A Comparison of Different Pulse Sequences in the Nondestructive Estimation of Seed Oil by Pulsed Nuclear Magnetic Resonance Technique

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ABSTRACT

Three basic pulse sequences, *viz.*, Free Induction Decay (FID), Spin Echo (SE) and Carr-Purcell-Meiboom-Gill (CPMG), available in the pulsed nuclear magnetic resonance technique have been used to estimate the relative oil content in single as well as bulk samples of oil-bearing seeds. Conclusive evidence has been presented to show that the CPMG sequence is vastly superior to the other two as far as the effects of seed orientation and moisture content are concerned.

INTRODUCTION

Nuclear Magnetic Resonance (NMR) spectroscopy provides a quick and convenient method for the nondestructive estimation of oil content in seeds (1-6). So far as pulsed NMR technique is concerned, there are basically three pulse sequences available (7), *viz.*, Free Induction Decay (FID), Spin Echo (SE) and Carr-Purcell-Meiboom-Gill (CPMG), for monitoring the NMR signal intensity of any sample. In principle, any one of the sequences could be used to estimate the oil content. The FID sequence had been used earlier in testing this technique (6). The present article deals with the application of these three pulse sequences in seed oil estimation and provides conclusive evidence to demonstrate the superiority of the CPMG sequence as far as the effects of moisture content and seed orientation (in single seed analysis) are concerned.

BASIC PRINCIPLE

The underlying idea in the estimation of oil content in seeds consists in evaluating the number of oil protons present in the seed and comparing it with that in a known amount of seed oil under identical experimental settings.

In the pulsed NMR technique, the sample is kept in a steady magnetic field so that the protons in the sample are polarized resulting in a net magnetic moment along the field direction. This magnetic moment is proportional to the total number of protons in the sample. The sample can then be subjected to different sequences of r.f. pulses satisfying the resonant condition.

$$\omega = \gamma H \qquad 1$$

ω — angular frequence of r.f. radiation

H — steady magnetic field

γ — magnetogyric ratio of protons

Thus, in the FID method, a single 90 pulse (so-called because it rotates the magnetic moment through 90 from the field direction) is applied. After the end of the pulse, the magnetic moment, which is now in a plane perpendicular to the magnetic field, gets dephased with a characteristic time constant T_2^* given by

$$1/T_2^* = 1/T_2 + \gamma \Delta H/2 \qquad 2$$

T_2 — transverse relaxation time of the sample

ΔH — magnetic field inhomogeneity

This magnetic moment induces a voltage in the coil surrounding the sample. The decay of this induced voltage as a function of time is called the free induction signal, so-called since the nuclei precess "freely" in the absence of any r.f. The intensity of this NMR signal can be measured at any desired instant with a sampling pulse. The time interval between the 90 pulse and the sampling pulse is called the delay time. The magnetic moment also reverts back to the field direction with a time constant T_1 called the longitudinal relaxation time.

In the SE method (8), the 90 pulse is followed by a 180 pulse after a time interval ζ so that a spin echo signal is formed at time 2ζ. The effect of magnetic field inhomogeneity in dephasing the magnetic moment is neutralized by the 180 pulse. But diffusion during the interval ζ may cause nuclei to move from one part of an inhomogenous field to another resulting in a decrease in the spin echo amplitude from the value one would expect on the basis of spin-spin interaction only.

In addition to the elimination of magnetic field inhomogeneity effects, the problem of diffusion is also overcome in the CPMG program (9,10), which consists of the sequence 90-ζ-180-2ζ-180-2ζ-180, etc., with a 90 phase difference between the 90 and 180 pulses. The CPMG signal as seen on the oscilloscope comprises of a series of spin echo signals at 2ζ, 4ζ, 6ζ, etc. By keeping ζ small, the time available for diffusion is reduced so that the spin echo amplitude decays exponentially with the time constant T_2.

MATERIALS AND METHODS

Seed Samples

For single seed analysis, groundnut and sunflower were used. To study the effect of seed orientation on the signal intensity, the single seed was tightly packed with glasswool in the NMR sample tube (6 mm diameter) and the initial position was arbitrarily taken as O. The sample tube was then rotated through 90, 180 and 270 and at each orientation, the signal intensity was measured. For each delay time (FID) or ζ value (SE), the coefficient of variation was calculated by dividing the standard deviation of the four observations by the mean.

$$\text{Coefficient of Variation (C.V.)} = \text{s.d./mean} \times 100 \qquad 3$$

To study the effect of moisture content on the oil estimation, linseeds with an initial moisture content of 4.3% were used. Four samples of the same variety, each weighing ca. 80 mg, were taken. One sample was retained as the control and the other three samples were hydrated to different extents by suspending them inside wet tissue paper kept in a closed container saturated with water vapor. Corresponding to each value of hydration, the signal intensities were measured by the various pulse methods. Assuming the oil content in the control sample to be 100%, the oil contents in the other samples were calculated according to the relation shown below.

$$\text{Percent oil content in sample A} = 100\,(s_A/m_A)(s_C/m_C)^{-1} \qquad 4$$

s_A and s_C – signal intensities of sample A and the undried control sample under identical spectrometer conditions.

m_A and m_C – dry weights of sample A and the Control sample as obtained after drying the samples at 60 C till a constant weight was reached.

For absolute oil estimations, a pure oil sample should be used as the control. Since we are now interested only in the effect of moisture on the estimated oil content, relative values should suffice. Accordingly, the seed sample itself is used as the standard which also takes into account the day-to-day variation in the sensitivity of the spectrometer.

Pulsed NMR Spectrometer

The coherent, pulsed NMR spectrometer fabricated at the Jozef Stefan Institute, Ljubljana, Yugoslavia, was operated at 32 MHz. The 90 pulse width was 4 μsec and the r.f. coil size was 6 x 6 mm. However, in order to reduce the effect of r.f. field inhomogeneity, the sample height was restricted to 4 mm. The intensity of the signal at any desired instant could be averaged over 10 or more repetitions and recorded on a four digit display. In the present work, each signal intensity measured is an average of ten repetitions.

The spectrometer also has a hardwired program to measure the intensity of the CPMG signal at eight different time positions and processes them so as to yield the signal intensity at zero time.

RESULTS AND DISCUSSION

The measured signal intensities are not only due to the protons from the oil but also from those present in the solid moiety of the seed (carbohydrates, proteins etc.), as well as from the protons of moisture that may be associated with the seeds. The fact that $(T_2)_{solid} \ll (T_2)_{bound\ water} \ll (T_2)_{oil}$ forms the basis for the discrimination between the oil signal and those from other components. Typical values are (11):

$(T_2)_{solid}$ – μsec

$(T_2)_{bound\ water}$ – msec

$(T_2)_{oil}$ – 100 msec

The free precession signal intensity can be written as (11)

$$G(t) = A \exp[-t/(T_2)_\zeta]^2 + B \exp[-t/(T_2)_w] + C \exp[-t/(T_2)_o] \qquad 5$$

At t = O, the signal intensity is A + B + C so that it is not possible to discriminate between the oil signal and those from other components. However, within 30 μsec, the signal due to solid component (first term in relation 5) would decay to 0.01% of its initial value so that its contribution could be completely neglected. It is not so easy to eliminate the contribution from the moisture whose signal intensity (second term in relation 5) would reduce to only 0.67% of its initial value even after 5 msec (assuming T_2 of water to be 1 msec). But during these 5 msec the signal intensity of the oil compont itself would decrease appreciably as the FID signal decay is mainly governed by the magnetic field inhomogeneities ($T_2^* \approx 3$ msec in the present studies). Thus, the signal to noise ratio of the FID signal at 5 msec would be too poor to carry out any worthwhile measurements.

On the other hand, the SE method can be expected to yield better results as the elimination of the effect of magnetic field inhomogeneity in this method enables one to observe the spin echo signal at 10 or even 20 msec after the application of the first pulse. During this time interval, the intensity of water signal would have decayed to 0.2% of its initial value.

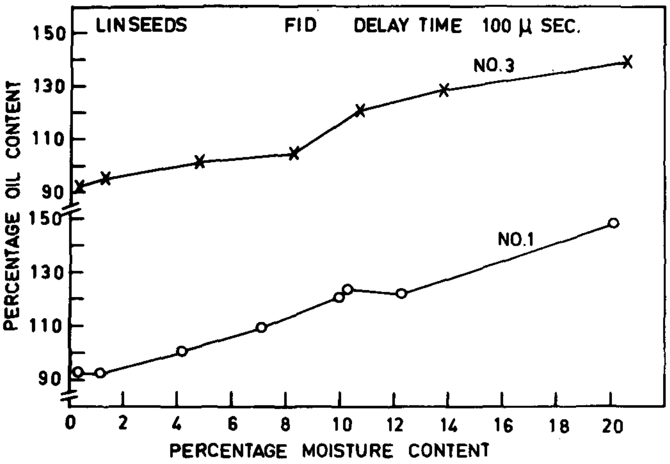

FIG. 1. Effect of seed moisture on the oil content as estimated by free induction decay method corresponding to a delay time of 100 μsec. Sample, linseeds. Numbers 1 and 3 denote two different samples.

The experimental results obtained in the present studies are in accordance with the above arguments. As the four samples have been drawn from the same variety, a value of 100% should be obtained for the oil content. But the oil content as estimated by the FID method corresponding to a delay time of 100 μsec increases continuously from 93% to 140% when the moisture content was varied from 0 to 20% (Fig. 1). Similar behavior is observed even when the signal intensities, measured at a longer delay time of 500 μsec, are used to calculate the oil content. This is because of the increased contribution of water to the signal intensity. The influence of moisture content is considerably reduced in the SE method as can be seen in Figure 2. At 20% moisture content, the estimated oil content corresponding to ζ = 10 msec is 106%. This 6% increase from the expected value of 100% obviously arises out of the water moiety indicating that a time interval as long as 20 msec (2ζ = 20 msec) is not sufficient for the water signal intensity to be eliminated completely. The T_2 values of water in oil-bearing seeds at different moisture contents are not known. However, in plant tissues, e.g., peas, T_2 of water has been reported to be 71 msec at 83% moisture content (12). In the present case T_2 of water may not be so high but can be expected to be much larger than 1 msec so that the water moiety contributes significantly to the signal

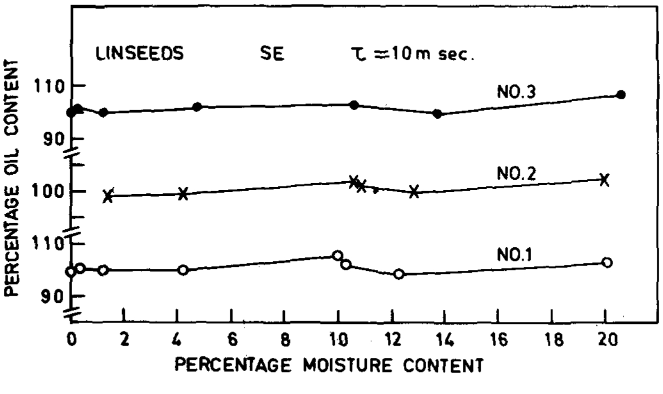

FIG. 2. Effect of seed moisture on the oil content as estimated by spin echo method correponding to a ζ value of 10 msec. Sample, linseeds. Numbers 1, 2 and 3 denote three different samples.

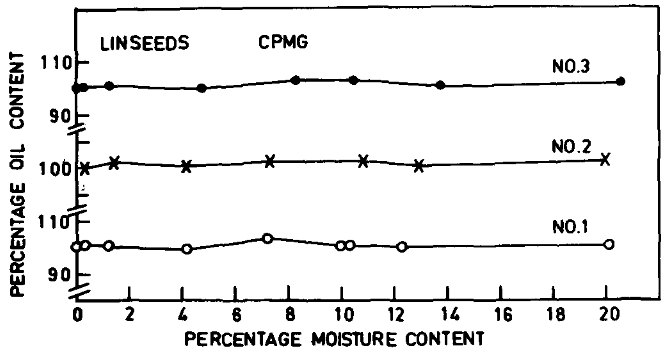

FIG. 3. Effect of seed moisture on the oil content as estimated by Carr-Purcell-Meiboom-Gill method. Sample linseeds. Number 1, 2 and 3 denote three different samples.

intensity even at 10 msec, thereby elevating the estimated oil content.

Even this 6% contribution is not observed with the CPMG method as can be seed from the fact that the oil content is 102% even at 20% moisture content (Fig. 3). This is to be expected as in this program, the signal intensities are measured at eight different time positions spanning over 180 msec so that the contribution due to moisture gets reduced to a minimum.

Regarding the problem of oil estimation in single seeds, the coefficient of variation is a measure of the effect of seed orientation on the signal intensity. Figure 4 shows that for a single groundnut seed, this coefficient as obtained in the FID method increases continuously from ca. 1% at a delay time of 100 μsec to ca. 60% corresponding to a delay time of 1400 μsec. Similar behavior, though somewhat to a lesser extent, is exhibited by a single sunflower seed (Fig. 5). This effect is comparatively less in the SE method where the maximum value is 6% (Fig. 4 and 5), while in the CPMG method it turns out to be 1.9, 0.3 and 0.4% for groundnut, sunflower and mustard respectively.

The signal intensity in the FID method has been shown to depend on the seed orientation (6). But at a delay time of 200 μsec, these authors found the signal intensity to be the same for all angular positions. However, we have not observed this latter behavior in any of the seeds investigated so far (groundnut, soybean, sunflower and maize).

The angular dependence of the signal intensity obviously arises out of the inhomogeneous distribution of oil in the seed so that the oil in different parts of the seed experiences different fields. This effect is therefore maximum in the FID method as the decay of the free precession signal is mainly decided by the magnetic field inhomogeneities. This angular dependence can be expected to decrease in a sample where the oil is distributed more uniformly. This is borne out by our own observations as well as those of others (6) that in mustard seeds, the coefficient of variation remains practically the same up to a delay time of 1200 μsec (Fig. 6). However, the increase at higher delay times cannot be fully ascribed to the heterogeneity of the oil in the seeds because even in a pure oil sample a similar increase is observed (Fig. 4).

Thus, if the FID method has to be used for oil estimation, then the delay time should be as small as possible in order to avoid the effect of seed orientation. However, this would introduce considerable error in the estimated oil content due to the presence of moisture as discussed earlier.

The effect of angular dependence on the signal intensity is considerably reduced in the SE method because the magnetic field inhomogeneities do not influence the spin echo amplitudes. However, even with this sequence the effect is not altogether avoided presumably because of diffusion effects. When these diffusion effects are eliminated as in the CPMG method, the coefficient of variation is less than 2% as mentioned earlier.

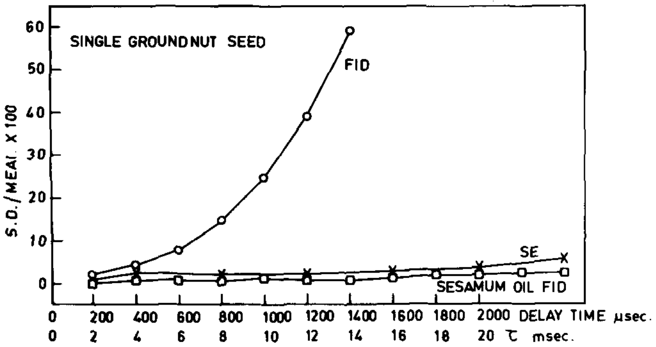

FIG. 4. Effect of seed orientation on signal intensity in a single groundnut seed as determined by FID (open circles) and SE (crosses) methods. Open squares – FID method for a sample of pure sesamum oil.

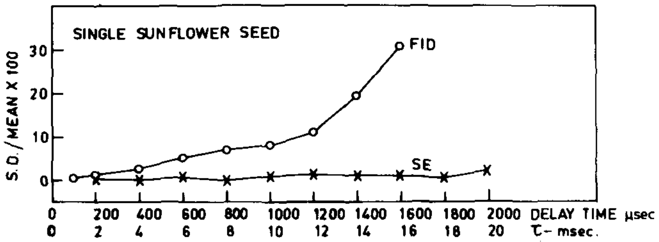

FIG. 5. Effect of seed orientation on signal intensity in a single sunflower seed as determined by FID (open circles) and SE (crosses)methods.

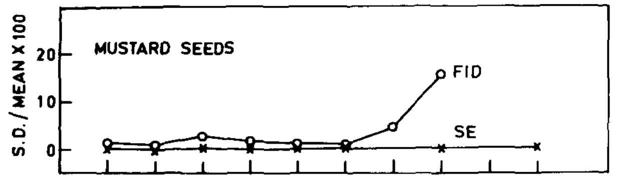

FIG. 6. Effect of seed orientation on signal intensity in a sample of mustard seeds as determined by FID (open circles) and SE (crosses) methods.

Thus, the CPMG method is decidedly superior to both FID and SE methods as the effects of both seed orientation and moisture content are eliminated. The fact that the moisture content does not influence the estimated oil content is of special importance since the process of drying the samples is avoided, thus maintaining the seed viability and thereby making this method truly nondestructive. Furthermore, in the estimation of absolute oil content in seeds it would be necessary to compare the signal intensity of the seed samples with that from an oil sample. The relaxation times of the oil in the seeds need not be the same as that of pure oil. In fact, the relaxation times of seed oil have been shown to vary from sample to sample (11) and even from seed to seed of the same variety (6). But the signal intensities as displayed by the CPMG method are independent of T_2 values as the measured intensities are extrapolated to yield the value at $t = O$, which is the best estimate of all the oil protons.

It remains now to establish a one-to-one correspondence between the values as obtained by this method and those determined by conventional chemical techniques such as Soxhlet extraction. Such studies are currently in progress.

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