KJM 9250

Proton T$_2$ Spectra on the AVI-600 and AVII-600

Version 1.0

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1.0 Introduction

An **awprotont2** parameter set and linked **VCLIST** file from which a **VDLIST** file _must_ be calculated prior to processing a T$_2$ data set have been set up on the AVI-600 and AVII-600 spectrometers running under TS2.1 and TS3.2 respectively.
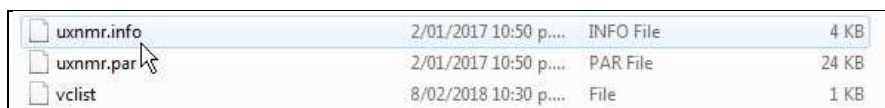
The parameter sets have **D1 = 10 sec** and 8 x **VCLIST** values. The T$_2$ data set is acquired using the _smallest vclist value first. Prior to processing T$_2$ data sets variable constant (VC) values must be converted to time domain variable delay (VD) values using the vctovd command (see instructions 15 and 16.)_

D1 and the longest **VD** time derived from the largest VC **value** should be 3-5 times the longest T$_2$ in the sample compound. T$_2$ is always shorter than T$_1$.

$$VD_{max} = [P2 + (2*D20)]* \ VC_{max}$$

T$_2$ data sets can be processed on the spectrometer terminal or off line using any version of Topspin provided the linked **VCLIST** file is either resident in the experiment's top level folder (= default setting: see below) or recreated or copied into the off line terminal's _C:\Bruker\TopspinX.X\exp\stan\nmr\lists\vc_ folder.

If required VCLIST file that is present in the experiment's top level folder can be opened with **WordPad** and the **VC** values in it can be viewed and used to recreate the original named **VCLIST** file from which the **VDLIST** file can be created using the **vctovd** command.

NB: The experimental copy of the variable constant file is named as **vclist** irrespective of the name of the source vclist file.

2.0 Experiment Set Up

1) Create an experiment and read in the **awprotont2** parameter set (+ **getprosol).**
    Pulse programme = **cpmg**

2) Review default settings. These settings can be adjusted if required.
    TD(F2) = 16K or **32K, TD(F1) = 8**
    SW = 14 ppm, O1P = 6.3 ppm.
    D1 = 10 sec.
    D20 = 400 usec = 0.000400 sec
        D20 should be approx 40-60% of 50*P2 (eg 50 *16.2 usec = 810 usec)
    NS = **multiple of 4 or 8, DS = 4 or 8.**

3) Type **ased** (enter) and review other parameters used in the job including the linked
   **VCLIST** file = **AWPROTONT2-8.**

4) The **VCLIST** file should have the entries shown below. Do not alter the values in this file. An alternatively named **VCLIST** file should be created if different **VC** and **D1** values are required for a particular compound. See the Appendix to this document for the **VD** values that arise from the **VC** values below when **P2 = (180 degree pulse) = 16.2 usec** and **D20 = 400 usec = 0.0004 sec.**

5) Set receiver gain using **RGA** *(important!)*.

6) Type **edp** (enter) and check that **SI(F2) = 32K, SI(F1) = 8, WDW(F2) = EM, LB (F2) = 0.3-0.5 Hz** or other value of your choice.

7) Start the acquisition using the **ZG** command

8) When the experiment has run type **rser 1** (enter) to read in the first serial file which will appear in a TEMP screen display window.

9) Type **EFP** (return) to transform it and phase it as per a normal proton spectrum.
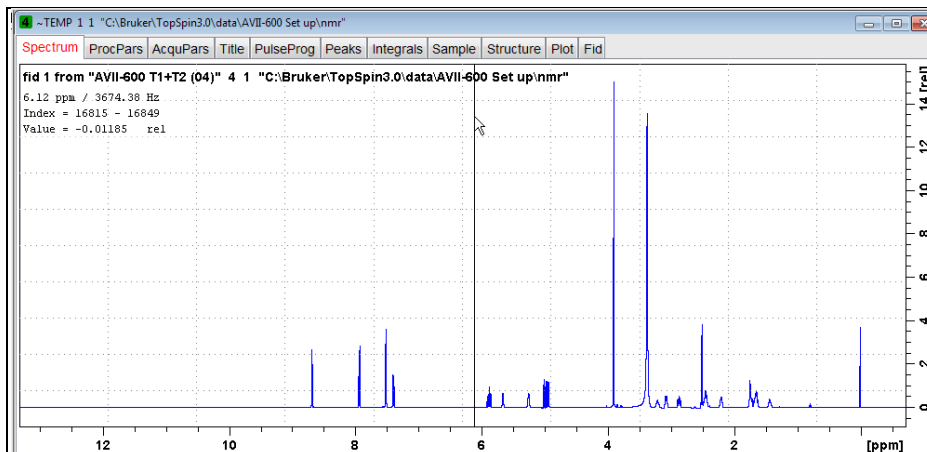
10) Type **edp** (enter) and note the phase constants for this spectrum.

|	
PHC0 [degrees]	21.062
PHC1 [degrees]	-40.841
PH_mod	pk

11) Close the **TEMP** window and reload the T_2 data set file.

12) Type **edp** (enter) and enter the phase constants noted in step 10 above into the **F2 PHC0** and **PHC1** cells and check **PH_MOD** = pk. **F1** cell info is not used.

⌃ Phase correction

PHC0 [degrees]	21.062	0	0th order correction for pk
PHC1 [degrees]	-40.841	0	1st order correction for pk
PH_mod	pk	mc	Phasing modes for trf, xfb, ...

13 Type **xf2** (return) to transform the 2D data set followed by **abs2** (return) to baseline smooth it.

14) Provided phase constants have been correctly set up the transformed data set plot should resemble that shown below. Signals intensities go from their maximum positive value to zero intensity.
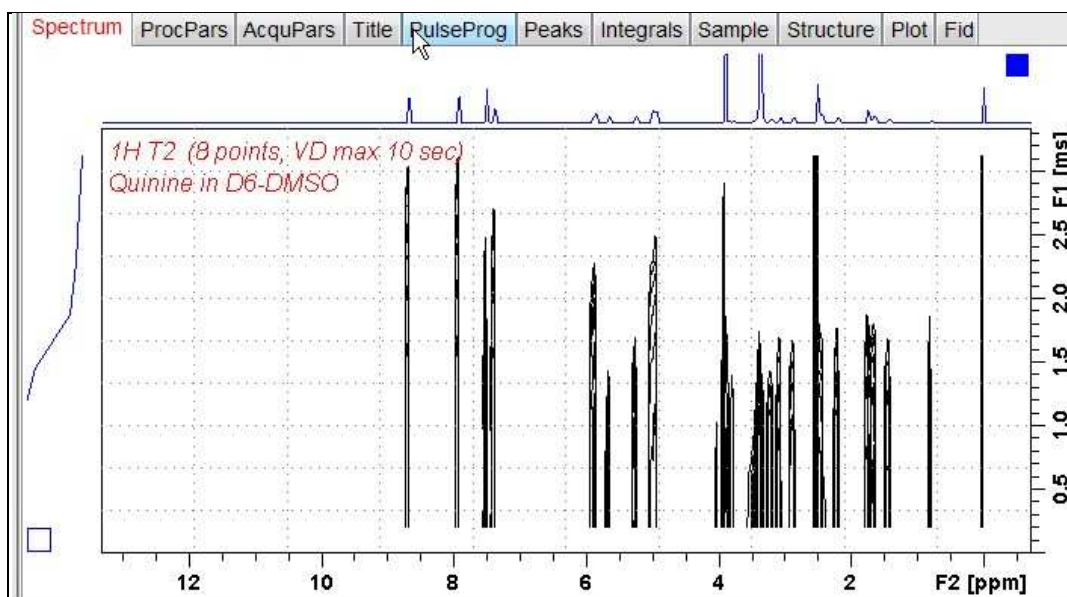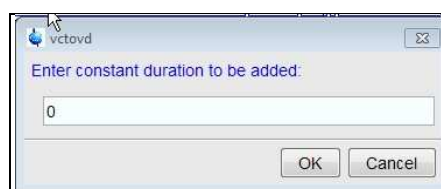
3.0 T₂ Data Set Processing

15) _Prior to processing a T₂ data set it is essential that variable constant (VC) values are converted to time domain variable delay (VD) values._

**16)** Type **vctovd** (enter) and click OK on the display panels that appear.
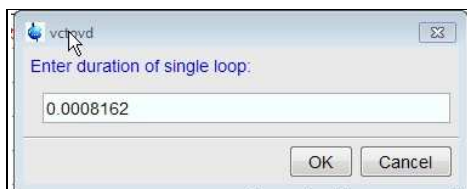

See the Appendix for the calculation of this value   Not used. .

17) Open Topspin's **Analysis** menu and click its **T1/T2 Relaxation** tab. If other Bruker processing software has been installed on the spectrometer terminal, as may be the case on the AVII-600, select the **Analysis** menu's **Topspin T1/T2 module** tab and open its **T1/T2 Relaxation** sub-menu tab.
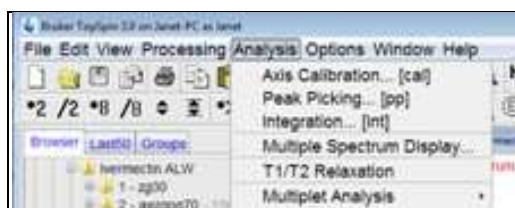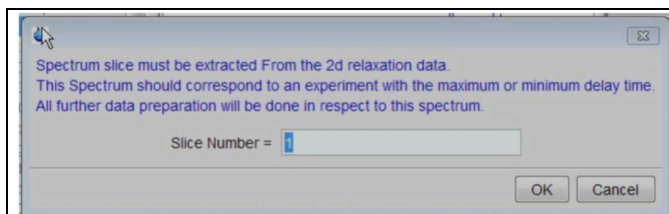

This will open up the screen display shown below.
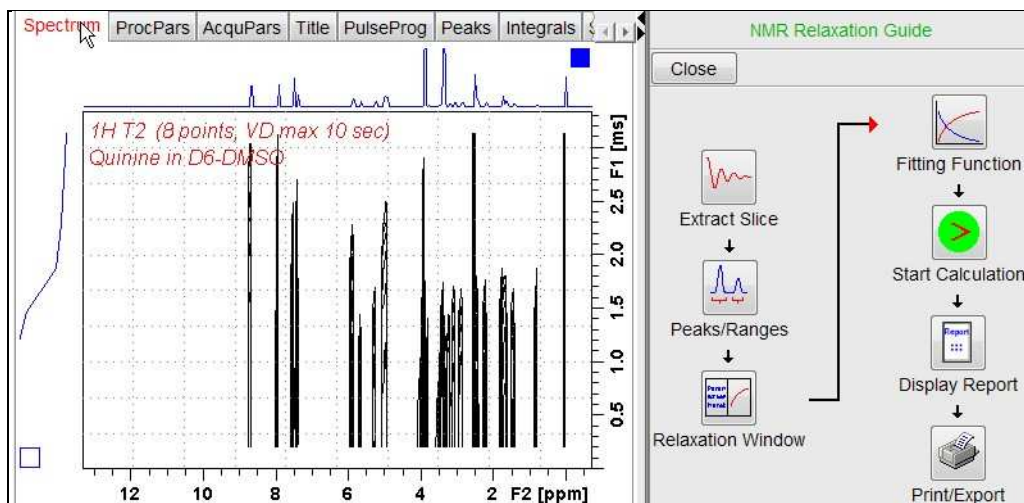
18) Click the **Extract Slice** button and then the click **Spectrum** button in the panel that appears.
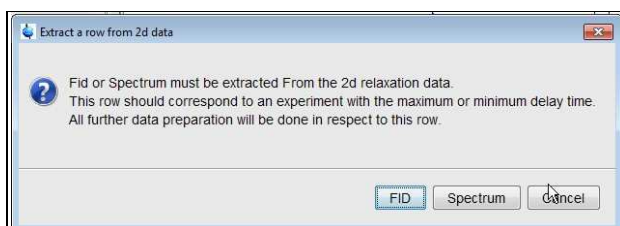
19) Enter **1** in the **Slice Number** cell and click the **OK** button. This will display the transformed spectrum ex the first (shortest) **VCLIST → VDLIST** value = the one that was phased via the **rser 1** routine in steps 8 and 9.

20) Expand the spectrum that appears using Topspin's **"E"** menu bar button and integrate selected peaks in it as per standard ^{1}H spectrum processing. You may need to click the integration button in Topspin's menu bar to start the integration protocol.

21) Click the "**Save Region As**" icon button (= 3rd from the right in the upper menu bar: *the one with the floppy disk icon + A below it*) and then its **Export Regions to Relaxation Module and .ret**. tab.
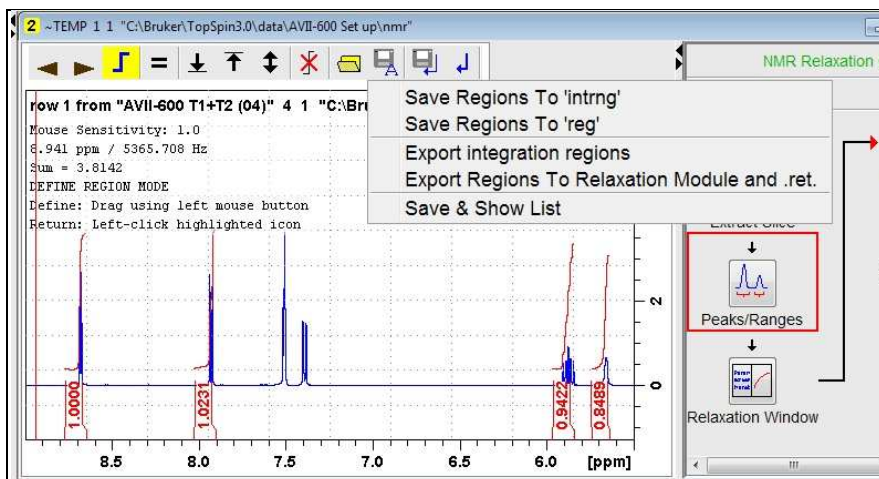

22) Click the **Relaxation Window** button and select **Intensity** or **Area** in the plot window that appears. **Area** is usually the better choice.
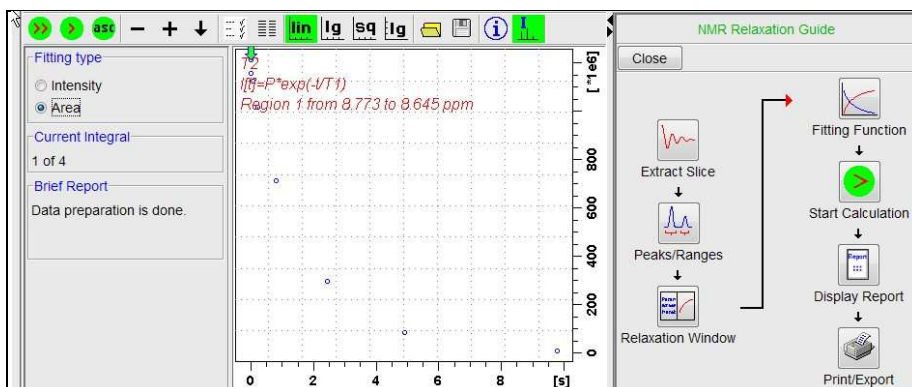

. *The plot's upper title line correctly has T$_2$, but the T$_1$ equation incorrectly appears below it. This is a long standing Topspin bug.*

23) Click the **Fitting Function** button and note the comments about **intensity** or **are**a options in the screen display that appears and close it.
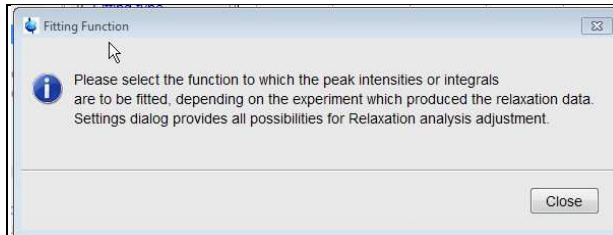

24) When the Fitting Function message screen is closed a panel with relaxation parameters (split into two screen captures below) will appear.
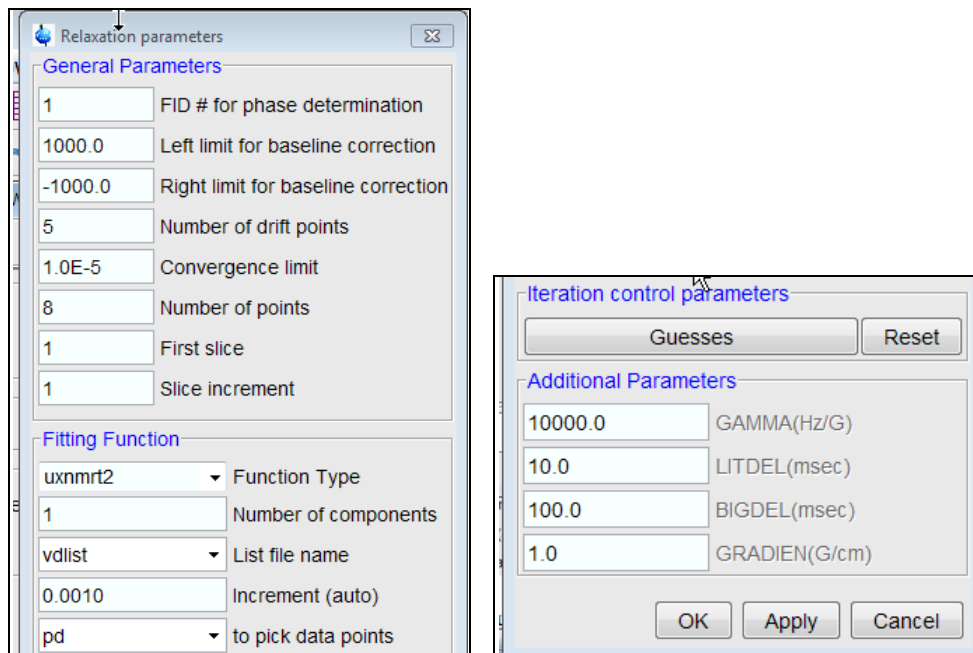
25) Check **Function Type = uxnmrt2** *(important!)* and **List file name = vdlist**.
Other cells/values can be left as they are (= default settings).
Click **Apply** and **OK** and close the window.

→ *next page......*

26) Click the **double red arrow** in the menu bar at the top left of the plot window and **NOT** the single red Start Calculation arrow button in the NMR Relaxation Guide menu below the Fitting Function button.
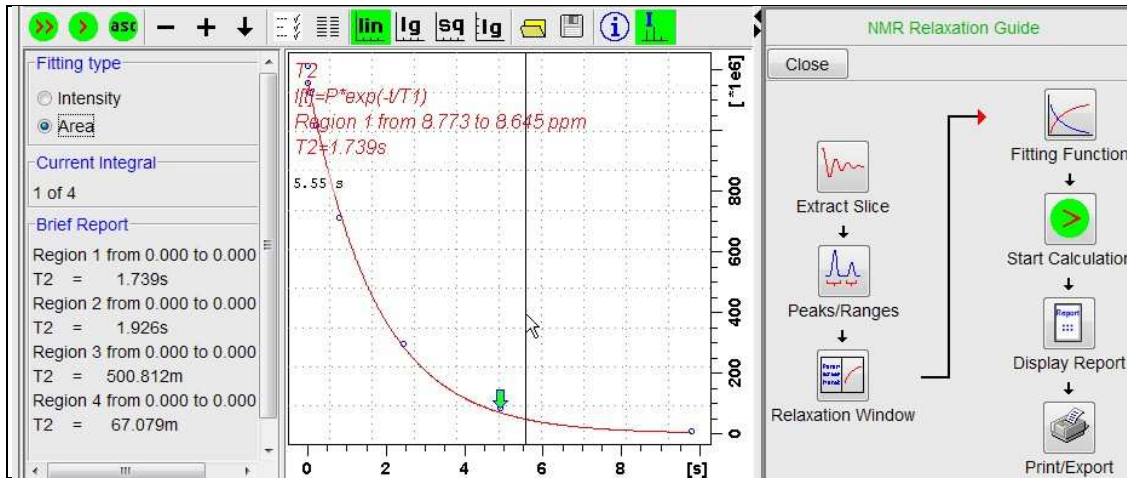
The appearance of the T_1 equation in this screen is a long standing Topspin error

27) The + and - buttons in the upper menu bar can be used move through the series of T_2 plots.

28) Fitting type (**Intensity** or **Area**) can be changed in the plot display. If this is done clicking the **double red arrow** will recalculate the T_2 results and update their plots.

29) The NMR Relaxation Guide has buttons which can be used to display and/or print T_2 results. A sample report for one signal is shown below. Zero value points (= completely T_2 relaxed) will be eliminated.

Appendix

VD values derived from **VC** values when **P2 = (180 degree pulse time) = 16.2 usec**
and **D20 = 400 usec = 0.0004 sec.**

$$VD = [P2 + (2*D20)] * VC$$

	A	B	C	D	E	F
1						
2	p2	16.2	usec	180 degree pulse time		
3						
4	d20	0.0004	sec			
5		0.400	msec	50 x p2		
6		400	usec	810	usec	
7				d20 should be 40-60% 50 x p2		
8						
9	p2+(2*d20)	816.2000000	usec			
10		0.8162000	msec			
11		0.0008162	sec			
12						
13	VC	VD usec	VD msec	VD sec		
14	4	3265	3.26	0.0033		
15	16	13059	13.06	0.0131		
16	64	52237	52.24	0.052		
17	256	208947	208.95	0.209		
18	1000	816200	816.20	0.816		
19	3000	2448600	2448.6	2.45		
20	6000	4897200	4897.2	4.90		
21	12000	9794400	9794.4	9.79		