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Experiment #2: Nuclear Magnetic Resonance**MASSACHUSETTS INSTITUTE OF TECHNOLOGY**

Department of Chemistry
5.311 Introductory Chemical Experimentation

Experiment #2
NUCLEAR MAGNETIC RESONANCE**I. Purpose**

This experiment is designed to introduce the basic concepts of nuclear magnetic resonance (NMR) spectroscopy – spin, energy levels, absorption of radiation, and several NMR spectral parameters, and to provide experience in identification of unknowns via ^1H (proton) NMR spectra. A series of **known** samples will be used to introduce methods of sample preparation, operation of the NMR spectrometers, and ^1H -NMR spectra from which students will measure chemical shifts, J-couplings and spectral intensities. Subsequently, students will record spectra of **three unknowns** and will use these spectra to determine the structure and identity of the compounds.

II. Safety

II.A. Chemicals: A number of different chemicals are involved in this experiment and should be handled with care to avoid harm to yourself or your colleagues. Because the presence of solvent protons would obscure the NMR signals of the desired compound, NMR measurements are commonly carried out in deuterated solvents such as acetone- d_6 or chloroform- d_3 .

II.B. Glass: In preparing NMR samples, you will use glass pipettes and NMR sample tubes. In placing a rubber bulb on the pipette or plastic cap on the sample tube, the experimenter should hold the tube immediately below the point of attachment to avoid breakage of the glass.

II.C. Magnetic Fields: The field generated by an NMR magnet can have deleterious effects on watches (battery-powered watches with liquid crystal displays are an exception), magnetic credit cards (VISA, Mastercharge, American Express, etc.), and cardiac pacemakers. Thus, when working in the vicinity of an NMR spectrometer, leave these items in an alternative location.

III. Reading

1. Hore, P. J. *Nuclear Magnetic Resonance*, Oxford University Press, Oxford, 1998. A delightful introduction to NMR Spectroscopy.
2. Mohrig, J.R.; Hammond, C.N.; Schatz, P.F.; Morrill, T.C. *Techniques In Organic Chemistry* W.H. Freeman: New York, 2003. Chapter 19.
3. Pavia, D. I.; Lampman, G. M.; Kriz, G. S. *Introduction to Spectroscopy: A Guide for*

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Students of Organic Chemistry, Saunders: Fort Worth, 1996. Chapters 3-5.

4. Canet, D. *Nuclear Magnetic Resonance: Concepts and Methods*, Wiley: New York, 1996. Enables students to understand the physical and mathematical background which underlines liquid state NMR.
5. Derome, A. E. *Modern NMR Techniques for Chemistry Research*, Pergamon Press: 1987.

IV. Theory

IV.A. Energy Levels of Nuclei in a Magnetic Field

Many isotopes of elements in the periodic table possess nuclear spin angular momenta, which results from coupling the spin and orbital angular momenta of the protons and neutrons in the nucleus. In 5.11, you learned that the orbital angular momentum, l , of an electron can be oriented in $2l + 1$ discrete states (e.g. a p electron ($l = 1$) can have $m_l = +1, 0$, or -1 orientation states). Nuclear spin angular momenta behave the same way. Nuclei with spin I can assume $2I + 1$ values of the spin orientation, characterized by quantum numbers, m_I , which are $[I, I-1, \dots, -(I-1), -I]$. For $I = 1/2$ the allowed m_I are $+1/2$ and $-1/2$, and for $I = 1$ the allowed m_I are $-1, 0$, and $+1$. If I is non-zero, the nucleus has a magnetic moment, and when placed in a magnetic field, the component of the moment along the field direction (conventionally taken as the z -direction) is $\mu_z = \gamma \hbar m_I$, where γ , which is a constant specific for each isotope, is termed the gyromagnetic ratio; \hbar is the Planck constant divided by 2π ($\hbar = 1.055 \times 10^{-34}$ J s), and m_I is the quantum number. Some γ values for chemically important nuclei are given in Table 1. Note that nuclei such as ^{12}C and ^{16}O are not included in the table, since they have $I=0$.

Table 1. Spin, gyromagnetic ratios, NMR frequencies (in a 1 T (10 kG) field) and the natural abundance of selected nuclides)*

Nucleus	Spin	Gyromagnetic Ratio, γ ($10^7 \text{T}^{-1} \text{s}^{-1}$)	ν (MHz)	Natural Abundance (%)
^1H	1/2	26.75	42.576	99.985
^2H	1	4.11	6.536	0.015
^{13}C	1/2	6.73	10.705	1.108
^{19}F	1/2	25.18	40.054	100.00
^{14}N	1	1.93	3.076	99.63
^{15}N	1/2	-2.71**	4.31	0.37
^{31}P	1/2	10.84	17.238	100.0

* T stands for Tesla (1 Tesla = 10^4 Gauss. The earth's magnetic field is around 0.5 Gauss)

** What is the physical meaning of a negative gyromagnetic constant?

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In a laboratory field B_0 , the nuclei can assume $2I+1$ orientations corresponding to the values of m_I . Each value of m_I corresponds to an energy given by (Figure 1)

$$E_{m_I} = -\boldsymbol{\mu} \mathbf{B}_0 = -\mu_z B_0 = -\hbar \gamma B_0 m_I \quad (1)$$

which can be rewritten as

$$E_{m_I} = -m_I \hbar \omega_0 \quad (2)$$

where the **Larmor frequency** is $\omega_0 = \gamma B_0$.

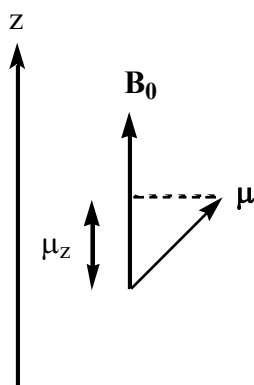


Figure 1. The relationship between the laboratory magnetic field B_0 and, the magnetic moment of the nucleus, μ , and its μ_z component (μ component along the z axis)

As shown in Figure 2, the energy separation between the two levels α and β of an $I=1/2$ system is then

$$\Delta E = E_\beta - E_\alpha = (1/2) \hbar \gamma B_0 - (-1/2) \hbar \gamma B_0 = \hbar \gamma B_0 \quad (3)$$

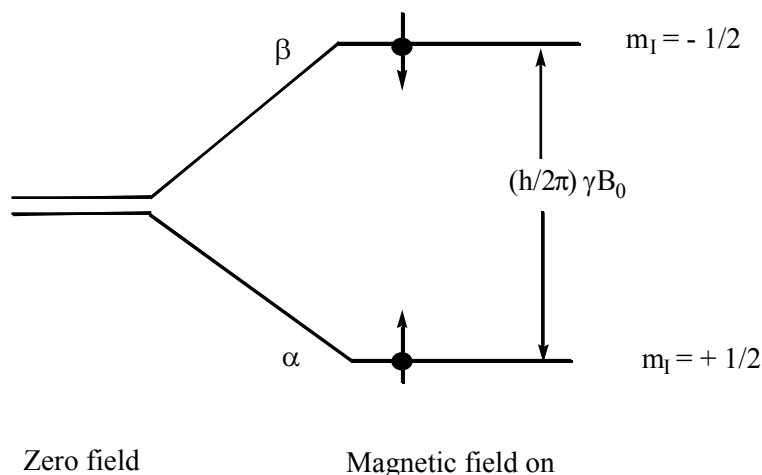


Figure 2. The nuclear spin energy levels of a spin-1/2 nucleus (e.g. ^1H or ^{13}C) in a magnetic field. Resonance occurs when the energy separation of the levels, as determined

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by the magnetic field strength, matches the energy of the photons in the electromagnetic field.

Thus, when the sample resides in a magnetic field and is bathed with radiation of frequency $\omega_0/2\pi = \nu_0$ (energy $\hbar\omega_0$) matching the difference between the levels resonance occurs -- i.e. energy is absorbed, as illustrated schematically in Figure 2. Equation (3) is the **resonance condition**. Using the values for γ in Table 1, we can calculate that for ^1H in a 4.7 T field, the resonance frequency is 200 MHz, and for an 11.7 T field $\nu_0 = 500$ MHz.

The amount of energy absorbed (and therefore the NMR signal strength) is proportional to the population difference between the α and β levels. In Experiment 1, you used the Lambert-Beer law to calculate the amount of light transmitted by an absorbing sample:

$$\% T = I/I_0 = e^{-A} = e^{-\epsilon cl}$$

The absorbance $A = \epsilon cl$ can be written equivalently as $A = k_{abs} N_{abs} l$ where k_{abs} is an absorption coefficient and N_{abs} is the number of absorbing molecules in the beam path. Because excited molecules can re-radiate light, the effective N_{abs} is actually $N(\text{lower state}) - N(\text{upper state})$. The ratio $N(\text{upper})/N(\text{lower})$ can be calculated from the formula

$$P = \frac{N_{upper}}{N_{lower}} = e^{-\frac{\Delta E}{kT}} \quad (4)$$

where ΔE equals the energy difference, k is the Boltzmann constant (1.380×10^{-23} J/K.mole), and T is the absolute temperature. For an optical transition, there are essentially no molecules normally present in the excited state, and N_{abs} is equal to the species concentration. For a proton, in an applied field with a strength of 7.0 T (e.g. the magnetic field of the Varian 300 MHz), using $\Delta E = h\nu$, the difference $\hbar \gamma B_0$ between two spin states of the proton is 1.99×10^{-25} J per molecule. The kT at room temperature ($T = 298$ K) is 4.11×10^{-21} J, therefore $\Delta E/kT = 4.84 \times 10^{-5}$. With such a small value of $\Delta E/kT$, equation (3), may be simplified using $e^{-x} \approx 1-x$:

$$\frac{N_{upper}}{N_{lower}} = e^{-\frac{\Delta E}{kT}} \approx 1 - \frac{\Delta E}{kT} \quad (5)$$

or

$$\frac{N_{lower} - N_{upper}}{N_{lower} + N_{upper}} \approx \frac{\Delta E}{2kT} \approx 2.42 \times 10^{-5} \quad (6)$$

With such small population differences, highly sensitive detection techniques are required.

IV.B. CHEMICAL SHIFTS

Every nucleus in a molecule is surrounded by an electron cloud, which possesses an electronic angular momentum induced by the laboratory field, B_0 . This angular momentum results in a local magnetic field,

$$\delta B_0 = -\sigma B \quad (5)$$

at the nucleus, which is opposed to the applied field because it arises from negatively charged electrons. Thus, the electrons **shield** the nucleus from the applied field and σ is called the **shielding constant**. The total field at each nucleus is therefore

$$B_{\text{loc}} = B_0 + \delta B = (1 - \sigma)B_0 \quad (6)$$

The feature that makes NMR so interesting and extremely important to chemists is that σ varies markedly from chemical group to chemical group. Thus, σ for a $-C^1H_3$ is different from σ for a $-C^1H_2-$, and that in turn is different from σ for an $-O^1H$. These features of NMR spectra, illustrated for the 1H 's in ethanol in Figure 3, are referred to as **chemical shifts**. They are important since they facilitate the identification of each type of 1H (or ^{13}C) present in a molecule.

All of the shifts in Figure 3 are referenced to the shift of the standard tetramethylsilane (TMS) and are calculated from the formula:

$$\delta = \frac{\nu - \nu_0}{\nu_0} \times 10^6 \quad (7)$$

where ν and ν_0 are the frequency of the unknown and reference (TMS), respectively. As mentioned above, the amount of shielding, and therefore the frequency separation between two lines, will increase with increasing field. However, the chemical shifts calculated from Equation (7) are field independent. Thus, shifts measured at 100 MHz can be compared directly with those obtained at 500 MHz. Note that positive δ values correspond to higher frequencies or lower magnetic fields.

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Figure 3 – The proton NMR spectrum of ethanol. TMS (the rightmost signal) stands for tetramethylsilane ($\text{Si}(\text{CH}_3)_4$). TMS is the widely used reference signal. The step-like curve is the integrated signal, proportional to the number of spins in each signal. This spectrum was first recorded by J.T. Arnold and co-workers in 1951 [*Journal of Chemical Physics* **19**, p. 507].

Figure 4 illustrates the dependence of proton chemical shifts on the various functional groups in more detail. Note that Figure 4b also shows chemical shifts for ^{13}C , another important NMR nucleus.

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(a)

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(b)

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Figure 4: Bar graph illustrating the range of chemical shifts expected for various types of (a) ^1H resonances and (b) ^{13}C resonances, referenced to TMS.

IV.C. J-Couplings and Spectral Intensities

The spectral lines of ethanol in Figure 3 exhibit fine structure -- i.e. the $-\text{CH}_3$ line consists of three lines and the $-\text{CH}_2-$ has four components¹. This coupling occurs because each magnetic nucleus also contributes to the local field of adjacent nuclei -- an effect referred to as **spin-spin coupling**. The strength of this coupling is described by a **field independent** coupling constant, J , measured in Hertz (Hz).

¹ J.T. Arnold, Phys. Rev. **102**, 136 (1955).

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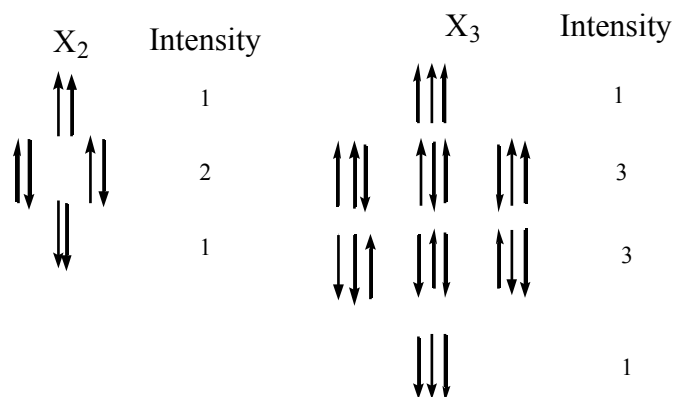


Figure 5: (a) Orientations of the spins in the X_2 group ($-\text{CH}_2-$) of an AX_2 spin system which give rise to a 1:2:1 triplet in the A resonance. The two nuclei may have the $2^2 = 4$ spin arrangements shown. (b) The $2^3 = 8$ arrangements of the three spins in an AX_3 group which lead to the 1:3:3:1 quartet in the A resonance.

The spectral pattern in Figure 3 can be understood by examining the simple diagrams shown in Figure 5. If two spins that correspond to the two ^1H 's on a $-\text{CH}_2-$ are present, they can be aligned in four different ways. Each spin is either parallel or opposed to the field. Therefore, two spins can be aligned both up, both down, and one up and one down (in two different ways: spin one up/spin two down, and spin one down/spin two up). Thus, the local field at the $-\text{CH}_3$ due to the adjacent $-\text{CH}_2-$ should have three components with an intensity ratio of 1:2:1, corresponding to both up, one up and one down (2 ways), and both down. Correspondingly, there are eight ways to align the three spins on the methyl group, as illustrated in the figure, resulting in a quartet for the $-\text{CH}_2-$ with intensities of 1:3:3:1. By extending these arguments to larger numbers of spins, it is possible to show that N equivalent spins split the resonance of a coupled group into $N+1$ lines, with intensities corresponding to the coefficients of a **binomial expansion** or **Pascal's Triangle**. A Pascal's triangle is shown on the following page, where N is the total number of equivalent spins.

The size of J-couplings depends on the structure and the nuclei involved. $^1\text{H}-^1\text{H}$ couplings are ~ 10 Hz, while $^1\text{H}-^{13}\text{C}$ and $^{31}\text{P}-^1\text{H}$ couplings can be ~ 100 Hz. and ~ 600 Hz, respectively.

Another noteworthy feature of Figure 3 is the integrated spectral intensity of each set of lines. The integrals, which are performed electronically in an experiment, are shown as the step-like curves in the figure and are in the ratio 1:2:3, corresponding to the number of each type of ^1H present. Thus, intensities in ^1H spectra can be employed to determine the relative numbers of each type of ^1H present in a molecule.

In summary, chemical shifts and spectral intensities provide information on the types and number of nuclei, respectively, associated with each functional group present in a molecule. Further J-couplings permit a determination of which groups are chemically bonded. Together

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these parameters permit the structures of most organic molecules to be determined by simply recording and interpreting their NMR spectra.

PASCAL'S TRIANGLE

<u>N</u>	<u>INTENSITY DISTRIBUTION</u>						
0			1				
1			1	1			
2			1	2	1		
3			1	3	3	1	
4			1	4	6	4	1
.							
.							
N	Expand $(1+x)^N$ and select the coefficients						

V. Experimental Procedures

V.A. Sample Preparation

V.A.1 General

The NMR tubes for the known and unknown samples should be clean and free of dust and particulate matter. Solid samples should be dissolved in a deuterated solvent and liquid samples can be run neat or diluted in a deuterated solvent as described further below.

An NMR sample tube is typically 175 mm in length with a 5 mm O.D. Minimum filling level is a distance of approximately 2 cm up from the bottom of the tube, which is equal to a volume of about 0.3 mL. However, the optimum filling level is a distance of approximately 4 - 5 cm up from the bottom of the tube (0.6-7.5 mL). Over filling can distort the homogeneity and lead to lower resolution. When the tube is inserted into the probe, the position of the transmitter coil is about 1 cm from the bottom of the tube.

As mentioned above, the solvent must dissolve the sample material. It is generally desirable to use as concentrated a solution as possible -- i.e. about 10%. In difficult cases where the sample is not very soluble, it may be necessary to find an alternative solvent. **Hydrogen-containing solvents** should be avoided whenever possible. The selected solvent **should not produce** strong signals of its own in the spectral region of interest. Some solvents commonly employed for proton NMR spectroscopy are:

deuteriochloroform -- CDCl_3

hexadeuteroacetone (acetone- d_6) -- $(\text{CD}_3)_2\text{C}=\text{O}$

hexadeuterodimethylsulfoxide (DMSO- d_6) -- $(\text{CD}_3)_2\text{S}=\text{O}$

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It is most important that the sample tube to be absolutely clean within and without; its surfaces must be completely dust-free. Particles just visible with the unaided eye may degrade the resolution if they are within the irradiation region.

V.A.2. Reference Material

It is usual to provide a reference line in each spectrum from which the chemical shifts of other signals can be measured. The normal method is to add a reference material (known as an **internal standard**) directly to the sample. The reference material should not interact with the test sample. If a symmetrical and non-polar type of molecule is used as the reference material, these effects will be very small. For this purpose tetramethylsilane (TMS) is most suitable. An additional advantage of using TMS is that the spectral line it produces is at a higher field position than almost all other signals, so that the chemical shifts of nearly all lines measured with respect to TMS are of the same low field direction. As an alternative method, the reference material may be contained in a sealed capillary tube, which is inserted into the sample tube. There can be no intermolecular effects by this method.

V.B. Standard (Known) Samples.

Using four separate NMR tubes prepare samples of the following standard samples:

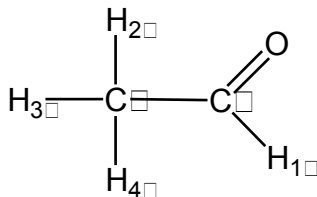
1. Acetaldehyde
2. Ethylbenzene
3. Vinyl acetate
4. 1,1,1,3,3,3-hexafluoroisopropanol (HFIP)
5. Simulation (with iteration) of crotonaldehyde H-NMR spectrum.

Record the spectra, measure the chemical shifts, J-couplings, and integrate the spectral lines.

Answer the following sets of questions for each of these systems:

V.B.1 Acetaldehyde.

1. Based on information provided in Figure 4a, predict the chemical shifts of protons H₁ through H₄ and the coupling pattern among the protons H₁-H₄.
2. Record the ¹H NMR of acetaldehyde (dissolved in CDCl₃). Calculate the chemical shift of H₁ and CH₃ protons (in ppm units).



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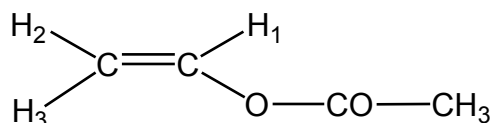
3. Explain the coupling pattern among the acetaldehyde protons. Calculate the coupling constant (in Hz) the aldehyde proton (H_1) and the methyl group protons.
4. Using your spectral parameters (chemical shifts and coupling constant) simulate (with gNMR, see the Appendix) the 1H NMR of acetaldehyde.

V.B.2 Ethylbenzene.

The spectrum of ethylbenzene ($C_6H_5CH_2CH_3$) exhibits a triplet, a quartet, and a third line with unresolved fine structure. Find the chemical shift for CH_3 , CH_2 , and aromatic protons. Explain these patterns and spectral intensities.

V.B.3 Vinyl acetate

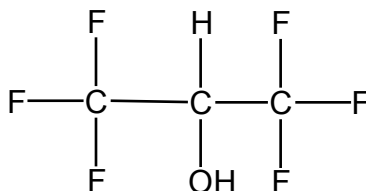
1. Predict the H-NMR of vinyl acetate (chemical shifts and coupling patterns).



2. Record the 1H NMR of vinyl acetate in $CDCl_3$.
3. Examine the coupling pattern for each of the 3 groups of signals. What are the values for J_{trans} (H_1 and H_3), J_{cis} (H_1 and H_2) and J_{gem} (H_2 and H_3)? Note: The magnitude of the J coupling is pivotal in elucidating the stereochemistry around a CC double bond.
4. Assign the calculated chemical shifts to H_1 , H_2 and H_3 , respectively. Rationalize the relative chemical shift by invoking the electron density (write a resonance structure) on the two CC double bond carbon atoms.
5. Simulate the H-NMR spectrum (with iteration, see the Appendix 1).

V.B.4 Hexafluoroisopropanol (HFIP)

The spectrum of HFIP is an example illustrating heteronuclear J-couplings -- between 1H and ^{19}F .



In the 1H NMR of HFIP you will notice only two signals: one assigned to OH proton and the second assigned to the CH proton.

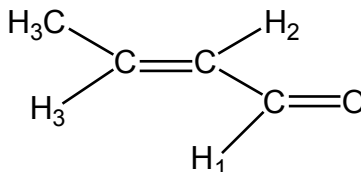
1. How many lines do you expect in the 1H spectrum from coupling to ^{19}F ($I=1/2$)? Calculate from the experimental spectrum the coupling J_{HF} .
2. Determine the intensity of each line in the spectrum. Do the intensities match those expected from a binomial distribution?

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3. What is the chemical shift for the CH proton?

V.B.5 Simulation of the ^1H NMR spectrum of crotonaldehyde.

Your TA will provide a copy of the ^1H -NMR spectrum of crotonaldehyde and a printout of the signal frequencies (ppm and Hz). Assign the signals (H_1 , H_2 , H_3 , CH_3) first by hand and then refine computationally (iteration-gNMR, see the Appendix 1) and the spectral parameters (chemical shift and all coupling constants). Provide a brief justification for your findings.



V.C. Unknown Samples

You will be given three unknown samples. Prepare the NMR tubes in the same way as the known samples and record the spectra.

- Measure chemical shifts, J-couplings, and spectral intensities.
- Refine the spectral parameters with gNMR with iteration.
- From these measurements, the shifts in Figure 4, and supplementary information provided by your TAs, deduce as much as possible about the identity of the compounds.
- How would you check the proposed structures?