

EXPERIMENTS TO DO:

1. Measure T_1 relaxation time for ^1H – use 2% 2-ethyl-1-indanone in CDCl_3
2. Quantitative ^1H NMR – use 2% 2-ethyl-1-indanone in CDCl_3
3. Quantitative ^{13}C NMR – use 1M 2-ethyl-1-indanone in CDCl_3
4. Proton-Coupled ^{13}C NMR – use 1M 2-ethyl-1-indanone in CDCl_3

For students doing research, please feel free to bring your own samples from your own laboratory. For the ^{13}C NMR, you will want to use a sample concentrated enough to get a nice spectrum in ~10 minutes or less. Since we have cryoprobes on the Bruker instruments, this means ~5-10 mg of sample in 600 μL of solvent.

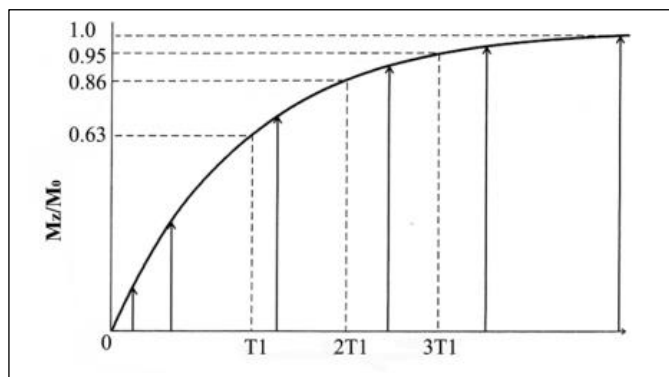
1. T_1 Relaxation Time: ^1H NMR

Introduction to T_1 Relaxation

1. Spin-lattice, or T_1 , relaxation is the mechanism by which the magnetization vector along the static field reaches thermal equilibrium with its surroundings. The T_1 relaxation time characterizes the rate at which the longitudinal M_z component of the magnetization vector exponentially recovers back to equilibrium, according to the following equation:

$$M_z(t) = M_z(0)\left[1 - \exp\left(-\frac{t}{T_1}\right)\right].$$

2. Thus, the time T_1 it takes for the longitudinal magnetization to recover is approximately 63% of its initial value after being flipped into the transverse plane by the first 90° pulse. $2 \times T_1$ and $3 \times T_1$ recover approximately 86% and 95% of the initial magnetization respectively.



After $5 \times T_1$, the magnetization will return to equilibrium (>99%). T_1 values are affected by dynamics in the system.

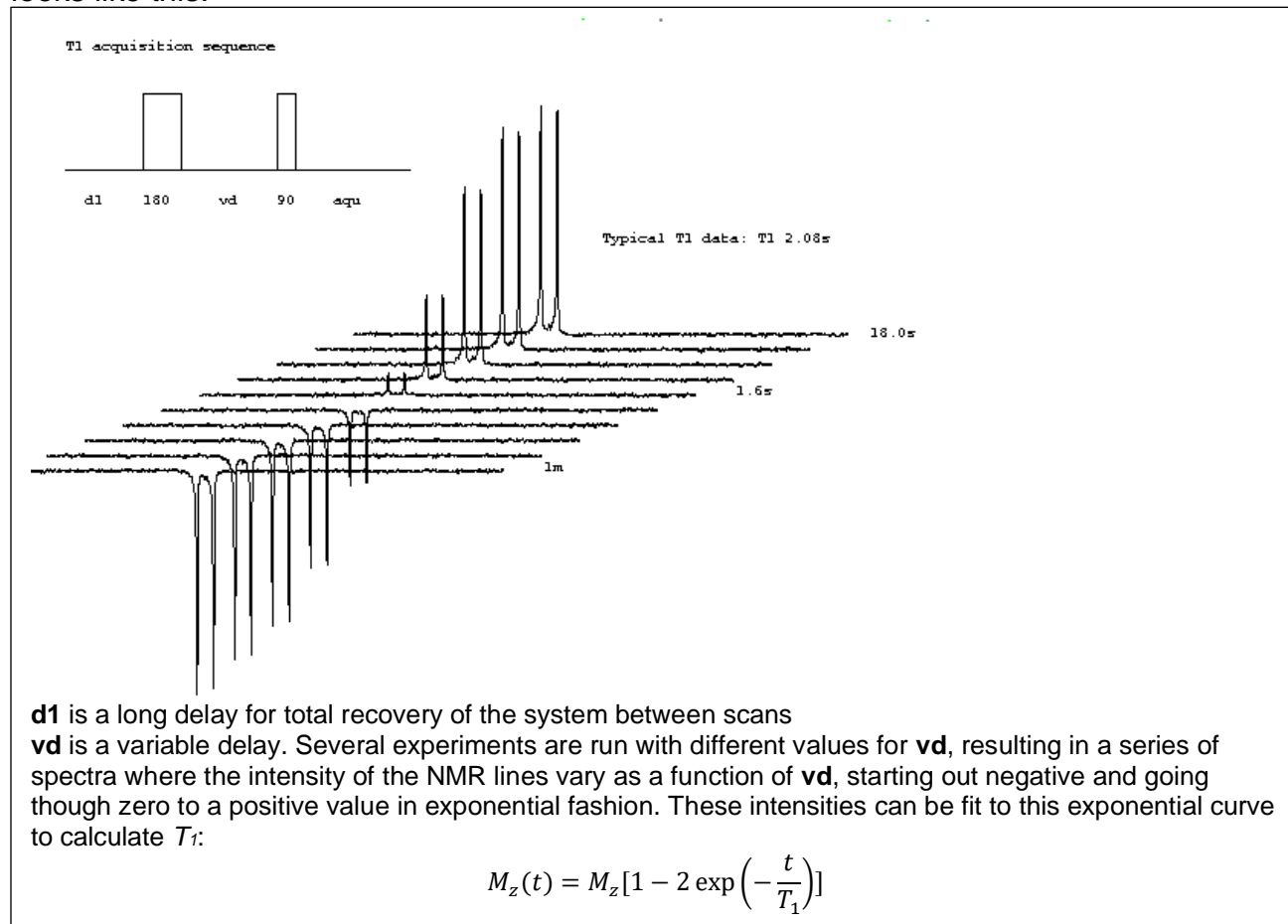
3. Why measure T_1 ? For example, T_1 determines the optimal repetition time for 1D acquisition to maximize SNR per acquisition time, as well as the mixing time for small molecule NOESY and EXSY experiments, and diffusion time for DOSY experiments. Most importantly, the accuracy for quantitative NMR (qNMR) measurements will depend on the repetition rate of the 1D acquisition. Optimal relaxation delay, depending on the parameters **d1** and **aq** for 1D qNMR measurements, is $\sim 5 \times T_1$ of the slowest relaxing resonance. This will minimize the measurement error to <1%.

4. Measuring T_1 quickly requires that you make an initial guess about the order of magnitude of the result. You must use this guess to select delay times for this measurement. If you guess well, only a small number of measurements will be needed to obtain a reasonable estimate. If you guess poorly, you will be able to tell from your initial result how to proceed with a new guess.

5. In this class, we will only run a "quick T_1 " inversion recovery (T1IR) measurement to save time. However, it will give you an idea of how the full inversion recovery experiment works. In the future, if you need to perform a full inversion recovery

experiment, it is a good idea to make a measurement with "quick T_1 " to make sure your initial guess is not way off.

The quickt1 experiment measures part of the inversion recovery. A truly accurate T_1 measurement requires fitting the T_1 constant to a full inversion recovery curve, which looks like this:



Quick T_1 Data Acquisition

FYI: The pulse program "quickt1" is designed for proton, but similar measurements could be done heteronuclei (with or without proton decoupling).

- 1) Type **new** to set up a new experiment and read in the proton parameters (**1h.av500** or **1h.av600**). Do the usual set-up routine: **lock** to chloroform, **atma** and tune the probe, **rsh LAST** and **topshim** in order to shim.
- 2) Before you start the T_1 measurement, you will need to calibrate the 90 degree pulse. You can use **pulsecal** for this if you'd like or do it manually.
- 3) You will need to change the **pulprog** to **quickt1.av500** or **quickt1.av600**.
- 4) Enter your calibrated 90° pulse as **p1**. You can check to make sure the 180° is correct. (**p2** should be **2xp1**).
- 5) Enter a small value for **d7**, the delay between pulses. 10 μ s should be OK.
- 6) Set the receiver gain by typing **rga**.

- 7) Change **ns** to 1 scan and type **zg** to acquire your data.
- 8) Process your data. Use **ef** for the apodization and Fourier transform. Rather than **apk**, manually phase the data with **.ph** so that the spectrum is upside down.
- 9) Vary **d7** and run the experiment again with **zg;efp**. Continue to adjust **d7** until the NMR lines of interest are nulled (it will look similar to the 180° or the 360° when you did the pulse calibration experiment last week).
 - If the desired peak is phased down, increase **d7** incrementally until you obtain a null signal (the signal is phased half up/half down)
 - If the desired peak is phased up, decrease **d7** until you obtain the null.
- 10) $T_1 \approx 1.44 \times d7$, where **d7** is the value that gives a null signal.

Note: Different types of protons have different T_1 relaxation times, so your aromatic protons relax at a different rate compared to your aliphatics.

Data Processing

For the homework assignment, please indicate the approximate T_1 relaxation time

2. Quantitative ^1H NMR

A quantitative NMR measurement requires appropriate values for the **d1** recycle delay (the time between scans) and a calibrated **p1** 90° pulse.

Note: In general, if you want to do quantitative NMR in which you get an absolute concentration, you will need an internal reference standard. If you use an insert for the reference standard, you need to account for the difference in volume of the solutions of the compound of interest vs the reference. We provide the qNMR advanced plug-in for MestReNova, which can handle this absolute quantitation in data processing.

In class, we will be performing a relative quantitation for the sake of time.

Quantitative ^1H NMR Data Acquisition

- 1) Type **new** to set up a new experiment and read in the proton parameters (**1h.av500** or **1h.av600**).
- 2) Set the pulse program **pulprog** to **zg**. (The default is **zg30**). Set **ns** to 8.
- 3) Set **p1** to your calibrated 90° pulse. Set **d1** to 5 x your approximate T_1 . Type **expt** to see how long the experiment will take. (Longer than the standard proton experiment, right?)
- 4) Set the receiver gain with **rga**, then **zg;ef;apk;abs n** to acquire and process your data.

Data Processing

**For the homework assignment, please integrate the same peaks you did as Lab 1. Compare the two—are they relatively similar?*

3. Quantitative ^{13}C NMR Spectrum

^{13}C NMR Spectrum – Data Acquisition

Most carbon NMR are qualitative experiments that give you information about the environment of the observed atoms, but do not provide an exact measure of how many

carbons are present. The two main reasons that a "routine" carbon NMR experiment is not quantitative are: 1) signal enhancement from the nuclear Overhauser effect (NOE), and 2) carbon T_1 relaxation times are often significantly longer than proton relaxation times. NOE is simple to overcome by changing your decoupler settings from **power-gated** to **inverse-gated**. To overcome the spin relaxation problem, a long relaxation delay (**d1**) during which all of the carbon nuclei in a molecule can return to the ground state is required. This can be a very long time, meaning that to ensure a reasonable experiment time a VERY concentrated sample is required. Additionally, a paramagnetic additive can be used to increase the rate of relaxation (in our case $\text{Cr}(\text{AcAc})_3$). The paramagnetic additives have an additional effect of decreasing the spin-spin (T_2) relaxation time, which broadens the NMR linewidths.

- 1) Insert the 1 M 2-ethyl-1-indanone sample. Type **new** to set up a new experiment and read in the carbon parameters (**13c.av500** or **13c.av600**). Do the usual set-up routine: **lock** to chloroform, **atma** and tune the probe, **rsh LAST** and **topshim** in order to shim.
- 2) **Lock** on to your deuterated solvent.
- 3) Tune the probe by typing **atma**.
- 4) Read in the default shim file: **rsh LAST**. Then run **topshim**.
- 5) Type **ased** to check your acquisition conditions. You can use **ns = 32**, **ds = 4**. The default pulse sequence is **zgpg30** (power-gated 30° pulse). Change the pulse sequence to **zgig** (inverse-gated, 90° pulse). Set **d1** to 15 seconds. This number would be MUCH larger without $\text{Cr}(\text{AcAc})_3$.
- 6) Due to its naturally low sensitivity (compared to ^1H), the receiver gain is already set to the maximum value. Do NOT type rga!
- 7) Type **zg** to acquire your data.
- 8) Process your data: **ef** apodizes and Fourier transforms the time domain data, **apk** automatically phase corrects the data, and **abs n** corrects the baseline for more accurate integrations.

^{13}C NMR Spectrum – Data Processing

Integrate this spectrum and compare it to your data from last week. **For the homework, briefly discuss the differences in integration ratios for the power-gated and the inverse-gated experiment.**

4. Proton-Coupled ^{13}C NMR Spectrum

^{13}C NMR Spectrum – Data Acquisition

- 1) Type **new** to set up a new experiment and read in the carbon parameters (**13c.av500** or **13c.av600**). You are already locked, tuned and shimmed, so you don't need to do those things.
- 2) Change the pulse sequence to **zg30** (it should have been set to **zgpg30** by default). **zg30** is a 30° pulse on ^{13}C before acquisition; **zgpg30** is a 30° pulse on ^{13}C before acquisition with power-gated decoupling.

- 3) Type **ased** to check your acquisition conditions. You can use **ns = 32**.
- 4) Due to its naturally low sensitivity (compared to ^1H), the receiver gain is already set to the maximum value. Do NOT type rga!
- 5) Type **zg** to acquire your data.
- 6) Process your data: **ef** apodizes and Fourier transforms the time domain data, **apk** automatically phase corrects the data, and **abs n** corrects the baseline for more accurate integrations.

^{13}C NMR Spectrum – Data Processing

****For the homework, plot the proton-coupled, proton-decoupled (both power-gated and inverse-gated) ^{13}C stacked horizontally. Briefly discuss the effect of each decoupling type (as well as no-decoupling) on the spectra. Why are these effects observed?*****

When you are done in the lab, make sure to log out. Type **CTRL + ALT + BACKSPACE** to log out of the computer.

What to turn in:

- Include a comparison plot where you stack the ^1H spectra of 2-ethyl-1-indanone from the "routine" ^1H (from lab 1) and "quantitative" ^1H (from this week). Make sure these are properly phased and the baseline has been corrected. Integrate the peaks and compare the "routine" vs "quantitative".
- Include a comparison plot where you stack the ^{13}C spectra of ethyl-indanone for each of the experiments: "routine" ^{13}C (with power-gated decoupling), "quantitative" ^{13}C (with inverse-gated decoupling), and proton-coupled ^{13}C . Make sure these are properly phased and the baseline has been corrected.
- Answers to the following questions:

Questions

1. What is the ^1H T_1 relaxation time of your sample?
2. We added a paramagnetic additive to our ^{13}C sample in order to reduce the relaxation time. Based on $d_1 = 15$ seconds, we make an assumption about what the ^{13}C T_1 is for our molecule. What is the assumed value of our ^{13}C T_1 relaxation time?
3. Broadly speaking, what are the steps to make a quantitative NMR measurement? Why is a "routine" NMR experiment not typically considered quantitative?
4. What is the difference between inverse-gated and power-gated decoupling? What is the effect of the different decoupling schemes (including no-decoupling) on your NMR spectra?