Experiment #20

Useful reference: Fukushima, Eiichi (1981). Experimental Pulse NMR: A Nuts and Bolts Approach. Addison-Wesley. UW Davis Centre Call #: QC762.F85 1981

Description of Spectrometer

The NMR spectrometer is a self contained pulse NMR spectrometer consisting of the units :

- pulse programmer
- frequency source
- radio frequency pulse modulator
- transmitter
- NMR probe
- NMR receiver and detector
- permanent magnet (no cooling required)
- power supply

The parameters relevant for NMR experiments are varied by the operator from the front panel of the spectrometer, shown in Figure 1.

Figure 1: NMR spectrometer. Numbers shown are referred to in the text as, for example, #1.

Pulse programmer

The built-in pulse programmer allows for five independent experiments :

- free induction decay (FID), or T_2^*
- inversion recovery (IR)
- spin-echo (SE)
- Carr-Purcell (CP)
- gated Carr-Purcell (CPS)

The type of experiment performed is selected by knob #1 (Note: all #'s refer to the labels found in Figure 1). The pulse sequence is either automatically repeated, with a repetition time selected by knob #2, or is triggered manually using the single experiment push button #3. The experimental operation mode, automatic or manual, is selected by means of switch #4.

The length of the r.f. pulses applied is determined by potentiometers #5, for the 90° pulse, and #6, for the 180° pulse. Variation of the spacing between pulses, where applicable, is determined by potentiometer #7, whose numeric value is shown on its face in milliseconds, and by multiplier knob #8.

Resonance

The magnetic field value, as determined by the permanent magnet of the spectrometer, determines the Larmor frequency of the precessing spins. The spectrometer is operating at a fixed frequency of 9 MHz but does allow slight frequency adjustment (± 60 kHz) to match the applied pulse frequency to the Larmor frequency determined by the magnet. Frequency adjustment is accomplished by adjusting potentiometer #9.

NMR probe head

The sample is inserted into the probe holder (#10) positioned at the top of the spectrometer, and is as close as possible to the exact centre of the pole faces of the permanent magnet.

<u>DO NOT DROP SAMPLES INTO THE SAMPLE HOLDER.</u> If a sample will not fit into the holder, call the demonstrator. <u>DO NOT FORCE ANYTHING INTO THE R.F. SAMPLE HOLDER AS THE R.F. COIL MAY BE DAMAGED.</u>

NMR Receiver

The NMR signal is generated by the spins inside the sample, inside the probe head, and is amplified and detected in the receiver section of the spectrometer. The controls for the receiver parameters are found in the portion of spectrometer front panel labeled DETECTION. The variable receiver gain allows for adapting to a very broad range of samples, ie. samples of varying nucleic abundance. The gain can be varied by adjusting control knob #11. Diode or phase sensitive detection can be selected using switch #12.

The Reference Phase

In phase-sensitive detection, the FID is seen at points of constant phase. The reference phase of the NMR signal is adjusted using potentiometer #13. Changing the setting of the reference knob will change the signal being output from the spectrometer. The signal is filtered to improve the signal-to-noise ratio. The bandwidth of the output amplifier can be varied in steps, using potentiometer #14, from ~ 200 kHz to ~ 3 kHz.

Gradient

A gradient can be applied across the sample by adjusting potentiometer #15. This is used for imaging experiments. For spectroscopy the gradient knob should be turned to 0.

The Experiment

I. <u>Finding Resonance</u>

Sample : Water

Spectrometer settings :
- Place the water sample in the magnet.
- Selection knob #1 set to FID
- Select the appropriate repetition time (about $5 \cdot T_1$) to maximize the signal.
- Adjust the receiver phase to maximize the signal
- Adjust the receiver gain so that a signal of approximately 1 V appears on the oscilloscope.
- Adjust the pulse to maximize the signal, by using potentiometer #5. This is a 90° pulse.

Explain what is happening as you make the above adjustments. Why is it called a 90° pulse?

With the settings as above you should see a resonance curve, or free induction decay (FID), on the screen. Adjust the NMR frequency, potentiometer #9, until the NMR signal is reasonably exponential – at this point the Larmor frequency, $\omega_o = \gamma H_o$, is identical to the r.f. frequency of the spectrometer. Once you obtain an exponential NMR signal, the spin system is on resonance.

Obviously if you set the pulse width to twice its length for the 90° pulse you obtain a 180° pulse. Adjust potentiometer #5 until you obtain a 180° pulse. The 180° pulse flips the macroscopic magnetization vector along the negative z axis. Since there is no magnetic vector in the XY plane, there should be no signal. You may see a small signal due to the inhomogeneity of the magnetic field.

What happens to the signal as you keep increasing the pulse width? With a pulse length of 3 x 90° = 270° the magnetic field vector would have been flipped 270° and the signal would appear as the negative of the 90° pulse signal. Explain this.

Save representative data acquired for each sample and discuss (the details of saving data to a PC for later analysis will be covered by the demonstrator at the start of the lab).

II. The FID and T_2

Samples : water, alcohol, rubber

Spectrometer settings :
- Selection knob #1 set to FID
- Select the appropriate repetition time to maximize the signal.
- Adjust the receiver gain to an appropriate value
- Adjust the receiver phase to maximize the signal
- Adjust the pulse to maximize the signal, by using potentiometer #5. This is a 90° pulse.

Adjust the NMR signal appropriately to obtain an exponential FID. The equation that gives the magnetization as a function of time and T_2^* is :

$$M_y(t) = M_o\, e^{-t/T_2^*}$$

Save the data set and plot on a semi-log graph. Find T_2^* from the slope of the line as indicated by the above equation. How well does the line match the values of the points plotted? Comment on any significant deviation from exponential behaviour. Do this for each sample and discuss.

It is useful to be able to estimate T_2^* directly from the FID on the screen. From the above equation we see that for $t = T_2^*$, the ratio $M_y(t = T_2^*)/M_o = 1/e \approx 0.37$. Thus, for an exponential decay, the time t where $M_y(t) \approx M_o/3$ is approximately equal to T_2^*. Estimate T_2^* this way for each sample.

III. Measurement of T_1

Samples : water, alcohol, rubber

Spectrometer settings :
- Selection knob #1 set to IR
- Select the appropriate repetition time to maximize the signal.
- Adjust the receiver gain to an appropriate value
- Adjust the receiver phase to maximize the signal
- Use the setting for the 90° pulse obtained previously. In this experiment two 90° pulses are used which are controlled by the potentiometers # 5 and #6. Potentiometer #6 is the first pulse and potentiometer #5 is the second pulse.

Obtain a 90° pulse for the water sample. Note that at $t = 5 \cdot T_2^*$ there is virtually no signal. Set τ, the time interval between the two pulses, by adjusting potentiometer #7, equal to $5 \cdot T_2^*$. You should see no signal. The second pulse flips whatever magnetization vector has regrown along the +Z axis towards thermal equilibrium with time constant T_1, according to :

$$M_z(\tau) = M_o\,[1 - e^{-\tau/T1}]$$

At $\tau << T_1$, the amount of regrowth is negligible and essentially zero signal is observed. The maximum amplitude of the FID signal varies directly with the amount of regrowth in time τ.

It may be helpful to consider the 2nd 90° pulse as a "Read Statement", the output of which is the magnitude of the magnetization vector initially along the +Z axis.

Now scan through τ by adjusting the potentiometer labeled #7, and find τ for which $M_z(\tau) = M_o/2$. Use the above equation to solve for T_1. Repeat for each sample and compare the T_1 values obtained. The important concept is that, while the signal has decayed in the X-Y plane rather quickly, the regrowth along the Z-axis is much slower.

The reason for needing a long repetition rate in some cases to maximize the signal should now be obvious. If the signal repeats faster than $5 \cdot T_1$, the system does not re-establish thermal equilibrium between pulses. If you were looking for the FID after a 90° pulse, the signal would not be maximal. This suggests a quick method of estimating T_1 for a sample. Set up a single 90° pulse (knob #1 to FID). As the repetition time is decreased (ie. the time between subsequent pulses is shortened) the amplitude of the FID will be found to drop significantly at a particular setting. The last setting before this drop occurs can be used as a rough estimate of $5 \cdot T_1$. Use this technique to estimate T_1. How does it compare with the previous value? Estimate T_1 using this method for the other samples.

Save representative data for each sample for inclusion with your report.

IV. Measurement of T_1 with inversion-recovery sequence :

Samples : water, alcohol, rubber

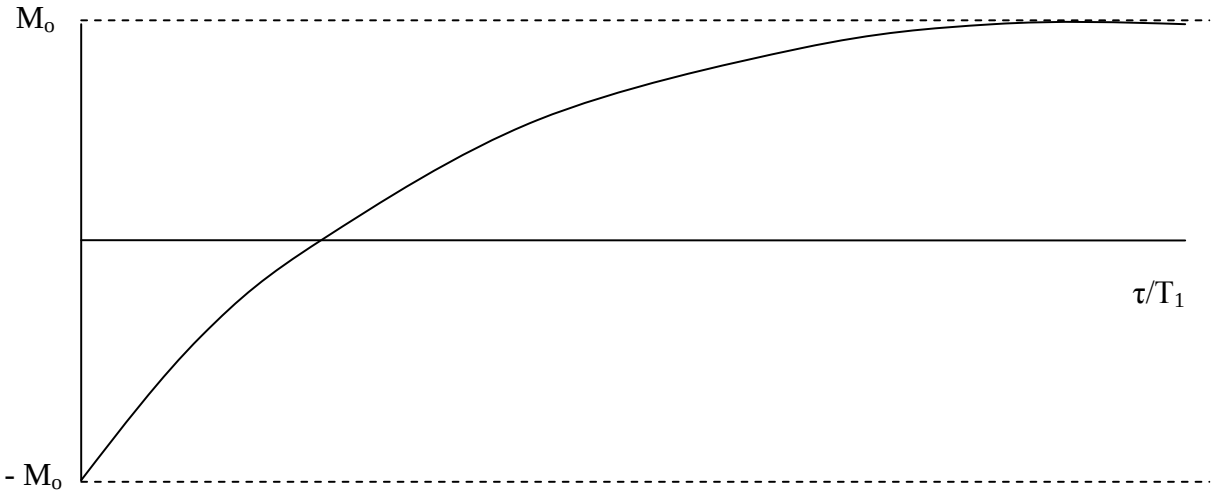
Spectrometer settings :
- Selection knob #1 set to IR
- Select the appropriate repetition time to maximize the signal.
- Adjust the receiver gain to an appropriate value
- Adjust the receiver phase to maximize the signal
- Use the setting for the 90° pulse obtained previously. In this experiment a 180° pulse and a 90° are used. Potentiometer #6 controls the first pulse (180° pulse) and potentiometer #5 is the second pulse (90° pulse).

Here the 180 - τ - 90 sequence is involved. The regrowth of the magnetic vector along the z-axis is given by :

$$M_z(\tau) = M_o \left[1 - 2 \cdot e^{-\tau/T_1}\right]$$

For the doped water sample, set up this sequence and record the FID following the 90° pulse for different values of τ, being careful to maintain good resonance throughout the experiment. Make sure the repetition time is at least $5 \cdot T_1$.

If the resonance was constant throughout the experiment, then each FID should have roughly the same T_2^* measured in section II. From each FID, extract the initial magnetization. These values plotted on a linear scale as a function of τ should yield the following graph:



Arrange the parameters in the above equation so that the linear slope of a semi-log plot of your results can be used to determine T_1.

Also determine T_1 using the zero-crossing method (use value of τ for which $M_z=0$ in the above equation to find T_1, using the above figure). How does it compare with the results obtained from the inversion-recovery experiment?

Using the inversion-recovery experiment determine T_1 for the 3 different samples.

Why is this sequence called the "inversion-recovery sequence"?

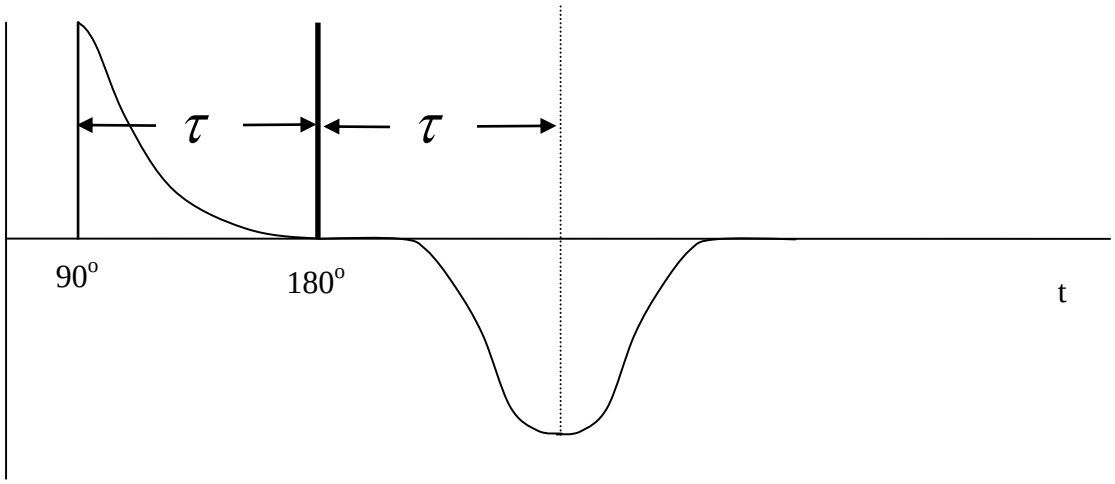
V. <u>Hahn Echo</u>

Samples : water, alcohol, rubber

Spectrometer settings :
- Selection knob #1 set to SE
- Select the appropriate repetition time to maximize the signal.
- Adjust the receiver gain to an appropriate value
- Adjust the receiver phase to maximize the signal
- Use the setting for the 90° pulse obtained previously. In this experiment a 90° pulse and a 180° pulse are used which are controlled by the potentiometers # 5 and #6 respectively. Potentiometer #5 is the first pulse (90° pulse) and potentiometer #6 is the second pulse (180° pulse).
- Before beginning the experiment, make sure the τ value is reset to 0 by adjusting the potentiometer labeled #7.

Set up a 90° pulse for the water sample (using the selection knob #1 set to FID). By now you may have noticed that every time you change samples you have to re-adjust your pulse length and reference phase.

Once you are set-up and have selected the selection knob #1 to SE, you should see the echo, which should look something like this :



Since the magnetization is being flipped to reform on the –Y axis, it will appear negative (i.e. 180° out of phase with a 90° FID). The echo will appear at $2 \cdot \tau$ after the 90° pulse, where τ is the time between the 90° and 180° pulse. Record the echo for various τ and extract the maximum amplitude of each echo from your data. The echo peaks decay according to the equation :

$$M(\tau) = M_o \cdot e^{-t/T_2}$$

Where $t = 2 \cdot \tau$. Find T_2 using the above equation for the 3 samples.

VI. <u>Freezing</u>

Samples : water

Spectrometer settings :
- Selection knob #1 set to FID
- Select the appropriate repetition time to maximize the signal.
- Adjust the receiver gain to an appropriate value
- Adjust the receiver phase to maximize the signal
- Adjust the pulse to maximize the signal, by using potentiometer #5. This is a 90° pulse.

Adjust the NMR signal appropriately to obtain an exponential FID. Now get the lab demonstrator to freeze the water sample in liquid nitrogen and return the sample tube to the spectrometer. What has happened to the signal? Explain.

REPORT

Your write-up should include a discussion of the theory as it relates to this experiment, equipment, comments on what you have seen, done, had trouble with, and an error analysis. Include all graphs.

When several methods for finding a particular quantity (i.e. T_1) have been mentioned in the experiment, you will be expected to compare them. Can you make any suggestions about the systems under investigation (water, alcohol, rubber) from a comparison of T_1 or T_2 values in the given samples? (i.e. Discuss how the relaxation times relate to the state or phase of a sample, to the relative rates of molecular motions in a sample and to the homogeneous or inhomogeneous nature of a sample. In this discussion you may find it useful to compare T_1 and T_2 between different samples as well as T_2 to T_1 of a particular sample.) If you have trouble getting readings for a given sample using a particular technique, you will then comment on your difficulties and the error involved.