

# KJM 9250

## **ProtonT2 Spectra on the AVIII800HD Spectrometer**

Version 5.0

# Topspin 3.5 Windows 7 AVIII HD 800



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### **ProtonT<sub>2</sub> Spectra on the AVIII800HD**

### **1.0 Introduction**

An **awprotont2** parameter set and linked **VCLIST** file from which a **VDLIST** file <u>must</u> be generated prior to processing a  $T_2$  data set have been set up on the AVIII800HD spectrometer running under TS3.5 respectively.

The parameter set have D1 = 10 sec and 8 x VCLIST values. The T<sub>2</sub> data set is acquired using the *smallest VCLIST value first. Prior to processing T<sub>2</sub> 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 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 use the **rpar** command to read in the following parameter set: **awprotont2** + **getprosol**,

pulse programme = **cpmgpg**.

- 2) Review default settings. These settings can be adjusted if required. TD(F2) = 64K or 32K, TD(F1) = 8 SW = 14 ppm, O1P = 6.3 ppm. D1 = 10 sec D20 = 400 usec = 0.000400 sec NS = a multiple of 2, 4 or 8, DS = 0, 2 or 4.
- 3) Type **ased** (enter) and review other parameters used in the job including the linked **VCLIST** file = **AWPROTONNT2-8**.
- 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 AWPROTNOT2-8 file's VC values using the vctovd command (see steps 15 and 16) when P2 = 16 usec (180 degree pulse) and D20 = 400 usec = 0.0004 sec are shown below.

Fi	le Edit Searck	Fi	le Edit Search
1	4	1	0.0032648
2	16	2	0.0130592
3	64	3	0.0522368
4	256	4	0.2089472
5	1000	5	0.8162000
6	3000	6	2.4486001
7	6000	7	4.8972001
8	12000	1 8	9.7944002

- 5) Set receiver gain using RGA(*important*!).
- 6) Type edp (enter) and check that SI(F2) = 32 or 64K, SI(F1) = 8, WDW(F2) = EM, LB (F2) = 0.3-0.5 Hz or other value of your choice.

SI	65536	8	Size of real spectrum
SF [MHz]	800.0300000	800.0272727	Spectrometer frequency
OFFSET [ppm]	13.27518	9.70900	Low field limit of spectrum
SR [Hz]	0	0	Spectrum reference frequency
HZpPT [Hz]	0.170299	1.251948	Spectral resolution
SPECTYP	UNDEFINED	•	Type of spectrum e.g. COSY, HI
Window function	n		
WDW	EM 👻	SINE -	Window functions for trf, xfb,
LB [Hz]	0.30	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.

Phase correc	tion
PHC0 [degrees]	30.204
PHCk [degrees]	-102.711
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 correctio	<u>n</u>		
PHC0 [degrees]	30.204	0	0th order correction for pk
PHC1 [degrees]	-102.711	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. Black = a positively phased signal. Signals will drop off to zero intensity as VD increases.



#### 3.0 T<sub>1</sub> Data Set Processing

- 15) <u>Prior to processing a T<sub>2</sub> 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.





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

Not used.

17) Click Topspin's Analyse menu tab then its Dynamics sub-menu tab and click its T1T2 tab.



18) This will open the T1/T2 processing menu bar shown below.

ſ	🖕 Bruker To	opSpin 3.5 pl	7 on KJEMI-NMR1	8 as norskvi						
		<u>S</u> tart	<u>A</u> cquire	Process	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	0	
	G Bac	k		<u>₩ _ </u> Eid	A Peaks/Ran	ges 🛃 Re	elaxation	Fitting	Calculation	Report

Click the **FID** tab and click the **Spectrum** button in the screen panel that opens.



19) The screen panel shown below will appear.

<b>4</b>	
Spectrum slice must be extracted Fin This Spectrum should correspond to All further data preparation will be do Slice Number =	om the 2d relaxation data. an experiment with the maximum or minimum delay time. one in respect to this spectrum.
	OK Cancel

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

(....next page)

SNectrum	ProcPars	AcquPars	Title	PulseProg	Peaks	Integrals	Sample	Structure	Plot Fi	d Acqu	
fid 1 from	"800 T1+T	2" 204 1	D:\uio	AVIIIHD800	\data\n	orskvi∖nn	nr	i.			
								a <mark>s is</mark> a sea a c		1	
								(4 (a) - 2 (a) (a) (a) (a) (a) (a) (a) (a)			anandaraa
										1	1
											a sua sul a cara E
								nut instremente			
											-
un in i					mana		an an a C	ninana	maha		nanahan
						11			u ha	di n	

**20)** Expand the spectrum that appears in the usual way and click the **Peaks/Ranges** menu bar tab.



Select the **Manual integration** tab, close any screen messages that appear, and integrate selected peaks in the usual way .



21) Click the "Save Region As" icon men bar button (= *the floppy disk icon with A below it*) and then its Export Regions to Relaxation Module and .ret. tab.



**22**) Click the **Relaxation** menu tab button, note any screen messages that may appear and close them. Select **Intensity** or **Area** in the plot window that appears. **Area** is invariably the better choice.

<u>A</u> cquire <u>P</u> roce	ess A <u>n</u> alyse	P <u>u</u> blish	<u>∨</u> iew <u>N</u>	<u>l</u> anage	0	
₩ <u>F</u> id	Peaks/Ranges	Relaxation	n Kritting	g 😕 <u>C</u> al	culation	<u>R</u> eport
2 Relaxation "800 T1+T2" 2	04 1 D:\uio\AVIIIHD800\dat	a\norskvi\nmr				
<mark>≫ ≫ asc</mark> – + ↓	≣≬≣≣ <mark>lin</mark> lg sq	lg 🗔 🖪 🚺	I.			
Fitting type Intensity Area	T2° I[t]=P*exp(-t/T1) Region 1 from 8.700	0 to 8.672 ppm				1.5 [ 1e9]
1 of 4 Data preparation is dor						0
						- - - -
		0	ō			6
۰ III ۲		2 4		1 I I 6	8	[s]

23) Click the Fitting menu bar tab and note the comments about intensity or area options in the screen display that appears and close it

<u>A</u> cquire	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	Manag	ge 🕜	
W~ <u>F</u>	id 🌆 <u>P</u> eak	s/Ranges	Relaxation	Fi	tting	<u>Calculation</u>	Report
(	<b>XA SUXA</b>	ti da	ter and the second s				
Fitting Functi	on 3				23		
Please are to	select the function be fitted, depending	to which the peak on the experimen	intensities or integrals t which produced the rel	axation data			
Setting	is dialog provides a	II possibilities for R	elaxation analysis adjust	ment.			
-				Close			
				Close			

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

(...next page)

1	FID # for phase determination				
-3.0629	Left limit for baseline correction				
-9.7074	Right limit for baseline correction				
5	Number of drift points				
1.0E-5	Convergence limit				
8	Number of points				
1	First slice	rlteration contr	ol parameters		
1	Slice increment		Guesses Reset		
SECAN	Deak consitivity	Additional Parameters			
1.4	Peak sensitivity				
1.4	Peak sensitivity	10000.0	GAMMA(Hz/G)		
1.4 uxnmrt2	■ Function Type	10000.0	GAMMA(Hz/G) LITDEL(msec)		
1.4 uxnmrt2 1		10000.0 10.0 100.0	GAMMA(Hz/G) LITDEL(msec) BIGDEL(msec)		
1.4 uxnmrt2 1 vdlist		10000.0 10.0 100.0 1.0	GAMMA(Hz/G) LITDEL(msec) BIGDEL(msec) GRADIEN(G/cm)		
1.4 uxnmrt2 1 vdlist 0.001		10000.0 10.0 100.0 1.0	GAMMA(Hz/G) LITDEL(msec) BIGDEL(msec) GRADIEN(G/cm)		

- 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 T2 plot's menu bar and <u>NOT</u> the **Calculation** tab in the upper menu bar.



- <u>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.</u>
- 27) The + and buttons in the upper menu bar can be used to advance (or reverse) the individual  $T_1$  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<sub>1</sub> results and update their plots.
- **29)** The **Report** tab in the .menu bar can be used to view fitting report. A sample report for one signal is shown below.

```
Publish
                                                       2
Acquire
         Process
                   A<u>n</u>alyse
                                      View
                                             Manage
   🧅 Fitting report
    File Edit Search
     1
      Dataset :
        D:/uio/AVIIIHD800/data/norskvi/nmr/800 T1+T2/204/pdata/1
     2
     3
       AREA fit :
        I[t] = P + exp(-t/T2)
     4
     5
     6
       8 points for Integral 1, Integral Region from 0.000 to 0.000 ppm
     7
       Results
                  Comp. 1
    8
    9
       P
             =
                9.613e-001
    10
      т2
                   1.607s
            =
    11 SD
            =
                1.858e-002
    12
    13
                        integral
                                   intensity
           tau
                ppm
    14
    15
                    8.681 1.7976e+009 1.0212e+008
          3.264m
    16
          13.056m
                    8.681 1.6649e+009 9.8701e+007
    17
          52.224m
                    8.681 1.6924e+009 1.0112e+008
    18
         208.896m
                    8.681 1.4943e+009 8.9044e+007
    19
         816.000m
                    8.681 1.033e+009 5.9991e+007
    20
           2.448s
                    8.681 3.8428e+008 2.2185e+007
    21
           4.896s
                    8.681 9.3812e+007 5.2976e+006
    22
           9.792s
                    8.681 6.7385e+006 4.1698e+005
```