**General Introduction**

Nuclear magnetic resonance (NMR) is a physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation. This energy is at a specific resonance frequency which depends on the strength of the magnetic field and the magnetic properties of the isotope of the atoms; in practical applications, the frequency is similar to VHF and UHF television broadcasts (60–1000 MHz). NMR allows the observation of specific quantum mechanical magnetic properties of the atomic nucleus. Many scientific techniques exploit NMR phenomena to study molecular physics, crystals, and non-crystalline materials through NMR spectroscopy. NMR is also routinely used in advanced medical imaging techniques, such as in magnetic resonance imaging (MRI).Most of the matter you can examine with NMR is composed of molecules. Molecules are composed of atoms. Here are a few water molecules. Each water molecule has one oxygen and two hydrogen atoms. Atoms contain nucleus. Nucleus composed of a single proton. The proton possesses a property called spin which:

1. can be thought of as a small magnetic field, and
2. Will cause the nucleus to produce an NMR signal.

\*Not all nuclei possess the property called spin.

**Spectroscopy**

Spectroscopy is the study of the interaction of electromagnetic radiation with matter. Nuclear magnetic resonance spectroscopy is the use of the NMR phenomenon to study physical, chemical, and biological properties of matter. NMR spectroscopy is routinely used by chemists to study chemical structure using simple one-dimensional techniques. Two-dimensional techniques are used to determine the structure of more complicated molecules. These techniques are replacing x-ray crystallography for the determination of protein structure. Time domain NMR spectroscopic techniques are used to probe molecular dynamics in solutions. Solid state NMR spectroscopy is used to determine the molecular structure of solids. Other scientists have developed NMR methods of measuring diffusion coefficients.

## Principles of NMR:

The principle of NMR usually involves two sequential steps:

* The alignment of the magnetic nuclear spins in an applied, constant magnetic field **H**0.
* The perturbation of this alignment of the nuclear spins by employing an electro-magnetic, usually radio frequency (RF) pulse. The required perturbing frequency is dependent upon the static magnetic field (**H**0) and the nuclei of observation.

The two fields are usually chosen to be perpendicular to each other as this maximizes the NMR signal strength. The resulting response by the total magnetization (**M**) of the nuclear spins is the phenomenon that is exploited in NMR spectroscopy and magnetic resonance imaging. Both use intense applied magnetic fields (**H**0) in order to achieve dispersion and very high stability to deliver spectral resolution.

All isotopes that contain an odd number of protons and of neutrons have an intrinsic magnetic moment and angular momentum, in other words a non zero spin, while all nuclides with even numbers of both have a total spin of zero. The most commonly studied nuclei are 1H and 13C, although nuclei from isotopes of many other elements (e.g. 1H, 13C, 15N, 19F, 31P).

A key feature of NMR is that the resonance frequency of a particular substance is directly proportional to the strength of the applied magnetic field. It is this feature that is exploited in imaging techniques; if a sample is placed in a non-uniform magnetic field then the resonance frequencies of the sample's nuclei depend on where in the field they are located. Since the resolution of the imaging technique depends on the magnitude of magnetic field gradient, many efforts are made to develop increased field strength, often using superconductors. The effectiveness of NMR can also be improved using hyper polarization, and/or using two-dimensional, three-dimensional and higher-dimensional multi-frequency techniques.

## Theory of nuclear magnetic resonance

**Nuclear spin and magnets:**

All nucleons, that are neutrons and protons, composing any atomic nucleus, have the intrinsic quantum property of spin. The overall spin of the nucleus is determined by the spin quantum number *S*. If the number of both the protons and neutrons in a given nuclide are even then *S* = 0, i.e. there is no overall spin. Then, just as electrons pair up in atomic orbitals, so do even numbers of protons or even numbers of neutrons (which are also spin-1⁄2 particles and hence fermions) pair up giving zero overall spin.

However, a proton and neutron will have lower energy when their spins are parallel, not anti-parallel, since this parallel spin alignment does not infringe upon the Pauli Exclusion Principle, but instead it has to do with the quark structure of these two nucleons. Therefore, the spin ground state for the deuteron (the deuterium nucleus, or the 2H isotope of hydrogen)—that has only a proton and a neutron—corresponds to a spin value of 1, not of zero. The single, isolated deuteron therefore exhibits an NMR absorption spectrum characteristic of a quadrupolar nucleus of spin 1, which in the "rigid" state at very low temperatures is a characteristic.

On the other hand, because of the Pauli Exclusion Principle, the tritium isotope of hydrogen must have a pair of anti-parallel spin neutrons (of total spin zero for the neutron-spin pair), plus a proton of spin 1/2. Therefore, the character of the tritium nucleus is again magnetic dipolar, not quadrupolar—like its non-radioactive deuteron cousin—and the tritium nucleus total spin value is again 1/2, abundant hydrogen isotope, 1H nucleus (the proton). The NMR absorption (radio) frequency for tritium is however slightly higher than that of 1H because the tritium nucleus has a slightly higher gyro magnetic ratio than 1H. In many other cases of non-radioactive nuclei, the overall spin is also non-zero. For example, the 27Al nucleus has an overall spin value *S* = 5⁄2.



**Values of spin angular momentum**

The angular momentum associated with nuclear spin is quantized. This means both that the magnitude of angular momentum is quantized (i.e. *S* can only take on a restricted range of values), and also that the orientation of the associated angular momentum is quantized. The associated quantum number is known as the magnetic quantum number, *m*, and can take values from +*S* to −*S*, in integer steps. Hence for any given nucleus, there are a total of 2*S* + 1 angular momentum states.

The z-component of the angular momentum vector (**S**) is therefore Sz = *mħ*, where *ħ* is the reduced Planck constant. The z-component of the magnetic moment is simply:



#### Spin behavior in a magnetic field

Consider nuclei which have a spin of one-half, like 1H, 13C or 19F. The nucleus has two possible spin states: *m* = 1⁄2 or *m* = −1⁄2. These states are degenerate that is they have the same energy. Hence the number of atoms in these two states will be approximately equal at thermal equilibrium.

If a nucleus is placed in a magnetic field, however, the interaction between the nuclear magnetic moment and the external magnetic field mean the two states no longer have the same energy. The energy of a magnetic moment **μ** when in a magnetic field **B**0 is given by:



Usually the *z* axis is chosen to be along **B**0, and the above expression reduces to:



Or alternatively:



As a result the different nuclear spin states have different energies in a non-zero magnetic field. In less formal language, we can talk about the two spin states of a spin 1⁄2 as being *aligned* either with or against the magnetic field. If γ is positive (true for most isotopes) then *m* = 1⁄2 is the lower energy state.

The energy difference between the two states is:



And this difference results in a small population bias toward the lower energy state.

#### Magnetic resonance by nuclei

Resonant absorption by nuclear spins will occur only when electromagnetic radiation of the correct frequency (e.g., equaling the Larmor precession rate) is being applied to match the energy difference between the nuclear spin levels in a constant magnetic field of the appropriate strength. The energy of an absorbed photon is then *E* = *h*ν0, where ν0 is the resonance radiofrequency that has to match (that is, it has to be equal to the Larmor precession frequency νL of the nuclear magnetization in the constant magnetic field **B**0). Hence, a magnetic resonance absorption will only occur when Δ*E* = *h*ν0, which is when ν0 = γ*B*0/(2π). Such magnetic resonance frequencies typically correspond to the radio frequency (RF) range of the electromagnetic spectrum for magnetic fields up to roughly 20 T. It is this magnetic resonant absorption which is detected in NMR.

## Instrumentation

There are two general types of NMR instrument;

* continuous wave and
* Fourier transforms.

Early experiments were conducted with continuous wave (C.W.) instruments, and in 1970 the first Fourier transform (F.T.) instruments became available. This type now dominates the market.

**Continuous wave NMR instruments**

In its first few decades, nuclear magnetic resonance spectrometers used a technique known as continuous-wave spectroscopy (CW spectroscopy). Although NMR spectra could be, obtained using a fixed magnetic field and sweeping the frequency of the electromagnetic radiation, this more typically involved using a fixed frequency source and varying the current (and hence magnetic field) in an electromagnet to observe the resonant absorption signals. This is the origin of the counterintuitive, but still common, "high field" and "low field" terminology for low frequency and high frequency regions respectively of the NMR spectrum.

CW spectroscopy is inefficient in comparison with Fourier analysis techniques since it probes the NMR response at individual frequencies in succession. Since the NMR signal is intrinsically weak, the observed spectrum suffers from a poor signal-to-noise ratio. This can be mitigated by signal averaging i.e. adding the spectra from repeated measurements. While the NMR signal is constant between scans and so adds linearly, the random noise adds more slowly – proportional to the square-root of the number of spectra (see random walk). Hence the overall signal-to-noise ratio increases as the square-root of the number of spectra measured

### Continuous wave NMR spectrometers are similar in principle to optical spectrometers. The sample is held in a strong magnetic field, and the frequency of the source is slowly scanned (in some instruments, the source frequency is held constant, and the field is scanned).

### cont wave nmr.jpg

**Fourier transform NMR instruments**

Most applications of NMR involve full NMR spectra, that is, the intensity of the NMR signal as a function of frequency. Early attempts to acquire the NMR spectrum more efficiently than simple CW methods involved illuminating the target simultaneously with more than one frequency. A revolution in NMR occurred when short pulses of radio-frequency radiation began to be used—centered at the middle of the NMR spectrum. In simple terms, a short pulse of a given "carrier" frequency "contains" a range of frequencies centered about the carrier frequency, with the range of excitation (bandwidth) being inversely proportional to the pulse duration, i.e. the Fourier transform of a short pulse contains contributions from all the frequencies in the neighborhood of the principal frequency. The restricted range of the NMR frequencies made it relatively easy to use short (millisecond to microsecond) radio frequency pulses to excite the entire NMR spectrum. The magnitude of the energy changes involved in NMR spectroscopy is small. This means that sensitivity is a major limitation. One way to increase sensitivity would be to record many spectra, and then add them together; because noise is random, it adds as the square root of the number of spectra recorded. For example, if one hundred spectra of a compound were recorded and summed, then the noise would increase by a factor of ten, but the signal would increase in magnitude by a factor of one hundred - giving a large increase in sensitivity. However, if this is done using a continuous wave instrument, the time needed to collect the spectra is very large (one scan takes two to eight minutes).

In FT-NMR, all frequencies in a spectrum are irradiated simultaneously with a radio frequency pulse. Following the pulse, the nuclei return to thermal equilibrium. A time domain emission signal is recorded by the instrument as the nuclei relax. A frequency domain spectrum is obtained by Fourier transformation.

Applying such a pulse to a set of nuclear spins simultaneously excites all the single-quantum NMR transitions. In terms of the net magnetization vector, this corresponds to tilting the magnetization vector away from its equilibrium position (aligned along the external magnetic field). The out-of-equilibrium magnetization vector processes about the external magnetic field vector at the NMR frequency of the spins. This oscillating magnetization vector induces a current in a nearby pickup coil, creating an electrical signal oscillating at the NMR frequency. This signal is known as the free induction decay (FID), and it contains the vector sum of the NMR responses from all the excited spins. In order to obtain the frequency-domain NMR spectrum (NMR absorption intensity vs. NMR frequency) this time-domain signal (intensity vs. time) must be Fourier transformed. Fortunately the development of Fourier Transform NMR coincided with the development of digital computers and the digital Fast Fourier Transform. Fourier methods can be applied to many types of spectroscopy.



### Experimental Considerations for NMR

### Temperature, Tuning, Locking, Shimming

The sample for an NMR experiment should not contain any particulate matter that may affect the field homogeneity within the sample. After the sample is stopped in the magnet it is necessary to tune the probe to get the most effective power transferred to the sample, and the most effective detection of the signal. Tuning the probe involves altering the complex impedance of the coil to minimize the reflected power. (This needs to be performed once during installation). After the probe is tuned it is necessary to "lock" the spectrometer on the external LiCl sample.

**Adjusting the Transmitter**

The next step is set the frequency of the pulses and to adjust the sweep (or spectral width). In general, spectrometer frequencies are specified using two parameters, a fixed number that depends on the magnetic field strength of the instrument and the observed nuclei, and a user adjustable offset that is added to the fixed number to give the frequency of the transmitted pulse. At the beginning the user defined offset is set to zero and a very large spectral width is used (e.g. 20 KHz). With such a wide window all of the resonance lines should fall in this frequency range. Since the time required for the 90o pulse is not yet known, the first spectrum is obtained using a short (6 μs) pulse. Once the position of the resonance peaks has been determined, the offset is moved to the middle of the spectrum and the spectral width is adjusted to be just large enough to span all of the resonance lines in the spectrum. The 90o pulse length is determined by observing the effect of the pulse length on the spectral intensity or on the FID signal intensity (RMS – root mean signal). The spectrometer frequency is set to be 300-500 Hz from the resonance frequency of the main resonances in the spectrum. The pulse length is set to 4 μs (e.g. << 90o) and a spectrum is obtained. The spectrum is phased to produce a positive absorption line. The pulse length is increased until the line goes through a maximum value of RMS or signal intensity (90o) and then reaches zero (180o). The pulse length is then doubled to produce a 360o pulse. The pulse length is slightly modified to produce a null signal. Final adjustment of the pulse length using 360o pulses avoids the necessity of waiting for the spins to relax.

**Shim System**

The shim system consists of a number of small coils that surround the area of the sample, which is contained within the most homogeneous region of the magnet. The purpose of the shim coils is to provide a means to make slight adjustments to the static magnetic field to increase its homogeneity. There can be a large number of coils, each of which generates a small magnetic field, which is shaped like a spherical harmonic. Since these functions are orthogonal the field generated by each shim coil is, in theory, independent of the fields generated by the other shim coils. However, in practice there can be considerable interaction and it is usually necessary to adjust several coils at the same time. All magnets show drift or a change in field strength, over time. These changes are usually small enough that they can be compensated by adjusting the H transmitter frequency to match the magnetic field / shim changes.

**The Lock**

Changes in the magnetic field strength are detected by measuring the resonance position of Li in the external lock reference. Both the absorptive and dispersive components of the Li resonance line are used in adjusting for field in homogeneity. Based on the absolute frequency of the Li resonance the transmitter frequency is adjusted to compensate for changes in magnet, sample, or shim.

**Generating the Pulse**

The actual frequency of the RF pulse is generated by mixing two frequencies together. The first, ωsyn is adjustable and is generated from the frequency synthesizer under computer control. The second is an internal constant frequency called the intermediate frequency, IF. After mixing of these two frequencies and amplification the signal is sent to the probe. Since the same sample coil is used to both send the RF pulse and receive the FID it is necessary to route the RF pulse to the coil and not to the pre-amplifier. Otherwise the rather intense RF power may damage sensitive components in the preamplifier. This routing is accomplished by grounding the circuit (using diodes) 1/4λ from the junction point. Under these conditions the pre-amplifier side of the circuit appears as an infinite resistance and most of the RF pulse goes to the sample coil.

**Receiving the NMR Signal**

The induced transverse magnetization is detected using the same coil that carried the RF pulse to the sample. However, since the induced EMF in the coil is quite weak, the grounded point in the pre-amplifier is not seen as a ground and the signal can pass the 1/4λ coil. The frequency of the induced RF is quite high. This is an extremely fast rate (i.e. 58 MHz) and there are practical problems associated with operating analog to digital converters at this frequency. Instead of trying to sample the magnetization at 58 MHz, the frequency of the signal is reduced to that in the rotating frame (audio range) by mixing the signal first with ωsyn and then with the IF.

**How to Obtain Quantitative NMR Spectra**

The quantitative information of NMR is contained in the area under the various resonances. A quantitative spectrum is simply a spectrum where you can trust the integral values and ratios. In other words, if the integral of resonance *A* is twice the height of the integral of resonance *B*, you can say with certainty that resonance *A* is due to twice the number of nuclei as resonance *B*. In the DPS process NMR instrument all spectra are converted to integral values during the data processing step. We use integrals because it is the areaof the resonances that is proportional to the number nuclei. The height of a broad line may be less than that of a sharp line, but its area may be greater

## Basic NMR techniques

When placed in a magnetic field, NMR active nuclei (such as 1H or 13C) absorb electromagnetic radiation at a frequency characteristic of the isotope. The resonant frequency, energy of the absorption, and the intensity of the signal are proportional to the strength of the magnetic field. For example, in a 21 Tesla magnetic field, protons resonate at 900 MHz. It is common to refer to a 21 T magnet as a 900 MHz magnet, although different nuclei resonate at a different frequency at this field strength in proportion to their nuclear magnetic moments.

### Chemical Shift

A spinning charge generates a magnetic field that results in a magnetic moment proportional to the spin. In the presence of an external magnetic field, two spin states exist (for a spin 1/2 nucleus):

* spin up
* spin down

Where one aligns with the magnetic field and the other opposes it. The difference in energy (ΔE) between the two spin states increases as the strength of the field increases, but this difference is usually very small, leading to the requirement for strong NMR magnets (1-20 T for modern NMR instruments). Irradiation of the sample with energy corresponding to the exact spin state separation of a specific set of nuclei will cause excitation of those set of nuclei in the lower energy state to the higher energy state.

For spin 1/2 nuclei, the energy difference between the two spin states at a given magnetic field strength are proportional to their magnetic moments. However, even if all protons have the same magnetic moments, they do not give resonant signals at the same field/frequency values. This difference arises from the differing electronic environments of the proton. Upon application of an external magnetic field, these electrons move in response to the field and generate local magnetic fields that oppose the much stronger applied field. This local field thus "shields" the proton from the applied magnetic field, which must therefore be increased in order to achieve resonance (absorption of rf energy). Such increments are very small, usually in parts per million (ppm). The difference between 2.3487T and 2.3488T is therefore about 42ppm. However a frequency scale is commonly used to designate the NMR signals, even though the spectrometer may operate by sweeping the magnetic field, and thus the 42 ppm is 4200 Hz for a 100 MHz reference frequency (rf).

However the location of different NMR signals is depend on the external magnetic field strength and the reference frequency, the signals are usually reported relative to a reference signal, usually that of TMS (tetramethylsilane). Additionally, since the distribution of NMR signals is field dependent, these frequencies are divided by the spectrometer frequency. However since we are dividing Hz by MHz, the resulting number would be too small, and thus it is multiplied by a million. This operation therefore gives a locator number called the "chemical shift" with units of parts per million. To detect such small frequency differences the applied magnetic field must be constant throughout the sample volume. High resolution NMR spectrometers use shims to adjust the homogeneity of the magnetic field to parts per billion (ppb) in a volume of a few cubic centimeters. In general, chemical shifts for protons are highly predictable since the shifts are primarily determined by simpler shielding effects (electron density), but the chemical shifts for many heavier nuclei are more strongly influenced by other factors including excited states ("paramagnetic" contribution to shielding tensor).



Example of the chemical shift: NMR spectrum of hexaborane B6H10 showing peaks shifted in frequency, which give clues as to the molecular structure.

The chemical shift provides information about the structure of the molecule. The conversion of the raw data to this information is called *assigning* the spectrum. For example, for the 1H-NMR spectrum for ethanol (CH3CH2OH), one would expect signals at each of three specific chemical shifts: one for the C*H*3 group, one for the C*H*2 group and one for the O*H* group. A typical CH3 group has a shift around 1 ppm, a CH2 attached to an OH has a shift of around 4 ppm and an OH has a shift around 2–3 ppm depending on the solvent used.

### Spin-Spin Coupling

### Nuclei experiencing the same chemical environment or chemical shift are called equivalent. Those nuclei experiencing different environment or having different chemical shifts are nonequivalent. Nuclei which are close to one another exert an influence on each other's effective magnetic field. This effect shows up in the NMR spectrum when the nuclei are nonequivalent. If the distance between non-equivalent nuclei is less than or equal to three bond lengths, this effect is observable. This effect is called spin-spin coupling.

**J-coupling (special case of Spin-Spin coupling)**

Some of the most useful information for structure determination in a one-dimensional NMR spectrum comes from **J-coupling** or **scalar coupling** between NMR active nuclei. This coupling arises from the interaction of different spin states through the chemical bonds of a molecule and results in the splitting of NMR signals. These splitting patterns can be complex or simple and, likewise, can be straightforwardly interpretable or deceptive. This coupling provides detailed insight into the connectivity of atoms in a molecule.

Coupling to *n* equivalent (spin ½) nuclei splits the signal into an *n*+1 multiplet with intensity ratios following Pascal's triangle. coupling to additional spins will lead to further splitting of each component of the multiplet e.g. coupling to two different spin ½ nuclei with significantly different coupling constants will lead to a *doublet of doublets*. Note that coupling between nuclei that are chemically equivalent (that is, have the same chemical shift) has no effect on the NMR spectra and couplings between nuclei that are distant are usually too small to cause observable splittings. *Long-range* couplings over more than three bonds can often be observed in cyclic and aromatic compounds, leading to more complex splitting patterns.

For example, in the proton spectrum for ethanol, the CH3 group is split into a *triplet* with an intensity ratio of 1:2:1 by the two neighboring CH2 protons. Similarly, the CH2 is split into a *quartet* with an intensity ratio of 1:3:3:1 by the three neighboring CH3 protons. In principle, the two CH2 protons would also be split again into a *doublet* to form a *doublet of quartets* by the hydroxyl proton, but intermolecular exchange of the acidic hydroxyl proton often results in a loss of coupling information.

Coupling combined with the chemical shift (and the integration for protons) tells us not only about the chemical environment of the nuclei, but also the number of *neighboring* NMR active nuclei within the molecule. In more complex spectra with multiple peaks at similar chemical shifts or in spectra of nuclei other than hydrogen, coupling is often the only way to distinguish different nuclei.

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#### Second-order (or strong) coupling

The coupling constant is small in comparison with the difference in NMR frequencies between the in equivalent spins. If the shift separation decreases (or the coupling strength increases), the multiplet intensity patterns are first distorted, and then become more complex and less easily analyzed (especially if more than two spins are involved). Intensification of some peaks in a multiplet is achieved at the expense of the remainder, which sometimes almost disappear in the background noise, although the integrated area under the peaks remains constant. In most high-field NMR, however, the distortions are usually modest and the characteristic distortions (*roofing*) can in fact help to identify related peaks.

Second-order effects decrease as the frequency difference between multiplets increases, so that high-field (i.e. high-frequency) NMR spectra display less distortion than lower frequency spectra. Early spectra at 60 MHz were more prone to distortion than spectra from later machines typically operating at frequencies at 200 MHz or above.

## FOURIER TRANSFORMS

A Fourier transform is an operation which converts functions from time to frequency domains. An inverse Fourier transform (IFT) converts from the frequency domain to the time domain.

**The + and - Frequency Problem**- A magnetization vector, starting at +x, is rotating about the Z axis in a clockwise direction. The plot of Mx as a function of time is a cosine wave. Fourier transforming this gives peaks at both + and - because the FT cannot distinguish between a + and a - rotation of the vector from the data supplied.

A plot of My as a function of time is a -sine function. Fourier transforming this gives peaks at + and - because the FT cannot distinguish between a positive vector rotating at + and a negative vector rotating at - from the data supplied.

The solution is to input both the Mx and My into the FT. The FT is designed to handle two orthogonal input functions called the real and imaginary components.

Detecting just the Mx or My component for input into the FT is called linear detection. This was the detection scheme on many older NMR spectrometers and some magnetic resonance imagers. It required the computer to discard half of the frequency domain data.

Detection of both Mx and My is called quadrature detection and is the method of detection on modern spectrometers and imagers. It is the method of choice since now the FT can distinguish between + and -, and all of the frequency domain data be used.

**The Two-Dimensional FT** - The two-dimensional Fourier transform (2-DFT) is an FT performed on a two dimensional array of data.

Consider the two-dimensional array of data depicted in the animation window. This data has a t' and a t" dimension. A FT is first performed on the data in one dimension and then in the second. The first set of Fourier transforms are performed in the t' dimension to yield an f' by t" set of data. The second set of Fourier transforms is performed in the t" dimension to yield an f' by f" set of data.

The 2-DFT is required to perform state-of-the-art MRI. In MRI, data is collected in the equivalent of the t' and t" dimensions, called k-space. This raw data is Fourier transformed to yield the image which is the equivalent of the f' by f"

## CARBON-13 NMR

Many of the molecules studied by NMR contain carbon. The carbon-12 nucleus does not have a nuclear spin, but the carbon-13 (C-13) nucleus does due to the presence of an unpaired neutron. Carbon-13 nuclei make up approximately one percent of the carbon nuclei on earth. Therefore, carbon-13 NMR spectroscopy will be less sensitive (have a poorer SNR) than hydrogen NMR spectroscopy. With the appropriate concentration, field strength, and pulse sequences, however, carbon-13 NMR spectroscopy can be used to supplement the previously described hydrogen NMR information. Advances in superconducting magnet design and RF sample coil efficiency have helped make carbon-13 spectroscopy routine on most NMR spectrometers.

The *sensitivity* of an NMR spectrometer is a measure of the minimum number of spins detectable by the spectrometer. Since the NMR signal increases as the population difference between the energy levels increases, the sensitivity improves as the field strength increases. The sensitivity of carbon-13 spectroscopy can be increased by any technique which increases the population difference between the lower and upper energy levels, or increases the density of spins in the sample. The population difference can be increased by decreasing the sample temperature or by increasing the field strength. Several techniques for increasing the carbon-13 signal have been reported in the NMR literature. The presence of spin-spin coupling between a carbon-13 nucleus and the nuclei of the hydrogen atoms bonded to the carbon-13, splits the carbon-13 peaks and causes an even poorer signal-to-noise ratio. This problem can be addressed by the use of a technique known as decoupling.

### Decoupling - The signal-to-noise ratio in an NMR spectrometer is related to the population difference between the lower and upper spin state. The larger this difference the larger the signal. This difference is proportional to the strength of the magnetic field.

### *Example :* consider the familiar hydrogen NMR spectrum of HC-(CH2CH3)3. The HC hydrogen peaks are difficult to see in the spectrum due to the splitting from the 6 -CH2- hydrogens. If the effect of the 6 -CH2- hydrogens could be removed, we would lose the 1:6:15:20:15:6:1 splitting for the HC hydrogen and get one peak. We would also lose the 1:3:1 splitting for the CH3 hydrogens and get one peak. The process of removing the spin-spin splitting between spins is called decoupling. Decoupling is achieved with the aid of a saturation pulse. If the affect of the HC hydrogen is removed, we see the following spectrum. Similarly, if the affect of the -CH3 hydrogens is removed, we see this spectrum. A saturation pulse is a relatively low power B1 field left on long enough for all magnetization to disappear. A saturation pulse applied along X' rotates magnetization clockwise about X' several times. As the magnetization is rotating, T2 processes cause the magnetization to dephase. At the end of the pulse there is no net Z, X, or Y magnetization. It is easier to see this behavior with the use of plots of MZ, MX', and MY' as a function of time. Since the B1 pulse is long, its frequency content is small. It therefore can be set to coincide with the location of the -CH2- quartet and saturate the -CH2- spin system. By saturating the -CH2- spins, the -CH2- peaks and the splittings disappear, causing the height of the now unsplit HC- and -CH3 peaks to be enhanced.

**Population Inversion**- Another method of improving the NMR signal in systems with spin-spin coupling is population inversion. Boltzmann statistics tell us that there are more spins in the lower spin state than the upper one of a two spin state system. Population inversion is the interchange of the populations of these two spin states so that there are more spins in the upper state then the lower one. Consider the CHI3 molecule. CHI3 will have four energy levels (L1, L2, L3, and L4) due to C-H spin-spin coupling. There are two carbon-13 absorption frequencies f1 and f2 and two hydrogen absorption frequencies f3 and f4. The population distribution between the four levels is such that the lowest state has the greatest population and the highest the lowest population. The two intermediate states will have populations between the outer two as indicated by the thickness of the levels in the accompanying diagram. The four lines in the spectrum will have intensities related to the population difference between the two levels spanned by the frequency. The two carbon-13 absorption lines (f1 and f2) will have a lower intensity than the hydrogen lines (f3 and f4) due to the smaller population difference between the two states joined by f1 and f2.

If the populations of L3 and L1 are inverted or interchanged with a frequency selective 180 degree pulse at f3, the signal at f2 will be enhanced because of the greater population difference between the states joined by f2. It should be noted that the signal at f1 will be inverted because the upper state of the two joined by f1 has a greater population than the lower one. An example of a population inverting pulse sequence designed to enhance the carbon-13 spectral lines is depicted in the animation window. The 180 degree pulse at f3 has a narrow band of frequencies centered on f3 that selectively rotates only the magnetization at f3 by 180 degrees.

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|  **NMR Applications*** **Solution structure** The only method for atomic-resolution structure determination of biomacromolecules in aqueous solutions under near physiological conditions or membrane mimeric environments.
* **Molecular dynamics** The most powerful technique for quantifying motional properties of biomacromolecules.
* **Protein folding** The most powerful tool for determining the residual structures of unfolded proteins and the structures of folding intermediates.
* **Ionization state** The most powerful tool for determining the chemical properties of functional groups in biomacromolecules, such as the ionization states of ionizable groups at the active sites of enzymes.
* **Weak intermolecular interactions** Allowing weak functional interactions between macrobiomolecules (e.g., those with dissociation constants in the micromolar to millimolar range) to be studied, which is not possible with other technologies.
* **Protein hydration** A power tool for the detection of interior water and its interaction with biomacromolecules.
* **Hydrogen bonding** A unique technique for the DIRECT detection of hydrogen bonding interactions.
* **Drug screening and design** Particularly useful for identifying drug leads and determining the conformations of the compounds bound to enzymes, receptors, and other proteins.
* **Native membrane protein** Solid state NMR has the potential for determining atomic-resolution structures of domains of membrane proteins in their native membrane environments, including those with bound ligands.
* **Metabolite analysis** A very powerful technology for metabolite analysis.
* **Chemical analysis** A matured technique for chemical identification and conformational analysis of chemicals whether synthetic or natural.
* **Material science** A powerful tool in the research of polymer chemistry and physics.
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