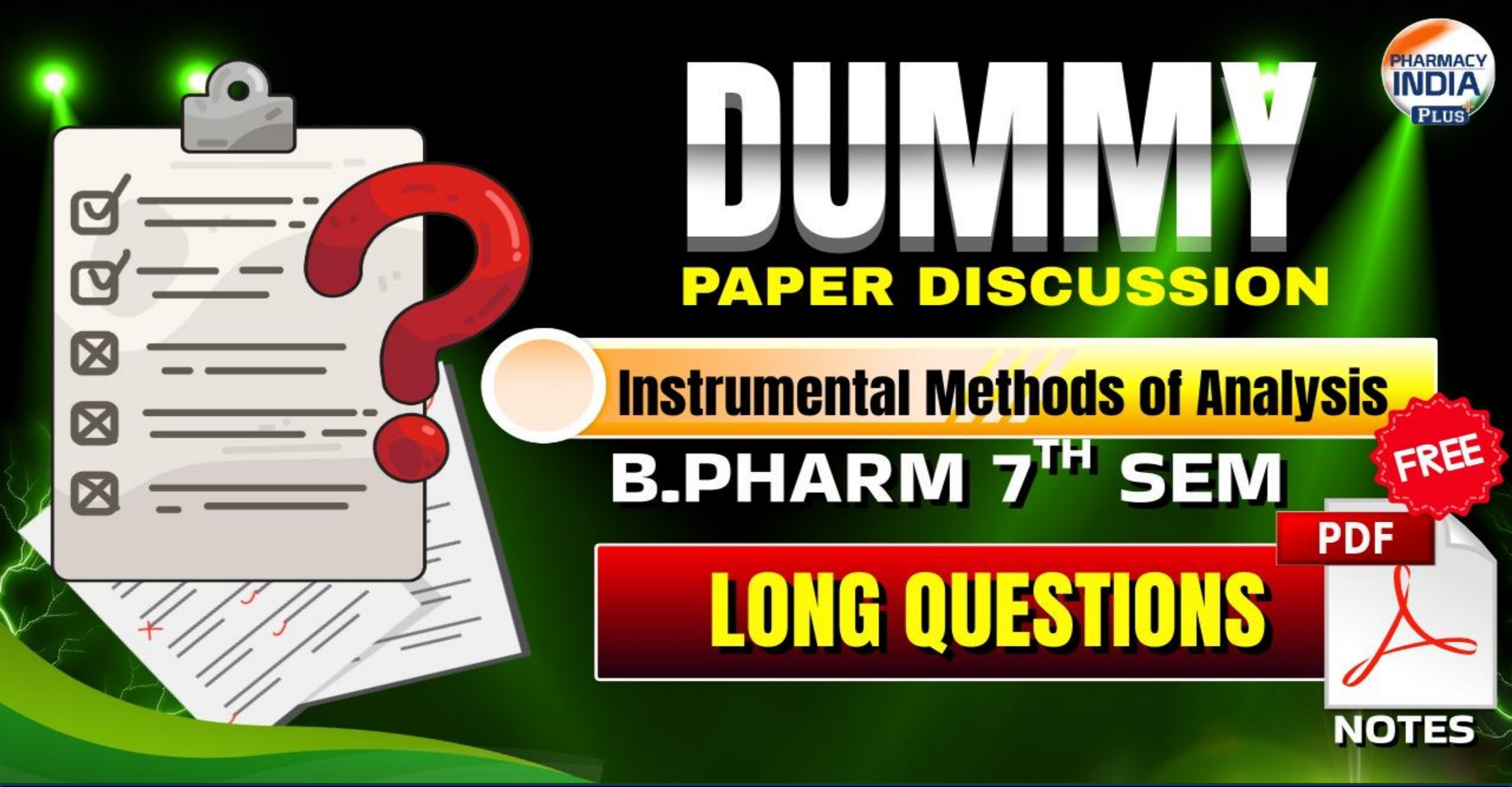

CLICK ON BANNER TO WATCH VIDEO

DUMMY PAPER DISCUSSION

INSTRUMENTAL METHODS OF ANALYSIS

B.PHARM | SEMESTER 7

LONG QUESTIONS (10 x2)

@PharmacyIndiaPlus

Download Lecture Notes - www.pharmacyindia.in

DOWNLOAD "PHARMACY INDIA" MOBILE APP

SCAN ME

Mobile Phone Par Click karein

Download Pharmacy India Mobile App from Play Store

DAILY UPDATES

जुड़िए PHARMACY INDIA

के साथ.....

WHATSAPP & TELEGRAM SE JUDNE KE LIYE ICONS PAR CLICK KARE

SCAN ME

Join Pharmacy India Plus

WhatsApp

SCAN ME

Join Pharmacy India Plus

1. Describe the principle, procedure for development and application of paper chromatography.

Principle of Paper Chromatography:

Paper chromatography is a **type of partition chromatography** used for separating and identifying mixtures that are or can be colored, especially pigments.

- The technique works on the **principle of partition** between two phases:
  - **Stationary phase:** A sheet of paper (typically cellulose) that holds water in its pores.
  - **Mobile phase:** A solvent or mixture of solvents that moves over the paper via capillary action.
- Components of the mixture partition between the stationary phase (water in the paper) and the mobile phase **solubility** and **affinity** for each phase.
- Components with **higher solubility** in the solvent and **lower affinity** for the paper move faster, and those with **greater affinity** for the stationary phase move slower.

2. Procedure of Paper Chromatography:

Materials Required:

- Chromatography paper (e.g., Whatman filter paper)
- Solvent or solvent mixture (mobile phase)
- Sample solution
- Capillary tubes or micropipette
- Beaker or chromatography chamber
- Pencil and ruler
- Glass rod or support

Steps:

Preparation of Chromatography Paper:

Draw a **pencil line** about 2 cm from the bottom of the paper. This is the **origin line**.

Spot a small amount of the sample mixture on the origin line using a capillary tube or micropipette. Allow it to dry and repeat for concentration if needed.

Preparation of Solvent System:

- Prepare a suitable mobile phase (e.g., a mixture of water and alcohol or other solvents depending on the sample).
- Pour the solvent into the chromatography chamber to a depth below the pencil line on the paper.

Development of Chromatogram:

- Place the paper vertically into the chamber so that the solvent touches the bottom edge but **not the sample spot**.
- Close the chamber to saturate it with solvent vapor.
- Allow the solvent to rise up the paper by **capillary action**.

1. Drying and Visualization:

- When the solvent front reaches near the top, remove the paper and **mark the solvent front** immediately with a pencil.
- Dry the paper.
- Visualize the spots:
  - Colored compounds are visible directly.
  - Colorless compounds may require spraying with reagents (e.g., ninhydrin for amino acids) or viewing under UV light.

2. Calculation of Rf Value:

Rf=Distance traveled by the solvent front/Distance traveled by the solute

2. Explain principle, instrumentation, and application of flame photometry.

Principle of Flame Photometry (Flame Atomic Emission Spectroscopy):

Flame photometry is based on the **principle of atomic emission spectroscopy,** used primarily for **quantitative analysis of alkali and alkaline earth metals** (e.g., Na, K, Ca, Li).

- When a sample solution is sprayed into a flame, **metal ions** in the sample get **excited** due to the flame's thermal energy.
- These **excited atoms** emit light of **characteristic wavelengths** as they return to their **ground state**.
- The **intensity of emitted light** is directly **proportional to the concentration** of the metal ion in the sample.

2. Instrumentation of Flame Photometer:

1. Sample Introduction System:

- **Nebulizer** converts the liquid sample into a fine mist.
- The mist is carried into the flame by a **carrier gas** (usually air-acetylene or air-propane).

2. Flame (Atomizer):

- Provides the energy required to **excite the atoms**.
- Common flames:
  - **Air-Acetylene**: for Na, K, Ca, Li
  - **Air-Propane**: safer but cooler flame

3. Optical System:

- Includes:
  - **Lens/slits** to focus the emitted light
  - **Monochromator or optical filters** to select the specific **wavelength** corresponding to the metal ion.

4. Detector:

- Usually a **photocell or photomultiplier tube** that detects the light intensity and converts it to an electrical signal.

Readout Device:

- The signal is displayed as:
  - Analog meter
  - Digital display

Applications of Flame Photometry:

- Clinical Applications
- Agricultural and Soil Analysis
- Food and Beverage Industry
- Pharmaceutical Industry

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

Aspect	Atomic Absorption Spectroscopy (AAS)	Atomic Emission Spectroscopy (AES)
Basic Principle	Measures light absorbed by atoms in ground state.	Measures light emitted by atoms in excited state.
Source of Energy	External light source (usually a hollow cathode lamp).	High-energy source (e.g., flame, plasma) excites the atoms.
Detection	Detects decrease in intensity of light (absorption).	Detects emitted light from excited atoms.
State of Atoms	Atoms absorb energy and move from ground to excited state.	Atoms emit energy while returning from excited to ground state.
Spectral Line Used	Specific wavelength of the element being analyzed.	Emission spectrum characteristic of the element.
Sensitivity	Generally more sensitive for trace analysis.	Less sensitive than AAS for some elements.
Common Use	Trace metal analysis in samples like water, blood, soil, etc.	Emission analysis of alkali and alkaline earth metals.
Instrument Example	Flame AAS, Graphite Furnace AAS	Flame Photometer, Inductively Coupled Plasma-Emission (ICP-OES)

Interferences in Atomic Absorption Spectroscopy (AAS):

A. Spectral Interference:

- **Cause:** Overlapping of absorption lines from different elements or molecular bands.
- **Effect:** False readings due to absorption by species other than the analyte.
- **Solution:**
  - Use a **high-resolution monochromator**.
  - Choose an **alternate absorption line** or background correction methods.

B. Chemical Interference:

- **Cause:** Formation of stable compounds (e.g., oxides, sulfates) that do not atomize efficiently in the flame.
- **Effect:** Reduces free atom population, lowering absorbance.

- **Solution:**
  - Add **releasing agents** (e.g., La^{3+} for calcium to prevent phosphate interference).
  - Use **higher temperature flames** to break down stable compounds.
  - Add **protective agents** or **ionization buffers**.

C. Ionization Interference:

- **Cause:** At high temperatures, atoms may ionize instead of staying neutral.
- **Effect:** Reduces the number of neutral atoms available to absorb radiation.
- **Solution:**
  - Add **ionization suppressors** like potassium or cesium, which preferentially ionize.
  - Use **lower flame temperatures** if possible.

D. Matrix Interference (Physical Interference):

- **Cause:** Differences in viscosity, surface tension, or density between the sample and standards.
- **Effect:** Affects **nebulization**, **aspiration rate**, and atomization efficiency.
- **Solution:**
  - Use **matrix-matched standards** or **standard addition method**.
  - **Dilute** viscous samples or modify sample preparation.

3. Explain the principle, types, and pharmaceutical applications of Potentiometry.

Principle:

Potentiometry is based on the measurement of potential (voltage) of an electrochemical cell without drawing significant current.

- The measured potential is related to the concentration of the analyte by the **Nernst equation:**

$$E=E0 + 2.303RT / nF \log[C]$$

Where:

- E = Electrode potential
- E⁰ = Standard electrode potential
- R = Gas constant (8.314 $J \cdot K^{-1} \cdot mol^{-1}$)
- T = Temperature (K)
- n = Number of electrons involved
- F = Faraday's constant (96,500 $C \cdot mol^{-1}$)
- C = Concentration of ionic species

In potentiometric titrations, instead of using chemical indicators (color change), the end point is determined by a sharp change in potential.

2. Types of Electrodes Used in Potentiometry

A. Reference Electrodes

These provide a constant, stable potential against which changes are measured.

- Standard Hydrogen Electrode (SHE) – primary reference, rarely used in practice.
- Calomel Electrode (Hg/Hg_2Cl_2) – widely used.
- Silver-Silver Chloride Electrode (Ag/AgCl) – stable and common.

B. Indicator (Measuring) Electrodes

These respond to the activity of specific ions in the solution.

- Glass Electrode – for H^+ ion concentration (pH measurement).
- Ion-Selective Electrodes (ISEs) – selective for Ca^{2+}, Na^+, K^+, F^-, Cl^- etc.
- Redox Electrodes – for oxidation-reduction titrations.

3. Instrumentation of Potentiometer

- Reference Electrode – stable potential.
- Indicator Electrode – responds to analyte.
- High Resistance Voltmeter – measures potential difference without drawing current.
- Titration Cell – where titration is performed.
- Computer/Recorder – plots potential vs volume of titrant.
- End-point is identified by the inflection point on the titration curve.

4. Types of Potentiometric Titrations

I. Acid–Base Titrations – determination of strong/weak acids or bases using glass electrode.
II. Redox Titrations – e.g., Fe^{2+} vs $KMnO_4$, involving electron transfer.
III. Precipitation Titrations – e.g., Cl^- with $AgNO_3$.
IV. Complexometric Titrations – e.g., EDTA titration for Ca^{2+}, Mg^{2+}.
V. Non-aqueous Titrations – useful for drugs insoluble in water.

1. Pharmaceutical Applications

- Drug Assays:
  - ✓ Alkaloids (e.g., atropine, ephedrine, quinine).
  - ✓ Sulfonamides, barbiturates, aspirin, and other APIs.
- pKa Determination: Helps in drug ionization and solubility studies.

5. Explain the principle, instrumentation, and pharmaceutical applications of X-Ray Diffraction (XRD).

Principle of X-Ray Diffraction (XRD)

- ✓ XRD is based on the interaction of X-rays with crystalline substances.
- ✓ When a crystal is irradiated with monochromatic X-rays, the atoms in the lattice planes diffract the X-rays in specific directions.
- ✓ The condition for constructive interference is given by Bragg's Law:

$$n\lambda = 2d \sin\theta$$

Where:

n = Order of reflection (integer)

λ= Wavelength of incident X-rays

d = Distance between crystal lattice planes

θ= Angle of incidence

Thus, by measuring θand intensity of diffracted beams, the arrangement of atoms in a crystal can be determined.

Instrumentation of XRD

A typical powder X-ray diffractometer consists of:

(i) X-Ray Source

- ✓ Produces X-rays, usually via an X-ray tube (Cu, Mo targets).
- ✓ Cu Kα radiation (λ = 1.5418 Å) is commonly used.

(ii) Collimator/Monochromator

- ✓ Ensures that the X-rays are parallel and monochromatic before striking the sample.

(iii) Sample Holder

- ✓ Holds the crystalline sample in the path of X-rays.
- ✓ Powdered solid samples are most commonly analyzed.

Monochromator:

- Filters the X-rays to produce **monochromatic radiation** for accurate results.

Goniometer:

- Precisely rotates the sample and detector at defined angles (θand 2θ).
- Helps in scanning across different diffraction angles.

Detector:

- Usually scintillation or proportional counters.
- Detects diffracted X-rays and converts them into electronic signals.

Pharmaceutical Applications of XRD:

XRD plays a **crucial role in drug development, formulation, and quality control**. Key uses include:

1. Polymorphism Identification:

- Many drugs exist in different **polymorphic forms** with different solubility and bioavailability.
- XRD helps to identify and differentiate these polymorphs (e.g., Ritonavir, Carbamazepine).

2. Crystallinity vs. Amorphous Content:

- Differentiates between crystalline and amorphous forms.
- Amorphous drugs often have higher solubility but less stability.