2D-NOESY Spectra of Small Molecules - Hints And Tips

One of the most common questions I hear is "why didn't I get a good NOESY spectrum?" This document aims to provide some comments on this experiment and its results along with some tips to help NMR users get the best possible 2D-NOESY data for small-organic-molecule samples. The discussion here assumes the reader is familiar both with the basics of 2D NMR and with obtaining routine 2D NMR spectra on our NMR Facility's Bruker Avance spectrometers.

The NOESY spectrum uses the nuclear Overhauser effect (nOe) to provide information about which proton (typically) resonances are from protons which are close together in space. This is distinct from COSY-type spectra which use the J-coupling interaction to report on which proton resonances are located on the same or adjacent carbon nuclei. While it is true that most protons which are on adjacent carbons are close enough to give a NOESY cross-peak, it is not necessarily true that protons which give a NOESY cross-peak are J-coupled.

One of the main practical differences between NOESY spectra and COSY/TOCSY spectra is the fact that the nOe interaction is relatively weak. This results in NOESY spectra having much lower-intensity cross-peaks than one would expect from a COSY/TOCSY spectrum of the same sample, even if the NOESY spectrum is a "good" spectrum with no obvious problems. This results in a situation were there is not the same type of linear relationship between the "results" of a NOESY spectrum (usually considered to be the overall signal intensities of the cross-peaks) and the quality of the sample and experimental setup as there is for COSY-type spectra. In other words, if one runs a COSY experiment on a decent sample, barring instrument problems or gross errors in experiment setup, one can reasonably expect to see cross-peaks of decent intensity in the resulting spectrum. However, this is not always the case with NOESY spectra.

There are several steps one can take to maximize the chances of getting the best possible NOESY data. These steps involve both the sample itself and the experimental setup. We shall discuss these below.

Sample-Related Considerations

1. Purity. While it is possible to run NOESY experiments on impure samples or on mixtures, typically the best results will be obtained for pure, single-component, samples. This is in part due to the fact that because NOESY cross-peaks can be weak, it is easy for the desired cross-peaks to be obscured by those from an impurity or another mixture component. Also, if the components of a multi-component sample have many overlapping resonances, assigning the cross-peaks to the desired component may be difficult. Sample purity also relates to the issue of concentration: if a sample is only 50% pure in the desired component, the the concentration of the desired component is only one-half of the entire sample concentration by weight.

2. Concentration. The optimum concentration for a NOESY sample is about the same as that needed to obtain a decent proton NMR spectrum in 8 - 16 scans. If the sample concentration is

too high, spectroscopic resolution can be degraded and the nOe interaction can be damped. If it is too low, weaker cross-peaks may not be found. A good estimate of a reasonable concentration is that which results in the sample peaks being of roughly (within 0.5 - 2.0 times) the same intensity as the residual proton signal from the solvent. If the sample can only be obtained in much lower concentration than that, then it would be of benefit to run the NOESY spectrum using an indirect-detection probe. If this is the case, contact the NMR Facility staff for assistance.

3. Quality. Foreign matter, such as silica gel, powder from molecular seives, or other solids, in the sample can have a detrimental effect both upon spectroscopic resolution and upon the efficiency of the nOe interaction. Sample tube quality also can affect resolution. Thus, for NOESY spectra, always use a high-quality NMR tube (Wilmad 528PP or better) and always filter the sample (a plug of Kimwipe inserted into a Pasteur pipette is an easy way to do this) into the NMR tube. A good NOESY sample should be free of residual water or other extraneous peaks such as TMS, residual protonated solvent, etc. Finally, the sample volume should be at least 0.6 mL in order to minimize problems related to sample shimming.

4. Solvent. While $CDCl_3$ is usually the solvent of choice for organic samples, it often contains traces of acid which can cause reactions to occur with some samples, thus interfering with the stability of the sample. It has been my own experience that, when possible, the use of other solvents such as CD_3OD and Acetone-d6 can give better spectroscopic dispersion and better NOESY spectra than that obtained with $CDCl_3$. Considerations such as solvent viscosity and the width of the solvent deuterium peak (relevant for spectrometer lock performance) sometimes are important also.

Experimental-Related Considerations

1. General. NOESY spectra should be run on samples that are well-shimmed and that have a strong, stable lock. The NOESY spectrum should be obtained without sample spinning. If the NOESY spectrum will be run for more than about four hours, use the sample temperature controller to keep the sample temperature constant; usually a setting of 298 - 300K works well. Be sure to take an adequate number of scans (NS, default is 16). It is better for a given amount of instrument time to take more scans and acquire less increments (1_TD, default is 256). If you have to finish your experiment before all of the increments are completed don't worry, the software can handle this during the data processing. Finally, check the probe tuning on your NOESY sample prior to starting the experiment.

2. Delay values D1 and D8. The delay D1 is the time between each scan where relaxation of all the spins occurs. The delay D8 is the time period during each execution of the pulse sequence in the NOESY experiment where cross-relaxation (the actual nOe interaction) takes place. When you load the default NOESY parameters (e.g. rpar noesy.bbo all) these delays are set to 4.0 sec. and 0.5 sec., respectively. These values are approximations that should give acceptable results for most good-quality solutions of small molecules, however, these values are almost certainly not going to be optimum for any given sample.

If you do not get a usable spectrum using the default values, you may find it advantageous to re-run the NOESY spectrum using a different value of D8; the typical range of D8 values for small organic molecules is 0.1 to 0.8 seconds, with the upper limit of D8 being on the order of the T1 (spin-lattice relaxation time) of the proton resonance in question. The D1 value is related to the T1 relaxation times of the protons in your sample; ideally D1 should be 2*T1 to 4*T1. Proton T1's are usually 1 second to a few seconds, if your sample has very long T1's it might be worth lengthening the D1 value and re-running the spectrum. It is possible to determine the T1 values for each proton resonance in your sample, but this is time-consuming and usually not necessary. The final point regarding these variables is that it is not possible to predict the best values for a given sample, so it is not unusual to have to run multiple NOESY spectra to obtain the best results.

3. Data processing. As with the delay values, the standard NOESY parameter set includes processing parameters that should give reasonable results for most good-quality samples. In order to change the windowing functions for data processing, one needs to open the processing parameters menu (command `edg`). The default window function for both dimensions is Gaussian multiplication (GM), you can see this shown in the pulldown menu adjacent to the WDW entry in the columns for each dimension. The parameters GB and LB also are relevant. The parameter GB varies between 0 and 1, with lower values favoring sensitivity over resolution. The LB parameter controls the "intensity" of the Gaussian function, making the value more negative increases the strength of the function. If your sample shows only weak cross-peaks, it can be advantageous to re-process the data with slightly smaller values of GB in both F1 and F2 (e.g., changing GB from 0.3 to 0.2) and using slightly larger negative values of LB (e.g. changing from -10 to -12). Note that if you change any of these parameters and reprocess the spectrum (command `xfb`), the original 2D FID is not disturbed - you can reprocess your data as many times as you want. You may also care to try different windowing functions, with sine-squared (QSINE in the WDW choice pulldown) often being useful to improve the resolution of the 2D NOESY data. This change will sacrifice some of the available signal-to-noise ratio, however.

It can happen also that the phasing of the 2D spectrum might need additional work beyond the simple method giving in the 2D-Training Guide. This is accessed via the PHASE button to the side of the 2D spectrum display. It is beyond the scope of this article to discuss the 2D phasing routine, suffice it to say that the software manual presentation is recommended reading if you care to try this. Also, facility staff can assist with the 2D phasing routine. Finally, I do not recommend using symmetrization (command `syma`) as a general rule. It can make the 2D spectrum look better, but it actually often makes the interpretation process more difficult.

Additional Reading

The Nuclear Overhauser Effect in Structural and Conformational Analysis, D. Neuhaus and M. Williamson, VCH, New York 1989(ISBN 1-56081-616-3, paper).