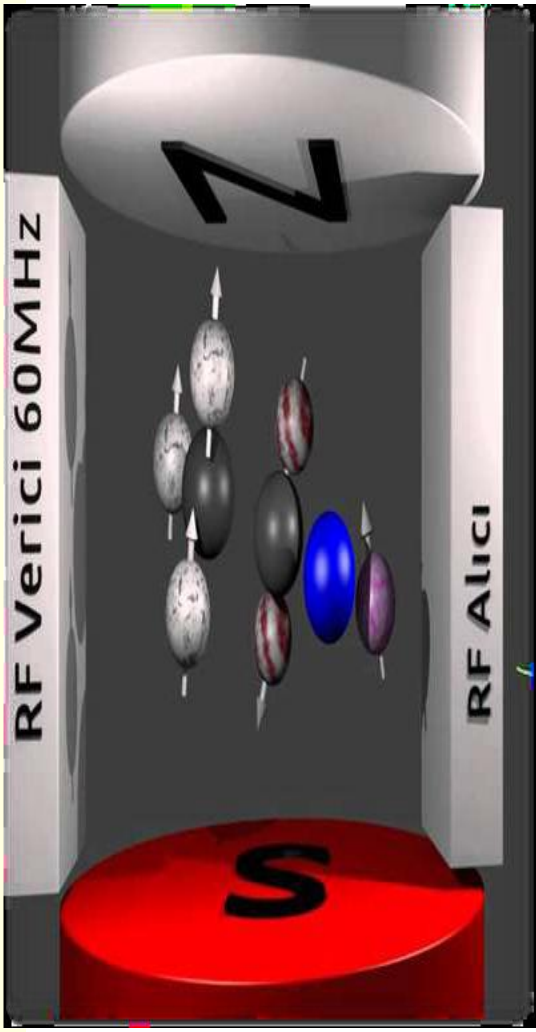
Introduction

- Nuclear magnetic resonance spectroscopy(NMR) is a powerful analytical technique used to characterize organic molecules by identifying carbon-hydrogen frameworks within molecules.
- It is a research technique that exploits the magnetic properties of certain atomic nuclei.
- It determines the physical and chemical properties of atoms or the molecules in which they are contained.



z

NMR History

- 1937 Rabi's prediction and observation of nuclear magnetic resonance
- 1945 First NMR of solution (Bloch et al for $H2O$) and solids (Purcell et al for parafin)!
- 1953 **Overhauser** NOE (nuclear Overhauser effect)
- 1966 Ernst, Anderson Fourier transform NMR
- 1975 Jeener, Ernst 2D NMR
- 1980 NMR protein structure by Wuthrich
- 1990 3D and 1H/15N/13C Triple resonance
- 1997 Ultra high field (~800 MHz) & TROSY(MW 100K)

Continuation of NMR History

2002 *Chemistry* Wüthrich (ETH)

"for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution"

2003 *Medicine* Lauterbur (University of Illinois in Urbana), Mansfield (University of Nottingham)

"for their discoveries concerning magnetic resonance imaging"

Types of NMR

- Two common types of NMR spectroscopy are used to characterize organic structure:
  - ^{1}H NMR:- Used to determine the type and number of H atoms in a molecule
  - ^{13}C NMR:- Used to determine the type of carbon atoms in the molecule

Source of NMR

- The source of energy in NMR is radio waves which have long wavelengths having more than 10^7nm, 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 ^1H and ^{13}C.

Theory of NMR

- In a magnetic field, there are now two energy states for a proton: a lower energy state with the nucleus aligned in the same direction as B_0, and a higher energy state in which the nucleus aligned against B_0.

- When an external energy source that matches the energy difference between these two states is applied, energy is absorbed, causing the nucleus to "spin flip" from one orientation to another.

- The energy difference between these two nuclear spin states corresponds to the low frequency RF region of the electromagnetic spectrum.

Theory of NMR(Contd…)

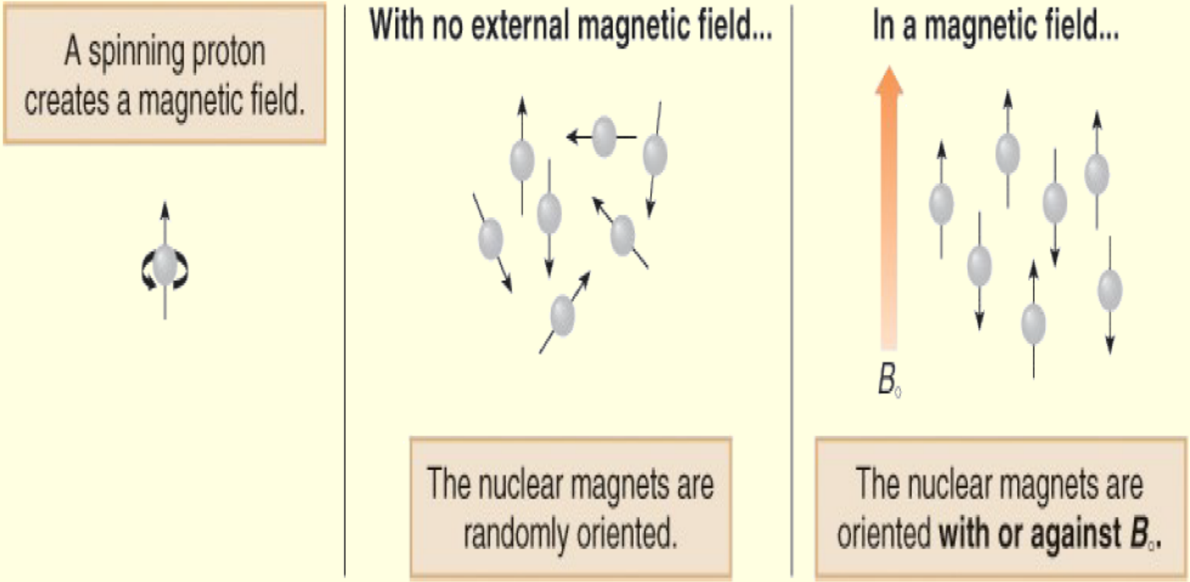
- When a charged particle such as a proton spins on its axis, it creates a magnetic field. Thus, the nucleus can be considered to be a tiny bar magnet.

- 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).

Effect of Magnetic field…



A spinning proton creates a magnetic field.

With no external magnetic field...

The nuclear magnets are randomly oriented.

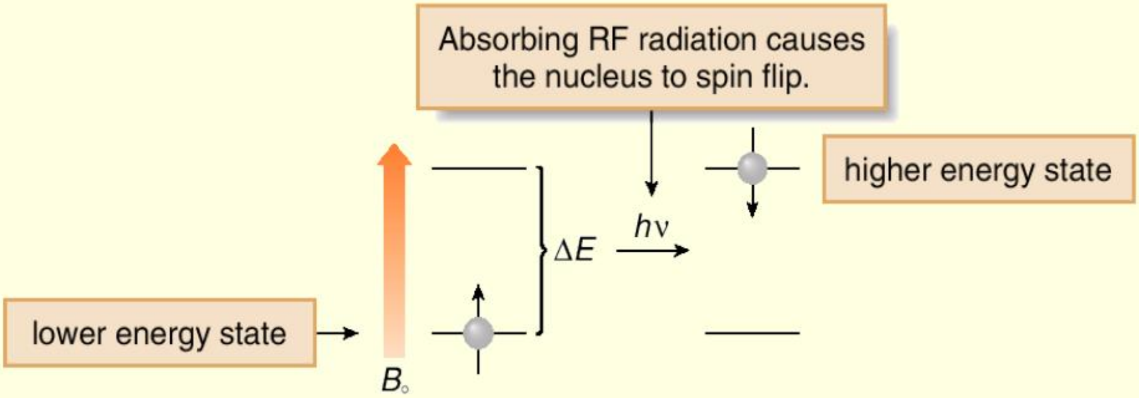
In a magnetic field...

The nuclear magnets are oriented **with or against B_0.**

Effect of Magnetic field(Contd..)

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

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



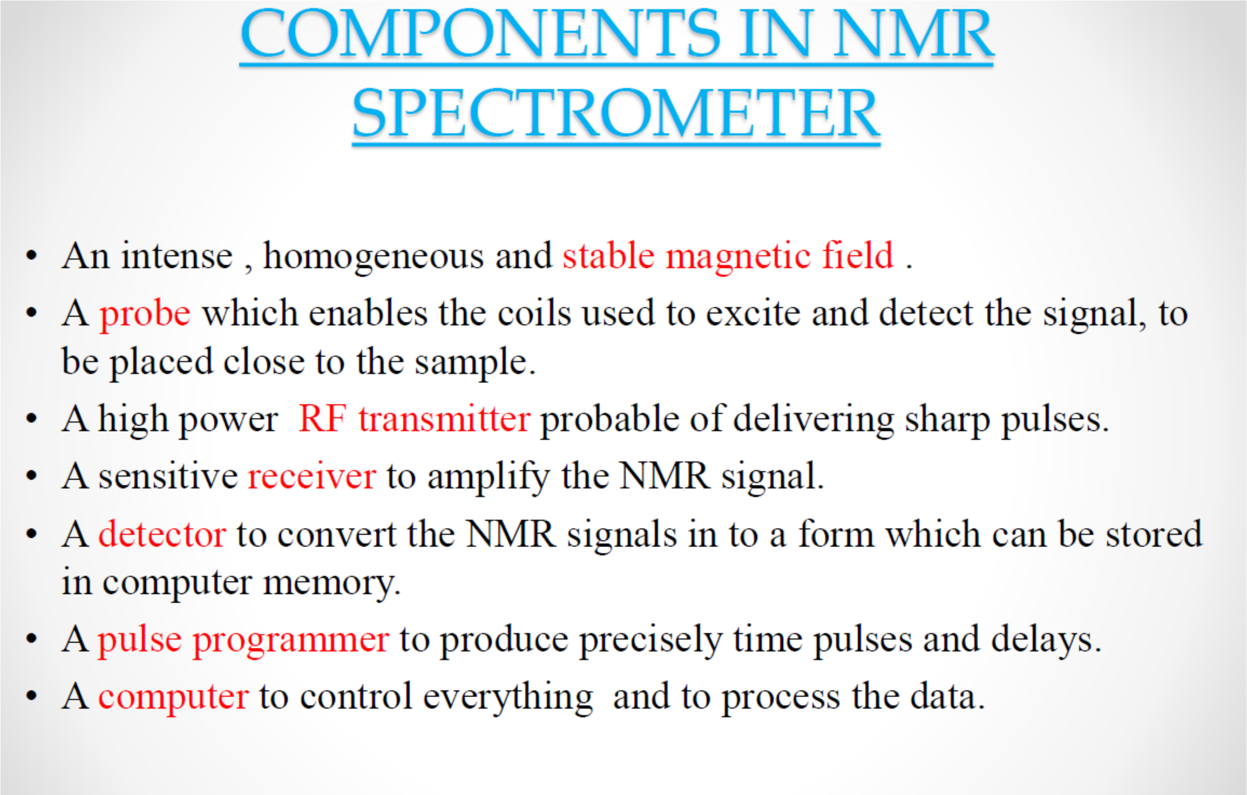
Absorbing RF radiation causes the nucleus to spin flip.

Effect of Magnetic field(Contd..)

- The frequency needed for resonance and the applied magnetic field strength are proportionally related:

$$\boxed{\nu \;\alpha\; B_O}$$

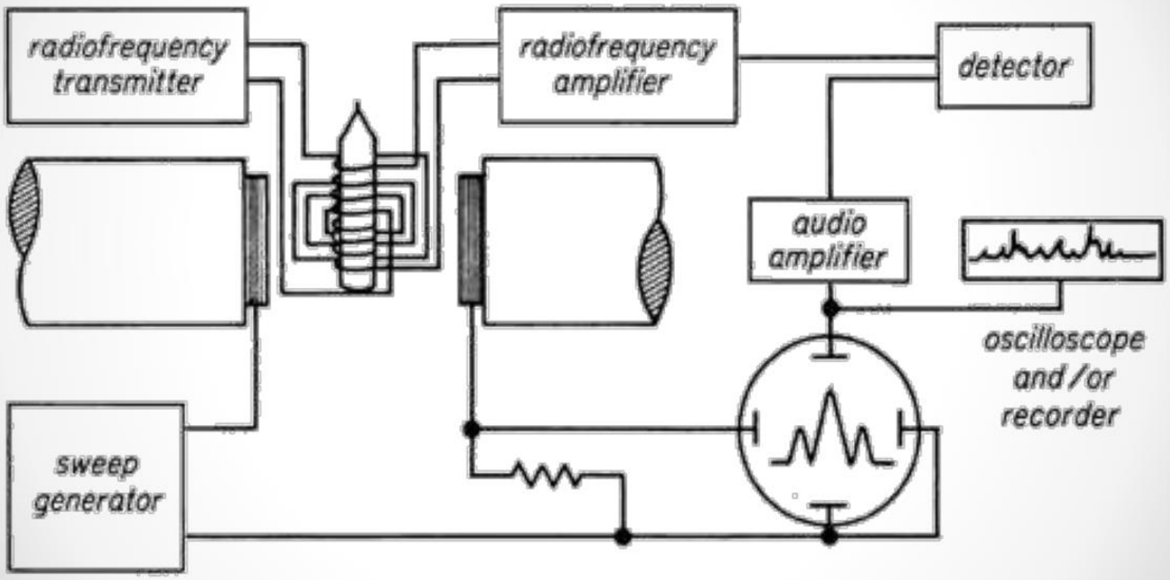
- The stronger the magnetic field, the larger energy difference between two nuclear spin states and higher the ν needed for the resonance.

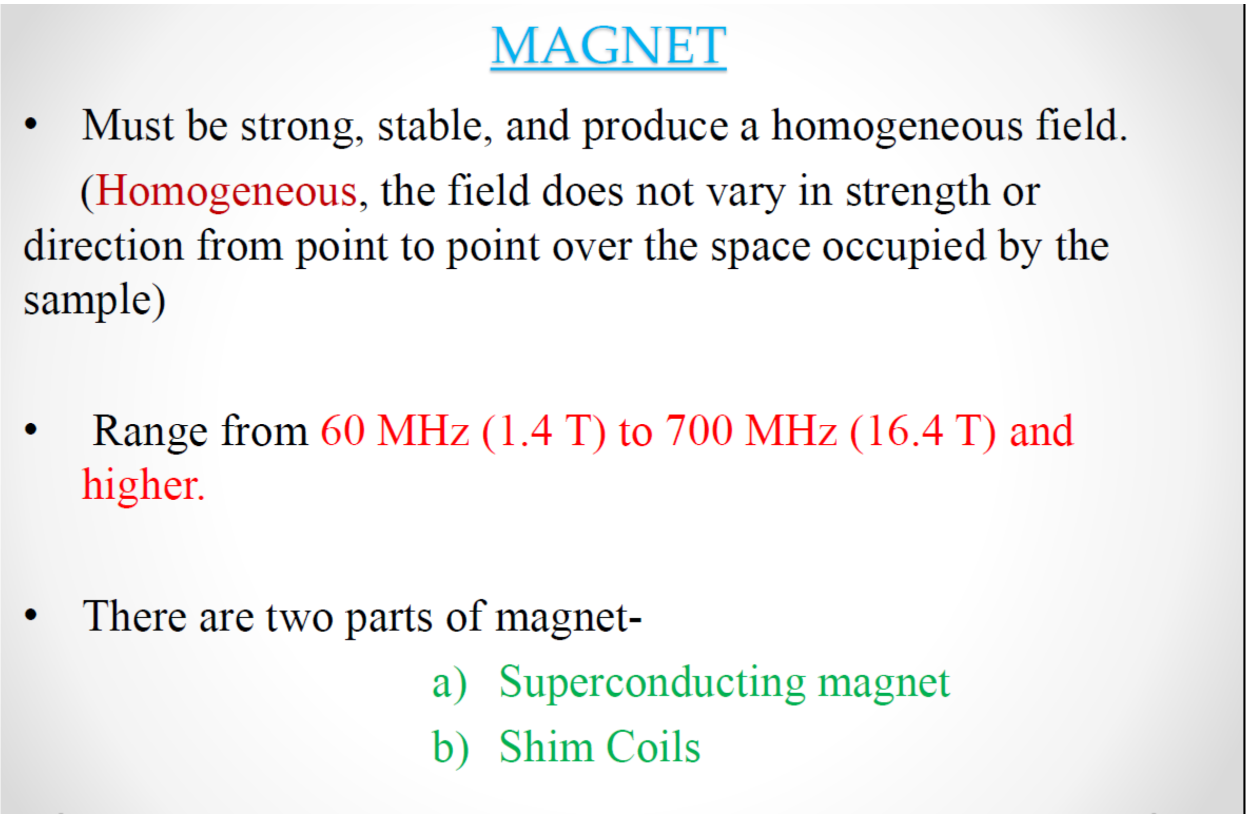


COMPONENTS IN NMR SPECTROMETER

- An intense , homogeneous and stable magnetic field .
- A probe which enables the coils used to excite and detect the signal, to be placed close to the sample.
- A high power RF transmitter probable of delivering sharp pulses.
- A sensitive receiver to amplify the NMR signal.
- A detector to convert the NMR signals in to a form which can be stored in computer memory.
- A pulse programmer to produce precisely time pulses and delays.
- A computer to control everything and to process the data.

BLOCK DIAGRAM





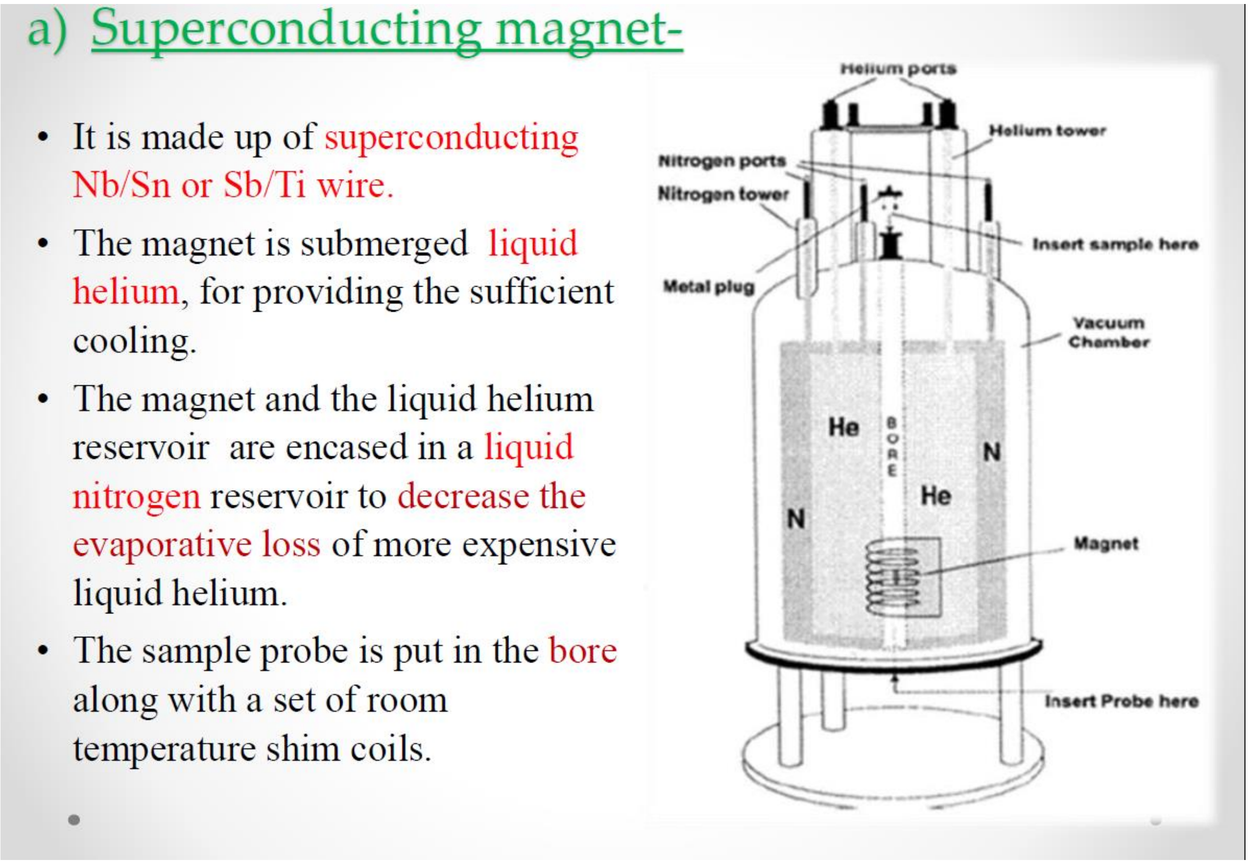
MAGNET

- Must be strong, stable, and produce a homogeneous field.

(Homogeneous, the field does not vary in strength or direction from point to point over the space occupied by the sample)

- Range from 60 MHz (1.4 T) to 700 MHz (16.4 T) and higher.

- There are two parts of magnet-
  a) Superconducting magnet
  b) Shim Coils

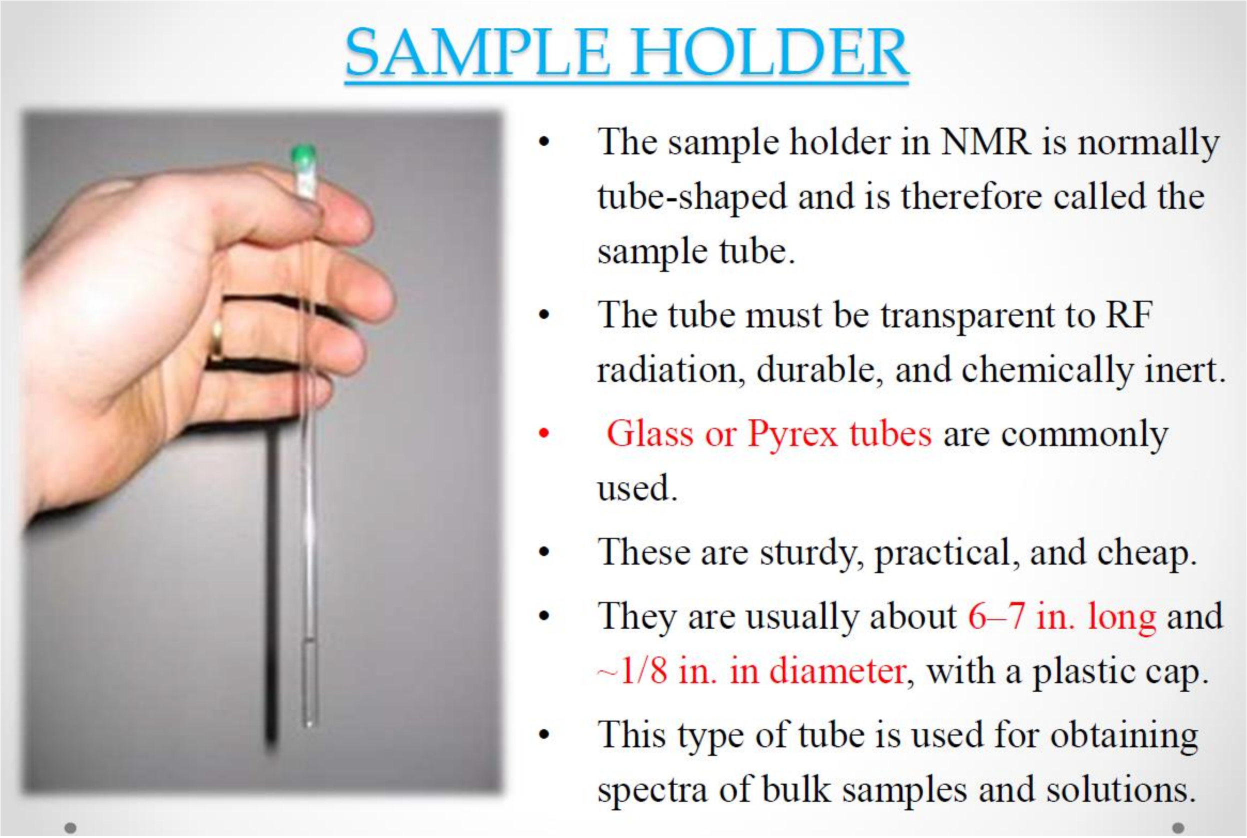


a) Superconducting magnet-

- It is made up of superconducting Nb/Sn or Sb/Ti wire.
- The magnet is submerged liquid helium, for providing the sufficient cooling.
- The magnet and the liquid helium reservoir are encased in a liquid nitrogen reservoir to decrease the evaporative loss of more expensive liquid helium.
- The sample probe is put in the bore along with a set of room temperature shim coils.

b) Shim Coils-

- Shim coils are used for making magnetic field homogeneous, provided by the magnets.
- Through these coils current is adjusted until the magnetic field has the required homogeneity.
- Magnetic field produced by the shim coils cancel the small residual inhomogeneities in the main magnetic field.
- Naming of shim coils is done on the basis, that on which direction they generate the corresponding magnetic field.



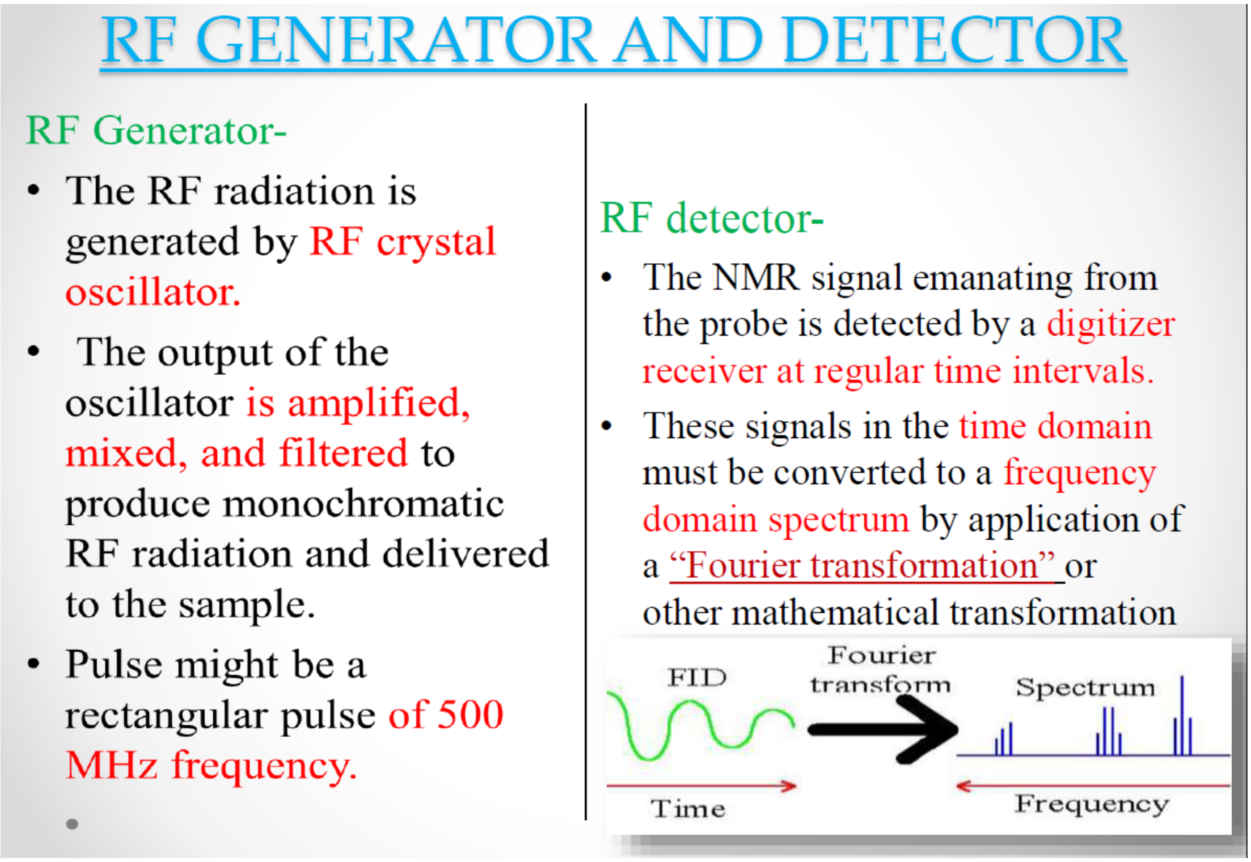
SAMPLE HOLDER

- The sample holder in NMR is normally tube-shaped and is therefore called the sample tube.
- The tube must be transparent to RF radiation, durable, and chemically inert.
- Glass or Pyrex tubes are commonly used.
- These are sturdy, practical, and cheap.
- They are usually about 6–7 in. long and ~1/8 in. in diameter, with a plastic cap.
- This type of tube is used for obtaining spectra of bulk samples and solutions.


Sample tube
Sample holder
Sample depth gauge
Sample length (5cm)
Coil length
varian
the first name in NMR

PROBE

- In which the sample holder is placed.
- Contains an air turbine to spin the sample holder, while the spectrum is collect.
- used to excite and detect the magnetization in radio-frequency of sample.
- The most essential component is the RF transmitting and receiving coil.
- For maximum sensitivity, a fixed frequency probe is needed (mean: a separate probe is required for each nucleus like1H, 13C, 19F).



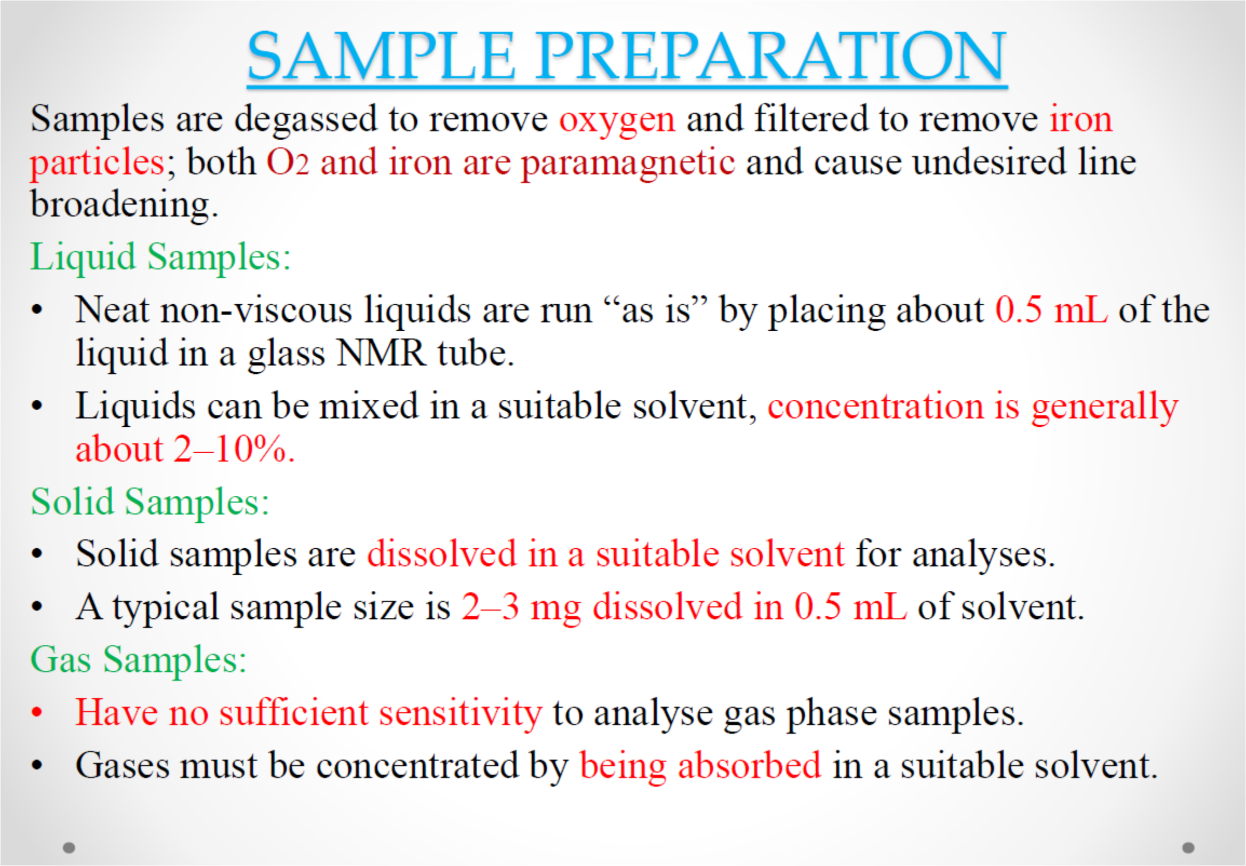
RF GENERATOR AND DETECTOR

RF Generator-

- The RF radiation is generated by RF crystal oscillator.
- The output of the oscillator is amplified, mixed, and filtered to produce monochromatic RF radiation and delivered to the sample.
- Pulse might be a rectangular pulse of 500 MHz frequency.

RF detector-

- The NMR signal emanating from the probe is detected by a digitizer receiver at regular time intervals.
- These signals in the time domain must be converted to a frequency domain spectrum by application of a "Fourier transformation" or other mathematical transformation



SAMPLE PREPARATION

Samples are degassed to remove oxygen and filtered to remove iron particles; both O_2 and iron are paramagnetic and cause undesired line broadening.

Liquid Samples:

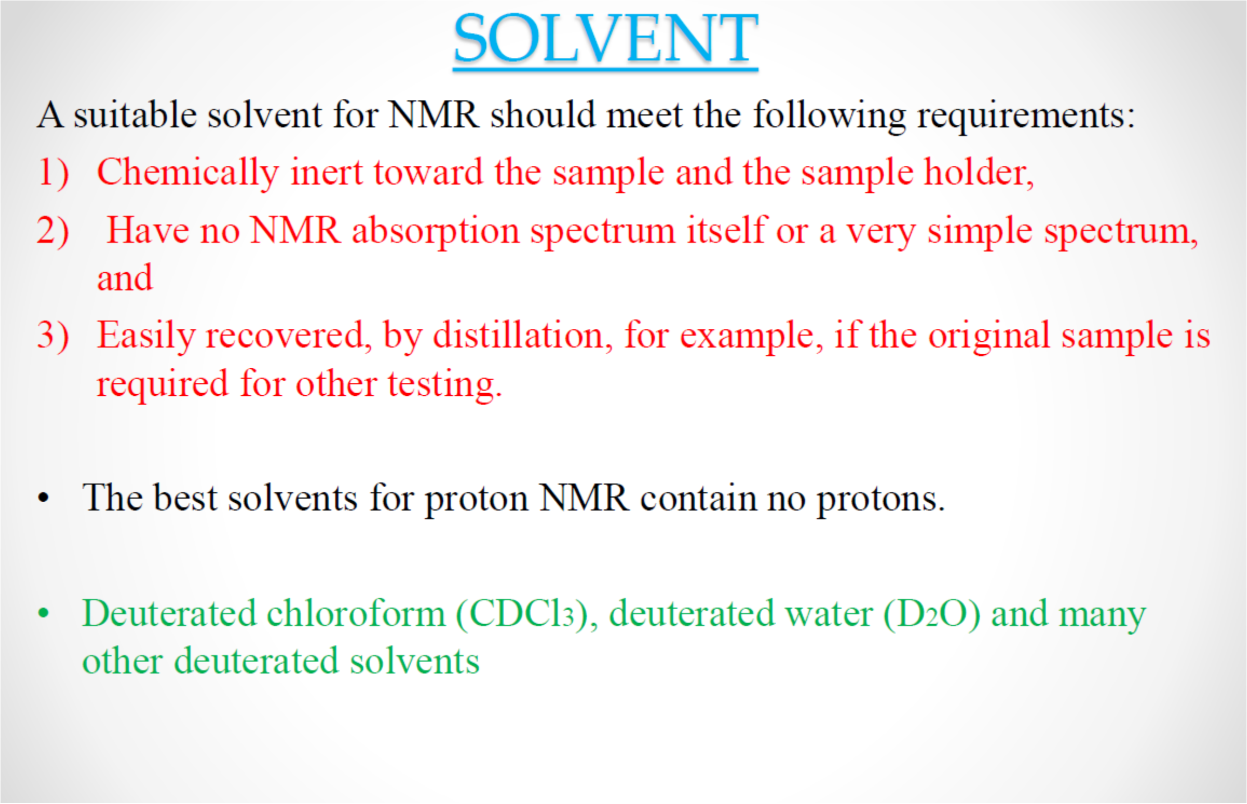
- Neat non-viscous liquids are run “as is” by placing about 0.5 mL of the liquid in a glass NMR tube.
- Liquids can be mixed in a suitable solvent, concentration is generally about 2–10%.

Solid Samples:

- Solid samples are dissolved in a suitable solvent for analyses.
- A typical sample size is 2–3 mg dissolved in 0.5 mL of solvent.

Gas Samples:

- Have no sufficient sensitivity to analyse gas phase samples.
- Gases must be concentrated by being absorbed in a suitable solvent.



SOLVENT

A suitable solvent for NMR should meet the following requirements:

1) Chemically inert toward the sample and the sample holder,
2) Have no NMR absorption spectrum itself or a very simple spectrum, and
3) Easily recovered, by distillation, for example, if the original sample is required for other testing.

- The best solvents for proton NMR contain no protons.

- Deuterated chloroform ($CDCl_3$), deuterated water (D_2O) and many other deuterated solvents

Spin of Nuclei

Fermions : Odd mass nuclei with an odd number of nucleons have fractional spins.

I = 1/2 (^{1}H, ^{13}C, ^{19}F, ^{31}P), I = 3/2 (^{11}B, ^{33}S) & I = 5/2 (^{17}O).

Bosons : Even mass nuclei with odd numbers of protons and neutrons have integral spins.

I = 1 (^{2}H, ^{14}N)

Even mass nuclei composed of even numbers of protons and neutrons have zero spin

I = 0 (^{12}C, and ^{16}O, ^{32}S)

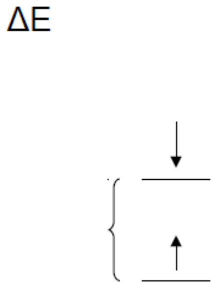
Nuclear Magnetic Resonance (nmr)

-the nuclei of some atoms spin: ^{1}H, ^{13}C, ^{19}F, …

-the nuclei of many atoms do not spin: ^{2}H, ^{12}C, ^{16}O, …

-moving charged particles generate a magnetic field (↗)

-when placed between the poles of a powerful magnet, spinning nuclei will align with or against the applied field creating an energy difference. Using a fixed radio frequency, the magnetic field is changed until the $\Delta E = E_{EM}$. When the energies match, the nuclei can change spin states (resonate) and give off a magnetic signal.



Types of samples

- Both liquid and solid type of samples can be used in NMR spectroscopy.

- For liquid sample, conventional solution-state NMR spectroscopy is used for analysing where as for solid type sample, solid-state spectroscopy NMR is used.

- In solid-phase media, samples like crystals, microcrystalline powders, gels, anisotropic solutions, proteins, protein fibrils or all kinds of polymers etc. can be used.

- In liquid phase, different types of liquid solutions, nucleic acid, protein, carbohydrates etc. can be used.

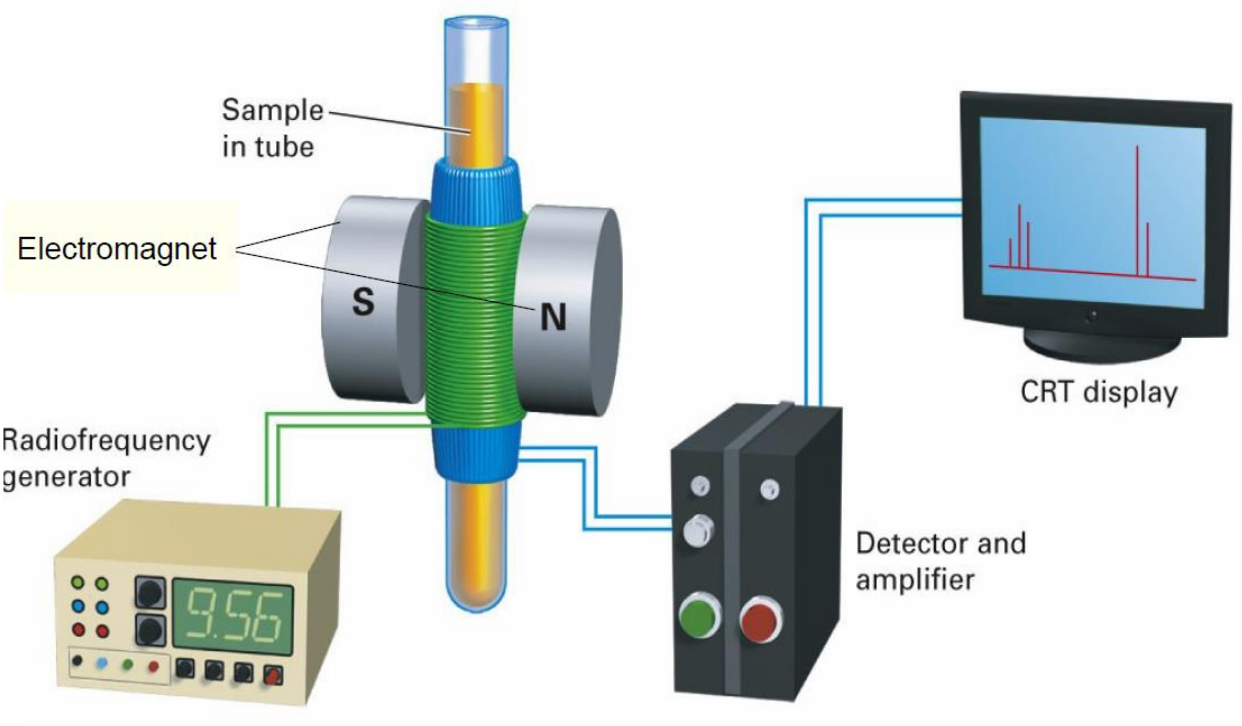
Principle of NMR

- The sample is dissolved in a solvent, usually $CDCl_3$(deutero-chloroform), and placed in a magnetic field.

- A radiofrequency generator then irradiates the sample with a short pulse of radiation, causing resonance.

- When the nuclei fall back to their lower energy state, the detector measures the energy released and a spectrum is recorded.

Schematic diagram of NMR set-up



© 2007 Thomson Higher Education

Principle of NMR(Contd…)

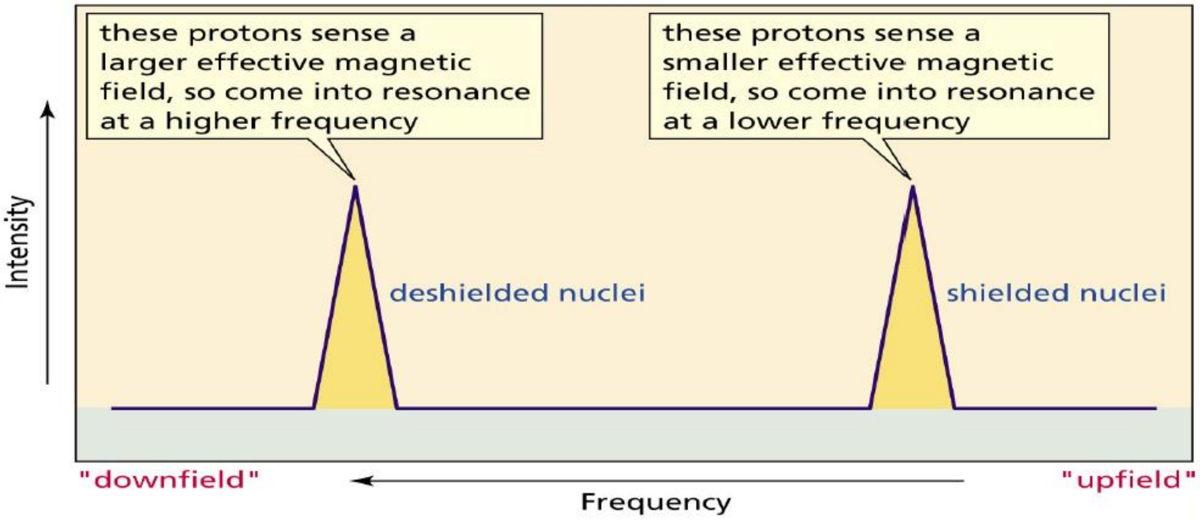
- Protons in different environments absorb at slightly different frequencies, so they are distinguishable by NMR.
- The frequency at which a particular proton absorbs is determined by its electronic environment.
- The size of the magnetic field generated by the electrons around a proton determines where it absorbs.

Chemical shift

- The relative energy of resonance of a particular nucleus resulting from its local environment is called chemical shift.
- NMR spectra show applied field strength increasing from left to right.
- Left part is downfield, the right is upfield.
- Nuclei that absorb on upfield side are strongly shielded where nuclei that absorb on downfield side is weakly shielded.
- Chart calibrated versus a reference point, set as 0, tetramethylsilane [TMS].

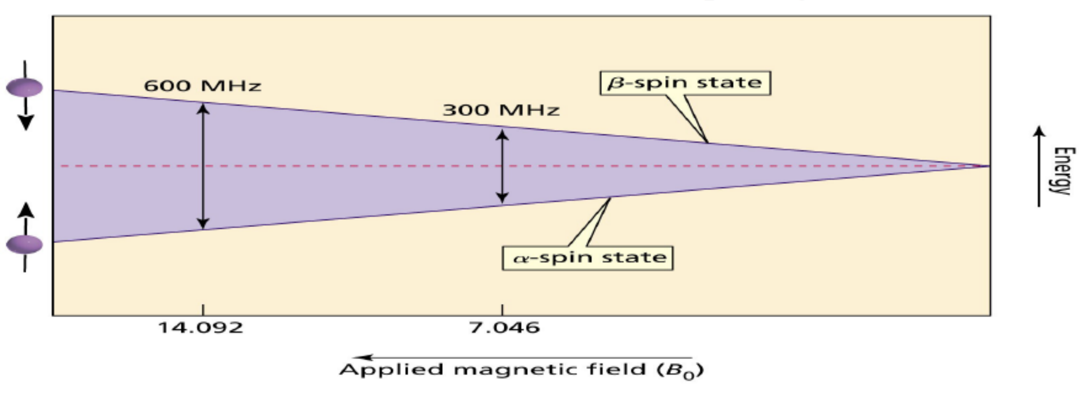
Chemical shift(Contd…)

- The electrons surrounding a nucleus affect the effective magnetic field sensed by the nucleus.



Chemical shift(Contd…)

- Shielded nuclei do not ‘sense’ as large a magnetic field as deshielded nuclei do. As a result, the energy difference between the α- and β-spin states is much lower in energy for shielded nuclei and resonate at a lower frequency.



- Deshielded nuclei have a much higher energy difference between the α- and β-spin states and these resonate at a much higher frequency.

Measurement of Chemical Shift

- Numeric value of chemical shift: difference between strength of magnetic field at which the observed nucleus resonates and field strength for resonance of a reference.
  - Difference is very small but can be accurately measured
  - Taken as a ratio to the total field and multiplied by 10^6 so the shift is in parts per million (ppm)
- Absorptions normally occur downfield of TMS, to the left on the chart.

Acquisition of spectra

- The received nuclear magnetic resonance response is very weak in signal and requires a sensitive radio receiver to pick up.

- A Fourier transform is done to extract the frequency-domain spectrum from the raw time-domain spectrum.

- Good ^{1}H NMR spectra can be acquired with 16 repeats, which takes only minutes.

- However, for heavier elements than hydrogen, acquisition of quantitative heavy-element spectra can be time-consuming, taking tens of minutes to hours.

- Then a average of all the acquired spectrum will be generated and displayed through the graph.

^{13}C NMR Spectroscopy

- Carbon-13: only carbon isotope with a nuclear spin
  - Natural abundance 1.1% of C's in molecules
  - Sample is thus very dilute in this isotope

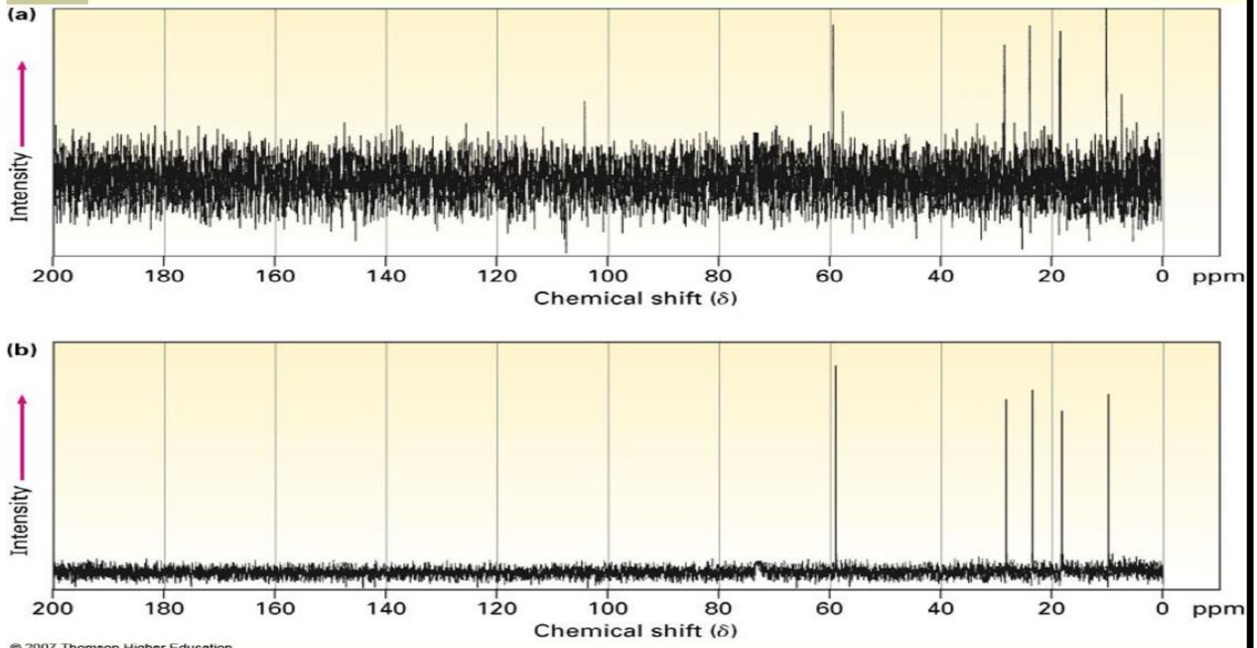
- Sample is measured using repeated accumulation of data and averaging of signals, incorporating pulse and the operation of Fourier transform (FT-NMR).

- All signals are obtained simultaneously using a broad pulse of energy and resonance recorded.

- Frequent repeated pulses give many sets of data that are averaged to eliminate noise .

- Fourier-transform of averaged pulsed data gives spectrum shown in next slide.

^{13}C NMR Spectroscopy(Contd…)



Carbon-13 NMR spectra of 1-pentanol, $CH_3CH_2CH_2CH_2CH_2OH$

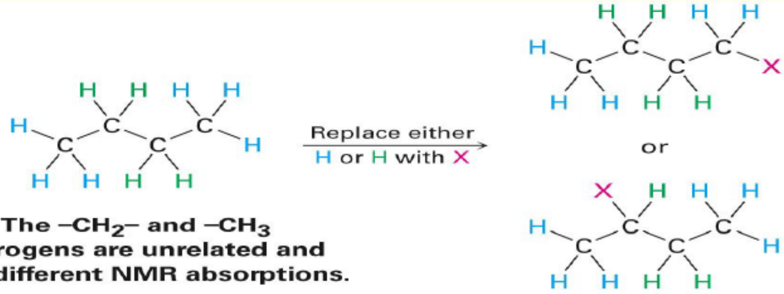
^{1}H NMR Spectroscopy

- Proton NMR is much more sensitive than ^{13}C and the active nucleus (^{1}H) is nearly 100 % of the natural abundance.
- Shows how many kinds of nonequivalent hydrogens are in a compound.
- Theoretical equivalence can be predicted by seeing if replacing each H with “X” gives the same or different outcome.
- Equivalent H’s have the same signal while nonequivalent are “different” and as such may cause additional splitting (diastereotopic effect).

^{1}H NMR Spectroscopy(Contd…)

- Replacement of each H with "X" gives a different constitutional isomer.

- Then the H's are in constitutionally heterotopic environments and will have different chemical shifts – they are nonequivalent under all circumstances.



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

The two replacement products are constitutional isomers.

© 2007 Thomson Higher Education