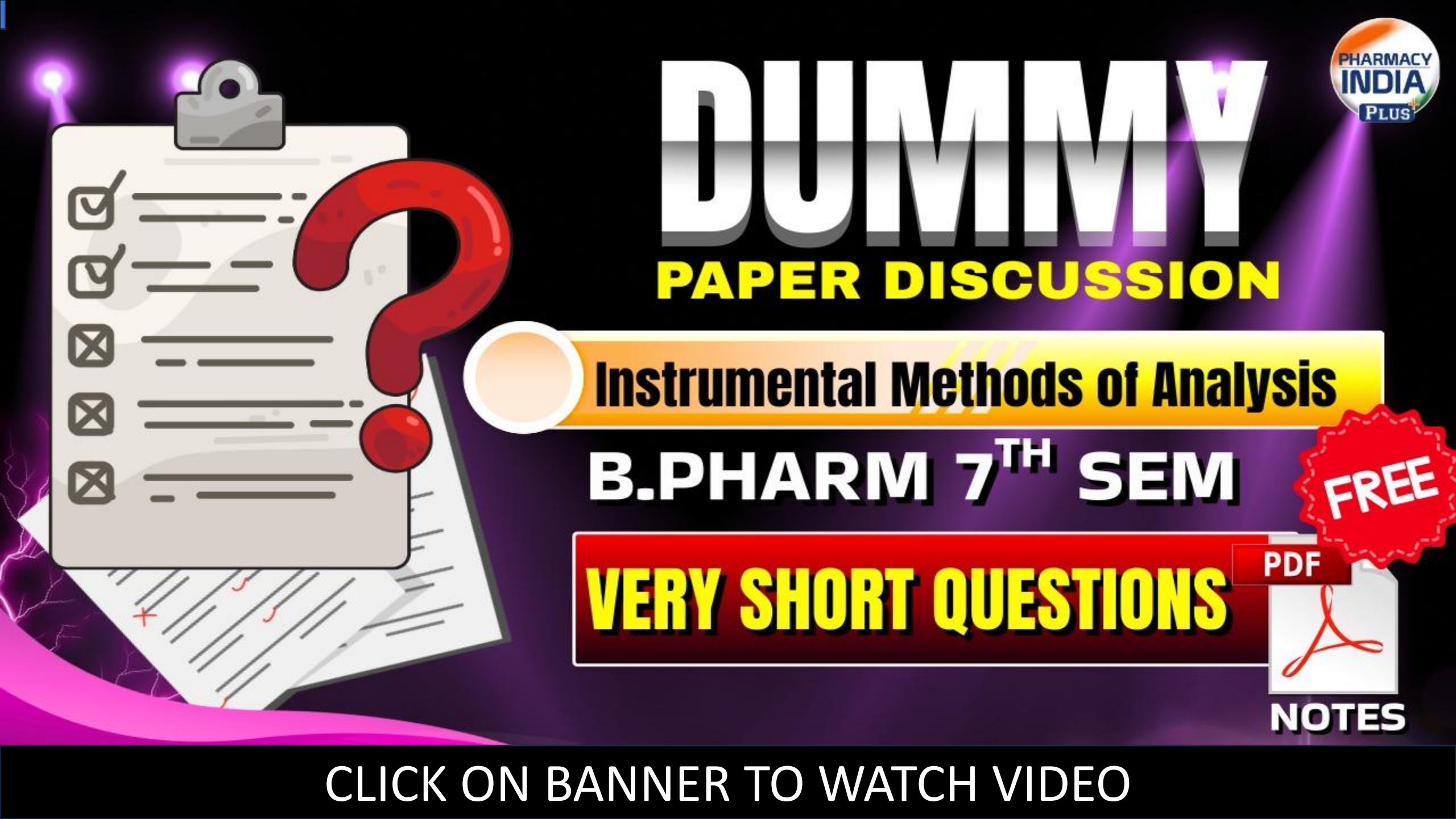
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INSTRUMENTAL METHODS OF ANALYSIS

B.PHARM | SEMESTER 7

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1. How does temperature programming affect the separation process in GC.

Temperature programming in GC improves separation by gradually increasing the column temperature, which:

- Speeds up elution of late-eluting (less volatile) compounds.
- Reduces peak broadening and tailing.
- Enhances resolution between compounds with different volatilities.
- Shortens overall analysis time compared to isothermal runs.

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2. What is the Principle of Nephelo-turbidometry.

Principle of Nephelo-turbidometry:

It is based on the scattering of light by suspended particles in a solution; the intensity of scattered (nephelometry) or transmitted (turbidimetry) light is proportional to the concentration of the particles.

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3. Discuss the Principle behind gel chromatography.

Principle of Gel Chromatography (Size-Exclusion Chromatography):

Gel chromatography separates molecules based on their size (and shape) using a porous stationary phase (gel beads).

- Large molecules cannot enter the pores and elute first.
- Small molecules enter the pores, take a longer path, and elute later.

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4. Write the use of thermocouples.

Uses of Thermocouples:

- Measurement of temperature in laboratories, industries, and households.
- Monitoring furnace, engine, and exhaust temperatures.
- Used in GC, HPLC detectors, and other scientific instruments.
- Employed in safety devices (e.g., gas appliances) to detect flame presence.

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5. Enumerate few applications of Flame photometry.

Applications of Flame Photometry:

- Determination of alkali metals (Na, K, Li) in biological fluids.
- Measurement of calcium and magnesium in clinical samples.
- Analysis of soil, fertilizers, and plant materials.
- Quality control in food and beverages (e.g., salt content).
- Detection of metal impurities in pharmaceuticals and chemicals.

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6. Write the various pumps used in HPLC.

Pumps used in HPLC:

- **Reciprocating pump** – most common, delivers constant flow at high pressure.
- **Syringe (displacement) pump** – delivers precise, pulse-free flow, used in research.
- **Pneumatic (gas pressure) pump** – uses compressed gas to drive solvent, less common.

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2. Write a note on stationary phases used in Gel chromatography.

Stationary Phases in Gel Chromatography:

- **The stationary phase consists of porous, cross-linked polymer or gel beads that allow size-based separation.**
- **Common materials used:**
  - **Sephadex (cross-linked dextran)**
  - **Sepharose (agarose-based)**
  - **Biogel P (polyacrylamide-based)**
  - **Porous silica gels**

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- **The pore size of the gel is carefully controlled to separate molecules of different sizes.**
- **Function: Large molecules are excluded (elute first), while small molecules enter the pores (elute later).**

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8. Write an example each for cation exchange resin and anion exchange resin.

Examples:

- Cation exchange resin: Dowex 50 (sulfonated polystyrene resin)
- Anion exchange resin: Dowex 1 (quaternary ammonium resin)

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9. What is electrophoretic mobility?

Electrophoretic mobility:

It is the velocity of a charged particle under the influence of an electric field, per unit field strength.

Formula: μ=v\E

where,

- μ= electrophoretic mobility
- v= velocity of particle
- E= applied electric field strength

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10. What is HETP? Give its significance?

HETP (Height Equivalent to a Theoretical Plate):

- It is the length of the chromatography column that corresponds to one theoretical plate (a hypothetical zone where solute equilibration occurs between stationary and mobile phases).
- **Mathematically:**

$$HETP = L \backslash N$$

where L = column length, N = number of theoretical plates.

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Significance:

- Smaller HETP → higher column efficiency.
- **Helps compare performance of different columns.**
- Determines the resolution power of a chromatographic system.

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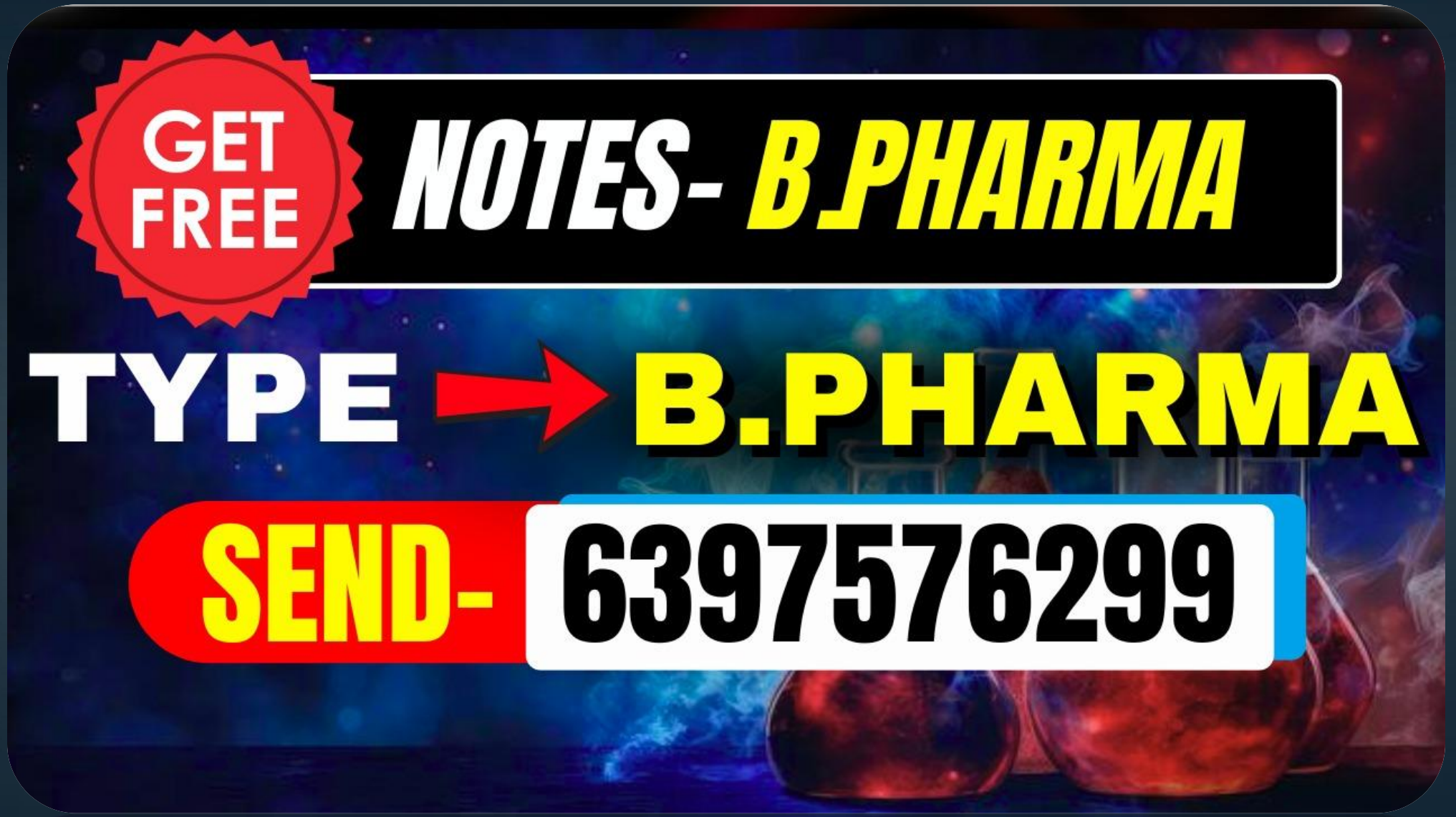

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