

Assignment No:-05

23AK1AO453

Questions:-

1. Explain UV-visible Spectroscopy?
2. Explain IR-Spectroscopy?
3. Explain High Performance liquid Chromatography [HPLC]?

Answers:-

JAns:- UV-VISIBLE SPECTROSCOPY:-

This is also known as electronic spectroscopy since it involves the promotion of electrons from ground state to higher energy state.

Principle of UV-Spectroscopy:-

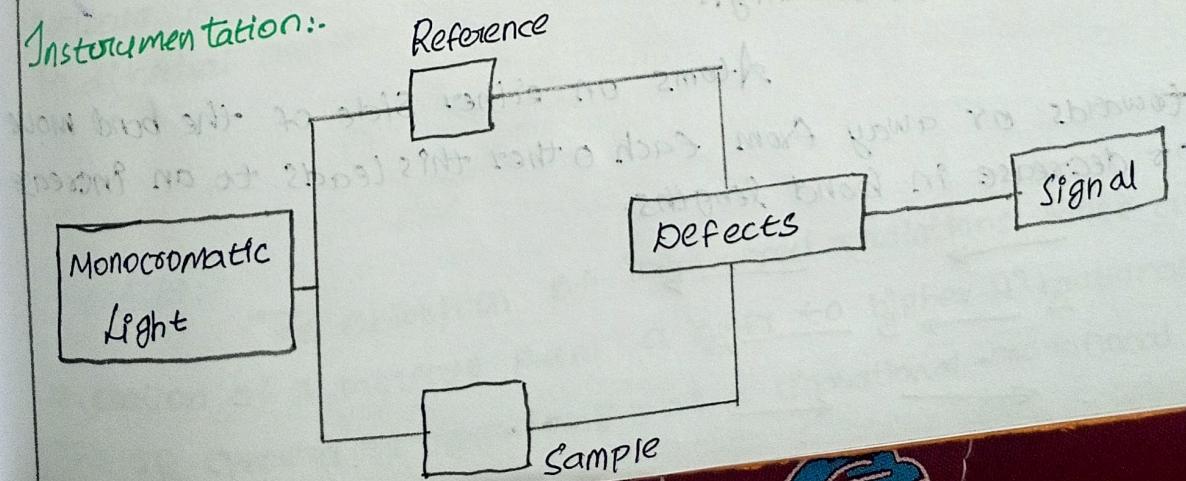
It follows Beer's Lambert's law, this law states that whenever a beam of monochromatic light is passed through a solution the rate of decreasing intensity of a radiation with thickness is proportional to the concentration of the solution and the incident radiation.

$$A = \epsilon CL$$

The absorption of visible light or UV light by chemical compound which produce distinct spectrum UV radiation are absorbed this results in the excitation of electrons from the ground state and the higher energy state.

The energy absorbed by the substance is equal to the energy difference b/w higher energy state and the ground state.

Instrumentation:-



Applications:-

- * This is used to detect the functional group.
- * used to detect the extent of configuration in polymers.
- * It is used to identify unknown compounds.
- * used to determine the configuration of geometrical isomers.
- * It can be used to determine the purity of a substance.
- * Analyze food colorings, quality of wine.
- * used to determine the rate constant.
- * used to determine the metal ions like iron, manganese in cement.

QAns:- IR-Spectroscopy:-

- * It is a technique used to study molecular vibrations.
- * It measures the absorption of infrared radiation, by the molecules providing information about functional groups and molecular structure.

Fundamental Modes:-

It refers basic vibration motion of a molecule these vibrations involve change in bond lengths, bond angles and dihedral angles.

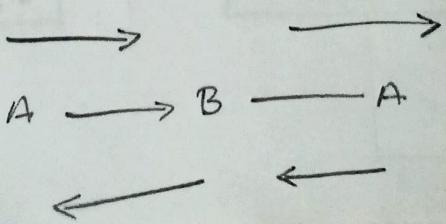
There are 3 types of fundamental modes

1. Stretching Mode:-

It involves the vibrational stretching or a compression of chemical bonds within a molecule. It is of two types.
1. Symmetric stretching.
2. Asymmetric stretching.

a) Symmetric stretching:-

Atoms on either side of the bond move towards or away from each other this leads to an increase or decrease in bond lengths.



b) Asymmetric stretching

bond move in
other while
change in bo

2. Bending mode

of chemical
a) Symmetric

move toward
change in

b) Asymmetric

bonding
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3. Torsion

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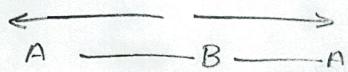
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b) Asymmetric Stretching:-

Here atoms are on either side of the bond move in opposite direction. One atom moves away the other while the other moves closer causing a change in bond length.

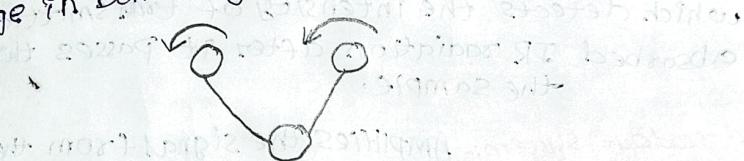


2. Bending Mode:-

It involves the vibrational bending or deformation of chemical bonds within a molecule. It is of two types.

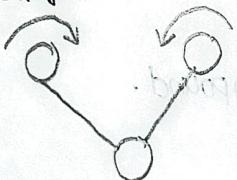
a) Symmetric Bending:-

In this mode atoms on both sides of a bond move towards or away from the central axis causing a symmetrical change in bond angle.



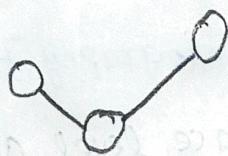
b) Asymmetric Bending:-

Here atoms are in either side of the bond move in opposite direction leading to an asymmetrical change in bond angle.



3. Torsional Mode:-

It involves twisting or rotation of parts of a molecule around a bond axis. It is associated with changes in dihedral angles.



Principal:-

The absorption of infra-red radiations cause an increase in energy level of a molecule from a lower to higher vibrational level. The IR spectra is considerable as a vibrational-rotational spectra.

- * All bonds in a molecule are not capable of absorbing the IR energy by only those bonds which are accompanied by a changing dipole moment will absorb in IR region.
- * thus these vibrational transitions are responsible for absorption of energy in the IR region.

Instrumentation:-

1. **IR Light source:** - Nernst glower.
2. **Sample compartment:** where the sample is placed.
3. **Monochromator:** separates the various wavelengths of IR radiation, allowing only a narrow range to reach the sample.
4. **Sample holder:** which holds the sample.
5. **Detector:** - which detects the intensity of transmitted or absorbed IR radiation after it passes through the sample.
6. **Amplifier and readout system:** - Amplifies the signal from the detector and provides a readable output in the form of spectrum.
7. **Data Processing System:** - Analysis and process the raw data to generate an IR spectrum.

Applications:-

1. Study of geometrical isomer.
2. Detection of impurity in a compound.
3. Study on rotational isomerism.
4. Quantitative analysis.
5. Determination b/w two types of hydrogen bonding:- Intermolecular and Intramolecular hydrogen bonding.
6. Identification of an organic compound:- used to detect the functional groups.

ANS:- High Performance liquid chromatography [HPLC]:-

It's used for the trace level analysis of variety of compounds in a short time.

Principle:-

The principle of separation is normal phase mode and reverse phase mode is adsorption.

- * When a mixed of components travel according to stationary field.
- * No two components travel faster.
- * The components with stationary phase travel faster.

Solvent reservoir:-

Phase it must be

Pump:- If one is available due to illusion.

Injectors:-

The sheodyne bulb loop using a

the sample

Column:-

which sep

Detector:-

the samples are high

Applications:-

* In case of drugs

* During

* Nutri

* Separation

* When a mixture of components are introduced into HPLC column they travel according to their relative affinities towards the stationary phase.

* No two components have the same affinity.

* The components which has more affinity towards the stationary phase travels slower and which has less affinity travels faster.

Solvent reservoir:-

A solvent reservoir is used to store the mobile phase it must be inert solvent.

Pump:- If one pump is used the composition of mobile phase is variable during the experiment it is called as gradient pump.

Injectors:-

The most useful and widely used injector is six-Port syringe type injector. the sample is introduced into the sample loop using a special syringe.

A anticlockwise rotation of the valve rotor places the sample into the mobile phase.

Column:-

It is the heart of the HPLC instrument. A column in which separation of the sample will take place.

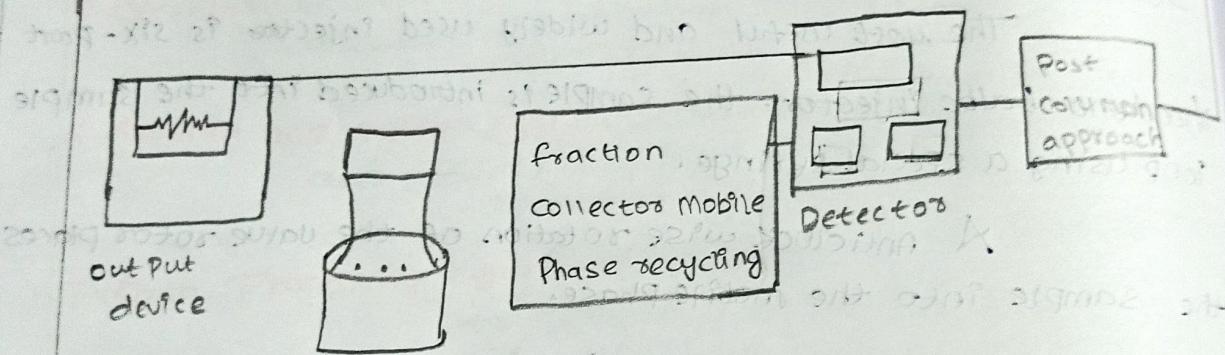
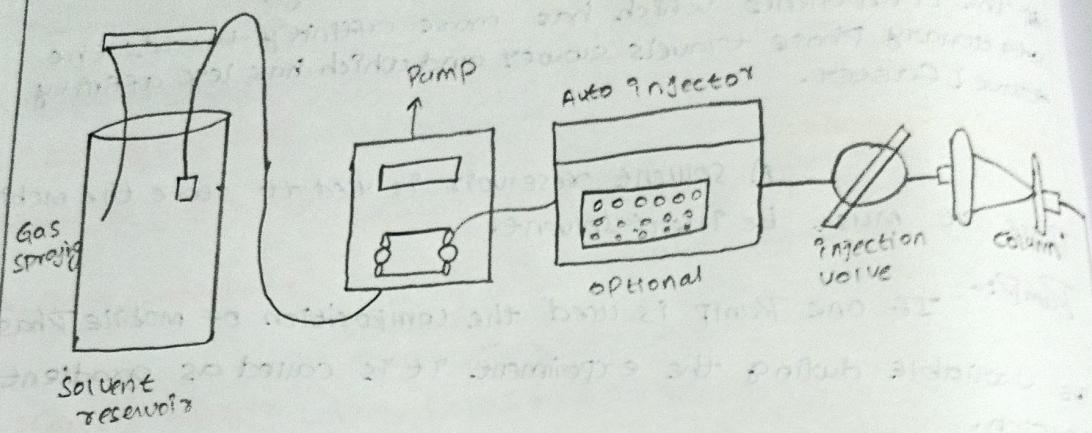
Detectors:-

A detector using to detect the concentration of the sample components as they come out of the column. These are highly sensitive.

Applications:-

- * In clinical science, pharmaceutical development, used to detect drugs in urine.
- * Purity of Products.
- * Nutrition analysis.
- * Separation of proteins, nucleic acid, amino acid, Pesticides,

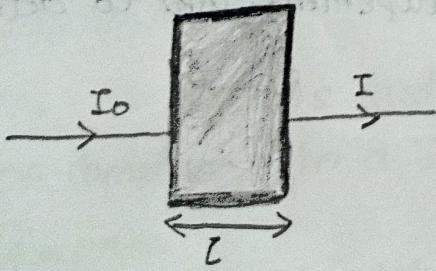
Hormones, Steroids and Steroids.



Q Marks

Q:- What is Beer-Lambert's Law?

Ans:- When a monochromatic light is passed through a solution containing the absorbing substance, the decrease in the intensity of light with Path length is proportional to the concentration of the solution and the intensity of Light.



$-dI/dI \propto I$ [Intensity]

$-dI/dc \propto c$ [Concentration]

$-dI/dL \propto L$ [Path length]

$-dI/dI \propto CI$

$-dI/dI = KCl$

$K = \text{Proportionality constant.}$