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UNIT-4

INSTRUMENTAL METHODS OF ANALYSIS

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INSTRUMENTAL METHODS OF ANALYSIS

B.Pharm | Sem -7

Unit – 4

Question Bank

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1 Explain the principle of Gas Chromatography.

Gas Chromatography is a **separation technique** used to analyze **volatile compounds** in a mixture.
It separates the components based on their **partition between a mobile phase (gas)** and a **stationary phase (liquid or solid)**.

- Principle of Gas Chromatography:

Gas Chromatography works on the principle of **partitioning** – that is, **distribution of compounds between two phases:**

- **Mobile Phase:** An inert gas (like Helium, Nitrogen, or Hydrogen) that carries the sample through the column.
- **Stationary Phase:** A liquid or solid material coated inside the

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

In Gas Chromatography, temperature affects the separation and analysis time of components in a sample. For mixtures with compounds of varying boiling points, a constant oven temperature may not give good separation.

Applications:

1. Improves Separation of Compounds:
   - Low boiling compounds elute quickly at low temperature.
   - High boiling compounds require higher temperatures to elute.
   - Gradually increasing the temperature helps separate all components efficiently.

2. Better Peak Shape and Resolution:
   - Prevents peak tailing and broadening.
   - Gives sharper, well-defined peaks in the chromatogram.

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Methodology of Gas Chromatography (GC):

1. Sample Injection:
- The sample (liquid or gas) is injected using a micro-syringe into the injection port.
- It is instantly vaporized due to high temperature.

2. Mobile Phase (Carrier Gas):
- An inert gas (e.g., Helium, Nitrogen, Hydrogen) carries the vaporized sample through the column.

3. Column:
- Long, narrow tube (made of glass or stainless steel).
- Coated with the stationary phase (liquid or solid).

4. Separation:
- Inside the column, components interact with the stationary phase.
- Based on their affinity and boiling point, they travel at different speeds and get separated.

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Methodology of Gas Chromatography (GC):

5. Detection:

- As each compound exits the column, it is detected by a detector (e.g., Flame Ionization Detector - FID).
- The detector generates a signal which is recorded as a peak on the chromatogram.

6. Data Output:

- The chromatogram shows peaks corresponding to each component.
- The area under the peak is proportional to the concentration.

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3 What is the importance of column selection in GC? Mention the types of columns used.

The **column is the heart of a GC system**. The choice of column **directly affects the separation, resolution, analysis time**, and **sensitivity** of the method.

Importance of Selecting the Right Column:

- Better Separation of Components
- Improved Resolution and Peak Shape
- Reduces Analysis Time
- Compatibility with Analyte
- Thermal Stability and Durability

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GC columns are classified mainly into two types:

1. Packed Columns

Structure: Stainless steel or glass tubes packed with a solid support coated with liquid stationary phase.
Dimensions: Shorter in length (1–3 meters), wider diameter (2–4 mm).
Use: Older technology, used for **gases and simple mixtures**.

Advantages:

- Durable and cheap.
- Can handle larger sample volumes.

Disadvantages:

- Lower efficiency and resolution compared to capillary columns.

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GC columns are classified mainly into two types:

2. Capillary Columns (Open Tubular Columns)

Structure: Long, narrow tubes with stationary phase coated on the inner wall.
Dimensions: Long (15–60 meters), narrow (0.1–0.53 mm internal diameter).

Types: WCOT (Wall-Coated Open Tubular): Thin film of liquid stationary phase on wall.
SCOT (Support-Coated Open Tubular): Support material coated with stationary phase.

Fused Silica Columns: Most commonly used; flexible, with protective polyimide coating.

Use:

- Widely used in modern GC systems for complex and trace analysis.

Advantages:

- High efficiency and sensitivity.
- Better separation and faster analysis.

Disadvantages:

- Fragile and more expensive.

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4 Write a short note on derivatization in GC.

- Derivatization in Gas Chromatography is a chemical process used to modify a compound in order to make it more suitable for GC analysis.
- Many compounds, especially those with polar functional groups like –OH, –COOH, or $–NH_2$, are either non-volatile or thermally unstable and cannot be directly analyzed by GC.
- Derivatization helps by converting these compounds into more volatile, thermally stable, and detectable derivatives. It also improves peak shape and resolution by reducing tailing.
- Common types of derivatization reactions include silylation, acylation, and alkylation. For example, silylation using reagents like BSTFA or MSTFA replaces active hydrogens with a trimethylsilyl (TMS) group, increasing volatility.
- Derivatization is widely applied in pharmaceutical analysis for the detection and quantification of non-volatile substances such as amino acids, sugars, steroids, and drug metabolites.

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5 Explain the mobile and stationary phases used in HPLC.

Mobile Phase:

It is the **liquid solvent** that flows through the column and carries the sample.
Can be **polar or non-polar**, depending on the type of HPLC.

Common solvents:

Polar: Water, methanol, acetonitrile
Non-polar: Hexane (less common)

In **Reverse Phase HPLC (RP-HPLC)**:

- Mobile phase is usually a **mixture of water (or buffer) and organic solvents** (e.g., methanol, acetonitrile).
- Must be **filtered and degassed** to remove particles and air bubbles.
- Plays a key role in **controlling retention time and resolution**.

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5 Explain the mobile and stationary phases used in HPLC.

Stationary Phase:

It is the material packed inside the column and remains fixed.
Usually made of silica particles coated with various functional groups.

Types:

Normal Phase HPLC: Stationary phase is polar (e.g., plain silica).
Reverse Phase HPLC (most common): Stationary phase is non-polar, typically C18 (octadecyl) or C8 (octyl) bonded silica.

- The sample components interact differently with the stationary phase, leading to separation.
- Compounds with stronger interaction with the stationary phase elute slower.
- Compounds more soluble in the mobile phase elute faster.

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6 How does temperature programming affect the separation process in GC?

1. It improves Separation of Compounds with Wide Boiling Points:

- Low boiling compounds elute quickly at lower temperatures.
- High boiling compounds require higher temperatures to elute.
- A temperature ramp allows **both low and high boiling compounds** to be separated efficiently in one run.

2. Reduces Analysis Time:

- If the temperature is kept constant (isothermal), high boiling compounds take a long time to elute.
- Temperature programming **speeds up elution**, reducing total analysis time.

3. Enhances Peak Shape and Resolution:

- Gradual heating improves peak sharpness and minimizes tailing or broadening, especially for late-eluting compounds.

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7 Discuss the advantages and limitations of HPLC.

Advantages	Limitations
High resolution and sensitivity	High cost of equipment and maintenance
Fast and efficient separation	Requires skilled operators
Can analyze both polar and non-polar compounds	Limited to compounds soluble in liquid phase
Suitable for thermally unstable compounds	Mobile phase solvents may be toxic or expensive
Accurate qualitative and quantitative analysis	Sample preparation may be time-consuming
Compatible with a wide range of detectors	Columns are expensive and may have limited life
Reproducible results	Not ideal for gas-phase or highly volatile compounds
Can be automated for high-throughput analysis	Waste disposal of solvents is required

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8 Compare GC vs HPLC.

Feature	Gas Chromatography (GC)	High-Performance Liquid Chromatography (HPLC)
Mobile Phase	Inert gas (e.g., Helium, Nitrogen)	Liquid solvent (e.g., water, methanol, acetonitrile)
Stationary Phase	Solid or liquid on solid support	Solid (silica-based), often coated or bonded
Sample Type	Volatile and thermally stable compounds	Non-volatile, thermally unstable, or polar compounds
Temperature	Requires high temperature (oven-controlled)	Performed at ambient or slightly elevated temperatures
Detection Methods	FID, TCD, ECD, MS	UV, PDA, RI, Fluorescence, MS
Analysis Speed	Faster for volatile compounds	Slower than GC but better for complex samples
Resolution and Sensitivity	High for volatile analytes	High for a wide range of compounds
Column Type	Capillary or packed columns	Mostly packed columns
Sample Preparation	Simple, but requires volatility	May involve more steps like filtration or derivatization

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9 Describe the instrumentation of HPLC with a labeled diagram.

HPLC consists of several essential components working together to separate and analyze compounds in a liquid sample.

1. Solvent Reservoir (Mobile Phase)

- Contains the liquid solvents (mobile phase).
- Solvents must be pure and degassed.

2. Pump

- Delivers the mobile phase at a constant flow rate and high pressure (up to 6000 psi or more).
- Ensures steady and reproducible solvent flow through the column.

3. Injector

- Introduces the liquid sample into the mobile phase stream.
- Usually a manual or automatic injector with a fixed volume loop.

4. Column

- Packed with stationary phase particles (e.g., silica with C18 bonded phase).
- The site where separation occurs based on interactions between analytes and stationary phase.

5. Detector

- Detects separated compounds as they elute from the column.
- Common detectors: UV-Visible, Photodiode Array (PDA), Refractive Index (RI), Fluorescence.

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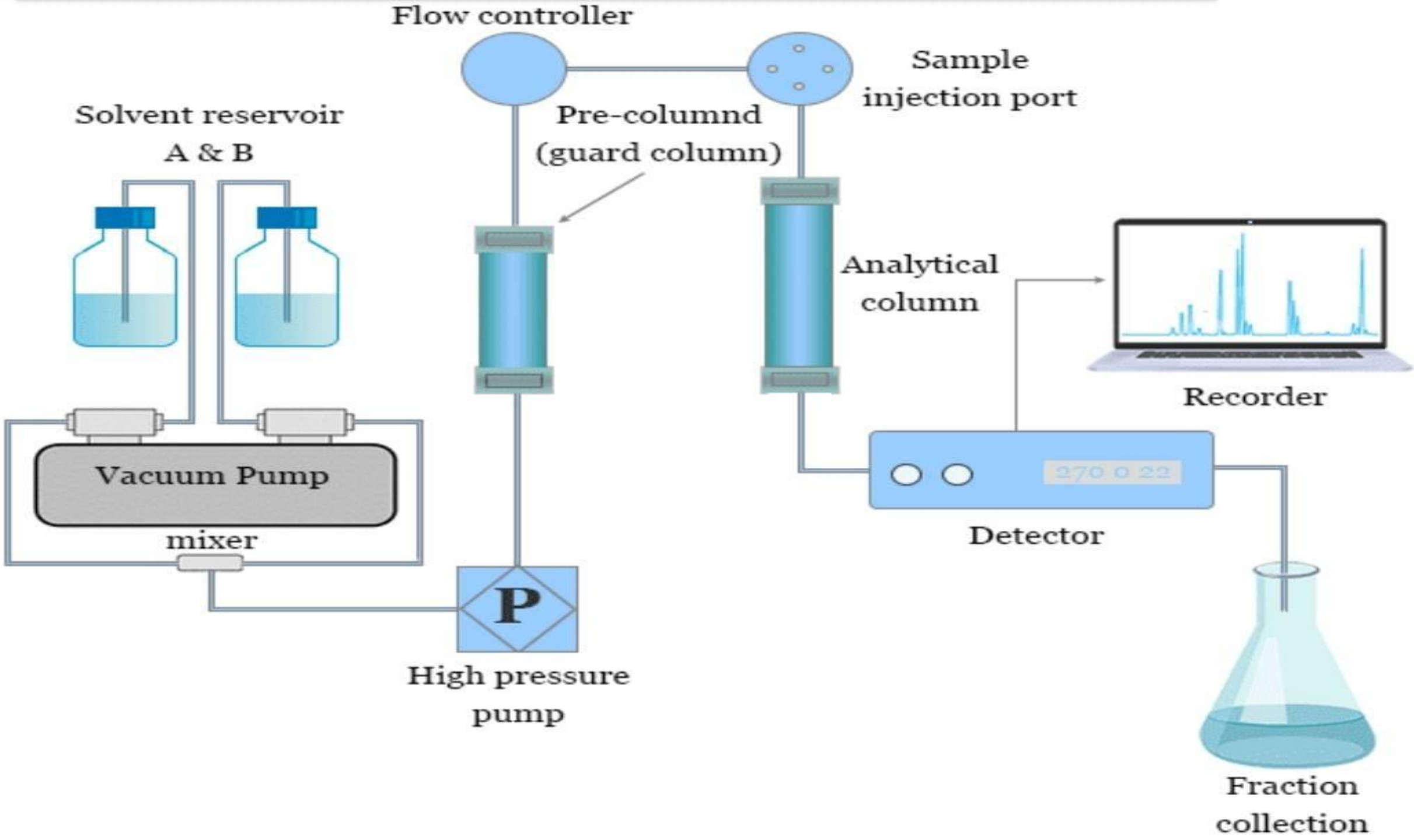
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10 Explain Isocratic and Gradient Elution in HPLC.

1. Isocratic Elution:

The mobile phase composition **remains constant** throughout the entire chromatographic run.

Example: 70% water + 30% methanol from start to finish.

- **Simple and easy to operate.**
- Suitable for samples with components having **similar polarity or retention times**.

Disadvantages:

- Longer analysis time for complex mixtures.
- Poor separation of components with widely different polarities.

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10 Explain Isocratic and Gradient Elution in HPLC.

2. Gradient Elution:

The mobile phase composition changes gradually during the run.
Usually starts with a higher proportion of polar solvent and gradually increases the proportion of less polar solvent (or vice versa).

Example: Starting at 90% water + 10% methanol, changing to 50% water + 50% methanol over time.

Advantages:

- Better separation of complex mixtures.
- Shorter analysis time.
- Improved peak shape and resolution for components with wide polarity differences.
- Often used in reverse-phase HPLC for pharmaceuticals, proteins, and complex natural products.

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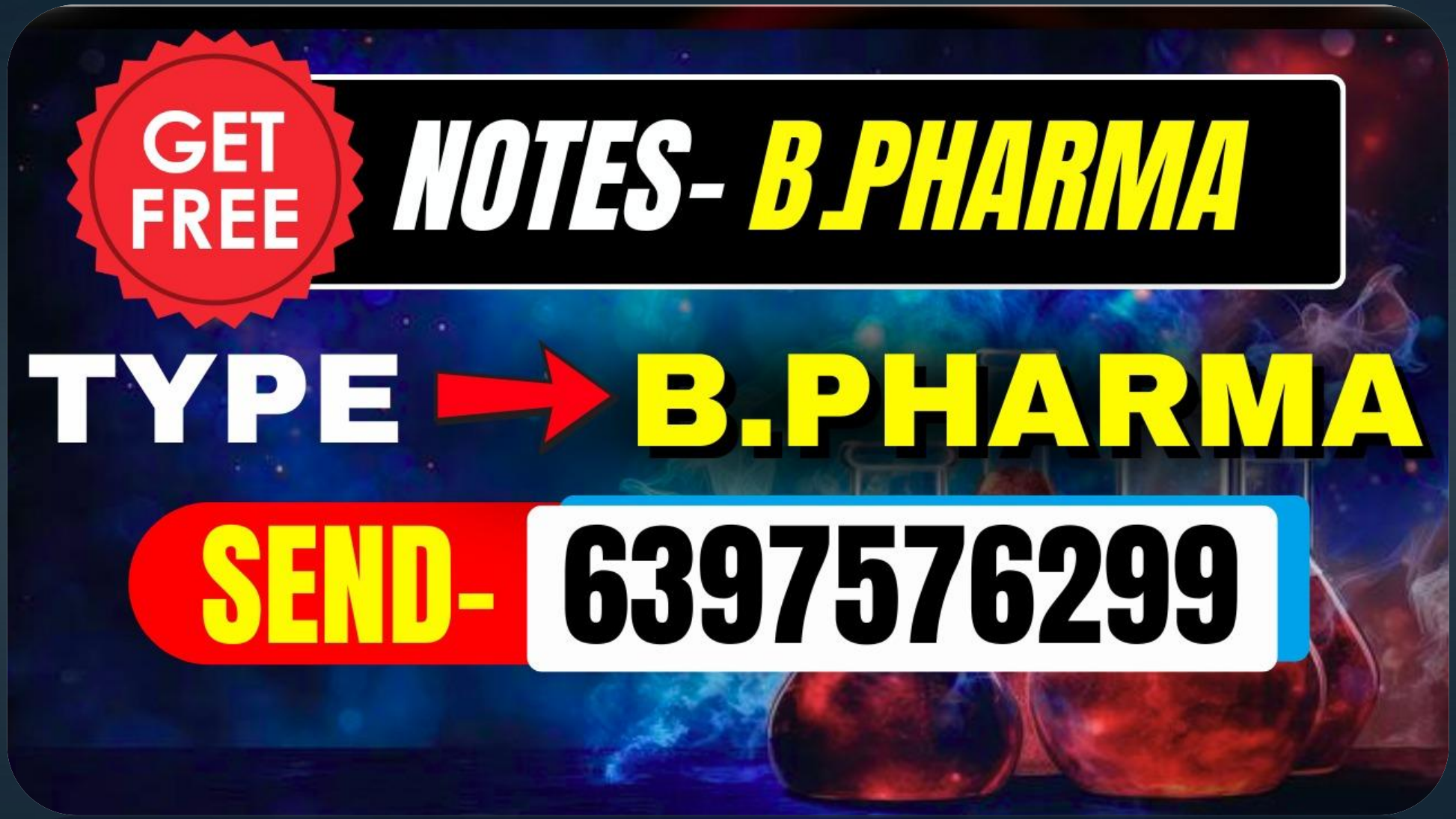

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