Nuclear Magnetic Resonance – Part II Experiment

Aim

To understand the basic principles of nuclear magnetic resonance (NMR) by measuring the characteristic relaxation properties of a number of samples then identifying an unknown sample using fluorine NMR spectroscopy.

Suggested Schedule

Monday, Tuesday:	Familiarise yourself with the machine. Tune to resonance using a sample of mineral oil, obtain 90° and 180° pulses, and learn how to measure T_1 and T_2.
Wednesday, Thursday:	Locate the 'sweet spot' of the magnet. Measure T_1 and T_2 of mineral oil.
Friday onwards:	Compare your results for T_1 and T_2 to those that you measure for $CuSO_4$ and Glycerol solutions; investigate trends including sample concentration, temperature etc. Investigate other potentially interesting samples. At some point in week 2, you will spend a day or two on the PS2/B machine and perform NMR spectroscopy on the available samples. Identify the samples using the chemical shifts you have obtained.

Warnings

DO NOT PLACE ANY METALLIC OBJECTS INSIDE THE MAGNET.

DO NOT FORCE NMR TUBES INSIDE THE MAGNET – IF IT DOES NOT FIT EASILY THEN CHECK YOU HAVE THE CORRECT SIZE TUBE

N.B. Please report any typos or errors to Dr Bohndiek via email: seb53@cam.ac.uk

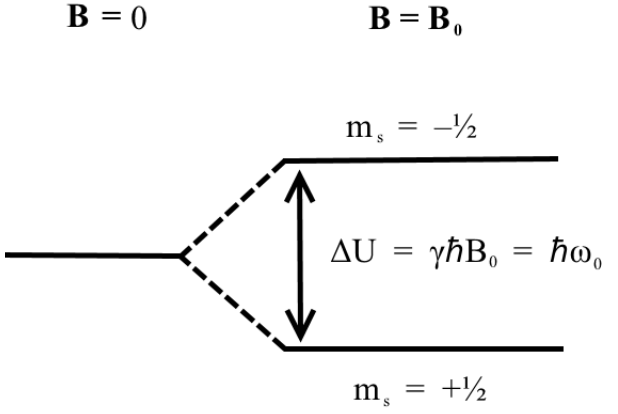
Part I

Theoretical background

Consider a nucleus with angular momentum **J** and magnetic moment **μ** = **γJ**, where **γ** is the gyromagnetic ratio. The nuclear angular momentum **J** is quantized in units of Planck's constant $\hbar$ as **J** = $\hbar$**S**, with **S** the spin of the nucleus.

The internal energy of this nucleus changes by U = $-\boldsymbol{\mu}\cdot\mathbf{B}$ when it is placed in an external magnetic field **B**; for a z-pointing magnetic field, this magnetic energy is:

$$U = -\mu_z B_0 = -\gamma\hbar m_s B_0 \qquad (1)$$



We will be dealing with hydrogen and fluorine nuclei, both of which have spin S = ½; m_s can therefore take two values: ±½. The energy separation between these two states in the external field **B** is:

$$\Delta U = \gamma\hbar B_0 = \hbar\omega_0 \qquad (2)$$

In a macroscopic sample containing many such nuclei, thermal equilibrium is given by a Boltzmann distribution. The number of nuclei in the m_s = +½ state will therefore be larger than in the m_s = −½ state, resulting in a net magnetisation aligned with the external field **B**. This magnetisation does not appear instantaneously, but instead grows exponentially upon application of the external field. The rate of change of z-aligned magnetisation M_z is given by:

$$\frac{dM_z}{dt} = \frac{M_0 - M_z}{T_1} \qquad (3)$$

where **T_1 is the spin-lattice relaxation time** and M_0 is the equilibrium magnetisation value.

The decay of any net x-y magnetisation as a sample returns to equilibrium obeys the differential equation:

$$\frac{dM_{xy}}{dt} = \frac{M_0 - M_{xy}}{T_2} \qquad (4)$$

where **T_2 is the spin-spin relaxation time**.

Q: Why are T_1 and T_2 be referred to as 'spin-lattice' and 'spin-spin' respectively?

You will measure both T_1 and T_2 using pulsed NMR. This involves applying short bursts (pulses) of a rotating magnetic field to the sample. As discussed above, the sample acquires a net magnetisation when inside an external magnetic field. The short magnetic pulses you will apply are used to manipulate this net magnetisation.

The response of the magnetisation to these short pulses can be modelled both classically and quantum mechanically; both approaches yield the same result (see AQP lectures). For simplicity,

we will discuss the classical result: the net magnetisation precesses around the applied magnetic field at angular frequency $\omega_0 = \gamma B_0$.

The magnetic pulses mentioned above (henceforth referred to as B_1) rotate around the z-axis at an adjustable frequency. Consider a Cartesian reference frame, described by x*, y* and z, rotating around the z-axis; this frame rotates at ω_{rf} such that the magnetic pulse B_1 is stationary within it (always aligned with, say, the x*axis). Initially the rotating field B_1 is not applied; in the lab reference frame the net magnetisation precesses around B_0 at angular frequency ω_0. In our new reference frame this magnetisation precesses at $\omega_{eff} = \omega_0 - \omega_{rf}$, implying that there is a new effective z-pointing magnetic field given by:

$$B_{z,eff} = \frac{\omega_0 - \omega_{rf}}{\gamma} \quad (5)$$

If the rotational frequency of B_1 is adjusted such that $\omega_{rf} = \omega_0$, this z-pointing effective field vanishes, and the system is said to be at **resonance**.

When B_1 is applied, the net magnetisation precesses around the x* axis. By applying this field for a finite time, the magnetisation can be rotated by any angle. A burst of B_1 that results in the net magnetisation lying along the y* axis is known as a **90° pulse**. Applying B_1 for twice as long results in a **180° pulse**; the resulting net magnetisation points in the negative z direction. These pulses are used to measure T_1 and T_2.

The Experiments

T_1 measurement: 180° - τ - 90°

T_1 characterises the return to thermal equilibrium of the z-pointing magnetisation, and is measured as follows:

(a) Apply 180° pulse: the magnetisation is rotated to point in the negative z direction.
(b) Wait time τ: the magnetisation begins to return to its equilibrium value for a time τ.
(c) Apply 90° pulse: the magnetisation is rotated from the z-axis onto the y*axis.
(d) Record the magnetisation in the x*-y* plane

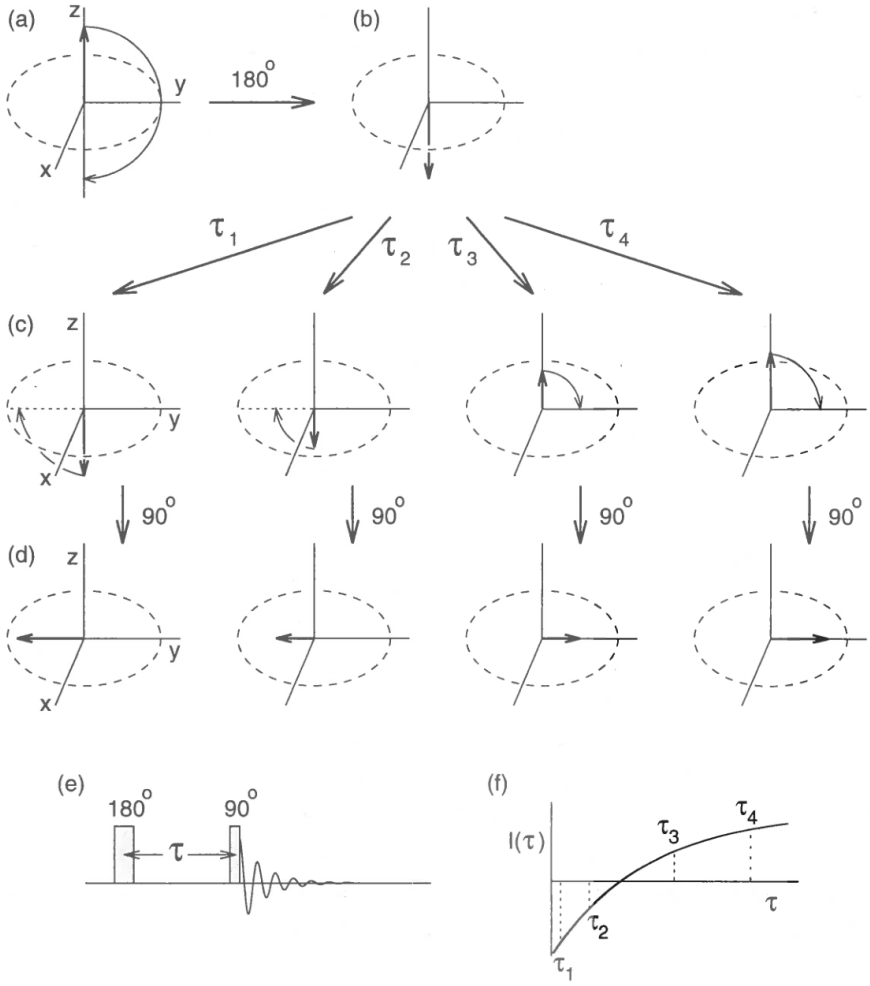


Figure 1 – T_1 measurement procedure
(a)-(d) see narrative, (e)the pulse sequence,
(f) the observed NMR signal intensity I(τ) as a function of the delay (τ)
(Hore, P. J. Nuclear Magnetic Resonance. 1995, page 79)

The instrument can only detect magnetisation precessing in the x-y plane. The 90° pulse brings the z-magnetisation into this plane so that its return to equilibrium can be measured.

Q: Can you think of another way of measuring T1?

T_2 measurement: 90° - τ - 180° - 2τ - 180° - 2τ - 180° etc.

T_2 characterises the return to thermal equilibrium of the magnetisation in the x-y plane, and is measured as follows:

(a) Apply 90° pulse: the magnetisation is rotated into the x-y plane.
(b) Wait time τ: the excited spins dephase rapidly during this time.
(c) Apply 180° pulse: the magnetisation in the x-y plane is rotated around x* back into the x-y plane.
(d) After a time τ a "spin-echo" is measured.
(e) After another time τ (2τ from last pulse) another 180° pulse is applied. 180° pulses separated by 2τ continue to be applied.

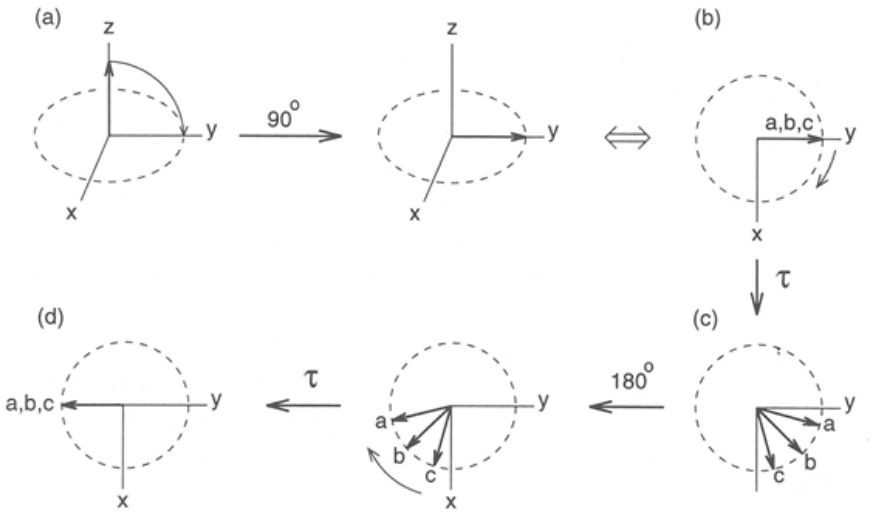


Figure 2 – T_2 measurement procedure
(a)-(d) see narrative
(Hore, P. J. Nuclear Magnetic Resonance. 1995, page 80)

After being brought into the x-y plane, the spins are initially in phase. This coherence decays rapidly. In most samples you will investigate, this decay is not primarily caused by spin-spin relaxation. The dominant decay mechanism is characterised by an additional decay time T_2*, which arises from inhomogeneities in the permanent field B_0. If B_0 varies across the sample then so too does ω_0. As a result of this varying rate of precession, each localised region of B_0 comes out of phase with its neighbours, and the net magnetisation in the x-y plane tends to zero. In order to measure T_2 instead of T_2* you will use the Carr-Purcell-Meiboom-Gill pulse sequence described above and shown in Figure 2.

The 180° pulse in this sequence produces "spin echoes" as seen in (c) and (d) in figure 2. In the above Figure there are three sample regions (a, b, and c) in different magnetic fields; the magnetisations of these three regions are originally aligned but slowly dephase as they precess

at different rates. Region c is shown to be precessing at the highest rate and must therefore be in the strongest magnetic field. After the magnetisations have dephased for a time τ, a 180° pulse is applied which rotates all three magnetisations around the x axis and, as shown in (c), results in the fastest precessing magnetisation lying 'behind' the others. As time passes the faster magnetisations 'catch up' with the slower ones and, after a time τ, all re-align along the negative y axis. This coming back into phase causes a second signal known as a spin echo. Once the spin echo has occurred, the magnetisation once again begins dephasing. Another 180° pulse is applied and another spin echo is formed. This is repeated to produce a long chain of such signals; the decrease in amplitude of subsequent spin echoes is characterised by T_2 rather than T_2^*.

Further reading

There are many very good textbooks covering NMR. Below is a small list of books that you may find helpful:.

- Hore, P. J. *Nuclear Magnetic Resonance*. (Oxford Chemistry Primers 32, 1995) – A very good introduction to the topic with clear explanations of the pulse sequences used. Also contains information on why different samples have different relaxation times.
- Keeler, J. *Understanding NMR Spectroscopy*. (Wiley-Blackwell, 2010) – A good overview of the theory discussed above, as well as a discussion of spectroscopy.
- Sanders, J. K. M. & Hunter, B. K. *Modern NMR Spectroscopy: A Guide for Chemists*. (Oxford University Press, 1993) – Another discussion of magnetisation, precession, and the rotating frame of reference.
- Derome, A. E. *Modern NMR Techniques for Chemistry Research*. (Pergamon Pr, 1987) – A more detailed in-depth discussion of the practicalities of performing 'real' NMR experiments, and the associated errors.
- Graaf, R. A. de. *In Vivo NMR Spectroscopy: Principles and Techniques.* (Wiley-Blackwell, 2007) – Another summary of the basic theory. This book also contains a detailed explanation of the tuning and matching procedure performed in the spectroscopy part of the experiment.
- Gadian, D. G. *NMR and its Applications to Living Systems*. (Oxford University Press, 1996) – Yet another overview of the theory. Also contains a description of the molecular mechanisms that control T_1/T_2.
- Slichter, C. P. *Principles of Magnetic Resonance*. (Springer, 1996) – A much more rigorous mathematical overview of all of the relevant theory. This level of detail is not expected from you, but explore this if you are interested.

The Instrumentation

You will be using two machines: PS1/A (metal) and PS2/B (wood). The PS2/B is an updated version of the PS1/A and will be used in the second week for spectroscopy. Both of these machines operate on the same principles and can be represented in block diagram form as:

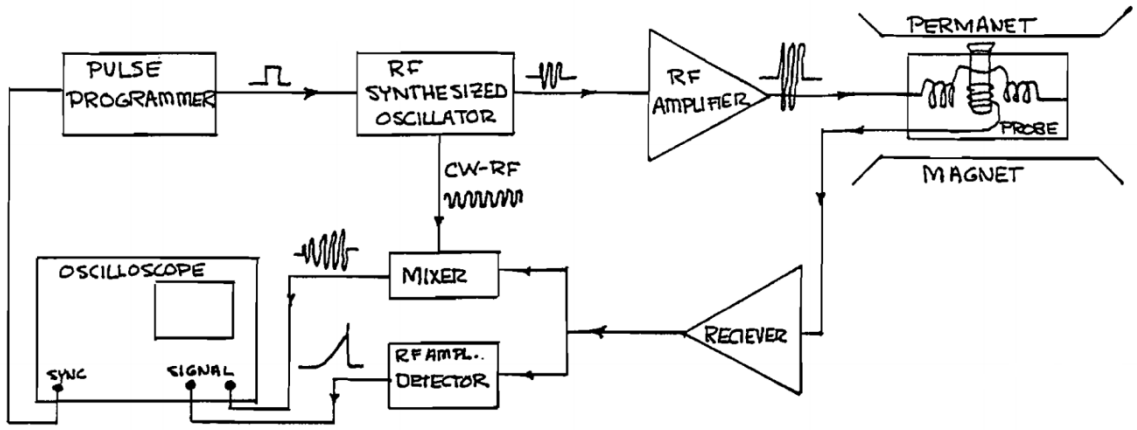


Figure 3 – Block diagram of the TeachSpin pulsed NMR instruments (TeachSpin PS1/A Manual)

Samples are placed in NMR tubes inside the permanent magnet, and acquire a net magnetisation aligned with B_0, the magnetic field of the magnet. In the PS1/A, the sample's position within the magnet can be changed; the dials on the front and side of magnet control the vertical and horizontal positions respectively.

The pulse programmer triggers radio frequency current bursts in the transmitter coils. These coils produce a rotating magnetic field B_1 perpendicular to the permanent field B_0. As discussed above, the net magnetisation of the sample can be rotated into the x-y plane by applying B_1 for a short, precise burst (known as a 90° pulse). The precession of any net magnetisation in the x-y plane is detected by the receiver coil.

For most experiments you will only need three outputs from the instrument:

RF amplitude signal: [Detector out on PS1/A, Env. Out on PS2/B] This signal is proportional to the peak amplitude of receiver coil signal. This is used to observe the magnetisation in the x-y plane.

Mixing signal: [Mixer out on PS1/A, Q Out on PS2/B] The frequency of this signal gives the difference in frequency between the applied RF pulse and the sample's magnetisation precession frequency. When the system is operating at resonance these two frequencies are the same and the mixing signal should resemble the RF amplitude signal, i.e. it shouldn't contain any 'beats'.

Sync. signal: [Sync Out on both PS1/A and PS2/B] This signal takes the form of a short top-hat signal whenever the pulse programmer triggers a

pulse. This signal should always be connected via a monostable to the Ext. input of the PicoScope, and any triggering in the PicoScope software should be done on this channel.

These signals are fed into a PicoScope and recorded using the PicoScope software. The software is freely available online if you wish to bring your own laptop.

Getting started

You cannot break anything by changing settings or flipping switches, although it may make a loud beeping noise if you trip the electronics. Power cycle the system if this happens.

The aim of these first couple of days is to learn how to operate the instrument and optimise your measurements. You can and should play around! If you unplug cables, make a note of the original connections.

Don't forget to think about errors!

Familiarisation

Start by investigating a 30-70µL sample of mineral oil; smaller samples yield weaker NMR signals whereas larger samples are more affected by B_0 inhomogeneities. Pipette the mineral oil into an NMR tube, seal the tube with a bung, and place an O-ring near the top. The O-ring stops the tube from falling into the magnet, and its position affects the sample height in the magnet.

Place the sample inside the magnet, and connect Detector out to PicoScope channel A and Mixer out to PicoScope channel B. On the pulse programmer, switch A ON and B OFF to apply a single 'A' pulse.

- Adjust the frequency and observe the effect on the Mixer out signal, keeping in mind (as detailed in the instrument section above) what this signal shows you. Attempt to 'tune to resonance'.
- Adjust A width. This changes the length of time for which the magnetic pulse is being applied. *Remembering that the output of Detector out is proportional to the magnetisation* ***in the x-y plane****, can you produce a 90° pulse? What about 180°? 270°?*
- Adjust the position of the sample using the controls on the side and front of the magnet. *Does this have an effect on the signal you observe? Are there positions which are 'better' than others? What makes these 'better'?*
- Adjust the Repetition time. This changes the time between a pulse sequence (in this case only one 'A' pulse) starting and it being repeated. *What effect does this have on the Detector out signal? How might you select and appropriate repetition time?* Remember that the magnetisation does not return instantly to equilibrium.

Now switch B ON and set Number of B pulses to 1. The Delay time (referred to as τ above) is the time between pulse A and pulse B.

Can you set up a method to measure T_1 and T_2 by using the descriptions above? Do not worry about recording your data at this stage, as you will need to redo these measurements more precisely.

Magnet sweet-spot

Before taking any precise measurements of T_1 and T_2, you need to find your magnet's 'sweet spot'. You could do this either by eye or by making a map of your magnetic field. How might you measure the magnetic field?

It is worth spending some time on this step, as any future measurements you take will depend on the homogeneity of the magnetic field around your sample. When you feel confident in your ability to take precise measurements of T_1 and T_2, you can move on. For the rest of your time doing this experiment you should always ensure that before taking any measurements:

- You are tuned to resonance.
    - The resonant frequency of each sample will drift over time, and different samples may have different resonant frequencies.
- Your repetition time is long enough.
    - Short repetition times do not allow the system to return to thermal equilibrium in between magnetic pulses. How might you decide how long is long enough?

T_1 : 180° - τ - 90°

Now move on to measuring T_1 of mineral oil. Solve the differential equation (3) above to find a relationship between τ, M, and T_1.

Tune your system to resonance and set up pulse A to be 180° and pulse B to be 90°. Vary τ and measure the resulting magnetisation after the 90° pulse. You should be recording:

1. M_0, the equilibrium magnetisation value – this is proportional to the peak voltage following a single 90° pulse. *Does this make sense?*
2. M, the magnetisation after a time τ – this is proportional to the peak voltage following the 90° pulse that follows your 180° pulse. Remember that some of your recorded magnetisation values should be *negative*.
3. τ, the delay time.

Think carefully about how you record 1-3 and what the errors are on these measurements. *Is the systematic or random error larger?*

Your system will drift away from resonance over time, and you should therefore try to take each set of measurements in as short a time as possible. You may also find that your measurements are temperature dependent*. Can you think why this might be?*

Your measured value of T_1 should be roughly **20-40ms**.

T_2 : 90° - τ - 180° - 2τ - 180° - 2τ - 180° etc.

You should now measure T_2 for mineral oil. Solve equation (4) to find the relationship between τ, M, and T_2. Set up the pulse train needed to measure T_2 and select an appropriate value of τ. If τ

is too short, the signals from adjacent spin echoes may overlap and interfere with each other, masking the true value of the peak signal. Increasing τ reduces this error but also reduces the total number of spin echo data points. You should use a delay time τ which gives a satisfactory (to you) balance of data quantity and quality.

Once you have set up your pulse train, save the waveform using the PicoScope software. You should record the magnetisation (peak height) of each spin echo along with the time elapsed between it and the 90° pulse. Use your solution to equation (4), together with the magnetisation following the first 90° pulse (giving M_0), to find a value for T_2.

Now, with your pulse-train set up, turn the Meiboom-Gill setting off. *What differences do you observe in your PicoScope trace? Try and find out what is happening and what the Meiboom-Gill switch is doing.*

Your measured value for T_2 should be roughly **10-50ms**.

Further experiments

Once you feel confident in your ability to measure T_1 and T_2, you can investigate how these relaxation times vary between different types of samples. Consider the most effective method for recording and analysing these data based on your initial experience. You should investigate any trends you find between T_1/T_2 and:

- Glycerol concentration
- $CuSO_4$ concentration
- Sample temperature

You may also want to investigate how ambient temperature and resonance drifts affect your measurements.

If you manage to finish all of the above, feel free to bring in and investigate any other samples. Students in the past have had success with shampoo, hair gel, olive oil etc.

Q: What are the fundamental limits on T_1 and T_2? How are these parameters used in the "real world"?

Part II

Spectroscopy

In practice, NMR is most widely used as a spectroscopic tool to identify and analyse the composition of chemical samples. Up until now you have been investigating macroscopic properties (T_1/T_2) and have been treating each sample as a 'blob' that is homogeneous but distinct from other samples. In reality, different nuclei within a molecule may be surrounded by varying degrees of electron density. This electron density shields the nuclei from external magnetic fields and, therefore, as the electron density varies, so too does the magnetic field that the nuclei experience. This results in one molecule having multiple resonant frequencies within it. It is these differences in resonant frequency, known as chemical shifts, which are measured and used in NMR spectroscopy to characterise the chemical composition of samples.

The variation in the magnetic field across a molecule is generally of the order of 1 part in 10^6, and a strong and homogeneous magnet is required to resolve the chemical shifts. The PS1/A that you have been using does not satisfy these criteria, and you will therefore use an updated instrument, the PS2/B. Other than a stronger permanent magnet, the PS2/B has 3 features not present on the instrument you have used so far:

Field homogeneity: Instead of changing the sample position to find the permanent magnet's 'sweet spot', the PS2/B has a fixed sample and four field gradient coils. These coils produce either linear (x, y, z) or quadratic (z^2) field gradients around the sample, the magnitude of which is controlled by the dials on the PS2 Controller module. The magnetic field around the sample can be homogenised by adjusting these gradients (a process known as shimming).

Temperature stability: The magnet is made up of two NdFeB disks, one on either side of the sample; each disk is surrounded by a thermoelectric cooler which acts to fix its temperature. The cooler is controlled via the PS2 Controller module. The setpoint temperature (i.e. the temperature the cooler will maintain) is controlled by the dials labelled Right/Left magnet temperature; the adjacent LEDs will shine red or green if the setpoint is higher or lower (respectively) than the current magnet temperature. When the setpoint matches the current temperature, the LEDs will turn off. At this point the Feedback loops should be closed to engage the stabilising mechanism. The heat sinks for the coolers are incorporated into the sides of the magnet. **Do not leave objects resting against the sides or place warm objects (like hands) on them during important measurements.**

Tuning and matching: The magnetic pulses are produced by transmitter coils around the sample. The transmitter coils can be thought of as LCR circuits being driven by a time alternating voltage of frequency ω_{rf}. The current within these coils is maximised when the driving frequency ω_{rf} is equal to the circuit's resonant frequency ω_{res}, which itself is

dependent on the inductance and capacitance of the RF coil. The tuning capacitor adjusts the capacitance of the RF coil such that ω_{rf} can be matched to ω_{res}. The RF coils are connected to the signal generator on the PS2/B by 50Ω coaxial cables, and the matching capacitor acts to match the impedance of the RF coil to the 50Ω coaxial cable. The role of these two capacitors is explained in much further detail in the de Graaf book listed under "Further reading" on the first handout.

You will use this instrument to find the fluorine spectra of compounds A and B, and then use these spectra to identify the two compounds.

Q: Why are these three factors important for making NMR spectroscopy measurements?

Warnings

BE CAREFUL WITH THE TUNING/MATCHING CAPACITORS ON THE PS2/B MAGNET, THEY ARE FRAGILE.

DO NOT LEAVE OBJECTS RESTING AGAINST THE SIDES OF THE PS2/B MAGNET.

DO NOT LEAVE THE THERMAL CONTROL LOOPS ON THE PS2/B MAGNET CLOSED OVERNIGHT.

The Experiment

As discussed above, each chemical environment within a sample has its own distinct resonant frequency. The RF amplitude signal is made up of these frequencies, and its frequency spectrum contains a peak for each chemical environment. On the PS2/B instrument these fluorine resonant frequencies are roughly 20MHz and are separated from each other by at most a few kHz. This scale renders it impossible to accurately distinguish the peaks.

To see how we resolve this problem, consider the frequency components of the mixing signal (the product of Env_Out and $\sin(\omega_0)$). Begin by Fourier decomposing Env_Out; the result is a sum of sine waves, each one oscillating at the resonant frequency of a specific chemical environment. Now multiply each sine component by $\sin(\omega_0)$, recalling that the product of two sine waves is:

$$\sin(\omega_0)\sin(\omega) = \frac{1}{2}(\cos(\omega_0 - \omega) - \cos(\omega_0 + \omega)) \quad (6)$$

Each chemical environment, with its distinct value of ω, contributes these cosine terms to the mixing signal.

You can now see that we have solved our problem; the first cosine term oscillates at the frequency difference we are interested in. The frequency spectrum of the mixing signal contains a peak for each chemical environment just like that of Env_Out, but this time the frequencies in question are low (a few kHz) and can be easily distinguished.

These frequency differences scale linearly with ω_0; stronger permanent magnetic fields result in larger resonant frequencies (see equation 2) and therefore larger frequency shifts. To compare data from different instruments and magnetic fields the frequency differences are converted into dimensionless chemical shifts:

$$\delta = \frac{frequency\ difference \times 10^6}{resonant\ frequency} \quad (7)$$

As well as finding the spectra (in dimensionless units of chemical shift δ) for compounds A and B, you will also obtain the spectrum for a reference compound (C_6H_5F). The spectrum of this reference compound can be used to calibrate the data you obtain with online tabulated fluorine chemical shifts. This will enable you to identify the samples measured.

Because you have limited time on this machine, what follows is a very detailed step-by-step guide on how to obtain the spectra that you need.

When you arrive in the morning, turn on the temperature control on the PS2 controller:

1. Ensure both loops are OPEN *[this means temperature control is off].*
2. Rotate the temperature dials until the error lights turn off. If red turn clockwise, if green turn anticlockwise. When both lights are off, flip both loop switches to CLOSED *[this turns on the temperature control].*
3. You may now need to wait up to 30 minutes for the magnet temperature to stabilise.

Initial setup

Adjusting the tuning capacitance to be close to resonance

Place 3 large O-rings around the RF probe. Fit a small O-ring after them (tight fit) and push the 4 rings as ar as they will go to the end of the probe. This makes the RF probe sit at the correct height inside the magnet.

1. Place RF probe into the sample holder and connect it to PicoScope channel A.
2. Turn on the instrument and change settings:
    - Frequency (F on synthesizer) to **21.67 MHz**
    - A_len (A on pulse programmer) to **3.1 µs**
    - Period (P on pulse programmer) to **400 ms**
    - Pulse A ON, Pulse B OFF (switches on a single pulse)
3. Should obtain pulse lasting 3-5µs. Maximise peak to peak amplitude of this pulse as follows:
    - PicoScope software:
        - Channel A ON, channel B OFF
        - Trigger to repeat. Trigger on Ext, trigger level at 200mV.
    - Adjust fine tuning capacitor (FTC) (screw on the magnet closest to the sample on the side of the grey cable. **Make sure to use the yellow plastic screwdriver**) to maximise signal. If maximum not obtained within 3 turns of the screw proceed to:

    - Adjust coarse tuning capacitor (CTC), which is the 3rd screw from the sample towards the grey cable. Do not unscrew all the way! Needs minimal screwing and is fragile. Once roughly found maximum, repeat above process with FTC.
- Should obtain ~40V peak-to-peak signal. If this is not achieved, check the height of the RF probe inside the magnet.

Setting the instrument up for a measurement

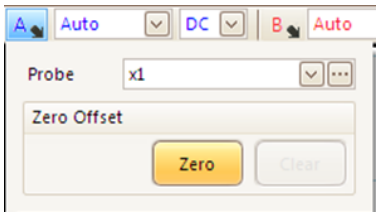
1. Change settings from default:
    - Synthesizer
        - F (Frequency) to **21.67 MHz** *[this begins tuning to proton resonance]*
        - P (Ref Phase) to **-180°** *[changes the phase of Q_Out (matching signal) so that it overlaps with Env_Out (sample signal) for easier tuning to resonance]*
    - Pulse programmer
        - A (A_len) to **3.1 µs** *[preliminary 90° pulse]*
        - B (B_len) to **6.2 µs** *[preliminary 180° pulse]*
        - N (Num_B) to 1 *[number of B pulses – require one 180° pulse]*
        - P (Period) to **500 ms** or higher *[equivalent to repetition time on PS1/A]*
        - A pulse ON, B pulse OFF (switches under selection knob)
    - Receiver
        - Filter TC (ms) to **0.01**
        - Band to **p** *[sets bandpass filter in instrument to proton frequency]*
        - CW In disconnected
        - Pulsed RF In connected to Pulsed RF Out
    - PicoScope hardware
        - Env. Out (on Receiver module) → channel A on PicoScope
        - Q Out (on Receiver module) → channel B on PicoScope
        - Sync Out (on Programmer module) → Monostable → Ext. on PicoScope
    - PicoScope software
        - Channel A ON, Channel B ON
        - Trigger to Repeat *[In Auto mode, if a trigger event does not occur within a given timeframe, the PicoScope records data regardless. This becomes an issue for longer repetition times. Repeat mode waits for a trigger event before recording]*
        - Trigger on Ext. (ensure Sync out → Monostable → Ext on PicoScope) *[Triggers on the Sync out signal which is synchronised with the applied pulses. Can adjust the Sync switch on the instrument to trigger on A or B pulse]*
        - Trigger threshold to 200 mV
2. Prepare sample
    - Place **50µL** of mineral oil into an NMR tube

- Position the bottom of a small O-ring **10.3 mm** from top lip of glass tube *[this ensures the sample lies in the centre of the magnet]*
- Place sample in magnet. Adjust height of the sample (by changing O-ring position) such that the FID (channel A signal) is maximised.

3. Refine settings
    - Field gradients
        - Adjust gain (on receiver) such that FID peaks at just under 1V.
        - Change frequency (on synthesizer) until Q_Out matches Env_Out (i.e. no beats in Q_Out). If you cannot get Q_out to resemble Env_Out you may need to adjust the coarse matching capacitor. This is the screw closest to the blue cable (symmetrically opposite to the coarse tuning capacitor). Adjust the coarse matching capacitor until the Q_out mixing signal has a positive peak at the same time as the FID peak.
        - Adjust the field gradients on PS2 controller from left to right to maximise area under the FID. +/- changes the sign of the gradient. Repeat this process from left to right at least 3 times.
        - Re-tune the frequency until there are again no beats in Q_Out (may not be necessary) *[the field gradients change B field of magnet and therefore also change the resonant frequency]*

Steps needed to perform NMR Spectroscopy

1. Adjusting tuning
    - Multiply the proton resonant frequency above by **0.9408**. This is the fluorine nucleus resonant frequency. Change F (frequency) to this calculated value. *[the ratio of the fluorine to proton gyromagnetic ratios is 0.9408. Since ω = γB, this is also the ratio of the resonant frequencies of the two nuclei]*
    - Switch Band from **p** to **f** on Receiver *[this changes the bandpass filter to the fluorine NMR frequency]*
    - Insert the RF probe, connect to PicoScope, and adjust tuning capacitors as before to maximise peak-to-peak signal. You will need to CAREFULLY adjust the coarse tuning capacitor (CTC).
2. Field homogeneity
    - Insert 50µl of either sample A or B into the magnet
    - Connect Env Out to channel A
    - Apply 3.1µs pulse and iteratively adjust the field gradients (as before) to maximise the FID.
        - The FID will have beats/oscillations and not be a clean exponential decay. This is normal. When adjusting the field gradients, it is easiest to look for effects in the 'tail' of the FID.
3. Precise impedance matching *[the motivation for this procedure is described inthe instrument manual under "CW experiments"]*
    - Receiver
        - Disconnect Pulsed RF In from Pulsed RF Out
        - Connect CW Out (on Synthesizer) to CW In on Receiver
        - Change Filter TC dial from 0.01ms to **3.3ms**
    - Synthesizer
        - Change A (CW Power) to **-65 dBm**
        - Switch CW Out to ON
    - PicoScope hardware
        - Disconnect both channel A and B. Enter PicoScope software and zero both channels A and B:



Then connect:

- I Out → Channel A on PicoScope
- Q Out → Channel B on PicoScope
- Monostable → Ext on PicoScope

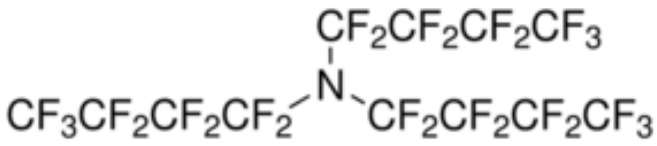
- PicoScope software
    - Turn off triggering
    - Turn on both channels A and B
    - Views dropdown menu → X-axis → A

- Iteratively adjust (fine) tuning and (coarse) matching capacitors until 'spot' on PicoScope is as close to 0 on both axes as possible. Can easily become trapped in local minima; if you become stuck far from the origin, move away from the minimum and try again. The matching capacitors are located on the other side of the sample as the tuning capacitors. They have the same appearance and 'mirrored' locations.
- Increase A (CW Pwr) on the Synthesizer in 5dBm increments and repeat the iterative matching process above. Repeat up to roughly -40dBM.

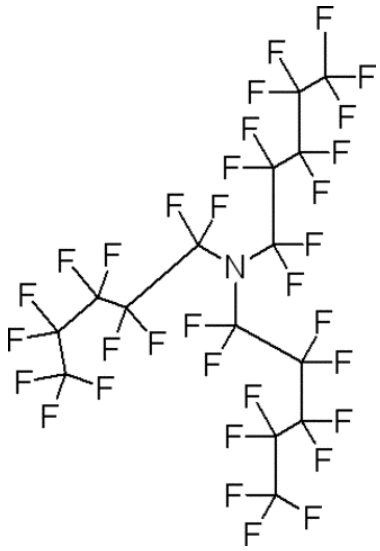
4. Obtaining spectra
    - Receiver
        - o Disconnect CW In from CW Out
        - o Connect Pulsed RF Out (on Synthesizer) to Pulsed RF In on Receiver
        - o Change Filter TC dial from 3.3ms to **0.01ms**
    - Synthesizer
        - o Switch CW Out to OFF
    - PicoScope hardware
        - o Env_Out → Channel A on PicoScope
        - o Q_Out → Channel B on PicoScope
        - o Monostable → Ext on PicoScope
    - PicoScope software
        - o Views → X-Axis → Time
        - o Trigger to Repeat
        - o Trigger on Ext.
        - o Trigger threshold to 200 mV
        - o Channel A OFF, Channel B AUTO
        - o Change from Scope Mode to Spectrum Mode (top left of screen)
        - o Change spectrum range to 3.052 kHz
    - You should now obtain a spectrum for the sample you have placed in your magnet.

You can now change your sample from mineral oil to the fluorine samples. Without adjusting any settings, compare different spectra in real time. *What are the differences between the spectra of samples A and B?*

Record the spectra of the two unknown chemicals as well as that of 50μL of the reference compound (C_6H_5F), and convert them into dimensionless units as described above. Overleaf are the structures of two chemicals C and D. *Can you match them up to your samples A and B?*



Chemical C



Chemical D

Once you have identified which is which, *can you match each peak in the spectra to its corresponding chemical environment in the molecules?* You should be able to find tabulated chemical shifts online; *are your chemical shifts comparable to what you find?* Think about how you would convert your values of δ into something useful using your reference compound. Note that your reference is only one of many currently in use. *Finally, do the chemical shifts you have measured agree with what you find online?* You might need to recall part IA Chemistry or read up on the effect nearby functional groups have on chemical shifts.