B.PHARMA
7th SEMESTER

BP-701T

INSTRUMENTAL METHODS OF ANALYSIS

INSTRUMENTAL METHOD OF ANALYSIS MODEL PAPER

SECTION A

VERY SHORT ANSWERS TYPE QUESTIONS (10 × 2 = 20)

1. Give the principle of UV Spectroscopy.

Answer

- When UV or Visible radiation is passed through a substance, absorption of energy results in the promotion of electron from the ground electronic state to the excited electronic state. Theamount of absorption of energy depends upon **wavelength of the radiation** & the **structureof compound**

2. Describe quenching with examples.

Answer

- In chemistry, quenching refers to any process which decreases the fluorescent intensity of a given substance. A variety of processes can result in quenching, such as excited state reactions, energy transfer, complex-formation and collisions.

3. Explain principle of Flame Photometry.

Answer

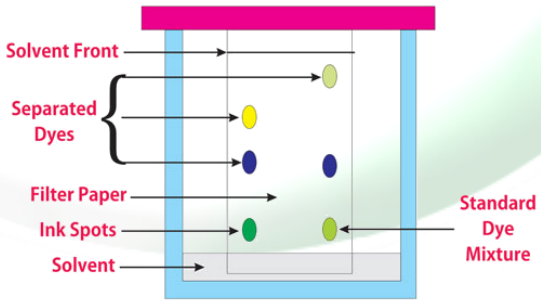
- The principle of flame photometer is based on the measurement of the emitted light intensity when a metal is introduced into the flame. The wavelength of the colour gives information about the element and the colour of the flame gives information about the amount of the element present in the sample.

4. What are various methods for preparation of TLC plates?

Answer

Various methods for preparation of TLC Plates

1. Stationary Phase
2. Mobile Phase
3. Activation of plates
4. Application of samples
5. TLC column development
6. Detection and visualization of spots



5. Give significance of Fermi Resonance.

Answer

- It is useful to understand Fermi resonance because it helps assign and identify peaks within vibrational spectra (i.e., IR and Raman) that may not otherwise be accounted for, however, it should not be used lightly when assigning spectra. Fermi resonance is a common phenomenon both in IR and Raman

spectra.

6. Define Chromophores with examples.

Answer

- The part of a molecule responsible for imparting colour, are called as chromophore. Or
- The functional group containing multiple bonds capable of absorbing radiations above 200nm due to n → π^* and $\pi \rightarrow \pi^*$ transitions
- E.g., – NO_2, N=O, C=O, C=N, C≡N, CC, CS, etc.

7. Give names of detectors used in HPLC.

Answer

Detectors used in HPLC

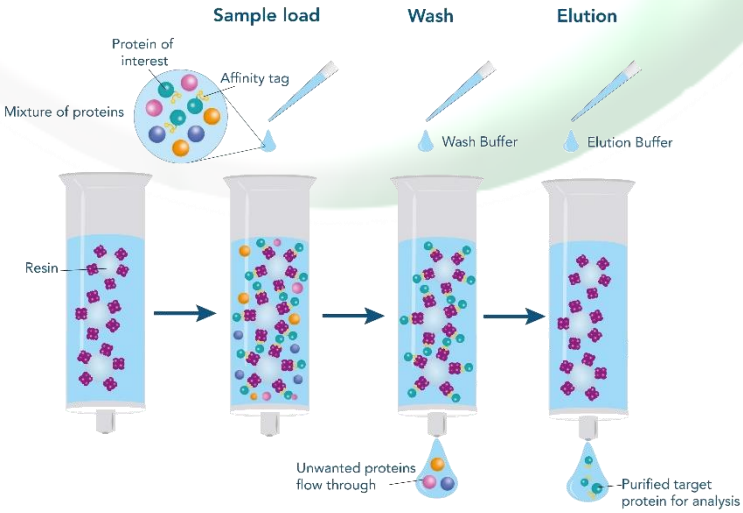
- Ultra-Violet absorption
- Photodiode array detector
- Fluorescence spectrometer
- Refractive index detector
- Differential refractometer

8. Describe principle of Affinity Chromatography.

Answer

Principle of Affinity Chromatography

- Based on selective non-covalent interaction between an analyte and specific molecules.

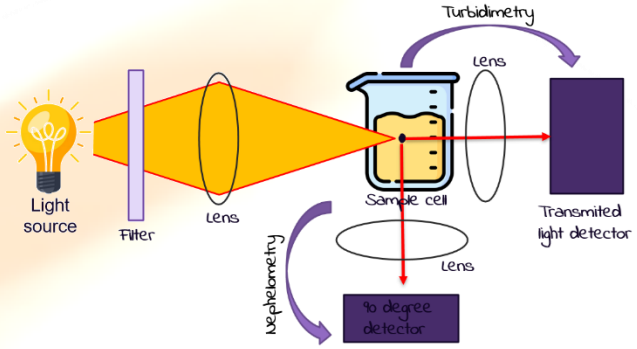


9. Explain applications of Nephelometry.

Answer

Applications of Nephelometry

- For determination of ions like sulphate, chloride, carbonate, magnesium, calcium, etc.
- In determination of impurities in pharmacopoeial substances.
- In determination of growth of micro-organism in vitamin and antibiotic assays.



10. Discuss factors affecting Vibrational frequency in IR spectroscopy.

Answer

Factors affecting Vibrational frequency in IR Spectroscopy

a) **Vibrational coupling -** The following four vibrations may be observed in the high-resolution spectra of compounds containing both —CH2 and —CH3 groups.

b) **Hydrogen bonding**
   - In general, the hydrogen bonding present in O—H and N—H compounds give rise to a number of effects in the IR-spectra.

c) **Electronic Effects**
   - **Conjugation Effect –**
     - Conjugation lowers the stretching frequency of carbonyl group by 15-40cm^{-1}. This is because carbonyl bond order is reduced and hence, the force constant reduces.

SECTION B

LONG ANSWERS TYPE QUESTIONS (2 × 10 = 20)

1. Discuss theory involved in IR Spectroscopy. Explain instrumentation of IR spectrophotometer with applications.

Answer

Theory of IR Spectroscopy

- The underlying principle of infrared spectroscopy is based upon the molecular vibrations which are further composed of the stretching and the bending vibrations of a molecule.
- **MOLECULAR VIBRATIONS**
  - The vibrations for molecules are of two types, namely :
    - Stretching, and
    - Bending (or deformation)

- **Stretching -** Vibration causes stretching whereby the distance between the two atoms increases or decreases, but the atoms remain in the same bond axis.
    - **Symmetrical Stretching -** In this case, the two hydrogen atoms either move towards or away from the central carbon atom in unison, thereby either altering the interatomic distance or causing no change in valence angle.
    - **Asymmetrical Stretching -** In this instance, one hydrogen atom approaches the carbon atom while the other moves away from the carbon atom.

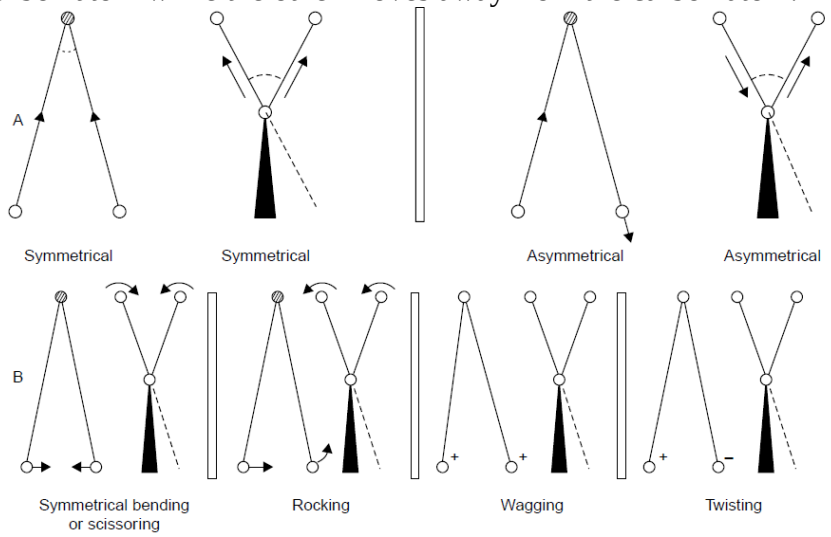


Figure: (A) Stretching (B) Bending

- **Bending (or Deformation) -** Vibration causes bending whereby the position of the atom changes relative to the original bond axis.
- **In-Plane Bending Vibrations**
    a. **Scissoring or Symmetrical Bending -** In this case, the two atoms connected to a central atom either moves toward or away from each other with certain deformation of the valence angle.
    b. **Rocking -** In this case, the structural unit swings back and forth in the plane of the molecule.
- **Out-of Plane Bending Vibrations**
    a. **Wagging -** In this case the structural unit swings back and forth out of the plane of the molecule.
    b. **Twisting -** In this case the structural unit rotates about the bond that joins it to the rest of the molecule.

Instrumentation

IR radiation source	**Incandescent lamp**	• Visible –Near IR
	Nerst glower	• Composed of zirconium, yttrium, thorium, heated at Max radiation at 1.4μ temperature range = 1000 - 18000C • **Disadvantage:** emits IR over wide wavelength.
	Globar cell	• Intered silicon carbide, heated at 1300-17000C, max radiation at 1.9μ
	Mercury arc	• Far IR, made up of quartz.

INSTRUMENTAL METHOD OF ANALYSIS MODEL PAPER

Mono chromators (Grating + prism → order sorter)	**Prism**	• NaCl (IR) • Lithium/Ca fluoride (region of stretching)
	Grating	• Aluminium, not attacked by moisture. • Use over considerable wavelength.
Sample cells	NaCl, KBr, LiBr, Thorium bromide: Transparent PTFE (Polytetrafluoroethylene).	
Sampling	**Solid run in solution**	• Solid dissolved in non aq solvent.
	Solid film	• (amorphous solids) deposited in cell by evaporation
	Nujol mull	• crystalline solids
		• Solid sample + nujol (mineral oil) → paste • Paste is separated between IR windows • Nujol has IR absorption at 719, 1376, 1462, 2915 cm-
	Pressed pellet	• Solid sample + KBr → pressed at (25000) • Pellet (up to 2mm thick (0.3mm), (1 cm diameter (13mm) • Always has a band at 3450 cm-1 from OH group.
Detectors	**Thermocouple**	• IR ↑ temp of junction. Due to temp difference between two points potential difference is created leads to flow of electricity. • Two dissimilar metal → bismuth and antimony.
	Bolometer	• Made of 1arm of wheat stone bridge IR falls on conductor, temp changes. • As temp changes, resistance changes → determined by current. • For 10C ↑ in temp → resistance ↑ by C%
	Thermistors	• Made of fused mixture of metal oxides. • As temp ↑, electrical resistance ↓. 10C ↑ temp → resistance ↓ 5%
	Golay cells	• IR → falls on metal plate → heat gas →expand → deform diaphragm. • Light from lamp is made to fall on diaphragm → reflect on photocell (any deviation is • determined)
	Semiconductor	• Lead sulphide/ lead telluride. Insensitive to longer wavelength.

		• As IR falls→ it is displaced → conductivity change
	Pyroelectric	• a dielectric placed in an E.F becomes polarized depending on the dielectric constant, if the field is removed, the polarization usually disappear, except with ferroelectric compounds, which retain strong residual polarization. Sometimes their residual polarization is temperature sensitive known as pyroelectric. • Eg: **Triglycerine sulphate (TGS)** cooled in liq N2 since response deteriorates above 450 and lost at 490 (Curie point).

Applications

- Determination of drugs in formulations
- Determination of cis-trans isomer
- Distinguish and characterize the pri-, sec-and tert-amine salts from one another
- Study of complex formations
- In quantitative reaction sequence study
- In the identification of functional groups

2. Describe theory, principle, instrumentation and application of Gas Chromatography.

Answer

Gas Chromatography

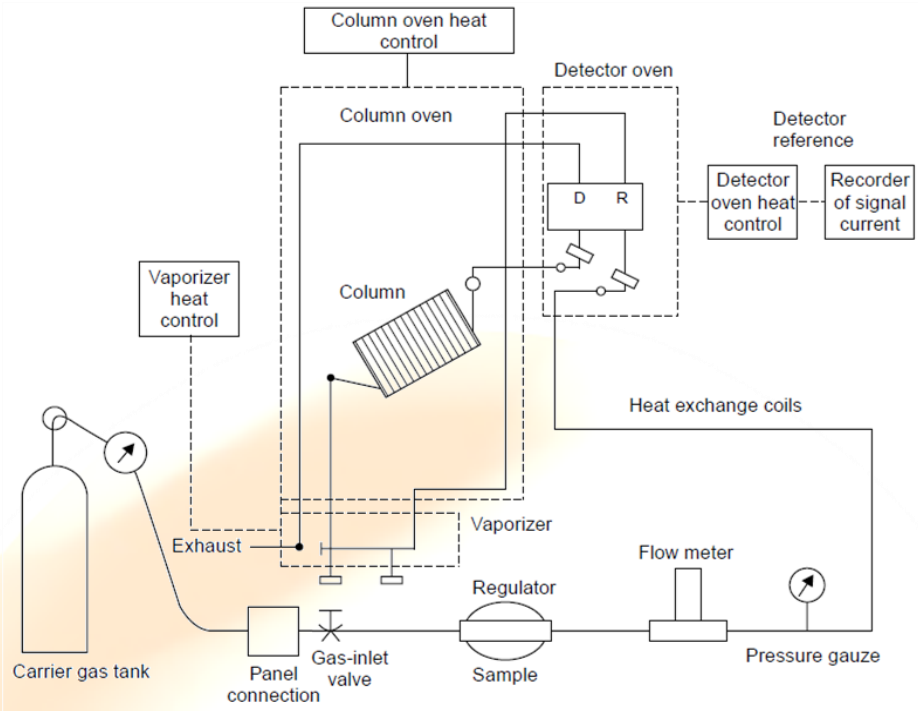
Theory

- In gas chromatography, the components of a sample are dissolved in a solvent and vaporized in order to separate the analytes by distributing the sample between two phases: a stationary phase and a mobile phase.

Principle

- The equilibrium for gas chromatography is partitioning, and the components of the sample will partition (i.e. distribute) between the two phases: the stationary phase and the mobile phase.
- Compounds that have a greater affinity for the stationary phase spend more time in the column and thus elute later and have a longer retention time (Rt) than samples that have a higher affinity for the mobile phase.
- Affinity for the stationary phase is driven mainly by intermolecular interactions and the polarity of the stationary phase can be chosen to maximize interactions and thus the separation.

Instrumentation



- **Carrier gas**
  - The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide.
  - The choice of carrier gas often depends upon the type of detector used.
- **Sample injection port**
  - The most common injection method is where a micro syringe is used to inject sample through a rubber septum into aflash vaporizer port at the head of the column.
  - The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample.
  - For packed columns, sample size ranges from tenths of a microliter up to 20 microliter.
  - Capillary columns, on the other hand, need much less sample, typically around 10–3 microliter.
  - For capillary GC, split/splitless injection is used.
- **Columns**

  There are two general types of column → **packed** and **capillary** (also known as open tubular).
  - **Packed columns**
    a. Contain a finely divided, inert, solid support material (commonly based on diatomaceous earth) coated with liquid stationary phase.
    b. Most packed columns are 1.5–10m in length and have an internal diameter of 2–4mm.
  - **Capillary columns**
    a. Have an internal diameter of a few tenths of a millimeter.
    b. They can be of one of the two types:
       - **Wall-coated open tubular (WCOT)**
       - **Support-coated open tubular (SCOT).**

- **Wall-coated columns** →consist of a capillary tube whose walls are coated with liquid stationary phase.
- **Support-coated columns** →the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. It is also known as PLOT (Porous layer open tubular column).
- SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.
- **Tubular Column**
    - These have much thinner walls than the glass capillary columns, and are given strength by the polyimide coating.
    - These columns are flexible and can be wound into coils.
    - They have the advantages of physical strength, flexibility and low reactivity.

Column temperature

- For precise work, column temperature must be controlled to within 10 ^{o}C.
- The optimum column temperature is depends upon the boiling point of the sample.
- As a rule of thumb, a temperature slightly above the average boiling point of the sample results in an elution time of 2–30 minutes.
- Minimal temperatures give good resolution, but increase elution times.
- If a sample has a wide boiling range, then **temperature programming** can be useful.
- The column temperature is increased (either continuously or in steps) as separation proceeds.

Detectors

- A non-selective detector responds to all compounds except the carrier gas, a selective detector responds to a range of compounds with a common physical or chemical property and a specific detector responds to a single chemical compound.

DETECTORS	TYPE	SUPPORT GASES	SELECTIVITY	DETECTABILITY
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic compounds	100pg
Thermal conductivity (TCD)	Concentration	Reference	Universal	1ng
Electron capture	Concentration	Make up	Halides, nitrates, nitriles, peroxides, anhydrides, organometallics	50fg
Nitrogen-phosphorus	Mass flow	Hydrogen and air	Nitrogen, phosphorus	10pg
Flame photometric	Mass flow	Hydrogen and air possibly oxygen	Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium	100pg

Photoionization	Concentration	Make up	Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics	2pg
Hall electrolytic conductivity	Mass flow	Hydrogen, oxygen	Halide, nitrogen, nitrosamine, sulphur	-

Application

1. **Qualitative Analysis**
   - When 2 substance gives coincident peak (one known and one unknown), it is evidence that the compounds may same.
   - Retention characterstics of unknown compound determined by:

a) Specific Retention volume (Vg) –
   - Flow rate of carrier gas X Adjusted Retention time)
   - But in this, reproducibility is very low due to varying packing density,liquid loading, activity of support, age etc.

b) Relative retention (rA / B) –
   - Adjusted retention volume of substance A related to that of reference standard B.
   - Here reproducibility is good.

2. **Quantitative analysis**
   - Size of the chromatographic peak is propotional to amount of the compound.
   - By measuring accurately the peak area or hight, quantitative analysis can be done.
3. **Presence of impurites.**
4. **Used in the quality control of :**
   - Antibiotics - Pencillin, Gentamycin
   - Anti T.B drugs - Isoniazid, Ethambutol
   - Antivirals - Amantadine, Idoxuridine
   - Anti neoplastics - Flurouracil, Doxorubin

3. Differentiate between Atomic absorption and atomic emission. Describe various interferences involved in Atomic Absorption Spectroscopy.

Answer

Differentiate between Atomic Absorption and Atomic emission

Atomic Absorption Spectroscopy (AAS)	Atomic Emission Spectroscopy (AES)
Atomic absorption spectroscopy is used to find the concentration of metals atom in a solution	Atomic emission spectroscopy is used to find out the concentration of the analyte by emission of light
A fixed amount of energy is absorbed by the electrons of an atom	The discrete energy emitted during de excitation
From ground state to an excited state	From excited state to ground state

Electromagnetic radiation is absorbed	Electromagnetic radiation is emitted
It can not be used for direct analysis of solid samples	It can be used for the direct analysis of solid samples
The spectrum obtained is dark lines are gaps	Colored spectrum is obtained
It requires a light source	It does not require a light source
It depends upon a number of ground-state atoms	It depends upon the number of excited-state atoms

Interferences involved in Atomic Absorption Spectroscopy

Spectral interferences

- Spectral interferences occur due to two reasons: flame and the matrix.
- **Due to flame:** When we use a flame atomizer in atomic absorption spectroscopy, then the light from the flame and the light source both reach the detector. To measure a small signal coming from the sample, we need to subtract the signal coming from the flame. If not, we get spectral interferences in the final spectra.
- **Due to matrix:** The matrix contains molecular species which can show molecular spectra. This might result in various spectral interferences such as:
    - **Overlapping of two lines:** molecular spectra is much broader with a greater net absorbance. So, it may overlap with the actual atomic spectra.
    - **Presence of combustion products:** The radiation may scatter due to this particulate matter and we get less absorption due to atomic species.
    - Absorption of radiation by matrix components.

Methods of correction

Two line method

- For this method, we need to know the absorption wavelength of interfering species.
- We will choose two wavelengths close together. One wavelength is the absorption wavelength of the analyte and the other wavelength would be the absorption wavelength of the interfering species. But the analyte must not absorb at the wavelength of the interfering species. The interference may absorb at the wavelength of the analyte.
- Since the two wavelengths are closer, the absorbance and absorptivities will be constant.
- By comparing the total absorbance at the first wavelength with the absorbance at a second wavelength, we can eliminate the interference due to molecular species.
  Aλ1 (atom + molecule) – Aλ2 (molecule) = A (atom)

Continuous source method

- Deuterium lamps can be used to capture molecular absorbance.
- Place a hollow cathode lamp and a continuous source Deuterium lamp and let the radiations meet and pass through the sample.
- Hollow cathode lamp will record the absorbance of atoms and molecules.
- The Deuterium lamp will record the absorbance only due to molecules.

Zeeman effect

- When the gaseous atoms are placed in a magnetic field, they orient themselves in the direction of the magnetic field.
- But this orientation is not seen for molecular species.
- So, the electronic energy levels get split. This is called the Zeeman effect which can be used to remove background from the spectra.

Chemical interferences

1. **Formation of components of low volatility:** In atomic absorption spectroscopy, we convert the sample into its atomic form. Sometimes, the anions and cations can react and form a salt that has low volatility. This decreases the number of atoms of the sample present in the flame. For example., in the estimation of calcium in the sample, the Ca2+ ions may react with SO42- ions to form CaSO4 which is a salt.
2. **Formation of oxides and hydroxides:** Due to the presence of air and oxides in the flame, the analyte may form oxides and hydroxides which might show intense molecular absorption.
3. **Ionization:** The absorption due to pure metal atoms and its ion is not the same. For atomic absorption spectroscopy, we need the atomic form of the analyte.

SECTION C

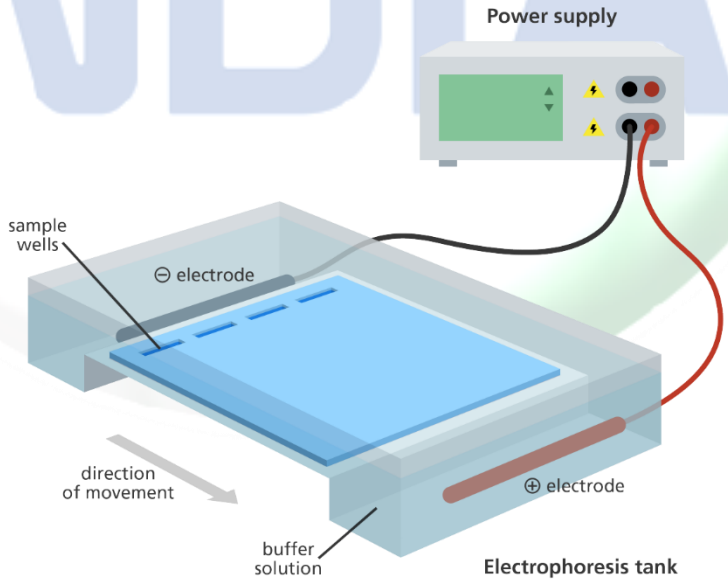
SHORT ANSWERS TYPE QUESTIONS (5 × 7 = 35)

1. Give theory of Gel Electrophoresis. Explain factors affecting electrophoretic mobility.

Answer

Theory of Gel Electrophoresis

- Gel electrophoresis separates DNA fragments by size in a solid support medium such as an agarose gel.



- Sample (DNA) are pipetted into the sample wells, followed by the application of an electric current which causes the negatively-charged DNA to migrate (electrophorese) towards the anodal, positive (+ve) end.

- The rate of migration is proportional to size: smaller fragments move more quickly and wind up at the bottom of the gel.
- DNA is visualized by including in the gel an intercalating dye, ethidium bromide.
- DNA fragments take up the dye as they migrate through the gel. Illumination with ultraviolet light causes the intercalated dye to fluoresce.
- The larger fragments fluoresce more intensely. Although each of the fragments of a single class of molecule is present in equimolar proportions, the smaller fragments include less mass of DNA, take up less dye, and therefore fluoresce less intensely.
- A "ladder" set of DNA fragments of known size can be run simultaneously and used to estimate the sizes of the other unknown fragments.

Factors affecting Electrophoretic mobility

a. **Electrolysis-** Electrophoresis is accompanied by electrolysis which causes microscopic bubbles to accumulate on the electrodes. When a bubble is formed. The electrical contact with buffer is lost.

b. **Ionic Strength-** The activity coefficients of ionic substances in solution are influenced by the concentration of the solution and by the valency of the ions. When the solution is a buffer, the weakly ionized component makes virtually no contribution to the ionic strength of the solution.

c. **pH and other Chemical Characteristics-** The electrophoretic mobility is greatly affected by the pH of a buffer, particularly when the sample is either a weak acid or a weak base, because the pH establishes its degree of ionization.

d. **Electro-osmosis-** during electrophoresis, there is often a flow of water under the influence of the voltage gradient. This is called electro-osmosis, the rate of which is influenced by the species and concentration of ionic solutes in water.

e. **Interaction with supporting medium-** Electrophoretic migration is slower in stabilizing medium than in free solution. If the supporting medium has ionic side chains (e.g., sulphate groups in agar or carboxylate groups in paper), these can interact with the particles being separated by electrophoresis. Ionic side chains are usually undesirable because they provide trailing.

f. **Heat-** The unavoidable electrical heating that accompanies the process of electrophoresis has a number of adverse effects. In free solution electrophoresis, heat causes convection currents. As a result, electrophoretic pattern is disrupted. Supporting media are intended to prevent this by impeding liquid flow. The adverse effects of heat are most evident in gel beds, where convection is virtually eliminated.

2. What is fingerprint region? Explain fundamental modes of vibrations in polyatomic molecules.

Answer

Fingerprint Region

- The entire IR spectrum can be used as a fingerprint for the purposes of comparing molecules, the 600 - 1400 cm-1 range is called the fingerprint region. This is normally a complex area showing many bands, frequently overlapping each other.

Fundamental modes of Vibration in Polyatomic molecules

- The underlying principle of infrared spectroscopy is based upon the molecular vibrations which are further composed of the stretching and the bending vibrations of a molecule.
- **MOLECULAR VIBRATIONS**
  - The vibrations for molecules are of two types, namely :
    - Stretching, and
    - Bending (or deformation)
- **Stretching -** Vibration causes stretching whereby the distance between the two atoms increases or decreases, but the atoms remain in the same bond axis.
  - **Symmetrical Stretching -** In this case, the two hydrogen atoms either move towards or away from the central carbon atom in unison, thereby either altering the interatomic distance or causing no change in valence angle.
  - **Asymmetrical Stretching -** In this instance, one hydrogen atom approaches the carbon atom while the other moves away from the carbon atom.

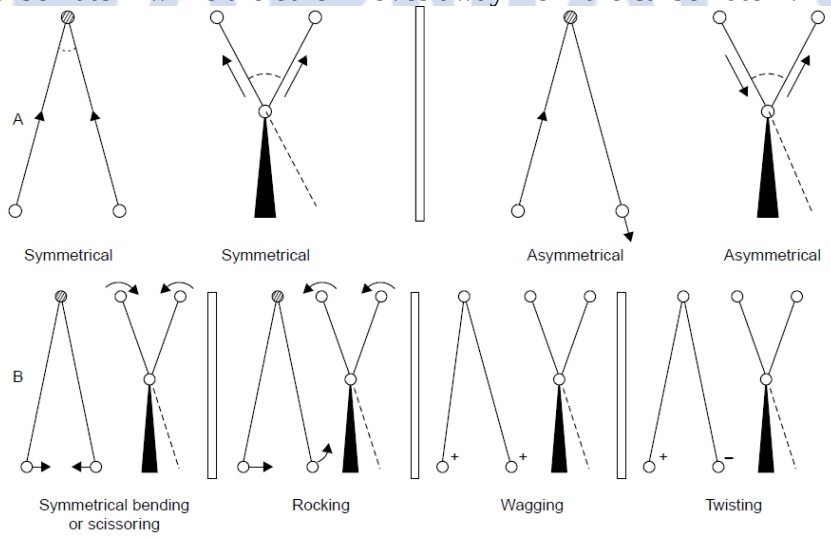


Figure: (A) Stretching (B) Bending

- **Bending (or Deformation) -** Vibration causes bending whereby the position of the atom changes relative to the original bond axis.
- **In-Plane Bending Vibrations**
  - c. **Scissoring or Symmetrical Bending -** In this case, the two atoms connected to a central atom either moves toward or away from each other with certain deformation of the valence angle.
  - d. **Rocking -** In this case, the structural unit swings back and forth in the plane of the molecule.
- **Out-of Plane Bending Vibrations**
  - c. **Wagging -** In this case the structural unit swings back and forth out of the plane of the molecule.

d. **Twisting -** In this case the structural unit rotates about the bond that joins it to the rest of the molecule.

3. Explain applications of spectrofluorometry.

Answer

Applications of Spectrofluorometry

1. Determination of ruthenium

- It is determined with platinum metal present. With the reagent, palladium forms a precipitate that can be separated by centrifugation.
- Because iron produces a compound that quenches fluorescence, it should be missing.

2. Determination of boron in steel

- It is identified through the formation of a compound with benzoin. Boron in the sample's acid solution is first transformed to boric acid, which is then co-distilled with methyl alcohol to separate it from the other components.
- The resultant distillate, which contains boric acid, is neutralised with NaOH, and methyl alcohol is evaporated.

3. Determination of aluminium in alloys

- The utilised reagent is the dye pontachrome blue black F, which is used in a buffered solution with a p H of 4.8. It is suitable for acid-soluble aluminium concentrations ranging from 0.01% to 1.00% in steel.

4. Determination of chromium and manganese in steel

- Steel is dissolved in acid, which is then oxidised using persulphate. CrO7 2- and MnO4 – are ions that absorb violet and yellow-green light, respectively.

5. Determination of uranium salts

- The sample is initially heated with nitric acid, followed by fusion with sodium fluoride and uranium fluoride. Upon cooling, the molten substance hardens into glass, which may be directly viewed with a fluorometer.
- With the reagent, palladium forms a precipitate that can be separated by centrifugation. Because it produces a compound that quenches fluorescence, iron should be absent.

6. Estimation of rare earth terbium

- Formation of a fluorescent compound with EDTA and sulpho salicylic acid. The spectrum of excitation correlates to the spectrum of absorption of sulpho salicylic acid.

7. Estimation of bismuth

- In order to absorb the radiation, the solutions are evaporated in an argon-hydrogen flame and then irradiated with the iodine emission line.

8. Determination of beryllium in silicates

- The development of a luminous beryllium-morin complex. Mercury cathode electrolysis removes interferences such as iron and rare earths; the fluorescence of the complex is complexing with triethanolamine and diethylene triamine pentaacetate.

4. Describe mechanism of ion exchange process in Ion Exchange

Chromatography.

Answer

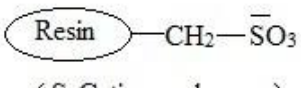
Mechanism of Ion exchange process in Ion Exchange Chromatography

- The mechanism of separation is by reversible exchange of ions between the ions present inthe solution and those present in the ion exchange resin.
- Ion exchange separations are mainly carried out in columns packed with an ion exchanger.

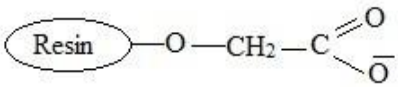
- There are two types of ion exchanger, as follows:

a) Cationic exchangers:

- It possesses negatively charged groups and these will attract positively charged groups.
- These exchangers are also called acidic ion exchange materials since their negative charges result from the proteolysis of acidic groups.
- Commonly used cation exchange resins are S-resin, sulfate derivatives; and CM resins, carboxylate derived ions



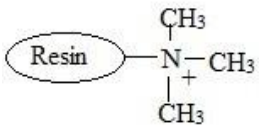
(S-Cation exchanger)



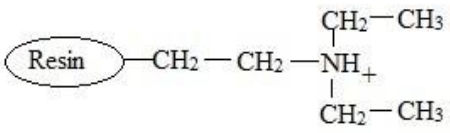
(CM-Cation exchanger)

b) Anionic exchangers:

- It has positively charged groups, which will attract negatively charged molecules.
- This exchanger is termed as basic ion exchange materials since their positive charges generally result from the association of protons with basic groups.
- Based upon the affinity of ions towards the matrix the ions like cation and anion are separated. The ions that have less affinity towards matrices will elute first and the ions that have more affinity towards matrices it will elute later.
- Commonly used anion exchange resins are Q-resin, a Quaternary amine; and DEAE resin, Di Ethyl Amino Ethane.



(Q-Anion exchanger)



(DEAE-Anion exchanger)

- The actual ion exchange mechanism is thought to be composed of five distinct steps:
  1. Diffusion of the ion to the exchanger surface. This occurs very quickly in homogeneoussolutions.
  2. Diffusion of the ion through the matrix to the exchanger site.

This is dependent upon the degree of cross linkage of the exchanger and the concentration of the solution.

3. Exchange of ions at the exchange site occurs. This occur instantaneously in an equilibriumprocess as follows:

   $Resin - SO_3H + Na^+ \longrightarrow Resins - SO_3Na + H^+$

   $Resin - N(CH_3)_3OH + Cl^- \longrightarrow Resin - N(CH_3)_3Cl + OH^-$

4. Diffusion of the exchanged ion through the exchanger to the surface
5. Selective desorption by the eluent and diffusion of the molecule into the external solutiontakes places.

5. Explain isocratic and gradient elution in HPLC.

Answer

Isocratic Elution in HPLC

- Isocratic elution is a term used in chromatography when the mobile stage has a recurring concentration. Here, the concentration of the mobile stage is recurrent throughout the entire chromatographic run. In this development, we have the possibility to see that the peak width increases with retention time in a linear fashion in the chromatogram. However, this brings a disadvantage: the late eluting peaks become very flat and broad. Then, these broad peaks become difficult to admit as peaks.
- Furthermore, in isocratic elution(IE), the selectivity does not change in functionality of the column dimensions. This means that selectivity is not dependent on changes in column dimensions. Here, length and diameter are thought of as column dimensions. Then, the peaks elute in the same order.

Gradient Elution in HPLC

- Step elution is a term used in chromatography when the mobile stage has a variable concentration. In other words, the concentration of the mobile step does not have to be kept recurrent. Among other things, in HPLC, a common splitting procedure uses 10% methanol at the beginning and ends at 90%, with the concentration gradually increasing.
- The mobile stage has two components: a weak solvent and a strong solvent. The weak solvent makes it easier for the solute to elute slowly, while the strong solvent makes the solute elute slightly. In reverse-stage

chromatography, we use water as the weak solvent and organic solvent as the strong solvent.

- In addition, the gradient elution procedure decreases the later eluting elements to make them elute more rapidly, giving a tight peak in the chromatogram. This procedure optimizes the shape and height of the peaks. Furthermore, in the gradient elution technique, the order of elution changes with changes in column dimensions.

6. Discuss derivatization in Gas Chromatography.

Answer

Derivatization in Gas Chromatography

- Derivatization reactions transform an analyte for detectability in gas chromatography or other instrumental analytical methods.
- Derivatization in gas chromatography analysis is a technique that modifies the functionality of an analyte to allow chromatographic separations.
- The resultant modified analyte is the product, and is known as the derivative, whose structure may be similar or closely related to but not the same as the original non-modified chemical compound.

Derivatization Reagent

- Derivatization reagent is a substance used for chemically modifying a compound so that a new compound, with properties suitable for analysis in gas and liquid chromatography, can be produced.
- A suitable derivatization reagent for gas chromatography should be selected based on the following criteria:

1) It should produce more than 95% complete derivatives.
2) It should not cause any rearrangements or structural alterations in the compounds during the formation of a derivative.
3) It should not cause any sample loss during the reaction.
4) It should produce a derivative that will not interact with the column used in gas chromatography.
5) It should produce a derivative that remains stable with time.

Objectives for Derivatization

1. It improves resolution and reduces tailing of polar compounds containing –OH, –COOH, –NH, –NH2, –SH, and other functional groups.
2. It enables the analysis of relatively non-volatile compounds.
3. It reduces the volatility of compounds prior to gas chromatography analysis.
4. It improves the analytical efficiency and increases detectability.
5. It causes stabilization of compounds for gas chromatography analysis.

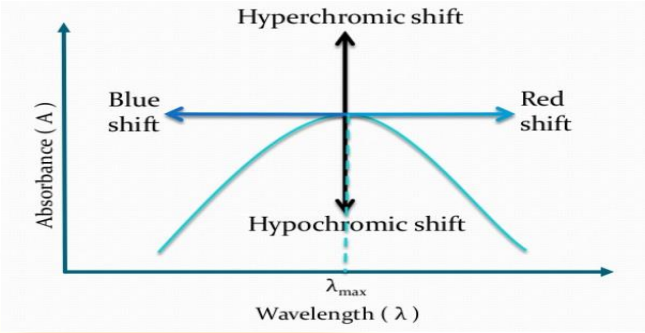
7. Describe spectral shifts and solvent effect on absorption spectra in UV

Spectroscopy.

Answer

UV Spectroscopy

Spectral Shifts on absorption spectra



- **Bathochromic shift (red shift) –** When an absorption maximum of a compound shifts to longer wavelength, it is known **as bathochromic shift or red shift**.
- **Hypsochromic shift (blue shift) –** When an absorption maximum of a compound shifts to shorter wavelength, it is known **as hypsochromic shift or blue shift**.
- **Hyperchromic shift –** When absorption intensity of a compound increased, it is known as hyperchromic shift.
- **Hypochromic shift –** When absorption intensity of a compound decreased, it is known as hypochromic shift.

Solvent effects on absorption spectra

- The solvent exerts a profound influence on the quality and shape of spectrum.
- The absorption spectrum of pharmaceutical substance depends practically upon the solvent that has been employed to solubilize the substance.
- A drug may absorb a maximum radiation energy at particular wavelength in one solvent but shall absorb partially at the same wavelength in another solvent.
- Polarity plays an important role in the position and intensity of absorption maximum of a
- particular chromophore.
- E.g. In case of non-polar solvents e.g. Iodine solution (purple color) the absorption maxima occur at almost the same wavelength as in iodine vapour (518 nm), whereas in case of polar solvents, a brownish color is obtained instead of purple color, because the absorption occurs at shorter wavelengths.
- Purified and certified solvents for spectroscopy should be used as we are looking for the “smooth” absorbance curve of solvent.
- Absorption bands of many substances are relatively sharper and may also exhibit fine structure when measured in solvents of low dipole moment.
- By increasing the polarity of the solvent, compounds like dienes & conjugated hydrocarbons do not experience any appreciable shift.

PHARMACY INDIA

JOIN US

IF YOU ARE B.PHARMA STUDENT

GET IT ON Google Play

Download PHARMACY INDIA App from play store

WHATSAPP

TYPE "PINDIA" & SEND US ON 8006781759 FOR PHARMA UPDATES

TELEGRAM

SCAN QR CODE TO JOIN BIGGEST PHARMA TELEGRAM GROUP (10000+ STUDENTS)

Instagram

FOLLOW PHARMAINDIA24 GET RECENT PHARMA JOBS UPDATES

WEBSITE

pharmacyindia.co.in GET LATEST PHARMA JOBS UPDATES

Download PHARMACY INDIA Mobile App From Play Store

GET B. PHARMA NOTES