University of Wyoming NMR Facility. Alexander K. Goroncy

Basic Experiment Setup and Basic NMR Spectrometer Operation Setup for Bruker 300 MHz, 400 MHz, and 600 MHz NMR spectrometers:

Safety in the NMR laboratory

There are federal training requirements about the dangers of the NMR magnets and the danger of helium asphyxiation that need to be taught to anybody entering the NMR room PS13.

Unauthorized persons need to be supervised at all times by a trained individual who need to instruct them about the dangers. Supervisors assume responsibility for encouraging untrained individuals to circumvent these mandatory requirements.

Please be aware that the superconducting magnets produce very strong magnetic fields that can strongly attract metals and can erase credit cards or other magnetic media. Superconducting magnets are always on. Do not approach the magnets with anything that can influence the magnetic field such as iron keys, tools, wallets, trolleys. People with metallic implants such as heart pacemakers should not approach the magnet without extensive additional safety training. The magnetic field may influence the proper operation of the pacemaker. The magnets in the facility are shielded in such a way that the magnetic field drops off to less than 1 Gauss (0.1 mT) in 2 m. That is considered pretty safe as the earth magnetic field ranges from 0.25 to 0.65 Gauss at the surface. If the magnetic field of the superconducting magnet is disturbed too much, the magnet may suddenly leave the superconducting state and becomes a normal conductor. If such a quench happens, helium evaporates and quickly fills the top of the room, the replacement of oxygen may lead to suffocation. Although the NMR laboratory has been designed in such a way that even the simultaneous quench of all superconducting magnets in the room will leave a layer of breathable air in the lower part of the room, it is strongly recommended to leave the NMR room as soon as possible and to notify the manager. While the temperatures of liquid helium is 4 K (-269 °C), the quench gas temperatures are around 70 - 90 K (600 MHz NMR). Helium gas spreads quickly over the whole room area under the ceiling, and then diffuses away. Due to the low heat capacity of the helium gas, the overall room temperature drop will be barely noticeable. To freeze a pipe, a distance less than 2 - 3 m is needed, less if any media flows in the pipes, or the helium gas does not blow directly on them. Only items directly installed above the magnet or in the path of the quench gas are susceptible to freezing.

Never put any object into the magnet except the NMR tube and spinner. No magnetic stirrer.

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Solution Sample Preparation

Please prepare your samples outside the NMR facilities if possible. If you must use the NMR facility, please prepare the samples inside the hood and not on top of the NMR console.

Generally, for 1H-NMR 0.1 mg of sample is sufficent although < 1 mg is usually enough (0.001 mmol / 5 mM) ; for 13C-NMR, 10 mg is sufficient although 0.015 mg is often enough (30 nmol / 0.05 mM). To perform solution-state NMR, the samples are prepared in an appropriate NMR tube and about 0.5-0.6 ml of dissolved sample solution is usually enough. The NMR tubes are commonly 5 mm tubes or 1.7 mm tubes and are available from the Chemistry stockroom. Normally you want to use deuterated solvents such as $CDCl_3$ or D_2O to simplify the spectra and to obtain a better signal to noise ratio as protonated solvents show up prominently on proton spectra and may mask the presence of your compound of interest. Also, deuterated solvents can be used to lock the sample (see below) and be useful as an internal reference (see a solvent list here). If the primary objective is not to observe the protons, but 31P for example, then 5-10% of deuterated solvent is enough (to obtain a lock). Use about 650 ul for common 5 mm NMR tubes (about 51 mm sample height. If it is impractical to use that much, special Shigemi tubes are available that can be filled to 200 ul. Or use 1.7 mm tubes for even less volume (35 ul). It is usually better to not under-fill NMR tubes as NMR setup (shimming) takes much longer and the signal to noise ratio is usually much worse. But over-filling NMR tubes is not a good idea either as it won't improve the signal to noise ratio and consumes laboratory resources needlessly. The sample won't be consumed by the NMR experiment and can be used for other purposes later. The solution should be clear of floating material and well-mixed. If the NMR data shows lines that are broad and/or that have unusual shapes, the main reasons are 1. Insufficient quantity of sample; 2. Significant concentration gradient across sample (inadequate mixing); 3. Floating material (use less concentration or use a better solvent); 4. Presence of paramagnetic compounds (e.g., iron, try to minimize those if possible); 5. Inadequate shimming (don't skip step 8 below).

To clean NMR tubes after the NMR experiments are over, rinse them with water or an organic solvent. Do not use a brush or another abrasive materials. To speed up the drying process, it may be a good idea to use a volatile solvent such as ethanol in the final washing cycle and the NMR tube can be air-dried. It is not recommended to bake the tubes in an oven at more than 45 Degree Celsius as the tubes may deform. A vacuum oven is better. If it is not practical to wait until the NMR tubes are completely dry, use a deuterated solvent such as D_2O in the final washing cycle. Sample degradation or precipitation may cause material to adhere to the inner walls of the tube. In that case, strong acids such as nitric acid (soaking for 1-3 days) may be employed. Chromic acid is not recommended as the residual chromium often adversely affect NMR experiments. If chromic acid is used nevertheless, nitric acid may remove the residual chromium. For samples that are not dissolved by acid (e.g., some polymers), a solvent that swells the sample may be used and a pipe cleaner might to sufficient to remove the softened material. Agitation in an ultrasonic bath with an appropriate solvent might be useful.

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Measuring your sample with the NMR spectrometers

1-Dimensional NMR

Log-on into the NMR computer. If you do not have an account, please contact Dr. Alexander Goroncy to get one. Type topspin<enter> into a terminal program or click the topspin-icon. Please do not log-on to the NMR computer remotely to run topspin as this prevents the user to operate the NMR instrument on-site due to licensing issues!

Before you come anywhere near the magnet, please be sure that you don't carry anything magnetic such as keys, credit cards, screw drivers, pace makers or metallic implants! The magnets may look innocent but they are very powerful indeed and always on!

1. Remove the black cap from the top of the NMR spectrometer.

2. ej<enter> to turn on the eject air flow. Wait until you hear the air flow before proceeding to the next step.

3. Place your NMR tube with spinner in the air flow. Use an appropriate spinner (blue one for most experiments, ceramic one for specialized experiments such as variable temperature experiments). When inserting the NMR tube into the spinner, grap the tube close to the spinner. This will avoid applying a torque that can easily break a tube and drive into a finger. Use the gauge to find the correct sample height in the spinner.Place sample tube in the spinner and the spinner in the same depth gauge. Push or pull the sample tube so that the depth of the sample above and below the center line of the sample depth gauge is equal. However, never exceed the lower limit (position of the adjustable white platform which should be at the line marked 3 mm - 10 mm; do not adjust the height of this white platform). Wipe the tube with tissue such as Kimwipe and after that grap the NMR/spinner combination at the top.

4. ij<enter> to stop the air flow and thereby lower the NMR tube inside the NMR magnet. Wait until it arrives there; wait until topspin says: finished.

5. Open one of your previous experiments in topspin or you can skip this step. It is usually recommended that the first experiment that you do for each new sample is a simple proton experiment such as zg30. There is a long list of possible pulse programs, either in the /opt/topspin3.x./exp/stan/nmr/par folder or in its subfolders. x can be 0, 2, or 5. A list of recommended pulse programs is below.

6. edc<enter> to create a new file. Choose the appropriate pulseprogram (zg30 is for a simple proton experiment) if you want to change it.

7. You may always change the pulse program parameters by typing "rpar" and choosing the desired program. In that case, also type: getprosol<enter>. This loads the default parameters for the particular probe and instrument. The parameters and the pulse program can be inspected under the "AcquPars" tab.

8. rsh<enter> and read a previous shim-file (if you don't know which one, use a recent one with the ending AG).

9. lockdisp<enter> to see the lock display if you don't see it already.

10. lock<enter> and choose solvent from pop-up window. Watch the lock display. When the sample is unlocked, the red/green line will appear at the bottom of the lockdisplay window. When the spectrometer is locking, an FID will appear when the locking finds the correct field setting, and it it will gradually increase to find the correct lock gain settings. The red/green line should be stable in the upper half of the lockdisplay window. You can adjust the position of the line by increasing or decrease the lock gain on the BSMS window (type bsmsdisp<enter>).

11. atma<enter> to tune probe. Also can do it manually with atmm<enter> and use the controls until you get the dip as low as possible (good matching) and directly on the vertical line (good tuning). Manual tuning is recommended for anything other than ^{1}H and ^{13}C. Manually tuning usually gives the best results in terms of sensitivity and lineshape but for routine samples the difference is slight. For non-automated tuning probes (like for our 300 MHz NMR spectrometer), the command to use is: wobb<enter>. The capacitors at the probe under the magnet need to be adjusted manually. The NMR probehead must be tuned and matched because it is a resonance circuit. If its resonance frequency and impedance are the same as the transmitter frequency and impedance, respectively, the full transmitter power is transferred to the probehead. However, if either or both are different, part of the transmitter power is reflected by the probehead. This results in a longer 90 Degree pulse. A multi-nuclear probehead has a resonance circuit for each nucleus and each nucleus must be tuned and matched separately. This needs to be done for each sample. Unless the physical conditions of the sample are changing (e.g., chemical reaction, decomposition, temperature), this has to be done only once for each sample.

12. It is generally not recommended to spin the samples. If you do wish to spin it, type "ro on"<enter> or to turn spinning off, "ro off: <enter>. Please be aware that spinning side bands may appear. For multi-dimensional NMR experiment, additional complications appear with spinning samples.

13. topshim<enter> will start the autoshimming routine. For more options: topshim gui<enter>. When Topshim is finished, there will be a message. That may take 2 minutes. For manual shimming: bsmsdisp<enter> and press: shim and adjust the shims manually by observing the red/green line on the lockdisplay. This line generally needs to be as high as possible. If the red/green line reaches the top, it might be necessary to bring it down by reducing the LOCK GAIN. The purpose of shimming is to maximize the magnetic field homogeneity, which depends on probehead and sample geometry. In general, it is necessary to shim the magnetic field after each sample change.

14. getprosol<enter> if you have loaded a default programs above and haven't copied the parameters from an old data set. Prosol is a feature that allows the software to communicate with the probe to determine which probe is installed and to use the standard power and pulse durations for that specific probe.

15. Check parameters such as ns<enter>, td<enter>, o1p<enter>, sw<enter> for number of scans, number of acquired points, center of the spectrum in ppm, width of the spectrum in ppm. Press the "AcquPars" tab for more settings and press the "Show pulse program parameters" for still more options (second bottom on top left). Both ns, ds should be a multiple of the longest phase cycle in the pulse program to avoid phasing errors after processing. If you are unsure about what that means, either look in the pulseprogram tab and read the description, or at least use multiples of 2, or 4, or 8, or 16, or 32, or 64. For simple 1H-NMR, NS=8 is usually sufficient, and for 13C-NMR, NS=128, for small concentrated chemicals.

16. pulsecal<enter> to calibrate the 90 Degree pulse length. This can be considered optional for a simple proton spectrum, but is important if you wish to do anything more complicated than that. When the pulsecal-routine is finished, it displays the result of the 90 Degree pulse length calibration. If the number is much larger than about 20 ms, then either some of the above steps have been omitted, or, the sample is not of good quality. Often, a substantial amount of undissolved material is the problem.

17. rga<enter> performs auto receiver gain. An abnormal result (e.g., too low, gain=0 will result in no signal) may be due to incorrect parameters settings. Type rg<enter> and type in a lower number in the field such as 2, 4, 8, 16, 32, 64, 128, 256, 512. This is especially important for multi-dimensional experiments to avoid overloading the detector.

18. zg<enter> starts the experiments.

19. Wait until the experiment is finished, or, if you cannot wait that long, type tr<enter> to transfer preliminary data during acquisition. You may type: tr xxx <enter> (such as tr 1024). In that case, the data will be transferred after scan number xxx (such as 1024).

20. efp<enter> and apk<enter> to process the data. More options can be found in "ProcPars" and "Analyse" in the TopSpin menu bar and should be used if for some reason the automated phase and baseline correction is not working properly. In that case go to "Analyse" and click "Phase", click on the "0" for first order correction and keep it pressed while moving the mouse up and down. Repeat for "1", the first order correction. Don't forget to click "Save".Zooming in can be done with the mouse and the left mouse bottom. To perform an exact zoom via a dialog, type: .zx <enter> and enter the coordinates of the desired region in the dialog box.

For peak integration, the "Integrate" button can be found under "Process". Set the cursor line, starting at the left of the spectrum, to the left of the first peak to be integrated, click the left mouse button and drag the cursor line to the right of the peak, then release the mouse button. Repeat this process to integrate all peaks of interest. It is possible to normalize the integration area. The "Save" button saves the analysis. It is possible to export the spectrum as a PDF or image file by clicking the hard drive button (top left) and choosing

The FID can be manipulated in various ways for sensitivity enhancement or resolution enhancement. This also runs under apodization and window function. Some suggestions how to get started with this are described below. Please type the commands in the command line in topspin.

For *sensitivity enhancement*: Apply line broadening, enhances first part of FID

lb 2 (positive line broadening, the more positive, the more sensitivity enhancement)

em (exponential weighted FID)

ft (Fourier transform)

apk (automatic phase correction)

For *resolution enhancement*: Use Gaussian or sine bell function, changes the shape of the lines of the spectrum, make them narrower, enhances later part of the FID (if used with negative line broadening)

lb -1 (negative line broadening, the more negative, the more resolution enhancement)

gm 0.4 (0<gb<1, the larger gb, the more resolution enhancement)

gm (Gaussian weighted FID)

ft (Fourier transform)

apk (automatic phase correction)

"Export". It is also possible to print the spectrum by clicking the hard drive button and "Print".

Most data, including spectrum, FID, peaks, etc., but not parameters can be exported to other applications by selecting the appropriate tab and selecting Edit/Copy from the Topspin menu, the Edit/Paste into the other application.

To export the data (numbers) into another applications, there are several methods. One method is to type "convbin2asc" and the result will be written to the same directory as the original spectrum, e.g. /opt/topspin3.0/data/<user_name>/<sample_name>/<experiment_number>/pdata/ <processing_number>/ascii-spec.txt (an example location might be: /home/topspin3.0/data/student/sample/23/pdata/1/ascii-spec.txt).

Another method to export the data (numbers) is to process the data, then right-click -> Save Display Region to -> A text file for use with other programs -> Please specify destination and type the desired location of your choice. A short way to accomplish this is to simply enter the command "totxt" in the Topspin command line. This will take you directly to the "Please specificy destination" window. This file contains a header with information and only the y values (intensities) are shown and the x values must be generated separately using the information in the header. This is in contrast with the file that is generated using the "convbin2asc" command that has both x- and y- values generated.

21. halt<enter> will stop the running experiment. This will only be necessary if you wish to prematurely end the experiment. Data is not saved automatically. In case the experiment is run in a repeated loop configuration, the data is saved until the end of the last loop.

22. If you wish to do another experiment with your sample, go back to step 5. You can skip tuning and shimming unless you change the conditions of the sample (e.g., different temperature).

23. If you are finished with your sample, type ej<enter>, take out your sample, type ij<enter>, replace the black cap on top of the spectrometer.

24. Exit topspin and log-out of the computer.

Some pulse programs for routine NMR experiments:

1D proton: zg30, PROTON

1D carbon: zgpg30, C13CPD

1D DEPT-135: dept135, C13DEPT135

1D DEPT-90: dept90, C13DEPT90

You may need to modify the parameters d1 (repetition rate), ns (number of scans), o1p (offset), sw (sweepwidth).

In the Topspin 3 flow interface that is installed in all systems now, there are icons that simply the above steps. In the ACQUIRE tab, there are icons for SAMPLE (insert / eject sample), LOCK (solvent lock), TUNE (tune nuclei), SPIN (turn spin on/off), SHIM (shimming with topshim), PROSOL (getprosol), Gain (automated receiver gain rga), GO (for starting the experiment, zg). For processing, there is a PROCESS icon. If you elect the go this route, please allow sufficient time to finish a job (such as shimming) before going to the next step. Data acquired on the 400 MHz and 600 MHz NMR spectrometers are accessible from the avserver.uwyo.edu computer (in the center of the NMR facility). Please use it to process the data.

2-Dimensional NMR:

All 2D experiments are a series of 1D experiments collected with different timing. They can be divided into two types: homonuclear and heteronuclear. Each type can provide either through-bond (COSY-type) or through space (NOESY-type) decoupling information. They are processed with a Fourier transform in both dimensions.

Experimental setup:

1 Acquire a proton spectrum first and make sure you have recorded the 90 Degree pulse length (step 16 above).

2. Reference the proton spectra and write down the value for the sweep width and center of spectrum. This information is needed for the 2D experiment.

3. If a hetero-nuclear 2D experiment, such as 1H-13C-HSQC or 1H-13C-HMBC is desired, the sweep width for the 13C and the center of the sweep width needs to be set. A 1D 13C-spectrum may be useful to acquire this information.

4. Create a new data set (see step 6 above), and in the "AcquPars" tab load the 2D experiment of your choice from the drop down menu, or copy from a previously acquired dataset.

5. getprosol<enter> if you have loaded a default programs above and haven't copied the parameters from an old data set.

6. Change the parameters for sweep width and offset (o1p and o2p) in both dimensions with appropriate numbers obtained from the earlier steps. It is always advantageous to reduce the spectra width in both dimensional to the minimum practical value. For the time domain F2, most likely 1H nucleus, you may set 1024, and for the F1 dimension, perhaps 13C, set 128 or 256 if your sample contains many different atoms. Set the 90 Degree 1H pulse width as well. Both ns, ds should be a multiple of the number of phases in the phase program to avoid phasing errors after processing. If you are unsure about what it means, either look in the pulseprogram tab and read the description, or use multiples of 8 or 16.

7. atma<enter> to tune probe. This is only necessary if you haven't tuned the probe with this sample using all the required nuclei before. For example, if you haven't tuned the carbon channel, yet, it is necessary to tune it. For non-automated tuning probes (like for our 300 MHz NMR spectrometer), the command to use is: wobb<enter>. The capacitors at the probe under the magnet need to be adjusted manually.

8. Unless you know what you are doing, don't spin the sample (type "ro off" <enter> if unsure) as otherwise the results may be more complicated to interpret.

9. rga<enter> performs auto receiver gain. An abnormal result (e.g., too low, gain=0 will result in no signal) may be due to incorrect parameters settings. Type rg<enter> and type in a lower number in the field such as 2, 4, 8, 16, 32, 64, 128, 256, 512. This is especially important for multi-dimensional experiments to avoid overloading the detector. Sometimes the automatic receiver gain optimization does not work very well for 2D experiments as it uses only the first increment to test the receiver gain. Often the signals for subsequent increments are larger and can saturate the receiver. Thus, it is best to use adjust the receiver gain to half or a quarter of the receiver gain that was "optimised" by rga.

10. zg<enter> starts the experiments.

11. Wait until the experiment is finished, or if you cannot wait that long you can get a preview by processing the data and updating the results as new data becomes available.

12. xfb<enter> to process the data. For more options change the processing parameters in "ProcPars".

13. halt<enter> will stop the running experiment. This will only be necessary if you wish to prematurely end the experiment. Data is saved automatically.

Some useful pulse programs for routine 2-Dimensional NMR experiments:

1H-1H-COSY: cosygpqf

1H-1H-NOESY: noesygpppph

1H-13C-HMQC: hmqcgpqf

1H-13C-HSQC: hsqcetgp, hsqcetgpsp, hsqcgpph

1H-13C-HMBC: hmbcgplpndq