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MICROSTRUCTURAL CHARACTERIZATION OF STARCH SYSTEMS BY NMR RELAXATION AND Q-SPACE MICROSCOPY

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Nuclear magnetic resonance (NMR) relaxation and q-space diffusion measurements have been used to probe the microscopic water distribution in a variety of starch-based systems, including packed beds of native starch granules with varying water contents, starch gels, and freeze-dried starch gels. The q-space data for the granular beds is compared with a variety of theoretical models and conforms best to unbounded diffusion in a lower dimensional space. In contrast to some earlier reports, the data for the gelatinized samples are not anomalous and conform to simple unrestricted diffusion in a three-dimensional space. The paper concludes with a novel method for probing pore size distribution in freeze-dried starch gels by infusion of acetone. **© 1998 Elsevier Science Inc.**

Keywords: **Nuclear magnetic resonance relaxation; Water diffusion; Q-Space microscopy; Starch; Freeze-drying; Gelatinization.**

INTRODUCTION

The combination of nuclear magnetic resonance (NMR) relaxometry and q-space microscopy has great potential for noninvasively probing microstructure in heterogeneous materials. To date, these techniques have been applied mostly to simple model systems such as beds of polystyrene or glass microspheres or to porous rocks of interest to the oil industry.[1–3] There have been relatively few attempts to apply these methods to heterogeneous biopolymer systems or food materials. In this paper we therefore explore to what extent microstructure and the microscopic water distribution in various heterogeneous starch systems can be ascertained by NMR relaxometry and q-space diffusometry.

MATERIALS AND METHODS

The pulsed-gradient stimulated-echo (PGSTE) diffusion measurements were undertaken on a Resonance Instruments Maran spectrometer operating at 23.5 MHz and thermostatted at 20°C. Diffusion times (Δ) were varied up to 500 ms, and the wavevector (q) was varied up to 3.5×10^4 m^{-1}. The water proton transverse relaxation measurements were undertaken on a Bruker MSL100 spectrometer operating at 100 MHz with the Carr–Purcell–Meiboom–Gill (CPMG) sequence with a 90–180° pulse spacing of 200 μs and a 90° pulse width of 2 μs. Acetone relaxation measurements were made at 300 MHz on a Bruker MSL300 spectrometer.

Packed beds of native potato starch granules were prepared by addition of the calculated amount of water to the granules. Because starch granules swell slightly in water, these beds were equilibrated at 5°C for up to 72 h, and the NMR behaviour was checked periodically. Within experimental error, the data were independent of storage time. Triplicate repeat samples were used at water contents of 30%, 35%, and 40% up to saturation (45%). Here water contents (W%) are defined as the weight of water per total weight of starch and water. Gelatinization was performed by heating the starch paste of known water content (listed in Table 1) in a special sealed polytetrafluor-ethylene-lined metal screw-top cylinder in an oil bath at 130°C for periods of up to 3 h. The experimental details of the freeze-drying procedure were previously described.[4]

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Table 1. Dependence of the water self-diffusion coefficient on water content (W%) in fully gelatinized potato starch gels

Water content (%)	Diffusion coefficient ($m^2\ s^{-1} \times 10^{-9}$)
85	1.66
85	1.63
80	1.51
80	1.49
75	1.37
75	1.44
70	1.21
70	1.26
65	1.12
65	1.09

RESULTS

Water Proton Transverse and Longitudinal Relaxation

Figure 1 shows the dependence of the distribution of water proton transverse relaxation times on water content for a randomly packed bed of native potato starch granules measured at 100 MHz with the CPMG pulse sequence. Consider first the water-saturated bed, which corresponds to a water content of 45%. The two peaks in the top distribution in Fig. 1 correspond, very approximately, to water inside and outside the granules. The fact that the long relaxation time peak is centred at ~30 ms and not at the bulk water value (~2.5 s) indicates that the exchange is in the intermediate, rather than the slow, regimen. Reducing the water content to 35–40% removes most of the bulk water (middle, Fig. 1). At 30%, only water inside the granules is observed, and the relaxation is single exponential (bottom, Fig. 1) and centred at ~2 ms. Further removal of water causes shrinkage of the granules and this shifts the relaxation time to shorter values. Unlike the transverse relaxation, the longitudinal relaxation is single exponential at all water contents. This is consistent with the smaller difference in intrinsic T_1 values and a longer observational time scale.

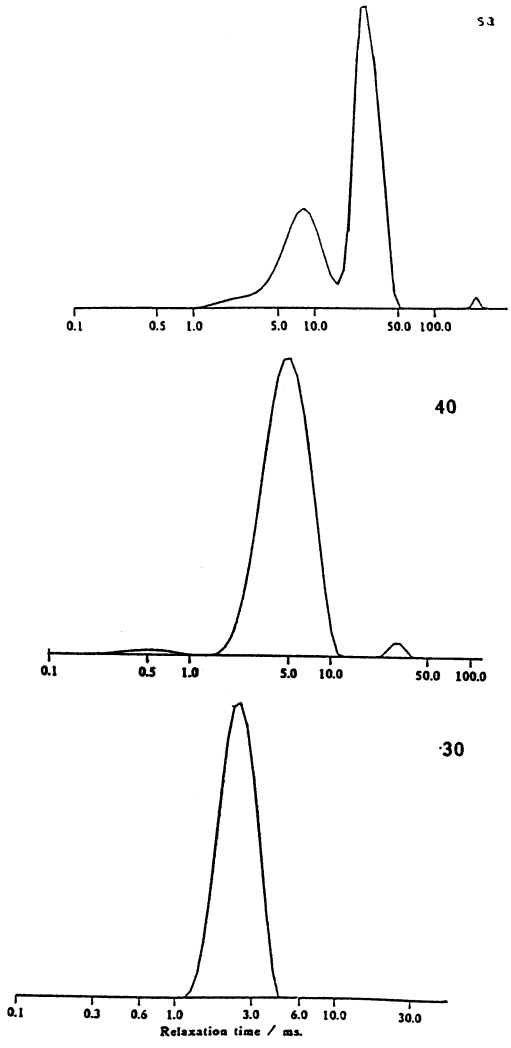

Fig. 1. The distribution of water proton transverse relaxation times for packed beds of native potato starch granules at the indicated water contents. (Top) the water-saturated bed corresponding to a water content of 45%; (middle) a water content of 40%; (bottom) a water content of 30%.

PFGSTE Diffusion Measurements

Before considering detailed results it is important to estimate the diffusion distance being probed in the stimulated echo experiment. Assuming the water self-diffusion coefficient within a water-saturated starch granule is similar to that in a concentrated starch gel ($\sim 10^{-9}$ m^2 s^{-1}; see Table 1) and a maximum diffusion time, Δ, of 200 ms, the root mean square displacement in three-dimensional diffusion, $(6D\Delta)^{1/2}$, is ~33 μm.

Because the starch granules vary in radius between 5 and 20 μm, this diffusion distance should be sufficient to observed exchange between water inside and outside the granules and the effects of restricted diffusion in packed starch granule beds. At first sight the results appear to support this prediction. Figure 2 (top) shows the dependence of the attenuation of the stimulated echo, E(q, Δ), on the product $q^2\Delta$ for various wavevectors, q, and diffusion times, Δ, at a water content of 45%, and Fig. 2 (bottom) shows the same data for a 30% water content. Two points are particularly noteworthy about these data. First is the observation that, within experimental error, variations in q^2 or Δ are equivalent. This is found to be true at all water contents between 30% and saturation. The second feature is the curvature in the data, which is also observed at all water contents and prevents a simple interpretation in terms of an apparent water diffusivity.

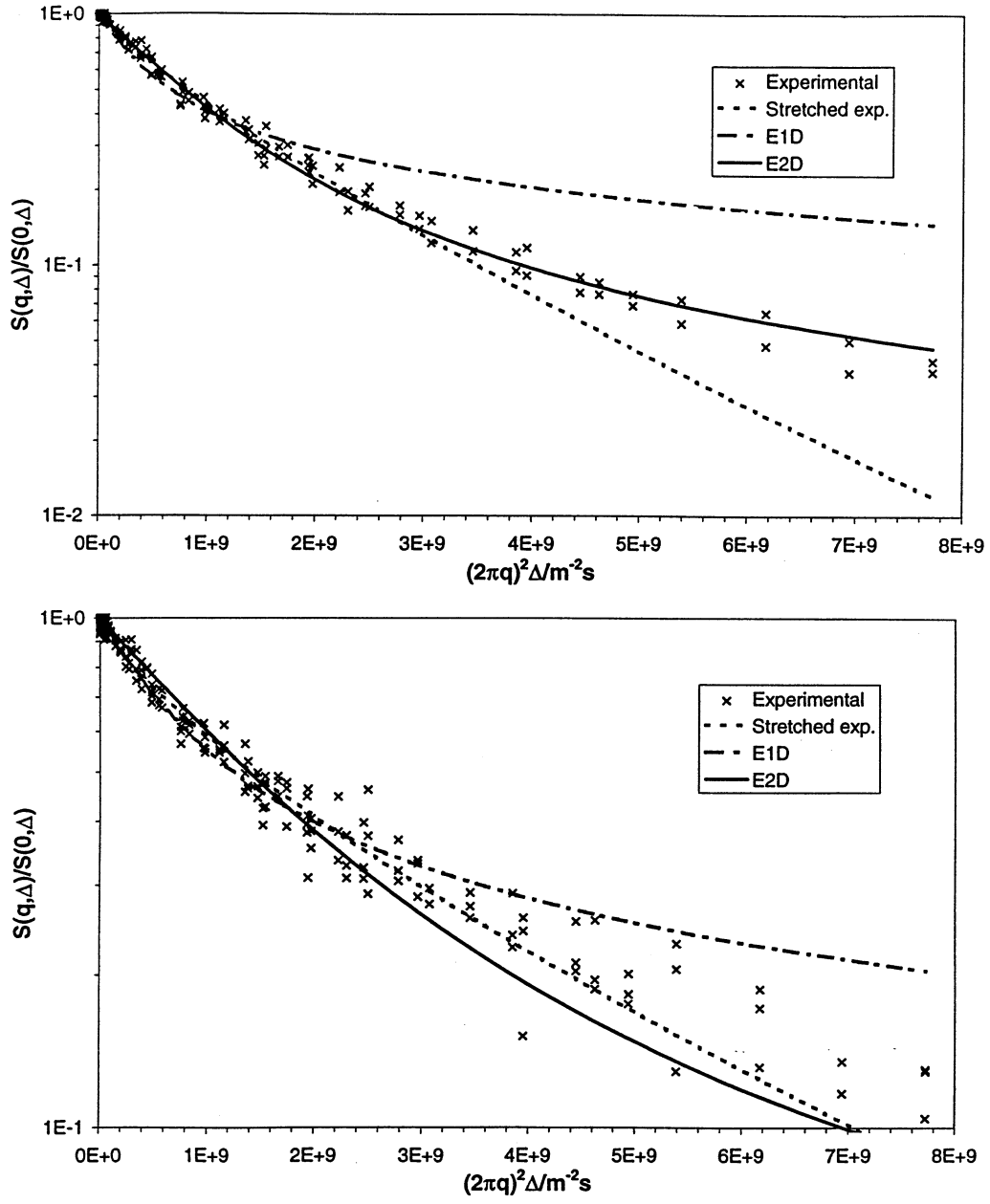

Fig. 2. The dependence of echo attenuation on $q^2\Delta$ for packed beds of native potato starch granules. (Top) The water-saturated bed. The lines show the best fits of the one- and two-dimensional diffusion models and for a stretched exponential. (Bottom) A water content of 30%. The results for three independent sample replicates are superimposed in these plots.

THEORETICAL INTERPRETATION

The observation that E(q, Δ) is a function of $q^2\Delta$ so that variation in q^2 or Δ is equivalent is particularly noteworthy and can be used to eliminate a number of theoretical models of the system microstructure based on exchange and restricted diffusion. Each model is considered in turn.

The Two-Phase Model[5]

A water content of 45% corresponds to a water-saturated bed, so it might be supposed that the two-phase exchange model of Karger[5] would provide a reasonable description of the diffusive exchange of water between the inside of the starch granules and the bulk water outside. These exchange equations characterize the water inside the granule with transverse and longitudinal relax-

ation times (T_{2a}, T_{1a}) and diffusion coefficient (D_a) and bulk water outside the granules with corresponding values (T_{2b}, T_{1b} and D_b). Diffusion of water between the inside and outside compartments is modelled as an instantaneous jump process characterized by exchange lifetimes, τ_a and τ_b. However, the exchange equations show that variations in q^2 and Δ are not equivalent operations, so the echo attenuation cannot depend universally on the product $q^2\Delta$. Moreover, a strong biexponential dependence on $q^2\Delta$ is predicted for all ranges of parameters compatible with bulk water outside and water inside the granule, considered as a concentrated starch gel. This is certainly not observed in Fig. 2, so this simple two-phase exchange model cannot account for the data even in the water-saturated state. Possible reasons for this failure may be the known polydispersity in the radius (and shape) of native potato starch granules, requiring a continuous distribution of exchange lifetime. As we shall demonstrate, microstructure within the starch granules also needs to be taken into account.

The Connected Pore Glass Model[6]

At a slightly lower water content of ~40%, a pendular state might be expected, whereby water-saturated starch granules would be connected by small water "bridges" at the point of contact between granules. Accordingly, the data were compared with the pore-glass model of Callaghan et al.[6] Applied to the starch system, this models assumes diffusive equilibration within spherical starch granules and unbounded diffusion between granules via water bridges. However, there are a number of distinctive features of the pore-glass model that are not seen in the data. First, variation in Δ at fixed q gives straight line plots with variable intercept when plotted against $q^2\Delta$ (see Fig. 3, top). Moreover, variation of q at fixed Δ is seen to give a very different plot with a common intercept (see Fig. 3, bottom). Neither situation is remotely observed in the experimental data, so, despite its elegance, we are also forced to eliminate this model as a viable description of the starch system.

Restricted Diffusion in a Sphere[7]

At a lower water content of 30%, the distribution of water proton transverse relaxation times (see Fig. 1) shows that all water outside the granules has been replaced by air, so if an isolated, water-saturated starch granule is considered as an isolated, structureless gel sphere, one might expect the data to conform to restricted diffusion inside a sphere. The corresponding theoretical plots for this model for variation of q^2 at fixed Δ and of Δ at fixed q^2 are shown in the top and bottom parts of Fig. 4, respectively. The dramatic differences between these two plots shows that this model also fails to explain the data. It should be noted that at sufficiently large wavevectors and diffusion times, this model predicts diffraction-like behaviour.[7] However, the range of wave vectors and diffusion times accessible in our experiments limits us to the top left corner of this diagram, well outside the range of the first diffraction peak. For this reason attempts are in progress to extend the range with fringe field diffusion measurements.

One- and Two-Dimensional Diffusion[8] *and Fractal Geometries*[9]

Because neither of the above models succeeds in describing the q-space data, we are led to consider the internal structure within the starch granules and compare the data with lower-dimensional diffusion models and fractal diffusion. Figure 2 (top) shows that a two-dimensional model provides a reasonable fit to the 45% water-saturated system, whereas Fig. 2 (bottom) shows that the data at the lowest water content (30%) falls between the predictions of one- and two-dimensional diffusions. Clearly, the apparent dimensionality of the diffusion increases with increasing water content, which is consistent with increasing surface coverage and water connectivity throughout the starch matrix. The echo attenuation in these lower-dimensional models is a function only of the product, $q^2\Delta$, so that variations of q^2 and Δ are equivalent, consistent with observation. Another well-known function of the product $q^2\Delta$ is the stretched exponential, and Fig. 2 shows that this also can be used to fit the data. However, a stretched exponential is, in this case, merely a convenient fitting function because it does not emerge from any specific model of the system microstructure. It is tempting to compare the data with diffusion in a fractal geometry. However, fractal geometries predict an echo attenuation scaling as exp $[-q^2D\Delta^{\alpha}]$, where α is related to the fractal dimension, so that q^2 and Δ variations are no longer equivalent. Fractal models are therefore also incompatible with the data.

THE EFFECT OF GELATION

Ohtsuka et al.[10] reported extensive stimulated echo pulsed field gradient measurements of diffusion in potato starch gels as a function of water content and storage time. The gels were prepared by heating starch pastes at 98.7 ± 0.4°C followed by storage at 5°C for various periods up to 4 days. LogE(q, Δ) was observed to decay linearly with $q^2\Delta$, permitting an apparent diffusion coefficient to be extracted. Surprisingly, the diffusion coefficient was found to depend on both Δ and storage time, indicative of microstructure and retrogradation within the starch gels. These authors fitted the data with the von-Meerwall-Ferguson model[11] and deduced that there were pores on the order of 10–20 μm within the gel. These pores are at least an order of magnitude larger than

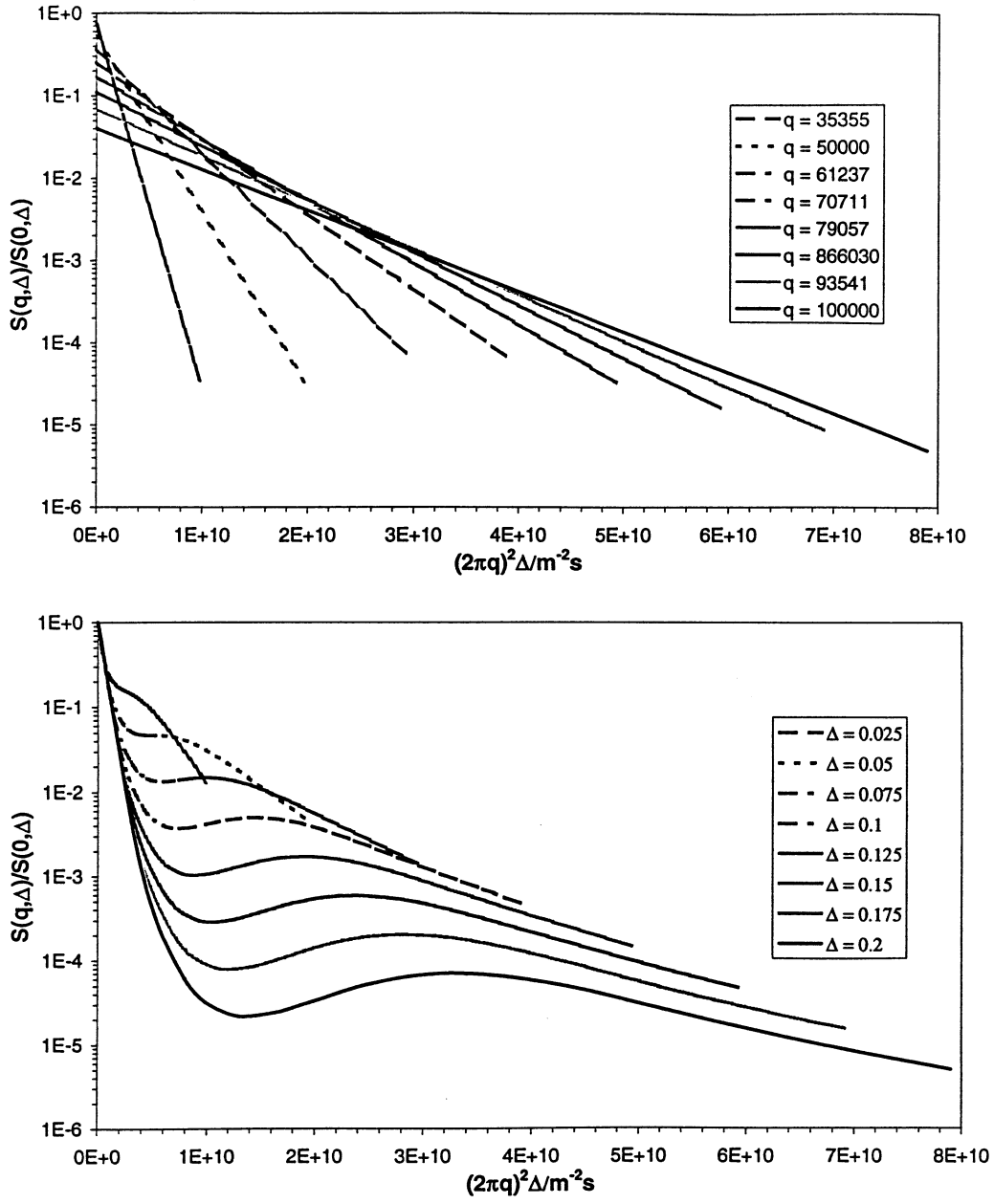

Fig. 3. Dependence of echo attenuation on $q^2\Delta$ predicted by the connected pore model.[6] (Top) Variation in Δ at the indicated wavevectors. Note the wave vector-dependent intercept. (Bottom) Variation in q^2 at the indicated diffusion times, Δ.

any observed with electron microscopy and stimulated us to repeat their experiments. We also found that log E(q, Δ) decays linearly with $q^2\Delta$, but our diffusion coefficient showed no anomalous dependence on Δ, q, or storage time. Table 1 shows that the decrease in the apparent water diffusion coefficient with decreasing water content agrees with the expected phase I behaviour reported by Kimmich et al.[12] for bovine serum albumin and gelatin gels. Our observations therefore suggest that the preparation procedure used by Ohtsuka et al.[10] failed to fully gelatinize the starch granules, so that the observed Δ dependence resulted from residual granules or "granule ghosts" embedded within a gel matrix. Indeed, attempts to repeat their preparation procedures failed to produce visibly homogeneous gels, and we had to resort to extended heating for 3 h in a specially sealed, polytetrafluor-ethylene-lined steel cylinder in an oil bath at temperatures of 130°C for complete sample gelatinization.

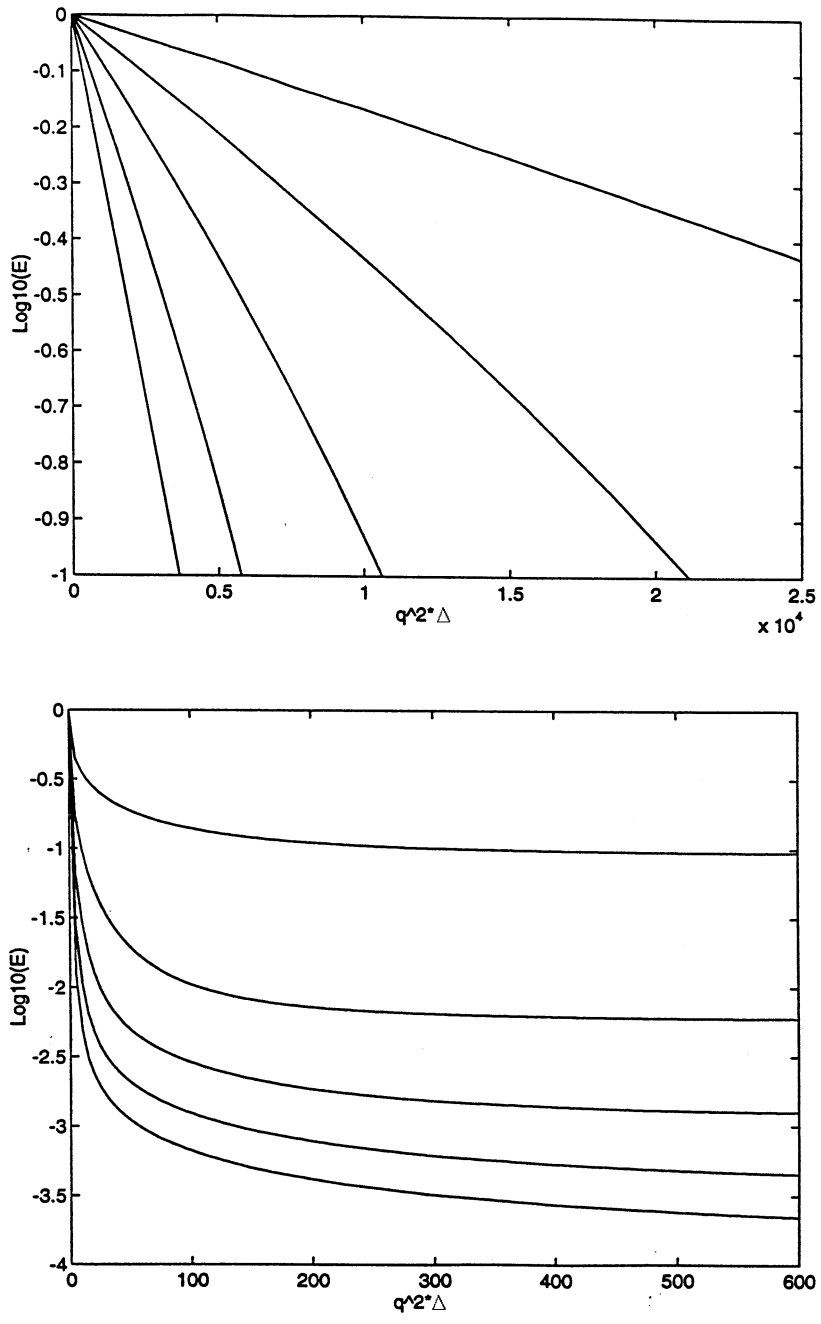

Fig. 4. Dependence of echo attenuation on $q^2\Delta$ ($cm^2\ s^{-1}$) predicted by the isolated spherical pore model.[7] (Top) Variation in q^2 at various diffusion times, Δ. From top to bottom, the curves correspond to $D\Delta/a^2$ values of 5, 2, 1, 0.5, and 0.2, respectively. (Bottom) Variation in Δ at various wave vectors, q. From top to bottom, the curves correspond to q values of 500, 1000, 1500, 2000, and 2500 cm^{-1}.

THE EFFECT OF FREEZING AND FREEZE-DRYING

The pores in a freeze-dried starch gel result from sublimation of ice crystals in the frozen gel. A determination of the pore size distribution in the freeze-dried gel is therefore also a measurement of the ice crystal size distribution in the frozen gel. Both are important for optimizing freezing and freeze-drying processes and for ensuring satisfactory product quality. The methodology used to determine pore size distribution and pore con-

nectivity in porous rocks from the distribution of water proton relaxation times is well known[3] and is widely used in the oil industry. Unfortunately, the same method cannot be applied to freeze-dried foods because addition of water collapses the food matrix. We have therefore explored the potential of infusing acetone into the matrix and exploiting the distribution of acetone transverse proton relaxation times instead of that for water. Our relaxation measurements are encouraging because they show a significant acetone-starch surface relaxation strength and an almost linear relationship between the acetone proton transverse relaxation rate and reciprocal pore size. This was done by measuring the average pore size at various radial distances within a freeze-dried cylinder of potato starch gel with scanning electron microscopy and comparing these with the acetone transverse relaxation times at the same radial positions. The data for starch gels have been reported in detail,[4] and a similar approach may permit the determination of ice crystal size distributions in frozen foods. One obvious complication in the more widespread application of the method is that acetone will dissolve lipids in fat-containing foods. In such cases it may be possible to circumvent this difficulty with an initial lipid extraction step, but this remains to be investigated.

CONCLUSION

It is at first sight very surprising that our q-space diffusion data are not described by simple models of the water distribution in the starch granule beds. The water-saturated beds undoubtedly contain at least two water compartments, yet the data do not conform with the standard two-phase diffusive exchange model.[5] It is conceivable that polydispersity in the starch granule size is obscuring the sharp biexponential predicted by the exchange model. However, the exchange model also predicts nonequivalence between variation of q^2 and Δ, which is not observed. At a lower water content, when the relaxation data confirm that water only resides within starch granules, the q-space data clearly do not comply with the "restricted diffusion in a sphere" model.[7] Moreover, at water contents between these cases, when it is expected that water bridges will connect the water-saturated starch granules, the data do not comply with the "connected-pore" model.[6] Instead, we are forced to concede that a lower, one- or two-dimensional diffusion model, whose dimensionality increases with water content, provides the most reasonable theoretical model for the randomly packed beds. Evidently the microstructure within native potato starch granules is the factor determining water diffusivity, and this internal structure is forcing the water to diffuse over surfaces and along starch gel chains in a process reminiscent of one- or two-dimensional diffusion. The shorter diffusion distance predicted by lower-dimensional diffusion compared to three-dimensional diffusion in the same diffusion time might also explain why exchange and restricted diffusion effects are not apparent in the data. If the water self-diffusion coefficient within a starch granule is actually much lower than 10^{-9} m^2 s^{-1}, say 10^{-10} m^2 s^{-1}, then the root mean square diffusion distance in one-dimensional diffusion, $(2D\Delta)^{1/2}$, is only 6–7 μm instead of the 33 μm predicted previously. This is less than the mean granule size and confines measurements to within a granule. It is gratifying that the destruction of internal granule structure by gelatinization restores the diffusion to a normal three-dimensional, unbounded geometry, and we conclude that previous observations[10] of restricted diffusion within starch gels were a consequence of incomplete gelatinization. It would be of interest to extend our measurements to higher wave vectors with fringe-field diffusion techniques,[12] and these experiments are currently underway.

It appears that acetone relaxometry may be a powerful new tool for probing both ice crystal-size distributions in frozen biopolymer (and food) systems and pore-size distributions in freeze-dried biopolymers systems. This aspect of the work has been more fully reported elsewhere[4] and should be of particular value in relating ice crystal size distribution to microstructure, composition, and freezing rate, especially if it is implemented in a spatially resolved form using transverse acetone relaxation time maps measured by CPMG-weighted magnetic resonance imaging.

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