

Advanced 1D Experiments

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1. Arrayed Experiments

It is often useful to acquire several spectra that differ only in the value of a single parameter such as a pulse width or delay length. This type of experiment is called an arrayed experiment. The two examples shown here are a 90° pulse width determination and a T₁ relaxation time determination, though there are many other experiments where arraying a parameter is useful, e.g., kinetics.

Arrayed experiments cannot use autogain. After taking a 1D spectrum using autogain, set **gain** to the value found by the autogain procedure by typing **gain='y'**. If you then type **gain?**, the value of **gain** will be displayed. In subsequent acquisitions, if the error message "ADC Overflow" is seen, reduce the gain until the message is no longer displayed.

There are three ways to array a parameter:

METHOD 1: Type **array** and let the spectrometer prompt you with questions. For instance, if you would like to collect NOEs at 4 different mixing times, from 250 ms to 1.75s, you should input the following:

```
parameter to be arrayed: mix
enter number of steps in array: 4
enter starting value: 0.25
enter array increment: 0.5
```

METHOD 2: Type **array('parameter',<number of steps>,<starting value>,<stepsize>)**. For the above array, this would be: **array('mix',4,0.25,1.75)**

METHOD 3: Type **param=val1,val2,val3,val4,...** where **param** is the name of the parameter to be arrayed and **valx** are the values that parameter should take in successive experiments. This is useful if you do not want to use fixed intervals for the array, e.g. **mix=0.25,0.5,1.0,2.0**.

PROCESSING AND DISPLAYING ARRAYED DATA

wft will process all the fids in the array

wft(1) will process just the first fid, **wft(2)** will process the second, etc.

ds(1) will display the first fid, **ds(2)** will display the second, etc

dssa will display all fids stacked vertically

dssh will display fids in a line horizontally

PLOTTING ARRAYED DATA

To plot the entire array as displayed on the screen, type: **pl('all') pscale page**

To plot individual spectra, display the spectrum with the **ds(#)** command, then **pl pscale page**

1.1. 90° Pulse Width Determination

For many experiments with multiple pulses, the value of the 90 degree pulse is critical for success of the experiment. The 90° pulse width is the duration of radio-frequency pulse at a given transmitter power (**tpwr**) that gives maximum signal from a single pulse. This value is periodically calibrated by NMR lab personnel and stored in the parameter **pw90**, but the exact value is solvent and sample dependent. The easiest way to determine the 90° pulse for a sample is to set up an arrayed experiment where the value of **pw**, the pulse width, is varied.

While a 90° pulse gives a maximum in the signal intensity, it is usually easier to look for the place where the peaks near the center of the spectrum pass through a null. This occurs at the 180° and 360° pulse widths. In practice, the 360° pulse width is the easiest to find as it is not as prone to artifacts. *The 360° pulse width will be the null point going from negative to positive intensity.*

- Collect a 1D spectrum with **nt=1** and phase it properly.
- Determine the expected 360° pulsewidth (4 times the 90° pulse). If you have no idea where the 360° pulse should be, you can run an array of 20 steps, starting at 3, and incrementing by 4, as follows:
- Type **array**.

The spectrometer will prompt you with the following questions:

parameter to array: **pw**

enter number of steps in array: **20**

enter starting value: **3**

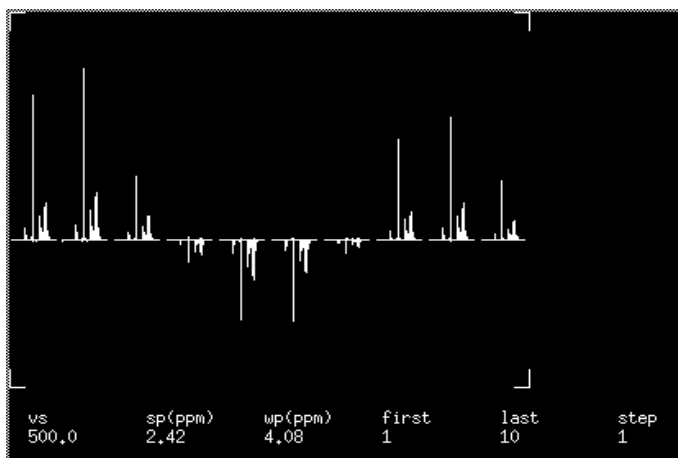
enter array increment: **4**

Upon completion of this script, a display of your arrayed values will appear on the screen. If not, go to the [**Text**] window and type **da**.

- Set **nt=1** and type **ai** to enter the absolute intensity mode.
- Run an experiment (**ga**).
- Type **dssh** to display all the spectra and **dssl** to display the spectrum number. (Optional: adjust the vertical scale (**vs**) and vertical position (**vp**) so all the peaks are on scale, eg., **vs=vs/2** and **vp=50**)

The 360° pulse will be the null going from negative intensity to positive intensity.

Shown below are the results from an array of **pw** values displayed with the **dssh** command. The 360 degree pulse is somewhere between the 7th and 8th spectra.



g. Adjust your array to find a more accurate value of **pw** once you have a general idea of where it is. In the above example, you would want to array again between the 7th and 8th spectra, using smaller increments. See step c.

h. Set the 90° pulse to this value: **pw=360°/4** where 360° is the value you found in step g. **pw?** will tell you the value. For example, if you obtain a 360° pulse width of 28.4 microseconds, the 90° pulse width will be 7.1 microseconds. You can let the computer do the math by typing **pw=28.4/4**.

i. Run another 1D experiment with this value of the 90° pulse.

j. To make the spectrum full scale again, type **f full**. Also reset **vp=12** if you changed it in step f.

Calibration of a 90° pulse width for ¹³C is not as easy, since your sample will probably not be concentrated enough to go through the above procedure quickly. If you would still like to calibrate the ¹³C 90° pulse width, it is best to use either the solvent ¹³C resonance(s) in your sample or a concentrated standard sample that is similar to your sample. Ideally, the ¹³C signal should be observable with a few scans, or this procedure will take a very long time. You will probably also have to increase **dl** for carbon spectra. Try **dl=10**. If the value of **dl** used is not large enough (3 to 5 times the longest T₁) the nulls in the series of spectra will be difficult to determine. If you use a standard sample to calibrate the 90° pulse width, the value of 90° pulse width obtained will not be as accurate as if it was obtained on your sample, but the closer the standard is to your sample, the better the results will be.

1.2. T_1 Determination

The T_1 relaxation time for a nuclear spin is the inverse of the rate constant for exponential relaxation back to equilibrium after excitation by an RF pulse. The value of T_1 for a given nucleus can give some information about molecular structure.

T_1 's for protons in small organic molecules are typically 1-5 seconds, while T_1 's for carbons range from milliseconds to 100 seconds or more, depending primarily on the number of protons attached to the carbon atom -- quaternary or carbonyl carbons have longer relaxation times than protonated carbons.

The T_1 values for the nuclei in a sample also determine how quickly the experiment can be repeated when signal averaging to increase signal-to-noise, however it is usually not worth the effort to determine T_1 values for every sample you run. If a 90° pulse is used to excite the nuclear spins, a delay of $5 * T_1$ should ideally be used between repetitions of the experiment. This delay allows > 99% of the spins to relax back to equilibrium between successive scans so that each scan gives maximum signal. For a given amount of spectrometer time, however, it has been found to be more efficient to use a pulse width that is less than 90° and a delay less than $5 * T_1$. To determine the optimum parameters to use, set **dl** so that **dl + at** is around $2 * T_1$ or $3 * T_1$, and type **ernst(T_1)**. This macro will set the pulse width, **pw**, to the optimum value to obtain the best signal-to-noise per unit time based on the value of the parameter **pw90**, the delays and acquisition time in the experiment, and the value of T_1 supplied when the macro is run. Of course, you may have to guess at the value of T_1 if you haven't measured it.

T_1 's are also important when quantitative integration is desired. In this case it is important to set **dl** so that **dl + at** is $= 5 * T_1$ of the nucleus with the longest T_1 . If this condition is not satisfied, then all nuclei will not relax fully back to equilibrium between scans, and the integrals of peaks with longer T_1 values will be smaller than they should be.

The T_1 inversion recovery pulse sequence consists of a 180° pulse followed by a variable delay, a 90° pulse and acquisition. By setting up an arrayed experiment with different values of the delay, it is possible to map out the exponential T_1 relaxation.

1. Acquire a 1D spectrum with **nt=1**.
2. Set **gain='y'**. In subsequent acquisitions, if you get a Receiver Overflow or ADC Overflow error, reduce the gain. [Typing **gain?** will tell you the current gain value.]
3. Calibrate the 90° pulse (see section 1.1) and collect another 1D spectrum with **nt=1**.
4. Type **dot1** (do T_1). You will be asked to enter guesses for the shortest and longest T_1 's expected and the amount of time (in hours) that you want the experiment to take.

The macro then sets up the T_1 experiment by arraying **d2** and setting **nt** so that the experiment takes approximately the time you requested. If you guessed incorrectly on your estimates of minimum and maximum values of T_1 , it will be apparent when the data is analyzed, and you will have to run the experiment again with different estimates. For protons, a reasonable first guess for minimum and maximum T_1 's might be 0.5 seconds and 4 seconds.

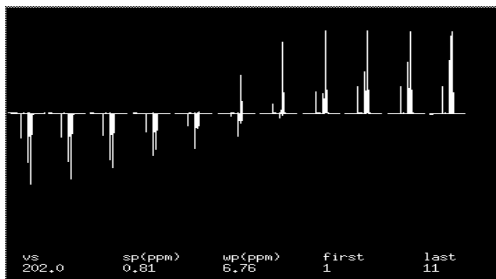
5. Set **pw** and **pw90** to the value you obtained in step 3.
6. Set **p1=2*pw** (i.e., a 180° pulse). [Note: p1 = p one, not L.]
7. Type **go** and save the experiment when it finishes.

PROCESSING T₁ DATA

8. When the experiment is complete, type **wft(1)** and phase this spectrum. Set the peak picking threshold to a level where all the peaks of interest are picked.

9. Then type **phase(180)** and **wft dssh**.

If your estimates of minimum and maximum T₁ values are reasonable, you should see spectra somewhat like those shown below for sucrose in D₂O with the peak heights changing from negative to positive.



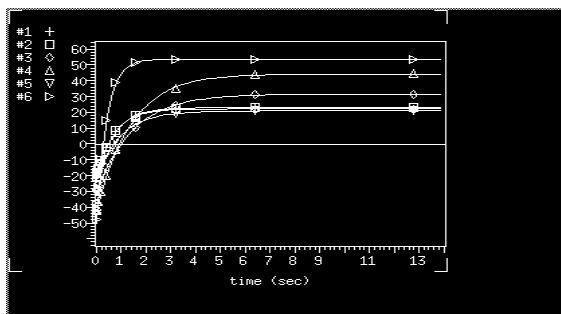
10. If the peak-picking threshold is set properly, typing **dll** will display a list of peaks in the text window and typing **fp** will create a peak file in a format that can be used by the exponential analysis software. Print this by typing **printon dll printoff**.

11. Exponential fitting of the peak heights can be done by typing **t1**. The result is a list of T₁ values and error estimates that are printed to the text window. Print this by typing **printon t1 printoff**

```
Exponential data analysis:
```

peak	T1	error
1	0.7069	0.007621
2	0.7172	0.006263
3	1.452	0.0184
4	1.367	0.01939
5	1.014	0.01359
6	0.4064	0.00231
7	0.3822	0.00254
8	0.3337	0.002789
9	0.3225	0.004422
10	1.402	0.0275
11	1.319	0.02562
12	0.3111	0.004339
13	0.9024	0.009524
14	0.9068	0.009284
15	1.086	0.01341

12. It is also possible to get a graphical plot of the T₁ fits by typing **expl**:



The **expl** command can only plot six lines at a time. To see fits for other peaks besides 1 through 6, you can type **expl(7,8,9,10,11,12)**, for example, to see lines 7 through 12. To plot this graph use **pexpl** instead of **expl** followed by **page**.

2. Solvent Suppression

2.1. Using the Decoupler (good)

Removal of an undesired solvent peak (like the HDO peak in D₂O) that is much larger than the peaks of interest greatly improves the signal-to-noise of the smaller peaks. This method is available on all instruments.

1. Set up a normal proton experiment with **nt=1** and acquire (**ga**).
2. Expand the display to show the solvent peak and place the cursor on the center of the solvent peak. Type **nl** to bring the cursor to the nearest line and type **sd** to set the decoupler to this frequency (**dof**).
3. Then set up the rest of the decoupling parameters:

dm='nyn' This is the decoupler mode. The pulse sequence is divided into three status periods - A, B, and C, which correspond to the times **d1**, **d2**, and **at**. The parameter **dm** is set to a string of three characters giving the on/off status of the decoupler during each of these periods. Setting **dm='nyn'** has the decoupler on during **d2** for presaturation.

dpwr=20 This is the decoupler power; start at 20; do not exceed 40.

homo='y' for homonuclear decoupling.

Other parameters that you do not have to set:

dmm='c' Set to **c** (continuous wave decoupling) for presaturation.

dfrq - decoupler frequency in MHz.

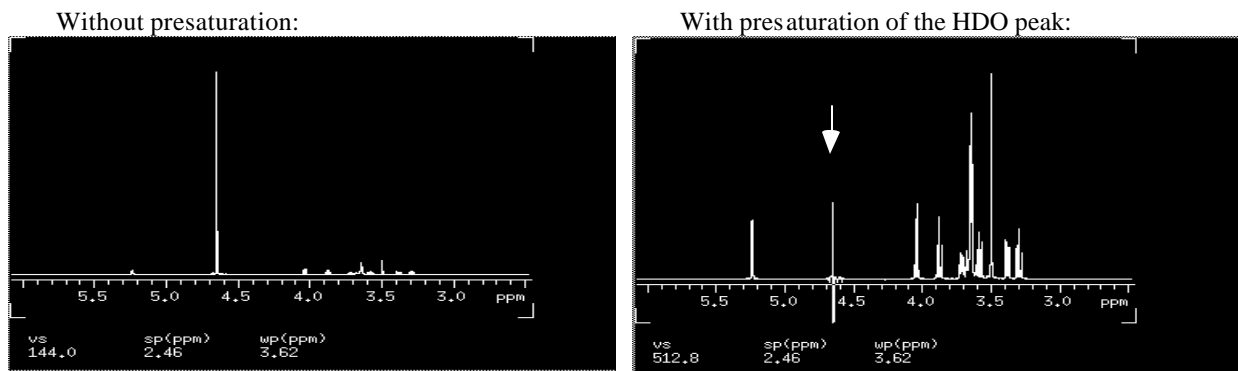
dn - decoupler nucleus.

dmf - decoupler modulation frequency.

dof - decoupler offset in Hz. Determines the frequency of the decoupler. Larger values move the decoupler downfield. This is set using the **sd** command.

4. Set **d2=2** for 2 seconds of presaturation, and set **d1=0** (since the delay **d2** of 2 seconds takes the place of the **d1** relaxation delay).

5. Acquire a spectrum, and the peak you selected for presaturation should be reduced in intensity.



- (Optional) For optimal presaturation, you may need to adjust **dpwr** and **dof**.

To find the best **dpwr**, set up an arrayed experiment by typing **gain='y' dpwr=16,20,24** and **d1=5**. Keep **nt=1** so the experiment will not take very long. Type **ga** to start the experiment, and when it is finished, type **dssa** to look at the three spectra taken with different values of **dpwr**. (The bottom spectrum corresponds to the first value in the **dpwr** array, in this case 16.) Decide which **dpwr** gives best suppression of the solvent signal and set **dpwr** to that value, or try another array of **dpwr** values. Generally the broader the peak, the larger **dpwr** must be. Do not use too large of a value of **dpwr** or you may also saturate other peaks near the solvent peak. Do not set **dpwr** > 40, since long pulses at high power may damage the probe.

To optimize **dof**, check the current value of **dof** and set up an array of values of **dof**: type **dof=dof-2,dof,dof+2**. Start the experiment (**ga**), and after the experiment finishes, type **dssa** to look at the spectra. If you find that the third one (on top) looks best, type **dof=dof[3]** to set the decoupler offset to the value used for the third spectrum. Any time you have set up an arrayed experiment, you can type **da** to see the parameter that is arrayed and the values that parameter will take.

- Set **nt** to the desired value and start the experiment (**ga**).

2.2. Using presaturation (better)

A different and more efficient method of doing solvent presaturation is with the presat pulse sequence which does not rely on the decoupler. This is available on all instruments.

- Collect a 1D spectrum with **nt=1**.
- Place the cursor on the peak to be presaturated and type **nl**.
- Type **sd**. This sets the **dof** value.
- Type **presat** to call up the parameters for water presaturation. You will have 5 saturation parameters:
sspul='n'
satpwr=2
satfrq should be set to the **dof** frequency
satdly=1.5
satmode='ynn'
- Adjust **satdly** (saturation delay) for optimum saturation. Try **satdly=1.5** and **d1=0**. The **satdly** is part of the relaxation time.
- Adjust **satpwr** (saturation power) for optimum saturation. Do not go above 25. Try values of 1 or 2 first.
- Collect another 1D spectrum with **nt=1**. If the solvent peak is sufficiently saturated, increase the **nt** and collect your spectrum. If not, repeat steps 5 and 6 or adjust **satfrq** as in section 2.1, step 6).

2.3. Using gradients (best)

The zotH₂O sequence uses gradients for solvent suppression. This sequence is available on the VI-500 and the VXR-300 which have gradient capability.

1. Collect a 1D spectrum (**nt=1**) with your sample not spinning.
2. Find the 90 degree pulse and set **pw** to that value (see section 1.1).
3. Collect another 1D (**nt=1**). Move the cursor to the peak you want to irradiate. Type **nl**.
4. Type **sd** to set the **dof** value.
5. Type **zot** to recall the gradient suppression sequence.
6. Set **solvent='solvent'** if your sample is not in D₂O.
7. Collect your spectrum.
8. For optimum suppression, array p1 ± 500 around the current p1 value. The default values are optimized for water samples.

2.4. Suppressing one or more! peaks using gradients

The `wet1d` sequence uses shaped pulses and gradients to suppress the peaks. The shaped pulse allows you to suppress more than one peak at a time (although you can use this for single peak suppression as well). Again, this sequence is only available on the VI-500 and the VXR-300 which have gradient capability.

1. Collect a 1D spectrum (**nt=1**) with your sample not spinning.
2. Find the 90 degree pulse and set **pw** to that value.
3. Click on **[Pbox]-[90]**
4. Put the cursors around the first peak to be saturated
5. Click on **[e-snob]**
6. Put the cursors around the second peak to be saturated
7. Click on **[e-snob]**
{repeat steps 6 and 7 if you have more peaks to suppress}
8. Click on **[close]**
9. Click on **[name]**--give a name to this shape (used in step 15)
10. Click on **[close]**
11. Answer the questions about **ref_pw90** and **ref_pwr** (the 90 degree pulse you determined in step 2 and **58** for the reference power level on the VI-500). If you make multiple shapes, this question is only asked the first time.
12. Click on **[Text]** in the right-hand scroll bar and scroll down near the bottom. Make note of the values in the text window for **pwwet** (in ms) and **wetpwr** (in dB). You will see something like this:
“Set pulse width to 6.3900 ms”
“Set pulse power to 23 dB”
13. (optional) move to another experiment, e.g.: **jexp2 mp(1,2) mf(1,2) wft**
14. Type **wet1d**
15. Change the parameters for the wet sequence to use the pulse shape you just made:
wetshape='name_of_shapefile' (from step 9)
pwwet=6390 (now in μ s: multiply the value in step 12 by 1000)
wetpwr=23 (dB) (from step 12)
axis='d'
16. Collect your spectrum (**ga**).
17. (Optional) Choose a different shaped pulse instead of `[e-snob]` if the desired results are not obtained.

3. 1-D NOE Experiments

The Nuclear Overhauser Effect (NOE) is a short-range ($< 5 \text{ \AA}$), through-space interaction between nuclei. It is *not* a through-bond interaction like J-coupling and can be seen between protons on different parts of a molecule that are in spatial proximity. This can be very useful in determining the conformation of a molecule. Typically, it is possible to see an NOE between protons that are less than 4-5 \AA apart.

3.1 Subtraction NOE Experiment

Subtraction NOE experiments are set up just like decoupling experiments (see section 4), except that the peak that is decoupled is the peak corresponding to the proton to which NOEs are to be seen. Typically the peak to be irradiated in the NOE experiment must be $> 0.2 \text{ ppm}$ away from all other peaks for the irradiation to not affect neighboring peaks in the spectrum. If an NOE is present between the irradiated proton and another proton, the spectrum will show a change in the intensity of the affected resonance. By subtracting a reference spectrum from the NOE spectrum, any changes in the intensity of peaks because of NOEs to the irradiated proton will be readily apparent. Because this is a subtraction experiment, spectrometer instabilities can dramatically affect the quality of the results you obtain.

1. Collect a 1D spectrum (**nt=1**) with the spinner off (turn the spinner off in the ACQI window), and the temperature regulated (type **temp=23 su**).
2. Set up the presaturation parameters:
dm='nyn'
dmm='c'
dpwr=20
homo='y'
d2=2
d1=6
3. Place the cursor on an empty region of the spectrum and type **sd**. This will be the reference spectrum needed for the subtraction routine.
4. Place the cursor on each peak in the spectrum that you want to presaturate and type **sda** for each of these peaks to set up an array of experiments.
5. Set **nt** to the desired number of transients and type **ga** to collect the spectra.
6. Save the data: **svf('filename')** and recall the data on the *offline* workstations. The subtraction macros are not available on the spectrometer computers.
7. Turn off the temperature control by typing **temp='n' su**.

PROCESSING NOE DATA

8. Using an offline workstation, load the data in experiment 1 (jexp1) and type **wft**.
9. Type: **clradd select(#) spadd** where # is the irradiated spectrum. This spectrum is added to experiment 5.
10. Type: **select(#) spsub** where # is the reference spectrum. This spectrum is subtracted from experiment 5.

11. Type: **jexp5 ds** to display the NOE difference spectrum.
12. Use **pl pscale pirn page** (not plot) to plot the NOE spectrum.

3.2. Gradient 1-D NOE Experiment (goesy)

The **goesy** is a gradient version of the 1D NOE and does not involve subtraction, thus eliminating subtraction artifacts. Because this is a gradient experiment, it is available only on the VI-500 and VXR-300.

1. Collect a 1D spectrum (nt=1) with your sample not spinning.
2. Set the cursors around your spectrum and move the sweepwidth by typing **movesw**
3. Collect another 1D (nt=1) to make sure all peaks can be phased properly.
4. Find the 90 degree pulse (see section 1.1).
5. Collect another 1D (nt=1). Save this spectrum (**svf**) for reference purposes.
6. Move the cursor to the peak you want to irradiate. Type **sd**
Repeat this step if you want to irradiate more than one peak. Record the value of **dof** displayed at the top of the screen for each peak. You will need these values in step 8.
7. Type **goesy**
8. Set the following parameters:
newtof=dof or **newtof=##,##,##** if you are irradiating multiple peaks.
d1=6
gain='y'
lb=1
nt=*multiple of 4*
9. Set the 90 degree pulse to the value you determined.
10. Collect your spectrum (**ga**).
11. Array the variable **mix** if you are not satisfied with your results, e.g., **mix=0.5,1.0,1.5**. The mixing time is the time you allow the NOE to buildup. For small molecules, this could be from 0.5 sec (default) to 2 seconds.

3.3. Gradient 1-D ROE Experiment (goesytr)

The **goesytr** is a 1D gradient version of the ROESY experiment. This is useful for molecules in the 1000-2000 molecular weight range that may not exhibit traditional NOE peaks. Available only on VI-500.

1. Collect a 1D spectrum (nt=1) with your sample not spinning.
2. Set the cursors around your spectrum and move the sweepwidth by typing **movesw**
3. Collect another 1D (nt=1) to make sure all peaks can be phased properly.
4. Find the 90 degree pulse (see section 1.1).

5. Collect another 1D (nt=1). Save this spectrum (**svf**) for reference purposes.
6. Type **goesytr**
7. Set the following parameters:
 - d1=6**
 - nt=***multiple of 4*
 - tpwr=58**
 - pw90** to the value you determined for the 90 degree pulse.
8. Place the cursors around the peak you want to irradiate.
9. Type **setshape**
10. Collect your spectrum (**ga**).
11. Array the variable **mix** if you are not satisfied with your results, e.g., **mix=0.5,1.0,1.5**. The mixing time is the time you allow the ROE to buildup. For small molecules, this could be from 0.3 sec (default) to 2 seconds.

4. Selective Decoupling

Selective decoupling experiments are performed to determine which protons are J-coupled to each other and to simplify multiplets so that coupling constants can be more accurately determined. Selective decoupling is done during the acquisition period of the experiment so that splittings due to J-couplings involving the decoupled proton are not observed.

1. Collect a 1D spectrum with **nt=1**. Set **gain='y'**.
2. Expand the display to show the multiplet you wish to decouple and place the cursor on the center of that multiplet. Type **sd** to set the decoupler to this frequency.
3. Then set up the rest of the decoupling parameters:

dm='nny' This is the decoupler mode. The pulse sequence is divided into three status periods - A, B, and C, which correspond to the times **d1**, **d2**, and **at**. The parameter **dm** is set to a string of three characters giving the on/off status of the decoupler during each of these periods. Setting **dm='nny'** has the decoupler on during acquisition to decouple the proton of interest.

dpwr=10 This is the decoupler power; start at 10; do not exceed 40.
homo='y' for homonuclear decoupling.

Other parameters that you do not have to set:

dmm='c' Set to **c** (continuous wave decoupling).

dfrq - decoupler frequency in Mhz.

dn - decoupler nucleus.

dmf - decoupler modulation frequency.

dof - decoupler offset in Hz. Determines the frequency of the decoupler. Larger values move the decoupler downfield. Set using the **sd** command.

4. Set **nt** to the desired number of scans and collect your spectrum (**ga**).

You may need to adjust **dpwr** and/or **dof** to optimize the decoupling by setting up arrayed experiments involving **dpwr** or **dof** as described above for presaturation experiments (section 2.1).

5. 1D TOCSY

The 1D version of the TOCSY experiment is useful to determine which spins are connected within a spin system.

1. In experiment 1, collect a 1D spectrum (nt=1) with your sample not spinning.
2. Find the 90 degree pulse at tpwr=58 (the default).
3. Collect another 1D spectrum.
5. Move the cursor to the peak of interest. Type **sd**
6. Join another experiment, e.g., **jexp2 mp(1,2)**
7. Find the 90 degree pulse at a lower power level. Try **tpwr=52** for D₂O or **tpwr=48** for organic solvents (pw should be around 25 μ s). Remember the 360 degree pulse is 4x this, so array around 100. These values will become slpw (pw) and slpwr (tpwr).
8. Go back to experiment 1 (**jexp1**) and type **TOCSY1D**
(Ignore the warning about the probe file.)
9. Set the following parameters:
tpwr=58
pw = the value you determined for the 90 degree pulse at tpwr=58 (step 2).
slpwr = lower power level used in step 7 (48 or 52).
slpw = to the value you determined for the 90 degree pulse at the lower power level in step 7
selfrq=dof
10. Array the mixing time. Type **array**.

The spectrometer will prompt you with the following questions:
parameter to array: **mix**
enter number of steps in array: **11**
enter starting value: **0**
enter array increment: **0.015**
11. Collect your spectrum (**ga**).
12. Type **dssa** to display the array of spectra. See page 2 for tips on displaying and plotting arrayed spectra.
13. If you do not get selective irradiation, try **selpwr=5**. The first spectrum should only exhibit the peak that you irradiated.

Trick: If you wish to selectively irradiate a peak that is within 0.2ppm of other peaks and reducing the selpwr does not work, move the decoupler location (by moving the cursor and typing **sd**) ~0.1ppm away from the desired peak (on the side opposite from those other peaks).

6.1 Gradient Shimming on non-Deuterated Solvents

If you are running samples in non-deuterated solvents, you can use the gradients to shim on your sample instead of monitoring the deuterium lock signal. Gradient shimming is commonly performed on samples in water (e.g., 90% H₂O and 10% D₂O), although other non-deuterated solvents have also given good results.

*This method does not work on deuterated solvents because the solvent signal is too weak.
The instruments equipped with gradients are the VI-500 and VXR-300.*

1. Insert sample, lock and shim (if possible); do not spin!
2. Recall standard ¹H parameters for your solvent.
3. Set **gain=0** and **nt=1** and collect a spectrum (**ga**).
4. (Optional, but may improve results) Calibrate the 90 degree pulse.

Making a Shim Map

The first time you attempt gradient shimming, you will need to create a map of the shims. This map will be instrument-dependent and probe-dependent.

5. Type **gmapsys**. This will call up the menu buttons for the gradient shim routine.
6. Select [**Set Params**] - [**Go, DSSH**]
You should see a 2 profile-type spectra. The amplitude of the 2nd may be smaller than the first. [**Return**]
7. Type **gmapsys** again.
8. Select [**Shimmaps**] - [**Automake Shimmap**] and give it a name at the prompt. Wait for the shimmap to finish.
9. Select [**return**] - [**Display**] - [**Display Shimmap**]. This will show you the shimmap you just created.
10. Select [**return**] - [**Autoshim on Z**]. This will do gradient shimming based on the shimmap. [**Return**]

For future shimming, you don't need to create another shimmap (unless you are using a different probe and/or instrument). However, if gradient shimming is unsuccessful, you might want to create another shimmap and try again.

Follow steps 1-3, then:

11. Type **gmapsys**.
12. Select [**Autoshim on Z**].

6.2 Shimming non-Deuterated Solvents using the FID

1. Insert sample.
2. Run a one scan (**nt=1**) spectrum and expand on a singlet. You may have to set gain=0.
2. Type **gf**
3. Open [**Acqi**] window.
4. Select [**FID**]
5. Select [**FID**] or [**Spectrum**]. The [**up**]/[**down**] buttons adjust magnification level.
6. Adjust shims slowly and monitor the fid level (maximize it) and fid shape (if shimming on the fid) or peak max and lineshape (if shimming on the spectrum).