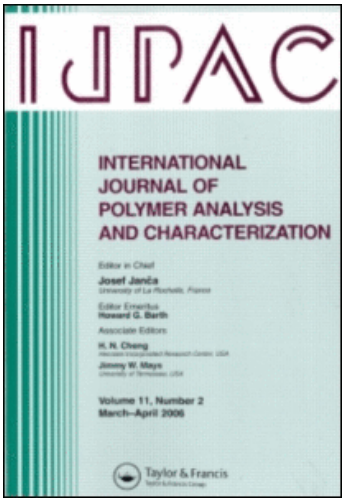

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Solid-state ^{13}C NMR Study of Scleroglucan Polysaccharide. Effect of the Drying Process and Hydration on Scleroglucan Structure and Dynamics*

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High-resolution solid-state ^{13}C CP/MAS NMR was used to study the evolution of a polysaccharide (scleroglucan) conformation from the anhydrous to the hydrated form. The influence of a thermo-mechanical treatment applied during the drying process of scleroglucan is analyzed both on the dried and rehydrated product. ^{13}C NMR spectra, ^{13}C relaxation times (T_{1C}) and ^{1}H relaxation times in the rotating frame ($T_{1\rho H}$) of scleroglucan dried by using instantaneous controlled pressure drop (Détente Instantanée Controlée®) were analyzed in order to explain the observed differences of rehydration capacity. Although the scleroglucan treated at 6 bar has the same conformational state (triple-helix) as the one treated at 1 bar, it shows two different relaxation times T_{1C} for the C-3 carbon involved in the interglycosidic linkage. The magnetization decay of the hydrated sample exhibits a decrease of two time constants with significant shortening of the spin-lattice relaxation times T_{1C} that accounts for the higher mobility of the chains. High-pressure treatment creates highly rigid and compact domains. Consequently, water molecules cannot readily access the inside of the triple-helix and relax the interchain hydrogen bonds.

Keywords: Scleroglucan; 13C CP/MAS NMR; Relaxation times

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INTRODUCTION

Scleroglucan is a polysaccharide with a regular structure based on repeating units of four D-glucose residues with three $\beta(1-3)$ and four $\beta(1-4)$ glycosidic linkages (Fig. 1). Like most polysaccharides, it is a highly polar polymer, which can easily trap water molecules. It is soluble in water and generally yields highly viscous solutions even at low concentration due to its high hydration capacity.[1,2]

In most applications, the dissolution rate of water-soluble polymers is of great importance.[3–6] In fact, most high-molecular-weight water-soluble polymers, such as xanthan gum, guar gum, carboxymethylcellulose and scleroglucan, generally require vigorous stirring and/or long stirring times to produce a uniform solution and to improve hydration capacity.[7]

In a previous study,[8] we have treated scleroglucan using a new drying-texturation process (Instantaneous Controlled Pressure Drop Process, "Détente Instantanée Controlée® D.I.C.") with the aim of improving its dissolution rate. It is based on the thermo-mechanical processing induced upon subjecting the product to a high-steam

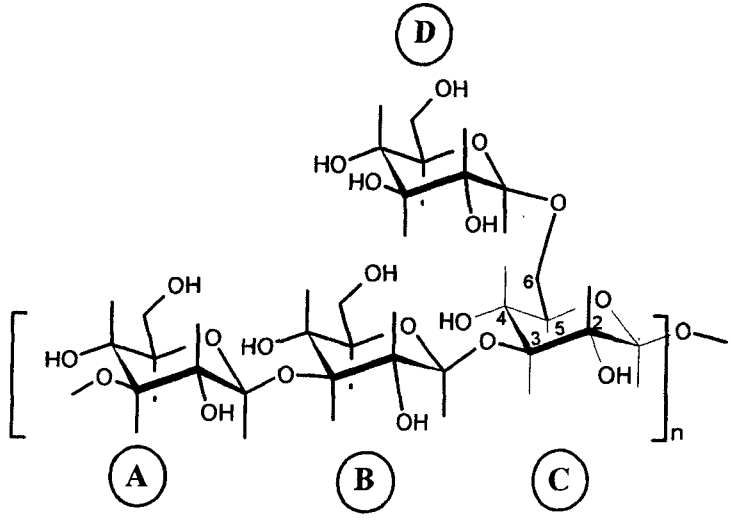

FIGURE 1 Scleroglucan repeat unit.

pressure to vacuum transition. High-steam pressure varies from 1 to 6 bar in contrast to the puffing techniques that require higher pressure.[9] This work demonstrate the feasibility of using the D.I.C. process to improve the solubility of scleroglucan and to optimize experimental conditions. The processing time and initial humidity of the samples have no significant effects on the dissolution rate. The most important parameter in D.I.C. process is pressure.

The previous NMR studies of scleroglucan concerned both solution and solid state.[10–12] We have used solid-state cross polarization magic angle spinning (CP/MAS) ^{13}C NMR in order to investigate scleroglucan in the solid state and to explain the dependence of hydration capacity on the parameters of the drying processes (D.I.C., hot air drying, and industrial vacuum drying). Indeed, scleroglucan treated at low pressure with D.I.C. (1 bar) gives rise to a better hydration capacity compared with the high-pressure process (6 bar) and samples dried using an air stream.[8] Hydration capacity was measured in a previous study[8] by using a Bohling V88 viscosimeter equiped with a turbine stirrer plunged into water. Figure 2 shows the evolution of the developed torque during the dissolution of

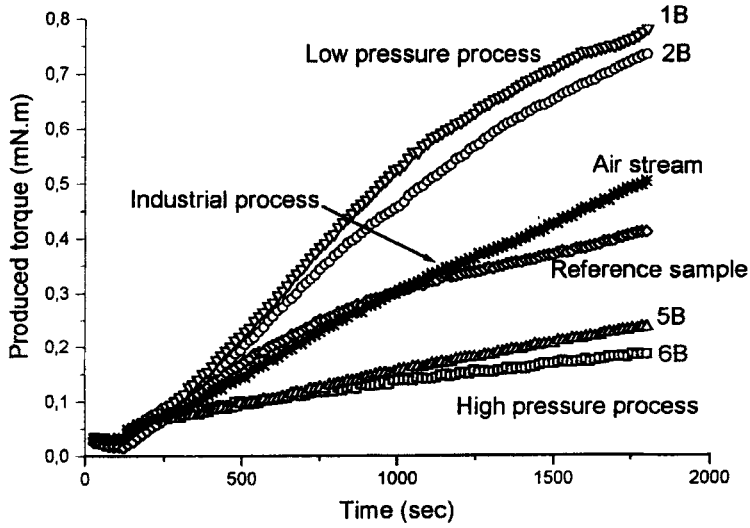

FIGURE 2 Evolution of the developed torque during the dissolution of scleroglucan.

scleroglucan. Low-pressure samples developed a torque four times higher than for high pressure samples.

In this paper, we wanted to determine if process-induced conformational changes of the polysaccharide chains were caused by changes in the triple-helix backbone,[10,11] or due to crystalline and amorphous domains. Solid-state spectra and relaxation time measurements were performed on dried and hydrated samples for this study.

EXPERIMENTAL

Sample Treatment[8]

Scleroglucan was provided by SKW Biosystems (Bauvais, France) in a coagulum form at 2.5 g of moist fraction/g of dry material. Before treatment, the samples were dried at 50°C under air flow until a constant moisture content (between 0.3 and 0.5 g H_2O/g dry material) was attained for each thermo-mechanical treatment induced by the D.I.C. process (Tab. I). After the introduction of the sample in the autoclave (~ 150 g), we introduced an atmosphere of overheated steam under pressure, which was released during 10 to 30 s and the pressure reduced instantaneously to 15 mbar. Samples were then dried under an air stream at 50°C to reach 0.1 g H_2O/g dry material. The reference sample was dried in a rotary vacuum dryer and the air stream sample was dried under an air stream of 70 L/h at 70°C.

Hydrated samples were obtained by adding a corresponding volume of water (20 and 40%) to scleroglucan. The homogeneity the hydration was assured by stirring the humidified product and by rapid rotation of the NMR rotor.

TABLE I Parameters of the D.I.C. drying processes

Sample name	*Pressure (bar)*	*Time (s)*	*Relative humidity (g H_2O/g dried material)*
Run 9 = 1B	0.97	20	0.4
Run 8 = 2B	2	10	0.3
Run 1 = 5B	5	30	0.5
Run 10 = 6B	6	20	0.4

NMR Experiments

Solid-state CP/MAS ^{13}C NMR spectra were obtained on a JEOL (Japan) Lambda 400 NMR spectrometer operating at 100 MHz equiped with an accessory for cross polarization. An amount of 200 to 250 mg of sample was placed in a double-bearing rotor made of zirconia oxide. The spinning speed was set in the range 3200 to 3500 Hz; faster spinning did not improve the quality of the spectra. The ^{1}H radio-frequency field strength was set to give a 90° pulse duration of the order of 6 μs; the same value was used for the dipolar decoupling process. The ^{13}C radio-frequency field strength was obtained by matching the Hartmann-Hahn condition. The contact times and recycle delays were 1.2 ms and 10 s, respectively for dry samples and 2 ms and 5 s for hydrated samples. For each spectrum, 500 transients were collected and ^{13}C chemical shifts were measured relative to carbon chemical shift of glycine carboxyl group (176.03 ppm).

^{13}C spin-lattice relaxation times in the laboratory frame were measured by the Torchia pulse sequence.[13] ^{1}H spin-lattice relaxation time in the rotating frame were measured by varying the delay between the beginning of the saturation pulse and the contact time. No special care was taken to maintain the desired relative humidity of the hydrated samples during acquisition and both NMR spectra and relaxation times were measured at ambient temperature.

RESULTS AND DISCUSSION

Solution NMR

In order to obtain information on the primary structure of scleroglucan and to check possible deterioration of polymer chains due to high-pressure treatment, we performed ^{13}C NMR spectra of three representative samples in solution.

Figure 3 shows the ^{13}C scleroglucan NMR spectra in DMSO-d_6 at 80°C for 1B, reference, and 6B samples. These spectra are identical and represent a peak distribution of regular conformation having four sugar units in its repeating sequence with no interactions between the individual chains and which exhibit a mono-chain random-coil

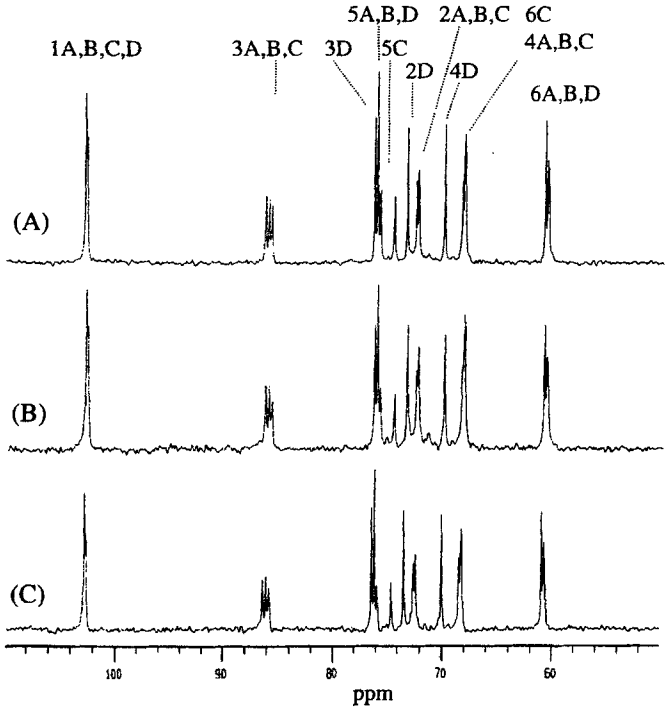

FIGURE 3 100 MHz ^{13}C NMR spectra of scleroglucan in DMSO-d_6 solution at 80°C. (A) 1B sample; (B) reference sample; (C) 6B sample.

conformation.[11,12] No residual peaks were observed in the background signal that could indicate a deterioration of the chains due to drying process.

Solid-state NMR

In order to obtain information on the chain conformation, we performed ^{13}C solid-state NMR on dry and hydrated scleroglucan. Previous solid-state ^{13}C NMR studies[10,11] showed the existence of three types of conformations: form I (lower molecular weight oligomers), form II (single chain) and form III (triple helix), which are distinguishable in linear and branched $\beta(1-3)$-D-glucans. The conformation dependence of the shifts of the C-3 ^{13}C NMR peak at the glycosidic linkages (which can vary over 3 ppm range) as well as

peak profile of C-2, C-4 and C-5 carbons among several types of samples were examined.

The ^{13}C NMR spectra of two samples dried by the D.I.C. process (1B and 6B) compared with the reference sample are presented in Figure 4. These spectra show six broad signals arising from the six carbon nuclei of the polymer chain repeating unit. As a general rule, amorphous polymers generate broad NMR resonance lines that result from the distribution of local magnetic environments arising from differences in conformational states. In contrast, crystalline polymers generally generate NMR spectra with narrow signals, owing to the ordered solid matrix and magnetic environment. These spectra are typical of a partially crystalline polymer, and similar to already published ones.[11,12] They present the same features and with no differences in peak chemical shifts even for the C-3. This observation is in favour of a unique conformation state independently of the drying process: a triple helix conformation.

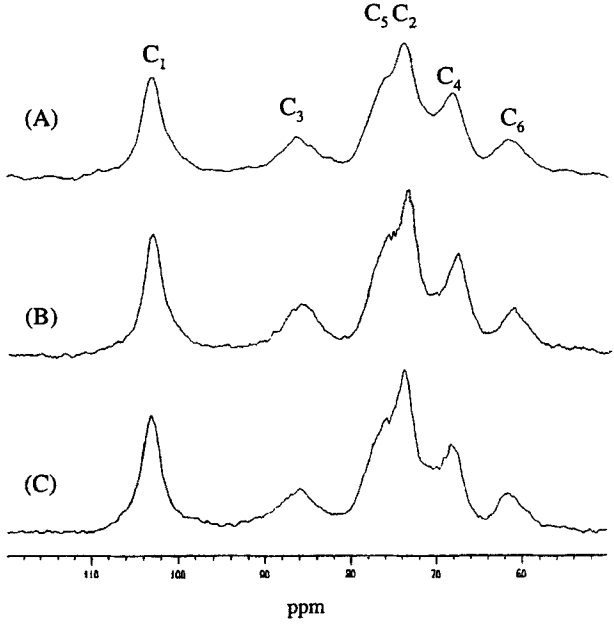

FIGURE 4 100 MHz solid-state CP/MAS ^{13}C NMR spectra of anhydrous scleroglucan. (A) 1B sample; (B) reference sample; (C) 6B sample.

The solid-state ^{13}C NMR spectra of hydrated 2B scleroglucan (Fig. 5) is better resolved, with an important decrease in the chemical shift dispersion. Two new signals appear in the spectra at 69 ppm and 77 ppm originate from the pendant D-unit. Nevertheless, no spectral changes were observed between the anhydrous and hydrated states of scleroglucan 2B.

Hydration has been recognized as a very important process in order to obtain the most favorable thermodynamic conformation (conformational stabilization) of variety of biological macromolecules. Previous ^{13}C NMR studies[11, 12, 14–16] have demonstrated that the hydration of amylose, starch or $\beta(1-3)$-D-glucans caused either substantial narrowing or displacements of ^{13}C NMR peaks as a result of conformational stabilization or change, respectively, depending on the type of the molecular structure. In the case of hydrated scleroglucan, water molecules can relax the internal strains associated with molecular packing or drying. It has also been shown that an increase in molecular or segmental motion can average the chemical shift anisotropy, and thus contribute to line narrowing.

Table II gives the chemical shifts of the main signals for anhydrous and hydrated scleroglucan. The δ values are identical for each type of

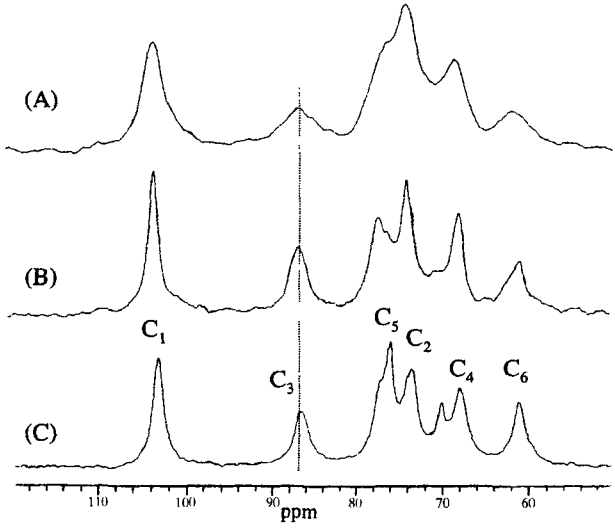

FIGURE 5 100 MHz solid-state CP/MAS ^{13}C NMR spectra of 2B scleroglucan. (A) anhydrous form; (B) hydrated sample with 20% water; (C) hydrated sample with 40% water.

TABLE II Chemical shifts (ppm) of anhydrous and hydrated 2B scleroglucan

Carbon	*Anhydrous scleroglucan*	*Scleroglucan+ 20% water*	*Scleroglucan+ 40% water*
C-1	103.3	103.4	103.4
C-2	74.1	74	73.5
C-3	86.5	86.5	86.7
C-4	68.5	68	68
C-5		77.2	77.2
C-6	62.2	61.2	61.2

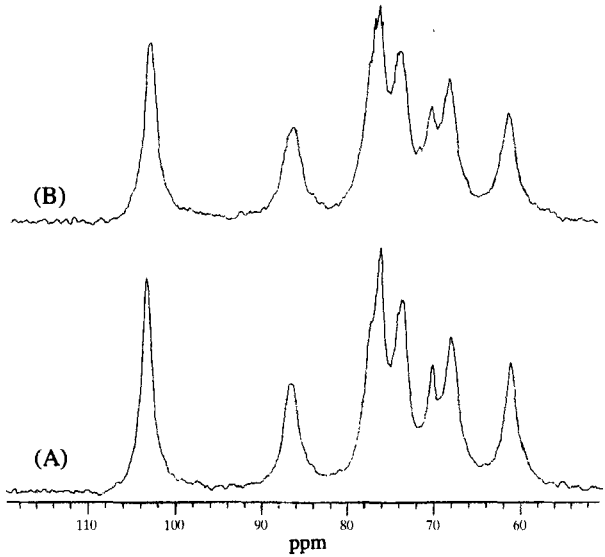

FIGURE 6 100 MHz solid-state CP/MAS ^{13}C NMR spectra of hydrated scleroglucan with 40% water. (A) 2B sample; (B) 5B sample.

carbons, except for the hydroxymethyl C-6 signal, which is shifted towards lower δ values ($\Delta\delta = -1$ ppm) on going from the anhydrous to hydrated form. This observation, as mentioned by Bardet *et al.*,[12] is in favor of a major *gauche–gauche* conformer for the hydrated scleroglucan. Water acts as a plasticizer, allowing a more stable conformation (triple helix), yielding a higher short-range order and, at the same time, a higher mobility of the polymer chain. This finding was confirmed by the relaxation time measurements.

Figure 6 compares the ^{13}C NMR spectra of 2B and 5B scleroglucan with the same hydration capacity. Here again, sample 5B spectrum is better resolved than the anhydrous sample with a decreasing chemical

shift dispersion due to the presence of water molecules. No chemical shift displacement is observed between the dry (Fig. 3) and hydrated 5B product except for C-6 carbons. However, the spectra of 2B and 5B hydrated scleroglucan can clearly be distinguished by the half width of the peaks. 5B sample shows broader peaks, specially concerning C-3 peak, which could be the result of two effects. Firstly, part of the scleroglucan is less hydrated than the 2B sample giving rise to broader line widths. Consequently, as the percentage of water is the same as in the 2B sample, the rest of the sample should be more hydrated. However, an excess in hydration of this type of polymer gives rise to higher mobility of the chains and, therefore, a short-range disorder close to the gel state. The consequence of this disorder is peak broadening in the spectrum due to the aggregates of triplex and entanglements of polymer chains.[12]

Relaxation Times Measurements

By measuring spin-lattice relaxation times, NMR can probe the motion at several places in a repeating unit if the time scale of this motion corresponds to the frequency experienced. In order to monitor segmental vibrational or rotational motions of the chain segments, we measured the ^{13}C spin-lattice relaxation time in the laboratory frame at 100 MHz. The results are presented in Table III (anhydrous samples) and Table IV (hydrated samples).

The T_{1C} values given in Tables III and IV were calculated by using a software to fit the data to a sum of two different exponential functions for dry and hydrated samples.

For dry scleroglucan, the decay curve of the magnetization was composed of two components (except for the C-6 signal) which correspond to two T_{1C} values. The shorter one ($T_{1C} \leq 6$ s) contributes to 20–30% of the total, whereas the longer one ($30 \leq T_{1C} \leq 80$ s) corresponds to major contribution. For C-1, C-2 and C-4 signals, these long T_{1C} values arise from the carbon atoms of the main linear chain, whereas the short T_{1C} values originate from both the more mobile lateral units and amorphous domains. The C-6 carbon represent a single and short relaxation time due to a rapid rotation of the hydroxymethyl groups A, B, D.

TABLE III Carbon spin-lattice relaxation T_{1C} (s) and degree of crystallinity for anhydrous scleroglucans

Carbon	*1B*	*2B*	*Air stream sample*	*Reference sample*	*5B*	*6B*
C-1	47	46	48	49	43	52
	7	6	10	5	3	5
C-2[a]	40	39	45	39	38	40
	2.8	4	3.5	4	3	4
C-3	**34**	**34**	**44**	**47**	**47**	**50**
	0.4	**2**	**1.2**	**1.8**	**1**	**4.8**
C-4	35	31	63	30	50	87
	5	5	6	4	6	8
C-6	0.7	0.7	0.7	0.7	0.8	0.9
Degree of crystallinity	90%	88%	76%	85%	73%	73%

[a] Average value from two or three peaks.

TABLE IV Carbon spin-lattice relaxation T_{1C} (s) and degree of crystallinity for 2B and 5B hydrated scleroglucan

Carbon	*2B+40% H_2O*	*5B+40 H_2O*
C-1	18	30
	3	2.2
C-2	13.3	19.5
	1.2	1.5
C-3	**12.9**	**24**
	2.5	**2.3**
C-4	5.5	12
	1.7	1.5
C-5	6.6	12.2
	2	1
C-6	0.27	0.4
Degree of crystallinity	70%	55%

Nevertheless, for the C-3 signal at 86 ppm, the peak intensity, arising only from the carbon of the linear chain, is also composed of two different exponential decays. This polymer being semicrystalline, the longer T_{1C} values correspond to ordered triple helix domains and the shorter one to amorphous domains. The relaxation times of rigid segments (long T_{1C}) increase with the processing pressure that indicates an increase of the rigidity of the ordered triple helix, but, at the same time, a decrease of the degree of crystallinity from 90 to

73%. This degree of crystallinity was measured by the long T_{1C} contribution to the total magnetization. These results prove that the high-pressure process induce the formation of a highly ordered conformation or compact domains.

For hydrated scleroglucan, the decay curve also was found to be composed of two components except for the C-6 signal (Tab. IV). An important shortening of the carbon spin lattice relaxation time T_{1C} was observed for 2B scleroglucan (long T_{1C} divided by 2.6) which accounts for the higher mobility of hydrated scleroglucan chains. In fact, T_{1C} values are mainly influenced by dipolar interaction with the hydroxymethyl protons undergoing rapid reorientation. As mentioned above, this increase in molecular motion is due to the water molecules inside the hydrated scleroglucan which relax the inter-chain hydrogen bonds. A shortening was also observed for the 5B sample (long T_{1C} divided by 2) but 55% of the chains maintain a long T_{1C} as compared to 2B sample ($T_{1C} = 24$ s), thus indicating that a large part of the hydrogen bonds are not relaxed and, consequently, the product is not fully hydrated.

The measurements of the ^1H relaxation times in the rotating frame ($T_{1\rho H}$) in a proton radio-frequency strength of 42 kHz were performed to check the homogeneity of chain packing. By assuming a single exponential decay of the magnetization, a fairly constant $T_{1\rho H}$ for all the carbon sites can be seen (Tab. V), in relation to a constant proton spin diffusion for both 2B and 5B samples. The single exponential decay is in favor of a semicrystalline polymer having small ordered triple-helix domains, allowing a homogeneous diffusion of proton spins. However, we can observe a very small variation between two different samples. $T_{1\rho H}$ seems to decrease with the processing pressure.

TABLE V Proton spin-lattice relaxation in the rotating frame $T_{1\rho H}$ (ms) for anhydrous scleroglucan samples

Carbon	*1B*	*2B*	*Air stream sample*	*Reference sample*	*5B*	*6B*
C-1	4.3	4.35	4.1	4.3	4.1	4
C-2[a]	4	4.1	4	4	4	4.1
C-3	**5.5**	**4.3**	**4**	**3.9**	**3.9**	**3.8**
C-4	4.4	4	3.9	4.1	4.1	4.1
C-6	4.6	4.5	4	3.8	3.8	3.8

[a] Average value from two or three peaks.

TABLE VI Proton spin-lattice relaxation in the rotating frame $T_{1\rho H}$ (ms) for 2B and 5B anhydrous scleroglucans

Carbon	*2B+40% H_2O*	*5B+40 H_2O*
C-1	9.9	9.4
C-2	9.2	7.7
C-3	**10.2**	**8.5**
C-4	9.6	7.7
C-5	9.1	8.3
C-6	8.5	7.5

Assuming that we are in the high-temperature range of the relaxation curve, an increase in the $T_{1\rho H}$ indicates a decrease in the H/C dipolar coupling due to chain mobility, thus confirming the results of the T_{1C} measurements, *i.e.*, the chains of high-pressure samples are packed more compactly than low-pressure samples.

The hydrated samples present a net increase of the $T_{1\rho H}$, always with only one exponential decay that indicates a homogeneous spin diffusion. Here again, the increase of $T_{1\rho H}$ is due to a decrease in C/H dipolar interactions. This decrease is caused by the insertion of water inside the interchain spaces. This can lead to an increase in the interchain average distance and to higher molecular motion. But 5B sleroglucan shows lower values of $T_{1\rho H}$ as compared to 2B sample which confirms that one part of the product is not hydrated. The spin diffusion remains homogeneous due to probably small hydrated and nonhydrated domains.

CONCLUSION

The different NMR experiments carried out in this study provided interesting information about the effects of the new drying technique (D.I.C.) on the solid-state conformation of scleroglucan. Although the solid-state CP/MAS ^{13}C NMR spectra for dry products did not reveal conformational changes in high-pressure samples, the hydrated form of these samples showed broader peaks indicating a nonhomogeneous distribution of water molecules inside the polymer chains. All anhydrous samples present the same triple-helix conformation but with differences observed on the mobility of the ordered conformation (C-3 T_{1C} increase from 34 to 50 s with the processing pressure of the

drying technique). This interpretation was confirmed by the $T_{1\rho H}$ values that are lower for the 5B sample than for 2B. The C/H dipolar coupling seems more effective for 5B indicating stronger rigidity of the chains.

The relaxation measurements show that hydration is not homogeneous for high-pressure samples. There are probably ordered triple-helix domains where water molecules can not relax the hydrogen bonds. It could explain the divergence of the viscosity measured by a turbine stirrer (see Fig. 2). Furthermore, these domains are small (single-exponential decay for $T_{1\rho H}$) and should be dispersed in the polymer.

The explanation of the effect of this new drying technique on the structure of scleroglucan could be based on the well-known effect of vacuum drying process.[17, 18] For porous media, the vacuum process collapses pores, giving rise to a highly compact system. For D.I.C. treatment, the initial water molecules from moisture are vaporized. The pressure drop induces a flux of water molecules from the bulk to the particles surface. For high-pressure treatment, this flux gives rise to a good vacuum inside the particles, packing the chains in a rigid-ordered triple helix. For low-pressure samples (1B and 2B), the pressure drop is lower and the mechanical strain on the polymer chains are not sufficient to create the ordered conformation of the triple helix.

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