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Solid State ^{1}H-NMR Study of β-Cyclodextrin–Water Complexes

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SUMMARY

Crystalline *β-cyclodextrin–water inclusion complexes were studied by continuous wave (CW) and pulse NMR methods. A series of samples with different water contents was prepared by vacuum drying. The CW results show decreasing motion of the carbohydrate macro-ring with decreasing water content. The experimental data suggest the existence of different water fractions which are characterized by different molecular mobilities. The dynamic exchange between the fractions depends on the temperature and water content of the samples.*

INTRODUCTION

The cyclodextrins (CD) are cyclic oligosaccharides composed of six, seven or eight D-glucopyranose units (α-, β- and γ-cyclodextrins). On account of their industrial potential as host molecules for inclusion complexes, these compounds have been studied extensively (Szejtli, 1981, 1982; Osa *et al.*, 1984) both in solution and in the solid state. NMR and X-ray studies are the most informative techniques dealing with the steric relations between host and guest molecules. High resolution NMR spectra provide data only for dissolved substances (Bergeron *et al.*, 1978; Poh & Saenger, 1983). Recently, some ^{13}C CP/MAS NMR investigations have been reported on crystalline complexes too (Saito *et al.*, 1982; Okazaki & McDowell, 1983; Hall & Lim, 1984; Inoue *et al.*, 1984). Some of these studies were concerned with molecular motion.

Many X-ray and neutron scattering studies have been reported on the CD–water complexes ('empty' CDs), which represent the most simple crystalline systems in CD chemistry (Manor & Saenger, 1972; Hingerty & Saenger, 1975; Tokuoka *et al.*, 1980; Lindner & Saenger, 1982; Saenger *et al.*, 1982; Fujiwara *et al.*, 1983). These crystallographic studies revealed the more or less defined positions of H-bonded water molecules and the conformation of the carbohydrate rings.

A vast part of the NMR literature is dedicated to the state of water in organic systems (Zeidler, 1973; Cooke & Kuntz, 1974). Special attention has been devoted to the hydration of low and high molecular carbohydrates (Suggett, 1974*a*,*b*; Hsi *et al.*, 1979). The aim of our present work was to gather further information concerning the microscopic motion of the carbohydrate macro-ring and the water molecules within or around it. Solid state NMR is an appropriate technique for such studies and is able to promote an improved understanding of the properties of aqueous cyclodextrin systems.

MATERIALS AND METHODS

The β-CD 9·4 hydrate used was a commercial product of Chinoin, Budapest. A series of samples with different water contents was prepared by vacuum drying at room tmeperature. The water content was controlled by mass measurement, and the samples were stored in sealed glass tubes.

The CW measurements were carried out at 8·5 MHz on a home-built NMR spectrometer (Tompa & Tóth, 1963) at room temperature. Two different modulation levels ($H_{\text{mod}} = 0{\cdot}3$ and 0·01 mT) and sweep speeds were used enabling the partially separated detection of the narrow and wide parts of the cyclodextrin CW spectra.

The pulsed NMR measurements were carried out on a Bruker SXP-100 spectrometer, between 220 and 300 K, at 90 MHz. The T_2^* relaxation times were calculated from the free induction decays after a $\pi/2$ pulse; the T_1 relaxation times were measured by $\pi - \tau - \pi/2$ pulse sequences.

RESULTS

The CW measurements were carried out at two different modulation levels. The higher level of modulation results in the intensity enhancement of the broader region of the spectrum; a typical spectrum is presented in Fig. 1. The narrow spectral region results from water protons, the broader one from carbohydrate protons. The pure carbohydrate signal can be obtained by a linear truncation of the narrower part of the spectrum. The most characteristic and physically interpretable item of data of such separated wide NMR spectra is the so-called second moment, M_2. The modulation corrected values of M_2 are presented as a function of the water content in Fig. 2.

The carbohydrate signal is negligible at the lower level of modulation, but that portion of the spectrum obtained from the more quickly moving water molecules is well measurable. Such spectra have neither a simple Gaussian nor a Lorentzian shape. The complex form is attributable to the presence of different water fractions characterized by different motional correlation times. Another possible explanation might be an anisotropic chemical shift tensor for those water protons participating in hydrogen bonds (Robert & Wiesenfeld, 1982).

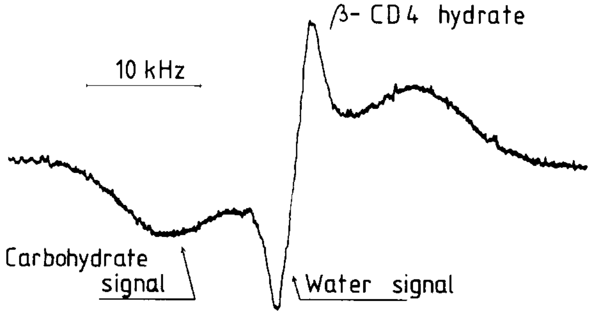

Fig. 1. Wide-line ^{1}H-NMR spectra of β-cyclodextrin $(H_2O)_4$.

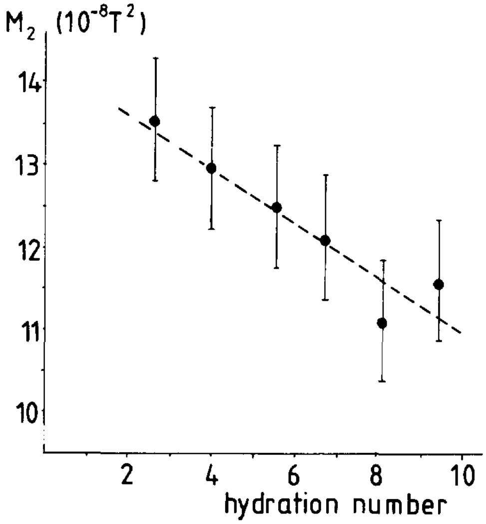

Fig. 2. Second moment of carbohydrate spectral part versus water content.

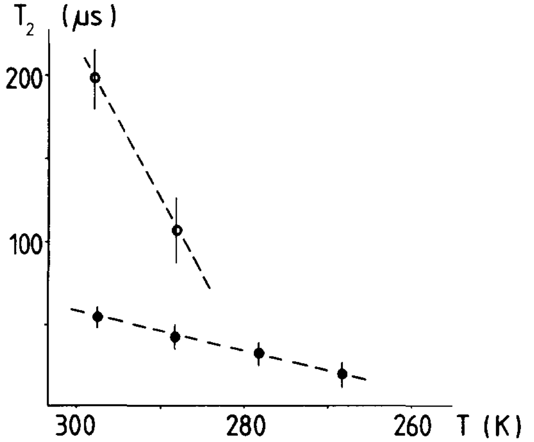

Fig. 3. T_2^* relaxation times of water in β-CD-$(H_2O)_{5\cdot5}$ at different temperatures. The two T_2^* values at the same temperature were obtained by fitting of the two exponentials; the full circles belong to the main relaxation processes.

Some results obtained by pulsed NMR relaxation time measurements are presented in Figs 3 to 6. The T_2^* relaxation times measured at different temperatures on β-CD 5·5 hydrate can be seen in Fig. 3. The free induction decays consist of two relaxation processes above 280 K; therefore two T_2^* values are obtained at these tempera-

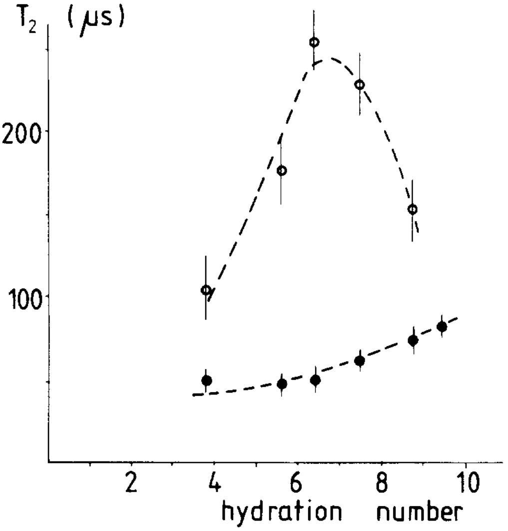

Fig. 4. T_2^* relaxation times of water at room temperature versus water content. Key as in Fig. 3.

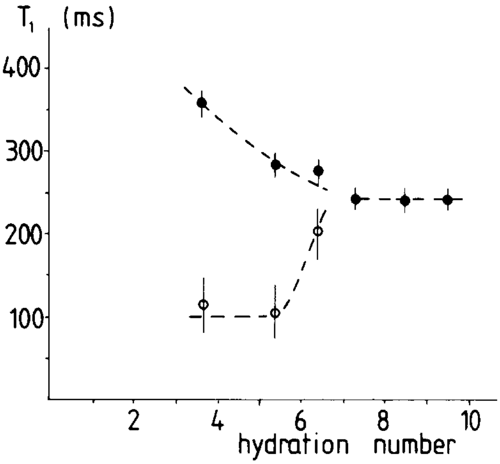

Fig. 5. T_1 relaxation times at room temperature versus water content. Key as in Fig. 3.

tures. Figure 4 shows water content dependence of T_2^* at room temperature. The T_1 values show interesting splitting phenomena, moving toward lower water content (Fig. 5) or lower temperature ranges (Fig. 6).

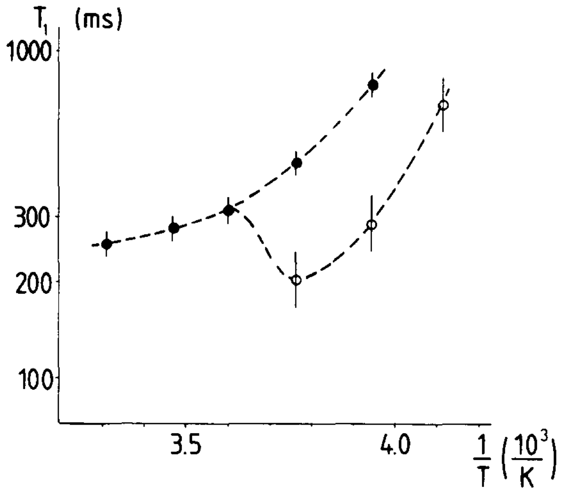

Fig. 6. T_1 values of β-cyclodextrin-$(H_2O)_{9.4}$ versus temperature. Key as in Fig. 3.

DISCUSSION

The data of Fig. 2 relate to the carbohydrate ring, even though some very rigidly bound water molecules may contribute to the measured second moment values. This contribution must be negligible because the highest possible proton number of the very rigidly bound water molecules is considerably smaller than that of the carbohydrate ring.

A significant increase of M_2 at lower water contents is seen in Fig. 2. The second moment values reflect the changes in the microscopic mobility of the protons in the sample. The second moment increases if the motion is less intense, i.e. the distribution function of motional correlation times is shifted toward lower frequencies. It seems that the freedom of molecular motion of the carbohydrate ring decreases with decreasing water content. The original H-bond network existing at higher degrees of hydration facilitates the ring vibrations; the partial breakdown of this network makes the whole system more rigid. A similar phenomenon is exhibited by biomolecular systems (Pócsik *et al.*, 1986).

Earlier papers dealing with crystallographic investigations on β-CD hydrates (Lindner & Saenger, 1982; Saenger *et al.*, 1982; Fujiwara *et al.*, 1983) share a common feature, namely locational disorder of the water molecules compared with the rigid and well-defined water structure of α-CD hydrates (Manor & Saenger, 1972; Hingerty & Saenger, 1975). In the β-CD dodecahydrate, only one of the included water molecules has ideally arranged tetrahedral surroundings, thereby providing the strongest possible hydrogen bond configuration (Lindner & Saenger, 1982). There are more internal and external sites than water molecules, so these sites are partially or fully occupied (Lindner & Saenger, 1982; Fujiwara *et al.*, 1983).

Moreover, Fujiwara *et al.* (1983) have suggested that the hydrate structure of β-CD may differ even at the same water content. In these systems the occupancy of different molecular sites varies, both for internal and external water molecules. These structures had shown strongly dissimilar thermal behaviour, especially in DSC and DTA measurements.

Two different interpretations can be given for the computer fitting results of our CW water spectra measured at the lower modulation level. This is a consequence of the lower signal/noise ratio of these spectra, which causes inconsistencies in computer fits when working with a large number of parameters. One possible interpretation is the existence of at least three motionally different water fractions with isotropic chemical shifts. The other possibility is the existence of at least two water fractions with an anisotropic chemical shift tensor in one of them ($\Delta\sigma = 10$–20 ppm). Because a proton, forming part of a hydrogen bond, has an anisotropic chemical shift tensor the second choice is the more likely.

The T_2^* relaxation times plotted in Figs 3 and 4 show the existence of at least two water fractions as well. The β-CD-$(H_2O)_{5\cdot5}$ has a two-exponential spin–spin relaxation process. The higher T_2^* values characteristic of the more freely moving fraction disappear at lower temperatures (see Fig. 3). The lower T_2^* values maintain their original slope. This suggests the 'freezing' of the motion of the less bound fraction. Figure 4 also shows split T_2^* values except for the 9·4 hydrate. At present, no explanation can be given for the slope of the higher T_2^* but an estimate of the exchange time between the fractions can be made from these data. It can be seen that the 9·4 hydrate represents a limit; in this hydrate the exchange is quick enough to mix the fractions during the measured free induction decay. Thus, the exchange time is estimated to be of the order of 100 μs. This exchange consists of the motion of whole water molecules, the proton exchange and the cross-relaxation.

The T_1 data plotted in Figs 5 and 6 indicate that splittings appear as we move towards lower temperatures and water contents. These splittings reflect the slowing down of the cross-relaxation between the water and carbohydrate protons. This slowing down is caused by a strong change in the relative motional states of the water and carbohydrate systems.

Because the T_2 relaxation time values are close to T_2^* ones, the T_1/T_2^* ratios are informative too. These ratios were calculated using the relaxation times belonging to the main parts of the relaxation processes. The values of the ratios were found to be more than 100 at the highest measured temperatures, close to the temperature of T_1 minimum values. This suggests wide distributions of motional correlation times.

If we summarize our results on the water systems of β-CD hydrates, then we see that the existence of at least two water fractions has been demonstrated. The water molecules are mobile in both fractions, with different distributions of optional correlation times. All these motions are much slower than the observed ones in liquid water. The fully and partially occupied molecular sites shown by crystallographic investigations (Lindner & Saenger, 1982; Fujiwara *et al.*, 1983) are at the lower and higher energy minima of the potential surface of these systems, respectively. Because the probabilities and thus the correlation times of microscopic jumps depend on these energies, the different water fractions may be formed by the molecules populating the differently occupied molecular sites.

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