

Fall 2014 Chem 636 – Lab #3

Assignment due at beginning of lab, week 4 (Sept 22 – 25).

Use Artemis (AVANCE-400) for this HW.

Always login to your own account when working. Although you may work in groups, each of you should setup and acquire your own data, as well as process and hand in that *individual* set of data. We'll use the same 3 samples this week as used in HW#2.

There's a chance that you'll collect data in this HW, and then decide you need to do it again. So don't wait until the last minute to start....

Reading – A primary reference for this lab is the guide “Acquisition Step-by-Step” located on the facility website in the User Guides → Avance section.

- Introduction: Zerbe&Jurt [ZJ] Chapter 1 [10 pgs; if the 1D section is really mysterious after reading, you're background likely is not commensurate the basic class requisites]
- Basic NMR theory: Claridge [Clar] 2.2.1, 2.2.2, 2.2.3, 2.3; ZJ 2.1-2.4
- Basics of Data Acquisition and Processing: Clar 3.2.1, 3.2.2, 3.2.3, 3.2.4; ZJ Chap 11
- Fourier Transform: Clar 2.3; ZJ 11.2, 11.2.6, 11.2.8, 11.2.9
- Apodization and Truncation Artifacts: Clar 3.2.7; ZJ 11.2.4, 11.4.3
- Phase correction: Clar 3.2.8; ZJ 11.2.3, 11.4.4
- Baseline correction: ZJ 11.4.6
- Preparing Samples: Clar 3.3 pgs 59-65; ZJ 2.5.1, 2.5.2

Goals – Use the Bruker Avance 400 (Artemis) for ^1H NMR data collection using Topspin.

Experiment with parameters that affect spectral resolution.

Experiment with the spectral window.

Where indicated in this homework, experiments should be run on facility samples provided in the SampleJet as documented on the sheet next to the spectrometer host computer. Some experiments can be run using your own sample(s).

The most important task in this homework is to insure you have a solid basis for using the 400 for acquisition of high-quality ^1H 1D NMR data.

1. Only bring clean samples tubes (the exteriors; *duh*) to the lab, and keep them upright (all NMR caps will leak on occasion). Clean with kimwipe and ethanol if you've gotten fingerprints or sweat on them.
2. Use the proper caps correctly placed on pristine 7" or 4" tubes; the caps and tubes should be undamaged (throw chipped or scratched tubes away!). Firmly seat the caps onto the tube.
3. Make sure the tube is properly placed into the robot. If you remove a 4" sample tray, insure that it is placed back into its proper position. Errors here under IconNMR will stop the queue.
4. Keep your thinking cap on when setting up and running experiments, please.

5. Ask questions! And document and report — email is usually best — any problems you observe while using the instrument. Don't assume staff will already be aware of the problem.

Three sets of parameters critically affect data quality in all NMR experiments. The following exercises will have you experiment with these parameters.

Experiment with parameters that affect resolution in ^1H spectra

The primary parameter affecting resolution is the acquisition time, **AQ**.¹ Other important parameters are:

SW – *sweep width* in Hz

TD – *time domain size*; number of points acquired ($\text{TD}/2 = \#$ complex pairs); stays constant when **SW** is changed

DW – *dwell time*; time increment between each acquired point \rightarrow

$$\text{DW} = \frac{1}{2\text{SW}} \quad \left[= \frac{1}{\text{SW}} \text{ on Varian/Agilent spectrometers} \right]$$

$$\text{AQ} \text{ – } \textit{acquisition time} \rightarrow \text{AQ} = \text{TD} \cdot \text{DW} = \frac{\text{TD}}{2\text{SW}}$$

AQ therefore changes when **SW** or **TD** is changed.

The key relationship is the following:

$$[\text{obtainable resolution in Hz}] \sim 1/\text{AQ}$$

The best resolution one can achieve on a 400 MHz magnet is ~ 0.05 Hz; that's as good as technology (shims) can manage. Perhaps as important, most natural linewidths in NMR are larger than 0.05Hz .² So $\text{AQ} \leq 20$ sec is an upper limit. The spectrometer will allow you to enter larger values (and may make **AQ** very large when you make **SW** small). But a large **AQ** values *waste of time and disk space*.

I. Resolution Assignment Using Artemis (the Bruker AVANCE-400):

Follow the one-page guide “Acquisition Step-by-Step” located on the facility website in the User Guides \rightarrow Avance section to assist you in completing this task.

Setup your Unix and TopSpin accounts as described in the lab demo session. Right-click on Unix icons to get a menu to place TopSpin and other apps onto the desktop or upper bar.

¹ You might be wondering about shimming: yes, this procedure is absolutely critical to obtaining good resolution.

When we talk about **AQ** being the “primary parameter affecting resolution”, this is *after* we've obtained a good shim.

² Natural linewidths, $\Delta\nu$, are related to relaxation, T_2 . For small molecules, it is often true that $\Delta\nu = 1/T_2$, and the smaller the molecule, the larger the T_2 . Thus the common observation is that proteins gives quite broad NMR lines (makes them easier to shim), moderate sized peptides shim better (and are significantly more difficult to shim), complex organic molecules have spectra with quite good resolution, but it is really small molecules like TMS that can be shimmed to the best homogeneity the magnet can provide.

a) Acquire a standard ^1H spectrum of rotenone in CDCl_3 . Process in a normal fashion, similar to that done in HW#2.

- Fix you DIR in the **new** panel (first time only): `/home/username/av400`
- It is recommended that you read in a new shim file at the beginning of your session. On the 400, use the command: `rsh bbfo.shim`
- Be patient with **amta** and **topshim** commands. Typing on the Bruker command line, or clicking elsewhere prior to finishing may freeze or discombobulate TopSpin.

☞ If TopSpin freezes:

1. Close out completely from TopSpin.
2. After 5s, restart TopSpin.
3. Enter `ii restart` ;wait for this to finish, approx 30s

Usually this procedure will cure the problem.

- Check parameters in the ACQPARS panel. Click on  (or equivalently `ased`) to view those used for the experiment chosen.

☞ Always hit `ENTER` or click elsewhere for a change in parameter to take.

- Click on PULSEPROG panel to see a listing of the pulseprogram. The comments listing at the bottom will provide information for minimum **NS**.

☞ *More useless NMR-geek trivia?* Think back to last week's HW#2: it is important on the AC-300 to always take data in multiples of 8 scans ($\text{NS} = i \times 8$). The new Avance III spectrometers reduce this requirement for routine 1D experiments to $\text{NS} \geq 2$ (which can be reduced to $\text{NS} = 1$ for fast kinetics studies). But even on the newest spectrometers, some of the more advanced 1D experiments require multiples of 8 scans: e.g., when acquiring a NOESY1D experiment, stopping at 26 scans will give terrible data. An interpretation based on that data could easily be very wrong! Suddenly the trivia is really important, but not necessarily any easier to remember. Here it helps to get into a good habit: we recommend acquiring all 1D experiments with the multiple-of-8 rule-of-thumb. It isn't like waiting for the extra 6 scans is gonna be all that hard to suffer through....

Work up this spectrum performing the “standard” processing protocol:

- one to two zerofills
- matched filter (exponential or MNova-gaussian multiplication with $\text{lb}=1/\text{AQ}$)
- phase correction
- baseline correction
- major multiplets integrated with normalized values (\approx # of protons in multiplet)

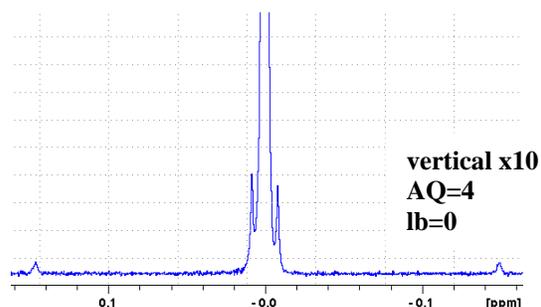
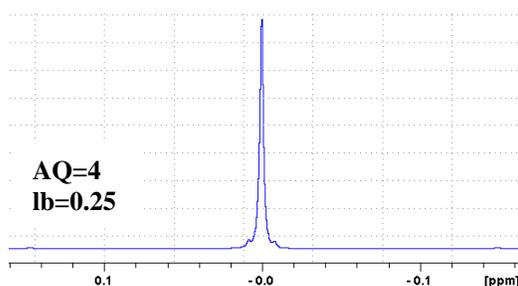
Remember to correctly annotate your plots.

☞ Plot the rotenone spectrum acquired on the 400 and the spectrum you acquired on the AC-300 in a stack plot. Save as .mnova and .pdf files and perform a normal upload.

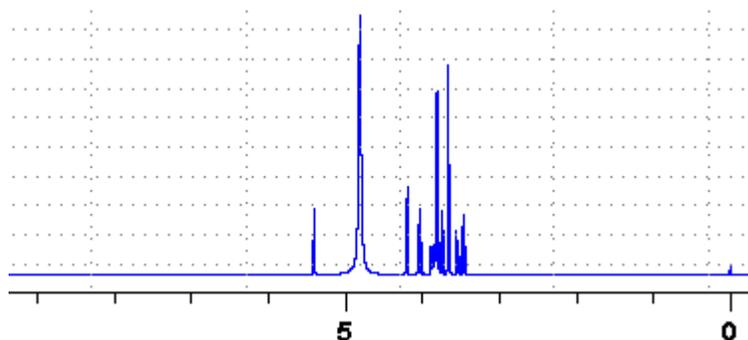
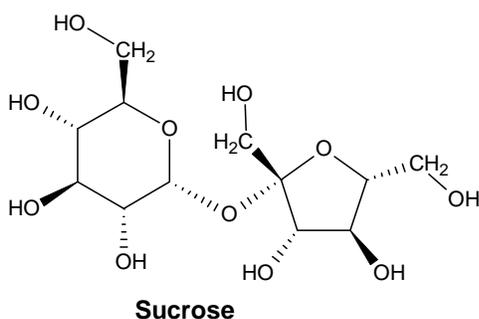
b) Acquire a standard ^1H spectrum of 3-heptanone in acetone- d_6 . Again process “normally”.

2☞ Plot the 3-heptanone spectrum acquired on the 400 and the spectrum you acquired on the AC-300 in a stack plot. Save as .mnova and .pdf files and perform a normal upload.

3☞ As done in the previous HW, expand your spectrum to show the TMS peak similar to that shown above; use $\text{lb}=0$ and increase the vertical scale. Plot it and turn in.



c) Acquire a “standard” ^1H spectrum of sucrose in D_2O sample.



4☞ Plot the sucrose spectrum acquired on the 400 and the spectrum you acquired on the AC-300 in a stack plot. Save as .mnova and .pdf files and perform a normal upload.

d) Acquire another spectrum but with **AQ** now 2 or 3× as long.

Compare this spectrum to the one acquired above. After adjusting the apodization to match the new **AQ**, can you see any improvements in resolution? [The standard parameters often give “best” quality data. Special sample preparation is often required to do better; i.e., don’t be surprised if the longer **AQ** doesn’t seem to help the resolution.]

e) Take two more spectra with much smaller **AQ**, one perhaps around 0.5 or 1s, and the other 0.1s or so. Go down to **AQ** = 0.05 if you wish. Process these data with $\text{lb}=0$.

These kinds of spectra are often best compared using stack plots. Make a stack of the four spectra (c, d, e) processed without apodization ($\text{lb}=0$).

5. Now process the data using a matched filter, $lb=1/AQ$, and make another stack plot. Save as .mnova and .pdf files and upload.

Q1 You will observe additional “noise” or “artifacts” in spectra acquired with smaller **AQ** and **lb = 0** (or no apodization applied). Suggest why these are present, and why they get bigger as AQ gets smaller?

Q2 Why does the matched filter remove this artifact? Is it really removed, or just reduced?

Q3 What does the matched filter do to the resulting linewidth of the spectra peaks?

Upload 5 plots as .mnova and .pdf files, and hand in answers to the 3 questions.