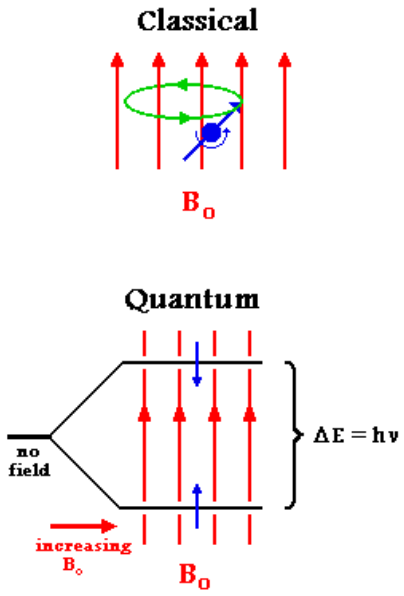
Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR is a spectroscopic technique which relies on the magnetic properties of the atomic nucleus. When placed in a strong magnetic field, certain nuclei resonate at a characteristic frequency in the radio frequency range of the electromagnetic spectrum. Slight variations in this resonant frequency give us detailed information about the molecular structure in which the atom resides.

The Classical Model. Many atoms (e.g., ^{1}H, ^{13}C, ^{15}N, ^{31}P) behave as if the positively charged nucleus were spinning on an axis. The spinning charge, like an electric current, creates a tiny magnetic field. When placed in a strong external magnetic field, the magnetic nucleus tries to align with it like a compass needle in the earth's magnetic field. Because the nucleus is spinning and has angular momentum, however, the torque exerted by the external field results in a circular motion called precession, just like a spinning top in the earth's gravitational field. The rate of this precession is proportional to the external magnetic field strength and to the strength of the nuclear magnet. This resonant frequency is in the radio frequency range for strong magnetic fields, and can be measured by applying a radio frequency signal to the sample and varying the frequency until absorbance of energy is detected.



The Quantum Model. This classical view of magnetic resonance, in which the nucleus is treated as a macroscopic object like a billiard ball, is insufficient to explain all aspects of the NMR phenomenon. We must also consider the quantum mechanical picture of the nucleus in a magnetic field. For the most useful nuclei, which are called "spin 1/2" nuclei, there are two quantum states which can be visualized as having the spin axis pointing "up" or "down". In the absence of an external magnetic field, these two states have the same energy and at thermal equilibrium exactly one-half of a large population of nuclei will be in the "up" state and one-half will be in the "down" state. In a magnetic field, however, the "up" state, which is aligned with the magnetic field, is lower in energy that the "down" state, which is opposed to the magnetic field. Because this is a quantum phenomenon, there are no possible states in between. This energy separation or "gap" between the two quantum states is proportional to the strength of the external magnetic field, increasing as the field strength is increased. In a large population of nuclei in thermal equilibrium, slightly more than half will reside in the "up" (lower energy) state, and slightly less than half will reside in the "down" (higher energy) state. As in all forms of spectroscopy, it is possible for a nucleus in the lower energy state to absorb a photon of electromagnetic energy and be promoted to the higher energy state. The energy of the photon must exactly match the energy "gap" (ΔE) between the two states, and this energy corresponds to a specific frequency of electromagnetic radiation:

$$\Delta E = h\nu$$

where h is Planck's constant. The resonant frequency, ν, is in the radio frequency range, identical to the precession frequency predicted by the classical model.

Useful Nuclei for NMR. The resonant frequencies of some important nuclei are shown below for the magnetic field strength of a typical NMR spectrometer (Varian Gemini-200):

Nucleus	Abundance	Sensitivity	Frequency
^{1}H	100%	1.0	200 MHz
^{13}C	1.1%	0.016	50 MHz
^{15}N	0.37%	0.001	20 MHz
^{19}F	100%	0.83	188 MHz
^{31}P	100%	0.066	81 MHz
^{57}Fe	2.2%	3.4×10^{-5}	6.5 MHz

Since the resonant frequency is proportional to the external magnetic field strength, all of the resonant frequencies above would be increased by the same factor with a stronger magnetic field. The relative sensitivity is a direct result of the strength of the nuclear magnet, and the effective sensitivity is further reduced for those nuclei which occur at low natural abundance. For example, ^{13}C at natural abundance is 5,700 times less sensitive (1/(0.011*0.016)) than ^{1}H when both factors are taken into consideration.

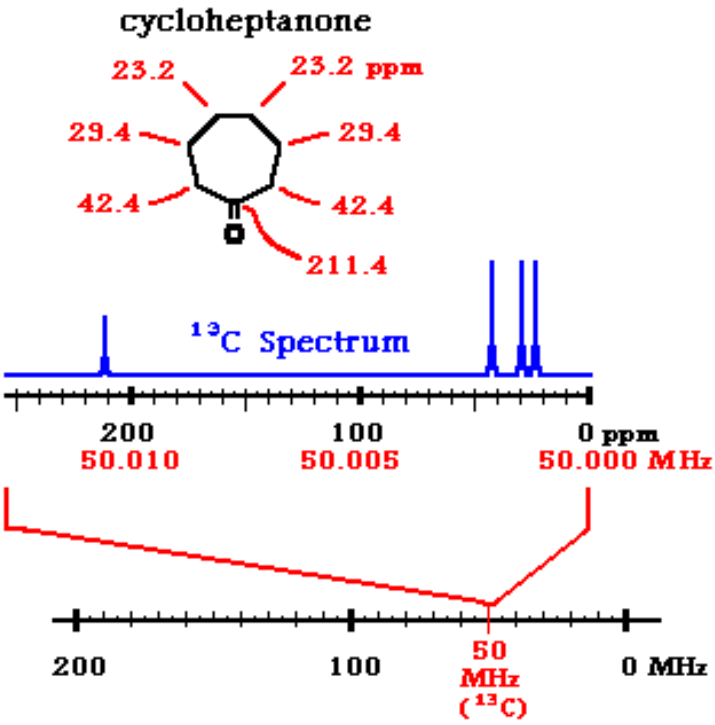
The Chemical Shift. The resonant frequency is not only a characteristic of the type of nucleus, but also varies slightly depending on the position of that atom within a molecule (the "chemical environment"). This occurs because the bonding electrons create their own small magnetic field which modifies the external magnetic field in the vicinity of the nucleus. This subtle variation, on the order of one part in a million, is called the chemical shift and provides detailed information about the structure of molecules. Different atoms within a molecule can be identified by their chemical shift, based on molecular symmetry and the predictable effects of nearby electronegative atoms and unsaturated groups.

The chemical shift is measured in parts per million and is designated by the greek letter delta (δ). The resonant frequency for a particular nucleus at a specific position within a molecule is then equal to the fundamental resonant frequency of that isotope (e.g., 50.000 MHz for ^{13}C) times a factor which is slightly greater than 1.0 due to the chemical shift:

$$\text{resonant frequency} = \nu_0 \, (\, 1.0 + \delta \times 10^{-6})$$

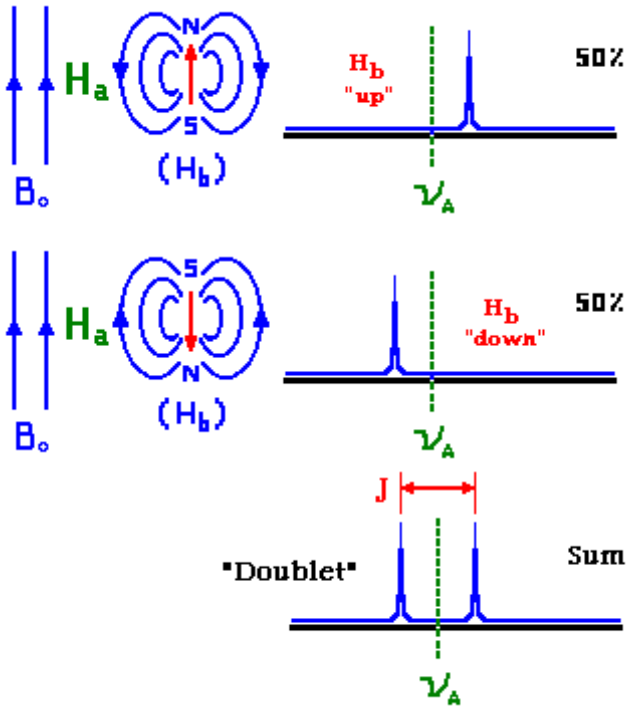
For example, a ^{13}C nucleus at the C-4 position of cycloheptanone (δ 23.3 ppm) resonates at a frequency of:

$$50.000 \text{ MHz} \, (\, 1.0 + \mathbf{23.2} \times 10^{-6}) = 50.000(1.0000232) = 50{,}001{,}160 \text{ Hz}$$



A graph of the resonant frequencies over a very narrow range of frequencies centered on the fundamental resonant frequency of the nucleus of interest (e.g. ^{13}C at 50.000 MHz) is called a **spectrum**, and each peak in the spectrum represents a unique chemical environment within the molecule being studied. For example, cycloheptanone has four peaks due to the four unique carbon positions in the molecule. Note that symmetry in a molecule can make the number of unique positions less than the total number of carbons.

Spin-Spin Splitting. Another valuable piece of information about molecular structure is obtained from the phenomenon of spin-spin splitting. Consider two protons (^1H_aC-C^1H_b) with different chemical shifts on two adjacent carbon atoms in an organic molecule. The magnetic nucleus of H_b can be either aligned with ("up") or against ("down") the magnetic field of the spectrometer. From the point of view of H_a, the H_b nucleus magnetic field perturbs the external magnetic field, adding a slight amount to it or subtracting a slight amount from it, depending on the orientation of the H_b nucleus ("up" or "down"). This changes the H_a chemical shift so that it now resonates at one of two frequencies very close together. Since roughly 50% of the H_b nuclei are in the "up" state and roughly 50% are in the "down" state, the H_a resonance is "split" by H_b into a pair of resonance peaks of equal intensity (a "doublet"). The relationship is mutual, so that H_b experiences the same splitting effect from H_a. This effect is transmitted through bonds and operates only when the two nuclei are very close (three bonds or less) in the bonding network. If there is more than one "neighbor" proton, more



complicated splitting occurs so that the number of peaks is equal to one more than the number of neighboring protons doing the splitting. For example, if there are two neighboring protons (H_aC-CH^b_2) there are four possibilities for the H_b protons, just like the possible outcomes of flipping two coins: both "up", the first "up" and the second "down", the first "down" and the second "up", and both "down". If one is "up" and one "down" the effects cancel each other and the H_a proton absorbs at its normal chemical shift position (ν_a). If both H_b spins are "up", the H_a resonance is shifted to the right by J Hz. If both are "down", the H_a resonance occurs J Hz to the left of ν_a. Because there are two ways it can happen, the central resonance at ν_a is twice as intense as the outer resonances, giving a "triplet" pattern with intensity ratio 1 : 2 : 1. Similar arguments for larger numbers of neighboring spins lead to the general case of *n* neighboring spins, which split the H_a resonance peak into *n + 1* peaks with an intensity ratio determined by *Pascal's triangle*. This triangle of numbers is created by adding each adjacent pair of numbers to get the value below it in the triangle:

```
                1
              1   1
            1   2   1
          1   3   3   1
        1   4   6   4   1
      1   5  10  10  5   1
    1   6  15  20  15  6   1
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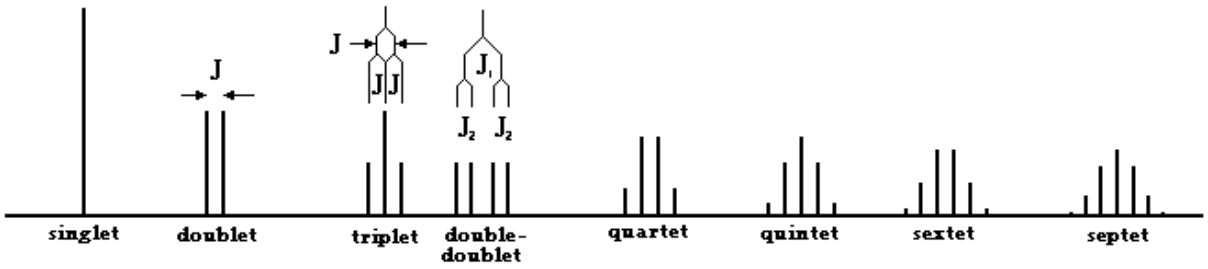
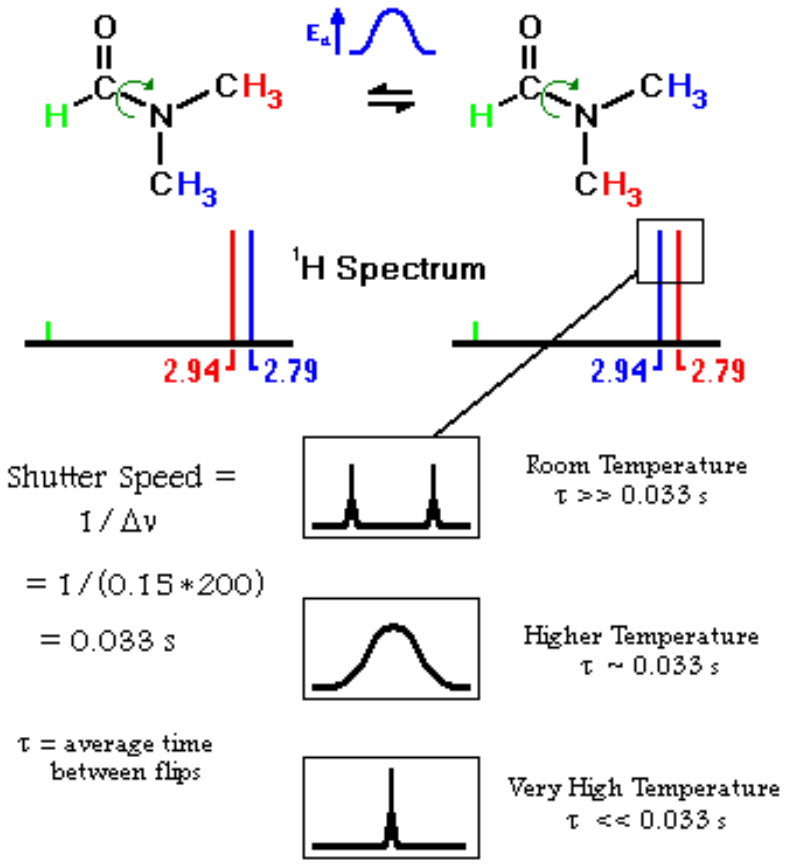
The strength of the spin-spin splitting interaction, measured by the peak separation ("J value") in units of Hz, depends in a predictable way on the dihedral angle defined by H_a-C-C-H_b, so that information can be obtained about the conformation of molecules in solution.



The NOE. A third type of information available from NMR comes from the nuclear Overhauser enhancement or NOE. This is a direct through-space interaction of two nuclei. Irradiation of one nucleus with a weak radio-frequency signal at its resonant frequency will equalize the populuations in its two energy levels. This perturbation of population levels disturbs the populations of nearby nuclei so as to enhance the intensity of absorbance at the resonant frequency of the nearby nuclei. This effect depends only on the distance between the two nuclei, even if they are far apart in the bonding network, and varies in intensity as the inverse sixth power of the distance. Generally the NOE can only be detected between protons (^{1}H nuclei) which are separated by 5 Angstroms or less in distance. These measured distances are used to determine accurate three-dimensional structures of proteins and nucleic acids.

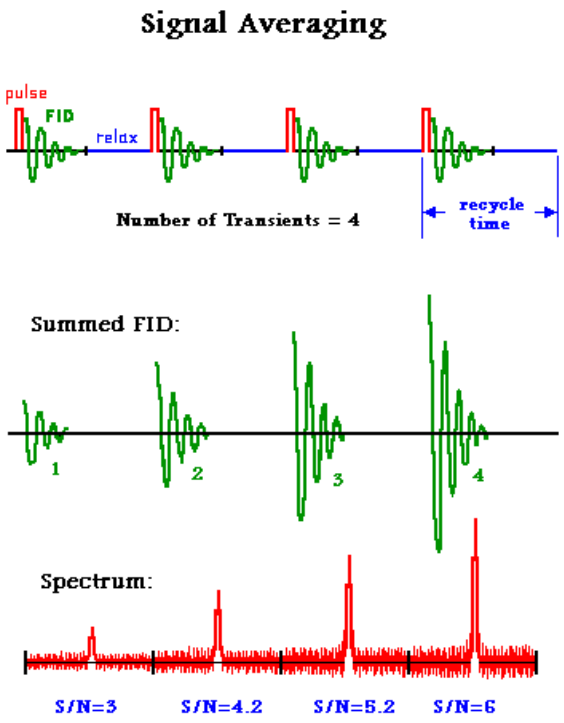


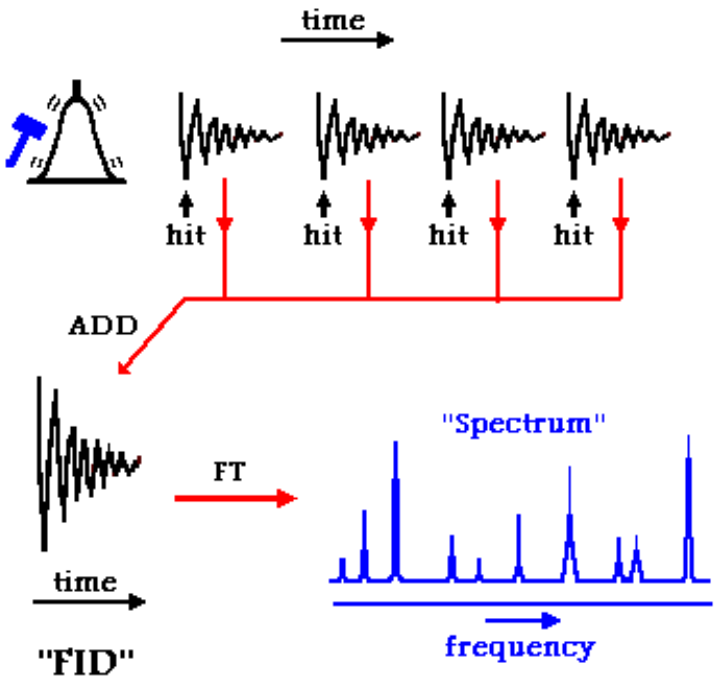
Dynamic NMR. NMR spectroscopy can also yield information about the motions of molecules in solution, including the overall tumbling of the molecule as well as conformational changes and bond rotation. There are many ways in which molecular motions on a number of different time scales can affect NMR relaxation rates and the appearance of NMR resonance peaks. The simplest effect occurs when a given nucleus in a molecule changes its magnetic environment, and thus its chemical shift, as a result of a simple molecular motion. For example, the methyl groups in N,N-dimethylformamide (DMF) change places as a result of the relatively slow rotation about the amide bond. The protons of the methyl group closer to the carbonyl oxygen have a larger chemical shift (2.94 ppm) than the other site (2.79 ppm) so that the resonant frequency of a given nucleus is bouncing back and forth between these two chemical shifts as the bond rotates. A "shutter speed" can be defined for the NMR experiment as the reciprocal of the difference in chemical shift (in Hz) between the two environments:

"shutter speed" = 1 / ((2.94 ppm - 2.79 ppm) x (200 Hz / ppm)) = 1 / (30 Hz) = 0.033 s

Slow exhange means that each nucleus is entirely in one environment during the shutter time, so that the motion is "frozen" and two sharp peaks are observed for different nuclei in the two environments. Heating the sample speeds up the exchange so that a blur is observed as nuclei move back and forth between chemical environments during the shutter time. At even higher temperature, the average nucleus moves back and forth so many times during the shutter time that a single sharp peak is observed at the average of the two chemical shifts (fast exchange). Study of this behavior as a function of temperature leads to determination of the rate constant and the activation energy for the bond rotation.

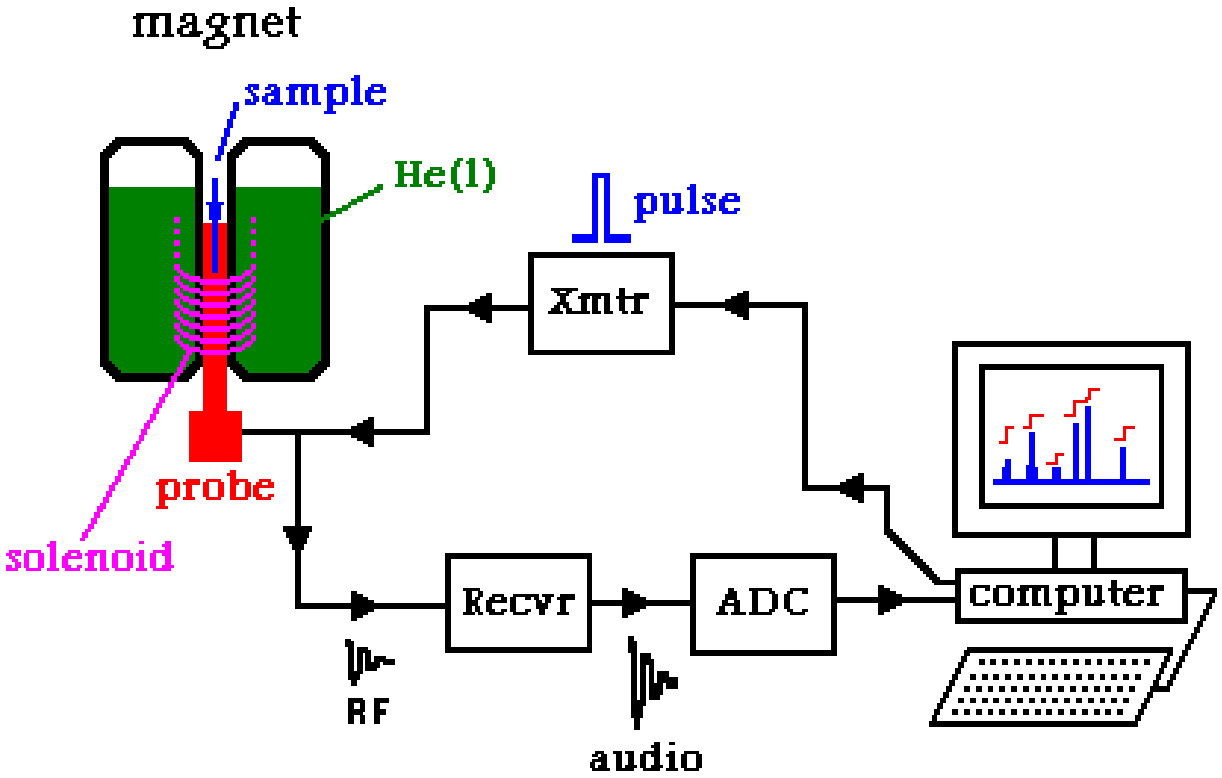
Pulsed Fourier Transform (FT) NMR. The first NMR spectrometers recorded a spectrum by slowly changing the frequency of a radio frequency signal fed into a coil near the sample. During this gradual "sweep" of frequencies the absorption of energy by the sample was recorded by a pen in a chart recorder. When the frequency passed through a resonant frequency for a group of nuclei in the sample, the pen went up and recorded a "peak" in the spectrum. This type of spectrometer, now obsolete, is called "Continuous Wave" or CW. Modern NMR spectrometers operate in the "pulsed Fourier-Transform" (FT) mode, permitting the entire spectrum to be recorded in 2-3 seconds rather than 5 minutes. The collection of nuclei (sample) is given a strong radio-frequency pulse which aligns the nuclei so that they precess in unison, each pointing in the same direction at the same time. The individual magnetic fields of the nuclei add together to give a measureable rotating magnetic field which induces an electrical voltage in a coil placed next to the sample. Over a period of a second or two the individual nuclei get out of synch and the macroscopic signal dies down. This "echo" observed in the coil is called the Free Induction Decay (FID), and it contains all of the resonant frequencies of the sample nuclei combined in one cacaphonous reply. This data is digitized and a computer performs a Fast Fourier Transform to convert it from an FID signal as a function of time (time domain) to a plot of intensity as a function of frequency (frequency domain). This "spectrum" has one peak for each resonant frequency in the sample. The real advantage of the pulsed-FT method is that, because the data is recorded so rapidly, the process of pulse excitation and recording the FID can be repeated many times, each time adding the FID data to a sum stored in the computer. The signal intensity increases in direct proportion to the number of repeats or "transients", but the random noise tends to cancel because it can be either negative or positive, resulting in a noise level proportional to the square root of the number of transients. Thus the signal-to-noise ratio increases with the square root of the number of transients. This signal-averaging process results in vastly improved sensitivity over the old frequency sweep method.





The process is analogous to hitting a bell with a hammer and recording the signal from the decaying sound coming out of a microphone. If the sound signal is digitized and summed for numerous repeated hammer blows, the resulting time domain signal contains all of the resonant frequencies of the bell. A Fourier transform will then convert the data to a "spectrum" - a graph of signal intensity as a function of frequency, revealing all of the resonant frequencies of the bell as well as their relative intensities.

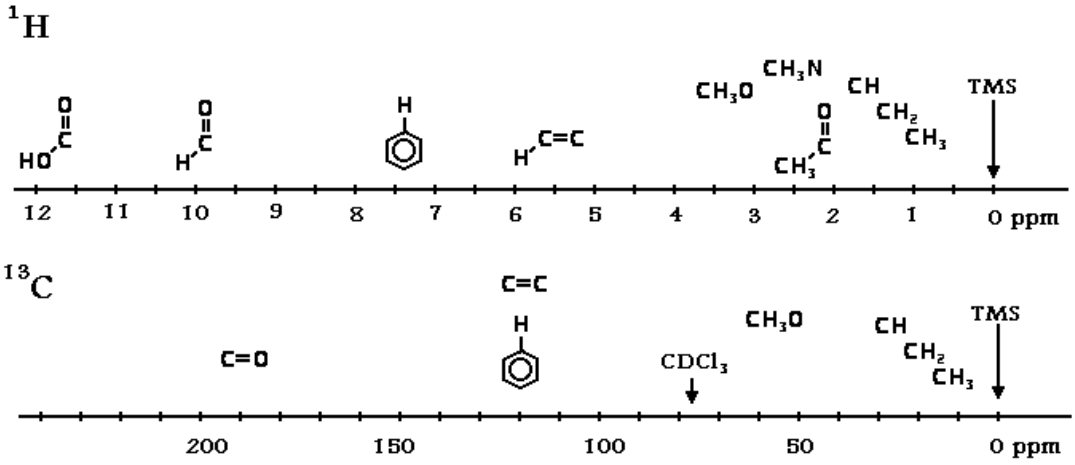
NMR Hardware. An NMR spectrometer consists of a superconducting magnet, a probe, a radio transmitter, a radio receiver, an analog-to-digital converter (ADC) and a computer. The magnet consists of a closed loop ("solenoid") of superconducting Nb/Ti alloy wire immersed in a bath of liquid helium (4°K). A large current flows effortlessly around the loop, creating a strong



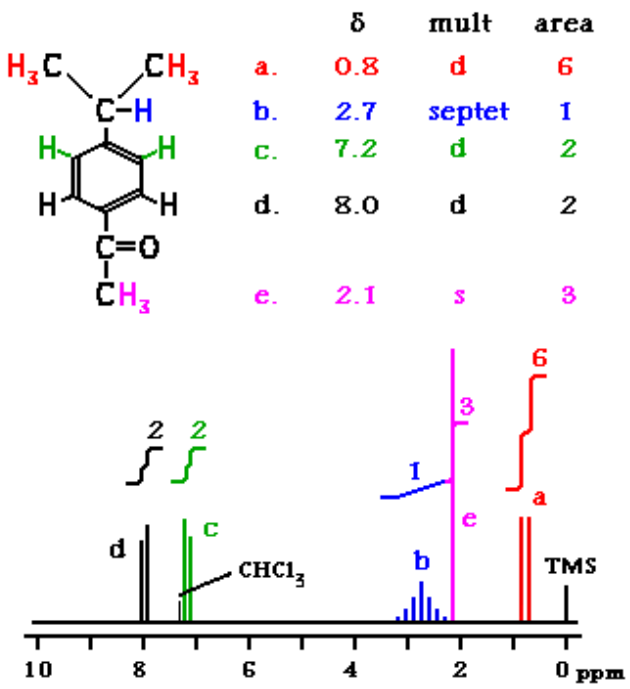
continuous magnetic field with no external power supply. The helium can ("dewar") is insulated with a vacuum jacket and further cooled by an outer dewar of liquid nitrogen (77°K). The probe is basically a coil of wire positioned around the sample which alternately transmits and receives radio-frequency signals. The weak signal (FID) received by the probe coil is amplified, converted to an audio frequency signal, and sampled at regular intervals of time by the analog-to-digital converter to produce a digital FID signal, which is really just a list of numbers. The computer determines the timing and intensity of pulses output by the transmitter, and receives and processes the digital information supplied by the analog-to-digital converter. The spectrum can be displayed on the computer monitor and plotted on paper with a digital plotter. The cost of an NMR instrument is $120,000 to $3,000,000, depending on the strength of the magnetic field (200 to 750 MHz proton frequency).

Sample Preparation. NMR spectra are usually measured using a solution of the compound of interest. For ^{1}H NMR, the solvent must be modified so that the solvent ^{1}H signal does not overwhelm the solute signals. Many solvents are available in deuterated form, such that all the ^{1}H atoms are replaced by ^{2}H (deuterium). The deuterium nuclei resonate at 30.7 MHz in a 200 MHz magnet, so they are effectively invisible to ^{1}H NMR spectroscopy. Commonly used solvents include deuterated water (D_2O), chloroform ($CDCl_3$), acetone (CD_3COCD_3), DMSO (CD_3SOCD_3), methanol (CD_3OD) and benzene (C_6D_6). These solvents are 99% or more deuterium at each site, but the residual ^{1}H still shows up as a peak in the proton spectrum. The ^{2}H signal from the solvent is used by the spectrometer as a "lock" signal to prevent the magnetic field from changing during the experiment. Tetramethylsilane ($(CH3)_4Si$, "TMS") is often added to the solvent to provide a reference peak at zero ppm for ^{1}H and ^{13}C. Samples should be prepared as homogeneous solutions with 5-10 mg (^{1}H spectra) or 30-50 mg (^{13}C spectra) of solute in a total volume of about 0.7 mL (4.5 cm deep in a 5-mm NMR tube). Larger volumes are wasteful of deuterated solvent, and smaller volumes make it very difficult to obtain a homogenous magnetic field ("shim").

Solvent	Deuterated solvent	Residual proton	Peak shape
$CHCl_3$	$CDCl_3$	7.26	singlet
acetone	CD_3COCD_3	2.04	quintet
DMSO	CD_3SOCD_3	2.49	quintet
water	D_2O	4.6	singlet
methanol	CD_3OD	3.31	quintet
benzene	C_6D_6	7.15	broad



The chemical shifts of ^{1}H and ^{13}C signals is affected by the proximity of electronegative atoms (O, N, Cl, etc.) in the bonding network and by the proximity to unsaturated groups (C=C, C=O, aromatic) in space. Electronegative groups shift resonances downfield, while unsaturated groups shift downfield when the affected nucleus is in the plane of the unsaturation, but have the opposite effect in regions above and below this plane. Nuclei can be equivalent (same chemical shift) by symmetry within a molecule (e.g., the two methyl carbons in CH_3COCH_3), or by rapid rotation around single bonds (e.g., the three methyl protons in CH_3CO_2H). The intensity (integrated peak area) of ^{1}H signals is directly proportional to the number of equivalent nuclei represented by that peak. For example, a CH_3 peak in a molecule would have 3 times the integrated peak area of a CH peak in the same molecule.



Examples. An example of a ^{1}H (proton) NMR spectrum is shown for 4-isopropylacetophenone. The two isopropyl methyl groups are equivalent by symmetry, and each methyl group has three protons made equivalent by rapid rotation about the C-C bond. This makes all six H_a (red) protons equivalent. Because they are far from any electronegative atom, these protons have a chemical shift typical of an isolated CH_3 group: 0.8 ppm (see diagram of typical shift values for ^{1}H). The absorbance is split into two peaks (a doublet) by the single neighboring H_b proton (blue). The six H_a (red) protons do not split each other because they are equivalent. The integrated area of the doublet is 6.0, since there are six H_a protons in the molecule. The H_b (blue) proton is split by all six of the H_a (red)

protons, so its absorbance shows up as a septet (seven peaks with intensity ratio 1:6:15:20:15:6:1). Its integrated area is 1.0, and its chemical shift is downfield of an isolated CH_2 (1.2 ppm) because of its proximity to the unsaturated aromatic ring (close to the plane of the aromatic ring so the effect is a downfield shift). The H_e (purple) methyl group protons are all equivalent due to rapid rotation of the CH_3 group, and their chemical shift is typical for a methyl group adjacent to the unsaturated C=O group. There are no neighboring protons (the H_d (black) proton is 5 bonds away from it, and the maximum distance for splitting is 3 bonds) so the absorbance appears as a single peak ("singlet") with an integrated area of 3.0. The H_c (green) and H_d (black) protons on the aromatic ring appear at a chemical shift typical for protons bound directly to an aromatic ring, with the H_d (black) protons shifted further downfield by proximity to the unsaturated C=O group. Each pair of aromatic protons is equivalent due to the symmetry of the aromatic ring. The H_c (green) absorbance is split into a doublet by the neighboring H_d (black) proton (note that from the point of view of either of the H_c (green) protons, only one of the H_d (black) protons is close enough to cause splitting) and the H_d (black) absorbance is split in the same way. Note that the J value (separation of split peaks) is the same for the H_c and H_d doublets, but slightly different for the H_a - H_b splitting. In this way we know, for example, that H_a is not split by either H_c or H_d.

The ^{13}C spectrum of the same compound is diagrammed below. Several differences can be seen in comparison with the ^{1}H spectrum. First, there is no spin-spin splitting due to adjacent carbons. This is because of the low natural abundance of ^{13}C, which is only 1.1%. Thus the probability of a ^{13}C occuring next to another ^{13}C is very low and splitting is not observed since ^{12}C has no magnetic properties. Second, there is no spin-spin splitting due to the protons attached to each carbon. This is prevented intentionally by a process called **decoupling**, in which all the protons in the molecule are simultaneously irradiated with continuous low-power radio frequency energy at the proton resonance frequency. This causes each proton to flip rapidly between the upper and lower (disaligned and aligned) energy states, so that the ^{13}C nucleus sees only the average of the two states and appears as a singlet, regardless of the number of attached protons. The lack of any spin-spin splitting in decoupled ^{13}C spectra means that each carbon always appears as a singlet. The multiplicity (s, d, t, q) indicated for each carbon in the diagram is observed only with the decoupler turned off and is not shown in the spectrum. Third, the peaks are not integrated because the peak area does not indicate the number of carbon atoms accurately. This is because ^{13}C nuclei **relax** more slowly than protons, so that unless a very long relaxation delay between repetitive pulses is used, the population difference between the two energy states of ^{13}C is not re-established before the next pulse arrives. Quaternary carbons, which have no attached protons, relax particularly slowly and thus show up with very low intensity.

The molecular symmetry, indicated by a dotted line where the mirror plane intersects the plane of the paper, makes the two isopropyl methyl carbons C_a (red) equivalent. Their chemical shift is a bit downfield of an isolated methyl group due to the steric crowding of the isopropyl group. Unlike protons, ^{13}C nuclei are sensitive to the degree of substitution or branching in the immediate vicinity, generally being shifted downfield by increased branching. C_b (blue) is shifted further downfield because of direct substitution (it is attached to three other carbons) and proximity to the aromatic ring. C_h (purple) is in a relatively uncrowded environment, but is shifted downfield by proximity to the unsaturated and electronegative carbonyl group. With the decoupler turned off, CH_3 carbons appear as quartets because of the three neighboring protons.

The aromatic CH carbons C_d (dark green) and C_e (black) are in nearly identical environments typical of aromatic carbons, and each resonance peak represents two carbons due to molecular symmetry. With the decoupler turned off, these peaks turn into doublets due to the presence of a single attached proton. The two quaternary aromatic carbons C_c (light green) and C_f (yellow) are shifted further downfield by greater direct substitution (they are attached to three other carbons) and by steric crowding (greater remote substitution) in the case of C_c and proximity to a carbonyl group in the case of C_f. The chemical shift of the carbonyl carbon C_g (light blue) is typical for a ketone. All three of the quaternary carbons C_c, C_f and C_g have low peak intensities due to slow relaxation (re-establishment of population difference) in the absence of directly attached protons.