Advance pharmaceutical analysis:

- The aim of this course to teach the students how to identify organic compounds from the:

synergistic information afforded by the combination of mass (MS), infrared (IR), nuclear magnetic resonance (NMR), and ultraviolet (UV) spectra. Essentially, the molecule is perturbed by these energy probes and the molecule's responses are recorded as spectra.

The pharmaceutical analyses remain unchanged, but remarkable evolution of instrumentation has been done.

In comparison, ultraviolet spectrometry has become relatively less useful for our purpose.

NMR, without question, has become the most sophisticated tool available to the organic chemist, in comparison; ultraviolet spectrometry has become relatively less useful for our purpose.

Mass spectrometry	Molecular size and formula
Infrared spectroscopy	Functional groups
NMR spectroscopy	Map of carbon–hydrogen framework

Absorption of UV-Visible light is chiefly caused by electronic excitation; the spectrum provides limited information about the structure of the molecule.

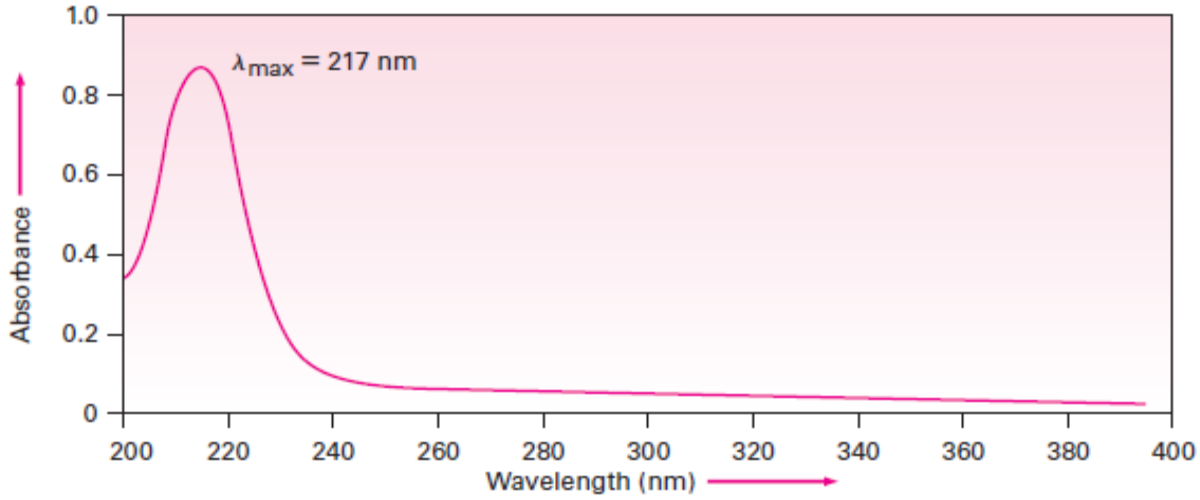


Figure 14.12 The ultraviolet spectrum of 1,3-butadiene, λ_{max} = 217 nm.

Absorption in the infrared region is due to molecular vibrations of one kind or another, the spectrum is generally very complicated and contains many absorption peaks, relatively few of which can be interpreted with a high degree of assurance.

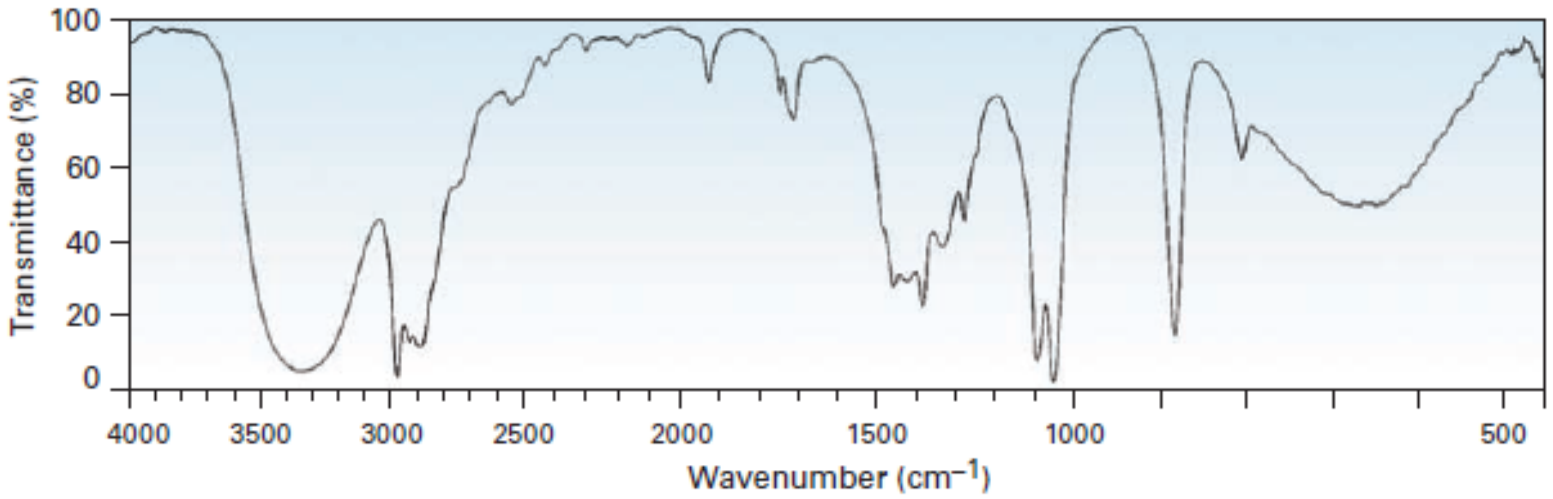
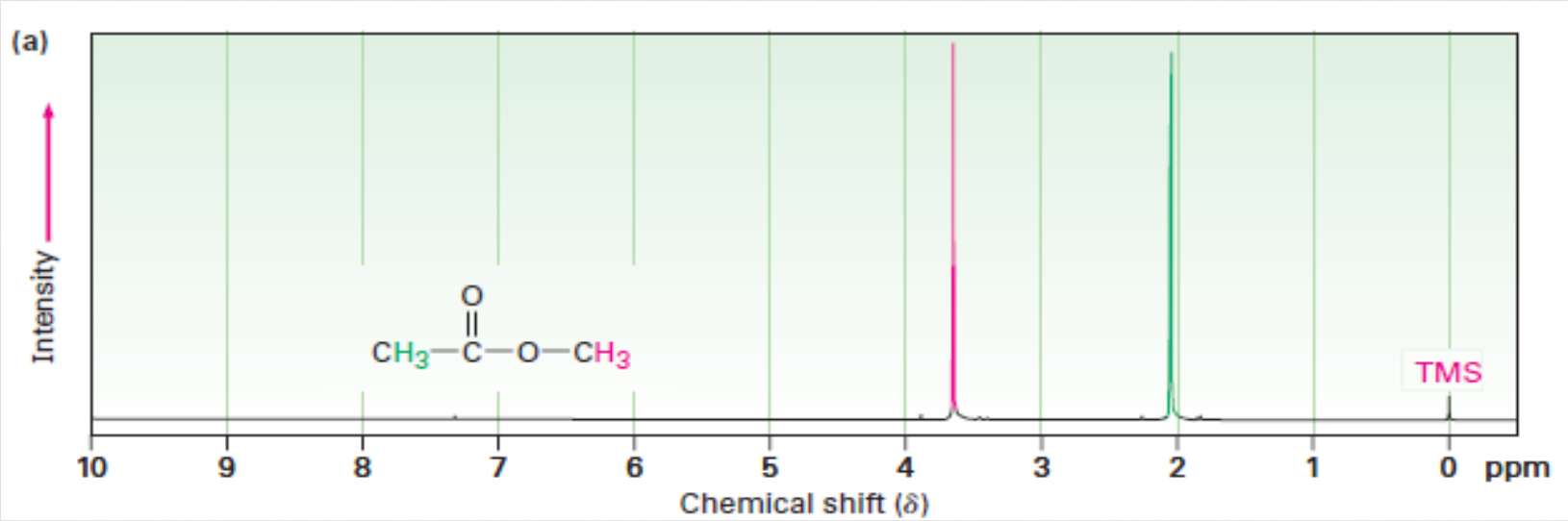


Figure 12.12 An infrared absorption spectrum of ethanol, CH_3CH_2OH. A transmittance of 100% means that all the energy is passing through the sample, whereas a lower transmittance means that some energy is being absorbed. Thus, each downward spike corresponds to an energy absorption.

On the other hand, the NMR spectrum of a compound, owing to nuclear spin transitions, can usually be completely interpreted, and it provides information about the number, nature, and environment of all of the protons in the molecule.



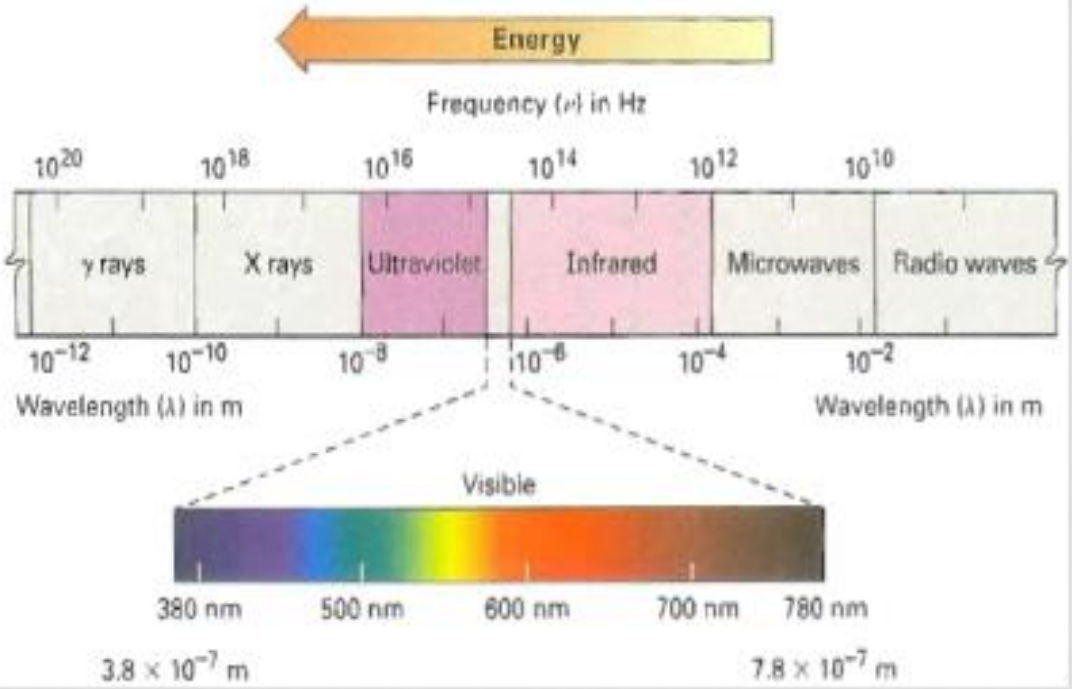
SPECTROSCOPY AND THE ELECTROMAGNETIC SPECTRUM

Infrared, ultraviolet, and nuclear magnetic resonance spectroscopies differ from mass spectrometry in that they are nondestructive and involve the interaction of molecules with electromagnetic energy rather than with an ionizing source.

Electromagnetic radiations:

Electromagnetic radiation is an electric and magnetic disturbance that is propagated through space at the speed of light. This type of radiation has no mass and is unaffected by either an electrical or magnetic field because it has no charge.

Figure 12.10 The electromagnetic spectrum covers a continuous range of wavelengths and frequencies, from radio waves at the low-frequency end to gamma (γ) rays at the high-frequency end. The familiar visible region accounts for only a small portion near the middle of the spectrum.

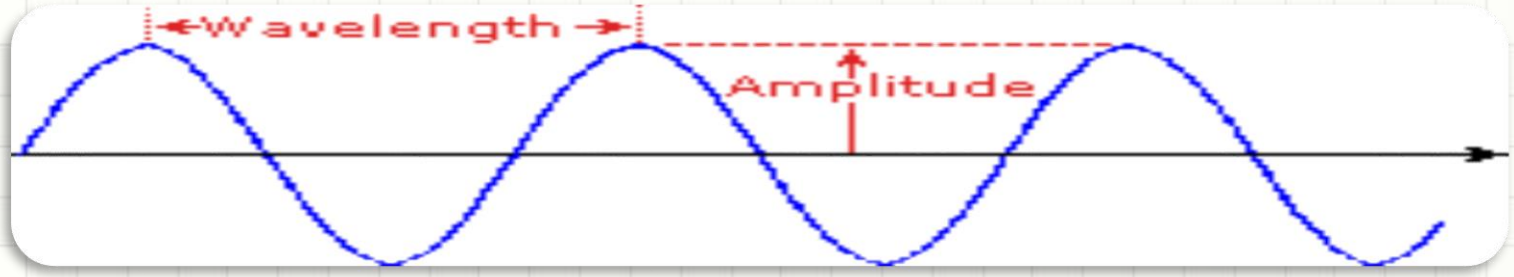


Electromagnetic radiation is often said to have dual behavior. In some respects, it has the properties of a particle, called a *photon,* yet in other respects it behaves as an energy wave.

Like all waves, electromagnetic radiation is characterized by:

- The wavelength, λ (Greek lambda), is the distance from one wave maximum to the next.
- The frequency, υ (Greek nu), is the number of waves that pass by a fixed point per unit time, usually given in reciprocal seconds (s^{-1}), or hertz, Hz (1 Hz = s -1).
- The amplitude is the height of a wave, measured from midpoint to peak.

Electromagnetic Radiation



The various forms of electromagnetic radiation differ in their frequency and, therefore, their energy. The energy of electromagnetic radiation can be calculated in electron volts from the following equation:

- The relationship between wavelength & frequency can be written as:

$$c = v \lambda$$

- As photon is subjected to energy, so

$$\varepsilon = h\nu = \frac{hc}{\lambda}$$

where h = Planck's constant (6.62×10^{-34} J · s = 1.58×10^{-34} cal · s).

C= speed of the light (3* 10^{10}) cm /second

V = the frequency (hertz), and λ is the wavelength (cm).

UV SPECTROSCOPY

Principles

Radiation in the wavelength range 200-700 nm is passed through a solution of a compound. The electrons in the bonds within the molecule become excited so that they occupy a higher quantum state and in the process absorb some of the energy passing through the solution. The more loosely held the electrons are within the bonds of the molecule the longer the wavelength (lower the energy) of the radiation absorbed.

Applications in pharmaceutical analysis

- A robust, workhorse method for the quantification of drugs in formulations where there is no interference from excipients.
- Determination of the p*K*a values of some drugs.
- Determination of partition coefficients and solubilities of drugs.
- Used to determine the release of drugs from formulations with time, e.g. in dissolution testing.
- Can be used to monitor the reaction kinetics of drug degradation.
- The UV spectrum of a drug is often used as one of a number of pharmacopoeial identity checks.

Strengths

- An easy-to-use, cheap and robust method offering good precision for making quantitative measurements of drugs in formulations
- Routine method for determining some of the physico-chemical properties of drugs which need to be known for the purposes of formulation
- Some of the problems of the basic method can be solved by the use of derivative spectra.

Limitations

- Only moderately selective.The selectivity of the method depends on the chromophore of the individual drugs, e.g a coloured drug with an extended chromophore is more distinctive than a drug with a simple benzene ring chromophore
- Not readily applicable to the analysis of mixtures.

The measurement of light absorption by a solution of molecules is governed by the Beer-Lambert Law, which is written as follows:

$$\log I_o/I_t = A = \varepsilon\, b\, c$$

where I_o is the intensity of incident radiation, I_t is the intensity of transmitted radiation; A is known as the absorbance and is a measure of the amount of light absorbed by the sample; ε is a constant known as the molar extinction coefficient and is the absorbance of a 1M solution of the analyte, b is the pathlength of the cell in cm, usually 1 cm and c is the concentration of the analyte in moles liter^{-1}.

Calculate the percentage of the incident radiation absorbed by a sample with an absorbance of (i) 2; (ii) 0.1.

Answers: (i) 99.0%; (ii) 20.6%

In pharmaceutical products, concentrations and amounts are usually expressed in grams or milligrams rather than in moles and thus for the purposes of the analysis of these products, the Beer-Lambert equation is written in the following form:

$$A = A\ (1\%,\ 1\ \text{cm})\ \text{b}\ \text{c}$$

A is the measured absorbance; A (1%, 1 cm) is the absorbance of a 1% w/v (1 g/100 ml) solution in a 1 cm cell; b is the pathlength in cm (usually 1 cm); and c is the concentration of the sample in g/100 ml. Since measurements are usually made in a 1 cm cell the equation can be written:

$$\text{c} = \frac{A}{A\ (1\%,\ 1\ \text{cm})}$$ which gives the concentration of the analyte in g/100 ml

BP monographs often quote a standard A (1%,1 cm) value for a drug which is to be used in its quantitation.

UV / Visible Spectroscopy

Spectroscopy

- It is the branch of science that deals with the study of interaction of matter with light.

OR

- It is the branch of science that deals with the study of interaction of electromagnetic radiation with matter.

Principles of Spectroscopy

Principles of Spectroscopy

- The principle is based on the measurement of spectrum of a sample containing atoms / molecules.
- Spectrum is a graph of intensity of absorbed or emitted radiation by sample verses frequency (ν) or wavelength (λ).
- Spectrometer is an instrument design to measure the spectrum of a compound.

Principles of Spectroscopy

1. Absorption Spectroscopy:

- An analytical technique which concerns with the measurement of absorption of electromagnetic radiation.

- e.g. UV (185 - 400 nm) / Visible (400 - 800 nm) Spectroscopy, IR Spectroscopy (0.76 - 15 µm)

Principles of Spectroscopy

2. Emission Spectroscopy:
   - An analytical technique in which emission (of a particle or radiation) is dispersed according to some property of the emission & the amount of dispersion is measured.
   - e.g. Mass Spectroscopy

Interaction of EMR with Matter

Interaction of EMR with matter

1. Electronic Energy Levels:

- At room temperature the molecules are in the lowest energy levels E_0.

- When the molecules absorb UV-visible light from EMR, one of the outermost bond / lone pair electron is promoted to higher energy state such as E_1, E_2, …E_n, etc is called as electronic transition and the difference is as:

$$\Delta E = h\nu = E_n - E_0 \quad \text{where } (n = 1, 2, 3, \ldots \text{ etc})$$

$$\Delta E = 35 \text{ to } 71 \text{ kcal/mole}$$

Interaction of EMR with matter

2. Vibrational Energy Levels:

- These are less energy level than electronic energy levels.
- The spacing between energy levels are relatively small i.e. 0.01 to 10 kcal/mole.
- e.g. when IR radiation is absorbed, molecules are excited from one vibrational level to another or it vibrates with higher amplitude.

Interaction of EMR with matter

3. Rotational Energy Levels:

- These energy levels are quantized & discrete.
- The spacing between energy levels are even smaller than vibrational energy levels.

$$\Delta E_{rotational} < \Delta E_{vibrational} < \Delta E_{electronic}$$

Principles of UV - Visible Spectroscopy

Principle

- The UV radiation region extends from 10 nm to 400 nm and the visible radiation region extends from 400 nm to 800 nm.

  Near UV Region: 200 nm to 400 nm

  Far UV Region: below 200 nm

- Far UV spectroscopy is studied under vacuum condition.
- The common solvent used for preparing sample to be analyzed is either ethyl alcohol 95%, or hexane.

The useful information obtained from the UV _Visible spectrum of any compound are:

1. The wave length of maximum absorption λmax .
2. The intensity of absorption.

The compound should be dissolved in some suitable solvent that doesn't itself absorb light in the region under investigation.

The position of the absorption peaks of a compound may be shifted if different solvents are used.

However, the λmax for non polar compounds is generally the same in alcohol and hexane, while λmax for polar compounds is usually shifted dependent on the polarity of the solvent used

THEORY INVOLVED

- When a beam of light falls on a solution or homogenous media ,a portion of light is reflected ,from the surface of the media, a portion is absorbed within the medium and remaining is transmitted through the medium.
- Thus if I_0 is the intensity of radiation falling on the media
- I_r is the amount of radiations reflected,
- I_a is the amount of radiation absorbed &
- I_t the amount of radiation transmitted then

$$I_0 = I_r + I_a + I_t$$

- The absorbance A or optical density is given by:
- $A = \log I_0 / I$
- The range of absorbance commonly recorded is 0 to 2
- Transmittion is the ratio of the transmitted light to incident light: $T = I / I_0$
- $A = \log 1/T$

ABSORPTION LAWS

- Lambert's law
- Beer's law
- Beer-lambert's law

- Lambert's law: the intensity of the transmitted light decreases as the thickness of the layer increases
- Beer's law: the absorption is proportion to the numbers of the absorbing molecules.

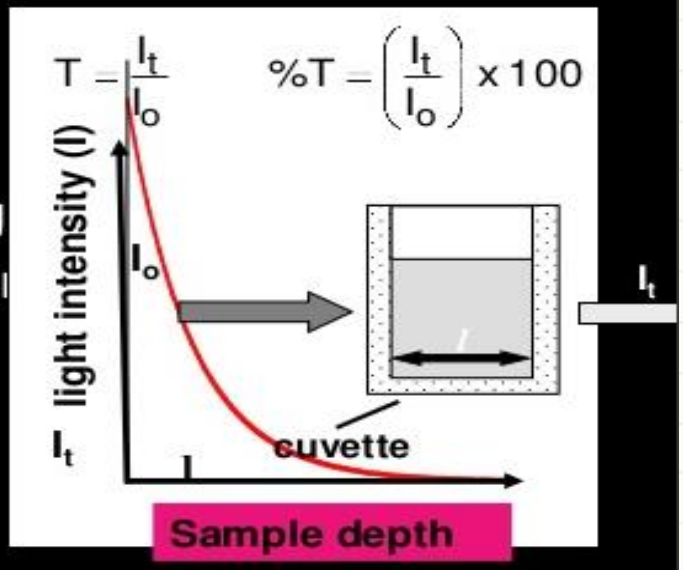
- Beer's law doesn't hold over the entire concentration range, it's operate in very dilute solution only.
- At concentrated solution we have +ve or _ve deviation which may be due to:

1. Association of the molecules.
2. The formation of the complex
3. Change in the refracting index of the solution.

These laws can be represented by this relationship:

BEER-LAMBERT'S LAW

- On combing the two laws, the beer-lambert law can be formulated as below
- $\log I_0/I = \epsilon.c.l = A$
- I_0 =intensity of incident light
- I = intensity of transmitted lig
- € =molar extinction co-efficie
- C=conc. Of solution
- L= path length of sample
- A = absorbance



$E_{1cm}^{1\%}$?

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

- **Nuclear magnetic resonance spectroscopy is a powerful analytical technique used to characterize organic molecules by identifying carbon-hydrogen frameworks within molecules.**

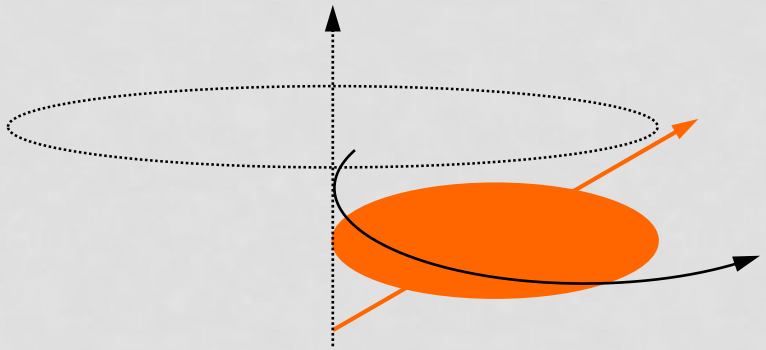
Nuclear Magnetic Resonance Spectroscopy

Introduction to NMR Spectroscopy

- **Two common types of NMR spectroscopy are used to characterize organic structure: ^{1}H NMR is used to determine the type and number of H atoms in a molecule; ^{13}C NMR is used to determine the type of carbon atoms in the molecule.**
- **The source of energy in NMR is radio waves which have long wavelengths, and thus low energy and frequency.**
- **When low-energy radio waves interact with a molecule, they can change the nuclear spins of some elements, including ^{1}H and ^{13}C.**

Introduction to NMR Spectroscopy

We begin by describing some magnetic properties of nuclei. All nuclei carry a charge. In some nuclei this charge "spins" on the nuclear axis, and this circulation of nuclear charge generates a magnetic dipole along the axis (Fig. 4.1). The angular momentum of the spinning charge can be described in terms of quantum spin numbers I; these numbers have values of 0, $\frac{1}{2}$, 1, $\frac{3}{2}$, and so on ($I = 0$ denotes no spin). The intrinsic magnitude of the generated dipole is expressed in terms of nuclear magnetic moment, μ.



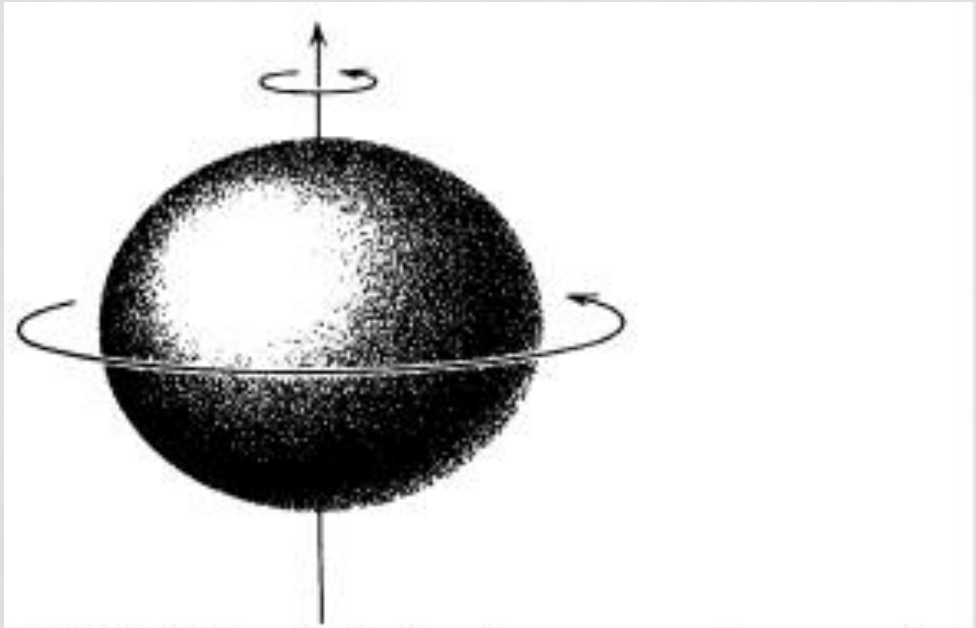


FIGURE 4.1. Spinning charge on proton generates magnetic dipole.

Relevant properties, including the spin number I, of several nuclei are given in Appendix H. The spin number I can be determined from the atomic mass and the atomic number as shown in the next column.

Spectra of several nuclei can be readily obtained (e.g., ${}^{1}_{1}\mathrm{H}$, ${}^{3}_{1}\mathrm{H}$, ${}^{13}_{6}\mathrm{C}$, ${}^{15}_{7}\mathrm{N}$, ${}^{19}_{9}\mathrm{F}$, ${}^{31}_{15}\mathrm{P}$) since they have spin numbers I of $\frac{1}{2}$ and a uniform spherical charge distribution

Nuclei with a spin number I of 1 or higher have a non-spherical charge distribution. This asymmetry is de-

the spin number I determines the number of orientations a nucleus may assume in an external uniform magnetic field in accordance with the formulas $2I + 1$. We are concerned with the proton whose spin number I is $\frac{1}{2}$.

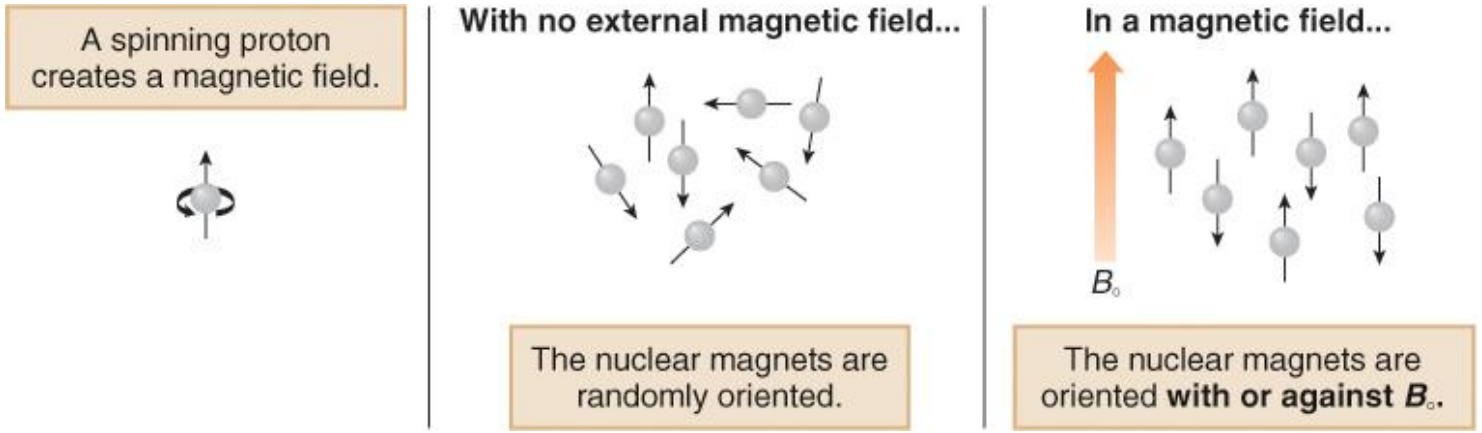
I	Atomic Mass	Atomic Number	Example (I)
Half-integer	Odd	Odd or even	${}^{1}_{1}H(\frac{1}{2})$, ${}^{17}_{8}O(\frac{5}{2})$, ${}^{15}_{7}N(\frac{1}{2})$
Integer	Even	Odd	${}^{2}_{1}H(1)$, ${}^{14}_{7}N(1)$, ${}^{10}_{5}B(3)$
Zero	Even	Even	${}^{12}_{6}C(0)$, ${}^{16}_{8}O(0)$, ${}^{34}_{16}S(0)$

In the case of ^{1}H nuclei only two orientations are allowed; the nuclear magnetic moments may be aligned with or aligned against the direction of the applied magnetic field.

Nuclear Magnetic Resonance Spectroscopy

Introduction to NMR Spectroscopy

- **When a charged particle such as a proton spins on its axis, it creates a magnetic field. Thus, the nucleus can be considered to be a tiny bar magnet.**
- **Normally, these tiny bar magnets are randomly oriented in space. However, in the presence of a magnetic field B_0, they are oriented with or against this applied field. More nuclei are oriented with the applied field because this arrangement is lower in energy.**
- **The energy difference between these two states is very small (<0.1 cal).**



Energy Differentiation

Difference in energy between the two states is given by:

$$\Delta E = \gamma h B_o / 2\pi$$

where:

B_o – external magnetic field
h – Planck's constant
γ – gyromagnetic ratio

When the energy of the photon matches the energy difference between the two spin states , an absorption of energy occurs. We call that phenomenon *Resonance*

$$\Delta E = h\upsilon = \gamma h B_o / 2\pi \qquad \text{So, } \upsilon = \gamma B_o / 2\pi$$

THE ORIGIN OF NMR SIGNALS:

(THE NUCLEI OF NMR –"ACTIVE NUCLEI " BEHAVE LIKE TINY BAR MAGNETS)

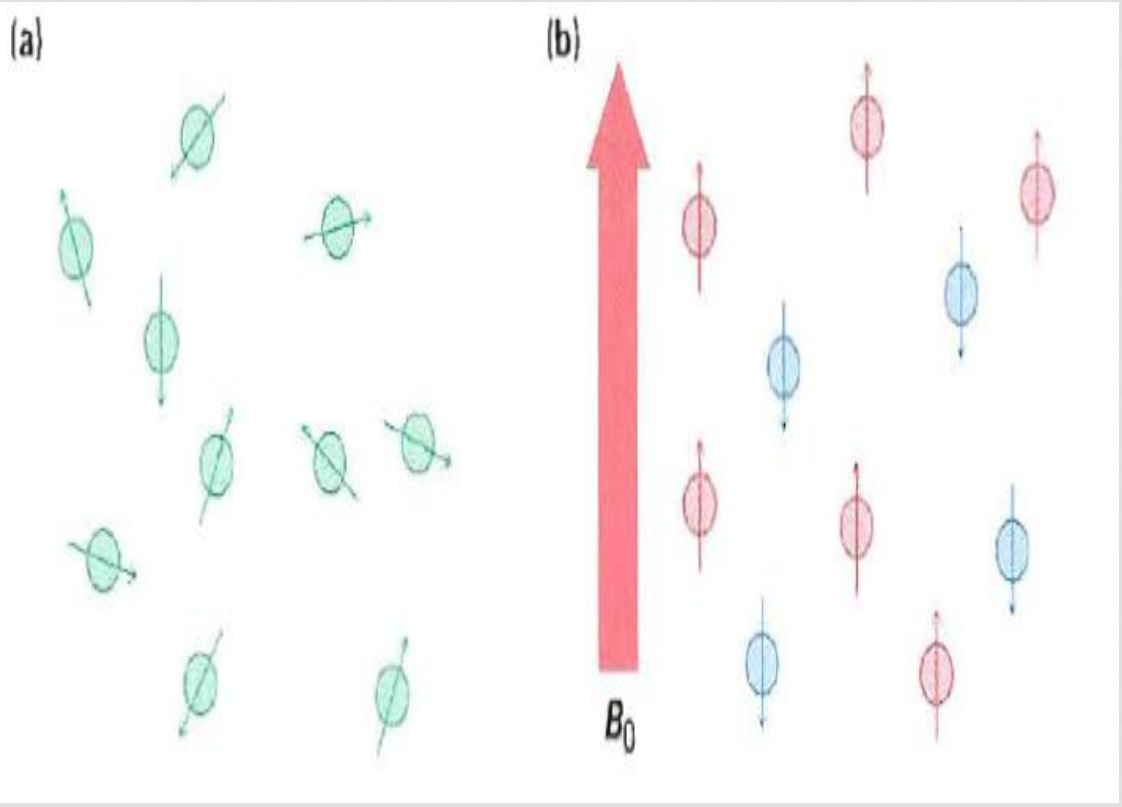


Figure 13.1 (a) Nuclear spins are oriented randomly in the absence of an external magnetic field but (b) have a specific orientation in the presence of an external field, B_0. Some of the spins (red) are aligned parallel to the external field while others (blue) are antiparallel. The parallel spin state is slightly lower in energy and therefore favored.

- Nuclei aligned with the magnetic field are lower in energy than those aligned against the field.
- The nuclei aligned with the magnetic field can be flipped "spin-flip" to the higher-energy state if the right amount of energy is added (DE).
- When this spin-flip occurs, the magnetic nuclei are said to be in resonance with the applied radiation hence the name nuclear magnetic resonance
- The amount of energy required depends on:
- the strength of the external magnetic field
- The identity of the nuclei.

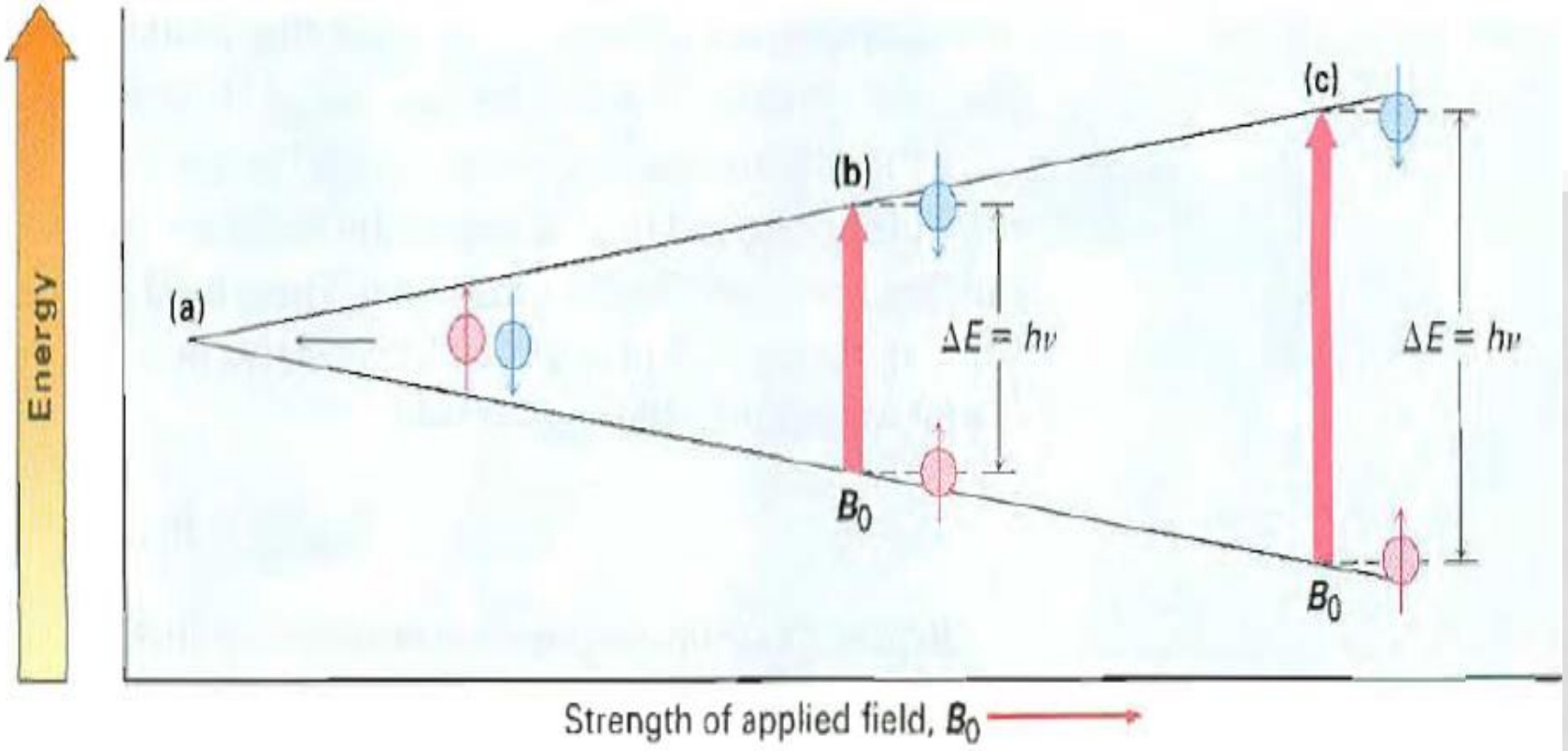
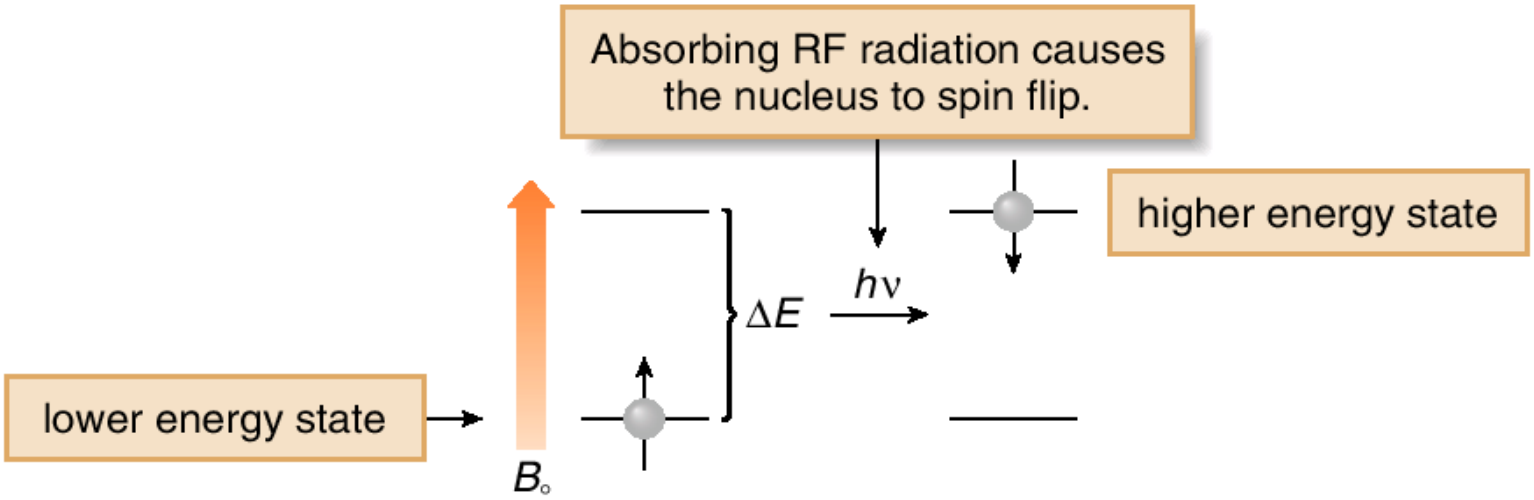


Figure 13.2 The energy difference ΔE between nuclear spin states depends on the strength of the applied magnetic field. Absorption of energy with frequency ν converts a nucleus from a lower spin state to a higher spin state. Spin states (a) have equal energies in the absence of an applied magnetic field but (b) have unequal energies in the presence of a magnetic field. At $\nu = 200$ MHz, $\Delta E = 8.0 \times 10^{-5}$ kJ/mol (1.9×10^{-5} kcal/mol). (c) The energy difference between spin states is greater at larger applied fields. At $\nu = 500$ MHz, $\Delta E = 2.0 \times 10^{-4}$ kJ/mol.

- For NMR spectroscopy the frequencies of interest are in the radio frequency (RF) range, typically 60-500 MHz depending upon the strength of Bo.
- The absorption of energy creates an excited state of the system. The process whereby the system returns to its lowest energy state, i.e. its ground state, is called relaxation.
- One way for the system to relax to the ground state is for it to emit radiation. If a suitable detector is available, e.g. an RF receiver, the emitted radiation may be recorded as a peak on a graph.

Introduction to NMR Spectroscopy

- **Thus, two variables characterize NMR:**
- **an applied magnetic field B_0, the strength of which is measured in tesla (T), and**
- **the frequency ν of radiation used for resonance, measured in hertz (Hz), or megahertz (MHz)—(1 MHz = 10^6 Hz).**



- A nucleus is in *resonance* when it absorbs RF radiation and "spin flips" to a higher energy state.

The radiofrequency ν_1 can be introduced either by continuous-wave (CW) scanning or by a radiofrequency pulse.

There are two types of NMR spectrometers continuous wave (CW) and pulsed Fourier transform (FT)

In the CW instruments, the oscillator frequency is kept constant while the magnetic field is changed gradually.

Limitation of the CW NMR spectrometers:

- 1. At any given moment, only protons resonating at a particular chemical shift can be subjected to excitation at the appropriate value of the magnetic field, and it is therefore necessary to *sequentially* excite the protons that have differing processional frequencies in a given molecule.
- 2. Time limitation, since it is often necessary to distinguish splitting that is only a fraction of a hertz in width; a serious time constraint is introduced.
- 3. Small "spinning side bands" are sometimes seen symmetrically disposed on both sides of a strong absorption peak; these result from in homogeneities in the magnetic field and in the spinning tube.
- 4. The oscillations seen only in scanned (CW) spectra at the low-frequency end of a strong sharp peak are called "ringing" (Fig. 4.11). These are "beat" frequencies resulting from passage through the absorption peak.

FOURIER TRANSFORM (FT) NMR SPECTROMETERS

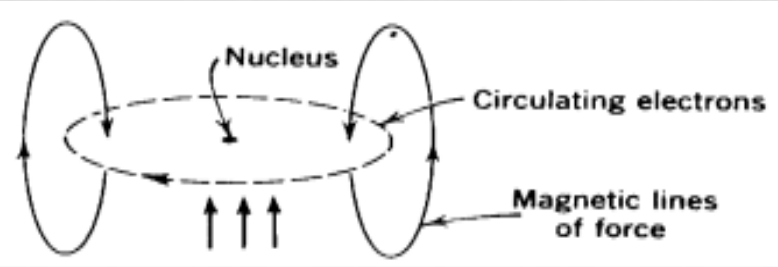
- The sample is placed in a constant, very strong magnetic field
- The sample is irradiated with a short broad pulse of radio frequency energy that excites all nuclei at once
- The resulting signal contains information about all of the absorbing nuclei at once
- This signal is converted to a spectrum by a Fourier transformation
- FT NMR allows signal-averaging, which leads to enhancement of real spectral signals versus noise
- The strong, superconducting magnets used in FTNMR spectrometers lead to greater sensitivity and much higher resolution than continuous wave instruments

The Nature of NMR Absorptions

- The absorption frequency is not the same for all ^{1}H or ^{13}C nuclei in a molecule:

When an atom is placed in a magnetic field, its electrons circulate about the direction of the applied magnetic field. This circulation causes a small magnetic field at the nucleus which opposes the externally applied field
The magnetic field at the nucleus (the effective field) is therefore generally less than the applied field by a fraction :

- $B_{effective} = B_{applied} - B_{local}$



Diamagnetic shielding of nucleus by circulating electrons.

In describing this effect of local fields, we say that nuclei are shielded from the full effect of the applied field by the surrounding electrons. Because each specific nucleus in a molecule is in a slightly different electronic environment, each nucleus is shielded to a slightly different extent and the effective magnetic field felt by each is slightly different. These tiny differences in the effective magnetic fields experienced by different nuclei can be detected, and we thus see a distinct NMR signal for each chemically distinct ^{13}C or ^{1}H nucleus in a molecule. As a result, an NMR spectrum effectively maps the carbon–hydrogen framework of an organic molecule. With practice, it's possible to read the map and derive structural information.

Chemical Shift-δ

The electron density around each nucleus in a molecule varies according to the types of nuclei and bonds in the molecule. The opposing field and therefore the effective field at each nucleus will vary. This is called the chemical shift phenomenon.

As we can tell from $\nu = \gamma B_0 (1-\sigma) / 2\pi$, the greater the value of B_o, the greater the frequency difference.
This relationship could make it difficult to compare NMR spectra taken on spectrometers operating at different field strengths.

The term chemical shift was developed to avoid this problem. The chemical shift of a nucleus is the difference between the resonance frequency of the nucleus and a standard, relative to the standard.

The operation of a basic NMR spectrometer is illustrated in Figure 13.4. An organic sample is dissolved in a suitable solvent (usually deuteriochloroform, $CDCl_3$, which has no hydrogens) and placed in a thin glass tube between the poles of a magnet. The strong magnetic field causes the ^1H and ^{13}C nuclei in the molecule to align in one of the two possible orientations, and the sample is irradiated with rf energy. If the frequency of the rf irradiation is held constant and the strength of the applied magnetic field is varied, each nucleus comes into resonance at a slightly different field strength. A sensitive detector monitors the absorption of rf energy, and the electronic signal is then amplified and displayed as a peak.

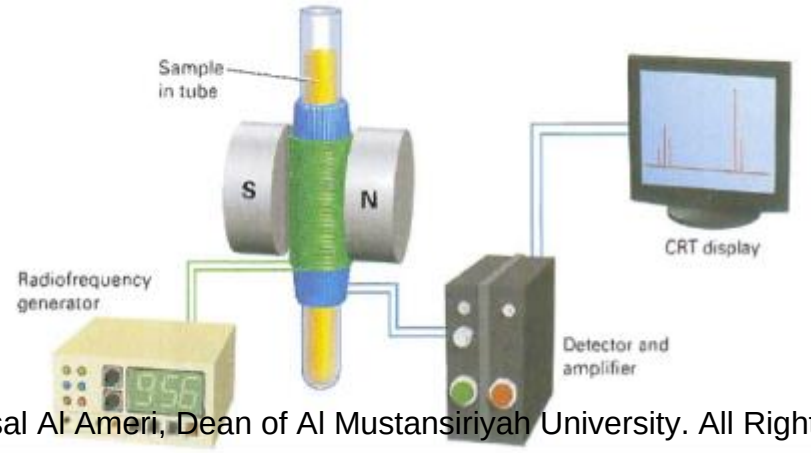


Figure 13.4 Schematic operation of an NMR spectrometer. A thin glass tube containing the sample solution is placed between the poles of a strong magnet and irradiated with rf energy.

NMR SPECTROSCOPY DIFFER FROM" IR" SPECTROSCOPY:

1. **The energy needed for NMR is much smaller than that required for IR Spectroscopy.**
2. **Time scales of the two techniques are quite different.**

(NMR about 10^{-3} second, while IR about 10^{-13} second)

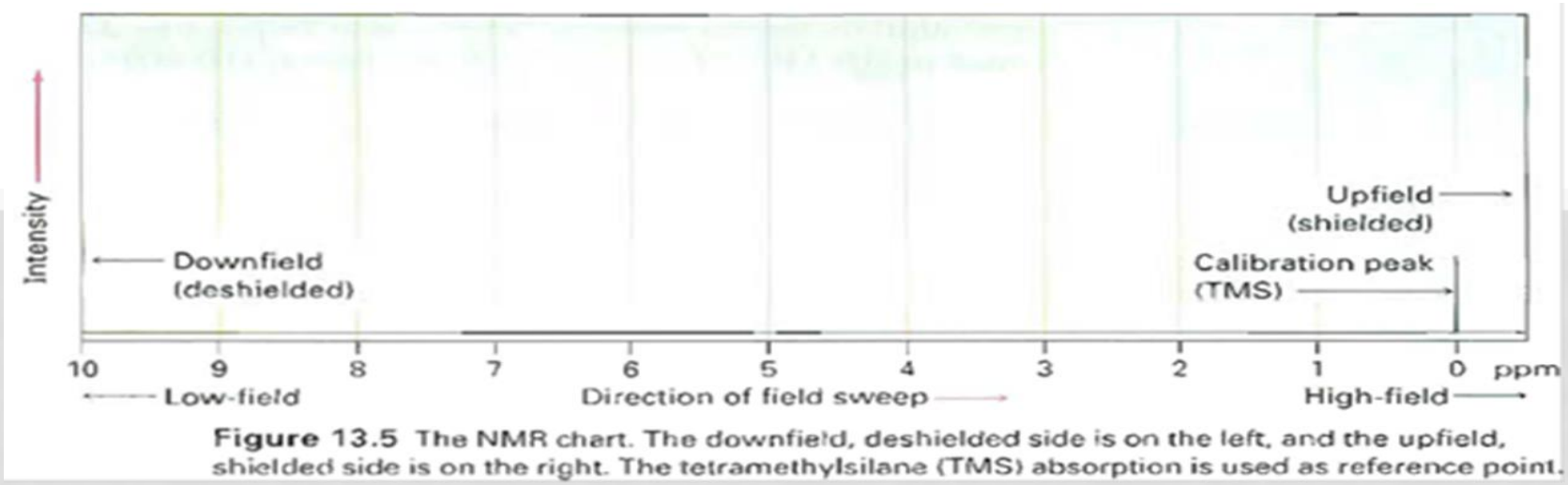


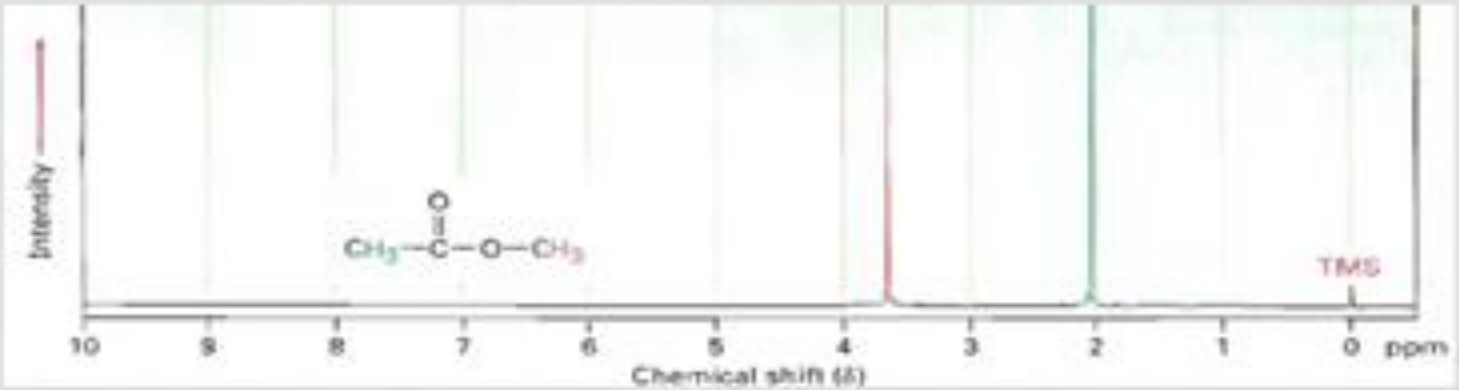
Figure 13.5 The NMR chart. The downfield, deshielded side is on the left, and the upfield, shielded side is on the right. The tetramethylsilane (TMS) absorption is used as reference point.

Shielding requires a higher magnetic field to bring the nucleus into resonance - the signals are up field in the NMR spectrum

Lower electron density around a nucleus deshields the nucleus from the external magnetic field

Deshielding causes absorption of energy at lower frequencies – the signals are downfield in the NMR spectrum

^{1}H NMR spectrum in Figure 13.3a shows only two peaks, however, even though methyl acetate has six hydrogens. One peak is due to the $CH_3C{=}O$ hydrogens, and the other to the $-OCH_3$ hydrogens. Because the three hydrogens in each methyl group have the same electronic environment, they are shielded to the same extent and are said to be *equivalent*. *Chemically equivalent nuclei always show a single absorption.* The two methyl groups themselves, however, are nonequivalent, so the two sets of hydrogens absorb at different positions.



To define the position of an absorption, the NMR chart is calibrated and a reference point is used. In practice, a small amount of tetramethylsilane [TMS; $(CH_3)_4Si$] is added to the sample so that a reference absorption peak is produced when the spectrum is run. TMS is used as reference for both ^1H and ^{13}C mea-

This material has several advantages: it is chemically inert, symmetrical, volatile (bp 27°C), and soluble in most organic solvents; it gives a single, intense, sharp, absorption peak, and its protons are more "shielded" than almost all organic protons. When water or deuterium oxide is the solvent, TMS can be used as an "external reference" in a concentric capillary or the methyl protons of the water-soluble sodium 2, 2-dimethyl-2-silapentane-5-sulfonate (DSS), $(CH_3)_3SiCH_2CH_2CH_2SO_3Na$, are used as an internal reference (0.015 ppm).

The position on the chart at which a nucleus absorbs is called its **chemical shift**. The chemical shift of TMS is set as the zero point, and other absorptions normally occur downfield, to the left on the chart. NMR charts are calibrated using an arbitrary scale called the **delta (δ) scale**, where 1 δ equals 1 part per

1δ = 1 ppm

ppm (part of the million) of the operating frequency of the instrument. e.g. if we use spectrometer operating at

200 MHz, 1 δ would be 1 millionth of 200,000,000 Hz, or 200 Hz. If we were measuring the spectrum using a 500 MHz instrument, 1 δ = 500 Hz. The following equation can be used for any absorption:

$$\delta = \frac{\text{Observed chemical shift (number of Hz away from TMS)}}{\text{Spectrometer frequency in MHz}}$$

Although this method of calibrating NMR charts may seem complex, there's a good reason for it. As we saw earlier, the rf frequency required to bring a given nucleus into resonance depends on the spectrometer's magnetic field strength. But because there are many different kinds of spectrometers with many different magnetic field strengths available, chemical shifts given in frequency units (Hz) vary from one instrument to another. Thus, a resonance that occurs at 120 Hz downfield from TMS on one spectrometer might occur at 600 Hz downfield from TMS on another spectrometer with a more powerful magnet.

By using a system of measurement in which NMR absorptions are expressed in relative terms (parts per million relative to spectrometer frequency) rather than absolute terms (Hz), it's possible to compare spectra obtained on different instruments. *The chemical shift of an NMR absorption in δ units is constant, regardless of the operating frequency of the spectrometer.* A ^{1}H nucleus that absorbs at 2.0 δ on a 200 MHz instrument also absorbs at 2.0 δ on a 500 MHz instrument.

The range in which most NMR absorptions occur is quite narrow. Almost all ^{1}H NMR absorptions occur 0 to 10 δ downfield from the proton absorption of TMS,

Thus there is a considerable accidental overlap of non equivalent signal will occur.

The advantages of using an instrument with higher field strength (say, 500 MHz) rather than lower field strength (200 MHz) is that different NMR absorptions are more widely separated at the higher field strength. The chances that two signals will accidentally overlap are therefore lessened, and interpretation of spectra becomes easier. For example, two signals that are only 20 Hz apart at 200 MHz (0.1 ppm) are 50 Hz apart at 500 MHz (still 0.1 ppm).

DIAMAGNETIC ANISOTROPY:

- It means that shielding and deshielding depend on the orientation of the molecule with respect to applied magnetic field. For example:
  1. The NMR peak of the proton of acetylene is found further to the right than electronegativity would predict, (peak = 1.8 **δ**) more shielded than ethylene protons (peak = 5.25 **δ**).

- Note: electron with drawing effect = SP > SP^2 > SP^3

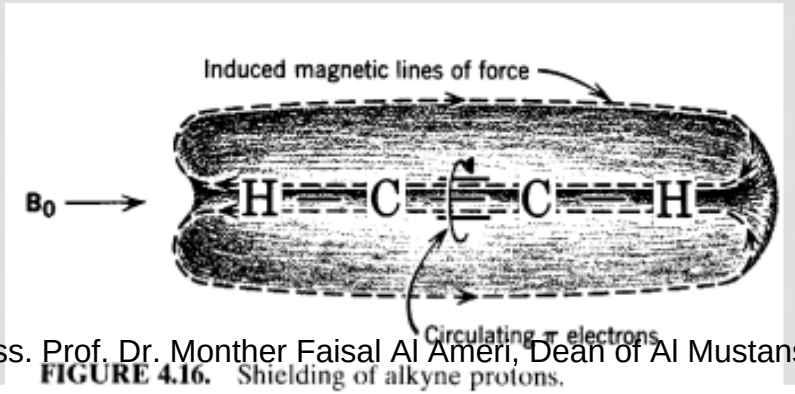


FIGURE 4.16. Shielding of alkyne protons.

2. Unexpected deshielding position of the aldehydic proton. (9.7- 10)

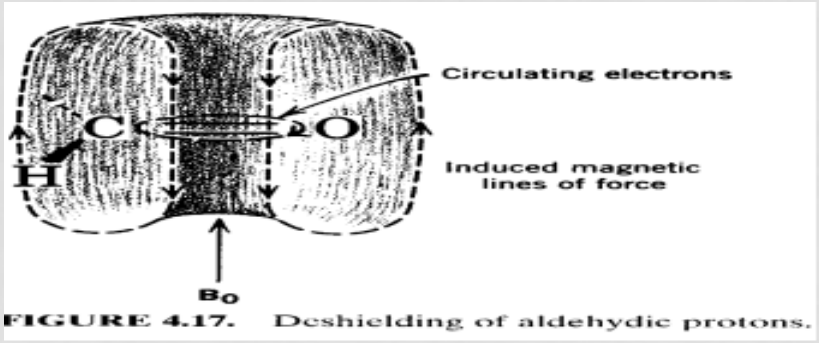


FIGURE 4.17. Deshielding of aldehydic protons.

3. Large deshielding of alkene protons.

4. Large deshielding of benzene ring protons, which is called "ring current effect" (6.5_ 8).

Note: a proton held directly above or below the aromatic ring is shielded.

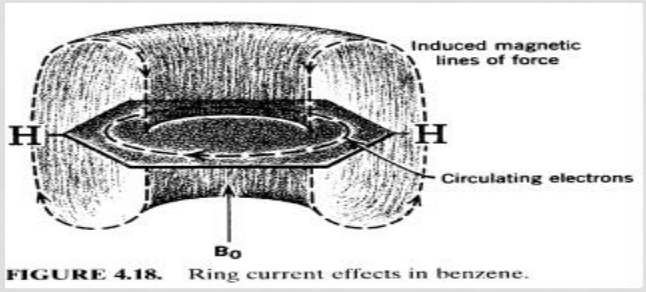
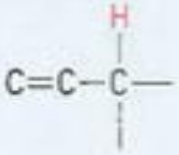
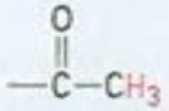
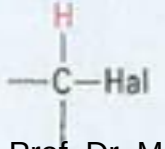
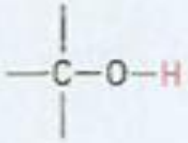
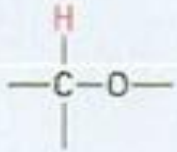
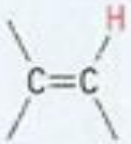
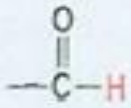
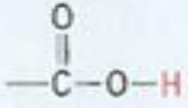


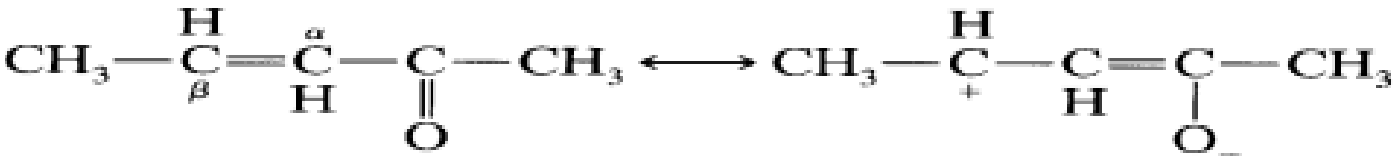
FIGURE 4.18. Ring current effects in benzene.

Table 13.3 | Correlation of ^1H Chemical Shift with Environment

Type of hydrogen		Chemical shift (δ)
Reference	$Si(CH_3)_4$	0
Alkyl (primary)	$-CH_3$	0.7–1.3
Alkyl (secondary)	$-CH_2-$	1.2–1.6
Alkyl (tertiary)		1.4–1.8
Allylic		1.6–2.2
Methyl ketone		2.0–2.4
Aromatic methyl	$Ar-CH_3$	2.4–2.7
Alkynyl	$-C\equiv C-H$	2.5–3.0
Alkyl halide		2.5–4.0
Alcohol		2.5–5.0
Alcohol, ether		3.3–4.5
Vinylic		4.5–6.5
Aryl	$Ar-H$	
Aldehyde		9.7–10.0
Carboxylic acid		11.0–12.0

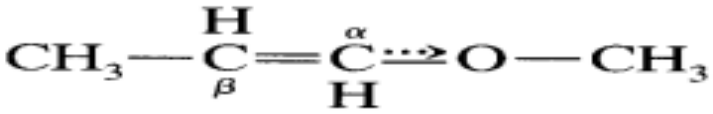
RATIONALIZATION AND PREDICTION OF APPROXIMATE CHEMICAL SHIFT DEPENDING ON INDUCTIVE EFFECT & DIAMAGNETIC ANISOTROPY

1. In an α,β-unsaturated ketone, resonance deshields the β-proton;



α-proton = $\delta \sim 6.2$
β-proton = $\delta \sim 6.8$

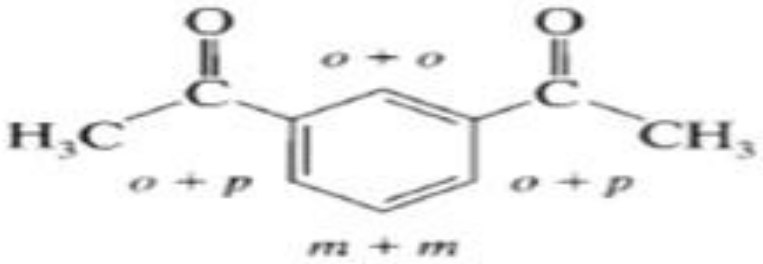
2. In a substituted vinyl ether, the oxygen atom deshields the α-proton by an inductive effect and shields the β-proton by resonance.



α-proton = $\delta \sim 6.2$
β-proton = $\delta \sim 4.6$

3. The shifts of protons *ortho*, *meta*, or *para* to a substituent on an aromatic ring are correlated with electron densities and with the effects of electrophilic reagents (Appendix Chart D.1). For example, the *ortho* and *para* protons of phenol are shielded because of the higher electron density that also accounts for the predominance of *ortho* and *para* substitution by electrophilic reagents. Conversely, the *ortho* and *para* protons of nitrobenzene are deshielded, the *ortho* protons more so (see Figure 3.23).

Since chemical shift increments are approximately additive, it is possible to calculate the ring proton shifts in polysubstituted benzene rings from the monosubstituted values in Appendix Chart D.1. The chemical shift increments for the ring protons of *m*-diacetylbenzene:



SOLVENT SELECTION:

- Characteristic of the ideal solvent:
- 1. should contain no protons
- 2. inert
- 3. low boiling
- 4. inexpensive
- 5. Deuterated solvents are necessary for modern instruments because they depend on a deuterium signal to lock or stabilize the B° field of the magnet.
- Solvent used in NMR spectroscopy:
- 1. CCl_4: because a/ it is contain no hydrogen.
- b/ good solvent for many organic compounds.
- c/ cheap and readily available.
- 2. $CDCl_3$

INFORMATION OBTAINED FROM NMR SPECTRA:

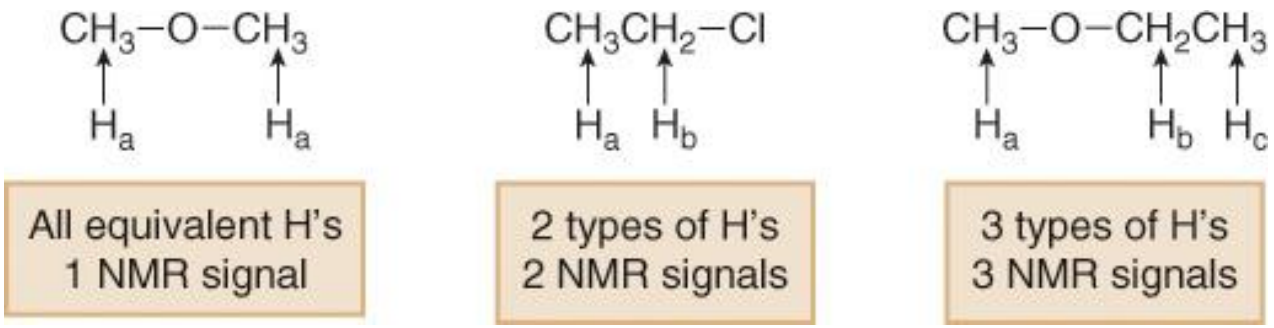
- 1. Number of signal: → number of sets of chemically equivalent protons.
- The equivalence or non-equivalence of two protons can be determined by seeing whether the same or different structures would result, if some group X were substituted for one of protons.
- If the protons are chemically equivalent → same product will be formed regardless of which protons is replaced, if the protons are chemically non equivalent → different product.
- e.g. 2,3- dimethyl butene →
- all 12 protons are equivalent $(CH3)_2C = C(CH3)_2$ → 4 methyl are equivalent

e.g. 2- methyl ,2-butene → are not equivalent

Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—Number of Signals

- **The number of NMR signals equals the number of different types of protons in a compound.**
- **Protons in different environments give different NMR signals.**
- **Equivalent protons give the same NMR signal.**

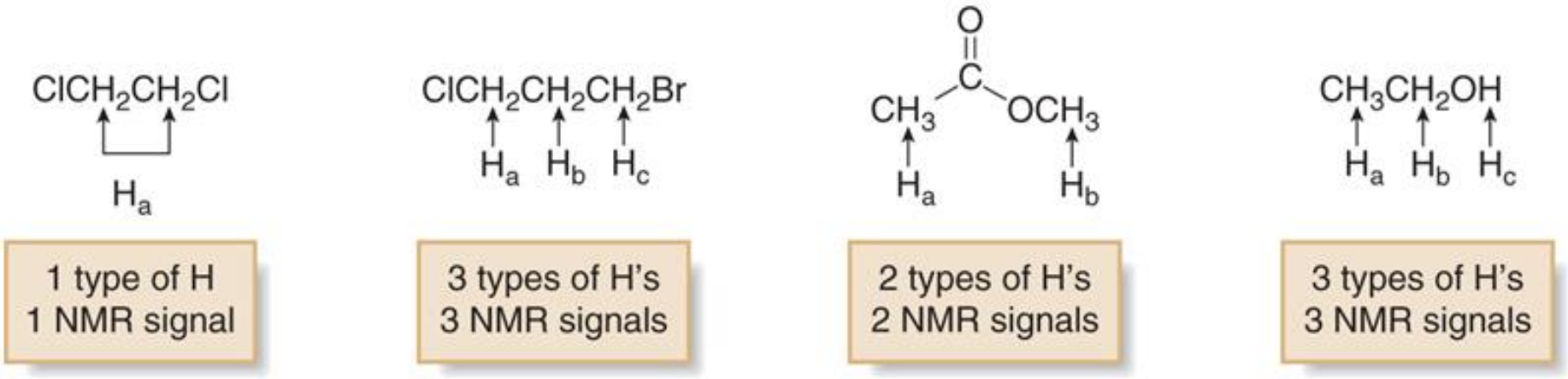


- **To determine equivalent protons in cycloalkanes and alkenes, always draw all bonds to hydrogen.**

Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—Number of Signals

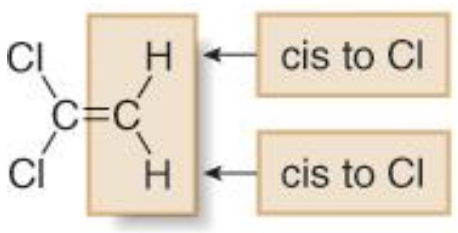
Figure 14.2
The number of ^{1}H NMR signals of some representative organic compounds



Nuclear Magnetic Resonance Spectroscopy

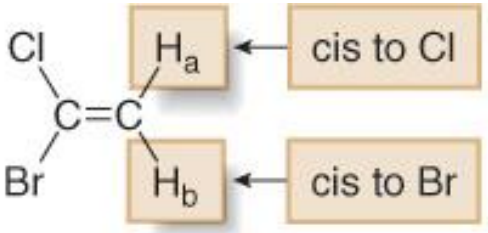
^{1}H NMR—Number of Signals

- **In comparing two H atoms on a ring or double bond, two protons are equivalent only if they are cis (or trans) to the same groups.**



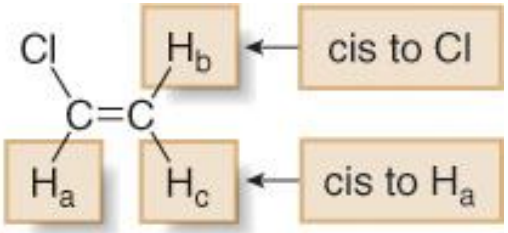
1,1-dichloroethylene

1 type of H
1 NMR signal



1-bromo-1-chloroethylene

2 types of H's
2 NMR signals

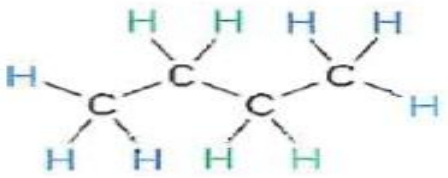


chloroethylene

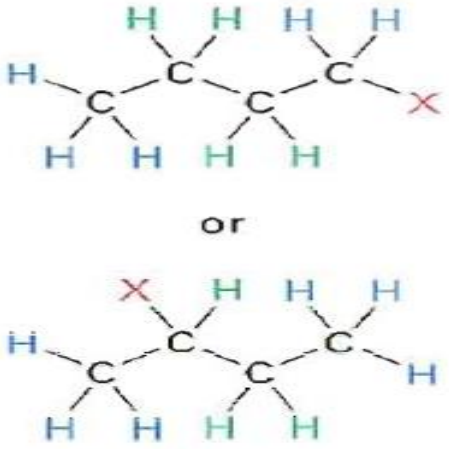
3 types of H's
3 NMR signals

- To predict the number of signals, must determine how many sets of protons are in unique environments:

One possibility is that the protons are chemically unrelated and thus non-equivalent. If so, the products formed on replacement of H by X would be different constitutional isomers. In butane, for instance, the $-CH_3$ protons are



The $-CH_2-$ and $-CH_3$ hydrogens are unrelated and have different NMR absorptions.



The two replacement products are constitutional isomers.

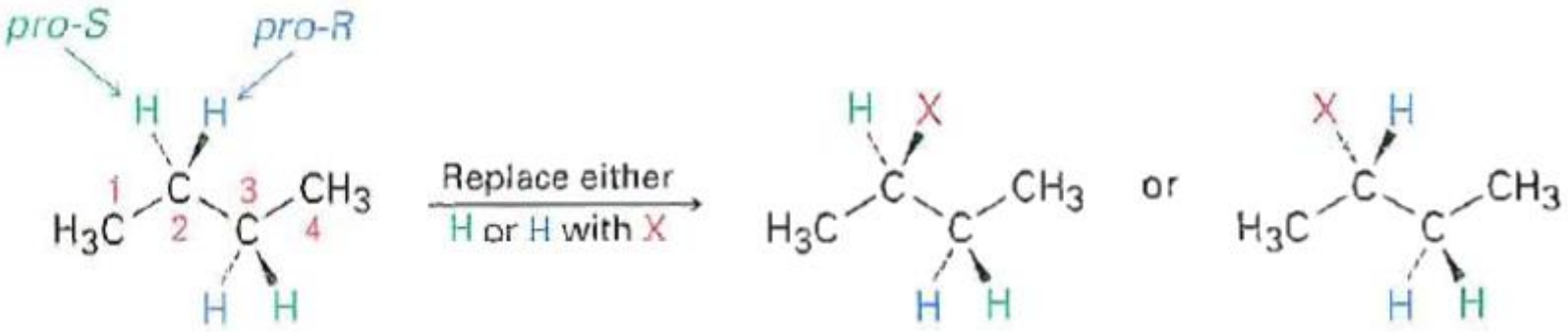
- 2. Homotopic Hydrogens (equivalent)
- Hydrogens are chemically equivalent or homotopic if replacing each in turn by the same group would lead to an identical compound.

The 6 $-CH_3$ hydrogens are *homotopic* and have the same NMR absorptions.

Only one replacement product is possible.

- 3. EnantiotopicHydrogens

If replacement of each of two hydrogens by some group leads to enantiomers, those hydrogens are enantiotopic

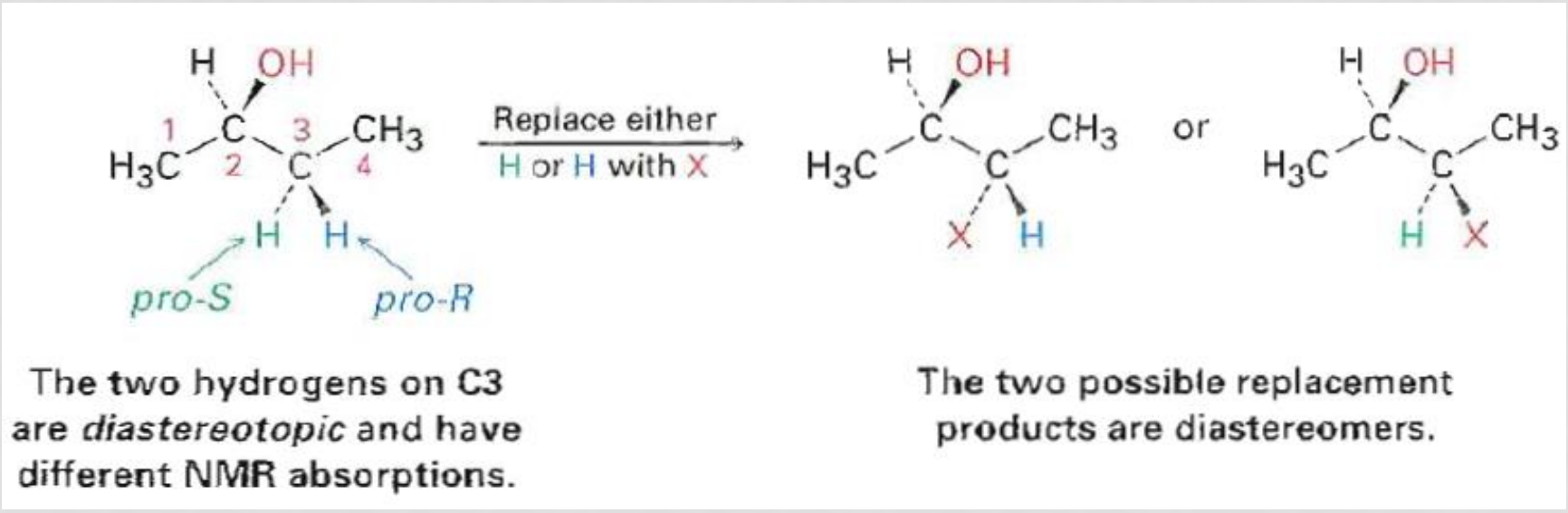


The two hydrogens on C2 (and the two hydrogens on C3) are *enantiotopic* and have the same NMR absorption.

The two possible replacement products are enantiomers.

- **4. Diastereotopic Hydrogens**

- **If replacement of each of two hydrogens by some group leads to diastereomers, the hydrogens are diastereotopic**
- **H Diastereotopic hydrogens have different chemical shifts and will give different signals**

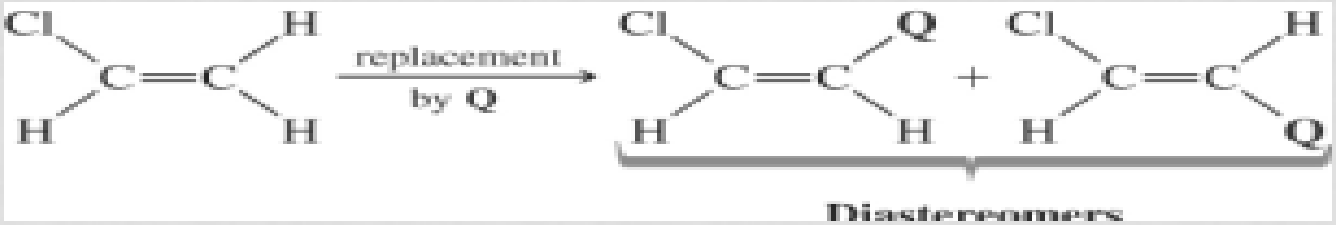


The two hydrogens on C3 are *diastereotopic* and have different NMR absorptions.

The two possible replacement products are diastereomers.

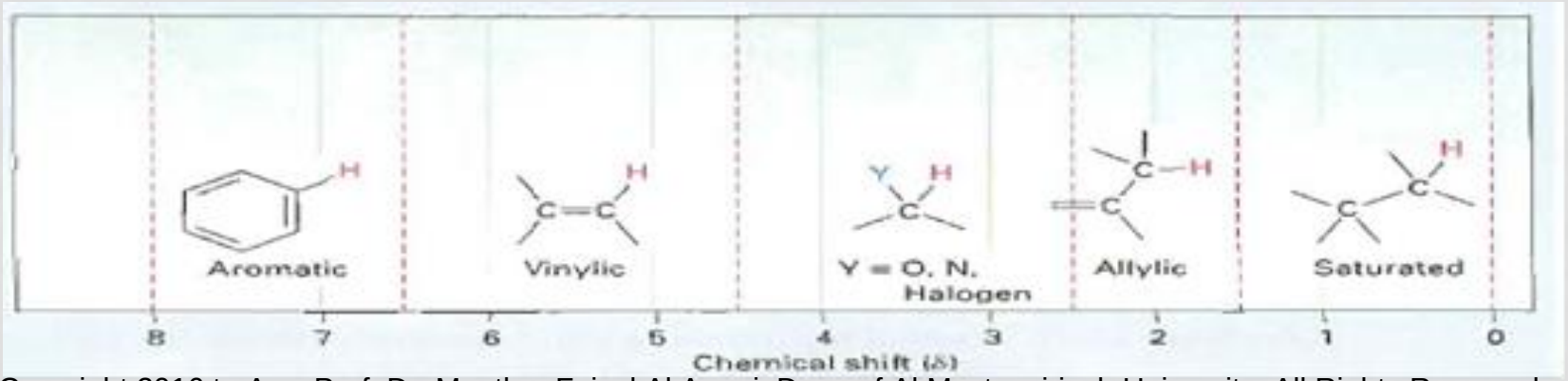
- The protons on a CH_2 group are usually diastereotopic if:

- On an unsymmetrical double bond
- t On opposite sides of a substituted ring
- t There is a chiral center in the molecule



- 2. the position of the signals (chemical shift in H-NMR Spectroscopy)

We said previously that differences in chemical shifts are caused by the small local magnetic fields of electrons surrounding the different nuclei. Nuclei that are more strongly shielded by electrons require a higher applied field to bring them into resonance and therefore absorb on the right side of the NMR chart. Nuclei that are less strongly shielded need a lower applied field for resonance and therefore absorb on the left of the NMR chart.



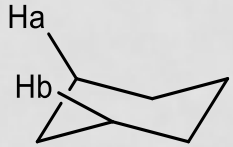
FACTORS EFFECTING CHEMICAL SHIFT:

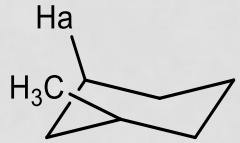
1. inductive effect:

Table 13.3 shows the correlation of ^1H chemical shift with electronic environment in more detail. In general, protons bonded to saturated, sp^3-hybridized carbons absorb at higher fields, whereas protons bonded to sp^2-hybridized carbons absorb at lower fields. Protons on carbons that are bonded to electronegative atoms, such as N, O, or halogen, also absorb at lower fields.

2. anisotropic effect

3. vanderwaals deshielding: proton (a) is not effected by proton (b), but if we substituted proton (b) by CH3 → repulsive because of steric effect with the proton (a) → deshielding → down field.





- 4. Hydrogen bonding:

They cause decrease the density around the hydrogen → deshielded by inductive effect.

The intensity of intermolecular hydrogen bonding depending on:

- The concentration: increase concentration → increase H-bonding → increase deshielding.
- The temperature: increase temperature → breakdown the H-bonding → high field.
- The purity.
- Polarity of the solvent: the solvent should be deuterated ($CDCl_3$) and also should not polar.

- Intramolecular H_ bonds are less affected by their environment than are intermolecular H _ bonds.

3. ^{1}H NMR—Intensity of Signals (integration of ^{1}H NMR absorption, proton counting)

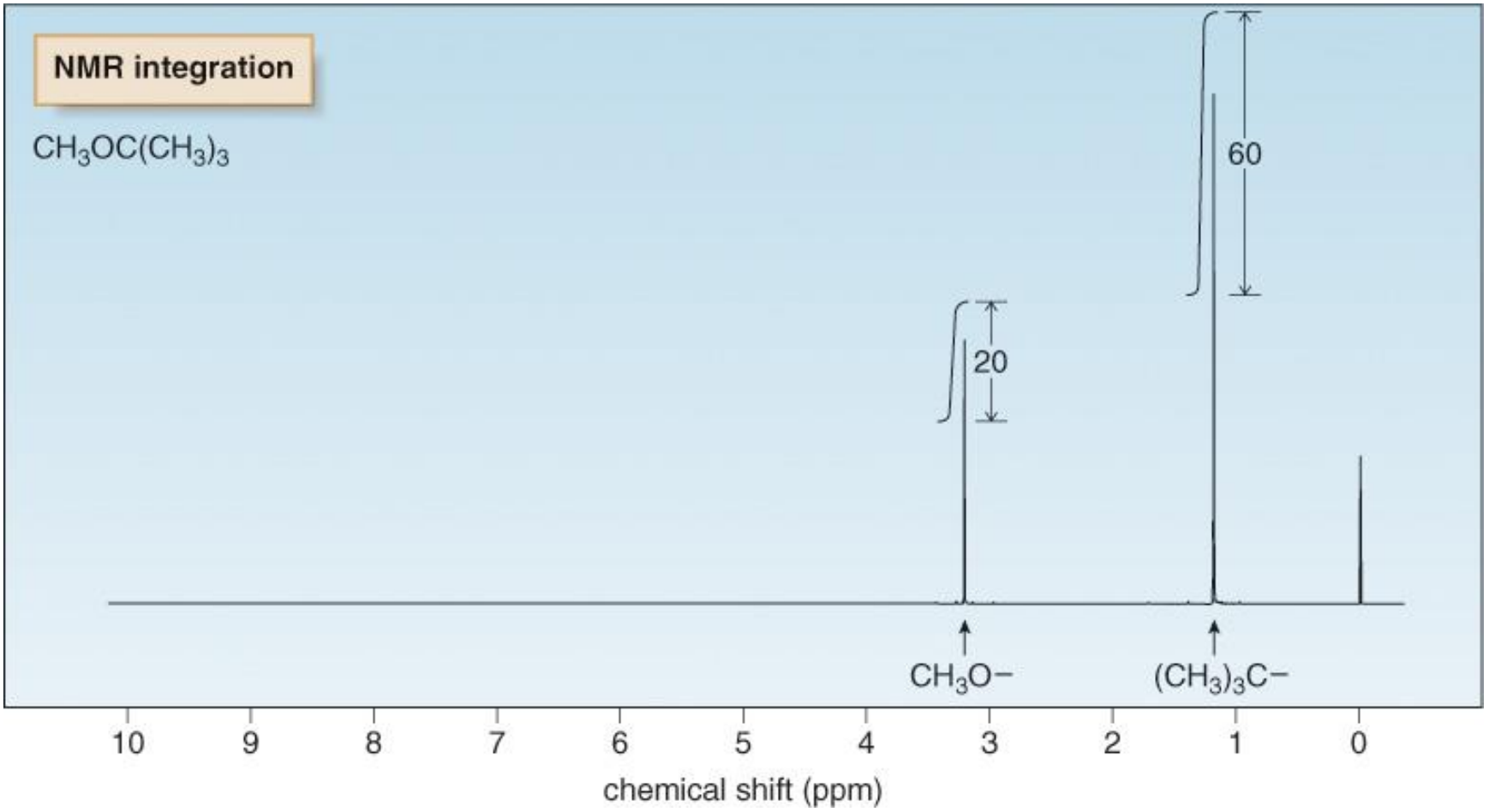
- **The area under an NMR signal is proportional to the number of absorbing protons.**
- **An NMR spectrometer automatically integrates the area under the peaks, and prints out a stepped curve (integral) on the spectrum.**
- **The height of each step is proportional to the area under the peak, which in turn is proportional to the number of absorbing protons.**
- **Modern NMR spectrometers automatically calculate and plot the value of each integral in arbitrary units.**
- **The ratio of integrals to one another gives the ratio of absorbing protons in a spectrum. Note that this gives a ratio, and not the absolute number, of absorbing protons.**

3. INTEGRATION OF H NMR ABSORPTION: PROTON COUNTING

The area under each peak is proportional to the number of protons causing that peak. By electronically measuring, or **integrating**, the area under each peak, it's possible to measure the relative numbers of the different kinds of protons in a molecule. If desired, the integrated peak area can be superimposed over the spectrum as a "stair-step" line, with the height of each step proportional to the area under the peak, and therefore proportional to the relative number of protons causing the peak. To compare the size of one peak against another, simply take a ruler and measure the heights of the various steps. For example, the two steps for the peaks in methyl 2,2-dimethylpropanoate are found to have a 1:3 (or 3:9) height ratio when integrated—exactly what we expect since the three $-OCH_3$ protons are equivalent and the nine $(CH_3)_3C-$ protons are equivalent.

Nuclear Magnetic Resonance Spectroscopy

^{1}H NMR—Intensity of Signals



4. MULTIPLICITY: "SPIN_ SPIN COUPLING" OR SPIN_ SPIN SPLITTING IN H NMR SPECTRA:

pling. This can be described as the indirect coupling of proton spins through the intervening bonding electrons. Very briefly, it occurs because there is some tendency for a bonding electron to pair its spin with the spin of the nearest proton; the spin of a bonding electron having been thus influenced, the electron will affect the spin of the other bonding electron, and so on, through to the next proton. Coupling is ordinarily not important beyond three bonds unless there is ring strain as in small rings or bridged systems, delocalization as in aromatic or unsaturated systems, or four connecting bonds in a W configuration.

As a general rule, called the n + 1 rule, protons that have n equivalent neighboring protons show n + 1 peaks in their NMR spectrum.

In the ^{1}H NMR spectra we've seen thus far, each different kind of proton in a molecule has given rise to a single peak. It often happens, though, that the absorption of a proton splits into multiple peaks, called a **multiplet**. For example, in the ^{1}H NMR spectrum of bromoethane shown in Figure 13.13, the $-CH_2Br$ protons appear as four peaks (a *quartet*) centered at 3.42 δ and the $-CH_3$ protons appear as three peaks (a *triplet*) centered at 1.68 δ.

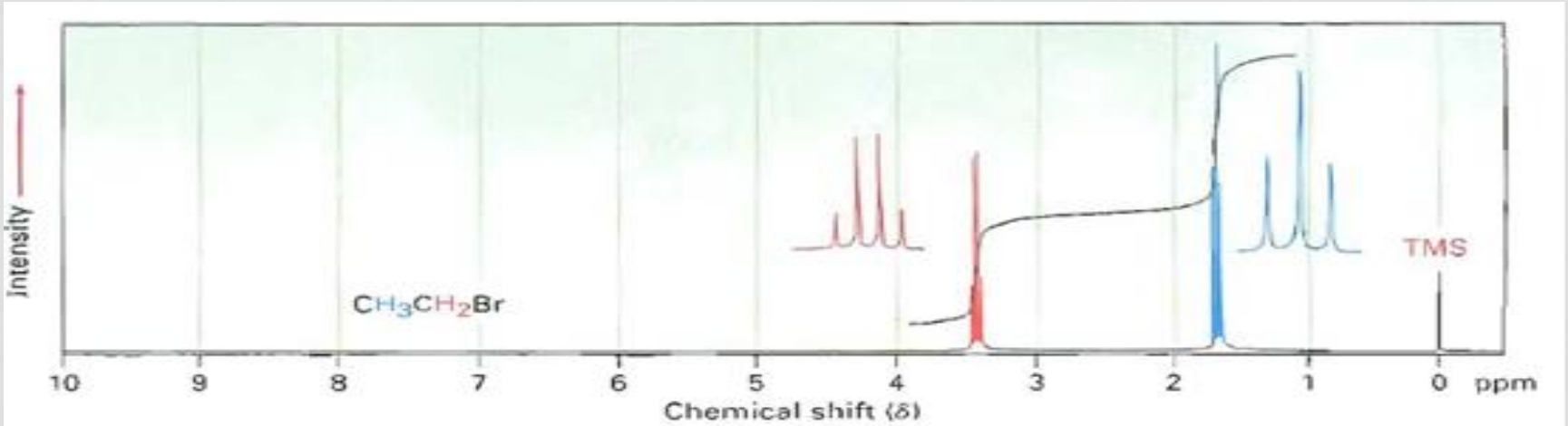


Figure 13.13 The ^{1}H NMR spectrum of bromoethane, CH_3CH_2Br. The $-CH_2Br$ protons appear as a quartet at 3.42 δ, and the $-CH_3$ protons appear as a triplet at 1.68 δ.

spectrum of 2-bromopropane in Figure 13.15 shows a doublet at 1.71 δ and a seven-line multiplet, or *septet*, at 4.28 δ. The septet is caused by splitting of the −CHBr− proton signal by six equivalent neighboring protons on the two methyl groups ($n = 6$ leads to $6 + 1 = 7$ peaks). The doublet is due to signal splitting of the six equivalent methyl protons by the single −CHBr− proton ($n = 1$ leads to 2 peaks). Integration confirms the expected 6:1 ratio.

The distance between peaks in a multiplet is called the **coupling constant**, denoted J. Coupling constants are measured in hertz and generally fall in the range 0 to 18 Hz. The exact value of the coupling constant between two neighboring protons depends on the geometry of the molecule, but a typical value for an open-chain alkane is $J = 6$ to 8 Hz. The same coupling constant is shared by both groups of hydrogens whose spins are coupled and is independent of

Spectrometer field strength

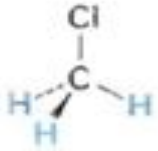
Two_ bond coupling is termed geminal; three _ bond coupling is termed, vicinal;

H—C—H	H—C—C—H
Geminal coupling	Vicinal coupling
2 bonds (^2J)	3 bonds (^3J)

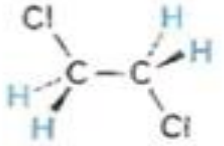
Spin- spin splitting in H NMR can be summarized by:

Rule1:

Chemically equivalent protons do not show spin–spin splitting. The equivalent protons may be on the same carbon or on different carbons, but their signals don't split.

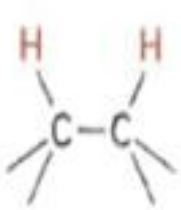


Three C–H protons are chemically equivalent; no splitting occurs.

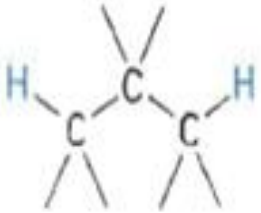


Four C–H protons are chemically equivalent; no splitting occurs.

Rule2: The signal of a proton that has n equivalent neighboring protons is split into a multiplet of $n + 1$ peaks with coupling constant J. Protons that are farther than two carbon atoms apart don't usually couple, although they sometimes show small coupling when they are separated by a π bond.



Splitting observed



Splitting not usually observed

Rule3: Two groups of protons coupled to each other have the same coupling constant, *J*.

e.g. CH_3CH_2Br multiplets of CH_2Br and CH_3 have the same J value.

- If V1 = chem. Shift of CH_3
  V2 = chem. Shift of CH_2
  ΔV = distance between chem. Shift of non equivalent protons in the molecule.

As ΔV/ J goes down → the distance between different peaks of 2 non equivalent protons smaller (peaks approach each other)

When $\Delta V = J$ → the two peaks of two protons are the same, i,e the two protons are equivalent.

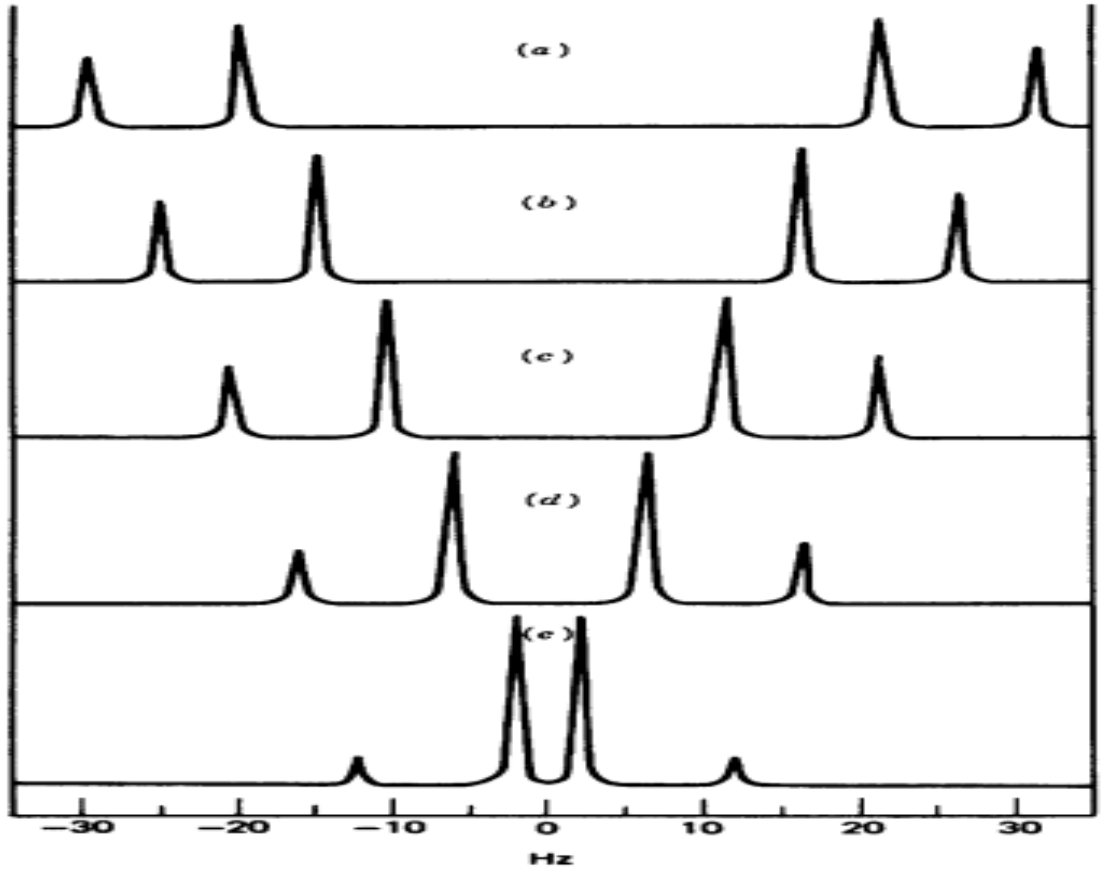


FIGURE 4.24. A two-proton system, spin coupling with a decreasing difference in chemical shifts and a large J value (10 Hz); the difference between AB and AX notation is explained in the text.

Rule4: The relative intensities of the peaks of a multiplet depend on (n):

Table 13.4 Some Common Spin Multiplicities

Number of equivalent adjacent protons	Multiplet	Ratio of intensities
0	Singlet	1
1	Doublet	1:1
2	Triplet	1:2:1
3	Quartet	1:3:3:1
4	Quintet	1:4:6:4:1
6	Septet	1:6:15:20:15:6:1

- The multiplicity and relative intensities may be easily obtained from Pascal's triangle:

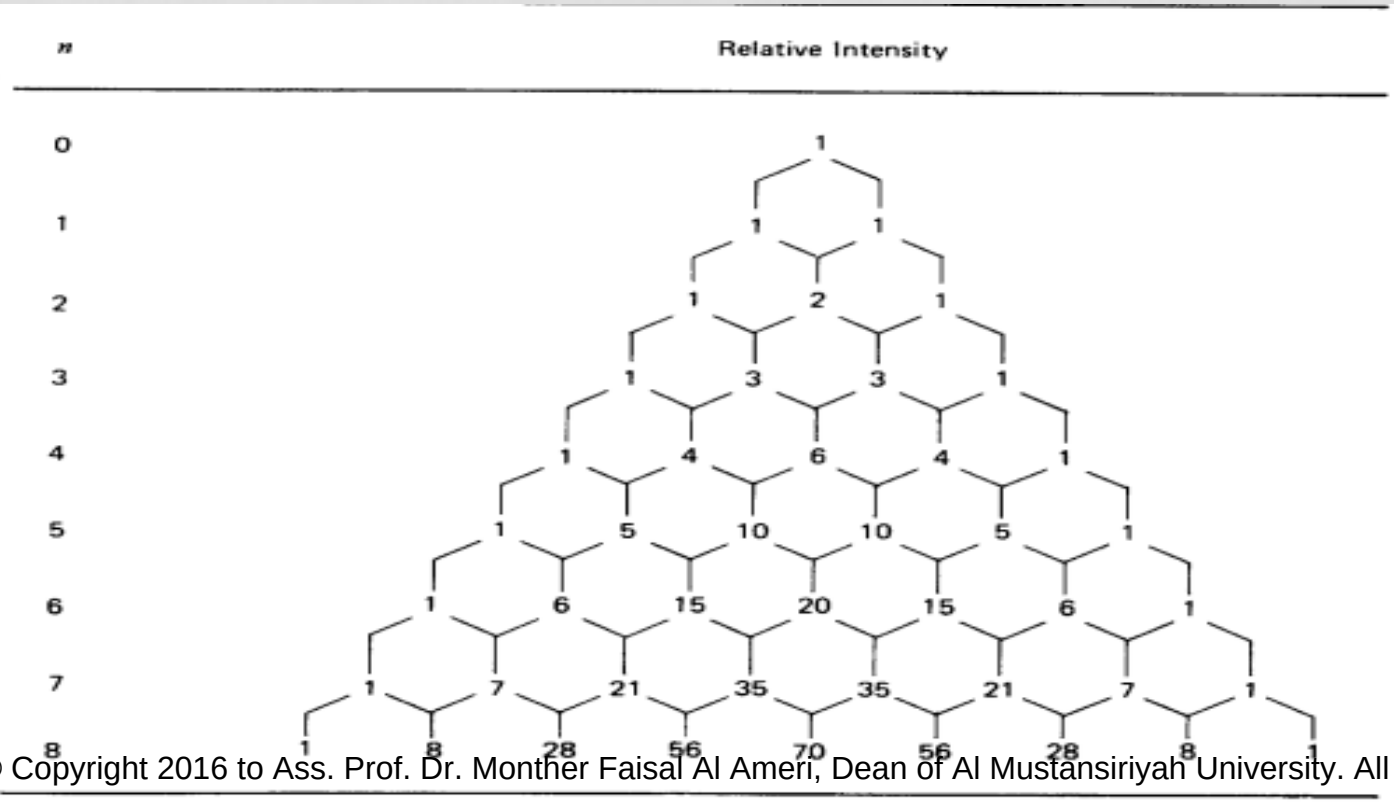


FIGURE 4.29. Pascal's triangle. Relative intensities of first-order multiplets; n = number of equivalent coupling nuclei of spin $\frac{1}{2}$ (e.g., protons).

The requirements for a simple, first-order multiplet can be summarized:

- The ratio $\Delta\nu/J$ must be larger than about 8; $\Delta\nu$ is the distance in Hz between the mid-points of the coupled multiplets. J is the coupling constant.
- The number of peaks in the multiplet is $n + I$, where n is the number of neighboring protons with the same coupling constant.
- The distance in Hz between the individual peaks of a simple, first-order multiplet represents the coupling constant.
- The simple, first-order multiplet is centrosymmetric, with the most intense peak(s) central (see the Pascal triangle, Figure 3.32).

A complex, first-order multiplet differs from a simple, first-order multiplet in that several different coupling constants are involved in the complex multiplet. The requirement that $\Delta\nu/J$ be greater than about 8 still holds, but Pascal's triangle does not hold for the complex multiplet. An example is presented later in

A system of three sets of protons, each set separated by a large chemical shift, can be designated $A_aM_mX_x$. If two sets are separated from each other by a small chemical shift, and the third set is widely separated from the other two, we use an $A_aB_bX_x$ designation. If all shift positions are close, the system is $A_aB_bC_c$. Both end sets are coupled to the middle set with different coupling constants, whereas the end sets may or may not be coupled to one another. The AMX systems are first-order; ABX systems can be approximated by using first-order rules, but ABC systems cannot be analyzed by inspection. These more complex patterns are treated in Section 4.12.

We can now appreciate the three main features of an NMR spectrum: chemical shifts, peak intensities, and spin splittings that are first order or that approximate first-order patterns. The term "weakly coupled" is used for first-order coupling ($\Delta\nu/J > \sim 8$) and "strongly coupled" is used for couplings whose $\Delta\nu/J$ ratio is less than about eight.

Example: Compound (A) has the chemical formula of $C_{10}H_{12}O_2$:

NMR spectrum showed the following signals (δ) :

7.93 (doublet), 6.91 (doublet), 3.84 (singlet), 2.93(quartet), & 1.20(triplet). The integration for the peaks is as follows: 20:20:30:20:30 respectively. Find the structure of compound (A), and then draw its spectrum chart.

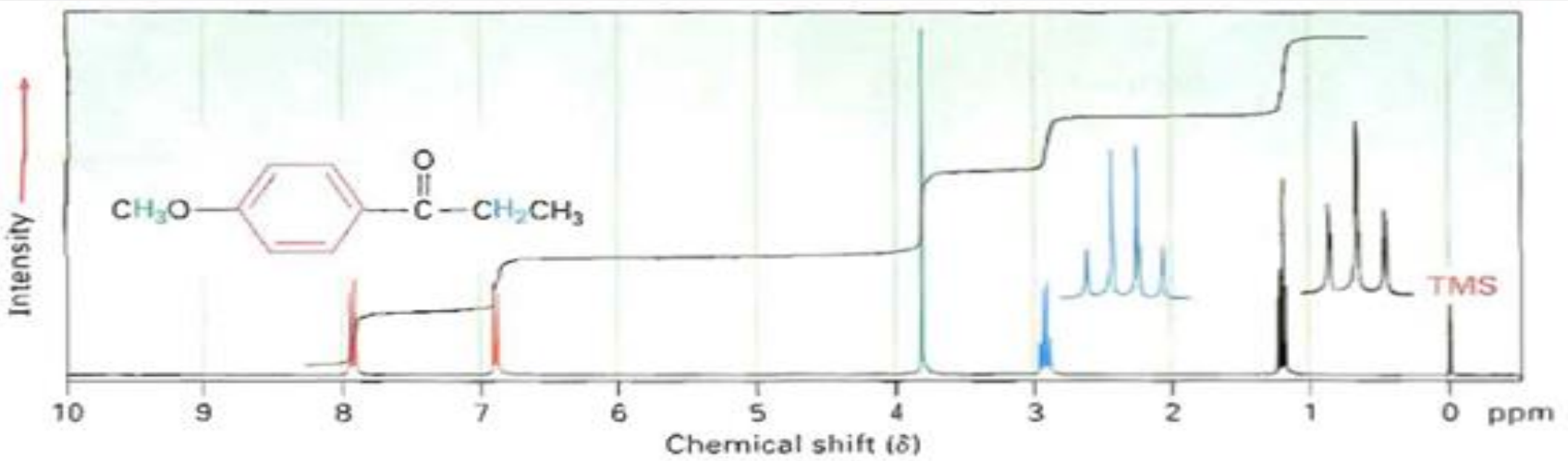


Figure 13.16 The ^{1}H NMR spectrum of *para*-methoxypropiophenone.

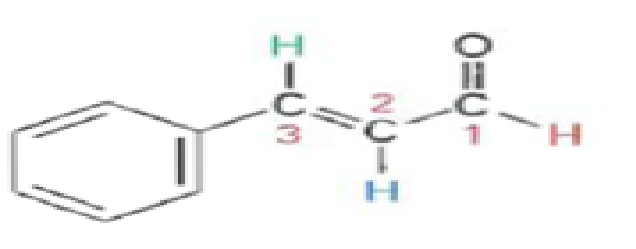
Exercise:

Propose a structure for a compound, $C_5H_{12}O$, that fits the following ^{1}H NMR data: 0.92 δ (3 H, triplet, J = 7 Hz), 1.20 δ (6 H, singlet), 1.50 δ (2 H, quartet, J = 7 Hz), 1.64 δ (1 H, broad singlet).

MORE COMPLEX SPIN-SPIN SPLITTING PATTERNS:

In the ^{1}H NMR spectra we've seen so far, the chemical shifts of different protons have been distinct and the spin–spin splitting patterns have been straightforward. It often happens, however, that different kinds of hydrogens in a molecule have accidentally *overlapping* signals. The spectrum of toluene (methylbenzene) in Figure 13.18, for example, shows that the five aromatic ring protons give a complex, overlapping pattern, even though they aren't all equivalent.

Yet another complication in ^{1}H NMR spectroscopy arises when a signal is split by two or more *nonequivalent* kinds of protons, as is the case with *trans*-cinnamaldehyde, isolated from oil of cinnamon (Figure 13.19). Although the $n + 1$ rule predicts splitting caused by equivalent protons, splittings caused by nonequivalent protons are more complex.

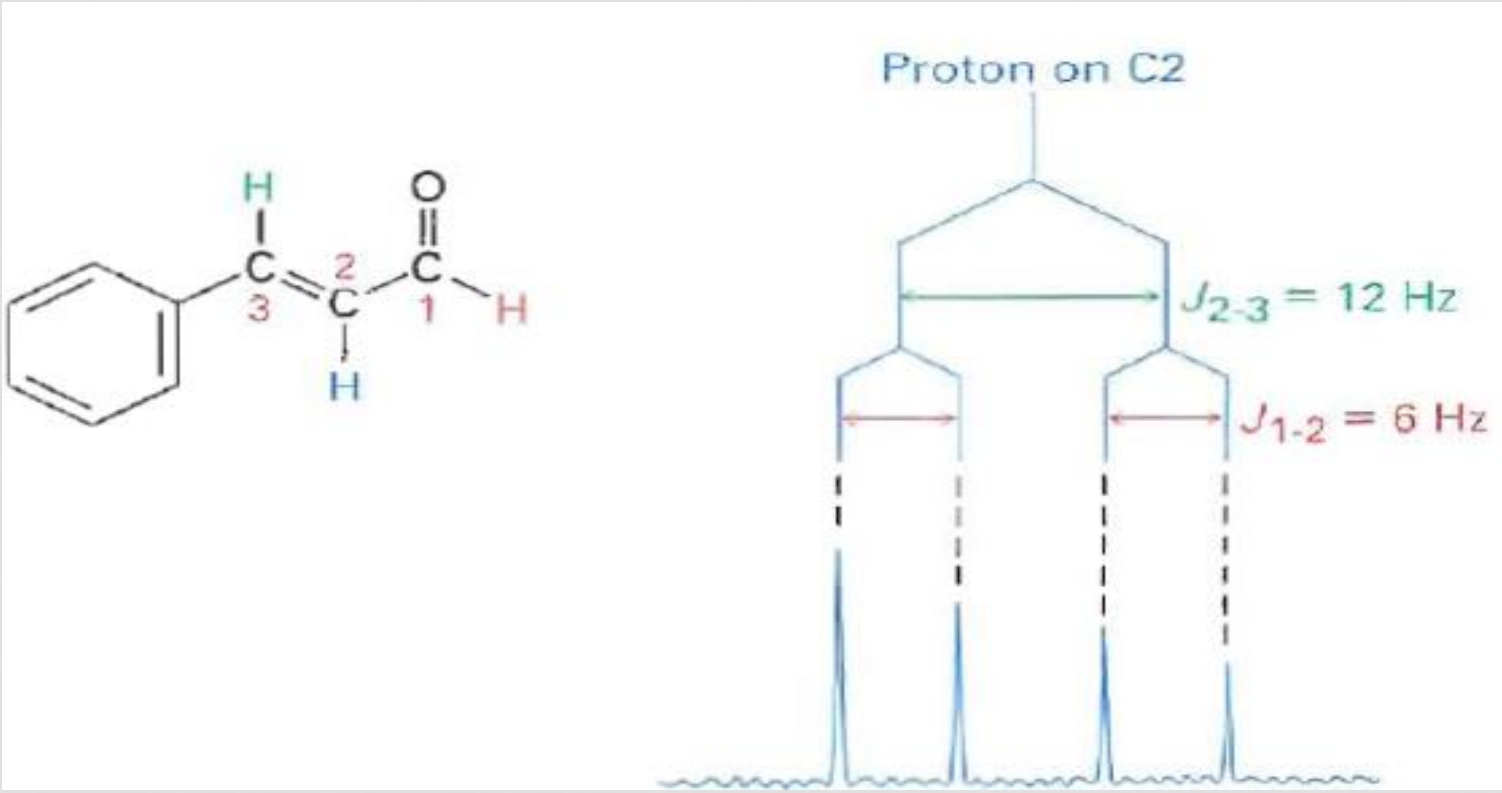


The five aromatic proton signals (black in Figure 13.19) overlap into a complex pattern with a large peak at 7.42 δ and a broad absorption at 7.57 δ.

The aldehyde proton signal at C1 (red) appears in the normal downfield position at 9.69 δ and is split into a doublet with J = 6 Hz by the adjacent proton at C2.

The vinylic proton at C3 (green) is next to the aromatic ring and is therefore shifted downfield from the normal vinylic region. This C3 proton signal appears as a doublet centered at 7.49 δ. Because it has one neighbor proton at C2, its signal is split into a doublet, with J = 12 Hz.

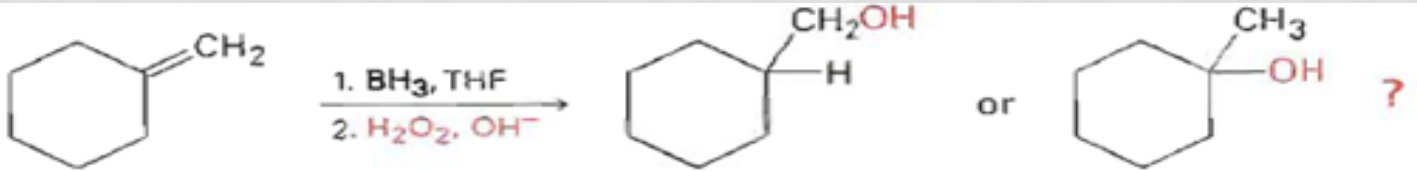
The C2 vinylic proton signal (blue) appears at 6.73 δ and shows an interesting four-line absorption pattern. It is coupled to the two nonequivalent protons at C1 and C3 with two different coupling constants: $J_{1\text{-}2}$ = 6 Hz and $J_{2\text{-}3}$ = 12 Hz.



USES OF 1H NMR SPECTROSCOPY:

NMR can be used to help identify the product of nearly every reaction run in the laboratory. For example, we said in Section 7.5 that hydroboration/oxidation of alkenes occurs with non-Markovnikov regiochemistry to yield the less highly substituted alcohol. With the help of NMR, we can now prove this statement.

Does hydroboration/oxidation of methylenecyclohexane yield cyclohexylmethanol or 1-methylcyclohexanol?



Methylenecyclohexane | Cyclohexylmethanol | 1-Methylcyclohexanol

The ^{1}H NMR spectrum of the reaction product is shown in Figure 13.21a. The spectrum shows a two-proton peak at 3.40 δ, indicating that the product has a $-CH_2-$ group bonded to an electronegative oxygen atom ($-CH_2OH$). Furthermore, the spectrum shows *no* large three-proton singlet absorption near 1 δ, where we would expect the signal of a quaternary $-CH_3$ group to appear. (Fig.

PROTONS ON OXYGEN, NITROGEN, AND SULFUR ATOMS

Protons directly bonded to an oxygen, nitrogen, or sulfur atom differ from protons on a carbon atom in that:

1. They are exchangeable.
2. They are subject to hydrogen bonding.
3. Those on a nitrogen (^{14}N) atom are subject to partial or complete decoupling by the electrical quadrupole moment of the ^{14}N nucleus.

Protons on Oxygen Atoms:

4.9.1.1 Alcohols Depending on concentration, the hydroxylic peak in alcohols is found between ~ δ 0.5 and ~ δ 4.0. A change in temperature or solvent will also shift the peak position.

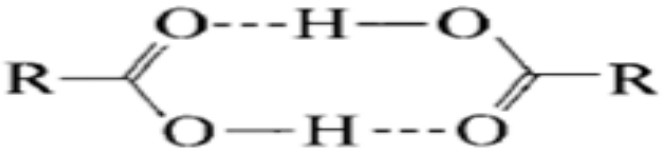
Rapid exchangeability explains why the hydroxylic peak of ethanol is usually seen as a singlet (Fig. 4.32). The OH proton shows a singlet, the CH_2 a quartet, and the CH_3 a triplet.

THE RATE OF EXCHANGE CAN BE DECREASED BY:

1. • Lowering the temperature.
2. • Using a dilute solution.
3. • Treating the solvent with anhydrous sodium carbonate or anhydrous alumina (basic impurities), then filtering through a pad of dry glass wool in a Pasteur pipette immediately before obtaining the spectrum.
4. • Grade 3A or 4A molecular sieves, immediately before obtaining the spectrum.

4.9.1.3 Phenols The behavior of a phenolic proton resembles that of an alcoholic proton. The phenolic proton peak is usually a sharp singlet (rapid exchange, no coupling), and its range, depending on concentration, solvent, and temperature, is generally to the left ($\delta \sim 7.5$ to $\delta \sim 4.0$) compared with the alcoholic proton. A car-

3.6.1.5 Carboxylic Acids Carboxylic acids exist as stable hydrogen-bonded dimers in nonpolar solvents even at high dilution. The carboxylic proton therefore absorbs in a characteristic range $\delta \sim 13.2 - \delta \sim 10.0$ and is affected only slightly by concentration. Polar solvents partially disrupt the dimer and shift the peak accordingly.



PROTONS ON NITROGEN ATOMS:

The common ^{14}N nucleus* has a spin number I of 1 and, in accordance with the formula $2I + 1$, should cause a proton attached to it and a proton on an adjacent carbon atom to show three equally intense peaks. There are two factors, however, that complicate the picture: the rate of exchange of the proton on the nitrogen atom and the electrical quadrupole moment of the ^{14}N nucleus (see Section 3.2.1).

Protons on nitrogen may undergo rapid, intermediate, or slow exchange. If the exchange is rapid, the

TABLE 3.5 Effect of NH exchange rate on coupling.

	Rate of NH Exchange		
	Fast	**Intermediate**	**Slow**
Effect on N—H	Singlet, sharp	Singlet, broad	Singlet, broad
Effect on C—H	No coupling	No coupling	Coupling
Examples	most aliphatic amines	N-methyl-p-nitroaniline	pyrroles, indoles 2°&1° amides and carbamates

Note that H—N—C—H coupling takes place through the C—H, C—N, and N—H bonds, but coupling between nitrogen and protons on adjacent carbon atoms is negligible. The proton–proton coupling is observed in the signal caused by hydrogen on carbon; the N—H proton signal is severely broadened by the quadrupolar interaction.

Protons on the nitrogen atom of an amine salt exchange at a moderate rate; they are seen as a broad peak, (δ ~8.5 to δ −6.0), and they are coupled to protons on adjacent carbon atoms (J ~7 Hz).

The use of trifluoroacetic acid as both a protonating agent and a solvent frequently allows classification of amines as primary, secondary, or tertiary. This is illustrated in Table 3.4 in which the number of protons on nitrogen determines the multiplicity of the methylene

TABLE 3.4 Classification of Amines by NMR of their Ammonium Salts in Trifluoroacetic Acid.

Amine Precursor Class	Ammonium Salt Structure	Multiplicity of Methylene unit
Primary	$C_6H_5CH_2NH_3^+$	Quartet (Fig. 3.41)
Secondary	$C_6H_5CH_2NH_2R^+$	Triplet
Tertiary	$C_6H_5CH_2NHR_2^+$	Doublet

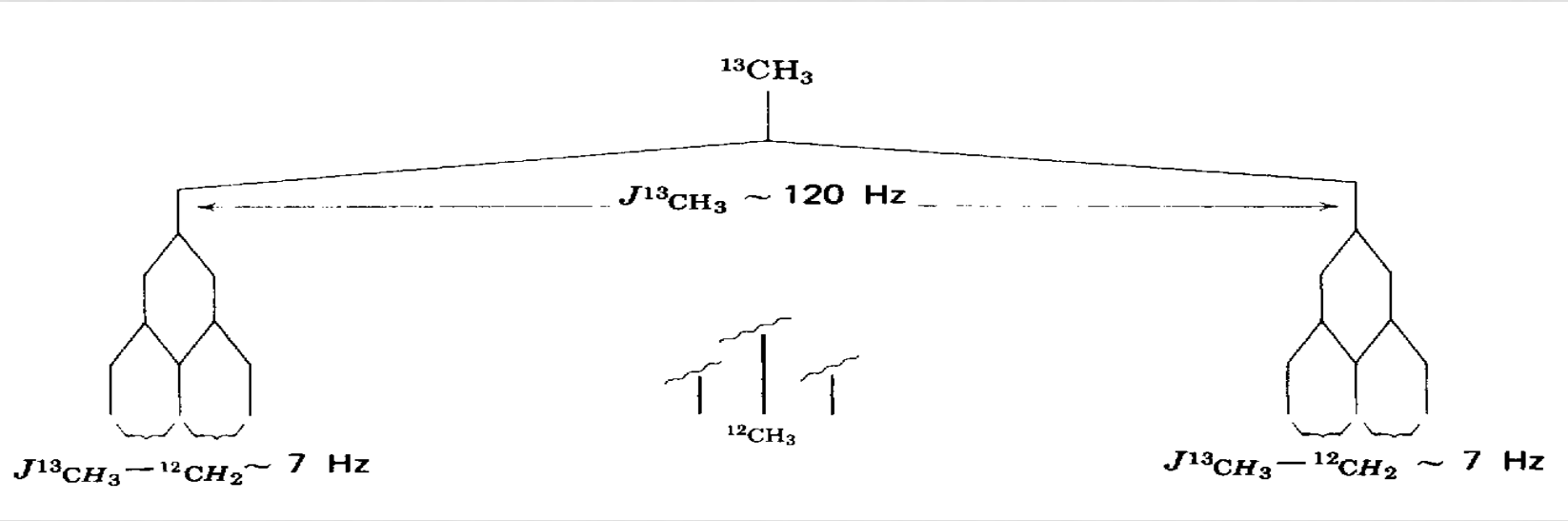
PROTON ON SULFUR:

Sulfhydryl protons usually exchange at a low rate so that at room temperature they are coupled to protons on adjacent carbon atoms (J ~8 Hz). They do not exchange rapidly with hydroxyl, carboxylic, or enolic protons on the same or on other molecules; thus, separate peaks are seen. However, exchange is rapid

COUPLING OF PROTONS TO ^{13}C:

The isotope ^{13}C has a natural abundance relative to ^{12}C of 1.1% and a spin number of $\frac{1}{2}$. Protons directly attached to ^{13}C are split into a doublet with a large coupling constant, about 115–270 Hz for ^{13}C—H. The CH_3—CH_2 group, for example, is predominantly $^{12}CH_3$—$^{12}CH_2$ but contains a small amount of $^{13}CH_3$—$^{12}CH_2$ and of $^{12}CH_3$—$^{13}CH_2$. Thus, the $^{13}CH_3$ protons are split into a doublet by ^{13}C ($J \sim 120$ Hz), and each peak of the doublet is split into a triplet by the $^{12}CH_2$ protons ($J \sim 7$ Hz) as shown below. These "^{13}C

satellite" peaks are small because of the small number of molecules containing the $^{13}CH_3$ group and can usually be seen disposed on both sides of a large $^{12}CH_3$ peak (e.g., the large $^{12}CH_3$ triplet shown below). The chemical shift of the $^{12}CH_3$ protons is midway between the satellites. See Chapter 5 for ^{13}C NMR spectrometry.



Magnetic Resonance Imaging (MRI)

Like NMR spectroscopy, MRI takes advantage of the magnetic properties of certain nuclei, typically hydrogen, and of the signals emitted when those nuclei are stimulated by radiofrequency energy. Unlike what happens in NMR spectroscopy, though, MRI instruments use data manipulation techniques to look at the three-dimensional *location* of magnetic nuclei in the body rather than at the chemical nature of the nuclei. As noted, most MRI instruments currently look at hydrogen, present in abundance wherever there is water or fat in the body.

The signals detected by MRI vary with the density of hydrogen atoms and with the nature of their surroundings, allowing identification of different types of tissue and even allowing the visualization of motion. For example, the volume of blood leaving the heart in a single stroke can be measured, and heart motion can be observed. Soft tissues that don't show up well on X rays can be seen clearly, allowing diagnosis of brain tumors, strokes, and other conditions. The technique is also valuable in diagnosing damage to knees or other joints and is a noninvasive alternative to surgical explorations.

Several types of atoms in addition to hydrogen can be detected by MRI, and the applications of images based on ^{31}P atoms are being explored. The technique holds great promise for studies of metabolism.

^{13}C NMR Spectrometry

The ^{12}C nucleus is not magnetically "active" (spin number, *I*, is zero), but the ^{13}C nucleus, like the ^{1}H nucleus, has a spin number of 1/2. However, since the natural abundance is only 1.1%. Thus, only about 1 of every 100 carbons in organic sample is observed by NMR.

The problem of low abundance has been overcome by:

1. The use of signal averaging. (increases instrument sensitivity)
2. Fourier-transform NMR (FT-NMR) (increases instrument speed)

The earlier, continuous-wave, slow-scan procedure requires a large sample and a prohibitively long time to obtain a ^{13}C spectrum, but the availability of pulsed FT instrumentation, which permits simultaneous irradiation of all ^{13}C nuclei, has resulted in an increased activity in ^{13}C spectrometry, beginning in the early 1970's, comparable to the burst of activity in ^{1}H spectrometry that began in the late 1950's.

Characteristics of ^{13}C NMR Spectroscopy

Samples for ^{13}C spectrometry are usually dissolved in $CDCl_3$, and the ^{13}C peak of tetramethylsilane (TMS) is used as the internal reference.* A list of the common deuterated solvents is given in Appendix A. The scale is in δ units (ppm). The shifts in routine ^{13}C spectra range over about 240 ppm from TMS—about 20 times that of routine ^{1}H spectra (~ 12 ppm). As a result of the large range and the sharpness of the decoupled peaks, impurities are readily detected and mixtures may be readily analyzed. Even stereoisomers that are difficult to analyze by means of ^{1}H spectrometry usually show discrete ^{13}C peaks.

^{1}H and ^{13}C do split each other the same (n+1) rule is used so:

- Signals due to methyl groups become quartets - coupled to 3 hydrogens.
- methylene groups become triplets - coupled to 2 hydrogens.
- methine groups become doublets - coupled to 1 hydrogen.
- quaternary or carbonyl carbons remain singlets.

this splitting can b eliminated by adjusting the instrument.

- **The technique of removing the coupling of 1H to an attached carbon is called broadband (BB) proton decoupling.**
- **Most 13C NMR, therefore, consist of a single peak for each unique carbon**

Can we observe ^{13}C nuclei independent of ^{1}H nuclei?

Yes, because 13C nuclei absorb at a different frequency than 1H nuclei. Simply use a different Rf generator tuned to a different frequency

Why is spin-spin coupling between adjacent ^{13}C's not observed?

Since the natural abundance of this isotope is so low, the chance of finding two ^{13}C's next to each other is practically nil. However, if a compound were synthesized with only ^{13}C's, then coupling would be observed.

Most ^{13}C resonances are between 0 and 220 ppm downfield from the TMS reference line, with the exact chemical shift of each ^{13}C resonance dependent on that carbon's electronic environment within the molecule. Figure 13.7 shows the correlation of chemical shift with environment.

13 C CHEMICAL SHIFTS

- Just as in 1H NMR spectroscopy, chemical shifts in 13C NMR depend on the electron density around the carbon nucleus
- Decreased electron density causes the signal to move downfield (deshielding)
- Increased electron density causes the signal to move up field (shielding)
- Because of the wide range of chemical shifts, it is rare to have two13C peaks coincidentally overlap

Some of the principal aspects of C NMR to consider that differ from H NMR are as follows:

- In the commonly used CPD or broadband proton-decoupled ^{13}C spectrum (see Section 4.2.1), the peaks are singlets unless the molecule contains other magnetically active nuclei such as ^{2}H, ^{31}P, or ^{19}F.
- The ^{13}C peaks are distributed over a larger chemical-shift range in comparison with the proton range.
- ^{13}C peak intensities do not correlate with the number of carbon atoms in a given peak in routine spectra, due to longer T_1 values and NOE.
- The ^{13}C nuclei are much less abundant and much less sensitive than protons. Larger samples and longer times are needed.
- For a given deuterated solvent, the ^{13}C and ^{1}H solvent peaks differ in multiplicities

- Factors determined the chemical shifts in13C-NMR:

1. Carbons chemical shift is effected by the electronegativity of nearby atoms.
2. Sp^3-hybridized carbons generally absorbed from 0 to 90 δ, while sp^2 carbons absorbed from 110 to 220.
3. Carbonyl carbons (C=O) are particularly distinct in ^{13}C NMR and always found at the low field end of the spectrum, from 160 to 180.

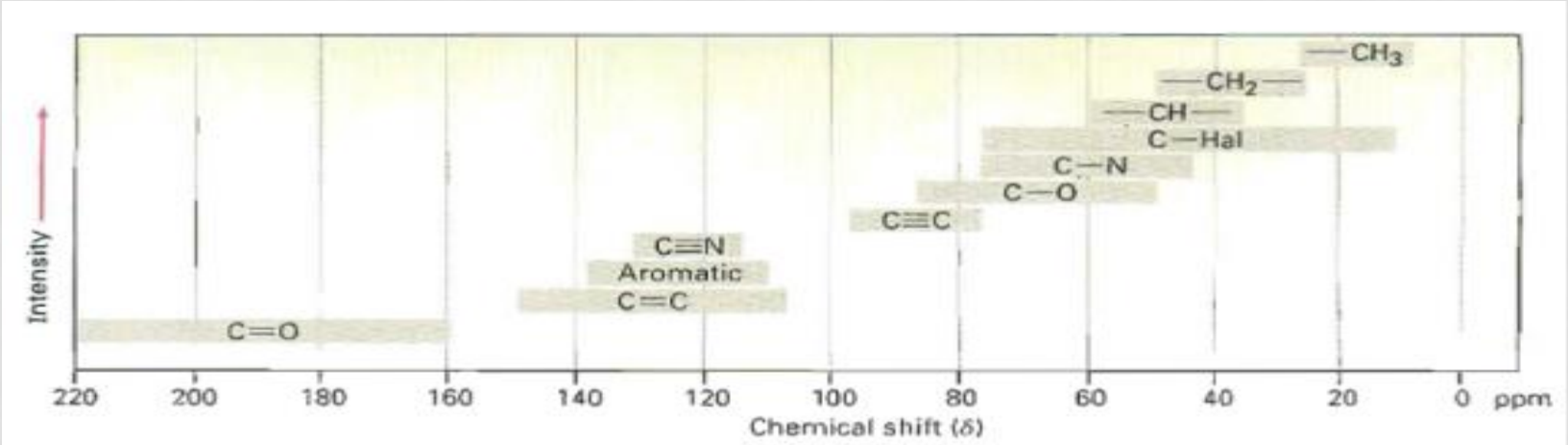
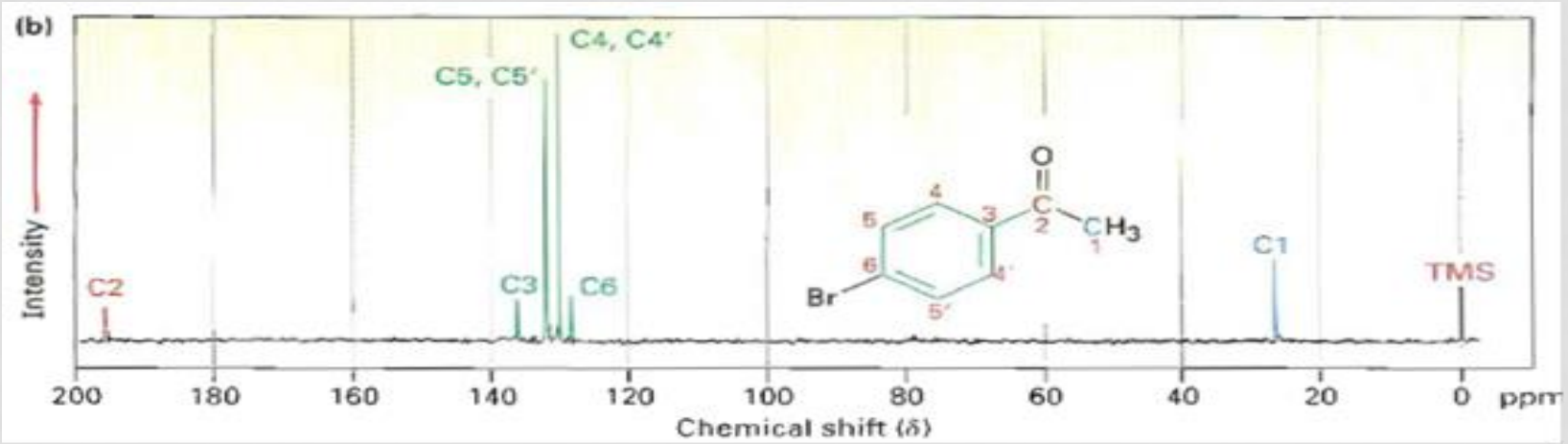


Figure 13.7 Chemical shift correlations for ^{13}C NMR.

particularly distinct in ^{13}C NMR and are always found at the low-field end of the spectrum, from 160 to 220 δ. Figure 13.8 shows the ^{13}C NMR spectra of 2-butanone and *para*-bromoacetophenone and indicates the peak assignments. Note that the C=O carbons are at the left edge of the spectrum in each case.



^{1}H Decoupling Techniques

As mentioned in Section 3.7.5, the ^{13}C nucleus does not show coupling in ^{1}H NMR spectra (except for ^{13}C satellites) due to the low natural abundance of ^{13}C (1.1%); however, the same cannot be said about the reverse. The ^{1}H nucleus is >99% in natural abundance and effectively couples to the ^{13}C nuclei. Because of the large $^{1}J_{CH}$ values for $^{13}C—^{1}H$ (~110–320 Hz) and appreciable $^{2}J_{CH}$, $^{3}J_{CH}$ values for $^{13}C—C—^{1}H$ and $^{13}C—C—C—^{1}H$ (~0–60 Hz) couplings, proton-coupled ^{13}C spectra usually show complex overlapping multiplets that are difficult to interpret (Figure 4.1a); the proton-coupled spectrum of choles-

DEPT ^{13}C NMR Spectroscopy

Techniques developed in recent years make it possible to obtain large amounts of information from ^{13}C NMR spectra. For example, *DEPT–NMR*, for *distortionless enhancement by polarization transfer*, allows us to determine the number of hydrogens attached to each carbon in a molecule.

A DEPT experiment is usually done in three stages, as shown in Figure 13.10 for 6-methyl-5-hepten-2-ol. The first stage is to run an ordinary spectrum (called a *broadband-decoupled spectrum*) to locate the chemical shifts of all carbons.

Next, a second spectrum called a DEPT-90 is run, using special conditions under which only signals due to CH carbons appear. Signals due to CH_3, CH_2, and quaternary carbons are absent. Finally, a third spectrum called a DEPT-135 is run, using conditions under which CH_3 and CH resonances appear as positive signals, CH_2 resonances appear as *negative* signals—that is, as peaks below the baseline—and quaternary carbons are again absent.

Broadband-decoupled	DEPT-90	DEPT-135
C, CH, CH_2, CH_3	CH	CH_3, CH are positive CH_2 is negative

C	Subtract DEPT-135 from broadband-decoupled spectrum
CH	DEPT-90
CH_2	Negative DEPT-135
CH_3	Subtract DEPT-90 from positive DEPT-135

Propose a structure for an alcohol, $C_4H_{10}O$, that has the following ^{13}CNMR spectral data:
Broadband _ decoupled ^{13}CNMR: 19.0, 31.7, 69.5 б
DEPT _90: 31.7 б
DEPT _ 135: positive peak at 19.0 & 31.7 б, negative peak at 69.5 б

2-Methyl-1-propanol

Propose a structure for an aromatic hydrocarbon, $C_{11}H_{16}$, that has the following ^{13}C NMR spectral data:

Broadband-decoupled ^{13}C NMR: 29.5, 31.8, 50.2, 125.5, 127.5, 130.3, 139.8 δ
DEPT-90: 125.5, 127.5, 130.3 δ
DEPT-135: positive peaks at 29.5, 125.5, 127.5, 130.3 δ; negative peak at 50.2 δ