DIFFUSION OF WATER IN THE ENDOSPERM TISSUE OF WHEAT GRAINS AS STUDIED BY PULSED FIELD GRADIENT NUCLEAR MAGNETIC RESONANCE

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ABSTRACT Pulsed field gradient nuclear magnetic resonance has been used to measure water self-diffusion coefficients in the endosperm tissue of wheat grains as a function of the tissue water content. A model that confines the water molecules to a randomly oriented array of capillaries with both transverse dimensions < 100 nm has been used to fit the data and give a unique diffusion coefficient at each water content. The diffusion rates vary from 1.8×10^{-10} m^2s^{-1} at the lowest to $1.2 \times 10^{-9} m^2s^{-1}$ at the highest moisture content. This variation can be explained in terms of an increase in water film thickness from ~ 0.5 to ~ 2.5 nm over the moisture range investigated (200–360 mg g^{-1}).

INTRODUCTION

The behavior of water in the tissue of wheat grains is of considerable practical importance. For example, the state of water in grains has a significant influence on their storage properties, whereas the uptake of water and its subsequent movement in the kernel are of concern in germination (Shellenberger, 1971).

The equilibrium hydration of wheat kernels has been thermodynamically characterized in studies of water sorption isotherms (Babbitt, 1945). Wheat has a porous structure and moisture is adsorbed onto the matrix of polymer chains that comprise the surface of the capillaries, the thickness of the water film being a function of the water content (Bushuk and Winkler, 1957).

Nuclear magnetic resonance (NMR) has long been used to investigate water in disperse systems (Clifford, 1975). Measurement of proton spin-lattice and spin-spin relaxation times have provided valuable information on the dynamic properties of water in these systems, whereas the pulsed field gradient technique has been employed to determine the diffusion coefficients of cellular water (Tanner and Stejskal, 1968) and water trapped in thin films in some clays (Boss and Stejskal, 1968). It has been recently shown that accurate diffusion coefficients can be measured using samples containing small quantities of slowly diffusing material by using the data accumulation and reduction features of a commercial pulsed-fourier transform spectrometer (Callaghan et al., 1979). In the present paper we report on measurements of the self-diffusion coefficients of water in the endosperm tissue of wheat grains as a function of the water content (film thickness).

THEORY

The pulsed field gradient technique has been described elsewhere (Stejskal and Tanner, 1965; Stejskal, 1965). The pulse sequence used is shown in Fig. 1, and a simple semiclassical explanation in the case of short gradient pulses may be given as follows.

On application along the sample z axis of the first gradient pulse of magnitude $G(T\text{m}^{-1})$ and duration $\delta(s)$, a spin with magnetogyric ratio γ, displaced by a distance z, will dephase by an angle $\gamma\delta Gz$ compared with spins at the origin. If a second and identical pulse is applied after the 180° inverting pulse, an equal rephasing will occur so as to enable an unattenuated echo to form. If, however, the spin has moved a distance Δz in the time between the two gradient pulses, its rephasing will be incomplete and there will be a permanent angular displacement given by:

$$\Delta\Theta = \gamma\delta G\Delta z. \tag{1}$$

For unrestricted diffusion in the duration Λ between the gradient pulses, the probability distribution for such a system is given by:

$$P(\Delta z) = \sqrt{\pi}\sigma \exp\left(-\Delta z^2/\sigma^2\right), \tag{2A}$$

where

$$\sigma^2 = 4D\Lambda, \tag{2B}$$

and the mean square distance traveled along the z axis is

$$\overline{z^2} = 2D\Lambda. \tag{2C}$$

The net effect of diffusion over the entire sample may be obtained by summing the

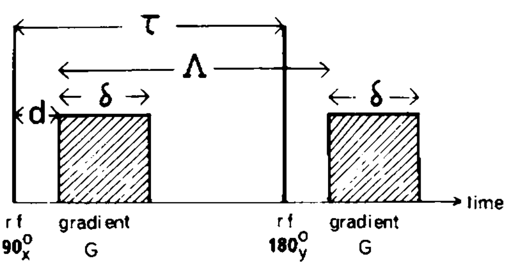

FIGURE 1

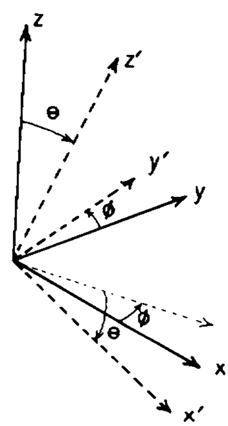

FIGURE 2

FIGURE 1 rf and field gradient pulse sequence showing the experimental times, τ, Λ, and δ.

FIGURE 2 Axis system showing the polar and azimuthal angles Θ and ϕ. z is the laboratory gradient axis and z' the symmetry axis of the element to which the diffusing molecule is confined.

magnetization components along the rf field direction, giving the normalized echo amplitude, R.

$$R = \frac{\int_{-\infty}^{\infty} \cos(\Delta\Theta) \exp(-\Delta\Theta^2/\gamma^2\delta^2G^2\sigma^2) d(\Delta\Theta)}{\int_{-\infty}^{\infty} \exp(-\Delta\Theta^2/\gamma^2\delta^2G^2\sigma^2) d(\Delta\Theta)}$$
$$= \exp(-\gamma^2\delta^2G^2\sigma^2/4).$$

Making use of Eq. 2B this may be written,

$$R = \exp(-\gamma^2\delta^2G^2D\Delta).$$

An exact expression where δ is finite is given by Stejskal and Tanner, 1965.

$$R = \exp(-\gamma^2\delta^2G^2D[\Delta - \delta/3]), \quad (3)$$

where $(\Delta - \delta/3)$ is therefore regarded as the effective diffusion time for the experiment. The experimental mean square distance traveled thus becomes:

$$\overline{z^2} = 2D(\Delta - \delta/3). \quad (4)$$

In practice $\overline{z^2}$ is required to be $> (100 \text{ nm})^2$ for any significant attenuation to be observed.

For diffusion measurements made in bulk solution displacements of order 100 nm or greater are unrestricted, and diffusion may be regarded as taking place in three dimensions. For water in some biological systems, however, this may not be the case. If, for example, the water is associated with a matrix of long polymer chains and is only a few layers thick, then it is quite possible that only one dimension (the long axis of the chain) will extend for > 100 nm and the field gradient experiment will observe one dimensional diffusion. For the purposes of a theoretical model the water may be thought of as being confined to thin capillaries. Another possibility is that the water is present as a thin film in which two dimensions will extend for the required distance and the field gradient experiment will observe two dimensional diffusion. A further consideration is that in systems such as the wheat endosperm tissue these capillaries or thin films are numerous and randomly oriented over the sample. It is therefore necessary to extend the theory to take into account these factors. A randomly oriented array of elements each with an axis of cylindrical symmetry, in which the longitudinal and transverse diffusion coefficients differ will be considered.

The axis system used is shown in Fig. 2, where z is the laboratory field gradient axis and z' is the longitudinal axis of the element in which the axial and transverse diffusion coefficients are $D_\parallel$ and $D_\perp$, respectively. Capillary behavior in which only one dimension extends for distances > 100 nm would be described by setting $D_\parallel = D$ and $D_\perp = 0$. Thin film behavior where two dimensions were extended would be characterized by $D_\parallel = 0$ and $D_\perp = D$.

Consider a displacement (x', y', z') in the element coordinate system. Only the mean square displacement along z, the laboratory field gradient axis, can influence the NMR experiment. For all azimuthal orientations it can be shown that

$$\overline{z^2} = \overline{z'^2}\cos^2\Theta + \overline{x'^2}\sin^2\Theta,$$

and hence from Eq. 4:

$$\overline{z^2} = 2D_{\parallel} (\Lambda - \delta/3) \cos^2\Theta + 2D_{\perp} (\Delta - \delta/3) \sin^2 \Theta. \tag{5}$$

The resultant echo attenuation, R, is obtained by averaging element orientations over all solid angles.

$$R = \frac{\int_0^{\pi} \exp(-k[D_{\parallel} \cos^2 \Theta + D_{\perp} \sin^2 \Theta] \sin \Theta) d\Theta}{\int_0^{\pi} \sin \Theta d\Theta}$$

$$= \exp(-kD_{\perp}) \int_0^1 \exp(-k [D_{\parallel} - D_{\perp}] x^2) dx \tag{6}$$

where $$k = \gamma^2\delta^2G^2(\Lambda - \delta/3).$$

For diffusion in a randomly oriented array of capillaries, the echo attenuation, R, given by Eq. 6 can therefore be expressed as:

$$R_{1D} = \int_0^1 \exp(-kDx^2) dx \tag{7}$$

In a thin film it may be written,

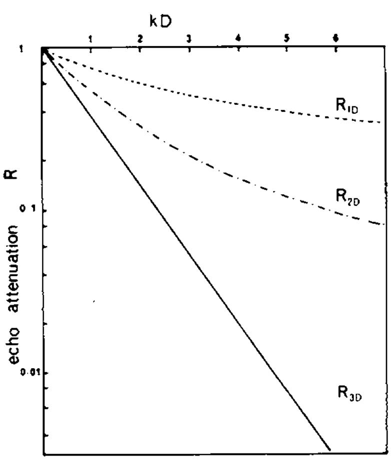

FIGURE 3

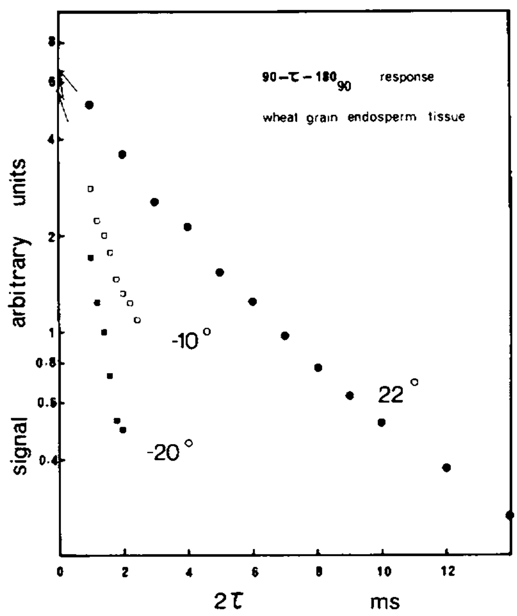

FIGURE 4

FIGURE 3 Theoretical echo attenuations for one (1D), two (2D), and three dimensional (3D) diffusion in a randomly oriented array of elements.
FIGURE 4 Spin echo response for water in wheat grain endosperm equilibrated to 98% RH. The temperatures shown are in degrees Celsius. The shortest echo interval available is 1 ms and data are extrapolated to $2\tau = 0$.

$$R_{2D} = \exp(-kD) \int_0^1 \exp(kDx^2)\, dx, \tag{8}$$

and for unrestricted three dimensional diffusion it becomes the familiar Eq. 3. These attenuations are shown in Fig. 3 as a function of kD.

As would be expected from physical considerations, it can be shown that in the limit $kD \ll 1$, the one and two dimensional attenuations are singly exponential and are equal to exp $(-kD/3)$ and exp $(-2kD/3)$, respectively.

EXPERIMENTAL

Endosperm tissue was obtained from wheat grains (c.v. Aotea) by dissection. Samples were equilibrated at the required relative humidity to a variety of moisture contents. Equilibration was carried out at 22°C for 6 wk (Pixton and Warburton, 1968). Moisture contents were determined gravimetrically using a two stage drying process (American Association of Cereal Chemists, 1969).

In each of the NMR experiments the endosperm tissue of a single grain (approximate weight, 0.02 g) was used. All diffusion measurements were carried out at 22°C and at 60 MHz, the proton resonance frequency. The pulsed field gradient device has been described elsewhere (Callaghan et al., 1979). It employs a 0.15-T m^{-1} A^{-1} Helmholtz pair with a uniformity better than 1% over the sample space. Current pulses of duration >0.5 ms and of up to 10 A magnitude were employed, the system having an accuracy of ~1% over the entire operating range. Typically between 100 and 400 accumulations were used in the experiments reported here.

RESULTS AND DISCUSSION

Fig. 4 shows a logarithmic plot of the proton spin echo signal as a function of 2 τ obtained at 22°C for a grain endosperm equilibrated in a 98% RH atmosphere. The data was

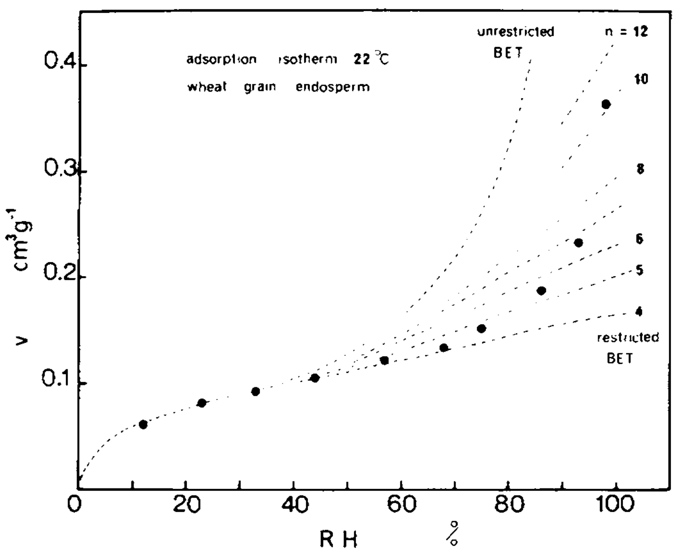

FIGURE 5 Adsorption isotherm at 22°C for wheat grain endosperm tissue. The theoretical curves have been calculated using the generalized BET equation where one of the parameters, n, is the number of multilayers to which adsorption is restricted. The BET parameters Vm and C are found to be 77 cm^3 g^{-1} and 26, respectively, in agreement with Bushuk and Winkler, 1957.

accumulated using a 90 − τ − $^{180}\!/_{90}$ sequence. Comparison with experiments on dry tissue shows that the signal arises entirely from moisture, the protein and starch proton signals having sufficiently short T_2's to be entirely attenuated for $2\tau > 1$ ms. For $2\tau > 30$ ms, where the water signal is significantly attenuated, a weak intensity slowly relaxing component is observed. We attribute this signal to traces of grain lipid whose polymethylene chain proteins undergo rapid rotational reorientation.

Considerable evidence suggests that the water giving rise to the NMR signal is located in thin films and capillaries of molecular dimensions. The intercepts at $\tau = 0$ of the spin echo plots for the 98% RH sample at −10 and −20°C (Fig. 4) correspond to 85 ± 7% and 98 ± 7% of the 22°C intercept, respectively, indicating that the greater part of the water does not freeze. This behavior was also shown for samples equilibrated at humidities below 98%. There is evidence that a depression of freezing point by 20°C or more indicates that water is in capillaries or thin films with a thickness of ~2 nm (Bakaev et al., 1959).

Additional evidence is provided by adsorption studies in which the degree of adsorption can be modeled by the molecular kinetic approach of Brunauer et al., 1938. In this model (BET) a distribution of molecular layer thickness exists over a given liquid film. The generalized BET theory allows for restriction of the maximum available layer thickness by some external geometrical constraint. The adsorption isotherm for endosperm tissue is shown in Fig. 5 together with the theoretical curves from the generalized BET equation in which the adsorbed water is restricted to a finite number of molecular layers. Similar results have been obtained

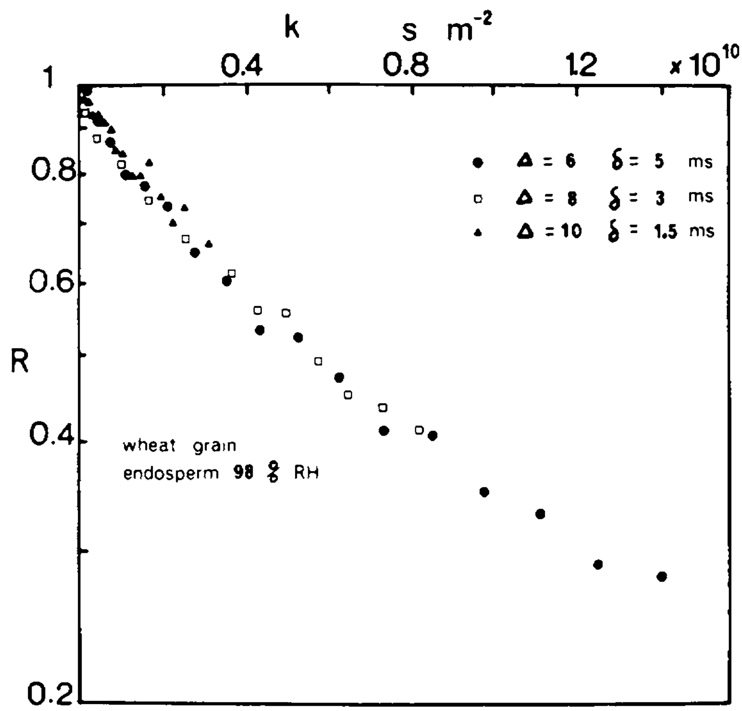

FIGURE 6 Echo attenuation vs. k for water in wheat grain endosperm equilibrated to 98% RH at 22°C. Three different sets of experimental parameters, Δ and δ, have been used, and in each case k was changed by varying the field gradient magnitude, G.

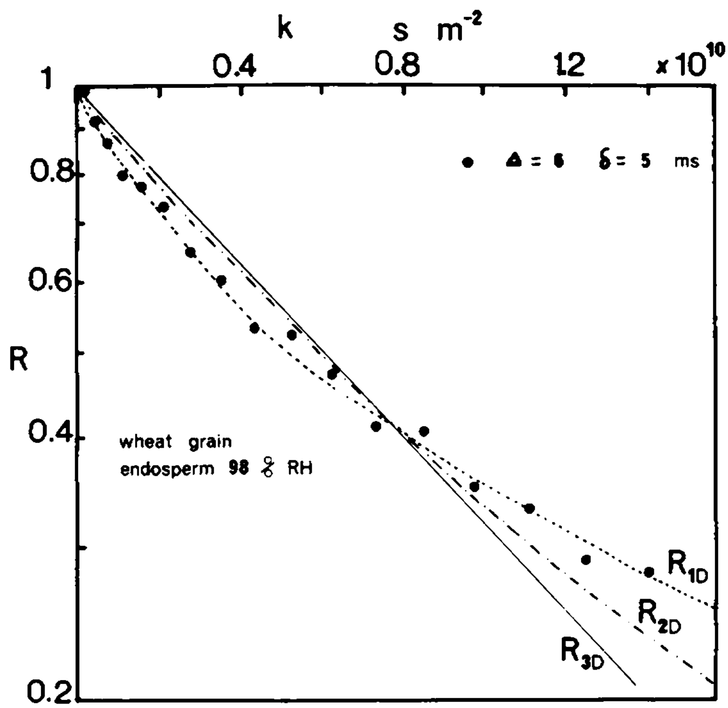

FIGURE 7 Echo attenuation for the $\Delta = 6$, $\delta = 5$ ms experiment showing the theoretical attenuations using equations 3, 7, and 8. These curves have been plotted to pass through the midpoint of the data. The best fit using R_{1D} yields $D = (6.1 \pm 0.2) \times 10^{-10}\ m^2\ s^{-1}$.

by other investigators (Bushuk and Winkler, 1957). It is clear that according to the BET theory most of the isotherm is represented by the curves corresponding to approximately a four or five molecular layer limiting thickness. However, above ~85% RH this layer thickness increases to a maximum value of ~10.

The Kelvin equation of capillary condensation (Hückel, 1929) may also be used in an attempt to fit adsorption isotherms at higher RH values (Clifford, 1975). This equation indicates that at 88% RH capillaries with radii <10 nm fill with water, whereas at 98% RH the corresponding value is 60 nm.

Thus, although the lack of rigorous theory precludes a complete description of multilayer adsorption, all the evidence obtained in the present study indicates that the tissue water is sited in thin films of molecular dimensions. Clearly the diffusion of water in wheat endosperm tissue is likely to be either one or two dimensional with respect to the pulsed field gradient experiment.

The R, k data points from pulsed field gradient experiments on the grain endosperm (98% RH) are shown in Fig. 6 for $2\tau = 12$ ms and three combinations of Δ and δ. The data is plotted with R on a logarithmic scale that would yield a straight line in the case of unrestricted three dimensional diffusion with a unique diffusion coefficient The multiexponential character of the data therefore requires a wide distribution of diffusion coefficients for the endosperm water if the three dimensional model is to be used. We in fact reject this model for reasons given above.

In applying the one and two dimensional models an attempt was made to fit the data using a single diffusion coefficient. Fig. 7 shows the best one and two dimensional fits to the $\Delta = 6$ ms, $\delta = 5$ ms data, with the three dimensional fit shown for comparison. The one dimensional model with a unique diffusion coefficient of $(6.1 \pm 0.2) \times 10^{-10}$ m^2s^{-1} fits the data satisfactorily and gives consistent values for D for the other combinations of Δ and δ. The model works equally well for grain endosperms equilibrated at lower RH values.

Fig. 8 shows the dependence of the water diffusion rate on moisture content in the range 88–99% RH. All the results were analyzed using the one dimensional model. The rapid decrease in the diffusion coefficient with decreasing water content precludes measurement below 88% RH. To measure smaller diffusion coefficients it is necessary to increase the observation time, $\Delta - \delta/3$, and, hence, 2τ. The rapid attenuation in the water signal (Fig. 4) makes this impractical.

Although it is possible, given an appropriate distribution of diffusion rates, that the two or three dimensional models can be used to fit the data, these data are excellently represented at each RH by a one dimensional model using a unique diffusion coefficient. Such one dimensional behavior is consistent with a confinement of the water molecules to a randomly oriented array of capillaries with both transverse dimensions <100 nm. In view of the freezing and adsorption experiments it seems likely that at least one of these dimensions is of the order of a few molecular diameters.

A unique diffusion coefficient associated with the adsorbed water at a given RH level can

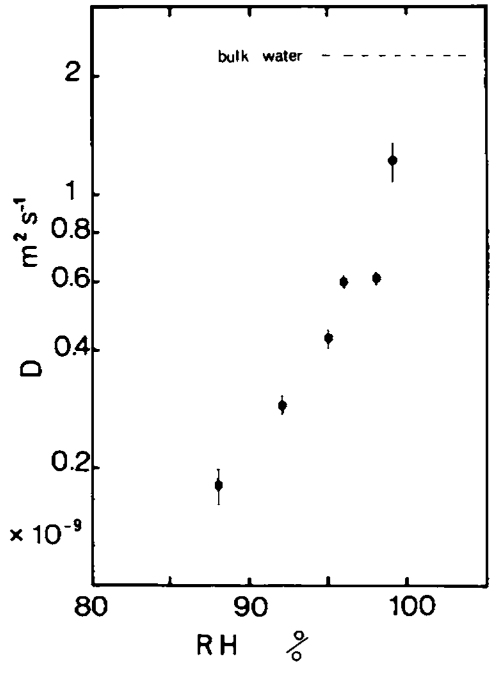

FIGURE 8 Diffusion coefficients for water at 22°C in wheat grain tissues as a function of equilibration RH. The corresponding hydration values can be obtained from Fig. 5. The data has been obtained using a one dimensional model.

be explained if all water layers throughout the grain have the same characteristic thickness; for example, the same molecular layer constraint of the BET description.

It can be agreed that in capillary multilayers water molecules will sample and hence average over a number of layers along with their local diffusion rates in the experimental interval of a few milliseconds. Furthermore, neutron scattering experiments on water (Olejnik and White, 1972) trapped in thin films suggest that the layer thickness determines the diffusion rate. If a uniform diffusion rate is found for the endosperm tissue a layer thickness that is uniform throughout the grain is indicated. Use of the diffusion coefficients measured in this study in conjunction with the neutron diffraction data indicates a film thickness of ~0.5 nm at 88% RH rising to 2.5 nm at 99% RH, a result consistent with the adsorption and freezing experiments. The change in diffusion rate with RH level indicates that the constraint on the film thickness changes. This is no doubt associated with the swelling of the protein matrix as the grain water content increases.

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