

MEASURING T_1 RELAXATION TIMES (ARX spectrometers)

OVERVIEW

1. Measuring a T_1 value requires that you make an initial guess about the order of magnitude of the result. You must use this guess to select delay times for this measurement. If you guess well, only a small number of measurements will be needed to obtain a reasonable estimate. If you guess poorly, you will be able to tell from your initial result how to proceed with a new guess.
2. It is always a good idea to do a very crude measurement first to make sure your initial guess is not way off. Use just a few delays (e.g. 4), including one that should be $\sim 5 \times T_1$. Use the minimum number of scans that will give reasonable signal to noise, preferably only one scan where possible.

DETAILED SET-UP

1. Obtain a spectrum of the sample using a 90 degree pulse. To do so, set PULPROG to **zg**, **zgdc**, **zgf**, or **zgfdc**, as appropriate. (See acquisition write-up.) Make sure you have set the receiver gain (with **rga**) using a 90 degree pulse.
2. If you do not wish to overwrite your original spectrum, create a new data set.
 - a. Type **new** to edit the name of the current data set.
 - b. Whenever a data set is entered that does not already exist, a new data set is created that contains parameters identical to those displayed at the time it was created.
3. Set up a variable delay list. This is called a "vdlist". The vdlist must have one delay time (in seconds) per line. Type **edlist**. Select **vd**. Either select the name of a list that you have used previously or select **Type New Name** (at the bottom of the edlist window). This will put you into the **xedit** editor. Enter the "infinity" value as the first line. Take note of how many delays you have in your list. When finished select **save** then **quit**. You should write down the values or print out the list from a unix window with the command **lpvdlist filename** (where you substitute the vdlist name that you used for *filename*).
4. Select an inversion recovery pulse program. The appropriate choice corresponds to the appropriate choice of pulse program above in the following way: **zg**→**t1ir.js**; **zgdc**→**t1irdc.js**; **zgf**→**t1irf.js**; **zgfdc**→**t1irfdc.js** (If you need to use delays in excess of ~ 55 seconds, you will require yet a different pulse program. In that event, see the NMR lab personnel for help.)
 - a. It is always a good idea to have a hard copy of the pulse program you are using. To obtain one, type **edpul pulse program name** (substituting the appropriate pulse program for *pulse program name*). The pulse program will be displayed. Select **print** to obtain a hard copy, then **OK** to exit from the display.

- b. Type **eda**, select the PULPROG parameter (top left). Type in the appropriate pulse program as described above.
 - c. From the pulse program listing, you can see that the data are to be acquired into a 2-D data set. Select **PARMODE**, then select **2D**. Then **SAVE** the entire menu. Reply **OK** to the message "Delete 'meta.ext' files?". This will change the look of the processing window. Type **eda**. There are now 2 columns of parameters, one for the **F2** dimension (the acquisition dimension) and one for the **F1** dimension. The only parameter of interest for the **F1** dimension is **TD**. It must be set to the number of different delay times you have in your vdlst.
 - d. You must also set the parameter **VDLIST** to the name of the list that you edited above. This parameter is located near the end of the list of parameters in the eda menu. Either select **VDLIST** with the left mouse button and type in the name or select **VDLIST** then select the small "button" on the right side of the VDLIST value to bring up the list of choices.
5. Type **ased**. This command is very useful in that it displays acquisition parameters as does the **eda** command, but it will display only those parameters relevant to the chosen pulse program. (With ¹⁹F observation, you will get 2 odd error messages that can be ignored.)
- a. The pulse program listing has comments about several parameters. The parameters that were already used for the normal spectrum mostly can be used as is. The only ones you need to be concerned with are those that are additional (or more critical) for the inversion recovery pulse programs.
 - b. These are:
 - d1** - (this is the number *one*, not the letter *l*) Use a value that you guess to be at least 5 times T₁.
 - p1** - (this is the number *one*, not the letter *l*) This is the 90 degree pulse time for the transmitter power output level that you are using. In the inversion recovery pulse programs (and **zg** and **zgdc**) the transmitter power output is determined by **t10**.
 - p2** - This is the 180 degree pulse time for the transmitter power output level that you are using.
 - l4** - (this is the letter *l*) - Set it equal to the number of different delay times you have in your vdlst. (This is also equal to **TD** in the **F1** dimension).
6. Type **expt**. A message will be displayed with the following information: 1) the total experiment time; 2) the size of the file that will be created in kbytes and blocks (1 kbyte = 2 blocks); and 3) available disk space in blocks. (The unix command **df** gives the available disk space in kbytes.) (As with **ased**, ¹⁹F observation gives 2 odd error messages that can be ignored.)
7. Type **ii**. Start the data acquisition with **zg**. This will acquire all spectra into a single "ser" file.

PROCESSING

1. Type **edp** to bring up the processing parameters. **SI** must be equal to 2ⁿ, where n is an integer ≥2. Set the value of **SI** in the **F1** dimension to the smallest value that is greater than or equal to **TD** in the **F1** dimension (i.e. the number of delays in the vdlst). For example, if you use 6 delay times, SI must be set to 8. (If you have an odd number of delay values, you will have to set the parameter **MC2** in the F1

dimension to **QF**). The rest of the processing parameters of interest will be as they were in the 1-D spectrum already obtained and need not be changed. **SAVE** the menu.

2. Type **xf2** to do the Fourier transformation in the **F2** dimension only. To display both positive and negative data, select the "button" labeled +/- 2 times. Then select **DefPlot** and input returns in reply to all the questions.
3. There are 2 ways to do the phasing. Here is one of them.
 - a. Type **rsr 1**. This reads the first fid from the 2-D data matrix. It should be data acquired with the "infinity" value for the delay. Do the phasing in the usual way. Select **return**, then **Save as 2D & return**. Return to the full data set by selecting the "button" labeled **2D**.
 - b. To apply the phasing just determined to all the spectra, type **xf2p**.
4. To obtain a reasonable estimate of the T_1 's rather than a quantitative measure, there is a command that will extract the individual spectra (rows in the processed data set) and place them into a series of process numbers (**procno**'s).
 - a. Type **split2D**. Reply **r** to the first question since we want rows not columns. The second question concerns the number of rows you wish to extract. Reply with the number of different delay values (τ values) that you used for the acquisition. The third question asks you to input the process number (**procno**) where the first row extracted should be placed. All subsequent rows will be placed in increasing process numbers. You can input any process number other than the one where you are viewing the 2D contour display (usually proc no. 1). (It can be helpful to start at something like 101, so that process no. 101 contains row #1.)
 - b. The individual spectra may then be viewed in the usual 1-D mode. Type **rep n** where **n** is the process number of the "first target procno" specified in **split2D**. A convenient way to step through the spectra from here is to use the dual display. Select the "button" labeled **dual**. A window will appear in which you must specify the name of the second data set for display. In this case, it defaults to what you want (i.e. the next higher procno) so all you have to do is select **SAVE**. In the dual display, the "buttons" on the upper left affect both spectra. The "buttons" below that area affect spectrum 1 (i.e. the one from which we entered the dual display) and the next set of "buttons" below that affect spectrum 2. Initially they will be on top of each other so move them so you can see both well. Step through the rest of the rows that have been extracted by selecting the "button" labeled **P↑**.
 - c. The delay time that produces a null of the signal of interest will be approximately $0.693T_1$ for that signal.
5. If the intensities of the peaks of interest do not go from negative to positive, then you must re-acquire the data with better choices for the delay times. Use **edlist** to edit the list. If the number of delays in the list is different from the last acquisition, you will have to change the value of TD in the F1 dimension (most easily done in the **eda** menu). Recall that the parameter I4 (this is the letter *l*) must also be set to the number of delays in the list. (See item 5b in the section entitled "Detailed Set-up"). In light of your results, consider whether the choice for **d1** was appropriate or should be changed before re-acquiring a data set. Type **zg** to re-acquire the data, then **xf2** to re-transform it. If your original

choice of **d1** was appropriate, then you will not have to re-determine the phase constants. In the **edp** menu, set the parameter **PH_mod** in the **F2** dimension to **pk**. The command **xf2** will include the phasing and you will not need to separately type **xf2p**.

6. To obtain a stacked plot of the individual spectra that have been placed into a series of process numbers with **split2D**, type **rep n** where **n** is the process number of the "first target procno" specified in **split2D**. Expand the region to be plotted and save it by selecting the "button" labeled **DP1**. Input a title for the plot in the usual way. Type **edg** and turn off the plotting of integral trails, integral labels, and peak labels, then **SAVE** the menu. Type **stack1d**. The following dialog will occur (sample answers in italics were used to obtain the stacked plot reproduced at the end of this write-up):

Expno/Procno Stack Plot 1.1 95/01/16 - Note

- run from EXPNO/PROCNO with largest positive intensity peak (for scaling)
- Set plot pars (title, axis, F1/F2, etc.) first!

(Select OK or Cancel depending on whether you have set these parameters)

Plot expnos (e) or procnos (p):	<i>p</i>
Enter first expno/procno to plot:	<i>19</i>
Enter number of expnos/procnos to plot:	<i>9</i>
Enter expno/procno increment/decrement:	<i>-1</i>
Enter number of expnos/procnos per page:	<i>9</i>

Full dimension available for plot

CX: 20.0
CY: 17.0

(Select OK)

Enter width for each plot:	<i>15</i>
Optimum x-increment (return if OK):	<i>0.5</i>
Enter plot height for largest peak in spectrum:	<i>5</i>
Enter offset to x-axis (-ve intensities?):	<i>5</i>
Optimum y-increment (return if OK):	<i>0.75</i>

7. To calculate T_1 values:

- a. From the 2-D contour display, select **Analysis**, then **Relaxation (T1/T2)**. Select **Process** on the bar at the top to see the commands that are available in the T1/T2 routine. All of the T1/T2 commands can either be selected from this menu or typed on the command line.
- b. You must first create a file of peak positions to use for the T_1 calculations. Type **rspc**. This will select the first row of the transformed spectra and convert the display to the usual 1-D mode. Expand the region of interest enough that you will be able to accurately select peaks with the mouse. Select **utilities**, then **defpoints**. If a file has already been created for this processed data set, you will be given the choice of append (**a**), overwrite (**o**), or quit (**q**). To create a new file, select **o**; to add more peak positions to an existing file, select **a**. Position the arrow on the peak of

interest, then press the middle mouse button, move to the next peak of interest, press the middle mouse button again, etc. When all peaks of interest in the displayed region have been selected, press the left mouse button. If peaks in other regions of the spectrum are to be included, expand the display of the region of interest, and repeat the above being sure to select **a** to append to the current file. Select **return**. Again select **Analysis**, then **Relaxation (T1/T2)**.

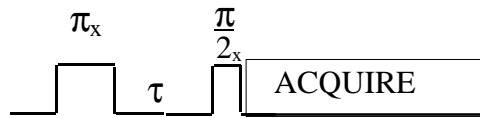
- c. Type **edt1**. The main parameter that may require adjustment is **DRIFT**. This parameter is the number of data points that peak maxima may vary from the maximum position in the first spectrum in the peak picking routine (see below). The choice of appropriate value is strongly dependent on the digital resolution in the **F2** dimension. (Type **edp** and examine the value for **Hz/pt** in the **F2** dimension to see the digital resolution of your spectra.) Select **SAVE** to exit from **edt1**.
- d. Type **pd**. This is a "peak picking" routine that will produce a table of peak intensities in all spectra acquired for each peak that you selected in step **6.b.** above. Ignore the message about integration. When the peak picking is finished, a display of the individual data points for the first peak should be visible. If you see no data points, select the button on the upper right of the buttons to the left of the data area. This should re-scale the data so the points are displayed.
- e. The next step will send the output to the printer. If you would like to view it on the display first, type **edo**, select **CURPRIN**, and type in **\$screen**, then select **SAVE**. Type **dat1** to calculate the T_1 's of all selected peaks. The message "cannot read integral range file No such directory" can be ignored. When this routine is finished the intensity data and calculated T_1 's will be displayed. If you wish to print it out, select **List**. If there are entries under the "Intensity" heading that are *********, try increasing the value of **DRIFT** in the **edt1** menu. You must repeat **pd** to peak pick the spectra using the wider drift tolerance. Then type **dat1** again to recalculate the T_1 's. (Any peak that has 2 or fewer intensities that are not ********* will produce an error message during the T_1 calculation. The T_1 reported for peaks that produce this error is always 1 sec. Be sure to ignore that result!) Select **return** to exit the T_1 calculation display. You should then be back in the contour display.
- [f. Sometimes the **rspc** command that extracted the first row of transformed data (see **7.b.** above) also converts the display parameters to 1-D mode. If you wish to re-display the entire data set in the 2-D mode, type **rpar standard2D plot**. (Be aware that this will delete the "baseline points" file. You would have to repeat step **7.b.** if you wish to repeat the T_1 calculation.)]

Printout of vdlst used to obtain data for stacked plot on the last page:

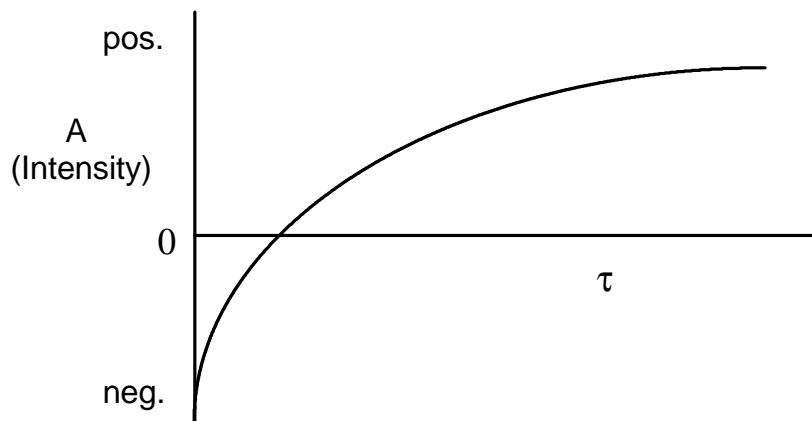
Jan 9 09:59 1995 /u/exp/stan/nmr/lists/vd/gramicidin Page 1

10
6.4
3.2
1.6
.8
.4
.2
.1
.04

Measuring T_1 Relaxation Times



Inversion Recovery



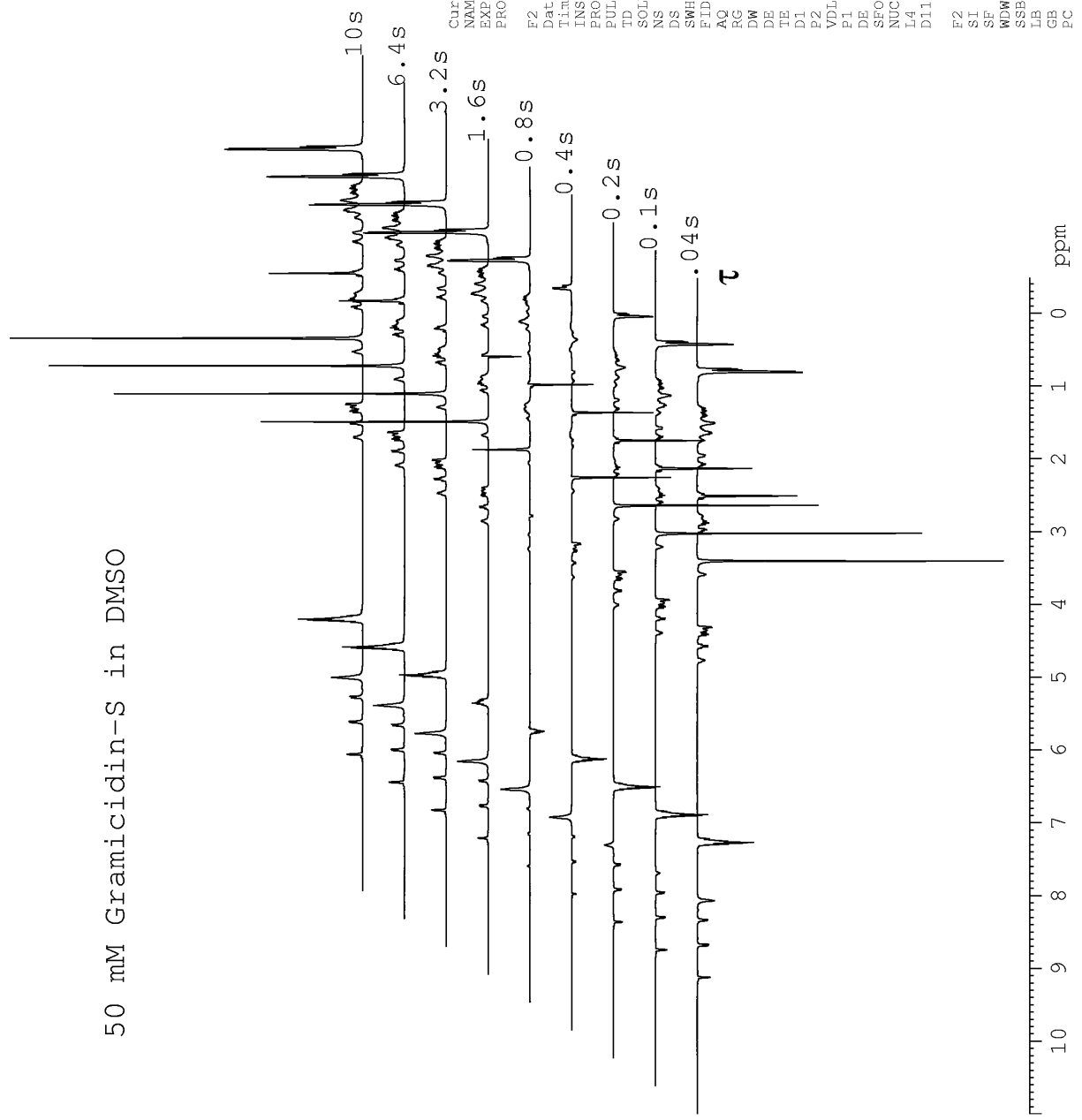
$$A_\tau = A_\infty (1 - 2e^{-\tau/T_1})$$

(assuming ideal 180° and 90° pulses)

$$A_\tau=0 \text{ when } \tau = \ln 2 T_1 = .693 T_1$$

As a rough estimate, the value for the delay at which a peak is nulled is ~70% of the T_1 value.

50 mM Grammicidin-S in DMSO



Current Data Parameters
 NAME grammicidin-S
 EXPNO 17
 PROCNO 19

F2 - Acquisition Parameters
 Date_ 950109
 Time 10.02
 INSTRUM arx500
 PROBHD 10 mm 11B
 PULPROG t1irp2
 TD 32768
 SOLVENT DMSO
 NS 8
 DS 0
 SWH 5747.126 Hz
 FIDRES 0.175388 Hz
 AQ 2.8508661 sec
 RG 128
 DW 87.000 usec
 DE 108.75 usec
 TE 300.0 K
 D1 10.0000000 sec
 P2 16.00 usec
 VDLIST grammicidin
 P1 8.00 usec
 DE 108.75 usec
 SF01 500.1326288 MHz
 NUCLEUS 1H
 L4 9
 D11 0.0300000 sec

F2 - Processing parameters
 SI 32768
 SF 500.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00