

Appendix A: Instructions for Using the Varian EM360A Nuclear Magnetic Resonance (NMR) Spectrometer

Written by
Danielle M. Solano
Department of Chemistry & Biochemistry
California State University, Bakersfield

Recommended Resources

- Website ~ The Basics of NMR, by Josef P. Hornak
<http://www.cis.rit.edu/htbooks/nmr/>
- Website ~ Nuclear Magnetic Resonance Spectroscopy
<http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtjml/Spectrpy/nmr/nmr1.htm>
- Video ~ NMR Made Easy! Part 6A - NMR to Molecule Structure - Organic Chemistry
<http://www.youtube.com/watch?v=5Uy7GiRaV2A>

Safety Notes

- Do not take any metal objects into the NMR room.
- Do not approach the NMR if you have a pacemaker or any medical implants.
- The magnetic field may damage watches, calculators, cell phones, or credit cards.

Directions

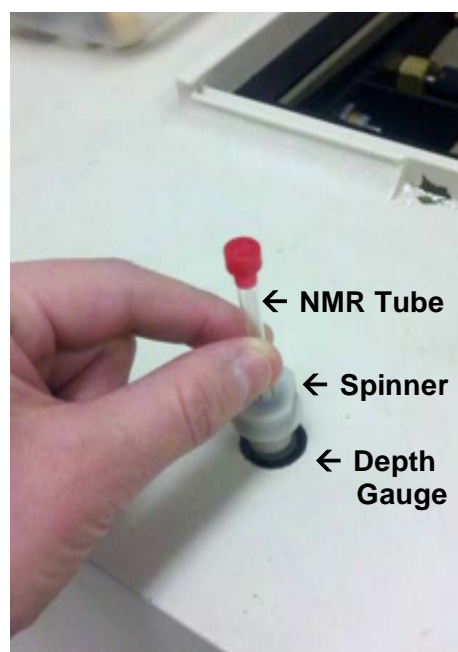
1. Prepare your sample.
 - a. Obtain a clean NMR tube. (NMR tubes can be checked out from the stockroom.)
 - b. Dissolve 50-100 mg of your sample in 0.7-0.8 mL of deuterated chloroform (CDCl_3). If your sample does not dissolve, you may need to try another NMR solvent such as deuterated dimethylsulfoxide ($\text{DMSO-}d_6$).
 - c. Add your sample to the NMR tube.
 - d. Check to see if the NMR solvent you are using contains TMS. If it does not, then add 1 drop of tetramethylsilane (TMS) to the tube. (Note: TMS is extremely volatile. Return it to the fridge after you are done using it.) Your tube should be approximately 1/3 full.
 - e. Cap your NMR tube and invert the tube slowly several times to mix the contents.
 - f. Use a Kimwipe to clean the outside of your NMR tube.
2. Remove the sample currently in the machine and load your sample into the machine.
 - a. Open the lid to the spectrometer.

- b. In the lower left corner is the sample eject button (see Figure 1). Press and hold the button and the sample in the instrument will be ejected on a cushion of air. Remove the sample and then release the button.
- c. Carefully remove the NMR tube from the spinner using slow, constant, even pressure.
- d. Insert your sample carefully into the spinner (**Do not insert a sample into the instrument without the spinner!**).
- e. Adjust the position of the tube in the spinner with the depth gauge (see Figure 2).
- f. Press and hold the sample eject button. Place your tube (with the spinner) on the cushion of air (see Figure 1). Slowly release the sample eject button to insert your sample.
- g. Look inside the instrument to make sure your sample is spinning. If it is not, eject the sample and reinsert it.

Figure 1: What the NMR spectrometer looks like from the top



Figure 2: Example of using the depth gauge



3. Obtain the proton NMR spectrum.
 - a. Open the WinPNMR Program.
 - b. Check to make sure you are in H1 mode. (You should see a H1> in the lower left-hand corner of the screen.)
 - c. Set the number of scans to 4. (If the number of scans is already set to 4, skip to step 3d.) Type "ns" and hit enter. Type "4" and hit enter.
 - d. Optimize the magnet for your sample and solvent. (If someone ran a sample just before you and used the same sample and NMR solvent, skip to step 3e.) Type "shim" and hit

- enter. You will be asked to enter a relaxation delay. Type "5" and hit enter. Once the shim is completed (the gray dialog box in the upper right corner of the screen will disappear), proceed to the next step.
- e. Collect the raw NMR data. Type "zg" and hit enter. You will be asked to enter a filename. Enter your name or something that describes your sample and hit enter.
 - f. The gray dialog box in the upper right corner of the screen will disappear once your sample collection is completed. If the raw data is yellow, proceed to step 4.
 - g. If the raw data is red, the receiver gain is set too high. To lower it, type "rg" and hit enter. Then enter a lower number than the rg value currently listed on the screen, and hit enter. Return to step 3e to rerun your sample.
4. View your NMR spectrum.
- a. Open the WinNuts Program (you can quickly switch to WinNuts by holding down the ALT key and then pressing the TAB key).
 - b. Type "a2" to load your spectrum. If your spectrum looks good, skip to step 5.
 - c. If your spectrum looks noisy, either your sample is not concentrated enough or you need to increase the number of scans. To increase the number of scans, return to the WinPNMR Program, then type "ns" and hit enter. Type "32" and hit enter. Return to step 3e to rerun your sample. (If increasing the number of scans does not solve the problem, you will need to make your sample more concentrated.)
5. Set the TMS peak to a chemical shift (δ) of 0 ppm.
- a. In the WinNuts Program, click and hold the left mouse button to show the cursor. Use the cursor and find the chemical shift of the TMS peak (the far right hand peak). If it is already at zero, skip to step 6.
 - b. Return to the WinPNMR Program (ALT+TAB).
 - c. Type "fo" and hit enter. You will be asked to enter the current position of the reference. Enter the chemical shift of the TMS peak that you just determined. Then you will be asked to enter the desired position of the reference. Type "0" and hit enter.
 - d. Return to step 3d to rerun your sample.
6. **Peak Picking:** In the WinNuts Program, label the peaks on your NMR Spectrum by clicking the PP button on the toolbar.
- a. If too few peaks are labeled, go to the "Tools" menu and select "Define Peaks". Now move the cursor to the top of each peak you want labeled and then left click. When you are finished, go to the "Edit" menu and make sure that "Peak label on" is checked. Then go to the "File" menu and select "Exit DP mode".
 - b. If too many peaks are labeled, go to the "Tools" menu and select "Define Peaks". Then go to the "Edit" menu and select "Clear selected peaks". Now move the cursor to the top of each peak you want labeled and then left click. When you are finished, go to the "Edit" menu and make sure that "Peak Labels On" has a check mark next to it. Hit enter to exit DP mode.

7. **Phasing:** Ensure that the baseline of your spectrum is flat. (If the baseline of your spectrum is already flat, skip to step 8.)
 - a. Click the "ZO" button on the toolbar to enter zoom mode.
 - b. Select the far left peak by clicking the left mouse button and dragging to highlight the peak in red.
 - c. Type "1".
 - d. Select the far right peak using the same technique and type "2".
 - e. Hit enter to exit zoom mode.
 - f. Type "pe".
 - g. Click and hold the left mouse button and move the mouse up and down to adjust the phase and make the baseline flat.
 - h. Hit enter to exit phase mode.
8. **Integration:** Determine the integration of each peak in your spectrum.
 - a. Under the "Process" menu, click "Fit Baseline". A screen will appear with many vertical red bars.
 - b. Use the left mouse button to add or delete red bars by clicking on the bar or empty space.
 - c. Fill in all the space that does not include your peaks.
 - d. When you are done, go to the "Edit" menu and select "Calculate Fit by Least Square to Polynomial".
 - e. Click the disk button on the toolbar to save. The bars will disappear.
 - f. Go the "Process" menu and select "Integrate Display".
 - g. Then go to the "Edit" menu and select "Clear all broken integrals".
 - h. Starting on the left side of the screen, left click twice before each peak.
 - i. Then move the cursor to the right just beyond the peak and left click again. Do this for each set of peaks you want to integrate (you do not need to integrate the TMS peak).
 - j. If you don't see any numbers, or aren't happy with their values, you can adjust them by clicking once on a peak and then typing "V". Enter the number of protons you think the peak contains in the box provided. Click the "OK" button.
 - k. Hit enter to exit integration mode. (If you don't see the integrals after you exit integration mode, then go to the "View" menu and select "Show Integrals".)
9. **Zoom:** If desired, you can zoom in on peaks of interest.
 - a. Click the "ZO" button on the toolbar. You are now in zoom mode.
 - b. Click the left mouse button and drag across the area you want to expand to highlight it in red.
 - c. Right click somewhere in the red region of the spectrum. If you want to return to the full view, go to the "Display" menu and select "All Reals".

d. When you are ready to move on, hit enter to exit zoom mode.

10. **Printing:** If desired, you can print your spectrum.

a. Go to the "File" menu and select "Printer Dialog".

b. Click the "Print" button. You should also save your file in case you would like to return to it in the future.