

Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy involves absorption of radiation in the radio-frequency region (about 4 to 900 MHz). In this type of spectroscopy, nuclei of atoms rather than outer electrons are affected, and in order for the nuclei to have the energy states for the energy to be absorbed, they must be placed in an intense magnetic field.

The theoretical foundations of this type of spectroscopy were laid by Pauli in 1924 when he suggested that certain nuclei would have spin and magnetic moment properties that would lead to splitting of their energy levels in a magnetic field. These postulates were experimentally verified in the following decade, but it was not until 1946 that Bloch and Purcell independently demonstrated that nuclei absorb electromagnetic radiation when placed in a strong magnetic field due to energy level splitting induced by that field, for which they were jointly awarded the Nobel prize in 1952. The first commercially available high-resolution instrument appeared the next year, and the growth of the field has been explosive ever since. The applications of NMR spectroscopy are both in chemical and biochemical structure elucidation and in quantitative determination of absorbing species.

The types of spectrometers available are *continuous-wave* (CW) and *pulsed* or *Fourier transform* (FT NMR) instruments. The former type, the only type available before about 1970, monitors absorption of the sample as either the frequency of the source or the strength of the magnetic field is scanned. In the pulsed instruments, the sample is irradiated with periodic pulses of RF energy directed through the sample at right angles to the magnetic field. These pulses produce a time-domain signal that decays in the interval between pulses. This signal is converted to a frequency-domain signal by Fourier transformation, and the resulting spectrum looks like that produced on a CW instrument.

Theory of Nuclear Magnetic Resonance

It is useful to use both classical and quantum mechanical explanations in discussing the NMR phenomenon. Quantum mechanics relates absorption frequencies to nuclear energy states, while classical mechanics gives a clear picture of the absorption process and how it is measured.

Quantum Mechanical Description

We assume that certain nuclei spin and thus have an angular momentum p . The maximum observable component of this angular momentum is *quantized* and is an integral or half-integral multiple of $h/2\pi$ (h is Planck's constant). The maximum number of spin components for p is based on its spin quantum number I . Thus the nucleus will have $2I + 1$ discrete states, whose angular momentum component in any given direction has values of $I, I-1, I-2, \dots, -I$. In the absence of an external field, the *energies* of the various states are *identical*. The four nuclei that have been of greatest value to researchers are ^1H , ^{13}C , ^{19}F , and ^{31}P , all of which have $I = 1/2$, and thus two spin states corresponding to $I = +1/2$ and $I = -1/2$. Heavier nuclei have spin quantum numbers ranging from *zero* to $9/2$, but we shall limit our discussion to nuclei with $I = 1/2$.

A spinning, charged nucleus creates a magnetic field analogous to that created by a current in a coil of wire. The magnetic moment μ is oriented along the axis of spin and is proportional to the angular momentum p :

$$\mu = \gamma p$$

where γ is a proportionality constant called the *magnetogyric ratio* dependent on the nucleus involved, and which will be shown to affect both frequency of absorbed energy and field strength.

[Magnetic properties of some nuclei with spin quantum number of $\frac{1}{2}$: Table 19-1 p. 500]

Energy Levels in a Magnetic Field

For nuclei with a spin quantum number of $\frac{1}{2}$, there are two magnetic quantum numbers m : $m = +\frac{1}{2}$ and $-\frac{1}{2}$. When the nucleus is brought into an external magnetic field B_0 , its magnetic moment becomes aligned in one of two directions with respect to the field. The potential energy E of a given quantum state is given by

[eqn. 19-3 p. 500]

In this case, the energies of the two quantum states are

$$E_{+\frac{1}{2}} = -(\gamma h/4\pi)B_0 \quad E_{-\frac{1}{2}} = (\gamma h/4\pi)B_0$$

The *difference* in the energy states is

[eqn. 19-4 p. 500]

[Fig. 19-1 p. 500]

Absorption of RF by Nuclei in a Magnetic Field

As we have seen in other types of spectroscopy, transitions between energy states can be brought about by absorption or emission of radiation of a frequency ν_0 with energy corresponding to the ΔE of the transition. Combining the Planck relationship ($E = h\nu$) with the above equation for ΔE , we obtain the frequency required to bring about the transition

[eqn. 19-5 p. 500]

The above equation allows us to determine the frequency required to bring about the transition in a given magnetic field for a given type of nucleus.

Distribution of Nuclei between Magnetic Quantum States

In the *absence* of a magnetic field, the energies of the magnetic quantum states of a nucleus are identical, thus a large number of protons will have an equal number of nuclei with magnetic quantum numbers of $m = +\frac{1}{2}$ and $m = -\frac{1}{2}$. However, when placed in a field, the nuclei will tend to orient so that the *lower* energy state ($m = +\frac{1}{2}$) predominates. We can readily calculate the extent of this predominance of the lower energy nuclei by combining the equation for ΔE with the Boltzmann equation

[eqn. 19-6 p. 501]

to get the relationship

[eqn. 19-7 p. 501]

Classical Description of NMR

A magnet spinning about its north-south axis, when subjected to a force from an applied magnetic field will experience a circular motion of its axis (*precession*) about the vector representing the applied field. The angular velocity of this motion (in radians per second) is given by

$$\omega_0 = \gamma B_0$$

This angular velocity can be converted to the frequency of precession ν_0 (the *Larmor frequency* ν_0) by dividing by 2π , thus

$$\nu_0 = \gamma B_0 / 2\pi$$

[Fig. 19-2 p. 502]

Classical Model of Absorption

Note that the Larmor frequency is identical to the frequency of absorbed radiation derived from a quantum mechanical treatment. The potential energy E of the precessing nucleus is

$$E = -\mu_z B_0 = -\mu B_0 \cos\theta$$

where μ is the magnetic moment of the particle, μ_z is the component of μ in the direction of the magnetic field, and θ is the angle between the magnetic field vector and the spin axis of the particle. Thus, when radio frequency energy is absorbed by the nucleus, the angle of precession θ must change. So for a nucleus with a spin quantum number of $\frac{1}{2}$, we can imagine that the absorption involves flipping the magnetic moment so that it is oriented in the direction *opposite* the field.

[Fig. 19-3 p. 503]

Absorption of Radiation in NMR

The criteria necessary for absorption of radiation to reverse the magnetic dipole are that it be a *magnetic force at right angles* to the fixed field that moves in a *circular path in phase with the precessing dipole*. The type of radiation that meets these criteria is *circularly polarized radiation* of an appropriate frequency. If the rotational frequency of the magnetic vector of the exciting radiation matches the precessional frequency of a nucleus, absorption and flipping of the magnetic dipole of the nucleus can occur.

The radiation produced by the RF coil that serves as a source for an NMR instrument is *plane polarized*. However, plane-polarized radiation consists of *d* and *l* circularly polarized radiation. The vector of the *d* component rotates in a clockwise direction, and the vector of the *l* component rotates in a counter-clockwise direction. The addition of these two vectors results in a vector *sum* that vibrates in a single plane.

[Fig. 19-4 p. 503]

Only the magnetic component of the exciting radiation that rotates in the precessional direction is absorbed.

Relaxation Processes in NMR

When nuclei of one type are exposed to radiation of a suitable frequency, absorption occurs because of the slight excess of those in the lower energy state when exposed to a strong magnetic field. Because this excess is small there is a risk that the absorption process will equalize the number of nuclei in the two states and cause the absorption *signal* to decrease and approach zero. In order to avoid this state of *saturation* of the spin system, the rate of relaxation of excited nuclei to their lower energy state must be as great or greater than the rate of absorption. Since radiation theory shows that the *probability* of spontaneous reemission of photons varies as the *cube* of frequency and that at radio frequencies this process does not occur to any great extent, *nonradiative* relaxation processes are important in NMR. To reduce saturation and produce a detectable absorption signal, relaxation time should be as short as possible (small lifetime of the excited state), but *width* of the absorption line is *inversely proportional* to lifetime, so higher relaxation rates lead to line-broadening, which degrades the resolution. These two opposing factors give optimum lifetimes of excited states of from 0.1 to 10 s for NMR spectroscopy.

Spin-Lattice Relaxation

Spin-lattice or *longitudinal relaxation* involves interaction of the excited nuclei with appropriate *magnetic components* of the field produced by the collection of all nuclei of the sample (the *lattice*). This lattice contains a continuum of magnetic components arising from the vibrational and rotation motion of its nuclei. Spin-lattice relaxation is a first-order decay characterized by a relaxation time T_1 , which is a measure of the *average* lifetime of an excited state. T_1 decreases as the mobility of lattice increases down to a minimum when further increase in lattice mobility *decreases* the possibility of a spin-lattice transition.

Spin-Spin Relaxation

Several other effects tend to reduce relaxation times and thus *broaden* NMR lines. These effects are grouped together as *spin-spin* or *transverse relaxation*, with a relaxation time T_2 . Two neighboring nuclei with identical *precession rates* but different magnetic quantum states can undergo an interchange of states due to interaction of magnetic fields. This does not change the *relative* population of the spin states, but does reduce the *average lifetime* of a *particular* excited nucleus, which results in line-broadening. T_2 values for crystalline or viscous samples are usually so small as to preclude them as samples unless special techniques are used.

Fourier Transform NMR

In *pulsed* NMR measurements, nuclei in a strong magnetic field are subjected *periodically* to very brief (1 to 10 μs) pulses of intense radio-frequency radiation (the frequency generally is on the order of 10^2 to 10^3 MHz). The interval T between the pulses is typically one to several seconds, during which a time-domain radio frequency signal (a *free-induction decay* or FID) is emitted by the excited nuclei as they relax. This FID signal is detected using a radio receiver coil that is perpendicular to the static magnetic field. Quite often the same coil is used to both pulse the sample and detect the decay signal. This detected signal is digitized and stored, and the time-domain decay signals from several pulses are usually averaged to improve the S/N ratio. These signal-averaged data are converted to a frequency-domain signal by Fourier transformation. Finally, digital filtering may be used to further improve the signal-to-noise ratio.

[Fig. 19-5 p. 505]

Pulsed Excitation

In the following discussion, it is helpful to view the effect of pulsed excitation on the *net* magnetic moment of the excess nuclei in the lower energy ($m = +\frac{1}{2}$) state in a *rotating frame of reference*. In this case the frame is rotating in the same direction as the *individual* magnetic moment vectors at the Larmor frequency. Before the pulse (a), the net magnetic moment M is on the z -axis. As the pulse is applied (b), the *magnetic* field (B_1) of the incident radiation interacts with the sample magnetization vector M (both are seen as *static*, the former along the x -axis and the latter along the z -axis, in the rotating frame of reference) in such a way that M experiences a torque that tips it off the z -axis in the y - z plane (c). The extent of that rotation α depends on the duration of the pulse τ .

$$\alpha = \gamma B_1 \tau$$

where α is the angle of rotation in radians.

[Fig. 19-6 a, b, & c: p. 506]

Pulsed Excitation (cont'd)

For many Fourier transform experiments, the pulse length is chosen so that α is 90° or $\pi/2$ radians (d). Typically, the pulse interval needed to accomplish this is about 1 to 10 μs , depending on the magnetic field of the incident radiation. Once the pulse is terminated (e), the nuclei begin to relax (through *spin-lattice* and *spin-spin* interactions) and return to their equilibrium positions. As a nucleus returns, there is a *reduction* in the M_y component of the magnetic moment and an *increase* in the M_z component.

[Fig. 19-6 d & e: p. 506]

Relaxation in a Rotating Frame of Reference

Spin-Lattice

Spin-Spin

[Fig. 19-7 p. 507]

Free Induction Decay (FID)

At the end of the pulse, when B_1 goes to zero, it becomes more convenient to look at the magnetic moment in a *fixed* (as opposed to rotating) coordinate system. Now the magnetic moment is rotating about the z -axis at the Larmor frequency, and the resulting radio frequency can be detected by a coil along the x -axis. As relaxation proceeds, this signal decays exponentially and approaches zero as the magnetic moment vector approaches the z -axis. This time-domain signal is stored and converted to a frequency-domain signal by Fourier transformation.

[Fig. 19-8 p. 508]

Free Induction Decay (cont'd)

If the exciting frequency ν differs from the Larmor frequency $\omega_0/2\pi$, as it often does, then the FID will be modulated by a sine wave of frequency $|\nu - \omega_0/2\pi|$.

[Fig. 19-9 p. 509]

Free Induction Decay (cont'd 2)

When two or more magnetically different nuclei are present, the FID signal develops a distinctive *beat* pattern as seen below in the time-domain ^{13}C spectrum of cyclohexene.

[Fig. 19-10 p. 510]

Effect of Chemical Environment on NMR

The frequency of RF radiation absorbed by a given nucleus is affected by its chemical environment (i.e., electrons and other nearby nuclei). These effects are actually what make NMR such a powerful tool for structure elucidation. The two main types of environmental effects are *chemical shift* and *spin-spin splitting*.

[Fig. 19-12 p. 511]

In the low-resolution spectrum (a) of ethanol above, there are three distinct signals for the three types of protons (-OH, -CH₂-, and -CH₃) in the molecule. The difference in the frequencies of absorption is called *chemical shift*. Note that the areas under the peaks correlate with the relative number of protons in each group. In the higher resolution spectrum (b), a secondary environmental effect is noted in the splitting of two of the low-resolution peaks into additional peaks, which is termed *spin-spin splitting*.

Chemical Shift

Chemical shift is caused by small magnetic fields due to the circulation of electrons about the nucleus, usually *in opposition* to the applied field. Thus the nucleus is exposed to a field B_0 *less* than the applied field B_{app} .

$$B_0 = B_{\text{app}} - B_{\text{app}}\sigma = B_{\text{app}}(1 - \sigma)$$

where σ is the *screening constant*, and is determined by both the *electron density* and its *spatial distribution* around the nucleus. The frequency of excitation ν_0 for that nucleus becomes

[eqn. 19-14 p. 511]

where $k = \gamma B_0 / 2\pi$. From the above spectra for ethanol, we can see that σ for $-\text{CH}_3 > \sigma$ for $-\text{CH}_2- > \sigma$ for $-\text{OH}$.

Spin-Spin Splitting

Spin-spin splitting results from interaction of the magnetic moment of one nucleus with that of a nucleus *immediately adjacent*. The magnetic fields of the adjacent nuclei affect the electron distribution in the bonds to a nucleus, which in turn causes splitting in its energy levels. The splitting pattern of a peak is related to the number of nearby nuclei.

Abscissa Scales for NMR Spectra

Since it is difficult to measure the absolute strength of a magnetic field with the accuracy needed for a high-resolution spectrum, the *change in field strength* relative to some *internal standard* is used instead. The most common standard is tetramethylsilane [TMS: $(\text{CH}_3)_4\text{Si}$]. Its advantages are that its protons are identical and the screening constant is large enough that its single, sharp peak is isolated at *higher* applied field than most protons of interest. Its main drawback is that it is not soluble in water, so for aqueous sample another standard must be used, usually the sodium salt of 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS). Peak positions are given relative to that of the standard, either in Hz, or in *chemical shift* δ .

[Fig. 19-13 p. 512]

One advantage of δ is that it is independent of magnetic field strength.

Chemical Shift

As noted before, the phenomenon of chemical shift arises from the interaction of the electrons about a given nucleus with the magnetic field. In its simplest form we may view the shift as due to the secondary magnetic field created when the electrons in a magnetic field precess about the nucleus in a plane perpendicular to the magnetic field. This secondary field *opposes* the primary, so the nucleus experiences a net magnetic field slightly *less* than the applied field. That is, it is *shielded* from the applied field to varying degrees, based on the electron environment, which is different for different chemical structures.

[Fig. 19-14 p.514]

One example of the effect of structure on chemical shift is that of substitution of various halides on methyl protons ($X-CH_3$, where $X = F, Cl, Br, \text{ or } I$), where δ decreases with electronegativity. This is because the more highly electronegative substituents decrease the electron density (and thus the shielding) of the methyl protons.

Magnetic Anisotropy

Many compounds with double or triple bonds have chemical shifts that cannot be explained by local diamagnetic effects. To account for the δ values of these compounds we must consider the *anisotropic* magnetic properties of the compounds, or the effect of orientation in the field. As we see below, if we orient the molecule so that electron currents in the multiple bonds are perpendicular to the magnetic field, the induced *magnetic* fields can either augment or decrease the net magnetic field experienced by the protons. In other words, magnetic anisotropy can result in *either* shielding *or* deshielding.

[Figs. 19-15 p. 514 & 19-16 p. 515]

Chemical shift is used to identify functional groups and aid in determining their arrangements. Correlation charts are used in conjunction with the spectrum itself, but we must bear in mind that exact values for δ may depend on such factors as solvent and sample concentration.

[Approx. chemical shifts for some methyl, methylene, and methane protons: Table 19-2 p. 517]

[Absorption positions of protons in various structural environments: Fig. 19-17 p. 516]

Spin-Spin Splitting

As we saw in the high-resolution spectrum of ethanol, the peaks for the methyl and methylene protons were each split into several narrow peaks, and furthermore, the spacing between the narrow peaks was the same. This spacing (in Hz) is called the *coupling constant J*.

These effects may be attributed to the effect that the spins of one group of protons have on the other. If we remember that the ratio between the two possible spin states of a proton is very nearly *one*, even in a strong field, we can see that the distribution of possible spin states in methyl and methylene groups would be as follows:

methylene
p. 515, column 2, bottom

methyl
p. 517 column 1, bottom

**[Relative peak intensities in 1st order multiplets:
Table 19-3 p. 518]**

Interpretation of First Order NMR Spectra

Interpretation of spin-spin splitting patterns is relatively straightforward for *first-order* spectra, that is, those in which the chemical shift δ between interacting groups is large compared to their coupling constant J . *Rigorous* first order behavior requires the ratio $J/\Delta\nu$ be 0.05 or less, but in practice analysis of spectra by first order techniques can often be done at $J/\Delta\nu$ ratios up to about 0.1. Also it should be noted that because chemical shift values in $\Delta\nu$ changes with the field strength of the instrument but spin-spin coupling constants (J) do not, in many cases a first order spectrum can be obtained with an instrument with a larger magnet.

[List of six rules: p. 518]

Splitting by Two Sets of *Nonequivalent* Protons

The splitting pattern generated by two or more sets of *equivalent* protons is usually straightforward. But if the protons in question are split by two or more sets of *nonequivalent* protons, the resulting splitting pattern can become quite complex. For example, the spectrum of 1-iodopropane, $\text{CH}_3\text{CH}_2\text{CH}_2\text{I}$, will have chemical shift bands at $\delta = 1.02$ (methyl), $\delta = 1.86$ (central methylene), and $\delta = 3.17$ (terminal methylene with iodine). The bands for the protons on either end of the molecule will be split into triplets with relative areas of 1:2:1 by the central methylene protons. The band centered on $\delta = 1.86$ will, however, be split by two *nonequivalent* groups of three and two protons. Experimental coupling constants for these interactions are $J = 7.3$ Hz for $-\text{CH}_3$ and $J = 6.8$ for $-\text{CH}_2\text{I}$, and by the rules, the band should be split into $(2 + 1)(3 + 1)$ or 12 separate peaks.

[Fig. 19-18 p.519]

Effect of Chemical Exchange

In the high-resolution spectrum of ethanol seen earlier, we saw no spin-spin coupling between the -OH and $\text{-CH}_2\text{-}$ protons, although from the rules, coupling would be expected (the two groups of protons are separated by only three bonds). As we can see below, if a spectrum is taken of a *highly purified* sample of ethanol, the expected splitting *does* appear. However, if a trace of acid or base is added to the pure sample, the splitting pattern reverts to what was seen earlier.

[Fig. 19-19 p. 520]

Acids, bases, and other likely impurities are known to catalyze proton exchange in alcohols, and thus it is plausible to attribute the spin *decoupling* to this proton exchange. If proton exchange is rapid, any -OH group will have a number of different protons associated with it in a brief time interval. Each of these protons will be affected by the three energy states of the methylene protons, thus *averaging* the effect on the alcoholic proton, so it gives a single sharp signal. Chemical exchange can even cause changes in the *chemical shift*, as when impurities in alcohol-water mixtures cause the water and alcoholic proton peaks to coalesce.

Double Resonance Techniques

Double resonance experiments in NMR spectroscopy include techniques in which samples are irradiated with two or more signals of *different* radio frequency. For example, if we irradiate with a second signal that causes *saturation* of a particular group of protons, we can *decouple* those protons from any other nearby protons. This technique is called *homonuclear spin decoupling*.

[Fig. 19-20 p. 521]

Block Diagram of an FT NMR Spectrometer

[Fig. 19-21 p. 522]

Components of Fourier Transform NMR Instruments

In the following figure, the main components of a Fourier transform NMR spectrometer are shown. The central component of the instrument is a highly stable magnet in which the sample is placed. This sample is also surrounded by a transmitter/receiver *coil*. Radio frequency (RF) radiation is generated by a frequency synthesizer with an output of ν_c . This signal passes into a pulser switch and RF transmitter, which creates an intense and reproducible pulse of radio-frequency current in the transmitter coil. The resulting RF radiation impinges on the sample, which is placed inside the coil. The length, amplitude, shape, and phase of the pulse are chosen by the operator at the computer console. (A pulse of $5 \mu\text{s}$ is shown in the figure.) The resulting FID signal is picked up by the same coil (now a receiver), sent to an RF amplifier, and then transmitted to a phase-sensitive detector. This detector produces the *difference* between the nuclear signal, ν_n , and the crystal oscillator output, ν_c , which results in the low-frequency, *time domain* signal, which is then digitized and collected in the memory of the computer for analysis by a Fourier transform program and other data analysis software. This program produces the *frequency domain* spectrum as its final output.

Magnets: the magnet is the heart of any NMR instrument, either continuous wave or Fourier transform. As both sensitivity and resolution increase with magnetic field strength, a magnet that produces the highest field strength possible is desirable. Also the field must be homogeneous and reproducible. For these reasons, the magnet is by far the most expensive component of an NMR instrument.

Modern instruments employ superconducting solenoids to produce the magnetic field up to 21 T (900 MHz for protons). The solenoid is wound from niobium/tin or niobium/titanium wire and is bathed in liquid helium to keep it at 4 K. The helium dewar is in turn held in a liquid nitrogen dewar.

Corrections for Field Inhomogeneities

Even the superconducting solenoid magnets just described will not produce fields of the needed homogeneity (to a few ppb) of themselves (variations up to 100 ppb being observed over the period of one hour). Several strategies are used to produce the required stability: *field/frequency lock systems* (which monitor a reference nucleus and use changes in intensity of absorption of this reference to generate a feedback signal to correct the drift), *shim coils* through which carefully controlled currents are passed to produce small magnetic fields to compensate for small inhomogeneities in the primary field, and *sample spinning* along its longitudinal axis using a stream of air against a small plastic turbine that slips over the sample tube.

Applications of ^1H NMR

One of the most important *qualitative* applications of proton NMR spectroscopy for chemists is structure elucidation, especially with *organic* compounds. Together with data from other sources, such as atomic mass spectroscopy, an NMR spectrum for an unknown compound can often allow one to determine its chemical structure. This is done mainly through interpretation of peak positions and fine structure based on a combination of *chemical shift* and *spin-spin splitting patterns*.

Quantitative information can also be derived from NMR spectra as well, as we have seen in the laboratory experiment involving NMR in this course. The basis of quantitative applications is the fact that the strength of a given NMR absorption signal is proportional to the number of protons in that state, i.e., peak area is proportional to the number of protons *with a given chemical environment* on a particular molecule. If the peaks for protons on two different molecules in a sample are separated enough, the relative concentrations of the two molecules can be determined from the ratios of peak areas.

[Ex. 19-4, see Fig. 19-24 p. 527]

[Ex. 19-5, see Fig. 19-25 p. 527]

[Ex. 19-6, see Fig. 19-26 p. 528]

Carbon-13 NMR

Carbon-13 NMR was first studied in 1957, but did not come into widespread use until the 1970's. The problem that had to be overcome was the low sensitivity of the technique due to both the low natural abundance of the isotope (1.1%) and the relatively small magnetogyric ratio (roughly 0.25 that of the proton). Signal enhancement due to more powerful magnets and employment of Fourier transform design (higher throughput, once again) has made ^{13}C magnetic resonance spectroscopy an important field today.

Carbon-13 NMR has some important advantages over proton NMR, especially as a tool for structure elucidation. First, it produces information about the *backbone* of an organic molecule, rather than about the periphery. Secondly, the shift range for ^{13}C is about 200 ppm (compared to 10-15 ppm for protons), so there is less overlap of peaks in ^{13}C spectra than in proton spectra. Thirdly, homonuclear spin-spin coupling between ^{13}C atoms is *not* observed in natural abundance samples because the probability of *two* ^{13}C atoms occurring next to each other in the same molecule is negligible. *Heteronuclear* spin-spin coupling between ^{12}C and ^{13}C does not occur because the spin quantum number for ^{12}C is *zero*. Finally, there are a number of excellent methods of decoupling the interaction between ^{13}C and protons.

Proton Decoupling in ^{13}C NMR

Broad-band decoupling: heteronuclear spin-spin splitting between ^{13}C and protons can be totally eliminated by irradiating the sample with broad-band radio-frequency radiation covering the range that encompasses the entire *proton* spectral region, while at the same time collecting the ^{13}C spectrum in the usual manner. In effect, we are *saturating* the protons. The proton signal is usually produced by a second coil in the sample probe.

[Fig. 19-27 p. 530]

Proton Decoupling in ^{13}C NMR

Off-resonance decoupling: if the decoupling frequency is set 1000 to 2000 Hz *above* the proton spectral region, the spectrum produced will be *partially* decoupled so that all but the largest spin-spin shifts are absent. Under these circumstances only the multiplicity of the protons on that carbon-13 atom are seen, so the peaks give more detail about the individual carbons (primary carbons appear as *quartets*, secondary as *triplets*, tertiary as *doublets*, and quaternary as *singlets*).

[Fig. 19-28 p. 531]

Nuclear Overhauser Enhancement

Under conditions of broad-band coupling, it has been found that the areas of ^{13}C peaks are enhanced by a factor that is significantly greater than what would be expected from the collapse of the multiplets to single lines. This is an example of a phenomenon known as *nuclear Overhauser enhancement* (NOE), which is a general effect encountered in decoupling experiments. It arises from direct magnetic coupling between a decoupled proton and a neighboring ^{13}C atom, which results in an increased population of the *lower* energy state of the ^{13}C nucleus over that predicted from the Boltzmann relationship. The ^{13}C signal can be increased by as much as a factor of *three*. While this NOE does increase the sensitivity of ^{13}C measurements, it has the disadvantage that the proportionality peak areas and the number of equivalent ^{13}C nuclei may be lost.

Application of NMR to Other Nuclei

While over 200 isotopes have magnetic moments and are thus susceptible to study by NMR techniques, the most important to date (besides protons and ^{13}C) are ^{31}P and ^{19}F , the former for its importance in biochemical applications, and the latter for its similarity to proton NMR behavior.