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^{1}H and ^{2}H NMR mobility in cellulose

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Abstract

Mobility of water in cellulose was studied by solid-state ^{1}H and high-resolution ^{2}H NMR as a function of moisture content within the unfreezable moisture range (0–19% dry basis). Measurements of relative mobilities were based on relative intensities, transverse and longitudinal relaxation times and lineshape analysis. At 2–16% moisture content (dry basis), water molecules reoriented anisotropically, suggesting an interaction with cellulose fibers. At moisture content below the monolayer value (2.8%, dry basis), 90% of the protons were immobile and no liquid deuterium signal was detected. A sharp increase in liquid or mobile ^{1}H intensity (accompanied by a decreased LW) and increases in ^{2}H NMR T_1 and T_2 relaxation times were observed as moisture increased above 9% (dry basis). At this moisture content the molecular mobility approached the fast exchange regime. The data confirmed earlier reports that unfreezable water could be highly mobile and not in a rigid state.

No glass transition was observed by DSC. However, NMR showed a significant mobility transition as the material transformed from a slow exchange (retarded mobility) to a fast exchange regime.

Keywords: Water mobility; Proton NMR; Deuterium NMR; Cellulose; Relaxation

1. Introduction

It has been recognized that many properties of food, such as microbial spoilage, enzyme activity, degradative reactions (e.g. lipid oxidation) are strongly dependent on the presence of water in the system. Water becomes "available" to chemical reactions and microbial growth when the molecules are adequately mobile. Thus, characterization of the molecular mobility of water is one of the key parameters to control food stability.

Nuclear magnetic resonance (NMR) has been used to characterize water mobility in foods (Richardson & Steinberg, 1987) by using oxygen-17 (^{17}O), proton (^{1}H) and deuterium (^{2}H) NMR. ^{17}O nucleus is more commonly used for the study of water molecular mobility owing to its simpler interpretation. Unfortunately, the lower ^{17}O NMR sensitivity compared to ^{1}H and ^{2}H NMR limits its use in the study of adsorbed water. ^{1}H and ^{2}H NMR have been used to study water in semisolid and solid systems, but its interpretation is complicated by the contributions from chemical exchange and/or cross-relaxation processes (Belton, 1990; Hills, 1992; Koenig, Bryant, Hallenga & Jacob, 1978; Shirley & Bryant, 1982). Solid-state NMR using ^{1}H and ^{2}H nuclei has been applied in characterizing water mobility in polymer solids (Li, Dickinson & Chinachoti, 1998; Tanner, Hills & Packer, 1991).

Cellulose was used in this study because it is a polymer present in plant tissue and its functionality is highly influenced by water in both native and modified forms. Natural cellulose is a structurally heterogeneous polymer, consisting of paracrystalline and amorphous domains. The glass transition temperature of dry cellulose has been reported to be extremely high (220–250°C, Akim, 1977; Back & Salmen, 1982; Kalashnik, Papkov, Rudinskaya & Milkova, 1991; Salmen & Back, 1977). Water can interact with the amorphous domains, but it is excluded almost completely from the crystalline regions (Child, 1972; Hatakeyama, Ikeda & Hatakeyama, 1987; Nakamura, Hatakeyama & Hatakeyama, 1981, 1983). Crystallinity has been reported to be a function of moisture (Creely & Tripp, 1971; Nakamura et al., 1983; Ray, 1969; Ray & Bandyopadhyay, 1975; Seitsonen & Mikkonen, 1972). Water sorption isotherms have been reported, suggesting that inter-molecular H-bond interactions are present among cellulose fibers and also among the active surface of cellulose and neighboring water

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molecules (Child, 1972; Froix & Nelson, 1975; Tsaryuk & Frantesson, 1991; Yano & Hatakeyama, 1988). Sorbed water can cause an increase in cellulose chain mobility by opening the intermolecular space and allowing more water molecules to enter and to form H-bonds with cellulose. At saturation or at the plasticization point (corresponding to the upward turn in the water sorption isotherm, Froix & Nelson, 1975), water sorption in the amorphous structure leads to swelling and corresponding formation of capillary or trapped water (Boesen, 1970). This spatially confined water has the properties similar to bulk water, e.g. freezable and rotationally mobile water (Boesen, 1970; Child, 1972; Froix & Nelson, 1975).

^{1}H and ^{2}H NMR spectra of cellulose at 0–10% water content (Capitani, Seagre, Attanasio, Blicharska, Focher & Capretti, 1995; MacKay, Bloom, Tepfer & Taylor, 1982; Wong, 1983) gave more informative lineshapes than ^{17}O NMR spectra that are extremely broad because of a strong quadrupolar interaction (Wong, 1983; Wong & Ang, 1985).

Molecular mobility of water has been addressed often as food stability indicator, but very little experimental data is available (van den Berg, 1991; Duckworth, 1981; Roos, Karel & Kokini, 1996; Slade & Levine, 1991). Plasticization of food matrices by water is an important phenomenon. A polymer may show its backbone chain and side-chain mobility increase with plasticization, but they may not agree with the motions of the diluents (Cauley, Cipriani, Ellis, Roy, Jones & Inglefield, 1991; McBrierty & Packer, 1993). Different analytical techniques vary their sensitivity range from the long-range relaxation or "structural relaxation" to the short-range motions or "molecular relaxation". For instance, thermal and thermomechanical relaxation measures mobility in a 20–300-nm range, while NMR measures molecular relaxation in a 1–2-nm range. Therefore, data on bulk properties alone (e.g. bulk viscosity, modulus, glassy/rubbery mechanical relaxation) cannot be used in some cases to describe directly the molecular changes. In a simple, low-MW system, correlation between structural (e.g. glass transition temperature) and molecular mobility (e.g. molecular mobility onset temperature as detected by electron spin resonance) can be found (Hemminga, Roozen & Walstra, 1993; Roozen, Hemminga & Walstra, 1991).

However, this may not be the case in more complex and heterogeneous systems. For instance, it has been reported that not all molecules or groups are rigid in glassy state. Water, for example, has been found to be highly mobile even in a glassy state of waxy corn starch (9.3% moisture content; Li et al. (1998); Schmidt, personal communication) and water–maltose solutions ($T < 285$ K, Hills & Pardoe, 1995). High (isotropic) molecular mobility of diluents in a glassy state of synthetic polymer blend has also been reported (Cauley et al., 1991). In the case of starch, although changes in the backbone mobility of starch can be observed during a glass transition (Vodovotz, 1996), this does not necessarily mean that smaller components such as water are also in a glassy, vitrified state. In fact, water (although unfreezable) has been reported to be very mobile (liquid-like) even at temperature ~100°C below the system T_g (Li et al., 1998). In one case, ^{2}H NMR mobile signals were observed in glassy gluten (Cherian & Chinachoti, 1996) and in another case, ^{1}H NMR mobility onset was observed at a temperature far below T_g of the waxy maize starch (Tanner et al., 1991). The objective of this work was to investigate changes in ^{1}H and ^{2}H NMR water mobility in cellulose undergoing hydration and plasticization.

2. Materials and methods

2.1. Sample preparation

Cellulose (Sigma Chemicals, St. Louis, MO) was mixed with double-distilled water to 30% solids, quench cooled with liquid nitrogen and then freeze dried (15°C, 15 atm vacuum). The freeze dried cellulose was then equilibrated at 25°C at different water activities by placing the sample in a mini-desiccator over a saturated salt solution of known a_w (ranging from 0.07 to 0.97a_w, Greenspan, 1977) or over deionized, distilled water. The saturated salt solutions were prepared either with H_2O or H_2O/D_2O mixture (1:1). The samples reached equilibrium (no further water uptake) in 10 days.

2.2. NMR analysis

2.2.1. Proton solid-state NMR

Samples were packed in a ZrO_2 rotor (7-mm OD Bruker Instruments Inc., Billerica, MA). Samples were analyzed with an ASX 300 spectrometer (Bruker Instruments Inc., Billerica, MA). A DEPTH pulse sequence (Bendall & Gordon, 1983) was used in order to minimize the signal arising from extraneous sources such as probe components. The data were acquired with an acquisition time of 18.1–67.2 ms (depending on moisture content), 90° pulse width of 5.75–6.00 μs, receiver gain of 256, spin rate of 4000 rpm, 64 scans and 50–200 kHz spectral width (depending on moisture content). The FIDs were analyzed using WIN-NMR® (Bruker Instruments Inc., Billerica, MA) to obtain Fourier transformed spectra. In case of overlapping peaks, the spectra were deconvoluted using Peakfit® (Jandel Scientific, San Rafael, CA) to obtain the intensity (area) and line width (LW). Samples were run at least in duplicate.

2.2.2. Deuterium high-resolution NMR

^{2}H NMR determination was carried out using an MSL 300 spectrometer (Bruker Instruments Inc., Billerica, MA). 0.2–0.3 g of sample were packed into 10-mm NMR tubes (8–10 mm in height). The probe was tuned each day of acquisition with the sample of higher moisture content analyzed that day. 90° WALTZ pulse sequence (Shaka, Keeler, Frenkiel & Freeman, 1983) was applied using 90° pulse width of 7.45–7.80 μs. The data were acquired with

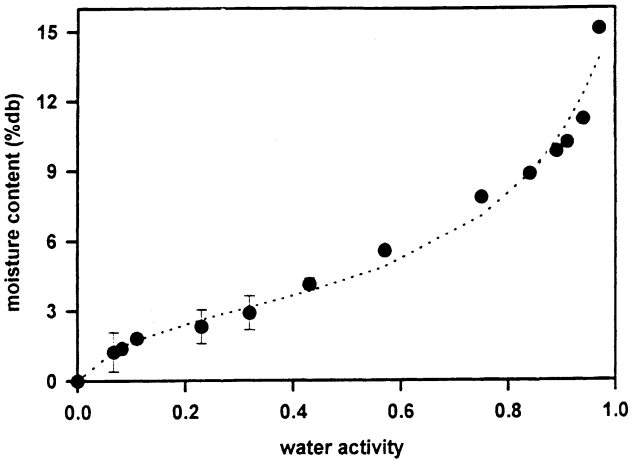

Fig. 1. Water sorption isotherm of cellulose at 25°C. The dotted line is the G.A.B. fitted curve ($R^2 = 0.98$).

12 ms acquisition time, 500 ms recycle delay ($>5T_1$), 20,000 Hz spectral width, 256 scans and the samples were run unlocked and at 16 rps spinning. At least duplicate samples were run.

Spin lattice relaxation time (T_1) was determined by inversion recovery with an inter-pulse spacing (τ) ranging from 50 μs to 1 s depending on the sample relaxation time. Data points were collected over at least eight different τ values and with a recycle time of 500 ms ($>5T_1$). The FID was analyzed from peak height (M_t) as a function of τ by fitting a single- (Eq. (1)) or a double- (Eq. (2)) exponential model (SYSTAT Inc., Evanston, IL)

$$M_t = M_0[1 - 2F \exp(-\tau/T_1)] \quad (1)$$

$$M_t = M_{0a}[1 - 2F_a \exp(-\tau/T_{1a})] + M_{0b}[1 - 2F_b \exp(-\tau/T_{1b})] \quad (2)$$

where M_t is peak height; M_0, M_{0a} and M_{0b} are equilibrium magnetization; τ is inter-pulse spacing; F, F_a and F_b are correction factors for M_0, M_{0a} and M_{0b}, respectively, for the relaxation process at lowest τ and 180° pulse imperfection; T_1, T_{1a} and T_{1b} are the spin lattice relaxation times. The a and b subscripts refer to the two components of the relaxation process.

Spin–spin relaxation time (T_2) was determined with a Carr Purcell Meiboom Gill (CPMG) pulse sequence (Carr & Purcell, 1954; Meiboom & Gill, 1958). The inter-pulse spacing (τ) is of 5–500 μs range. At least eight different τ values were used for each T_2 determination. The acquired FID was Fourier transformed to give a spectrum. Peak height was measured and analyzed as a function of $2\tau n$, where n is the number of echoes (2 in this case). Single- (Eq. (3)) and double- (Eq. (4)) exponential fitting was done by non-linear curve fitting (SYSTAT Inc., Evanston, IL).

$$M_t = M_0 \exp(-2\tau n/T_2) \quad (3)$$

$$M_t = M_{0a} \exp(-2\tau n/T_{2a}) + M_{0b} \exp(-2\tau n/T_{2b}) \quad (4)$$

Terms were defined above and T_2, T_{2a} and T_{2b} are the spin–spin relaxation times

2.3. Differential scanning calorimetry analysis

A known amount (8–10 mg) of sample was placed in sample pans (stainless steel hermetically sealed, Perkin Elmer, Somerset, NJ), and analyzed by DSC 100 (Seiko Instruments, Terrance, CA) or DSC 4 (Perkin Elmer, Somerset, NJ). An empty pan was used as reference. The samples were cooled with liquid nitrogen to −80°C, then heated to 200°C at a rate of 2°C/min, immediately recooled (to −80°C), and then rescanned at the same conditions. Glassy–rubbery transition was analyzed based on an endothermic baseline shift arising from heat capacity change (Riva & Schiraldi, 1992; Wunderlich, 1994). The samples were analyzed also for their freezable water content, in a −30 to 80°C temperature range at a heating rate of 5°C/min. The presence of an ice melting peak was taken as evidence of the presence of freezable water.

3. Results

3.1. Water sorption behavior

Cellulose was equilibrated at various water activities (a_w) at 25°C to give equilibrated moisture contents, which were plotted as a function of a_w. The resulting water sorption isotherm of cellulose is shown in Fig. 1. The sigmoidal curve was fitted according to the Guggenheim Anderson de Boer (GAB) equation (Eq. (5); Rizvi (1986).

$$M/M_0 = (C_g K a_w)/[(1 - K a_w)(1 - K a_w + C_g K a_w)] \quad (5)$$

where M is the equilibrated moisture content (% dry basis), M_0 and K are constants.

The fitting (R^2 of 0.98) gave an M_0 of 2.8 ± 0.6%

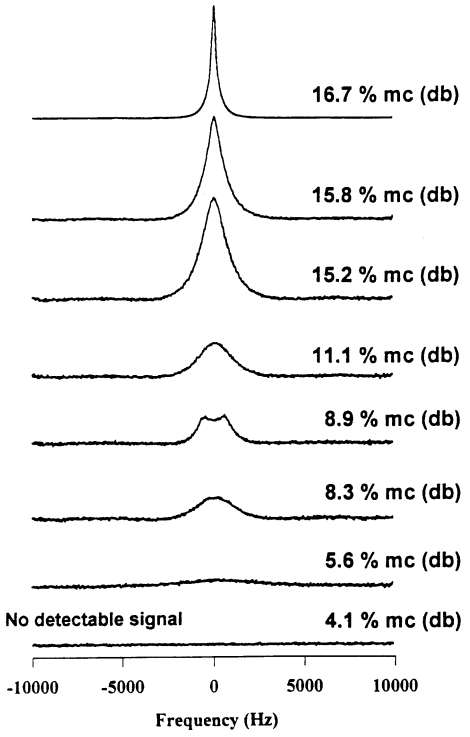

Fig. 2. ^{2}H high-resolution NMR spectra for cellulose at variable moisture content. The moisture content (% dry basis) is reported for each spectrum.

(g water/100 g solids) in agreement with the literature (Hernadi, 1984). This water was reported to have rotational correlation time (τ_c) in the order of 10^{-5}–10^{-7} s, as compared to 10^{-12} s for bulk water (Yano & Hatakeyama, 1988).

3.2. Unfreezable water content and glassy to rubbery transition analysis

The presence of freezable water in cellulose samples was determined by DSC analysis. The unfreezable water content was plotted against moisture content and extrapolated to the moisture content for 0% freezable water. The amount of unfreezable water was found to be ~19% moisture content (dry basis). All the water detected at $a_w \leq 0.97$ was unfreezable in the DSC time frame.

From DSC data, no evidence of a glassy to rubbery transition was observed. Cellulose has been reported earlier to show a high glassy to rubbery transition temperature in the range of 220–250°C for dry samples (Akim, 1977; Back & Salmen, 1982; Kalashnik et al., 1991; Salmen & Back, 1977), which is well above its decomposition temperature (Akim, 1977). T_g for pure cellulose has been approximated by extrapolation from mixtures (Nishio & Manley, 1988, 1990; Nishio, Roy & Manley, 1987; Zelenev & Glazkov, 1972). When cellulose is hydrated, T_g is expected to be below the decomposition temperature, but it was not observable in our experimental conditions. DSC is not sensitive to the small changes in heat capacity (Cauley et al., 1991; Quinn, Kampff, Smyth & McBrierty, 1988). To our knowledge, there are no published experimental data related to the glass transition of cellulose.

3.3. High-resolution ^{2}H NMR

Fig. 2 shows ^{2}H NMR spectra of cellulose over 4.1–16.7% moisture content (dry basis) exhibiting complex spectra. Very little or no signal was detected at moisture contents ≤5.6% dry basis (or $a_w \leq 0.56$). Since the NMR spectrometer used was designed to detect liquid components ($T_2 > 100$ μs), the lack of signal detection below 5.6% moisture content indicated that up to this moisture level water was relatively less mobile ($T_2 < 100$ μs). As a result, the ^{2}H signal intensity (data not shown) increased with the amount of sorbed moisture very little at first (low moisture contents) and at beyond 5.6% moisture content it increased exponentially. The curvilinear change indicated that the observed deuterium population only accounted for the more mobile one ($T_2 > 100$ μs). Hence, the information obtainable from this NMR experiment could only be applied to the more mobile deuterium population.

The lineshape of the ^{2}H peak changed with moisture content as shown in Fig. 2. At moisture contents 11.1% (dry basis) the Lorentzian lineshape was observed. At 8.9% moisture content, a doublet composed of two Lorentzian (almost identical) peaks was observed (Fig. 2). At moisture contents ≤8.3% dry basis, one peak (broad) was observed.

The peculiar splitting of the ^{2}H peak only at 8.9% moisture is a phenomenon associated with anisotropic motion in fibrous materials (Dehl & Hoeve, 1968; Woessner, 1980) and it has been previously reported also in oriented cellulose (Matsumura, Hayamizu, Nakane & Yakumoto, 1987; Wong, 1983). This quadrupolar splitting is an evidence for anisotropic motion of water in the system. The quadrupolar splitting is related to the order parameter, S (Wong, 1983), which describes the ordering of water molecules relative to the fiber axis. S is affected by the lateral motions of water molecules along the fiber surface (Matsumura et al., 1987; Wong, 1983; Wong & Ang, 1985). Water molecules strongly absorbed to a heterogeneous system tend to orient in specific, preferential manners creating different spin populations. When the ^{2}H spin populations exchange at a slow rate within the NMR time frame, splitting can be observed. On the contrary, if the exchange among the ^{2}H populations is rapid, a single Lorentzian peak could result since all different motions would average within the NMR time frame (Matsumura et al., 1987). Quadrupolar splitting has been studied previously in cellulose oriented samples of variable moisture contents (Matsumura et al., 1987) and temperatures (Wong, 1983). The quadrupolar splitting was reported to decrease with increasing moisture content and temperature and it disappeared when fast exchange conditions were met. Below 0.75 water activity the quadrupolar splitting reached a constant value.

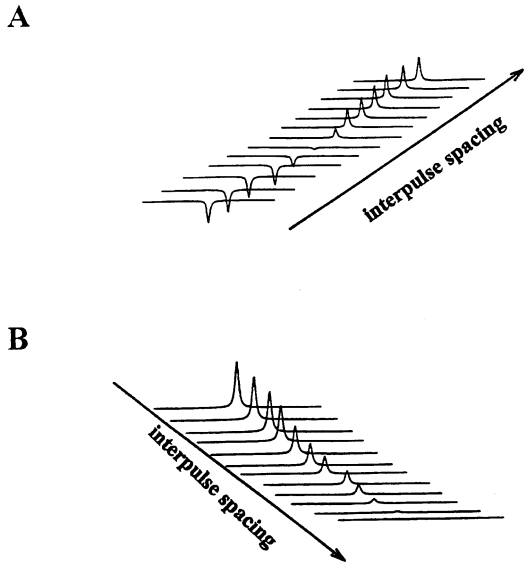

Fig. 3. (A) Longitudinal relaxation time determination from inverse-recovery method at variable inter-pulse spacing. (B) Transverse relaxation time determination with a CPMG pulse sequence at variable inter-pulse spacing.

In our experiment, the residual quadrupolar splitting was observed undoubtedly at an intermediate moisture, i.e. $a_w = 0.81$ (8.9% moisture, dry basis) indicating the existence of one deuterium population encompassing two spin populations. These two deuterium spin populations differed only because of their different orientation on the cellulose fiber. They had equal mobility as speculated from the equal LW of the constituent peaks and as measured by T_2 relaxation time (see below). The two spin populations exchanged slowly within the NMR time frame and were, therefore, distinguishable. At lower moisture contents ($\leq$8.3%, dry basis), a quadrupolar splitting was not evident, but might exist as two broad spectra overlapping showing only one broader peak. Additionally, random packing of cellulose fibers in the NMR tube might not favor splitting detection. The spectra in fact could be forced into two overlapping Lorentzian peaks, but the splitting was not obvious because of substantial overlap arising from line broadening associated with the lower molecular motions at relatively lower moisture contents.

Above 11.1% moisture content a single Lorentzian peak was observed, indicating that all the deuterons are in fast exchange. Multiple and complex phenomena might affect the detected signal (e.g. cross-relaxation, diffusive exchange), but cannot specifically be accounted for because of lack of simple interpretative models. The single Lorentzian peak was observed at moisture contents corresponding to the upward concavity in the water sorption isotherm. This point has been considered by some as the polymer plasticization point (Froix & Nelson, 1975).

Deuterium inversion recovery T_1 and CPMG T_2 relaxation times (Fig. 3) were approximated with a single-exponential model. R^2 of the fitting was $>$0.90 at $a_w > 0.75$ and $>$0.96 at $a_w > 0.75$. T_2 was determined by CPMG analysis in order to avoid the effect of magnetic field inhomogeneities (Canet & Robert, 1995; Delpuech, 1995; Meiboom & Gill, 1958; Pople, Schneider & Bernstein, 1959). When dual peaks were observed (8.9% moisture), the LWs from each peak were similar or identical.

Both T_1 and T_2 of ^{2}H NMR data increased with increasing moisture content in all the samples, as expected (Fig. 4). T_1 increased from 8 ms at 6% moisture content (dry basis) to 43 ms at 18% moisture content (dry basis, Fig. 4). T_1 relaxation times in the order of tens of milliseconds are expected for most food systems (Belton, 1995). T_2 increased from $\sim$100 to 700 μs over the same moisture content range of T_1 (Fig. 4). Both T_1 and T_2 increased almost correspondingly with the water sorption data, i.e. only slightly at 5–11% moisture content (dry basis) range followed by a sharp increase at moisture contents $>$11% Fig. 4).

The relatively shorter values of T_2 as compared to T_1 ($T_1 \gg T_2$; Fig. 4) indicated that the system was retarded in mobility and not in an extreme narrowing range, suggesting

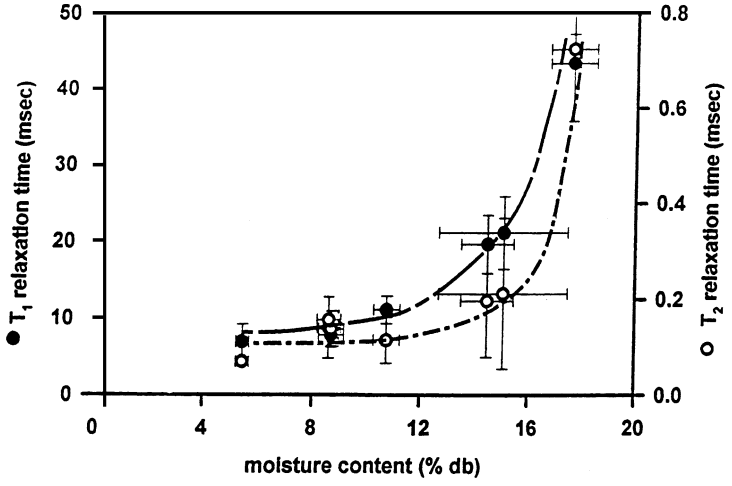

Fig. 4. Changes of deuterium T_1 and $\mathbf{T}_2$ relaxation times for cellulose as a function of moisture content.

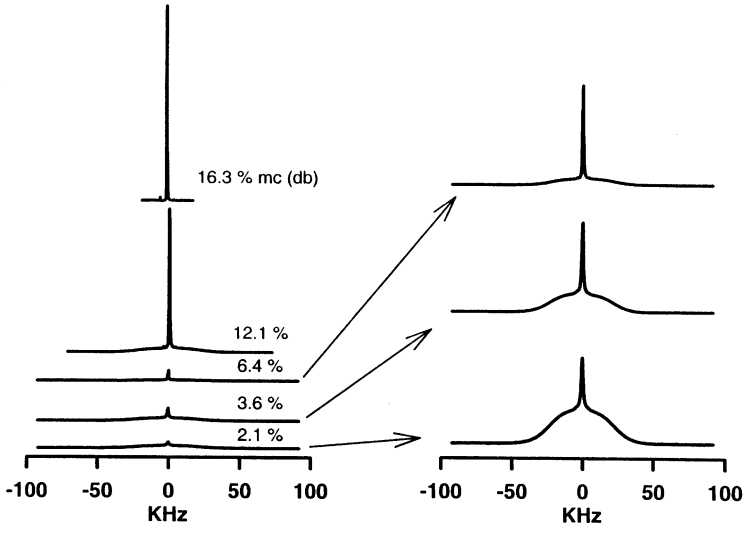

Fig. 5. ^{1}H solid-state NMR spectra for cellulose at variable moisture content. The moisture content (% dry basis) is reported for each spectrum. Some enlarged spectra are also shown on the right.

that water molecular motion was strongly affected by the presence of cellulose fibers. The anisotropic reorientation arising from the presence of cellulose fibers might be possible either by an interaction or an entrapment of water in the fibrous structure. Water–cellulose interactions could possibly prevail over water–water interactions and promote the fast relaxation ($T_2 < T_1$) observed. Quantitative interpretation of the spectra is extremely difficult because of the non-extreme narrowing limit condition. The spectra observed are the average of the contributions of deuterons on both solids and water and they undergo multiple complex phenomena (e.g. cross-relaxation, chemical and diffusive exchange, etc.). At 11% moisture content there was a significant increase in the amount and the mobility of the mobile fraction of the deuterons.

3.4. ^{1}H solid-state NMR

^{1}H solid-state NMR spectra of cellulose at variable moisture content is shown in Fig. 5. Both narrow and wide components were detected for samples of moisture contents ≤12% (dry basis). The narrow component represented protons (water molecules) reorienting rapidly and "liquid-like" (Capitani et al., 1995; Tanner et al., 1991), while the wide component was due to the immobile (rigid) protons of the "bound" water or cellulose (Capitani et al., 1995; MacKay et al., 1982; Tanner et al., 1991). At moisture contents of 12% (dry basis), only the narrow liquid-like component was observable. Deconvolution ($R^2 \geq 0.995$) decomposed the spectra into a Lorentzian narrow component and a Gaussian wide component, as observed in starch

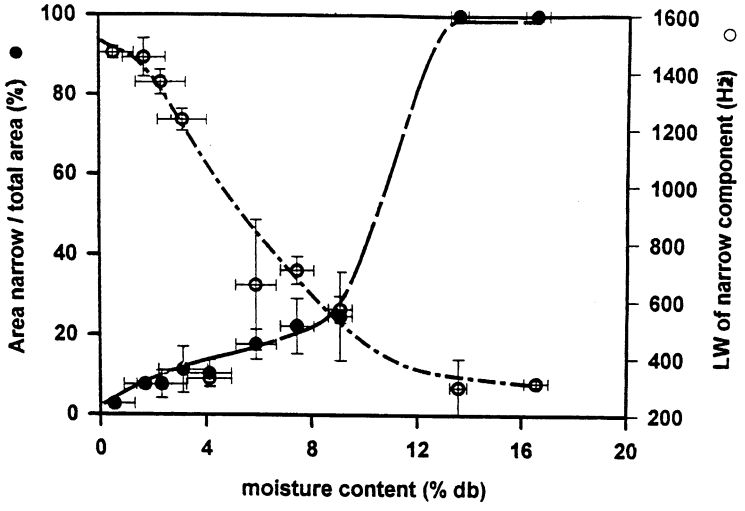

Fig. 6. Changes in the relative ratio of the narrow/wide component areas (solid circles) and LW of the narrow component (open circles) of ^{1}H solid-state NMR spectra at increasing moisture content.

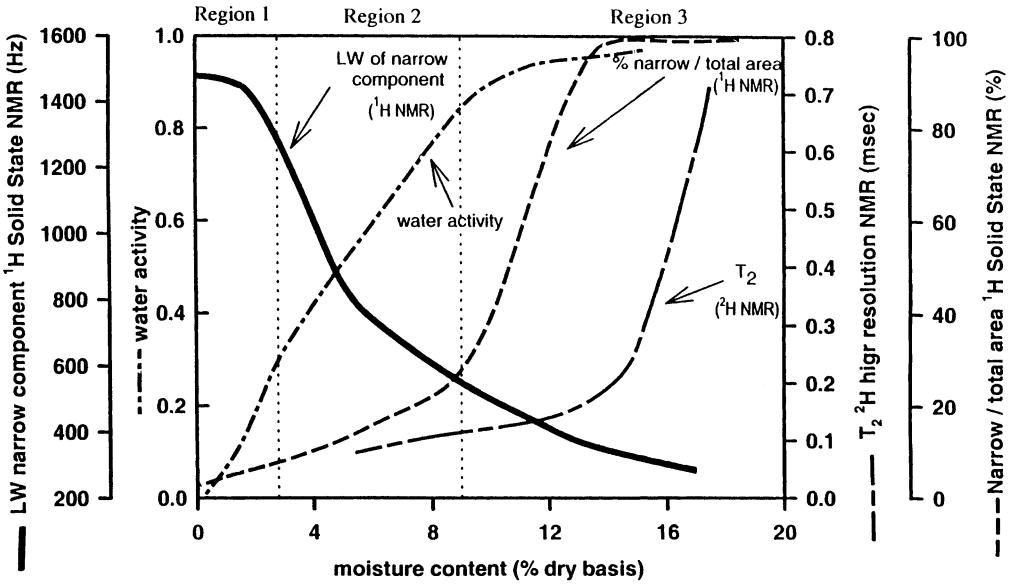

Fig. 7. Summarized chart for cellulose changes in water activity, high-resolution ^{2}H NMR T_2 relaxation times, LW of ^{1}H solid-state NMR narrow component and ^{1}H solid-state NMR relative ratio of the narrow/wide component. Dotted lines indicate the three sorption regions.

(Li et al., 1998; Tanner et al., 1991). The wide Gaussian peak predominated (higher relative area) at lower moisture content (decreased mobility; Fig. 6). At 1% moisture content (dry basis), 3% of the total ^{1}H were in a narrow, liquid state (Fig. 6). At 5% moisture content (dry basis) the mobile ^{1}H increased to 15% and at >12% moisture, all protons were narrow in LW (Fig. 6). This change is also temperature dependent; protons became more immobilized at lower temperature (Capitani et al., 1995).

The Gaussian peak (wide component) was approximately 39–45 kHz wide, independent of moisture content as also observed in waxy corn starch (Li et al., 1998), but moisture dependence was also reported (Tanner et al., 1991). The rigid protons are composed of the cellulose protons and the protons of immobilized water. The critical moisture where solid Gaussian protons were observed (9% moisture content, dry basis) agreed well with that for the shift from slow to fast deuterium exchange (~8.9% moisture content, dry basis, Figs. 2 and 4).

LW of the narrow component decreased with increasing moisture content, as expected, from 1500 Hz at 1% moisture content (dry basis) to 300 Hz at moisture content ≥13% (dry basis, Fig. 6). Increased LW at lower moisture content was due not only to reduced water mobility, but also to other phenomena, such as chemical exchange, diffusion, and chemical shift anisotropy (Canet & Robert, 1995; Delpuech, 1995; Meiboom & Gill, 1958; Pople et al., 1959). ^{1}H solid-state analysis showed a slow increase in mobile protons with added water (at moisture content <9%; Fig. 6), strongly suggesting that the water–cellulose interaction and the "bound" water at the surface of cellulose fibers did not give a mobile proton signal. The significant increase in mobility above 9% moisture content was possibly related to penetration of water molecules in narrow interstices between amorphous cellulose fibers. Whether this is relevant to a glass transition remains speculative. This work showed no evidence of glass transition from thermal or thermomechanical relaxation, but strong evidence of molecular mobility changes. This suggests that in a biopolymer system, NMR can serve as a more sensitive method for molecular mobility change than the originally proposed glass transition method (Slade & Levine, 1991). The ability to distinguish the narrow and the wide component with various hydration levels offers a quasi-quantitative comparison of molecular motion in a short experimental scale.

4. Discussion

A chart summarizing the ^{1}H and ^{2}H NMR water mobility parameters and water activity as a function of moisture content is shown in Fig. 7. The ^{1}H signal showed high immobility (90% of the protons were solid-like) in region 1 (0–3% moisture content) and even mobility of the liquid-like component was retarded (line broadening of the ^{1}H narrow component). Water was strongly associated to the cellulose fibers, e.g. by H-bonding to the OH groups as previously proposed (Child, 1972; Froix & Nelson, 1975). In region 2 (moisture content 3–9%), ^{1}H NMR showed significant rigidity (70–90% of the protons are immobile), but the mobility of the mobile component increased based on LW measurements and ^{2}H NMR T_2 in the 0.1–0.2 ms range. In this region, a liquid deuterium signal showed that the deuterated water molecules gave a split resonance at 8.9 moisture content as reported earlier (Wong, 1983) corresponding to the point where the wide proton component disappeared, the water sorption isotherm showed an upward concavity, line narrowing was significant, ^{2}H T_2 and ^{2}H area

increased. In region 3 (moisture content >9%), the system was in fast exchange conditions (narrowing limit conditions were not met), water molecules were highly mobile, but their T_2 was 300 times shorter than T_2 of bulk water. Even in region 3, water molecules were still unfreezable (up to 19% moisture content), suggesting that they were not phase separated and did not form large ice crystals detectable by DSC. However, in a molecular range (NMR), this water is highly mobile (0.2–0.7 ms ^{2}H T_2 range) and therefore not vitrified in a viscous-glassy state as earlier suggested in many biopolymers (Slade & Levine, 1991). NMR selectively detected mobile and immobile protons over a lower concentration (even in the monolayer region). Water molecules, although affected by the presence of cellulose, exhibited a significant mobility at >9% moisture as observed by large mobile protons and deuteron signals. Water in this region was potentially available to chemical reactions and microbial activity, depending on the system (Lang, 1980).

Cellulose fibers have been reported to exhibit a glassy–rubbery transition of 220–250°C at 0% moisture content (Akim, 1977; Back & Salmen, 1982; Kalashnik et al., 1991; Salmen & Back, 1977). If so, in regions 2 and 3, a significant structural (glass) transition in the amorphous domains of cellulose would be expected, but was not observed experimentally by DSC. On the other end, significant molecular mobility changes were observed by NMR (short-range). NMR selectively detected mobile and immobile protons over a lower concentration (even in the monolayer region). Water molecules, although affected by the presence of cellulose, exhibited a significant mobility at >9% moisture as observed by large mobile proton and deuteron signals. The experimental evidence reported in this work strongly supports the need to differentiate between structural and molecular mobility.

5. Conclusions

This work clearly shows that unfreezable water can be significantly mobile (T_2 in ms range) and that molecular mobility transitions over the 0–19% moisture (dry basis) was significant and strongly related to changes in a_w. Molecular transition was observed even though a glass transition was not detected by DSC. These data strongly show that structural immobility does not necessarily indicate system immobility at a molecular level.

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References

Akim, E. L. (1977). Changes in cellulose structure during manufacture and converting of paper. *Cellulose chemistry and technology*, Washington, DC: Jett CA (pp. 153–172).

Back, E. L., & Salmen, N. L. (1982). Glass transition of wood components hold implications for molding and pulping processes. *TAPPI*, *65*, 107–110.

Belton, P. S. (1990). Can nuclear magnetic resonance give useful information about the state of water in food stuffs? *Comments on Agriculture and Food Chemistry*, *2*, 179–209.

Belton, P. S. (1995). NMR in context. In G. A. Web, P. S. Belton & M. J. McCarthy, *Annual reports on NMR spectroscopy* (pp. 1–18). London: Academic Press.

Bendall, M. R., & Gordon, R. E. (1983). DEPTH and refocusing pulses designed for multipulse NMR with surface coils. *Journal of Magnetic Resonance*, *53*, 365–385.

van den Berg, C. (1991). Food–water relations: progress and integration, comments and thoughts. In H. A. Levine & L. Slade, *Water relationship in food* (pp. 21–28). New York: Plenum Press.

Boesen, C. E. (1970). Bound water. *Cellulose Chemistry and Technology*, *4*, 149–164.

Canet, D., & Robert, J. B. (1995). Timescales in NMR: relaxation phenomena in relation with molecular reorientation. In J. J. Delpuech, *Dynamics of solutions and fluid mixtures by NMR* (pp. 19–72). Chichester, UK: Wiley.

Capitani, D., Segre, A. L., Attanasio, D., Blicharska, B., Focher, B., & Capretti, G. (1995). ^{1}H NMR relaxation study of paper as a system of cellulose and water. *TAPPI*, *79*, 113–122.

Carr, H. Y., & Purcell, E. M. (1954). Effects of diffusion on free precession in nuclear magnetic resonance experiments. *Physical Review*, *94*, 630–638.

Cauley, B. J., Cipriani, C., Ellis, K., Roy, A. K., Jones, A. A., & Inglefield, P. T. (1991). Glass transition and dynamics of phosphate esters dissolved in two glassy polymer matrices. *Macromolecules*, *24*, 403–409.

Cherian, G., & Chinachoti, P. (1996). ^{2}H and ^{17}O nuclear magnetic resonance study of water in gluten in the glassy and rubbery state. *Cereal Chemistry*, *73*, 618–624.

Child, T. F. (1972). Pulsed NMR study of molecular motion and environment of sorbed water on cellulose. *Polymer*, *13*, 259–264.

Creely, J. J., & Tripp, V. W. (1971). Effect of humidity on the crystallinity of cellulose. *Textile Research*, 371–373.

Dehl, R. E., & Hoeve, C. A. (1968). Broad-line NMR study of H_2O and D_2O in collagen fibers. *The Journal of Chemical Physics*, *50*, 3245–3251.

Delpuech, J. J. (1995). Timescales in NMR: nuclear site exchange and dynamic NMR. In J. J. Delpuech, *Dynamics of solutions and fluid mixtures by NMR* (pp. 73–172). Chichester, UK: Wiley.

Duckworth, R. B. (1981). *Solute mobility in relation to water content and water activity*, *Water activity: influences on food quality*. New York: Academic Press (pp. 295–317).

Froix, M. F., & Nelson, R. (1975). The interaction of water with cellulose from nuclear magnetic resonance relaxation times. *Macromolecules*, *8*, 726–740.

Greenspan, L. (1977). Humidity fixed points of binary saturated aqueous solutions. *Journal of Research of the National Bureau of Standards — A. Physics and Chemistry*, *81A*, 89–96.

Hatakeyama, T., Ikeda, Y., & Hatakeyama, H. (1987). Effect of bound water on structural change of regenerated cellulose. *Makromolekulare Chemie*, *188*, 1875–1884.

Hemminga, M. A., Roozen, M. J. G. W., & Walstra, P. (1993). Molecular motions and the glassy state. In J. M. V. Blanshard & P. J. Lillford, *The glassy state in foods* (pp. 157–171). Nottingham: Nottingham University Press.

Hernadi, A. (1984). Accessability and specific surface of cellulose

measured by water vapour sorption. *Cellulose Chemistry and Technology*, *18*, 115–124.

Hills, B. P. (1992). The proton exchange cross-relaxation model of water relaxation in biopolymer systems. II. The sol and gel state of gelatine. *Molecular Physics*, *76*, 509–523.

Hills, B. P., & Pardoe, K. (1995). Proton and deuterium NMR studies of the glassy transition in a 10% water–maltose solution. *Journal of Molecular Liquids*, *63*, 229–237.

Kalashnik, A. T., Papkov, S. P., Rudinskaya, G. V., & Milkova, L. P. (1991). Liquid crystal state of cellulose. *Polymer Science U.S.S.R.*, *33*, 107–112.

Koenig, S. H., Bryant, R. G., Hallenga, K., & Jacob, G. S. (1978). Magnetic cross-relaxation among protons in protein solutions. *Biochemistry*, *17*, 4348–4358.

Lang, K. (1980). *Physical, chemical and microbiological characteristics of polymer and solute bound water*. PhD dissertation, University of Illinois at Urbana-Champaign.

Li, S., Dickinson, L. C., & Chinachoti, P. (1998). Mobility of "Unfreezable" and "Freezable" water in waxy corn starch by ^{2}H and ^{1}H NMR. *Journal of Agriculture and Food Chemistry*, *46*, 62–71.

MacKay, A. L., Bloom, M., Tepfer, M., & Taylor, I. E. P. (1982). Broadline proton magnetic resonance study of cellulose, pectin, and bean cell walls. *Biopolymers*, *21*, 1521–1534.

Matsumura, K., Hayamizu, K., Nakane, T., & Yakumoto, O. (1987). Angular dependence of ^{1}H- and ^{2}H-NMR spectra of H_2O and D_2O absorbed in cellulose acetate film. *Journal of Polymer Science: Part B. Polymer Physics*, *25*, 2149–2163.

McBrierty, V. J., & Packer, K. J. (1993). *Nuclear magnetic resonance in solid polymers*, Cambridge: Cambridge University Press.

Meiboom, S., & Gill, D. (1958). Modified spin–echo method for measuring nuclear magnetic relaxation times. *The Review of Scientific Instruments*, *29*, 688–691.

Nakamura, K., Hatakeyama, T., & Hatakeyama, H. (1981). Studies on bound water of cellulose by differential scanning calorimetry. *Textile Research Institute*, *9*, 607–613.

Nakamura, K., Hatakeyama, T., & Hatakeyama, H. (1983). Effect of bound water on tensile properties of native cellulose. *Textile Research Journal*, *11*, 682–688.

Nishio, Y., & Manley, R. S. J. (1988). Cellulose/poly(vinyl alcohol) blends prepared from solutions in *N*,*N*-dimethylacetamide–lithium chloride. *Macromolecules*, *21*, 1270–1277.

Nishio, Y., & Manley, R. S. J. (1990). Blends of cellulose with nylon 6 and poly(e-caprolactone) prepared by a solution coagulation method. *Polymer Engineering and Science*, *30*, 71–82.

Nishio, Y., Roy, S. K., & Manley, R. S. J. (1987). Blends of cellulose with polyacrylonitrile prepared from *N*,*N*-dimethylacetamide–lithium chloride solutions. *Polymer*, *28*, 1385–1390.

Pople, J. A., Schneider, W. G., & Bernstein, H. J. (1959). *High-resolution nuclear magnetic resonance*, New York: McGraw-Hill.

Quinn, F. X., Kampff, E., Smyth, G., & McBrierty, V. J. (1988). Water in hydrogels. 1. A study of water in poly(*N*-vinyl-2-pyrrolidone/methyl methacrylate) copolymer. *Macromolecules*, *21*, 3191–3198.

Ray, P. K. (1969). On the degree of crystallinity in jute and mesta fibers in different states of purification and moisture content. *Journal of Applied Polymer Science*, *13*, 2593–2600.

Ray, P. K., & Bandyopadhyay, S. B. (1975). Some observation on the concept of the degree of crystallinity in cellulose fibers with reference to moisture effects. *Journal of Applied Polymer Science*, *19*, 729–733.

Richardson, S. J., & Steinberg, M. P. (1987). Applications of nuclear magnetic resonance. In L. B. Rockland & L. R. Beuchat, *Water activity: theory and applications to food*, New York: Marcel-Dekker.

Riva, M. & Schiraldi, A. (1992). *Applicazioni della calorimetria ai prodotti alimentari.* Sottoprogetto 4, Monografia RAISA 1.

Rizvi, S. S. H. (1986). Thermodynamic properties of foods in dehydration. In M. A. Rao & S. S. H. Rizvi, *Engineering properties of foods* (pp. 133–180). New York: Marcel-Dekker.

Roos, Y. H., Karel, M., & Kokini, J. L. (1996). Glass transitions in low moisture and frozen foods. *Food Technology*, *11*, 95–108.

Roozen, M. J. G. W., Hemminga, M. A., & Walstra, P. (1991). Molecular motion in glassy water–malto-oligosaccharide (maltodextrin) as studied by conventional and saturation-transfer spin-probe ESR spectroscopy. *Carbohydrate Research*, *215*, 229–237.

Salmen, N. L., & Back, E. L. (1977). The influence of water on the glass transition temperature of cellulose. *TAPPI*, *60*, 137–140.

Seitsonen, S., & Mikkonen, I. (1972). X-ray study on the effect of moisture content on the crystalline state of cellulose. *Journal of Polymer Science*, *10*, 1743–1748.

Shaka, A. J., Keeler, J., Frenkiel, T., & Freeman, R. (1983). An improved sequence for broadband decoupling: WALTZ 16. *Journal of Magnetic Resonance*, *52*, 335–338.

Shirley, W. M., & Bryant, R. G. (1982). Proton nuclear spin-relaxation and molecular dynamics in the lysozyme–water system. *Journal of the American Chemical Society*, *104*, 2910–2918.

Slade, L., & Levine, H. (1991). Beyond water activity; recent advances based on alternative approach to the assessment of food quality and safety. *Critical Reviews in Food Science and Nutrition*, *30*, 115–360.

Tanner, S. F., Hills, B. P., & Packer, R. (1991). Interactions of sorbed water with starch studied using proton nuclear magnetic resonance spectroscopy. *Journal of the Chemical Society Faraday Transactions*, *87*, 2613–2621.

Tsaryuk, V. I., & Frantesson, A. V. (1991). Infrared study of the water contained in cellulose. *Polymer Science*, *33*, 260–267.

Vodovotz, Y. (1996). *Aging of starch and bread as studied by DSC, DMA, NMR and confocal microscopy*, University of Massachusetts.

Woessner, D. E. (1980). An NMR investigation into the range of the surface effect on the rotation of water molecules. *Journal of Magnetic Resonance*, *39*, 297–308.

Wong, T. C. (1983). Deuteron nuclear magnetic relaxation of water adsorbed on oriented cellulose. *Molecular Physics*, *48*, 495–502.

Wong, T. C., & Ang, T. T. (1985). Study of molecular dynamics of water adsorbed on macroscopically oriented cellulose by deuterium nuclear magnetic resonance. *Journal of Physical Chemistry*, *89*, 4047–4051.

Wunderlich, B. (1994). The nature of the glass transition and its determination by thermal analysis. In R. J. Seyler, *Assignment of the glass transition* (pp. 17–31). Philadelphia: ASTM.

Yano, S., & Hatakeyama, H. (1988). Dynamic viscoelasticity and structural changes of regenerated cellulose during water sorption. *Polymer*, *29*, 566–570.

Zelenev, Y. V., & Glazkov, V. I. (1972). Relaxation processes in cellulose and its derivatives. *Vysokomolekulyarnye Soedineniya*, 1–15.