Chem 636, Spring 2008 HW #10

Due at the beginning of lab week of 18 Nov 2008

Use Hermes (Varian Mercury-300) for this lab.
Refer back to HW#2 for detailed information on setting up an automation experiment on Hermes.
See, for additional reading, Claridge sections 5.1–5.3, and 5.6 (esp. 5.6.1, 5.6.3, 5.6.4).

Note: Although Claridge is a very good textbook for the introduction of theoretical and practical aspects of NMR, section 5.3.4 discussing 2D COSY data acquisition lacks in its discussions of evolution and J_{obs}. The next section introduces these important topics.

^1H-^1H Correlation Spectroscopy, COSY, on Hermes

This homework set has a primary goal of introducing you to the technique of proton-proton (homonuclear) COSY experiments. Since these are the first 2D experiments you will perform in any detail, there are new details to learn. It is important to understand that much of 2D NMR extends straightforwardly from 1D NMR. ***Evolution*** during the new added dimension is the new and, in some ways, fundamentally new concept. In 2D COSY, evolution determines the information content, not resolution. The most important concepts in this lab are the following:

1. 2D spectra are acquired in a fashion that is completely analogous to kinetics or variable temperature data sets, where many 1D spectra are acquired sequentially. These 1D spectra are often presented as ***stacked plots***. A 2D experiment is always a simple stack of 1D spectra, where typically one parameter, a time delay **d2** [on Varian spectrometers; **D0** on Bruker spectrometers], is incremented for each successive spectrum. The number of 1D spectra that are acquired is set by the parameter **ni** [or NE=TD1 for Bruker].

   The only change in a COSY experiment from one 1D spectrum to the next is the delay time **d2** ($\equiv t_1$) that separates the two 90° rf pulses.

2. In 2D data sets, spectral resolution is an important quantity, but in a different way than with 1D data. For 1D data, the obtainable resolution ≈ 1/**at** [1/AQ] is fundamental to our ability to observe *J*-couplings. In 2D COSY data sets, the obtainable resolution is important primarily with regard to observing different chemical shifts. This reduced requirement allows much smaller **at** to be useful in 2D experiments (**at** is often 10× smaller in 2D experiments as compared to 1D experiments)

3. *J*-couplings are observed in COSY spectra not as resolved multiplets, but rather as crosspeaks arising between different chemically shifted protons. ***Evolution*** of the *J*-couplings during the first time period, t_1, is the new and crucial aspect of COSY experiments. The total evolution time is:

$$at1 = \frac{ni}{2 \cdot sw} \tag{1}$$

   Increasing **ni** or reducing **sw** thus provides longer evolution. The former is easy to do, but increases the overall length of the experiment. **sw1** (which always = **sw** in COSY experiments) is optimized in all 2D experiments (this is done automatically on hermes), so reducing **sw** further is usually not possible.

4. Crosspeak intensity arises from creation of *antiphase* magnetization, $I_Z S_Z$, during the evolution period:

$$\text{crosspeak intensity} \propto I_Z S_Z \propto \sin(\pi \cdot at1 \cdot J) \quad (2)$$

Maximum crosspeaks will therefore be observed when *at*1 = 1/2J. For J_{HH} = 10 Hz, maximum intensity would occur for $at1_{max}$ = 50ms. For J_{HH} = 5 Hz, $at1_{max}$ = 100 ms. For **sw** = 8 ppm on a 300 MHz spectrometer, **ni** = *at*1×2×sw = 0.1s×2×8ppm×300Hz/ppm = 480. This is value that is easy to achieve in COSY. The number of transients, **nt** [NS] = 1 works fine (**nt**=2 is recommended). If **d1** [RD/D1] = 2 and **at** = 0.2, the total length of the experiment would be:

total time ~ ni × nt × (d1+at) = 480 × 1 × 2.2 = 1056s = 18 min

Smaller *J*-couplings may not produce identifiable crosspeaks, however.

Q1 → What value of **ni** would be needed to produce maximum crosspeaks for a J_{HH} = 3 Hz? How long would the experiment take on a 300 MHz spectrometer for **sw**=8ppm using **nt**=2, **d1**=2, **at**=0.2?

Q2 → What values of **ni** and total experiment time would be needed on a 500 MHz spectrometer?

5. Fortunately, crosspeaks do not have to maximized (i.e., when they would be roughly as intense as the 1D proton multiplets) to be unambiguously identified; if the signal-to-noise is very good, 1/10th maximum might be sufficient; in this case, *at*1 = 1/(30J), so 15× shorter evolution (i.e., **ni** could be this much smaller). Empirically, the following equation has been found to work well for the default setup of COSY in our laboratory:

$$J_{obs} \geq \frac{1}{6 \cdot \boldsymbol{at}1} = \frac{\boldsymbol{sw1}}{6 \cdot \boldsymbol{ni}} \quad (3)$$

J_{obs} is the smallest coupling where you can trust that an identifiable crosspeak will be observed. Crosspeaks may appear between protons that have a smaller coupling, but you may have to run additional experiments to be assured of the coupling.

Q3 → What is J_{obs} for the examples given above in Q1 and Q2?

6. One trick used to speed the observation of small *J*-couplings (≤ 2 Hz) in COSY spectra is to insert a fixed delay, **tau**, before and after the 2nd rf pulse, which adds to *at*1. This is the basis of the long-range COSY experiment, available on our 500 MHz spectrometers.

Perform the following three experiments on the sample changer on Hermes, but do only one session on any single night. Take all data on your unknown sample.

Standard COSY on Unknown Compound

7. Setup and acquire a standard "fast" Gcosy spectrum of your unknown compound. Change **d1**=2, but otherwise accept the default parameters. Interrogate the values of **nt** and **ni**; these can be found in a number of ways, but typing **nt? ni?↵** in the command bar is the simplest.

   VNMRJ automatically adds a standard **Proton** experiment, if one hasn't already been requested. A proton 1D is a required for COSY experiments, in allowing the software to automatically optimize the spectral window (**sw** and **tof** [O1]) for the COSY.

Higher-Resolution COSY

8. Add another Gcosy experiment to your current Study.

   • Before making any changes to this experiment's parameters, request an update of the time of this experiment. The simplest method is to type **time**↵ in the command line (after double-clicking to read in the parameters). Also note the current values of: **np fn fn1 sw sw1 ni at.**

9. To improve the obtainable resolution, **np** [TD] can be increased. In VNMRJ, the more natural alternative, directly making **at** larger can be done; one method of doubling **at** would be to type **at=at*2**↵ in the command line. VNMRJ will automatically adjust **np** when **at** is changed, since **at** = **np**/(2×**sw)**.

   → Double the size of either **np** or **at**.

   • Now request the new time of the experiment. Note that it changed only a little. In a 2D COSY experiment, the total time of the experiment is roughly the following:

   total time = [time per scan] × [# scans per spectrum] × [# spectra]

   total time ~ [d1+at] × [nt] × [ni] (4)

   The 2nd line is a bit off as it doesn't include the evolution time, t_1. But it's usually good enough. Increasing **np** and thus **at** is a small effect, as **d1** will usually dominate the time per scan in 2D experiments.

Q4 → What is the obtainable resolution for this experiment? Give your answer in both Hz and in ppm.

Since VNMRJ will change/optimize **sw** following the **Proton** experiment, the obtainable resolution could only be estimated. But by doubling **np**, the (F2) resolution will be twice as good as in the standard COSY.

Why improve the F2 resolution? If multiplets have close to the same chemical shift (e.g., are strongly overlapping), better resolution can assist in determining which of the two protons crosspeaks are coming from.

• Simply increasing **np** is not sufficient. The next two steps are required to improve overall resolution in the 2D spectrum.

10. After increasing **np**, make the same adjustment to the Fourier number (the size of the spectrum). COSY spectra are always square in Fourier numbers, so:

    → Double the size of both **fn** and **fn1**.

11. Last, but definitely not least, increase **ni** by at least the same amount. This will increase the evolution time, allowing smaller *J*-couplings to be observed. Increasing **ni** also improves the overall resolution, since the obtainable resolution in the F1 dimension = 1/*at*1.

    → Set **ni**=512.

    A common rule-of-thumb for changes in **ni** and **np** is to set **np** > 4×**ni**. If **ni**=128 → **np**=1024; if **ni** =200 → **np** = 2048 [**np** usually is set to a power of 2]. In these cases, processing of the data will require two or three zero-fills, respectively. On a 300, however, it is best to keep **np** ≤ 2048 (otherwise observing large couplings becomes problematic), thus the setting used above.

12. When done with everything, recheck the sample information and solvent for the Study, and submit everything to the NightQueue.

Processing – Resolution Enhancement via Sinebell Apodization

$$\textbf{obtainable resolution in indirect dimension} \sim \frac{1}{\boldsymbol{at1}} = 2\frac{\boldsymbol{sw1}}{\boldsymbol{ni}} \qquad (5)$$

where the addition of the number 1 to the symbols **at** and **sw** denotes the acquisition time and sweep width acquired in the indirect, or t_1, dimension. **ni** is the t_1 analog of **np**. It is nearly always true in COSY spectra that **sw1** = **sw** (this must be true to symmetrize the data). Thus, for the conditions used above (**sw**=8ppm) and **ni**=128,

$$1/\textbf{at1} \;=\; 2 \times 8\text{ppm} \times 300\text{Hz/ppm} / 128 \;=\; 75\text{ Hz}.$$

This seemingly terrible resolution in the standard COSY setup is alleviated by four factors:

a) We zero-fill, usually twice, improving the effective resolution by roughly a factor of 2× (zero-filling more than two full times is not useful). The effective resolution in the example above would therefore be ~ 37 Hz.

b) 2D contours provide better resolution (by perhaps another factor of two) than the calculation shown above. The center-of-mass of the 2D contours, and the addition of the 2nd dimension provides real gains in usable resolution.

c) We need to only resolve chemically shifts between proton multiplets. A single multiplet being an unresolved "lump" is OK (as is usually the case with 2D spectra; the *J*-couplings within any one multiplet are unresolved): crosspeaks now provide the information about *J*-couplings.

d) Strong resolution-enhancement is applied using sinebell, or sinebell-squared, *apodization* [see Claridge, section 3.2.7, for a good discussion of commonly used apodization functions]. Sinebell apodization definitely improves spectra resolution, but it also introduces phase distortions and significantly reduces signal-to-noise.

To observe the effects of this function on a standard ^1H 1D spectrum, import the **Proton_Minsw_01.fid** acquired above into NUTS. Perform normal processing:

BC LB=0.3 **EM ZF ZF FT** and phase.

Save this spectrum in NUTS format (it will be used later). Place the spectrum into the NUTS buffer (BU). Re-import and reprocess using:

BC MS ZF ZF FT PS

MS is the NUTS command to **m**ultiply a **s**inebell, and **PS** applies the **s**ame **p**hasing as used previously. The negative-going parts of each line (which should be symmetric on each side of the positive peak, since a PS has been applied) are normal for a sinebell-processed spectrum, and can make phasing difficult. Note also the loss in sensitivity. Zooming about any multiplet, on the other

hand, will show clearly the enhancement in resolution. Sinebell multiplication of 1D spectra is occasionally used to assist in resolving complex multiplets.

Adjust the "Amplitude" (in the Buffer Properties box that comes up by right-clicking on the buffer when inside the BU routine) of the normally processed spectrum; set it to 0.2 to get the normal and sinebell-processed spectrum to have similar intensities.

PLOT1 → Plot these two spectra displayed on one page and hand in.

In COSY spectra, phasing issues arising from the sinebell apodization are avoided by displaying the magnitude values of the data, $\sqrt{\mathrm{Re}^2 + \mathrm{Im}^2}$. [See Claridge, Fig. 5.22 on pg. 166 and accompanying text, for more detail on how sinebell and magnitude processing improve resolution by decreasing the "star" pattern of 2D peaks.]

The **MS** apodization degrades the ability to perform quantitative analysis. For this reason (and others), **MS** and functions similar to it are not used for routine workup of ^1H data. For COSY spectra, on the other hand, sinebell or sinebell-squared apodization works nicely for both improving resolution and removing truncation artifacts. The latter would be particular egregious in 2D data, since **at** is very much smaller—so the effective truncation is very much larger—in 2D compared to 1D data.

Processing 2D COSY Data

The NMR facility has a limited set of licenses to the 2D processing portion of NUTS that cover only the PCs in the facility proper. You will have to perform the following tasks, therefore, on a PC in the facility.

It is usually best to start the processing of a 2D dataset in a new NUTS window. Starting Arrayed Mode, for example, is simple in a new window, and is a bother otherwise. If you see a "Danger Will Robinson" message, close the current NUTS window and re-start in a new one.

Save data (e.g., the 1H 1D spectrum used for the 2D border displays) onto a memory key, or to the R: drive on the facility PCs. The S: drive does not allow writes/saves.

Macro Processing (RU) of COSY Spectra

12. In a new NUTS window, type **RU**↵

13. Select varian_mag_cosy_sb2_2zf.mac to process the standard Gcosy dataset, from 7 above.

14. The macro will request you to select the data set, either from your memory key or the S: drive.

15. Once selected, the macro will perform the following processing steps:

    a) **AR** ; arrayed mode, enabling fast processing (we always want this)

    b) **BC MS MS FT** ; standard processing of the stack of t_2 data (each FID in a data row) using sinebell-squared apodization; F2 spectra after the FT

    c) **TD** ; transposed the data; rows become columns, and columns become rows t_2 (F2) was rows before TD, t_1 (F1) are rows after

d) **MS MS ZF ZF FT** ; sinebell-squared applied to t_1 FIDs, then double zero-fill, and finish with 2nd FT to get F1 spectra

e) **MC SR SS** ; magnitude calculation, spectrum reverse, spectrum scaling

16. The data is now ready to be viewed using the command

2D ; display the 2D specrum; use **1D** to return to 1D mode, which you must do to save the 2D spectrum (yes, this is weird)

CP **;** use **c**ontour **p**lot for careful inspection, and all plotting

Viewing the 2D data prior to symmetrization can be useful to insure the t_1-noise is not abnormally large, which would indicate some serious problem with the data. If the sample were spinning—the spinner should turn off automatically—as one example, the t_1-noise would be very large.

17. Symmetrize: **SY**

18. Add 1D borders at the Top and Left using the Borders menu items.

PLOT2 → Add proper annotations—in addition to sample, name, date, etc., include **ni** and **d1**—and plot this spectrum out.

The only tricky part in the processing used above is the selection of the macro to do double zero-filling, rather than some other number of zero-fills (one or three would also not be uncommon). The apodization could also be changed: sinebell, rather than sinebell-squared, which is better for signal-to-noise but provides less resolution enhancement. But the zero-fills have to be done properly or symmetrization will not be possible. The number of points in F2 and F1 (i.e., following both FTs) must be identical to perform **SY**.

19. **# Zero-Fills Needed:** Look at the data prior to doing any processing steps: use **View → Spectra Parameters**, and **Number of Points** in the F1 and F2 columns. Do this for the High-Res Gcosy you acquired in sections 8-11 above. The zero-fills, always performed on the F1 data, will have to make:

#pts in F2 = #pts in F1 after zero-fills

Most likely, you will need just one zero-fill for this dataset; e.g., if

#pts in F2 = 1024 and #pts in F1 = 512 → one zero-fill is required

[Note: #pts in F2 in NUTS are *complex pairs* of points, whereas **np** in VNMRJ gives the total # points; NUTS thus shows ½ **np**. So for the dataset above, **np**=2048.] If instead

#pts in F2 = 501 and #pts in F1 = 128 → two zero-fills are required

The 1st ZF increases #pts in F1 to 256, and 2nd gets it to 512. The #pts in F2 is always rounded to the next biggest power of 2, in this case 512.

20. To process the High-Res Gcosy then, start in a new NUTS window, enter **RU** and select the macro **varian_mag_cosy_sb2_?zf.mac** with the correct number of **zf**'s in it. If you choose the correct macro, finishing with **2D CP SY** should work OK.

PLOT3 → Add borders, proper annotations, and plot this spectrum out.

Q5 → Using the actual **sw** used for this dataset, show the calculation for J_{obs}.

Manual Processing of COSY Spectra

21. Inspection of the processing commands listed in section 15 above shows that steps b) and d) are standard 1D processing, with **MS** being used instead of **EM**. To see how this works, try the following:

    a) **GA** the standard Gcosy data in a new NUTS session.

    b) Perform the commands listed in 15 a) and b).

    Note that you could decide in this step to perform only sinebell rather than sinebell-squared, by performing one MS rather than two. Or you could choose a different apodization altogether. [Usually the same apodization is then performed in 15 d).]

    c) At this point, the 2D dataset has been processed identically to a stacked set of 1D spectra (which is what a COSY dataset is!). To see that this is true, try the command:

    SP ; stack plot mode (note in status at lower mid-right of screen)

    The 1D spectra are rows, with the first (smallest **d2**) at the bottom.

    W ; whitewash

    can help visualize the data. Try also **O** and change the Y-offset to 10. This will show the first 20 or so spectra. Reduce the vertical scaling to see each spectrum. As is typical for COSY data processed with MS, the phases will be changing dramatically through the data.

    As normal in NUTS, hit **Enter↵** to exit the SP routine.

    d) Now perform the steps 15 c) and d). Finish with the **MC** (magnitude calc) command.

    SP will now show the 2D spectrum as a stack, rather than in contour mode. You will want to do **O** and set Y-offset back to ~ 2. **W** (whitewash mode) on will often be best.

    e) Note that the data are "backwards". The **SR** command (you have to exit SP to perform SR, then re-enter SP) is required to give the normal presentation (δ increasing from right to left).

PLOT4 → Plot the stack plot, and annotate by hand (just need your name and date).

Displays of 2D data at this point (or following **SY**) in **SP** mode are sometime desirable, in presenting some aspect of the data in a preferable manner.

Turn in 4 plots and answers to 5 questions.