# BRUKER AC 200 NMR SPECTROMETER

# $^1$H ACQUISITION WITH DISNMR SOFTWARE

## I. Introduction

A. Every user of the AC 200 NMR spectrometer must be checked out on the instrument before beginning routine use. Users will have access subject to the current signup rules and schedules. These procedures must be followed to avoid very expensive damage to the spectrometer.

B. Although this manual is moderately detailed, it is not intended to be comprehensive. See the Bruker System Software Manual, DISNMR Reference Manual and Practical Guide to 1-D and 2-D Experiments for AC/AM Systems for much more information. Do not remove these manuals from the Science 4304 NMR lab.

C. For convenience, commands using the "control" key are written in this manual with the prefix, **[ctrl]**. For example, **[ctrl] A** means hold the "control" key down and simultaneously press the "A" key. Some commands are entered only after the "return" key is pressed. For example, to plot a spectrum from within the EP routine, you press the "X" key followed by the "return" key. This action is signified in this manual as **X [return]**.

## II. Signup

A. Safety/Personal Belongings

1. **Nobody with a pacemaker should ever enter the NMR lab! No exceptions!**
2. The strong magnetic field can erase credit and library cards! Take off mechanical watches, remove wallets with credit and library cards, and place them on the counter by the sink.

B. Availability of the Instrument

1. If the red, "Experiment in Progress" sign is displayed and the pulse light on the console flashes regularly, sign up for a later session.
2. If the red, "Experiment in Progress" sign is not displayed and the pulse light on the console does not flash, press the "**LINE/RGB**" button on the monitor to activate the screen.

C. Signup

1. Record your name and lab phone number in the signup binder.
2. Place the red, "Experiment in Progress" sign face up on the console.

## III. Sample Loading

A. Press the "**spin**" button on the System Control Microprocessor (SCM) unit to turn off the spinner.

B. Press the "**lock**" button on the SCM to turn off the lock. Turn off the "**auto lock**" and "**auto shim**" lights on the SCM, if they are on.

C. Press the orange, "**2nd**" button followed by the "**lift**" button on the SCM to eject the sample.

D. Remove the sample and **carefully** take the reference NMR tube out of the spinner turbine. Put the reference NMR tube in the NMR tube rack on the counter.

E. Insert your NMR tube **carefully** into the spinner turbine. Use the Plexiglas sample positioner to position the tube in the spinner turbine.

F. Place the sample into the sample cylinder.

G. Press the "**lift off**" button to lower the sample into the probe. Listen for the sample tube to click into the probe.

H. Press the "**spin**" button to obtain a spin rate of 18 - 20 rps at equilibrium.

## IV. Software Setup

A. There are three blocks in the Aspect 3000 computer's RAM. Type **1** to enter block #1.

B. Type **RJ PROTON.001 [return]** to load default $^{1}H$ acquisition parameters ($CDCl_3$ solvent).

C. If the "X" light on the console is lit, type **II [return]** to initialize the interface.

D. O1, the observe offset, which positions the transmitter frequency to the center of the spectrum, has a default value of 3346 for $CDCl_3$ and benzene-$d_6$. The value of O1 is solvent dependent. Change O1 and the field value if you are using a solvent other than $CDCl_3$ or benzene-$d_6$.

| lock solvent | O1 value | field value |
|---|---|---|
| acetone-$d_6$ | 4380 | 3460 |
| $CDCl_3$ | 3346 | 3682 |
| benzene-$d_6$ | 3346 | 3682 |
| $D_2O$ | 3825 | 3557 |
| DMSO-$d_6$ | 4289 | 3457 |

E. Type **2** to enter block #2. Type **PJ PROTON.001 [return]** to load default $^{1}H$ processing and plotting parameters ($CDCl_3$ solvent). Type **1** to return to block #1.

## V. Magnetic Field Lock

A. If necessary, press the "**sweep ampl**" button on the SCM and set the sweep amplitude to 200 with the SCM's wheel.

B. If necessary, press the "**lock power**" button on the SCM and set the lock power to 150 - 450 with the SCM's wheel so that the deuterium resonance is seen clearly. The optimum lock power depends upon the lock solvent that is used. The value for $CDCl_3$ is 380.

C. The deuterium resonance should appear as one resonance per scan: 1 going left and 1 going right. Press the "**field**" button on the SCM and adjust the field with the SCM's wheel to center these resonances so that they are equally disposed about the vertical center line of the monitor.

D. If you do not see these resonances, press the "**lock gain**" button on the SCM and increase its value with the SCM's wheel. If you still do not see the signal, use the SCM to turn up the lock power. If still nothing is seen, make a note of the field setting and change the field until you find the signal.

E. If the line shape of the signals is not approximately symmetrical, press the "**lock phase**" button on the SCM and adjust the lock phase with the SCM's wheel. Don't be too fussy about making the signals symmetrical.

F. Press the "**lock**" button on the SCM. The resonances will no longer appear. Instead, a noisy line will appear, traced back and forth. When the machine locks on the deuterium signal, this line will rise on the screen (it may go off the top of the screen) and the lock light will come on.

G. If the signal is not visible, use the "**lock gain**" button to bring the signal down onto the screen. Position the signal so that it is approximately 1 cm from the top of the screen.

## VI. Magnetic Field Shims

A. If the lock signal is extremely noisy, type **RSH SHIM.396 [return]** to load default magnetic field shim values. Otherwise, continue with step VI.B.

B. Press the "**Z**" button on the SCM and note the Z shim value.

C. Use the SCM's wheel to alter the Z shim value so that the signal rises on the monitor. If, during this process, the signal goes off the top of the monitor, use "**lock gain**" to bring the signal back to about 1 cm from the top. Don't bring the signal too low on the monitor or you will lose the lock.

D. Repeat this process with the "**$Z^2$**" button on the SCM to alter the $Z^2$ shim value.

E. Repeat steps VI.B, VI.C and VI.D, above, to maximize the height of the signal.

F. Press the "**std by**" button on the SCM to leave the SCM in standby mode.

## VII. Receiver Gain

A. Type **RGA [return]**. The spectrometer will set the receiver gain automatically. When this process is complete the computer will display the "Auto RG Finished" message on the liquid crystal display.

B. Type **RG [return]** to confirm the value. Then press **[return]**.

## VIII. Acquisition

A. Type **ZG [return]** to begin accumulation of data. Data will collect in block #1.

B. For overnight acquisitions, press the "**LINE/RGB**" button on the monitor to deactivate the screen.

## IX. Data Manipulation

A. After a number of scans (the exact number will depend upon sample concentration), type **TR [return]** The computer will respond with "1," "2" or "3." Type **2 [return]** to transfer data to block #2. Accumulation will continue in block #1. Type **EF [return]**. This command performs an exponential multiplication of the FID, followed by a Fourier transform of the data. When the "FT In Progress" message disappears, type **EP [return]**. An expanded version of your (unphased) spectrum will appear in light blue. (Note: "EP" is a computer routine used to prepare your data for output. "EP" has various subroutines: "P, G, F, X, I," etc. If you press the [return] key too many times you will exit from the EP routine and you will see the spectrum in green. To get back to EP , type **EP [return]**.) Use the "**A**" and "**B**" knobs to move and expand/contract the spectrum. (Note: Knobs A, B, C and D change function depending upon which subroutine of EP is in use.) Use the "**- / +**" vertical display buttons to the left of the keyboard to decrease or increase the amplitude of the spectrum to a desirable level.

B. Find the largest peak in the spectrum and type **P** to enter the phasing subroutine. Use the "**C**" knob to phase the peak. Use the "**A**" knob to move to another part of the spectrum and use the "**D**" knob to phase that region. If necessary, use **[ctrl] C** and **[ctrl] D** to reverse the effect of knobs **C** and **D**. Use the "**A**" knob to return to the original large peak to make certain that it is still phased properly. Once you are satisfied with the phase, type **M** to memorize the phase and exit from the phasing subroutine. (Note: It is a good idea to use the "+" vertical display button to increase the spectrum's vertical amplitude during phasing. Reduce the vertical amplitude when phasing is done.)

C. If the signal-to-noise ratio is acceptable, proceed to step IX.D. If the signal-to-noise ratio is not acceptable, type **1**, to return to block #1. Then return to step IX.A and transfer more data to block #2.

D. Use the "**A**" knob to scan through the spectrum to find the TMS or other reference peak to set the chemical shift scale. Use the "**C**" knob (coarse) and the "**D**" knob (fine) to position the cursor exactly on the maximum of the reference peak. Type **G**. The computer will respond with the value in ppm where the cursor is located. If this value is correct, press **[return]**. If not, type the value **[return]**.

E. If you want to pick a smallest peak for peak picking, position the cursor at the vertical level of your choice, then type **M [return]**. The computer will memorize the vertical amplitude of this setting. The chemical shifts of all peaks taller than this threshhold value will be printed.

F. Set the frequency limits of the spectrum to be plotted (frequency or ppm limits) with the "F" command. (Note: Press the "**:**" key to switch between Hz and ppm.)

1. Type **F**.
2. The computer will respond with its default value of F1. Type, e.g., **10.5 [return]**.
3. The computer will respond with its default value of F2. Type, e.g., **-0.5 [return]**.
4. The computer will respond with the corresponding ppm/cm that was set by your F limits.

G. If you want to pick a peak to set the vertical amplitude of the spectrum, position the cursor on the peak of your choice and type **Y**. The computer will display the current value of the Y amplitude in cm. Type the desired value (for 11" x 17" paper, 18.5 cm is appropriate) **[return]**. Then the computer will display the current value of the length of the X-axis in cm. Type the desired value (for 11" x 17" paper, 35 cm is appropriate) **[return]**. (Note: If you don't do the above procedure, the tallest peak in the spectrum will be picked to determine the vertical amplitude of the plot.)

H. Spectrum Title

1. Press **[return]** to exit from the EP routine. The spectrum will reappear in green.
2. Type **TI [return]**, then your spectrum title **[return]**.

I. Automatic or Manual Integration

1. Automatic integration
    a. Type **AZF [return]**. The computer will display its default integral filename, INT1.001. Press **[return]** to accept the integral filename. Then the computer will perform the integration and store the integral file as INT1.001. Wait for the "AZF In Progress" message to disappear before proceeding to step X.B.

2. Manual integration

   a. From within EP, move the cursor to the left side of the peak of choice.

   b. Type **I** to enter the integration subroutine. The integration curve will appear. Adjust the amplitude of the curve with the "**- / +**" vertical display buttons on the keyboard.

   c. Type **M** to enter the phasing subroutine. Use the "**C**" and "**D**" knobs to phase the curve. Use **[ctrl] C** and **[ctrl] D** to reverse the effect of knobs **C** and **D**. (Watch out! These knobs are so sensitive that it is very easy to mess up the integration curve. If this situation occurs, it is a good idea to exit from and restart the integration subroutine.) Once you finish phasing, type **M** to exit from the phasing subroutine.

   d. Use the "**C**" and "**D**" knobs to move the cursor just to the left of the first peak you want to integrate. Type **Z**. This will set the area to zero and begin the integration.

   e. Move the cursor just to the right of the peak and type **Z**.

   f. Repeat steps d. and e. with other resonances in the spectrum, as appropriate.

   g. To define the absolute value of the integral, move the cursor to a peak of choice. Then adjust the cursor's position to be several data points to the left of the right end of the integration curve. Type **A**. The computer will print the current value of the area. Type the desired value **[return]**.

   h. Type **[ctrl] R** to show the entire spectrum and integration curve. Use the "**- / +**" keys on the keyboard to adjust the height of the integration curve.

   i. Type **E** to store the integration data. The computer will print the integral filename. Press **[return]** to confirm the filename and exit from the integration subroutine. If you want to change the integral filename, type the new integral filename **[return]**.

## X. Data Output

A. If you still are in the EP routine, press **[return]** to exit.

B. Type **PXB [return]** to plot both the spectrum and the integral. The computer will ask for the integral filename on the liquid crystal display under the monitor. Type **INT1.001 [return]**. To plot the spectrum without an integral, type **PX [return]**.

## XI. Standby

A. Type **1** to return to block #1.

B. Type **[ctrl] H** to halt data acquisition. The "pulse" light and the "adc" light should go out.

C. Press the "**spin**" button on the SCM to turn off the spinner.

D. Press the "**lock**" button on the SCM to turn off the lock. Turn off the "**auto lock**" and "**auto shim**" lights on the SCM, if they are on.

E. Press the orange, "**2nd**" button followed by the "**lift**" button on the SCM to eject the sample.

F. Remove the sample and **carefully** take the sample NMR tube out of the spinner turbine. Put the sample NMR tube in the NMR tube rack on the counter.

G. Put the reference NMR tube **carefully** in the spinner turbine. Use the Plexiglas sample positioner to position the tube in the spinner turbine. Then place the reference into the sample cylinder.

H. Press the "**lift off**" button to lower the reference into the probe. Listen for the reference tube to click into the probe.

I. Press the "**spin**" button to obtain a spin rate of 18 - 20 rps at equilibrium.

J. Lock the instrument, as described in Section V. Make certain that the lock light stays on.

K. Shim the magnet, as described in Section VI.

L. Press the "**std by**" button to leave the SCM in standby mode.

M. Type **NP [return]** to eject the plotted spectrum from the plotter.

N. Press the "**LINE/RGB**" button on the monitor to deactivate the screen.

O. Place the red, "Experiment in Progress" sign face down on the console.

P. Notify the next user that the instrument is available.

Q. Take your wallet, watch, sample and spectrum.