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Solid state NMR spectroscopy study of molecular motion in cyclomaltoheptaose (β-cyclodextrin) crosslinked with epichlorohydrin[1]

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Abstract

Dry and hydrated insoluble cyclomaltoheptaose (β-cyclodextrin, β-CD) polymers have been investigated by solid state ^{13}C NMR spectroscopy techniques such as cross polarization/magic angle spinning with dipolar decoupling (CP/MAS), magic angle spinning both with (DD-MAS) and without (MAS) dipolar decoupling and CP/MAS dipolar dephasing (dd-CP/MAS) to allow the assignment of the main ^{13}C signals. In the solid state, the presence of water in the samples resulted in a better resolution reflecting increased mobility. Two distinct components (crosslinked β-CD and polymerized epichlorohydrin) have been found. The molecular mobility of these two components has been analyzed in terms of relaxation parameters such as ^{13}C spin lattice relaxation (T_1) and ^1H spin lattice relaxation in the rotating frame ($T_{1\rho}$). The T_1 values of the polymers show that the β-CD trapped inside the polymers does not seem to undergo changes in its mobility whatever the amount of epichlorohydrin. The addition of water to β-CD significantly increases the T_1 values reflecting strong interaction between β-CD and the solvent. The $T_{1\rho}$ values obtained reflect the homogeneous nature of the materials.

Keywords: Cyclomaltoheptaose (β-cyclodextrin); Cyclodextrin polymers; Epichlorohydrin; Solid state NMR spectroscopy; Water

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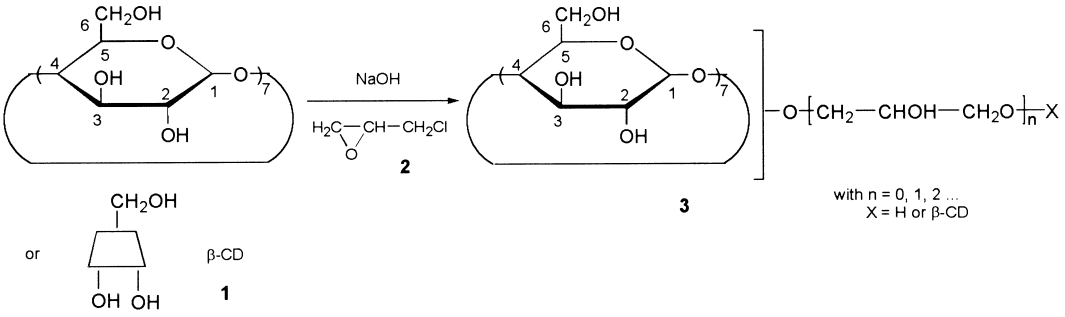


Scheme 1. Synthesis of cyclomaltoheptaose polymers.

1. Introduction

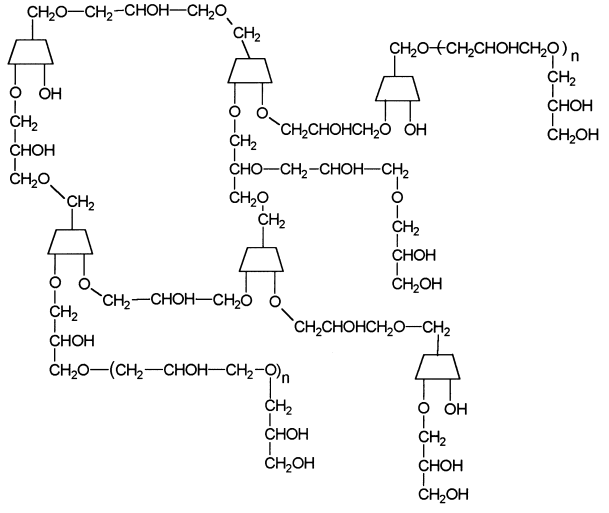
There has been considerable interest in the preparation, properties and applications of cyclomaltoheptaose (**1**) (β-cyclodextrin, β-CD) polymerized with epichlorohydrin (**2**) to form the insoluble crosslinked polymer **3** [1–7]. This entity is a polymeric mixture containing β-CD units joined by repeating glyceryl linkers (Scheme 1). These polymers have been used in chromatography [2], in catalysis [4] and in other fields such as the pharmaceutical and food industries [5–7]. They also have a potential use in waste water treatment as a result of the ability of β-CD to form inclusion complexes with many pollutants [8–12].

As little information is available on the NMR spectroscopy characterization and structure of these insoluble polymers, we have undertaken investigations based on solid state NMR spectroscopy techniques. The aim of this work was to investigate the molecular mobility of the components which could provide a better understanding of the influence of the structure on the complexing capacity of the materials. In this paper are reported the preparation and an NMR spectroscopy study of the molecular motion of β-CD polymers having different CD/epichlorhydrin ratios. Solid state ^{13}C NMR techniques such as cross polarization/magic angle spinning with dipolar decoupling (CP/MAS), magic angle spinning both with (DD-MAS) and without (MAS) dipolar decoupling and CP/MAS dipolar dephasing (dd-CP/MAS) have been used to differentiate between sections of these materials with different molecular mobilities. Two components have been found and analyzed in terms of relaxation parameters such as ^{13}C spin lattice relaxation (T_1) and ^{1}H spin lattice relaxation in the rotating frame ($T_{1\rho}$).

2. Results and discussion

Epichlorohydrin, which contains two reactive functional groups, can react with β-CD molecules (crosslinking step) and/or itself (polymerization step). The possible structure of β-CD polymer **3** obtained by condensation between β-CD and epichlorhydrin is shown in Scheme 2. Several samples with β-CD contents ranging from 20 to 80% w/w have been prepared (Table 1). In these samples, two kinds of structure could be expected: β-CD crosslinked by epichlorhydrin and polymerized epichlorhydrin. In order to confirm this assumption, we have investigated the structure of these insoluble polymers by solid state NMR spectroscopy.

Generally speaking, the use of the carbon–proton dipolar interaction in a CP/MAS experiment is a very efficient tool for the analysis of rigid structures, but becomes less effective when the mobility of the sample increases. Moreover, if the sample is



Scheme 2. A possible structure for a cyclomaltoheptaose polymer.

Table 1
Experimental conditions and amount of β-CD incorporated in the polymers

Polymer	Epichlorhydrin [a]	T (°C)	Time (h)	aq NaOH [b]	β-CD [c]
1	10	50	2	50	290
2	15	50	2	50	310
3	17	50	5	23	700
4	20	50	2	50	200
5	20	50	24	50	420
6	20	50	8	50	150
7	30	80	2	50	110
8	59	22	24	23	800

[a] In molar ratio epichlorhydrin/CD.
[b] % in w/w.
[c] From spectrophotometric method, in mg/g.

not homogeneous, the different phases can respond differently to the imposed conditions. In this case, the use of the solid state high resolution NMR DD-MAS technique both with and without CP, gives additional information on the rigid and mobile phases [13–15]. All these methods have been used in the present work.

Prior to the study of the polymer samples, free β-CD was investigated as a dry and hydrated sample. The dry β-CD sample was obtained by recrystallization from water and heating overnight under vacuum at 110 °C. The CP/MAS ^{13}C NMR spectrum of β-CD is very sensitive to its hydration level as shown in Fig. 1A and 1A′. The dry β-CD sample shows different degrees of crystallinity (which may also include different forms) as revealed by the number of resonances due to C-1, C-4 and C-6 atoms of the glucopyranose unit. The hyperfine structure of the β-CD spectrum is dependent on the conditions of crystallization used as shown in previous work [16–18]. The addition of water to the β-CD sample decreases the resolution of the spectrum (Fig. 1A′). The chemical shift data obtained for the two hydration states of β-CD are presented in Table 2.

The CP/MAS spectrum of the dry insoluble polymer **4** (kept under vacuum at 60 °C for 20 h) shows the peaks of a disordered β-CD (broad signals) in the range 50–110 ppm (Fig. 1B). This

Table 2
^{13}C spin lattice relaxation (T_1 in s) of the carbon atoms of β-CD, β-CD polymers and potato starch both of dry (see Experimental) and hydrated (33% w/w of water) samples

	Chemical shift (ppm)	β-CD		Polymer 4		Polymer 3		Potato starch	
		Dry	Hydrated	Dry	Hydrated	Dry	Hydrated	Dry	Hydrated
		T_1	T_1	T_1	T_1	T_1	T_1	T_1	T_1
C-1	103.9	13.6							
	102.9	20.9							
	102.1	21.2							
	101.6	22.5	34						
	100.4			22	1.6	20.1	2.7	34.6	27.8
	99.6							38.2	34.7
C-4	84.3	16.9							
	82.8	15.5							
	81.3	17.6	27.6	16.4	1.2	16.7	2.6		
	78.0	18.5							
	74.7							27.3	26.4
C-2	76.9	11.6							
C-3	76	10.8							
C-5	74.8	12.2							
+ Epichlorhydrin	73.4	11.4							
	72.9								26.1
	72.4	13.5	22.7	9.9	0.6	13.1	1.1	23.4	
	71.9								24.7
	70.6				0.6				28.7
	69.8								21.1
C-6	63.9					5.9	0.4		
	62.5			nd [a]	0.4				
	61.4							6.9	6
	60.1	3.3							

[a] nd = not determined.

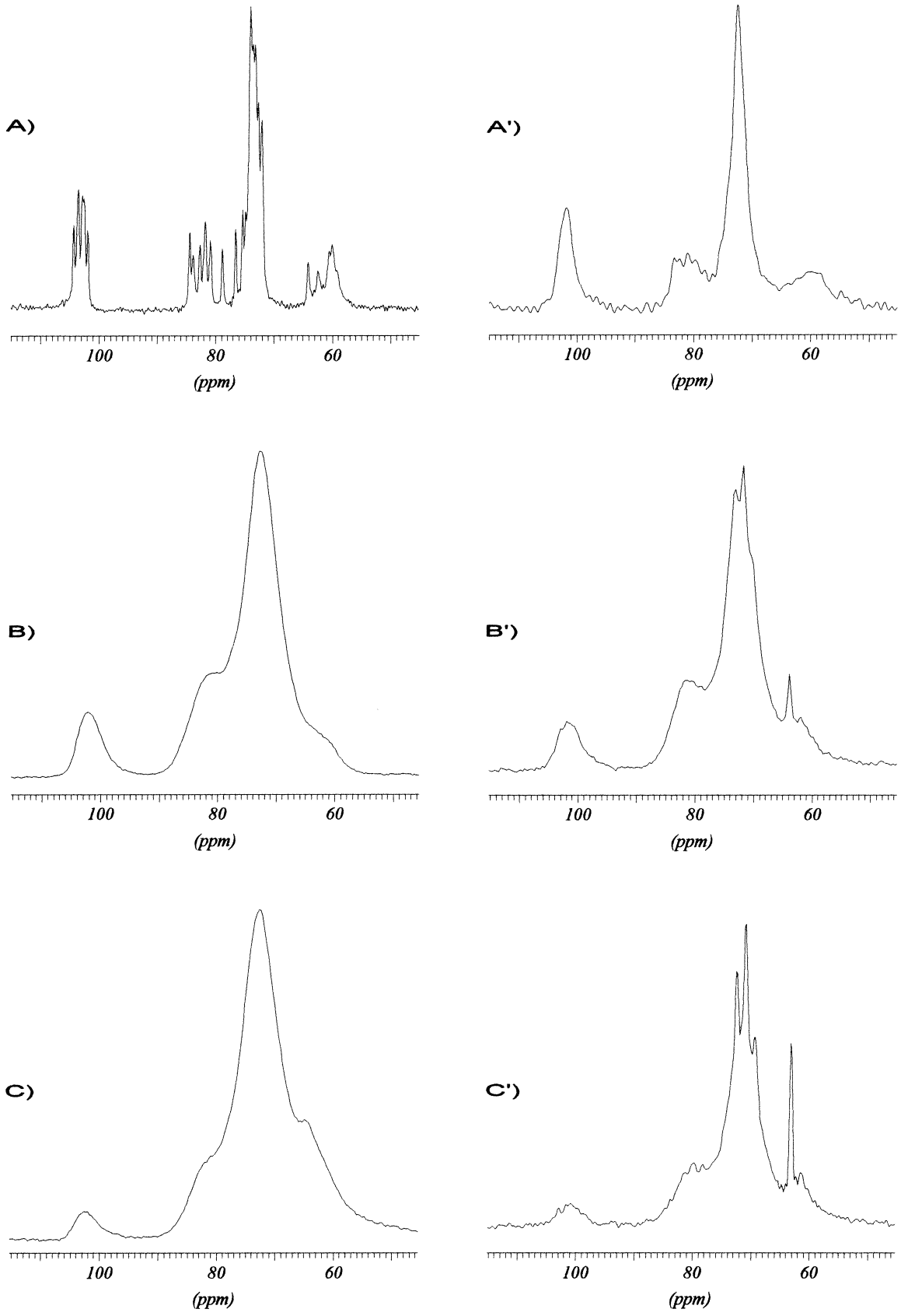


Fig. 1. (A) and (A′) CP/MAS spectra of dry and hydrated* β-CD respectively; (B) and (B′) CP/MAS spectra of dry and hydrated* β-CD Polymer 4 respectively; (C) and (C′) MAS spectra of dry and hydrated* β-CD Polymer 4 respectively [* hydrated with (33% w/w of water)].

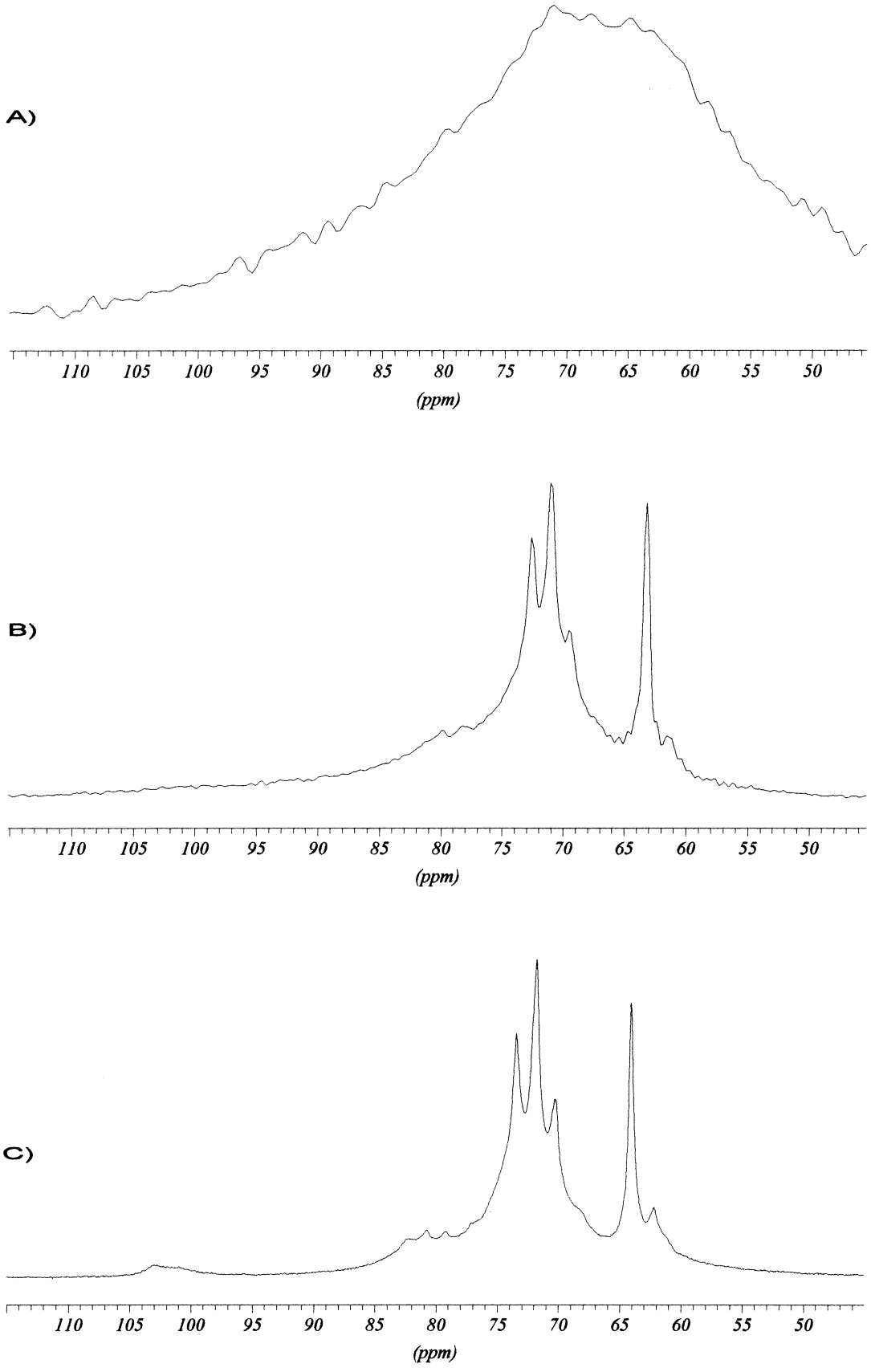


Fig. 2. (A) and (B) MAS spectra without dipolar decoupling of dry and hydrated* β-CD Polymer 4 respectively; (C) ^{13}C NMR spectrum of β-CD Polymer 4 (80% w/w of D_2O, $T = 313$ K) [* hydrated with (33% w/w of water)].

polymer contains about 80% (w/w) of the crosslinking agent (see Table 1) the signals of which are completely hidden by the C-2, 3 and -5 β-CD peaks. In the DD-MAS spectrum of the polymer (Fig. 1C), a different ratio between the area of the signals is observed. Noteworthy is the decrease of the signals of β-CD C-1 carbon and the increase of the signals in the region between 60 and 70 ppm attributable either to the hydroxymethyl group at C-6 in the glucose unit of β-CD or to the hydroxymethyl group of epichlorohydrin. A higher contribution to the total signal intensity of the most mobile carbons, as expected, can account for this behavior. This observation confirms the existence of two components with different molecular mobilities in the polymer. This can be emphasized by the addition of water which interacts with the system and changes its mobility and proton distribution. In Fig. 1B′ and 1C′, the CP/MAS and DD-MAS spectra of the polymer hydrated with about 33% w/w of water are shown. A signal at 62.5 ppm is now evident and is attributable to the hydroxymethyl group of the epichlorohydrin terminal residue, as demonstrated in a similar soluble structure by the H/D isotopic effect in the ^{13}C spectrum (not shown). A small signal at about 62 ppm has been assigned to C-6 of β-CD. The mobile parts of the crosslinking agent are still emphasized in the DD-MAS spectrum. The spectra of both a dry and a hydrated sample have also been recorded without dipolar decoupling (MAS). In this case, only the low power decoupling generally used for solutions was used. The MAS spectrum of the dry sample shows only a very broad band typical of solids (Fig. 2A), while for the hydrated sample sharp resonances are detected (Fig. 2B). In these conditions, the signals of β-CD decay and only the peaks of the crosslinking agent can be observed. In this case, the interaction of the epichlorohydrin, or part of it, with the solvent is comparable with that observed in solution. The β-CD is still the stiffer part even if the interactions with the solvent decrease the relaxation time (Table 1).

As the final aim was to use these materials as sorbents for water pollutants, the hydration was increased to about 80% (w/w of D_2O). This sample resembles a gel more than a solid. The ^{13}C spectrum of this sample, recorded in solution, is shown in Fig. 2C. It is similar to the spectrum in Fig. 2B (33% w/w), but the anomeric C-1 signal of the crosslinked β-CD is now detectable. This means that, in these conditions, the interaction of β-CD with water becomes comparable to the one observed in a soluble β-CD polymer which has been used as a model compound. The DEPT spectrum (not shown) of the same sample revealed three CH_2 signals at 62, 62.5 and 74 ppm corresponding to the C-6 of β-CD, the CH_2OH of the epichlorhydrin terminal residue and the CH_2OR of the epichlorhydrin group. The use of another technique, ^{13}C CP/MAS dipolar dephasing (dd-CP/MAS) at different delay times gave useful information on the magnitude of ^{13}C–^{1}H dipole–dipole interactions [19,20]. The dd-CP/MAS spectra, run on a hydrated sample of β-CD polymer, showed results comparable to those obtained with DD-MAS techniques. As the dipolar dephasing time increased, the spectra showed a decrease in intensity of the signals arising from the most rigid carbons (Fig. 3). At long dephasing times, dispersion of the signals at 100.4 ppm (anomeric C-1 of β-CD) and between 75 and 85 ppm (C-4 of the glucose unit), was observed. After 50 μs of dephasing, the

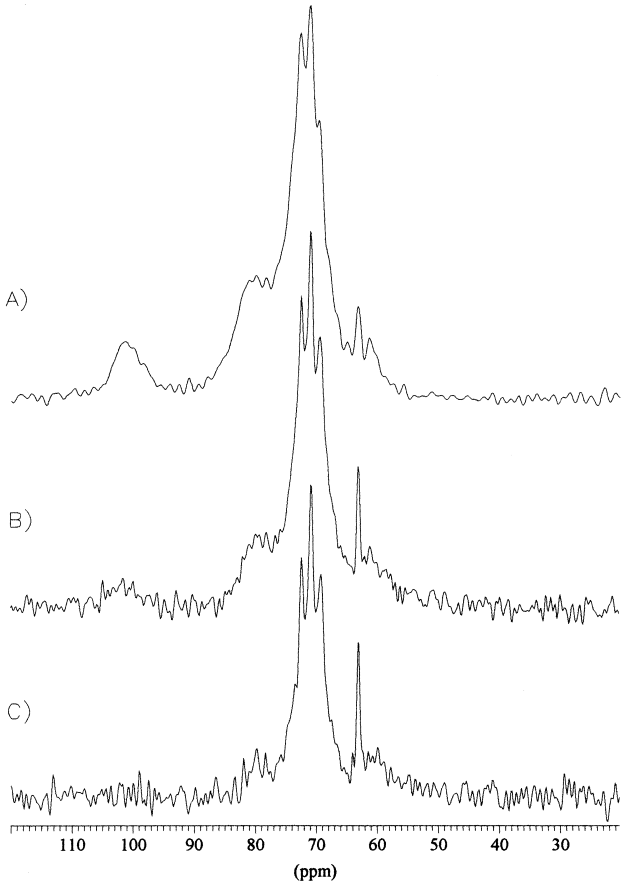


Fig. 3. CP/MAS spectra with the dipolar dephasing technique of hydrated * β-CD Polymer 4 using different delay times: (A) 5 μs; (B) 20 μs; and (C) 50 μs [* hydrated with (33% w/w of water)].

spectrum resembled the one obtained without DD. By the use of these techniques it was possible to distinguish between two structures with overlapping signals and different mobilities within the same polymer. Furthermore, different interactions with water could be highlighted.

The values of ^{13}C spin lattice relaxation times (T_1) and ^{1}H spin lattice relaxation in the rotating frame ($T_{1\rho}$) have been calculated in order to quantify the molecular mobility of the materials examined and to check possible differences in this property for polymers characterized by different CD/epichlorhydrin ratios. These parameters have been measured at room temperature for dry and hydrated samples of free β-CD and β-CD polymers. T_1 values were obtained with the Torchia sequence [21]. The T_1 values of the crosslinked β-CD were very similar to those of the crystalline β-CD form (Table 2). The β-CD trapped inside the polymer did not seem to change its mobility whatever the amount of epichlorohydrin. The addition of water to β-CD significantly increased the T_1 values, consistent with a change of the crystalline cell [17,18]. In the case of starch, the addition of water to the system which results in an increase of crystalline as compared to amorphous material, showed essentially identical values for T_1 (Table 2 [22]). The addition of water to β-CD polymers decreases the values of T_1 more than 10 times. This means that not only does the epichlorohydrin component interact with water as previously observed, but the β-CD also experiences a very strong interaction with the solvent to the detriment of CD–CD interactions. The importance of this kind of interaction appears to be greater in the samples containing more β-CD (slight decrease of T_1 values). The $T_{1\rho}$ values given in Table 3 are the proton spin lattice relaxation times observed by ^{13}C NMR spectroscopy. This technique offers a better resolution over direct pulsed ^{1}H NMR spectroscopy. The $T_{1\rho}$ values were obtained by measuring ^{13}C magnetization as a function of a variable delay, and were determined from the same expression used for the standard inversion recovery

Table 3
^{1}H spin lattice relaxation in the rotating frame ($T_{1\rho}$ in ms) of the carbon atoms of β-CD, the β-CD polymers and potato starch both of dry (see Experimental) and hydrated (33% w/w of water) samples

	Chemical shift (ppm)	β-CD		Polymer 4		Polymer 3	Potato starch	
		Dry	Hydrated	Dry	Hydrated	Dry	Dry	Hydrated
		$T_{1\rho}$	$T_{1\rho}$	$T_{1\rho}$	$T_{1\rho}$	$T_{1\rho}$	$T_{1\rho}$	$T_{1\rho}$
C-1	103.9	2.6						
	102.9	2.6						
	102.1	2.6						
	101.6	2.5	4.7					
	100.4			1.4	1.5	2.6		15.3
	99.6						6.8	18.8
C-4	84.3	2.6						
	82.8	2.4						
	81.3	2.4	4.5	1.5	1.4	2.5	6.6	
	78.0	2.4						
	74.7							15.9
C-2	76.9	2.4						
C-3	76.0	2.2						
C-5	74.8	2.5						
+ Epichlorhydrin	73.4	2.6						
	72.4	2.6	4.6	1.8	1.5	2.4	7.1	
	71.9							15.5
	71.1							15.5
	70.6				1.7			
C-6	63.9					2.2		
	62.5			nd [a]	2.8			
	61.4						7.5	16.4
	60.1	2.7	4.2					

[a] nd = not determined.

experiment [23,24]. The $T_{1\rho}$ values reflect, among other things, the rate of the spin diffusion which is inversely related to the proton–proton intermolecular distance and to the degree of molecular motions [15]. All the carbon signals detected for each sample examined showed equivalent $T_{1\rho}$ values (Table 3). This finding, typical of organic solids, is indicative of the homogeneous nature of the samples. The low values of $T_{1\rho}$, although different, reflect spin diffusion in a domain of very small dimension (probably 1 nm) [23]. The values of $T_{1\rho}$ of the hydrated samples did not substantially change in the crosslinked samples. The contact alone with water does not change the dimensions of the 'crystallite'. On the contrary, addition of water to the β-CD and to a potato starch sample is eventually enough to increase the crystallinity and the $T_{1\rho}$ values 2–3 times.

3. Conclusion

This study was carried out in order to investigate the structure of β-CD using ^{13}C solid state NMR spectroscopy techniques. In the prepared materials, there is evidence of two kinds of structure, crosslinked β-CD and polymerized epichlorohydrin, with different molecular mobilities as indicated by ^{13}C NMR experiments. The T_1 values of the polymers are very similar to those of the crystalline β-CD form. The β-CD trapped inside the polymer does not seem to change its mobility whatever the amount of epichlorohydrin. The addition of water to β-CD resulted in better resolution in the spectra of the polymers and significantly increased the T_1 values reflecting strong interaction between β-CD and the solvent. The $T_{1\rho}$ values are equivalent, therefore indicating the homogeneous nature of the samples.

4. Experimental

General.—β-CD supplied by Janssen Chimica (Beerse, Belgium) was recrystallized from water and dried overnight under vacuum at 110 °C. Other compounds were of the highest quality available and were purchased from various suppliers. These reagents were used without further purification.

NMR spectroscopy.—Solid state ^{13}C CP/MAS NMR spectra were recorded with a Bruker AC-300 spectrometer operating at 75.47 MHz and 303 K. The following conditions were applied: repetition time 4 s, ^{1}H 90° pulse length 5.6 μs, contact time 1 ms and spin rate 4200 Hz. The compounds were placed in a zirconium rotor, 7 mm in diameter and 21 mm high. The chemical shifts were recorded relative to tetramethylsilane via benzene as secondary reference. For the evaluation T_1 the Torchia sequence was used [21]. The ^{13}C NMR spectrum in D_2O was measured at 100.61 MHz on a Bruker AMX 400 spectrometer fitted with a 10 mm BB probe. This measurement was performed at 313 K and the chemical shift values were referenced to external sodium 3-(trimethylsilyl) propionate-d_4.

General procedure for the preparation of the cyclodextrin polymer.—The procedure introduced by Solms and Egli [1], and extended by Komiyama et al. [4] was used with some minor modifications consisting of an increase in the amount of crosslinking agent in order to obtain mechanically stable polymers containing different amounts of β-CD. Composition of the various reaction mixtures is given in Table 1. A typical polymerization reaction was carried out as follows (polymer 1 in Table 1). In a thermostated reactor vessel, NaOH (40 mL, 50% w/w) containing $NaBH_4$ (5 mg) was heated to 50 °C. In this soln, β-CD (25 g) was dissolved. The desired amount of epichlorohydrin was added slowly dropwise and the mixture was vigorously stirred with a magnetic stirrer. The viscosity of the soln started to increase, and 2 h later a solid could be observed. Acetone (80 mL) was added and stirring and heating were continued for 10 min. After cooling, the insoluble polymer was poured into a large beaker of water, filtered and the resulting solid was purified by several Soxhlet extractions with acetone and water for 3 d. The polymer was then filtered and dried under vacuum at 60 °C for 20 h, crushed, and finally granulated to particle sizes of 1–2 mm in diameter. A series of polymers was prepared with different molar ratios of epichlorohydrin and β-CD. The results are summarized in Table 1. Preliminary experiments showed that the best method for the determination of the amount of β-CD in the material was the determination of reducing sugars with tetrazolium blue after acidic hydrolysis as previously reported [25]. The materials prepared have different β-CD contents (ranging from 20 to 80% w/w) as described in Table 1.

Acknowledgements

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