MEASURING T_1 RELAXATION TIMES
-TOPSPIN-

<u>OVERVIEW</u>

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 ~5 X T_1 . Use the minimum number of scans that will give reasonable signal to noise, preferably only one scan where possible.

3. <u>These directions presume that you know the things in the data acquisition and data processing write-ups. Please consult them if you do not know how to do something that these directions tell you to do.</u>

<u>DATA ACQUISITION DETAILS</u> (Based on Topspin 1.3)

1. Acquire a spectrum of the sample **<u>using a 90 degree pulse</u>**. To do so, set PULPROG to **zg,** or **zgdc**, as appropriate. (See acquisition write-up.) Type **gpro** and tune the probe, as usual, before a data acquisition. Make sure you have set the receiver gain (with **rga**) using a 90 degreee pulse and have the spectral width set to the value you intend to use for the T_1 measurement. Process the spectrum as usual, i.e. Fourier transform and phase the data set.

2. If you do not wish to overwrite the spectrum in step 1, 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. For “Experiment”, choose “Use current parameters”. This will assure that you have an appropriate value for **rg** if you did step 1. (above) correctly.

3. Set up a variable delay list (vdlist). Type **edlist**. Under “Options”, select “Edit parameter list”. Under “Required parameters”, “List type=”, select “Variable delay (vd)”, then select OK. Either select the name of a list that you have used previously or under “Newfile:” (at the bottom), type in a filename, then select OK.

   a. The vdlist must have one delay time (in seconds) per line. Each delay value must be on a separate line. You must use a leading zero for entries less than 1 second, e.g. use 0.5, not .5.

   b. Enter the "infinity" value as the first line. This is a value (in seconds) that you feel reasonably certain will be at least 5 times the longest T_1 value anticipated. (You may want to change this value after your first crude measurement.)

c. Take note of how many delays you have in your list. It is probably a good idea to write down the values if you are just making a crude estimate. If you do the actual calculation described later, the values will be a part of the report that is generated.

d. When finished select OK.

4. Type **eda** or select the **AcquPars** tab. Select the **PULPROG** parameter (first parameter in the list). Type in the appropriate pulse program as described below or select it from the sub-menu of pulse programs obtained by selecting the small "?" button on the right.

   a. The appropriate choice depends on the pulse program used for the normal 1D spectrum. If it was **zg**, use **t1ir**; if it was **zgdc**, use **t1irdc.js**.

   b. The data are to be acquired into a 2-D data set. On the icon bar in the parameter menu, select the **Change acquisition dimension of current data set** button. From the pop-up menu, select **Change dimension from 1D to 2D**, then select OK. 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 <u>must</u> be set to the number of different delay times you have in your vdlist.

   c. 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 the LISTS category on the left side of the parameters or use the **Search parameter** "button" to find **VDLIST.** Then select the small "button" on the right side of the VDLIST value to bring up the list of choices. Select the list that you created. You will be able to view or edit it from here if needed.

5. Type **ased** or select the **Show pulse program parameters** "button" on the icon bar in the parameter menu. This command is very useful in that it displays only those parameters relevant to the chosen pulse program.

   a. The most important parameter to consider changing is the relaxation delay, **d1**. Use a value that you guess to be at least 5 times T_1, i.e. the value you used for the "infinity" value in the vdlist. You can view and edit your vdlist from here if needed.

   b. When done, select the **Show all acquisition parameters** "button".

6. Type **expt** or select the **Estimate experiment time [expt]** "button" on the icon bar in the parameter menu. A message will be displayed with the following information: 1) the total experiment time; 2) the size of the file that will be created; and 3) available disk space. For an initial experiment using ns=1 and only ~ 4 delay values, the total experiment time should be only a few minutes. There is no need to start with many delay values and more scans than necessary to get a reasonable estimate.

7. Type **zg** or select the **Start acquisition** "button" on the 2^{nd} line from the title bar to start the data acquisition. This will acquire all spectra (i.e. one for each delay value) into a single "ser" file.

<u>DATA PROCESSING DETAILS</u> (Based on Topspin 2.1 pl1)

1. Type **edp** or select the **ProcPars** tab to bring up the processing parameters.

a. Set **SI** in the **F1** dimension to the number of delay values in the vdlist. (**SI** must be equal to 2^n, where n is an integer >2. From the value you input, the data system usually sets the value for **SI** to the smallest value that is greater than or equal to **TD** in the **F1** dimension, i.e. the number of delays in the vdlist. For example, if you use 6 delay times, **SI** will default to 8. If it sets it less than the number of delays, then enter the next larger allowed value.)

b. If you have an odd number of delay values, you will have to make sure the parameter **MC2** in the **F1** dimension is set to **QF**.

c. Set **PH_mod** in the **F2** dimension to **pk**.

d. 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.

2. Type **xf2** to do the Fourier transformation in the **F2** dimension only. If you followed all the directions in the data acquisition steps and turned on the phasing as described in step 1.c. above, your individual spectra will be phased properly.

3. If you have not followed all the directions in the data acquisition section, you may need to phase the T_1 data. (Please be aware that some lack of following directions may require that you reacquire the data.) To phase the T_1 data, type **rsr 1**. This reads the first spectrum from the 2-D data matrix. It should have been data acquired with the "infinity" value for the delay.

a. Type **ef**, then use the phase subroutine to do the phasing in the usual way. On the phase subroutine menu bar, select the **Save for 2D spectrum [.s2d]** "button", then the usual **Return & save phased spectrum [.sret]** "button".

b. Return to the 2D data set by selecting the **To last 2D data [.2d]** "button".

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. 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 data 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, etc.)

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 multiple display feature. Select the **Multiple display [.md]** "button" on the 2^{nd} row down from the title bar. In the Topspin "Browser" area at the left, expand the experiment numbers to see all the process numbers that were created by the **split2D** command. Drag (one at a time) the rest of the rows that were split out of the 2D data set. If the spectra are displayed on top of each other, select the **Toggle the display layout** "button" on the multiple display subroutine menu bar to separate them.

c. The delay time that produces a null of the signal of interest will be approximately $0.693T_1$ for that signal. If there is not a spectrum where it is nulled, make a guess based on the values where the signal changes sign. Select the **Return [.ret]** “button” on the subroutine menu bar to exit.

5. If the intensities of the peaks of interest do not change sign, then you must re-acquire the data with better choices for the delay times. Return to the 2D display of the data by selecting the button labeled the **To last 2D data [.2d]** “button”. Use **edlist** to edit the list.

   a. If the number of delays in the list is different from the previous data acquisition, you will have to change the value of **TD** in the **F1** dimension (most easily done in the **eda** menu; see item **4.b.** in the “Detailed Set-up” section above)

   b. In light of your results, consider whether the choice for **d1** was appropriate or should be changed before re-acquiring a data set. (Remember that **d1** should always be $\underset{\sim}{>}5XT_1$.)

   c. Type **zg** to re-acquire the data.

   d. If the number of delays in the vdlist has changed, enter the value for **SI** in the **F1** dimension as in processing step 1 above. Repeat data processing steps 2 through 4 above to process the data and look at the individual rows. If your original choice of **d1** was appropriate, then you will not need to re-determine the phase constants in step 2, just use **efp**.

6. To calculate T_1 values:

   a. Return to the 2D display of the data by selecting the button labeled the **To last 2D data [.2d]** “button”.

   b. Select the **Analysis** menu in the middle of the 1st row below the title bar. From the pull-down menu, select **T1/T2 Relaxation**. This will open the “NMR Relaxation Guide” on the right side of the Topspin data window. The guide tells you what to do, but mostly does not actually do anything.

      1. Select the **Extract Slice** “button” in the guide. Since you have already done the Fourier transform, you can choose “Spectrum” from the window that comes up. Choose Slice Number 1. (This is the equivalent of the **rsr** command.)

      2. Select the **Peaks/Ranges** “button” in the guide. Select **Manual Integration**. After you have defined the integrals of the peaks of interest, you must select the **Save region as…** icon on the subroutine menu bar, then select **Export Regions To Relaxation Module and .ret.** to exit the subroutine.

      3. Select the **Relaxation Window** “button”. This opens a window that shows the default parameters for T_1 calculations. (If that window doesn’t open automatically, you can open it by selecting the **Settings** “button” on the subroutine menu bar.) Most are reasonable choices. Change is the **Function Type** in the “Fitting Function” section to **invrec**, then select **OK**. Under “**Fitting type**” to the left of the data displayed, select **Area**.

      4. Select the green >> “button” on the left end of the subroutine menu bar. That will calculate the T_1 values for all of your peaks. The “Brief Report” area on the left of the data will display the T_1 values for all of the peaks you selected. You can step through the display of the data points for each peak by using the **-** & **+** “buttons” on the subroutine menu bar. The plot displayed is not necessarily

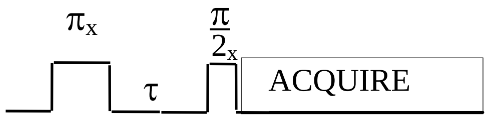
the same region that you acquired unless you select the **Show full spectrum, leave intensity scale [.hr]** “button” on the third line below the title bar.

5. Select the **Display Report** “button’ in the guide. This opens a separate window that has all the information about all the calculations. You can print the report or save it to a text file from this window.

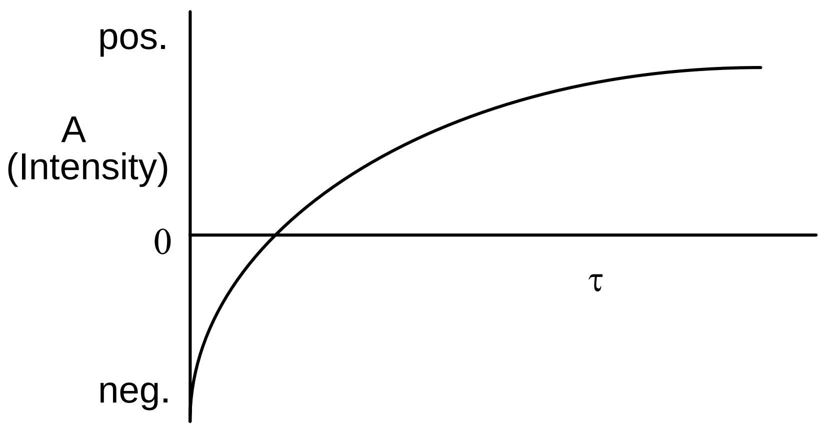
6. The **Print/Export** “button” in the guide tells how to print or export the T_1 plot displayed. To export the data, select an appropriate directory, then input a filename that has one of the listed extensions.

7. Close the window when you are done by selecting the x in the upper right corner of the data area. This will return you to the 2D display of the data. To return to your T_1 display, select **Analysis** on the main menu bar, then **T1/T2 Relaxation** as above. From the NMR Relaxation Guide, select **Relaxation Window**.

Measuring T_1 Relaxation Times



Inversion Recovery



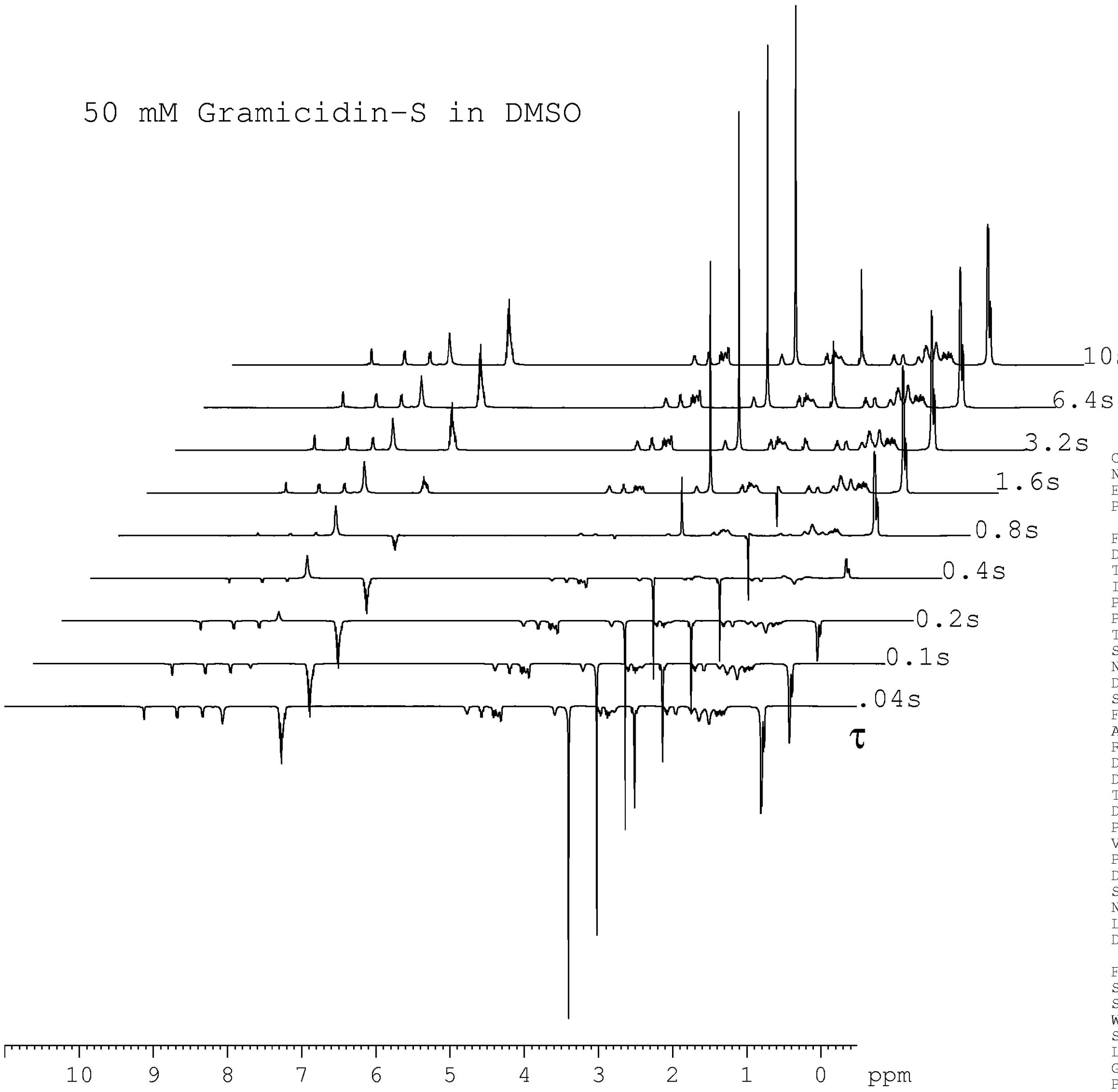
$$\mathbf{A_\tau = A_\infty (1-2e^{-\tau/T_1})}$$

(assuming ideal 180° and 90° pulses)

A_τ=0 when τ= ln2T_1=.693T_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 Gramicidin-S in DMSO



Current Data Parameters		
NAME	gramicidin-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	10.00000000	sec
P2	16.00	usec
VDLIST	gramicidin	
P1	8.00	usec
DE	108.75	usec
SFO1	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	