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PAPER DISCUSSION



Instrumental Methods of Analysis

B.PHARM 7TH SEM

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INSTRUMENTAL METHODS OF ANALYSIS B.PHARM | SEMESTER 7

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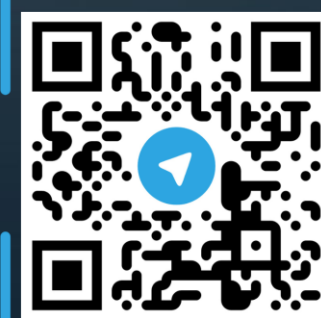


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1. What is Mull Technique?

- The **Mull Technique** is a method used in **IR spectroscopy** to analyze solid samples.
- The solid is ground into a fine powder and mixed with a drop of **mineral oil (like Nujol)** to form a **paste (mull)**.
- This paste is placed between two **IR-transparent plates (usually NaCl)** and then analyzed.
- It's useful for **insoluble or thermally unstable** compounds, but the oil can interfere with parts of the spectrum.



2. Give principle of UV spectroscopy.

Principle of UV Spectroscopy:

- UV spectroscopy is based on the absorption of ultraviolet or visible light by molecules. When UV light passes through a compound, electrons in π (π) and non-bonding (n) orbitals absorb energy and get excited to higher antibonding orbitals (π^* or σ^*).
- The amount of light absorbed is measured and is directly related to the concentration of the compound (Beer–Lambert's law).

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

Methods for Preparation of TLC Plates

TLC plates are thin layers of adsorbent coated on a support material (glass, plastic, or aluminum). Two main methods are used:

1. Laboratory-Coated Plates

- **Preparation of slurry:** Adsorbent (silica gel G, alumina, etc.) is mixed with a binder like calcium sulfate and water to form a uniform slurry.
- **Coating:** The slurry is spread evenly on clean glass plates (usually 0.25–0.5 mm thickness) using a spreader or applicator.

- **Drying:** Plates are air-dried and then heated in an oven at 100–110 °C to remove moisture and activate the adsorbent.
- **Use:** These plates are economical but require time and skill for uniform coating.

2. Pre-coated Commercial Plates

- Ready-to-use plates available with adsorbent already coated on glass, aluminum, or plastic sheets.
- They are uniform in thickness, reproducible, and save time.
- Often contain fluorescent indicators for easy visualization.

4. Give principle of UV spectroscopy.

Principle of UV Spectroscopy

UV spectroscopy is based on the **absorption of ultraviolet (200–400 nm) or visible (400–800 nm) light** by molecules. When UV light passes through a compound, electrons in **π (pi) bonds or non-bonding (n) orbitals** absorb energy and undergo electronic transitions to higher energy antibonding orbitals (π^* or σ^*).

The amount of light absorbed is measured, and according to **Beer–Lambert's law**, the absorbance is directly proportional to the concentration of the absorbing species and the path length.

5. Give significance of Fermi Resonance.

Definition: Fermi resonance occurs when a **fundamental vibration** and a **overtone/combination band** have nearly the same energy, causing them to interact.

Significance:

- 1. Band Splitting:** Leads to splitting of absorption bands in IR spectra, giving two peaks instead of one.
Band Intensity Change: Alters the intensity of bands (one becomes stronger, the other weaker).
- 2. Spectral Interpretation:** Helps in identifying overtones and combination bands, making spectral analysis more accurate.
- 3. Structural Information:** Provides information about coupling between vibrational modes and molecular symmetry.



6. Discuss factors affecting Vibrational frequency in IR spectroscopy.

The vibrational frequency of a bond depends mainly on the **bond strength** and the **masses of the atoms** involved. The following factors influence it:

1. Bond Strength

- Stronger bonds vibrate at higher frequencies.
- Example: $\text{C}\equiv\text{C}$ (triple bond) > $\text{C}=\text{C}$ (double bond) > $\text{C}-\text{C}$ (single bond).

2. Reduced Mass of Atoms

- Lighter atoms vibrate at higher frequencies.
- Example: $\text{C}-\text{H}$ ($\sim 3000\text{ cm}^{-1}$) > $\text{C}-\text{D}$ ($\sim 2200\text{ cm}^{-1}$).

3. Electronegativity of Atoms

- More electronegative atoms pull electrons strongly, increasing bond polarity and vibrational frequency.
- Example: C–F ($\sim 1100\text{ cm}^{-1}$) > C–Cl ($\sim 700\text{ cm}^{-1}$).

4. Resonance and Conjugation

- Delocalization of electrons weakens the bond, lowering vibrational frequency.
- Example: C=O in conjugated ketone ($\sim 1680\text{ cm}^{-1}$) < simple ketone ($\sim 1720\text{ cm}^{-1}$).

7. Differentiate between Fluorescence vs Phosphorescence.

Feature	Fluorescence	Phosphorescence
Excited State Involved	Singlet $\rightarrow$ Singlet transition ($S_1 \rightarrow S_0$)	Triplet $\rightarrow$ Singlet transition ($T_1 \rightarrow S_0$)
Spin Nature	Allowed transition	Forbidden transition
Lifetime of Emission	Very short ($10^{-9} - 10^{-6}$ sec)	Longer (10^{-3} sec to several minutes or hours)
Persistence of Glow	Stops immediately when excitation source is removed	Continues even after excitation source is removed
Energy of Emission	Higher (shorter wavelength)	Lower (longer wavelength)
Example	Fluorescein, Quinine	Zinc sulfide, Glow-in-the-dark materials

8. Mention the Advantages of Thin Layer Chromatography (TLC).

- ❑ **Simple and Fast** – Easy to perform with rapid separation.
- ❑ **Low Cost** – Requires less equipment and cheaper than HPLC or GC.
- ❑ **High Sensitivity** – Detects small quantities (nanogram level with fluorescence/chemical reagents).
- ❑ **Versatility** – Can be used for separation of a wide variety of compounds (polar, non-polar, organic, inorganic).
- ❑ **Multiple Sample Analysis** – Several samples can be analyzed simultaneously on a single plate.

9. Write the various pumps used in HPLC.

Pumps in HPLC

1. **Syringe Pump** – Delivers fixed volume at constant flow; used for micro-HPLC.
2. **Reciprocating Pump** – Piston moves back and forth; gives high pressure and constant flow; most commonly used.
3. **Peristaltic Pump** – Rollers compress tube; simple but low pressure; rarely used in HPLC.
4. **Hydraulic Pump** – Uses hydraulic amplification for pulse-free flow; less common.

10. What do you understand by quenching?

Definition: Quenching is the **process of decreasing or suppressing the fluorescence** of a molecule due to interaction with another substance (quencher).

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