(Multiple–) Solvent Suppression Using WET

updated: 25 July 2011

The following is largely based on notes from the University of Minnesota's Biomedical NMR facility located at: http://www1.umn.edu/nmr/UserDocs_NHH/Wet1d.pdf, but is specific to the setup here at the UWChemMRF. This procedure has been tested and works fine on our INOVA-500 and 600 spectrometers, currently running VNMR 6.1C. This will not work as described here on any of our other spectrometers.

I. Initial calibrations

1. Acquire a normal ^{1}H 1D spectrum, and perform the normal **pw90** calibration. If receiver gain clipping occurs with **gain=0** [this is not unusual, because of the protonated solvent(s)], continue anyway with the calibration. The crossover spectra when **pw** is close to 360° should be acquired without clipping. Set **pw90 = pw$_{360°null}$ / 4** and **pw=pw90**.

2. The next step will generate the shaped rf pulse needed by the WET sequence to suppress your solvent signal(s). That procedure will request that you enter a

   reference pw90 ≡ **pw90** × **tpwr_cf**.

   The compression factor, tpwr_cf, can be found in the facility probe file at: /vnmr/probes/*probename/probename*. Use the File Manager to get to that file; the parameter will be listed about 25 lines down from the top as **H1tpwr_cf**. Make sure you also note **tpwr** that goes with this **pw90** value, as that will also be requested.

   You now should have the following written down from your calibration experiment:
   pw90
   tpwr_cf
   tpwr

II. Generating the shaped rf pulse using Pbox

1. It is easy to mess something up in the following. Recommended is to keep the 1H 1D data intact, e.g., in exp1, and do the following in another exp#. This is easily accomplished with

   e.g., **mf(1,2) jexp2 dsx**

2. Expand the region of the spectrum that contains the solvent signal(s) to be suppressed. Finish is a **ds**. This will have the menus setup correctly to then:

   click MORE(INT) ; in the menu bar
   click PBOX ; program that generates shaped rf
   click 90 ; we want an excitation pulse

3. In the messages area, "Place cursors around region to be excited" is displayed. This selection often entails a compromise: a broader box will produces a shorter rf pulse, but signals

roughly 3× the width of box will be affected by that pulse. Thus one wants to keep the box narrow for selectivity, but not too narrow to keep the pulse from getting too long.

a) Set your box now (left-click and place, then right-click and place).
The cursors can be set precisely using something like **cr=7.625p delta=100**. **cr** places the left (downfield) cursor (here in ppm), and **delta** sets the width in Hz.

b) Choose a shape from the list. **e-Burp1** and **G4** are high quality, but long. Use **G4** if more than 2 peaks need suppression. **e-Snob**, and (More More) **u-Burp** are reasonable alternatives. Do not use (More More Other) **seduce**, regularly used for NOESY1D, for this application.

After clicking on the shape, Pbox will display the shape name, and bandwidth **bw** and offset **off**, both in Hz. Writing down **off** may be useful during post-processing (see III.3 below).

c) If you want to suppress more than one solvent peaks, repeat a) and b) above for each additional peak: i.e., place the box on the next solvent peak, and click a shape (the same as for the first; don't know if shapes can be mixed). This can be done many times (certainly up to four peaks).

d) After selecting all solvent peaks of interest,

click CLOSE.
click NAME

Enter a Shapefile name. Keep it short, do not start with a number, do not use special characters of any type, and note it for later. Hit ↵ (RETURN) at the end.

click CLOSE

e) At "Enter reference 90 degrees pulse width (usec):" enter the value of **pw90 × tpwr_cf** from the calibration step I.2 above.

f) At "Enter reference power level:" enter **tpwr** from step I.2 above.

The shape will be generated and displayed graphically (amplitude vs time).

4. Once again, additional information must be found and written down, that will be needed to finalize the setup of the WET sequence. Pbox has written a file

/export/home/*username*/vnmrsys/shapelib/*Shapefile*.RF

Open this file using the CDE File Manager, and write down the first two numbers following Pbox in the 1st line. The first is **pw (us)** and the 2nd **pwr** (as denoted in the 2nd line).

III. Setting up the WET (wet1d.c) sequence

1. Pbox did not modify your ^{1}H 1D dataset. But you could redo **mf(1,2) jexp2 dsx** . In any event, start with the ^{1}H 1D data.

2. Enter: **wet1d**↵

3. This macro will setup the experiment based on the ^{1}H 1D set: **sw, tof, solvent,** etc. will be preserved. You must change the following parameters:

   pwwet = pw (us) from step II.4 above
   wetpwr = pwr from step II.4 above
   wetshape = '*Shapefile*' from step II.3.d above

4. The experiment should now work. Note that **dz** can be arrayed to optimize (minimize) the residual solvent level left. **ss≠0** will provide better results, as will **nt=*n*×4**.

5. Post-processing **Solvent Subtract**(ion), available from the PROCESS2 panel can also be used on one peak (note that the offset must be input correctly for this to work; see section II.3.b above). This can be particularly useful in improving baseline distortions (but typically WET leaves baselines in pretty good shape).