

KJM 9250

$Carbon T_2 \, Spectra \, on \, the \, AVI600 \, and \, AVII \, Spectrometers$

Version 5.0

Topspin 1.3 Windows XP AVI600 Topspin 2.1 Windows 7 AVII600



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Carbon T₂ Spectra on the AVI-600 and AVII-600

1.0 Introduction

An **awcarbont2** parameter set and linked **VCLIST** file from which a **VDLIST** file <u>must</u> 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 T₂ data set is acquired using the <u>smallest vclist value first</u>. *Prior to processing T₂ data sets variable constant* (VC) values must be converted to time domain variable delay (VD) values using the <u>vctovd command (see instructions 15 and 16.)</u>

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 the 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.

uxnmr.info	2/01/2017 10:50 p	INFO File	4 KB
🗋 uxnmr.par 🗟	2/01/2017 10:50 p	PAR File	24 KB
📄 vclist	8/02/2018 10:30 p	File	1 KB

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

- Create an experiment and read in the awcarbont2 parameter set (+ getprosol).
 Pulse programme = awcpmgpg
- 2) Review default settings. These settings can be adjusted if required. TD(F2) = 32K, TD(F1) = 8 SW = 240 ppm, O1P = 110 ppm. D1 = 10 sec. D20 = 800 usec = 0.000800 sec 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 = **AWCARBONT2-8**.

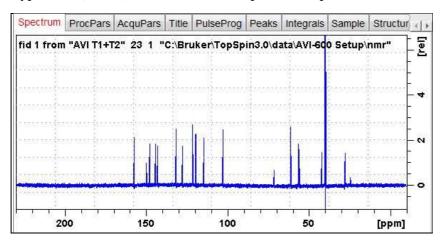
4) The VCLIST file should have the entries shown below. Do not alter the values in this file. An alternatively named VCLIST file should be created if different VC and D1 values are required for a particular compound. The VD values calculated from the AWCARBONT2-8 file's VC values using the vctovd command (see steps 15 and 16) when P2 = (180 degree pulse) = 27.7 usec on AVI-600 and D20 = 800 usec= 0.0008 sec are shown below.

Fil	e Edit Search		Fi	ile Edit Search
1	16		1	0.0260432
2	32		2	0.0520864
3	64		3	0.1041728
4	128		4	0.2083456
5	300		5	0.4883100
6	1200		6	1.9532400
7	3000		7	4.8831000
8	6000	1000	8	9.7662001
		~	9	an a

- 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) = 2 Hz or other value of your choice.

sı 🗼	32768	8	Size of real spectrum			
SF [MHz]	150.9028090	600.1300000	Spectrometer frequency			
OFFSET [ppm]	230.05040	220.01100	Low field limit of spectrum			
SR [Hz]	0	0	Spectrum reference frequency			
HZpPT [Hz]	1.105709	1.290504	Spectral resolution			
SPECTYP	UNDEFINED		Type of spectrum e.g. COSY, HMQC			
Window function						
WDW	EM -	SINE	Window functions for trf, xfb,			
LB [Hz]	2.00	0.30	Line broadening for em			

- 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 carbon spectrum.



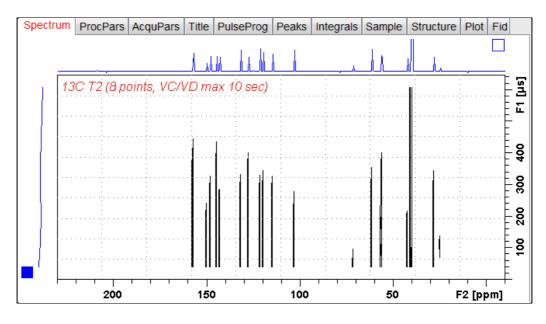
10) Type **edp** (enter) and note the phase constants for this spectrum. As per AVI and AVII ¹³C spectra a large negative PHC1 value will be require

Phase correction					
PHC0 [degrees]	-133.289				
PHC1 [degrees]	-603.435				
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.

Contraction							
PHC0 [degrees]	-133.289	0	Oth order correction				
PHC1 [degrees]	-603.435	0	1st order correction				
PH_mod	pk 🔻	mc 🔹	Phasing modes for t				

- **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) <u>Prior to processing a T₂ data set it is essential that variable constant (VC) values</u> are converted to time domain variable delay (VD) values.
- 16) Type vctovd (enter) and click OK on the display panels that appear.

🤹 vctovd	×
Enter duration of single loop:	
0.0016277	
	OK Cancel
	OK
Single VC loop value =	$n^2 \perp 2 \ge d^{20}$

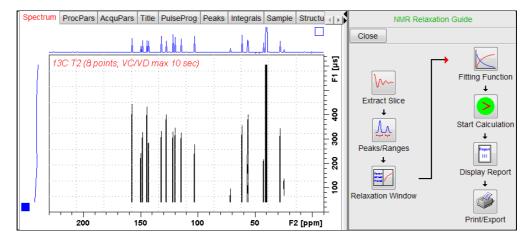
vctovd	Σ
Enter constant dura	ation to be added:
0	
I.,	
	OK Cancel

Single VC loop value = $p^2 + 2 \ge d^2 0$

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.

File Edit View Processing A	nalysis Options Window Hel	P
·2 /2 •8 /8 ◆ ¥ •3	Axis Calibration [cal] Peak Picking [pp] Integration [int]	1
Browser Last50 Groups	Multiple Spectrum Display.	
A hermectin ALW	T1/T2 Relaxation	- Jun
1 - 2250	Multiplet Analysis	

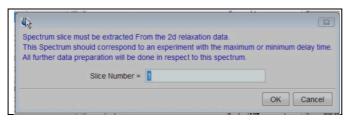
This will open up the screen display shown below.



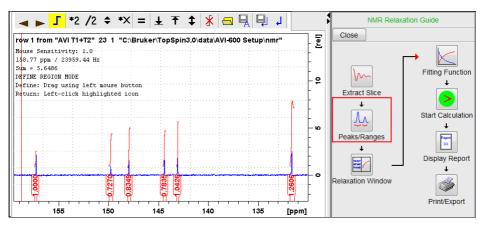
18) Click the Extract Slice button and then the click Spectrum button in the panel that appears.



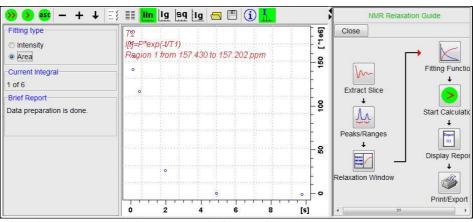
19) Enter 1 in the Slice Number cell and click the OK button. This will display the transformed spectrum ex the first (shortest) VCLIST → VDLIST value = the one that was phased via the rser 1 routine in steps 8 and 9.



- **20)** Expand the spectrum that appears in the usual way and integrate selected peaks in it. Integrals should start and terminate as close as possible to the edge of a peak.
- 21) Click the "Save Region As" icon button (= 3rd from the right in TS3.2's the upper menu bar, or towards the left hand side of the menu bar in TS2.1) = *the one with the floppy disk icon + A below it*) and then its Export Regions to Relaxation Module and .ret. tab..

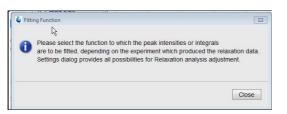


22) Click the **Relaxation Window** button and select **Intensity** or **Area** in the plot window that appears. **Area** is the best choice for carbon T₂ values.



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.

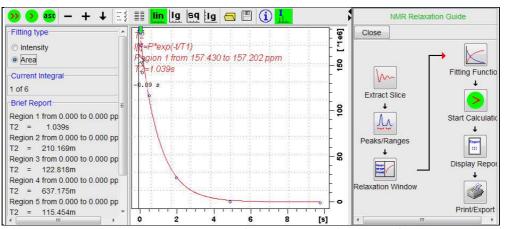
23) Click the **Fitting Function** button and note the comments about **intensity** or **are**a options in the screen display that appears and close it.



24) When the Fitting Function message screen is closed a panel with relaxation parameters (split into two screen captures below) will appear.

	e Relaxation p							
	General Par	ameters						
	1	FID # for phase determination						
	1000.0	Left limit for baseline correction						
^	-1000.0 Right limit for baseline correction							
	5	Number of drift points						
	1.0E-5	Convergence limit						
	8	Number of points				NC		
	1	First slice				rol parameters		
	1	1 Slice increment				Guesses	Reset	
				Additional Parameters				
	Fitting Funct	tion			10000.0	GAMMA(Hz/G)		
	uxnmrt2	 Function Type 			10.0	LITDEL(msec)		
E	1	Number of components		r	i	100.0	BIGDEL(msec)	
	vdlist	 List file name 				1.0	GRADIEN(G/cm)
	0.0010	Increment (auto)						
	pd	▼ to pick data points				OK Apply	Cancel	

- 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.
- 26) Click the double red arrow in the menu bar at the top left of the plot window and <u>NOT</u> 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 T₂ 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) or negative artifact peaks will be eliminated.

```
1
   Dataset :
    C:\Bruker\TopSpin3.0\data\AVI-600 Setup\nmr/AVI T1+T2/23/pdata/1
 2
3
   AREA fit :
4
    I[t]= P*exp(-t/T2)
5
 6 8 points for Integral 1, Integral Region from 0.000 to 0.000 ppm
7
   Results
             Comp. 1
8
9 P
         = 9.816e-001
10 T2 =
              1.039s
11 SD = 4.819e-002
12
13
       tau ppm integral intensity
14
15
      26.043m 157.226 1.8464e+008 4.3523e+007
     52.086m157.2251.7182e+0084.3054e+007104.173m157.2241.4193e+0084.2111e+007
16
17
     208.346m 157.226 1.5655e+008 3.6103e+007
18
     488.310m 157.226 1.1662e+008 2.8708e+007
19
      1.953s 157.225 2.6262e+007 8.0794e+006
20
21
       4.883s 157.232 -2.8243e+005 eliminated
22
       9.766s 157.232 -1.0611e+006 eliminated
23
```