Experiments on Unity–500

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I. Normal 1d ^1H Acquisition

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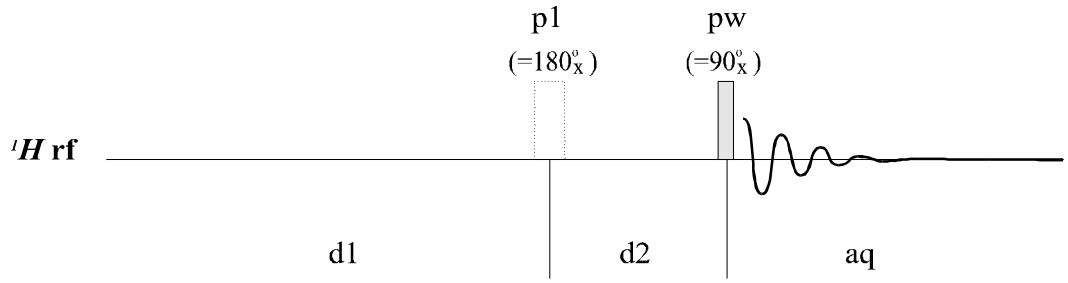
A. Discussion

- 1d ^1H acquisition on the Unity is simplified by the **solvent='*solventname*'** command. When set appropriately, **tof=0** should center the transmitter correctly.
- With identical transmitter and decouple channels, it is simple to decouple *X* nuclei on the Unity-500 while observing ^1H; e.g., for ^{31}P set **dn='P31'** and other parameters correctly (see later section).
- **calfa** is an important baseline flattening (timing correction) macro on the Unity when setting up 2d experiments. Note the use of this command in the acquisition section.
- Spin-lattice relaxation (T_1) can be measured/estimated with this sequence: set **p1=2*pw90** **pw=pw90** and **d2** appropriately (both p1 and d2 are normally are set =0; see later section for details on T_1 estimates).
- Note that Varian sequences commonly 'hide' some delays. In this sequence, a delay **rof1** prior to, and delays **rof2** and **alfa** following **pw** are not shown. See Varian's documentation on pulse programming for more details.

1d ^1H Acquisition (s2pul)



B. Critical Parameters

d1 – relaxation delay; assuming pw~30° d1>T_1 to obtain quantitative integrations
aq – acquisition time ✹ determines (ignoring sample effects) resolution ~ 1/aq
pw & **twpr** – critical for pw90 calibration and many other experiments

C. 1d 1H Acquisition

- to setup parameters (method **b** is recommended)
  a) either read in a data file (**rt** or FILE *left-click* LOAD) or parameter file (**rtp**) for normal ^1H acquisition, or
- ☞ b) click on MAIN MENU, SETUP, and select nucleus and solvent
  c) alternatively, run s2pul macro (but normally will need to reset **spin='n'**, **gain=0**; check temp and vttype, and check tpwr (=58 typically) and pw (=5-8µs typically).

- use UWMACRO TUNE PROBE and tune probe
- use **go** to start acquisition
  - **ga** will automatically apply a **wft** (weighted Fourier transform) following acquisition
  - use **wft** to manually transform
  - **dscale** will display the axis
  - **dsx** will apply **wft dscale(-3)**
- optimize **sw** by setting *right-click* or *-drag* (right mouse button) and *left-drag* (left mouse button) cursors, and use **movesw**
- set reference by placing cursor close to peak, then type **nl** and **rl** command, e.g., **rl(7.24p)**
- if always getting ADC OVERLOAD beep, then receiver gain is too high; **gain** can be set three ways (number **1** recommended):

☞ 1. – simply lower **gain** until ADC OVERLOAD warning goes away; if warning stays with **gain=0**, insert attenuators into ***bottom*** BNC (preamp output), not into the probe connections
  – make sure **gain** is within 10B of overload warning

2. – reduce **nt=1** and enter **gf** ; make sure to wait a few seconds (menu will flash) before
  – click on acqi and then **FID**; make sure SPECTRUM is not selected and click on **DOWN** until you see the red horizontal lines that indicate the ADC clipping limits; adjust **gain** until the fid fills ~1/2 of the region to the clipping voltage
  – switch **nt** back to original value
3. set **gain='n'** which will implement autogain adjustment (***not*** recommended, especially for 2d where huge artifacts can result)

D. ^{1}H *pw90 Calibration*

- normally can perform calibration on sample; best to *not* perform it on a clean solvent since T_1 for solvents can be very long
- set **tpwr** to desired setting; typically 58 for 5mm probes, 52 for 3mm probes
- set **d1=5-10s** depending on sample T_1 (**d1=5** is usually ok)

☞ recommended: perform a coarse check of pulse widths using:

array<ret>
variable to array: **pw**
number of increments: **20**
starting value: **3**
size of increment: **3**

- set **nt=1** and use **go**; should see a sinusoidal response; if not, usually **d1** needs to be longer
- to obtain an accurate **pw90**, check about the 360° value with an array increment size of 0.5 to 1 μs
- plot arrayed spectra using the following commands:

wft dssh ; transforms all spectra and displays in a horizontal stack
full ; resets plot area to full screen

E. 2nd Order Shimming on a 500 MHz Spectrometer

- Make sure the lock is not saturating. Check by watching that the lock level increases consistently with increasing LOCK POWER. Once the lock level drops or stays steady with increasing power, back off the power by roughly 20%. I've heard some Varian chemists look for a 50% decrease when dropping LOCK POWER by 6 dB when LOCK POWER is ok (i.e. low enough to not be saturating), but I've not seen consistent results doing this. I simply look for a "bounce" in the lock level, and go 20% below the setting at which the bounce is last observed (can be difficult for fast-relaxing solvents like D_2O).
- Adjust LOCK GAIN to give lock level in 25-65 range. Optimize lock level using LOCK PHASE. Optimize lock level with **Z** and **Z2**.
- Change **Z2** in one direction enough to change lock level by 5-10% (since optimized in previous step, level will decrease). Re-optimize lock level again with **Z**. If newly optimized lock level is lower than the previous one, try changing **Z2** in the other direction; otherwise continue changing **Z2** in same direction until re-optimization does not improve the lock level.
- Keep changing **Z2** in the same direction and optimizing lock level with **Z**, until an overall maximum has been found. Set **Z** and **Z2** to this maximum setting.
- Keep lock signal value between 25-65 using the LOCK GAIN; check that LOCK PHASE is set correctly on regular intervals (especially after any large changes in **Z2**).

- Other 2nd order shim combinations that would require a similar shimming iterative scheme such as described above (i.e. find a simple maximum in lock level, change the high order shim in a particular direction—lock level decreases—then re-optimize with lower order shims to see if lock level improves from what started with):
  Z^3: Z^2, Z: will require 2nd order corrections at Z^2 to obtain overall maximum in lock signal
  Z^4: Z^3 2nd order correction involving 2nd order optimization of Z^2 (thus each change in Z^4 can require a significant effort to see if there's any improvement at all).
  XZ: X
  YZ: Y
  XZ^2: XZ(2nd order), X
  YZ^2: YZ(2nd order), Y

F. 1d Data Workup and Plotting

- **dc** will correct any linear baseline shifts
- **bc(5)** will correct fifth-order baseline, assuming **region** command (run automatically on a **bc**) can find peak and baseline regions; **bc** is *not* recommended for 1d, but is ok for 2d workup (default spline fit; see manual for details)
- display the axis using **dscale(-3)** and **axis='p'**, or use the macro **dsx**
- plot spectra using the following commands:
  pl ; plots spectrum with **sc** being cm in from *right* side **wc** being width in cm
  pap ; plots all parameters along left hand side, or **ppa** for the major parameters only; does plot out **text**, which can be entered manually, but easiest way is to use the CDE File Manager to go to ~/vnmrsys/exp1 (assuming working in exp1) and double click on **text** file; simple editing of the file can then be performed
  ppf ; plots peak pick
  th controls threshold height used);
  dpf will show peak picks on screen

dll lists peak picks with intensities (**printon dll printoff** will print)

pirn ; prints integral values under axis:
– start with **cz** to clear integrals
– click MAIN MENU DISPLAY INTERACTIVE PARTIAL INTEGRAL
– type **region** (if you don't like the regions, start again with **cz** and use the RESETS button to enter regions with the mouse manually)
– type **intnorm** or use UWMACRO MORE NORMINT or DISPLAY NORMINT [**adjism** is Varian's not-so-good command] to adjust the peak amplitude
– enter **vp=12** to get axis out of list area
– integrals will plot with **pl** when on screen; **pirn** is needed to list values below axis