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ARTICLE

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NMR stray-field analysis of oil drop size distribution in peanut cotyledons

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Abstract The technique of stray-field NMR has been applied to the study of an oil-bearing seed. It is found to provide additional information about the smallest size of oil drops within the cotyledon not easily measurable by other methods. A peanut was chosen as a convenient seed to investigate so as to allow comparison with previously published NMR pulsed field gradient data. We find a broad distribution of oil drop sizes ranging from a lower limit of order 0.26 μm up to a maximum of approximately 1.3 μm.

Key words Diffusion-weighted NMR · Stray-field NMR · Pulsed field gradient · Peanut · Spherosome

Introduction

The application of NMR techniques to oil seeds

NMR has been routinely used in recent years to analyse the oil content of some seeds. It has the advantage over traditional methods, in which the seeds are pulped to extract the oil, in that NMR measurements can be made non-destructively, leaving the seeds viable for future planting.

There are three major components of seeds which produce an NMR signal. These are oil, water and solid material, including proteins. These components can be readily distinguished by their different characteristic NMR signals. The water is often present in an adsorbed condition, whereas the oil exists in distinct droplets inside structures known as spherosomes. For this reason the transverse relaxation time-constant, T_2, of the water tends to be ~1 ms whereas that of the oil is ~100 ms. The protons in the solid material have a relaxation time ~100 μs.

Early pulsed NMR experiments consisted of observations of a simple free induction decay and a comparison of the results with a calibration curve obtained using pure oil. This enabled the quantity of oil inside a given sample to be measured. The excellent reproducibility of this NMR technique has been noted by Gambhir (1992). However, the method gives no indication of the size of the oil drops within the seed, and the information useful for judging the maturity of the seed is unavailable.

The application of NMR pulsed field gradient (PFG) and NMR PFG stimulated echo techniques allows the physical effects of diffusion to be used to analyse the oil and water signals. In these experiments, the amplitude of the NMR echo depends on how far the protons have diffused since the initial 90° radio frequency (RF) pulse. The behaviour of a freely diffusing species under these conditions is well known, as is the behaviour under conditions of bounded diffusion where the oil or water is confined in small drops (Stejskal and Tanner 1968).

One of the first applications of PFG NMR to the study of seeds was by Fleischer et al. (1990), who examined rape seeds. Although their primary aim was to develop an NMR technique for the routine measurement of oil and water content, they reported interesting bounded diffusion effects from which they were able to conclude something of the structure of the seed cells. Their results showed that the echo amplitude became independent of the pulse separation time, Δ, at values greater than approximately 30 ms. This indicated that the oil was contained in drops of order $(2D\Delta)^{1/2}$ in size where $\Delta = 30$ ms and D is the diffusion coefficient of the pure extracted rape seed oil. From a separate measurement of D they were able to conclude that the oil drop radius was approximately 0.7 μm. Closer analysis of the results indicated that a Gaussian spread of drop sizes was required to fit the PFG results. They also reported

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the existence of a constant signal of order 5–10% from what appeared to be a very slowly diffusing or immobile species which they were unable to identify.

In a similar PFG study, Maphossa and Halse (1996) examined peanut seeds. They also found bounded diffusion behaviour, indicating an oil drop size in peanuts of approximately 1.1 μm. As with rape seeds, a significant constant proton signal (~40% of the total) was also noted. They suggested that this signal was also due to oil, but contained in such small drops that the oil would exhibit bounded diffusion even for the smallest value of Δ (20 ms) used in their experiments.

The work on peanuts was continued by Zakhartchenko et al. (1997), who were able to extend the PFG measurements to much higher pulse amplitudes and Δ values using the PFG stimulated echo technique. They confirmed that most of the oil was contained in drops approximately 1.2 μm in size, but concluded that the distribution of drop sizes was so narrow that no significant numbers of oil drops were to be found below about 0.95 μm.

In order to examine the structure of a peanut cell on a smaller size scale, we have used the newer stray-field technique generally known as STRAFI. The method exploits the large, stable field gradient which is available near the edge of a conventional solenoidal superconducting magnet. For a recent account of this method see, for example, McDonald and Newling (1998) and Carlton et al. (2000). We also carried out PFG experiments on the same peanut to compare with our stray-field measurements and to compare with the earlier published PFG results.

Theory

Constant gradient (stray-field) spin echo

In these experiments, a large constant field gradient G is assumed to be present, and the pulse sequence (90-τ-180-τ-echo) is that of a simple gradient echo. The theoretical expression for the echo amplitude in the case of bounded diffusion under these conditions has been given by Neuman (1974). In the case of a spherical boundary of radius R, a spin echo suffers an attenuation given by:

$$\ln\left(\frac{M(2\tau,G)}{M_0}\right) = -\frac{2\gamma^2G^2}{D}\sum_{m=1}^{\infty}\frac{\alpha_m^{-4}}{\alpha_m^2R^2-2} \times\left(2\tau-\frac{3-4\exp(-\alpha_m^2D\tau)+\exp(-\alpha_m^2D2\tau)}{\alpha_m^2D}\right) \quad (1)$$

where the α_m are derived from the mth root of the Bessel function equation:

$$\alpha_m R J'_{\frac{3}{2}}(\alpha_m R) - \frac{1}{2}J_{\frac{3}{2}}(\alpha_m R) = 0 \quad (2)$$

and M_0 is the amplitude of the echo in the limit as $\tau \to 0$. When $\tau \ll R^2/D$, this equation reduces to the well-known free diffusion limit given by:

$$\ln\left(\frac{M(2\tau,G)}{M_0}\right) = -\frac{2\gamma^2G^2D\tau^3}{3} \quad (3)$$

while for long diffusion times $\tau \gg R^2/D$, when diffusion is bounded, this equation reduces to:

$$\ln\left(\frac{M(2\tau,G)}{M_0}\right) = -\frac{R^4\gamma^2G^2}{D}\cdot\frac{8}{175}\cdot\left(2\tau-\frac{581R^2}{840D}\right) \quad (4)$$

Thus unbounded diffusion [Eq. (3)] is characterised by a τ^3 dependence, while bounded diffusion depends on τ. Note also that for bounded diffusion, the gradient of the log plot of signal amplitude versus τ depends on R^4 and so is very sensitive to the radius of the diffusion boundary.

Pulsed field gradient experiments

A full analysis of a PFG experiment in which diffusion takes place in spherical pores of radius R has been derived by Murday and Cotts (1968). In the experiment a 90° RF pulse is followed at a time τ by a 180° pulse and an echo results at a time 2τ. To make the amplitude of the echo sensitive to diffusion, two equal gradient pulses of magnitude g and duration δ are included either side of the 180° pulse and separated in time by Δ. In our experiment, Δ was made equal to τ. In this case the ratio of the NMR echo amplitudes with and without the gradient pulses is given by:

$$\ln\left(\frac{M(2\Delta,\delta,g)}{M_0}\right) = -2\gamma^2g^2\sum_{m=1}^{\infty}\left[\alpha_m^2\left(\alpha_m^2R^2-2\right)\right]^{-1} \times\left(\frac{2\delta}{\alpha_m^2D}-\frac{\left\{2+\exp\left[-\alpha_m^2D(\Delta-\delta)\right]-2\exp\left(-\alpha_m^2D\delta\right)-2\exp\left(\alpha_m^2D\Delta\right)+\exp\left(-\alpha_m^2D(\Delta+\delta)\right)\right\}}{\left(\alpha_m^2D\right)^2}\right) \quad (5)$$

where the α_m are again given by Eq. (2) and M_0 is the amplitude of the echo in the limit as $q \to 0$, where $q = \gamma g\delta$. Equation (5) has two well-known limiting forms. For small Δ, where the diffusion is unbounded, Eq. (5) reduces to the Stejskal-Tanner equation:

$$\ln\left(\frac{M(2\Delta)}{M_0}\right) = -\gamma^2g^2\delta^2D(\Delta-\frac{1}{3}\delta) = -q^2D(\Delta-\frac{1}{3}\delta) \quad (6)$$

while in the long Δ limit when the diffusing species exhibit bounded diffusion, Eq. (5) reduces to the well-known result:

$$\ln\left(\frac{M(\delta,g)}{M_0}\right) = -\frac{R^2\gamma^2 g^2\delta^2}{5} = -\frac{R^2q^2}{5} \quad (7)$$

In the analysis which follows, the full Eq. (5) was used throughout in order to preserve accuracy.

Experimental

The stray-field experiments were carried out at a point along the axis of a 4.7 T (200 MHz) horizontal bore superconducting magnet near the end of the magnet where the field was approximately 2.7 T and the field gradient was 18.3 T m^{-1}. A solenoidal coil, approximately 9 mm in diameter and 30 mm long and tuned to 117 MHz, was used as the RF coil. A single peanut was placed inside a glass tube and inserted into the RF coil. The 90° RF pulse length was approximately 5 μs. Spin echoes were observed at τ values ranging from 40 μs to 6 ms.

For the PFG experiments, the same peanut was placed inside an RF coil consisting of a birdcage resonator approximately 20 mm in diameter and 50 mm in length positioned at the centre of the 4.7 T magnet and tuned to 200 MHz. Gradient pulses of duration $\delta = 4$ ms were used throughout. For PFG experiments to be effective at investigating systems where diffusion is bounded within structures, the diffusion time, Δ, must be sufficiently long for the molecules within to be able to diffuse across the structure. For diffusion times below this minimum, the size of the echo will be dependent upon Δ, making analysis unnecessarily complex. For times in excess of this minimum the echo height will be independent of Δ. Preliminary PFG experiments were carried out using a range of Δ values, and it was found that bounded diffusion was assured for a value of Δ of 150 ms or greater. This diffusion time was used in all our subsequent PFG experiments.

Results

According to the information provided by the supplier, the oil concentration in the peanuts we used was of the order of 48% by mass. This figure was checked by measuring the relative amplitudes of the long (oil) and short (water + solids) T_2 components of the NMR free induction decay and also independently by crushing and extraction of the oil. A dessication experiment showed a water content of approximately 6%. The remaining 46% was made up of solid material (protein, carbohydrate and fibre).

The results of the stray-field experiments on a peanut are shown in Fig. 1 in which the normalised proton spin echo amplitude is plotted, on a logarithmic scale, as a function of τ. The signal from the oil at zero echo time, M_0, was estimated from an analysis of the echo heights for the first few values of τ up to $\tau \approx 100$ μs. These first few readings contain contributions from the water and solid material content whereas M_0 refers only to the oil. The water and solid signals decay very rapidly with τ and consequently do not significantly affect the graph beyond these first few readings. In the case of the solid component, this is simply because of the very short $T_2 \approx 100$ μs. In the case of the water content, where the T_2 may be as much as ~1 ms, it is the diffusion of the water in the large stray field gradient which kills the signal. Even for water in gels, the diffusion coefficient is known to be ~10^{-9} m^2 s^{-1} (approximately one half that of pure water) and so is 100 times greater than the oil. Equation (3) therefore gives an extremely rapid decay for the water signal.

The early part of the graph in Fig. 1, up to $\tau \approx 2.5$ ms, varies as τ^3, indicating the presence of freely diffusing oil. Analysis of this portion of the graph using Eq. (3) enables the value of the diffusion coefficient of the oil to be calculated. With G = 18.3 T m^{-1}, a value of D = 1.0×10^{-11} m^2 s^{-1} at 20 °C was obtained. This is consistent with the value for bulk peanut oil (1.3×10^{-11} m^2 s^{-1} at 30 °C) obtained by Zakhartchenko et al. (1997), and therefore good evidence that the signal

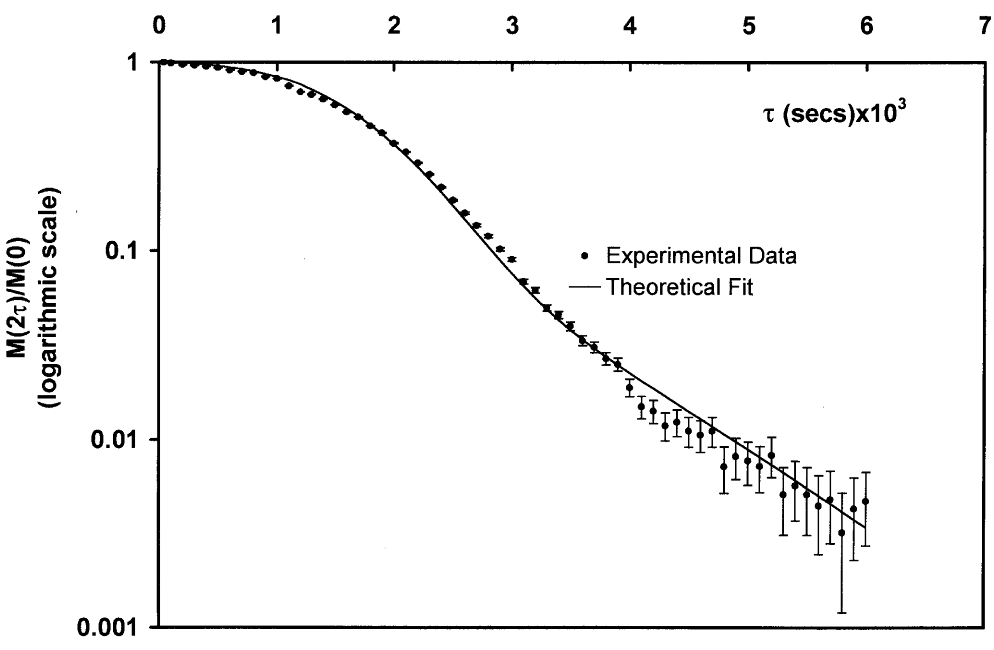

Fig. 1 NMR stray-field measurements of a peanut, taken at 117 MHz, showing the normalised proton spin echo amplitude plotted on a logarithmic scale as a function of RF pulse separation τ. Also shown is a theoretical fit [Eq. (1)] based on the oil drop size distribution shown in Table 1

Table 1 Peanut oil volume distribution as a function of drop radius

Histogram radius (μm)	0.26	0.52	0.78	1.04	1.3	1.56
Volume proportion %	15 ± 1	18 ± 3	0 ± 7	26 ± 7	41 ± 10	0 ± 9

originates from the oil component of the cotyledon. At echo times greater than $\tau \approx 2.5$ ms, the graph changes to a linear dependence on τ [Eq. (4)], showing that oil in the smallest drops has now come within the limit of bounded diffusion.

The fact that the first indications of bounded diffusion occur at approximately 2.5 ms can be used to estimate the size of the smallest oil drops. From the well-known result for a random walk in one dimension we have:

$$\overline{R^2} = 2D2\tau \qquad (8)$$

where 2τ is the time between the initial RF pulse and the formation of the echo. With $D = 1.0 \times 10^{-11}$ m^2 s^{-1} and $2\tau = 5$ ms, this provides an estimate of the minimum oil drop radius of order 0.3 μm.

As τ increases beyond ~2.5 ms, bounded diffusion starts to occur in successively larger drops. However, as can be seen from Eq. (4), the R^4 factor weights the signal very heavily in favour of the smallest drop sizes.

The PFG results obtained from the same peanut as used in the stray-field experiment are shown in Fig. 2. In this figure the normalised experimental echo amplitude is plotted, on a logarithmic scale, as a function of q^2 $(=\gamma^2\delta^2g^2)$ for a value of $\Delta = 150$ ms (in a PFG experiment the echo time is kept constant in order to eliminate T_2). For a peanut cotyledon containing oil drops of only one radius, this graph would be a straight line. The curved nature of Fig. 2 indicates a distribution of oil drop sizes.

Theoretical fitting

In order to fit the experimental results (Figs. 1 and 2), various models for the oil drop size distribution within the peanut were considered. Computations involving a continuous size distribution proved too lengthy, even when specified in analytic form. It was therefore decided to model the oil drop radius distribution with six equally spaced histogram ranges. Since the fitting of the linear portion of the stray-field graph in Fig. 1 is very sensitive to the smallest drop radius (0.26 μm), this value was taken as the smallest radius.

The corresponding volume proportions were treated as fitting parameters for the full Eqs. (1) and (5). A least squares procedure was used to determine the best overall fit to the PFG and stray-field data taken together. The result (continuous lines, Figs. 1 and 2) demonstrates that it is possible to find one set of parameters (Table 1) which fits both sets of experimental data.

Note that the different estimated errors in the volume proportions reflect their different sensitivities in the fitting to the experimental data.

Discussion

From Table 1 we can see that most of the oil is found to exist in drops of size between 1.0 and 1.3 μm, in agreement with the previous work of Zakhartchenko et al. (1997) and Maphossa and Halse (1996). However, approximately 30% of the oil exists in smaller drops at radii of 0.26 μm and 0.52 μm. Despite the very coarse quantisation of radius values, we suggest that these smaller drops are the immobile fraction observed by Fleischer et al. (1990) in rape seeds and the 40% fraction of small drops proposed by Maphossa and Halse (1996). This conclusion is consistent with the work of Jacks

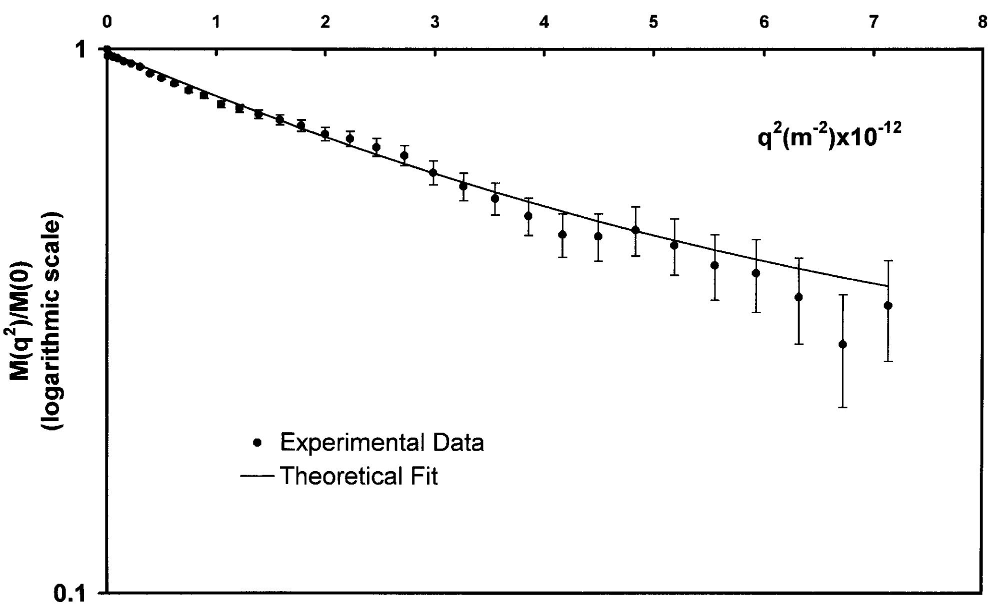

Fig. 2 NMR PFG measurements taken at 200 MHz using the same peanut as in Fig. 1. In this figure, the normalised spin echo amplitude is plotted, on a logarithmic scale, as a function of q^2 $(=\gamma^2\delta^2g^2)$. A gradient pulse separation of $\Delta = 150$ ms ensured bounded diffusion for all oil drop sizes. The theoretical fit [Eq. (5)] is based on the same oil drop size distribution (Table 1) as that used in Fig. 1

et al. (1967, p 587, fig. 1) in which an electron micrograph of a collection of peanut spherosomes is reproduced showing a range of oil drop sizes from 0.3 μm up to approximately 1.0 μm. More recent scanning electron microscope studies of oil bodies in seeds by Leprince et al. (1998) unfortunately does not include data on peanuts. Their measured radii for rapeseeds are, however, the same order as our results.

Conclusion

NMR PFG is a well-established technique which has been applied by several authors to the study of oil-bearing seeds. The amplitude of the signal from a large drop initially exceeds that from a small drop because of its greater volume. However, it also decays more quickly because of the R^2 dependence [Eq. (7)], so that the PFG experiment is sensitive to both large and small oil drops.

The stray-field technique, on the other hand, provides an effective filter to the larger drops. The signal from freely diffusing species decays rapidly as τ^3, whereas the signal from a species subject to bounded diffusion exhibits a linear τ dependence. The oil in the smallest drops reaches this bounded stage at an earlier value of τ and from then on decays much less rapidly. This results in the signal from the smallest drops eventually dominating over the larger ones. When using NMR to study oil drops in seeds, therefore, the stray-field experiment provides useful additional information about the smallest drops, enabling an accurate lower limit to be placed on their size.

Investigation of a peanut cotyledon using both of these NMR techniques has shown the results to be consistent with the same oil drop size distribution. Analysis of the PFG and stray-field techniques show them to be complementary. Together they provide a more detailed picture of the oil drop distribution than can be found by the use of just one technique alone.

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