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The distribution of water in native starch granules—a multinuclear NMR study

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Abstract

The microscopic distribution and dynamic state of water in native potato, maize and pea starch granules are investigated with NMR relaxometry and diffusometry. Besides extra-granular water, three water populations can be identified inside native potato starch granules. These are assigned to water in the amorphous growth rings; water in the semi-crystalline lamellae and "channel water", which is located in the hexagonal channels within the B-type amylopectin crystals. The first two water populations are orientationally disordered and exchange with each other on a millisecond timescale at 290 K. NMR diffusometry shows that the water in packed granule beds is undergoing translational diffusion in a 2-dimensional space, either in thin layers between granules and/or in amorphous growth rings within the granules. The "channel water" is uniquely characterised by a 1 kHz deuterium doublet splitting and is in slow exchange with water in the other compartments on the NMR timescale. In the smaller maize granules all intra-granular water populations are in fast exchange and there is no evidence for "channel water" in the A-type crystal lattice. The NMR water proton and deuterium data for pea starch are consistent with a composite A and B-type crystal structure.

Keywords: Starch; NMR; Water; Potato; Maize; Pea

1. Introduction

The structure of native starch granules has been researched intensively for many years using a variety of microscopic and scattering techniques, including optical and electron microscopy, light, X-ray and neutron scattering. The results have been extensively reviewed (Frazier, Donald & Richmond, 1997; Gallant, Bouchet & Baldwin, 1997) and used to construct models of starch granule structure such as that shown in Fig. 1, reproduced from Bogracheva, Morris, Ring and Hedley (1998). What is less well researched, or understood, is the distribution and dynamic state of water within starch granules. This is important because water exercises a crucial role in determining the response of starch granules to processing treatments such as heating, microwaving, high pressure or chemical processing. Small angle neutron scattering (SANS) has been used to probe the water distribution inside maize starch granules during gelatinisation (Donald, Waigh, Jenkins, Gidley, Debet & Smith, 1997), but this technique relies on simplified models for data interpretation and is not widely available.

NMR is undoubtedly the technique of choice for studying the state of water in starch granules, and this has been recognised since the early work of Leckert (1976) and Mousseri, Steinberg, Nelson and Wei (1974). These exploratory studies were undertaken on starch powders containing less than 33% water, when most of the bulk water has been removed from the granule, and were limited by the use of continuous wave NMR spectrometers and relatively unsophisticated data analysis. Nevertheless they showed that the water could be characterised by two, or possibly three, transverse proton relaxation times. The interpretation of the NMR relaxation data therefore focused on the exchange dynamics of various states of "bound water" in the granule. More recently we have extended these NMR studies on starch powders to include solid state techniques such as wideline proton lineshape analysis, longitudinal cross-relaxation, rotating frame relaxation and magic angle spinning (MAS). These methods succeeded in characterising the dynamic state of water adsorbed on the surface of waxy-maize starch at water contents less than 20% (Tanner, Hills & Parker, 1991). The dynamic state of this surface adsorbed water is, of course, insensitive to granule microstructure and is determined by water–starch

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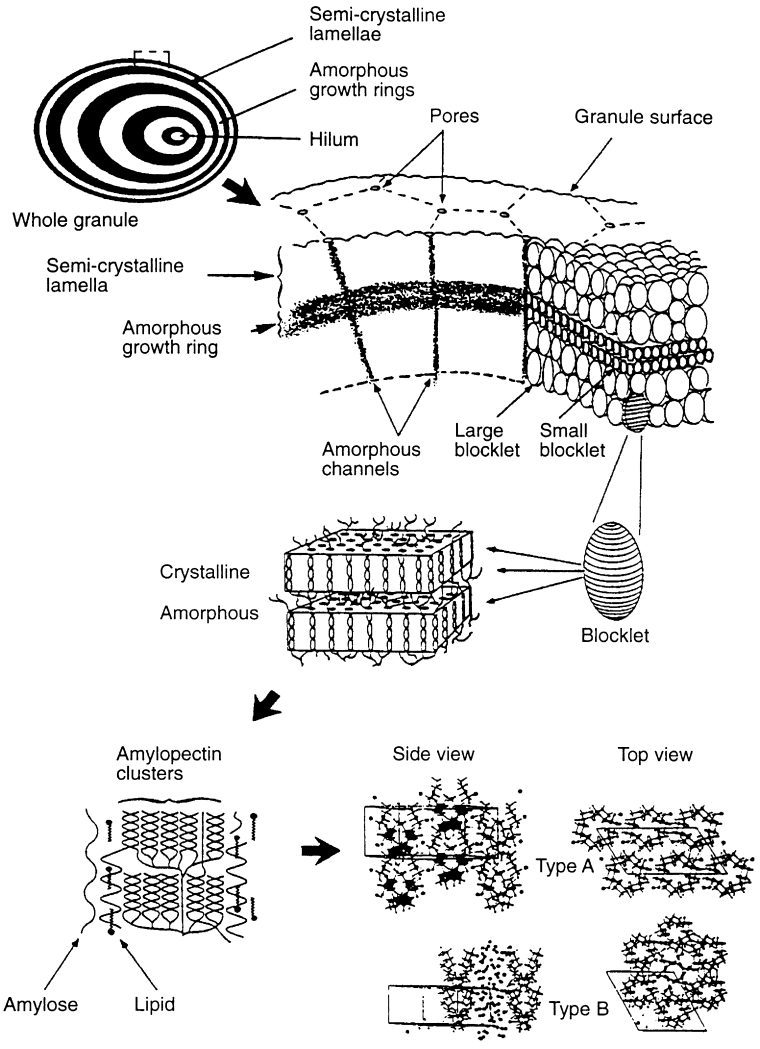

Fig. 1. The idealised structure of a starch granule redrawn from Gallant et al. (1997). The dots in the type A and B amylopectin lattices correspond to positions of water molecules deduced from X-ray scattering data.

interactions at the molecular level. The role of granule microstructure in determining water relaxation at high water contents when all sub-granule compartments are water-saturated was not explored.

The structure and dynamics of the amylose and amylopectin components of native starch granules has been studied with a variety of high-resolution solid state NMR techniques, especially CPMAS (Morgan, Furneaux & Larsen, 1995). This identified three distinct dynamic components in the starch component, namely a highly crystalline region formed from amylopectin double helices; a more mobile, rubber-like amorphous region associated with amylopectin branch points and solid-like regions formed from lipid inclusion complexes of amylose (see Fig. 1). A 2-dimensional $^{1}H-^{13}C$ wideline separation (WISE) solid-state NMR technique has been used to probe water–starch interactions on the molecular distance scale (Kulik, Chris de Costa & Haverkamp, 1994). The results confirmed that water organisation in type A starch differs from that in type B and suggested that water in type A was more mobile than in type B. Although such solid-state NMR techniques are valuable for probing very short range water–starch interactions on the (sub-)nanometer distance scale they are of little value for investigating the distribution and dynamic state of water in starch granules on the microscopic distance scale.

Deuterium lineshape analysis has been applied to starch granules at high water contents. A deuterium doublet with a splitting of approximately 1 kHz was discovered in the deuterium spectra of D_2O in potato starch as early as 1976 (Leckert, 1976) and assigned to a unique type of slowly exchanging water inside the granule. Unfortunately the relevance of this observation to granule microstructure was not appreciated because it was thought to be related to both the large size of the potato granule and the presence of calcium or phosphate ions (Yakubu, Baianu & Orr, 1990). More recently the freezing behaviour of water in waxy maize starch was investigated with deuterium NMR and this

identified a fraction of isotropically reorienting, mobile, water in the granule which apparently failed to freeze even at −32°C (Li, Dickinson & Chinachoti, 1998).

A combined multinuclear, water oxygen-17, deuterium and proton relaxation study has been reported for dilute suspensions of potato granules in water (Yakubu, Ozu, Baianu & Orr, 1993). In these dilute suspensions the relaxation is single exponential and was explained in terms of the fast exchange of bulk and "weakly bound" water at the granule surface. An average correlation time of 20 ps was deduced for the weakly bound surface water in the range of 2–40% by weight of starch. Unfortunately the role of intragranular water was not investigated and it is unclear how the dilute starch suspensions were stabilised, because the granules settle under gravity in bulk water.

Given the variety of NMR studies on native starch it is surprising that the potential of water proton NMR relaxometry and diffusometry for probing the microscopic distribution of water inside starch granules appears to have been largely overlooked. This is best done by working, not with dilute starch suspensions or starch powders but with water-saturated packed beds of native starch granules. The relationship between water proton relaxometry and the microscopic distribution of water in randomly packed particulate beds has been explored in a variety of non-starch systems in our earlier papers. These include the microscopic water distribution in randomly packed beds of Sephadex microspheres (Hills & Babonneau, 1994), porous silica (Hills & Quantin, 1993; Hills, Belton & Quantin, 1993) and monodisperse glass microspheres (Snaar & Hills, 1995). Similar methods have also been applied to water compartmentalised in sub-cellular structures (Hills & LeFloc'h, 1994). Water proton diffusion techniques, such as q-space microscopy, have also been developed as microstructural probes (Callaghan, 1991) and modifications of this technique have been used to study water distribution among the pores of packed silica beds (Hills, Wright & Snaar, 1996). The use of water relaxometry and diffusometry as indirect probes of microstructure in food science has been reviewed (Hills, 1998).

In this paper we therefore extend the application of these NMR techniques to packed beds of native starch granules and explore the microscopic distribution of water among the various sub-granular compartments. Three types of native starch granule, namely maize, potato and pea starch granules have been chosen because they are representative of A, B and C type starches, respectively (Bogracheva et al., 1998). As we shall show, the microstructural complexity of the native starch granule makes assignment of NMR signals to sub-granular compartments very difficult. For this reason the major effort in this paper has had to be devoted to solving this assignment problem. Nevertheless it is our intention to use the same NMR techniques to investigate the response of the granules to processing operations, such as heat treatment, microwaving and Lintnerisation and this will be the subject of future papers. The effects of freezing and drying will be included in this paper because they help resolve the NMR assignment problem.

This assignment difficulty has, in the past, lead to quite different interpretations of NMR relaxation data such as that involving differing amounts of 'weakly' and 'strongly' bound water in starch systems. This interpretation continues even in the recent literature (Le Botlan, Rugraff, Martin, & Colonna, 1998). For this reason we have used a variety of different experimental techniques to verify that our peak assignments are related to granule structure and not to so-called 'bound' water.

2. Experimental

NMR water proton transverse relaxation times were acquired on a Bruker MSL100 spectrometer operating at a resonance frequency of 100.13 MHz. A solenoid RF coil was used with horizontal, 5 mm internal diameter tubes. This gave short 90° pulses of 1–2 μs. Free Induction Decays were determined with a single 90° pulse of 2 μs duration and a dwell time of 4 μs. Transverse relaxation times were measured with the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence with a 90–180° pulse spacing of 150 μs and full phase cycling. A recycle delay of 10–15 s was used to avoid saturation effects and 48–64 acquisitions were accumulated. The FID and CPMG echo decay envelopes were analysed as a continuous distribution of exponentials with Resonance Instruments WINDXP software. To avoid over-processing of data, the regularisation parameter in this inversion was determined from the amount of noise in the baseline. Temperatures were thermostated in the usual way using a liquid nitrogen evaporator.

NMR water deuterium spectra were acquired on a Bruker MSL300 spectrometer operating at a deuterium resonance frequency of 46 MHz with a 90° pulse of 2.1–2.6 μs. The quadrupolar echo pulse sequence was used with a delay of 40 μs. Below 270 K a short dwell time of 0.2–0.4 μs was used to enable ice signals to be recorded and, above 270 K a longer dwell of 4 μs was used to resolve smaller splittings of ca. 1 kHz. Deuterium exchanged starch granules were prepared by suspending the granules in excess D_2O, allowing them to settle under gravity, and replacing the supernatant with pure D_2O. This cycle was repeated three times.

NMR water diffusion measurements were undertaken on a Resonance Instruments MARAN spectrometer operating at 22MHz with 90° pulse lengths of 5.2 μs. Sample tubes were 18 mm internal diameter and were inserted vertically into the magnet. The stimulated echo pulse sequence was used with variable gradient amplitudes and diffusion times up to 200 ms. The temperature was thermostatted at 293 K by flow of nitrogen gas through the probe.

The samples of potato starch were obtained from BDH, maize starch from Sigma and Pea starch from Nutrio, Danisco Grinsted products Ltd. Water saturated suspensions were made by addition of excess cold water, gentle hand

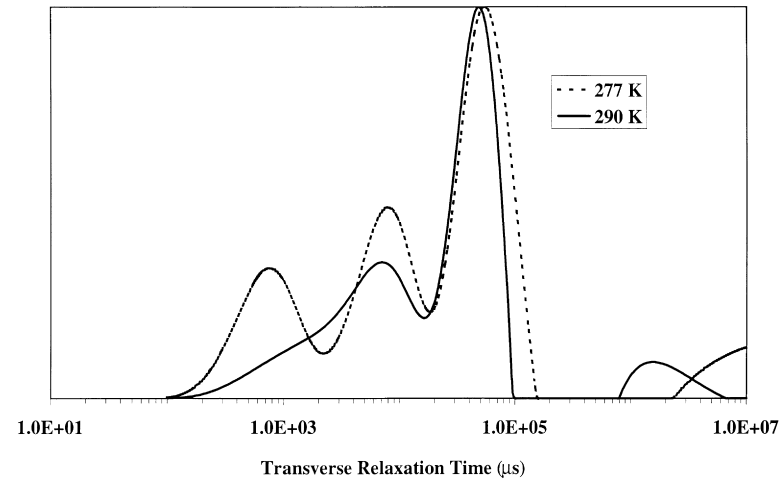

Fig. 2. The distribution of water proton transverse relaxation times for a water-saturated, packed bed of potato starch granules at the indicated temperatures.

centrifugation followed by removal of supernatant water. Freeze-thawing experiments were performed by rapidly freezing the sample in liquid nitrogen and then gradually raising the temperature in the NMR probe. No difference was found between the frozen–thawed and untreated sample. Packed beds at water contents below saturation were prepared by adding the calculated weight of water to starch granules that had been dried in a desiccator over P_2O_5. Electrical conductivities were determined by placing the sample in a conductivity meter (Jenway, Dunmow, Essex, UK). To give a measureable conductivity water was replaced by a 1 M KCl solution.

3. Results and discussion

3.1. Water compartmentation in a starch granule

Fig. 1 depicts the idealised structure of a native starch granule as deduced from microscopy and scattering data (Gallant et al., 1997). If this model is correct then there should be several sub-populations of water within a water-saturated granule. On the largest distance scale there will be two populations corresponding to water in the amorphous growth rings and to water in the semi-crystalline lamellae. Within the semi-crystalline lamellae there will be water in the amorphous channels separating large and small blocklets as well as water inside the amylopectin blocklets. Finally, on the smaller distance scale corresponding to the macromolecular internal structure of a blocklet, there will be water in the crystalline lattice of the amylopectin clusters and water in the amorphous branch-points separating the crystalline clusters. Because of differences in amylose and amylopectin concentration and chain mobility, water protons in each of these sub-granular compartments are expected to be characterised by different intrinsic relaxation times. The distribution of water relaxation times, such as that shown in Fig. 2, should therefore be equivalent to the distribution of water among the various compartments. Unfortunately diffusion of water between neighbouring compartments averages their NMR signals to an extent that depends on the magnitude of the water self-diffusion coefficient, the compartment morphology and the difference in intrinsic relaxation rates. As a consequence most compartments will not be distinguishable in NMR relaxometry, especially the smaller compartments, unless microstructural barriers hinder inter-compartment water diffusion. Fortunately several distinct water populations can be distinguished in the NMR relaxation behaviour of water in native starch granules and these provide a unique insight into the structure–function behaviour of the granule.

3.2. Water proton transverse relaxation time distributions for water-saturated starch granule beds

As a first step to exploring water distribution in starch granules the distribution of water proton transverse relaxation times was obtained using the CPMG pulse sequence with a 90–180° pulse spacing of 150 μs at a spectrometer frequency of 100 MHz. The results for a water-saturated, packed bed of potato starch granules are shown in Fig. 2. At 290 K two peaks centred at ca 8 and 50 ms are observed in the relaxation time distribution, with an obvious shoulder on the 8 ms peak. The much smaller intensity peak centred at approximately 3 s can be ignored as it arises from residual supernatant bulk water in the meniscus, the excess of the supernatant water having been removed from the top of the starch granule bed before measurement.

The more difficult problem is assigning the 50 and 8 ms relaxation peaks to particular water compartments. As a provisional assignment we assume that the 50 ms relaxation time peak arises from bulk water in external interstitial spaces between the granules. Bulk water has a relaxation time of about 3 s, so if this is the correct assignment it implies that the extra-granular water exists as a very thin layer between closely packed granules and that its relatively

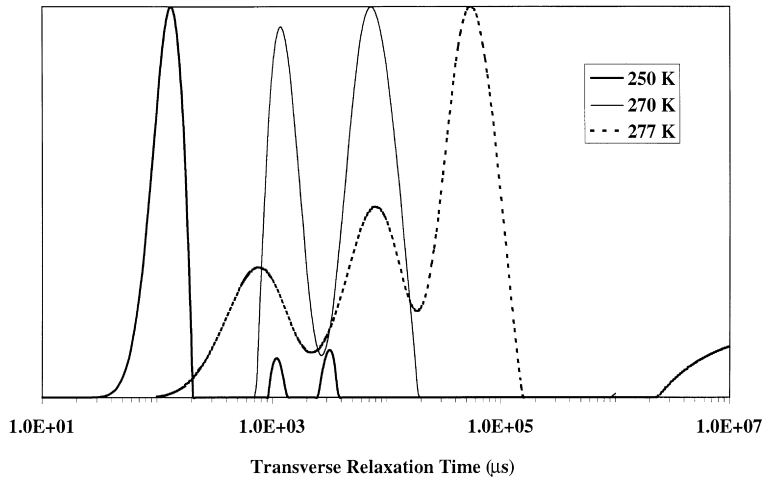

Fig. 3. The effect of freezing on the distributions in Fig. 2.

short relaxation time of 50 ms is caused by fast proton exchange with hydroxyl protons in the amylopectin and amylose molecules on the surface. It is surprising that inter-granular water-filled pores do not appear as longer relaxation time peaks in the water-saturated packed bed as we observed in Sephadex and silica beds (Hills & Quantin, 1993). However repeated attempts using long acquisition times of up to 10 s in the CPMG sequence failed to detect any peak with a relaxation time longer than 50 ms except that of meniscus water at approximately 3 s. According to this hypothesis the 8 ms relaxation peak is assigned to water inside the granules, and this needs to be in slow exchange with the outside water on the NMR measurement timescale of a few 100 ms.

These assignments can be tested by investigating freezing and drying behaviour.

3.3. The freeze-thawing behaviour of water-saturated starch granule beds

Lowering the temperature to just above freezing reduces the water self-diffusion coefficient and slows the exchange of water molecules between the various compartments. This helps to shift the water relaxation time distribution into the slow exchange regime so that compartments are more easily resolved. Freeze-thawing behaviour is another helpful assignment tool because differences in amylose and amylopectin concentration can result in different compartments having different freeze-thawing behaviour. Ice, which has a short transverse proton relaxation time of just a few microseconds, is not detected on the millisecond timescale of the CPMG pulse sequence so the freezing of water in a particular compartment is detected by the disappearance of its associated relaxation time peak.

Figs. 2 and 3 show the result of rapidly freezing a water-saturated potato starch sample in liquid nitrogen then warming to the indicated temperatures. Freezing the sample at 250 K causes a new peak with a short relaxation time of only ca 100 μs to appear while the intensity of the peaks at 1 and 8 ms is greatly reduced and that at 50 ms has vanished. The 100 μs peak is characteristic of non-freezing water closely interacting with the surface of amylose and

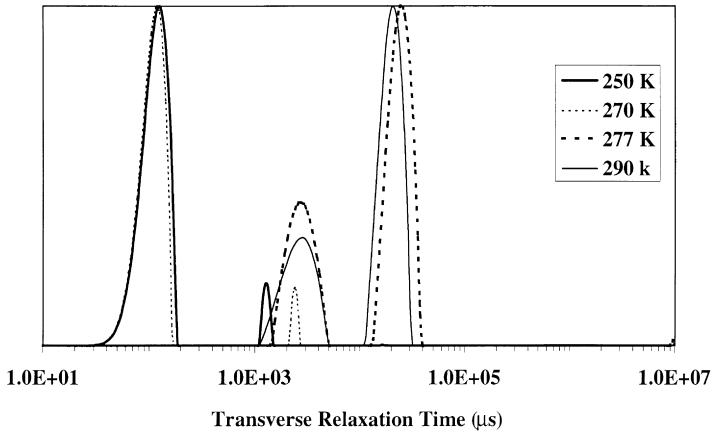

Fig. 4. The temperature dependence of the water proton transverse relaxation time distributions for a water-saturated packed bed of maize starch granules.

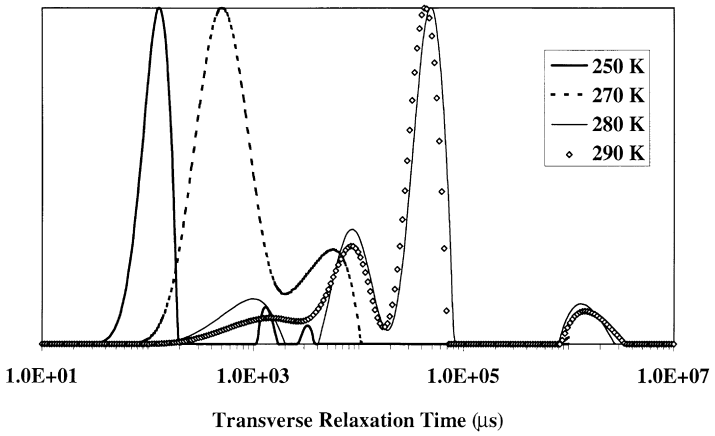

Fig. 5. The water proton transverse relaxation time distributions for a water-saturated bed of pea starch at the indicated temperatures.

amylopectin molecules so this is consistent with the freezing of most of the water in the 1 and 8 ms compartments. It can be seen that just above freezing (277 K) the peak at 8 ms can be resolved into two water populations with relaxation times of 8 and 1 ms. At 270 K new peaks at 1 and 8 ms have appeared showing the presence of unfrozen water inside the granule. However the absence of the 50 ms peak at 270 K shows that the bulk water outside the granules remains frozen. Warming to 277 K melts the extragranular water so the 50 ms peak appears. At 290 K the 1 and 8 ms peaks have merged into a broad peak centred at about 8 ms, presumably by the process of exchange averaging by rapid molecular diffusion. Note that only relative peak intensities are significant in Figs. 2–6 so the plots have been scaled to the highest intensity peak.

A rough estimate of the exchange lifetime, τ_{ex}, of the two water populations associated with the 1 and 8 ms peaks can be made by noting that, for exchange averaging of two populations with intrinsic relaxation rates R_a and R_b, the product $\tau_{ex}|R_a - R_b|$ must be less than unity. Taking R_a as $10^3\ s^{-1}$ and R_b as $33\ s^{-1}$ the exchange lifetime will need to be of the order of 1 ms for onset of exchange coalescence of the two relaxation time peaks. Equating this exchange lifetime to the diffusion time (a^2/D) gives a diffusion distance, a, of the order of 1μm for a water self diffusion coefficient, D, of $10^{-9}\ m^2\ s^{-1}$. It could be argued that a smaller value of the water self-diffusion coefficient should be used in this order-of-magnitude estimate, but the situation is complicated by the fact that water diffusion inside the granule is restricted by the granule microstructure. We are therefore assuming that the intrinsic water self-diffusion coefficient in the amorphous growth rings is approximately that of bulk water.

Fig. 4 shows similar results for maize starch. The peaks are shifted slightly to shorter relaxation times (25 and 3 ms instead of 50 and 8 ms, respectively) with the important difference that there is no shoulder on the 3 ms peak and, below 277 K, the ca 3 ms relaxation peak does not split. It is interesting to note that, in contrast to potato starch, the intragranular water in the smaller maize granule remains frozen at 270 K, though it not clear why this should be the case.

These observations support the previous assignments: The peak at 50 ms is extra-granular bulk water so it would be expected to freeze and melt at 273 K. The remaining

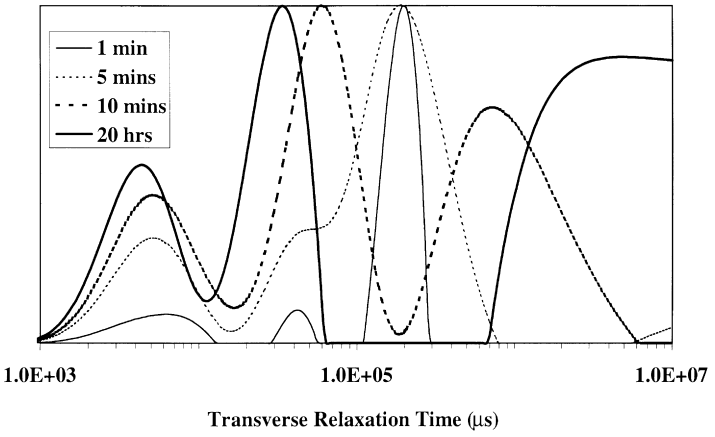

Fig. 6. The effect of reduced packing fraction on the water relaxation time distributions of concentrated suspensions of potato starch granules. The settling times after shaking the diluted suspension are indicated.

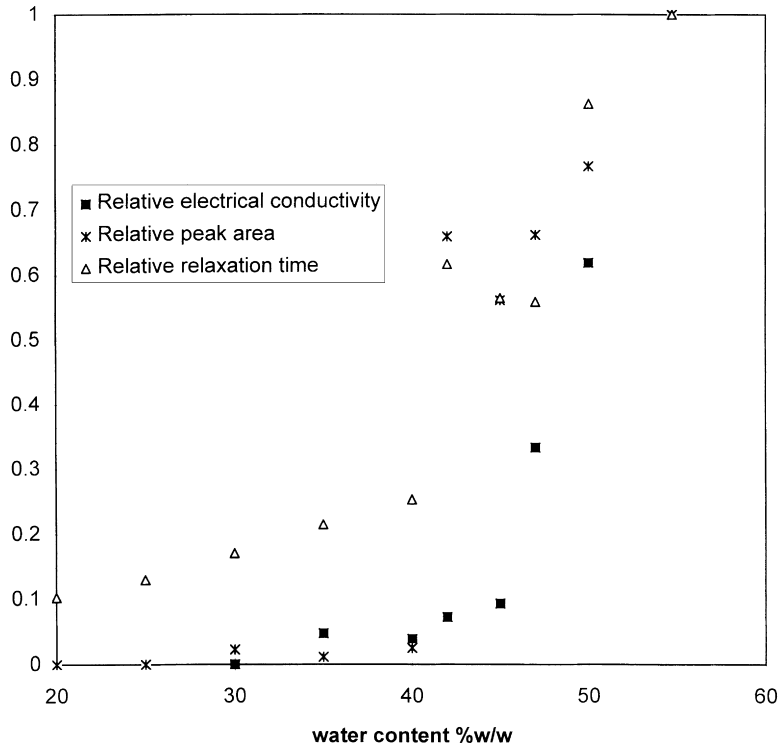

Fig. 7. The effect of lowering the water content on the distribution of water proton transverse relaxation times in a packed bed of potato starch at 298 K. For ease of comparison all data has been normalised to the value of the water-saturated bed at a water content of ca. 55%(w/w). Above 40%(w/w) water there are two peaks and the data refers only to the longest relaxation time peak believed to be extragranular water. Below 40% there is only a single peak believed to be intragranular water.

peaks correspond to water inside the granules, such as water in the amorphous growth rings, which exists in a rubbery state (Kulik et al., 1994) and is expected to melt below 273 K. The question then arises as to why the potato starch shows a splitting, whereas the maize starch does not. The most probable explanation is that the two exchanging populations correspond to water in the amorphous growth rings and water in the amorphous regions of the semi-crystalline lamellae. The estimated diffusion distance of 1 μm is compatible with the width of a growth ring in potato and the absence of a splitting in maize and the shift to shorter relaxation times is then explained by the smaller size of the granule and the thinner growth rings, leading to much faster exchange averaging. As we shall see, this assignment is also consistent with the deuterium lineshape data.

The relaxation data for pea are interesting because it is commonly designated a “type C” starch. Recent work suggests that this is not a third type of crystalline amylopectin but instead each pea starch granule has a composite structure of layers of A and B type amylopectin (Bogracheva et al., 1998). If so, the water relaxation time distribution of pea should resemble the combined relaxation time distributions of that for maize and potato. Fig. 5 shows that this is indeed the case. The relaxation distributions most closely resemble that of B-type starch with the important difference that at 280 K the three peaks are clearly separated indicating that they arise from water in well-separated regions of the granule. Moreover, at 270 K the relative intensity of the 1 ms peak is much greater than in B-type potato starch alone.

3.4. The effect of reduced packing density

The relaxation time distributions of a concentrated granule suspension were also compared with that of the water-saturated packed bed. The suspension was created by addition of a layer of bulk water on top of a water-saturated packed bed of potato starch granule bed. After NMR acquisition the sample was removed from the spectrometer, shaken to create a uniform suspension, quickly replaced in the spectrometer and relaxation time distributions measured with the CPMG sequence as a function of time. In this way the effects of gravimetric settling of the granules could be examined.

The results of the reduced packing density experiment are shown in Fig. 6. Before shaking the layer of bulk water appears as a large peak in the relaxation time distribution at approximately 3 s. The effect of lowering the packing density by shaking is dramatic. The 50 ms peak and the bulk water peak now combine into a single peak at about 200 ms; while the 8 ms peak remains essentially unaltered, though at an apparently lower relative intensity because of the normalisation to the peak height of the largest peak. The effects of gravimetric settling can be seen after only 1 min in

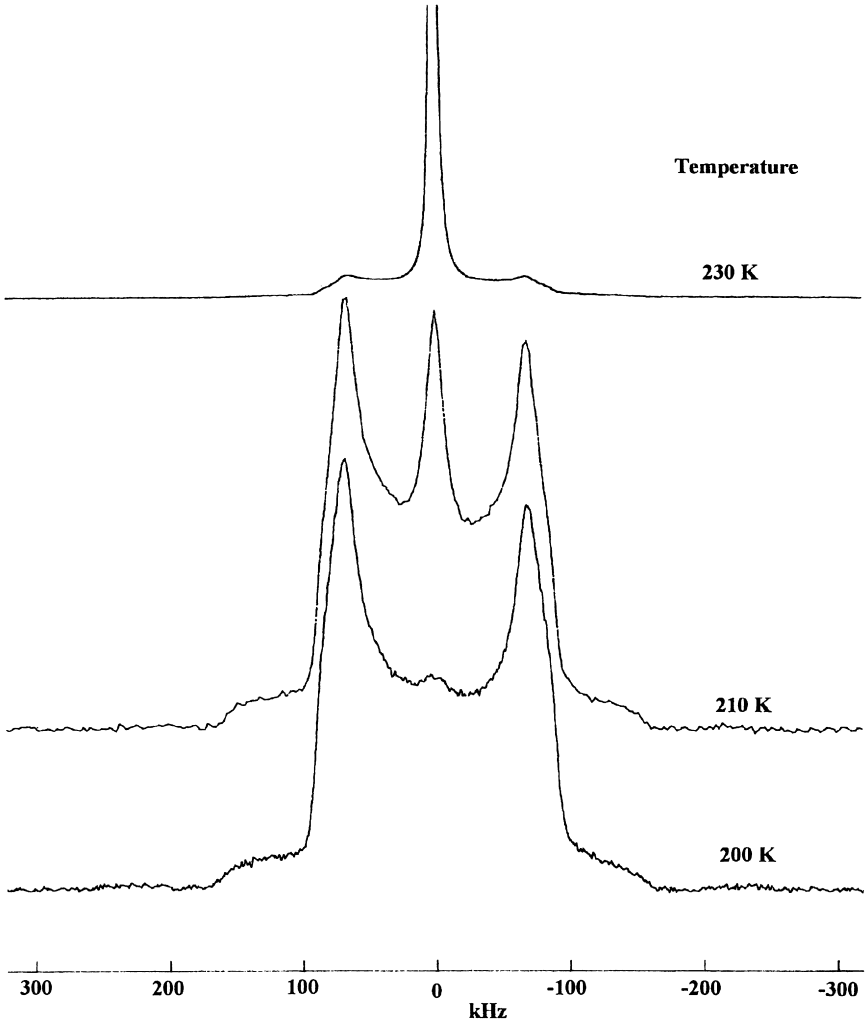

Fig. 8. The effect of temperature on the water deuterium spectrum for a frozen packed, D_2O-saturated bed of potato starch granules.

the reappearance of the relaxation time peak at 50 ms. After 10 min the relaxation time distribution shows intermediate peak positions corresponding to a loosely packed starch layer at the bottom of the tube and a more dilute suspension in the top. After 20 h the starch layer has again compacted and the initial distribution is recovered. These changes are strongly supportive of the previous assignments, according to which the 8 ms peak arises from water inside the granules and that at 50 ms to extra-granular water which is shifted to longer relaxation times as the granule separation increases.

3.5. The dependence of the water relaxation time distributions on water content

Provided the diffusive exchange of water molecules between the compartments is slow on the NMR timescale, the relative peak areas in the relaxation spectra are proportional to the relative water populations and can be used to monitor the changing distribution of water within the granule bed as the water content is progressively lowered (Hills & Babonneau, 1994). In this series of experiments we are concerned with the distribution of bulk water between granular compartments and not with the dynamics of surface adsorbed water. We therefore work at high water contents no lower than 20%(w/w). The relaxation behaviour of surface adsorbed water in starch at lower water contents has been the subject of an earlier paper (Tanner et al., 1991).

Because of powerful osmotic forces, lowering the water content should first remove extra-granular bulk water, then water from the amorphous growth rings, and finally water from the semi-crystalline lamellae which contain a denser network of amylopectin. Progressive removal of water from the amorphous growth rings would also be associated with granule shrinkage. The relaxation data is consistent with this picture. Lowering the water content first reduces the area of the 50 ms relaxation peak relative to the 8 ms peak until the 50 ms peak vanishes. Further drying shifts the 8 ms peak to progressively shorter relaxation times. Fig. 7 shows the decrease in the relative population of water in the 50 ms peak as the water content is lowered from saturation to 20%(w/w).

Lowering the water content also causes the relaxation

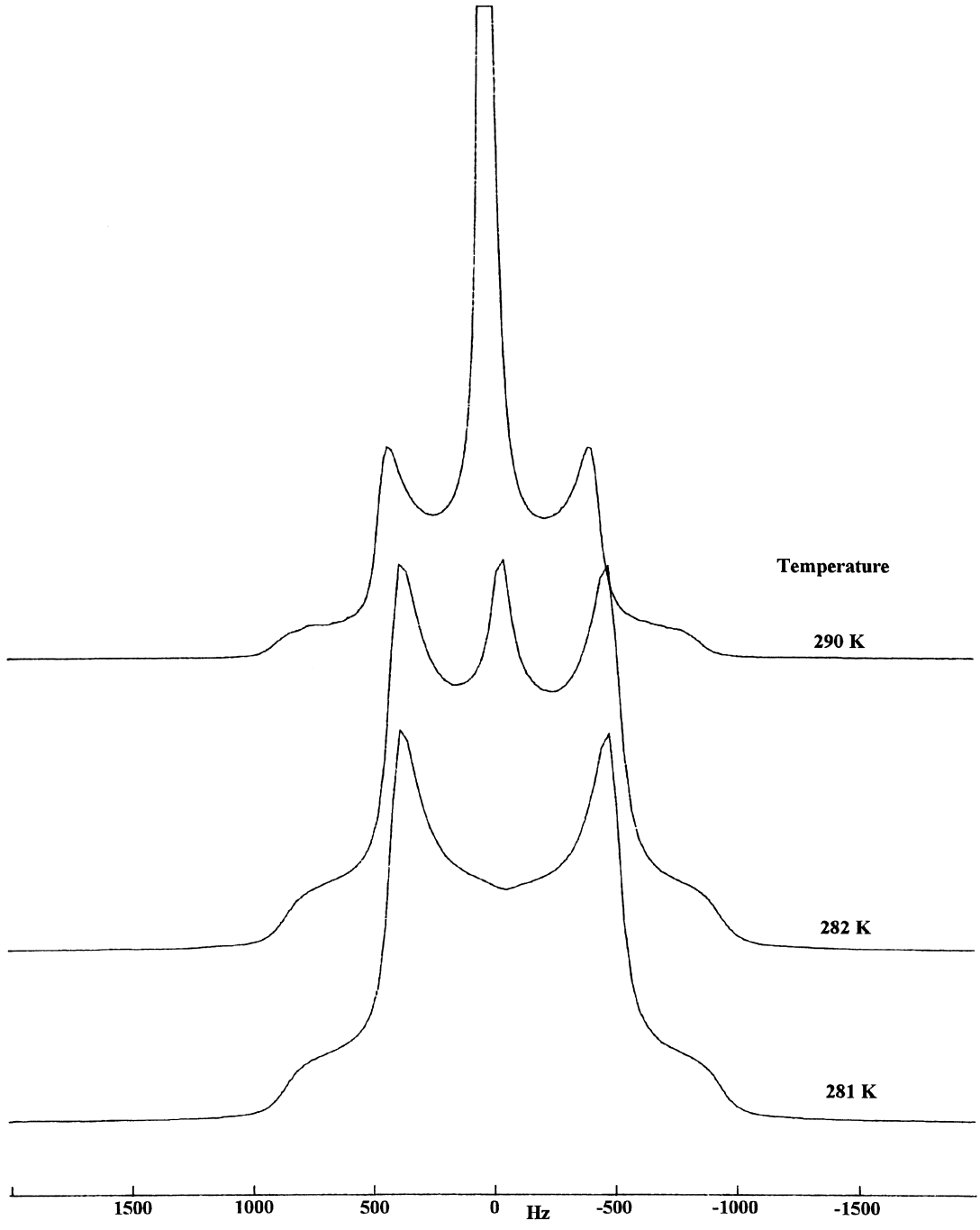

Fig. 9. The effect of temperature on the water deuterium spectrum for a frozen–thawed, packed, D_2O-saturated bed of potato starch granules.

time peaks to shift to shorter times. This is to be expected if the water proton transverse relaxation time is dominated by fast exchange of protons between water and starch hydroxyl groups. The long relaxation time peak at ca. 50 ms first begins to shift until its relative area is zero (at ca. 40% in Fig. 7). Then the peak at ca. 8 ms begins to shift as the water content is further reduced below 40%. Because the order for removal of water is first external bulk water, then water in the amorphous growth rings, then water in the semi-crystalline lamellae, these data are consistent with the previous compartmental assignments. Further support comes from electrical conductivity measurements.

3.6. Electrical conductivity of packed starch granule beds

If the 50 ms peak arises from bulk water outside the granules then the electrical conductivity of the packed bed made up with a dilute KCl solution should decrease in proportion to the relative area of the 50 ms peak as the degree of saturation is reduced. The relative electrical

conductivities plotted in Fig. 7 show that this is indeed the case.

3.7. Deuterium lineshape analysis

The deuterium nuclei in D_2O relax by intramolecular reorientational modulation of the electric quadrupole interaction. The deuterium spectral lineshape is therefore a useful probe of orientational ordering of water molecules. Reference has already been made to the discovery of a deuterium doublet with a splitting of ca. 1 kHz in the spectrum of D_2O in potato starch (Leckert, 1976; Yakubu et al., 1993). Representative spectra showing the temperature dependence of the deuterium wide-line spectrum as a frozen, D_2O-saturated, packed bed of native potato granules is progressively warmed from 200 K to room temperature are shown in Figs. 8 and 9. Below 273 K the deuterium powder pattern of ice dominates the spectrum with its large characteristic doublet splitting of ca. 140 kHz. At the lowest temperature of 200 K this ice pattern is all that can be observed, but as the temperature is raised above 210 K, a central peak of mobile unfrozen water with a width at half height of ca. 2 kHz appears and progressively increases in amplitude relative to the ice doublet. Just below freezing, at 270 K, this central line is sufficiently narrow to reveal the smaller doublet splitting of ca. 1 kHz. Above freezing (276.8 K for D_2O), the broad ice doublet vanishes leaving only the 1 kHz-split doublet. This is shown at a temperature of 281 K in Fig. 9. Curiously, Fig. 9 also shows that at temperatures above 281 K, a new peak appears in the centre of this doublet. At 290 K, the spectrum therefore consists of a central "isotropic" line together with the 1 kHz-split doublet. Similar changes with maize starch, except that there is no 1 kHz split narrow doublet, only a single central peak above freezing.

The observation that there is no 1 kHz split narrow doublet in maize, only in potato starch, strongly suggests that the narrow 1 kHz wide doublet is characteristic of partially oriented "channel" water located in the hexagonal-shaped central water channel of the B-type amylopectin crystal lattice. The small magnitude of the splitting can be explained in several ways. In the fast exchange regime the observed splitting, $\Delta\omega$, is the weighted average of the splittings associated with each site or sub-population. If the water fraction in site i is labelled P_i, we can write

$$\Delta\omega = \Sigma_i P_i(\Delta\omega)_i$$

The small splitting could therefore, in principle, be the result of fast exchange with another population of isotropically rotating water inside the channel and having zero splitting. The splitting, $(\Delta\omega)_i$, will also be reduced in each subpopulation, i, by reorientational motion. If the reorientational correlation time is τ_r, the quadrupolar splitting is given as

$$(\Delta\omega)_i = 0.75\chi S_i(\tau_r)_i \quad (1)$$

where χ is the quadrupole coupling constant (216 kHz for deuterium in D_2O). Unfortunately the magnitudes of both the fractions, P_i, the splittings, $(\Delta\omega)_i$, and the order parameters, S_i, are all unknown so it is not possible to proceed further in the analysis of the observed 1 kHz deuterium line splitting.

Besides the small splitting, $\Delta\omega$, it is also interesting to note that, on raising the temperature from just above freezing to 290 K, $\Delta\omega$ remains constant and a new central peak appears and progressively increases in amplitude. This shows that the population of isotropically reorienting water, giving rise to the central peak does not exchange with the channel water giving the deuterium splitting. In other words, if "channel water" gives rise to the deuterium splitting then it does not exchange with other water populations inside the granule on the NMR observational timescale of a few milliseconds. The appearance of the central peak must therefore be a result of motional narrowing of an isotropic population of water other than that giving rise to the deuterium splitting (provisionally assigned to channel water). A hint as to the nature of this water is the observation that the appearance of the central peak above 280 K coincides with the exchange averaging the two short relaxation time peaks in the proton transverse relaxation time in Figs. 2 and 3. This suggests that these two exchanging populations give rise to the central peak in the deuterium spectrum when they are sufficiently exchange narrowed. Since neither of these populations can be channel water, they must be assigned to other non-freezing water populations in the granule, such as water in the amorphous growth rings and water in the amorphous regions in the semi-crystalline lamellae. Presumably, the anisotropically reorienting "channel water" has such a short "solid-like" transverse proton relaxation time, of just a few tens of microseconds, that it cannot be observed with the CPMG sequence. This would be consistent with the results of the 2-dimensional "WISE" solid-state NMR experiment which suggests that the channel water in B-type starch is less mobile than the water in A-type starch (Kulik et al., 1994).

It appears, therefore, that the 1 kHz wide deuterium doublet is characteristic of water in B-type amylopectin crystals so that the spectrum of pea starch granules should be a weighted sum of the A- and B-type spectra and show a 1 kHz doublet and a central peak even at temperatures below 280 K when no central peak appears in the potato-B-type spectrum. Fig. 10 shows that this is indeed the case. Whether lineshape analysis can give the percentages of A and B type starch in the pea granule remains to be explored.

3.8. The starch proton free induction decay

Fig. 11 shows the temperature dependence of the distribution of proton transverse relaxation times obtained by multiexponential analysis of the Free Induction Decay (FID) for packed beds of potato starch saturated in D_2O. A similar result is obtained for maize. In this case the NMR signal originates from the non-exchanging CH

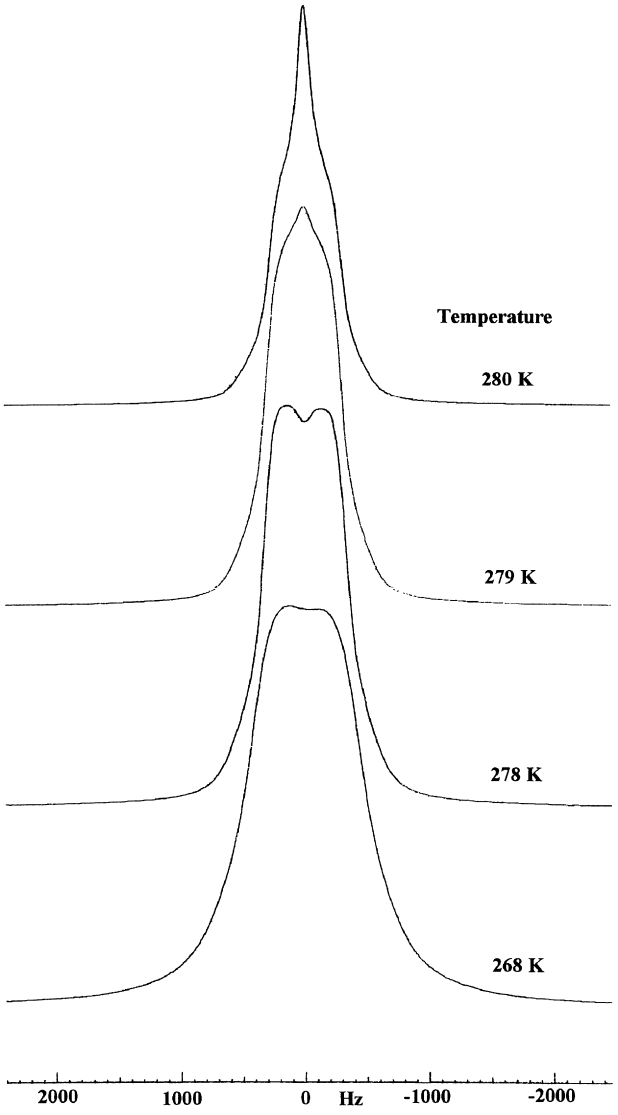

Fig. 10. The effect of temperature on the water deuterium spectra of packed, D_2O-saturated bed of pea starch granules.

protons on the amylose and amylopectin and from small amounts of residual HOD. The more rigid the polysaccharide chain, the shorter the associated CH proton relaxation time, so the relaxation time distribution reflects the spectrum of changing starch chain mobility within the granule. Two relaxation peaks are observed, at approximately 20 and 80 μs for both potato and maize but the fact that the much lower amplitude peak at 80 μs vanishes at temperatures below 273 K strongly suggests that it is simply residual HOD. The starch CH protons therefore merely contribute a single relaxation peak at ca. 20 μs in both potato and maize starch granules. This observation should have potential value in monitoring starch chain dynamics during starch processing.

3.9. NMR diffusometry—q-space microscopy

Although water diffusion is usually a hindrance to probing microstructure with relaxometry, in NMR diffusometry it is used as an indirect microstructural probe (Hills, 1998). This is possible because microstructural barriers restrict water self-diffusion and alter the water diffusion propagator. Sub-granular microstructure can therefore be probed using NMR measurements of the propagator. Details of the technique, sometimes called "q-space microscopy" can be found in a number of NMR texts (Callaghan, 1991; Hills, 1998).

Fig. 12 shows the dependence of the normalised amplitude of the stimulated echo, $S(q,\Delta)/S(q=0,\Delta)$ on the product $(2\pi q)^2\Delta$ in a pulsed gradient stimulated echo NMR diffusion experiment on a water-saturated packed potato granule bed. Here q is the wavevector $\gamma G\delta$ where γ is the proton gyromagnetic ratio, G the applied field gradient and δ the gradient pulse duration. Δ is the diffusion time defined by the time between the applied gradient pulses. For unrestricted diffusion of pure bulk water, the stimulated echo amplitude is given as

$$S(q,\Delta)/S(q=0,\Delta) = \exp[-(2\pi q)^2 D(\Delta - \delta/3)] \quad (2)$$

In this simple case a plot of $\ln S(q,\Delta)/S(q=0,\Delta)$ against $(2\pi q)^2(\Delta - \delta/3)$ gives a straight line whose slope is the water self diffusion coefficient, D. When microstructure restricts the water diffusion this simple relationship no longer applies and fitting the data with various models of restricted diffusion can give valuable microstructural information.

A key observation in the starch granule diffusion data is that, despite the curvature of the logarithmic plots, the echo amplitude, when $\delta \ll \Delta$, varies as the product $(2\pi q)^2\Delta$ so that variation of q^2 and Δ are equivalent. The common theoretical models consistent with these observations are those describing diffusion in sub-3-dimensional spaces. In fact the data for the water-saturated potato starch granules can be fitted satisfactorily if the water is confined to diffusion in a 2-dimensional space (see the continuous line in Fig. 12). Neither a one-dimensional space nor diffusion in a fractal space (corresponding to a stretched exponential in Fig. 12) can be fitted to the data. Removing the 50 ms relaxation peak by lowering the water content to 30% lowers the dimensionality of the diffusion to between 1 and 2. The diffusion data for packed beds of maize starch follow similar trends and can be fitted very well with the two dimensional model.

Sub-three dimensional water diffusion would be expected if the extragranular water exists as a thin interstitial film. In like manner, an amorphous growth ring has a two-dimensional (curvilinear) geometry, so, provided the water is restricted to the growth ring on the NMR measurement timescale of a few milliseconds, its diffusion characteristics will also be 2-dimensional. If "channel water" in the amylopectin crystals has a short transverse relaxation time of just a few microseconds then it will not, of course, be visible in the stimulated echo experiment.

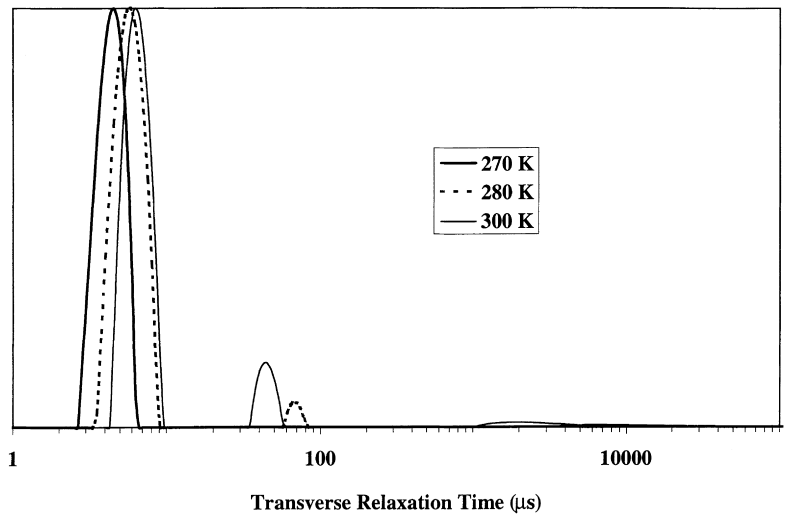

Fig. 11. The effect of temperature on the distribution of proton transverse relaxation times for a D_2O-saturated bed of potato starch granules.

4. Discussion and conclusion

In this paper we have used NMR relaxation, diffusion and multinuclear spectral data to probe the distribution and dynamic state of water in native, unprocessed starch granules. The major difficulty in this approach is the assignment of water proton relaxation time peaks to sub-granule water populations. Taken as a whole, the evidence favours the assignment of the longest water proton transverse relaxation peak at ca. 50 ms to extra-granular water. This is an unusually short relaxation time for bulk water, which normally has a relaxation time of about 2.5–3 s. We can only speculate that the close packing of the granules forces the external water into a very thin film, perhaps only a few hundred microns thick, so that the granule surface acts as an unusually effective relaxation sink. Another possibility is the leaching of amylose from the granules into the extra-granule water, which is known to occur slowly at room temperature

The shorter relaxation time peaks can therefore be assigned to water inside the starch granule. In B-type potato starch two intra-granular water populations can be distinguished. One population can be assigned to water in amorphous growth rings and another to water in the amorphous regions within the semi-crystalline lamellae. The coalescence of their proton relaxation time peaks and the gradual emergence of an unsplit central peak in the D_2O-deuterium spectrum above 280 K shows that these two water

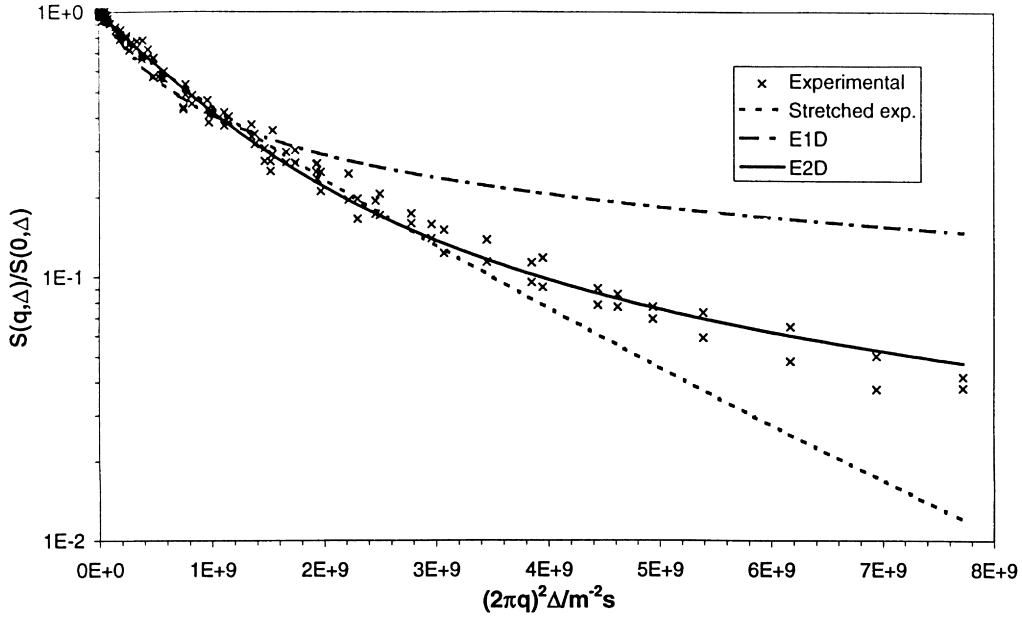

Fig. 12. The dependence of the normalised stimulated echo amplitude on $q^2\Delta$ for a water-saturated bed of potato starch granules at 298 K. The lines are theoretical best fits for the indicated functional forms.

populations are exchanging with each other. A simple exchange analysis gives a room-temperature exchange lifetime of roughly one millisecond for these two water populations and a diffusion distance of about 1 μm, corresponding, in order of magnitude, to the width of the growth rings. These two water populations also do not freeze at 270 K and they contain "orientationally disordered" water as shown by the absence of any associated deuterium spectral doublet.

In addition to these two water populations there is a third population of water in B-type potato starch, which has been assigned to "channel water". This is orientationally ordered water inside the hexagonal channels of B-type amylopectin crystal clusters within the blocklets. This gives rise to a characteristic deuterium doublet with a 1 kHz splitting and deuterium lineshape analysis shows that this is not exchanging with the other three water populations on the NMR observational timescale of a few milliseconds. The fact that the relaxation time distribution of pea starch resembles the sum of the potato and maize distributions supports its composite A and B type granule structure.

If these assignments are correct, then the areas in Fig. 3 at 270 K permit the ratio of water in the amorphous growth rings to non-channel water in the semi-crystalline lamellae to be determined for potato starch from the relative peak areas. These are about 3:2 for potato starch granules. Absolute values cannot be given because of the unknown amount of water in the B-type channels. Unfortunately, no independent information on water distribution in potato starch is available from other techniques. The only other data is the SANS study of the fraction of water in the crystalline lamellae of waxy maize starch (Donald et al., 1997), which gives a value of about 11%. Because the granule sizes of waxy maize and potato starch are so different, comparison of these figures is not particularly meaningful.

In A-type maize starch there is only one intragranular population of water that can be identified in the relaxation time distribution and deuterium spectrum. This is because the smaller granule size permits faster exchange averaging of the water between the amorphous growth rings and the semi-crystalline lamellae. Moreover the absence of "channel water" in the A-type amylopectin crystal structure means that there is no 1-kHz wide deuterium doublet in the deuterium spectrum of maize granules. The characteristic deuterium doublet splitting observed for water in B-type starch but not in A-type starch, provides a rapid and non-invasive method of distinguishing A, B and C-type starch. Indeed we have confirmed that pea starch is a composite of A and B type starch, though we have not yet made any attempt to use NMR to quantify the mixture composition.

It is interesting to compare our analysis with a recent water proton relaxation time analysis of wheat-starch suspensions (Le Botlan et al., 1998). These authors observed only two relaxation time peaks which they interpreted in terms of a two-site exchange model of "weakly bound" and "bound water". Unfortunately no account was taken of the different water populations associated with granule microstructure. Our results show the inadequacy of such a model. For example our observation of three relaxation time peaks in, for example, Fig. 2 at 277 K shows that a simple two-site analysis which ignores microstructure is wholly inappropriate.

In conclusion we have shown that NMR water proton relaxation and deuterium lineshape analysis can provide a simple means of measuring water distribution inside native starch granules and for distinguishing A, B and C type starches. In subsequent papers we will use these new NMR methods to study structure–function relationships in starch granules as they are subjected to different processing and storage conditions.

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