`

CHE301 final term notes

Regards By Admins:

VU Friends

Q.1 What is gravimetric analysis?

Gravimetric methods are quantitative methods that are based on determining the mass of a pure compound to which the analyte is chemically related.

• Cheap, easily available apparatus,

• Slow and accurate

• Wide range of sample conc.(ng- kg)

• No calibration required

Q.2 Write a note on classification of gravimetric analysis?

There are three classification of gravimetric analysis:

1. Precipitation gravimetry
2. Volatilization gravimetry
3. Electrogravimetry

1. Precipitation gravimetry.:
   the analyte is separated from a solution of the sample as a precipitate and is converted to a compound of known composition that can be weighed.

2. Volatilization gravimetry:

   , the analyte is separated from other constituents of asample by conversion to a gas of known chemical composition. The weight of this gasthen serves as a measure of the analyte concentration.

3. Electrogravimetry.:

the analyte is separated by deposition on an electrode by anelectrical current. The mass of this product then provides a measure of the analyte concentration.

Q.3 Which steps required in gravimetric analysis?

There are following steps required in gravimetric analysis:

- Preparation of the solution
- Washing
- Drying or igniting
- Weighing
- CalculationPrecipitation

- Washing drying origniting
- Weighing Calculation

Q.4 Write properties of good precipitates?

- Should be filtered and washed easily
- Very low solubility (no significant loss during filtration / washing)
- Unreactive with constituents of the atmosphere
- Chemical composition must be known, if necessarily ignited,

Q.5 Write types of precipitates (on basis of particle size)?

1-Colloidal suspensions:

- Particles are invisible to the naked eye (10−7 − 10−4 cm in diameter).
- Colloidal particles show no tendency to settle from solution.
- Not easily filtered.

2-Crystalline suspension:

- particles with dimensions on the order of tenths of a millimeter or greater.
- The temporary dispersion of such particles of a tend to settle spontaneously,
- Easily filtered.

Q. 6 Define coagulation of Colloids?

- By short period of heating to decreases the number of adsorbed ions and

thus the thickness tohfe double layer.

- Increase the electrolyte concentration of the solution
- In acidic or basic solution

Q.7 Define peptization of Colloids?

- Peptization is the process by which a coagulated colloid reverts to its original dispersed state.
- Peptization can be avoided by washing with an electrolyte that can be volatized by heating.

Q. 8 Write mechanism and types of precipitation?

Precipitation mechanism:

1. Induction period.
2. Nucleation.
3. Particle growth to form larger crystal
4. Adsorption
5. Electrostatic

NOTE:

- In nucleation, large number of small particles produced,
- If growth predominated, a smaller number of larger particles is produced.

Types of co-precipitation:

A. Surface adsorption
B. Mixed-crystal formation
C. Occlusion
D. Mechanical entrapment

Q.9 What is crystal Growth?

- Used for single crystal X-ray studies
- Separation soyfnthesized products
- Separation from impurities

Q.10 Define chromatography?

- Chromatography is a method of physically separating mixtures of gases,

liquids, or dissolved substances.

- Chromatography can be used to identify drugs, poisons and many other substances.
- Separation is determined by the molecular size and/or charge.

Q.11 Separation of molecules by distribution between?

Stationary phase:

- A stationary phase (absorbent) phase the material on which the separation takes place.can be solid, gel, or liquid. Also called matrix, resin, or beads.

Mobile phase:

- The mobile phase is the solvent transports the sample and it is usually a liquid, but may also be a gas. Also called eluting buffer.
- The compounds to be separated are considered solutes.

Important Note:

Mobile Phase: Non Polar
Stationary Phase: Polar

Q.12 Chromatography done by which phases?

➢ **By mobile phase:**

1. Liquid chromatography
2. Gas chromatography

Mobile Phases Used:

- Hexanes, ether, tetra chloromethane, Xylene, Toluene
- Chloroform, Dichloromethane Ethyl Acetate
- Water, Alcohol
- Buffer

➢ **By Stationary Phases:**

- Filter Paper, Cellulose
- Silica Gel, Alumina, Polyamide (in TLC)
- Squalene, carbowax M, Dimethylpolysiloxane (in GC)
- C-8, C-18, Licosorb, Silicone (in HPLC

Q.13 Explain chromatography..?

The mechanism that causes the stationary phase to retard the movement of molecules:

1. **Sieve mechanism:**

Separation according to size or MW. (molecular sieve = gel filtration = size exclusion = gel permeation).

2. **Charge interaction:**

Separation based on net charge.

3. **Solubility characteristics** :

Separation based on polarity Hydrophobic chromatography, reverse-phase chromatography, Adsorption or normal-phase

4. **Biological or Specific interaction**:

:

Capture any molecule that exhibit such property
affinity chromatography, dyechromatography
Antibody-antigen: (Immuno precipitations and other forms)

Q.14 Write name of chromatographic Techniques..?

- Paper Chromatography
- Thin layer chromatography
- Column Chromatography
- Electrophoresis
- High Performance
- Liquid Chromatography
- Gas Chromatography

Q.15 What is paper Chromatography?

- **Stationary Phase used:**

Filter Paper, Cellulose

- **Mobile Phase used:**

water: alcohol or organic solvents

- Use chromatographic tank, beaker covered with petri dish, any jar with lid
- To analyze: Ink, steroids, food colours, pigments and drugs
- A qualitative technique to calculate and compare *Rf* values
- *Rf* is ratio between distance traveled by individual component divided by the distance travel by solvent

Q.16 Explain thin layer chromatography?

- **Stationary Phase used:**
  Adsorbent silica pasted on glass or aluminum plates
- **Mobile Phase used:**
  Usually organic solvents (pure or mixed in variable ratios)

- TLC plates are made of glass or aluminum
- Solvent equilibrate the environment
- May be placed with wall so that it may not fall in the chamber

Developing agents:

- Ninhydrin (amino acids)
- Quantitative technique when scratched and quantified the analyte

Q.17 What is Column chromatography?

- **Stationary Phase used:**
  Silica gel, flash silica, alumina
- **Mobile Phase used:**
  organic solvents (starting from less polar to high polarity)

Stationary phase is held in a narrow
Mobile Phase tube through which the mobile phase is forced under pressure or under
the effect of gravity.

- Choice of stationary phase depends upon chemistry of analytes.
- Silica Gel used, is available in varied mesh sizes starting from 60 to 800 Mesh.
- Alumina (Al2O3) used as solid support, available in acidic basic and neutral phases.

Q.18 Write flash chromatography..?

In flash column chromatography the sample is pumped through the packed glass column. Flash silica has quite smaller mesh size.

Q.19 Define electrophoresis and its types and two dimensional electrophoresis.?

Define:

- Electrophoresis is a technique used in laboratories in order to separate macromolecules based on size or charge.
- The technique applies a charge so proteins move towards a positive charge electrode.

Types of electrophoresis:

Electrophoresis may be of two types:

1-Slab electrophoresis-TLC
2- Capillary electrophoresis-Column

- Velocity (v) of charged molecule in an electric field can be assessed with formula;

$$v = Eq/F$$

- where E is applied electric charge, q is net charge on a molecule, and F is frictional coefficient
- depends upon mass and shape of molecule.

Two dimensional electrophoresis?

- This technique separate the mixture of proteins according to charges (IEF) and
  second with the size (SDS-PAGE).

Q.20 Define high performance liquid chromatograph?

- It is normally used as reversed phase chromatographic technique
- Mobile phase is pumped into the column with high pressure
- Separation occur based on size, charge, affinity etc

Q.21 Write different types of detector?

- UV Detector:
  For coloured or UV active compounds
- PDA detector:
  UV based scanning
- RI:

Refractive Index detector for polysacharides

- **ELSD:**

  Evaporating light scattering detector

- **Conductivity:**

  For charged containing molecules

Q.22 Define HPLC columns and Theoretical plate?

HPLC columns:

- Columns, made of stainless steel of varying length and internal diameter (Ø) are used for analytical chromatography.
- Columns may be heated to give higher efficiency. However, not to be heated above 60°

Theoretical plate:

- A theoretical plate in many separation processes is a hypothetical zone or stage in which two phases, such as the liquid and vapor phases of a substance, establish an equilibrium with each other.
- Such equilibrium stages may also be referred to as an equilibrium stage, ideal stage, or a theoretical tray.
- No. of theoretical plates = 5.54*(Rt / Peak ½ width)2

Q.23 Write a note on types of Chromatography?

1. Adsorption
2. Partition
3. Ion- Exchange
4. Molecular
5. Exclusion Affinity

1. Adsorption chromatography:

Adsorption chromatography involves the analytical separation of a chemical mixture based on the interaction of the adsorbate with the adsorbant.

2.Partition chromatography:

This form of chromatography is based on a thin film formed on the surface of a solid support by a liquid stationary phase. Solute equilibrates between the mobile

phase and tshtaetionary liquid.

3.Ion Exchange Chromatography:

In this type of chromatography, the use of a resin (the stationary solid phase) is used to covalently attach anions or cations onto it. Solute ions of the opposite charge in the mobile liquid phase are attracted to the resin by electrostatic forces

4.Molecular Exclusion Chromatography:

Also known as gel permeation or gel filtration, based on interaction between the stationary phase and solute. While passing through a porous gel molecules separate according to their sizes. The pores are normally small and allows smaller molecules to enter the gel. The larger molecules pass through the column at a faster rate.

5.Affinity Chromatography:

This is the most selective type of chromatography employed. It utilizes the specific interaction between one kind of solute molecule and a second molecule that is immobilized on a stationary phase. For example, the immobilized molecule may be an antibody to some specific protein. When solute containing a mixture of proteins are passed by this molecule, only the specific protein is reacted to this antibody, binding it to the stationary phase. This protein is later extracted by changing the ionic strength or pH.

Q.24 Method development on HPLC?

- Followed previous guidelines we have to develop an analytical method.
- That method needs validation afterward.
- Only after validation it can be considered as an analytical method.

Q.25 Define Gas Chromatography and write common detector with GC?

- Partition of molecules between gas (mobile phase) and liquid or solid (stationary phase).

Common Detectors used with GC:

- Flame Ionization Detector
- Thermal Conductivity Detector
- Electron Capture Detector
- Mass Spectrometer Detector

Applications of GC:

- Perfume industry
- Oil industry
- Essential oils analysis

Q.26 Define following.....?

❖ **Filtration**

- To separate solid insoluble particles from solution.

For example

- separate sand from water,
- or purify salt from mixture of sand and salt or other impurities.

❖ **Sublimation**

- To separate one solid from other if it vaporize by heating.

For example

separate salt from benzoic acid or naphthalene.

❖ **Solvent extraction**

- Solvent extraction is liquid-liquid extraction....
- Liquid-liquid extraction is a useful method to separate components (compounds) of a mixture.

- To separate two materials (solid or liquid) from immiscible liquids. One of the two liquids is water and other is any organic solvent.
- Suppose we have a mixture of sugar in vegetable oil and want to separate the sugar from the oil. (the sugar particles are too tiny to filter and you suspect that the sugar is partially dissolved in the oil).
- Sugar is much more soluble in water than in vegetable oil, and, water is immiscible with oil.
- The water phase is in the bottom layer because water is denser than oil.
- By shaking the layers (phases) well, you increase the contact area between the two phases. The sugar will move to the phase in which it is most soluble:

the water layer.
- Remove the bottom water layer, evaporate water to obtain the sugar.

Q.27 What is batch extraction method?

- There are two possible ways to follow extraction procedure.
- One is to use high amount of solvent at one time.
- The second one is to use small amount of solvent in more than one extraction steps.

- **Partition Coefficient *Kp* (Distribution Coefficient *Kd*)**
- When a compound is shaken in a separatory funnel with two immiscible solvents, the compound will distribute itself between the two solvents.
- At a certain temperature, the ratio of concentrations of a solute in each solvent is always constant. And this ratio is called the distribution coefficient.

Distribution coefficient:

- Where solvent1 and solvent2 are immiscible liquids.
- By convention we consider the organic solvent as Solvent 1 and water as solvent 2.

Q.28 What happens if we extract twice with 100 mL of solvent 2?

If we use 100 mL of solvent2 at one time then 200 particles extracted. Now we add a second 100 mL volume of fresh solvent2. According to the distribution coefficient K=2, so we can extract 67 more particles from the remaining solution. So, total 267 particles extracted as compared to 240 particles if we use 200mL of solvent2 for one time extraction.

Q.29 Change in chemistry of solute?

Changing chemistry of solute we can extract organic compounds in water as well.

- Like dissolve like.
- Organic compounds dissolve in organic solvents.
- Inorganic salts dissolve in water.
- However, certain organic compounds can change their solubility from organic solvent to water.

What kind of Organic compounds can change their solubility?

- Organic acids include carboxylic acids (strong organic acids) and phenols (weak organic acids).
- Organic bases includes amines

Q.30 chemistry of solute sample problems?

First sample problem

- The phenols are weak acids and can reeaascilty with sbtarsoensg e.g. NaOH.
- But they can't react with weak bases like sodium carbonate.

Second sample problem.

- We have a mixture of benzoic acid and p- methoxyphenol, dissolved in dichloromethane.

Third sample problem.

- If we have a mixture of benzoic acid and p-chloroaniline, dissolved in dichloromethane. We can do the either way, add 5% HCl to make amine water soluble or add 5% NaOH to make acid water soluble.

Q.31 Extraction Scheme and its application?

- We can Separate a complex mixture by designing an extraction scheme.

Applications:

- For extraction of caffeine from lteaves
- .For extraction of nicotine from tobacco.

Q.32 Recovery of organic compounds from salts? And its application.?

- We can recover an organic acid or base from their salt by reverting the pH of solution.

Recovery of acids and bases from salts

- Acids can be recovered from their salts by treating with HCl
- Decrease pH to acidic range~2
- For salts of bases we have use solution of a base to increase pH~1
- Useacidic solution (HCl) or Basic solution (NaOH).

.

Solvent Extraction Applications:

- Extraction of food colours.

- Extraction of oil and grease from waste water
- Extraction of drugs from formulation to conduct assay.
- Extraction of benzoic acid (preservative) from samples.

Q.33 What is SPE or Solid Phase Extraction?

solid-phase extraction (SPE) is a sample preparation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties.

Sorbent: The solid phase used for extraction.
Matrix: Our sample.
Analyte: A compound or type of compound.
Washing: The solvent used for washing out the interferences.
Elution solvent: The solvent use to elute out the analyte.

Types of Sorbent:

1. Polar (Normal Phase)
2. Non-Polar (Reverse Phase)
3. Ion Exchange
4. Copolymeric
5. Size exclusion
6. Chiral

1..Polar interaction:

- Also called hydrophilic or normal phase
- Involves hydrogen bonding, pi-pi and dipole/ dipole interactions
- Sorbents – silica, diol, diethylamino, cyanopropyl
- Applications – lipids, oil additives, carbohydrates, phenols, oil soluble vitamins
- Analytes – amines, hydroxyls, carbonyls, aromatic rings, heteroatoms (O, S, N, P)
- Matrix – non-polar, organic
- Elution solvents – medium to high polarity

Applications:

- Vegetable oil, ointment, cereals, crude oil products, soil and sludge may use for pretreatment. dissolution of sample in non-polar solvent and removal of polar interfering compounds are needed before application.

Why we use non polar sorbent?

Some times we have to use non polar sorbent Because our analyte are polar and non-polar sorbents can adsorb unwanted materials. Washing is easy.

2..Non-Polar Sorbent:

- It is also called reverse phased.
- Same as in HPLC.
- Also called hydrophobic or reverse phase
- Involves van der Waals / dispersion forces
- Sorbents – C2, C3, C4, iC4, tC4, C5, C6, C7, C8, C10, C12, C18, C20, C30 phenyl and cyclohexyl
- Matrix – biologicals, water, aqueous buffers
- Elution solvents – typically non-polar to moderately Polar

Applications:

• Sample containing vitamins, steroids, pesticides, aflatoxins, antibiotics and other class of drugs may ibseolated from hospital waste, body fluids or irrigation water using this technique.

3..Ion exchange sorbent:

- Cation exchange resins are negatively charged and adsorb positively charged cations from matrix.
- Anion exchange resins are positively charged and adsorb negatively charged conjugate bases from matrix.

➢ **Cation exchange sorbents:**

- Cation exchange sorbents are negatively charged
- Basic analytes manipulated to carry positive charge
- Opposites attract forming strong bonds
- Sorbents
  - Benzenesulfonic acid (strong)
  - Propylsulfonic acid (strong)
  - Carboxylic acid (weak)

Applications:

- Include basic drugs, catecholamines, pharmaceuticals, herbicides
- Analytes: Amines, Pyrimidines (cations)
- Matrix: aqueous
- Elution: Basic elution solvents to neutralize analyte

➢ **Anion exchange sorbents:**

- Anion exchange sorbents positively charged
- Acidic analytes manipulated to carry negative charge

- Opposites attract forming strong bonds
- Sorbents
  - 1°, 2° amine
  - Aminopropyl (weak)
  - Quaternary amine (strong)
  - Diethylamino (weak)

Applications:

- Include phosphates, acidic drugs, organic acids, fatty acids, vitamins
- Analytes: Phosphates, Carboxylic acids, Sulfonic acids
- Matrix – aqueous
- Acidic elution solvents to neutralize analyte

Applications:

- Sample containing vitamins, caffeine, saccharine, amino acids, or food sample can be extracted using this technique.

4.. Copolymeric sorbent:

- Hydrophobic & ionic retention mechanisms
- Reverse phase sorbent with cation or anion exchange

- Acidic, basic & neutral analyte applications
- Matrix – aqueous
- Elution solvents mixture of organics with acid or base

5..Size exclusion:

- Porous silica or polymers, where separation is based on differences between the volumes of the molecules, correspondinMethod Selection

Guidelines:

- Based on above sketch we can easily frame the thodology of extraction for different kind of analytes.g to steric exclusion

Q.34 Discuss UV Spectroscopy with wavelength energy relation?

- Electromagnetic radiation
- Wavelength and energy
- Electronic transitions in organic molecules
- Conjugated and colours
- Chromophores and auxochrome
- Beer Lambert Law; explanation and application
- Effect of substituents on UV Spectrum
- Schematic diagram of UV Spectrophotometer

- Woodward Fieser Rule (theoretical calculation of wavelength maxima) for conjugated diene and –Unsaturated diene systems.
- **Amplitude (A) of the sinusoidal wave is defined as** the length of the electrical vector at the maximum in the wave.
- Frequency refers to the number of oscillations of the field per second

Wavelength and Energy relation:

- E = hv = hc/v
- Where E = energy
- h = Plank's constant
- Energy and frequency are directly proportional
- Wavelength and energy are inversely proportional

Q.35 Absorption of light by molecules or ion cause which types of energy?

Absorption of light by molecules or ions causes three types of energy changes:

1..Rotational (Low energy change):

Molecule rotates around a center of gravity

2..Vibrational (Low energy change):

Average internuclear distance of two or more atoms in the molecule changes

3.. Electronic (High energy change):

Change in energy of the electrons or transitions

- UV radiation bring electronic transitions in molecules.

Q.36 In organic compound which type of ground state electron involved?

In organic compounds, three types of ground state electrons may be involved:

sigma (bonding) electrons

* pi (bonding) electrons

o n (non-bonding) electrons

Wavelength and transitions:

- For sigma to sigma transition required energy can be achieved with electromagnetic radiation of < 100 nm.
- Pi to pi transition is possible with 171 nm (vacuum UV range)
- For an absorption in normal UV range the molecule must have conjugated system or non-bonding electron along with double bond.

Why molecules display colours:

- Transition metals are coloured because of d-d transitions while organic molecules are coloured because of conjugation.
- Higher wavelength or lower energy.

Complementary colours:

- When white light falls upon a sample, either reflection or absorption takes place
- If only a portion of Light is absorbed, the color of the sample is determined by the reflected light **(is called complementary colours).**

Q.37 Define chromophores and auxochromes?

- Color-bearing functional group
- Exhibits a characteristic absorption spectrum in the UV / Vis region
- Chromophoreconjugated with others, display more intense absorption band at a longer wavelength

Auxochromes

- Functional group that does not absorb in the UV/Vis region
- It has the effect of Shifting Chromophores wavelength as we increasing their intensities • For example -OH, - OR, -NHR, -SH, -Cl, -Br, -I

- Substitution of simple alkyl group produces a small bathochromic effect (red shift) along with hyperchromic shift.
- Substitution of auxochromic groups (OH, NH2 etc.) causes a pronounced red shift with large intensification (hyperchromic shift).
- Altering the availability of the non-bonding electrons (e.g. conversion of phenol to its anion) results in pronounced red shift and hyperchromic shift.
- Attachment of an unsaturated group such as ethylenic, acetylenic, nitrile, aldehydic, carboxylic and nitro to the benzene ring produces a red shift, often to a greater extent.
  - Analysis of an unknown is done by using a calibration curve of known concentration. Finding wavelength is maximum is a qualitative

estimation(spectrum mode) Analysis of unknown by using calibration curve is quantitative analysis (photometric mode).

Q.38 Write UV Spectrophotometer..?

1..Light source
2..Monochromator
3.. Sample compartment
4..Detector
5..Read out Device

1. **Light source:**
   - Tungsten lamp emit light of visible region (350-800 nm) while Deuterium lamp Emit 160nm to 400nm
   - UV spectrophotometer normally cover the range from 190 to 1100 nm.

2.Monochromators
- It used in UV Spectrophotometer
- Diffraction gratings
- Prism
- Filters
- In UV Spectrophotmeter the monochromators are installed before sample compartment.

• UV/V is Spectrophotometers Used in two ways;

1.Determination of wavelength maximum (where we have to scan full range spectrum).
2.To determine absorbance values at a particular wavelength.

Q.39 Write Photomultiplier and Woodward-Fieser Rule for conjugated rule?

Photomultiplier
Photomultiplier tubes (photomultipliers or PMTs), members of the class of vacuum tubes, and more specifically vacuum phototubes, are extremely sensitive detectors of light in the ultraviolet, visible, and near-infrared ranges of

the electromagnetic spectrum.

Woodward-Fieser Rule for Conjugated Dienes:

- For theoretical calculation of wavelength synthesized compound
- Used as QC tool.
- Calculated values may be checked and correlated with experimental values.
- Now we calculate some reported diene systems to make some understanding.
- And compare them with reported observed values.
- Woodward-Fieser Rule for Alpha, beta – Unsaturated Carbonyl Compounds
- For theoretical calculation of wavelength maxima of conjugated carbonyl systems.

Applications:

1. Structure Determination
2. Analytical applications
3. Kinetic measurements
4. Ionization constants
5. Stereochemical study

Analytical Applications

- **Food – color and consistency**
  In quality control testing of foods
- **Beverage – impurity and decomposition**

Stability of product with aging or help in determining expiry dates of products

- **Leaf analysis – chlorophyll and photosynthesis process**
  may evaluate ripening of fruits etc.
- **Pharmaceutical manufacturer – metabolic pathways of drugs, nucleic acid analysis, kinetics and dissolution testing**
  - Drug availability testing using dissolution apparatus
  - Drug availability in human body after infusion
  - Kinetic studies to monitor reaction speed and completion status

Academic and Government Application

- Environmental testing streams Medical testing
- Academic – students
-

Q.40 Explain IR Spectroscopy..?

- UV ranges from 190 to 350 nm
- Visible region extended from 350 (Violet) to 760 nm (Red)
- IR region is up to 1 mm wavelength of electromagnetic radiation
- (nu-bar) represents wavenumber; the number of wavelengths per cm (cm-1)
- Mid IR region represents 2.5 to 16 m (4000 to 625 cm-1) Vibrational Modes
- The total number of vibrations in a molecule depends upon the number of atoms present in the molecule.
- Each atom has three degrees of freedom because three coordinates (x, y, z) are
  required to describe itsposition relative to other atoms in a molecule.
- Only vibrations that cause a change in 'polarity' give rise to
  bands in IR spectra
- Instead Symmetric stretch the unsymmetrical stretches appear
  in IR spectra.

Vibrational Frequency

- We can calculate an approximation value for the stretching vibrational frequency of a bond by **HOOK's LAW**. Lower masses appear in left region of I(3700- 2500 cm-1).
- Less force number to right region of IR spectra (1300-800 cm-1)R spectra

Peaks value:

- We solve IR spectrafor major peaks of functional groups.
- IR Spectra contains many tiny peaks due to overtones.
- Finger print region is very important in differentiating two compounds with similar functional groups.
-

Q.41 Write features of IR Spectra..?

IR Spectra have three main features:

1. Position of peaks.
   Reduced mass
   Bond strength
2. Strength of peaks.
   Change in polarity
3. Width of peaks.
   Hydrogen bonding

Note:

- In IR Spectra the C=O shows most intense peaks.
- NH appear as one peak while NH2 as two peaks.

Q.42 Why benzene ring show multiple peaks?

- Benzene ring due to conjugation shows multiple (4) peaks around 1400 cm −1.
- C-C absorb around 1300- 800 cm-1 while C=C absorb at 1900-1600 cm-1.

Factors influencing IR absorption:

Additional to conjugation effect the other factors influence carbonyl absorption are

- Ring strain
- substitution effect
- Hydrogen bonding

If the bond angle increases above 120°, the opposite effect is observed, as for di-*tert*-butyl ketone the Absorption frequency of carbonyl is 1695 cm-1.

Strong hydrogen bonding in carboxylic acids leads to dimerization and due to this all bonds i.e.OH, C=O and CO appear at lower frequency in IR spectra.

Q.43 Interpretation of IR spectra..?

The most useful regions are as follows:

- 1680-1750 cm-1:C=O stretches feature very strongly in IR specta
- 2700-3100 cm-1: different types of C-H stretching vibrations.
- 3200-3700 cm-1: various types of O-H and N-H stretching vibrations.
- Too many bonds absorb in the region of 600- 1600 cm-1 to allow confident assignment of individual bands.
- However, this region is useful as a fingerprint a molecule, *i.e.* if spectrum is almost to an authentic reference spectrum then the structure can be assigned with some confidence.

Q.44 Write main components of IR Spectrophotometer?

Main components are:

1. IR radiation source
2. Sample compartment
3. Monochromator

4.Read out device

1..Radiation Source:

- Nernest filament is composed of a mixture of oxides of zirconium, thorium and yttrium, which is formed into a hollow rod about 2 mm in diameter and 2–5 cm in length.
- Globar is a bonded silicon carbide rod about 7 mm in diameter and 5 cm in length.
- Detector works on principle of a thermocouple, which converts the infrared (electromagnenergy energy electrical energy.
- Sample are placed as drop of liquid and solids can be mixed with nujol.

Q.45 Write Applications of IR Spectroscopy..?

1. Structure Determination:

- Search for benzene ring presence
- Search for functional group regions (carbonyl, NH, OH, CO etc.)
- Search for CH of different types

2. Identification of compound:

- Matching given structure (metal complex formation)
- Matching of standard through finger print region
- Differentiate if we have alcohol or ether

3. Detection of impurities:

If any additional peaks are observed in the IR spectrum then it is due to impurities present in the compound.

4. Progress of reaction:

- Oxidation of secondary alcohol to ketone
- Reduction of alcohol to alkene
- Hydrolysis of sugars

5. Presence of composition of mixture

- IR is a qualitative technique
- In rare cases it may be used as quantitative e.g. ortho, meta and para xylene

Q.46 Write detail on Atomic Structure or spectroscopy?

- Atom is a divisible particle that represents the properties of matter.
- It has positive charge confined in the centre.
- The negative charge particles are found around the nucleus in different energy
  orbits.
- When we give energy to the atom in any form, it could be electromagnetic radiation or thermal energy, electrons absorb that energy and jump in higher shell.
- They emit same amount of energy when come back to ground state.
- Bohr postulate that the electrons reside in different energy levels and they need a specific amount of energy to jump.
- When they come back to ground state same amount of energy emit.
- The colour in the spectrum correspond to that energy.

Q.47 Write Atomic absorption spectroscopy?

- Light source (HCL)
- Nebulizer
- Atomizers
- Monocromators
- Readout device
- Printer

- Flame photometers can be used for very few metals that burn with coloured flames (Na, K, Li etc.)w. ill discuss the detail of these parts one after another.

Absorption spectrum of different atoms:

These absorption or emission spectrum are different for different atom .Some of the spectral lines may be common but some other may be characteristic of that atom.

Hydrogen spectral lines

- Before Bohr different scientists have proved experimentally that hydrogen gas absorb or emit energy of different ranges i.e. UV range (Lymen series), Visible range (Balmer series), IR region (Paschen
  series).
- Bohr explained it.

Q.48 What is Hollow Cathode Lamp?

- HCL is a vacuumed tube bearing cylindrical cathode and some noble gas at very low pressure.
- It generates photons of particular metal for which cathode is made
- In cases where we have to analyze more volatile metals, the electrodeless discharge lamp (EDL) is used.

Q.49 What is nebulizers and atomizers and its uses?

Nebulizers:

- Nebulizers convert liquid into mist.
- Then burner remove heavy droplets and only allow the mist to pass through it.
- The remaining part move to waste while almost equal amount of sample reaches to flame.
- These nebulizers are pneumatic and work with help of pressurized gases.
- These gases push the sample into burner which atomize them.

Atomizers:

- Atomizers are used to atomize the sample.
- The sample contain the metal in ionic form (salt).
- Atomizers / burners heat the sample and convert them to atomic form.

Use of Atomizer:

- Metal like As and Hg are not atomized in this form so they are transformed to metal hydrides (gaseous form) that can be atomized in formal
- .Solid or biological samples have to digest properly to aspirate in flame.

Q.50 What is Flame Atomization?

- Flame atomizers transformed sample into atomic form.
- From their ionic solution
- From hydrides gases

Use of Flame atomization

- Most abundantly used flame is airacetylene. Graphite Furnace Atomizer
- Is a hollow graphite cylinder (5 cm length x 1 cm diameter).
- Tube is surrounded by a water circulation jacket separated by a gas space where an inert gas (Ar/ N) is circulated.
- A small amount of analyte sample solution(1–100 L) is introduced in the cell.

Graphite Furnace Atomizer

- Is a hollow graphite cylinder (5 cm length x 1 cm diameter).
- Tube is surrounded by a water circulation jacket separated by a gas space where an inert gas (Ar/ N) is circulated.
- A small amount of analyte sample solution (1–100 L) is introduced in the cell.

Different Types of zone:

- Preheating zone:

  Where combustion mixture is heated to the ignition temperature by thermal conduction from the primary reaction zone.

- Primary reaction zone:

  - This zone is about 0.1 mm thick at atmospheric pressure
  - There is no thermodynamic equilibrium in this zone and the concentration of ions and free radicals is very high.

- Interconal zone:

  Extend up to considerable height. The maximum temperature is achieved just above the tip of the inner zone. This zone is used for flame photometry.

- Secondary reaction zone :

In this zone, the products of the combustion processes are burnt to stable molecular species by the surrounding air.

Q.51 Comparison of FAAS with GFAAS?

- In FAAS only liquid sample can be injected.
- In GFAAS both liquid and solid samples qualitity.
- GEAAS is minimized as compared to FAAS.
- Analytical precision is good in FAAS (10%) but has low sensitivity.

Q.52 Write different types of Interferences? And removal of spectral

interferences.

1..Spectral interferences
2..Non-spectral interferences
3.. Matrix interferences
4.. Chemical interferences
5.. Ionization interferences

Type 1 interference: Spectral

- If emission line of two overlap, **a spectral interference** is said to exist (a rare case for wavelength-specific atomic absorption spectroscopy, as the typical emission line width may be only 0.002 nanometers, actual overlap is extremely rare).
- The chances for spectral interference increase when multi-element lamps are used, where the source may contain close emission lines for several elements.

Removal of spectral interferences:

1.Continuous source background correction. BGC.

2. The electronic energy levels of an atom, placed in a strong magnetic field, are changed.

Q.53 Describe Background correction and Zeeman background correction?

Background correction:

1. Requires additional continuum light source(s).
2. Requires the intensities of the primary and continuum sources to be similar.
3. Two continuum sources are required to cover the full wavelength range.
4. Requires critical alignment of the continuum and primary sources for accurate correction.

Zeeman background correction:

When an atom is placed in a magnetic field and its atomic absorption profile observed with polarized light, the normal single-line atomic absorption profile is split into two or more components symmetrically displaced about the normal position.

Type 3 interference: Matrix

Q.54 Define Matrix interferences with example?

- Occur at the nebulization step, if the sample is more viscous
- The sample uptake rate or nebulization efficiency is different

Example:

An example of this type of interference is the effect of acid concentration on absorbance (phosphoric acid concentration increases, change the sample viscosity).

- Matrix interferences can also cause positive error. The presence of an organic solvent in a sample will produce an enhanced nebulization efficiency.
- One way of compensating for this type of interference is to match the matrix (by blank preparation)

Q.55 Method of Standard Additions?

1. Aliquots of a standard are added to portions of the sample
2. Add standard solution
3. Make up the Volume The method of standard additions will not compensate for background absorption or other types of spectral interference, and normally will not compensate for chemical or ionization types of interference.
The method of standard additions will not compensate for background absorption orother types of spectral interference, and normally will not compensate for chemical or ionization types ofinterference.

Type 4 interference: Chemical

Q.56 What is Chemical interferences?

- In atomizer sufficient energy must be available to create free atoms.
- If the sample contains a component which forms a thermally stable compound with the analyte, will not decompose completely
- The effect of phosphate on calcium is an example of a chemical interference.
- Calcium phosphate does not totally dissociate in an air acetylene flame.
- Therefore, as phosphate concentration is increased, the absorbance due to calcium atoms decreases.
- There are two means of dealing with this problem.
  Use of Graphite Furnace:
- Graphite furnace can be used for higher atomization temperature.
- The sensitivity is also increased from mg/L to g/L.

Type 5 interference: Ionization

Q.57 What is Ionization interferences?

- In atomizer the dissociation process does not stop at the ground state atom.
- the ground state atom thermally raised to the excited state or an electron creating an ion.
- This electronic rearrangement deplete the number of ground state atoms
- It was observed in case of alkali and alkaline earth metal (e.g. sodium).
- Ionization interference can be eliminated by adding an excess of an element which is very easily ionized, creating a large number of free electrons in the flame and suppressing the ionization of the analyte.
- Potassium, rubidium, and cesium salts are commonly used as ionization suppressants.

Q.58 Write Uses of HVG or Hydride vapour generator?

- Arsenic salts are notatomized in air–acetylene or nitrous oxide–acetylene flame.
- Hydride vapour generator is used to convert arsenic, or antimony to respective
  hydride by reacting with $NaBH_4$ and HCl.
- Mercury can be atomized by cold vapour mercury technique

Q.59 What is Atomic Emission Spectroscopy? And working of ICP?

• ICP/OES and ICP MS
• Atomization sourcechanged to inductive coupled plasma
• Temperature of plasma can reach up to 10,000K
• 70 elements can be determined to ppb level
• Low chemical interferences

Working of ICP:
The sample is prepared inliquid form as in case of FAAS.
• Sample is injected throughnebulizer @ 0.5 to 1.5 mL/min.
• The emitted spectral lines pass through monochromator.
• The separation in MS is on basis of mass to charge ratio.

Q.60 Write Sample preparation?

Technique can be optimized for better extraction

- Normally biological sample wet digested while plant materials digested through
  ashing
- Glass can be solubilize in HF while metal in aqua regia.
- Microwave can safe time and sensitive material

Sample preparation techniques for atomic spectroscopy:

- Wet digestion
- Dry ashing
- Microwave dissolution

Q.61 Atomic Florescence Spectroscopy?

Photoluminescence and its Types

- Fluorescence
- Phosphorescence
- Instrumentation

Photoluminescence:

• Molecules that have an electronic excitation are *excited, with* vibrational excitation are *hot*

• When excited molecular states decay back to the ground state, resulting in the emission of light, undergoing a *luminescence* process

• If after de-excitation the emitting light is in visible range **called *photoluminescence (Fluorescence* and *Phosphorescence***

Important Note:

- **Absorption process** occurs over short time interval (10-15 s).
- **Vibrational relaxation** (emission of IR while lowering vibrational state) occurs in

~10-12 s. De-excitation to electronic ground state with emission of IR occurs in 10-9 s.

- **vibrational relaxation** occurs ~1000 times faster

Q.62 What is Phosphorescence?

Phosphorescence

- In the fluorescence process, the electron did not change its spin direction
- But under the appropriate conditions, a spin-flip can occur (during absorption or afterward)

- The light emission process must wait until electron undergoes a spin-flip to revert back to it original state May take 10-3 – 102 s
- Light emission is delayed long enough so that materials “glow in the dark” after exposure to light
- Because of the lower energy of triplet state, lower energy photon emission than incident or fluorescence photons
- Because of the long lifetimes, molecular triplet states are more often involved in photo-chemical and photo- biological reactions than singlet states in molecules

Fluorescence spectrophotometer:

- Measure the intensity of fluorescence light as a function of wavelength
- Light source is perpendicular to detector

REMEMBER US IN YOUR PRAYERS

REGARDS =

VU FRIENDS