

# Running an NOE-Difference on the Varian

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The Nuclear Overhauser Effect (NOE) arises through dipole-dipole relaxation. All other relaxation pathways contribute to *lessen* the overall magnitude of the NOE. If you plan on doing an NOE experiment you should degas your sample. This will help to minimize relaxation due to dissolved O<sub>2</sub>.

All parts written in **bold type** is direct input at the command prompt or a standard VNMR button

Note that the NOE-Difference experiment is best performed on the Varian Inova 500.

- Type **jexp1** (join experiment #1). **Do not** perform this experiment in experiment 5 or you will lose your data!
  - Some related commands you should know:
    - ✓ **cexp(#)** create experiment number #
    - ✓ **delexp(#)** delete experiment #
    - ✓ **unlock(#)** unlock experiment #
- Insert your sample into the magnet in the usual fashion. Choose the nucleus and solvent and type **su**. *DO NOT SPIN* the sample. Spinning of the irregularly shaped sample tubes degrades the quality of the NOE.
- Regulate the temperature at 22°C:
  - Type **vtype=2** (the default is '0') this will allow you to address the temperature controller.
  - Type **temp=22 su**. If this doesn't work go to the next step.
  - Type **temp**. In the Temperature Control Window select the 'Turn temperature control on at ...' radio button and use the slider bar to select the desired temperature. Maintaining a stable temperature is crucial for a successful NOE experiment.
- Lock the sample. Once you've determined the proper lock power, increase the power by 4db and decrease the lock gain such that the lock level is at ~80%. This is done to reduce deuterium lock jitter and noise, which can reduce the sensitivity of the NOE-Difference experiment.
- Tune the probe.
- Shim the sample. Since the shimming must be excellent, you should consider gradient shimming your sample. The gradient shimming tutorial can be found at [http://web.mit.edu/speclab/www/varian\\_gradient.pdf](http://web.mit.edu/speclab/www/varian_gradient.pdf). If you need assistance ask a staff member.
- Acquire and process a normal 1D-proton spectrum
  - Optimize desired spectral width (or sweep width) using the cursors and pressing the **[Expand]** button.
  - Note that you *must include all peaks including the solvent* in this new sweep width.
  - Type **movesw gain='y' ga** and then wait for the experiment to complete. Make sure the shimming is good. If not, keep shimming.
- Type **presat**. This will set up the parameters for the PRESAT pulse sequence.

- Using the mouse set the saturation frequency (**satfrq**) on the desired peak. (Type **ds** to make the cursor active.)
- The delay 'd1' should be set to  $5 \cdot T_1$ . If you don't know the value of  $T_1$  you may either run a  $T_1$  experiment (the tutorial is at [http://web.mit.edu/speclab/www/varian\\_T1.pdf](http://web.mit.edu/speclab/www/varian_T1.pdf)) or set the value of d1 to between 10 and 30 seconds (i.e. **d1=20**).
  - Type **d1=20** or whatever number you've determined
  - Type **ss=2** This will set the number of 'steady state' scans to 2
  - Type **dn='H1'** This sets the decoupler nucleus to proton
  - Type **sd** This sets the decoupler frequency to the cursor position.
  - Type **satfrq=dof** This sets the saturation frequency equal to the decoupler offset.
  - Type **satdly=5** Sets the saturation delay to 5 seconds
  - Type **satpwr=-15** Sets the saturation 'power' to -15db
- Array the saturation frequency.
  - Type **satfrq=satfrq,5000**. 5000 is generally an area of the spectrum with no peaks (~15ppm) and serves as a control.
- Verify that the 'target' peak is saturated.
  - Type **nt=1 au wft**. If the peak has been sufficiently suppressed, type **nt=16** (or whatever value you feel is necessary. 256 to 512 transients may be necessary to give rise to the NOE for your sample. But remember, this will require a correspondingly longer period of time) **au**. Type **time** to view the experimental time.
  - If the peak has not been sufficiently suppressed, increase the saturation power by typing **satpwr=-13 au wft**. Repeat as necessary until the peak is suppressed, but never increase the satpwr parameter over '+15'. Doing so **will** damage the probe.
- Once the acquisition has completed, phase and process the spectrum as usual. Then type **dodiff**. The dodiff macro will process the 'difference' spectrum.
  - **delexp(5)** (delete experiment #5) **df(1)** (display fid #1) **add** (adds fid #1 to add / subtract experiment) **df(2)** (display fid #2) **sub** (subtracts current (fid #2) from current content of add / subtract experiment (fid #1)) **jexp(5)** (joins experiment #5) **vp=wc2max/2** (adjusts vertical position to 50% of maximum) **wft** (weighted Fourier transform) **vsadj** (automatic vertical scale adjust) **dscale** (displays the reference scale)
  - Carefully inspect the difference spectrum to ensure that only one peak is saturated.
  - Integrate to quantify the NOE. Cut all of the integrals as usual, type **bc** to baseline correct and set the irradiated peak to 100. By doing so, the other integrated peaks will be in units of '% enhanced'.
- To run the experiment again you will need to rejoin the experiment where you started (in this case **jexp1**).

## Helpful hints for a successful experiment.

Common experimental problems are:

- Incomplete saturation (dpwr is too low)

- Failure to reach an equilibrium (or steady state)
- Competition from other relaxation sources

Hints in sample preparation:

Solvent tips:

- The solvent should be highly deuterated to decrease the intermolecular d-d relaxation.
- A good strong lock signal works best. DMSO-d<sub>6</sub> and acetone-d<sub>6</sub> both work extremely well. Avoid weak, broad, or temperature-dependant lock signals.

Solution tips:

- Do not 'over-load' the sample. Use a concentration low enough to avoid aggregation.

Purification tips:

- Filter the solution.
- Avoid paramagnetic contaminants.

Minimizing subtraction artifacts:

- Use a large number of transients (**nt**)
- Use steady-state pulses (**ss=2** or more)
- Use the VT controller. Small temperature fluctuations will introduce subtraction errors.
- In the difference spectrum, use a small amount of line broadening (**lb=0.5** or **lb=1**)