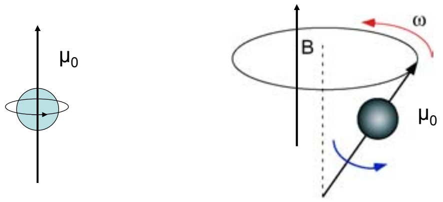
Nuclear Magnetic Resonance Spectroscopy

Basics

Most spinning nuclei *behave* like magnets.

However, as opposed to the behavior of a classical magnet the nuclear spin magnetic moment does not *always* align with the direction of the magnetic field B (quantization). NMR fundamentals are the same for all magnetic nuclei.



In the absence of a field the nuclear magnets orient in all possible directions in space.

Nuclear spin quantum number *I*

Depending on the nuclear constitution, they would possess a nuclear spin moment.

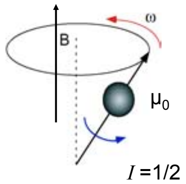
A Mass #	Z Atomic #	*I* (nuclear spin q.n.)
Odd	**Odd** or even	n/2 (n ≠ 0 & odd integer)
Even	Even	0
Even	**Odd**	n (integer)

Nuclei of interest ^1H, ^2H, ^{13}C, ^{19}F, ^{29}Si, ^{31}P, ^{127}I, etc.

Any nucleus with an odd Z or an odd A has a net spin.

Nuclear angular momentum of ρ_0 $\rho_0 = \sqrt{I(I+1)}\hbar$

Precessional movement



Bare proton

$$2\pi\nu = \omega$$

magnet of magnetic moment (dipole) μ_0

$$\mu_0 = \gamma\rho_0 = \gamma\sqrt{I(I+1)}\hbar$$

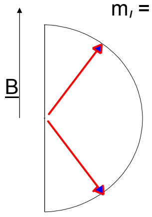
magnetogyric ratio of the nucleus

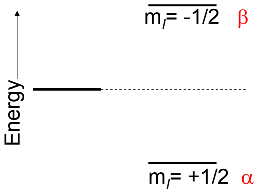
http://www.chem.queensu.ca/FACILITIES/NMR/nmr/webcourse/index.htm
http://www.eclipse.net/~numare/nsinmrpt.htm

orientations (states) in a **B** =2I+1
Each state characterized by $m_I = -I, -I+1, \ldots, +I$

I = 1/2

m_I = +1/2; α, ground state





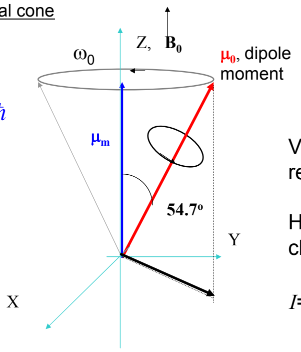
m_I = -1/2; β, excited state

In the presence of a B the two states differentiates.

Precessional cone
Of a single
nucleus

$$\mu_m = \gamma m_I \hbar$$

$$\omega_0 = \gamma B_0$$



Vertical component remains steady.

Horizontal component changes direction.

I=1/2 Ground state

ω_0= Larmor frequency, rad/s
The quantity NMR experiment ‘measures’.

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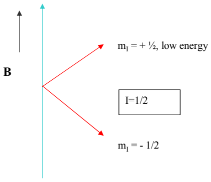
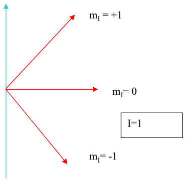
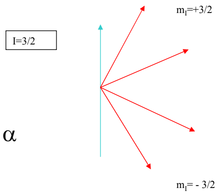
http://www.bruker-nmr.de/guide/eNMR/chem/NMRnuclei.html



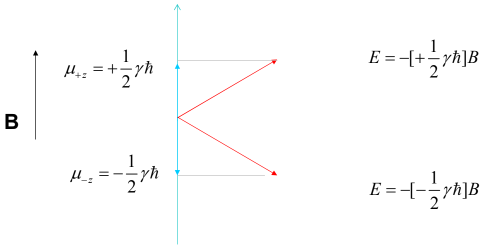




Selection rule:
$\Delta m_I = \pm 1$

$\alpha \rightarrow \beta$ or $\beta \rightarrow \alpha$

Transitions between states dictated by selection rules



$\mu_{+z} = +\frac{1}{2}\gamma\hbar$

$\mu_{-z} = -\frac{1}{2}\gamma\hbar$

$E = -[+\frac{1}{2}\gamma\hbar]B$

$E = -[-\frac{1}{2}\gamma\hbar]B$

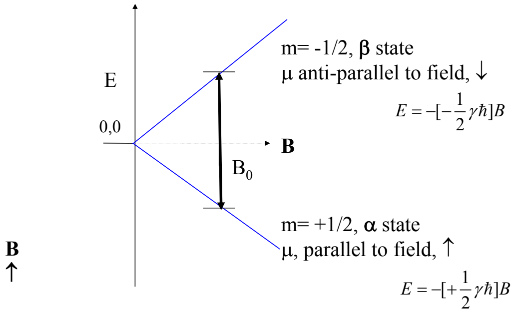
$$\Delta E = \gamma\hbar B$$

magnetogyric ratio of the nucleus

$\mu_z = m_I\gamma\hbar Z$

$E = -\mu B$

Energy Changes in a varying B



m= -1/2, **β** state
μ anti-parallel to field, ↓

$E = -[-\frac{1}{2}\gamma\hbar]B$

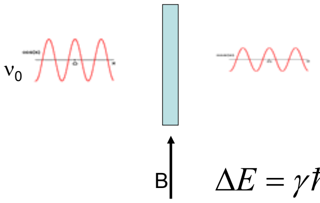
m= +1/2, **α** state
μ, parallel to field, ↑

$E = -[+\frac{1}{2}\gamma\hbar]B$

$$\Delta E = \gamma\hbar B_0 = h\nu$$

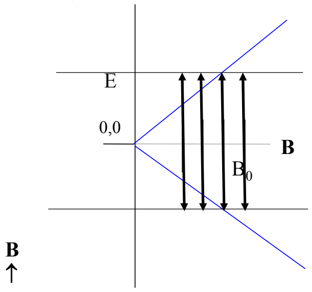
ν falls in the radio frequency region.

NMR Experiment; pseudo classical



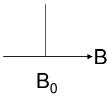
$$\Delta E = \gamma\hbar B_0 = h\nu_0$$

Continuous wave experiment; Field sweep
frequency constant



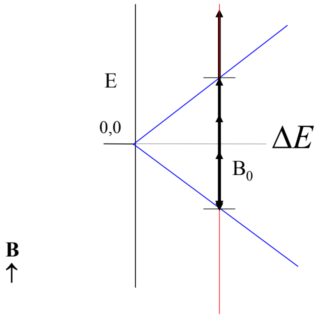
$$\Delta E = \gamma\hbar B_0 = h\nu_0$$

$$\nu_0 = \frac{\gamma B_0}{2\pi}$$



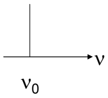
Continuous wave experiment; Frequency sweep

B constant



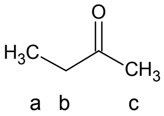
$$\Delta E = \gamma \hbar B_0 = h\nu_0$$

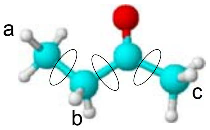
$$\nu_0 = \frac{\gamma B_0}{2\pi}$$

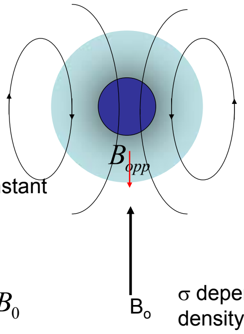


Proton in a sample are in a different electronic environment than a bare proton. Protons with different type of environments, characterized by σ, can be recognized.

Each type of proton has the ‘same environment if free rotation is possible.







B_{opp} shields the nucleus

Screening constant

$$B_{opp} = \sigma B_0$$

$$B = (1-\sigma)B_0$$

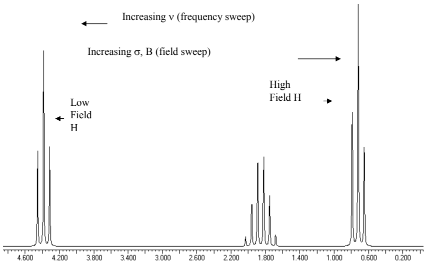
$$\nu = \frac{\gamma(1-\sigma)B_0}{2\pi}$$

σ depends on the electron density around the atom.

Therefore ν of absorption of each type of H is unique.

In the NMR experiment, the nuclear energy levels are excited and the “absorptions” of the nuclear excitations recorded.

Note the hyperfine splitting of NMR peaks.



The ‘pseudo’ NMR experiment is impractical, i.e. absorbance cannot be measured directly by experiment.

The reason for it is a population distribution issue and the nature of the interaction of electromagnetic radiation with matter (energy levels of matter).

very small

Boltzmann Law
$$\frac{N_\beta}{N_\alpha} = e^{-\frac{\Delta E}{kT}}$$

For protons $\gamma = 26.75 \times 10^7$ rad/T s; h-bar = 1.055×10^{-34} Js, k = 1.380658×10^{-23} J/K. At 298K and $B_0 = 7.05$T

$$\frac{N_\beta}{N_\alpha} = 0.99995 \approx 1 \qquad \Rightarrow N_{\beta-} \cong N_\alpha$$

$$\frac{N_\beta}{N_\alpha} = e^{-\frac{\Delta E}{kT}}$$

$$\frac{N_\beta}{N_\alpha} = 1 - \frac{\Delta E}{kT} \qquad \text{Using Taylor series for } \frac{\Delta E}{kT} << 1$$

$$\frac{N_\alpha - N_\beta}{N_\alpha} = \frac{\Delta E}{kT}$$

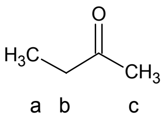
$$N_\alpha - N_\beta = N_\alpha \frac{\Delta E}{kT}$$

Population excess is linear in ΔE under this condition.

Samples contain millions of molecules. All nuclei of the same type would precess at the same Larmor frequency.

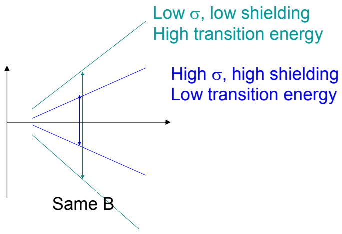
The vector sum of the magnetic moments of one type of nuclei is a measure of the number of protons of that type in the molecule.

The determination of the magnetic moment for protons in each type of electronic environment forms the essence of the NMR experiment.



M_a:M_b:M_c=3:2:3

Relationship between energy levels, magnetic field and shielding:


Low σ, low shielding
High transition energy

High σ, high shielding
Low transition energy

$$\nu = \frac{\gamma(1-\sigma)}{2\pi} \mathrm{B}_0 \qquad |\text{slope}| = \frac{\gamma(1-\sigma)}{2\pi} \qquad 2\pi\nu = \omega$$

Chemical Shift

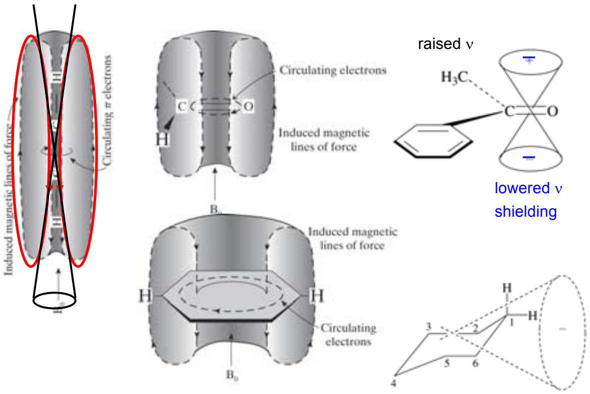
The resonance frequency (shifts) depends on the value of σ, the electronic environment around the nucleus,

Larger electron densities has smaller Larmour frequencies.

Factors determining the frequency shift: σ, B_{eff};

a. electro-negativity of the neighboring groups
b. electron currents (delocalization) in response to B_o leading to magnetic fields which are anisotropic that would enhance/diminish the effective field at the nucleus .

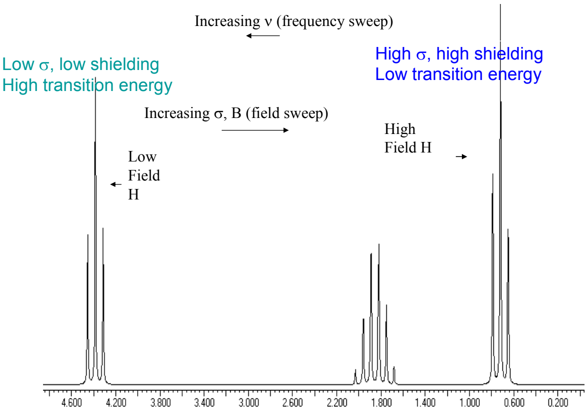
$$\nu = \frac{\gamma(1-\sigma)B_{eff}}{2\pi}$$



raised ν

lowered ν
shielding

$$\nu = \frac{\gamma(1-\sigma)B_{eff}}{2\pi}$$



Increasing ν (frequency sweep)

Low σ, low shielding
High transition energy

High σ, high shielding
Low transition energy

Increasing σ, B (field sweep)

Low
Field
H

High
Field H

In FTNMR vocabulary a x MHz instrument would mean an instrument where the applied field B_0 is such that the *bare protons* would rotate at a Larmor frequency of x MHz.

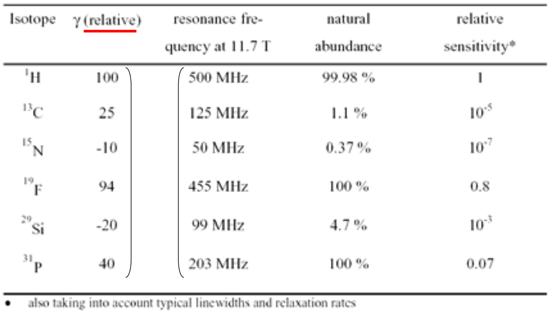
In such an instrument, the AC frequency of the pulse for excitation of bare protons is x MHz.

MSUM - 400MHz instrument.

The pulse generates a range of frequencies centered at 400MHz.

	^{1}H	^{2}H		^{13}C	^{14}N	^{15}N
Nuclear Spin, I	1/2	1		1/2	1	1/2
Nuclear magnetic moment, μ	+2.79284	+0.85743		+0.70241	0.40376	-0.28319
Gyromagnetic Ratio (rad T^{-1} s^{-1})	26.7510×10^{7}	4.1064×10^{7}		6.7263×10^{7}	1.9331×10^{7}	-2.7116×10^{7}
Quadrupole moment (m^2)		2.73×10^{-31}			1.6×10^{-30}	
Relative Sensitivity	1.00	9.65×10^{-3}		1.59×10^{-2}	1.01×10^{-3}	1.04×10^{-3}
Natural Abundance, %	99.985	0.015		1.10	99.63	0.37
Absolute Sensitivity	5680	8.2×10^{-6}		1.00	5.69	0.0219

$$\nu = \frac{\gamma(1-\sigma)B_0}{2\pi}$$



Isotope	γ (relative)	resonance frequency at 11.7 T	natural abundance	relative sensitivity*
^{1}H	100	500 MHz	99.98 %	1
^{13}C	25	125 MHz	1.1 %	10^{-5}
^{15}N	-10	50 MHz	0.37 %	10^{-7}
^{19}F	94	455 MHz	100 %	0.8
^{29}Si	-20	99 MHz	4.7 %	10^{-3}
^{31}P	40	203 MHz	100 %	0.07

- also taking into account typical linewidths and relaxation rates

$$\nu_0 = \frac{\gamma B_0}{2\pi}$$

For bare protons σ = 0. γ = 26.75 × 10^7 rad T^{-1} s^{-1} and B_0 = 7.05T the Larmor frequency is;

$$\nu = \frac{\gamma(1-\sigma)B_0}{2\pi}$$

$$\nu = \gamma(1-\sigma)\frac{B_0}{2\pi}$$

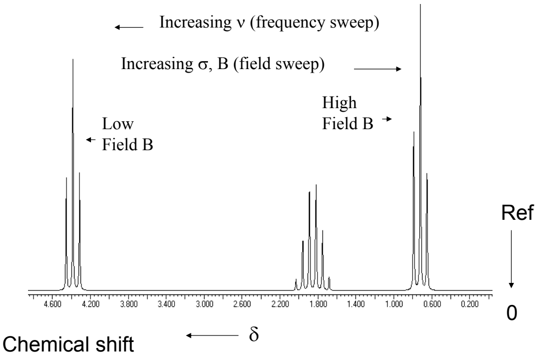
$$\nu = 3\times 10^8\,Hz$$

A bare ^{1}H(σ=0) at 2T field resonates at 90.0000 MHz;

A typical proton would have a frequency of 89.9995 MHz, In the same field, it is an unwieldy number to report, further in a different instrument (different field) the frequency will be different.

Thus absorption positions are reported with respect to a reference.

General presentation of NMR spectra:



The position of NMR resonance signals (frequency scale) is dependent on the external magnetic field strength, B_0 (as opposed to UV, IR, AA etc).

$$\omega_{sample} = \gamma_H (1 - \sigma_{sample}) \mathbf{B_0}$$

Since no two magnets in the spectrometers will have exactly the same field, resonance frequencies will vary accordingly. A method for specifying uniquely the position of NMR signals is needed.

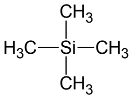
To express the position of NMR signals unequivocally, the signals are expressed relative to signal from a standard compound.

The reference compound is chosen so that the protons of it are well shielded than the typical protons in compounds of interest (so that reference absorptions does not interfere with the resonances normally observed for organic compounds .

Further it should give a single sharp nmr signal.

It should be chemically un-reactive and easily removable from the sample after the measurement.

TMS



$$H_3C{-}\overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{Si}}{-}CH_3$$

The most common reference compound used is tetramethyl silane. TMS is rich in H's(12) and methyl group H's are richer in electrons (larger σ) than in many organic compounds and they are structurally equivalent.

$$\omega_{ref} = \gamma_H (1 - \sigma_{ref}) \mathbf{B_0}$$

$$\omega_{sample} - \omega_{ref} = \gamma H (\sigma_{ref} - \sigma_{sample}) \mathbf{B_0}$$

$\sigma_{ref} - \sigma_{sample}$ is a very small quantity.

$$\omega_{ref} = \gamma_H(1-\sigma_{ref})\mathbf{B_0}$$

$$\omega_{sample} = \gamma_H(1-\sigma_{sample})\mathbf{B_0}$$

$$\omega_{sample} - \omega_{ref} = \gamma_H(\sigma_{ref} - \sigma_{sample})\mathbf{B_0}$$

$$\nu_{sample} - \nu_{ref} = \gamma_H\underbrace{(\sigma_{ref} - \sigma_{sample})}_{\text{very small}}\,\mathbf{B_0}/2\pi$$

Different kinds of protons usually appear at different chemical shifts makes it possible to distinguish one kind of proton from another. One reason for the different values of chemical shift is the difference in shielding. If there is more electron density around a proton, it sees a slightly lower magnetic field and vice versa.

<u>Chemical Shift</u>

$$\delta = \frac{\omega_{sample} - \omega_{reference}}{\omega_{rf}} \times 10^6$$

$$\delta = \frac{\nu_{sample} - \nu_{reference}}{\nu_{rf}} \times 10^6$$

<u>Chemical shift is independent of the instrument field strength.</u>

For bare $^1H(\sigma=0)$at 2T field resonate at 90.0000 MHz;

A typical proton would have a frequency of 89.9995 MHz, it is an unwieldy number to report, further if a different instrument (different field) is used the frequency will be different.

Thus absorption positions are reported with respect to a reference. If a reference compound resonates at 89.9990MHz.

Chemical shift: $\frac{89.9995\,10^6 - 89.9990\,10^6}{90\cdot 10^6}\cdot 10^6 = 5.556$

Chemical Shift (ppm): $\frac{89.9995\,10^6 - 89.9990\,10^6}{90\cdot 10^6}\cdot 10^6 = 5.556$

Frequency shift (Hz) $89.9995\,10^6 - 89.9990\,10^6 = 500$

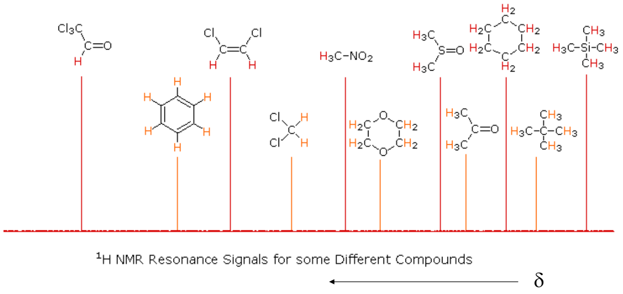
Frequency shift in a 300MHz instrument (Hz):

$$\frac{(5.556)\cdot 300\cdot 10^6}{10^6} = 1666.8$$

Entire proton range 300MHz instrument is 12ppm.
In (Hz):

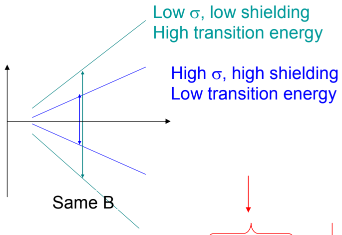
$$\frac{(12)\cdot 300\cdot 10^6}{10^6} = 3600$$

Chemical shift decreases with increasing shielding of the nucleus



¹H NMR Resonance Signals for some Different Compounds

The higher the field the larger the frequency range over which the spectrum spreads. This enhances the resolution of peaks.



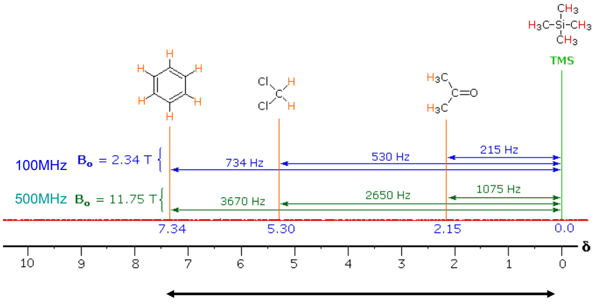
Low σ, low shielding
High transition energy

High σ, high shielding
Low transition energy

Same B

$$\nu - \nu_{ref} = \left[\frac{\overbrace{\gamma(\sigma - \sigma_{ref})}}{2\pi}\right]B$$

Frequency shift is dependent of the instrument field strength.



734Hz vs. 3670Hz (Benzene)

The resonance frequencies of nuclei in similar environments are the ‘same’.

‘same’ = the ‘mean’ of the frequencies.

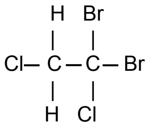
The necessity to look at a ‘mean’ arises from the fact that in general the spins of the nuclei in close proximity do influence each other and hence their resonant frequencies.

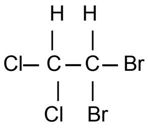
Such influences, termed as coupling (scalar coupling) results in the splitting of the spectral peaks. There are weakly coupled and strongly coupled systems.

Scalar (Spin) Coupling

Most of the time the NMR spectrum consists of groupings of multiple lines (multiplets). This is due to ^1H - ^1H coupling (spin-spin splitting/J-coupling/scalar coupling). The nuclei which are 1 to 3 bonds away (especially) would ‘feel’ the nuclear spin state of each other via the electron spins of the intervening bonds. That generates the splitting of spectral lines.

Geminal ^2J





Vicinal ^3J

Only magnetically nonequivalent nucleii couple.

Classification of Spin Systems

The chemical shifts of protons in a molecule can differ in various degrees.

Depending on the extent of coupling (J) between the nuclei, the splitting pattern changes.

Simple rules (n+1) and area ratios (Pascal triangle) is useful for weakly coupled systems (first order spectra).

The spin system, in general, for example is referred to as $\mathbf{A_nB_m}$ (second order spectra) if the chemical shifts are quite close together, and $\mathbf{A_nX_m}$ (first order spectra) if the chemical shifts are far apart relative to J.

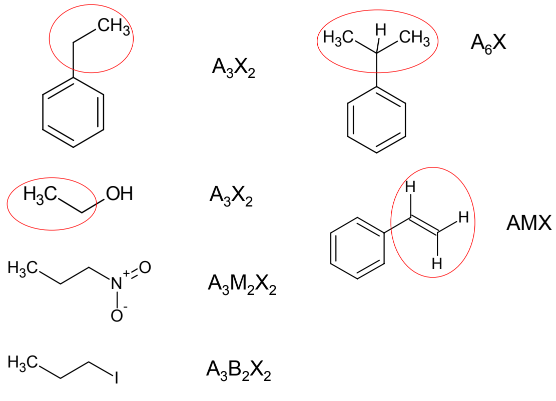
Pople Notation

Each chemically different type of proton is given a capital letter; A,B C …M,N…. X,Y,Z.
The number of protons in a group protons is indicated as a subscript; A_2, B_3 …
Protons with similar δ values are assigned letters close to one another in the alphabet; A,B,C; M,N; X,Y, Z.

Two protons same chemical shift but magnetically nonequivalent is assigned, for example AA’.

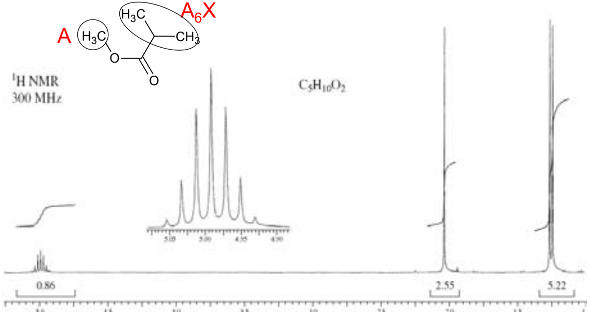
AX, AM weakly coupled, AB strongly coupled pair of protons.

Three protons very different in from each other (and therefore weakly coupled) would be like AMX, ABX, BX weakly coupled, AB strongly coupled.



First-order Scalar Coupling (weakly coupled systems)

Coupling of spins lead to peak splitting (hyperfine structure). In first order spectra the splitting of the peaks follows a simple pattern.

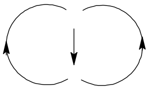


Consider an AX system:

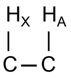
A molecule which contains a proton (H_A) attached to a C, and that this C is attached to another C carrying a proton (H_X). In this situation H_A feels the presence of H_X via bonding electrons.

Protons are ‘tiny little magnets’, that orients either parallel or anti-parallel the magnetic field B_0 of the instrument. When the field created by H_X effectively reinforces the magnetic field B_0 H_A feels a slightly stronger field, but when the field created by H_X opposes B_0, H_A feels a slightly weaker field.

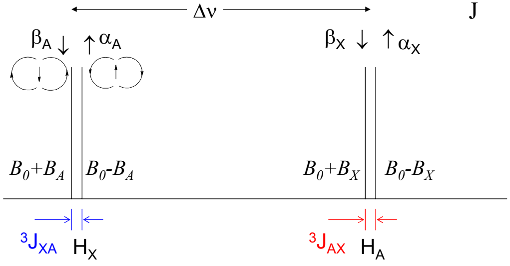
This leads to two signals for H_A depending on the alignment of H_X. Similarly H_X can feel either a slightly stronger or weaker field due to H_A.



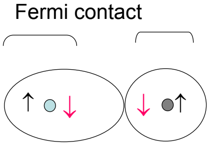
$$\nu = \frac{\gamma(1-\sigma)B_0}{2\pi}$$



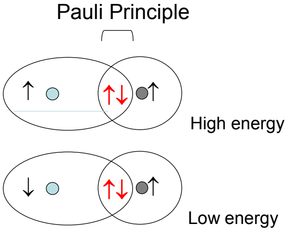
$$\frac{\Delta\nu}{J} > 8$$



^{13}C-^1H AX System ^1J

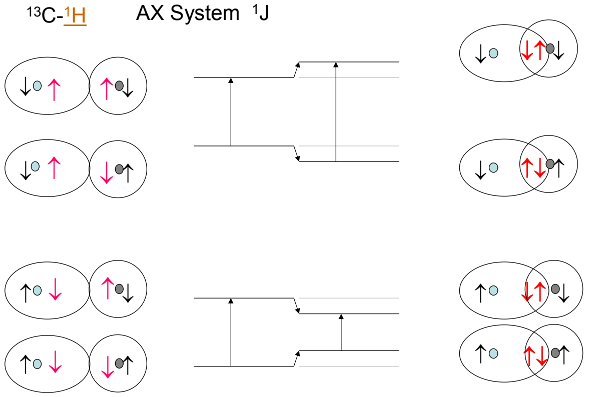


Isolated atoms – Fermi contact energetically favors anti-parallel orientation of spins.



Bonded atoms – Pauli Principle demands anti-parallel electron spins making nuclear and electron spins parallel, ….

$^{13}\text{C-}^{1}\text{H}$ AX System ^{1}J



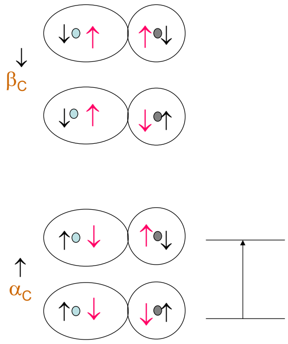
Eigen-states with no coupling

Effect on eigen-states with coupling

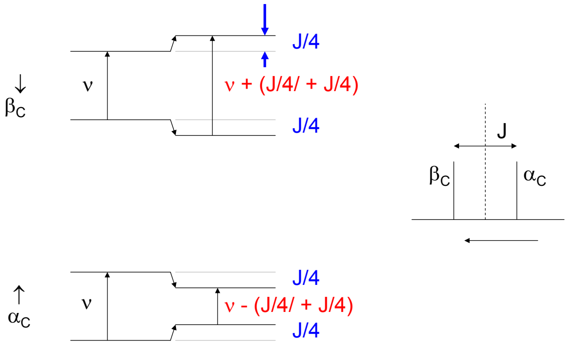
$^{13}\text{C-}^{1}\text{H}$ AX System ^{1}J

The difference in the nuclear spin of bonded atom (left) gave rise to four eigen states.

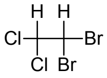
If otherwise it would be two eigen states and one frequency.



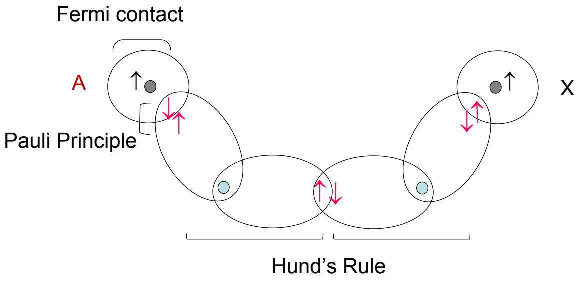
$^{13}\text{C-}^{1}\text{H}$ AX System ^{1}J



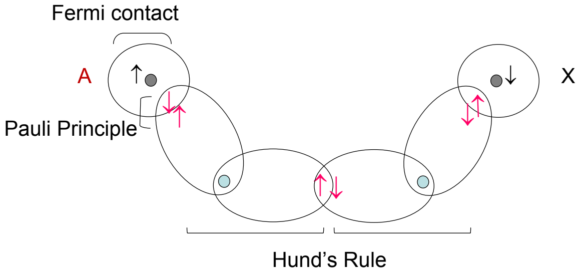
For the X peaks a similar situation (doublet) would form.



AX System ^{3}J

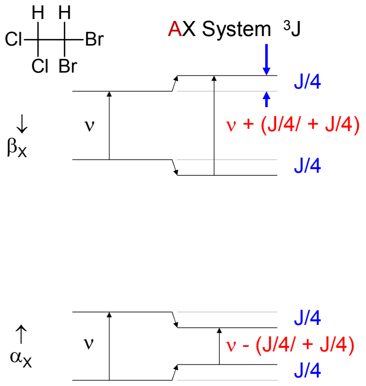


High energy



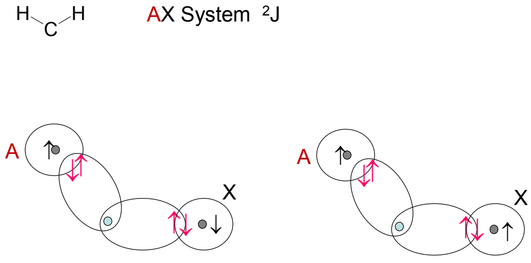
Low energy

AX System ^3J



For the X peaks a similar situation (doublet) would form.

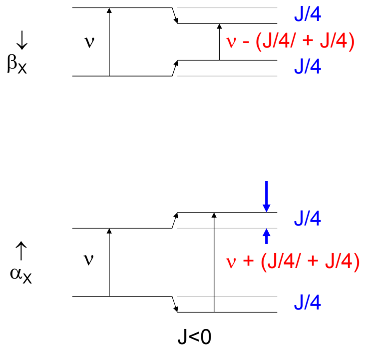
AX System ^2J



High energy

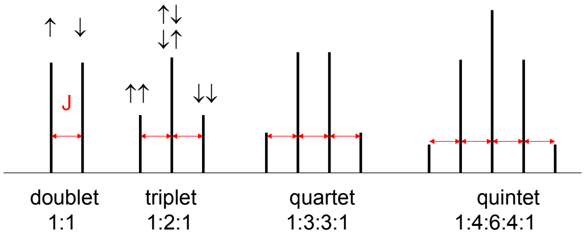
Low energy !!

AX System ^1J



Regardless of sign J the splitting pattern would be the same for same value of J.

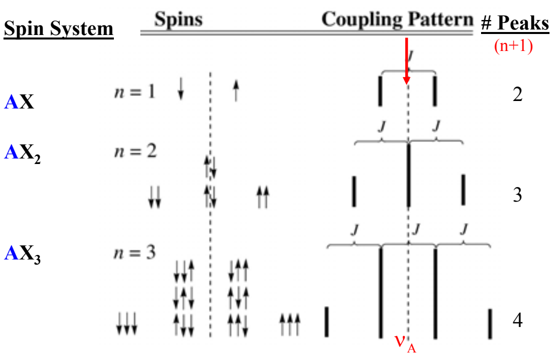
Stick Diagram – first order splitting due to scalar coupling



The splitting between peaks, J, are equal, the ratio of peaks conform to the Binomial coefficients (first order spectra).

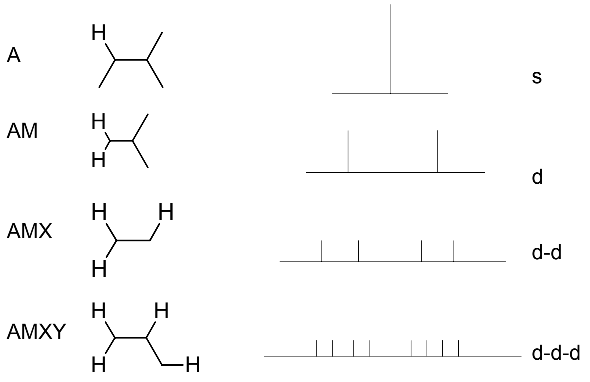
J is independent of the field strength of the instrument. Value of J (Hz) property of the spin system of the molecule.

Stick Diagram – first order splitting



Centro-symmetric, equally spaced peaks by J, with area ratios ⇒ Binomial coefficients!!.

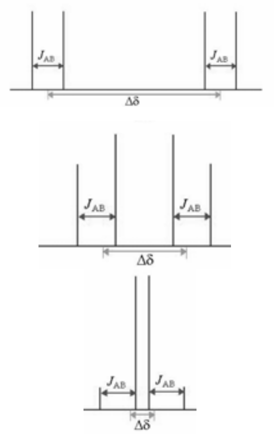
Stick Diagram – first order splitting



Second order spectra

If the chemical shifts are similar then distortions in peak height could occur. For more than two spins, extra signals may appear.

These effects are observed in second order coupling.



First order splitting: $\frac{\Delta \nu}{J} > 8$ AX, AMX, AX_2,...

Second order splitting: $\frac{\Delta \nu}{J} < 8$ AB, ABC, AB_2,...

$\Delta \nu =$ chemical shift difference of the coupling nuclei (Hz)

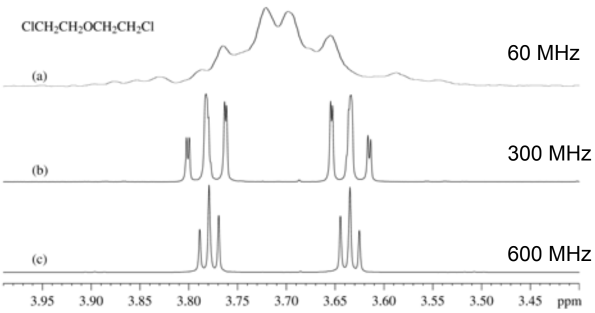
Splitting pattern of the first order spectra are extremely useful To extract information about the connectivity and the stereochemistry of the molecule.

Because chemical shift difference is dependent of the magnetic field (actually increases with the applied field) and the coupling constant is independent of the applied field, it is possible to change the ratio;

$$\frac{\Delta \nu}{J}$$

At larger fields $\Delta\nu$ would be larger while J remains the same.

Instruments with larger fields simplify the second order pattern to a first order pattern.



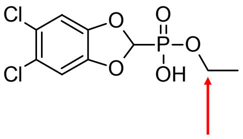
Coupling constant (J) has the same value regardless of the field strength.

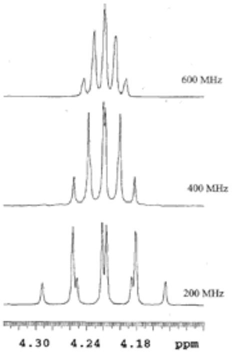
Both homonuclear and heteronuclear coupling folow the (n+1) rule.

J = f (bond angles, temperature and solvent -influence the conformation of the compound)

$^1J > {^2J} > {^3J}$ …..; ^1J , ^3J, ... > 0 ; ^2J, ^4J …<0

The appearance of the multiplet does not show the sign of J.





-CH_2-

Note: Methylene protons are magnetically non-equivalent. Each H⇨1:3:3:1 pattern & are overlapped.

Caution: Overlapped peaks ⇒ ratio not Binomial coefficients!!.

H-NMR Experiment: One Pulse Experiment

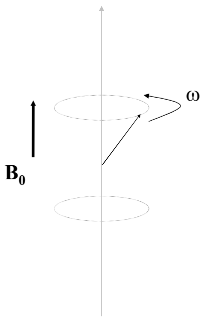
Place the sample in the magnetic field.

The nuclei attains equilibrium composition. Magnetic moments orient on z-axis.

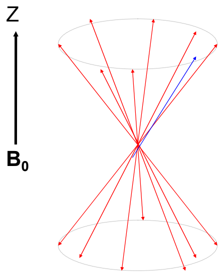
After a recycle delay, d1, apply a RF pulse ($\theta=\pi/2$).

The magnetic moments for each type of nuclei are moved onto the x-y plane.

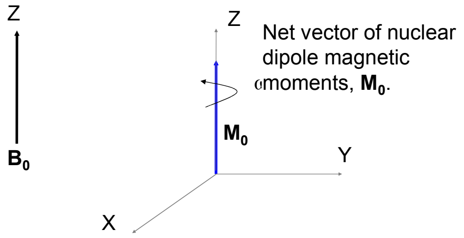
The FID collected.



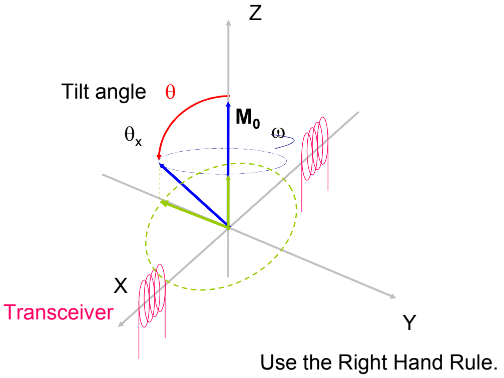
Magnetic moment vector, single nucleus



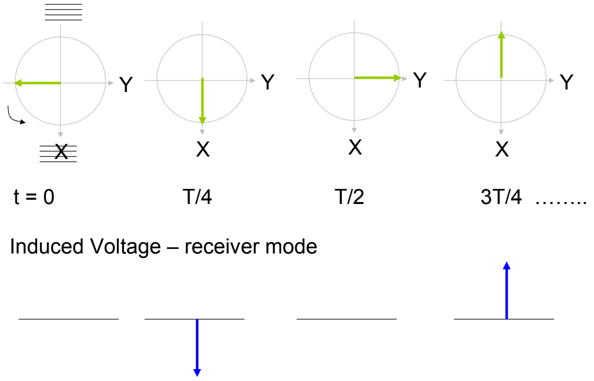
Collection of nuclear dipole magnetic moments - symmetrical components



Net vector of nuclear dipole magnetic moments, **M_0**.

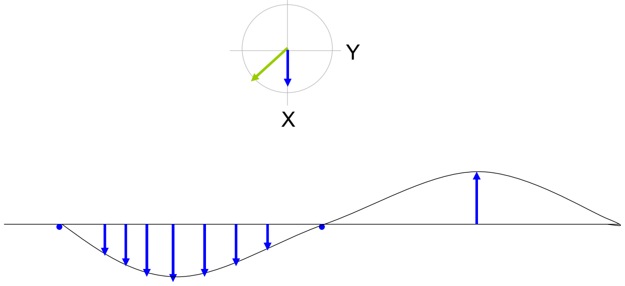


Use the Right Hand Rule.



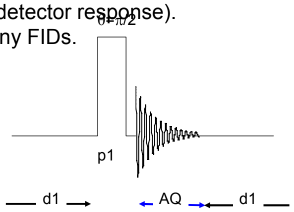
Induced Voltage – receiver mode

Each M with it’s Larmor frequency generates a time varying voltage at the ‘receiver’ coil. Sine/cosine curve.



1D-H-NMR Experiment: One Pulse Experiment (Single Channel)

Place the sample in the magnetic field.
The nuclei attains equilibrium composition. Net magnetic moments orient on z-axis.
After a recycle delay, d1, apply a RF pulse ($\theta=\pi/2$).
The magnetic moments for each type of nuclei are moved onto the x-y plane.
The FID collected (detector response).
Collect and add many FIDs.



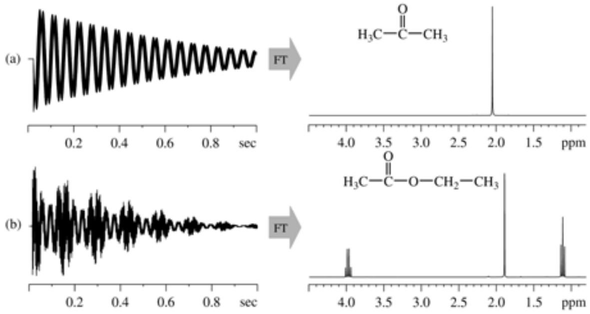
Fourier Transformation of FID

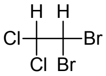
The signal detected is a time domain signal. The signal decays due to the T_2 relaxation and is therefore called free induction decay (FID). The equilibrium magnetization is directed along the external magnetic field in Z). The time-dependent signal can be converted into the frequency domain by the Fourier transformation

$$\overline{m}_p(\omega) = \int_0^\infty m_p(t)\exp(i\omega t)dt = \frac{[m_x(0)+im_y(0)]}{-i(\omega-\Omega_0)^2+1/T_2^2}$$

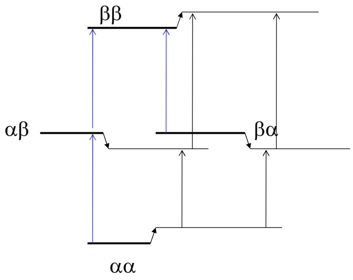
The intensity of the detected signal with the highest value at Ω_0 (resonance frequency)

$$\left|\overline{m}_p(\omega)\right|^2 = \frac{\left|m_p(0)\right|^2}{(\omega-\Omega_0)^2+1/T_2^2}$$





AX System ^3J



For the X peaks a similar situation (doublet) would form.