PINMRF

Bruker DPX / ARX NMR Spectrometers running XWinNMR Routine/Survey 2D Spectra using Standard Parameter Sets

INCLUDING:
DPX300 w/ 5mm QNP Probe – 408 HANS
ARX300 w/ 5mm QNP Probe – G43 RHPH
ARX400 w/ 5mm QNP Probe – 369 WTHR

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Routine/Survey 2D Spectra using Standard Parameter Sets - Overview

This booklet provides the information necessary to obtain routine 2D NMR spectra on routine samples using the Bruker DPX and ARX-series NMR spectrometers running XWinNMR. These experiments require a minimum of operator intervention or optimization, and as such they are appropriate for the non-expert NMR user to carry out. Note that not all experiments are available for every instrument or probe.

These experiments encompass two general classes of 2D spectra. These are 1) proton homonuclear spectra, such as magnitude COSY, phase-sensitive TOCSY and NOESY, and 2) carbon-13-detected heteronuclear spectra, such as the HETCOR experiment (please note that the vastly more-sensitive proton-detected $^{13}$C-$^1$H gradient-enhanced magnitude-mode HMQC and HMBC experiments are available on the Bruker Avance DRX500 and AV800 spectrometers).

These experiments are set up with dedicated parameter sets designed to provide good results, with minimal operator effort, for normal organic and organometallic samples in typical organic solvents or D$_2$O. However, if the ultimate in sensitivity or resolution is required in your work, or your sample falls into one of the categories mentioned below, please contact PINMRF staff for assistance with more highly specialised experiments.

The experiments presented here DO NOT encompass the following:
1. samples which need solvent or other peak suppression;
2. samples which need non-standard observation windows;
3. experiments involving selective or semi-selective pulses;
4. heteronuclear 2D experiments involving nuclei other than $^{13}$C and $^1$H;

Note that some of these experiments will give better results if probe tuning is carried out on all relevant nuclei. See pp. 14-17 of the PINMRF manual Bruker Avance / AV NMR Spectrometers - Training Guide for Basic 1D NMR Spectroscopy for information regarding probe tuning. Please ask PINMRF staff for assistance with probe tuning if you need it.
These notes assume that the reader is already checked out for basic 1D operation on PINMRF’s Bruker spectrometers running XWinNMR.

**Data Obtained from the Experiments**

The proton homonuclear spectra provide information on proton-proton coupling networks and the spatial proximity of protons to one another. The COSY spectrum shows couplings between protons when the J value between them is ca. 5 Hz. or higher; usually this results from 2-, 3- and 4-bond couplings. The TOCSY experiment shows couplings down to ca. 2 Hz., which can include 5- and occasionally 6-bond couplings. The NOESY experiment gives information about which protons are close together in space. The heteronuclear spectra relate information about proton-carbon J-couplings. The HETCOR (also called HXCO) experiment correlates carbons with their directly attached protons; the COLOC variant of this experiment shows which carbons have 2- or 3-bond couplings (ca. 10 Hz.) to a given proton, but setting up this experiment will require PINMRF staff assistance. Note that quaternary carbons do not show up in the HETCOR spectra. HETCOR usually requires several hours to run.

**NOTES**
Routine/Survey 2D Spectra using Standard Parameter Sets -
Acquisition and Processing Guidelines
Bruker DPX / ARX NMR Spectrometers
PINMRF

2D data acquisition using standard parameter sets

1. Create a new data set. All 1D and 2D files will be acquired using the SAME experiment NAME but DIFFERENT experiment NUMBERS.

2. Create/read experiment (expno) 1. Put in your sample, lock, spin (if desired) and shim.

3. Obtain a proton spectrum in expno 1 using the standard proton parameters (e.g. h1.prob.solv, where solv = acet, cdcl, d2o, dmso, meth according to the solvent you are using and prob refers to the sample probe currently in use). Check the probe tuning for proton.

4. If desired, obtain a $^{13}$C spectrum in expno 2 using standard parameters. In addition, if desired, obtain a DEPT spectrum in expno 3. Check the probe tuning for $^{13}$C.

NOTE: a $^{13}$C spectrum is not required if only $^1$H 2D spectra are to be obtained, but it is necessary prior to running a HETCOR experiment.

5. Create/read expno 4. We will run a COSY in this dataset. Type rpar cosy.prob.solv all to load the COSY parameters.

6. If your sample is extremely dilute, increase the number of scans using the ns parameter. To examine/change this or any other acquisition parameter you can also use either the eda (edit acquisition parameters) or the ased (acquisition setup editor) commands. Each brings up a window; after examining and changing anything (if necessary), click the SAVE button to close the window and save the changes, or click the CANCEL button to close the window without saving any changes.

7. Use the setti command to enter your title (as discussed in the 1D operation guidelines).

8. Turn off sample spinning and check that the lock is stable; adjust lock power and/or gain if necessary.

9. Type acqu to display the acquisition window and then use rga to set receiver gain - wait for message telling you it is finished.

10. Type zg to start acquisition. The default COSY experiment takes about 15 minutes.
NOTE: to run other 2D experiments, create/read other expno’s as needed. Repeat from step 5 above using the following parameter sets (instead of \texttt{cosy.prob.solv all}):

For TOCSY: type \texttt{rpar tocsy.prob.solv all} (default time: 20 min.);
For NOESY: type \texttt{rpar noesy.prob.solv all} (default time: 2.5 hr.);
For HETCOR: type \texttt{rpar hetcor.prob.solv all} (default time: 3.0 hr.);

2D processing for magnitude experiments (COSY, HETCOR)
11. When the experiment is finished, or after enough increments are completed, type \texttt{xfb} to do the 2D Fourier transformation. The 2D spectrum will be displayed in intensity image mode.

2D processing for phase-sensitive experiments (TOCSY, NOESY)
11. Determine phase correction and do the 2D processing as follows:
   a) Any time after the second FID has been completed and saved, type \texttt{rser 1} (for NOESY) or \texttt{rser 2} (for TOCSY) to read the first or second FID (as appropriate) into a separate 1D dataset.
   b) Type \texttt{lb} and enter 8 into the window. Then type \texttt{ef} to apply exponential multiplication and do the Fourier transformation.
   c) Now phase this spectrum as normal, EXCEPT instead of clicking the SAVE & RETURN choice when finished, click the SAVE AS 2D & RETURN choice instead.
   d) Type \texttt{to2d} to go back to the 2D experiment, then \texttt{acqu} to go back to the acquisition window.
   e) When the experiment is finished, or after enough increments are completed, type \texttt{xfb} to do the 2D Fourier transformation. The phasing parameters obtained from the 1D spectrum (above) will be used to phase the 2D data. The 2D spectrum will be displayed in phase-sensitive image mode. The red-yellow-green data is positive intensity and the blue-purple data is negative. If additional phase correction is needed, then the 2D phase routine will be used. Please contact PINMRF staff for assistance with this routine.

Expansion and plotting
12. \textbf{SPECTRUM MANIPULATION}: To expand the spectrum, move the mouse over the spectrum window and click the left mouse button. A crosshair will appear on the spectrum.
Move the crosshair with the mouse to the lower left edge of the region you want to expand. The middle mouse button will then freeze the crosshair in this location. Moving the mouse again will move a second crosshair; move this to the upper right edge of the region you want to expand and then click the middle mouse button again. Now click the left mouse button to release the cursor from the spectrum. This will leave a box on the spectrum showing the desired expansion area. Move the mouse to the left border buttons and click on the button close to the top that has the rectangle on it (this is the right-hand button on the fourth row down from the top). This will expand the selection region of the 2D spectrum. To go back to the full spectrum, click on the ALL button close to the expansion button; the EXP button will re-expand to the previously chosen region.

Other manipulations:
• *2, /2, *8, /8: click these buttons to adjust vertical scale up or down by factor 2 or 8;

13. **DEFINE PLOT REGION:** To define a region for plotting, expand the 2D spectrum and set the vertical scale as desired. Click on the DEFPLOT button to the left of the spectrum display. A window will appear asking for the type and number of levels you want. Enter the following:
For COSY, TOCSY, HETCOR: change levels?: y ; # of positive levels: 6 ; # of negative levels (if asked): 0 ; display contours?: n .
For NOESY: change levels?: y ; # of positive levels: 6 ; # of negative levels: 1 ; display contours?: n .

14. **PLOTTING:**
a) Prior to plotting the spectrum on paper, type view to see the plot output displayed on the screen (in a new window). Check that the plot is correct prior to plotting to paper. Click on the QUIT button in this new window to close the view window.
b) To plot the spectrum on paper using the default parameters, type plot. This will plot the 2D spectrum, the axes, internal 1D projections (see below about projections) along both axes, the title, and the parameters. If you want to alter this, type edg (edit graphics parameters). A window will appear with buttons for the choices regarding the items included in the plot; after examining and changing anything, click the SAVE button to close the window and save the changes, or click the CANCEL button to close the window without saving any changes.
15. **PROJECTIONS:**

Typically, when a 2D spectrum is plotted out, the X and Y axes of the 2D matrix are flanked by 1D spectra that represent each axis. These 1D spectra are either the actual 1D spectrum of that axis, taken from a separate 1D experiment (called the “external” projection), or they are the actual skyline projection of the 2D data matrix in that dimension (called the “internal” projection). The external projection has the same resolution as the normal 1D spectrum, whereas the internal projection has the resolution of the 2D spectrum, which is a lot lower. The 2D parameter sets used in this procedure are set up to use internal projections, because it is easy to set up the files to do so. To change the internal projection to an external projection, follow the procedure below.

**PLEASE READ THE ENTIRE PROCEDURE BELOW BEFORE ATTEMPTING IT:**

This will change the projection in the F2 dimension from internal to external. Type `edg (edit graphics parameters)`. A window will appear with buttons for the choices regarding the items included in the plot. Click on the EDPROJ2 button and a new window will open. Click on the PF2EXT button and choose the EXTERNAL(1R) choice in the pop-up menu. Then, in the PF2EXP field, enter the correct experiment number for the 1D spectrum you previously obtained of this sample (this should be “1”). Then, click on the SAVE button at the bottom left of the menu to exit the EDPROJ2 menu, then click on the SAVE button at the bottom left of the menu to exit the `edg` menu. If you are unsure of the changes you made, click the CANCEL button instead.

- For COSY, TOCSY and NOESY spectra, you may repeat the process in the same way for the F1 projection; just substitute EDPROJ1, PF1EXT and PF1EXP for the menu names/choices above.

- For HETCOR spectra, the F2 projection will be the $^{13}$C spectrum. IF YOU HAVE ALREADY OBTAINED A $^{13}$C SPECTRUM, follow the procedure for the F1 projection mentioned above, EXCEPT that in the PF2EXP field, enter the experiment number for the $^{13}$C spectrum, NOT the proton spectrum. The F1 projection will be the $^1$H spectrum.
XWINNMR

Commonly Used Keyboard Commands

NOTE THAT ALMOST ALL COMMANDS ARE AVAILABLE FROM THE PULL-DOWN MENUS AT THE TOP OF THE SPECTRUM DISPLAY

Parameter Setup
edc - edit current dataset - reads existing and/or creates new datasets
eda - edit acquisition parameters
ased, edasp - edit acquisition parameters - pulse program driven or nucleus-related
edte - set up temperature controller parameters
edp - edit processing parameters
edg - edit graphics parameters - controls plot output attributes
wrpa - copy current dataset to a new one - specify new name and experiment number
re - read new dataset - specify new name and experiment number
edpul_pulprog - display pulse sequence file for sequence "pulprog"

Data Acquisition
lockdisp - display lock window
lock - start autolocking routine using Bruker default parameters
rpar - read parameter set
rsh - read shim set
ii - initialize interfaces
acqu - display acquisition window - shows FID on screen
wobb - start wobble routine for probe tuning
rga - set receiver gain automatically
ns - number of scans
2s ns – display number of scans stored with current dataset
d(n) - enter value for pulse sequence parameter (delay) D(n) where n = 1 to 31; e.g.: d1
p(n) - enter value for pulse sequence parameter (pulse) P(n) where n = 1 to 31
zg - zero current data and start acquisition
ogo - start data acquisition
tr - transfer FID to disk for processing
halt - halt data acquisition after next scan
stop - stop data acquisition immediately
standard – set up spectrometer for CDCl₃ standard sample

Custom Acquisition AU Programs for Advanced 1D Spectra
deptjsh – turn current C13 dataset into DEPT experiment and acquire
homodecjsh – turn current H1 dataset into a homodecoupling experiment and acquire
noediffjsh – turn current H1 dataset into a 1D nOe-difference experiment and acquire
presatjsh – turn current H1 dataset into a presaturation experiment and acquire
Data Processing and Plotting
setti - enter title for plot
rser x - read 1D serial file #x out of 2D dataset
ft - Fourier transformation
ef - exponential multiplication and Fourier transformation
efp - carry out “ef” plus apply existing phase corrections to spectrum
multiefp - carry out “efp” on multiple spectra in sequential experiment numbers
xfb - 2D Fourier transformation
apk - automatic phase correction of 1D spectrum
abs - apply automatic baseline straightening to spectrum
lb - controls amount of exponential multiplication
sref – set chemical shift scale reference using TMS or default parameters
nzp - number of data points to zero at start of FID
zp - zero nzp points at start of FID
basl - enter baseline correction routine
pscal - define plot vertical scaling method
cy - plot vertical scaling
cx - plot horizontal scaling
mi - threshold for peak picking
pps - peak picking with output on screen
pp - peak picking with output on paper
view - view plot output on screen
plot - plot spectrum on paper
edc2 - enter parameters for second and third datasets (e.g. for dual display mode)
dual - dual display mode - EXPNO2 and PROCNO2 parameters must be set in the edc2 menu
xwinplot - start XwinPlot standalone program using current dataset
xwp - start XwinPlot standalone program using current plot region

Data Handling
dir – show a listing of your experiment names
dirf – show a listing of all 1D FID files (includes experiment names and numbers)
dirser – show a listing of all 2D SER files (includes experiment names and numbers)
dirs – show a listing of all 1D spectra (includes experiment names and numbers)
dir2d – show a listing of all 2D spectra (includes experiment names and numbers)
dels – delete 1D processed spectra (FID’s are not deleted)
del2d – delete 2D processed spectra (SER files are not deleted)

Notes
**NOTE:** “solv” = solvent = acet, cdcl, cd2cl2, d2o, dmso, or meth
(for C₆D₆ use cdcl, for CD₃CN use acet).

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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 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 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 sieves, 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. If you need to use a significantly smaller sample volume we recommend the use of a Shigemi-type NMR tube. Please contact PINMRF staff for assistance in this case.

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$_3$OD and acetone-d$_6$ 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 to acquire fewer 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.qnp.solv 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 edp). 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 reduce the 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 XWinNMR software manual 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