Solvent suppression with Vnmrj 2.2 / 2.3 and chempack

E. Alvarado, U of Michigan, 07/09/10

When recording the spectrum of solutions in non-deuterated solvents or when using deuterated solvents containing large amounts of protonated solutes, it is important to reduce the intensity of the largest signals. This will greatly improve the sensitivity and appearance of the dilute signals in solution. For example, in order to see the important amide hydrogen atoms, the spectra of proteins and peptides is usually acquired in a solution of 90% H_2O / 10% D_2O. In this solvent mixture, a good solvent suppression method is indispensable.

Two schemes for solvent suppression are readily available in vnmrj: **presaturation** of the solvent signal and the **WET** (Water suppression Enhanced through T_1 effects) pulse sequence. Both can be used together with most pulse sequences in vnmrj's library and both can simultaneously suppress more than one signal for solvents like THF. If you require a 2D spectrum with solvent suppression, first take a 1D spectrum with the method of your choice and then select the appropriate 2D experiment from vnmrj's menu Experiments > Convert current parameters to do... The suppression parameters selected in the 1D experiment will be used during acquisition of the 2D spectrum.

Using **presaturation** (**PRESAT**), the decoupler is turned on at the solvent's resonance for a short period of time, just before acquisition, to saturate its signal. Presaturation has been traditionally the easiest to set up, but has the disadvantages that it requires very good shimming, its selectivity is not the best, and exchangeable protons can also be saturated. The three parameters needed: decoupler offset, decoupler power, and saturation delay are established during the experiment setup.

The **WET** method uses selective pulses, applied at the solvent's resonance(s), followed by pulsed field gradients to dephase residual solvent magnetization. It provides very efficient suppression with excellent selectivity and is recommended when using solvents like 90% H_2O/10% D_2O or solvents with multiple resonances like THF. Since a waveform generator, a pulsed field gradient amplifier and a gradient-capable probe are required, this pulse sequence is not available in older instruments.

The procedure to measure a 1D proton spectrum with either one of these methods is essentially the same. But as usual with vnmrj, there are several ways to accomplish the same results. Here, we will show a couple of ways. If you haven't recorded a spectrum yet, start by reading the appropriate field (z0) and lock parameters for your solvent and shim the sample. For non-deuterated solvents, remember to turn the lock off and shim on the solvent's proton signal by selecting from the Main menu: Acquisition > Do Gradient Shimming... > Use 1H/gradient map, or with our macro gradshim('H1'). Make sure the probe is tuned to proton before you use gradient shimming on the solvent's proton signal. The document "Shimming and Locking", available in our web site, has more information on shimming of non-deuterated solvents. Next, select the method from the menu: Experiments > Setup NEW parameters to do... > 1H Observe – Suppress Defined Solvents. The relevant parameters are displayed in the Acquire, Default panel.
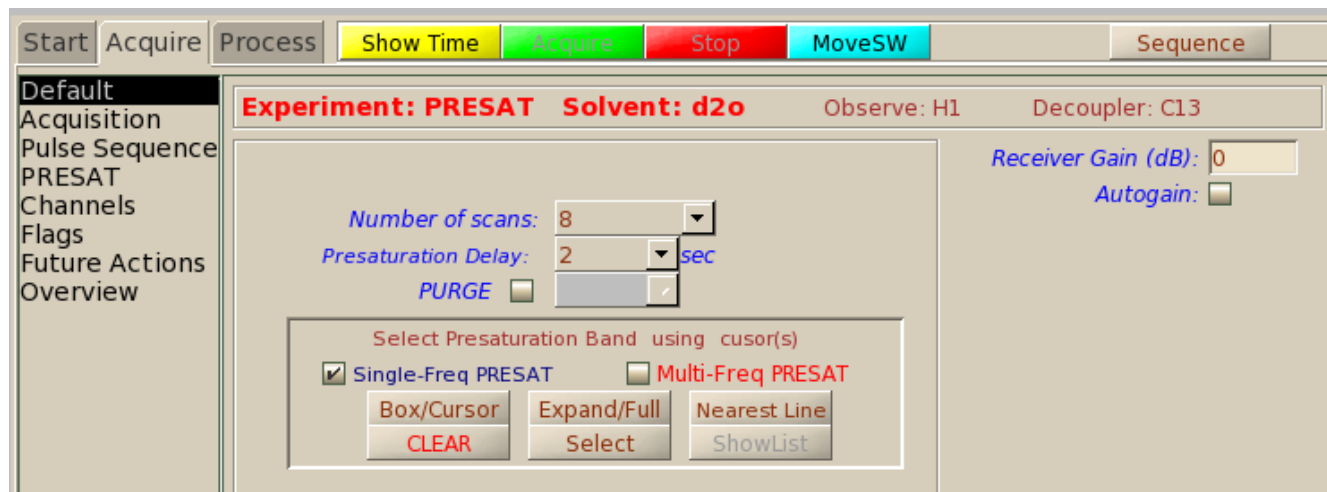
Using PRESAT

If **PRESAT** was chosen (Acquire, Default panel is shown below), after customizing the spectral width and number of scans, you can simply select one of the pre-defined solvents from the "Saturate?" drop down list (H_2O/D_2O, wet DMSO or 90% H_2O). The first entry, H_2O/D_2O, will suppress the residual solvent signal (HDO) of D_2O; the second is for the residual water signal commonly found in DMSO-d_6 at around 3.3 ppm; and the last is a more aggressive method designed to reduce the huge water peak in 90% H_2O. A second way of choosing the solvent peaks is to click the Suppress.. *0* tallest peaks check box and enter a number of peaks (1 for H_2O, 2 for THF, etc) to have vnmrj automatically find the tallest peaks and choose parameters to suppress them. And

a third way of selecting the peaks is to check the Select Later check box to have vnmrj take a "scout" spectrum and let you manually select the peaks. The PURGE option can help getting a cleaner residual signal after suppression. Do not select H2O peak (offset) unless you know the exact frequency offset of the peak to be saturated. This is likely to be different from the default value being offered. After you make your choices, click on [Acquire] to start the acquisition.

If Select Later was chosen, after clicking [Acquire], the panel show below will open up. You can now expand the scout spectrum and select with the cursors the peak or peaks that you want. You can select a peak using either one or two cursors. Click on [Box/Cursor] to display only one or two cursors. When using one cursor, position the cursor on top of the peak and click [Select]. That will use enough power to suppress a region five times the linewidth of the selected peak. If you want better control, you can use two cursors to define the region to be suppressed (the power used). The tallest peak inside the cursors will define the saturation frequency. Click [Select] to select that peak and width. If more peaks are to be suppressed, choose Multi-Freq PRESAT and continue selecting peaks in the same manner. When you are done, click on [Acquire]; do not type go or ga to start the acquisition.

Using WET

Defining peaks in **WET** follows a similar procedure to the one used in presat (please read "Using PRESAT" above for details). If **WET** is chosen, you can select one of the pre-defined solvents (see the parameter panel below). Or, select Suppress *0* tallest peaks (enter a number). Or check Select Later to select the peaks manually from a "scout" spectrum. Among the solvent choices you will see entries for both the deuterated and undeuteraded solvents; for example CD_3CN and CH_3CN. The first is meant for the removal of the residual proton peak of deuterated solvents; while the latter applies more vigorous conditions for the removal of the huge solvent peak in protonated solvents. After you make your choices, click on [Acquire] to start the acquisition.
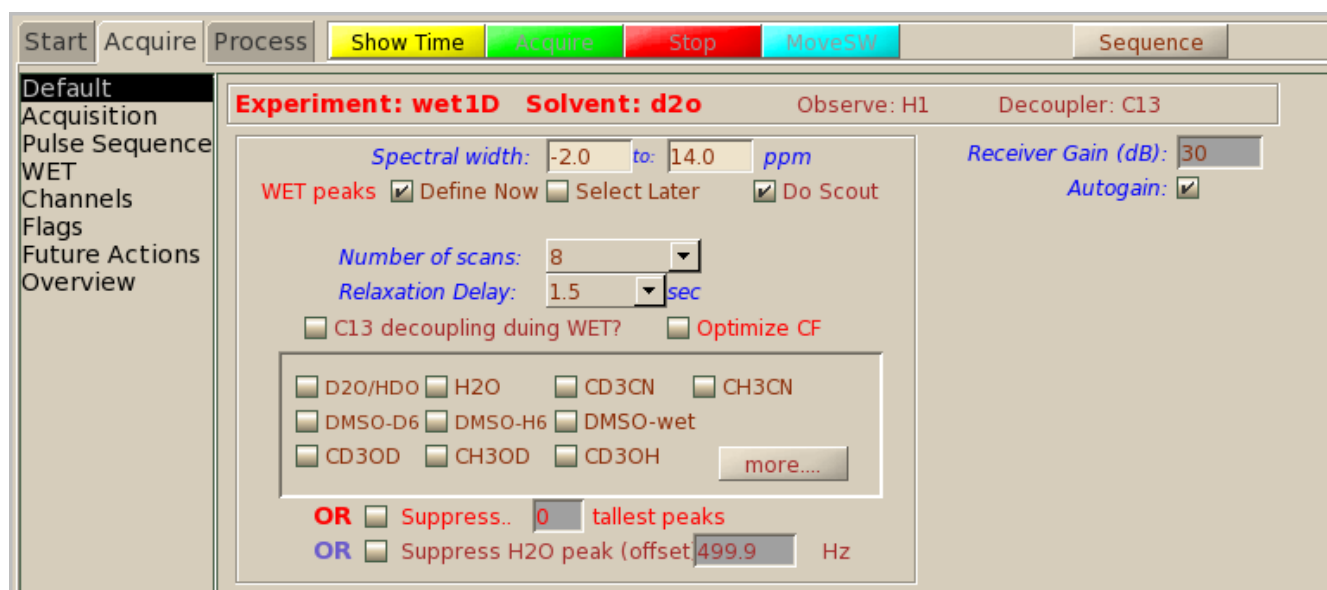
If Select Later was checked, the panel shown below opens up after the scout spectrum is measured. Expand the spectrum and select peaks in the same manner described for presat.   Remember to click on [Acquire] (do not type go or ga) to start the acquisition.
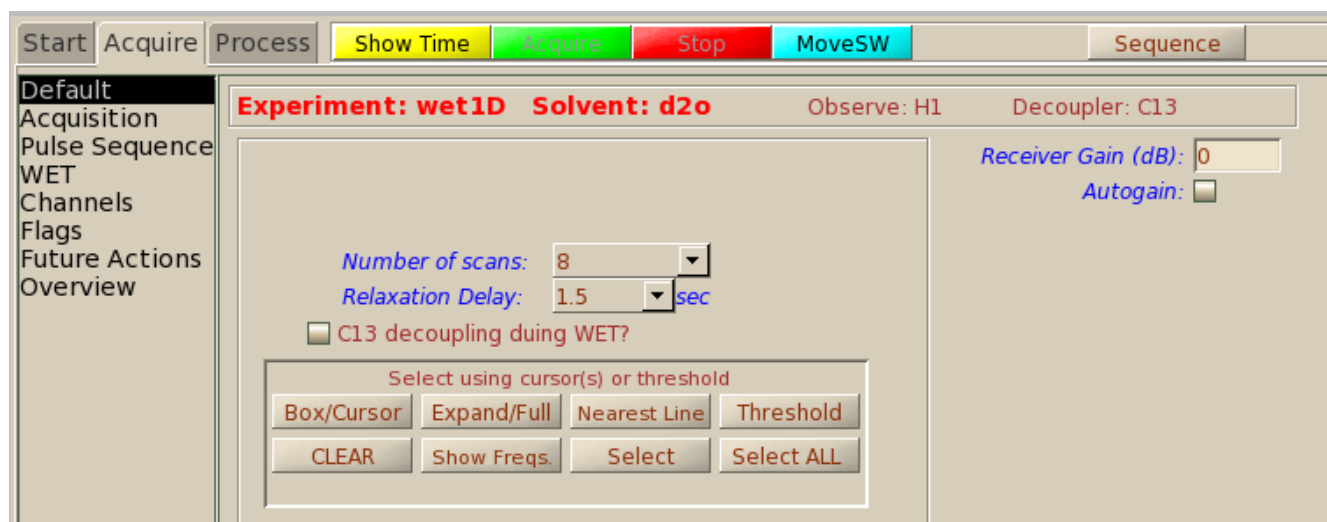
Hints

- If you already have recorded a "normal" proton spectrum, you can select Experiments > Convert current parameters to do... > Solvent Suppression – Select peaks to go directly to the manual peak selection phase described above (second and fourth figures shown).

- There will always be a residue of the solvent peak. This residual signal may be partly negative and may not be possible to phase completely. It is almost impossible to completely eliminate it with these pulse sequences. This is of no concern as long as the residual peak's height is not much higher (more than 10 times) than the peaks of interest.

- If the residual solvent peak is asymmetrical, try carefully shimming again.

- If the suppression is not enough, try manually selecting the peaks. If using presat, increasing the Presaturation Delay can help. The Power in the Acquire, PRESAT panel can also be increased slightly, by 3 or 6 units, but to no more than 12 units.

- If the suppression is not very clean, move the transmitter offset so the solvent peak is right in the center of the spectrum. Put the cursor over the solvent and select Process > Set Transmitter at Cursor, or type movetof. You may need to increase or modify the spectral width (sw) after this, and the resulting spectrum may also need to have its chemical shift reference re-set (setref macro, or enter a value in the Process, Default parameter panel).

- Increasing the number of steady state scans (ss) can also help in getting better suppression.

- The integration of peaks close to the suppressed peak(s) is not to be trusted.

Reference

S.H. Smallcombe, S.L. Patt and P.A. Keifer. *Wet solvent suppression and its applications to LC NMR and High-Resolution NMR Spectroscopy*. J. Magn. Reson. A **117**, 295 (1995).