Nuclear Magnetic Resonance

NMR is probably the most useful and powerful technique for identifying and characterizing organic compounds. Felix Bloch and Edward Mills Purcell were awarded the 1952 Nobel Prize for their work in the area, although the technique did not become practical for organic compound structure determination until the 1960s.

Analysis of the nuclear Schrödinger equation indicates that nuclei have spin quantum numbers. The nuclear spin quantum number, I (where I is *integer* or *half-integer),* has values that are related to the nuclear shape and to the number of the different types of nuclear particles.

Nuclei with even mass numbers and even numbers of protons have $I = 0$ (*e.g.*, ^{12}C, ^{16}O). These nuclei cannot be used for NMR. Nuclei with odd mass numbers (due to odd numbers of either protons or neutrons) have $I = {}^{n}/_{2}$ (*e.g.*, ^{1}H, ^{13}C, ^{19}F, ^{31}P, which are all $I = {}^{1}/_{2}$). Nuclei with even mass numbers as a result of odd numbers of both protons and neutrons have $I = n$, where n is an integer.

If I is non-zero, the nucleus is spinning and must have one of several possible spin states. All of the spin states are degenerate in the absence of an applied external magnetic field, but in an applied field, the spin states have different energies, because the spin can align with or against the applied magnetic field. The difference in energy between the spin states is small, even in a large magnetic field, and corresponds to the energy in the radiofrequency part of the electromagnetic spectrum.

The difference in energy between the spin states is: $\Delta E = \dfrac{\gamma h \mathbf{B}_0}{2\pi}$ where γ is the magnetogyric ratio of the nucleus (the ratio of the magnetic moment to the angular momentum, sometimes called the gyromagnetic ratio), h is Planck's constant, and $\mathbf{B}_0$ is the applied magnetic field. Because $E = h\nu$, the frequency of the radiation that corresponds to the energy difference is $\nu = \dfrac{\gamma \mathbf{B}_0}{2\pi}$

The higher field NMR at Rose-Hulman has a field strength $\mathbf{B}_0$ of 7 Tesla (corresponding to 300 MHz for proton NMR), generated by a superconducting electromagnet. The magnet itself is about 4 inches tall and 5 inches across, with hole about 1.5 inches across into which the sample is inserted. To maintain its superconducting properties, the magnet needs to be cooled to liquid helium temperature (~4 K). If the helium level drops too much, the top of the magnet will become exposed, and the resulting heat will boil off the helium, possibly suffocating anyone in the room. The magnetic field is strong enough to erase credit cards and damage watches within 1 meter of the instrument. (For comparison, the earth's magnetic field strength is ~5 x 10^{-5} Tesla.) Note that, because the magnetogyric ratio differs for each type of nucleus (see Table 4), the frequency range that corresponds to the difference in energy is also different. Thus, at a given field

strength, different nuclei will absorb at different frequencies, and so only one type of nucleus at a time will be observable.

Increasing $\mathbf{B}_0$ results in higher resolution (and requires more expensive instruments) because the energy difference between the spin up and spin down states increases, which makes the transitions easier to distinguish. In addition, the coupling constants between nuclei, which determine the splitting patterns, are due to the nuclear magnetic fields of nearby nuclei. Because these small magnetic fields are constant regardless of the imposed field, overlap of splitting from different resonances is less likely because the signals are effectively further apart.

Table 4. Properties of Selected Nuclei

Isotope	Spin	Abundance	Magnetogyric ratio ($\gamma/10^7$) (rad/sec•Tesla)	Comments
^{1}H	$I = {}^{1}/_{2}$	99.985%	26.7522128	Nearly all organic compounds contain hydrogen
^{2}H	$I = 1$	0.015%	4.1066	Quadripolar nucleus
^{3}H	$I = {}^{1}/_{2}$	0	28.5349779	
^{7}Li	$I = {}^{3}/_{2}$	92.41%	10.3977	Useful for organometallics
^{12}C	$I = 0$	98.9%	–	***Useless for NMR***
^{13}C	$I = {}^{1}/_{2}$	1.07%	6.728284	
^{14}N	$I = 1$	99.632%	1.93378	Quadripolar nucleus
^{15}N	$I = {}^{1}/_{2}$	0.368%	-2.712618	
^{19}F	$I = {}^{1}/_{2}$	100%	25.18148	
^{31}P	$I = {}^{1}/_{2}$	100%	10.8394	Nucleic acids, some organic reagents

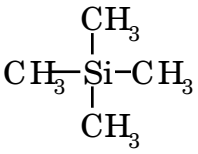
The NMR signal for a nucleus depends on the local environment within the molecule. "**Chemical shift**" is a small effect that results from the magnetic field of the electron cloud near the nucleus. Because electron clouds are asymmetric within molecules, the electron clouds interact with different nuclei differently. The electron clouds reorganize in the imposed magnetic field; the structure-dependent asymmetries in the clouds result in slight perturbations (a few parts per million for proton NMR) of the magnetic field experienced by the nuclei. These perturbations decrease the magnetic field perceived by the nucleus: $\mathbf{B}_{loc} = \mathbf{B}_0(1-\sigma)$.

Because the apparent field strength at the nucleus is different, the radiofrequency that is required for absorption will also be different. If a relatively dense electron cloud surrounds the nucleus, the opposing field induced in the electron cloud will have a relatively strong effect. This effect is called "**shielding**", and results in

smaller chemical shifts.

For a given chemical group (*e.g.*, a methyl group), the induced field is proportional to the strength of the external field. Chemical shift, δ, is the difference in hertz from the reference compound divided by the instrument frequency. Chemical shifts are given in ppm (parts per million) of the base radiofrequency used. This means that all instruments, regardless of field strength will have the same chemical shift.

Even with current instruments, the magnetic field drifts somewhat; an internal standard is necessary to calibrate the signals. TMS (tetramethylsilane, below) is in wide use as a standard, because it is the most strongly shielded compound commonly used. TMS has a δ of 0 (by definition). TMS has 12 hydrogens, all in the same environment, so it yields a strong signal.



Tetramethylsilane
(TMS)

The likely range of chemical shift for ^{1}H is ~15 ppm, which corresponds to a range of 4500 Hz for a 300 MHz instrument. A number of different terms are used to describe relative chemical shift (Figure 15). The terms "upfield" and "downfield" are from instruments that use variations in applied magnetic field to observe nuclei at different chemical shift; most modern instruments employ a constant magnetic field, and vary the frequency of electromagnetic radiation used to excite the nuclei.

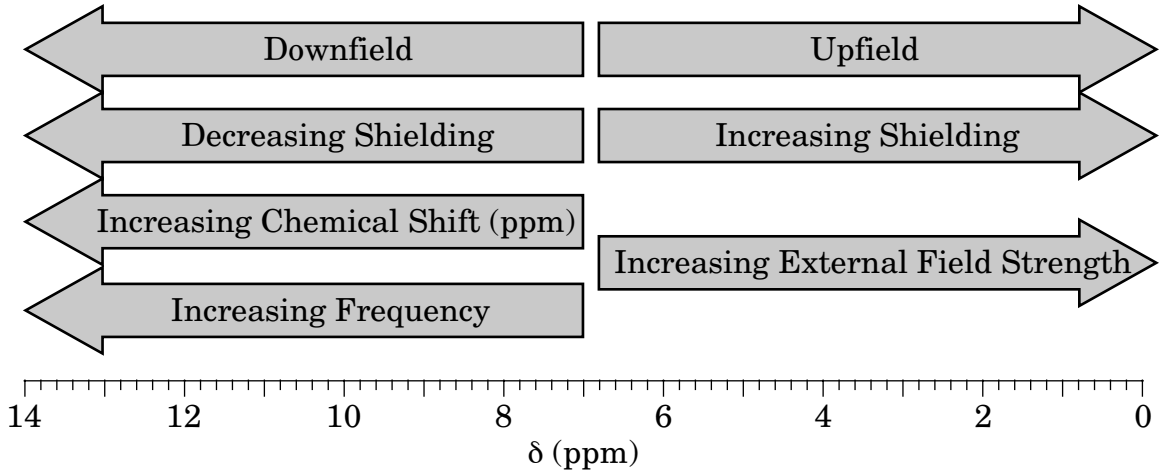


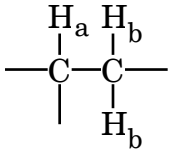
Figure 15. Definition of some of the terms used to compare chemical shifts.

Because ^{12}C has $I = 0$, it cannot be used for NMR. However, protons are found in essentially all organic molecules, and ^{1}H NMR is highly useful for organic structure determination. As mentioned, the field experienced by a nucleus depends on several factors: $\mathbf{B}_0 \pm \mathbf{E}_0 \pm \mathbf{P}$, where $\mathbf{B}_0$ is the applied field, $\mathbf{E}_0$ is the effect due to electron cloud of the attached carbon as modified by nearby functional groups, and **P** is the

coupling effect from protons on adjacent carbons. A single proton splits adjacent protons because it may exist with its spin axis "up" or "down", resulting in two possible states and therefore in a doublet of peaks. A pair of protons splits adjacent protons into three peaks: both protons may be spin up, both may be spin down, or (with twice the probability) one may be spin up while the other is spin down. In this case, the paired spin up/down state does not alter the δ; the other conditions result in a slight change upfield or downfield of the expected resonance.

Chemically equivalent protons do not split each other. Ethane has a single peak at δ = 0.9 ppm. Benzene has a single peak at δ = 6.9 ppm.

The coupling constant, **J**, determines the degree of splitting. Typical values of J for proton NMR are between 1 and 15 Hz, with J = ~7 Hz for alkanes. At least in simple cases, the coupling effect generally occurs over 3 bonds (*e.g.*, for the system below, the two H_b will split H_a, and H_a will split the two H_b).



A ^1H NMR spectrum thus contains three types of information that are useful in helping to determine the structure of the compound:

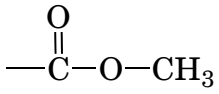
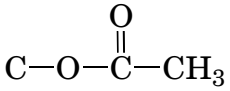
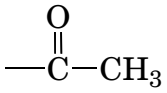
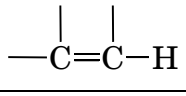
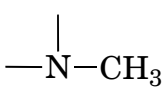
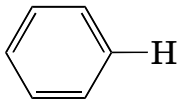
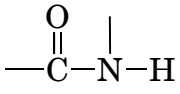
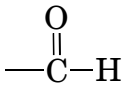
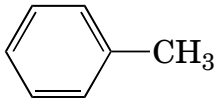
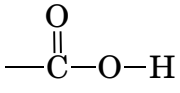
1. The **chemical shift**, which is the result of the function groups in close proximity to the proton.

2. The **splitting pattern**, which is the result of other hydrogens within three bonds of the hydrogen of interest.

3. The **peak area** for a particular resonance, which is proportional to the number of protons present in that environment.

A full description of the use of NMR to determine molecular structure is beyond the scope of this manual. However, a correlation table that summarizes the chemical shifts observed by different protons in different environments may be found on the next page (Table 5).

Table 5. Predicting Chemical Shifts for ^{1}H-NMR Spectra
(adapted from Table 12.4, J.M. Hornback *Organic Chemistry*, 1998)

Type of Hydrogen	Chemical Shift (δ)	Type of Hydrogen	Chemical Shift (δ)
—C—CH_3	0.9	Br—CH_3	2.7
C=C—CH_3	1.6	Cl—CH_3	3.0
—C≡CH	1.8	—O—CH_3	3.3
	Variable (1-4)		3.7
—O—H	Variable (2-5)	O_2N—CH_3	4.1
	2.0	F—CH_3	4.2
	2.2		5.5-6.5
	2.2		7-8
I—CH_3	2.2		Variable (6-8)
N≡C—CH_3	2.2		10
	2.3		12

Procedure: Find the functional group near the proton of interest. Take the chemical shift listed and:
Add 0.3 if CH_2 instead of CH_3, or add 0.7 if CH instead of CH_3.
Add 0.3 for electronegative group or atom attached to the adjacent carbon.
When two electronegative groups are attached to the same carbon, both groups affect the hydrogens: add the indicated values from the table together, add the correction for the methylenyl or methinyl hydrogen, and subtract 0.9 from the total.

The value obtained by these calculations is a **prediction**, and may deviate from observed values by 1 ppm or more.

Obtaining NMR Spectra

Two types of NMR instruments are available at Rose-Hulman. The higher field instrument is the 300 MHz NMR. It has a superconducting electromagnet. As with many instruments, the 300 MHz NMR uses deuterium from the sample as an internal standard; this means that ***you must use deuterated solvents with the 300 MHz NMR***. (If you do not have deuterium in your sample, parts of the instrument may burn out while trying to detect something that is not there.)

The lower field instrument is a 90 MHz NMR, which has a permanent magnet. The 90 MHz NMR does not have a deuterium channel; you may therefore use liquid samples without dilution. If you have a solid sample, you may use both deuterated and non-deuterated solvents (non-deuterated solvents have protons that will show up in the NMR spectrum; most people avoid these additional peaks by using deuterated solvents). The 90 MHz NMR is somewhat easier to operate than the 300 MHz NMR. In this course, the 90 MHz NMR will be used routinely.

Obtaining a Proton NMR spectrum using the 90 MHz NMR

1. **Preparing the sample**

If your sample is a ***liquid***, put 20 to 30 drops of sample and about 4 drops of TMS in a clean NMR tube with no additional solvent. (If you do not have enough sample to spare 20-30 drops, add a few drops of your sample and 20 to 30 drops of deuterated solvent (usually deuterochloroform, $CDCl_3$, unless your compound is not chloroform soluble) and ~1 drop of TMS.) Cap the NMR tube, and mix well using a vortex mixer.

If your sample is ***solid***, create a pile of the compound a few millimeters high in a clean NMR tube. Add 20 to 30 drops of deuterated solvent (usually deuterochloroform ($CDCl_3$) unless your compound is not chloroform soluble) and ~1 drop of TMS. Cap the NMR tube, and vortex to mix the sample.

2. **Inserting the sample into the NMR**
    a. Open the NMR chamber.
    b. Use the sample eject button to remove the previous sample.
    c. Carefully take the previous NMR tube out of the spinner.
    d. Carefully place your NMR tube in the spinner and use the template to adjust the insertion depth.
    e. Gently wipe the spinner and exposed NMR tube with a Kimwipe.
    f. Place the spinner in the instrument.
    g. Turn off and then turn on the spinner air supply. Check to make sure that your sample is spinning.
    h. Close the NMR chamber.

3. **Collecting the spectrum**
    a. In the program **WinPNMR**, the prompt should be H1 (type **nu H1 <Enter>** if necessary).
    b. Type **shim <Enter>** to shim the magnet for the sample. The program will ask for a receiver delay value; type **5 <Enter>**.

c. Type **zg <Enter>** to collect a spectrum. The program will ask for a file name; to use the default file (pnmrfid) press **<Enter>** a second time.
d. Once the spectrum is collected, switch to the program **NUTS**.
e. Type **a2** to look at the new spectrum. Use the mouse cursor to check the position of TMS. If the value is not satisfactory (within about 0.2 ppm of zero), switch back to WinPNMR, and type **fo <Enter>.** The program will ask for the observed position of TMS. Enter the observed chemical shift for TMS (including the sign) and click OK; the program will then ask for the desired value. Enter **0** and click OK. You will need to collect a new spectrum (**zg <Enter>**) for the altered field offset value to take effect. You will also need to use **a2** or **<Ctrl+F2>** to see the new spectrum in NUTS.

4. Processing the Proton NMR spectrum

a. In NUTS, type **<Ctrl+F2>**.[12] Select the file you wish to analyze; the default is the pnmrfid that you just collected.
b. In the Comment box, enter the name of the sample (usually in the form of "<compound> in <solvent>", or "<compound> (neat)", if you did not add any solvent).
c. Type **ap** to autophase
d. **Integrate the peaks**.
    i. Type **bc** to baseline correct the spectrum.
    ii. Type **id** to run the integration routine.
    iii. **Left-Click** twice to the left and once to the right of each peak.
    iv. If necessary, type the letter **l** to delete the last integral. To delete other integrals, click the mouse button once, and move the vertical curser so that it is in the middle of the integral you wish to remove, and type **d**.
    v. **Left-Click** once so that the vertical cursor is in the middle of an integral, and type **v**. Enter the number of protons for that integral; the other integrals will be recalculated relative to the value you input. (***Note: it is a big help if you know what peaks your compound should have!***)
    vi. Type **<Enter>** to exit id.
    vii. If necessary, type **<Ctrl+i>** to show integrals
e. **Pick peaks for ppm display**. Press and hold the mouse button. Move the mouse until the horizontal cursor describes the desired peak threshold, and type **m**. (Alternatively, type **dp** and click on the peaks you wish to label.)
f. **Select the options for printing or export**.
    i. Type **<Ctrl+i>** to toggle integrals.
    ii. Type **<Ctrl+b>** to toggle peak table.
    iii. Type **<Ctrl+p>** to toggle peak selections.
    iv. Type **pl** to print the spectrum.

[12]Note that in NUTS, the commands **a2** and <**Ctrl+F2**> are slightly different. The NUTS software does some additional processing when you use <Ctrl+F2>; among other things, it attempts to place the TMS peak at zero ppm if it can find the TMS peak.