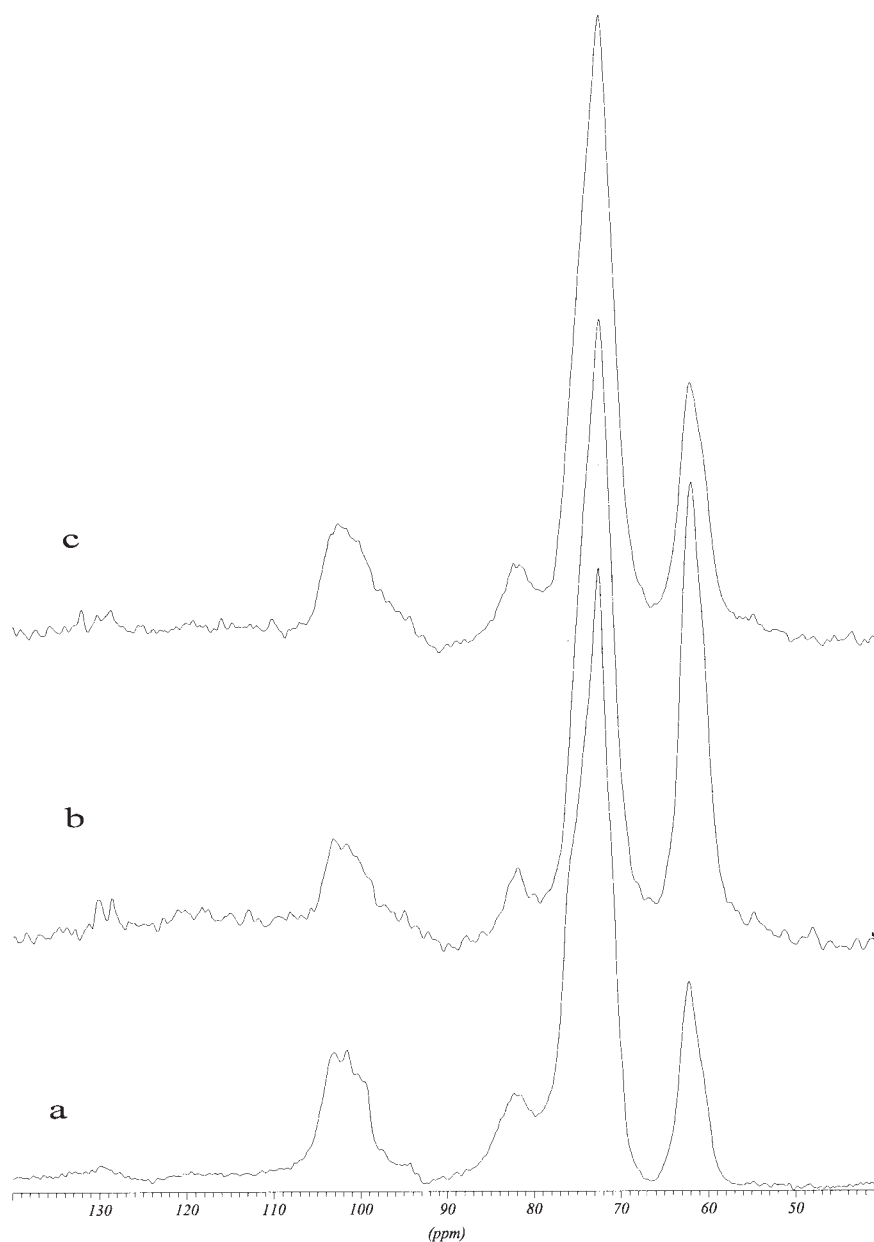


Figure 7 (a) CP-MAS spectrum and (b,c) MAS spectra of starch-enriched flour: (b) $D_1 = 4$ s and (c) $D_1 = 20$ s.

contact times. This result was confirmed with the T_{CH} and $T_{1\rho}^1H$ parameters, which corresponded to the polarization of proton spins that could be transferred to a dilute spin system with a rate of $1/T_{CH}$ and to the polarization of proton spins that could be dissipated to the lattice by relaxation under the spin-locked conditional rate $1/T_{1\rho}$, respectively. The T_{CH} values (Table III) were constant (45–60 μ s), and so the different CP-MAS spectra could be compared. Table IV provides the values of $T_{1\rho}^1H$ for the flour and different polymers. A big difference between the flour and the polymers, revealing the addition of a strong amorphous component into the polymers, was found. On the other hand, the $T_{1\rho}^1H$ values of the different car-

bon atoms for the polymers were similar, and so the samples were considered homogeneous; this confirmed that the different CP-MAS spectra could be compared. $T_{1\rho}^1H$ was not very sensitive to the motion of singular molecular groups because it was averaged within domains as large as 1–10 nm. Therefore, $T_{1\rho}^1H$ indicated the homogeneity of the phase in the spatial range.

We observed a broadening of the peaks in the range of 55–85 ppm with an increase in the amount of EPI used during the synthesis. Similar results were previously published.^{35,44} Therefore, this broadening could be due to the ordering change or to the mobility change of the material. The C-1 signal (100–105 ppm)

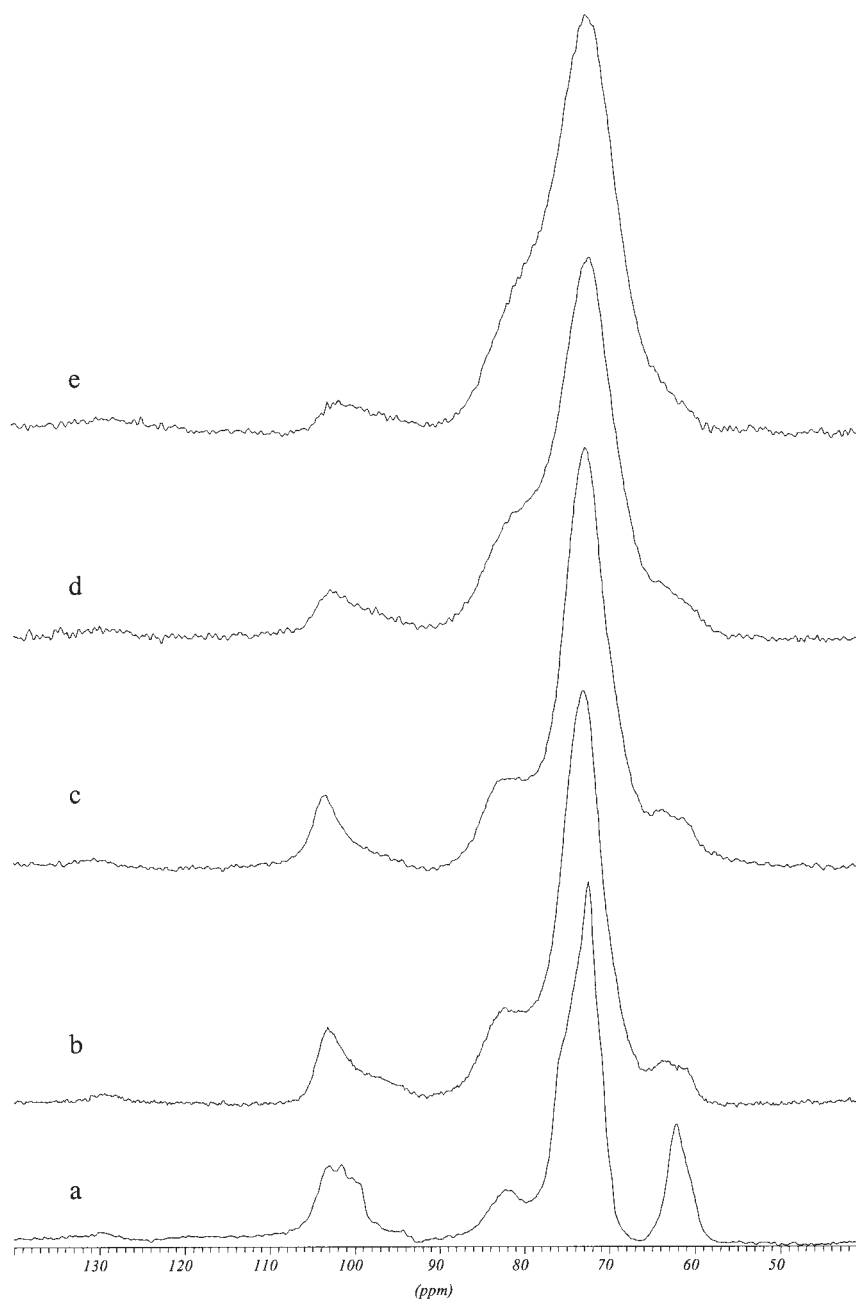


Figure 8 CP-MAS spectra of crosslinked starch derivatives of (a) starch-enriched flour, (b) polymer 1, (c) polymer 2, (d) polymer 3, and (e) polymer 6.

decreased when the amount of the crosslinking agent was increased. This could be explained by a reduction of the glucose quantity but also by an increase in the disorder introduced by the crosslinking. Furthermore, in the spectrum of polymer 1, which was synthesized with a small amount of EPI, the C-1 signal was still visible but showed a large loss of resolution. Before assigning the shoulder at the 95 ppm resonance, we should point out that a γ -gauche effect,²⁸ such as that encountered in the bending of sugar residues, produces exactly this type of upfield shift. This effect was previously observed in oligomeric products of cellu-

TABLE III
 T_{CH} Values for the Flour and Different Polymers

	C-1	C-4	C-2, C-3, and C-5	C-6
Industrial flour	56	46	59	39
Polymer 1	63	51	56	62
Polymer 2	65	47	50	51
Polymer 3	51	46	48	50
Polymer 4	59	50	53	51
Polymer 6	47	47	47	47

TABLE IV
 $T_{1\rho}$ ^1H Values for the Flour, Different Polymers, and
 Corn Starch as a Reference

	C-1	C-4	C-2, C-3, and C-5	C-6
Industrial flour	6.00	5.40	6.90	7.40
Polymer 1	3.00	2.80	2.90	2.50
Polymer 2	2.58	2.40	2.50	2.10
Polymer 3	2.80	2.50	2.80	2.7
Polymer 4	3.10	3.00	3.10	2.60
Polymer 6	3.30		2.90	
Corn starch	9.95	8.1	8.8	5.4

lose.⁴⁵ Thus, we could assume the occurrence of a conformational change that followed the crosslinking reaction according to the presence of this 95 ppm resonance.^{6,8} Indeed, the crosslinking strongly modified the flour structure, inducing the formation of a more amorphous structure; this could especially be seen for the C-1 signal.

Figure 9 compares the ^{13}C DD/CP-MAS spectra and ^{13}C DD/MAS spectra with D_1 values of 4 and 20 s for polymers 1–3 and 6. For the ^{13}C DD/MAS spectra with $D_1 = 4$ s, the signal at 63.5 ppm is the most intense for all the polymers. It is attributable to the hydroxymethyl groups of the EPI terminal residue.³⁵ In fact, for these short experimental delays, more intense signals were obtained because of the high mo-

bility of this group. A shoulder at 61.5 ppm can also be seen, and the same observation with minor intensity can be made, except for polymers 3 and 6, for which the important chemical derivatization of the glucose hydroxymethyl groups induced a loss of the signal. These can also be explained by the lower mobility of the C-6 hydroxymethyl groups in comparison with that of the hydroxymethyl groups of the EPI terminal residue.

With respect to the C-1 peak for all the polymers, two groups could be formed: one with polymers 1 and 2, for which all the resonances were always defined, and another with polymers 3 and 6, for which there was an important loss of resolution. For polymer 1, the C-1 resonance was pronounced in the ^{13}C DD/CP-MAS spectrum (1a), was reduced for the ^{13}C DD/MAS spectrum with $D_1 = 4$ s (1b), and almost reached the CP-MAS intensity with $D_1 = 20$ s (1c). The same evolution could be observed for polymer 2 with a loss of resonance intensity. This could be explained by a higher contribution to the total signal intensity of the most mobile carbons with a low value of D_1 . For $D_1 = 20$ s, a more rigid structure could be observed, and so a spectrum close to the one obtained with ^{13}C DD/CP-MAS was observed. For polymers 3 and 6, the C-1 resonance was not present for $D_1 = 4$ s or $D_1 = 20$ s. This fact could be explained by an increase in the rigidity of the polymer involving a loss of the

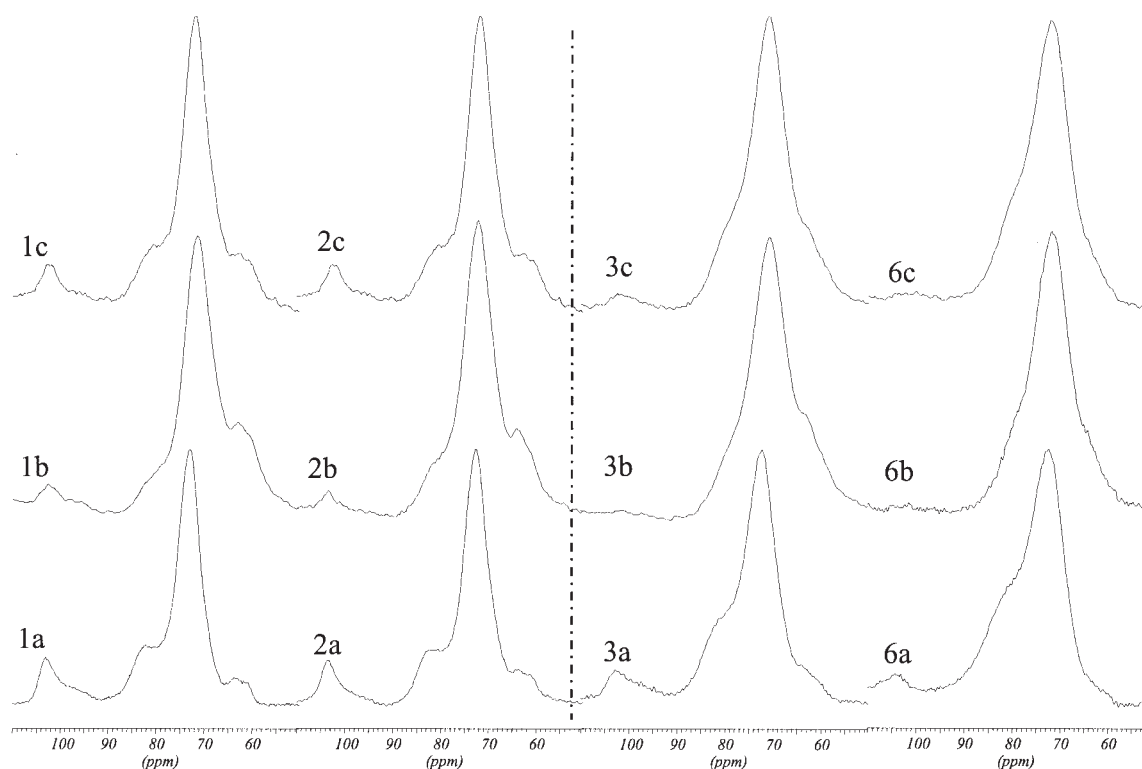


Figure 9 Influence of the crosslinking degree on (a) CP-MAS and (b,c) MAS spectra.

TABLE V
 T_1 ^1H (ms) Values for the Industrial Flour Before and After Being Mixed in an Aqueous Sodium Hydroxide Solution Without a Crosslinking Agent and for Polymers 1, 3, and 6

	C-1	C-4	C-2, C-3, and C-5	C-6
Industrial flour	0.93	1	0.938	0.94
Treated flour (with NaOH)	0.243	0.26	0.261	0.23
Polymer 1	0.474	0.468	0.471	0.466
Polymer 3	1.09	1.12	1.092	1.1
Polymer 6	1.35	1.7	1.7	1.7

signal for all the MAS experiments. These results were confirmed by the MAS spectra recorded at $D_1 = 120$ s (not shown), in which the C-1 signal was present, confirming the high rigidity of the starch. The previous conclusions confirmed that for the larger amounts of EPI used during the synthesis, the crosslinking step was more important, inducing more rigid and amorphous materials. This was also confirmed by the $T_1^1\text{H}$ values reported in Table V for the industrial flour investigated before and after being mixed in an aqueous sodium hydroxide solution without crosslinking agents and polymers 1, 3, and 6. $T_1^1\text{H}$ was an efficient parameter for translating the rigidity of the polymers.⁴⁶ We observed a reduction when the flour was mixed in basic media; this indicated a loss of the crystalline structure and thus a more mobile system. The increase between the treated flour and polymer 1 and especially polymers 3 and 6 confirmed the increase in the rigidity with the amount of the crosslinking agent used during the synthesis.

In our previous studies,^{18–20} we focused our attention on the determination of the sorption properties of crosslinked starch derivatives for dyes and phenolic compounds. One of our conclusions was that the crosslinking agent created a network structure in the polymer chain that induced a steric effect (physical adsorption), and in this case, the action of the crosslinking agent was predominant. In fact, when we increased the crosslinking degree, the sorption capacity was higher, except for the most crosslinked polymers (5 and 6). Therefore, it was not necessary to have a high degree of crosslinking to obtain the best results. Strong reticulation could induce a less accessible network and disadvantage the different interactions. In this study, we focused our attention on the increase in the rigidity with the amount of the crosslinking agent used during the synthesis. Therefore, we could confirm that the amount of the crosslinking agent added during the synthesis induced a more extensive crosslinking reaction. In this way, we could control the structures of the different polymers (which were directly related to the amount of the crosslinking agent added during the synthesis). A correlation was found

between the crosslinking degree, structure, and mobility of these crosslinked starch derivatives and their sorption properties for dyes and phenolic compounds.

CONCLUSIONS

Crosslinked starch materials with various compositions were characterized with different techniques. The determination of the amount of glucose in the polymers was made with the method described by Dubois et al.^{31,32} The results showed that a more important crosslinked step inducing a rigid structure could be expected when the amount of EPI used during the synthesis was more important. Spectroscopic studies were performed. Raman spectra proved the purification of the polymers after the synthesis step. FTIR spectra showed that the crosslinking reaction occurred and also demonstrated the gradual evolution with the amount of EPI added during the synthesis. Solution and solid-state NMR studies allowed the assignment of the principal ^{13}C signals. In addition, CP-MAS and MAS spectra and relaxation time measurements provided information about the structure, homogeneity, and mobility of the different polymers. We can conclude that increasing the amount of EPI added during the synthesis increased the amorphous content, and the material became more rigid because the increasing number of covalent bonds in the polymer network reduced their mobility. Finally, these conclusions can be related to the sorption properties, especially for dyes and phenolic compounds used in previous studies.^{18–20}

References

- Kuniak, L.; Marchessault, R. H. *Starch* 1972, 24, 110.
- Dumoulin, Y.; Alex, S.; Szabo, P.; Cartilier, L.; Mateescu, M. A. *Carbohydr Polym* 1998, 37, 361.
- Lenaerts, V.; Moussa, I.; Dumoulin, Y.; Mebsout, F.; Chouinard, F.; Szabo, P.; Mateescu, M. A.; Cartilier, L.; Marchessault, R. J *Controlled Release* 1998, 53, 225.
- Boutboul, A.; Giampaoli, P.; Feigenbaum, A.; Ducruet, V. *Carbohydr Polym* 2002, 47, 73.
- Zhang, L. M.; Chen, D. Q. *Colloid Surf A* 2002, 205, 231.
- Rong, Y. W.; Yuan, Y. H. *Starch* 2002, 54, 260.
- Kim, B. S.; Lim, S. T. *Carbohydr Polym* 1999, 39, 217.
- Chung, H. J.; Woo, K. S.; Lim, S. T. *Carbohydr Polym* 2004, 55, 9.
- Wesslen, K. B.; Wesslen, B. *Carbohydr Polym* 2002, 47, 303.
- Crini, G.; Morcellet, M. J *Sep Sci* 2002, 25, 789.
- Fang, J. M.; Fowler, P. A.; Tomkinson, J.; Hill, C. A. S. *Carbohydr Polym* 2002, 47, 245.
- Seidel, C.; Kulicke, W. M.; Heb, C.; Hartmann, B.; Lechner, M. D.; Lazik, W. *Starch* 2001, 42, 305.
- Shiftan, D.; Ravenelle, F.; Mateescu, M. A.; Marchessault, R. H. *Starch* 2000, 52, 186.
- Veiga, V.; Ryan, D. H.; Sourty, E.; Llanes, F.; Marchessault, R. H. *Carbohydr Polym* 2000, 42, 353.
- Hamdi, G.; Ponchel, G.; Duchêne, D. J *Controlled Release* 1998, 55, 193.
- Simkovic, I. *Carbohydr Polym* 1996, 31, 47.

17. Simkovic, I.; Laszlo, J. A.; Thompson, A. R. *Carbohydr Polym* 1996, 30, 25.
18. Delval, F.; Crini, G.; Vebrel, J.; Knorr, M.; Sauvin, G.; Conte, E. *Macromol Symp* 2003, 203, 165.
19. Delval, F.; Crini, G.; Morin, N.; Vebrel, J.; Bertini, S.; Torri, G. *Dyes Pigments* 2002, 53, 79.
20. Delval, F.; Vebrel, J.; Morcellet, M.; Janus, L.; Crini, G. *Polym Recycl* 2000, 5, 137.
21. Masaro, L.; Zhu, X. X. *Prog Polym Sci* 1999, 24, 731.
22. Tonelli, A. E. *NMR Spectroscopy and Polymer Structure: The Conformational Connection*; Wiley-VCH: New York, 1989.
23. McBrierty, V. *Solid State Nucl Magn Reson* 1997, 9, 21.
24. Mathur, A. M.; Scranton, A. B. *Biomaterials* 1996, 17, 547.
25. Schmidt-Rohr, K.; Spiess, H. W. *Multidimensional Solid-State NMR and Polymers*; Academic: New York, 1994.
26. McBrierty, V. J.; Packer, K. *NMR in Solid Polymers*; Cambridge University Press: Cambridge, England, 1993.
27. Sozzani, P. In *From Molecular Materials to Solids*; Morazzoni, F., Ed.; Polo Editoriale Chimico: Milan, 1993; p 47.
28. Koenig, J. L. *Spectroscopy of Polymers*; American Chemical Society: Washington, DC, 1992.
29. Bovey, F. A. In *Nuclear Magnetic Resonance Spectroscopy*, 2nd ed.; Academic: New York, 1988; p 399.
30. Schaefer, V. J.; Stejskal, E. O. *Top Carbon-13 NMR Solid Polym* 1978, 3, 283.
31. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Nature* 1951, 168, 167.
32. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal Chem* 1956, 28, 350.
33. Delval, F.; Crini, G.; Janus, L.; Vebrel, J.; Morcellet, M. *Macromol Symp* 2001, 166, 103.
34. Crini, G.; Bertini, S.; Torri, G.; Naggi, A.; Sforzini, D.; Vecchi, C.; Janus, L.; Lekchiri, Y.; Morcellet, M. *J Appl Polym Sci* 1998, 68, 1973.
35. Crini, G.; Cosentino, C.; Bertini, S.; Torri, G.; Vecchi, C.; Janus, L.; Morcellet, M. *Carbohydr Res* 1998, 308, 37.
36. Wiedenhof, N.; Lammers, J. N. J. J.; Van Panthaleon Van Eck, C. L. *Starch* 1969, 5, 119.
37. Casu, B.; Reggiani, M. *J Polym Sci* 1968, 24, 803.
38. Casu, B.; Reggiani, M.; Gallo, G. G.; Vigevani, A. *Tetrahedron* 1968, 24, 803.
39. Gidley, M. J. *Carbohydr Res* 1985, 139, 85.
40. Crini, G.; Bourdonneau, M.; Martel, B.; Piotto, M.; Morcellet, M.; Richert, T.; Vebrel, J.; Torri, G.; Morin, N. *J Appl Polym Sci* 2000, 75, 1288.
41. Torri, G.; Naggi, A.; Cosentino, C.; Pizzoferrato, L.; Capelloni, A. *Atti del II Congresso Nazionale di Chimica degli Alimenti*; Grafica Editoriale: Messina, Italy, 1995; p 1121.
42. Cheetham, N. W. H.; Tao, L. *Carbohydr Polym* 1998, 36, 285.
43. Horii, F.; Yamamoto, H.; Hirai, A.; Kitamaru, R. *Carbohydr Res* 1987, 160, 29.
44. White, J. W.; Ricciuti, C.; Mayer, J. *J Assoc Off Agro Chem* 1952, 35, 859.
45. Gast, J. C.; Atalla, R. H.; McKelvey, R. D. *Carbohydr Res* 1990, 200, 137.
46. Capitani, D.; De Angelis, A. A.; Crescenzi, V.; Masci, G.; Segre, A. L. *Carbohydr Polym* 2001, 45, 245.