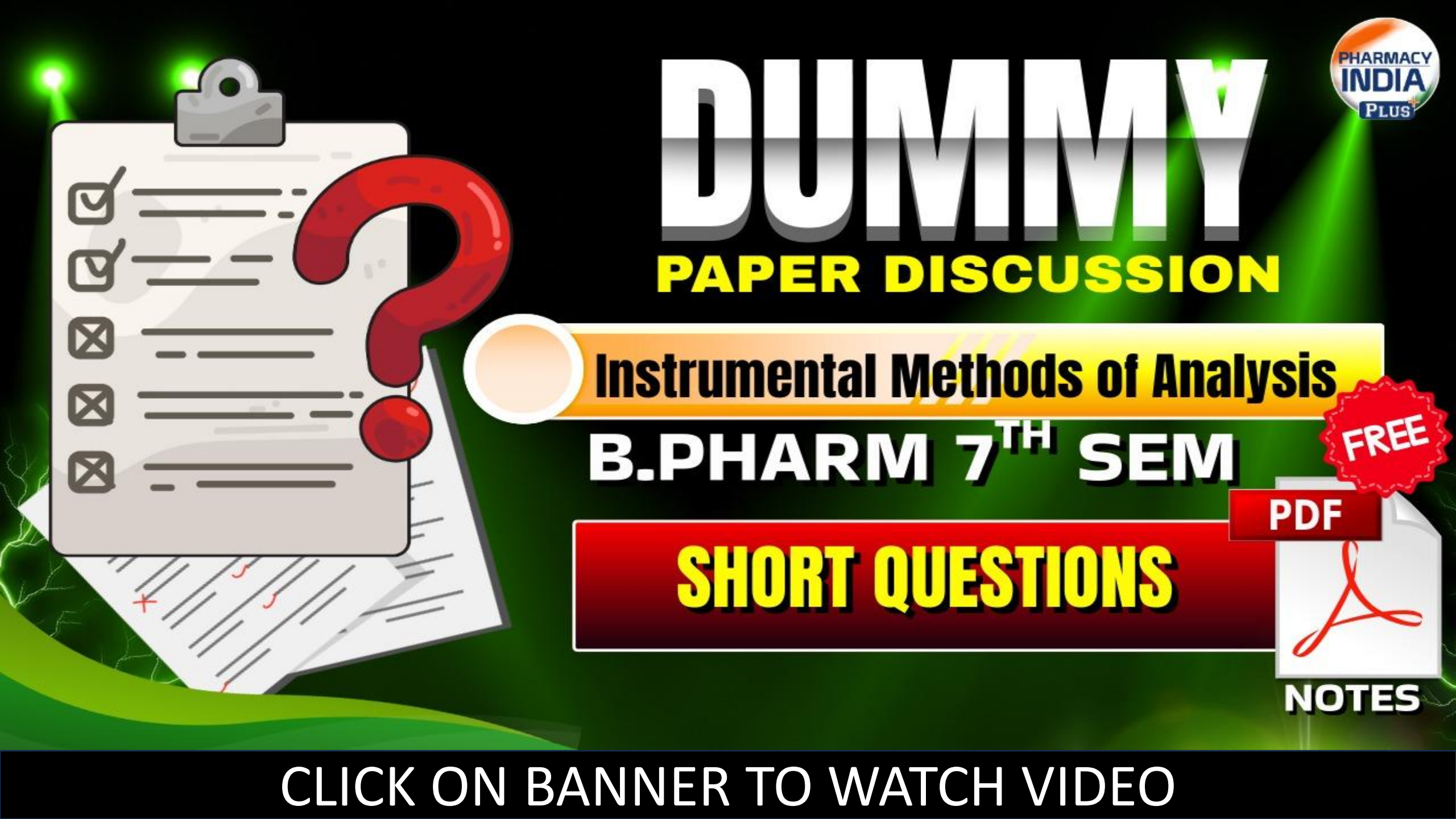
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INSTRUMENTAL METHODS OF ANALYSIS

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1. Discuss the principle and applications of ion exchange chromatography.

Principle:

Ion exchange chromatography (IEC) separates ions and polar molecules based on their **charge**.

- It works by **reversible exchange of ions** between the sample and a **stationary phase** (ion exchange resin).
- The resin contains **charged groups**:
  - **Cation exchange resin**: contains **negatively charged** groups (binds **cations**).
  - **Anion exchange resin**: contains **positively charged** groups (binds **anions**).
- Ions in the mobile phase (buffer) **compete** with sample ions for binding sites.
- Separation occurs based on **strength of interaction** (charge, size, and affinity).

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Applications:

1. **Water Softening and Purification :** Removal of **Ca^{2+} and Mg^{2+}** from hard water.
2. **Amino Acid and Protein Separation :** Based on differences in **net charge** at a given pH.
3. **Purification of Nucleotides and Peptides :** Useful in **biotechnology** and **pharmaceutical** industries.
4. **Ion Analysis in Environmental Samples :** Detection of **anions and cations** in water and soil.
5. **Separation of Metal Ions :** For **qualitative and quantitative** analysis in chemical labs.
6. **Blood and Serum Analysis :** Separation of charged biomolecules for clinical diagnostics.

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2. Write about principle, methods, and applications of TLC.

Principle:

Thin Layer Chromatography (TLC) is based on the principle of **adsorption chromatography**.

- A sample mixture is spotted on a **stationary phase** (a thin layer of silica gel or alumina coated on a glass/plastic/metal plate).
- The plate is placed in a **mobile phase** (solvent or solvent mixture).
- As the solvent rises by **capillary action**, components of the mixture move at different rates depending on their **affinity** for:
  - The **stationary phase** (adsorption)
  - The **mobile phase** (solubility)

  More strongly adsorbed compounds move **slower**, while weakly adsorbed compounds move **faster**, resulting in separation.

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Methods (Procedure):

1. **Preparation of TLC Plate:**
   - Use pre-coated TLC plates or prepare by spreading slurry of silica/alumina on glass/plastic.
2. **Sample Application:**
   - Spot the sample mixture near the bottom (about 1–2 cm from the edge) using a capillary tube.
3. **Development:**
   - Place the plate in a development chamber with solvent.
   - Solvent moves up by capillary action, separating components.
4. **Detection:**
   - Visualize spots under UV light or by spraying with a detecting reagent (e.g., iodine, ninhydrin).
5. **Rf Value Calculation:**

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Applications:

1. **Purity Checking:**
   - To assess purity of drugs and chemical compounds.
2. **Identification of Compounds:**
   - Compare Rf values with standard samples.
3. **Monitoring Reactions:**
   - Check progress of chemical reactions in labs.

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3. Explain Isocratic and Gradient Elution in HPLC.

Isocratic and Gradient Elution in HPLC

Isocratic Elution:

- The **mobile phase composition remains constant** throughout the chromatographic run.
- Suitable for **simple mixtures** where analytes have **similar polarities**.
- Provides a **stable baseline** and is easier to operate.
- Requires **less complex instrumentation**.
- May lead to **longer run times** or **poor separation** for complex samples.

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Gradient Elution:

- The **mobile phase composition changes gradually** during the run.
- Ideal for **complex mixtures** with components of **varying polarities**.
- Enhances **separation efficiency** and **reduces analysis time**.
- Provides **sharper peaks** and better resolution for late-eluting compounds.
- Requires **gradient-capable pumps** and more complex instrumentation.
- Can cause **baseline drift**, which needs to be managed during data analysis.

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4. Write the principle and instrumentation of Gel electrophoresis.

Principle:

Gel electrophoresis is a technique used to **separate charged molecules**, such as **DNA, RNA, or proteins**, based on their **size and charge**.

- When an electric field is applied, **ngaetively charged molecules** (like DNA) migrate towards the **positive electrode (anode)**.
- The molecules move through a **gel matrix** (typically agarose or polyacrylamide), which acts as a **molecular sieve**.
- **Smaller molecules** move **faster and farther** through the gel, while **larger molecules** move **slower**, allowing separation based on **size**.
- The rate of migration is influenced by:
  - **Size and shape** of the molecule
  - **Gel concentration**
  - **Voltage applied**

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Instrumentation:

1. **Power Supply:**
   - Provides the **electric field** required for migration.
   - Usually supplies **20–150 volts**, depending on the gel type and application.
2. **Electrophoresis Chamber (Tank):**
   - Holds the **gel** and **buffer solution**.
   - Contains **electrodes** at each end to create the electric field.
3. **Gel Casting Tray and Comb:**
   - Used to **cast the gel** and create **wells** for sample loading.

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4. Gel Matrix:

- **Agarose gel**: commonly used for DNA/RNA.
- **Polyacrylamide gel**: used for protein separation and high-resolution applications.

5. Buffer Solution:

- Maintains **pH** and **conductivity**.
- Common buffers: **TAE**, **TBE** for nucleic acids; **SDS-PAGE buffer** for proteins.

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5. Explain the importance of temperature programming along with methodology of GC.

Importance of Temperature Programming in GC:

Temperature programming gradually increases the oven temperature during the run. This helps separate compounds with different boiling points more efficiently, improves peak shapes, and reduces analysis time compared to keeping a constant temperature.

Methodology of Gas Chromatography:

- Sample is injected and vaporized.
- An inert carrier gas transports the sample through the column.
- Components separate based on their interaction with the stationary phase.
- Oven temperature is controlled (constant or programmed).
- Detector records the separated compounds as peaks.

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

Spectral Shifts:

- Spectral shifts refer to changes in the **absorption wavelength (λmax)** of a molecule when conditions change.
- Two main types of shifts occur:

1. Bathochromic shift (Red shift):

- ✓ Absorption moves to **longer wavelengths** (lower energy).
- ✓ Caused by increased conjugation or solvent interactions that stabilize the excited state more than the ground state.

2. Hypsochromic shift (Blue shift):

- ✓ Absorption moves to **shorter wavelengths** (higher energy).
- ✓ Occurs when solvent or molecular changes destabilize the excited state relative to the ground state.

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Solvent Effect on Absorption Spectra:

- Solvent polarity affects the electronic environment of the solute molecules.
- **Polar solvents** can stabilize excited or ground states differently, causing shifts in absorption peaks.
- Usually:
  - Polar solvents cause **bathochromic shifts** if they stabilize the excited state more.
  - Non-polar solvents may cause **hypsochromic shifts**.
- Solvent hydrogen bonding can also influence absorption intensity and position.

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7. Explain applications of Spectrofluorometry.

Applications of Spectrofluorometry

1. **Biochemical Analysis:**
   - Detecting and quantifying **proteins, nucleic acids**, and **enzymes**.
   - Studying **enzyme kinetics** and **binding interactions**.

2. **Medical Diagnostics:**
   - Measuring **biomarkers** and **drugs** in blood or urine.
   - Fluorescent labeling in **immunoassays** and **cell imaging**.

3. **Environmental Monitoring:**
   - Detecting trace amounts of **pollutants** like polycyclic aromatic hydrocarbons (PAHs) and pesticides.
   - Monitoring **water quality**.

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4. Pharmaceutical Industry:

- Drug analysis and **quality control**.
- Tracking **drug metabolism** and **pharmacokinetics**.

5. Forensic Science:

- Detecting biological fluids (blood, saliva) using fluorescence.
- Analysis of inks, fibers, and explosives.

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