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Cross-polarisation kinetics and proton NMR relaxation in polymers of *Citrus* cell walls

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Abstract

The kinetics of cross-polarisation in CP-MAS NMR are not well understood, especially for hydrated polymers, but have the potential to yield motional information. The mobility of polysaccharides within primary cell walls from *Citrus* (orange) mesocarp was studied by ^{13}C NMR CP-MAS experiments, in the dry state and with the addition of H_2O or D_2O (3 cm^3/g). D_2O was used to replace the hydroxyl protons of carbohydrates other than crystalline cellulose and thus to deduce their contribution to cross-polarisation and spin diffusion. The cross-polarisation process was modelled by an exponential function with two stages corresponding to polarisation transfer to ^{13}C from directly-bonded and more remote protons. In contrast to theoretical predictions the proportion of the total cross-polarisation achieved during the initial fast phase, from directly-bonded protons, was particularly sensitive to increased proton mobility resulting from hydration with H_2O, although not with D_2O. This effect was attributed to disruption of direct cross-polarisation by mobile hydroxyl and water protons. The slow phase of polarisation transfer was also retarded by methyl rotations and by segmental motions which increased with hydration. The proton T_1 values were closely similar to one another in dry cell walls but addition of D_2O greatly reduced the extent of averaging by spin diffusion and separated the T_1s into three groups corresponding to methylated pectins concentrated in the middle lamella, the more rigid remainder of the cell-wall matrix, and cellulose. © 1996 Elsevier Science Ltd.

Keywords: NMR; Cross-polarisation; Polarisation transfer kinetics; Polysaccharides; Cell walls

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1. Introduction

The natural materials that provide living organisms with their structural strength are hardly understood at all in terms of modern materials science, although their molecular complexity implies a high degree of sophistication and their mechanical properties compare favourably in certain respects — controlled, anisotropic flexibility, and exceptional resistance to fracture — with any materials made by man. They share enough common features with synthetic polymers to be studied by similar techniques, including the solid-state NMR methods that are the subject of this paper. However they also differ from synthetic materials in many ways. They are generally composites of very thin crystalline fibres, much thinner than those used in man-made composites, aligned and bonded within a partly ordered matrix. Unlike synthetic materials they are strongly hydrated, usually containing more water than polymer. Thus they comprise a range of microphases with properties varying from those of crystalline solids to those of viscous polymer solutions, but conventional boundaries between these phases are not always evident or, at least, are crossed by structurally important polymer chains.

It is evident, therefore, that methodologies such as solid-state NMR will need some adaptation before they can be transferred from the study of synthetic polymers to that of natural structures. This paper deals with the application of ^{1}H relaxation methods and of $^{1}H-^{13}C$ cross-polarisation kinetics, a relatively unexplored route to motional information [1,2], to plant cell walls. Primary cell walls are the main load-bearing structures in non-woody, growing plants. They are constructed of crystalline cellulose fibres, which in most species are coated with hydrogen-bonded xyloglucans and embedded in a matrix containing both xyloglucans and pectins [3]. Sheets of parallel fibres move apart when the cell walls are swollen by hydration, the inter-layer spacing being controlled by pectins [4]. Understanding the mechanical properties of plant cell walls in terms of their molecular architecture is a major challenge that will need to be met before the mechanisms of plant growth and fruit ripening can be understood.

Enough progress has been made in the micromechanical study of primary cell walls by solid-state NMR to demonstrate the potential of the technique. The pioneering studies by Irwin et al. [5] showed that molecular motion could be detected within apple cell walls, although he used rigorously dry samples that were not typical of their natural state. Broadline proton-NMR relaxation measurements by Taylor and co-workers [6,7] have confirmed that hydrated cell walls show a broad range of proton mobilities from crystal-like to liquid-like, although in broadline experiments direct assignment of these to specific polymers is not possible. Newman and co-workers [8] have used variations in proton relaxation behaviour to probe both the relative mobilities of polymers within fruit cell walls and their spatial arrangement.

In this paper we describe the characterisation of the molecular motions occurring within dry and hydrated primary cell walls isolated from *Citrus* mesocarp (orange peel), with two objectives. Our first aim was to explore the kinetics of cross-polarisation in a hydrated system. Cell walls are an excellent model system for this purpose because they contain carbon atoms varying greatly in mobility and bearing zero to three protons. Our second aim was to define those main-chain motions that are likely to be most significant in controlling the macroscopic mechanical properties of primary cell walls. We concen-

trate here on the kHz motions controlling the kinetics of cross-polarisation and on the MHz motions probed by the proton T_1. A related paper [9] will discuss proton T_2 experiments and the manipulation of cross-polarisation kinetics to characterise motions in the most liquid-like components of *Allium* cell walls. *Citrus* mesocarp cell walls are typical of the primary cell walls of dicotyledonous plants except in their unusually high content of methylated pectin, which gives them a wide range of internal polymer mobilities. Their composition has been described [10]. They are the most important source of pectin used in the food industry.

2. Theory

Kinetics of cross-polarisation.—In ^{13}C CP-MAS spectrometry of solid polymers, one way to measure the proton rotating-frame relaxation time $T_{1\rho}$ is by varying the contact time τ allowed for cross-polarisation. It is often assumed that magnetisation in ^{13}C rises exponentially with time constant T_{CH}, then decays with time constant $T_{1\rho}$, so that the two time constants can be extracted by fitting a dual exponential function [11]. It has become clear, however, that the rising stage is too complex to be described by a single exponential, but comprises a rapid and a slower phase [1,2]. Depolarisation of ^{13}C in the rotating frame is described by similar two-phase kinetics [12]. It is now common to estimate proton $T_{1\rho}$ values by the alternative delayed-contact procedure in order to avoid the complexities involved, but in principle a variable-contact experiment is capable of providing useful additional information on polymer motions from the initial stage of increasing magnetisation if the results can be interpreted. There is both theoretical and experimental evidence that at the limit of very short contact time, the magnetisation of each ^{13}C nucleus is derived by coherent transfer from protons covalently bonded to it. If the sample is a single crystal, the resulting initial increase in signal intensity S with contact time τ is given by the rising part of an oscillating function, damped by motional interactions with nonbonded protons. For a ^{13}C nucleus with a single covalently bonded proton this function has the form [1]:

$$S = S_0/2[1 - \exp(-3R\tau/2)\cos b\tau/2] \quad (1)$$

where S_0 is the theoretical maximum signal intensity, b is the dipolar coupling between the ^{13}C nucleus and the covalently bonded proton, and R is the rate of spin diffusion within the surrounding shell of nonbonded protons.

In powder samples, the variation in orientation of the C–H bonds results in a spread of values of b, which controls the oscillatory frequency. This leads to destructive interference and the oscillation is damped out much more quickly. Wu et al. [2] showed that under these conditions the oscillatory term, summed over all orientations, could be approximated by an exponential function of the form:

$$S = S_0/2[1 - \exp(-3\tau/2T_{CHR})\exp(-\tau^2/2T_{CHD}^2)] \quad (2)$$

For clarity we introduce some changes in the published notation [2] in eq (2): T_{CHR} and T_{CHD} are time constants related inversely to R and b, respectively.

At longer cross-polarisation times the dominant mode of polarisation transfer origi-

nates from the non-bonded proton pool. Its shows exponential behaviour with τ and its rate is controlled by spin diffusion between the non-bonded protons [1,13]. Inclusion of additional terms for this slow cross-polarisation phase [2] and for spin–lattice relaxation in the rotating frame gives:

$$S = S_0 \exp(-\tau/T_{1\rho})\left[1 - s\exp(-\tau/T_{CHR}) - (1-s)\exp(-3\tau/2T_{CHR})\exp(-\tau^2/2T_{CHD}^2)\right] \quad (3)$$

It was assumed [2] that for a ^{13}C nucleus with a single covalently bonded proton, $s = 0.5$ so that the fast and slow components of cross-polarisation are predicted to be equal in magnitude. This is because, from eqs (1) and (2), cross-polarisation from one covalently-bonded proton can produce only half of the theoretical maximum ^{13}C magnetisation: the rest must be supplied by non-bonded protons. If two or more protons are covalently bonded to a ^{13}C nucleus it seems reasonable that the magnitude of the fast component will be greater, other things being equal. The approximation 2/3:1/3 for the relative magnitudes of the fast and slow components of depolarisation ($s = 0.33$) within a CH_2 group in a powder sample has been suggested [2]. Lauprêtre [13,14] used an alternative damped-oscillation model derived from the kinetics of cross-polarisation in the solution state [15]. This model, although different in structure, can give almost identical curves to eq (3) if the rate of damping is great enough (data not shown). It incorporates a more detailed treatment of the cross-polarisation of ^{13}C nuclei with more than one covalently bonded proton.

A satisfactory fit to eq (3) demands a high signal:noise ratio because of the number of variables involved. When the data do not meet these requirements it is desirable to reduce the number of variables as far as possible, but a function with the entire rising phase, from zero initial ^{13}C magnetisation, described by T_{CH}, does not give convincing fits. This may be remedied [16] by regarding the initial, fast rising phase simply as an intercept $(1-s)S_0$ on the signal intensity axis, according to eq (3):

$$S = S_0\left[1 - s + se^{-\tau/T_{1\rho}}(1 - e^{-\tau/T_{CHR}})\right] \quad (4)$$

When using this approach it is necessary to avoid the inclusion of data points at short τ values within the slow phase of cross-polarisation, i.e. typically $\tau < 100$ μs.

In gels and hydrated composite materials like those examined here, there is a wide spread of cross-polarisation rates. This has unexpected consequences. Mobile components requiring a few ms for cross-polarisation will not be visible in a conventional CP-MAS spectrum with contact time $\tau = 0.5$–1 ms [9,17]. If the determination of proton $T_{1\rho}$ values is attempted by a variable-contact experiment, these mobile components contribute increasing signal intensity over the τ range 1–5 ms and thus reduce the *apparent* rate of $T_{1\rho}$ decay during that time period. The apparent value of T_{CHR} is reduced at the same time [9]. We have observed similar behaviour in variable-contact experiments on hydrated cell walls from *Lycopersicon* [16], *Apium* and *Cucumis* (unpublished).

Proton spin diffusion.—The proton spin diffusion coefficient D is given by:

$$D = 0.13d^2/T_2 \quad (5)$$

where d is the mean interproton distance [18].

We have shown that proton T_2 values in dry cell walls are about 10–20 μs but that hydration extends the T_2 range to some hundreds of μs [9]. This gives D values of ca. 10^{-15} cm^2 s^{-1} for dry cell walls and for the rigid microfibril phase in hydrated cell walls, decreasing to 10^{-16} cm^2 s^{-1} for the more mobile pectic constituents, and perhaps still further if there are diffusion barriers bridged by water between polymer molecules. Hydration with D_2O replaces the hydroxyl protons, i.e. about half of the total, in all the cell-wall polysaccharides except crystalline cellulose. The resulting increase in d will increase D by a further factor of two, but by less than that if bridging water molecules are involved. Thus in hydrated cell walls, proton spin diffusion is expected to be rapid and straightforward only within the microfibrils. Elsewhere in the cell wall it will be slow, variable, and restricted to complex paths between regions of high proton mobility that function as spin insulators.

3. Results and discussion

Dry cell walls: fitting to the model for cross-polarisation.—The CP-MAS spectrum of dry cell walls from *Citrus* mesocarp is shown in Fig. 1a. It shows the typical features of a primary cell-wall spectrum but with an unusually high content of methyl-esterified pectin. Fig. 2 and Table 1 show the results from a variable-contact experiment on these cell walls, analysed according to eq (3). A satisfactory fit to eq (3) was obtained and the behaviour of methine, methylene, and methyl groups can be compared. Eq (4) also modelled the observed behaviour of the signal intensity during cross-polarisation with reasonable accuracy (data not shown), provided that time points under 100 μs were excluded.

For all the resonances of CH carbons the fast and slow cross-polarisation phases were almost equal in magnitude ($s = 0.47 \pm 0.01$), as predicted from eqs (2) and (3). The slow phase was smaller in magnitude than the fast phase for CH_2 carbons (mean $s = 0.37$), but less so than predicted ($s = 0.33$ [1]). It was relatively large and very variable for CH_3 carbons ($s = 0.59$–0.82), and the fast phase was essentially absent ($s \approx 1$) for carboxyl carbons as would be expected from the fact that these carry no covalently bonded protons to provide rapid cross-polarisation.

The rate of the initial fast step was greater for CH_2 than for CH carbons as predicted by the model. The time taken for the 65 ppm and 89 ppm signals to reach half their maximum intensity was close to the rigid-lattice limits of 20 μs (CH_2) and 28 μs (CH) suggested and experimentally corroborated by Lauprêtre et al. [14], although their model is not adopted here. The similar rate of increase for the corresponding resonances from the ring carbons of non-cellulosic polysaccharides implies that there was little segmental chain mobility on the 10^{-5} s timescale to interfere with the direct spin transfer from bonded protons to ^{13}C.

It would be expected that the greater number of protons in a methyl group would lead to a more rapid initial cross-polarisation phase (an extension [15] of Lauprêtre et al.'s analysis [14] gives $t_{1/2}$ for a rigid methyl carbon = 17 μs). This was not observed, implying that methyl rotations disrupting the initial cross-polarisation phase are a prominent type of motion within dry cell walls.

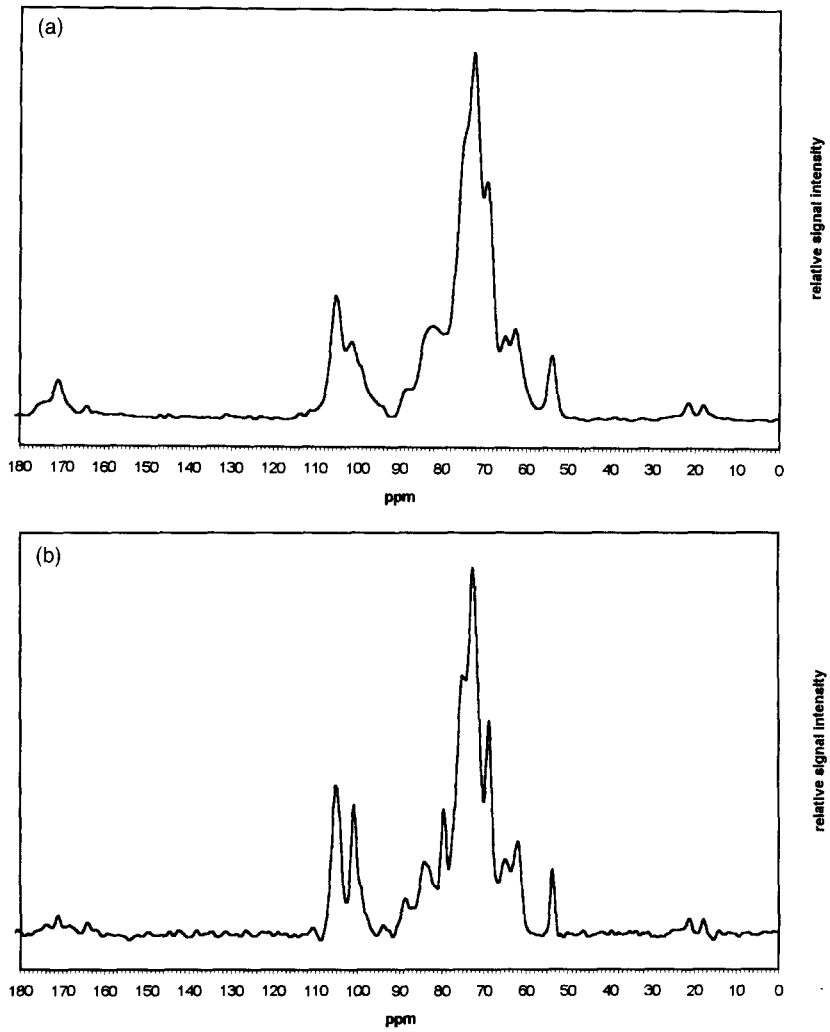

Fig. 1. CP-MAS spectra of orange peel cell walls: (a) dry, (b) hydrated with H_2O.

The rate of spin transfer amongst non-bonded protons close to a ^{13}C nucleus determines the rate of the slow second phase of polarisation transfer. Table 1 shows that T_{CHR} was similar for most CH and CH_2 groups but much longer for the methyl resonances, showing that spin exchange with non-bonded, as well as bonded, protons was retarded by methyl rotations. It was also long for the pectic carboxyl signals at 171 and 175 ppm. The possible structures for Ca-linked pectate (175 ppm) all lack protons (other than those of water) close to the carboxyl groups. The methylated carboxyl (171 ppm) lies in a relatively hydrophobic environment with a concentration of methyl groups in close proximity [17,19], which may facilitate methyl rotations.

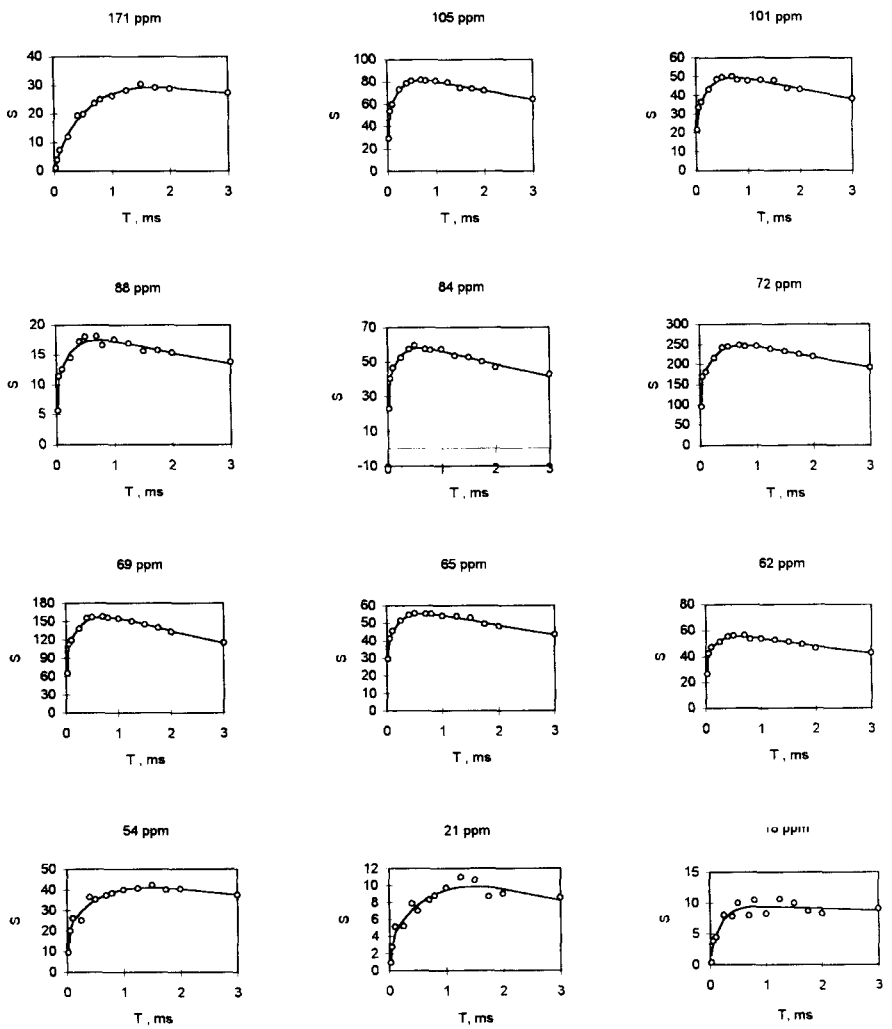

Fig. 2. Variation of signal intensity with contact time for dry cell walls. Fitted curves are derived from eq (3).

Spin–lattice relaxation.—Inversion-recovery experiments were used to determine the proton T_1 through the ^{13}C spectrum. The proton T_1s depend on motions on the 10^{-8} s timescale. The longer times over which T_1 decay is measured make spin diffusion possible over larger domains than those characterised by the ^1H $T_{1\rho}$. Dry cell walls gave proton T_1s that varied little throughout the spectrum (Table 2), with a very slight tendency towards shorter values for pectic resonances. Spin diffusion would therefore appear to be sufficient to average the T_1 values almost completely throughout the spectrum.

Effects of hydration with H_2O.—Hydration in either H_2O (Fig. 1b) or D_2O generally reduced linewidths, as is commonly observed with polysaccharides [8,18]. It also

Table 1
Cross-polarisation kinetics for dry cell walls [a]

Assignment	ppm	s	T_{CHD} (μs)	T_{CHR} (ms)
Pectic carboxyl, Ca form	175	0.87	50	0.82
Pectic carboxyl, Me ester	171	0.94	43	0.65
Cellulose C-1	105	0.48	22	0.24
Pectic and other C-1	101	0.49	18	0.27
Crystalline cellulose C-4	89	0.51	23	0.26
Crystal-surface cellulose C-4	84	0.44	22	0.24
Pectin C-4	80	0.45	22	0.26
General carbohydrate	72	0.47	21	0.30
Pectic C-2, C-3	69	0.46	20	0.31
Crystalline cellulose C-6	65	0.39	17	0.24
Other hexosan C-6	62	0.35	20	0.23
Pectic methoxyl	54	0.60	26	0.67
Acetyl CH_3	21	0.82	36	1.27
Rhamnose methyl	18	0.81	37	0.21

[a] Resonance assignments are based on published data [6,7]. The time constants T_{CHD} and T_{CHR} refer to the fast and slow phases of cross-polarisation respectively, and s is the fraction of the total cross-polarisation occurring in the slow phase.

reduced the relative intensity of the 54 ppm signal from methyl esters, and the 69, 80, and 101 ppm signals from ring carbons, of pectin, so that the contribution of the mobile esterified pectins was reduced in the spectrum. Hydration almost eliminated the carboxyl signals (171–177 ppm) and the 94–98 ppm upfield shoulder on the pectic C-1 signal, which has been tentatively assigned to galacturonan chains in conformations intermediate between the twofold and righthanded threefold helices. All these effects of hydration have been observed for pure galacturonans [17] and *Allium* and other cell walls [9,16].

Table 3 shows the $T_{1\rho}$ and cross-polarisation parameters for the *Citrus* cell walls after

Table 2
Laboratory-frame proton spin–lattice relaxation times (s) for dry and H_2O-swollen cell walls [a]

Assignment	(ppm)	Dry	H_2O
Pectic carboxyl, Me ester	171	0.35	–
Cellulose C-1	105	0.41	1.05
Pectic and other C-1	101	0.40	1.06
Crystalline cellulose C-4	89	0.36	1.2
Crystal-surface cellulose C-4	84	–	0.71
Pectin C-4	80	–	0.89
General carbohydrate	72	0.42	0.99
Pectic C-2, C-3	69	0.39	1.10
Crystalline cellulose C-6	65	0.44	0.8
Other hexosan C-6	62	0.42	1.2
Pectic methoxyl	54	0.39	1.4
Acetyl CH_3	21	0.43	–

[a] Resonance assignments are based on published data [6,7].

Table 3
CP-MAS experiment with variable contact time on cell walls in H_2O [a]

Assignment	ppm	$T_{1\rho}$ (ms)	s	T_{CHD} (μs)	T_{CHR} (ms)
Cellulose C-1	105	9	0.68	4	0.18
Pectic and other C-1	101	10	0.83	17	0.21
Crystal-surface cellulose C-4	84	5	0.68	5	0.31
Pectin C-4	80	9	0.71	7	0.28
General carbohydrate	72	8	0.64	11	0.30
Pectic C-2, C-3	69	10	0.69	15	0.32
Crystalline cellulose C-6	65	8	0.43	4	0.23
Other hexosan C-6	62	9	0.63	5	0.34
Pectic methoxyl	54	40	0.99	–	1.5

[a] Resonance assignments are based on published data [6,7]. The time constants T_{CHD} and T_{CHR} refer to the fast and slow phases of cross-polarisation respectively, and s is the fraction of the total cross-polarisation occurring in the slow phase.

hydration. If the only effect of hydration is to increase molecular motion, the theory of Wu et al. [2] as expressed in eq (3) predicts that both phases of cross-polarisation will be retarded by interfering motions but their relative magnitudes will be unchanged: for CH groups they will be equal ($s = 0.5$). Table 3 shows that this prediction is incorrect. The most obvious effect of hydration was a reduction in the relative magnitude of the fast initial phase ($s \approx 0.7$). There was little apparent effect on T_{CHR} for the visible part of the spectrum but this does not include the signals from the most mobile pectins, which have exceptionally long T_{CHR} values [9]. Thus the mean T_{CHR} values for the pectic fraction of the cell wall were increased, as predicted. There was little variation in T_{CHR} among the C-1, C-4, and C-3/C-5 pectic resonances after hydration. This suggests that most of the motions responsible were undulations or segmental rotations of the whole galacturonan chain, not rotations of individual functional groups: averaging of T_{CHR} by spin diffusion over significant distances is ruled out by the distinctive behaviour of methyl groups. It may be assumed that the reason for the disappearance of the carboxyl resonances from the spectra was that they could not be effectively cross-polarised from adjacent hydroxyl or bound water protons when these were in rapid motion.

The values of T_{CHD} appeared to be reduced by hydration, but with the number of data points available it was not clear if the fast initial phase of cross-polarisation remained genuinely exponential, and non-exponential behaviour may have affected the fitted T_{CHD}. The principal effect of hydration, however, was to reduce the magnitude of the fast phase rather than its rate, and this is not explained by existing models [1,2,14] of cross-polarisation.

The $T_{1\rho}$s for the pectic resonances might be expected to be reduced by the extra mobility permitted by hydration, but they were considerably increased, as were the $T_{1\rho}$s of other mobilised components. For comparison, the $T_{1\rho}$ of dry cell walls averaged 3 ms with little variation across the spectrum (data not shown). The long apparent $T_{1\rho}$ values for hydrated pectins may be attributed to the presence of highly mobile, methylated pectic polymers that cross-polarise so slowly that they begin to contribute to the spectrum only after 2–3 ms of contact time [9].

Hydration also lengthened the $T_{1\rho}$ of the cellulosic resonances, but this effect must have had a different origin. Hydration swells the matrix of the cell wall between the cellulose microfibrils [4] and would thus lengthen the spin diffusion path between cellulose and pectin. At the same time the spin diffusion coefficient would decrease with increasing pectin mobility (see theory section). Both of these effects would isolate the proton spin reservoir of the cellulose microfibrils and reduce the extent to which the long $T_{1\rho}$ of cellulose was averaged with the shorter $T_{1\rho}$ of the pectins.

Hydration considerably lengthened the proton T_1 values for all resonances (Table 2). The shorter values for pectic resonances show that the trend was for the T_1 to decrease with increasing mobility. However the value of the T_1 observed for any signal depends also on the degree to which it is averaged by spin diffusion. It may therefore be predicted that hydration in H_2O will generally decrease the proton T_1s of the more mobile cell-wall fractions by increasing their chain mobility on the 10^{-8} s timescale, but will also isolate them from the rest of the cell wall as motions on the 10^{-3} s timescale restrict spin diffusion and as the polymer chains move apart. Combining these predictions is made complicated by the fact that the most mobile, methylated pectins are under-represented in the ^{13}C spectra from which the proton T_1s of the hydrated cell walls were derived, since they would cross-polarise only to a small extent during the contact time of 1 ms, but they would nevertheless contribute to the T_1 values averaged by spin diffusion. It is likely that in the dry cell walls where the averaging process of spin diffusion was almost complete, the most important relaxation route for the whole cell wall was through these methylated pectins, and particularly through their rotating pectic methyl groups, but that after hydration this route was unavailable to the spins in the rigid part of the cell wall because of restricted spin diffusion into the domain occupied by the highly mobile, 'invisible' methylated pectins. This would explain the unexpected observation that hydration generally increased the proton T_1 of those polymers that appeared in the spectra.

Effects of hydration in D_2O.—Hydration with D_2O instead of H_2O produced quite different effects. D_2O replaces the bound water and hydroxyl protons of non-cellulosic polysaccharides with deuterium, but not the hydroxyl protons of crystalline cellulose, nor the carbon-bonded protons of any polysaccharide [20]. It was used here for two purposes: to clarify the role of the replaceable hydroxyl protons in cross-polarisation, and to reduce the effectiveness of proton spin diffusion by diluting the proton reservoirs. A direct effect on relaxation rates would also be expected if rotating hydroxyl groups that are capable of being deuterated contribute to the $T_{1\rho}$ and/or T_1 mechanisms of relaxation. We were unable to isolate this effect, however, due to the magnitude and complexity of the effects of deuteration on spin diffusion.

After hydration with D_2O the fast and slow phases of cross-polarisation were about equal in magnitude for the main 72 ppm resonance, as in the dry cell walls (an inferior signal/noise ratio made it difficult to estimate the cross-polarisation kinetics for the smaller signals). This suggests that many of the dipolar interactions that disrupted the initial cross-polarisation phase in H_2O were with exchangeable hydroxyl protons, implying a degree of mobility — presumably rotation of the hydroxyl groups — in excess of that shown by the C–H protons. Exchangeable hydroxyl protons could in principle either contribute to the slow phase of the cross-polarisation process, or disrupt

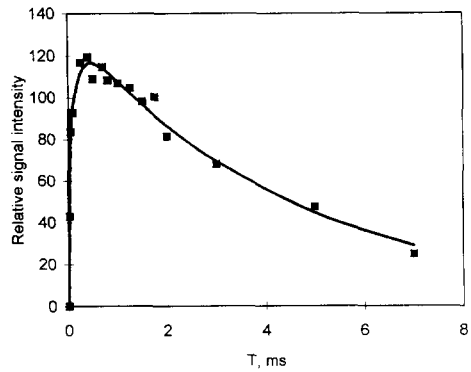

Fig. 3. Variation of signal intensity with contact time for 72 ppm resonance in spectrum of cell walls hydrated in D_2O. The fitted curve is derived from eq (3) with $T_{1\mu} = 4.5$ ms, $s = 0.45$, $T_{CHD} = 25$ μs, and $T_{CHR} = 0.19$ ms.

it by rotating and interfering with polarisation transfer to any one carbon from the protons in other C–H groups. The slightly reduced value of T_{CHR} in D_2O suggests that both effects may have been operating.

Hydration with D_2O resulted in shorter $T_{1\rho}$s for the pectic than for the cellulosic resonances, the reverse of what was observed after hydration with H_2O. This confirms that for all but the most mobile polymers in the cell wall, the true (rather than the apparent) proton $T_{1\rho}$ values were decreasing with increasing mobility.

Hydration in D_2O (Fig. 3) separated the T_1 values clearly into three groups, corresponding in order of increasing T_1 to (1) methylated pectins (54 ppm; 0.3 s), (2) other pectins (69, 80 ppm; 0.5–0.6 s), and (3) the rest of the polysaccharides including cellulose (1.2 s). In this experiment, therefore, spin diffusion was sufficiently retarded by deuteration to prevent spatial averaging of the T_1 across the whole cell wall, and there were no complicating effects of the highly mobile fraction as encountered in the $T_{1\rho}$ experiments.

Within the rigid microfibrils, where there was little deuteration, the proton spin diffusion coefficient D is estimated as 10^{-15} m^2 s^{-1} (see Theory section). The one-dimensional mean diffusion distance X is given by $X = \sqrt{2DT_1}$, which here is ca. 45 nm, much greater than the lateral dimensions of the microfibrils. However, in the mobile parts of the cell wall D is likely to be $5 \cdot 10^{-17}$ m^2 s^{-1} or less after deuteration, giving $X \approx 7$ nm; possibly an overestimate if gaps between molecules must be bridged by water molecules so mobile that they function as spin insulators. Cellulose is confined to the cell wall itself and pectins are not only present there but are the only component of the middle lamella, the cementing layer that joins the walls of two adjacent cells together [3]. The middle lamella in hydrated primary cell walls is typically some tens of nm thick, and the cell wall itself some hundreds of nm. Within the cell wall the microfibrils are 10–20 nm apart [3]. These dimensions are therefore sufficient to give distinct values of the proton T_1 for the polymers outside the microfibrils, and the highly methylated and low-methyl pectic polymers were clearly separated in space within this region.

4. Conclusions

For these complex and chemically diverse materials, it was possible to extract a considerable amount of motional information by studying the kinetics of cross-polarisation in variable-contact CP-MAS experiments. The CP kinetics for the dry cell walls were in good agreement with the two-phase exponential model of Wu and co-workers [2], although for hydrated samples the relative contributions of the covalently bonded and non-bonded protons differed from those predicted by the model and were sensitive to the presence of highly mobile protons. This allowed useful motional data to be obtained from cross-polarisation in hydrated samples, but the true proton $T_{1\rho}$ could not be effectively determined in the same experiment due to the complex influence of the most mobile polymer fractions.

From the cross-polarisation and proton T_1 data it is clear that there is a considerable diversity of motions within cell walls. Methyl rotations, particularly those of the pectic methyl ester groups, predominated in the dry cell walls, and probably also at the highest frequencies (10^8 Hz) after hydration. Hydroxyl rotations were present on a sufficient scale to influence the rapid phase of cross-polarisation in the more mobile pectic polymers of hydrated cell walls. The chain segmental motions that may be assumed to control the mechanical properties of the material were most evident in the pectic fraction and on the kHz frequency scale.

5. Methods

Cell walls were prepared from *Citrus* mesocarp as follows. The peel (140 g) from seven Jaffa oranges, with the cuticle removed by peeling to a depth of 0.5 mm, was homogenised in 0.5 L of 2 g/L sodium deoxycholate with the addition of 400 g of ice. The 0.15–0.85 mm size fraction was collected on a stainless steel sieve and washed extensively with cold water, then with 0.5 L of MeOH and 0.5 L of $CHCl_2$–MeOH (2:1 v:v). The cell walls were air-dried at room temperature to a moisture content of about 10%. For some experiments H_2O or D_2O was added in the ratio 3 cm^3:1 g cell walls. In the experiments with D_2O there was no prior exchange so that ca. 3% HOD was present.

NMR experiments were carried out on a Varian VXR-300 spectrometer operating at 75.34 MHz for ^{13}C. MAS rates varied between 3.5 and 4.3 kHz. The proton spin-lock and decoupling fields applied were close to 40 kHz except in the experiment on cross-polarisation kinetics of dry cell walls, where a 60 kHz field was used. For comparability with the data on hydrated cell walls the ^1H $T_{1\rho}$ of the dry cell walls was measured in a separate experiment with 40 kHz spin-lock and decoupling fields, giving very similar cross-polarisation kinetics (data not shown) but somewhat lower signal/noise. Between 10 and 16 values of the contact time, in the range 25 μs–7 ms, were used in different variable-contact experiments. In the inversion-recovery experiments the contact time was 1 ms and the delay after inversion varied between 50 ms and 2.5 s in 12 steps. Relaxation data were calculated from signal intensities at spectral peaks, except for the proton T_1 where it was possible to construct a complete plot across the spectrum (Fig. 4); this showed that except for the 60–65 ppm and 82–84 ppm

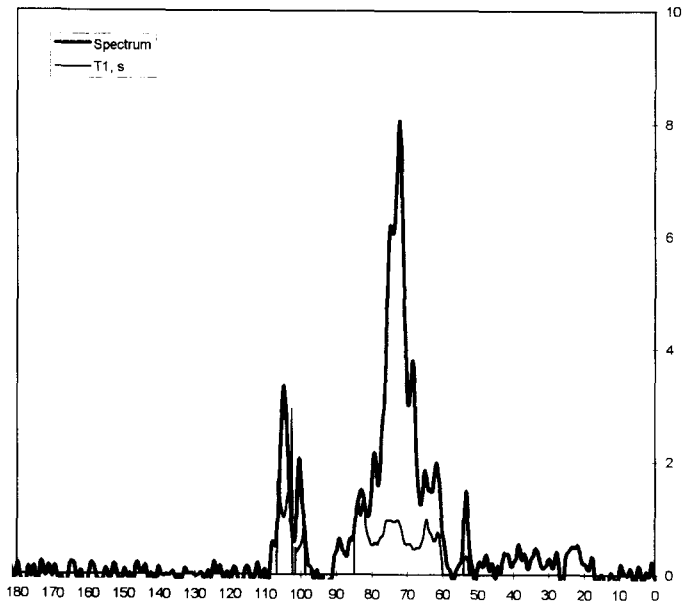

Fig. 4. Proton T_1 for cell walls hydrated in D_2O, plotted across the ^{13}C spectrum.

regions of the spectrum the errors introduced by signal overlap did not distort the analysis. Signal assignments were based on published data [21,22] (it should be noted that in ref. [21] the assignments of the 21 and 54 ppm resonances should be interchanged).

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