

Measuring T_1

Introduction

7.1

The spin-lattice relaxation time of the various ^1H nuclei of a molecule may be determined by using the inversion recovery pulse sequence. The pulse sequence begins with a recycle delay (t_{rd}) that is sufficiently long to ensure that all magnetization returns to equilibrium (i.e., pure z-magnetization). A 180° pulse is applied which inverts the magnetization. The recovery delay follows to allow varying degrees of T_1 relaxation (depending on the value of the recovery delay time). The final 90° pulse then converts any z-magnetization into observable transverse magnetization, which is detected during the acquisition period immediately following the final pulse. Notice that if the recovery delay time is very short, the pulse sequence is equivalent to $t_{rd}-270^\circ\text{-acq}$, and the detected signal has full, negative intensity. On the other hand, if the delay is very long, full T_1 relaxation occurs between the 180° and 90° pulses, and the detected signal has full positive intensity. T_1 can be determined by repeating the experiment with several different recovery delay values, processing the results identically, and plotting peak intensity with respect to recovery delay time. The resulting curve is an exponential with rate $1/T_1$. (Notice that for some intermediate value of the recovery delay, the peak intensity is zero and $T_1 = t_{null}/\ln 2$; however, the accuracy of this measure of T_1 is usually low.)

The procedure described in this chapter is for determining ^1H T_1 values. A similar procedure may be used for measuring ^{13}C T_1 values. However, for measuring ^{13}C T_1 's, it is important to use inverse-gated ^1H decoupling to improve the spectral signal-to-noise ratio without selectively enhancing peak intensities through NOE effects. It is also important to use a sufficiently long recycle delay (recall that ^{13}C T_1 's can be much longer than ^1H T_1 's).

Sample

The sample used to demonstrate a T_1 experiment in this chapter is 100 mM Pamoic Acid in DMSO-d₆.

Pulse Sequence Diagram

7.2

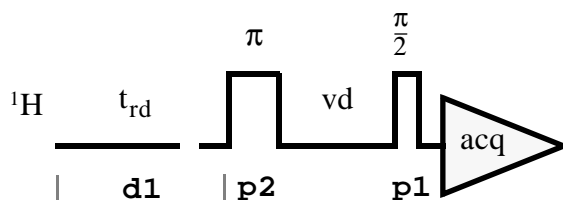
The inversion recovery pulse sequence is shown in Figure 20. Notice that the pulse **p1** must be set to the appropriate 90° time found in Chapter 5 'Pulse Calibration'. The 180° pulse length **p2** is determined by the pulse program itself.

The pulse sequence begins with the recycle delay **d1**. The 180° pulse **p2** is followed by the recovery delay **vd**. The value of **vd** is determined by the delays contained in the appropriate vclist, and is varied over the course of the experiment. Observable magnetization is created by the final 90° pulse **p1**, which is followed immediately by the acquisition period.

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A 1D spectrum is obtained for each value of νd , and the results are stored in a 2D data set. The 2D data set is used by the T_1 calculation routine, which allows the user to determine T_1 for any number of peaks of the 1D spectrum.

Figure 20: Inversion Recovery Pulse Sequence



Acquisition and Processing

7.3

Make sure the following preliminary steps have been completed: Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^1H observation.

Create a new file directory

Enter **re proton 2 1** to call up the data set proton/2/1. Enter **edc** and change the following parameters:

NAME	t1data
EXPNO	1
PROCNO	1 .

Click **SAVE** to create the data set t1data/1/1.

^1H reference spectrum

Enter **rga** to perform an automatic receiver gain adjustment. Acquire and process a standard ^1H spectrum, as described in Chapter 3 ‘Basic 1H Acquisition and Processing’ (notice that since the data set t1data/1/1 was created from proton/2/1, most acquisition parameters are already set). Calibrate the spectrum and optimize **sw** and **o1** so that the ^1H signals cover almost the entire spectral width. Acquire and process an optimized spectrum.

Create a new file directory for the 2D data set

From the data set t1data/1/1, enter **edc** and change EXPNO to 2. Click **SAVE** to create the data set t1data/2/1. This data set will be used for the inversion recovery experiment. Although inversion recovery is not technically a 2D experiment, it does generate an array of 1D spectra which are most easily handled as one 2D file. Thus, t1data/2/1 must be changed into a 2D data set as described below.

Enter **eda** and set PARMODE = 2D. Click on **SAVE** and ok the message “Delete ‘meta.ext’ files?”. The window now switches to a 2D display and the message “NEW 2D DATA SET” appears.

Write the variable delay list

The inversion recovery experiment requires a variable delay list to provide all the values of the recovery time **vd**. To create the variable delay list, first enter **edlist**. A menu of list types appears. Select **vd** from this menu. This calls up a menu of existing vdlst filenames and gives the user the option of creating a new file ('Type new name'). Simply type the name **t1delay**. This calls up the vi editor. Enter the delays desired, some appropriate values are listed below:

```
10 s
5 s
4 s
3 s
2 s
1 s
0.5 s
0.25 s
0.1 s
0.01 s .
```

When the list is complete, save the file and exit the editor. (Note that for best results, it is recommended to begin and end the list with the longest **vd** value (if all is working properly, then, the first and last spectra should be identical), and to scramble the order of the intermediate values.)

Set up the acquisition parameters

Enter **eda** and set the acquisition parameters as shown in Table 24. Use the values determined in Chapter 5 'Pulse Calibration' for the parameters **p11** and **p1** (¹H observe high power level and 90° pulse time). The relaxation delay time **d1** should be chosen to be longer than five times the longest T₁ value to be measured.

Be sure to set **vdlist** to the name of the appropriate variable delay list (here **t1delay**). This may be done in the **eda** menu either by typing **t1delay** in the box next to the parameter VDLIST, or by clicking with the right mouse button on this box to call up the menu of possible vdlsts and then selecting **t1delay** with the left mouse button. Also be sure to set **14** equal to the number of entries in the vdlst (here 10).

The F2 parameters **o1** and **sw** (not shown in the table) should be identical to the values used in the optimized ¹H reference spectrum (t1data/1/1). The only important F1 acquisition parameter is **td**, and this should be set to the number of delays in the vdlst.

Table 24. Inversion Recovery Acquisition Parameters

F2 Parameters		
Parameter	Value	Comments
PULPROG	t1ir	see Figure 20 for pulse sequence diagram.
TD	16k	
NS	8	the number of scans must be 8*n in order for the phase cycling to work properly.

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DS	4	number of dummy scans.
PL1		high power level on f1 channel (see “An Important Note on Power Levels” on page 7).
P1		90° ^1H high power pulse on f1 channel.
P2		180° ^1H high power pulse on f1 channel; calculated internally.
D1	10s	relaxation delay; must be longer than five times the longest T_1 to be measured.
D11	30msec	delay for disk I/O; predefined.
L4	10	loop counter; set to number of entries in vdlst.
VDLIST	t1delay	name of vdlst used to provide various recovery delays.
F1 Parameters		
Parameter	Value	Comments
TD	10	number of experiments.

Acquire the 2D data set

If this data set was created from the ^1H reference spectrum t1data/1/1, the receiver gain is already set correctly.

Enter **zg** to acquire the time domain data. The approximate experiment time for the inversion recovery experiment with the acquisition parameters set as shown above is 30 minutes.

Set up the processing parameters

Enter **edp** and set the processing parameters as shown in Table 25. Notice that the F1 parameter **si** must be set to a power of two greater than or equal to the number of delays in the vdlst used.

Table 25. Inversion Recovery Processing Parameters

F2 Parameters		
Parameter	Value	Comments
SI	8k	
SF		spectrum reference frequency (¹ H).
WDW	EM	
LB	1 Hz	
PH_mod	no	
PKNL	TRUE	necessary when using the digital filter.
BC_mod	quad	
F1 Parameters		
Parameter	Value	Comments
SI	16	select a power of two greater than or equal to the number of delays in vdlst.
BC_mod	no	
MC2	QF	

Process the spectra

The spectra will be processed by the automation program **proc_t1**. If desired, however, the spectra may be processed manually. Simply enter **xf2** to multiply the time domain data by the window function and also perform the Fourier transform in F2 only. The 2D data set is displayed automatically.

Write the integral range file and baseline point file

The automation program **proc_t1** which will be used to calculate T_1 for the defined peaks requires a predefined integral range file and baseline point file. These files must be written before running the automation program.

From the 2D data set, move to a 1D data set containing the row for which **vd** is a maximum (here, row 1). This may be accomplished by entering **rser 1**. This copies the FID of the first row into the data set ~TEMP/1/1.

Enter **ef** to apply line broadening. Manually phase correct the spectrum and store the correction.

Click on **integrate** to enter the integration subroutine. Click the left mouse button to tie the cursor to the spectrum and then use the middle mouse button to select the integration regions. Click once at the beginning and once at the end of each region. A separate region must be selected for each peak for which T_1 will be calculated. When all the desired regions have been defined, click the left mouse button to release the cursor from the spectrum. Click on **return** and select **Save as 'intrng' and return** to store the regions and return to the main 1D window. Enter **wmisc** to call up the menu of miscellaneous list types. Select **intrng** to select the integral

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range file type. This calls up the list of possible files. Simply type the new name **t1reg**. Now the integral regions selected above are written to the integral range file **t1reg**.

From the main 1D window, enter **bas1** to enter the baseline submenu and from here click on **def-pts** to enter the baseline point subroutine. In this subroutine, the cursor is tied to the spectrum. Use the middle mouse button to select the points for which T_1 will be calculated. One and only one point must be selected for each integral region defined above. Take care to select the point of maximum intensity for each peak (region). When finished, click the left-hand mouse button to release the cursor from the spectrum and store the baseline points. Next enter **wmisc** to call up the menu of miscellaneous list types. Select **bas1pnts** to select the baseline point file type. This calls up the list of possible files. Simply type the new name **t1bas**. Now the baseline points selected above are written to the baseline point file **t1bas**.

Click on **return** to return to the main 1D window. From here, click on **2D** with the left mouse button to return to the full 2D data set.

T_1 Calculation

7.4

Once the T_1 data have been acquired and the integral range and baseline point files have been defined, the data may be processed and the T_1 calculation carried out using the automation program **proc_t1**. This program first Fourier transforms and phase corrects the rows of the 2D T_1 data set. It then performs a T_1 calculation on all the peaks indicated by the integral range and baseline point files.

The automation program **proc_t1**

Start the automation program from the 2D data set by entering **xau proc_t1**. Answer the questions as follows:

```

Enter fid no. for phase determination: 1
Enter left limit for baseline correction: 1000
Enter right limit for baseline correction: -1000
Enter no. of drift points: 20
Enter name of baseline point file: t1bas
Enter name of integral range file: t1reg
Enter name of VD list to use: t1delay
Enter calc. type (T1=1, T2=2): 1 .

```

The FID corresponding to the largest value of **vd** (i.e., full relaxation between the 180° and 90° pulses) should be used for phase determination. The values for left and right limit for baseline correction are in ppm. The values ± 1000 are suggested merely to be sure that all spectra fall completely within these limits. The automation program applies a baseline correction in F2 (**abs2**) between these two limits, and it is important, obviously, to baseline correct the entire spectral width.

The number of drift points accounts for the fact that the maximum of a peak selected for a T_1 calculation is usually not at exactly the same position for each 1D spectrum. The number of drift points specifies how many digital points the peak maximum may vary. This parameter may need some optimization. It is important to select the number of drift points large enough so that you are always sure to find the peak maximum, yet small enough so that the maximum is always of the *same* peak. If the number of drift points is chosen incorrectly, peak picking will not work properly

and the T₁ curves will not all be smooth exponential curves. (Peak picking can also fail if the integral regions and baseline points are not selected carefully.)

When **proc_t1** is finished, the message “T1 result stored in t1r” appears. The full pathname of this file is /u/data/[user name]/nmr/t1data/2/pdata/1/t1r (i.e., it is in the same directory as the current processed data).

The peak intensity vs. **vd** time data are also gathered and plotted for each resonance. To view these results, select **Analysis->Relaxation** to enter the T₁/T₂ routine. The first T₁ curve should appear automatically in the window. Enter **nxtp** to view the T₁ curves for successive peaks.

Check T₁ curves

At this point it is a good idea to check the T₁ curve for each selected resonance to verify that the **vd** values were chosen so that all curves are clearly defined. For example, for a very short value of T₁, more short values of **vd** may be needed to define the beginning of the exponential curve, while for a very long value of T₁, more long values of **vd** will be required to define the “end” of the curve. If any T₁ curves are not well defined, it is necessary to edit the vlist **t1delay** and rerun the experiment so that reliable T₁ measurements for those resonances may be obtained.

Also check all T₁ curves to be sure that they are smooth exponentials. If not, although it may not be necessary to rerun the experiment, it is necessary to redo the T₁ calculation. First, if there are only a few bad points per T₁ curve, it is possible to remove the bad points and redo the T₁ calculation by hand. Points may be removed from a curve one at a time by typing **elim** and then selecting a point with the middle mouse button (click the left mouse button to quit without choosing a peak). Eliminated points may be restored by entering **rstp** (this restores all eliminated points to all T₁ curves).

Once the bad points have been removed from a curve, enter **ct1** to begin the T₁ calculation for that resonance. Enter **nxtp** to call up the next curve, remove the bad points, enter **ct1** to calculate T₁ for that peak, and so on. Alternatively, remove the unwanted points from all curves and then enter **dat1** to begin the T₁ calculation for all selected peaks. (Note that unless CURPRIN is changed *before* using **ct1** or **dat1** to recalculate T₁, the numerical results from **proc_t1** will be overwritten as discussed below.)

If there are too many bad points for a given T₁ curve to be a reliable fit, **proc_t1** should be rerun. It may be necessary to use a different number of drift points, or to redefine the integral range and baseline point files.

Check numerical results

The numerical results generated by the T₁ calculation routine may be stored in a file, displayed on the monitor, or sent directly to the printer. The automation program **proc_t1** automatically stores the results in the file t1r in the processed data subdirectory. (After running **proc_t1** enter **edo** to call up the plotter options menu and note that CURPRIN is set to t1r.) Each time a T₁ calculation is run with CURPRIN set to t1r, this file is overwritten. This means that it *is* overwritten each time **proc_t1** is run, and *may* be overwritten when **ct1** or **dat1** is used. However, before using **ct1** or **dat1**, the user also has the option to set CURPRIN to \$screen or to the appropriate printer.

To display the numerical results on the screen, set CURPRIN to \$screen as follows: enter **edo**, click the box next to CURPRIN with the left mouse button, and then

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enter \$screen. Click **SAVE** to exit the **edo** menu. To send the results directly to the printer, click the box next to CURPRIN with the right-hand mouse button to call up the menu of printer options, select one of these with the left-hand mouse button, and then click **SAVE** to exit the **edo** menu.

The numerical results consist of a table for each selected peak. These tables indicate TAU (i.e., **vd** value), CURSOR, FREQ, PPM, INTEGRAL, and INTENSITY for each point. Below each table is the statement “[n] intensities fit” or “[n] areas fit”. This is an indication of how well the peak picking worked. For example, here 10 values of **vd** were used, so if peak picking worked well, 10 intensities should have been fit for each peak selected. If 0 or very few intensities were fit for one or more peaks, it is a good idea to redefine the integral range and baseline point files and rerun **proc_t1**.

Finally, for each peak selected, there is a table indicating the T_1 and standard deviation calculated.

T_1 parameters

If necessary, the user may edit a number of parameters used in the T_1 calculation routine. In the T_1/T_2 menu, select **edt1** from the **T1cmds** pulldown menu, or simply enter **edt1**. Some appropriate values are indicated in Table 26.

Table 26. T_1 Parameters

Parameter	Value	Comments
NUMPTS	10	number of delays in vdlst.
FITTYPE	intensity	T_1 will be calculated from peak intensity (rather than integrated area).
CURSOR	1	start with the first peak chosen.
CONV	e^{-5}	convergence criterion for the fit algorithm.
DRIFT	20	allowed peak drift for peak picking.
START	1	starting spectrum for peak picking.
INC	1	increment for next spectrum used in peak picking.
NUMTERM	3	number of variables used in fitting routine.

Create a Stacked Plot

7.4.1

This section describes a method for obtaining a stacked plot of the 2D data set. The plot is created by the au program **stack2d**, which uses the plot parameter set **stackplot**. Note that **stackplot** is a 1D plot parameter set.

To create the 1D parameter set, first return to the reference spectrum (enter **re 1 1**). Use the mouse to select an appropriate region for plotting. Once the desired region is displayed, save it by clicking on **DP1** with the left mouse button and hitting return to answer the questions that follow.

Next create the plot parameter set to be used by the au program. Enter **edg** to call up the plot parameter menu. Make sure that SPECT is set to YES, but that XAXIS, YAXIS, TITLE, INTEGR, and PARAM are set to NO (see Appendix C, section C.2 '1D Plotting Parameters'). Click on the ED which appears next to the option ED-SPECT to call the submenu "Spectrum Plot Parameters". The following selected parameter values are suggested for A4 (8.5" × 11") paper.

Table 27. Spectrum Plot Parameters for Stacked Plot

Parameter Name	Value (A4)	Comments
SXLLEFT	2.0cm	
SYLLEFT	1.0cm	
CX	20.0cm	
SHEI	20.0cm	
FIP		These parameters were set when DP1 was used to define the plot region.
F1		
F2P		
F2		
PPMCM		
HZCM		
DHEI	19.0cm	
SZERO	4.0cm	
CY	6.0cm	

Click **SAVE** to save these changes and return to the main **edg** menu. There, click **SAVE** to save all changes and exit **edg**.

Next, save these parameters as the plot parameter file **stackpar**. This is done by entering **wpar stackpar plot**.

Once the 1D parameters are set, return to the 2D processing menu (enter **re 2 1**).

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Start the stacked plot automation program by entering **xau stack2d**. Answer the questions as follows:

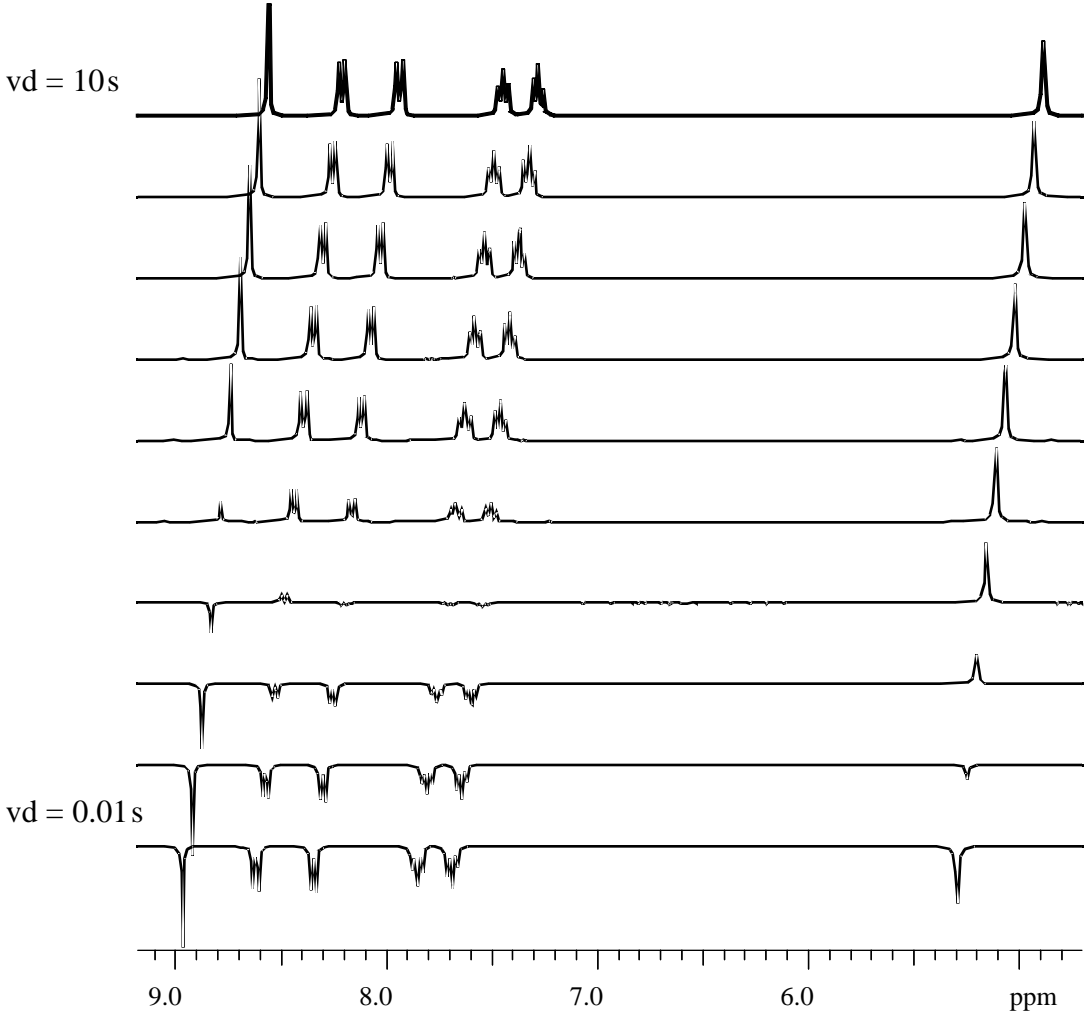
```
Enter NAME:                t1data
Enter EXPNO:                2
Enter PROCNO:              1
Enter USER:                [user name]
Enter DISK:                 u
Repeat dialog (r) or continue (c): c
Enter first row to plot:    10
Enter row increment:        -1
Enter number of rows:      10
Enter row for scaling:     1
Enter x increment [cm]:    0.2
Enter y increment [cm]:    1.2 .
```

The resulting stacked plot is sent to the plotter specified by the parameter CURPLOT. To check or change this parameter, enter **edo** to call up the output device parameter menu. Using the right-hand mouse button, click on the box next to CURPLOT to open the menu of plotter options. Select one of these with the left-hand mouse button, and then click **SAVE** to exit the **edo** menu.

A stacked plot of the results of the inversion recovery sequence run on 100mM Pamoic Acid in DMSO-d6 is shown in Figure 21. Notice that only a portion of the entire spectral width used is shown in the figure.

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Figure 21: Inversion Recovery Spectra of 100 mM Pamoic Acid in DMSO-d₆



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