

Bruker Avance NMR Spectrometers
UIC Chemistry / RRC-East NMR Lab

**Routine/Survey 2D Spectra using Standard Parameter Sets -
Acquisition and Processing Guidelines**

Table of Contents

1.	Overview of Experiments Covered.....	1
2.	Survey 2D Spectra Acquisition and Processing Guidelines.....	2
3.	XWINNMR Commonly Used Commands (incl. supplement for 2D experiments)...	6
4.	Parameter Sets	7
5.	Probe Tuning Guidelines.....	8
6.	Probe Tuning Matrix and Sensitivity Discussion.....	10

Updated 02-01-01: updates reflecting the addition of the BBO probe for the Avance-400; JSH.
Posted 02-28-00 JSH.

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 our new Bruker Avance NMR Spectrometers. The 2D experiments covered here are both robust and fast when run on our new NMR equipment with the Z-axis field gradient accessory. In addition, these experiments require a minimum of operator intervention or optimisation, and as such they are appropriate for the non-expert NMR user to carry out.

These experiments encompass two general classes of 2D spectra. These are 1) proton homonuclear spectra, such as fast gradient-enhanced magnitude COSY, phase-sensitive TOCSY and NOESY, and 2) proton-detected heteronuclear spectra, such as ^{13}C - ^1H gradient-enhanced magnitude HMQC and HMBC. 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_2O . 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 see the NMR Facility 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 ^1H ;
5. phase-sensitive experiments which require interactive phase correction or other data processing.

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 on proton-carbon J-couplings. The HMQC experiment correlates protons with their directly attached carbons; the HMBC experiment shows which carbons have 2- or 3-bond couplings (ca. 10 Hz.) to a given proton. Note that quaternary carbons do not show up in the HMQC spectrum.

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These notes assume that the reader is checked out for 1D operation
on the Bruker Avance Spectrometers.

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.bbo).
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 .
5. Create/read expno 4. We will run a COSY in this dataset. Type `rpar cosy.bbo_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. For COSY experiments only: after the RGA routine is finished,

type `rg` to find the current value of the receiver gain. Enter one-half of the current value into the input window (followed by the return key).

10. Type `zg` to start acquisition. The default COSY experiment takes about 10 minutes. For some of the 2D experiments you will see the lock signal drop periodically - this is normal.

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 `cosy.bbo_all`):

For HMQC: type `rpar hmqc.bbo_all` (default time: 40 min.);

For HMBC: type `rpar hmhc.bbo_all` (default time: 40 min.);

For TOCSY: type `rpar tocsy.bbo_all` (default time: 40 min.);

For NOESY: type `rpar noesy.bbo_all` (default time: 2.5 hr.).

2D processing for magnitude experiments (COSY, HMQC, HMBC)

11. When the experiment is finished, or after enough increments are completed, type `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 `rser 1` (for NOESY) or `rser 2` (for TOCSY) to read the first or second FID (as appropriate) into a separate 1D dataset.

b) Type `lb` and enter `8` into the window. Then type `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 `to2d` to go back to the 2D experiment, then `acqu` to go back to the acquisition window.

e) When the experiment is finished, or after enough increments are completed, type `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.

Expansion and plotting

12. **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 as and set the vertical scale as desired. Click on the DEF PLOT 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, HMQC, HMBC: 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 `HMQC` and `HMBC` spectra, the F1 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 `PF1EXP` field, enter the experiment number for the ^{13}C spectrum, **NOT** the proton spectrum.

XWINNMR

Commonly Used Keyboard Commands (expanded 9-99)

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
 edpul_pulprog - display pulse sequence file for sequence "pulprog"

Data Acquisition

lockdisp - display lock window
 lock - start autolocking routine
 rpar - read parameter set
 rsh - read shim set
 ii - initialize interfaces
 acqu - display acquisition window - shows FID on screen
 wobbb - start wobble routine for probe tuning
 rga - set receiver gain automatically
 ns - number of scans
 d(n) - enter value for pulse sequence parameter (delay) D(n) where n = 1 to 31; e.g.:
 d1 - enter value for pulse sequence parameter (delay) D1
 p(n) - enter value for pulse sequence parameter (pulse) P(n) where n = 1 to 31
 zg - zero current data and start acquisition
 go - start data acquisition
 tr - transfer FID to disk for processing
 halt - halt data acquisition after next scan
 stop - stop data acquisition immediately

Data Processing and Plotting

setti - enter title for plot
 ft - Fourier transformation
 ef - exponential multiplication and Fourier transformation
 em - exponential multiplication
 lb - controls amount of exponential multiplication
 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 - plot spectrum to screen
 plot - plot spectrum on paper

2D-Related Commands

mc2 - enter type of 2D acquisition (QF for magnitude 2D's, TPPI for phase-sensitive, usually)

1_td - enter number of t1 increments to be acquired (_ = space)

nd0 - enter value of nd0 parameter (set according to 2D pulse sequence)

in0 - enter value for 2D increment in seconds (set according to 2D pulse sequence)

to2d - go back to 2D dataset from related 1D dataset

Avance Spectrometer Parameter Files - 02/01/01DPX-400 (BBO probe)

h1.bbo
 homodec.bbo
 f19.bbo
 c13.bbo
 c13dept.bbo
 p31.bbo
 p31nd.bbo
 cosy.bbo
 hmqc.bbo
 hmbc.bbo
 tocsy.bbo
 noesy.bbo
 shims.bbo

DPX400 (BBI probe)

h1.bbi
 homodec.bbi
 c13.bbi
 c13dept.bbi
 p31.bbi
 p31nd.bbi
 pt195.bbi
 se77.bbi
 si29.bbi
 cosy.bbi
 hmqc.bbi
 hmbc.bbi
 tocsy.bbi
 noesy.bbi
 shims.bbi

DRX-500 (BBO probe)

h1.bbo
 homodec.bbo
 f19.bbo
 c13.bbo
 c13dept.bbo
 p31.bbo
 p31nd.bbo
 b11.bbo
 h2.bbo
 o17.bbo
 pt195.bbo
 si29.bbo
 cosy.bbo
 hmqc.bbo
 hmbc.bbo
 tocsy.bbo
 noesy.bbo
 shims.bbo

DRX-500 (TBI probe)

h1.tbi
 homodec.tbi
 c13.tbi
 c13dept.tbi
 p31.tbi
 p31nd.tbi
 cosy.tbi
 hmqc.tbi
 hmbc.tbi
 tocsy.tbi
 noesy.tbi
 shims.tbi
 shimsn2ns.tbi (for water suppression using N2 VT gas)

Probe Tuning Guidelines - BBI, TBI and BBO Probes
Bruker Avance NMR Spectrometers
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Spectrometer setup - ^1H tuning

1. Run a quick proton experiment to determine that the sample is correct.
2. We will tune the ^1H coil first. Turn off the sample spinning.
3. Type `acqu` to go to the acquisition window, then type `wobb` to start the probe tuning ("wobble") routine.

Tuning the ^1H coil

4. The computer screen will show a trace with a dip in it; hopefully the dip will be close to the correct frequency in the center of the screen. If there is no dip, click the `WOBB-SW` button to the left of the screen, then enter a new value in the correct field of the screen that pops up. The default SW is 4 MHz.; try 12 or 16 MHz. if it needs changing.
5. When you have confirmed that there is a dip present, go to the magnet and adjust the tune and match screws under the probe to bring the minimum of the dip to the center of the screen and also as close to the bottom of the display as possible. NOTE: the location of the dip left to right is referred to as the "tune" and the depth of the dip (closeness to the baseline) is referred to as the "match." The tune screw will tend to move the dip left - right and the match screw will improve the depth of the dip. The display on the preamp housing also shows the quality of the probe tuning and can be used in conjunction with or instead of the computer screen display. NOTE: on the TBI probe on the DRX-500 the proton coil tune and match is done using the small screwdriver-like rods and the special tool hanging off the probe.
6. When the tuning is OK, type `halt` to stop the wobble routine.
7. Restart sample spinning if desired. NOTE: leave spinning off for 2D experiments.
8. Re-acquire the proton spectrum if desired.

Spectrometer setup - X-nucleus tuning

1. Carry out steps 1 - 8 above to ensure the ^1H coil is tuned.

2. Use the `edc` command to read or create a new dataset.
3. Use the `rpar` command to read the correct parameter set for the X-nucleus you want to observe, then type `ii` to initialize the spectrometer. Turn off sample spinning.
4. Type `acqu` to go to the acquisition window, then type `wobb` to start the probe tuning ("wobble") routine.

Tuning the X-nucleus coil

5. X-nucleus coil tuning is done using gold-colored sliders visible on the bottom of the probe. The sliders are numbered, and there is a directory of numbers for the tune and match settings for most common X-nuclei hanging below the probe. Confirm that the tune and match settings are correct for the X-nucleus you want to observe. NOTE: on the TBI probe on the DRX-500, the X-BB coil is not used for ^{13}C - there is a dedicated ^{13}C coil for that. The ^{13}C coil is tuned using the single blue screw. The X-BB coil is tuned in the same fashion as the other probes. NOTE: For observing X nuclei other than ^{13}C with the TBI probe, the cable from the middle (vertical) connection on the preamplifier has to be connected to the X-BB coil connection on the probe.
6. The computer screen will show a trace with a dip in it; hopefully the dip will be close to the correct frequency in the center of the screen. If there is no dip, click the WOBB-SW button to the left of the screen, then enter a new value in the correct field of the screen that pops up. The default SW is 4 MHz.; try 12 or 16 MHz. if it needs changing.
7. When you have confirmed that there is a dip present, go to the magnet and adjust the gold tune and match sliders under the probe to bring the minimum of the dip to the center of the screen and also as close to the bottom of the display as possible. NOTE: the location of the dip left to right is referred to as the "tune" and the depth of the dip (closeness to the baseline) is referred to as the "match." The tune slider(s) will tend to move the dip left - right and the match slider(s) will improve the depth of the dip. The display on the preamp housing also shows the quality of the probe tuning and can be used in conjunction with or instead of the computer screen display.
8. When the tuning is OK, type `halt` to stop the wobble routine.
9. Restart sample spinning if desired. NOTE: leave spinning off for 2D experiments.
10. Be sure to tune the X-nucleus coil back to ^{13}C on the BBO and BBI probes before you leave the spectrometer. The X-BB coil on the TBI probe is tuned to ^{31}P by default.

Avance Spectrometers - Probe Tuning Matrix

Which experiments are likely to require probe tuning?

TYPE OF EXPERIMENT:	H/F NUCLEI:		X NUCLEI:		
	<u>1H</u>	<u>19F*</u>	<u>13C</u>	<u>31P</u>	<u>OTHER</u>
1D obs. no dec.	No	Yes	Maybe	Yes	Yes
1H homonuc. dec.	Yes	No	No	No	No
1D X obs. 1H dec.	Maybe	Yes	Maybe	Yes	Yes
1D X obs. DEPT	Yes	Yes	Yes	Yes	Yes
1D 1H obs. X dec.	Yes	Yes	Yes	Yes	Yes
2D Simple COSY	No	Yes	N/A	N/A	N/A
2D 1H-only other	Yes	Yes	N/A	N/A	N/A
2D 1H obs. X dec.	Yes	Yes	Yes	Yes	Yes
2D X obs. 1H dec.	Yes	Yes	Yes	Yes	Yes
3D, 4D any	Yes	Yes	Yes	Yes	Yes

*¹⁹F only possible with the BBO probes. ¹⁹F - ¹H experiments are not possible with our existing probes.

Instrumental Overview - Sensitivity (S/N), 5mm Probes

<u>Console/Probe</u>	<u>¹H sensitivity</u>	<u>¹³C sensitivity</u>
AM-400/Nalorac BB	160:1	130:1
Avance DPX-400/BBO-Z-grad	220:1	160:1
Avance DPX-400/BBI-Z-grad	650:1	110:1
Avance DRX-500/BBO-Z-grad	330:1	220:1
Avance DRX-500/TBI-Z-grad	750:1	90:1

As can be seen from the chart, the indirect-detection probes on both of the new instruments provide huge increases in sensitivity for ¹H observation. The BBI-Z-gradient (broadband-inverse) probe on the DPX-400 provides roughly the same ¹³C sensitivity as does the Nalorac BB probe on our old AM-400, even though the BBI-Z-gradient probe is not optimized for X-nucleus observation. The same is true of the TBI-Z-gradient (triple-resonance broadband-inverse ¹H-¹³C-X) probe on the DRX-500, except this probe suffers for ¹³C observation because the ¹³C coil is double-tuned and optimized for ¹H observation. The BBO (broadband-observe) probes offer a significant increase in ¹³C and other X nuclei sensitivity for direct X-nucleus observation. As of January 2001, the BBO probes will be the default probes for both spectrometers.