

Analytical Chemistry Sp-I

LONG QUESTIONS

— (1) —

Open Tubular Column in GIC :-

Capillary columns are made up of stainless steel tube, glass, copper or fused silica material which is usually between 5-500 m in length. These columns are kept in a suitable thermostate for maintaining a constant temperature. (150-300°C)

Most commonly used stationary phase are polyethylene glycols, zeolites, alumina etc.

Types :-

There are the following main types of Open Tubular Column.

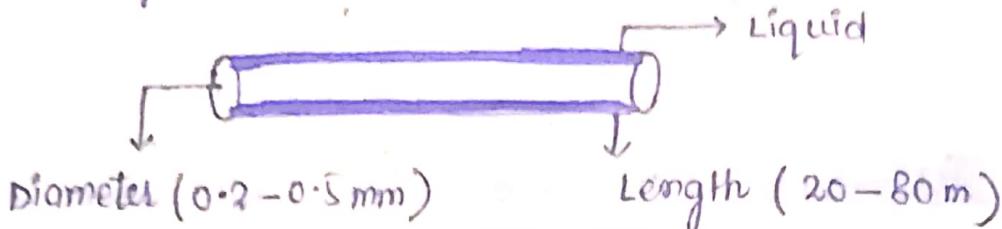
- Wall Coated Open Tubular Column (WCOT)
- Support Coated Open Tubular Column (SCOT)
- Porous Layer Open Tubular Column (PLOT)
- Fused Silica Open Tubular Column (FSOT)

WCOT :-

This column is used for only GIC.

Wall coated columns consist of a capillary tube whose walls are coated with liquid stationary phase.

In this case, stationary phase is liquid in capillary tube and it sticks with the walls of capillary tube and a small hole is left for entering gas and sample mixture as a mobile phase.



SCOT :-

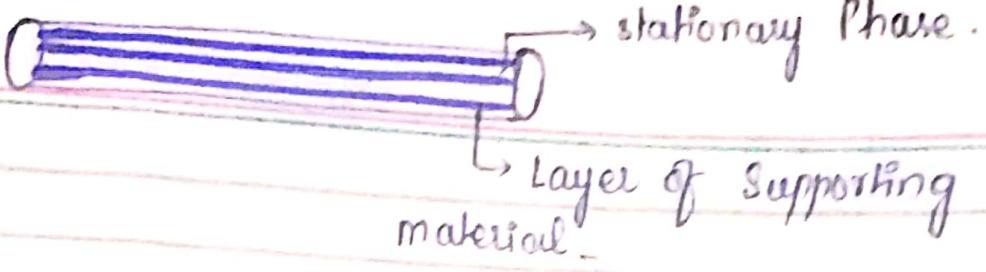
In Supported Coated Columns, the inner wall of the capillary is lined with a thin layer of support material onto which the stationary phase has been absorbed. It is also used for GLC.

In this case Liquid is also stationary phase but that Liquid is supported by a solid material which is dipped in liquid and then that material is packed in this capillary tube.



PLOT :-

This type of capillary tube is used for GSC. In this case stationary phase is a solid which is again mixed with another column of solid which is basically supporter but produce a solid layer as a solid. These are made by extending the inner wall of columns by substances such as glica.



FSOT :-

A new type of WCOT column was developed known as Fused Silica Open Tubular column. Fused silica is produced from purified quartz with a very low metal oxide contents.

Mostly long, narrow open tubular columns are made of fused silica and coated with polyimide.

These columns are flexible and can be shaped into coils. They have the advantage of flexibility, Low reactivity, physical strength are used for majority of analysis.

Advantages of Open Tubular Column :

Open Tubular column gives better resolution, larger theoretical plate number, greater sensitivity, smaller sample capacity and decrease analysis time than packed column.

Working Of Electron Capture Detector (ECD) in G.C:-

Principle :-

This detector is used in G.C to detect trace amount of chemical compounds in a sample.

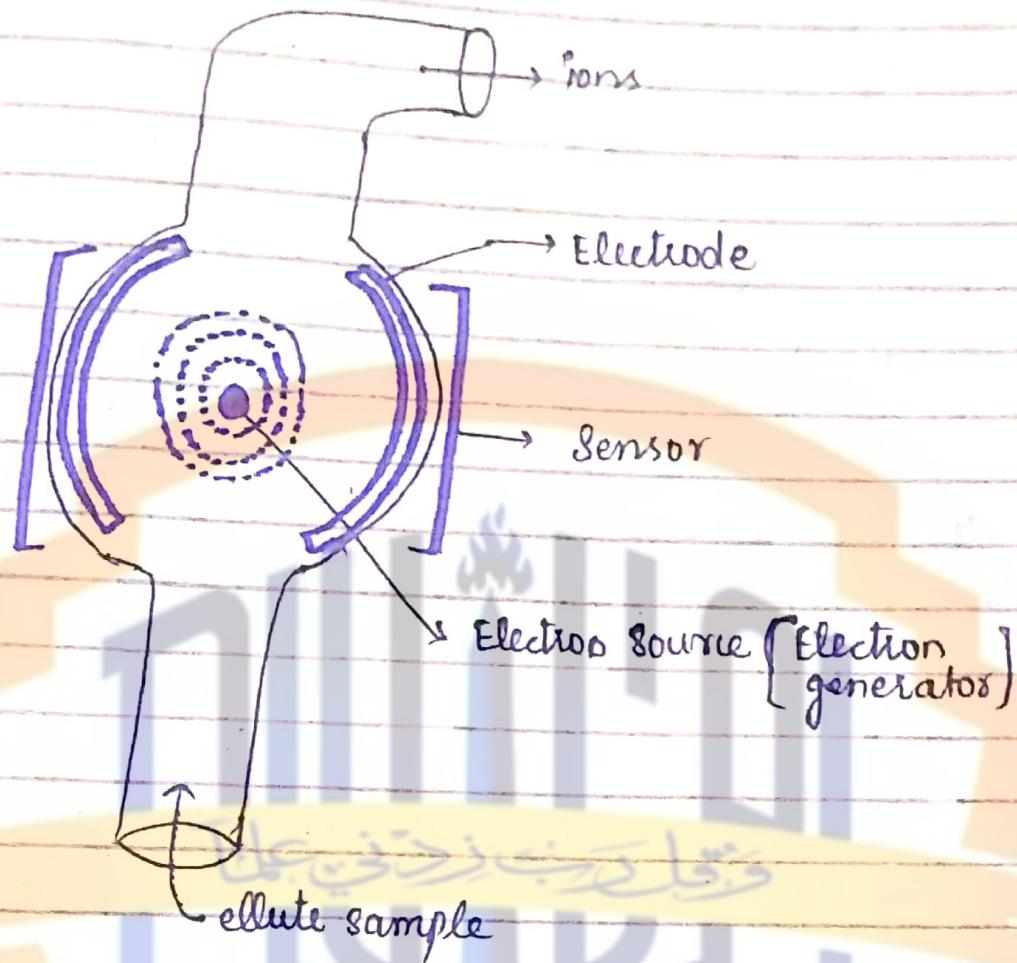
An electron capture detector is a device for detecting atoms and molecules in a gas through the attachment of electrons via electron capture ionization.

Working :-

The ECD uses a radioactive beta particle emitter in conjunction with a so called makeup gas flowing through the detector chamber. In the middle of the detector, an electron generator is made up by platinum wire which is coated by Ni_{63} . A particular number of electrons are present in this position. The electrons are accelerated towards a positively charged anode, generating a current.

As the sample is carried into the detector by carrier gas, electron absorbing analyte molecules capture electrons and reduces the current between the collector anode and cathode. The analyte concentration is proportional to the degree of

electron capture.



Advantages of ECD :-

This detector is particularly sensitive to halogens like chlorinated insecticides, polychlorinated biphenyls, carbonyls, peroxides, quinones, nitro compounds.

Disadvantages of ECD :-

It is insensitive to alcohols, amines and hydrocarbons.

The sensitivity of ECD decreases with moisture.

— (3) —

Various Types of Column in HPLC :-

columns are the main component in HPLC because the column is responsible for the separation of the sample components. The samples passes through the columns with the mobile phase and separates in its components when it comes out from the column.

Generally, silica gel is filled in HPLC columns because of its particle size and helps in separation of components. It is also an inert material that does not react with mobile phases.

Types :-

There are different types of chromatography columns on the basis of their composition and method of separation.

- Normal Phase column
- Reverse Phase column
- Ion Exchange column
- Size Exclusion column

Normal Phase HPLC column :-

This type of columns has more polar stationary phase than the mobile phase. The packing material of the column should be more polar than the mobile phase.

Separation of the sample components occurs on the basis of the polarity of the sample component. Sample components having more polarity interact more with polar stationary phase resulting in separation from less polar component. The chromatography column packing in which normal phase columns are used is known as "Normal Phase Chromatography".

Reverse Phase HPLC Column :-

It is reverse of normal Phase columns. It has a non-polar or less polar stationary phase than the more polar mobile phase. Bonded hydrocarbons like C₈ and C₁₈ and other non-polar hydrocarbons are used as stationary phase in reverse phase columns. This type of chromatography is known as Reverse Phase chromatography.

Ion Exchange HPLC Column :-

The compounds those can easily ionize are analyzed using these columns. Stationary phase in these columns remain acidic or basic having negative or positive charge while mobile phase is a polar liquid as the salt solution in water.

Separation of molecules occurs on the basis of attractive ionic force between

molecules and the charged stationary phase. Due to the exchange of ions during the separation of sample components, is known as Ion Exchange chromatography.

Size Exclusion HPLC column :-

Porous stationary Phase in these columns allows the separation of components according to their size combination of polymers like polysaccharides and silica is used as stationary phase in these columns.

Small sample molecules penetrate in the pores of stationary phase while the large molecules penetrate partially into the pores.

Therefore, the large molecules of the sample elute first than the small molecules and this chromatography is called size exclusion chromatography.

PUACP

— (1) —

Mobile Phase Delivery System :-

In Mobile Phase delivery system, different solvents like water, acetone, chloroform are taken in separate containers.

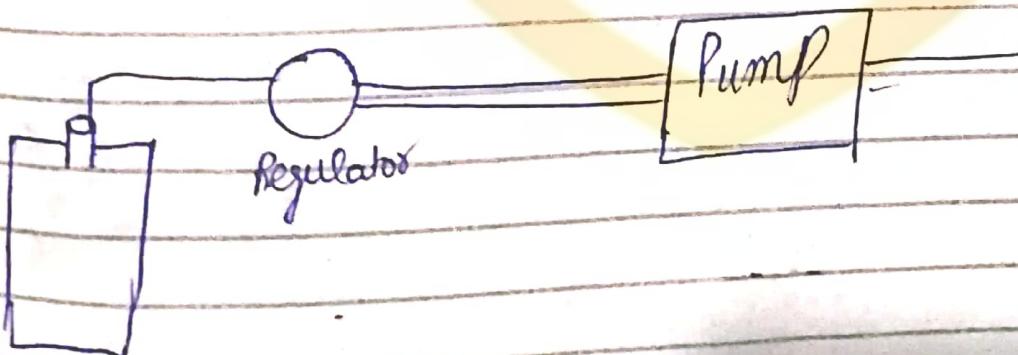
From each container, dissolved gases are removed by degasser in which vacuum pump is used. Then these almost pure gases are entered in a mixture.

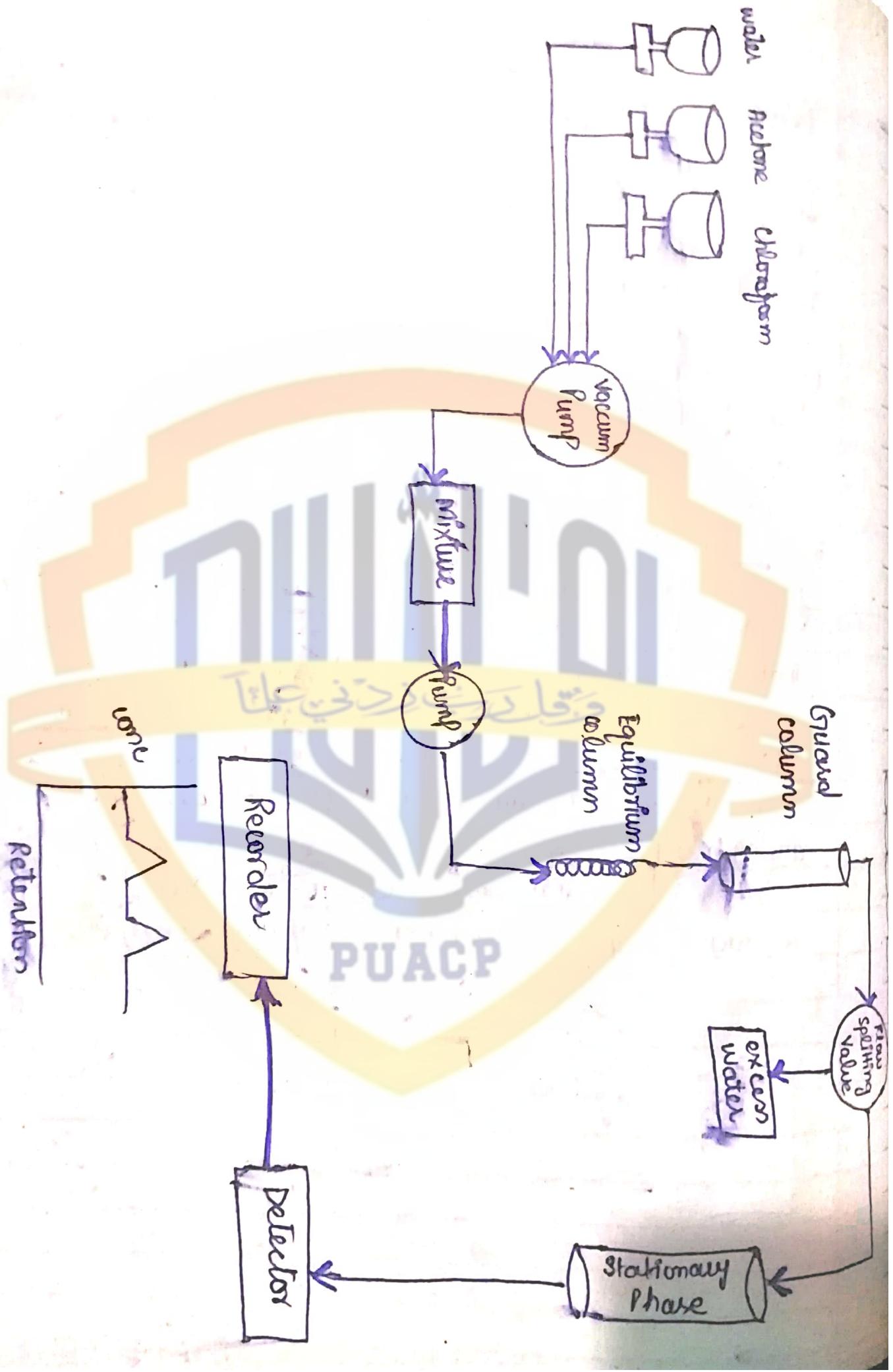
After getting a mixture, this mixture is introduced in Equilibrium column. This provides a chance to get equal and uniform concentration of all solvents.

In Guard column, if any impurity is present that is removed like N_2 , O_2 and vapours.

Then this solution is introduced in Flow Splitting Valve. It actually splits or separates excess solvent which is not in our use during HPLC.

Now this solvent is entered in a such compartment which contains sample.





Instrumentation in HPLC

HPLC system contain following components

- Mobile Phase Reservoir
- HPLC Pump
- Sample Injection System
- Chromatographic column
- Detector
- Data Processor
- Recording System

Instrumentation in GC

It have following components

- Carrier gas
- Sample Injection system
- chromatographic columns
- Column thermosetting
- Flow Meters
- Detectors
- Recorders.

Sample Injection
system

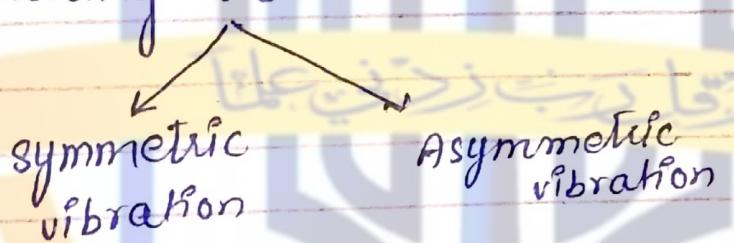
PUACP

① Applications of UV/Vis Spectroscopy - [Sp-II]

- ① Identification of a compound
- ② Determination of a structure
- ③ Study of chemical kinetics
- ④ Study of Isomerism
- ⑤ Quantitative Analysis
- ⑥ Determination of conjugation
- ⑦ Qualitative Analysis
- ⑧ Detection of Impurities.
- ⑨ Tautomeric equilibria

② Vibrational Modes in IR Spectroscopy

① Stretching vibration



② Bending vibration

Bending in
Plane

- ⇒ Scissoring
- ⇒ Rocking

Bending out of
Plane

⇒ wagging

⇒ Twisting

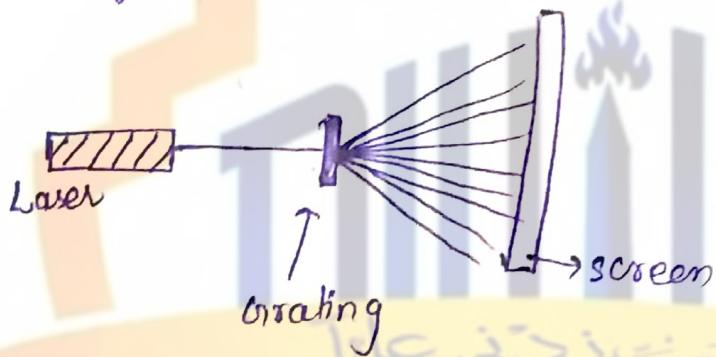
③ Types of Gratings:

- Diffraction Grating
- Transmission Grating

- Ruled Grating
- Holographic grating

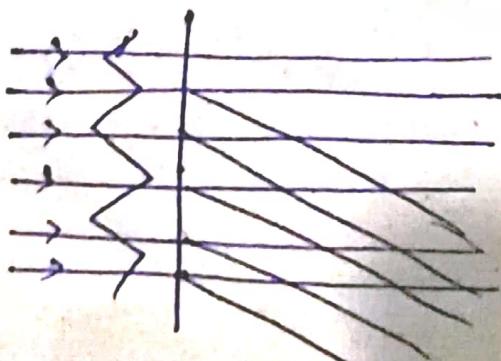
Diffraction Grating:

It gives more refined dispersion of light is obtained by means of diffraction grating. This consist of large number of parallel lines about 1500 - 3000 inch is ruled on highly polished surface of Aluminium.



Transmission Grating

It is similar to the diffraction grating but refraction take place instead of reflection. Refraction produces reinforcements, this occur when radiation transmitted through grating reinforces with the partially refracted radiation.



Radiation Sources of IR spectroscopy

IR instruments require energy which emit IR radiations which must be steady, intense, enough for detection and extended over the desired wavelength.

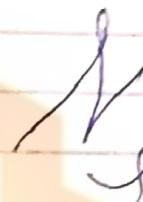
1- Incandescent Lamp

2- Nernst Glower

3- Incandescent wire

4- Globar Source

5- Mercury Arc



Incandescent Lamp

- Temp can be upto 1100°C
- Used in near IR region but failed in far IR region
- A closed wound Nichrome wire is used
- A black oxide film formed on the coil by heating

Nernst Glower

- Temp $1000 - 18000^{\circ}\text{C}$
- In IR region it is most common source
- Consist of hollow rod 2mm in diameter and 2-5 m in length
- Composed of mixture of oxides like Zirconium, Yttrium, Thorium

Globar Source • small rod of silicon carbide • $1300 - 1700^{\circ}\text{C}$

Mercury Arc

Heated Quartz — shorter wavelength

Mercury Plasma — longer wavelength

Incandescent wire

Heated by Passage of current to 1100°C

- It produces low intensity of radiation

3) Radiation Filters used in UV/vis Spectroscopy

The common Radiation filters are as follows:

- Prism
- Monochromator
- Interference filters
- Absorption filters
- Polarizing filters

L.08

① **Prism** - These are used to disperse light into its constituents wavelengths, allowing for selection of specific wavelength for analysis.

② **Monochromator** These devices uses a single wavelength of light from a broad spectrum. They often uses prism or diffraction gratings.

③ **Interference Filters** These are used in spectrophotometer for their narrow bandwidths and high transmission efficiencies.

④ **Absorption filters** These filters absorb specific wavelength of light, allowing only desired wavelengths to pass through.

⑤ **Polarizing filters** These filters allow only light waves oscillating in particular plane to pass through. They are used to control polarization effects in spectroscopy.

Components of Raman Spectrometers

- Light source
- Sample optics
- Monochromator
- Detector
- Recorder
- Filter
- Calibration
- Spectrometer

Source of Light For intense source of radiation a mercury lamp is used. Sometimes lasers are also used for this purpose. Laser light is highly monochromatic and plane polarized.

Sample Optics The sample optics must be arranged so that the scattered radiation is observed at 90° to incident light.

Filters These are made of nickel oxide, glass or quartz were used to eliminate unwanted radiation for getting monochromatic radiation.

Monochromator It must have excellent stray light characteristics. It has 2 functions

1- To increase the resolving power of spectrometer

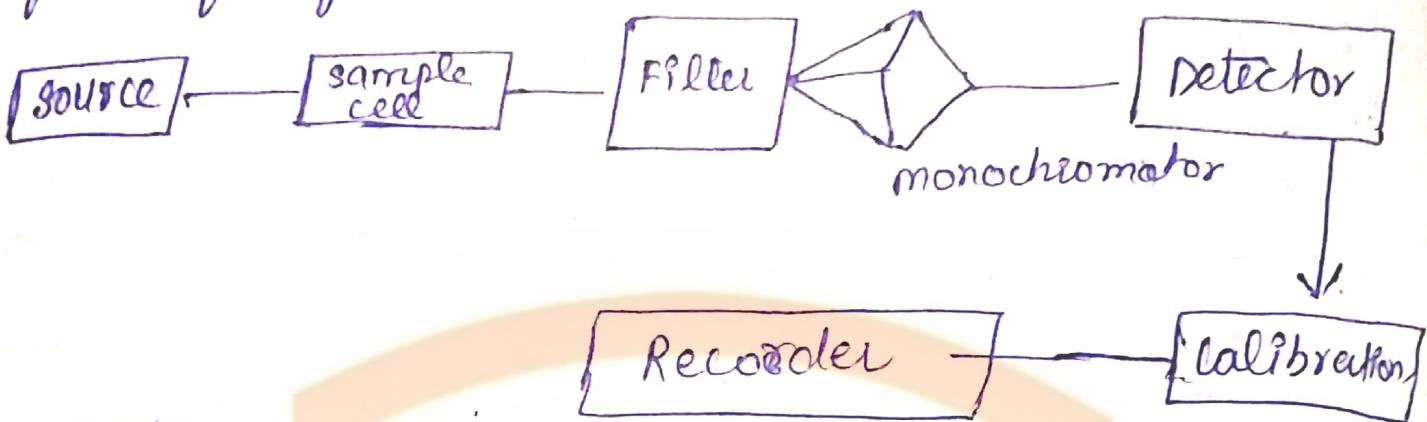
2- To reduce stray light due to exciting radiation

Detectors Photomultiplier is used as detectors in laser spectrometer. It causes the photons incident

or photocathode causes emission of electrons.

Calibration For routine calibration CCl_4 and iodine are used. It is used to record the emission light from laser source

Spectrometer A radiation beam from gas laser is incident on sample cell through lens, then moved to monochromator and after detection and calibration is recorded in the form of signals.



Recorder

It is used to record signals coming from detector.

ICP Inductively Coupled Plasma

Q: 01

1

Explain for sample Pumps And Spray chambers introduction in ICP-AES?

Ans:-
Pump and
In ICP-AES Pumps
liquid samples into
the reproducibility of

There are different
Peristaltic Pump

Diaphragm Pump

Centrifugal Pump

Electric Diaphragm Pump

Spray Chambers:-

ICP spray chambers removes larger sample
droplets from aerosols. Agilent offer a quartz
spray chamber as standard.

The Spray chamber provides maximum
sensitivity and efficiency. It is an integral part
of the sample introduction. It affects precision and
is responsible for filtering the sample mist
to promote the appropriate droplet size distribution
to reach the plasma.

There are different types of spray chambers
are used:

- Cyclonic Spray chamber
- Scott Spray chamber

- Baffled Cyclonic sc
- Ryon Scott sc

The spray chamber filters the aerosol created by the nebulizer. To ensure efficient energy transfer, the maximum size of droplets that enter the plasma is $10 \mu\text{m}$. Filtration is done using different phenomena according to the design of spray chamber.

Elimination of the biggest droplets can be done by gravity or centrifugal effect. Smaller droplets can be created by the evaporation effect or impact on walls of the spray chamber. This will be a competing effect that will create bigger droplets from small droplets.

8

Metal Isotope Spectroscopy

It is an analytical technique used to determine the composition and concentration of metal isotopes in a sample.

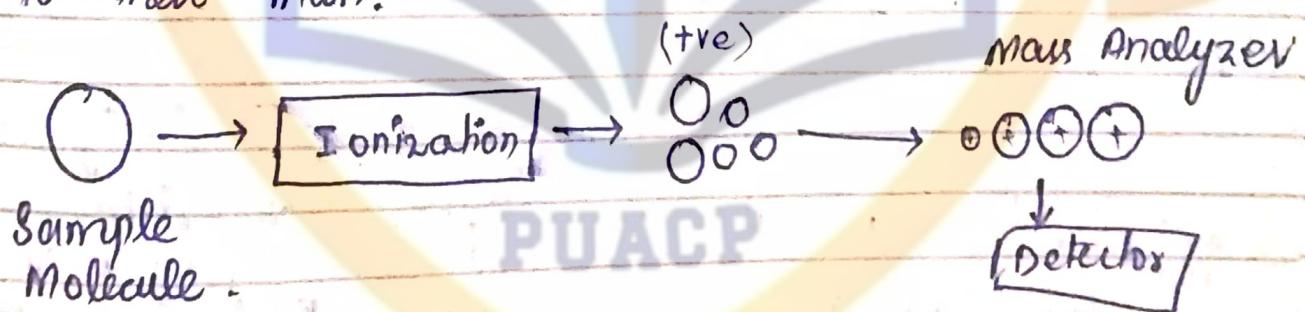
Principle:-

This method relies on principle that isotopes of a metal element have the same number of protons but different number of neutrons resulting in different atomic masses.

Phenomena :-

First, there is an ionization source, where the molecule is given a positive electrical charge either by removing an electron or by adding a proton.

Next in line there is a mass analyzer, where the cationic fragments are separated according to their mass.



Finally, there is a detector which detects and quantifies the separated ions.

In Laboratories

One of the most common type of it in Labs is "electron ionization".

In Ionization source, the sample molecule is bombarded by a high energy electron beam which has effect of knocking a valence electron of the molecule to form radical cation. Then cation breaks up into smaller fragments. Some are positive and others are neutral fragments. The neutral one will adsorb onto the wall of chamber and removed by vacuum source.

Then in Mass analyzer (+ve) charge fragments will move down a tube by electric field.

Then ions are deflected by strong magnetic field. The detector at the end of curved flight tube records and quantifies the stored ions.

a) Components Of Czerny-Turner Monochromator

The Czerny Turner monochromator is a widely used type of monochromator in analytical chemistry, particularly for its high wavelength resolution, and compact design.

The key components of Czerny-Turner Monochromator are:

- Entrance slit
- collimating mirror
- Diffracting grating
- Focusing Mirror
- Exit slit

Entrance Slit :-

This is where the light enters in the monochromator. The slit width determines the resolution and amount of light that can be processed.

Collimating Mirror

After passing through the entrance slit, light is directed onto a collimating mirror that produces parallel light beams.

Diffraction Grating

The parallel light then hits a diffraction grating, which disperses the light into its component wavelengths.

Focusing Mirror

The dispersed light is then reflected off a focusing mirror that directs the light towards exit slit.

Exit Slit

Finally, the exit slit selects a narrow band of the dispersed light allowing only the desired wavelength to pass through for analysis.

Jablonski Diagram

10

It is an energy diagram that represents the electronic states of a molecule and transition between them.

Phenomena

Here a concise explanation of phenomena illustrated in a Jablonski diagram.

Absorption :-

When a photon is absorbed the molecule transitions from the ground state (S_0) to an excited singlet state (S_1, S_2 etc).

Fluorescence :-

A molecule may turn to ground state (S_0) from an excited singlet state (S_1, S_2) by emitting a photon, resulting in fluorescence.

Internal Conversion :-

This is non-radioactive process where the molecule transitions between vibrational levels within the same electronic state.

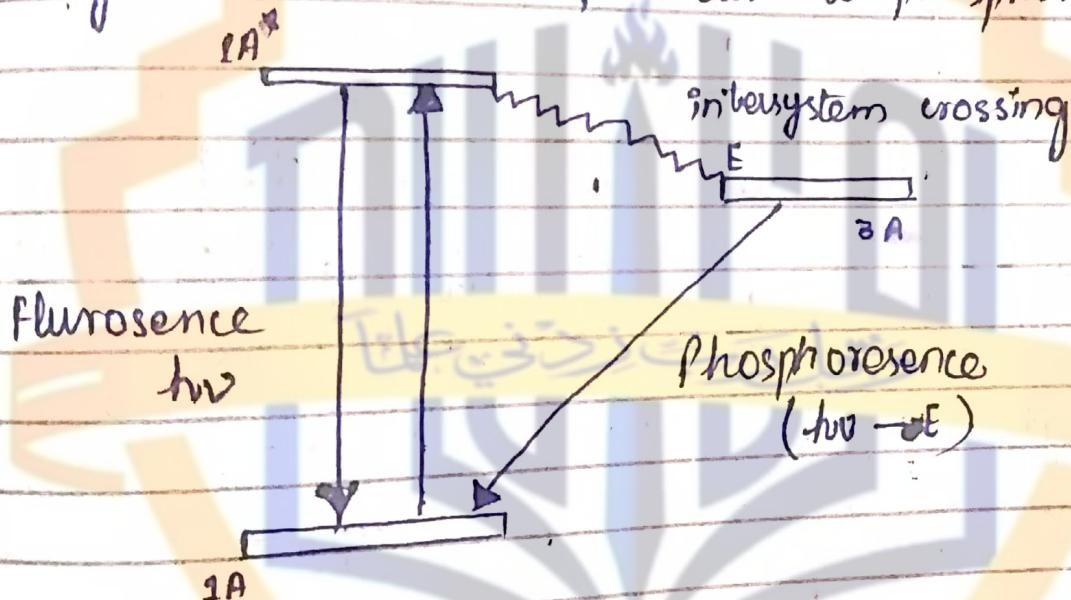
Intersystem Crossing :-

The molecule may cross over

from a singlet state to triplet state (T_1) which has different spin multiplicity.

Phosphorescence :-

If the molecule return to ground state from triplet state (T_1). it may emitted a photon over a longer time scale, known a phosphorescence.



Types of Emitted Raman Radiation Source

11

Raman Radiation sources can be understood through the classification of scattering events.

There are two primary classes of scattering relevant to Raman Spectroscopy-

Elastic Scattering (Rayleigh Scattering)

This occurs when the photon is absorbed by the particle and then re-emitted without any change in energy ($\Delta E = 0$)

The Scattered radiation remains the same wavelength as the incident radiation.

Inelastic Scattering (Raman Scattering)

In this case, the absorbed photon is emitted with change in energy ($\Delta E \neq 0$) resulting shift in wavelength.

Raman scattering can be further divided in two types

Stokes Scattering

The emitted photon has less energy than incident photon, resulting in longer wavelengths.

Antistokes Scattering

The emitted photons has more energy than incident photon, leading to shorter wavelength.

P

Working Of Pyroelectric detectors And Bolometer :-

Pyroelectric detectors and bolometers are both used to detect changes in energy but they operate on different principles and are suited for different applications.

Pyroelectric Detector :-

Pyroelectric detector work based on the pyroelectric effect, where temperature fluctuations in a pyroelectric crystal cause a change in polarization, resulting in a charge being generated. When the crystal absorbs light it heats up, creating a temperature gradient that leads to an electrical signal. They key material used in pyroelectric detectors include DLaTGS, LiTaO₃ and PZT, each with varying properties that affect their pyroelectric coefficient.

Bolometers

Bolometers, on other hand, measure radiant heat by detecting the temperature change caused by absorption of electromagnet radiation. They consists of an absorptive element such as a thin layer of metal, connected to thermal reservoir. The absorbed power raises the temperature of the absorptive element and this temperature change can be measured directly by thermometer.

Absorption Photometry

It is also known as spectrophotometry, is a fundamental analytical technique in chemistry that measures the amount of light absorbed by a substance.

Instrumentation

The key components of

Absorption photometer

include,

Light source :-

It provide light that passes through the sample.

Monochromator :-

It isolates the specific wavelength of light that is absorbed by analyte.

Sample Holder

It contains the sample solution.

Detector :-

It measures the intensity of light before and after passing through

sample.

Readout device

It displays the absorbance or transmittance values.

Principle

The operation of an absorption photometer is based on the Beer-Lambert Law, which states that the absorbance (A) is directly proportional to the concentration of absorbing species in the solution, the path length of the sample (l), and molar absorptivity (ϵ).

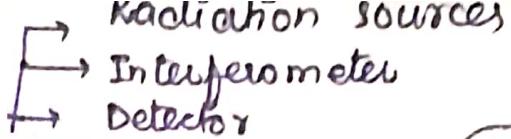
$$A = \epsilon l c$$

Applications

It is widely used in various fields for

- **Concentration Determination** :- To measure the concentration of a substance in a solution.
- **Kinetic Studies** :- To monitor reaction rates by measuring changes in absorbance over time.
- **Enzyme Assays** :- To determine enzyme activity.
- **Water Analysis** :- For testing the quality of water by detecting pollutants.
- **Pharmaceutical Analysis** :- To ensure the quality and quantity of active ingredients in drugs.
- **Medical Research** :- It helps to study the interaction of light with biological tissues.

L-9



Instrumentation of FTIR

14

The basic components of FTIR spectrometer are as follows.

- Source
- stationary Mirror
- Beam splitter
- Detector

1- [Radiation Sources]

- Incandescent Lamp
- Nernst glower
- Globar source
- High Pressure Mercury Arc

2- [Interferometer]

The basic component of FTIR is Michelson Interferometer.

The Monochromator is replaced by an interferometer, which divides radiation beam then recombines them in order to produce respective interference signals measured as a function of optical path difference.

It produces interference signals, which contains IR spectral information generated after passing through a sample.

It consists of three components

- Moving Mirror
- Fixed Mirror
- Beam Splitter

Mirrors They are generally made of metal.

The mirrors are polished on the front surface and may be gold coated to improve corrosion resistance.

Beam Splitter It can be constructed of a material such as Si or one deposited in a thin coating onto an IR-transparent substrate.

Construction

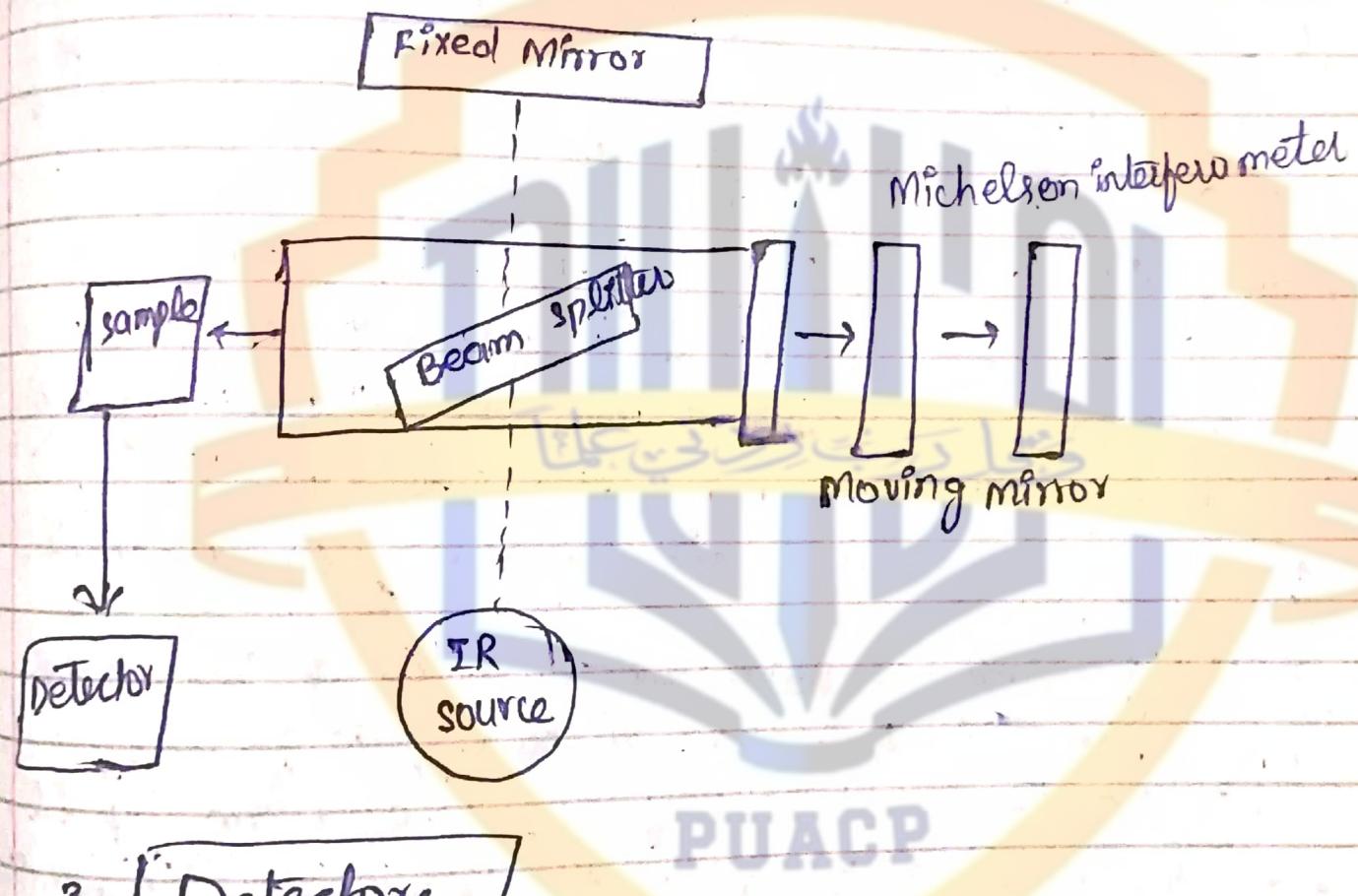
The two mirrors are perpendicular to each other. The beam splitter is a semi-reflecting device and is often may be deposited onto a thin film of Chromium onto a flat KBr substrate.

Working

Radiation from the broadband IR source is collimated and directed into the interferometer, and impinges on the beam splitter.

- At the beam splitter half of the IR beam is transmitted to the fixed mirror and the remaining half is reflected to the moving mirror.
- After the divided beams are reflected from the two mirrors, they are recombine at beam splitter

Due to the change in the relative position of mirror, an interference pattern is generated. The resulting beam then passes through the sample is eventually focused on the detector.



3- [Detectors]

- Pyroelectric detector
- Photonsensitive semiconducting detectors

Basic Principle of ICP-AES

(15)

ICP-AES is an emission spectrophotometric technique exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state after excitation by high temperature (Argon) Plasma.

It is also referred as (ICP-OES) optical emission spectroscopy. It is an analytical technique used to detect chemical elements.

It is a type of emission spectroscopy that uses the ICP to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The "plasma" is a high temperature source of ionized source gas.

The plasma is sustained and maintained by inductive coupling from electrical coils at megahertz frequencies.

The source of temperature is in the range from 6000 to 10,000 K. The intensity of emissions from various wavelengths of light are proportional to the concentration of elements within sample.

Applications Of IR on the basis of Qualitative and Quantitative Analysis

IR spectroscopy is a powerful tool in analytical chemistry, here we have its applications.

Qualitative Analysis

1- Identification Of Compound :-

It is primarily used to identify functional groups and characterized covalent bonds within molecules. By Analyzing the absorption spectra, we can determine the structure of unknown substance.

2- Functional Group Determination :-

The technique is sensitive to vibrational frequencies of molecular bonds which are unique to specific functional groups making it an essential method for identifying organic and inorganic compounds.

Quantitative Analysis

1. Concentration Measurement :-

IR spectroscopy can measure the concentration of substance by analyzing the intensity of absorption peaks. This is particularly useful in determine the purity of a sample or the percentage composition of a mixture.

2. Pharmaceutical application :-

In pharmaceutical industry, quantitative IR spectroscopy is used to analyze drug components, excipients and formulations. It is non-destructive method that give precise results.

Special Detection System & Readout devices used in ICP-AES

The detection system is used to convert photons in electric current. The most common system are :

- Photomultiplier tubes
- Solid State detector

1- Photomultiplier tubes

It is made of a cathode followed by several dynodes to amplify the signal. Photons reach the cathode through a window made of a material suitable for the wavelength range that has to be covered. Electrons are created by the cathode and amplified by dynodes.

2- Solid State Detectors

These are based on CCD (charge-coupled device) or CD (charge injection device) technology. These detectors use the photon silicon interactions to allow measuring the signal. The photons reaching the detector are converted into electrons through an electron-hole pair that can be transported to reading device and measured.

ReadOut devices

The readout devices responsible for processing and displaying the data obtained from detector.

- Analogue to Digital converter :-

It converts analogue signal from detector into digital form.

- Computer System :- It process the digital data, applies calibration curves and calculate concentration of element.

- Display :- The processed information is displayed in a readable format, often as spectrum or conc. readout.

Working Principle of Atomic Emission Spectroscopy

It is a method used to analyze the elemental composition of a sample by studying the wavelength of light emitted.

- ① Sample Preparation :- The sample is prepared in a way that it can be vaporized into free atoms or ions. This is often done by applying high temperature to the sample in flame, plasma or spark.

- ② Excitation When the atoms or ions in sample are then excited to higher energy levels. This excitation can be due to the thermal energy in the flame or plasma or through electrical energy in case of spark or arc methods.

③ Emission of Radiation

atoms returns to lower energy state, they emit radiation. The wavelength of this emitted radiation is characteristic of element.

④ Detection

The emitted light is passed through a spectrometer which disperses the light into its component wavelength. It measures the intensity of light at each wavelength.

⑤ Analysis

The intensity of emitted light at a specific wavelength

is proportional to the number of atoms at particular element in excited state.

By measuring the intensity, concentration of element in sample is measured.



Principle And Application of Mass Spectrometer

Principle :-

Mass Spectrometer is an analytical technique that separates ionized particles such as atoms, molecules and clusters by using differences in ratio of their charges to their respective masses and can be used to determine the molecular weight of the particles (m/z)

Applications of Mass Spectrometer

There are following applications of Mass spectrometer -

- Due to its capability to distinguish between substances, it is used to determine unknown substances.
- To identify the isotopes of a substance
- Elemental And Isotopic Analysis
- In analytical laboratories that study the chemical physical and biological properties of a substance it is favored over several other analytical techniques
- Mass Spectral Imaging
- Organic And Bio organic analysis
- Structural elucidation
- characterization of ionic species and chemical reactions.

(20) Advantages and Limitations of (AES) and (AAS)

These both are valuable techniques in analytical chemistry-

Advantages

• Low Interferences :-

AES typically has less interferences from other elements in sample due to higher temperatures.

• Multi-element Analysis :-

AES can detect multiple element at once, which is beneficial.

• Dynamic Ranges :-

AES offers a broad range of detection from very low to high concentrations.

• Chemical Interferences :-

AES experiences low chemical interferences from other elements.

• Stable Results :-

AES gives more reliable and consistent results.

PUACP

• Light Source :-

AAS uses a light source like Hollow Cathode Lamp and AES uses plasma or flame.

Limitations

- Spectral Interferences :-

AES suffers from spectral interferences due to presence of many emission lines.

- Cost And Operating Expenses :-

The equipment and operations costs for AES are generally higher than for AAS -

- Sample State,-

AES typically requires sample to be in a liquid solution which may necessitate additional sample preparation.

PUACP

Multichannel Spectrometer, used in ICP-AES

It is sophisticated instrument designed to detect and measure the presence of multiple element within a sample simultaneously.

Working:-

The liquid sample is nebulized into a fine aerosol and introduced into a plasma torch.

An inductively coupled Plasma is generated typically using argon gas. The excited atoms emit lights of characteristic wavelengths as they return to the ground state.

The emitted light is passed through the spectrometer which separates it into its component wavelength. Unlike singal channel spectrometer, a multichannel spectrometer can detect multiple wavelengths.

Advantages:-

- 1- High Selectivity
- 2- High Sensitivity
- 3- Large Dynamic Range
- 4- Lower Detection Limits
- 5- Multi-element detection
- 6- Fewer Matrix Interferences

(Sp - I)

①

Long Questions

— (1) —

Applications of Nernst Equation :

The Nernst equation can be used to calculate

- Single electrode reduction or oxidation potential at any conditions
- Standard electrode potentials
- Comparing the relative ability as a reductive or oxidative agent.
- Finding the feasibility of combining such single electrodes to produce electrical potential.
- Emf of an electrochemical cell
- Unknown ionic concentration
- The pH of solutions and stability of sparingly soluble salts can be measured with help of Nernst equation.

— (2) —

Derivitization in GC :-

Derivitization is the process of chemically modifying a compound to produce a new compound to produce a new compound which has properties that are suitable for analysis using a GC or HPLC

Why ?

- To permit analysis of compounds which are not directly analysable due to for example inadequate stability and volatility.
- To improve chromatographic behaviour or detectability.
- Many compounds do not produce a useable chromatography or the sample of interest goes undetected.

- The main reason for derivatizing is to impact volatility to other non-volatile compounds.
- It is done to change the analyte properties for a better separate and also for enhancing the method of sensitivity.
- To improve the chromatographic separation, peak shape or response of the analyte.

— (3) —

Applications of TGA Curves:-

- TGA is primarily used to characterize materials by measuring change in mass a function of temperature.
- It is used to study the kinetics of the reaction rate constant.
- From TGA, we can determine the purity and thermal stability of both primary and secondary standard.
- Used in the study of catalyst.
- Analysis of oxidation, evaporation or combustion.
- Oxidative stability of materials.
- Estimated life time of a product.
- It is especially useful for studying polymer based products that need to react.
- The effect of reactive or corrosive atmosphere on materials.

(4)

Problems in using HPLC :-

Most common problems are:

① HPLC Pressure Problems:-

The primary issues with pressure include.

Abnormal Pressure which generally means, there is no flow because there is no power, happens when there is leak or air trapped in the pump head.

Pressure cycling can be caused by air in the pump, faulty valves, a system leak, seal failure in pump

② HPLC Leakage Problems:-

Leaks can occur anywhere within HPLC.

Leaks at the fittings typically means the fitting needs to be tightened, cleaned or replaced.

Leak at the HPLC column can be caused by loose end fitting that needs to be tightened.

Leak at the Pump can be due to loose fittings or loose valves that must be tightened.

③ HPLC Chromatographic Problems:-

It can cause

Peak Tailing due to fit blockage, a column, sample interaction with active sites.

Peak Fronting due to low temperature using the wrong sample solvent.

Split Peaks due to contamination in the column inlet or on the guard.

Distortion of Large Peaks due to overloading sample

Distortion of Small Peaks due to using the wrong injection solvent.

Retention Time drift: due to poor control of temperature or column equilibrium or mobile phase changing.

Tailing of acidic or basic peaks due to inadequate buffering.

—(5)—

Potentiometric Acid Base Titration

An acid-base titration is a quantitative analysis used to determine the acid or base concentration by precisely neutralizing the acid or base with a known concentration standard solution.

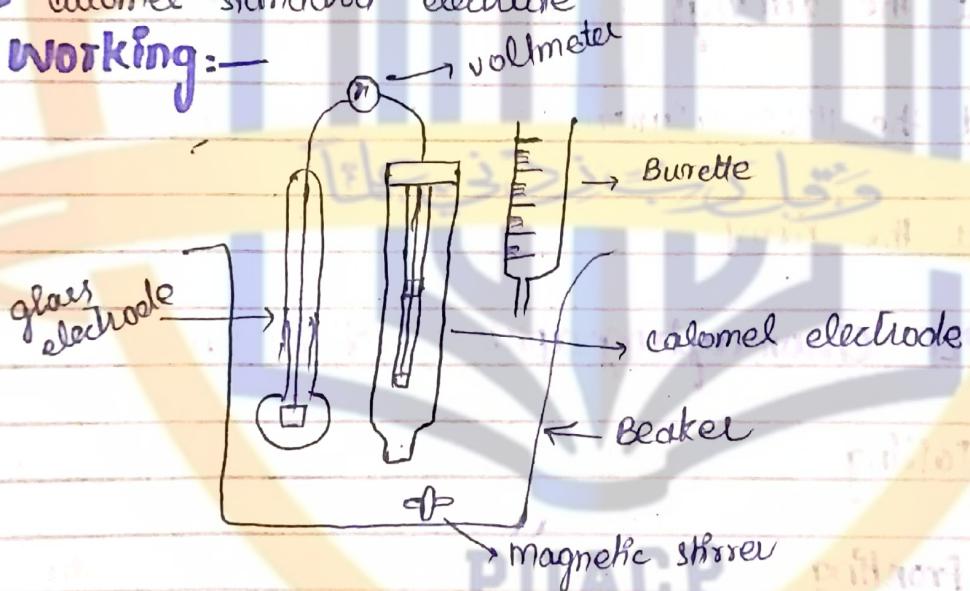
Example:- Titration of HCl with NaOH

Apparatus:-

The apparatus used for potentiometric acid-base titration is

- Burette
- Beaker
- Magnetic stirrer
- Glass electrode
- Electronic voltmeter
- calomel standard electrode

Working:-



A Hydrogen electrode or a glass electrode is immersed in solution of the acid whose strength is to be determined - The glass electrode is coupled with a standard calomel electrode.

The cell than connected with potentiometer or voltmeter - When a base is added , pH of the solution changes -

UV-Visible Spectroscopy

Long Questions

Question No : 01

Write down the Applications of UV-visible Spectroscopy ?

Ans :-

UV-Visible spectroscopy finds vast applications in the laboratory and industry, only some important applications play role in organic chemistry.

- 1- Identification of a compound
- 2- Determination of Structure
- 3- Study of Chemical Kinetics
- 4- Study of Isomerism
- 5- Quantitative Analysis
- 6- Detection of Impurities
- 7- Structure elucidation of organic compounds
- 8- Determination of Molecular weight
- 9- Qualitative Analysis
- 10- Detection of Functional groups
- 11- Detection of Conjugation

Identification of a Compound :-

The identity of a compound synthesized in the lab can be established with certainty by comparing its UV-visible spectrum with that of the standard sample.

Determination of Structure :-

In cases, where very little information about a compound is available on chemical grounds certain conclusions can be drawn by studying the

UV-visible spectra of a compound and correlating with extensive data available about the bands and intensities.

Study of Chemical Kinetics :-

Any chemical reaction characterized by disappearance of a functional group and appearance of a new functional group can be studied by studying the intensity of absorption maxima of disappearing functional groups.

Study of Isomerism :-

When a compound exhibits geometrical isomerism, the trans-isomers shows absorption at a higher wavelength with large values of extinction coefficient compared to cis-isomer.

Quantitative Analysis :-

UV-visible spectroscopy can also be used for quantitative analysis using Beer-Lambert's Law. The concentration of single absorbing substance in a solution can be readily determined using this law.

Detection of conjugation :-

With the help of UV spectrum, one can establish the presence of conjugation in a compound.

Detection of Impurities :-

UV absorption spectroscopy has one of the best method for determination of impurities in organic molecules. Additional peaks can be observed due to impurities in the sample and it can be compared with that of the standard raw material.

Structure Elucidation of Organic Compounds :-

UV-spectroscopy is useful in the structure elucidation of organic molecules, the presence or absence of unsaturation, the presence of hetero-atoms. From the location of peaks and combination of peaks, it can be concluded that whether the compound is saturated or unsaturated, heteroatoms are present or not etc.

Determination of Molecular weight :-

Molecular weights of compounds can be measured spectrophotometrically by preparing the suitable derivatives of these compounds. **For Example:** If we want to determine the molecular weight of amine then it converted into amine picrate.

The known concentration of amine picrate is dissolved in a litre of solution and its optical density is measured at

λ_{max} 380 nm.

Qualitative Analysis :-

UV-spectroscopy can characterize those types of compounds which absorb UV-radiation. Identification is done by comparing the absorption spectrum with the spectra of known concentrations.

Detection Of Functional groups :-

This technique is used to detect the presence or absence of functional groups in the compound. Absence of a band at particular wavelength regarded as evidence for absence of particular group.

Question No: 02

Describe Beer - Lambert's Law?

Ans:-

Beer-Lambert's Law :-

Beer's Law and Lambert's Law are usually combined and the combined law is known as Beer-Lambert's law. This law can be stated as :

"the rate of decrease of intensity of monochromatic beam of light with respect to the thickness of the medium is directly proportional to intensity of light and concentration of absorbing substance in solution."

Mathematically,

Beer-Lambert's law can be stated as :

$$-\frac{dI}{dl} \propto I \cdot C$$

- I → intensity of light
- C → concentration of absorbing substance in solution.
- $-\frac{dI}{dl}$ → rate of decrease of intensity of light with respect to thickness of medium or optical path length.

The above expression can be written as :

$$-\frac{dI}{dl} = K \cdot I \cdot C \quad \text{--- (1)}$$

- K → proportionality constant. Its value depends on the nature of absorbing material and wavelength of light used.

Integrate the Eq (1)

$$\int \frac{dI}{dl} = -K \cdot C \int dI$$

Separate variables :-

$$\ln I = -K \cdot C \cdot l + \text{constant} \quad \text{--- (2)}$$

For the value of constant :-

The value of constant of integration can be determined using initial conditions:

when, $t=0$ then $I=I_0$

$$\ln I_0 = \text{constant} \quad \textcircled{3}$$

Putting Eq (3) in Eq (2) :

$$\ln I = -K \cdot C \cdot J + \ln I_0$$

$$\ln I - \ln I_0 = -K \cdot C \cdot J$$

$$\ln \left(\frac{I}{I_0} \right) = -K \cdot C \cdot J$$

$$\ln \left(\frac{I_0}{I} \right) = K \cdot C \cdot J$$

Taking common log :-

$$2.303 \log \left(\frac{I_0}{I} \right) = K \cdot C \cdot J$$

$$\therefore \ln = 2.303 \log$$

$$\log \left(\frac{I_0}{I} \right) = \frac{K}{2.303} \cdot C \cdot J$$

$$\therefore \underline{K} = \underline{\epsilon}$$

$$\log \left(\frac{I_0}{I} \right) = \epsilon \cdot C \cdot J$$

$$2.303$$

- ϵ → It is called Molar extinction coefficient or molar absorption coefficient or molar absorptivity.

$$\log \left(\frac{I}{I_0} \right) = -\epsilon \cdot C \cdot J$$

$$\frac{I}{I_0} = e^{-\epsilon \cdot C \cdot J}$$

$$I = I_0 e^{-\epsilon \cdot C \cdot J}$$

- $\log \left(\frac{I_0}{I} \right)$ → Absorbance → It is a dimensionless quantity and denoted by 'A'. Its value depends upon the path length, concentration of absorbing substance and wavelength of light.

So,

$$A = \epsilon \cdot C \cdot J$$

$$\epsilon = \frac{A}{C \cdot J}$$

The value of 'B' can be determined from the slope of the plot.

If $C = 1 \text{ mol dm}^{-3}$ and $l = 1 \text{ cm}$, then

$$\epsilon = A \quad \text{--- (4)}$$

The greater the molar absorptivity, the greater the absorbance.
According to Beer-Lambert's law for Multicomponents system
absorbance of a mixture can be written as:

$$A = A_1 + A_2 + A_3 + \dots$$

$$A = \epsilon_1 \cdot I \cdot C_1 + \epsilon_2 \cdot I \cdot C_2 + \epsilon_3 \cdot I \cdot C_3 + \dots$$

Another important quantity related to this law is "Transmittance".
"Transmittance" is denoted by T . It is also dimensionless quantity.
It can be defined as:

$$T = \frac{I}{I_0}$$

- I_0 → intensity of incident light
- I → intensity of transmitted light

Mathematical Relation b/w Absorbance and Transmittance:

$$A = \log \left(\frac{I_0}{I} \right)$$

$$A = \log \left(\frac{1}{T} \right)$$

$$\therefore \frac{I_0}{I} = \frac{1}{T}$$

$$A = -\log T \quad \text{--- (5)}$$

Percent Transmittance :-

$$\text{Percent Transmittance } (\%T) = T \times 100 \quad \text{--- (6)}$$

- Hence Absorbance is equal to the negative logarithm of transmittance.
- Both quantities are interconvertable.
- $A \rightarrow$ varies from 0 to 2
- $\%T \rightarrow$ varies from 0 to 100.

Question No : 03

Write down the factors which affect the λ_{max} value?
Ans:-

There are the following factors which affect the λ_{max} value.

- Effect of Sample Temperature
- Effect of Sample Concentration
- Effect of Sample pH
- Effect of Solvent
- Effect of Conjugation
- Effect of Steric Hindrance

Effect of Conjugation :-

When two or more chromophores are conjugated, the absorption maxima is shifted to a larger wavelength or shorter frequency. Conjugation increase the energy of HOMO and decreases the energy of LUMO. As a result less energy is required for an electronic transition in a conjugated system.

As the number of conjugated double bonds increases, the value of λ_{max} also increases. The more conjugated double bonds in a compound, the less energy is required for electronic transition and therefore, the longer is the wavelength at which electronic transition occurs.

PUACP

Effect of Steric Hindrance :-

UV-Spectroscopy is very sensitive to the distortion of the chromophores. The position of absorption maximum and its intensity depend on the length and effectiveness of the conjugative system.

Steric hindrance is mostly occur in geometrical isomers. Trans-isomers exhibit absorption peaks at longer wavelength and absorptivity is higher than counterpart.

The position of absorption peak is dependent on the effectiveness and length of conjugation system.

Effect of Solvent :-

The absorption spectrum depends on the solvent in which the absorbing substance is dissolved. The choice of solvent can shift peaks to shorter or longer wavelengths.

This depends on the nature of interaction of the particular solvent with the environment of chromophore in the molecule under study.

It is usually observed that ethanol solutions give absorption maxima at longer wavelength than hexane solutions.

Effect of Sample PH :-

The pH of the sample solution also have a significant effect on absorption spectra. The absorption spectra of certain aromatic compounds such as phenols and anilines on changing the pH of the solution.

Extended conjugation leads to a decrease in the energy difference between the HOMO and LUMO orbitals which results in red or bathochromic shift along with the increase in the intensity of absorption.

Effect of Sample Temperature :-

The following three criteria may be given as general effect of temperature on solution spectra.

Band of sharpness increases with decreasing temperature.

Position of absorption maximum does not move or moves very little towards the longer wavelength side, with decreasing temperature.

The total absorption intensity is approximately independent of the temperature.

③

Effect of Sample Concentration :-

According to Beer-Lambert's law it might be expected that the sample concentration is directly proportional to the intensity of the absorption.

At high concentrations, molecular interaction can take place causing changes to the position and shape of absorption bands. Such effects need to be identified and take into consideration.

The solvent used affect also the fitness of absorption band in UV-spectrum. It has been observed that polar solvents give rise to broad bands while non-polar solvents show more resolution. While completely removing the solvent gives the best resolution. This is due to solvent-solute interaction.

Question No : 04

Describe the various types of Electronic Transition in UV/vis Spectroscopy?

Ans:-

Electronic Transition :-

Molecular electronic transitions take place when electrons in a molecule are excited from one energy level to a higher energy level.

OR

The jumping of an electrons from one energy level to another is called electronic transition.

Types of Electronic Transitions in UV/vis Spectroscopy

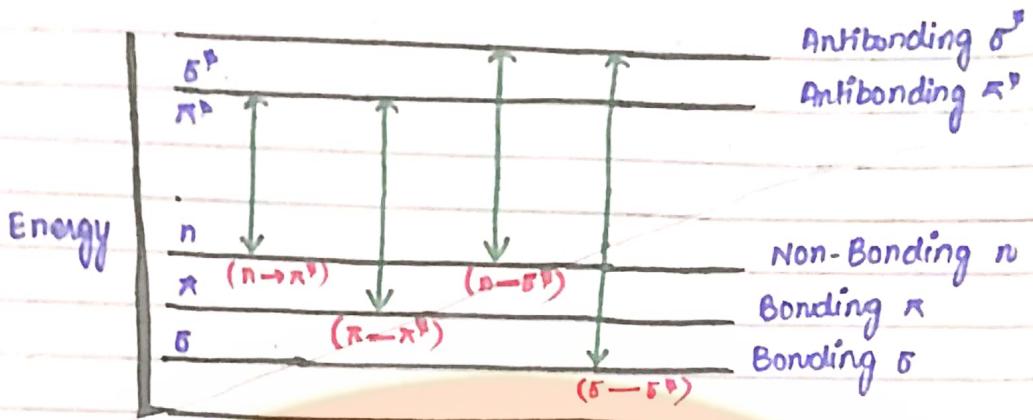
There are following types :

1- $\sigma \rightarrow \sigma^*$

2- $n \rightarrow \sigma^*$

3- $n \rightarrow \pi^*$

4- $\pi \rightarrow \pi^*$



$$\Delta E(n - \pi^*) < \Delta E(\pi - \pi^*) < \Delta E(n - \sigma^*) < \Delta E(\sigma - \sigma^*)$$

① $\sigma - \sigma^*$ Transition :-

- Transition in which a σ electron is excited to σ^* orbital is called $\sigma - \sigma^*$ transition.
- A high amount of energy is required to this transition.
- Energy supplied by UV-radiation of wavelength less than 200nm.
- Saturated hydrocarbons such as CH_4 , C_2H_6 , C_3H_8 , C_6H_{12} in which electrons are involved in the formation of σ -bond formation.

② $n - \sigma^*$ Transition :-

- It involves the excitation of non-bonding electron to σ^* orbital.
- The compounds like O, S, N are not participate in this bonding.
- Less amount of energy is required than $(\sigma - \sigma^*)$ transition.
- Energy gap is less as compared to $(\sigma - \sigma^*)$.
- Photons of lower energy is needed for longer wavelength.

③ $n - \pi^*$ Transition :-

- It involves the excitation of non-bonding electrons to π^* orbitals.
- It occurs in compounds having double or triple bond $\text{O}=\text{O}$, $\text{S}=\text{N}$.
- The small amount of energy is required than $(\sigma - \sigma^*)$, $(n - \sigma^*)$.
- Photons of higher wavelength are needed.

④ ($\pi - \pi^*$) transition :-

- It involves the excitation of π -electrons to π^* orbitals.
- Large amount of energy is required, but the intensity of absorption is very high.
- It occurs in unsaturated compounds containing at least one multiple bond.

