NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

All nuclei carry a charge. In some nuclei, this charge spins on the nuclear axis. A magnetic field is generated along the axis because of this spin and the associated charge and is given as

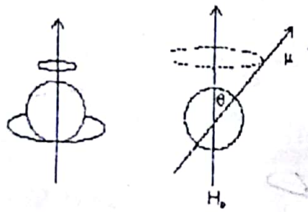
$\mu = [I(I+1)]^{1/2}\, h/2\pi$

where I is the nuclear spin number of a nucleus which is obtained by combining the spins of protons and neutrons, each of magnitude ½. Each nucleus, being composed of different numbers of protons and neutrons, will have its own total spin value. With regard to spin quantum numbers of nuclei, the following table can be considered.

No. of protons	No. of neutrons	Spin no., I	Examples
Even	Even	0	C^{12}, O^{16}, S^{32}
Odd	Even	1/2, 3/2	H^{1}, F^{19}, P^{31}, B^{11}, Br^{79}
Even	Odd	1/2, 3/2	C^{13}, I^{127}
Odd	Odd	1	H^{2}, N^{14}

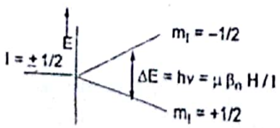
If a magnetic nucleus is placed in an external magnetic field of strength H, the nuclear spin will lie at an angle θ to the field and the nucleus will precess about the axis of the field. The frequency of the precession of the given nucleus is directly proportional to the strength of the applied field, H_o, and is given by the Larmor equation,

$\omega = 2\pi\nu = \gamma H$



where ω = angular velocity, ν = frequency of precession and γ = magnetogyric ratio, a constant for a given nucleus (for H, γ = 26750).

Under the influence of an applied magnetic field, the given magnetic nucleus can take up (2I+1) number of different orientations with respect to the applied field. For example, with I = 1/2, the possible spin states are −1/2 and +1/2. In the low energy state the magnetic moment of the nucleus is aligned parallel to the external magnetic field and in the high energy state the nuclear magnetic moment is aligned anti-parallel to the external field.



In the absence of external magnetic field, the energies of the spin states of protons are degenerate. As the field is applied on the nucleus, the spin states lose their degeneracy and become separated by an energy difference (ΔE), which is a function of the strength of applied field.

Now an alternating radio frequency (rf) is applied at right angles to the constant magnetic field. If the frequency of rf radiation and that of the precession of the magnetic nucleus are identical, they would be in resonance. Then, the radiation would be absorbed by the nucleus and it flips from the low energy spin state to the high energy state. The frequency of the radiation can be given as

$\Delta E = h\nu = \mu\beta_N H/I$

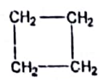
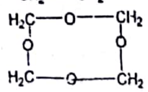
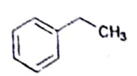
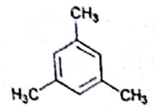
where *h* is Planck's constant, ν is the frequency of radiation, μ is the magnetic moment of the proton and β_N is the nuclear magneton.

Thus, the NMR technique consists in exposing the protons to a powerful magnetic field. The protons will precess at different frequencies. Now, we irradiate these precessing protons with steadily changing rf frequencies. It is generally more convenient to keep the radio frequency constant and the magnetic field is constantly varied. At some values of H the energy required to flip the protons matches the energy of the radiation. Absorbance occurs and a signal is observed.

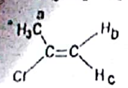
All protons do not absorb at the same applied field, but absorption depends upon the magnetic field a particular proton feels. Thus at a given radio frequency, different protons will require slightly different applied field strengths to produce the same effective field strength which causes absorption. The number of signals at different field strengths is equal to the different sets of equivalent protons.

Number of signals in NMR spectrum

The number of signals in the NMR spectrum reveals the number of different sets of equivalent protons present in a molecule. Each signal corresponds to a set of equivalent protons. It may be noted that magnetically equivalent protons are chemically equivalent

Molecule	No. of signals	Molecule	No. of signals
$(H_3C)_2C{=}O$	1	$(CH_3)_3C{-}NH_2$ (a: CH_3, b: NH_2)	2
	1	$CH_2Br{-}CH_2Br$	1
	1	$CH_3{-}CHBr_2$	2
$CH_3{-}OH$ (a: CH_3, b: OH)	2		3
$CH_3{-}CH_2{-}NH_2$ (a b c)	3		2
$CH_3{-}CH(OH){-}CH_3$ (a b a, c: OH)	3	$CH_3{-}CH_2{-}O{-}CH_2{-}COOH$	4

CH_3-CH_2-CH_2-Cl (a, b, c) 3



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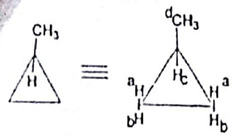

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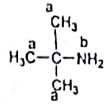

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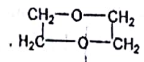
CH_3-CH_2-O-CH_2-CH_3 2

CH_3-CH_2-CH_2-COOH 4

CH_3-CH_2-CHO 3



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Relaxation processes

The population distribution between the two spin states of a nucleus is given by the Boltzmann equation $\frac{n_u}{n_l} = e^{-\Delta E/kT}$

where, n_u and n_l are number of nuclei in the upper and lower energy levels, ΔE is the energy difference between the two states, k is the Boltzmann constant and T is temperature in kelvin. In UV and IR spectroscopies, $\Delta E >> kT$, and hence most of the molecules exist in a lower energy state, i.e., the population in the ground state is so large that no appreciable change in the population occurs during absorption. But in NMR, since $\Delta E \leq kT$, there is only slightly excess of nuclei in the lower energy state. For protons the population distribution ratio is 0.99999/1.00001 at 14092 G and at room temperature. Nuclei in the lower energy state absorb radio frequency radiation and undergo transitions to the higher energy state. Thus initial absorption of energy occurs until equal population in the two states is attained, then further absorption ceases. This phenomenon is called saturation. But in practice the population excess in the ground state is maintained due to returning of nuclei from excited state to ground state, thereby causing continuous absorption. The various ways by which a nucleus returns to the ground state from the excited state without emitting radiation are known as relaxation processes.

Spin-spin or transverse relaxation occurs by the mutual exchange of spins by two precessing nuclei in close proximity to one another.

$H + H^1 \longrightarrow H^1 + H$

Although this mutual exchange of spins shortens the lifetime of an individual nucleus in the excited state, it does not contribute to the maintenance of the required excess of nuclei in the ground state.

Spin-Lattice or Longitudinal relaxation involves the transfer of the excess energy ΔE to the surrounding atoms either in the same molecule or in solvent molecule. This process maintains an excess of nuclei in lower state, which is the condition required for the observation of continuous absorption.

Spectral Line Width

The natural width of a spectral line is inversely proportional to the average time the system spends in the excited state. Thus, sharp resonance lines are observed for states of extended excitation, and broad lines are observed for short-lived excited states.

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i) Solids and viscous liquids usually display broad resonance lines.

ii) The presence of paramagnetic molecules (dissolved oxygen) or ions in the sample causes paramagnetic broadening.

iii) Resonance lines for protons attached to an element that has an electric quadrupole moment will be broadened (the electric quadrupole is a measure of the non-spherical nature of electrical charge distribution of the nucleus). Only nuclei with spin numbers greater than ½ have electrical quadrupole moments. The nitrogen nucleus (I = 1) is the common example of this phenomenon.

Chemical Shifts

In NMR spectroscopy, the resonance frequency required to attain the Larmor frequency of the precessing nucleus is dependent upon the electronic environment about the nucleus. The electrons, under the influence of the external magnetic field, circulate around nucleus, thereby producing a secondary or induced magnetic field, which may oppose or reinforce the external field, so that the magnitude of the field seen at the nucleus, H_N, is different from the applied field, H_o.

$H_N = H_o(1-\sigma)$

where, σ is the shielding constant, which depends on the hybridization and electronegativity of the groups attached to the atom containing the proton.

If the induced field opposes the applied field, then the proton will come to resonance only at a higher applied magnetic field (up field). In this case, the proton is said to be shielded. If the induced field reinforces the applied filed, the net magnetic field felt by the nucleus is larger than the applied field, and the proton will come to resonance at a lower applied field (down field). In this case, the proton is said to be deshielded.

Such shifts in the positions of nmr absorptions which arise due to the shielding or deshielding of protons by the electrons are called Chemical Shifts.

Accurate measurement of H_N and H_o is difficult. Hence a reference material is employed, and the difference between the field strengths at which the sample nucleus and the reference nucleus absorb is measured. For measuring chemical shifts of various protons in a molecule, the signal for tetramethylsilane (TMS) is taken as reference.

TMS has the following characteristics.

i) It contains 12 equivalent protons [$(CH_3)_4Si$] and it gives a single resonance signal at high field in NMR spectrum.

ii) It is miscible with almost all organic substance.

iii) It is highly volatile and is readily removed from the system.

iv) It is chemically inert.

v) It does not take part in inter molecular associations with the sample.

The difference in the absorption position of the proton with respect to TMS signal is called *chemical shift*. It is denoted as δ and defined as

$$\delta = \frac{\nu_{sample} - \nu_{ref}}{Operating\ frequency\ in\ MHz} \times 10^6 \text{ ppm} \quad \text{or}$$

$$\delta = \frac{H_{o(ref)} - H_{o(Sample)}}{H_{o(ref)}} \times 10^6 \text{ ppm (parts per million)}$$

where ν_{sample} refers to resonance frequency of the sample and ν_{ref} to the resonance frequency of TMS.

Most chemical shifts have δ values between 0 and 10

When Me_4Si is employed as a standard, ν_R is greater than ν_S and δ values are negative. Large negative numbers refer to lesser shielding. (For TMS, δ is arbitrarily taken as zero). Instead of δ, another scale, τ is used; τ = 10 – δ

The larger values for τ indicate a more shielded proton (For TMS, τ is 10).

Factors influencing chemical shift

The factors influencing the values of chemical shift are inductive effect, van der Waal's deshielding, anisotropic effects and hydrogen bonding.

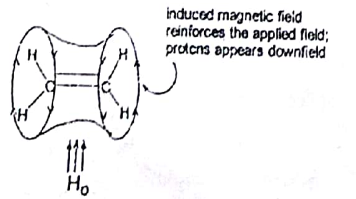
a) Inductive effect: A proton is said to be deshielded if it is attached with an electronegative atom or group. Greater the electronegativity of the atom, greater the deshielding caused to the proton. If the deshielding is more for a proton, then its δ value will also be more.

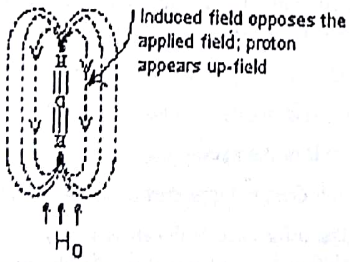
	TMS	CH_4	CH_3I	CH_3Br	CH_3Cl	CH_3F
δ	0	0.4	2.16	2.68	3.05	4.26

Likewise, the successive substitution of an electronegative atom for hydrogen will result in an increase in δ values. For example: CH_3Cl (δ = 3.05), CH_2Cl_2 (5.3), $CHCl_3$ (7.3)

b) van der Waal's Deshielding: In over-crowded molecules, it is possible that some protons may be occupying sterically hindered position. The electron cloud of a bulky group will tend to repel the electron cloud surrounding the proton. Thus, such a proton will be deshielded and will resonate at slightly higher values of δ.

c) Anisotropic effects (Space Effect): In molecules having π bonds, the deshielding or shielding effect on protons is higher than that can be accounted for by the inductive effect alone. Olefinic (δ = 4–8), aldehydic (δ = 9.5–10) and aromatic protons (δ = 7–9) are more deshielded and the alkyne protons (δ = 1.5–3.5) are more shielded.



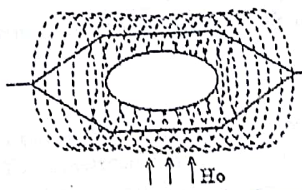


i) Anisotropic effect in alkenes: When an alkene group is so oriented that the plane of the double bond is at right angles to the applied field, induced circulation of π electrons generates induced magnetic field, which is diamagnetic around carbon atom and paramagnetic in the region of the alkene protons.

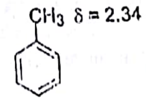
Thus the protons require a lower value of applied magnetic field to come to resonance, and therefore appear at lower filed (higher δ) than expected.

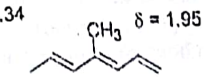
ii) Anisotropic effect in alkynes: When the axis of an alkyne group lies parallel to the direction of the applied field, the π electrons are induced to circulate around the axis in such away that the induced field opposes the applied field. Thus, protons feel diamagnetic shielding and hence resonance occurs at higher applied field (low δ value).

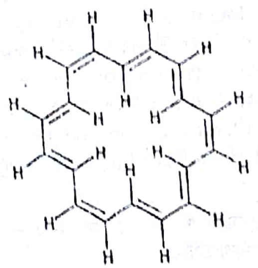
iii) Anisotropic effect in aromatic compounds: In case of benzene, loops of π electrons are delocalised cylindrically over the aromatic ring. These loops of electrons are induced to circulate in the presence of the applied field producing ring current. The induced current is diamagnetic in the centre of the ring and is para-magnetic outside the ring. Thus the aromatic protons experience a magnetic field greater in magnitude than the applied field and come to resonance at down field (higher δ).



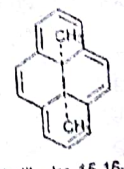
The deshielding caused by the ring current effects in aromatic compounds (cyclically delocalised π-electrons) is greater compared to deshielding of conjugated alkene groups having no cyclic delocalisation.







[18]-Annulene



15,16-dihydro-15,16-dimethylpyrene

This observation is greatly helpful in NMR in deciding aromaticity of an organic compound.

In [18]-annulene, the 12 peripheral protons are deshielded (δ = 8.9) and the six internal protons are shielded (δ = –1.8).

Similarly, in 15,16-dihydro-15,16-dimethyl-pyrene, the methyl groups appear at δ –4.2, showing the methyl groups are deep in the shielding zone of the ring current. The substantial ring current indicates aromatic character in a non-benzenoid ring system.

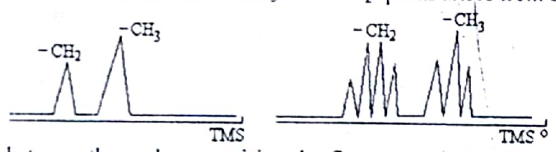
d) Hydrogen bonding: The hydrogen-bonded proton being attached to a highly electronegative atom will have smaller electron density around it. Being less shielded, the field felt by such a proton will be more and hence resonance will occur downfield. The downfield depends upon the strength of hydrogen bonding.

Inter- and intra-molecular hydrogen bondings can be easily distinguished. Intra-molecular hydrogen bonding does not show any shift in absorption due to the change either in concentration or temperature, i.e. the δ value is temperature- and concentration- independent in the case of intra-molecular H-bonding.

Spin-Spin Splitting

The interaction between the spins of the neighbouring nuclei in a molecule may cause the splitting of the lines in the NMR spectrum. This is known as Spin-Spin coupling or spin-spin interaction.

The high resolution HNMR spectrum of ethyl bromide shows a triplet and a quartet for $-CH_3$, and $-CH_2-$ protons. The fine structure in the $-CH_3$ and $-CH_2-$ peaks arises from Spin-spin splitting, and the

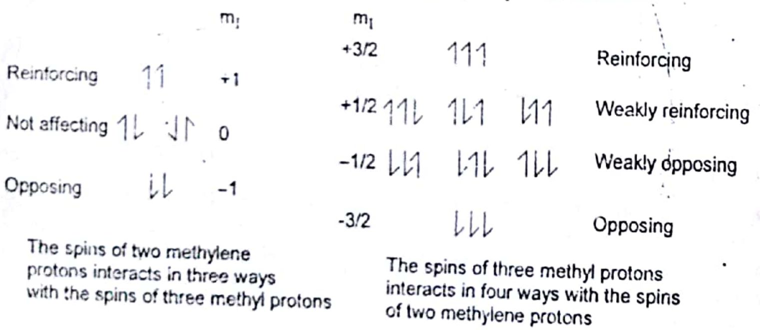


separation, J, between the peaks comprising the fine structure is referred to as spin-spin coupling constant.

The magnitude of δ depends upon the applied field strength and that of J is field independent.

The fine structure is due to the transmittance of the magnetic moment of the neighbouring proton through the intervening bonds. A proton can have spin numbers +1/2 and −1/2. In the +1/2 state the magnetic moment of the neighbouring proton aligns with the applied field while in the −1/2 state it opposes the applied field. As a result, the experimental proton comes to resonance two times.

The number of peaks in the fine structure (multiplicity) is given by the formula (2nI+1), where n is the number of equivalent neighbouring nucleus and I is the spin quantum number.



The fine structure in the methyl peak in the NMR spectrum of ethyl bromide is due to its spin interaction with that of the neighbouring methylene protons. The two methylene protons can have three spin arrangements, viz., 1, 0, -1 with relative ratios 1:2:1. As a result, the methyl peak is split into a triplet. Similarly, the quartet nature of methylene peak is due to the fact that the three neighbouring methyl protons can have four spin values, 3/2, 1/2, −1/2, −3/2, with relative intensity ratios 1:3:3:1.

i) The relative areas in a multiplet is obtained form the coefficients of binomial expansion $(x+y)^n$ or from the Pascal triangle.

ii) Equivalent nuclei do not split each other.

iii) The mutual magnetic influence between the neighbouring protons is transmitted via the electrons in the intervening bonds. This coupling operates strongly through one bond or two bonds, less strongly through three bonds, and rather weakly through four or more bonds.

iv) Rapid intermolecular chemical exchange has a pronounced effect on spin-spin splitting.

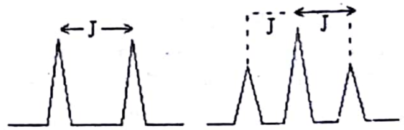
v) The peak area depends upon the number of the absorbing protons and the multiplicity of a signal depends upon the number of neighbouring protons.

Coupling Constants

1) The distance between the centres of the two adjacent peaks in a multiplet is constant and it is called coupling constant.

2) The value of the coupling constant is independent of the external field.

3) It is measured in *Hertz* (Hz) or in cps (cycles per second). It is denoted by the letter *J*.



4) The value of *J* remains the same whatever be the applied field. From the value of coupling constant one can distinguish between the two singlets and one doublet and also a quartet from two doublets.

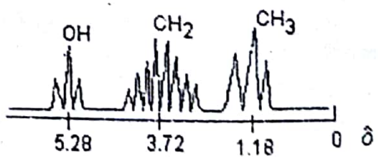
5) It can be done by simply recording the spectrum at two different radio frequencies. If the separation (in Hz) between the lines (value of J) does not change, the signal is a doublet.

6) If the separation between the lines increases with increasing frequency, then the signals may be considered as two singlets.

7) The value of J lies between 0 and 20Hz.

Chemical Exchange (Proton Exchange Reaction)

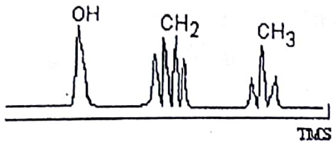
The phenomenon of chemical exchange can be explained by considering the nmr spectrum of anhydrous ethanol and also the spectrum of ethanol containing small quantities of water.



The fine spectrum of pure ethanol shows three peaks *viz.*, a triplet for CH_3 protons, an octet for $-CH_2$ protons and a triplet for hydroxyl proton. The octet nature of methylene protons is due to the splitting of the peak by the three methyl protons into a quartet and each peak in the quartet is further into a doublet by the hydroxyl proton. The OH proton appears as a triplet because of coupling to $-CH_2-$ protons. The splitting due to −OH proton is absent in commercial samples of ethanol. In the presence of acidic or basic impurities, the −OH proton of a molecule is exchanged with that of another molecule.

$$R-OH^{*} + H-OH \longrightarrow R-OH + {}^{*}H-OH$$

As a result of this fast exchange, the $-CH_2$ protons do not feel the spin interaction of $-OH$ proton. In the spectrum of ethyl alcohol containing water, the OH signal appears as a singlet and its coupling with adjacent $-CH_2-$ does not take place.



The proton exchange becomes faster as the water content is increased. The exchange of OH protons in presence of water or at high temperature or in acidic medium is so rapid that a particular proton does not reside on a particular oxygen atom long enough for the nuclear coupling to be observed. Rapid chemical exchange causes spin decoupling.

The proton exchange does not occur, i) If the sample is pure ii) If the spectrum is recorded at a low temperature iii) If the sample is dissolved in a highly polar solvent like dimethyl sulphoxide. Under all these conditions, the rate of exchange process is slowed down and as a result the OH proton can have enough time to couple with the neighbouring protons.

Deuterium Exchange

If a few drops of deuterium oxide are added in the sample, the D_2O exchanges with the labile protons such as $-OH$, $-NH_2$, $-SH$ and also with reactive methylene protons (those flanked by the carbonyl groups). The mechanism is similar to that of proton exchange reactions.

When a little D_2O is added to ROH, then due to rapid exchange, ROH becomes ROD.

$$ROH + D_2O \rightleftharpoons ROD + HOD$$

Thus, the signal for $-OH$ proton normally observed in ROH will be missing in the Hnmr spectrum and a signal for proton in H–OD will appear.

Similarly, if a little D_2O is added to RCOOH, then due to rapid exchange, it becomes RCOOD.

$$RCOOH + D_2O \rightleftharpoons RCOOD + HOD$$

The signal for the proton in RCOOH which normally appears at a negative tau values will be missing and a signal for proton corresponding to H–OD appears instead.

This technique which is employed for detecting the presence of OH,NH groups etc., is called deuterium exchange. For the deuterium exchange technique, two spectra are run.

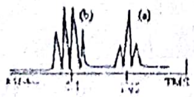
i) One with the sample dissolved in a solvent other than D_2O

ii) Second spectrum with the sample dissolved in the same solvent and containing a few drops of D_2O.

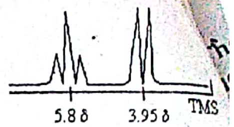
On comparing the two spectra, if the peak areas are seen to diminish, the sample may contain $-OH$, $-NH_2$, $-SH$ group in which deuterium exchange is possible.

NMR spectra of simple organic compounds:

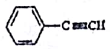
1) CH_3-CH_2-Br Shows two signals
a) A three protons triplet = 1.65 δ
b) a two protons quartet = 4.4 δ



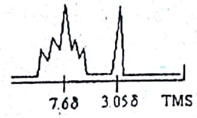
2) Cl_2CH-CH_2Cl shows two signals
a) a two proton doublet = 3.95 δ
b) a one proton triplet = 5.8 δ



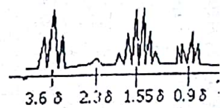
3) Shows two signals



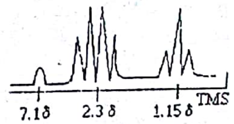
a) One proton singlet 3.05 δ
b) Five proton unsymmetrical multiplet = 7.6 δ



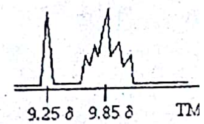
4) $CH_3CH_2CH_2OH$ shows four signals
a) Three proton triplet = 0.9 δ
b) Two proton sexlet ($-CH_2$) = 1.55 δ
c) Two proton triplet (CH_2-O) = 3.6 δ
d) One proton singlet due to $O-H$ = 2.3 δ



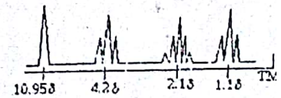
5) $CH_3CH_2CONH_2$ shows three signals
a) Three proton triplet = 1.15 δ
b) Two proton quartet = 2.3 δ
c) Two proton hum = 7.1 δ



6) C_6H_5 CHO shows two signals
1) Five proton unsymmetrical pattern = 9.25 δ
2) One proton singlet (CHO) = 9.85 δ

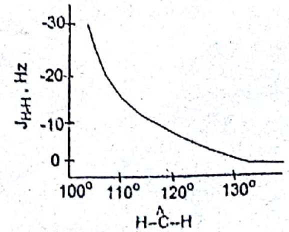


7) $CH_3-CH_2-CH(Br)-COOH$ shows four signals
i) One proton triplet ($-CHBr$) = 4.2 δ
ii) Three proton triplet (CH_3) = 1.1 δ
iii) Two proton pentet = 2.1 δ
iv) One proton singlet ($-COOH$) = 10.95 δ (off the scale)



Geminal coupling

In the case of geminal protons (protons attached on the same carbon atom having different chem environment) of a saturated compound, the value of J depends upon the bond angle. J can have sign. It is of greatest magnitude (10-14 Hz) in the strain-free cyclohexanes and cyclopentanes. W increasing angular strain the J_{gem} valus drops, being 8-14 Hz in cyclobutane and 4-9 Hz cyclopropane.



The Geminal Karplus correlation points out that when the bond angle is 105°, J is –25 cps. J becomes nearly –12 cps when the bond angle increases to 109°. When the bond angle is widened to 125°, the value of J increases to zero. If the bond angle is wider than 125°, we observe small positive values for the coupling constants. A plot showing the relationship between the values of J versus the bond angle is shown here.

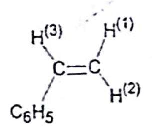
A few characteristics of geminal coupling constant are:

i) The value of coupling constant increases with the increase in bond angle. Example.: J_{gem} is –12.4 cps for methane and it is +2.5 cps for ethylene.

ii) The increase in electronegativity of the atom or group, which withdraws sigma electrons, increases the value of coupling constant. Example.: J_{gem} for CH_3Cl is –10.8 cps whereas it is –9.4 cps for CH_3F

iii) The value of J decreases if electronegative substituents withdraw electrons from the π bonds. Example.: J gem is +2.3 cps for ethane where as it is –3.3 cps for vinyl chloride.

iv) For mono substituted olefins, $J_{trans} > J_{cis} > J_{gem}$



J cis $(H^1, H^2) = 10.6$ cps

J trans $(H^2, H^3) = 17.4$ cps

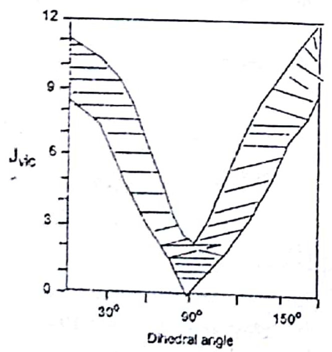
J gem $(H^1, H^2) = -1.4$ cps

Vicinal coupling

Vicinal protons are the protons which are separated by three bonds. The electronegativity of substituents attached to the neighbour carbon alters the value of J_{vic}. The more the electronegativity of the substituents, the smaller is the value of J_{vic}, so that in unhindered ethanes $J_{vic} = 8$ Hz while in halagenoethanes it is 6-7 Hz.

In compounds having restricted rotation, J_{vic} is affected by the H–C–C, C–C–H and also by the presence of small rings.

The dihedral angle φ between the two C–H vicinal bonds influence J_{vic} according to the *Karplus equation*:



φ between 0° and 90° : $J_{vic} = 8.5 \cos^2 \varphi - 0.28$

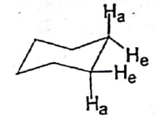
φ between 90° and 180° : $J_{vic} = 9.5 \cos^2 \varphi - 0.28$

The relationship between φ and J_{vic} can be represented graphically. J_{vic} is the largest for *tran* coplanar (φ =180°) and for *cis* coplanar (φ = 0°). When φ =90°, very small couplings arise.

For example: In cyclohexane, the φ between the axial protons is 180° and hence J_{vic} = 10-13 Hz. Fc diequatorial protons or for axial/equatorial protons, J_{vic} = 2-5 Hz corresponding to 60° orientation.

Long–range coupling

Usually no coupling is observed if the distance between the two absorbing nuclei is more than thre

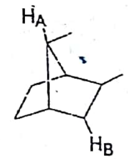


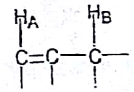
covalent bonds. But unsaturated compounds or in fluoro compounds, appreciable coupling i observed with the help of high resolution spectrometers even if the concerned nuclei are three bond apart. It is called long – range coupling.

Example: 1) Coupling through conjugated poly-acetylenic chains may occur through as many as nin bonds.

2) Meta coupling in a benzene ring is 1 to 3 Hz and para 0 to 1 Hz.

3) In 5-membered hetero-aromatic rings, coupling between 2 and 4 protons is 0 to 2 Hz.





4) J_{AB} in the bicyclo[2,1,1]hexane system is about 7 Hz.

5) In allyl systems, J_{AB} (four-bond coupling) is in the range 0-2 Hz

Trans coupling

In alkene groups, the trans coupling (J_{trans} = 11-19 Hz) is larger than the cis coupling (J_{cis} = 5-14 H:

Spin-Spin Splitting in Aromatic Systems

i) Benzene having a substituent with no strong shielding or deshielding effect give single pe Example: Toluene

ii) Compounds with two identical *p*-substituents give single line spectra because of molec symmetry. Example: *p*-Dinitrobenzene

iii) Compounds with two different *p*-substituents give two line spectra. Also due to coupling betw the ortho and meta protons, each line is further split. Example: *p*-Chloroaniline.

iv) Compounds with a single substituent that is either strongly shielding or deshielding usi causes the ortho protons to move upfield or downfield with respect to meta or para protons. We 2H complex multiplet and a 3H complex multiplet. Example: Acetophenone.

v) Identical groups ortho to each other produce symmetry in the molecule. NMR shows a single complex multiplet which is symmetrical about the mid-point Example: Diethylphthalate

vi) Highly substituted rings give very simple spectra and the coupling constants are in the order $J_{ortho} > J_{meta} > J_{para}$.

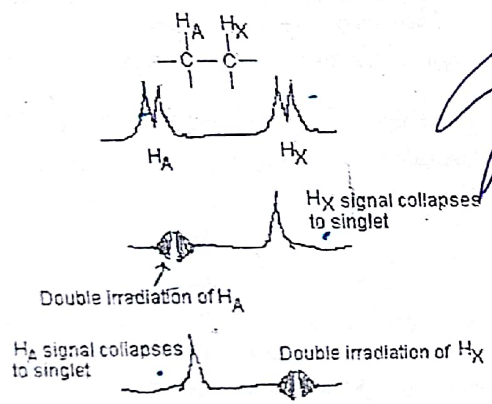
vii) Polynuclear aromatic hydrocarbons invariably give very complex spectra, δ in the range 7-9.

Simplification of Complex NMR Spectra

1) Increased Field Strength

Usually multiplicity is produced when $\Delta\delta > 6J$. δ is field dependent. For example, a δ value of 2 ppm in 60 MHz instrument corresponds to 120 Hz while in 100 MHz instrument, it corresponds to 200 Hz. But coupling constants are field independent. So, when we increase the field strength (ν_o), the ratio $\Delta\delta$:J is effectively increased. Therefore, the overlapping coupling multiplets can be pulled apart by increasing the field strength.

2) Double Resonance (Spin Decoupling)



This technique involves the irradiation of a proton or a group of equivalent protons with sufficiently intense radio frequency energy to eliminate completely the observed coupling to the neighbouring protons.

In the given example, H_A and H_X are in different environments. The nmr shows two doublets corresponding to each proton.

When H_A is irradiated with second radio frequency of appropriate energy, the time of this nucleus in any one spin state will be too short to resolve the coupling with H_X and rapid transitions between the two spin states of A will be stimulated. Hence proton A will come to resonance only once and H_X will appear as a singlet.

By the same argument, if we irradiate proton B with the correct radio frequency energy to cause it to undergo rapid transition between its two spin states, proton H_X will come to resonance only once, and H_A will appear only as a singlet.

In this technique, we make simultaneous use of two radio frequency sources. In addition to the basic NMR instrument, a second tuneable radio frequency source is needed to irradiate H_X at the nec... frequency. The recording of the spectrum is done in the same way. It is called double resonance or double irradiation. It is also called spin decoupling. It is a powerful technique for simplifying spectrum. It helps in the identification of coupled protons in spectra that are too complex for detailed analysis.

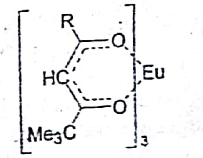
Application of this method to inorganic molecules can be explained by taking the example of diborane. The peaks for terminal and bridged protons are split by the ^{10}B nucleus (I = 3), thereby giving a complex spectrum. By applying a second rf, ^{10}B nuclei are saturated (decoupled) and the spectrum shows two sharp peaks with intensity ratio 2:1 corresponding to terminal and bridged protons.

3) Using Shift Reagents

Shift reagents provide a method for spreading out NMR absorption patterns without increasing the strength of the applied magnetic field.

Addition of shift reagents to sample which has functional group like $-NH_2$, -OH, -CO-, -CHO, -O-, -S-, -COOR, or -CN etc. results in substantial magnification of the chemical shift differences of non-equivalent protons.

The shift reagents are ions in rare earth (lanthanide) series co-ordinated to organic ligand.



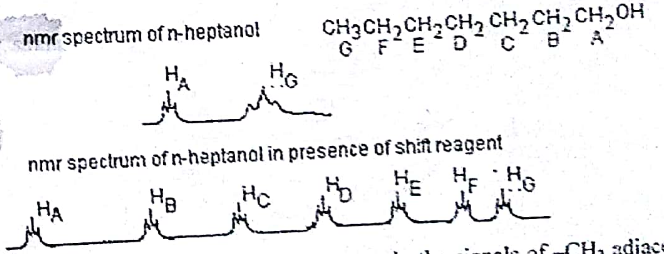
R = t-butyl: $Eu(dpm)_3$

R = $CF_2CF_2CF_3$: $Eu(fod)_3$

Example: $Eu(dpm)_3$ (tris(dipivalomethanato)europium(III)) and $Eu(fod)_3$ (tris-1,1,2,2,3,3-heptafluoro-7,7-dimethyl-3,5-octanedionato)europium(III)).

The lanthanide complex should be soluble in common NMR solvent.

In general, europium complexes produce shifts to higher δ, while praseodymium complexes produce shifts to lower δ.



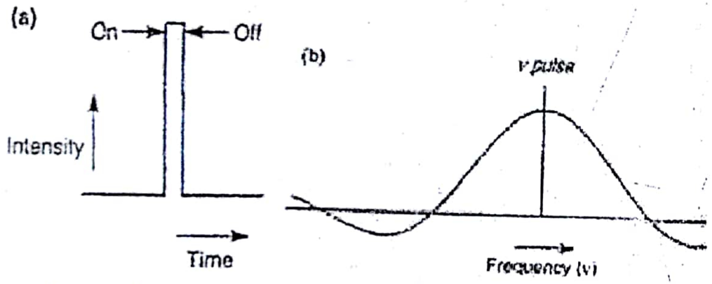
For example: The nmr spectrum of 1-heptanol shows only the signals of $-CH_2$ adjacent to -OH (A protons, δ 3.8, triplet) and the terminal $-CH_3$ (G protons, δ 0.9 triplet). Upon addition of shi...

ADVANCED NMR TECHNIQUES

Pulsed Fourier Transform NMR (FT-NMR)

The continuous wave (CW) type of NMR spectrometer operates by exciting the nuclei on type at a time, i.e., each distinct type of proton (phenyl, vinyl, methyl, and so on) is excited individually, and its resonance peak is observed and recorded independently of all the others. Efficiency and sensitivity of such a system are far from optimum. The time required to observe a NMR spectrum by CW method is Δ/r (in seconds), where Δ is the range of frequencies that must be scanned to cover the chemical shift range and *r* is the resolution desired. For C^{13} at 25 MHz, where Δ is typically about 5 kHz and linewidths are about 1 Hz, it needs to scan the 5-KHz region at a rate of 1 Hz sec^{-1}, or lower, working out for a minimum time of 5000 seconds (~83 minutes).

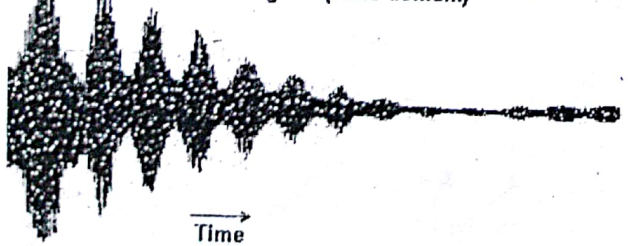
An alternate approach is to use a powerful but short burst of energy, called *a pulse*, that excites all of the magnetic nuclei in the molecule simultaneously. An instrument with a 2.1 Tesla magnetic field uses a short (1 – 10 μsec) burst of 90 MHz energy to accomplish this. The source is turned on and off very quickly, generating a pulse (figure a).



This pulse actually contains a range of frequencies centered about the fundamental (figure b) (Uncertainty principle – as the time is very short, the uncertainty in frequency is large). This range is great enough to excite all of the distinct types of hydrogens in the molecule at once.

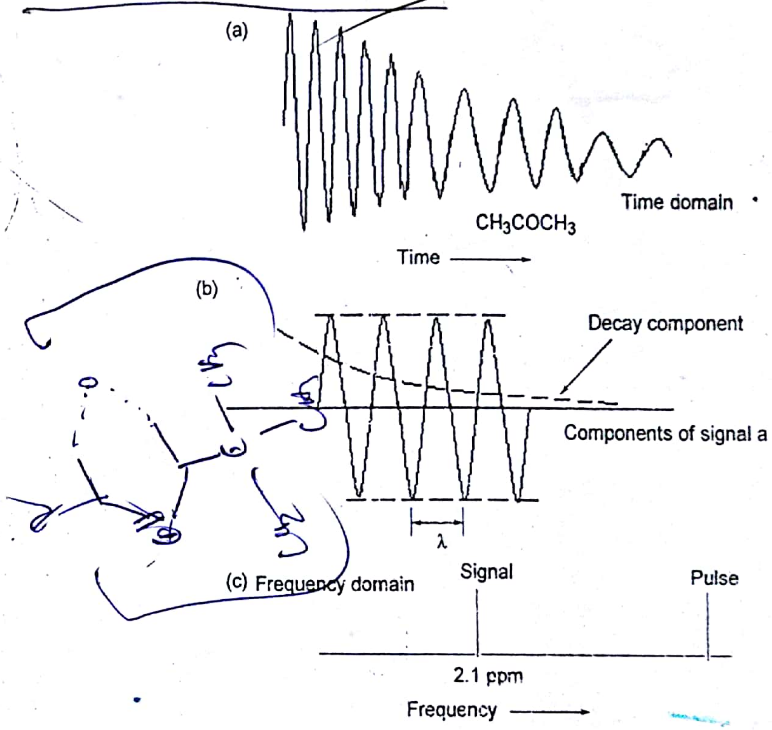
When the pulse is discontinued, the excited nuclei begin to lose their excitation energy and return to their original spin state, or relax, emitting electromagnetic radiation (emr). Since the molecule contains many different nuclei, many different frequencies of emr are emitted simultaneously. The emission is called a *free-induction decay* (FID) signal.

A FID signal (Time domain)



The intensity of the FID decays with time as all of the nuclei eventually lose their excitation. The FID is a superimposed combination of all the frequencies emitted and is quite complex, from which the individual frequencies due to different nuclei are extracted by using a computer and mathematical method, called a *Fourier Transform* (FT) analysis.

The figure shows the FID (not to scale) for the H-atoms in acetone, recorded using an instrum with 7.05 T magnet operating at 300 MHz.



Since acetone has only one type of hydrogens, the FID curve is composed of a single sinusoidal v (figure a). The signal decays exponentially with time as the nuclei relax and this signal dimini Since the horizontal axis is time, the FID is otherwise called a *time-domain signal.*

When the decay is removed, the FID would appear as a sine (or cosine) wave of constant inte (figure b). Frequency is calculated from the wavelength of this wave as $\lambda = \frac{1}{\nu_{acetone} - \nu_{pulse}}$ and chemical shift of acetone protons from the position of the pulse is given as

$$\delta'_{acetone} = \frac{\nu_{acetone} - \nu_{pulse}}{\nu_{pulse}}$$

The actual chemical shift of this peak is calculated as

$$\delta_{actual} = (\delta'_{acetone} - \delta'_{TMS})$$

This peak is now plotted as a chemical shift on a standard NMR spectrum chart (figure c). Thu time-domain signal is converted to a frequency-domain signal.

A complex molecule has many types of hydrogens, and the FID is the superimposition of many different frequencies, each of which will have a different decay rate. Fourier Transform analysis is applied to individual components to convert them to frequencies.

Advantages

i) More sensitive and weak signals can be measured.

ii) It is much faster, can be recorded in few seconds; CW method requires 5 – 10 minutes.

iii) Repeated recording is possible so that very small quantity of sample is enough.

iv) Noise is reduced to great extent as the signal-to-noise ratio improves as a function of square root of number of scans, n: $\frac{S}{N} = f\sqrt{n}$

v) FT-NMR is especially suitable for the examination of nuclei that are not very abundant in nature, nuclei that are not strongly magnetic, or very dilute samples.

Proton Decoupled ^{13}C Spectra

The decoupling technique eliminates all interactions between protons and ^{13}C nuclei; therefore, only singlets are observed. The disadvantage of this technique is the information on attached hydrogens is also lost.

Proton decoupling is accomplished in ^{13}Cnmr by simultaneously irradiating all of the protons in the molecule with a broad spectrum of frequencies in the proper range, generated by a second, tunable rf generator, *the decoupler*. In the ^{13}Cnmr spectrum of ethyl phenylacetate, every chemically and magnetically distinct carbon gives a single peak. The two ortho ring carbons and two meta ring carbons are equivalent by symmetry and each gives only a single peak.

Nuclear Overhauser Enhancement (NOE)

When a proton-decoupled ^{13}C spectrum is recorded, the intensities of many of the carbon resonances increase significantly above those observed in a proton-coupled experiment. Carbon atoms with hydrogen atoms directly attached are enhanced the most, and the enhancement increases (not linearly) as more hydrogens are attached. This effect is known as *Nuclear Overhauser Enhancement* (NOE).

The NOE effect is heteronuclear and positive, i.e., irradiating the hydrogens increases the intensities of the carbon signals. The maximum enhancement that can be observed is given as

$$NOE_{max} = \frac{1}{2}\left(\frac{\gamma_{irr}}{\gamma_{obs}}\right)$$

where γ_{irr} is the magnetogyric ratio of the nuclei being irradiated and γ_{obs} is that of the nucleus being observed. For a proton-decoupled ^{13}C spectrum,

$$NOE_{max} = \frac{1}{2}\left(\frac{267.5}{67.28}\right) = 1.988$$

indicating that ^{13}C signals can be enhanced upto 200% by irradiation of the hydrogens.

The reverse effect can also be observed. The hydrogen signals would increase when we irradiate ^{13}C nuclei. However, the effect would be very small, because there are few ^{13}C atoms in a molecule.

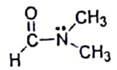
Signal enhancement due to NOE is explained as a result of cross-polarization in which a polarization of the spin states in one type of nucleus causes a polarization of the spin states in another nucleus. The interaction of the spin-spin dipoles operates through space, not through bonds, and its magnitude decreases as a function of the inverse of r^3, where r is the radial distance from the hydrogen of origin.

$$NOE = f\left(\frac{1}{r^3}\right) \qquad C \xrightarrow{r} H$$

Thus, nuclei must be rather close together in the molecule in order to exhibit the NOE effects. The effect is greatest for hydrogens that are directly attached to carbon.

NOE is sometimes used to verify peak assignments. Irradiation of a selected hydrogen or group of hydrogens leads to a greater enhancement in the signal of the closer of the two carbon atoms being considered.

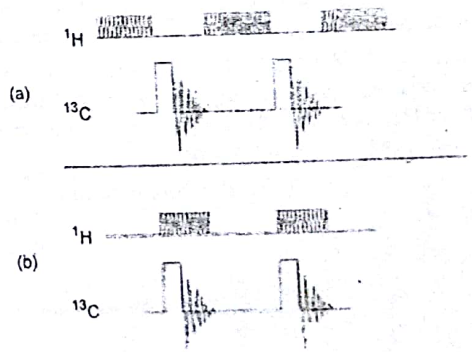
In dimethyl formamide, the two methyl groups are non-equivalent, showing two peaks at 31.1 and 36.2 ppm, because free rotation is restricted about the C–N bond due to resonance interaction between the lone pair on nitrogen and the π-bond of the carbonyl group.



Irradiation of the aldehyde hydrogen leads to a larger NOE for the carbon of the syn-methyl group that for that of the anti-methyl group.

NOE-Enhanced Proton-Decoupled Spectrum (Gated Decoupling Spectrum)

Decoupling is available only while the decoupler is in operation and stops immediately when the decoupler is switched off. This aspect is utilized in retaining the benefits of NOE even when determining a proton-decoupled ^{13}Cnmr spectrum that shows the attached hydrogen multiplets.



(a) Pulse sequence for gated decoupling
(b) Pulse sequence for inverse gated decoupling

This is achieved by a technique known as *pulse sequence*. NOE effect is built up by irradiating wi the decoupler during a period before the pulse and then turning off the decoupler during the pulse ai free-induction decay collection periods. The effect of this pulse-sequence is to allow the NOE develop while the decoupler is on. Because the decoupler is switched off during the excitation pul:

11.18.2 Applications

Solid state NMR spectroscopy has many wide-ranging applications. The high resolution solid state NMR spectra can give us information on bonding, structure and dynamic behaviour of solid state structures. Since the line width is a function of the internuclear distance, it has been possible in simple cases to measure proton-proton distances, which are difficult to measure by other techniques. The MAS or CP MAS technique is useful in following solid state reactions, phase changes and polymorphism. It is useful in the study of amorphous materials such as glasses and gels and also of silicates and zeolites using ^{29}Si and ^{27}Al NMR.

11.19 2D NMR SPECTROSCOPY

Since 1980, two-dimensional (2D) methods have revolutionalized the practice of NMR. It has resulted in the development of several techniques for the structural elucidation of complex organic molecules. 2D NMR comprises of a relatively new set of multipulse techniques that make it possible to unravel complex spectra. The two-dimensional techniques can identify resonances connected by through-bond coupling, by through space interactions or by chemical exchange. We restrict ourselves here to a brief introduction to the basic principles and a discussion of the most popular 2D experiments.

11.19.1 The Principles of 2D NMR

The conventional NMR spectrum is called as an one-dimensional spectrum because it has only one dimension in frequency; the chemical shift and the spin-spin couplings are displayed on the same axis. With larger molecules this can lead to very complicated spectra with many overlapping multiplets. Using a special pulse sequence, it is possible to obtain a spectrum in two dimensions so that the chemical shift is on the conventional axis and the coupling constant information on a different axis.

In the conventional 1D NMR discussed earlier, the FID is recorded immediately after the pulse and the only time domain involved (t_2) is the one during which FID is recorded. However, if the signal is not recorded immediately after the pulse and a certain time interval (t_1) was allowed to elapse before detection, then during this time interval (called the evolution period), the nuclei can interact with each other in different ways depending on the pulse sequences employed. Introduction of this second dimension led to the development of several NMR techniqes for the elucidation of structure of complex organic molecules.

All 2D NMR experiments use multipulse sequences. Initially the so-called 'preparation phase' involves equilibration of the sample in a magnetic field. It is followed by one or more pulses at the beginning of the subsequent evolution period. It is then allowed to evolve as a function of time (evolution phase). During this phase, the spin system is allowed to affect each others behaviour for example by spin-spin coupling or interact with each other through space.

...detection i.e. collection of FIDs then begins (acquisition phase). Acquisition ... is the time taken to digitise the FID. After the acquisition phase, the ... of preparation, evolution and detection continues.

There are many forms of 2D NMR spectra. Generally 2D NMR spectra would be required for systems for which 1D spectra are complicated. All 2D experiments are a series of simple 1D experiments collected with different timings. Each type of 2D NMR can provide either through bond or through space coupling information. In the 2D NMR spectroscopy, the data are collected in two different time domains: acquisition of FID (t_2) and a successively incremented delay (t_1). The resulting FID (data matrix) is accordingly subjected to two successive sets of Fourier transformations to obtain a 2D NMR spectrum in two frequency axes, F_1 and F_2. The pulse sequences used in a 1D and the simplest 2D NMR experiments are given in Fig. 11.70.

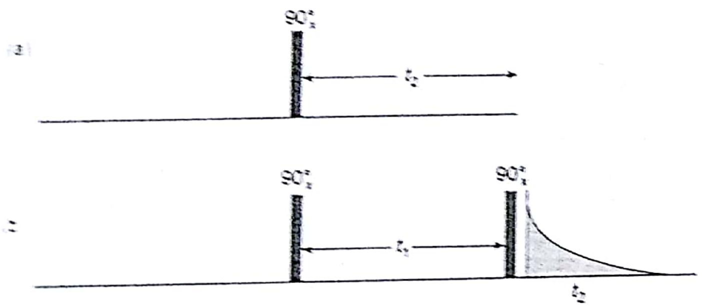


Fig. 11.70 Typical pulse sequence used for (a) 1D ^{1}H NMR and (b) 2D NMR experiment.

The preparation period allows the spin system to attain its equilibrium state by relaxation processes. The effect of t_1 can only be observed indirectly by noting its influence on t_2. It is therefore necessary to carry out F_2 transformation (across t_2) domain first in order to generate a series of spectra. The first set of Fourier transformation in 2D NMR experiments such as COSY discussed below produces signals in F_2 dimension. The second set of Fourier transformations across t_1 yields signals in F_1 dimension.

There are two basic methods of plotting a two-dimensional NMR spectrum (Fig. 11.71). The first is a stack plot which is difficult to analyze. The other is a contour plot which is a cross-section through the stack plot at a chosen height. The contour maps are quicker to plot than stacked plots and easier to analyze.

11.19.2 COSY

A 2D technique known as COrrelated SpectroscopY(COSY) gives information about spin-spin coupling between nuclei of a single isotope, say protons. The COSY experiment is one of the most important techniques of 2D NMR

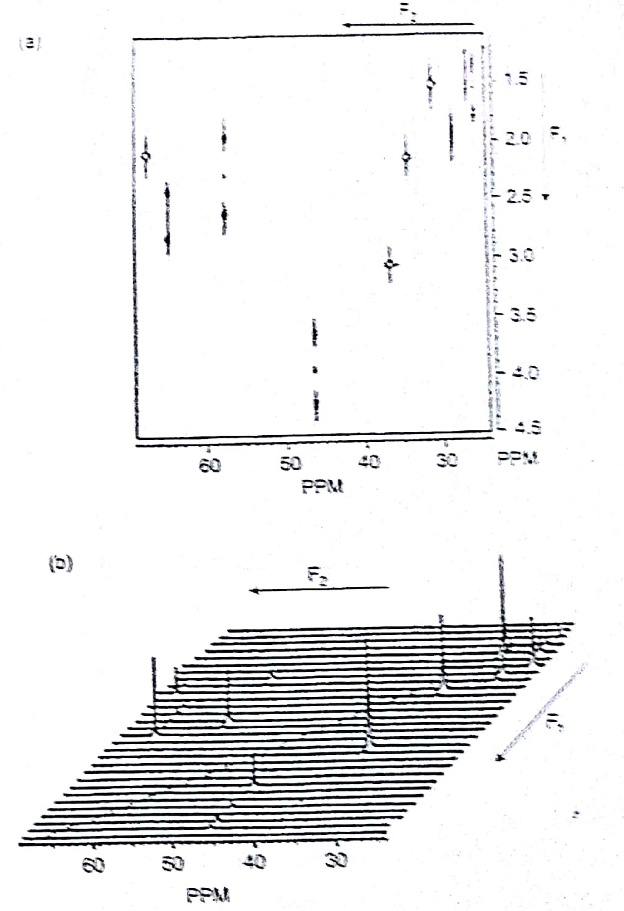


Fig. 11.71 Presentation of a 2D NMR spectrum: (a) contour plot and (b) Stack plot.

spectroscopy for establishing proton-proton couplings. Here both the frequ... axes relate to the chemical shifts and the so-called 'cross peaks' show the ... which are involved in scalar coupling. Thus the COSY spectrum prov... structural information through spin connectivities. The pulse sequence use... obtain the COSY spectrum is given in Fig. 11.72.

In Fig. 11.73, the 2D COSY spectrum of the sodium salt of n-butyric ... is displayed as an intensity contour map. In the spectrum, two identical pr... chemical shift axes (labeled as F_1 and F_2) are plotted orthogonally; F_2 on ... x-axis and F_1 on the right, the chemical shift scale running from top to bot...

11.19.5 NOESY

A 2D spectrum which serves to identify all the proton-proton NOEs occurring in a molecule in a single experiment is called a NOESY (Nuclear Overhauser Effect SpectroscopY). The NOESY technique has the advantage that it applies to molecules in solution and the compound need not have to be crystalline as in X-ray crystallography. The two dimensional NOESY is used extensively for stereochemical assignments. It provides an indirect way to extract information about internuclear distances. A typical NOESY spectrum is given in Fig. 11.78. The off-diagonal cross peaks represent the NOE interactions between various nuclei.

A large number of 1D NOE experiments may have to be carried out if the spatial relationships among several protons in a molecule are to be established.

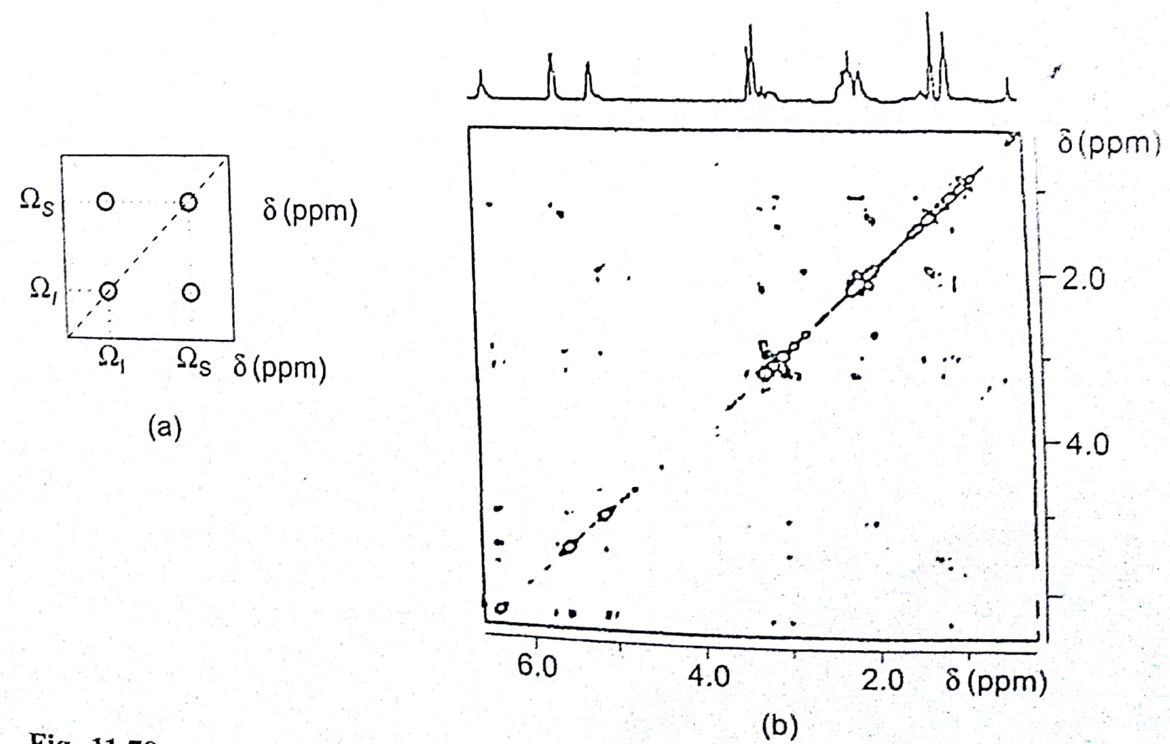


Fig. 11.78 (a) NOESY spectrum for a pair of dipolar coupled nuclei *I* and *S*, and (b) a typical NOESY spectrum.

In such cases, the use of NOESY is particularly advantageous since it may be difficult to carry out irradiation selectively in 1D experiments without affecting other nearby protons. In the NOESY spectrum all inter-proton NOE effects appear simultaneously and the spectral overlap is minimized due to the spread of the spectrum in two dimensions.

The NOSEY spectrum appears like a proton -proton COSY spectrum because each orthogonal axis is of the proton chemical shifts and the normal spectrum appears on the diagonal. However, the cross peaks indicate those protons that are closer in space i.e. they provide evidence of through-space interactions. Thus a NOSEY spectrum provides vital information about the geometry of the molecule. Larger molecules normally give much more intense NOE cross peaks and yield detailed information on their geometry. Since the contour plot along the diagonal which represents the normal one-dimensional spectrum is not the clearest way to follow the proton resonances, the one-dimensional spectrum is frequently reproduced along one axis of the two-dimensional contour plot. The cross peaks are symmetrically placed with respect to the diagonal. Overlapping resonances can often be eliminated by recording the spectrum again at different temperatures, pH, or in a different solvent.

D. Double Resonance

Though we have discussed the double resonance (or double irradiation) already, we shall give further remarks on it. As we have said, in a double-resonance experiment, the sample is simultaneously exposed to rf radiation of two different frequencies, one frequency being used to observe radiation absorption and the second frequency to produce a perturbation that affects the spectrum. For instance, in observing natural-abundance ^{13}C spectra in organic compounds, in addition to applying a pulse of radio frequency, radiation that covers the frequency range of ^{13}C absorptions, one also applies continuous strong rf radiation whose frequencies cover the range of proton absorption frequencies. This results in the removal of the spin-spin coupling between ^{1}H and ^{13}C nuclei (a process called **decoupling**). As a result, ^{1}H spins do not split the ^{13}C absorption lines. Since the probability that two C nuclei are both ^{13}C is very small, there is no $^{13}C - ^{13}C$ spin-spin splitting in natural-abundance ^{13}C NMR. With no spin-spin coupling, the ^{13}C natural-abundance spectrum contains one line for each set of non equivalent carbons. In ^{13}C NMR, the reference compound is TMS and the ^{13}C chemical shifts in organic compounds usually lie in the range of δ from – 10 to 230 ppm. As with protons, the δ-value is characteristic of the kind of carbon being observed. For example, δ for C=O carbon in ketones is usually between 200 and 225 ppm. The combination of proton and ^{13}C NMR is an extremely powerful method for structure determination. The convenient notation $^{13}C - \{^{1}H\}$ is used to identify **proton-decoupled carbon – 13 NMR spectra.** We usually double-irradiate *all* protons simultaneously while recording the ^{13}C spectrum. A decoupling signal is used that has all the ^{1}H frequencies spread around 80 MHz and is, therefore, a form of radio frequency noise. Spectra derived thus are ^{1}H–*decoupled* or *noise-decoupled.* Most ^{13}C spectra are recorded in this way.

E. The Nuclear Overhauser Effect (NOE)

Consider the following double-resonance experiment. The ^{1}H NMR spectrum of a molecule is recorded (using either a CW or FT spectrometer), while the sample is continuously irradiated with rf radiation of frequency ν_s that is the NMR absorption frequency of a specific set (which we call S) of chemically equivalent protons in the molecule. We then find that the intensities of all lines that are due to protons that are close to the set-S protons in the molecule are changed as compared with the spectrum taken without continuous radiation at ν_s. The radiation at ν_s changes the energy level population distribution of the set-S protons, and the magnetic dipole-dipole interaction between the set-S protons and nearby protons changes the populations of the nearby protons, thereby changing the intensities of their NMR lines. This intensity change is the **nuclear Overhauser effect (NOE).** The magnitude of NOE is usually proportional to $1/r^6$ where *r* is the distance between the set-S protons and protons producing the line whose intensity is changed. The NOE is negligible for $r > 4Å$. The NOE can be used to help assign spectra and to find internuclear distances in a molecule.

The magnitude of NOE depends on the balance of relaxation mechanisms, and its value for an A – {X} experiment, occurring when dipole-dipole mechanisms predominate, is given by

$$NOE_{max} = 1 + \gamma_X/2\gamma_A \qquad ...(21)$$

where γ_is are the magnetogyric ratios.

Simply stated, in decoupling experiments the ratio of the total integrated double resonance intensity to single resonance intensity is called the NOE. The NOE enhancement can give a useful gain in signal-to-noise ratio. Dipolar relaxation is particularly important for spin 1/2 nuclei and as the rate is inversely proportional to the sixth power of the distance between the nuclei, it is most significant where the nucleus being irradiated is directly bonded to the one being observed. For $^{13}C - \{^{1}H\}$, the maximum effect is nearly 3 and enhancements close to this are normally seen for all carbons bound to hydrogen. It should also be noted that Eq. 21 leads to *negative* enhancements if γ_X or γ_A is negative. For $^{29}Si - \{^{1}H\}$, the maximum effect is – 1·5 while for $^{15}N - \{^{1}H\}$, it is –4. But, as the minimum effect is +1, the actual effect may in practice be zero and expected resonances may be absent. Fig. 4 presents ^{29}Si spectra of $SiHPh_3$ showing the inversion of signals and the improvement in the signal-to-noise ratio obtained on proton decoupling.

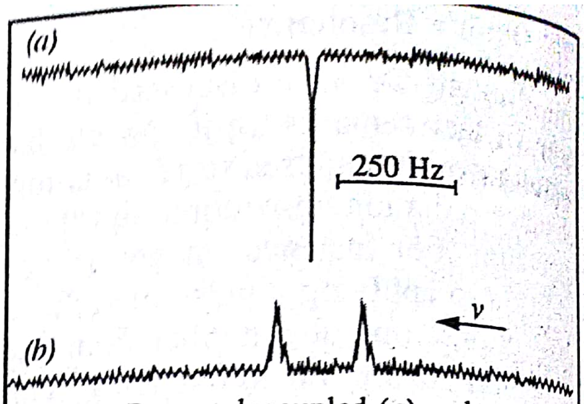


Fig. 4. Proton-decoupled (*a*) and coupled (*b*) ^{29}Si spectra of triphenylsilane.

F. Off-Resonance Proton Decoupling

Fully proton-decoupled ^{13}C NMR spectra offer a distinct advantage over fully coupled spectra (sometimes called *non-decoupled* spectra) The advantage is that the removal of coupling multiplicity makes the spectrum simpler in appearance and ensures almost no confusing overlap in adjacent signals, but there is a sensitivity bonus in addition. Thus, for example, the methyl carbon in p-hydroxyacetophenone would appear in a non-decoupled spectrum as a 1 : 3 : 3 : 1 quartet because of the three attached and coupling protons and, when this is decoupled, the whole of the signal intensity appears as a single line (of intensity 8 relative to the outside lines of the quartet). The fact that the signal is a quartet proves that it arises from a methyl group and unfortunately this valuable piece of information is lost in the fully decoupled $^{13}C - \{^1H\}$ NMR spectrum. There are several techniques which allow this information to be retained ; the simplest consists in carrying out the proton decoupling by irradiation of the sample with radiofrequency which is not quite exactly that of the protons but a few hundred hertz displaced. The consequence of this **off-resonance decoupling** is an incomplete collapse of the multiplicity, and vestigial quartets remain from methyl carbons, with triplets from CH_2, doublets from CH and singlets from fully substituted carbons. It is convenient to annotate signals in $^{13}C-\{^1H\}$ spectra to indicate multiplicity, with the abbreviation *q*, *t*, *d* and *s* for quartet, triplet, doublet and singlet, respectively.

G. Application of 2D NMR