

Method of Nuclear Magnetic Resonance Spectroscopy for application in bio-medical research

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25 March 2016

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Presentation outline

- Introduction
- Fundamental principles and theory
- Instrumentation
- Solvents in NMR spectroscopy
- Chemical shift
- Interpretation of Proton chemical shifts
- Applications NMR spectra

Introduction

- Nuclear magnetic resonance, or NMR is a phenomenon which occurs when the nuclei of certain atoms are immersed in a static magnetic field and exposed to a second oscillating magnetic field. Some nuclei experience this phenomenon, and others do not, dependent upon whether they possess a property called spin.
- The first nuclear magnetic resonance (NMR) was detected early in 1938 in a molecular beam, and the first studies of NMR in bulk materials were carried out about 8 years later.
- In 1952 F. Bloch and E. Purcel were awarded by the Nobel Prize.

The Nobel Prize in Physics 1952 was awarded jointly to Felix Bloch and Edward Mills Purcell *"for their development of new methods for nuclear magnetic precision measurements and discoveries in connection therewith"*

The screenshot shows the Nobelprize.org website. The header includes the logo, navigation links (Home, Nobel Prizes and Laureates, Nomination, Ceremonies, Alfred Nobel, Educational, Events), and utility links (Video, Podcast, About Us, Search). The main content area is titled "The Nobel Prize in Physics 1952" and lists the laureates: Felix Bloch and E. M. Purcell. Below the title are two portrait photos of the laureates, each with a "Prize share: 1/2" caption. A sidebar on the left provides navigation for "Nobel Prizes and Laureates" and "About the Nobel Prize in Physics 1952". On the right, there are promotional banners for "2016 NOBEL PRIZE ANNOUNCEMENTS", "Nobel Women", and "2015 Nobel Laureates".

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All Nobel Prizes in Physics
All Nobel Prizes in 1952

The Nobel Prize in Physics 1952
Felix Bloch, E. M. Purcell

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The Nobel Prize in Physics 1952


Felix Bloch
Prize share: 1/2


Edward Mills Purcell
Prize share: 1/2

2016 NOBEL PRIZE ANNOUNCEMENTS

Nobel Women

2015 Nobel Laureates
© The Nobel Foundation. Photo: Lovisa Engblom. ALFR. NOBEL

Application of NMR in medicine

MRI is specialist application of multi dimensional Fourier transformation NMR



- ✓ Anatomical imaging.
- ✓ Measuring physiological functions
- ✓ Flow measurements and angiography.
- ✓ Tissue perfusion studies.
- ✓ Tumors

Nuclear Magnetic Resonance (NMR) is a spectroscopy technique

Nuclear Magnetic Resonance (NMR) is a spectroscopy technique which is based on the absorption of electromagnetic radiation in the **radio frequency region 4 to 900 MHz** by nuclei of the atoms.

Proton Nuclear magnetic resonance spectroscopy is one of the most powerful tools for elucidating the number of hydrogen or proton in the compound.

It is used to study a wide variety of nuclei:

- ^1H ^{15}N
- ^{19}F ^{19}F
- ^{13}C ^{31}P



NMR spectroscops

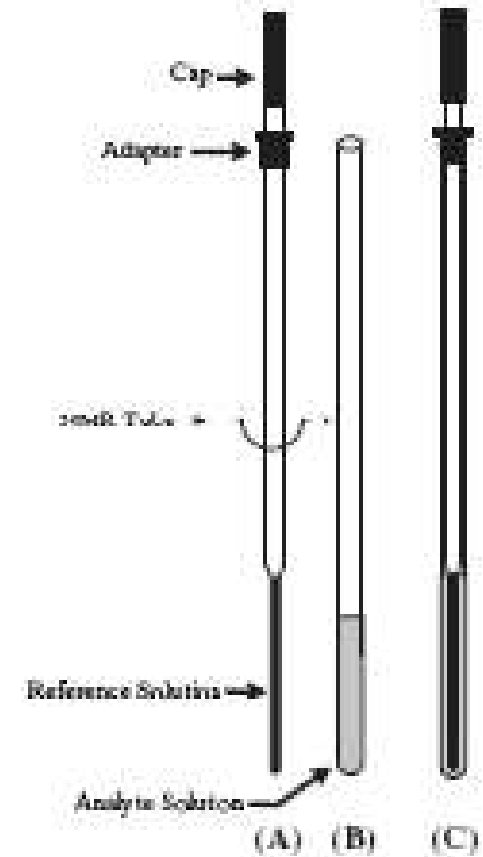


- Varian Inova 750 (CACTUS)
- HCN (1H /13C /15N) –
- http://www.usc.es/g/l/investigacion/riaidt/rm/rmn/modules/equipamento/equipamento_0008.html

- Bruker DRX-500 (CACTUS)
- 1H/13C/15N
http://www.usc.es/g/l/investigacion/riaidt/rm/rmn/modules/equipamento/equipamento_0007.html



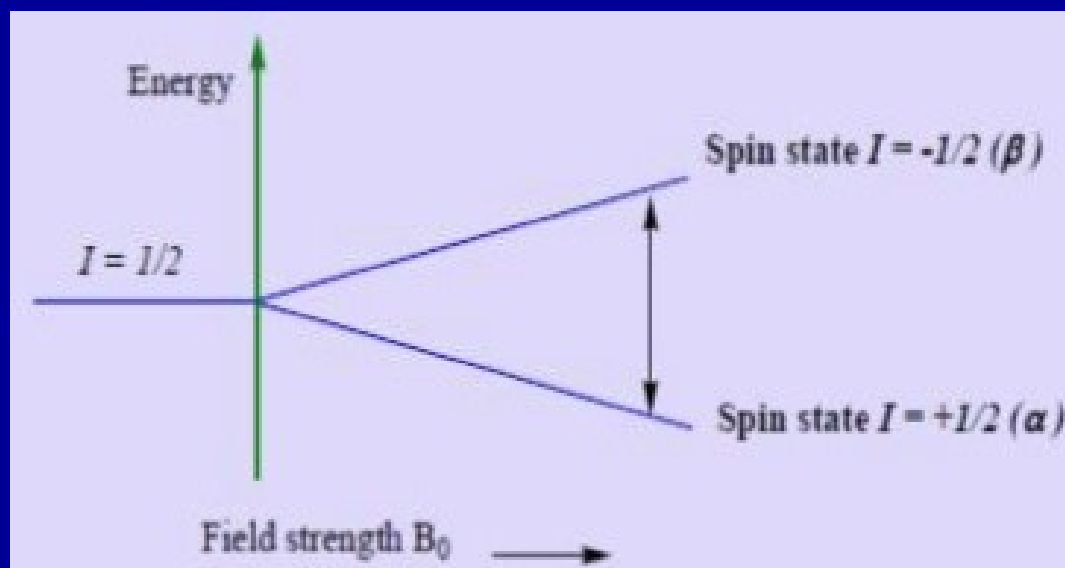
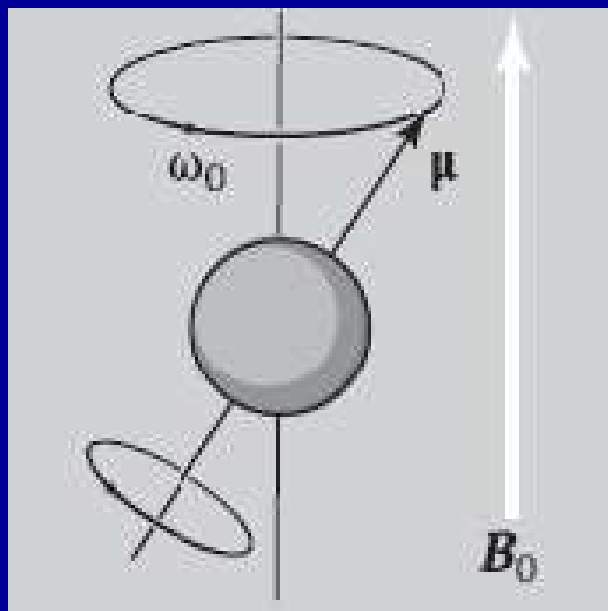
How to prepare sample for analysis



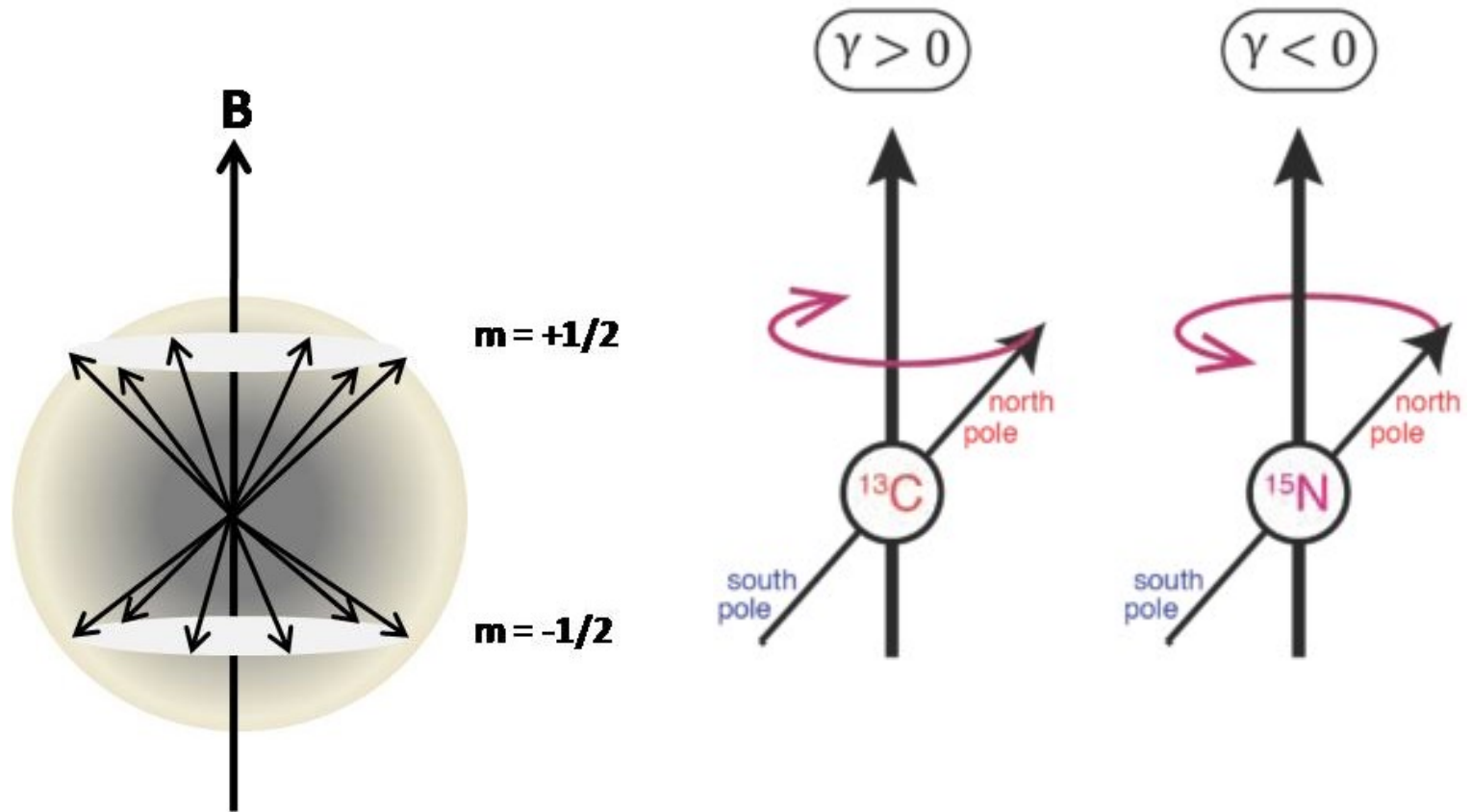
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- Refilling_Nitrogen_in_BRUKER_Magnets_V10_720p

- Nucleus with spin $I \neq 0$ in magnetic field (B_0) is rotating in the direction of axis. In case of spin $I = 1/2$, are aligned clockwise or counterclockwise to the B_0 .



Spin



Theory of NMR:-

Spin quantum number (I) is related to the atomic and mass number of the nucleus.

I	Atomic Mass	Atomic Number	Examples	
Half-integer	Odd	Odd	^1H (1/2)	NMR active
Half-integer	Odd	Even	^{13}C (1/2)	
Integer	Even	Odd	^2H (1)	
Zero	Even	Even	^{12}C (0)	Not NMR active

Elements with either **odd mass** or **odd atomic number** have the property of nuclear “spin”.

Nuclei with Spin

- The shell model for the nucleus tells us that nucleons, just like electrons, fill orbitals. When the number of protons or neutrons equals 2, 8, 20, 28, 50, 82, and 126, orbitals are filled. Because nucleons have spin, just like electrons do, their spin can pair up when the orbitals are being filled and cancel out. Almost every element in the periodic table has an isotope with a non zero nuclear spin.
- NMR can only be performed on isotopes whose natural abundance is high enough to be detected.
- Some of the nuclei routinely used in NMR are listed below.

Nuclei with Spin

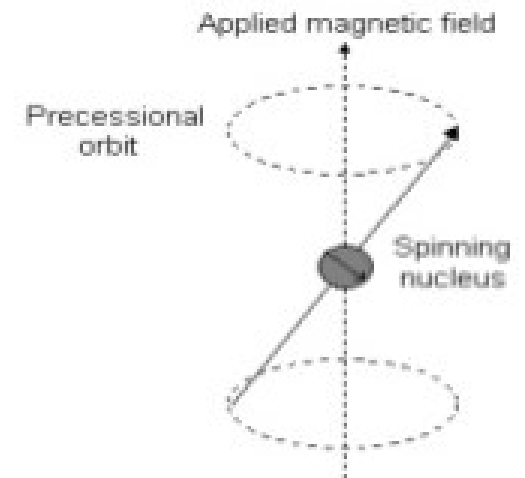
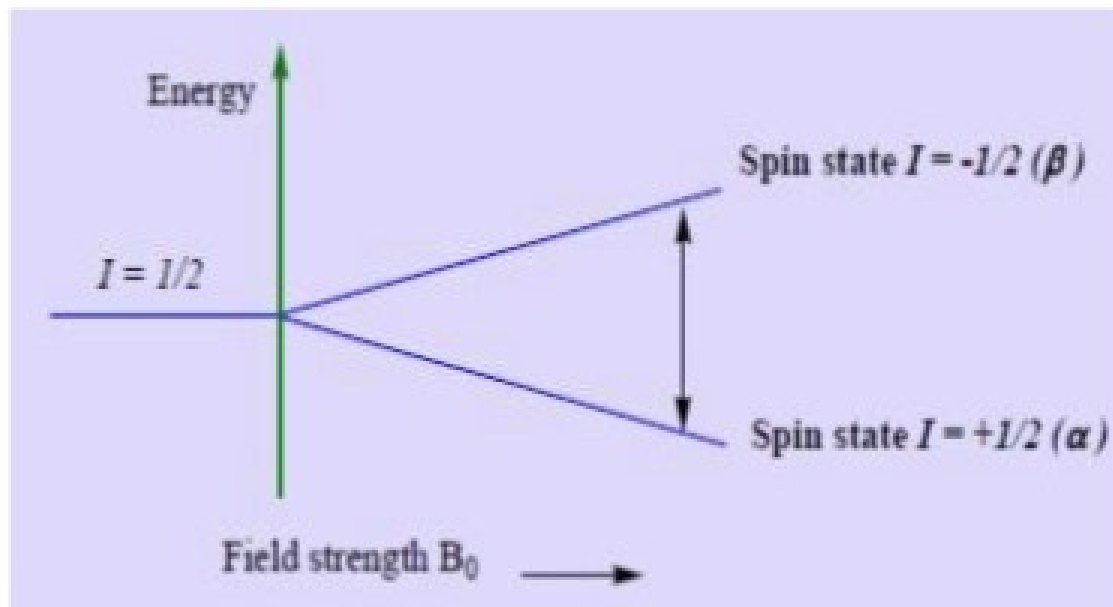
Nuclei	Unpaired Protons	Unpaired Neutrons	Net Spin	γ (MHz/T)
^1H	1	0	1/2	42.58
^2H	1	1	1	6.54
^{31}P	1	0	1/2	17.25
^{23}Na	1	2	3/2	11.27
^{14}N	1	1	1	3.08
^{13}C	0	1	1/2	10.71
^{19}F	1	0	1/2	40.08

Spin and other characteristics of main nuclei

Isotope	Occurrence in nature (%)	spin number I	Magnetic moment $\mu^{[5]}$	Electric quadrupole moment ($e \times 10^{-24} \text{ cm}^2$)	Operating frequency at 7 T (MHz)	Relative sensitivity
^1H	99.984	1/2	2.79628		300.13	1
^2H	0.016	1	0.85739	2.8×10^{-3}	46.07	0.0964
^{10}B	18.8	3	1.8005	7.4×10^{-2}	32.25	0.0199
^{11}B	81.2	3/2	2.6880	2.6×10^{-2}	96.29	0.165
^{12}C	98.9	0				
^{13}C	1.1	1/2	0.70220		75.47	0.0159
^{14}N	99.64	1	0.40358	7.1×10^{-2}	21.68	0.00101
^{15}N	0.37	1/2	-0.28304		30.41	0.00104
^{16}O	99.76	0				
^{17}O	0.0317	5/2	-1.8930	-4.0×10^{-3}	40.69	0.0291
^{19}F	100	1/2	2.6273		282.40	0.834
^{28}Si	92.28	0				
^{29}Si	4.70	1/2	-0.5548		59.63	0.0785
^{31}P	100	1/2	1.1205		121.49	0.0664
^{35}Cl	75.4	3/2	0.92091	-7.9×10^{-2}	29.41	0.0047
^{37}Cl	24.6	3/2	0.68330	-6.2×10^{-2}	24.48	0.0027

If an external magnetic field is applied, the number of possible orientations calculated by $(2I+1)$.

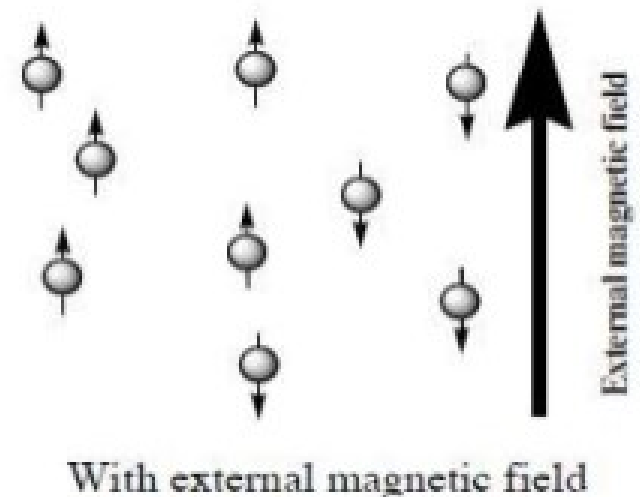
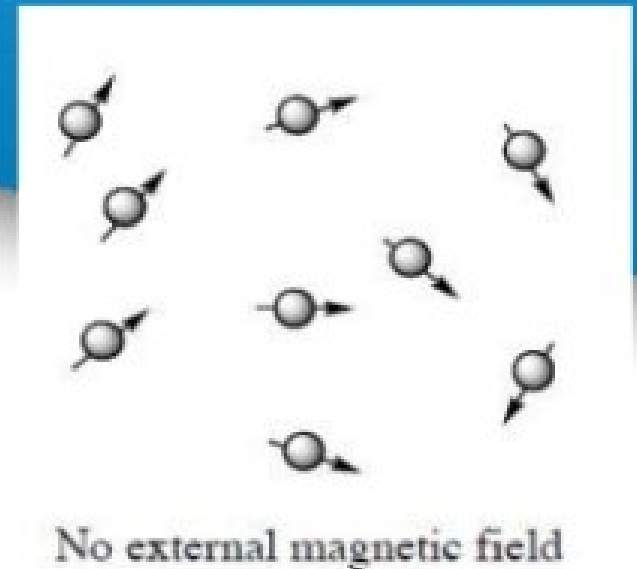
Hydrogen has spin quantum number $I=1/2$ and possible orientation is $(2*1/2+1=2)$ two $+1/2$ and $-1/2$.



Principles of NMR

The theory behind NMR comes from the spin of a nucleus and it generates a magnetic field. Without an external applied magnetic field, the nuclear spins are random in directions.

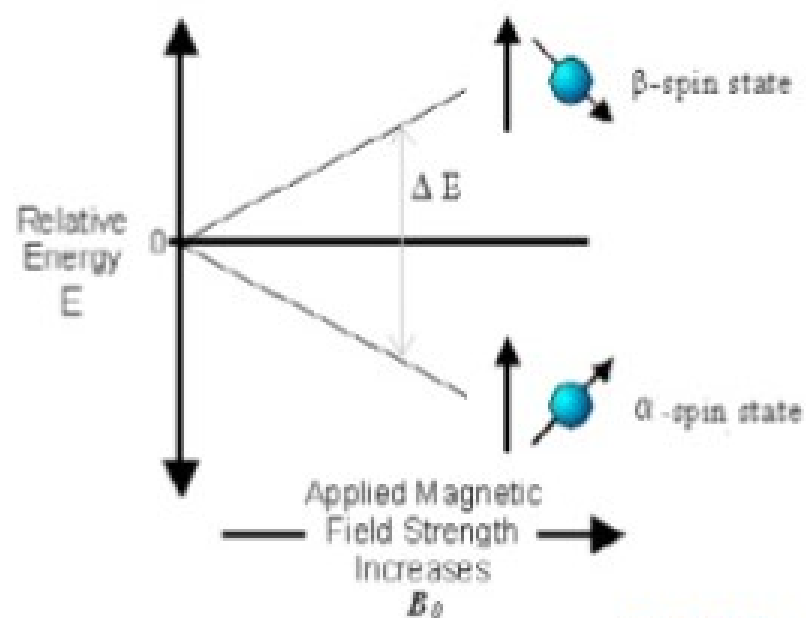
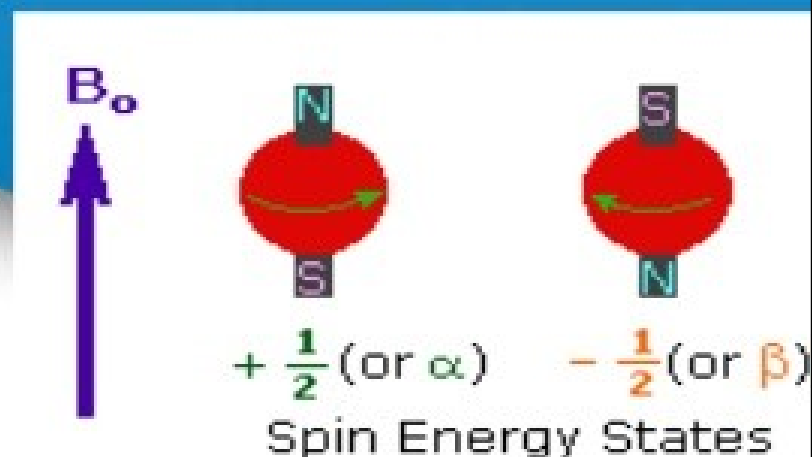
But when an external magnetic field (B_0), is present the nuclei align themselves either with or against the field of the external magnet.



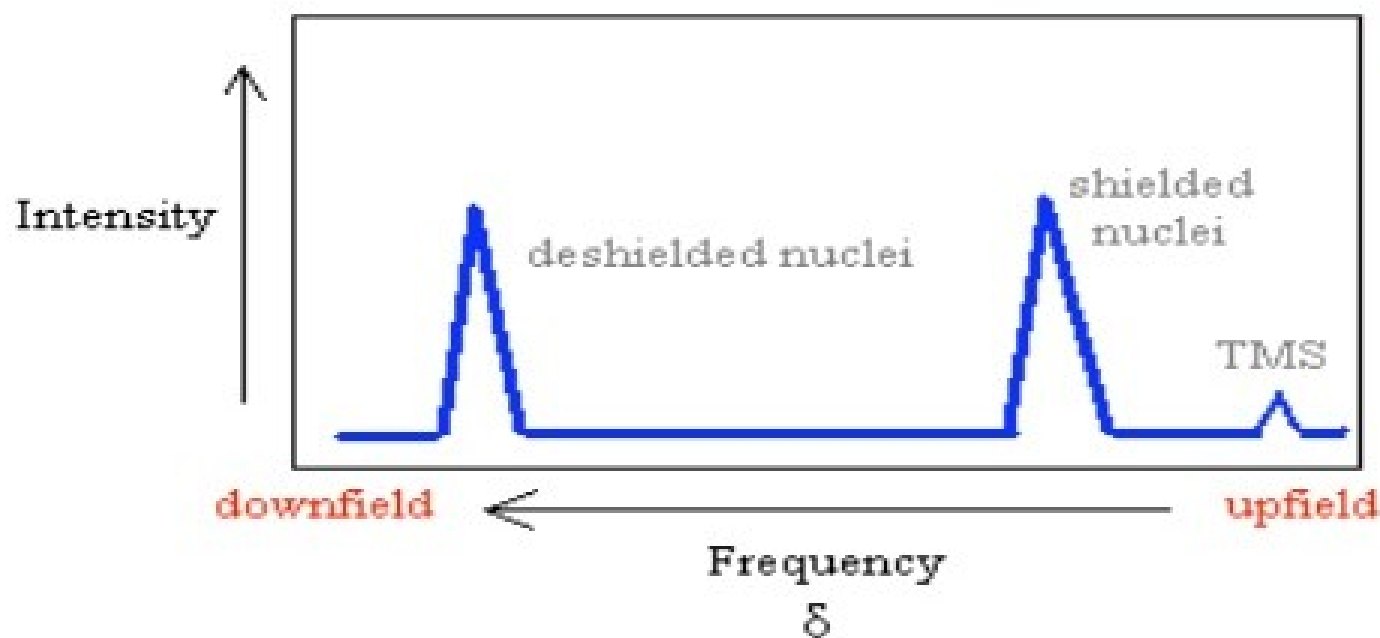
If an external magnetic field is applied, an energy transfer (ΔE) is possible between ground state to excited state.

when the spin returns to its ground state level, the absorbed radiofrequency energy is emitted at the same frequency level.

The emitted radiofrequency signal that give the NMR spectrum of the concerned nucleus.



NMR spectrum

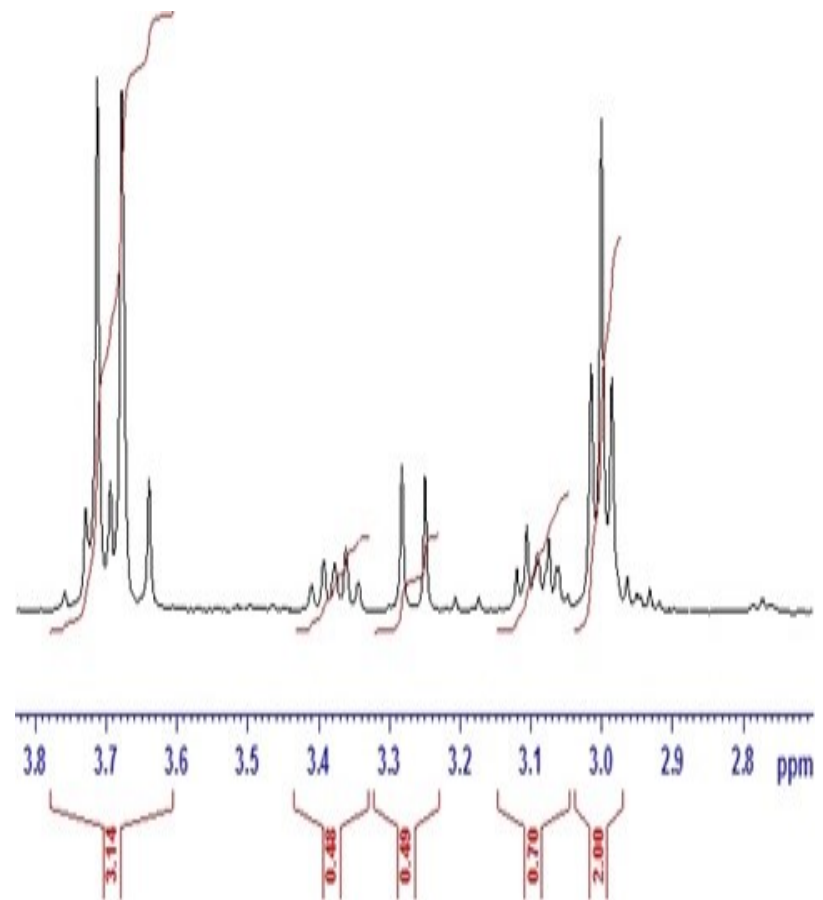
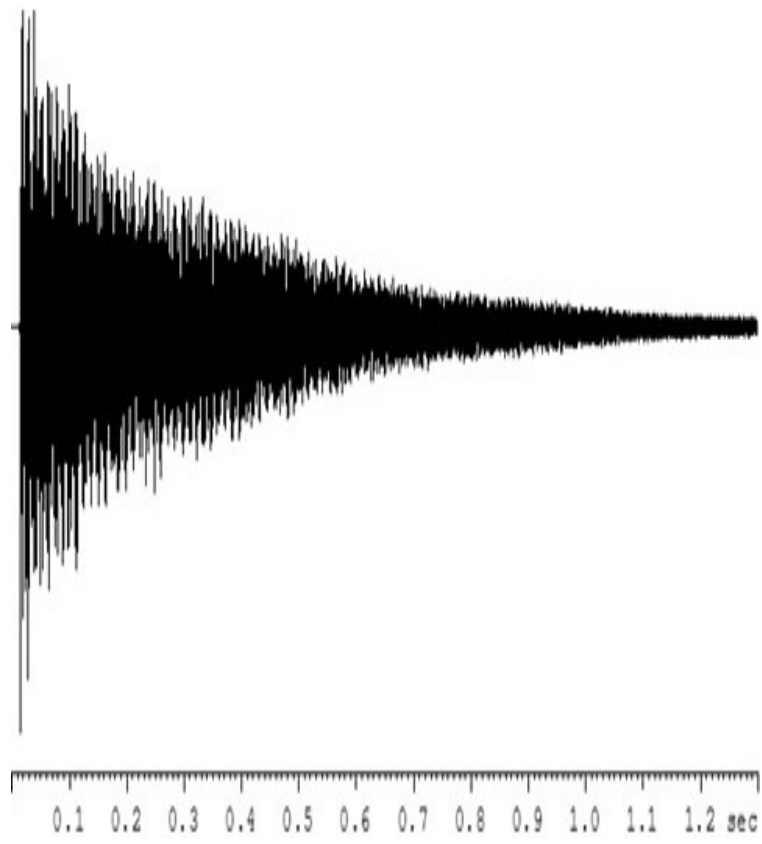


The NMR spectrum is a plot of intensity of NMR signals VS magnetic field (frequency) in reference to TMS

How to convert signal to spectra

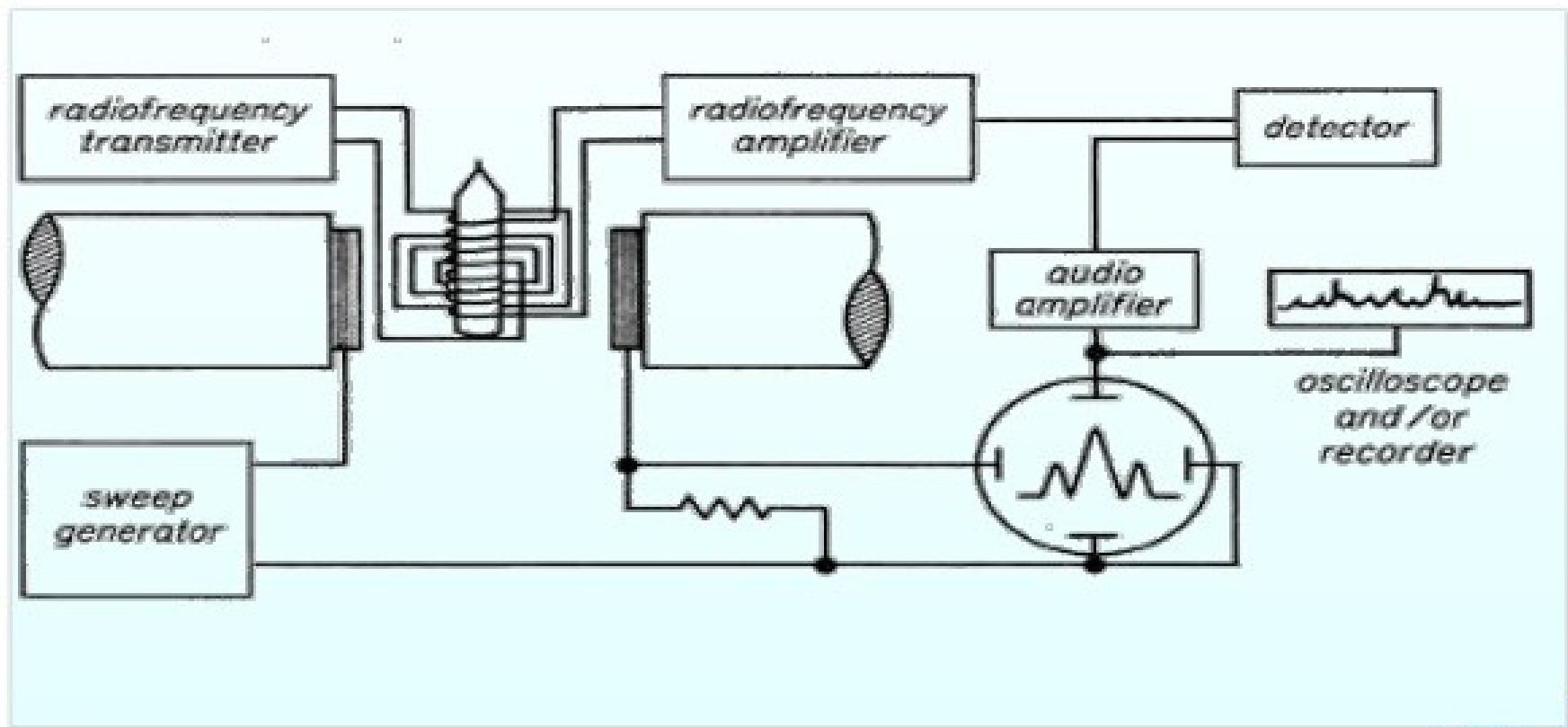
- As a result of impulse spectroscopy it is possible to obtain not only the usual spectrum with visible peaks of resonance, but the image of damped resonant oscillations, in which the signals are mixed from all the resonant nuclei. It is called the free induction decay (FID). In order to convert this spectrum, it is necessary to use mathematical methods, the so-called Fourier transformation, by which any function can be represented as the sum of the set of harmonic oscillations.
- In other words, "the required acquisition time depends on the smallest line width in the spectrum, and truncation of the NMR signal in the time domain [free induction decay (FID)] must be avoided.
- If truncation occurs, signal forms with (substantial) "wiggles" appear in the spectra, and, in combination with FID baseline correction modes, wrong intensities will result" (Diehl et al., 16).

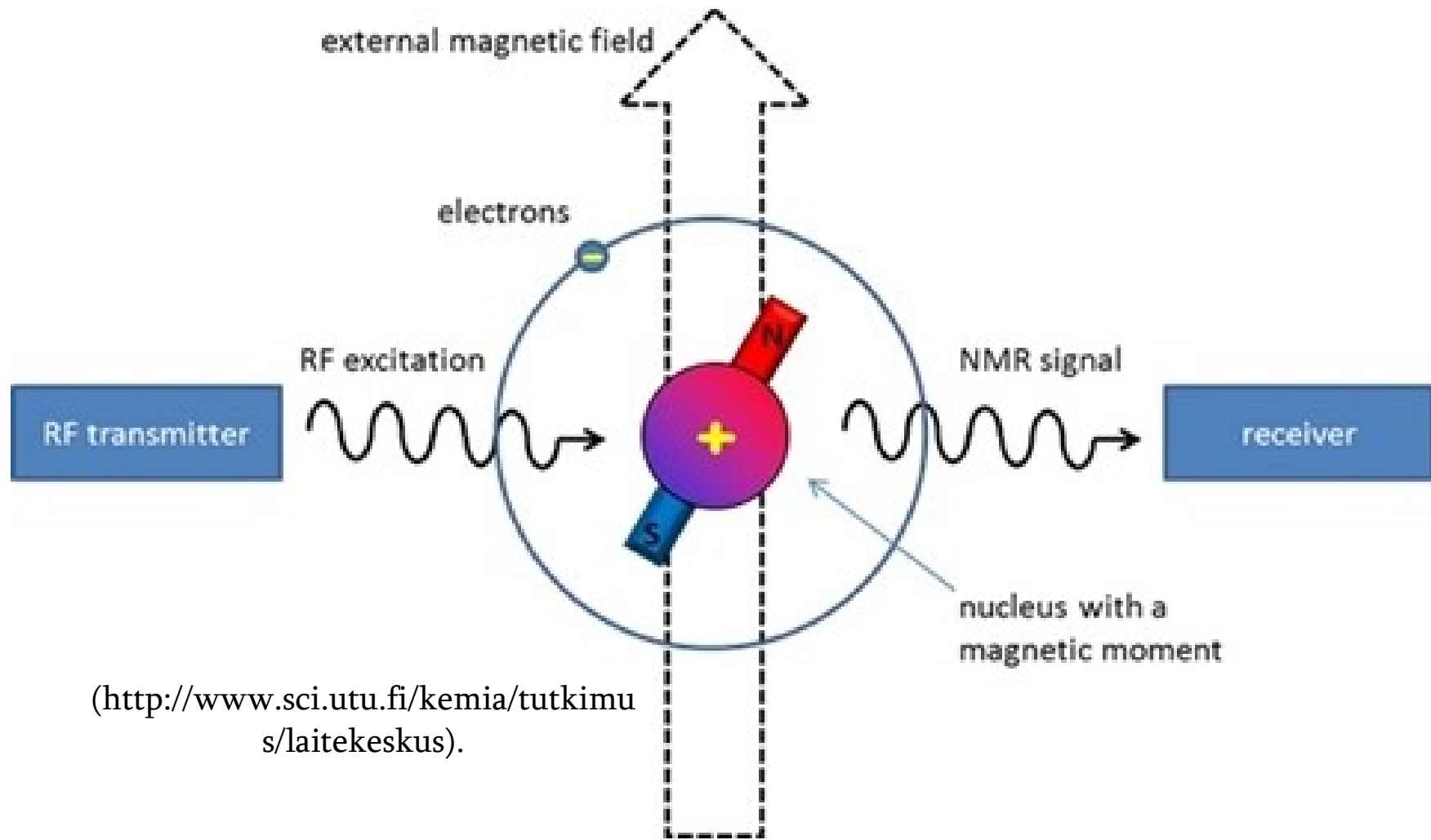
FID



NMR instrumentation

1. Sample holder
2. Permanent magnet
3. Magnetic coils
4. Sweep generator
5. Radio frequency transmitter
6. Radio frequency receiver
7. Read out systems





(<http://www.sci.utu.fi/kemia/tutkimus/laitekeskus>).

- 1. Sample holder** :- Glass tube with 8.5 cm long, 0.3 cm in diameter
- 2. Permanent magnet** :- It provides homogeneous magnetic field at 60-100 MHz
- 3. Magnetic coils** :- These coils induce magnetic field when current flows through them.
- 4. Sweep generator** :- To produce the equal amount of magnetic field pass through the sample

5. Radio frequency transmitter :- A radio transmitter coil that produces a short powerful pulse of radio waves

6. Radiofrequency Receiver :- A radio receiver coil that detects **Receiver** radio frequencies emitted as nuclei relax to a lower energy level

7. Readout system :- A computer that analyses and record the data

Solvents used in NMR

The following solvents are normally used in which hydrogen replaced by deuterium.

CCl_4 - carbon tetrachloride

CS_2 - carbon disulfide

CDCl_3 - Deuteriochloroform

C_6D_6 - Hexa deuteriobenzene

D_2O - Deuterium oxide

Chemical shift

A **chemical shift** is defined as the difference in parts per million (ppm) between the resonance frequency of the observed proton and tetramethylsilane (TMS) hydrogens.

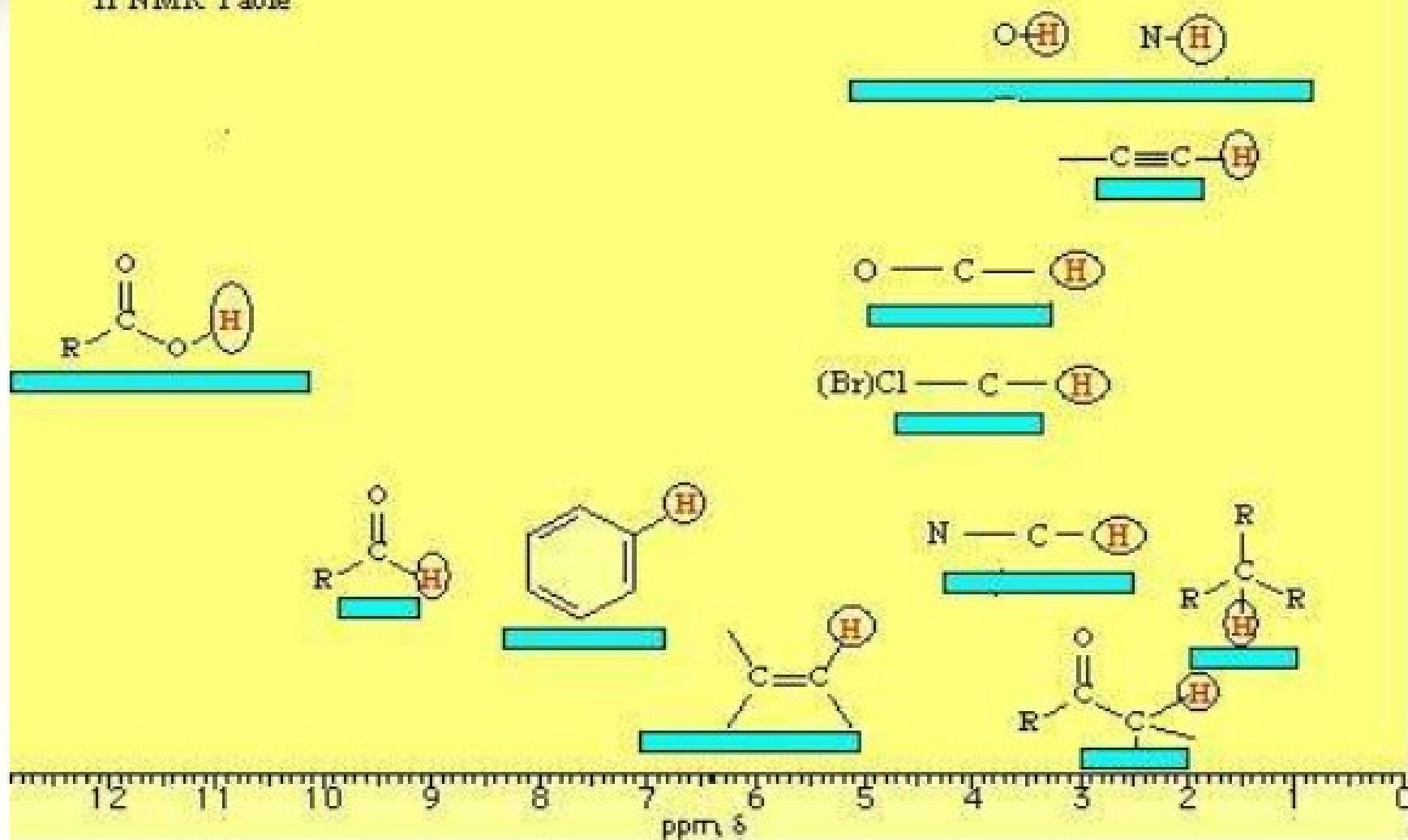
TMS is the most common reference compound in NMR, it is set at $\delta=0$ ppm

Proton NMR

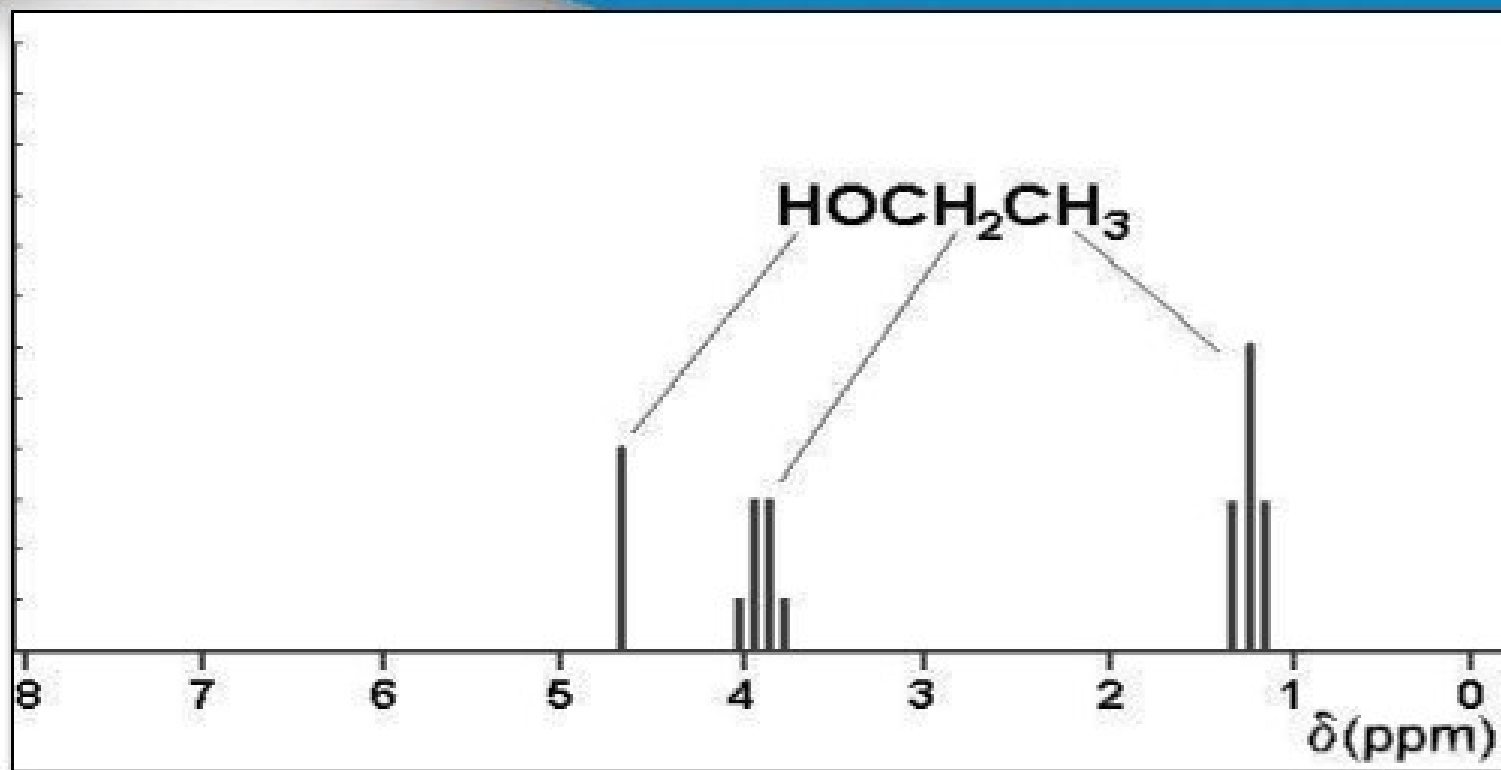
The most common form of NMR is based on the hydrogen-1 (^1H), nucleus or proton.

It can give information about the structure of any molecule containing hydrogen atoms.

H NMR Table



Proton NMR spectra of Ethanol:-



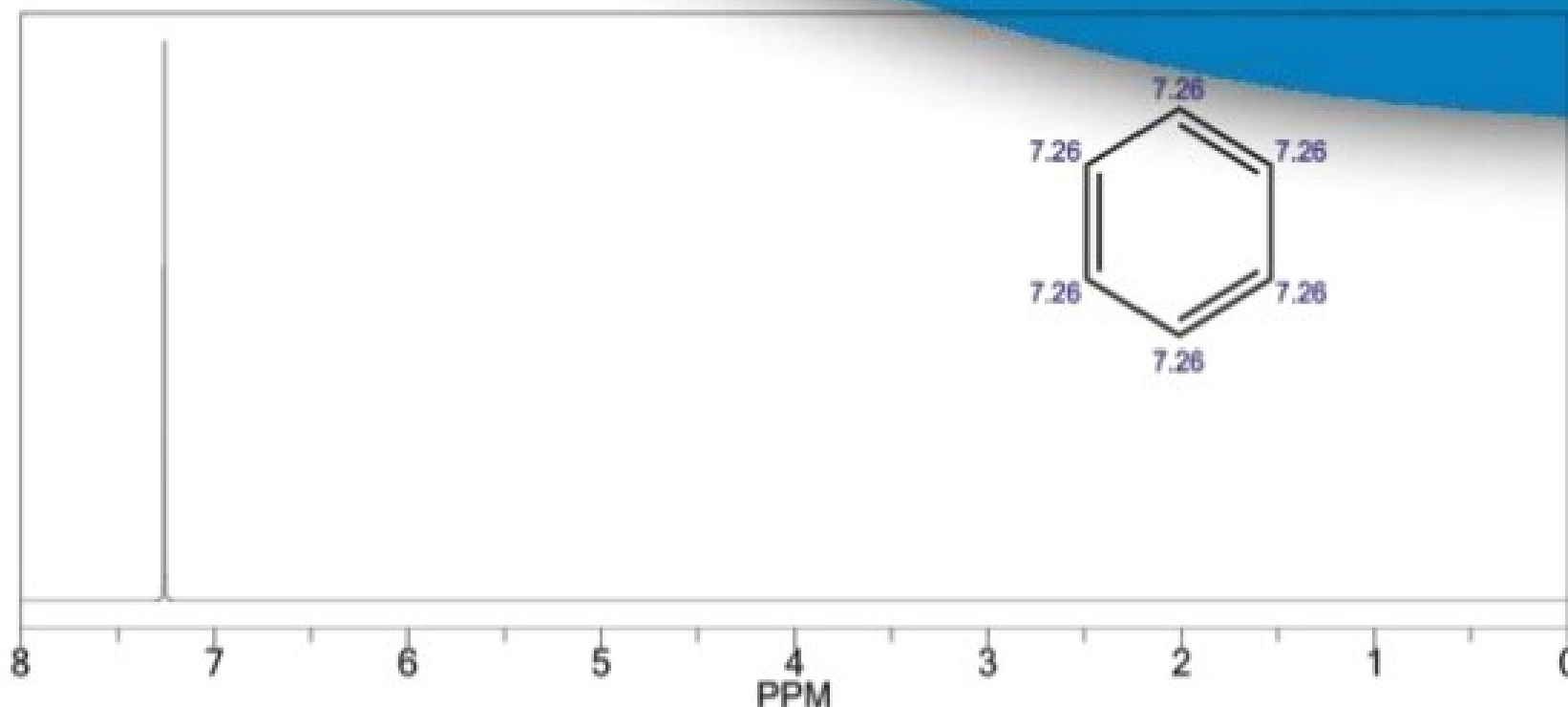
^1H spectrum of Ethanol:-

3 types of proton

$\text{CH}_3, \text{CH}_2, \text{OH}$

^1H NMR spectra of Benzene

ChemNMR ^1H Estimation



- Aromatic Hydrogen shows peak in the chemical shift scale 6.5-8.0 ppm.
- From the above spectrum Benzene has same type of protons and it shows single peak at 7.26

Interpretation of ^1H NMR spectra:-

- | | |
|---|---|
| Number of signals | - Indicates how many "different kinds" of protons are present. |
| Position of signals | - Indicates something about (chemical shift) magnetic (electronic) environment of protons |
| Relative intensity of signals | - Proportional to number of protons present |
| Splitting of signals (spin spin coupling) | - Indicates the number of nearby nuclei usually protons |

GENERAL APPLICATIONS OF NMR SPECTROSCOPY

✓ NMR is used in biology to study the Biofluids, Cells, Perfused organs and biomacromolecules such as Nucleic acids(DNA, RNA), carbohydrates Proteins and peptides. And also Labeling studies in biochemistry.

✓ NMR is used in food science.

Contd...

- ✓ NMR is used in pharmaceutical science to study Pharmaceuticals and Drug metabolism.

- ✓ NMR is used in chemistry to ;
 - Determine the Enantiomeric purity.
 - Elucidate Chemical structure of organic and inorganic compounds.
 - Macromolecules –ligand interaction.

¹H-NMR SPECTROSCOPY

applications

¹H widely used for structure elucidation.

Inorganic solids- Inorganic compounds are investigated by solid state ¹H-NMR. eg $\text{CaSO}_4 \cdot \text{H}_2\text{O}$.

Organic solids- Solid-state ¹H NMR constitutes a powerful approach to investigate the hydrogen-bonding and ionization states of small organic compounds.

- Direct correlation with hydrogen-bonding lengths could be demonstrated, e.g. for amino acid carboxyl groups.

Polymers and rubbers- Examine hydrogen bonding and acidity.

Peptides and proteins

Clinical and scientific research

In vivo NMR studies-

- concerned with ^1H NMR spectroscopy of human brain.
- Many studies are concerned with altered levels of metabolites in various brain diseases.
- To determine the spatial distribution of any given metabolite detected spectroscopically IS (image selected *in vivo* spectroscopy).

Recommended readings :

- Organic spectroscopy by William Kemp
- Instrumental methods of chemical analysis by Chatwal
- Instrumental methods of analysis by Willard

NMR Laboratory Instrumentation



Computing



NMReady is similar to desktop computers in the age of mainframes

Thank you! Questions?

