Longitudinal Relaxation Time (T_1) Measurement for ^{1}H on VnmrJ2.2D (use on Ra, Mut, and Isis)

Knowledge of the T_1's of a molecule is important in the setup of many 1D and 2D experiments, in which the relaxation delay (*d1*) must be set according to the T_1's of the signals of interest. In some instances, the T_1 values themselves are used as structural parameters in the characterization of a variety of compounds.

The T_1's of a sample can be measured using the inversion-recovery method. In the experiment, the time (*d2*) between the inversion (180° pulse) and the read pulse (90° pulse) is incremented. The signals will start out inverted (*d2* is very short, see Figure 1 below) and as the recovery time is increased (*d2*), the signals will recover their equilibrium magnetization and a typical 'positive' spectrum is obtained. The point when a given signal has zero intensity in this experiment is termed τ_{null}. The longitudinal relaxation time, T_1, is approximately $1.44\tau_{null}$.

For most applications, only a rough approximation of the longest T_1 is needed for setting *d1* or other parameters. In which case, no *pw90* measurement would be necessary and the default *pw90* can be used. However, if accurate T_1 measurements are required, you should determine the 90° pulse width on your sample and set *pw90* to the measured 90° pulse width.

If the measured T_1's fall outside the expected range, you should rerun the experiment with a new expected minimum and maximum T_1.
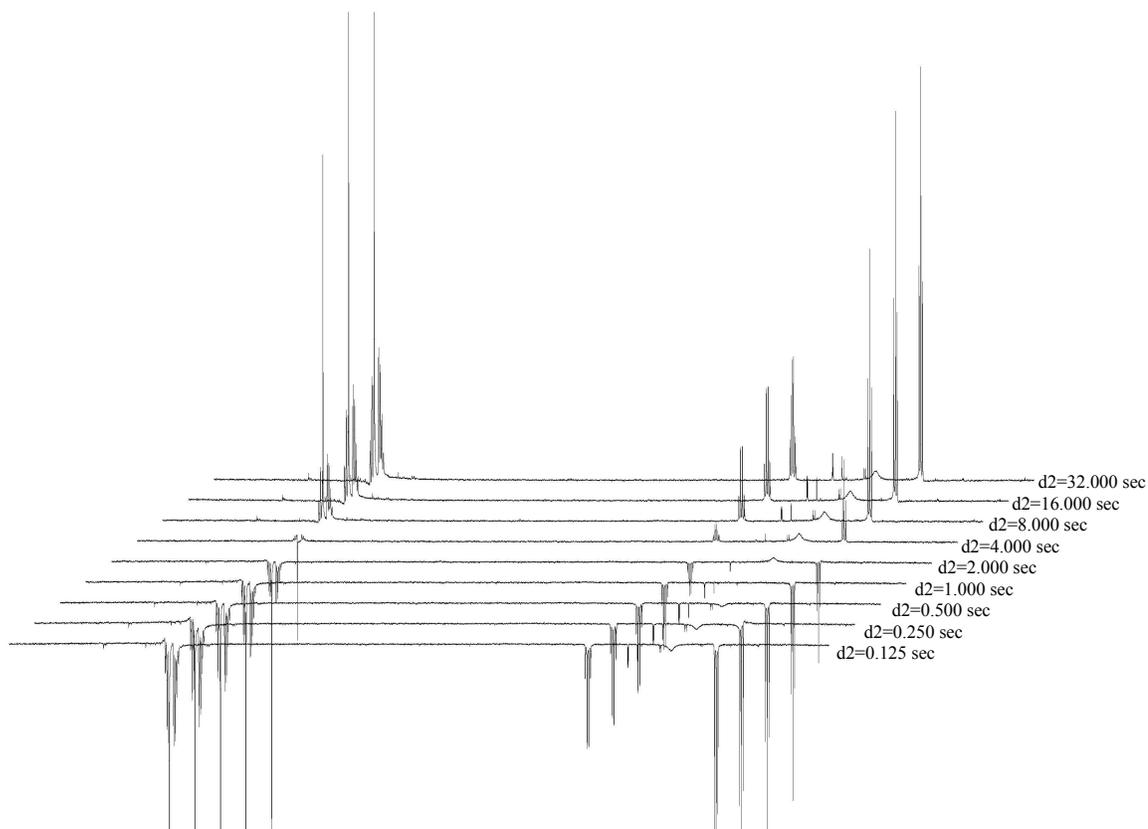

Figure 1. An example of the spectra from an Inversion Recovery experiment to determine the T_1's for ethylbenzene obtained on a Varian Unity 400 MHz spectrometer using the *dot1* macro.

Explanation of Types of Commands Found in this Handout:

1. The VNMR software and the UNIX operating system are both case sensitive. This means that the computer distinguishes whether the letters are entered in upper case (*i.e.* CAPITALS) or lower case. The user must be careful to type the correct case for each letter in a command.
    Example: **jexp1** is not the same as **JEXP1**

2. Some commands are line commands and are typed in by the user followed by a return (a Return↵ is assumed for typed **bold** text commands).
    Example: **su**

3. Some commands are executed by clicking a mouse button with its pointer on a "button" found on the screen. The execution of these commands are indicated by a two letter designation (LC {left click}, RC {right click}, or CC {center click}) followed by a word or words in shadow or **bold** that would appear in the button.
    Example: LC **Main Menu**

    This means to click the left mouse button with its pointer on the button that says "Main Menu".

4. Some commands are executed by the mouse itself. These commands are indicated by a two letter designation (LC, RC, or CC) and a description of what the user should do in parentheses.
    Example: LC (at 6 ppm)

    This means to click the left mouse button with the mouse cursor positioned at 6ppm.

5. Parameters are entered by typing the parameter name followed by a equal sign, the value, and a return.
    Example: **nt=16**

<p style="text-align:center">*   *   *   *   *</p>

IMPORTANT CONSIDERATIONS FOR T$_1$ MEASUREMENT

- Make sure that you are using the best quality tube possible.
- Filter the sample to remove any impurities that may affect the experiment.
- Oxygen is paramagnetic and will reduce T$_1$'s. Degas (freeze-pump-thaw) the sample to obtain the most accurate T$_1$'s. This is only necessary if you are determining T$_1$'s for NOESY, ROESY, or for dynamic information about your compound.
- T$_1$'s are temperature dependent. Consider running the experiment at a temperature slightly above room temperature.
- T$_1$'s are concentration dependent. Make sure to note the concentration.

ACQUIRE A STANDARD ^{1}H SPECTRUM

Setup a standard ^{1}H.

Lock and shim the sample as usual. When shimming is completed, enter:

pw=pw90   sets the pulse width to 90°.
nt=1 (or minimum *nt* that would give a spectrum with acceptable S/N)
ga   acquire the data and Fourier transform the result.

When acquisition is complete, phase, reference, and save the spectrum as usual.

gain='y'   turns off autogain and uses the gain setting from the last experiment.

DETERMINE 90° PULSE (OPTIONAL, BUT RECOMMENDED)

We are going to look for the 360° instead of a 90° pulse. We do this because the 360° pulse will produce a null signal and the factor of 4 gives us a little better accuracy.

pw90?   query the value of pw90.

What is the value of 4*pw90? This is the default 360° pulse. We will array the pulse width around this number to find the null point.

pw=8,*(360°pulse)-2, 360°pulse, (360°pulse)+2*   input the correct values for the 360° pulse. For example, if the 360° pulse is 40, then I would use pw =8, 38, 40, 42.

ai   set absolute intensity mode.
d1=10   set the recycle delay to 10 seconds.
nt=1   use one scan.
ga   acquire the data and Fourier transform the result.

When acquisition is complete,

ds(1)   displays first spectrum.
aph   automatic phasing. If this fails, manually phase.

Expand around peaks never the center of the spectrum.

dssh   display stacked spectra horizontally.

The first spectrum will be positive. The next should be negative and the remaining should be getting less negative until passing through zero to positive. If the peaks do not cross zero, rerun the experiment with longer 360º pulses (i.e. if your last spectrum is still negative at 48, then rerun with pw=50,52,54. Repeat until the null is reached).

Note the value of the null spectrum and divide it by 4; this is the true 90º pulse. If you don't know the number, click on the bottom of the two icons that look like, *, in the graphics toolbar. This will display the actual pw values underneath the spectra.*

pw90= *determined 360ºpulse/4*   set pw90 to true 90 pulse.

## AUTOMATED SETUP FOR THE T_1 EXPERIMENT (RECOMMENDED)

LC Experiments   opens experiments tab
LC Relaxation Measurements   opens Relaxation measurements sub-menu
LC T1 Measurement   selects the T1 Measurement experiment

In Parameter Panel,

LC Acquire, Defaults Tab   opens the T1 Measure parameter panel

In the middle of the Parameter pane, make sure T1 Mode is Inversion Recovery (see image below).
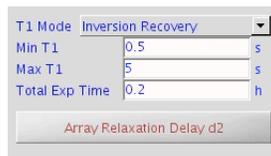
*In the **Min T1** field, enter your minimum expected T_1. In **Max T1** field, enter maximum expected T1. If unsure, use the default values. Use the default Total Exp Time.*

*LC on **Array Relaxation Delay d2***   sets up the arrayed values

Enter your determined pw90 value now.

pw=*determined pw90*   sets the 90° pulse to your determined value.
p1=2*pw   sets the 180° inversion pulse to twice the determined value.
ga   acquire the spectra and FT the results

VIEWING, PROCESSING, AND PLOTTING OF T$_1$ DATA

When acquisition is complete,

LC the *Process Tab* in the Bottom Parameter Panel
LC ***T1 Analysis***
LC ***Display Last Spectrum*** (see picture to right)
f aph   autophase spectrum. If it doesn't phase
                                                 properly, manually phase it.

Set Threshold such that all the peaks on which you'd like a T$_1$ analysis are picked.

LC ***Do T1 Analysis***   this performs the T$_1$ analysis and displays
                                                 the peak list and the full T$_1$ results.

LC ***Plot*** *in the current Process tab*   selects the Plot options menu
LC ***Auto Plot***   this plots the T$_1$ analysis and spectra. The
                                                 screen display will be distorted, but not the
                                                 plot.

pll page   prints a line list for identifying peaks in the
                                                 T$_1$ analysis.

If you are determining the longest T1 for a quantitative proton experiment, look for the
longest T1 that is not your solvent peak and use 5 x *(that value)* for your proton d1.