

## Operating Procedure for the INOVA 500 NMR

### I. Logging on spectrometer/Inserting sample

From the console, login with the appropriate user account and enter password.

Accounts:           organic (Chemistry Research Groups - walkup interface)  
                          leigh (Experimental, Biopak Interface)

\* If you are returned to the login screen, click on **Options, Session** and select **Common Desktop Environment** and login again.

At this point, a Desktop screen appears and on the bottom panel is an icon that looks like a tiny NMR spectrum, *single click* this to launch **vnmrj**.

In the bottom, center window, under the Start tab click the first menu item entitled **Study**, then click on **Eject**. Remove the standard and place the sample (use depth gauge to set proper depth) on the air stack. Return to the console and click the Insert button.

\* The PENTA probe, the primary probe for the 500 MHz, NMR, is particularly sensitive to changes in solvent. Therefore, it is recommended that you tune every time you get on, the instrument or change solvent.

### II. WALKUP User Setting up the experiment(s)

- a. choose the experiment(s) you wish from the protocol list on the left, e.g. Proton, Carbon, Dept, DQCOSY, etc. This creates a “new sample” line in the automation window (below the protocol window).
- b. Be sure that the **Probe** button (Bottom left screen) has "**penta7612**" selected.
- c. in the bottom, center window under the **Start** tab, click the first selection entitled **Study**. In this window, you must type in the sample name and select the solvent from the pulldown. Also in this window, make sure the boxes on the right for “Find z0” and “Gradient shim” are “checked”. Here is where you would also check “Plot all data” if you want plots to be initiated automatically after acquisition.
- d. If you wish to change any parameter for the experiment(s) prior to collecting data, you must do it now. To change any parameters, double click on the time field for the experiment in the protocol window (the field turns yellow with *italic* letters). To change acquisition parameters, click on the **Acquire** tab and then either the **Default** or **Acquisition** menu item and change the appropriate parameter. To change a processing parameter, click on the **Process** tab and then the Process menu item.

For example, some parameters you may wish to check and/or change:

Number of scans  
Block size (the smallest increment of acquisitions after which you can view spectrum as it collects)  
Line broadening (typically 0.5 Hz for proton, 2 Hz for carbon)  
Spectral width

- d. When the parameters are set correctly, click on Submit.

*While the experiment is running ...*

The “new sample” line changes to a sample name according to the date with ID# (001, 002, etc). Below that is a line the states the type of experiment (proton , carbon, etc), followed by a third line that begins with the length of time the experiment will take followed by the type of experiment. This third line turns yellow during the lock/shim procedure (automated), then turns

blue when the data is being collected, then turns back to yellow when the experiment is finished (and the time field turns into an icon of a lock). Also, at the bottom of the window, there is an acquisition status bar that turns blue when the experiment is in progress and turns green (with the word Idle) when the experiment is finished.

If you wish to abort an acquisition, click on the red Stop button next to the Process tab.

### III. Viewing/adjusting/plotting the spectrum

Most spectral adjustments are made using the column of icons to the left of the spectral window that will appear as you process the data.

- a. *view the entire spectrum*: double click on the line beneath the name of the file in the protocol window (the line begins with an icon of a lock). This will process the spectrum with the parameters listed in the Process tab and display it in the spectral window. For the time being, you can hide the integrals (for proton spectra) by clicking on the integration icon (top, green integral - click it twice to turn them off). To display the chemical shift scale under the spectrum, click on the scale icon (looks like a white ruler). You can adjust the vertical scale of the spectrum using the middle mouse "wheel" (click) or set to maximize the tallest peak by selecting the "Autoscale" button in the Process tab under the Default or Display menu item.
- b. *phasing the spectrum*: To autophase, click the Process tab, then the Process menu item, then click "Autophase Full". To phase the spectrum manually, click on the phasing icon that looks like a red clock. Then click and drag the left mouse button to phase the downfield region; click and drag the right mouse button to phase the upfield region. When finished phasing, click the cursor icon (very top icon with one or two vertical red lines).
- c. *expanding regions*: use the left and right mouse buttons in the spectral display window to place the red cursor lines on the downfield (left mouse button) and upfield (right mouse button), then click on the icon that looks like a magnifying glass with a "+" in it.
- d. *setting the reference peak*: having chosen the solvent in II.c., the reference should be automatically set. If an adjustment is needed, you can do this in the Process tab under the Display menu item.
- e. *displaying peak frequencies*: adjust the threshold for selecting peaks. Click on the icon with the yellow horizontal lines on it; click and drag the left mouse button to position the yellow bar lower than the smallest peak you wish to be "seen". Click on icon again to hide the bar. To display the frequencies on the screen, click on the "Peak frequencies" button in the Process tab under the Display menu item.

### IV. Integration

- a. Start by selecting "Clear Integral Resets" in the Process tab under the Cursors/Integration menu item.
- b. *Define the integration regions*: click on the integral resets icon (looks like an integral sign with ... on either side). Next, place the cursor arrow on the downfield side of the peak and click the left mouse button. Move to the upfield side of the peak and click the left mouse button again. Repeat for each peak of interest.
- c. *Correct the integral baselines*: Click on the integral phasing icon (under the integration and integration resets icons) and use the left/right mouse buttons to drag the phase and slope into adjustment (make the integral baselines parallel with the spectrum baseline).

### V. Plotting the spectrum

In the Process tab, under the Plot menu item you can select what you wish to plot. In general, click the following: "Plot spectrum", "Plot Spectrum Scale", "Normalized Integrals" (for proton spectra). If you wish the peak frequencies to be marked above each peak, select the box "On peaks" under "Plot Peak Frequencies". To generate the plot, click on "Plot Page".

#### VI. Shutdown

Remove sample by clicking on Eject in the Start tab under the Study menu item. Replace the standard (sucrose sample in D<sub>2</sub>O) into the sample holder and replace the sample on the stack. Click on Insert. Exit Vnmrj under the Utilities pulldown. When the window closes, click on the background with the right mouse button and select "Log out" and then click on OK to confirm.