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Chromatography

INTRODUCTION

Chromatography is considered as a group of techniques for the separation of the components (compounds) of mixtures by their continuous distribution between two phases, one of which is moving past the other. The systems are:

- A solid stationary phase and a liquid or gaseous mobile phase (adsorption chromatography, here the adsorption by the stationary phase is the main phenomenon for separation of the components, hence the name).
- A liquid stationary phase and a liquid or gaseous mobile phase (partition chromatography, here the partition between the stationary and mobile phases is the main cause for separation of the components, hence the name).
- A solid polymeric stationary phase containing replaceable ions, and an ionic liquid mobile phase (ion exchange chromatography, here the ion exchange is responsible for chromatographic separation, hence the name).
- An inert gel which acts as a molecular sieve, and a liquid mobile phase (gel chromatography).

The basis for the separation of the components of a mixture may be defined in terms of one of these four modes described above or by a combination.

Types of Chromatography

Type of chromatography	*Adsorbate phase*	*Adsorbent phase*
Gas-liquid	Gas	Liquid
Liquid-liquid	Liquid	Liquid
Gas-solid	Gas	Solid
Liquid-solid	Liquid	Solid

Column Chromatography

- **Adsorption chromatography**

  The technique was originally developed by the Russian botanist T.S. Wett in 1906.

Table 23.1 Adsorbents and solvents

Adsorbent (stationary phase)		*Adsorbate (mobile phase)*
Weak	Sucrose Starch Insulin Talc. Sodium carbonate	Petroleum ether Carbon tetrachloride Cyclohexane Carbon disulphide Ether Acetone Benzene Toluene Esters Acetonitrile Chloroform Alcohols Water Pyridine etc.
Medium	Calcium carbonate Calcium phosphate Magnesium carbonate Magnesium oxide Calcium hydroxide	
Strong	Activated magnesium silicate Activated alumina Activated charcoal Activated magnesia Activated silica	

Increasing eluting power ↓

Schematic representation of apparatus for column chromatography

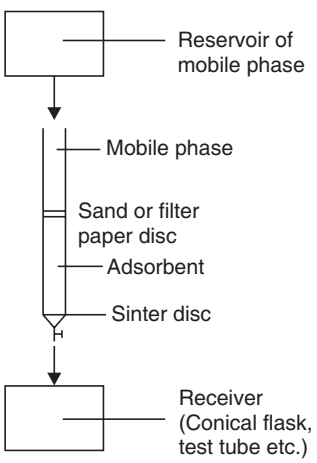

Fig. 23.1 A set-up for column chromatography.

Column Characteristics

Adsorbent/adsorbate weight ratio	*30:1*
Length/diameter ratio	*10-15:1*
Column length: (*i*) Multicomponent system (*ii*) Components with similar affinities for adsorbent (*iii*) Components with different affinities for adsorbent	 Long column Long column Short column

Detection and Recovery of Components

- For those mixtures which are coloured, visual examination is sufficient.
- Colourless components may be detected visually if they fluoresce normally or under UV light.

It is usual practice to complete the chromatogram by eluting the various components with solvents. For colourless compounds the eluate is collected as a large number of fractions, each of small volume.

Advantages of Column Chromatography

- Separation and collection of components of a mixture in the pure form both in laboratory and industry.
- Very efficient method of separation of mixtures of components.

Example. *Separation of leaf pigments of spinach leaves viz. chlorophyll and carotenoids.*

Paper Chromatography

Paper chromatography is a type of chromatography in which the stationary phase is water in the fibres of paper and the mobile phase is another solvent. During paper chromatography the chemicals in a mixture *partition* themselves between two solvents, *viz.* water and mobile solvent. Each component in the mixture has a different equilibrium constant (partition constant), which determines whether it has a greater tendency to dissolve in the stationary phase or in the moving phase. As a result the mixture separates as the chemicals move at different speeds. The technique is, therefore, closely related to column partition chromatography.

Partition constant (partition or distribution coefficient) is defined as: the ratio of two concentrations in the two layers is a constant when a solid is shaken with two immiscible liquids as an equilibrium is set up to which the equilibrium law applies.

$$K = \frac{\text{Concentration of the solid in solvent I}}{\text{Concentration of the solid in solvent II}}$$

Paper chromatography is employed to analyse mixtures such as inks, food, colours, dyes, and amino acids.

The movement of components on the paper depends on the amount and nature of stationary phase compared with the amount of mobile phase in the same part of the paper and also on the partition coefficient.

If the conditions are kept same, each component in a mixture moves a fixed fraction of the distance moved by the solvent. The R_f value for the substance is a measure of this fraction. R_f is defined as:

$$R_f = \frac{\text{Distance travelled by centre of component}}{\text{Distance travelled by the solvent front}}$$

R_f values are of considerable importance in paper chromatography and thin layer chromatography (tlc).

There are two types of paper chromatography:

- Ascending chromatography
- Descending chromatography

The origin of the name depends on the nature of solvent movement.

Method

Ascending chromatography

- A pencil line is drawn about 3 cm from one end of the selected paper.
- 2-5 µl volumes of solutions of sample and reference compounds at about 2 cm intervals of the line.

- The paper is folded perpendicular to the line drawn.
- The edges of the paper are held with clips.
- The tank is prepared by placing the mobile phase (about 1 cm depth of the tank) and the lid is placed.
- Some time is allowed to saturate the tank with solvent vapour.
- The folded paper is placed in the tank followed by placing the lid.
- The development is allowed to take place *i.e.,* eluted until the solvent reaches a suitable height (15-20 cm).
- The paper is removed, dried and now comes the question of location of the component.

Detection of components

If the components or substances in a mixture are colourless they are invisible on the paper. If so, the analyst has to '*develop*' the paper or plate in case of tlc with a suitable '*locating agent*'. Common developing agents are (*i*) iodine vapour (*ii*) $KMnO_4$ (1%) (*iii*) bromocresol green (0.05%) (*iv*) ninhydrin for amino acid etc. Amino acids give purple spot.

Advantages

- Rapid and reliable separation and identification.
- The technique may also be used for quantitative purpose.

Disadvantages

- Large scale separation of components is not possible.
- Time consuming, it takes much time to 'elute' compared to tlc.

Thin Layer Chromatography (tlc)

Here the stationary phase is a thin layer of a solid, generally silica gel or alumina, on a glass or plastic plate while the mobile phase is a solvent. The rate at which a component moves up a **tlc** plate depends on the *equilibrium* between adsorption on the solid (stationary phase) and solution in the solvent (mobile phase). The position of equilibrium varies from one substance to another as the components of a mixture separate.

Advantages of tlc

- tlc is quick and cheap.
- Only a very small sample is required.

The technique is widely used in laboratories as well as in industries. It can be used quickly to check whether a chemical reaction is taking place as expected. During purification of a product **tlc** can indicate whether or not all the impurities have been removed from the reaction product.

Detection of the components

- Coloured compounds are easy to detect on a tlc plate.
- A quick way of detecting the position of a colourless organic compound, to allow to stand the plate in a covered chamber with iodine crystals. The iodine is absorbed by the compound giving coloured spots.
- Other methods such as spraying with certain reagents are also employed.
- Alternatively a tlc plate may be impregnated with a fluorescent chemical. When the plate is placed under a UV lamp the whole plate glows except in the areas where organic compounds absorb radiation, so that they are located as dark spots.

R_f values are used to record the distances moved by component chemicals of a mixture relative to the distance moved by the solvent.

General method

- The size of the glass plate is 20 × 20, 20 × 10, or 20 × 5 cm.
- 30 g of adsorbent (stationary phase, generally silica gel) is made to a smooth paste with requisite amount of water or solvent specified (preparation of chromatoplate).
- The slurry is quickly poured to a spreader or applicator and is spread (thickness 0.25 mm).
- The slurry is allowed to set for 3-4 min.
- The plate is transferred to a drier and allowed to dry for 1 hr.
- The plate is transferred to a desiccator over silica gel.
- Spots are given as usual.
- Eluted with mobile phase in a chromatographic chamber.
- The solvent is dried, the plate is developed and the spots are located.
- R_f is measured to identify the components, *e.g.*, R_f for L-lysine is 0.14, DL-alanine is 0.36 and L-leucine is 0.65.

High Performance Liquid Chromatography (hplc)

hplc is a sophisticated technique of liquid chromatography. The mobile phase is a solvent of very high purity. The stationary phase consists of very small particles of a solid (silica gel) packed into a long steel tube. The use of fine particles increases the surface area helping to separate the components in a mixture efficiently. A pump provides very high pressure (1-550 bar, 0.1-55 Mpa, 14.6-8000 p.s.i) to maintain a flow rate of mobile phase at the rate of 0.01-10 ml min^{-1}. The material (15-25 µl) to be chromatographed is injected through a septum into the centre of the packing material with the help of a syringe. Various types of detectors are used.

One application of **hplc** is to study the fate of drugs administered in the body.

Gas-liquid Chromatography (glc)

glc is a sensitive analytical technique for analysing *mixtures of liquids*. In a modern gas-liquid apparatus, the stationary phase is a thin film of liquid adsorbed on the inside surface of a coiled capillary tube of about 30 m long inside an oven. Sample to be analysed is injected into the hot column with the help of a syringe. The mobile phase is a gas, which carries the vapours of the sample mixture through column. The components in the mixture separate as they pass through the column. The components are detected as they come out and the signal detector is fed to a chart recorder.

The chart recorder shows how long it takes for each component of the mixture to pass through the column. This time is called retention time (t_r).

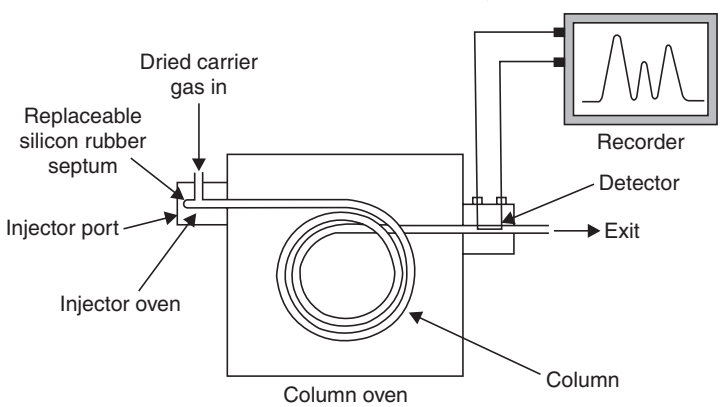

Fig. 23.2 The main features of gas-liquid chromatography.

The areas under the peaks on the print out give a measure of the proportions of the components of the mixture.

A glc machine can be calibrated by injection of known amount of compounds and recording their retention times.

Application of glc

(*i*) Source of oil pollution from the pattern of peaks which acts like a fingerprint for any batch of oil.

(*ii*) Measuring the level of alcohol in urine and blood samples.

(*iii*) Detection and measurement of pesticides in the river water.

Gas Chromatography (gc)

The separation of the components in a mixture in the gaseous state achieved by partition column chromatography using a gaseous mobile phase was first suggested by Martin and Synge in 1941.

The technique requires the vapourisation of the sample, which is carried through a prepared column, at a suitable temperature by a stream of carrier gas (mobile phase). During the passage of the vapour of the sample through the column, separation of the components of the mixture takes place by adsorption effects if the prepared column is of adsorbent only.

If the particles of adsorbent are coated with a liquid which forms a stationary phase then partition effects the separation process. It is better to use the term *support* for the liquid phase rather than *adsorbent* as adsorption effects are undesirable in partition columns.

It is essential that the sample is stable when vapourised and during its passage through prepared or *packed column* (packed with high polymer beads) in order to avoid decomposition of products and the presentation of a complex chromatogram as the carrier gas elutes the products of the column.

The basic apparatus for **gc** is shown diagrammatically.

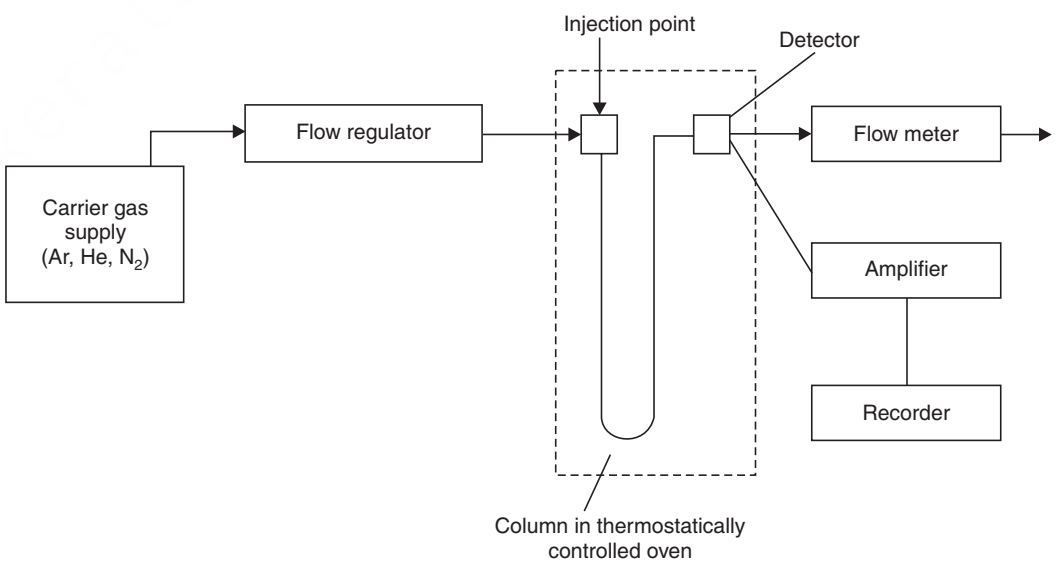

Fig. 23.3 gc apparatus.

Highlight:

- **Nernst's isotherm:**

$$K_A = \frac{C_s}{C_m}$$

where K_A = constant of distribution of component A at a given temperature.

C_s = concentration of component in stationary phase (Adsorbent)

C_m = concentration of component in mobile phase (Adsorbate)

Some parameters of gc

- **Retention time.** It refers to the time taken by the component to be extracted from the column and arrive at the detector. It is measured on the abscissa of the chromatogram from the start to the maximum peak.
- **Retention volume.** It refers to the volume of the solvent that is required to extract a component and bring it to the detector. It is measured from the start to the maximum of the peak on the abscissa of chromatogram.
- **Chromatogram.** It is a volume/speed curve which illustrates a chromatographic separation. The concentration component is found to be proportional to the signal. The signal illustrates the progress of a chromatographic separation.

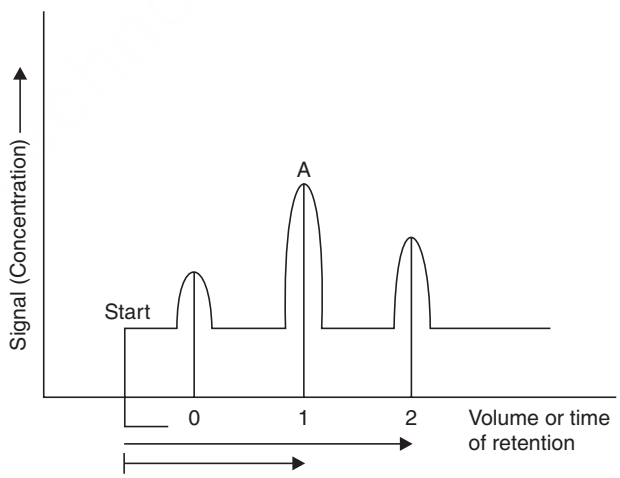

Fig. 23.4 Chromatogram.

- **Separation factor (α):**

$$\alpha = \frac{K_A}{K_B}$$

where K_A and K_B are the constants of distribution of two components A and B respectively.

- **Efficiency.** It refers to the capacity of column to separate the components.
- **Resolution.** It refers to the capacity of a column to separate two consecutive peaks of a chromatogram.

EXERCISES

1. What is chromatography?
2. Write notes on adsorption and partition chromatography.
3. What is meant by R_f in chromatography?
4. What are the advantages and disadvantages of paper chromatography?
5. What is thin layer chromatography (**tlc**)?
6. Mention the advantages of **tlc**.
7. What is column chromatography? Mention its importance.
8. What are:
   (*a*) **hplc** (*b*) **glc** (*c*) **gc?**

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Instrumental Methods of Analysis

INTRODUCTION

The field of spectroscopy is divided into two main classes:

(i) Emission spectroscopy

(ii) Absorption spectroscopy

An emission spectrum is obtained by some light source such as a flame or an electric arc. This spectrum is due to the excitation of atoms by thermal or electrical means. In case of absorption spectroscopy energy absorbed causes electrons in a ground state to be promoted to a higher excited state. The life-time of electrons in this excited state is short and they return to either a lower excited state or to the ground state. The absorbed energy is released as light. Fluorescent lights and colours obtained by heating salts of certain elements in a flame are very common examples of *emissions spectra.* In some cases the excited states may have appreciable life-times. In these cases the excited states usually have appreciable life times and emission of light starts after excitation has ceased. Such a phenomenon is called **phosphorescence**.

An absorption spectrum is obtained by placing the substance between the spectrometer and some source of energy, usually it is an electromagnetic radiation which is applied. The spectrometer analyses the transmitted energy related to the incident energy for a given frequency of the electromagnetic radiation. The regions of electromagnetic radiation of greatest interests to the organic chemists are 200-400 mμ (ultraviolet), 400-800 mμ (visible), and 2-16 μ (infrared).

The mechanisms of absorptions of energy are different in the ultraviolet, infrared and nuclear magnetic resonance regions, but the fundamental phenomenon is the absorption of a certain amount of energy. The energy absorbed is given by

$$E = h\nu$$

where h is Planck's constant and ν is the frequency of incident light (in cycles per second, *cps*). ν is related to the wavelength λ as follows,

$$\nu = \frac{c}{\lambda}$$

where c is the velocity of light, λ is the wavelength in cm.

The wave number is also used in the description of spectra. The wave number k is related to λ by

$$k = \frac{1}{\lambda}$$

i.e., k is the wave number in cm^{-1}.

Interpretations of molecular spectra by the organic chemists are based largely on empirical correlations with extensive compilations of data. At the present time, the various spectral methods are the more commonly used physical methods. Absorption of ultraviolet and visible light is chiefly caused by electronic excitation; the spectrum provides limited information about the structure of the molecule. 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. On the other hand, the proton magnetic resonance (*pmr*) 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 the protons in the molecule.

- **Ultraviolet spectroscopy**

A region of electromagnetic radiation whose interaction with a molecule gives rise to electronic transition exists at 100-8000 Å (10-800 mμ).

Visible Light and Electromagnetic Spectrum

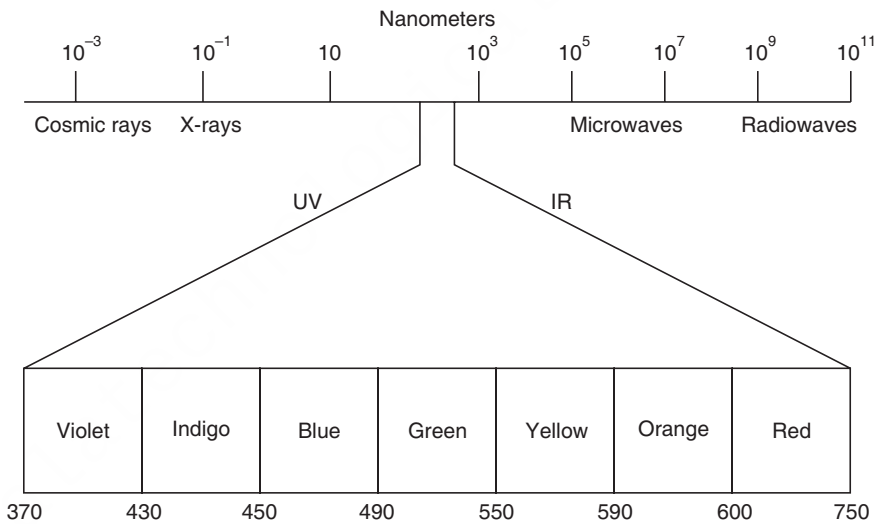


Fig. 24.1 Wavelengths of electromagnetic radiation.

The total energy (E_T) of a diatomic molecule is the sum of electronic energy (ε_e), vibrational energy (ε_v) and rotational energy (ε_r), *i.e.*,

$$E_T = \varepsilon_e + \varepsilon_r + \varepsilon_v$$

If the electromagnetic radiation in the region of 10-800 mμ interacts with a molecule, a change in the energy of the molecule from the ground state to a higher level *i.e.*, excited state occurs. The transition of energy, due to the displacement of a valence electron accompanied by the electronic excitation, is a change in ε_v and ε_r of the molecule. The energy requirements for the excitation of the latter two modes is comparatively less than that for electronic excitation.

Some terms concerning UV

Chromophore: A moiety of a molecule which is responsible for selective absorption of radiation in a given range of specially UV or visible region.

Auxochrome: A chemical group which does not give rise to an absorption band by itself, but upon being attached to a chromophore alters both the position and intensity of the absorption peak.

Bathochromic shift: It is a shift of the peak position (λ_{max}) to a higher wavelength due to the effect of a substituent group or solvent, it is also known as red shift.

Hypsochromic shift: It is a shift of λ_{max} to lower wavelength. It is also known as **blue shift.**

Hyperchromic and hypochromic effects: These terms refer to an increase and decrease in absorptivity of the molecule respectively.

Types of absorption bands: There are four types of absorption bands. They occur due to electronic transition of a molecule.

(*i*) **R-bands:** These are observed in compounds containing such groups as $>C=O$, $—NO_2$ etc. They involve $n - \pi^*$ transition. The ε_{max} value is less than 100. The band at 279 mμ observed in the UV spectrum of acetone is an example of an R-band.

(*ii*) **K-bands:** These arise from $\pi–\pi^*$ transition in π–π conjugated systems and show ε_{max} greater than 10,000. 1,3,5-hexatriene is an example of such a conjugated system.

(*iii*) **B-bands:** These are due to aromatic and heterochromatic systems. The λ_{max} values are between 230-270 mμ and ε_{max} less than 2000. These bands are called **benzenoid bands**. In the presence of K-bands the position of the B-band is shifted to larger wavelengths. The UV spectrum of benzaldehyde contains K, R and B bands.

(*iv*) **E-bands:** These are also known as ethylenic bands and are characteristic of the aromatic systems as are the B-bands. Only difference is: they occur at lower wavelengths.

The presence of an auxochromic group shifts an E-band to a higher wavelength. The ε_{max} values of these bands vary from 2000-14000. The bands at 210 mμ with ε_{max} of 6200 for phenol is an example of E-band.

Instrumentation for UV spectrum

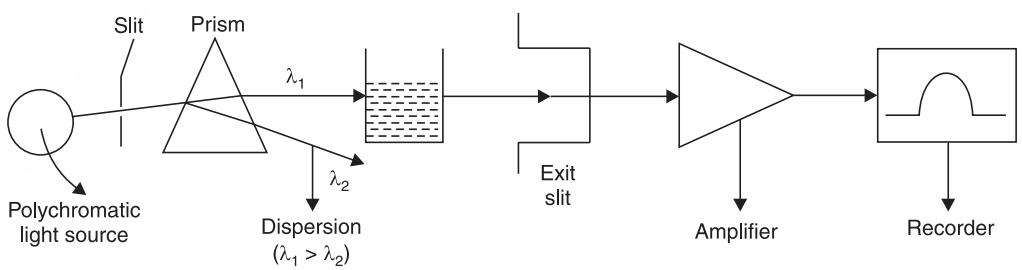


Fig. 24.2 A simplified diagram.

Radiation source. The source for the UV range is usually high pressure hydrogen or deuterium discharge lamp, which covers a range of 200-375 mμ. A xenon arc or, a mercury lamp provides a more intense radiation. The source employed for the visible range is 6 or 12V tungsten automobile head lamp bulb. UV is a plot of absorbance vs. wavelength.

Applications of UV in analytical chemistry

The working formula:

$$A = \varepsilon\, b\, c$$

is derived from Lambert-Beer's law, where A is the absorbance, E is molar extinction coefficient, *b* is the path length in cm and *c* is molar concentration (mol l^{-1}). The absorbance values of the

standard solutions of known concentrations are determined from the spectrophotometer and a calibration graph is constructed. Absorbance value of the test solution is determined from the instrument and the concentration of the test solution is determined from the calibration groups.

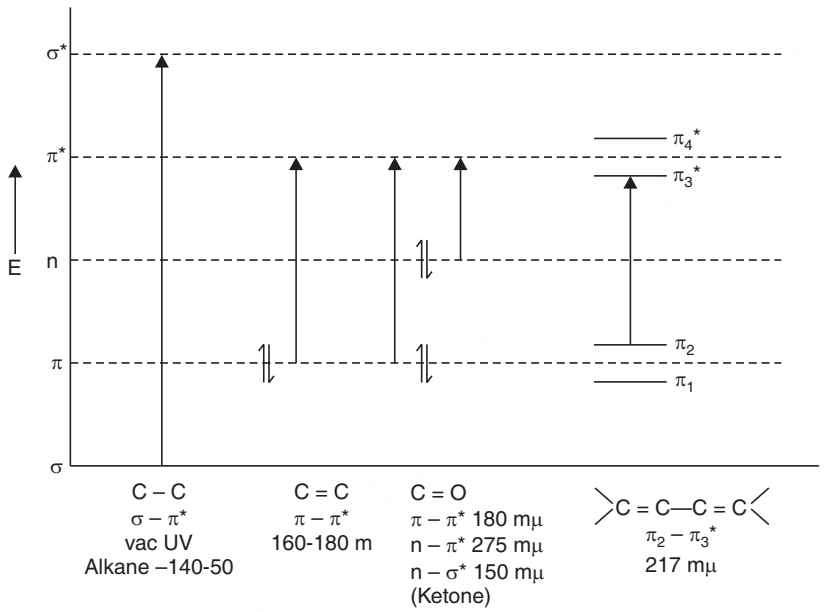


Fig. 24.3 Energy diagram for the electronic transitions.

Conjugated systems

If two or more chromophoric groups are present in a molecule and they are separated by two or more single bonds, the effect on the spectrum is additive. But there is little electronic interaction between isolated chromophoric groups. The λ_{max} values for α, β unsaturated ketones, α, β unsaturated aldehydes and conjugated dienes bear an excellent numerical correlation depending on the number of substituent groups. The rules for calculation of λ_{max} values of such compounds are relatively very simple and is exemplified below:

Calculation of λ_{max} values

- **For α, β unsaturated ketones:**

(*i*) In order to calculate the absorption (λ_{max}) of an α, β unsaturated ketones a base value of 215 mμ is assigned.

(*ii*) For each α-substituent 10 mμ are added to the base value.

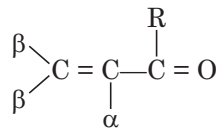
(*iii*) For each β substituent 12 mμ are added to the base value.

(*iv*) For each ring system (6 or 5 membered) to which the carbon-carbon double bond is exocyclic 5 mμ are added to the base value.

(*v*) If the carbon-carbon double bond and the carboxyl group are in a five-membered ring 10 mμ subtracted from the calculated value and if only the carbon-carbon double bond is in a five membered ring 5 mμ is added.

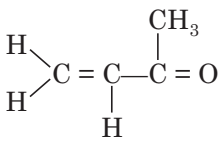
The correlation of calculated values and experimental values is within a permissible limit.

Example 1.



α, β-unsaturated ketone

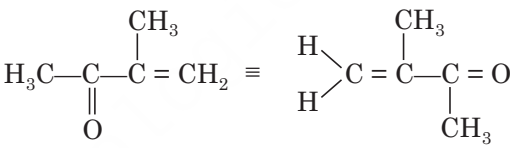
Methyl-vinyl-ketone:



In this case, there is no α and β substituent. Here base value is 215 mμ and no additions are required according to rule. So, the calculated λ_{max} value is 215 mμ. The observed value is 213 mμ.

Example 2.

2-Methyl-1-butene 3 one



Here, base value is 215 mμ,

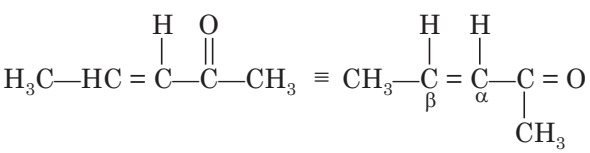
For α-substituent +10 mμ is added.

Calculated λ_{max} value = 225 mμ.

The observed value is 220 mμ.

Example 3.

3-pentene-2-one.



Here, base value is 215 mμ

There is no α substituent + 0 mμ

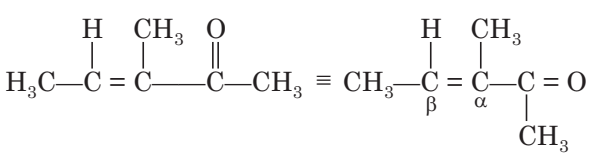
For one β substituent + 12 mμ

Calculated λ_{max} value = 227 mμ.

Observed λ_{max} value = 224 mμ.

Example 4.

3-Methyl-3 pentene-2 one.

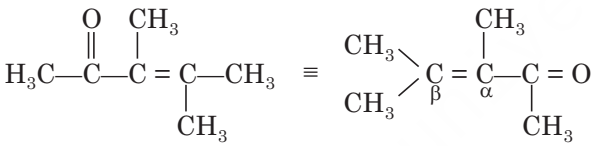


Here base value is 215 mμ
For one α substituent + 10 mμ is added
For one β substituent + 12 mμ is added.

Calculated λ_{max} value = 237 mμ.
Observed λ_{max} value = 236 mμ.

Example 5.

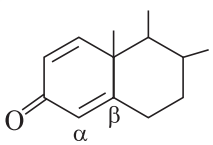
2,3-di methyl-2 pentene-4 one.



Here, base value is 215 mμ.
For one α substituent + 10 mμ.
For each β substituent (2 × 12) = + 24 mμ

Calculated λ_{max} value = 249 mμ.
Observed λ_{max} value = 246 mμ.

Example 6.



It is an α, β unsaturated ketone.

Here, the base value is 215 mμ
There is no α-substituent + 0 mμ
For each β-substituent + 24 mμ
For each ring to which
the double bond is exocylic + 5 mμ

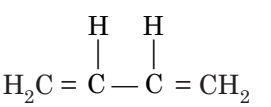
Calculated λ_{max} value = 244 mμ
Observed λ_{max} value = 246 mμ.

Conjugated di-enes (acylic)

For acylic conjugated dienes and cyclic conjugated dienes containing non-fused six-membered ring system a base value of 217 mμ is assigned. 5 mμ are added for each acylic alkyl substituent. 5 mμ is added for each ring to which the diene system is exocylic. Absorptions of non-polar compound such as unsaturated hydrocarbon are not changed with a change in solvent.

Example 1.

Butadiene



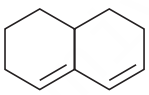
Here, the base value is 217 mμ

So, calculated value of λ_{max} is 217 mμ

Observed λ_{max} value is 217 mμ.

Conjugated dienes in which the double bonds are contained within rings absorb somewhat differently. If the conjugated double bonds are contained in separate, but fused, six-membered rings (a heteroannular diene), a base value of 214 mμ is used; if the conjugated double bonds are contained in the same ring (a homoannular diene), a base value of 253 mμ is used. For each alkyl substituent group on the diene system or, for each ring to which a carbon-carbon double bond is exocylic, 5 mμ are added to the calculated value.

Homoannular diene

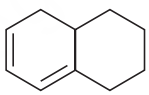


Heteroannular diene

For cyclic conjugated dienes:

Example 1.

Homoannular diene (I)



(I)

Calculated λ_{max}

Here, the base value is 253 mμ

For 3-ring residules + 5 × 3 mμ

For one ring to which

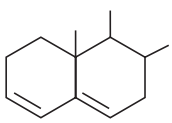
the carbon-carbon double bond is exocylic + 5 mμ

Calculated: λ_{max} value is 273 mμ.

Observed: λ_{max} value is 275 mμ.

Example 2.

Heteroannular diene (II)



(II)

Here, the base value is 214 mμ

For 3-ring residues + 15 mμ

For one ring to which the

Carbon-carbon double bond + 5 mμ is exocylic.

Calculated: λ_{max} value is 234 mμ

Observed: λ_{max} value is 235 mμ.

Quantitative application of UV spectroscopy

Absorption of light in both the ultraviolet and visible regions of the electromagnetic spectrum takes place when the energy of light matches that required to induce in the molecule and electronic transition and its associated vibrational and rotational transitions subsequently.

Beer-Lambert's law

When a beam of light is passed through a transparent cell containing a solution of an absorbing substance, then the intensity of the incident light may be reduced.

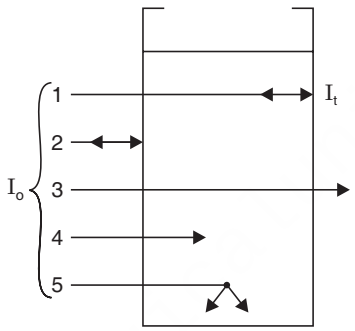


Fig. 24.4 Reduction of the intensity of light by reflection at cell faces (rays 1-2), absorption (ray 4) and scattering by particles (ray 5).

$I_{absorbed} = I_o - I_t$

$I_o \Rightarrow$ intensity of the incident light.

$I_t \Rightarrow$ intensity of the transmitted light.

Generally UV experiments are done in quartz cells as glass absorbs UV.

The transmittance (T) is:

$T = \frac{I_t}{I_o}$ and % T is given by % $T = \frac{100\, I_t}{I_o}$

In 1760 Lambert put forward the relation between I_t and I_o for various thicknesses (b) of the substance. This relationship is given in shape of a law, which is known as Lambert's law. The law is stated as follows:

The rate of decrease in intensity of light with thickness is proportional to the intensity of the incident light.

When the law is expressed mathematically

$$-\frac{dI}{db} \propto I$$

or $$-\frac{dI}{db} = k_1 I_t \qquad [k_1 \Rightarrow \text{proportionality constant}]$$

or $$-\frac{dI}{I_t} = k_1 db$$

Integrating both sides:

$$-I_t = k_1 b + c$$

when $b = 0$, $c = -\ln I_0$

or $\quad -\ln I_t = k_1 b - \ln I_0 \quad$ or $\ln \frac{I_0}{I_t} = k_1 b$

or $$\log_{10} \frac{I_0}{I_t} = \frac{k_1 b}{2.303}$$

where, $\log_{10} I_0/I_t$ is called the absorbance (A).

Absorbance is reciprocal of common logarithms of transmittance.

$$A = \log_{10} \frac{I_0}{I_t} = \log_{10} \left(\frac{1}{T}\right) = -\log_{10} T = 2 - \log (\% T).$$

Beer in 1852 put forward another relationship which is between absorbance and concentration which is mathematically expressed as:

$$\log_{10} \frac{I_0}{I_t} = \frac{k_2 C}{2.303}$$

Beer's law is defined as:

The intensity of a beam of parallel monochromatic radiation decreases exponentially with the number of absorbing molecules. It may be stated also as: the absorbance is proportional to the concentration.

The combination of these two laws leads to Beer–Lambert's law, which is mathematically expressed as:

$A = \log_{10} \frac{I_0}{I_t} = abc$ in which the proportionality constants $k_1/2.303$ and $k_2/2.303$ are combined to the single constant 'a' which is known as **absorptivity**. The value of 'a' as well as its name depend on units of concentration. When 'c' is in moles litre^{-1}, 'a' is called **molar absorptivity,** formerly known as **molar extinction coefficient (ε)**

then the equation takes the form:

$$A = \varepsilon.b.c.$$

When the absorbance is measured for 1% (w/v) solution in a 1 cm cell, then the equation takes the form

$$A = A^{1\%}_{1cm}.b.c.$$

$A^{1\%}_{1cm}$ is known as **specific absorbance**.

Colorimetric analysis is also based on Beer–Lambert's law. Here the light used is visible one and coloured samples are analysed.

Infrared Spectroscopy

When infrared light is passed through a sample of an organic compound, some of the frequencies are transmitted through the sample without being absorbed. If we plot the percent absorbance or percent transmittance against frequency the result is infrared (IR) spectrum.

Molecular vibration

At ordinary temperatures organic molecules are in a constant state of vibration each bond having its characteristic stretching and bending frequency and being capable of absorbing light of that frequency. The vibrations of two atoms joined together by a chemical bond can be compared to the vibrations of two balls joined by a spring, using this analogy we can justify several frequencies of infrared spectrum.

Two balls joined by a spring

For example to stretch spring requires more energy than to bend it; thus stretching energy of a bond is greater than the bending energy and stretching absorptions of a bond appear at higher frequencies in the infrared spectrum than the bending absorption of the same bond.

Calculation of vibrational frequencies

We can calculate the vibrational frequency of a bond with a reasonable accuracy, in the same way as we can calculate the vibrational frequency of a ball and spring system; the equation of calculation is Hook's law,

$$\nu = \frac{1}{2.\pi}\left(\frac{k}{m_1 m_2 / m_1 + m_2}\right)^{1/2}$$

where ν = frequency, k = a constant related to the strength of the spring (the force constant of the bond), m_1, m_2 = the masses of two balls or atoms, $m_1 m_2 / m_1 + m_2$ is known as reduced mass.

As an example, we can calculate the approximate frequency of C—H stretching vibration from the following data,

$$k = 500\ \text{Nm}^{-1} = 5 \times 10^5\ \text{gm s}^{-2} \times \frac{1}{m}$$

$$= 5 \times 10^5\ \text{gs}^{-2}$$

$$1\ \text{N} = 1\ \text{kg} \times \text{m/sec}^2 = 1000\ \text{gm s}^{-2}$$

$$m_C = \text{mass of C-atom} = 20 \times 10^{-24}\ \text{gm}$$

$$m_N = \text{mass of H-atom} = 1.6 \times 10^{-24}\ \text{gm}$$

$$\nu = \frac{7}{2 \times 22}\left[\frac{5 \times 10^5\ \text{gs}^{-2}}{(20 \times 10^{-24}\text{g})(1.6 \times 10^{-24}\text{g})/(20 + 1.6)10^{-24}\ \text{g}}\right]^{1/2} = 9.3 \times 10^{13}\ \text{s}^{-1}$$

To express this in wave numbers ($\bar{\nu}$) we use relationship,

$$\bar{\nu} = \frac{\nu}{c} = \frac{9.3 \times 10^{13}\ \text{s}^{-1}}{3 \times 10^8\ \text{ms}^{-1}} \text{ where } c = \text{velocity of light}$$

$$= 3.1 \times 10^5\ \text{m}^{-1} = 3100\ \text{cm}^{-1}$$

So, $\nu_{max.}$ for C—H (Str.) is 3100 cm^{-1}.

The vibrational frequency of a bond is expected to increase when the bond strength increases and also when the reduced mass of the system decreases. As for example,

Stretching : C = C	>	C—C	Stretching : $\rangle$C = O	>	C—O
1600 cm^{-1}			1700 cm^{-1}		1100 – 1300 cm^{-1}
Stretching : C—H	>	C—C	Stretching : O—H	>	C—O
3100 cm^{-1}			3500 cm^{-1}		1100–1300 cm^{-1}
$\frac{12 \times 1}{13}$	<	$\frac{12 \times 12}{24}$	$\frac{16 \times 1}{17}$	<	$\frac{12 \times 16}{28}$

Infrared spectroscopy

Infrared spectroscopy is dependent on modes of vibration of the atoms in a molecule and those have been shown in the following Fig. 24.5.

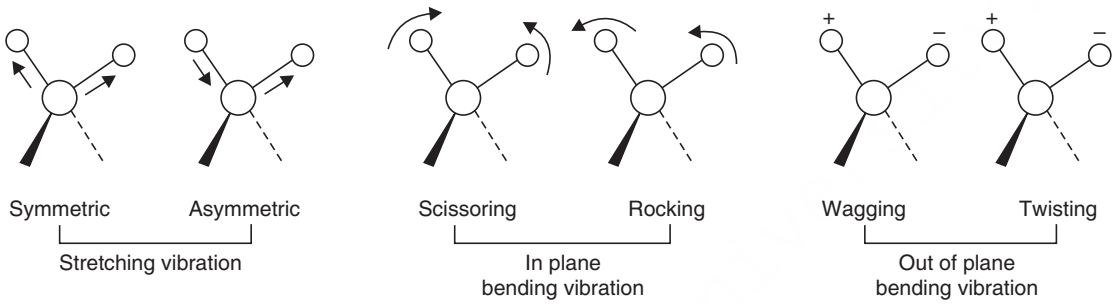


Fig. 24.5 Modes of vibration of the atoms in a molecule.

Applications of IR spectroscopy

- The IR spectrum cannot distinguish a pure sample from an impure sample.
- The progress of organic reactions can be studied with IR spectrum.
- Detection of functional groups in a molecule.
- Identity of two samples that have identical spectra, generally it occurs in Finger print region which is 1430 = 910 cm^{-1}

Examples of some stretching and bending vibrations in alkanes

			ν_{max}
C—H	stretching	—	2950 and 2820 cm^{-1}
—CH_2—	bending	—	1458 cm^{-1}
C—CH_3	bending	—	1458–1380 cm^{-1}

IR Spectrum for ether and alcohol:

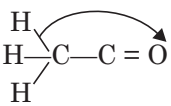
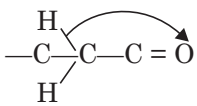
Replacement of —CH_2— in alkane by 'O' results in ether. There is appearance of C—O stretching vibration at 1110 cm^{-1} in the spectrum when an H atom of a hydrocarbon is exchanged for an '–OH' group, the spectrum changes in a very predictable way, it now shows absorptions owing to 'O—H' and 'C—O' stretching vibrations in addition to the hydrocarbon chromophoric groups present *i.e.* for alcohols:

O—H stretching vibration is at 3448 cm^{-1} (polymeric association of OH groups)

C—O stretching vibration is at 1053 cm^{-1}

In CCl_4 solution O—H stretching vibration is 3788 cm^{-1} (as the degree of association changes with dilution).

Aldehyde and ketones: IR spectra show absorption H—C = O (stretching) at 1740–20 cm^{-1} (strong); for a cyclic ketone stretching vibration is at 1725–1700 cm^{-1} (strong) (distinction from —CHO and $\rangle$C = O); C—H (stretching) in —CHO group is at 2880–2625 cm^{-1} (weak to medium)(distinction from $\rangle$C = O).

Absorption due to C—H (deformation) in  is at 1365–1355 cm^{-1} (shown by arrow) and in  is at 1435–1405 cm (strong).

Acids: IR spectra for —C(=O)—OH group, —C = O (stretching) is at 1725-1700 cm^{-1} (s) (as that of acylic $\rangle$C = O), —O—H (stretching) is at 3650-3500 cm^{-1} (s) (m) (lower than that for alcohols), stearic acid in CCl_4 solution gives ν_{max} at 2974 cm^{-1} (OH str. is hidden by C—H str), 1706 cm^{-1} for $\rangle$C = O str., C—H (def.) at 1460 cm^{-1}. Stearic acid in solid state gives λ_{max} at 2940, 1460 and 1370 cm^{-1} (bands for nujol) $\rangle$C = O stretching at 1709 and 1312, 1295, 1279, 1258, 1235, 1220 and 1188 cm^{-1}. These bands are characteristic of long chain *n*-alkyl compounds, in the solid state. The absorptions of stearic acid in CCl_4 solution and in solid state show a difference in IR spectrum of the compound.

Esters: For CH_3—C(=O)—OCH_2CH_3

ν_{max} are:

1742 cm^{-1} (C = O str. in solid aliphatic esters)

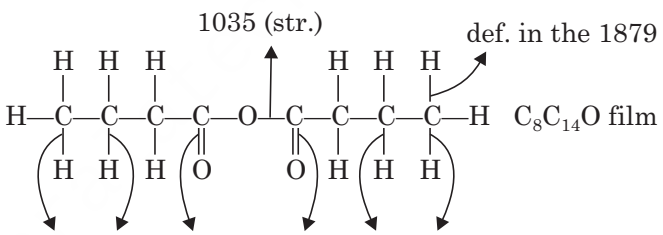
1241 cm^{-1} (C—O str. in acetate)

1370 cm^{-1} (C—H def. in CH_3)

3003 cm^{-1} (C—H str. in CH_3 or, CH_2)

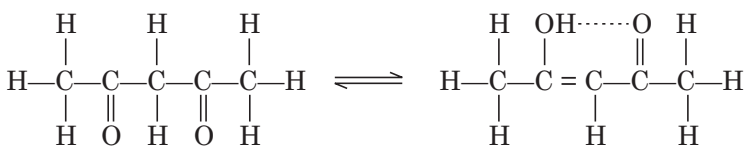
1449 cm^{-1} (C—H def. in CH_3 or, CH_2)

Acid anhydrides: C = O str. in acylic anhydride are 1840-1800 (s) and 1780-1740 cm^{-1} (s). There is also a band in the region 1180-1030 cm^{-1} (s) due to C—O str in the grouping —C—O—C—. ν_{max} for various str. and def. of bonds in an anhydride are shown below with the arrows.



All the values are in cm^{-1}.

β-diketones:



[1725-700 cm^{-1} single stretching with greater intensity (as there are two carbonyl groups) in IR spectrum]

Absorption at 1655 cm^{-1} (s) for $\rangle$C = O in enols

α, β unsaturated ketones contain grouping $\rangle$C = C—C = O (1685-1665 cm^{-1} (s)) enols contain this grouping,

but the shift to 1655 cm^{-1} that occurs in these compounds is attributed to H–bonding —O—H----O = C<. True alcoholic OH absorption band near 3700 cm^{-1} in enols are absent, but there is absorption band near 2700 cm^{-1} (*s*) which is attributed to the OH group (*s*) indicates strong absorption, (*m*) indicates medium absorption.

Amines: Primary and secondary amines contain N—H bond, both types absorb in the region 3500 – 3300 cm^{-1} (*v*) but the two are distinguished by the fact that primary amines show two bands in their region, whereas secondary amines generally show only one. If there is H-bonding, then the region is 3400 – 3100 cm^{-1} (*s*) C—N – str. 1200 – 1020 cm^{-1} in aliphatic amines (*w* – *m*), C—N – str. + 200 – 1020 cm^{-1} in aromatic amines (*s*), N—H – def 1650–1590 cm^{-1} (*s* – *m*) in primary amines, N—H – def 1650 – 1550 cm^{-1} (*s*).

Because of the overlap of these regions, it is not possible to distinguish the types of amines in this way.

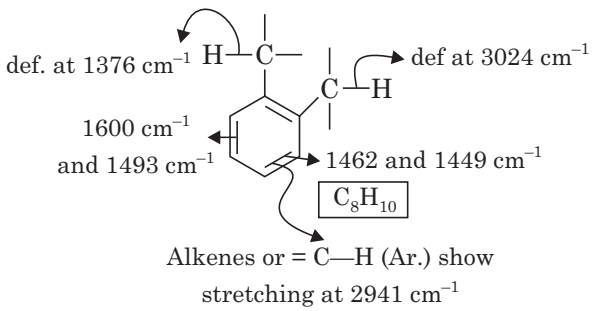
Aromatic Compounds: Aromatic compounds produce a large number of absorption bands in the IR region. The following regions are particularly useful for recognising the presence of benzene, polynuclear aromatics and benzene derivatives.

>C—H ⇒ 3080 – 3030 cm^{-1} (*w*) (str) and the bands for C = C (in plane vibration) are 1625 – 1600 cm^{-1} (*v*), 1590 – 1575 cm^{-1} (*v*) and 1525 – 1475 cm^{-1} (*v*).

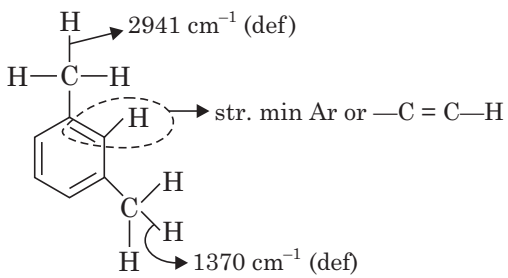
Substituent groups show very light effect on the above bands, but new bands are introduced according to orientation of the groups. However, because of a great deal of overlapping of the various bands in the region 1225–970 cm^{-1}; this region is not very useful.

On the other hand, these isomers in the aromatic system may be readily distinguished by the bands due to (C—H) (out of plane) deformation 1, 2 isomer shows ν_{max} 770 – 735 cm^{-1} (*s*) 1, 3 isomers at ν_{max} 800 – 750 cm^{-1} and 725 – 680 (m) ; 1, 4 isomer at ν_{max} 840 – 810 cm^{-1} (*s*); mono-substituted benzene ring shows ν_{max} at 770 – 730 cm^{-1} (*s*) and 710 – 690 cm^{-1} (*s*) (Two bands distinguish it from 1, 2 isomer): ν_{max} for str. and def. of the bonds are shown by the arrows.

1, 2-disubstituted benzene:



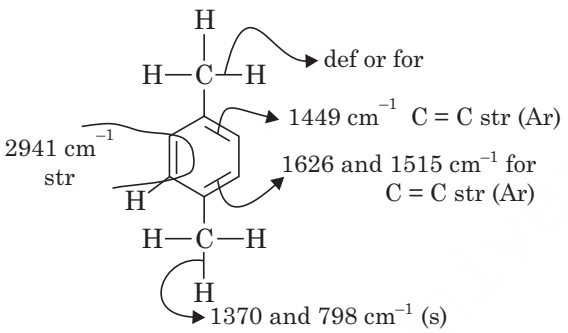
1, 3-disubstituted benzene:



C = C (Ar) str. is at 1613, 15878 and 1490 cm^{-1}

and 1450 cm^{-1} for C—H def in CH_3
and 772 and 654 cm^{-1} (*s*) for 1, 3-disubstitution

1, 4-di-substituted benzene:



1, 4 disubstituted benzene

Abbreviations: ν_{max}–wave number cm^{-1}, *s*-strong, *w*-weak, *m*-medium, str. (stretching), def. (deformation), *v* (variable)

Some IR Spectra:

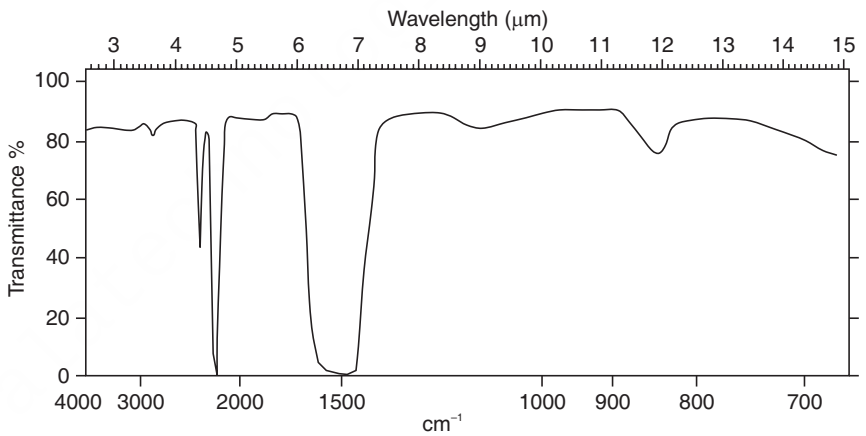


Fig. 24.6 IR spectrum of carbon disulphide (0.1 mm cell).

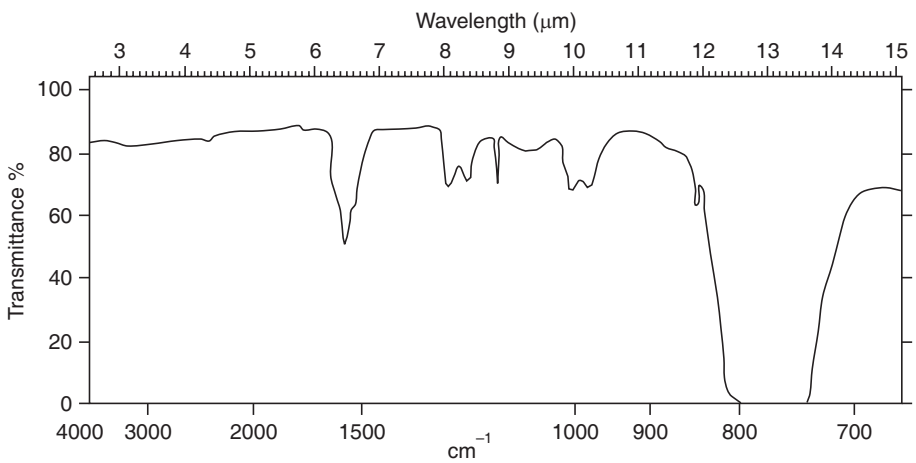


Fig. 24.7 IR spectrum of carbon tetrachloride (0.1 mm cell).

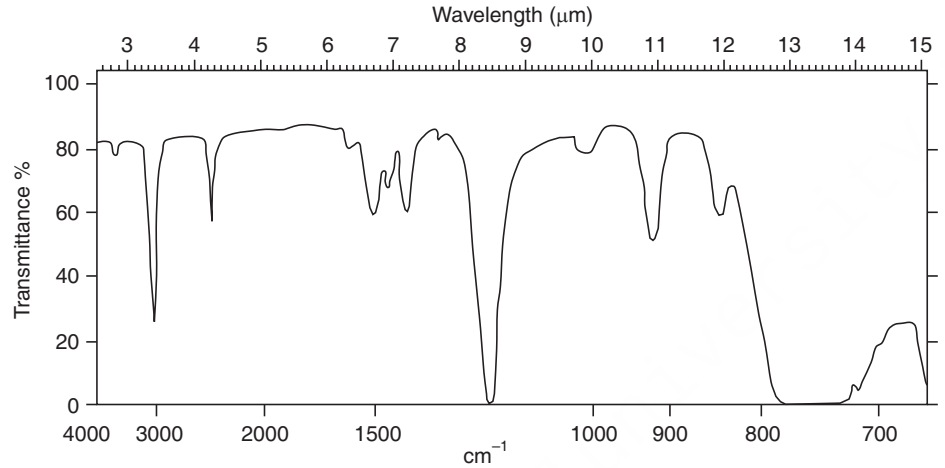


Fig. 24.8 IR spectrum of chloroform (ethanol-free) (0.1 mm cell).

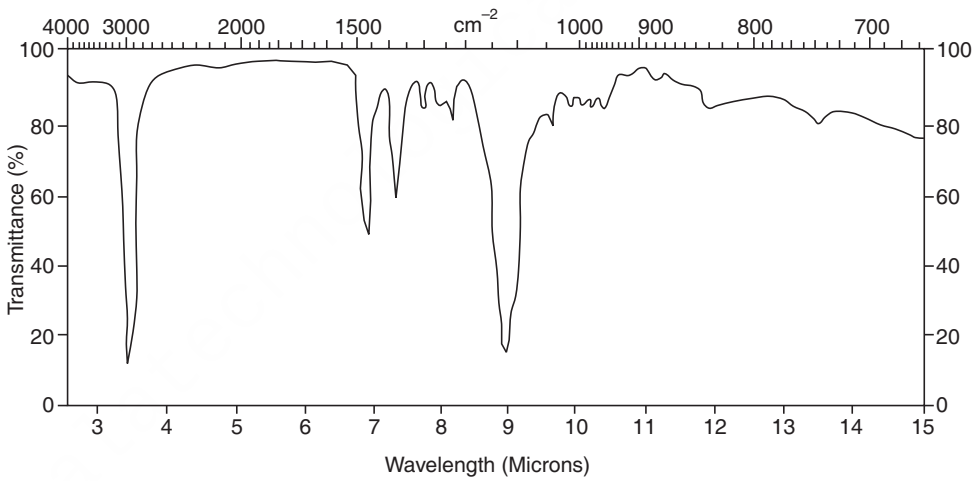


Fig. 24.9 Di-*n*-butyl ether, liquid film.

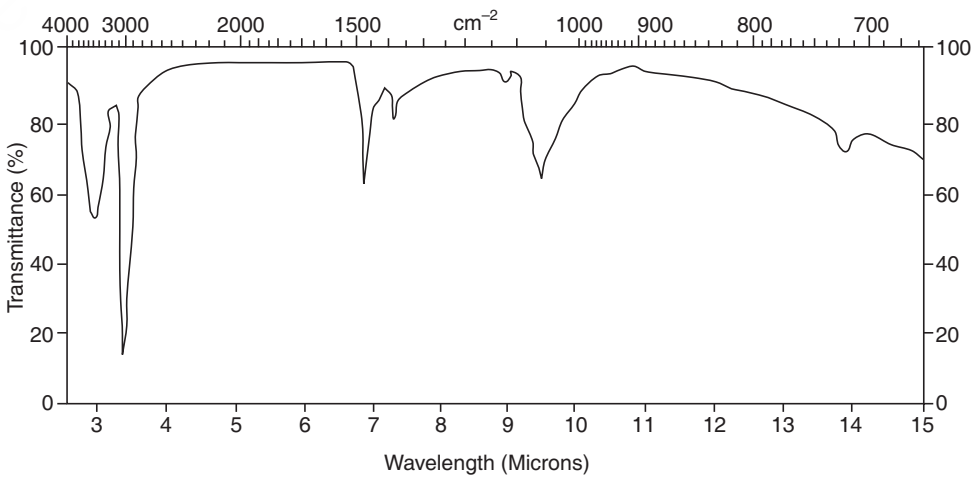


Fig. 24.10 Lauryl alcohol, liquid film.

Nuclear Magnetic Resonance (nmr) or Proton Magnetic Resonance (pmr) spectroscopy

nmr is a powerful analytical technique to evaluate the structures of carbon compounds. The technique is used (*i*) to identify unknown compounds, (*ii*) to check for impurities and (*iii*) to study the shapes of molecules. In medicine, magnetic resonance imaging uses *nmr* to detect the hydrogen nuclei in the human body, especially in water and lipids. The term 'nuclear' interprets the technique that detects nuclei of atoms such as hydrogen-1 (proton). The term 'magnetic' interprets the nuclei that act like tiny magnets that can line up either in the same direction or in the opposite direction to an external magnetic field. The term 'resonance' is the absorption of energy in the form of radio waves with the frequency corresponding to the size of energy jump as the nuclei flip from one alignment in a magnetic field to the other (Fig. 24.11).

The atomic nuclei spin about their axes. Now since a rotating charged sphere is always associated with it a magnetic moment, then all the charged particles in a nucleus will make that nucleus to behave like a tiny bar magnet, with its magnetic moment along the axis of rotation of the nucleus. The angular momentum of the spinning nucleus can be represented in terms of spin numbers (I). These numbers can attain the values 0, $\frac{1}{2}$, 1, $\frac{3}{2}$ etc. (I = 0 denotes no spin). The nuclear magnetic moment is denoted by the symbol 'μ'.

Each proton and neutron in a nucleus has its own spin and I is a resultant of these spins. If the sum of protons and neutrons is even, I is 0 or, integral (1, 2, etc.), if the sum is odd I is $\frac{1}{2}$ integral *i.e.,* $\frac{1}{2}$, $\frac{3}{2}$, $\frac{5}{2}$. If both protons and neutrons are even numbered then I is 0. That is why, both C^{12} and O^{16} have no resultant nuclear magnetic moment and give no *nmr* signal. Several nuclei H^1, F^{19}, C^{13} have a spin number I of $\frac{1}{2}$ and a uniform spherical charge distribution. Nuclei with spin number I of 1 or higher have a non-spherical charge distribution.

If a magnetic nucleus is placed in a uniform magnetic field it is seen that the magnetic dipole assumes only a discrete set of orientations. The system is said to be quantised. The magnetic nucleus can attain any one of the possible orientations indicated by (2I + 1).

Thus a proton ($I = \frac{1}{2}$) can assume only one of the two possible orientations corresponding to the energy level of $\pm \mu H_o$. One with respect to the direction of the applied magnetic field and the other is aligned against the direction of the applied field (Fig. 24.11).

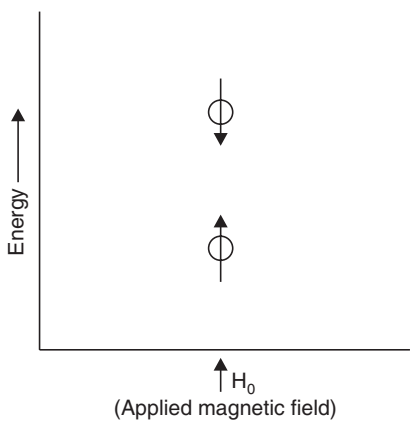


Fig. 24.11 Energy levels of a proton.

The transition of a proton from one possible orientation to another is effected by the absorption or emission of a discrete amount of energy such that,

$$E = h\nu = 2\,\mu H_o$$

where, ν = frequency of electromagnetic radion absorbed or emitted

h = Planck's constant

μ = nuclear magnetic moment

H_o = applied magnetic field.

For protons if a magnetic field 14000 Gauss is applied the frequency of such energy remains in the radio frequency region (60 megacycles per second).

Unless the axis of the nuclear magnet is oriented exactly parallel or antiparallel with the applied magnetic field (Fig. 24.11), there will be a certain force by external magnetic field to so orient the spinning nucleus. But because the nucleus is spinning, the effect is that its rotational axis draws a circle perpendicular to the applied field. Such an effect is shown in Fig. 24.12. This motion of the nucleus is called **Precession**.

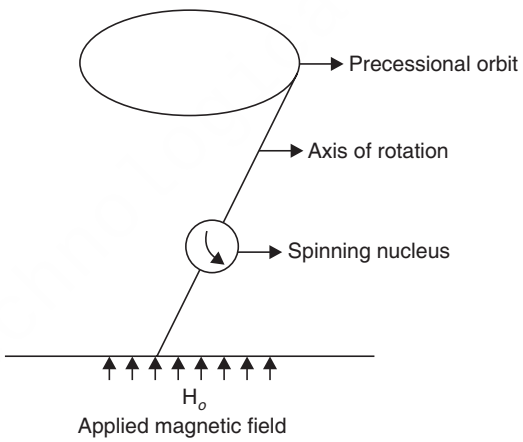


Fig. 24.12 Precession.

Theory of nuclear resonance

A proton in a static external magnetic field may assume only two possible orientations corresponding to the energies of $\pm\,\mu H_o$. The low energy orientation corresponds to that state in which the nuclear magnetic moment is aligned parallel to the external magnetic field, and the high energy orientation corresponds to that state in which the nuclear magnetic moment is aligned antiparallel (opposed) to the external magnetic field. It is possible to the induce transitions between these two orientations. The frequency ν of electromagnetic radiation necessary for such a transition is given by

$$\nu = 2\mu H_o/h$$

where H_o is the strength of the external or applied magnetic field and h is Planck's constant.

The precessional frequency of the spinning nucleus *i.e.,* nuclear magnet is exactly equal to the frequency of electromagnetic radiation necessary to induce a transition from one nuclear spin state to another. The nuclear transition corresponds to a change in the angle that the axis of the nuclear magnet makes with the applied magnetic field. This change can be brought about through the application of electromagnetic radiation whose magnetic vector component is rotating in a plane perpendicular to the main magnetic field. When the frequency of the

rotating magnetic field and the frequency of the precessing nucleus become equal, they are said to be in resonance, and the absorption or emission of energy by the spinning nucleus then occurs. Thus a **nuclear resonance** will occur when a nucleus (I > 0) is placed in a stable magnetic field and subjected to the electromagnetic radiation of appropriate energy.

The electromagnetic radiation is supplied by an oscillator with its magnetic field at right angles to the applied field and since the position of absorption peak, that is, where resonance occurs, depends on the frequency of the oscillator or the strength of the applied magnetic field. It is possible to change from the lower to higher energy level by employing a variable frequency with a fixed applied magnetic field or vice versa. In practice it is easier to vary the magnetic field rather than the frequency, the result is the *nmr* spectra which is usually a graph of signal intensity (ordinate) against the magnetic field (abscissa) expressed in milligauss at a fixed frequency (Fig. 24.17).

Instrumentation

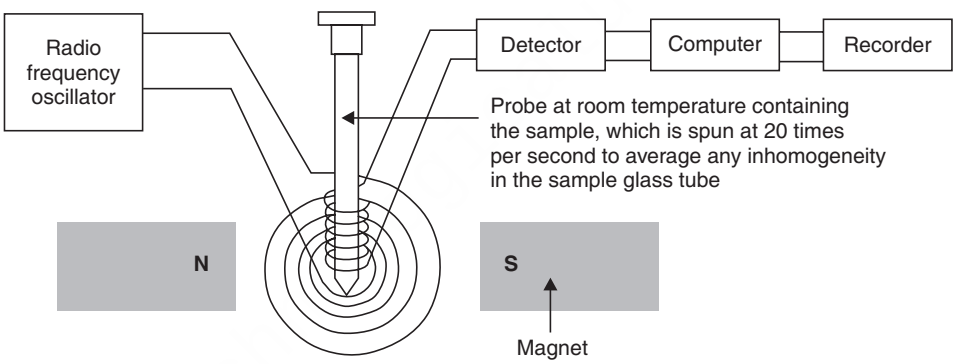


Fig. 24.13 Diagram of an *nmr* spectrometer.

Shielding, Deshielding and Chemical Shift

It might be expected that the resonance frequency for a given magnetic field depends only on the nature of atomic nucleus concerned. This, however, is not the case. The applied magnetic field causes electrons surrounding a nucleus to circulate in a plane perpendicular to the applied field, and this generates electric current to produce a magnetic field in opposition to the applied magnetic field (Fig. 24.13). Thus the effective magnetic field 'H' experienced by the nucleus is smaller than the applied magnetic field (H_o). The relationship between the two is expressed as:

$H = H_o(1 - \sigma)$ σ is called the **shielding or, screening constant** (which is nondimensional) and has a positive value. But it may be negative *i.e.,* then the effective magnetic field is larger than the applied magnetic field to orient the nuclear magnetic. In that case the proton is said to be **deshielded**. Since the numerical value of σ depends on the chemical environment of a given nucleus, the shielding or deshielding varies with its electronic environment. Then at a given radiofrequency, all protons absorb at the same effective field strength, but they absorb at different applied field strengths.

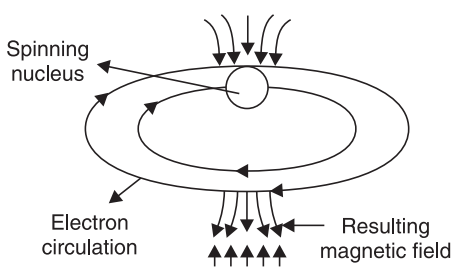


Fig. 24.14 Diamagnetic electronic circulation about a nucleus.

Each set of equivalent protons in a compound will have a slightly different electronic environment from other set of protons and hence will absorb at a slightly different applied field strength to produce the same effective field strength which can cause the 'resonance' to occur.

Shielding causes a shift of the resonance frequency to higher values of the applied field (H_o), that is , the shift is **upfield**. On the other hand, deshielding causes a shift of the resonance frequency to lower values of the applied field, that is, the shift is **downfield**. The magnitude of this shift is called **chemical shift**. Since the value of the applied field experienced by the organic compound cannot be determined accurately, chemical shifts are measured relative to some standard compound which contains the nucleus under consideration. Tetramethylsilane [TMS, $(CH_3)_4Si$] is particularly useful for proton magnetic resonance (*pmr*). TMS contains twelve equivalent protons. The *pmr* spectrum of this compound shows a single sharp line which occurs at higher field than any other protons in most of the common organic compounds. That is, most *pmr* signals occur downfield with respect to TMS.

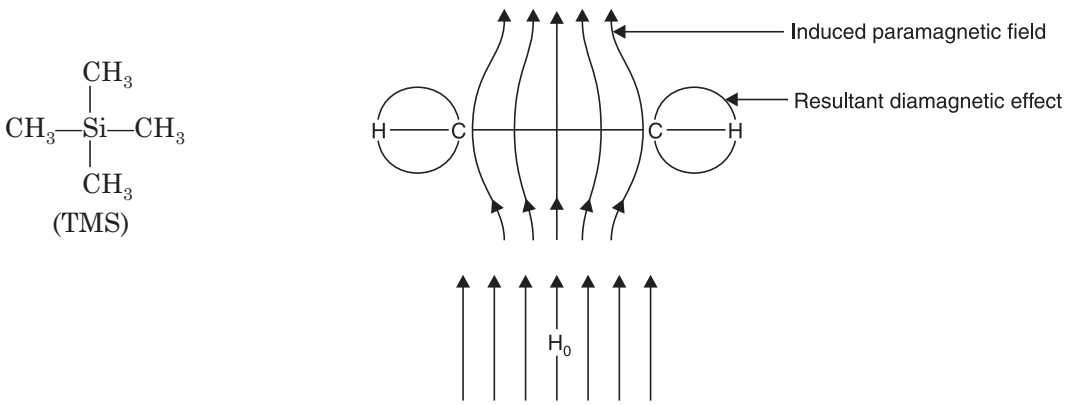


Fig. 24.15 Shielding of an acetylenic proton in terms of paramagnetic effects.

The chemical shift may be reported in various ways. The resonance frequency of the proton of the compound is dependent on the strength of the applied magnetic field. The shift may be reported as units of the magnetic field in milligauss. However, the magnetic field can also be expressed as Hertz (Hz) or cycles per second (cps). The separation in Hz is also proportional to the frequency of the oscillator, *e.g.*, if the separation between a proton signal and signal of TMS is 60 Hz at 40 MHz the separation at 60 MHz will be 90 Hz. Therefore it is desirable to repart chemical shifts in such units that are independent of the operating conditions of the spectrophotometer. This can be done very easily by defining chemical shift δ by the expression

$$\delta = \frac{\text{Separation in Hz}}{\text{Oscillator frequency}} \times 10^6$$

The factor 10^6 is introduced in order to record the chemical shift as a convenient value. This is usually in the range 1-10 and is expressed in ppm.

The dependence of the chemical shift of the oscillator frequency is shown in the following example,

$$\delta = \frac{60}{40 \times 10^6} \times 10^6 = 1.5 \text{ ppm.}$$

$$\delta = \frac{90 \times 10^6}{60 \times 10^6} = 1.5 \text{ ppm.}$$

It is now becoming common practice to express chemical shifts in τ (tau) values, defined by:

$$\tau = 10\text{-}\delta.$$

10 ppm is assigned to the line of TMS in *nmr* spectrum. Most protons have positive τ value *i.e.,* $\delta < 10$. Strong acidic protons have negative τ value *i.e.,* $\delta > 10$.

Position of signals (chemical shifts)

The greater the shielding of nucleus the larger is τ value (the smaller is δ). Since the degree of shielding depends on the electron density surrounding the proton any structural feature that decreases this electron density causes a decrease in shielding (deshielding) with consequent lowering of the τ value (the chemical shifts) movesdown field. Thus electronegativity

		τ(ppm)			τ(ppm)
CH_3I		7.83	CH_3—C		9.12
CH_3Br	deshielding	7.35	CH_3—N		7.85
CH_3Cl	↓	6.98	CH_3—O	↓	6.70
CH_3F		5.70			

of the atoms attached in the compounds in the column gradually increases as such there is gradual deshielding and τ value decreases in the column.

Si is less electronegative than carbon, so the protons of CH_3 groups in TMS are more shielded than those in C—CH_3.

Examples of Shielding and Deshielding

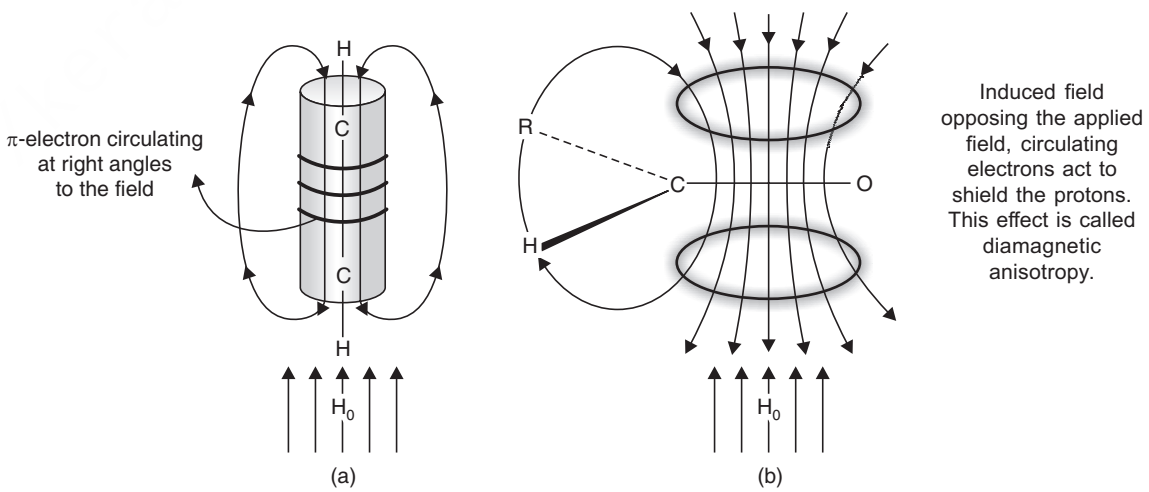


Fig. 24.16 (*a*) Shielding of an acetylenic proton and (*b*) deshielding of an aldehydic proton in terms of diamagnetic anisotropic effects.

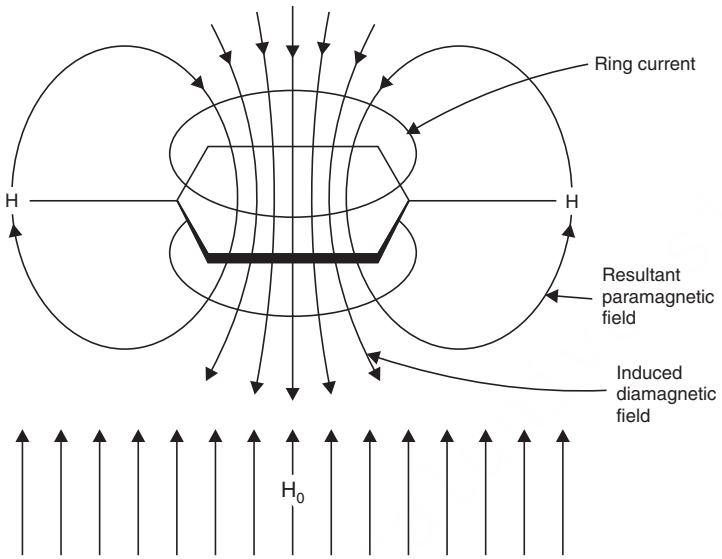


Fig. 24.17 The deshielding of aromatic protons due to a ring current effect.

The nuclear magnetic spectrum

At a given radio frequency, all the protons will absorb at a same effective field strength, but they also absorb at different applied field strengths. The applied field strength is measured and plotted against the absorption of radiation; the results are signals and the spectrum is called a nuclear magnetic resonance spectrum (Fig. 24.18(*a*) and (*b*)).

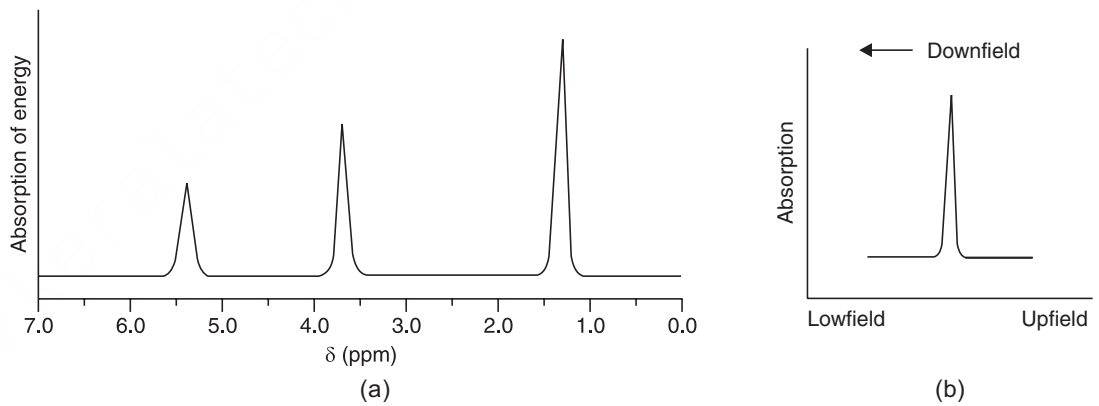


Fig. 24.18 (*a*) *NMR* spectrum for ethanol at low resolution, (*b*) a signal.

Informations predicted by the spectrum

- The number of signals indicate the different types or set of protons present in the molecule.
- The positions of signals predict something about the electronic environment of each kind of proton.
- The intensities of signals indicate how many protons of each kind is present.
- The splitting of the signals into several peaks indicates the environment of a proton with respect to other nearby protons.

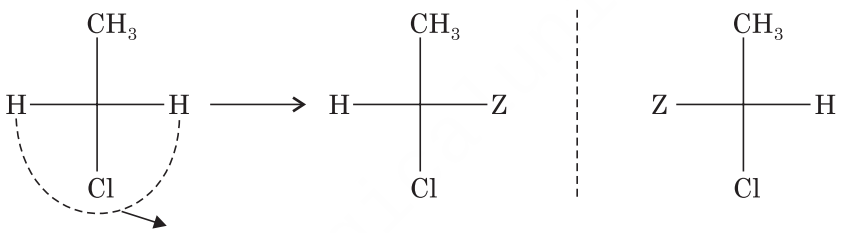
Number of signals

A set of protons with the same environment are said to be equivalent. The number of signals in the *nmr* spectrum tells us, therefore, how many sets of equivalent protons, how many kinds of protons.

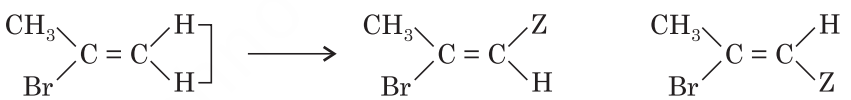
Judging the equivalent protons

Each proton in a molecule is in turn to be replaced by some other atom Z. If replacement of either of two protons by Z would yield the same product or enantiomeric products—then the two protons are chemically and magnetically equivalent.

Example. CH_3 CH_2—Cl (CH_3 protons are non-equivalent to the CH_2 protons)

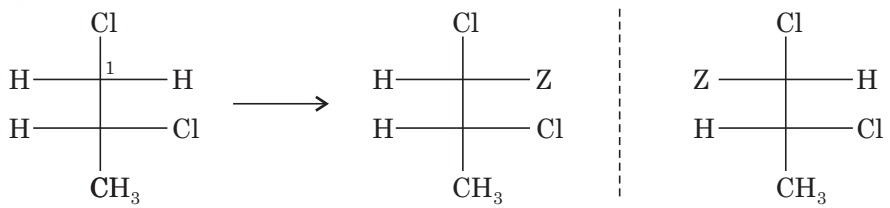


Enantiomeric protons (enantiomeric pairs)
Magnetically equivalent (one *nmr* signal) for the two protons



Diastereomeric
proton pair

The environment of these two protons are neither identical nor mirror images of each other, magnetically these protons are non-equivalent and we expect the *nmr* signal from each one.

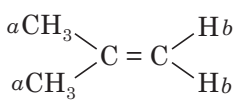


1, 2-dichloropropane (*a*) (*b*)

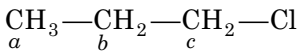
C–1 are diastereomeric, magnetically non-equivalent and give separate *nmr* signals

Examples: The number of signals indicate the presence of the number of sets of magnetically nonequivalent protons in the molecule of the compound.

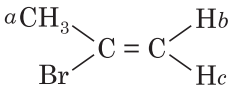
$\underset{a}{CH_3}—\underset{b}{CHCl}—\underset{a}{CH_3}$ → 2 nmr signals.



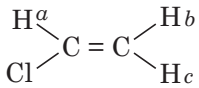
→ 2 nmr signals.



→ 3 nmr signals.

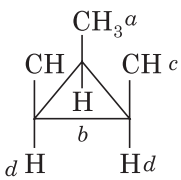


→ 3 nmr signals.



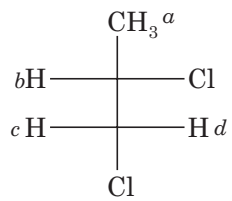
→ 3 nmr signals.

Vinyl chloride



→ 4 nmr signals.

Methyl cyclopropane



→ 4 nmr signals.

1, 2-dichloropropane

Peak area and Proton counting of a Compound

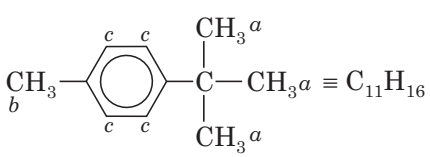
Total step heights (Fig. 24.19) for 16 protons of the compound 1, 4-methyl tertiary butylbenzene = $\underset{a}{8.8} + \underset{b}{2.9} + \underset{c}{3.8}$ = 15.5 units *i.e.,* the number of squares within an integration in an *nmr* spectra (Fig. 24.19) for the compound, $C_{11}H_{16}$ then $\frac{16H}{15.5}$ = 1.03 H is the number of protons per unit. Then number of *a* protons, *b* protons and *c* protons in the compound are:

$aH = 1.03 \times 8.8 = 9.1$

$bH = 2.9 \times 1.03 = 3$

$cH = 1.03 \times 3.8 = 3.914$

i.e.,



≡ $C_{11}H_{16}$

(I)

1,4-Methyl tertiary butylbenzene

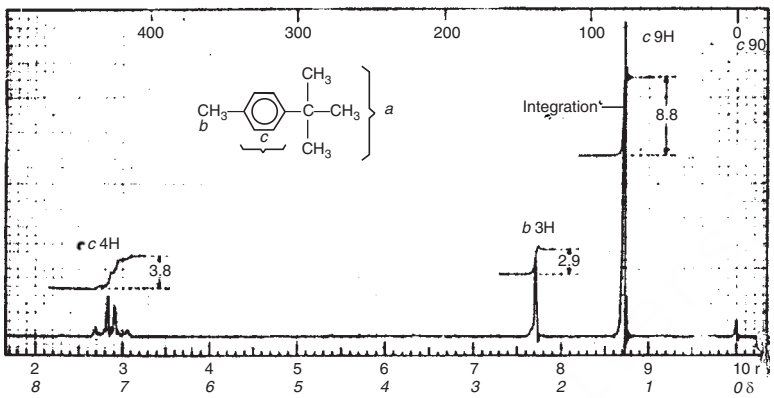
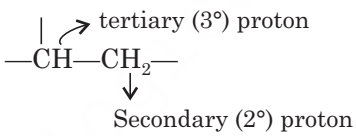
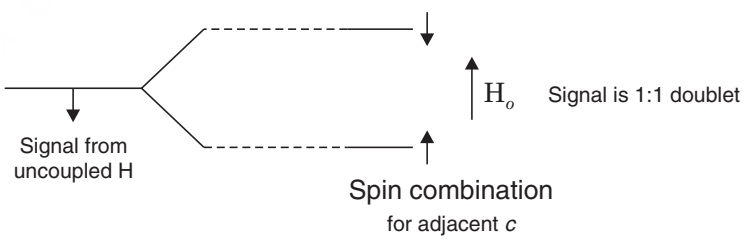


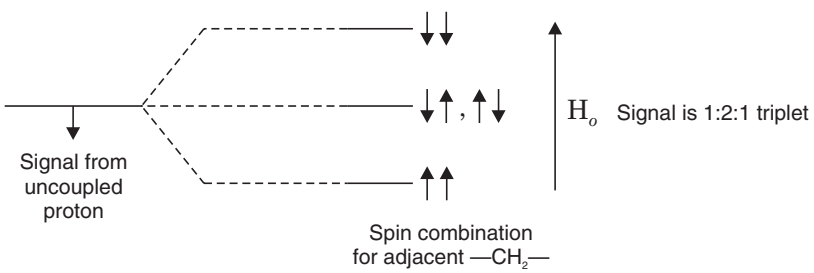
Fig. 24.19 NMR spectrum of 1, 4-methyl tertiary butylbenzene (Proton counting).

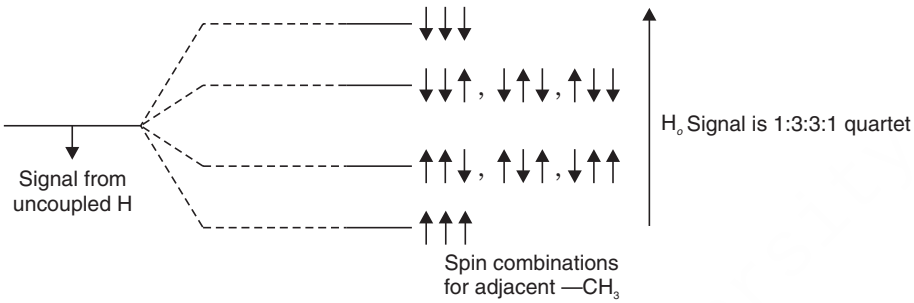
Splitting of signals; Spin–spin coupling



The magnetic field that a 2° proton (*s*H) feels at a particular instant during nmr is slightly increased tertiary or slightly decreased by the spin of neighbouring tertiary proton (*t*.H), increased if the *t*.H happens at that instant to be aligned with the applied field; or decreased if the *t*.H happens to be aligned against the applied field for half of the molecules. Then, absorption by a *s*H is shifted slightly downfield and for another half of the molecules the absorption is shifted slightly upfield. The signal is split into two peaks a doublet with equal peak intensities.







Examples:

Compound (A) $CHBr_2—CH_2Br$

$(n = 1)$ — Downfield triplet $(n + 1)$

$(n = 2)$ — Upfield doublet $(n + 1)$

Compound (B) $CH_3—CHBr_2$

$(n = 3)$ — Downfield quartet

$(n = 1)$ — Upfield doublet

Signal splitting is determined by the number of neighbouring protons (n). The number of peaks $b(n + 1)$.

Peak area reflects the number of absorbing protons and the multiplicity of splittings reflects the number of neighbouring protons (Figs. 24.19 and 24.20).

We may expect to observe spin splitting only gives the non-equivalent neighbouring protons. By non-equivalent protons, we mean protons with different 'chemical shifts'. By neighbouring protons we mean most commonly protons on adjacent carbons.

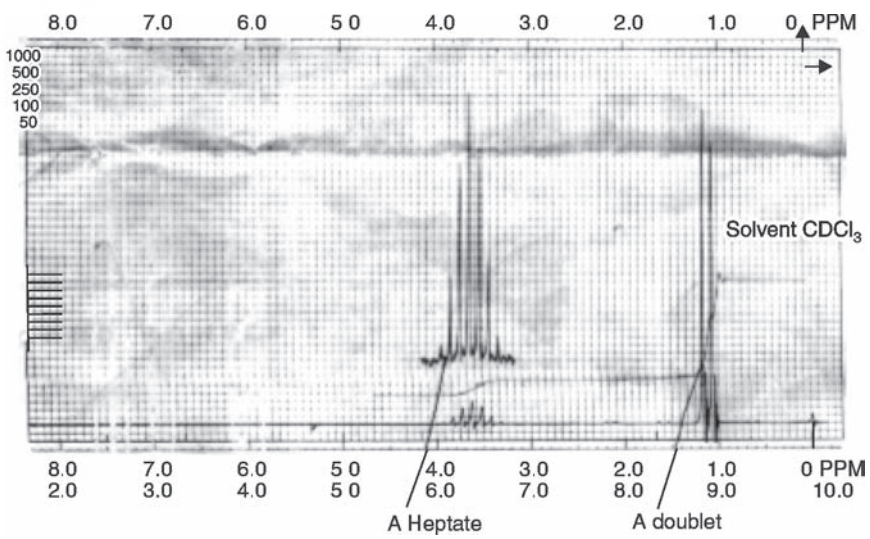


FIg. 24.20 The observed multiplicity of a given group of equivalent protons clearly depends on the number of proton on adjacent atom. The multiplicity of a given group by the expression $(n + 1)$, where n is the number of protons on adjacent atoms.

Mass spectrometry

Introduction: Mass spectrometry is an accurate instrumental technique for determination of **relative atomic masses** and relative molecular masses. **Mass spectrometry can also help to determine the molecular structures and to identify unknown compounds.**

Basic principles

In the simplest mass spectrometer, an organic molecule is bombarded with electrons and is converted to highly energetic positively charged ions (molecular ions or parent ions, $M^{+\cdot}$), which break up into smaller ions (daughter ions). The loss of an electron from a molecule leads to a radical cation ($M^{+\cdot}$); and the whole process can be represented as follows:

$$\underset{\text{(Organic molecule)}}{M} \longrightarrow \underset{\text{(Molecule ion)}}{M^{+\cdot}} + 2e$$

The molecule ion ($M^{+\cdot}$) generally decomposes to a **pair of fragments**, which may be either radical plus or a small molecule plus a radical cation. Thus,

$$\underset{\text{(Molecular ion)}}{M^{+\cdot}} \longrightarrow \underset{\text{(ion)}}{m_1^{+}} + \underset{\text{(radical)}}{m_2^{\cdot}} \quad \text{or} \quad \underset{\text{(radical cation)}}{m_1^{+\cdot}} + \underset{\text{(small molecule)}}{m_2}$$

The molecular ions, the fragment ions and fragment radical ions are separated by deflection in a variable magnetic field according to this mass and charge, and generate a current (the ion current) at the collector in proportion to their relative abundances. **A mass spectrum is a plot of relative abundance against the ratio mass to charge (the m/z value).** For singly charged ions, the lower the mass the more easily is the ions deflected in the magnetic field. Doubly charged ions are occasionally formed, they are deflected much more than singly charged ions of the same mass; and they appear in the mass spectrum at the same value as singly charged ions of the half, since,

$$\frac{2m}{2z} = \frac{m}{z}$$

Neutral particles produced in the fragmentation, whether uncharged molecules (m_2) or radicals ($m_2^{\cdot}$) cannot be detected directly in the mass spectrometer.

Instrumentation

There is a high vacuum inside a mass spectrometer to produce and study ionised atoms and molecules including fragments of molecules.

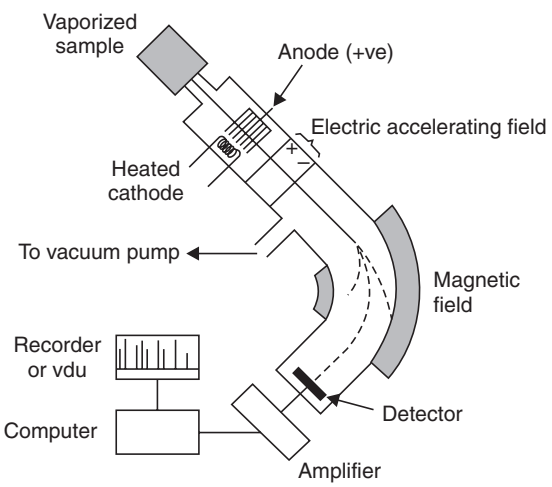


Fig. 24.21 Diagram of a mass spectrometer.

Schematic representation for production of a mass spectrum:

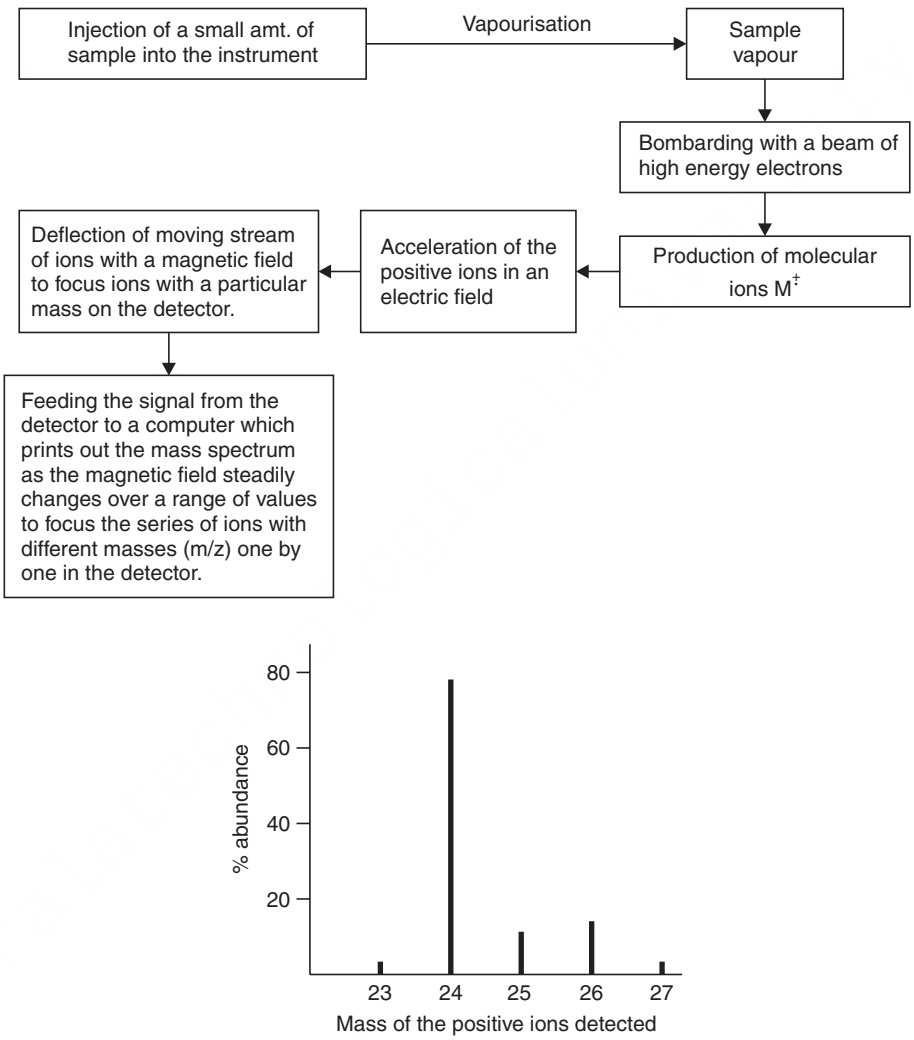


Fig. 24.22 Mass spectrum of magnesium.

The instrument needs calibration using a reference compound with a known structure and molecular mass so that the computer can print a scale on the mass spectrum.

The mass spectrum for an element displays the relative abundance of different isotopes of the element. This makes it possible to calculate the relative atomic mass for the element (see Fig. 24.22).

Applications

- **Structure elucidation**

When a molecular compound is being analysed, the peak of the ion with highest mass is usually the whole molecule ionised is known as 'molecular ion' or parent ion ($M^{+\cdot}$), which is the relative mass (molecular weight) of the compound.

Nitrogen rule. If a molecular ion has an even molecular weight, it possesses either no nitrogen or an even number of nitrogen atoms. An odd molecular weight compound requires an odd number of nitrogen atoms.

The **base peak** is the most intense line of the spectrum.

Metastable ions. Fragmentation ($m_1^+ \rightarrow m_2^+ + m_3$) in general occurs in the ion source of the mass spectrometer before the positive ions are accelerated and therefore a distinct peak results for each fragmentation.

So mass spectrum is a 'fragmentation pattern'. The computer has a database of mass spectra so it can identify an unknown compound by matching its spectrum with one in its database.

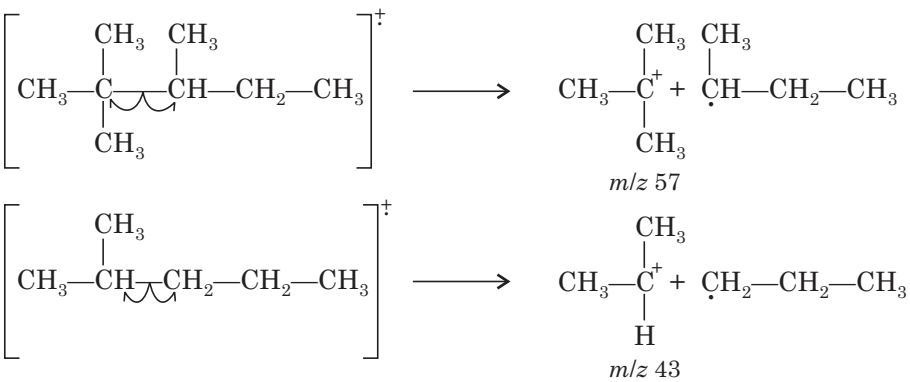
A chemist who synthesises new compounds can study their fragmentation pattern in a mass spectrometer and determine its structure with the help of other spectra like infrared spectroscopy (i.r.) and nuclear magnetic resonance (n.m.r.).

The combination of gas-liquid chromatography (glc) with mass spectrometry is of great importance in modern chemical analysis. First glc separates the components in an unknown mixture, such as a sample of polluted water, then mass spectrometry (ms) detects and identifies the components.

Highlights:

- The parent peak ($M^{+\cdot}$) is the most intense in straight-chain compounds, the intensity reduces with increased chain branching.
- In a homologous series, the intensity of M^+ decreases with increase in molecular weight.
- In branched chain hydrocarbons cleavage is preferred at the bond adjacent to the branch; thus giving rise to tertiary (3°) carbocations as 3° is more stable than 2°, which in turn is more stable than 1° carbocations.
- During representing the fragmentation, a one electron shift is represented by a 'fish hook' arrow ' ↷ ' and a two electron shift in the usual manner by ' ↷ ' .

Examples. For Methylpentanes:

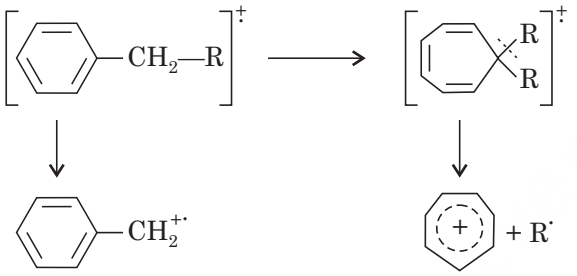


m/z 57

m/z 43

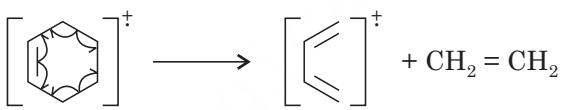
- The probability of the existence of a strong $M^{+\cdot}$ peak is high for unsaturated or cyclic systems present in the molecule. As in carbocation chemistry the carbocations formed are stabilised.

- Alkylbenzenes cleave at the C–C bond β to the aromatic ring resulting in a highly stabilised carbocation. Most alkylbenzenes give the more stable tropolium ion (I) rather than the Benzyl cation (II).



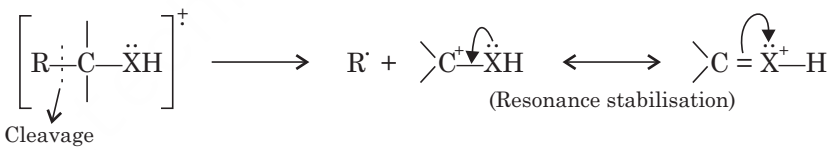
(II) (I)

- Cyclic alkenes often undergo generally a **retro-Diels-Alder fission**.



m/z 54

- Compounds such as alcohols, mercaptans, amines, esters cleave at C—C— bond β to the hetero atom.



SHORT QUESTIONS AND ANSWERS

Q. 1. What is atomic spectroscopy?

Ans. Ground state of an atom means it is with normal electronic configuration. In this state the atom remains in its lowest energy state and this is the most stable state of the atom. **Excited state of an atom** refers to the electronic configuration availed by an atom after absorbing certain definite amount of energy. The valence electrons are promoted to some higher permitted energy level by absorption of energy. In the excited state, the atom is unstable and the excited electron tends to come back to the original position *i.e.,* ground state. After about 10^{-4} sec. the electron returns to the ground state by emitting the amount of energy absorbed during excitation. The energy is emitted or absorbed in the form of electromagnetic waves of definite frequency *i.e*., of definite wavelengths. This process of excitation and return to the ground state is the basis of spectroscopic analysis of atomic absorption, emission and atomic fluorescence.

Q. 2. What is atomic absorption spectroscopy?

Ans. This is the analytical technique based on the phenomenon of light absorption (UV or visible). It is applicable both to qualitative and quantitative analyses.

Q. 3. What are the parameters for expressing the absorption?

Ans. Transmittance is the ratio of the intensity of light transmitted to the intensity of incident light.

$$\text{(Transmittance)} \quad T = \frac{I}{I_o} \qquad T\% \text{ (percent transmittance)} = \frac{100 \times I}{I_o}$$

$$A = \log_{10} \frac{I_o}{I} \quad \therefore \quad A = 2 - \log T\%$$

where A = Absorbance, I_o = Intensity of incident light,

I = Intensity of transmitted light.

· Combination of Lambert–Beer's law given

$$A = A_{1\,cm}^{1\%}\, bc.$$

A = Absorbance, $A_{1\,cm}^{1\%}$ = Absorbance of 1% (*w*/*v*) solution for path length of 1 cm,

b = thickness (cm), *c* = concentration (g/100 ml)

$$\varepsilon \text{ (molar extinction coefficient)} = \frac{A_{1\,cm}^{1\%} \times \text{molecular wt.}}{10}.$$

Q. 4. Calculate the concentration of a substance A in an ethanolic solution of which the absorbance in a 1 cm cell at its λ_{max} 241 nm was found to be 0.890. The A (1%, 1 cm) is 540 at 241 nm.

Ans. $A = A_{1\,cm}^{1\%}\, bc.$

$$0.890 = 540 \times 1 \times c$$

$\therefore$ C = 0.00165 g/100 ml.

Q. 5. Calculate the concentration in μg ml^{-1} of a solution of substance B (mol.wt. 204.2) in 0.1 (M) HCl, showing absorbance at its λ_{max} 277 nm of 0.613 in a 4 cm cell. The molar absorptivity (ε) at 277 nm is 5432.

Ans. Here the working formula is $A = \varepsilon bc$.

$$0.613 = 5432 \times 4 \times c$$

$\therefore$ $c = 2.82 \times 10^{-5}$ mol l^{-1}

$= 2.82 \times 10^{-5} \times 204.2$ gl^{-1}

$= 0.00576$ gl^{-1}

$= 5.76$ μg ml^{-1}.

Q. 6. What is molecular spectroscopy?

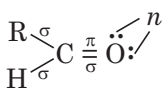
Ans. The internal energy (E) of a molecule is given by:

E = Kinetic energy + Potential energy + E_{rot} + $E_{vib.}$ + E_{trans}.

The molecular spectroscopy mainly deals with the study of interaction between radiant energy and the molecule. In general molecular spectroscopy is absorption spectroscopy.

Q. 7. What are the different electronic transitions that take place on absorption of UV light?

Ans. When a molecule, specially organic molecule, absorbs UV radiations the electrons are excited to higher energy levels. In the diagram below the electrons are represented



The following electron transitions take place:

$\sigma \rightarrow \sigma^*$ (antibonding), $n \rightarrow \sigma^*$, $n - \pi^*$ (antibonding)

$\pi \rightarrow \pi^*$

These electronic transitions are responsible for UV absorption of a molecule.

Q. 8. Mention the applications of UV.

Ans. (*a*) **Qualitative:**

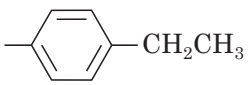
- Detection of conjugation
- Detection of functional groups
- Detection of geometrical isomers.

(*b*) **Quantitative:**

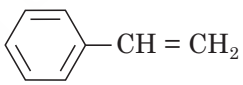
- Analysis of various samples (drugs, dyes etc.)

Q. 9. Can UV differentiate ethylbenzene from styrene?

Ans.



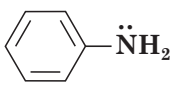
Ethylbenzene

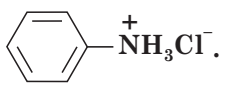


Styrene

Extended conjugation is maximum in case of styrene. So styrene will absorb at a higher wavelength.

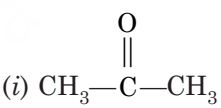
Q. 10. Which of the following compounds will have greater λ_{max}?

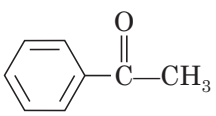
(*i*) 

(*ii*) 

Ans. Compound (*i*) shows greater λ_{max} value as the lone pair on N atom comes into resonance with the benzene ring to extend the conjugation. In (*ii*) there is no such electron pair.

Q. 11. Which of the following compounds will have greater λ_{max}?

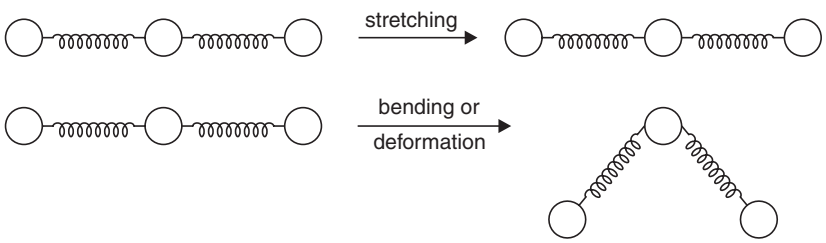
Ans. (*i*) 

(*ii*) 

Compound (*ii*) will show greater λ_{max} as there is conjugation in benzene ring as well as C = O group remains in conjugation with the benzene ring.

Q. 12. What are the principles of IR?

Ans. The atoms in a molecule bond are in a state of constant vibration and rotation. They may be compared with two balls (atoms) joined by spring (bond). On absorption of IR the bond may stretch, bend etc., as shown below. So stretching and bonding of bonds are responsible for IR absorption.



Q. 13. What is the range of infrared radiations? Correlate the different units used in I.R. spectroscopy.

Ans. I.R. region lies between 0.8 to 20 μm or 800–20,000 nm of electromagnetic radiation. The region between 0.8 to 2.5 μm is called the near I.R. region and 15 to 20 μm is called far I.R. region. The region between 2.5 – 1.5 μm is the proper I.R. region.

Generally wave number ν (Nu) which is the number of vibrations of the radiation per cm.

The correlation between the different units used in I.R. is given below:

$$1\ \mu m = 10^{-6}\ m = 10^{-4}\ cm$$

So, $$\nu = \frac{1}{\lambda\ \mu m} = \frac{10^4}{\lambda}\ cm^{-1}$$

Q. 14. What are functional group and fingerprint region in I.R.?

Ans. The **functional group region** is 2.5 to 7.4 μm (4000–1430 cm^{-1}). In this region functional groups of organic compound is detected. The **fingerprint region** is 7–11 μm (1430 – 910 cm^{-1}) gives a good deal of information about the molecule besides the functional groups of the compound. The fingerprint region can lead us to identify an organic compound.

Q. 15. How is an I.R. spectrum recorded?

Ans. (*a*) **IR source:** A Nernst glower, a rod of an allow of Zirconium, Yttrium and Erbium oxides. The rod is electrically heated to 1750 K.

(*b*) Rock salt disc or KBr disc is used as glass and quartz absorb I.R.

(*c*) **Sample preparation:** Either the sample with KBr is made to pellet or Nujol mull is used. Nujol is hydrocarbon in nature.

(*d*) **Recording of spectra:** The sample is placed in Rock salt cell in the path of I.R. The change of intensity of light transmitted draws a graph which is IR spectrum.

Q. 16. How will you distinguish CH_3COOH from CH_3COCH_3 with the help of I.R. spectra?

Ans. $>C = O$ (str.) peaks will be observed in both the spectra in the region of 1700 cm^{-1}. But an absorption bond at 2500–3000 cm^{-1} (broad) will be observed in spectrum of CH_3COOH due to dimeric association of CH_3COOH molecules through hydrogen bonding.

Q. 17. Give the structure of molecular formula C_3H_8O from the following data: I.R. (Nujol) ν_{max} – 2950 and 2820 cm^{-1}, 1110 cm^{-1}.

Ans. Three isomers for the molecular formula are:

(*i*) $CH_3CH_2\ CH_2OH$, (*ii*) $CH_3CHOH\ CH_3$ and (*iii*) $CH_3O\ CH_2CH_3$

As there is no peak near 3300 cm^{-1} the first two are ruled out. The peak at 1110 cm^{-1} is C—O (str.) for ether linkage and 2950 and 2820 cm^{-1} for C—H (str). So the compound is $CH_3OCH_2CH_3$.

Q. 18. What is nuclear magnetic resonance (n.m.r.)?

Ans. Protons and neutrons in the nucleus spin. If the particles in a nucleus don't have their spins paired, there is a net spin. Charged particles like proton when spin will generate magnetic field and magnetic moment along the axis of spin. Thus a proton or nucleus acts like a tiny bar magnet. Nuclei with even mass numbers ${}_8C^{12}$, ${}_8O^{16}$ have no resultant spin and hence the magnetic property. The magnetic property of the nucleus is responsible for nuclear magnetic resonance. The precissional frequency of the spinning nucleus when equals the frequency of the applied magnetic field (electromagnetic radiation), the nucleus changes its spin state from

one to the other and the absorption or emission of energy then takes place by the nucleus and nuclear resonance then occurs. The absorption of energy is recorded in a graph and is known as n.m.r.

Q. 19. What are equivalent and non-equivalent protons in n.m.r.?

Ans. Protons present in a molecule having the same environment absorb at the same magnetic field strength. Such protons are called equivalent protons.

And protons which have different environments in a molecule absorb at different magnetic fields, such protons are called non-equivalent protons. All equivalent protons give one signal in the n.m.r. spectrum. Non-equivalent protons give different signals.

Q. 20. Give a schematic diagram of pmr spectrum.

Ans. See text.

Q. 21. Identify equivalent and non-equivalent protons in the following molecules and hence the number of signals and splitting.

(*i*) $CH_3—CBr_2—CH_3$ **and** (*ii*) CH_3CH_2Cl

Ans. (*i*) $\overset{a}{C}H_3\,CBr_2\,\overset{a}{C}H_3$

All are equivalent protons. So n.m.r. spectrum will show one signal *i.e.*, a 6H, *s* (six proton, singlet)

(*ii*) $\underset{2}{\overset{a}{C}H_3}\,\underset{1}{\overset{b}{C}H_2}\,Cl$

2 protons in C—1 are equivalent and 3 protons in the C—2 are equivalent; but the protons of C—1 and C—2 are non-equivalent as designated by '*a*' and '*b*'. So two signals are obtained in the n.m.r. spectrum. The upfield signal is a triplet (3H, *t*). The downfield signal is quartet (2H, quartet). 'CH_2' protons give downfield signal as the C—2 is attached to electronegative 'Cl' atom (See text).

Q. 22. What are shielding and deshielding of protons?

Ans. In the NMR spectrum of a compound, the electrons around the protons also play their role. When a compound is placed in a magnetic field, the electrons around the protons generate also a magnetic field known as induced magnetic field. This induced magnetic field may reinforce or oppose the applied field. So two cases may arise:

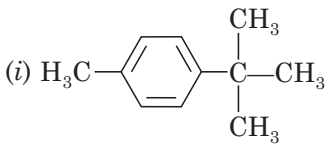
(*i*) If the induced field opposes the applied field and thus the effective magnetic field 'H' experienced by the nucleus is smaller than the applied magnetic field (H_o). H is related to H_o as $H = H_o (1 - \sigma)$ where, $\sigma \Rightarrow$ a nondimensional quantity known as shielding or screening constant and has a positive value.

The proton is said to be shielded by the electrons. A greater applied field is required for the excitation of protons.

(*ii*) If the induced field reinforces the applied field (here σ is negative), an enhanced field strength will be experienced by the proton and proton is said to be deshielded in this case.

Shielding causes a shift of the resonance frequency to higher values of the applied field (H_o) *i.e.,* the shift is upfield. On the other hand **deshielding** causes a shift of the resonance frequency to lower values of the applied field *i.e.,* the shift is downfield. The magnitude of this shift is called **chemical shift**.

Q. 23. Match the number of signals of the compounds given:

Compounds	*Signals*
(*i*) 	1
(*ii*) C_2H_5OH	3
(*iii*) $CH_3—O—CH_3$	3
(*iv*) $CH_3—O—CH_2CH_3$	3

Ans. Compound (*i*) will produce three signals because there are three types of protons *i.e.*, —CH_3 protons on the left side of the ring, methyl protons on the right side of the ring and ring protons. Since there is no non-equivalent protons in the molecule, all the peaks will be singlet and no splitting will take place.

Compound (*ii*) will produce three signals as there are three types of protons. It will be a triplet for —CH_3 protons, a quarlet for —CH_2 protons and a singlet for —OH proton.

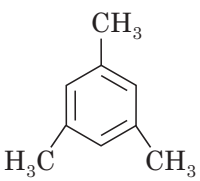
For compound (*iii*) only one signal will be obtained in case of 3rd compound as there are only one type of protons and hence it will be a singlet.

Compound (*iv*) will produce three signals. There are three types of protons. Hence a singlet for OCH_3 protons, a quartet for —CH_2 protons and a triplet for —CH_3 protons.

Q. 24. Translate the following set of NMR spectral data to a compound. Molecular formula C_9H_{12}, singlet τ 3.22, 3H; singlet τ 7.75, 9H.

Ans. (*i*) The formula C_9H_{12} corresponds to the general formula of aromatic hydrocarbon C_nH_{2n-6}. Thus the compound will contain a benzene ring with some other substitutions.

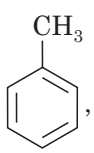
(*ii*) The compound gives two signals and thus it indicates the presence of two types of protons. These conditions are satisfied only by the structure:



1, 3, 5-trimethyl benzene

This structure explains the existence of singlet τ 3.22 due to three ring protons and singlet τ 7.75 due to nine —CH_3 protons.

Q. 25. Count the number of signals for the following compounds:

(1)  , (2) $CHCl_2—CH_3$

Ans. (*i*) The first compound contains two types of protons, the ring protons and —CH_3 protons, hence two signals will be observed.

(*ii*) The second compound contains two kinds of protons as indicated by *a* and *b* (below).

$$\overset{a}{\text{CH}}\text{Cl}_2\text{—}\overset{b}{\text{CH}}_3$$

So, two signals will be observed.

Q. 26. State the significance of peak area.

Ans. Peaks obtained in the NMR spectrum make different areas with the base line. It has been observed that area under an NMR signal is directly proportional to the number of equivalent protons that give rise to that signal. By comparing the area subtended by different signals, we can calculate the relative proportion of different types of protons. As for example, in the case of peaks obtained for benzyl alcohol, the ratios of the areas under the peaks are 1:2:5, indicating that the three types of protons are in the ratio 1:2:5. There are one —OH proton, two methylene protons and five ring protons.

Q. 27. Mention the various uses of n.m.r. spectrum.

Ans. NMR spectroscopic technique is applied in different cases.

(*i*) **For identification of functional groups.** Every functional group gives a characteristic signal in the NMR spectrum. By studying the chemical shift of compound, it becomes possible to establish what kind of functional group is present in the molecule.

(*ii*) **For structure determination of an unknown compound.** It is possible to elucidate the structure of an unknown compound from the n.m.r. studies. This is due to the protons which under different environments give different chemical shifts. By observing doublets, triplets or multiplets, it is possible to place hydrogens at appropriate place in the formula and hence a structure can be established.

(*iii*) **For comparison of two compounds.** NMR spectrum is like fingerprint of a compound. Two compounds which show same n.m.r. spectrum must be identical in structures.

Q. 28. Discuss the applications of mass spectroscopy.

Ans.

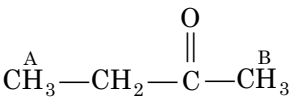
- Determination of molecular mass of a compound (known and unknown).
- Determination of molecular structure analysing the fragmentation pattern and taking the help of UV, I.R., nmr spectra.
- The combination of gas liquid chromatography (glc) with mass spectrometry (ms) is of great importance in modern chemical analysis. First glc separates the chemicals in an unknown mixture, as for example, polluted water, when the ms detects and identifies components.

Q. 29. Translate the following set of spectra to three isomeric organic compounds.

Description–Colourless liquid (b.p. – 80°C); molecular formula – C_4H_8O; I.R. ν_{max}^{film} (cm^{-1}) – 1715 (*s*), NMR (neat liquid) – δ 1.06 (3H, *t*), δ 2.14 (3H, *s*), δ 2.43 (2H, *q*).

Ans. The IR band at 1715 cm^{-1} indicates that the compound possesses a $\rangle C = O$ group. This evidence, taken with the molecular formula C_4H_8O, suggests that the compound is either an aliphatic aldehyde or a ketone.

The NMR spectrum readily excludes are aldehyde as there is no resonance at about δ 9.5 and confirms the structure as butan-2-one (methyl ethyl ketone).



Methyl group (A) and the methylene group appear respectively as the expected triplet and quartet. Methyl group (B) and methylene group are both deshielded (moved to downfield) by the adjacent $\rangle C = O$ group. The principal ions in the mass spectrum of butan-2-one are $CH_3—C \equiv O^+$ (m/z 43) and $CH_3CH_2—C \equiv O^+$ (m/z 57), and molecular weight $M^{\ddagger}$ – 72.

EXERCISES

1. (*a*) Mention the wavelengths for visible, UV and I.R. radiations.
(*b*) Name the different spectroscopic techniques which are employed for the elucidation of structures of organic compounds.

2. What do you mean by:
(*a*) absorption spectrum (*b*) emission spectrum?

3. State Beer-Lambert's law and deduce mathematical expression for the same.

4. What are the different electronic transitions that take place on the absorption of UV light?

5. Explain the terms:
(*i*) chromophore (*ii*) auxochrome (*iii*) blue shift (*iv*) red shift.

6. Mention the applications of UV spectroscopy.

7. Calculate the molar absorptivity of the solution of concentrated 1×10^{-4} mol l^{-1}, given A = 0.20 and path length = 2.5 cm. (**Ans.** 8000 dm^3 mol^{-1} cm^{-1})

8. Mention the principles of I.R. spectrum.

9. Indicate whether you will use I.R. or U.V. spectroscopy for distinguishing the following pairs of compounds:
(*a*) $CH_2 = CH—CH_2—O—CH_3$ and $CH_3\,CH_2\,COCH_3$
(*b*) $CH_3—O—CH_3$ and $CH_3\,CH_2\,OH$
(*c*) $CH_2 = CHCH_2OH$ and $CH_3\,CH_2\,CHO$

10. What is nuclear magnetic resonance?

11. What are equivalent and non-equivalent protons?

12. What is meant by shielding and deshielding of a proton?

13. What is chemical shift?

14. Why do we select tetramethyl silane (TMS) as a standard substance in n.m.r.?

15. What do you mean by splitting of signals?

16. What is mass spectroscopy? What is the principle of m.s.?

17. Give applications of:
(*i*) UV (*ii*) I.R. (*iii*) n.m.r. (*iv*) m.s.