

Operation of Bruker 400 MHz FT-NMR Spectrometer

Background Information

An FT-NMR spectrum is obtained by briefly exciting a sample with radio frequency radiation (*pulse*), and then recording energy emitted by the sample over time (*free induction decay* or *FID*). The shape of the FID curve contains information about all of the nuclei that absorbed energy from the initial pulse. This information is extracted by means of a Fast Fourier Transform (FFT or FT). This mathematical procedure converts the signal vs. time data that was collected after the pulse into signal vs. frequency data, i.e., an NMR spectrum.

A few quirks worth noting about the FT-NMR experiment:

- **Multiple pulses improve S/N.** Since noise (N) is random energy while signal (S) is not, we typically average FIDs obtained from multiple pulses. The S/N improves as the square root of the number of pulses. In other words, a 16 pulse spectrum will have S/N that is four times better than that of a 1 pulse spectrum.
- **Field homogeneity improves peak shape and resolution.** An ideal spectrum contains very narrow (sharp) peaks that are easily resolved from other nearby peaks. The peaks also have a symmetrical shape. These characteristics are achieved by applying the same applied magnetic field to all parts of the sample. Several tricks are used to create this uniform or homogeneous field:
 - o We spin the sample rapidly about the tube axis.
 - o We use small radio frequency coils to produce small magnetic fields that compensate for imperfections in the field produced by the superconducting magnet. These coils are called *shim* coils. Most of the shim coils require only infrequent adjustment. However, we adjust the power flowing through two *shim* coils, Z^1 and Z^2 , before collecting each NMR spectrum. This adjustment is called *shimming the field*.
 - o We use an electronic feedback loop to detect, and correct for, any variations in the overall strength of the field. This feedback loop is called a *lock circuit*. The lock circuit monitors the NMR signal produced by deuterium nuclei in the NMR solvent, and it adjusts the field if the *lock signal* (i.e., the deuterium signal) starts to drift. Obviously, the locking procedure makes it necessary to use a deuterated solvent (CDCl_3 , D_2O , CD_3SOCD_3 , etc.) for your NMR sample.
- **A large number of parameters control the NMR experiment. These parameters are solvent-, and to a lesser degree, sample-dependent.** Each part of the NMR experiment is defined by several parameters. The “pulse” is characterized by its signal power, frequency, duration, repetition rate, and so on. The “FID” is, likewise, characterized by amplifier settings, acquisition time period, time between signal measurements, number of data points in computer data file, and so on. You are not expected to know all of (or any of) these parameters. Standard sets of parameters have been created for each NMR solvent that you are likely to use. Your job is to make sure that you load the appropriate set of parameters into the computer before attempting to collect an NMR spectrum.

- **The NMR magnet is extremely cold and very powerful.** The 400 MHz magnet is an electromagnet based on a low-temperature superconducting material. This material is cooled with liquid helium (4 K), which is surrounded by a vacuum insulating layer, a liquid nitrogen layer (77 K), and another vacuum insulating layer. Damage to any part of the magnet can compromise its ability to maintain a low temperature, at which point it would no longer remain superconducting. The main result would be a quick rise in temperature and quick conversion of liquid coolant (helium + nitrogen) into a rapidly expanding, but still fairly cold gas. This process, which is called a *magnet quench*, can damage the magnet material and needs to be avoided at all costs. Therefore, you must take pains not to damage the outer shell of the magnet.

Probably the only way you might harm the magnet's shell is by bringing a metal object (keys, screwdriver, coins, etc.) into its vicinity. The magnet's powerful field can draw a metal object towards it, and the impact might compromise the magnet.

The magnet's powerful field can have other unexpected and undesirable effects too. **Please read and obey the warning signs on the door of the NMR lab and on the magnet itself.** As a rule, you should remove keys, cell phones, wallets (credit cards have magnetic strips!) from your pocket before approaching the magnet. Put these items on a table at least 6-8 feet from the magnet while you perform your NMR experiments. Remember to collect your belongings before leaving the NMR lab.

Overview

Standard NMR experiments consist of the following steps:

- Inserting your sample in the magnet (**lift on/off, spin on**)
- Setting the lock signal (**lock solvent**)
- Shimming the field (**Z¹ Z² standby**)
- Acquiring an NMR spectrum
 - o load parameters (**test.1H.solvent**)
 - o set receiver gain (**RGA**)
 - o acquire and check data (**ZG, NS, EF, APK**)
 - o save, print and/or email data (**File: Save or Send To**)
- Removing your sample (**spin off, lift on/off**)

Basic NMR Operation for Proton Acquisition

Insert Sample, Lock, and Shim

- 1 Insert NMR tube into spin collar
 - You can grasp the tube with your fingers, but only touch collar with a Kimwipe
- 2 Adjust depth with the depth gauge
- 3 Press **LIFT ON**, listen for “air,” place sample on top of probe, and press **LIFT OFF**
- 4 Press **SPIN ON**
 - The signal in the **Lock Display** window is your deuterium lock signal
 - If your solvent is different from the previous sample, a signal may not be visible
- 5 Type '**lock solvent**' (*solvent* should be **cdcl3**, **d2o**, or **dmsd** to match your NMR solvent)
 - The signal in the **Lock Display** windows changes to a (noisy) horizontal line. The vertical position of this line represents the strength (quality) of your lock signal, and by inference, the homogeneity of the magnetic field
- 6 Shim the lock signal by pressing **Z¹** and adjusting the wheel for maximum signal
- 7 Shim the lock signal by pressing **Z²** and adjusting the wheel for maximum signal
 - **DO NOT ADJUST ANY OTHER SHIMS. CHECK TO MAKE SURE YOU ARE ADJUSTING Z¹ & Z² ONLY**
- 8 Press **STANDBY**

Set spectrum parameters

- 9 Find your personal data folder in the browser window
- 10 Click-drag your **test.1H.solvent** folder into the data window
 - *solvent* should be **cdcl3**, **d2o**, or **dmsd** to match your NMR solvent
 - If you do not have a suitable *solvent* parameter folder, use another **test.1H.wrong solvent** folder instead, and perform the following steps
 - Type '**edc**'
 - Adjust the solvent in the parameter window
 - Save the new parameters as **test.1H.correct solvent**
- 11 Type '**gpro**' to identify the probe and load parameters
- 12 Type '**rga**' to set receiver gain. Wait until the 'RGA finished' statement appears in the data window

Acquire spectrum

- 13 Type '**ns**'. If necessary, type '**1**' and click on **OK**
- 14 Type '**zg**'. Wait until the 'acquisition finished' statement appears in the data window (an FID will also appear in the spectrum window)
 - The **test.1H.solvent** parameter files are set up to perform one pulse (NS = 1). This typically takes 7-8 seconds
- 15 Type '**ef**' to convert the FID into a standard NMR spectrum
- 16 Type '**apk**' to automatically correct the spectrum's phase.
 - At this point, it is a good idea to 1) check peak shapes (e.g. is TMS a single symmetric peak, or is it split, or lopsided? Imperfections can be corrected by

additional shimming.) and 2) check signal-to-noise (S/N). Remember that S/N improves as the square *root* of the number of scans (NS).

- 17 Type '**ns**', type '**8**' (or some multiple of 4), and click on **OK**. This resets the number of scans
- 18 Repeat '**zg**'
- 19 Repeat '**ef**'
- 20 Repeat '**apk**' (note: **efp** has the same effect as **ef** + **apk**)

Save/Email/Print spectrum

- 21 Use **File: Save** to save your spectrum
 - Select **Copy data set to a new destination**
 - Click **OK**
 - Set folder name
 - **Suggestion:** name your folder **INI.PGindex** where
 - **INI** are your initials
 - **PG** is the page number in your lab notebook that describes this sample
 - **index** is a letter 'A', 'B', etc. that indicates which sample this is.
 - For example, page 25 of my notebook might describe a chromatography experiment that produces three samples. In this case, I would write the following sample names – **AJS.25A**, **AJS.25B**, **AJS.25C** – in my notebook, on the cards labeling the NMR tubes, and on the folders on the NMR computer.
 - Click **OK**
- 22 You can also email the entire spectrum folder to yourself as a ZIP archive. To do this, select **File: Send To** and set your email address and all other necessary parameters
- 23 It is possible to calibrate and print your NMR spectrum at the NMR console, but these instructions are not included here.

Eject sample & insert a new one

- 24 Press **LIFT ON**, remove your sample when it appears (use Kimwipe to hold the collar) and repeat all of the steps above with your next sample
 - **LIFT ON** automatically turns off the lock circuit and the spin air flow

Closing up shop

- 25 After removing your last sample, press **LIFT OFF** (a softer "air" sound will persist – ignore it), and press the **SPIN** and **LOCK** buttons so that these lights stop blinking
- 26 Set the collar (and its Chemwipe) on the console
- 27 If necessary, click-*drag* a **test.1H.solvent** folder to the spectrum window so that the next user does not inadvertently overwrite your saved data file