COMPARISION BETWEEN ^{1}H & ^{13}C NMR

UNDER THE ESTEEMED GUIDANCE OF

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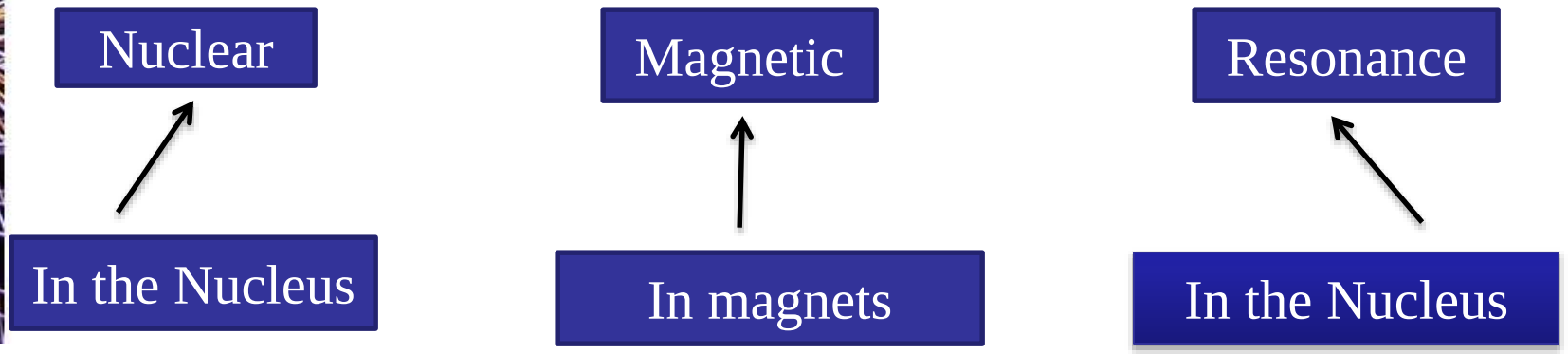
NUCLEAR MAGNETIC RESONANCE

(NMR Spectroscopy)

- A spectroscopic technique that gives us information about the number and types of atoms in a molecule.
- Nuclear magnetic resonance spectroscopy is a powerful analytical technique used to characterize organic molecules by identifying carbon-hydrogen frameworks within molecules.



- It is concerned with the magnetic properties of certain atomic nuclei.
- Involves change in the spin state at the nuclear level.

SPINNIG NUCLEUS:

✓ proton acts as a tiny spinning bar magnet and possesses both electrical charge and mechanical spin.

- NMR is the most powerful tool available for organic structure determination.
- It is used to study a wide variety of nuclei:

1. ^{1}H
2. ^{13}C
3. ^{15}N
4. ^{19}F
5. ^{31}P

THEORY:

- Two common types of NMR spectroscopy are used to characterize organic structure: 1H NMR is used to determine the type and number of H atoms in a molecule; 13C NMR is used to determine the type and number of C atoms in the molecule.
- The source of energy in NMR is radio waves which have long wavelengths, and thus low energy and frequency.
- All the atoms contains nuclei and all nuclei contains protons (+ve) charge in which some charge nuclei posses “Spin” on their own axis.

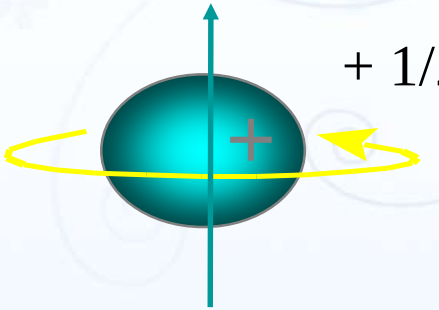
- Spin nuclei are those which contains either **Odd** atomic number or odd mass number or both e.g. 1H, 2H, 13C, 14N, 17O, 35Cl etc are useful for NMR.
- Those nuclei contains **Even** number of atomic and mass number are not useful for NMR e.g. 12C, 16O etc.
- The nuclei posses spin, they spin on their nuclear axis leads to generate magnetic dipole 'μ' so the angular momentum of this spinning charge is quantified and described by Quantum Spin Number " I ".

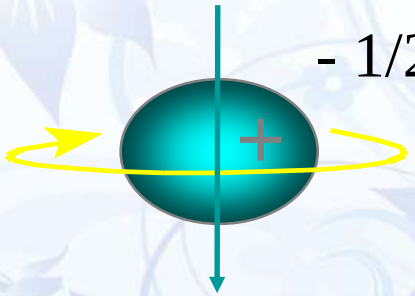
Element	^{1}H	^{2}H	^{12}C	^{13}C	^{14}N	^{15}N	^{16}O	^{19}F	^{31}P	^{32}S
Nuclear spin quantum number (*I*)	1/2	1	0	1/2	1	1/2	0	1/2	1/2	0
Number of spin states	2	3	1	2	3	2	1	2	2	1

SPIN QUANTUM OF VARIOUS NUCLEI

Atomic number	Mass number	Spin quantum number. (I)	Example
Even	Even	0	^{12}C, ^{16}O, ^{32}S
Odd/ Even	Even/ Odd	1/2,3/2,5/2	^{1}H, ^{19}F, ^{31}P, ^{11}B, ^{79}Br & ^{13}C, ^{127}I,
odd	odd	1	^{2}H, ^{14}N

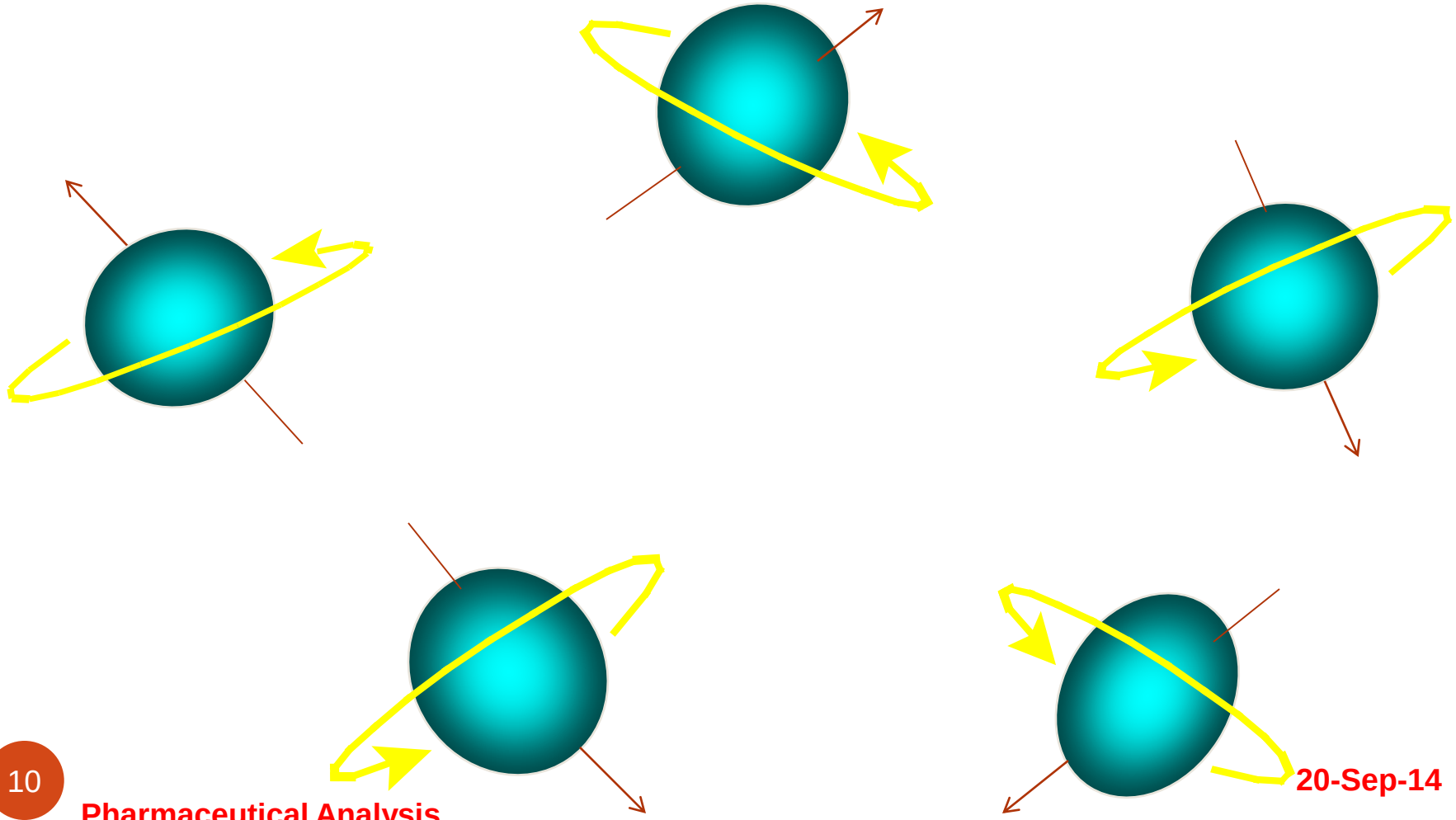
- The individual protons have spin quantum number +1/2 or -1/2.
  i.e. Hydrogen have spin quantum number (I) = +1/2 or -1/2.



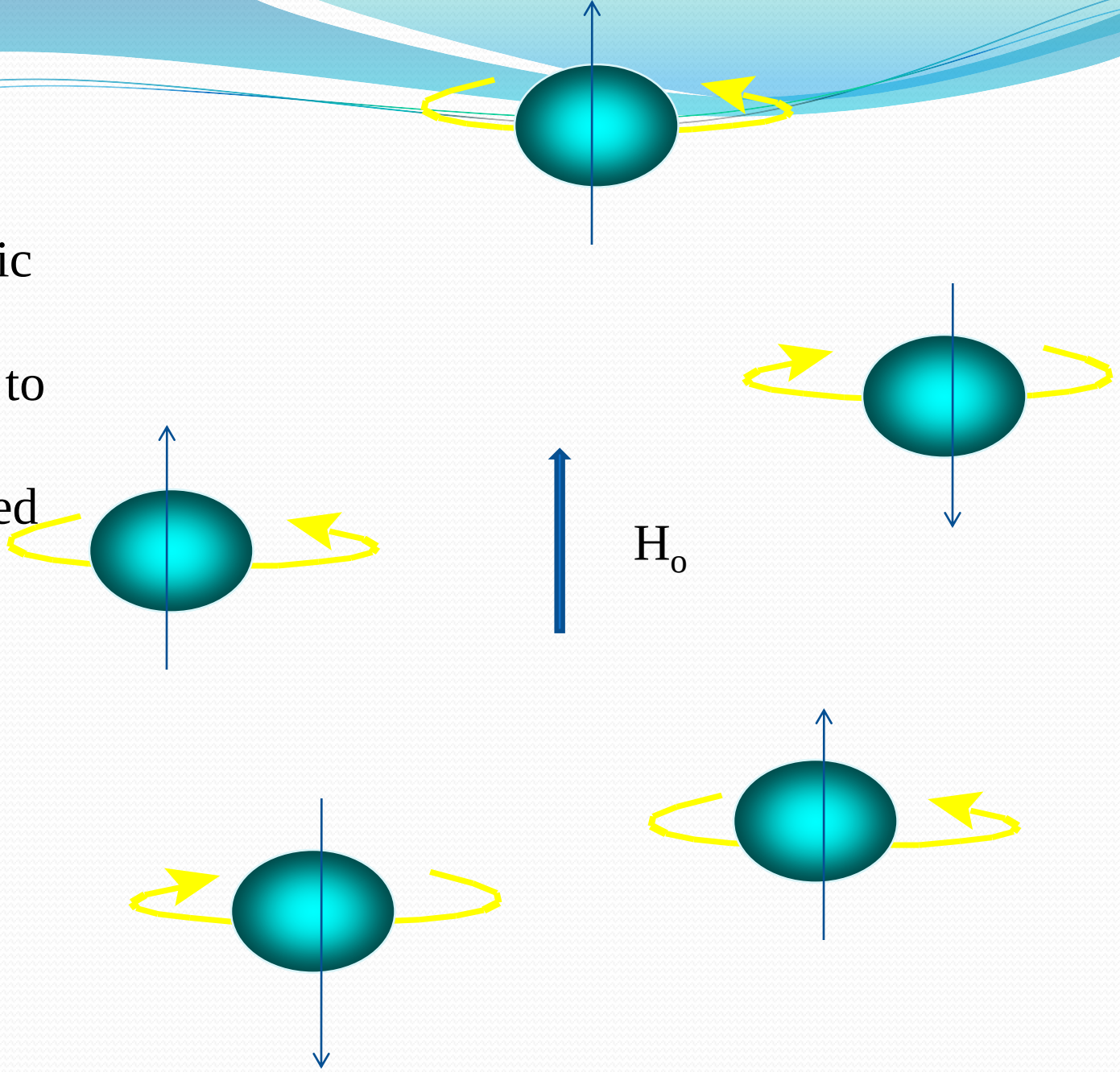


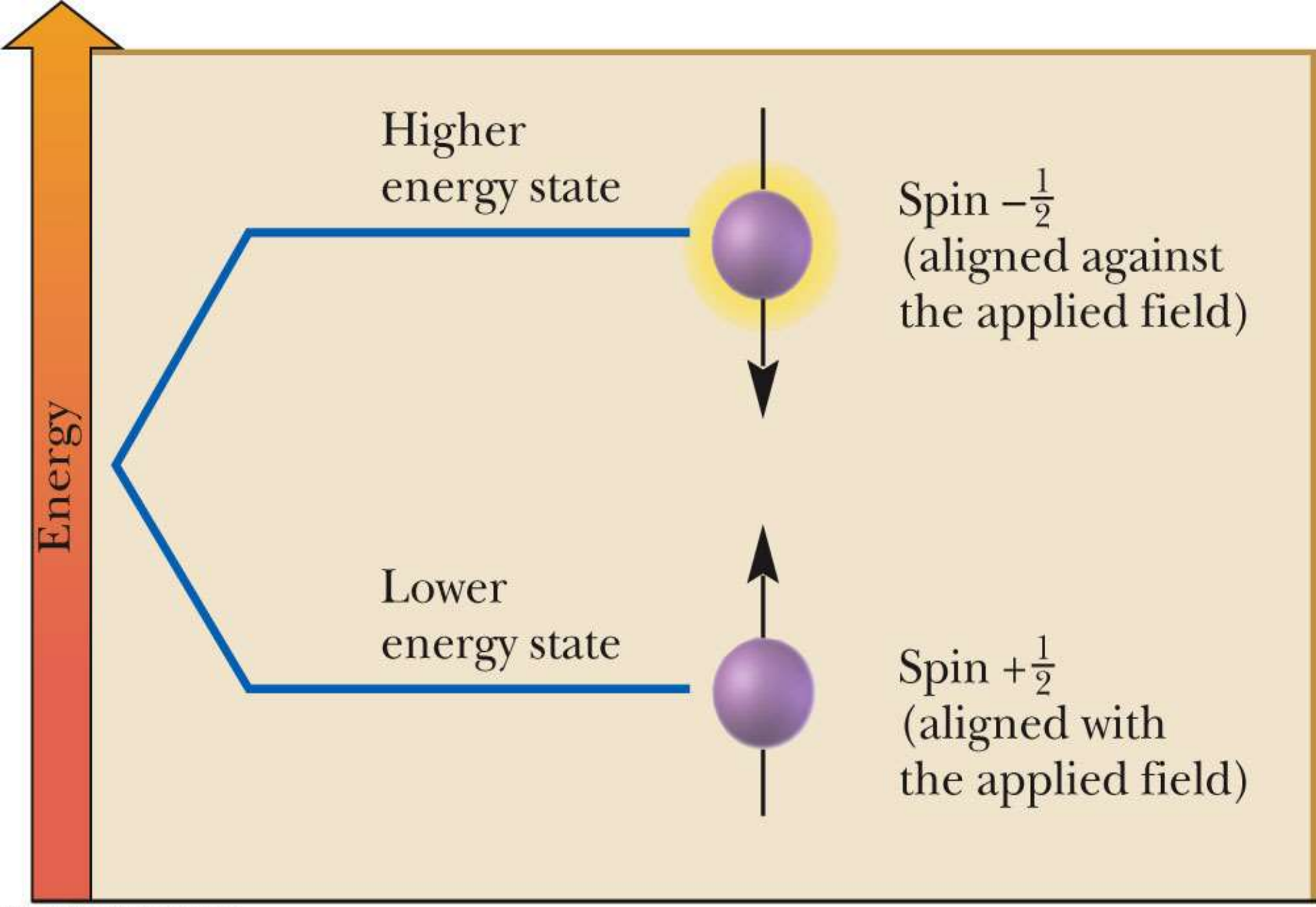
- These spin states have equal amount of energy (degenerated) in the absence of magnetic field.
- When a charged particle such as a proton spins on its axis, it creates a magnetic field. Thus, the nucleus can be considered to be a tiny bar magnet.

The distribution of nuclear spins is random in the absence of an external magnetic field.

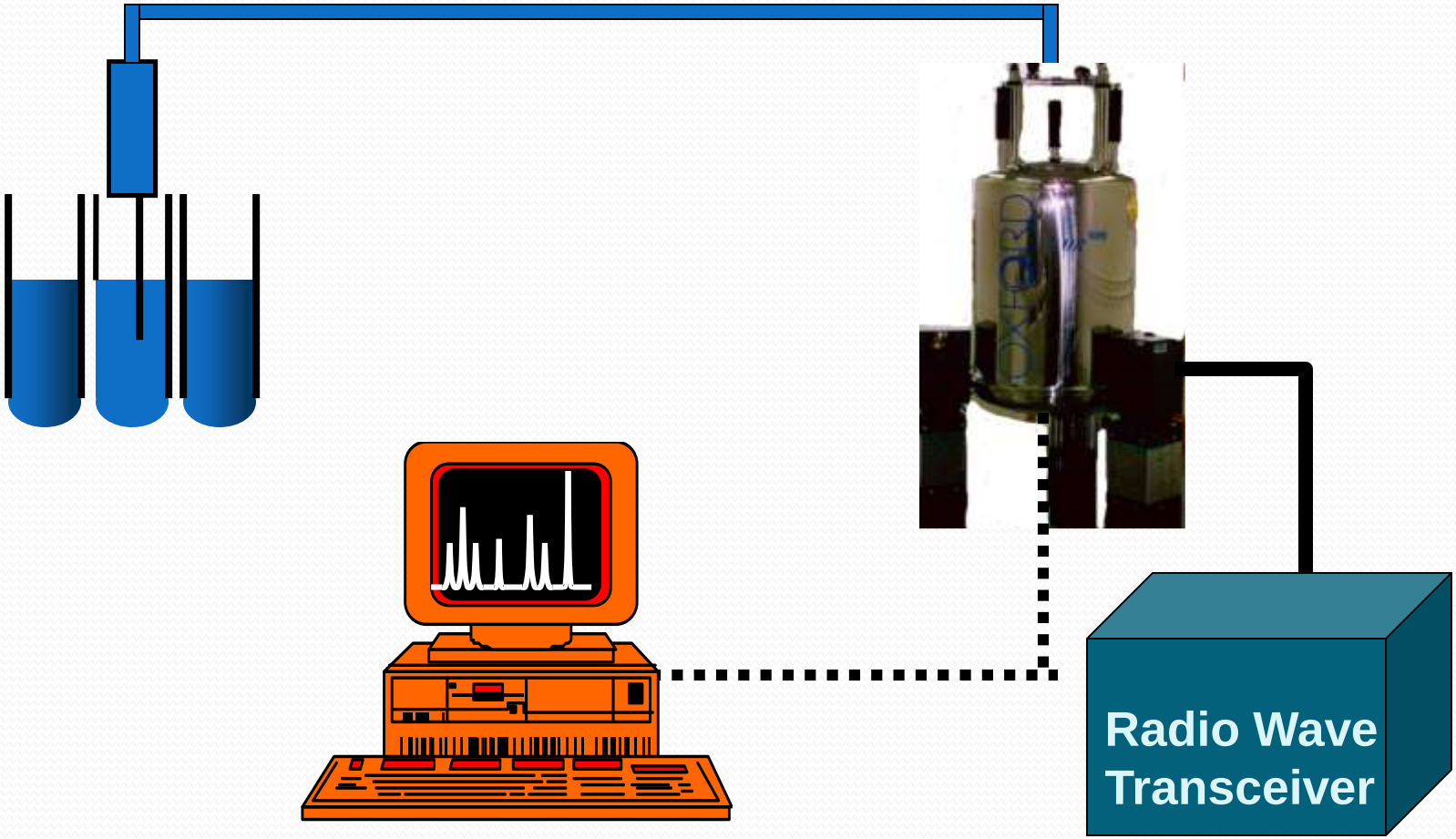


An external magnetic field causes nuclear magnetic moments to align parallel and antiparallel to applied field.





A Modern NMR Instrument



CONTINUOUS –WAVE (CW) INSTRUMENT

- In the CW spectrometers the spectra can be recorded either with field sweep or frequency sweep.
- Keeping the frequency constant, while the magnetic field is varied, (swept) is technically easier than holding the magnetic field constant and varying the frequency.
- Instruments which vary the magnetic field in a continuos fashion scanning from the downfield end to upfield end of the spectrum, are called **Continuous wave instruments**.
- The continuous wave type of NMR spectrometer operates by exciting the nuclei of the isotope under observation one type at a time.

THE CONTINUOUS –WAVE (CW) INSTRUMENT

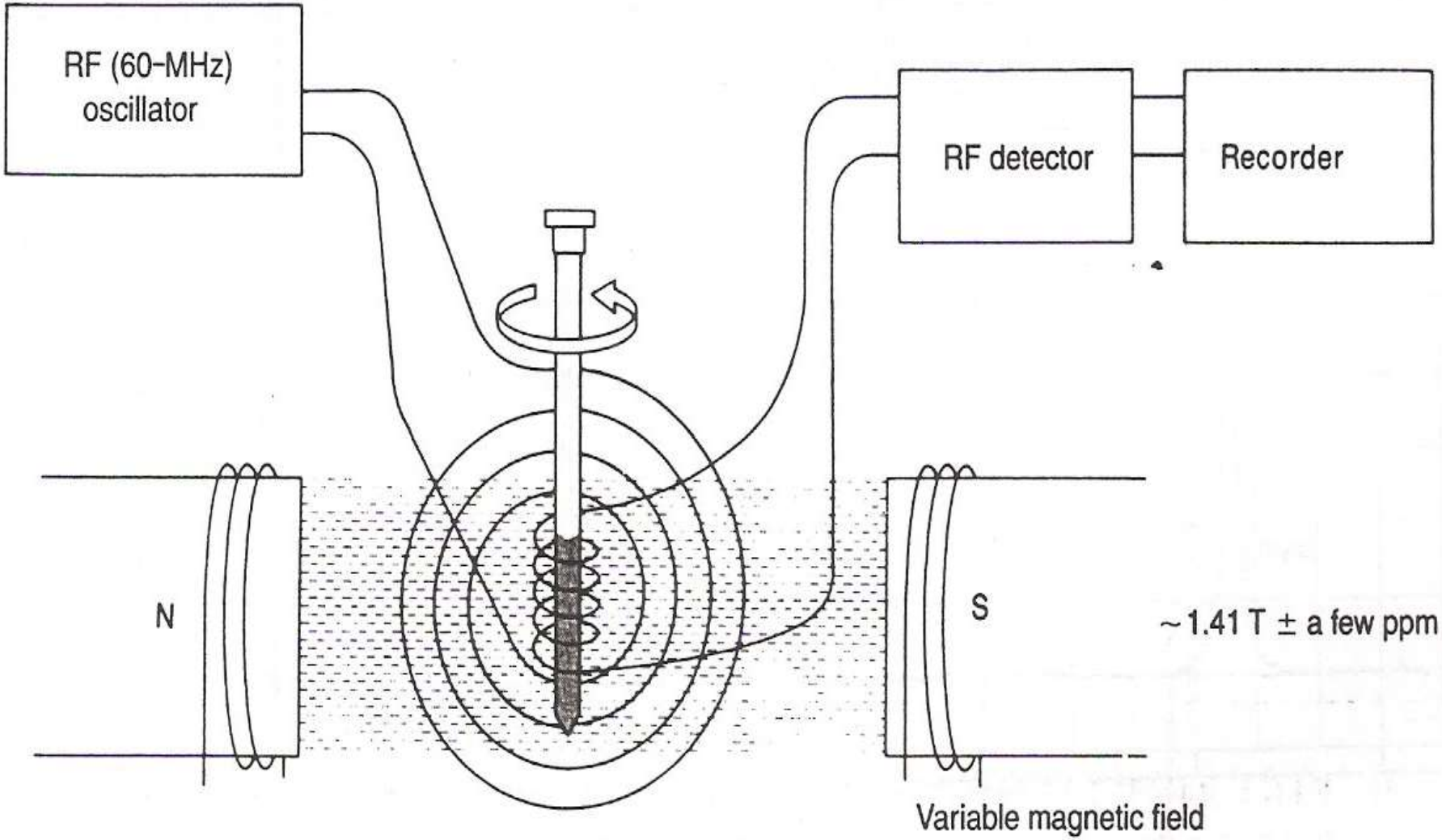


FIGURE 3.11 The basic elements of the classical nuclear magnetic resonance spectrometer.

THE PULSED FOURIER TRANSFORM (FT)

INSTRUMENT

- The continuous wave type of NMR spectrometer operates by exciting the nuclei of the isotope under observation one type at a time.

- An alternative approach to this modern sophisticated instrument is to use a powerful but short burst of energy called a pulse that excites all of the magnetic nuclei in the molecule simultaneously and all the signals are collected at the same time with a computer.

- In an organic molecule for instance all of the H1 nuclei are induced to undergo resonance at the same time.

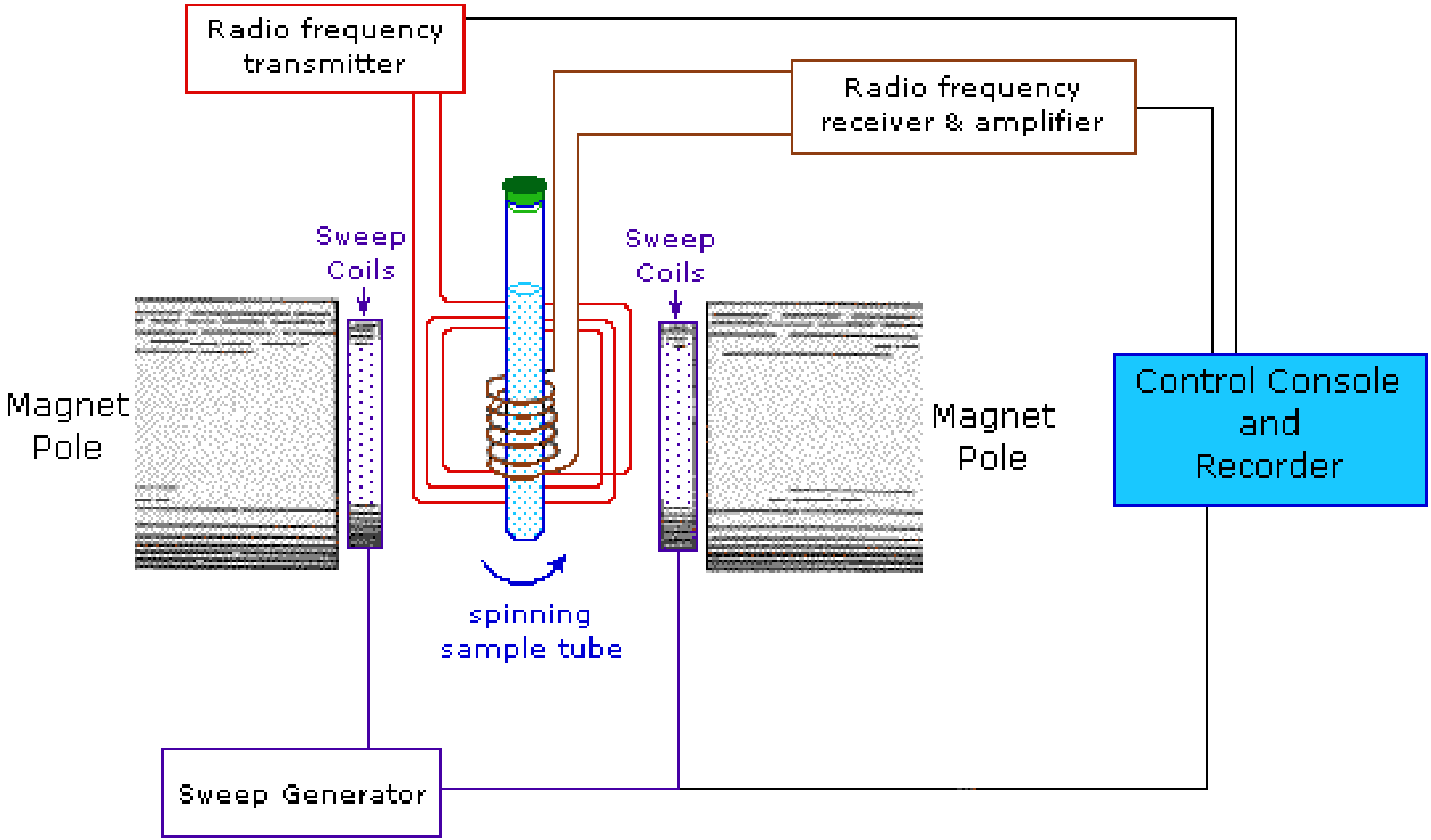
- The pulse actually contains a range of frequencies centered about the hydrogen in the molecule at once this signal burst of energy.
- When the pulse is discontinued the excited nuclei begin to lose their excitation energy and return to the original state or relax. As each excited nucleus relaxes it emits EMR.
- Since the molecule contains many different nuclei many different frequencies of EMR are emitted simultaneously. This emission is called a free-induction decay (FID) signal.

- The intensity of FID decays with the time as all of the frequencies emitted and can be quite complex. We usually extract individual frequencies due to different nuclei by using a computer and a mathematical method called a

Fourier-transform analysis.

- The Fourier transform breaks the FID into its separate since or cosine wave components. This procedure is too complex to be carried out by eye or by hand; it requires a computer.

FT NMR INSTRUMENTATION



ADVANTAGES OF FT-NMR

- FT-NMR is **more sensitive and can measure weaker signals**.

- The pulsed FT-NMR is much faster (seconds instead of min) as compared to continuous wave NMR.

- FT-NMR can be obtained with less than 0.5 mg of compound. This is important in the biological chemistry, where only μg quantities of the material may be available.

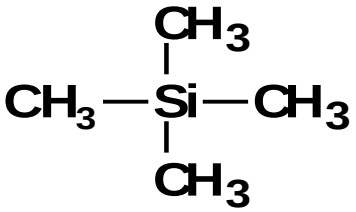
- The FT method also gives improved spectra for sparingly soluble compounds.

- Pulsed FT-NMR is therefore especially suitable for the examination of nuclei that are magnetic or very dilute samples.

Solvents

- The solvent used for dissolving sample should have following properties;
  - **Should not contain proton,**
  - **Inexpensive**
  - **Low boiling point and non polar in nature.**
- Generally deuterated chloroform $CDCl_3$ is used as solvent.
- If sample is soluble in polar solvent, then deuterium oxide (D_2O), DMSO, CCl_4, CS_2, Cf_3, COOH are used as solvent.

Internal Standard



Tetramethylsilane (TMS)

TMS (Tetra methyl silane) is most commonly used as IS for measuring the position of **1H, 13C** and in NMR spectroscopy. Due to following reasons;

- It is **chemically inert** and miscible with a large range of solvents.
- **Its twelve protons are all magnetically equivalent**.
- Its **protons are highly shielded** and gives a strong peak even small quantity.

- It is less electronegative than carbon.
- It is highly volatile and can be easily removed to get back sample.
- It does not take part in intermolecular associations with sample.

Chemical Shift

- Chemical shift is the difference between the absorption position of the sample proton and the absorption position of reference standard”

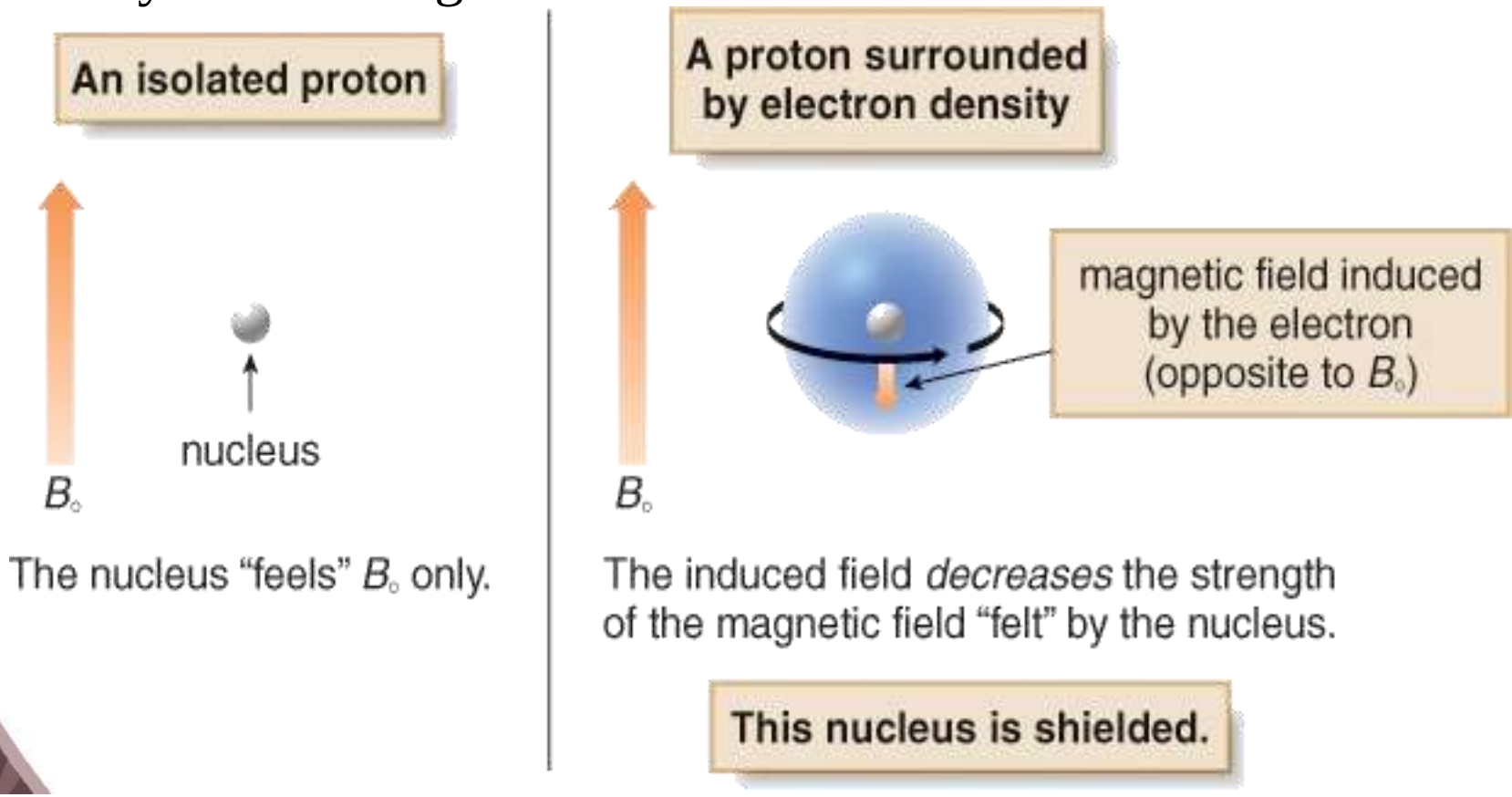
- Variations of the positions of NMR absorptions due to the electronic shielding and deshielding

- Measured in ***parts per million*** (ppm).
- The chemical shift is independent of the operating frequency of the spectrometer
- Same value for 60, 100, or 300 MHz machine.
- Common scale used is the delta (δ) scale.

$$\textbf{Chemical Shift, ppm } \delta = \frac{\textbf{Shift from TMS in Hz}}{\textbf{Spectrometer frequency (MHz)}} \times 10^6$$

Shielding or Deshielding Protons In Molecule

- Such shifting in position of NMR absorption signals which arise due to the **shielding or deshielding** of proton by surrounding electrons are called as **Chemical shift**.



Factors affecting chemical shift:

Following are the factors which influence the chemical shift;

- **Inductive effect**
- **Van der Waal's deshielding**
- **Anisotropic effect**
- **Hydrogen bonding**

This happens, whenever there is a long lecture

it’s better to be alert….

Difference between ^{1}H NMR& ^{13}C NMR

Proton NMR (PMR)	^{13}C NMR (CMR)
1. It is study of spin changes of **proton** nuclei.	1. It is study of spin changes of **carbon** nuclei.
2. Chemical shift range is **0-14 ppm.**	2. Chemical shift range is **0-240 ppm.**
3. **Continuous wave** method is used	3. **Fourier transform** Technique is used.
4.slow process.	4.Very fast process.

5. Coupling constant range is **0-15Hz**.	5. Coupling constant range is **125-250Hz.**
6. Solvent peak is not observed.	6. Solvent peak is observed.
7. Area under the peak is considered	7. Area under the peak is not considered.
8. **TMS** peak is singlet.	8. **TMS** peak is quartet.
9.Effect of substituent on adjacent carbon atom can varies chemical shift.	9. Effect of substitute on adjacent carbon atom cannot varies chemical shift.

Property	^{1}H	^{13}C
NMR frequency MHz for a 1-T Field	42.576	10.705
Natural abundance	99.9844	1.108
Relative Sensitivity at constant field	1.000	$1.59x10^{-5}$
Magnetic moment (μ)	2.79268	0.70220
Spin Number (I)	1/2	1/2
Magnetogyric ratio	26,753	6,728

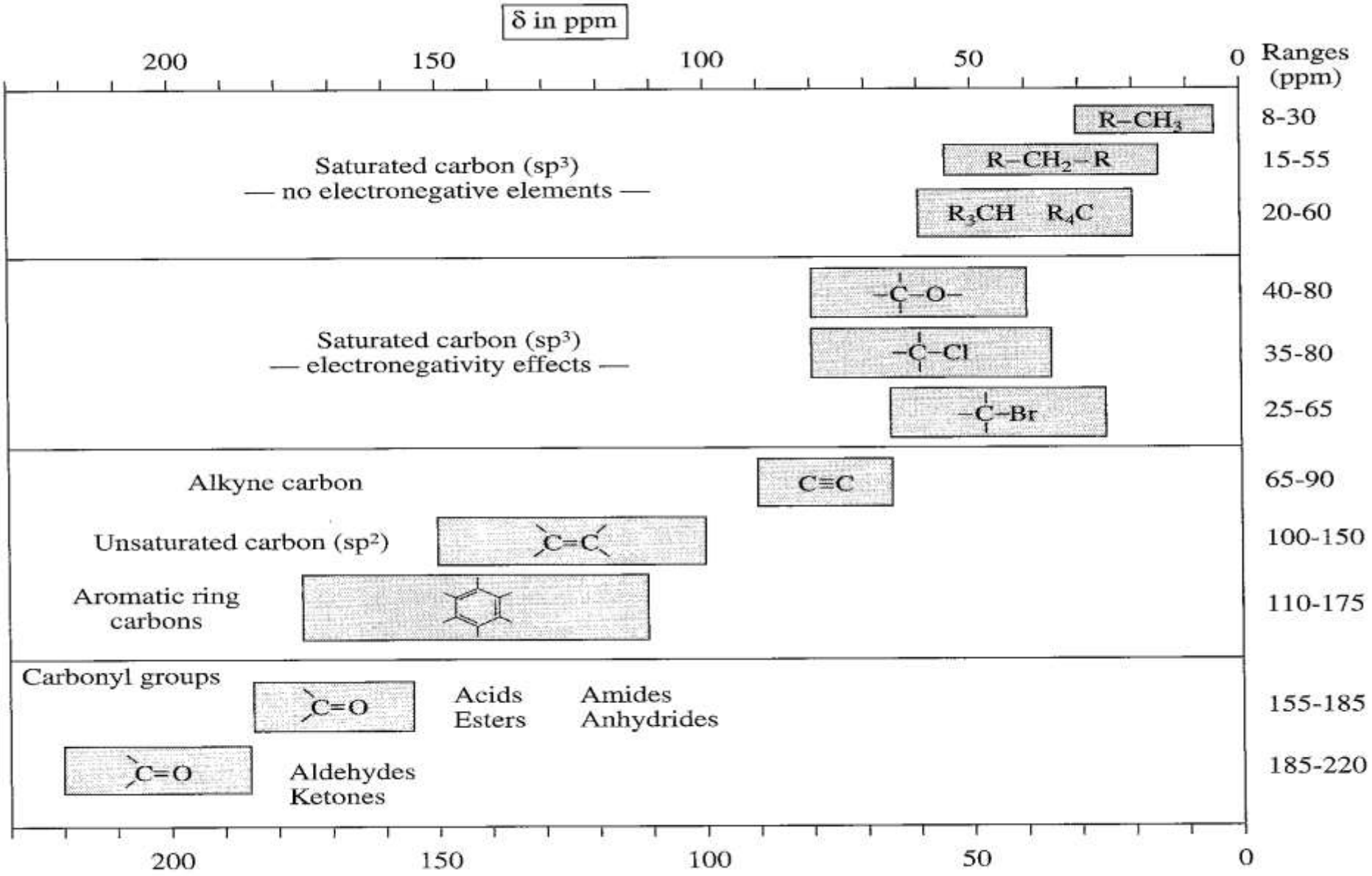


FIGURE 4.1 A correlation chart for ^{13}C chemical shifts (chemical shifts are listed in parts per million from TMS).

NATURAL ABUNDANCE:

- The most abundant isotope of carbon ^{12}C is not detected by NMR, as it is magnetically inactive (I=0).
- The low natural abundant isotope ^{13}C is magnetically active (I=1/2).
- The natural abundance of 1H is 99.9844%
- As a result of the natural abundance of ^{13}C is 1.1% , the sensitivity of ^{13}C nuclei is only 1.6% that of ^{1}H nuclei..

GYRO MAGNETIC RATIO:

- ^{13}C nucleus gyro magnetic ratio is much lesser than proton nucleus.

  C-6,728; H-26,753.

- This shows that CMR is more sensitive than PMR which is overcome by using FT-NMR technique

Signal Splitting of 1H: The (n + 1) Rule

- In a NMR spectrum all equivalent protons do not appear as a signal peak, e.g. **1,1,2 - tribromoethane** which has two types of equivalent protons, thus it shows two peaks in NMR spectrum.

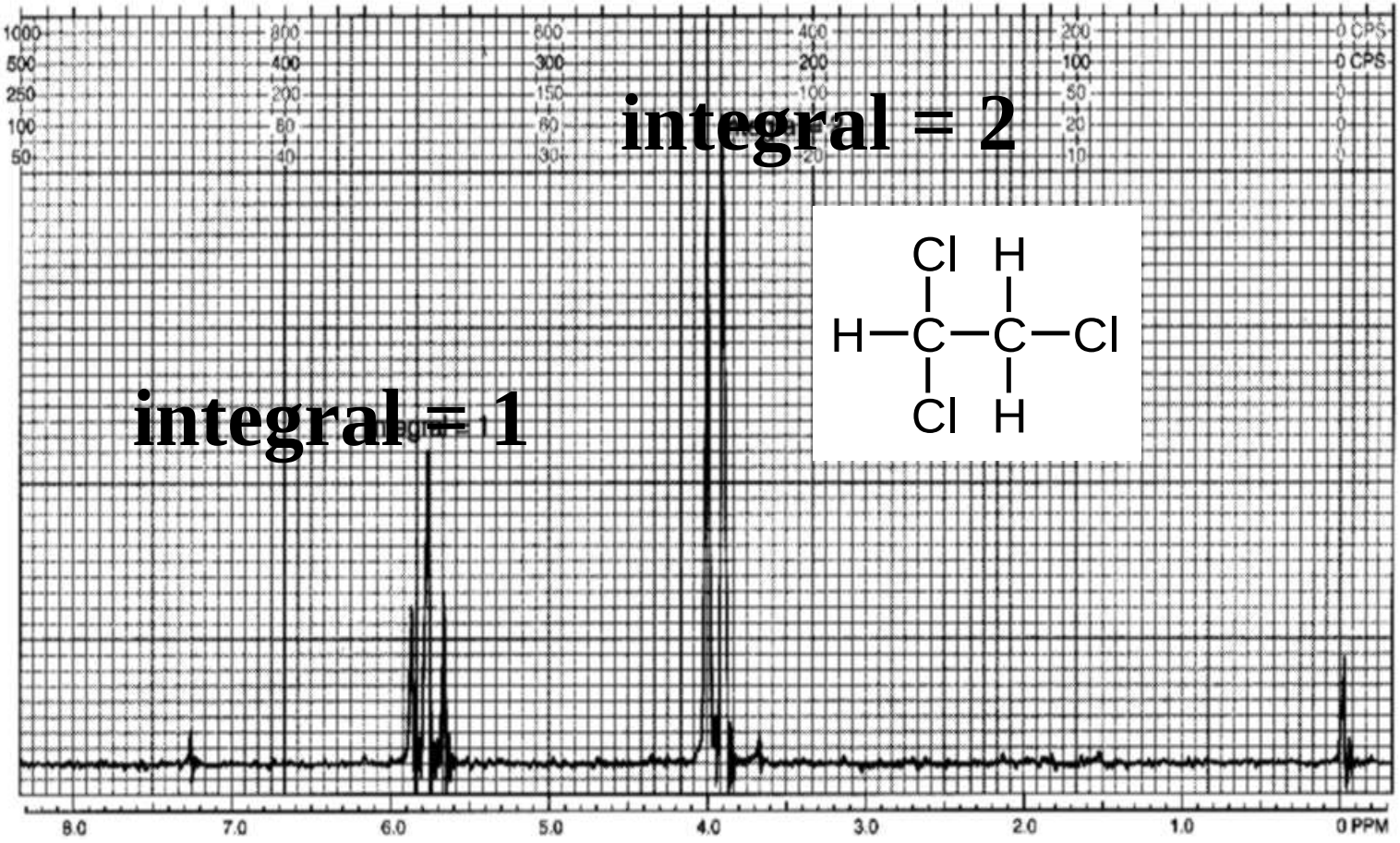
- But the actual spectrum consists of two peaks but subdivided into 3 and 2 sub peaks or splitting one for (CH) and two for (CH_2Br) protons respectively.

➢This phenomenon of splitting of equivalent protons into (n + 1) rule, where;

n is **the no. of equivalent protons attached to the adjacent carbon to which the protons under consideration is attached.**

➢ So as per the (n + 1) rule in **1,1,2- tribromoethane**, the (C_1) has two equivalent protons of Methylene on the carbon next to it, therefore, n = 2 and hence it will split into (2+1) = 3 peaks (Triplet).

1,1,2-Trichloroethane

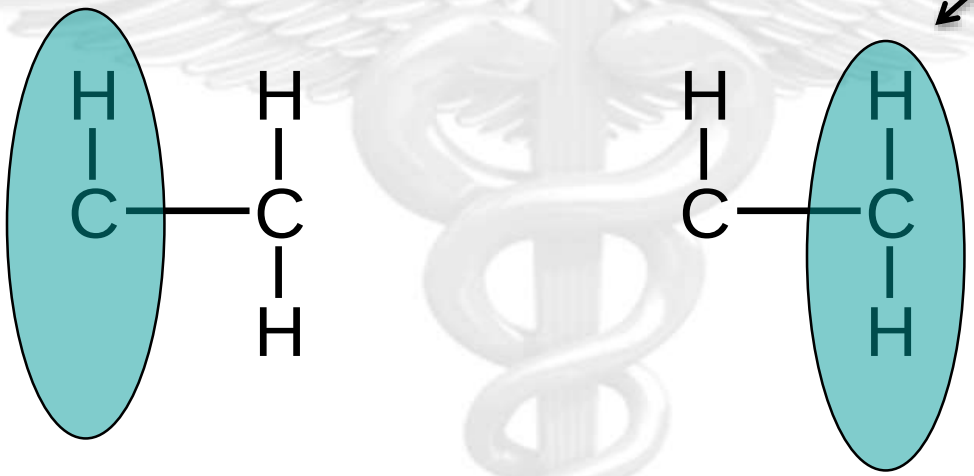


Where do these multiplets come from ?

interaction with neighbors

this hydrogen's peak
is split by its two neighbors

these hydrogens are
split by their single
neighbor



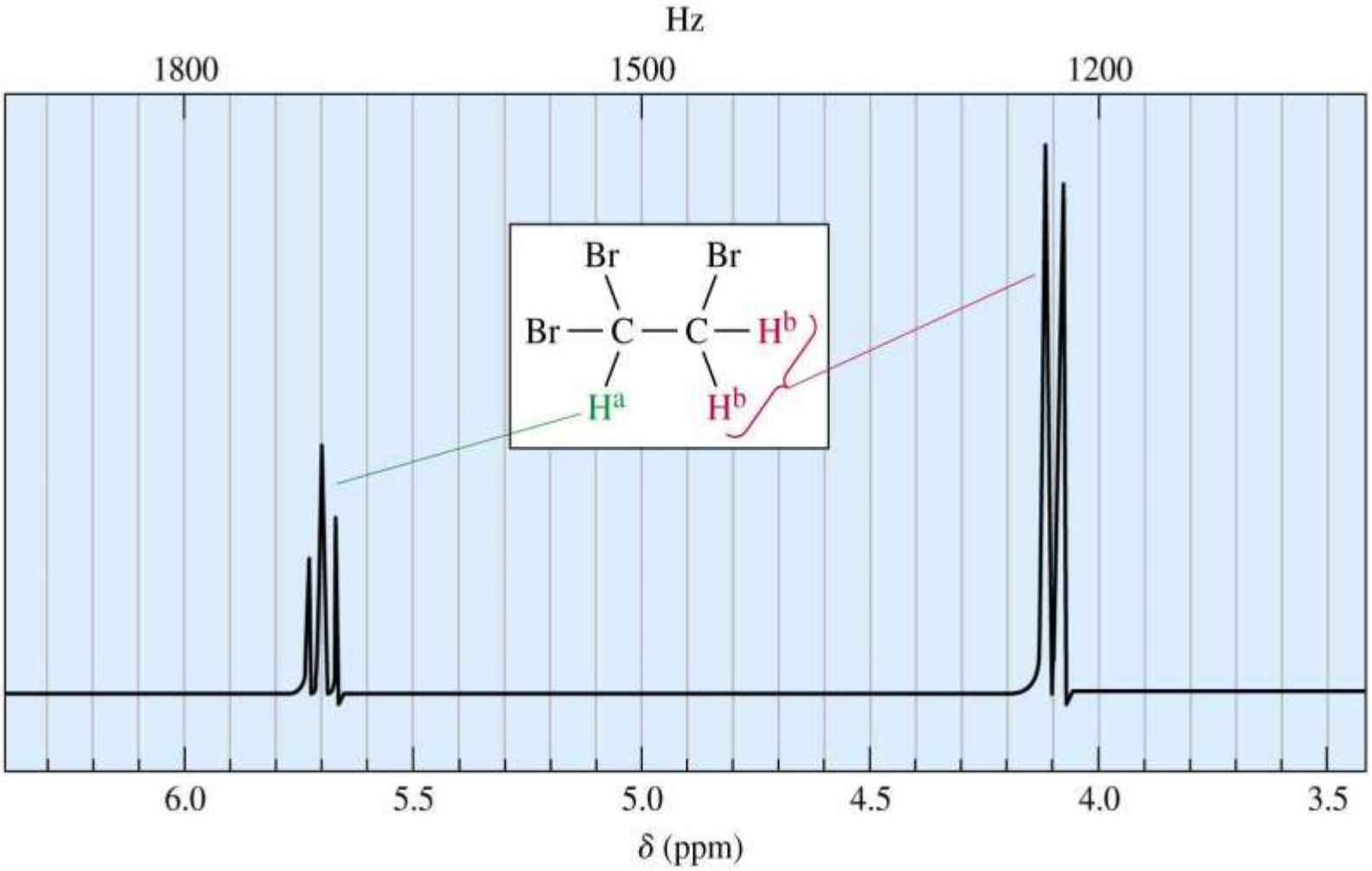
two neighbors
n+1 = 3
triplet

one neighbor
n+1 = 2
doublet

MULTIPLETS

- Singlet
- Doublet
- Triplet
- Quartet
- Quintet
- Sextet
- Septet

➢ The Methylene protons are having n = 1 therefore, it will split (1+1) = 2 peaks (Doublet).



SPLITTING OF ^{13}C SIGNALS:

- Splitting take place acc. to **2nI+1 rule**

  Where ***n= no. of nuclei***

  I=spin quantum number

  CH_3 = 3+1=4 quartet

  CH_2 = 2+1=3 triplet

  CH = 1+1=2 doublet

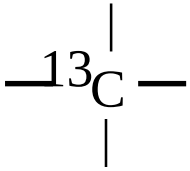
  C = 0+1=1 singlet

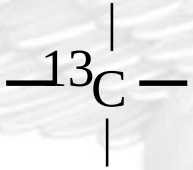
- **$CDCl_3$** gives three peaks because its I=1 so acc. to 2nI+1

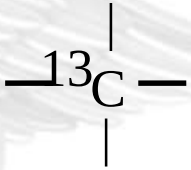
  2×1×1+1=3 so it gives **1:1:1 peaks**

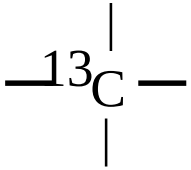
- Solvents used are **CDCl3, DMSO, $d_6$acetone, d_6 benzene**

COUPLING TO ATTACHED PROTONS

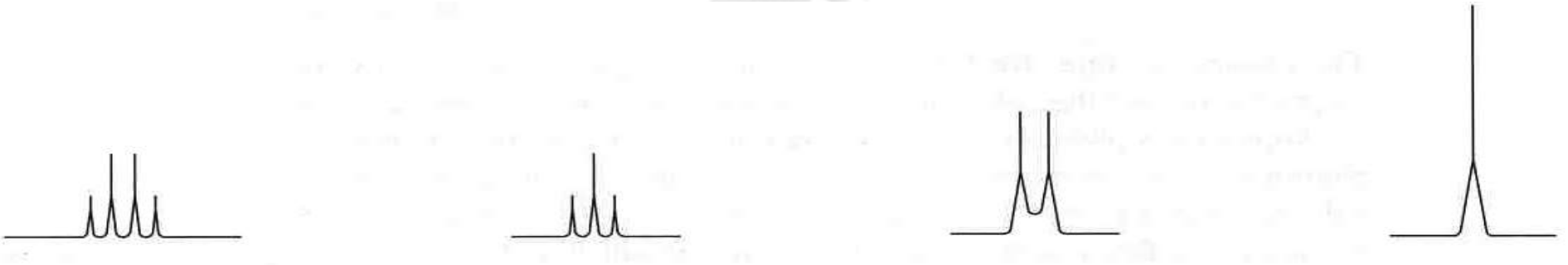








n+1 = 4 n+1 = 3 n+1 = 2 n+1 = 1



Methyl Carbon Methylene carbon Methylene carbon Quaternarym carbon

The effect of attached protons on ^{13}C resonances

Coupling phenomenon:

- Both ^{13}C and ^{1}H have I =0, so that we expect coupling in the spectrum between **^{13}C - ^{13}C and ^{13}C - ^{1}H.**
- The **probability of two ^{13}C nuclei adjacent** to each other in the same molecule is **extremely rare due to low natural abundance of ^{13}C.**
- So that ^{13}C- ^{13}C coupling will not usually exist. However the ^{13}C - ^{1}H coupling have observed in CMR spectrum.
- As a result of coupling makes the ^{13}C spectrum extremely complex , consequently there is an overlap of multiplets.

- These ^{13}C - ^{1}H coupling can be eliminated by adopting following techniques.

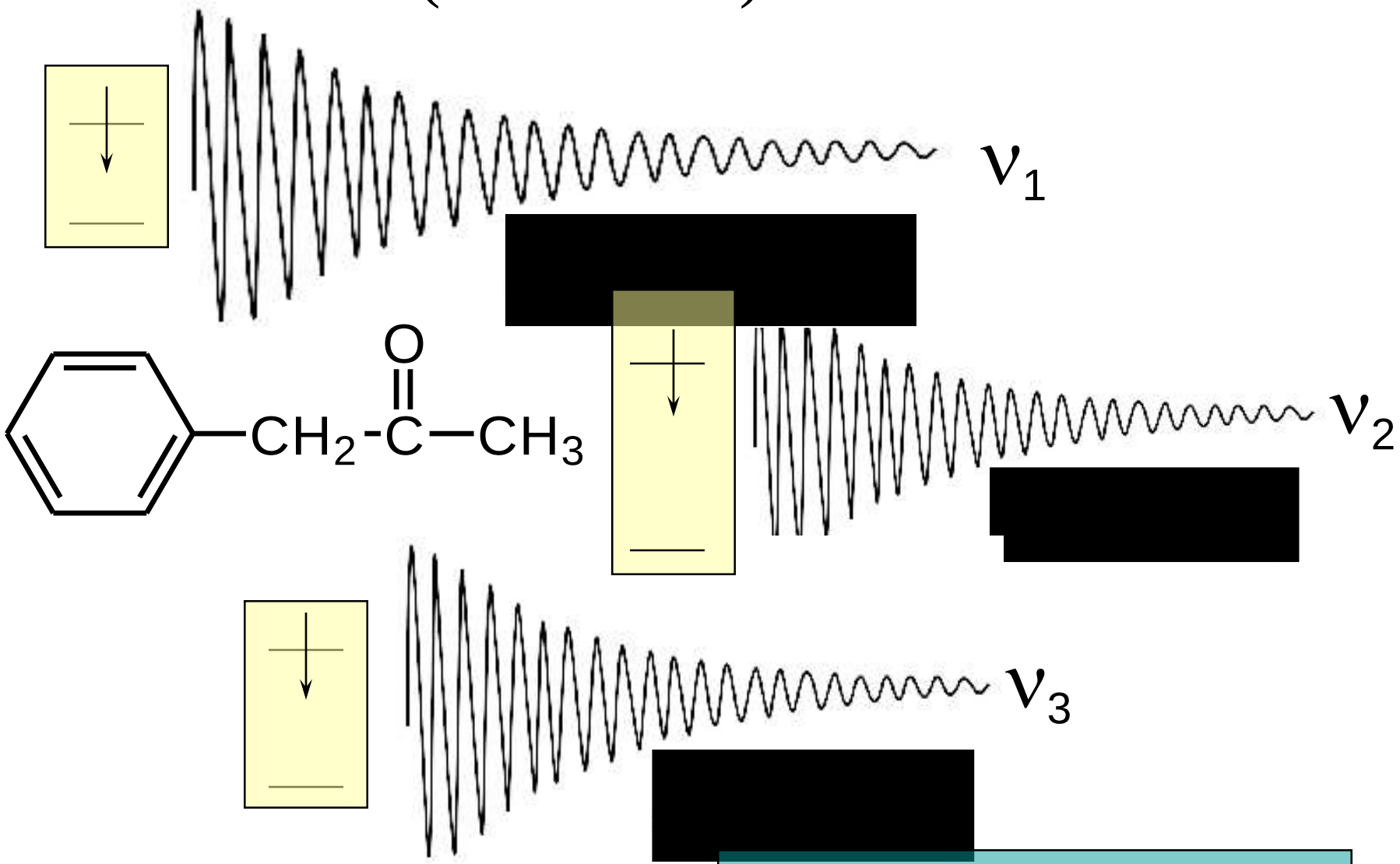
a) FT technique

b) Decoupling technique

c) Nuclear overhauser phenomenon for enrichment of the carbon signal.

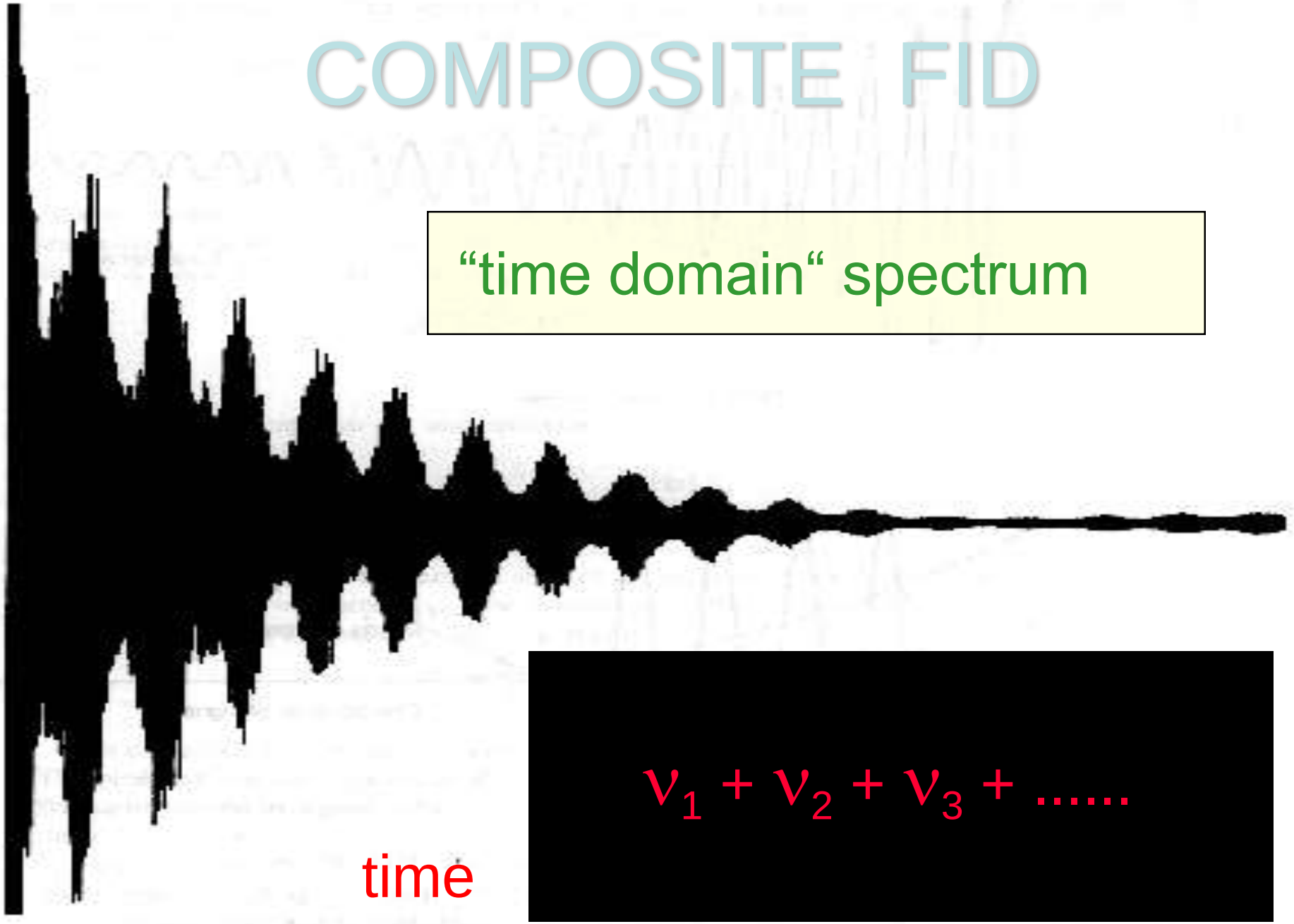
FREE INDUCTION DECAY

(relaxation)



ν_1, ν_2, ν_3 have different half lifes

COMPOSITE FID



"time domain" spectrum

time

$\nu_1 + \nu_2 + \nu_3 + ...$

FOURIER TRANSFORM

A mathematical technique that resolves a complex FID signal into the individual frequencies that add together to make it. (Details not given here.)

TIME DOMAIN → converted to → FREQUENCYDOMAIN

DOMAINS ARE MATHEMATICAL TERMS

FID

NMR SPECTRUM

COMPLEX SIGNAL → FT-NMR computer / Fourier Transform → $\nu_1 + \nu_2 + \nu_3 + ...$

a mixture of frequencies caying (with time)

individual frequencies

converted to a spectrum

DECOUPLING

PROTON OR NOISE DECOUPLING

COHERENT OR BROAD BAND DECOUPLING

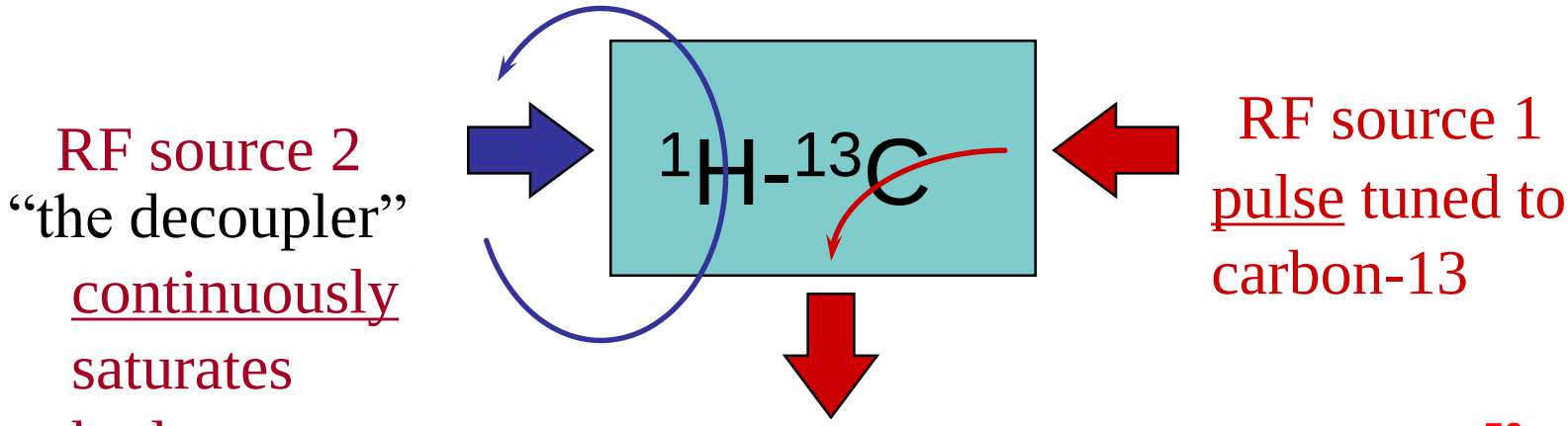
OFF-RESONANCE DECOUPLING

DECOUPLING THE PROTON SPINS

PROTON-DECOUPLED SPECTRA

A common method used in determining a carbon-13 NMR spectrum is to irradiate all of the hydrogen nuclei in the molecule at the same time the carbon resonances are being measured.

This requires a second radiofrequency (RF) source (the decoupler) tuned to the frequency of the hydrogen nuclei, while the primary RF source is tuned to the ^{13}C frequency.



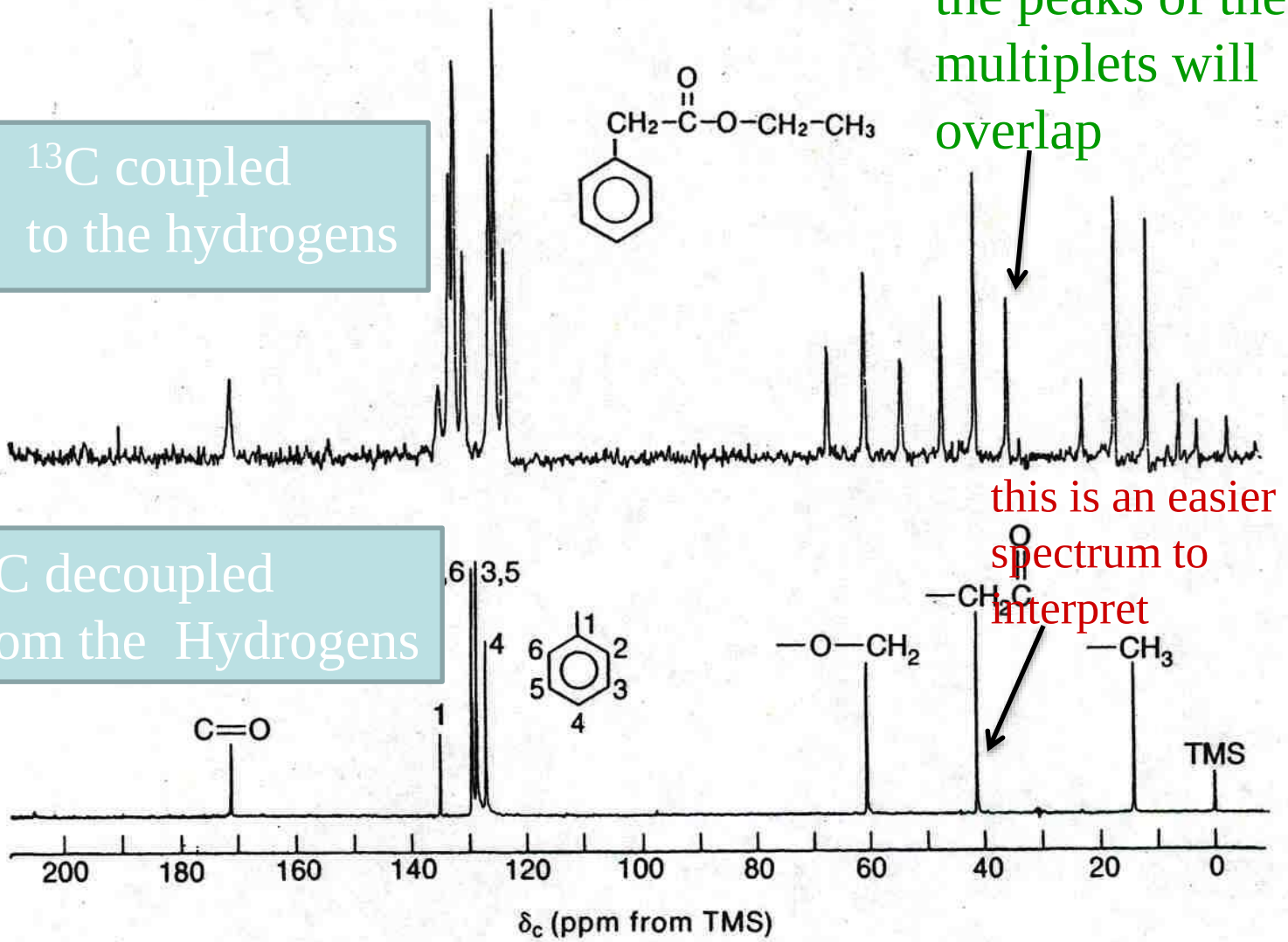
ETHYL PHENYLACETATE

in some cases the peaks of the multiplets will overlap

^{13}C coupled to the hydrogens

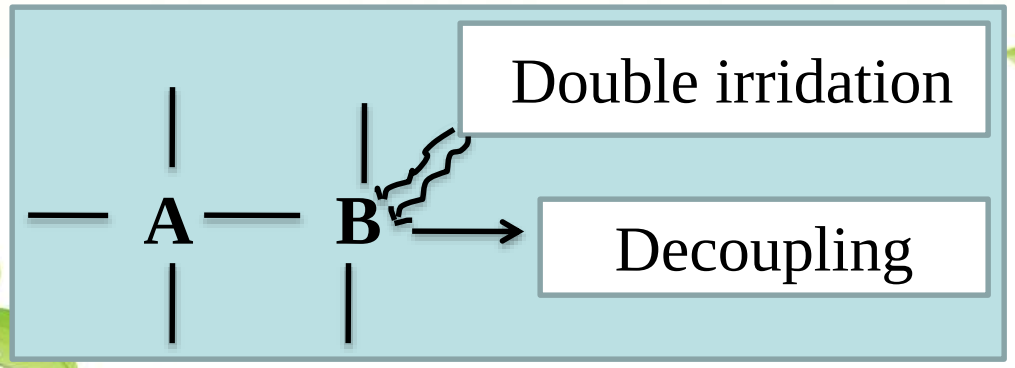
^{13}C decoupled from the Hydrogens

this is an easier spectrum to interpret



2) Broadband decoupling:

- In this technique, all the proton resonance can be reduced and to get sharp CMR spectral peaks, each directly reflecting a ^{13}C chemical shift.
- The NMR spectrum of nucleus A is split by nucleus B, because A can see B in different magnetic orientation.



3. Off resonance decoupling:

- 1000-2000 Hz above the spectral region.
- Partially decoupled spectrum are obtained
- Primary carbon nuclei- quartet
- Seconadry carbon nuclei- triplet
- Tertiary carbon nuclei- doublet
- Qurternary carbon nuclei- single line

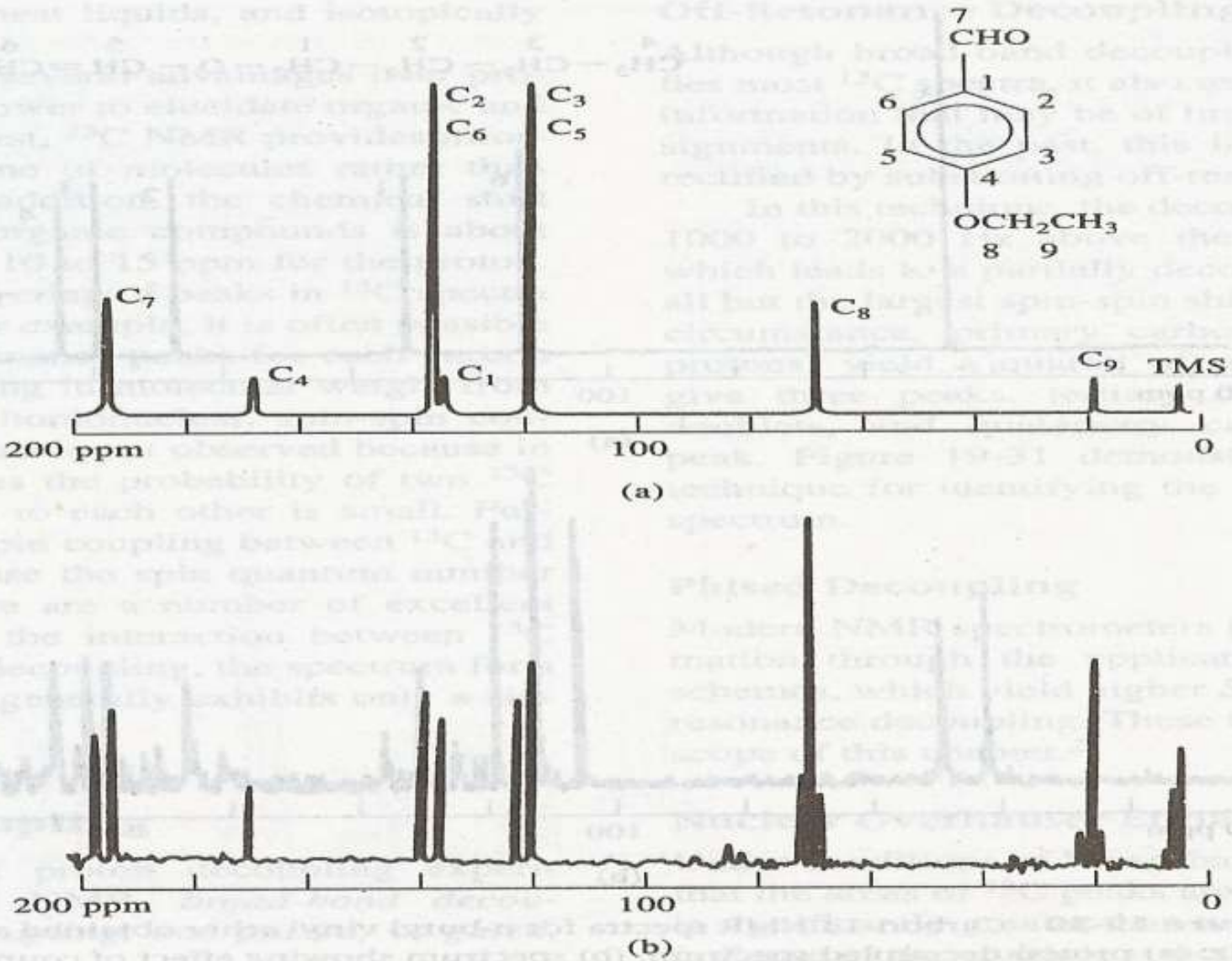


Figure 19-31 Comparison of (a) broad-band and (b) off-resonance decoupling in ^{13}C spectra of *p*-ethoxybenzaldehyde. *(From R. J. Abraham, J. Fisher, and P. Loftus, Introduction to NMR Spectroscopy, p. 106. New York: Wiley, 1988. With permission.)*

Applications:

- Identification of structural isomers
- Detection of hydrogen bonding
- Detection of aromaticity
- Distinction between Cis-trans isomers and conformers
- Detection of electronegative atoms or group
- Detection of some double bond character due to resonance
- Industrial applications in solids
- Metabolic studies

- Metabolic studies on human

1. Brain function
2. Glucose metabolism in liver
3. Glucose metabolism in muscle
4. Determination of degree of unsaturation of fatty acids in adipose tissue
5. Characteristic of body fluids and isolated tissues
6. In diseased state


conclusion

- Structure elucidation is most common application of ^{13}C spectroscopy PMR & CMR are often employed as complementary techniques.
- Although the order of accuracy achieved by ^{13}C NMR was a low relative to an high performance liquid chromatography technique, it was concluded that quantitative analysis by ^{13}C NMR had significant potential in pharmaceutical analysis.
- It is also used in the investigation of bio synthetic path ways, dynamic properties of molecules detection and identification of labeled sites may be accomplished by CMR directly or by examination of ^{13}C ^{1}H satellite signals.

  Example cephalosporin-C.

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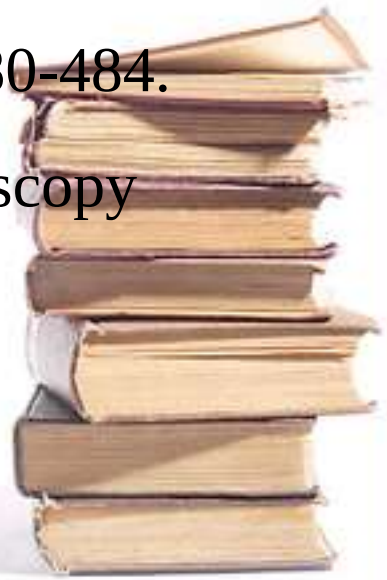

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