# Noesy1d experiments with Vnmrj 2.2 and chempack

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As usual, begin by acquiring a 1D spectrum in workspace 1.  Make sure the sample is properly shimmed and the spectrum is carefully phased.  For better results, reduce the spectral width (place cursors around the region of the spectrum where the signals are located, for example between 0.5 and 8.5 ppm, and type `movesw`) and take a new spectrum.  If solvent suppression is required, choose a method (PRESAT or WET) from the Experiments menu.

**Setup**

- Select from the Menu "Experiments > Convert current parameters to do... Selective Excitation Experiments > 1D NOESY".

- Display the "*Acquire, Defaults*" panel if not already displayed.

- Expand around the region of the spectrum you want to irradiate.  Place the cursors around the peak(s) to irradiate and click the [Select] button.  The cursors should contain the peak entirely, starting at the baseline and should be symmetrically positioned around the middle of the peak or multiplet as shown.  If you make a mistake during peak selection, click the [CLEAR] button to delete the last defined peak or multiplet.  You can define more than one peak to saturate by repeating this selection process as many times as desired.
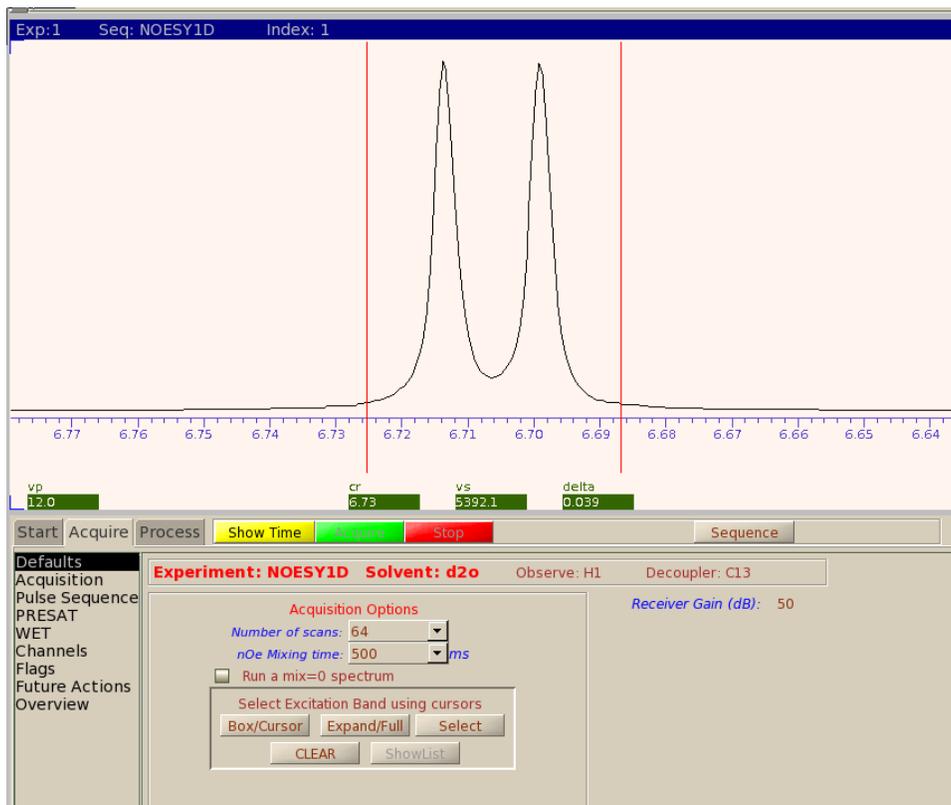


- Change the Number of scans if desired.  The default is 64 but 32 can be used on concentrated samples (>20 mg) and 128 scans or more will be needed on dilute samples or in instruments

equipped with a probe of low proton sensitivity.

- Change the nOe Mixing time if desired. The default is 500 ms. Choose a value in milliseconds from the drop down list or type in the command line, for example "`mixN=0.75`" (enter the value in seconds). The value can also be entered in the *Acquire, Pulse Sequence* panel. See the note below for hints on selecting mixing times.

- Click on [Show Time] to see how long the experiment will run.

- Click on [Acquire] to start. <u>Do not just type `go` or `ga`</u>. The pulse sequence requires that the shape of a selective pulse be calculated and this is done only when the [Acquire] button is clicked.

- Save the spectrum after the acquisition is finished if desired.

## Processing

- Process the spectra as usual with `wft`. A larger line broadening, for example `lb=1`, can be used on noisy spectra to reduce the noise. If the spectrum is not properly phased, carefully phase it keeping the saturated peak completely negative. The remaining peaks are usually positive, indicating positive NOEs, but can also be negative for negative or indirect NOEs, or partially out of phase when the peaks experiencing intensity enhancement are also strongly coupled to the saturated signal.

- Display the entire spectrum and the full integral and adjust the baseline (level and tilt) of the integral. The baseline should be as horizontal and straight as possible. Expand the region where the peaks are located and cut integral regions for each signal of interest. The saturated peak is usually assigned an integral value of -100. To to this, display the "*Process, Default*" panel, put one cursor on top of the integral of the saturated signal and click on [Set Norm to]. The value to the right of this button should be (positive) 100. Click [Integral Values] to display the values.

- If more than one peak was selected for saturation, two new buttons should appear on the vertical tool bar: and . These buttons are used to display the next or previous spectrum in the set.

## Selecting a mixing time

It is difficult to recommend a good, "generic" value for the mixing time since it depends on many factors as molecular weight, molecular symmetry, solvent, relaxation times, etc. However, on small molecules of molecular weight < 500, mixing times as large as 1.5 seconds can be used. With molecular weights between 500 and 1000, the mixing time can be 0.5 to 1.0 seconds. With molecular weights between 1000 and 3000, NOESY1D is not recommended as the enhancements will be close to zero; use the ROESY1D pulse sequence with a mixing time around 0.5 – 1.0 seconds. With molecular weights above 3000 the NOESY1D pulse sequence can be used again but with shorter mixing times of around 200 ms.

As an example, a series of NOESY1D spectra with different mixing times were collected on our Inova 500. The sample used was atropine, MW=289, in non-degassed $CDCl_3$. Saturation of a particular hydrogen atom (at around 3.0 ppm) gave an enhancement of the methyl group at 2.2 ppm that varied with the mixing time as follows:

| Mixing time, s | Enhancement, % | Signal to noise ratio |
|:---:|:---:|:---:|
| 0.2 | 0.9 | 4.0 |
| 0.5 | 1.8 | 8.0 |
| 0.8 | 2.5 | 9.0 |
| 1.1 | 3.4 | 9.6 |
| 1.4 | 4.0 | 9.0 |
| 1.8 | 4.6 | 8.7 |

For this hydrogen atom, a good mixing time is around 1.0 to 1.5 seconds; although larger mixing times may provide higher enhancements they also lead to a decrease in the signal to noise ratio of the signals in question.