

Quantitative ^1H NMR

In this lab, you will experiment with parameters on the Bruker AC (Homer) spectrometer that, in particular, affect quantitative and kinetic NMR data. Start by looking at sections I and IV of Chapter 2 of the Bruker AC User Guide: **Bruker AC Acquisition Basics**, which outlines the basic one-dimensional (1D) proton experiment and the acquisition parameters involved.

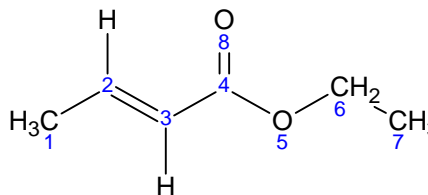
In this lab writeup, **Q#** \rightarrow indicates a question to be answered in the turned-in homework set. **P#** \rightarrow indicates a plot (and work in NUTS preceding the plot) to be handed in. *These sections are boxed, and should be done **after** you finish your session on Homer.*

Use **Ethyl crotonate in acetone- d_6** for this lab.

– $\delta(\text{ppm})$ [$T_1(\text{s})$] assignments:

1 – 1.86 [40] **2** – 6.92 [41] **3** – 5.84 [13]

6 – 4.12 [9] **7** – 1.23 [9]



A. Receiver Gain (RG) Clipping

With the standard parameter set modified to **NS=1 DS=0**, acquire a FID with the default **RG (=40)**.

*Note the severe “clipping” of the FID. The NMR signal comes to the detector as an oscillating analog voltage. After amplification (set by **RG**), the signal is digitized by an analog-to-digital converter (ADC). All ADCs have a limited range of amplitudes (voltages) that can be digitized. Bruker AC spectrometers use a 12-bit digitizer, so the largest values that can be represented are $\pm 2^{11} = \pm 2048$. Any voltage that is too large is simply set to the maximum value, ± 2048 .*

Perform a Fourier transform (FT) of the FID on the spectrometer and note the resulting spectrum. Reduce RG to 10, reacquire, and again note effect of the clipping in the spectrum in the spectrometer on the spectrometer.

FID#1: Acquire a FID with a proper setting of **RG** and **NS=1 DS=0**. Save this FID for processing in NUTS. Follow this **WR** by performing one last FT on the spectrometer. Satisfy yourself that the spectrum now has a normal appearance.

Q1 \rightarrow Describe the effect of **RG** clipping on an NMR spectrum.

B. 1-Scan versus 8-Scan Spectra: Speed and Sensitivity of Data Acquisitions, and Quadrature Artifacts

FID#2: Now use the more common settings of **NS=8 DS=2** (same RG as above), and reacquire. Save this FID for later processing.

After you finish taking all data for this HW on the spectrometer, process the FID#1 and FID#2 datasets in two separate NUTS windows. Process normally, using care when phasing. Do a baseline correction, and integrate the five proton multiplets. Normalize the integrals by setting the upfield methyl multiplet to 3. You should be able to observe at least three issues when comparing these two spectra:

- a) The 8-scan spectrum has better sensitivity, by a factor of $\sqrt{8}$, than the 1-scan spectrum. *Signal increases proportional to NS. The noise also increases, but only as \sqrt{NS} . Signal/noise thus increases as $NS/\sqrt{NS} = \sqrt{NS}$.* This effect is easiest to see by increasing the vertical scale in the two spectra to the point where the ^{13}C satellites can be observed. Note then the difference sizes of the stochastic (random) noise in the baselines.
- b) Quadrature artifacts are eliminated in the 8-scan spectrum. *Complex data—observing the signal amplitude as projected onto the X-axis, and separately the amplitude projected onto the Y-axis—cannot be acquired perfectly. Either the receiver is not perfectly orthogonal (Bruker), or two orthogonal receivers won't have identical gain (Varian), as each pair is acquired. Either case will produce small artifact peaks at locations exactly symmetric about the center of the spectrum.* In the 1-scan spectrum, you should be able to see the quad artifacts for the two methyl peaks at ~ 9.4 and 10.0 ppm. They are quite small ($\sim 0.2\%$), but such artifacts can be quite troublesome if they overlap peaks that need to be quantified (as in a kinetics run). 8-scan spectra eliminate such artifacts by cycling the receiver(s) through all quadrature variations (i.e., running a “quad cycle”). Unless fast data is needed, typically for a kinetics run, always take data with NS equaling a multiple of 8.
- c) The integrals for the 8-scan spectrum will show significant deviations from integer values. The values for the vinyl protons will be around 60% (i.e., 0.60). The 1-scan spectrum will likely be better, perhaps a lot better.

Quantitative data relies on the magnetization, M , of each proton being at the equilibrium Zeeman polarization prior to each scan. Protons relax to equilibrium at different rates, which are highly dependent on the proton's local environment. In general, high proton density causes faster relaxation, whereas protons in lower density environments relax slower: aliphatic protons will therefore tend toward faster relaxation (having more and closer neighboring protons), whereas vinyl and aromatic protons will often relax slower. This is case the ethyl crotonate. If we wait long enough between scans all protons will have relaxed to equilibrium, and the proton spectrum will be quantitative. Repeating the experiment too fast, however, will diminish the intensity of the slower-relaxing protons, the vinyl protons, relative to the faster relaxing protons. The proton relaxation—called spin-lattice relaxation, T_1 —can be quantified by more advanced NMR experiments, a topic we'll cover in a bit more detail later in the semester.

P1 & 2 → Plot the 1-scan and 8-scan spectra with accurate integrals. Include sufficient experimental details in the annotations so each spectrum can be easily identified.

Q2 → Think about how these two spectra were acquired. Why would the 1-scan spectrum have more accurate integrals than the 8-scan spectrum?

Q3 → If you check with other students in the class, you would find that the integrals for the 8-scan spectrum would be quite reproducible, but those on the 1-scan spectrum vary from one student to another by a considerable amount. Why might this be so?

C. Repetition Rate Method for Acquiring Quantitative Data

Measuring accurate T_1 values is the best method for properly setting up subsequent quantitative experiments: setting the “repetition delay” = $RD+AQ$) $> 5 \times T_1$ insures better than 99% accuracy in proton intensities. [^{13}C satellites, impurities, and peak overlap limit accuracies in routine spectra to approx. 5%.] Even so, the following method is useful in two important cases that arise fairly often:

- i. The ^1H spectrum of a sample (similar to ethyl crotonate) gives non-integer values on integrals, confusing assignments and interpretations. The researcher needs to acquire a new spectrum where the integral values can be relied on as being accurate.*
- ii. Quantitative work (e.g., a kinetics run) needs to be performed, but sensitivity is so low as to make measurement of T_1 values very difficult. This is usually not the case for ^1H data. When kinetics run are based on ^{13}C spectra, on the other hand, measuring T_1 values of the carbons can be very arduous.*

In such cases, the follow procedure can be used:

Take a standard data set (you've already done this with FID#2). Determine the repetition delay that was used: on a Bruker AC, this will usually equal **RD+AQ**.

FID#3: Acquire another FID with **RD** increased by an amount that doubles the repetition delay. Save this for later processing.

FID#4: Acquire a third FID with **RD** increased again, now such that the repetition delay is 4 \times that of the first data set.

FID#5: Acquire one last FID, but now with NS=1 DS=0 RD=120.

P3 & P4 & P5 \rightarrow Work up FID#3, FID#4 and FID#5 identically to FID#2. Again be careful with phasing, baseline corrections, and setting up the integrations. Provide annotations that include the changed parameters. Plot and turn in these three spectra. Note that the relative integrals improve with increases in RD, with only the final spectrum being accurate to $< 5\%$.

In normal practice, you would continue taking such data until increases in the repetition delay no longer change the integral values (which happens > 100 s for ethyl crotonate). At that point, you can be confident that the delay is long enough and the data are quantitative. The ethyl crotonate sample is unusual in being quite small (lower MW compounds tend to slower relaxation), and having been carefully degassed (O_2 dissolved in solution is a major cause of faster relaxation, since the ground state of O_2 is paramagnetic). For these reasons, the T_1 relaxation of the vinyl protons of ethyl crotonate are quite long, equaling ~ 20 s.

D. Avoiding Quadrature Artifacts in 1-Scan Spectra

Since quadrature artifacts always appear as mirror images about the center of a spectrum, moving all the observed peaks to one side of the spectrum center will insure that no artifacts are produced on top of real signals. The center of the spectrum on Bruker spectrometers is defined by the parameter O1. Moving it is a relatively simple task, performed in EP mode.

Change your experiment back to **NS=1 DS=0 RD=0**, and reacquire a FID. **FT** on the spectrometer, and inspect the spectrum in **EP** mode. Do a quick phase correction if needed using **P** (correct with the **C** knob), then **M** to memorize the correction.

Still EP mode, move the cursor so it is to the left (downfield) of the most downfield multiplet. Enter **O1** and then **M** to memorize the change. Note the new **O1** value.

FID#5: Reacquire the FID, and save for processing off-line.

FT on the spectrometer to make sure the spectral window is as desired (all peaks should now be to the right of spectrum center).

P6: Work up, plot and hand-in this spectrum. Make sure your annotation includes the new O1 parameter setting.

Setting O1 as described above for 1-scan kinetics data can be important for accurate quantitation. Modifying the spectrum center is important in other areas of NMR, and can be sometimes be quite challenging. The inorganic chemist trying to observe metal nuclei—which often have very large chemical ranges—is a prominent example. The known chemical shift range of ^{195}Pt is nearly 15,000 ppm, equaling nearly 1 MHz on a 300 MHz (^1H frequency) spectrometer. Liquids NMR probes can observe only ~100kHz in bandwidth, so the choice of the spectrum center is critical to being able to observe the nucleus in different compounds. Acquisition of up to many spectra with O1 values incremented by 100 kHz in each spectrum is not uncommon to insure observation of ^{195}Pt in a novel platinum-containing compound.

Another important example of modifying O1 (spectral center) occurs in the acquisition of 2D and 3D NMR data. ^{13}C and ^{15}N are two commonly nuclei observed “indirectly” in the new dimensions. The sweep widths and spectral centers for each nucleus must be setup properly to obtain optimized data.

E. Incorrect Spectral Windows (Folding), and Unusual Shifts and Nuclei

If a chemical shift falls outside the spectra region, the peak will be “folded” back into the spectrum (as described in more detail in lecture). In practice, two issues related to folding are quite important:

- i. Folded peaks will “move” in chemical shift as spectral region is changed: observing a constant chemical shift for a peak is the best proof that it is not being folded.*
- ii. Peaks too far from the spectral region will not be observed at all, due to attenuation of the signal by filtering. The analog filters on Bruker ACs will attenuate the intensity by $\frac{1}{2}$ when the signal is 20% outside the spectral region, and nearly complete attenuation for signals greater than the sweep width away from the edges. [New spectrometers use digital filters; the peak need only be slightly outside the spectral region to be completely attenuated.] The most straightforward method to insure detection of all nuclei in a compound is to open up the sweep width to a value larger than the possible chemical shift range. The default setting for ^1H spectra in the Chemistry facility is $\text{SW} = 20$ ppm, large enough for all normal organic compounds. But protons in some inorganic and organometallic compounds can run outside even this setting. The Berry group has synthesized compounds containing paramagnetic Fe where the protons ranged from +120 to -20 ppm.*

In this part of the lab, you will move the spectra region around to observe the effects of folding and spectral filtering. You will then “correct” the spectrum by opening the sweep width up quite wide, insuring observation of the complete spectrum, followed by optimizing the spectral region to re-obtain good resolution. This is a good general procedure for observing unusually shifted nuclei, and confirming that the observed peaks are not folded.

FID#6: Move O1 (the spectrum center) 3 ppm downfield, sufficient to push the methyl peaks off the right-hand side of the spectrum.

P7 → Process this spectrum in NUTS, and note how the folded methyl peaks will not phase with the other peaks that are still in the spectral region. Inability to phase peaks is one indicator of spectral folding. Plot and turn-in with proper annotations.

FID#7: Move O1 another 3 ppm downfield. At this point, the full ethyl crotonate spectrum has been pushed outside (to the right) of the spectral region.

P8 → Process this spectrum in NUTS, plot and turn-in with proper annotations. Note how the spectrum is both attenuated (by the spectral filters) and reversed in direction. The apparent chemical shifts are now nonsense.

To insure proper observation of the spectrum, open the sweep width up substantially by setting **SW = 200P** (make sure you type the **P** in when entering the value on the spectrometer; forgetting it will set SW to 200 Hz = 0.67 ppm). Write down the values for **SW**, **TD** and **AQ** to use in answering the next question. Also note the chemical shift—and poor resolution—of at least one of the peaks (it doesn't matter than it is not referenced; just write down the value).

Q4 → Show how the value of **AQ** was set on the spectrometer. Then show what the obtainable resolution is for this spectrum, based on that value of **AQ**.

FID#8: Optimize the spectral region by going into **EP** mode, and expanding the spectrum so the observed multiplets fill ~ 50% of and are reasonably centered on the screen. Enter **^O** while still in EP mode: this will set **SW** and **O1** to match what you displayed. Note the new setting of **AQ**, and reduce it to 4 (seconds) if it is larger. Acquire a new spectrum, and save it. FT and inspect the spectrum in EP mode. The chemical shift noted in the spectrum having SW=200p should be the same in this greatly resolution-improved spectrum.

P9 → Process, plot and turn-in with proper annotations.

The process you just performed is appropriate for the situations mentioned above: compounds having unusually shifted protons, or for the direct detection of other nuclei. We will return to this subject later in the course when acquiring ^{31}P and ^{19}F data.

Turn in 9 plots, and answers to 4 questions by 5pm on Monday, Oct 13.

You will have another assignment due the week of Oct 13, HW#6 involving the use of NUTS Data Tables to produce NMR listings for publications. So you should try to get most of this assignment done during the 1st week Sept 30 to Oct 8.