

Nuclear Magnetic Resonance Spectroscopy

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.

Types of NMR

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

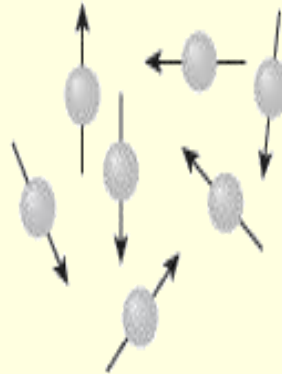
- ♣ When a charged particle such as a proton spins on its axis, it creates a **magnetic field**. Thus, the nucleus can be considered to be a tiny bar magnet.
- ♣ Normally, these tiny bar magnets are randomly oriented in space. However, in the presence of a magnetic field B_0 , they are oriented with 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.

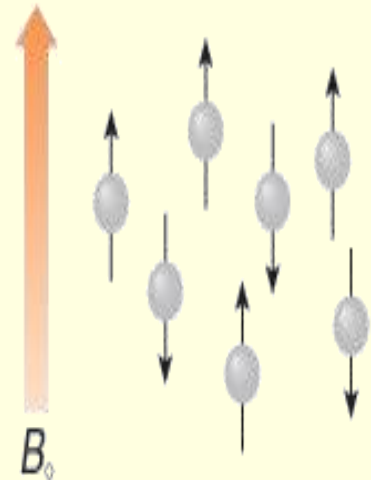


With no external magnetic field...



The nuclear magnets are
randomly oriented.

In a magnetic field...

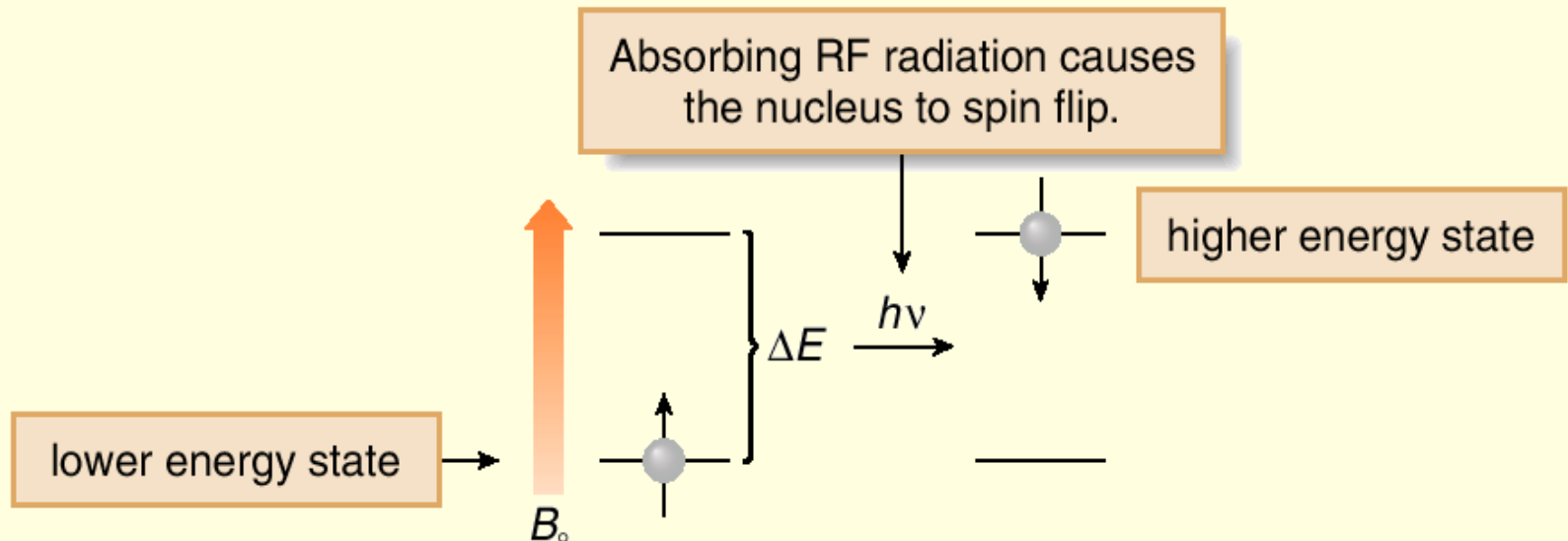


The nuclear magnets are
oriented **with or against** B_0 .

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 ν of radiation used for resonance, measured in hertz (Hz), or megahertz (MHz).



Effect of Magnetic field(Contd..)

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

$$\omega \propto B_0$$

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

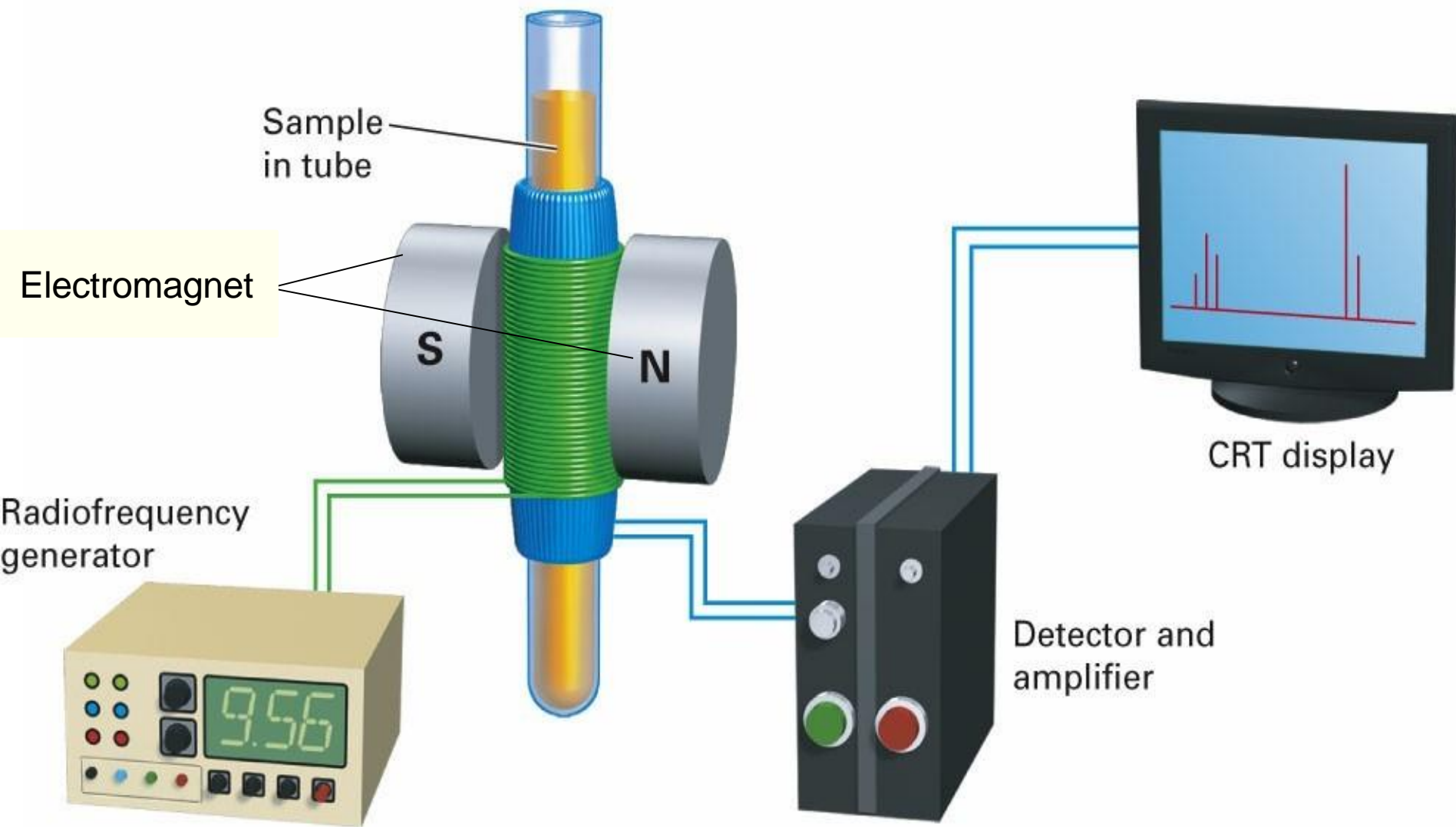
Types of samples

- v Both liquid and solid type of samples can be used in NMR spectroscopy.
- v For liquid sample, conventional **solution-state** NMR spectroscopy is used for analysing where as for solid type sample, **solid-state** spectroscopy NMR is used.
- v In solid-phase media, samples like crystals, microcrystalline powders, gels, anisotropic solutions, proteins, protein fibrils or all kinds of polymers etc. can be used.
- v 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



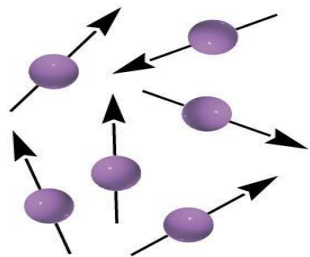
Principle of NMR(Contd...)

- ♣ 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.

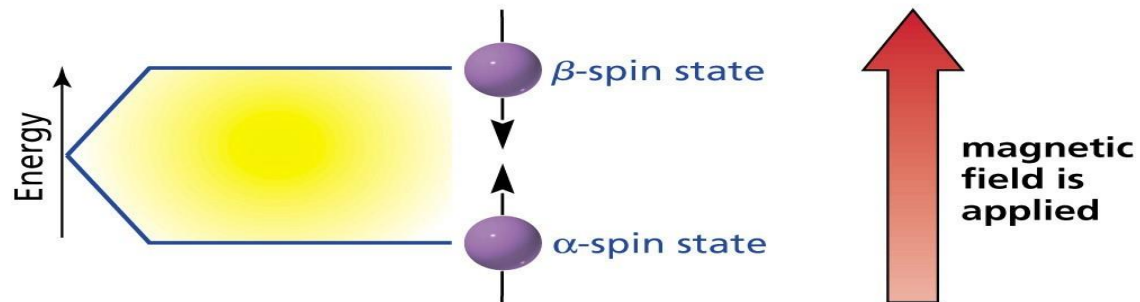
Principle of NMR(Contd...)

♣ Modern NMR spectrometers use a constant magnetic field strength B_0 , and then a narrow range of frequencies is applied to achieve the resonance of all protons.

♣ Only nuclei that contain **odd mass numbers** (such as ^1H , ^{13}C , ^{19}F and ^{31}P) or **odd atomic numbers** (such as ^2H and ^{14}N) give rise to NMR signals.

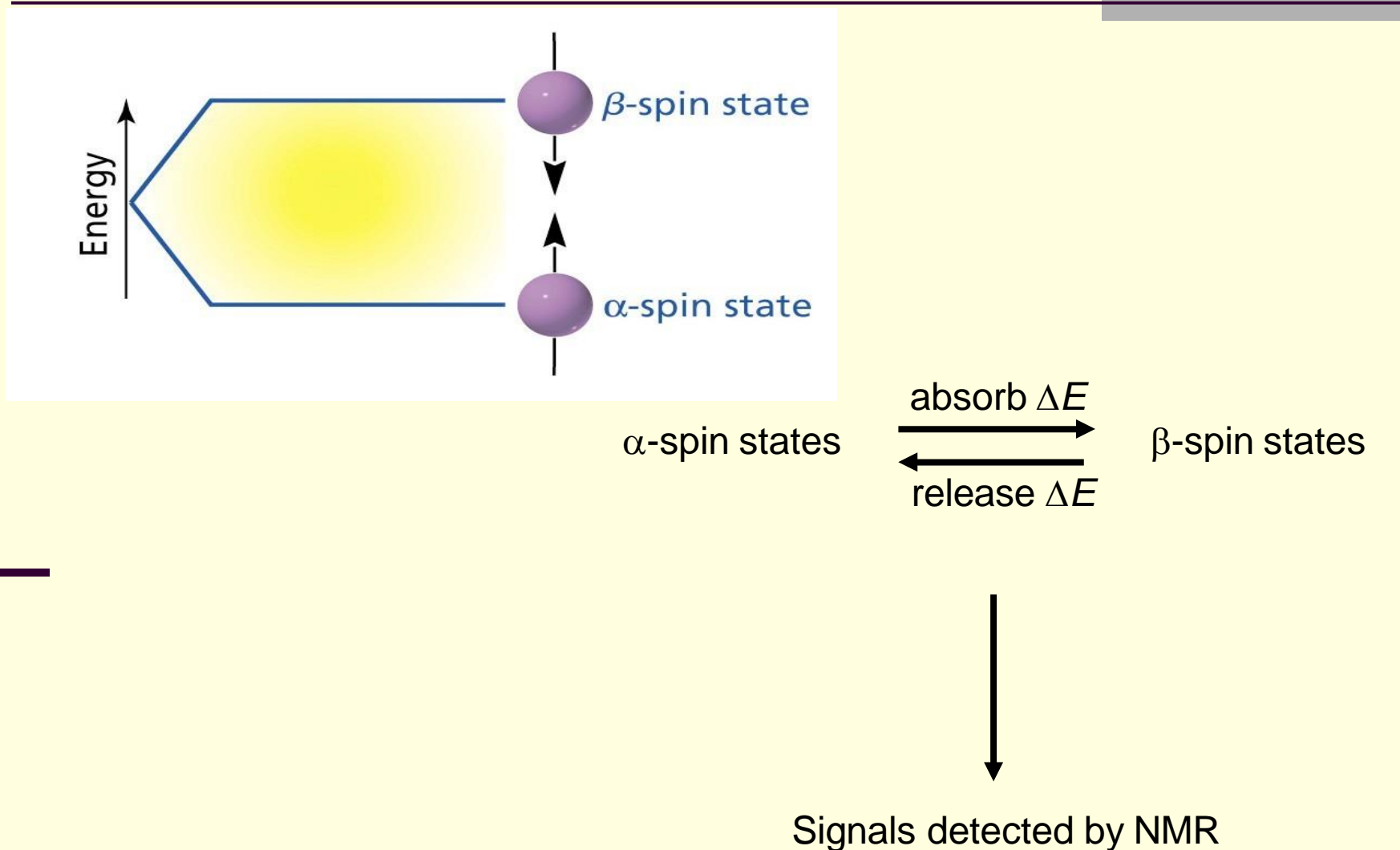


no applied magnetic field



The spin state of a nucleus is affected by an applied magnetic field....

Principle of NMR(Contd...)

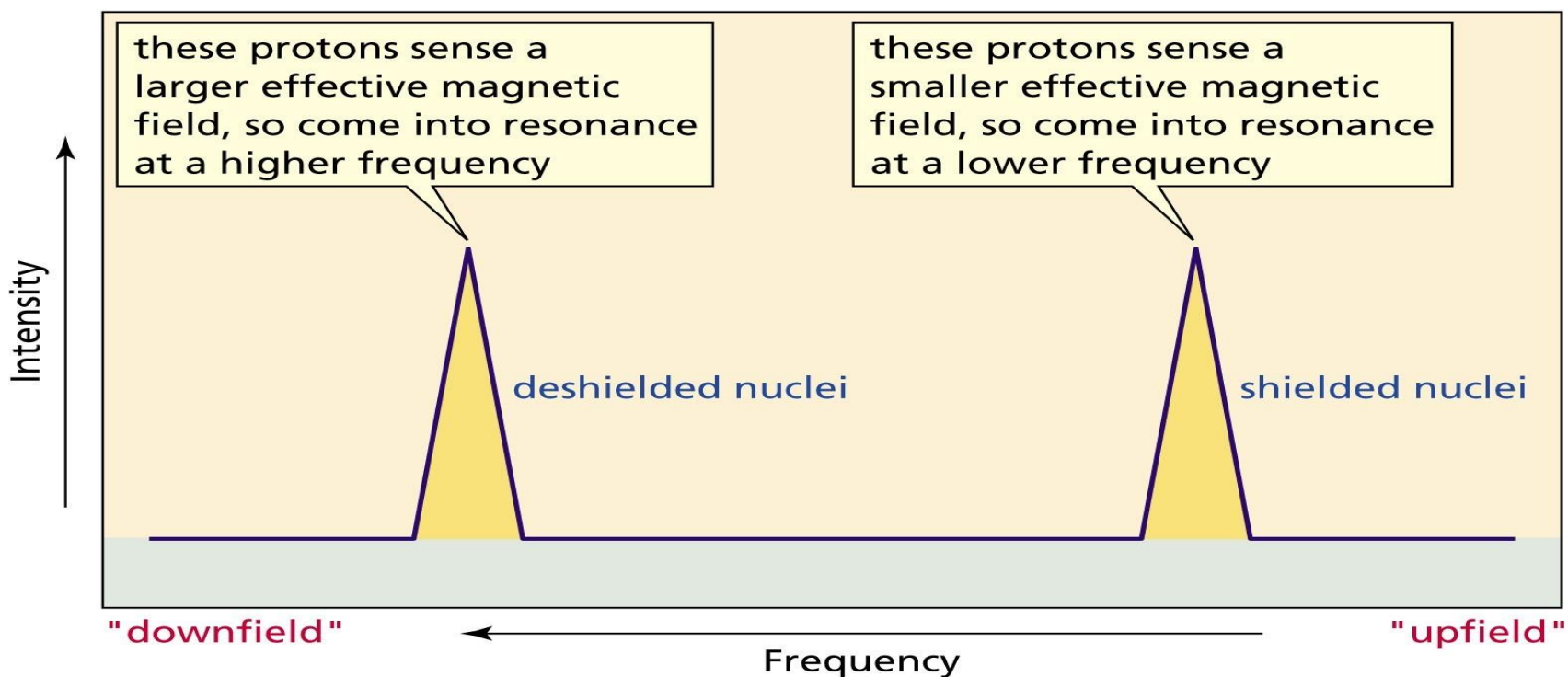


Chemical shift

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

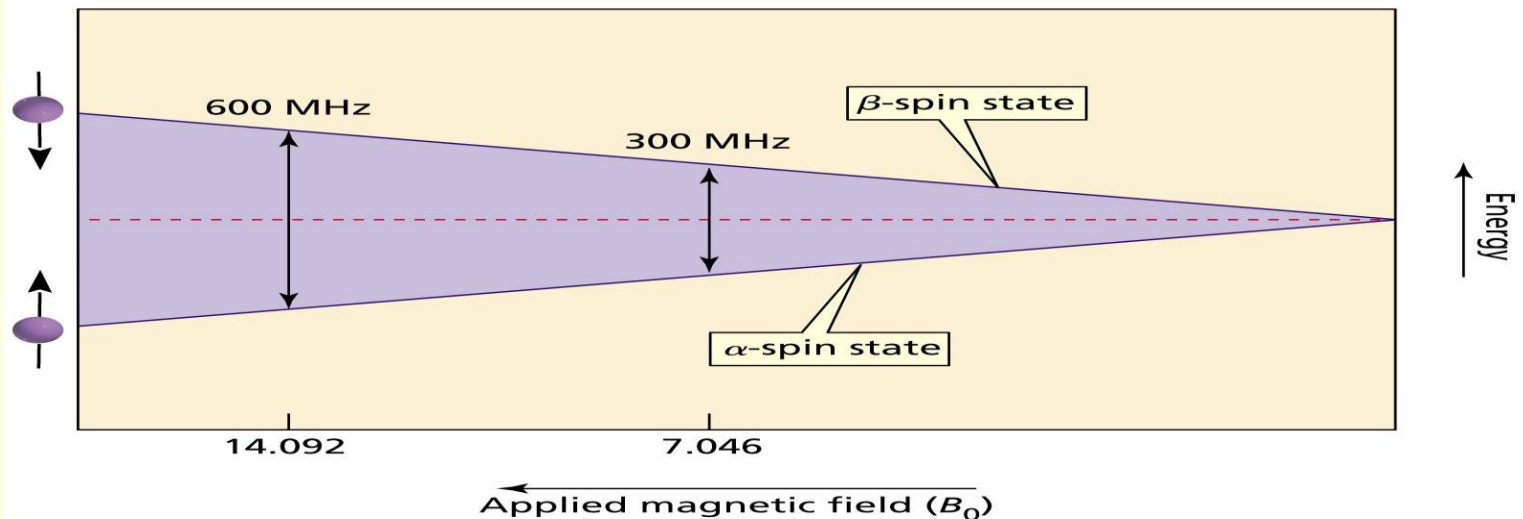
Chemical shift(Contd...)

- ♣ 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.

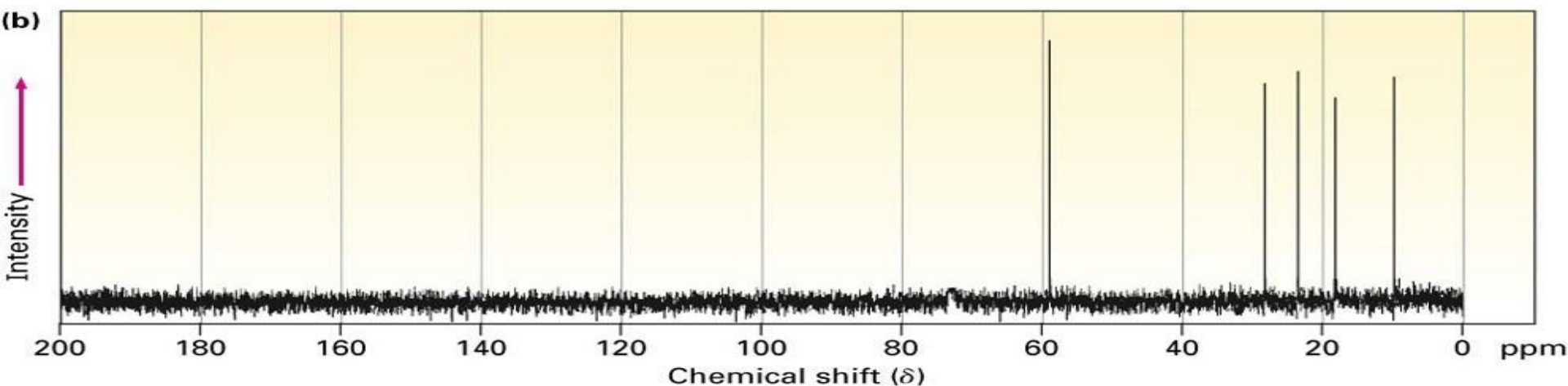
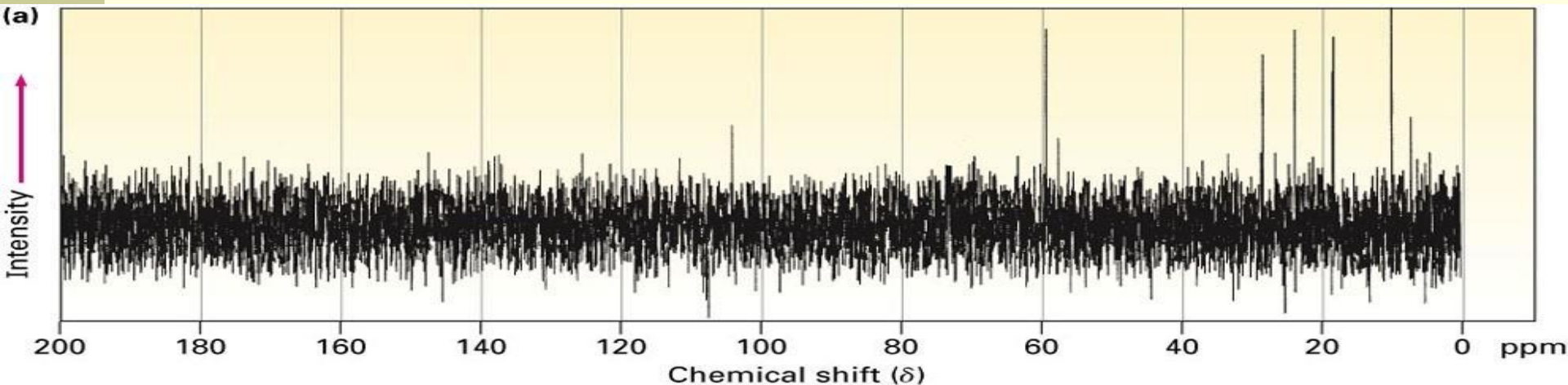
Acquisition of spectra

- ∨ 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 ^1H 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...)



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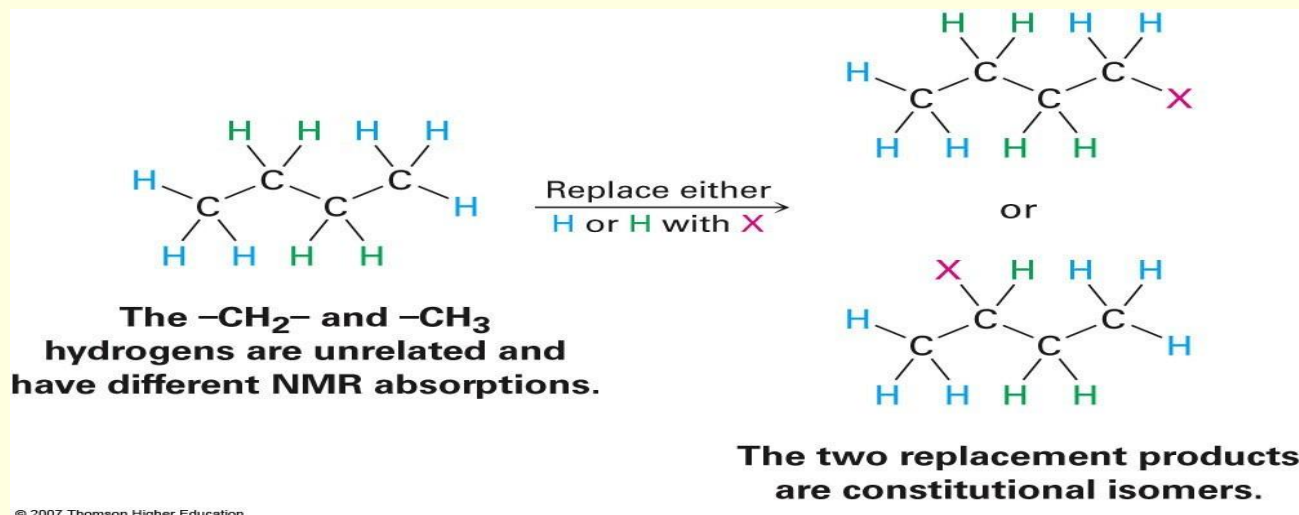
Carbon-13 NMR spectra of 1-pentanol, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$

^1H NMR Spectroscopy

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

^1H NMR Spectroscopy(Contd...)

- v Replacement of each H with “X” gives a different constitutional isomer.
- v Then the H’s are in **constitutionally heterotopic** environments and will have different chemical shifts – they are nonequivalent under all circumstances.



NMR machine at IICPT

- ✓ Type:- Bench top NMR spectrophotometer
- ✓ Frequency:- 60 MHz
- ✓ Magnet:- Permanent electromagnet
- ✓ Available nuclei:- ^1H , ^{19}F
- ✓ Sample:- Standard 5mm NMR glass tubes
- ✓ Field strength:- 1.41T
- ✓ Resolution:- 70 ppb
- ✓ Operating temperature:- 18-26°C
- ✓ Power supply:- 100-240 VAC(50-60 Hz)
- ✓ Dimensions:- 24×28×43 cm
- ✓ Weight:- 18kg
- ✓ Software:- Mnova software
- ✓ Cost:- 34,41,000—/



Summary

- ▼ Nuclear magnetic resonance spectroscopy basically provides the detailed information about the structure, dynamics, reaction state, and chemical environment of molecules.
- ▼ It has various applications in food industries, food science, chemical analysis of different products, pharmaceutical approach etc.
- ▼ To analyse the **carbon-hydrogen framework** in the molecule is the basic work of NMR technique.