## *CREATING AN ARRAYED EXPERIMENT FOR A KINETICS RUN*

Note: **Bold text** represent boxes you should click. *italic text* represent text you should type and hit RETURN.

**IMPORTANT TIPS FOR A KINETICS EXPERIMENT:**

**Internal Standard:**

1. You will need an internal standard that does not interfere with the kinetics of the reaction and that does not have peaks that overlap with your starting material or product.
2. Furthermore, you should use an internal standard in roughly a one-to-one proton equivalent to the substrate (a proton equivalent is equal to moles/# of protons).
3. It is also important that the internal standard be somewhat similar to the compounds being measured to ensure that the T1s are not too dissimilar.

**Remember those T1s:**

1. If you do not allow for complete relaxation between pulses, you will not get accurate integration and hence incorrect data. T1s for aromatic and alkene protons can be longer than for alkyl protons. It is always good to determine your T1's. Click here to learn how to determine T1's.
2. As a general rule, a delay of at least 30 seconds between scans should be enough for complete relaxation. If waiting 30 seconds between scans is not feasible, then you would use the Ernst command, which will calculate the appropriate pulse width based on a given pw90, at, d1, and T1. A good value for the T1 for Ernst would be 30 seconds; thus, you would type *ernst(30)*. This is not the best solution. The Ernst calculation is intended to give the best Signal-to-Noise for a given T1 and recycle delay. It is not designed for quantitative work.

### *Instructions for Kinetics Experiments Performed in NMR Probe:*

There are two possibilities for running a simple array: using a macro or using command line execution.

**USING THE ARRAY MACRO:** This will allow you to array parameters with set intervals between scans.

1. Lock and shim on your sample or a blank sample with the appropriate solvent. Locking and shimming on your sample is appropriate for reactions to be performed at temperatures well above room temperature.
2. Take a quick scan (nt=1) to ensure proper shimming.
3. Determine your T1 and set d1=5*T1. Set nt=8 or to another value which will be repeated in VIc. Type *su time*. Note the time, you will need this.
4. Set VT controller to desired temperature. Type *temp=# su*, where # is your desired temperature in °C. If this does not change the temperature, type *temp* and move the slider in the new pop-up to the appropriate temperature. Most instruments, except for the UnityPlus-300's, which can be set to 100 °C, have a temperature limit of between 0 and 50 °C for safety. If you need to do temperatures outside of this range, contact us at ext. 792. REMEMBER: DO NOT EXCEED THE BOILING POINT OF YOUR SOLVENT! Allow the temperature to equilibrate prior to beginning your kinetics

array. You will need to shim at the temperature of your kinetics run to ensure the best results.

5. Type *gain?*. Note the value of gain and type *gain=* that value (e.g. *gain=30*). Autogain is not allowed for arrayed experiments.
6. Setup the arrayed experiment:
    6.1. Type *array*. A set of questions will come up and must be answered.
    6.2. Parameter to be arrayed:
        6.2.1. Type *pad*. This is the preacquisition delay which will set the interval between successive scans.
    6.3. Number of increments:
        6.3.1. This is the total number of spectra or data points you want. If I want to monitor a reaction every 10 minutes for 3 hours, I would need 18, so I type '18'.
    6.4. Enter Starting Value:
        6.4.1. This is the time between each individual data point (a data point consists of 8 scans in this case) or spectrum. To calculate this value, take the time in seconds you want between spectra and subtract the time determined in step III. Thus, for an example, if I want to take spectra at 10 minute intervals, which is every 600 seconds. Step III gave me a value of 16 seconds; therefore, my pad should be 600 – 16 or 584 seconds. I type *584*.
    6.5. Enter Array increment:
        6.5.1. Type *0*. This sets the increment to zero, which means the preacquisition delay will always stay the same. If you typed 1, the pad would increase by 1 second each successive spectrum.
    6.6. Now you can run the experiment:
        6.6.1. Before starting, you may want to change the first increment to 0 so that there is no delay before the first data acquisition. To do this, type *pad[1]=0*. Type *da* and look at the bottom VNMR window to ensure that the array is correct.
        6.6.2. Insert your sample. Type *i* and *go*. You won't be allowed to lock your sample as it is already acquiring. By locking and shimming your sample on a blank prior to the arrayed experiment, you have set Z0 where it will lock. The instrument should lock after a short period. At this time you can shim to get the best possible spectrum. The first scan will occur after the pad you set. You can shim throughout the experiment if you desire. It's a good idea to check the timing between each data acquisition using a stopwatch.

***USING COMMAND LINE EXECUTION FOR AN ARRAY:*** This allows you to have differing values between each data point or spectrum.

♦ Follow steps I-V above.

♦ Type, for example, when you want to take a spectrum every 10 minutes (see VI. d. above) *pad=584,584,584,584,584,584* to take six spectra with about 10 minutes between each data point. Each number represents an experiment with the preacquisition delay (pad) in seconds set for that number. If I wanted to take 5 measurements at 10 minutes, 15 minutes, 17 minutes, 20 minutes, and 40 minutes, (assuming the actual acquisition takes 16 seconds, see VI. d.) I would type pad=584,284,104,174,1184. Remember that the pad is the time between each data point. Run the experiment:

♦ Insert your sample. Type *i* and *go*. You won't be allowed to lock your sample as it is

already acquiring. By locking and shimming your sample or a blank prior to the arrayed experiment, you have set Z0 where it will lock. The instrument should lock after a short period. At this time you can shim to get the best possible spectrum. The first scan will occur after the pad you set. You can shim throughout the experiment if you desire.

♦ Save your arrayed experiment as you would a normal experiment (i.e. *svf('filename')*).

**Important Commands for Arrayed Spectra:**

*ds(#)* - displays spectrum #, where # is the spectrum number in the array. The first spectrum is 1, the second is 2, etc. (e.g. *ds(1)* to display first spectrum).

*dssa* - displays all spectra vertically

*dssh* - displays all spectra horizontally

*dssl* - displays the spectrum number(#) to be used with ds(#)

*pl('all')* - print all stacked spectra

**Kinetics Data Analysis:**

1. Type *wft dssh full ds aph*.
2. Click on **Next=>Th**. Use the left mouse button to set the threshold.
3. Type *dll fp*.
4. Type one of the following depending on which type of analysis you want:
   4.1. *kind* - Kinetics analysis, decreasing peak intensity.
   4.2. *kinds* - Kinetics analysis, decreasing intensity, short form output.
   4.3. *kini* - Kinetics analysis, increasing peak intensity.
   4.4. *kinis* - Kinetics analysis, increasing intensity, short form output.
5. Type expl. This will perform an exponential anaylsis.

***Instructions for Determination of T1:***

The constant for relaxation, T1, is known as longitudinal or spin-lattice relaxation. This is the time for magnetization along the z-axis to relax. Many factors influence T1's including temperature, paramagnetic impurities, solvent viscosity and dielectric, molecular size and structure. Within a given molecule, the individual resonances will have different T1's depending on their molecular environment. As a rough rule of thumb, more protons for a resonance means shorter T1's. Thus, alkynes and aromatic protons will have longer T1's than alkyl groups. Carbon T1's are generally longer than a proton's.

If insufficient time is allowed for all protons to completely relax (5*T1) between pulses in an FT NMR experiment, one will obtain inaccurate integration data. Let's say, for example, that a molecule has two resonances possessing T1's of 1 and 10 seconds, respectively. We decide to pulse every 5 seconds (i.e. at=5 and d1=0). We will get all the

signal from the first resonance, but clearly not from the second (it still has 5 more seconds of relaxation and signal to give us). The result is incorrect integration!

**Important considerations for T1 measurements:**

1. Make sure that you are using the best quality tube possible.
2. Filter the sample to remove any impurities that may affect the experiment.
3. Oxygen is paramagnetic and will reduce T1's. Degas the sample to obtain the most accurate T1's.
4. T1's are temperature dependent. Run the experiment at a temperature slightly above room temperature.
5. T1's are concentration dependent. Make sure to note the concentration.

**T1 Measurement:**

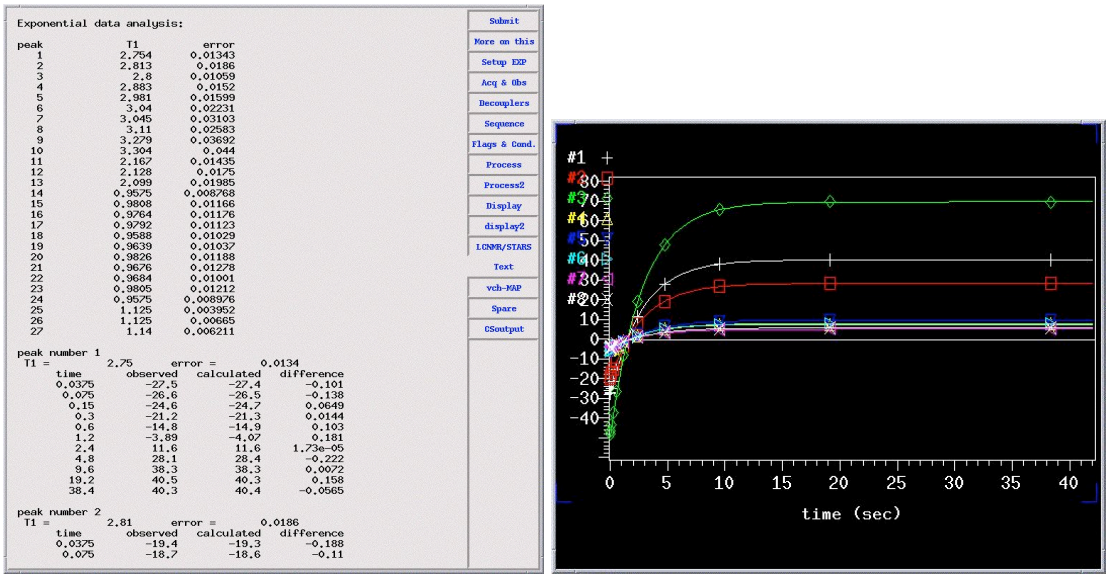
1. Type *dot1*.
2. Answer the questions:
    2.1. Minimum T1 estimate: enter the shortest T1 you expect.
    2.2. Maximum T1 estimate: enter the longest T1 you expect.
    2.3. Experiment time: This is the time you have to do the experiment. The longer, the better.
3. Type *go*.

***Data Processing:***

1. When completed, save the file and type *wft ds(arraydim)* to display the last spectrum acquired.
2. Phase the spectrum.
3. Select the threshold (click **Th**) and adjust to be below all the peaks you wish to analyze.
4. Type *dpf*.
5. Type *fp*.
6. Type *t1 center expl*.

The output you receive will look something like the following for 2-phenylbutanoic acid. The picture on the left shows a portion of the output after typing *t1*. The first table shows the determined T1 and its error. For example, peak 1, which is an aromatic peak, has a T1 of 2.754 seconds with an error of 0.01343 seconds. The methyl T1 is peaks 25-27 and has a T1 at 1.1(0.007) seconds.

The picture on the right shows the output from typing *expl*. This shows the behavior of the peaks with varying delays.




## *Instructions for Variable Temperature (VT) Operation:*

Variable temperature NMR is an excellent way to investigate dynamic behavior of molecules. Both qualitative and quantitative information about inversion, ring-flip, and other barriers can be extracted from a series of spectra at different temperatures.

VT operation is available on many of our spectrometers (click here to view capabilities of our instruments). We have set limits of 0 to 50 °C for most spectrometers. These temperatures were selected to protect the probes from damage due to either a solvent freezing and cracking the NMR tube (D20 at 0 °C) or a solvent boiling and spilling in the probe (CDCl3 at 62 °C). Both could cause significant probe damage. This has occurred, not because of the user who did the VT experiment, but because of the next user who was unaware of the probe temperature.

**IMPORTANT: BE SURE THAT YOU DO NOT GO ABOVE THE BOILING POINT OR BELOW THE FREEZING POINT OF YOUR SOLVENT. IF YOU DO NOT KNOW THESE VALUES, CLICK HERE TO FIND OUT. IF YOU REQUIRE TEMPERATURES OUTSIDE THESE RANGES, PLEASE CONTACT US (386, 387, OR 792).**

**YOU MUST ALLOW AMPLE TIME FOR THE PROBE TO RETURN TO ROOM TEMPERATURE (UP TO 15 MINUTES) PRIOR TO THE NEXT USER'S TIME. FAILURE TO DO SO MAY LEAD TO PROBE DAMAGE.**

### Temperature Calibration:

The temperature that is displayed on the instrument console and on the VNMR screen display is not necessarily the actual temperature. Therefore, it is important to calibrate the temperature. This calibration can be done a week in advance on the experiment or a week

after, but it is best done within a few days. If you will be using a series of temperature, you should obtain a calibration curve.

1. Lock and shim on your sample or on the standard.
2. Eject the sample and insert the ethylene glycol (for High temperature) or methanol (for Low temperature) sample. Be sure to gauge the sample properly. Since there is no deuterated solvent in the sample, you will not be able to lock or shim. It doesn't matter. You will still be able to acquire the spectrum.
3. Type *vttype=2 su*. Then type *aa* to abort current temperature control and finally type *temp*. A new window will pop-up. Drag the slider to the desired temperature.
4. Allow the probe and sample to equilibrate for at least 10 minutes.
5. Click **Setup**=>**H1,CDCl3**. Type *nt=1 ga*.
6. Type *aph*. Place the cursors on top of the 2 peaks.
7. Type *tempcal('e')* for ethylene glycol (high temp) or type *tempcal('m')* for methanol (low temp).
8. Repeat for the entire temperature range you require.
9. Plot the actual temperature vs. set temperature. The slope of this line will be the calibration factor.

Step by Step Instructions for **UnityPlus-500** (Anubis: B-7 subbasement): Click here for other instruments.

1. Choose a deuterated solvent that is a liquid in the temperature range you require.
2. Eject the standard sample and insert your sample.
3. For **high** temperature operation:
    3.1. Type *temp=your desired temperature*. Type *su*. For example, I may type *temp=40 su*. If this does not change the temperature, type *temp* and move the slider in the new pop-up to the appropriate temperature.
    3.2. Allow temperature to equilibrate for at least 10 minutes.
    3.3. Shim and acquire spectrum as usual.
    3.4. When completed, type *temp* and set temperature back to 25. IMPORTANT: RESET THE TEMPERATURE AT LEAST 10 MINUTES PRIOR TO THE NEXT USER'S TIME SLOT.
4. For **low** temperature operation:
    4.1. Type *temp=your desired temperature*. Type *su*. For example, I may type *temp=0 su*. If this does not change the temperature, type *temp* and move the slider in the new pop-up to the appropriate temperature.
    4.2. Go to the chiller located against the wall behind the magnet (when looking from the computer. It is the west wall).
    4.3. The setting should be 15.0. Using the down arrow key, set the temperature to 10 degrees below your desired temperature. For example, if I typed *temp* and slide the bar to 0, I would set the chiller to **-10**.
    4.4. The temperature should change rapidly. If there is little or no temperature change in 2 minutes, contact the NMR staff (792).
    4.5. Allow temperature to equilibrate for at least 10 minutes.
    4.6. Shim and acquire spectrum as usual.
    4.7. When completed, type *temp=25 su*. Reset the chiller to 15. IMPORTANT: RESET THE TEMPERATURE AT LEAST 10 MINUTES PRIOR TO THE NEXT USER'S TIME SLOT.

Step by Step Instructions for **Other** instruments:

1. For **high** temperature operation:
    1.1. Type *temp=your desired temperature*. Type *su*. For example, I may type *temp=40 su*. If this does not change the temperature, type *temp* and move the slider in the new pop-up to the appropriate temperature.
    1.2. Allow temperature to equilibrate for at least 10 minutes.
    1.3. Shim and acquire spectrum as usual.
    1.4. When completed, type *temp=25 su*. If this does not change the temperature, type *temp* and move the slider in the new pop-up to the appropriate temperature. IMPORTANT: RESET THE TEMPERATURE AT LEAST 10 MINUTES PRIOR TO THE NEXT USER'S TIME SLOT.
2. For **low** temperature operation: **WE HIGHLY RECOMMEND YOU CONTACT US (792) PRIOR TO DOING LOW TEMPERATURE WORK ON ANY INSTRUMENT OTHER THAN ANUBIS (B-7).**
    2.1. Type *temp=your desired temperature*. Type *su*. For example, I may type *temp=0 su*. If this does not change the temperature, type *temp* and move the slider in the new pop-up to the appropriate temperature.
    2.2. Remove the styrofoam bucket located on the leg of the magnet by twisting.
    2.3. Fill the bucket with liquid nitrogen and CAREFULLY place back around VT cooling coil.
    2.4. Allow temperature to equilibrate for at least 10 minutes.
    2.5. Calibrate if desired.
    2.6. Shim and acquire spectrum as usual.
    2.7. When completed, remove the cooling bucket, type *temp=25 su*. If this does not change the temperature, type *temp* and move the slider in the new pop-up to the appropriate temperature. Wait until the temperature has equilibrated to 25 °C. IMPORTANT: RESET THE TEMPERATURE AT LEAST 10 MINUTES PRIOR TO THE NEXT USER'S TIME SLOT.
    2.8. Type *temp='n'*.