

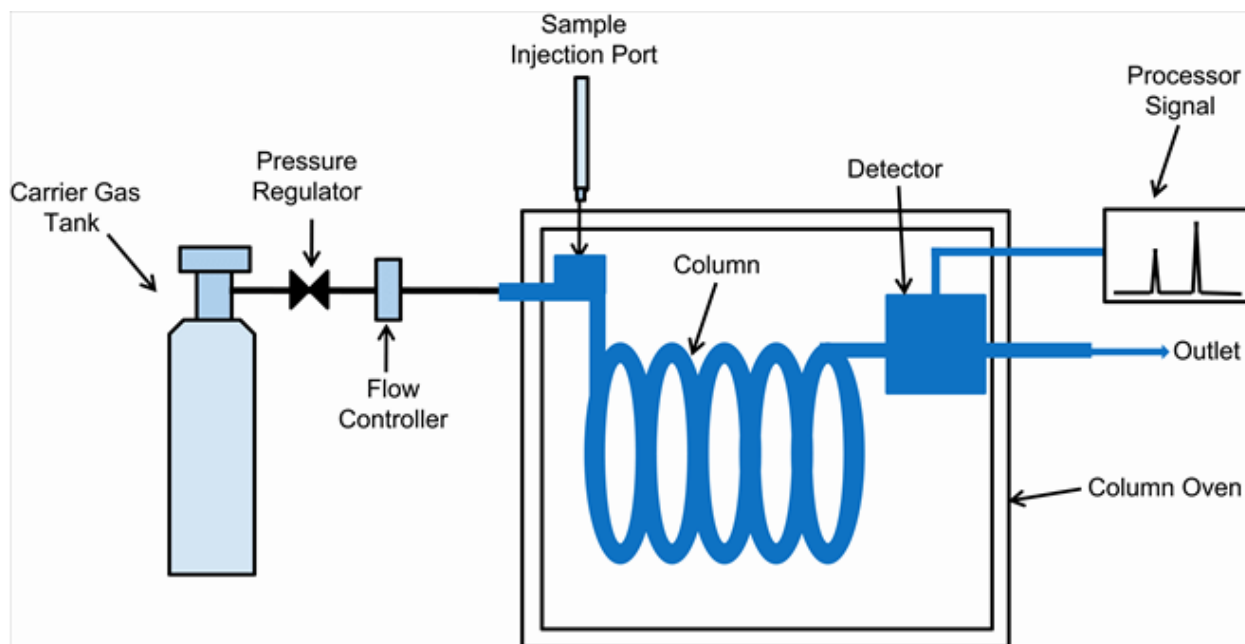
MOUDLE 6: Instrumental methods of chemical analysis

CHROMATOGRAPHY

Gas Chromatography:

Gas Chromatography is so called because the mobile phase is gas. It is two types depending upon whether the stationary phase is a liquid (e.g., Gas-liquid chromatography) or solid (e.g., Gas solid Chromatography). Thus for Gas liquid chromatography (GLC), the stationary phase is a high boiling liquid and the sorption process is predominantly one of partition. For, Gas Solid chromatography (GSC), the stationary phase is solid and adsorption plays major role.

Principle: When the vapors pressure of the sample in gas stream are allowed to pass through a column containing stationary liquid or solid phase, the components of mixtures migrate at different rate due to differences in boiling point, solubility or adsorption leading to separation of mixture in microgram quantities. A continuous flow of gases elutes the component from the column in order of increasing distribution ratios from where they pass to detector connected to recording system. Generally chemically inert carrier gases like He, N₂ or Ar. The temperature of separating column must be equal or slightly above the boiling point of the least volatile component of the sample.



Application:

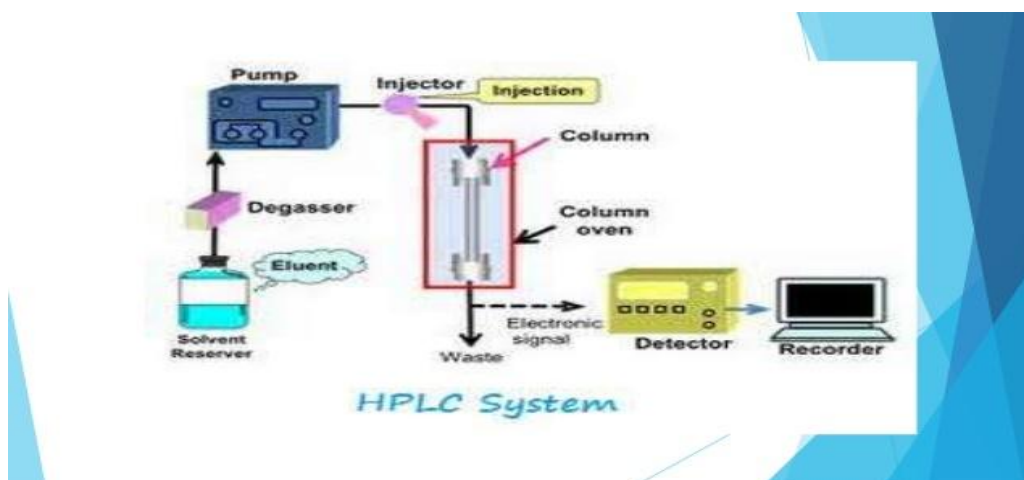
1. GC is used for the separation of thermally stable and volatile organic and inorganic compounds
2. It is used for the analysis of gaseous sample, liquid solution and volatile solid.
3. The determination of antioxidant, food preservatives, contaminant and adulterants are routine analysis by GC
4. It is used in quality control, analysis and monitoring of drugs and pharmaceuticals
5. GC is easily adapted to separate mixtures of air pollution.
6. GC is successfully used in separation and determination of many components in petroleum products.
7. Recently it has been utilized for elemental analysis of organic samples.

High performance liquid chromatography (HPLC):

HPLC has its origin in column chromatography. Much higher efficiencies and better resolution are achieved in HPLC through the use of smaller particles of stationary phase and pumping of the mobile phase through the column under pressure (2000 bar) for achieving higher flow rate ($1-5 \text{ cm}^3 \cdot \text{min}^{-1}$).

Principle:

Microgram to gram quantities of mixtures can be separated by passage of sample of pressured flow of a liquid mobile phase through a column containing stationary solid phase. components of mixtures migrate through the column at different rates due to different relative affinities for the mobile phase stationary phase based on charge, size and adsorption.



Application of HPLC:

1. It is used largely for the separation of non-volatile substances including ionic and polymeric substances
2. It is used in pharmaceutical, bio-medical and food product industries.
3. It is also used in forensic chemistry
4. It is used in pesticide industries.
5. It also extremely helpful in clinical and environmental studies.

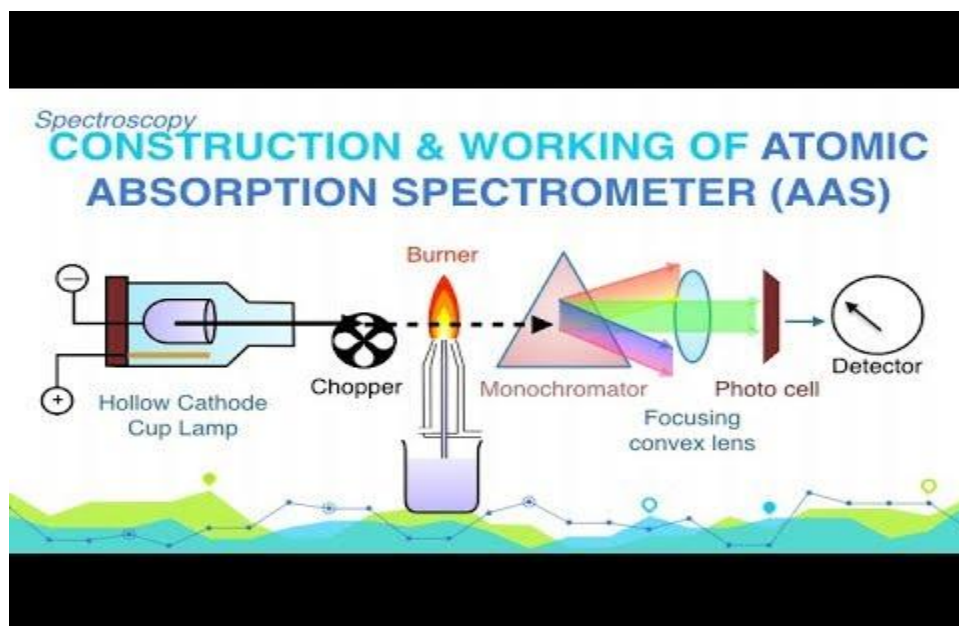
ATOMIC ABSORPTION SPECTROSCOPY

Q. Discuss the principle and application of Atomic Absorption Spectroscopy (AAS)

AAS is a procedure employed in analytical chemistry to quantify elements by absorption of light by free gaseous atoms

Principle:

- The technique uses basically the principle that free atoms (gases) generated in atomizer can absorb radiation at specific frequency.
- AAS quantifies the absorption of ground state atoms in the gaseous state.
- The atoms absorb UV or visible light and make transition to higher energy levels. The analyte concentration is determined from the amount of absorption.
- The absorbance is measured in the atomizer with the help of detector. Beer-Lambert law is then used to determine the analyte concentration.



Application of AAS:

- Estimation of trace elements in biological fluids like blood and urine.
- Estimation of trace elements like Cu, Ni and Zn in food products.
- Estimation of Zn in Zinc insulin solution.
- Estimation of lead in calcium carbonate and petrol
- Estimation of elements in soil samples, water supply, effluents etc.
- Used in forensic science

FLAME PHOTOMETRY

Q. Discuss the principle and application of Flame Photometry

Ans:

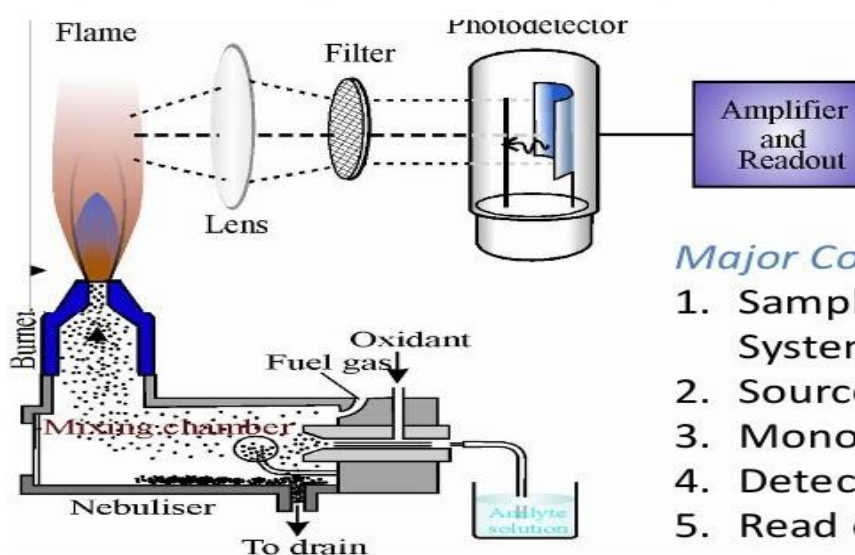
Flame photometry (also called flame atomic emission spectrometry) is a branch of atomic spectroscopy in which the species examined in the spectrometer are in the form of atoms. The atoms under investigation are excited by light.

Principle

- Liquid sample containing metal salt solution introduced into a flame
- Solvent is vaporized, leaving particles of solid salt which is then vaporized into gaseous state

- Gaseous molecule dissociates to give neutral atoms which can be excited (unstable atoms) by thermal energy of flame.
- The unstable excited atoms emit photons while returning to lower energy state
- The measurement of emitted photons forms the basis of flame photometry using photomultiplier tube detectors

Schematic Representation of the Flame Photometer



Major Components:

1. Sample Delivery System
2. Source
3. Monochromator
4. Detector
5. Read out device

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Application of Flame Photometry

- To estimate Na, K, Ca, Li etc level in sample of serum, urine, and other body fluid.
- Flame emission photometry is useful in determination of alkali and alkaline earth metals
- Used in determination of lead in patroles
- Used in determination of calcium and magnesium in cement
- Used in therapeutic monitoring

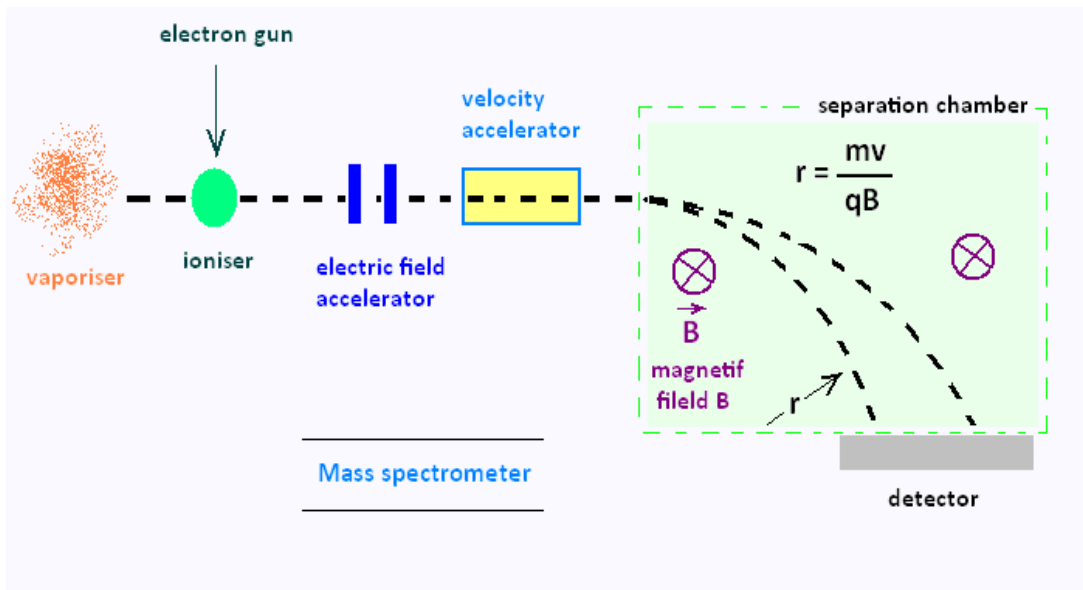
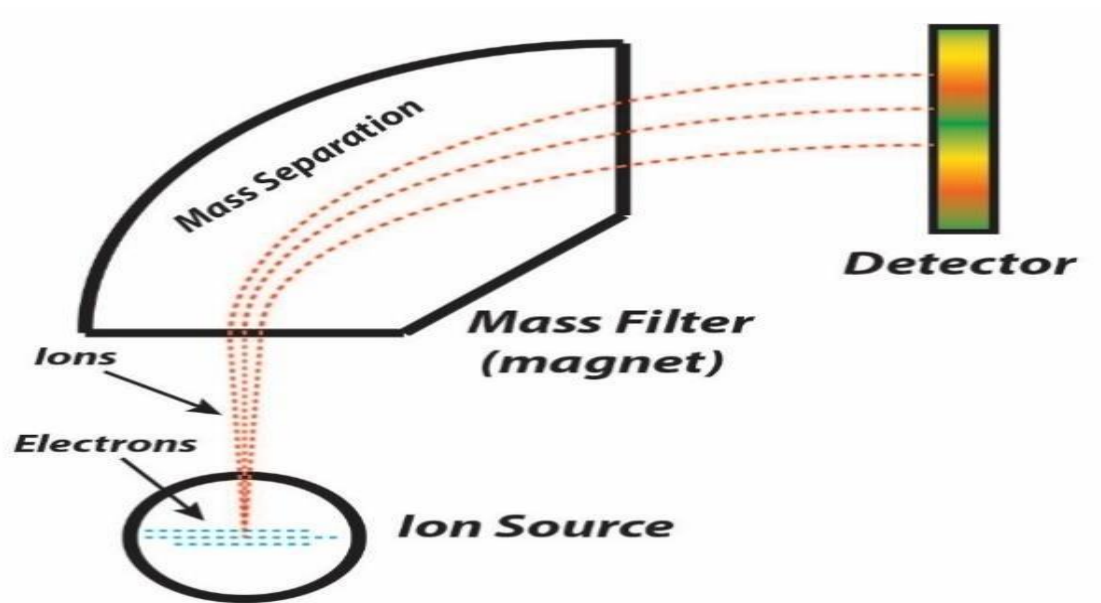
MASS SPECTROMETRY

Mass spectrometry is the most accurate method for determination of molecular mass of compounds

Principle

- Organic molecules are bombarded with electrons
- Molecules are converted into highly energetic positively charged ions

- The formed ions are separated by deflection in magnetic field according to their mass to charge (e/m) ratio and subsequently detect and record them.



Application of Mass spectrometry:

- Used in determination of molecular weight of organic compounds
- Used in drug metabolism studies, pharmacokinetics
- Used in identifying drugs kinetics
- Used in bio molecules characterization such as protein and peptide
- Used in environmental analysis like pesticides on food
- Used in soil and ground water contamination studies
- Used in forensic analysis

Nuclear Magnetic Resonance (NMR):

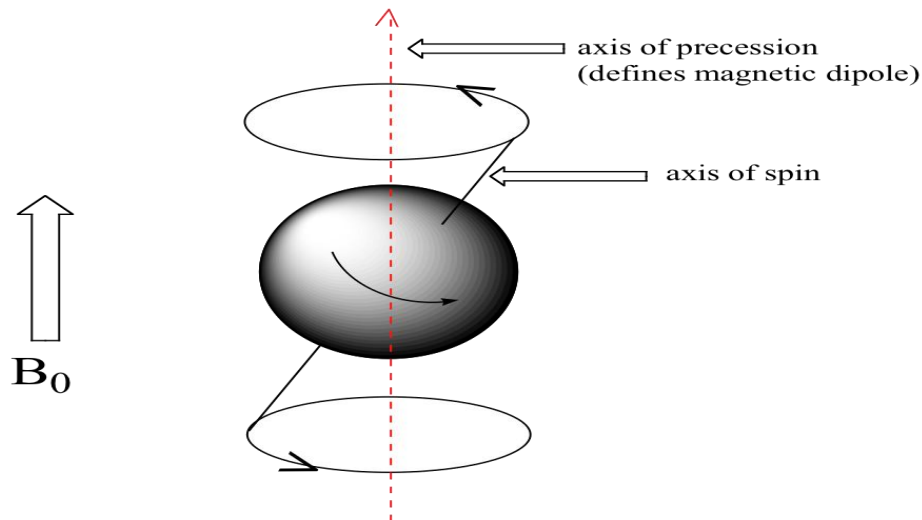
NMR is a form of absorption spectroscopy. The sample absorbs electromagnetic radiations in the radio frequency region. NMR spectrum is a plot of the frequencies of absorption peaks versus peak intensities.

Principle:

The theory behind NMR comes from the spin of the nucleus and it generates a magnetic field. Without an external applied magnetic field, the nuclear spins are random in directions. But when an external magnetic field is present the nuclei align themselves either with or against the field of external magnet.

Q. What is Larmour or precessional frequency? How the precessional frequency can be measured?

Ans. The movement of the nucleus is like that of spinning top. The top spins and also move around slowly. Similarly, the nucleus also performs a waltz like motion. This waltz like motion of the nucleus along the vertical axis is known as the precessional motion.



The frequency of precession of proton is proportional to the strength of applied external field (B_0) experienced by the proton.

Quantitatively,

$$\nu_0 = \frac{\gamma B_0}{2\pi}$$

The Larmor Equation!!!

$$\Delta E = k B_0 = h \nu$$

can be transformed into

$$\nu = \left(\frac{\gamma}{2\pi} \right) B_0$$

frequency of the incoming radiation that will cause a transition

gyromagnetic ratio γ

strength of the magnetic field

γ is a constant which is different for each atomic nucleus (H, C, N, etc)

Q. What is spin active nuclei?

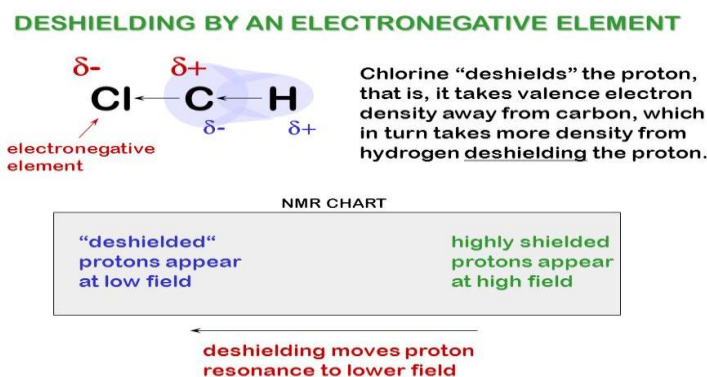
Ans: Those nuclei which exhibit NMR phenomenon are called spin active nuclei. All nuclei possess spin angular momentum. Only those nuclei with spin quantum number (I) greater than zero will precess along the axis of rotation. Nuclei like $^{12}_6\text{C}$, $^{16}_8\text{O}$, $^{32}_{16}\text{S}$ with $I=0$ are spin inactive. Nuclei ^1_1H , $^{13}_6\text{C}$, $^{19}_9\text{F}$ are spin active and exhibit NMR spectroscopy

Q. What is NMR Spectroscopy? Discuss the term chemical shift for a nucleus under different environment? What are the various application of NMR spectroscopy?

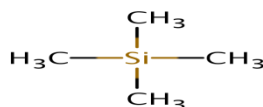
Answer:

It is branch of spectroscopy in which radio frequency waves induce transitions between magnetic energy level of nuclei of a molecule, which are created by keeping the nuclei in a magnetic field. NMR spectrum is a plot of the frequencies of absorption peaks versus peak intensities.

When a molecule is placed in an electric field, the electrons rotating around it generates their own electric field. This field may align with the field or may oppose it. If it align with the applied field, the protons feels a higher field strength and is said to be **deshielded**. If the induced magnetic field opposes the applied field, the proton experiences a lesser magnetic field and is said to be **shielded**. Shielding shift the absorption up field and deshielding shift the absorption down field to get effective field strength necessary for absorption. Such shift in position of NMR spectrum as compared to a standard reference which arise due to the shielding and deshielding of proton by the electron are called **Chemical shift**.



The standard used universally to measure chemical shift is tetra methyl silane(TMS). TMS has 12 equivalent protons and hence a sharp single signal is obtained.



The difference in absorption position of the proton with respect to TMS signal is called Chemical shift. TMS is chosen as reference because it is miscible with most organic solvents and it volatile. Hence can be easily removed from the system. It does not take part in intermolecular

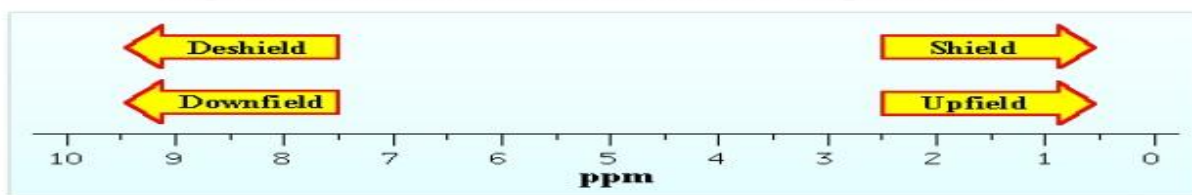
association with the sample. Chemical shift are usually expressed in δ (delta) units. The value of delta is expressed in ppm.

Shielding of protons:-

High electron density around a nucleus **shields** the nucleus from the external magnetic field and the signals are **upfield** in the NMR spectrum

Deshielding of protons:-

Lower electron density around a nucleus **deshields** the nucleus from the external magnetic field and the signals are **downfield** in the NMR spectrum



Factors influencing Chemical shift:

1. Shielding and deshielding effect: Shielding shift the absorption up field and deshielding shift the absorption down field to get effective field strength necessary for absorption.

2. Inductive effect: The $-I$ groups causes reduction in electron density around the proton(deshielding). Thus a lower field is needed to bring about resonance. Hence, the absorption occur downfield (higher δ values) far from the TMS signal.

Similarly, the electropositive atom like silicon pushes the electron towards the methyl groups. Hence, methyl groups are highly shielded and higher fields needed to bring about the resonance. Thus absorption occur at up field(lower δ value).

3. Anisotropic effects: Shielding and deshielding in unsaturated compounds depends upon the manner in which the pi electrons circulate under the influence of applied field. These field are diamagnetic (oppose to the field) in certain directions and paramagnetic (aligned with the field) in others. Hence these effects are called anisotropic effects.

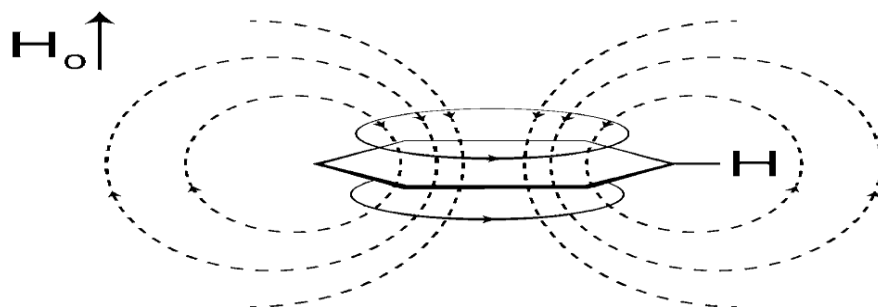


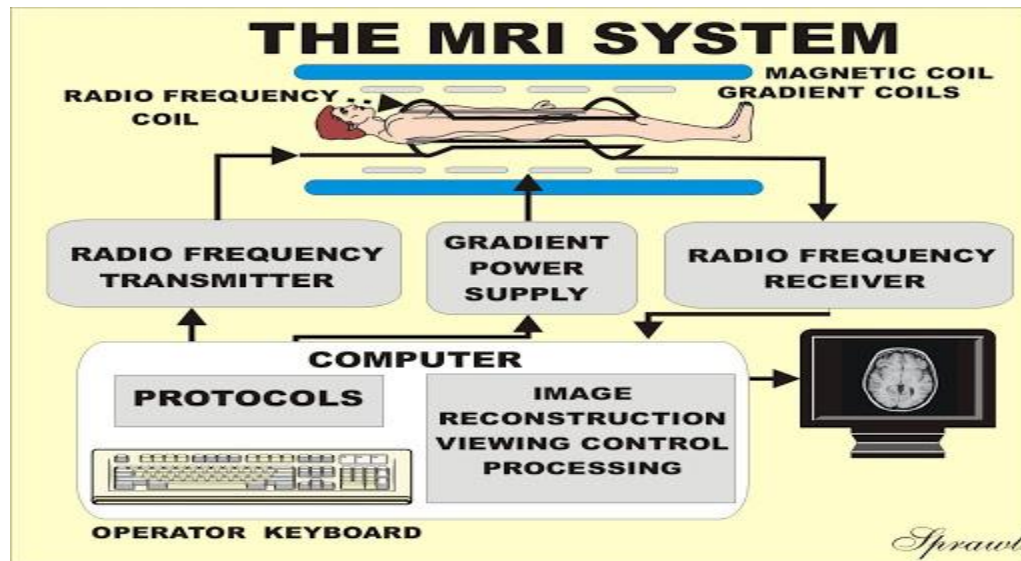
Fig: anisotropic effect of benzene ring

4. **Hydrogen bonding:** Hydrogen bonded protons are attached to a strongly electronegative atom and hence is highly deshielded. Therefore absorption occurs downfield.

Application of NMR spectroscopy

- Determination of structure of organic compounds
- A powerful technology for metabolite analysis
- A mature technique for chemical identification and conformation analysis of chemicals
- This technique is used in MRI scanning
- Used in drug screening and design
- Solid state NMR has the potential for determining atomic-resolution structures of domains of membrane protein

Q. write short note on MRI



Ans: Magnetic Resonance Imaging (MRI) is based upon the principle of nuclear magnetic resonance. When a human skull is subjected to a gradient field. The protons in a head (water and fat) will come to sharp resonance at a given frequency only when applied field causes them to precess at their resonance frequency. At only one particular point along this gradient field will the proton be in resonance. This distance across the head is therefore correlated with the field gradient. Then the gradient is altered, it causes the resonance to occur at a different point. The distance of this point along the gradient is again correlated. In this way an image of the protons present in the eyes or brain is constructed by scanning along several gradients applied across a network of axes through a slice of the head. By repeating the process across several slice through the head, the complete reconstructed image is defined.

Infra-red or Vibrational Spectroscopy:

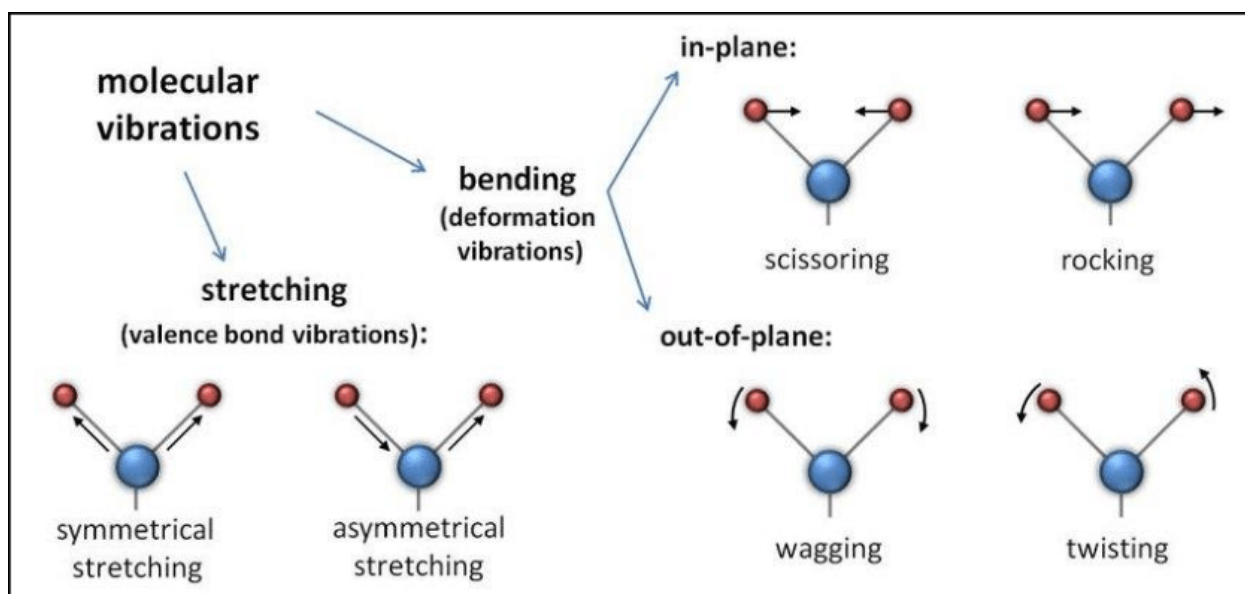
Vibrational spectroscopy involves the transitions between the vibrational energy levels of a molecule on the absorption of radiation falling in the spectral range of $500\text{--}4000\text{ cm}^{-1}$. A single vibrational energy change is accompanied by a large number of rotational energy changes. Thus the vibrational spectra appear as vibrational-rotational bands.

Principle : Atoms in the molecules do not remain in fixed position but vibrate. This vibrational motion is quantized. When infra-red radiation is passed through the sample, the molecule absorbs radiations of appropriate energy and gets excited to a higher vibration level. It is important to note that IR light is absorbed only when the dipole moment of the molecule is different in the two vibrational levels. And giving rise to closely packed absorption bands, called IR absorption bands. The bands correspond to the characteristic functional groups and the bonds present in the chemical substances.

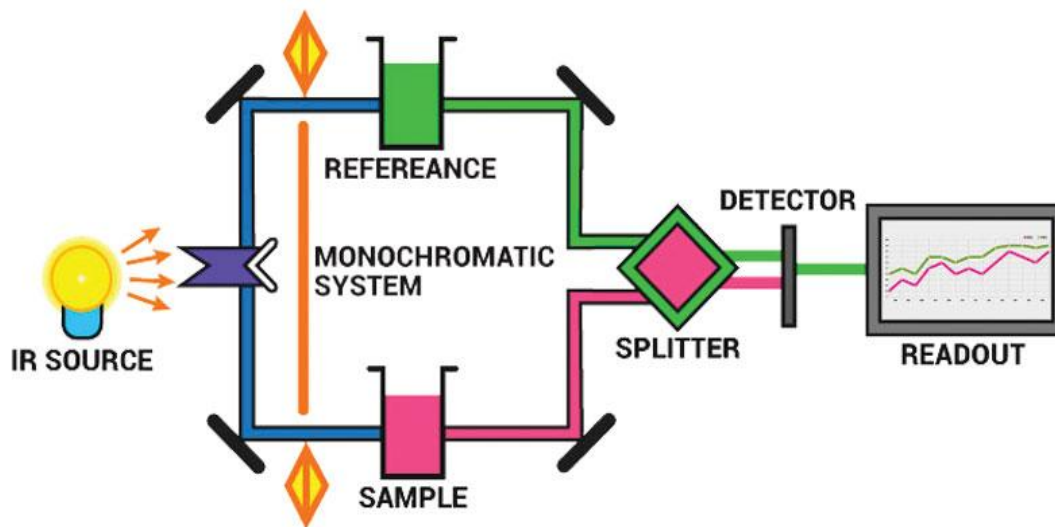
Modes of vibration: There are two modes of vibration

a) Stretching vibration

b) bending vibration



Instrumentation:



Dipole moment and infrared spectrum:

All types of molecule cannot interact with IR radiation. Only those molecules which undergo a net change in dipole moment because of its vibration and rotational motion absorb in the IR region. Such molecule are known as infrared active and other are known as infrared inactive.

e.g., cis-1,2-dichloroethene is infrared active

H₂, O₂, N₂ are infrared inactive

Application of Infra-red spectroscopy:

- Determination of force constant from vibrational spectrum
- Determination of purity of many chemicals
- Identification of unknown compounds
- Identification of functional groups in organic molecules
- To distinguish between intra and inter molecular hydrogen bonding
- Elucidation of structure is possible by IR spectroscopy

UV-Visible Spectroscopy

Application of UV-Visible spectroscopy:

- Extent of conjugation can be determined through UV-Visible spectroscopy

- Determination of geometrical isomer
- Identification of unknown compounds
- Distinction between conjugated and non-conjugated compounds
- Detection of impurities
- Detection of hydrogen bonding
- Quantitative analysis of unknown solution

Derivation of Beer's Law

The fraction of photons absorbed by the slab is: $\frac{dI}{I_z} = -\sigma N dz$
(negative since photons are removed)

Integrating both sides gives: $\ln I_z = -\sigma N z + C$

The difference in intensity between $z = 0$ and $z = b$ is therefore:

$$\ln I_0 - \ln I = (-\sigma N 0 + C) - (-\sigma N b + C) = \sigma N b$$

$$\Rightarrow \ln \left(\frac{I_0}{I} \right) = A = \sigma N b$$

Or for liquids: $A = \log_{10} \left(\frac{I_0}{I} \right) = \frac{\sigma N b}{2.303} = \epsilon c b$

Where ϵ = molar absorptivity ($\text{L mol}^{-1} \text{ cm}^{-1}$ or $\text{mol}^{-1} \text{ cm}^2$) and c = concentration (mol cm^{-3})

NOTE: theory and principle of uv-visible is given in the class, those were absent in class collect the notes from others.