

DUMMY

PAPER DISCUSSION



Instrumental Methods of Analysis

B.PHARM 7TH SEM

LONG QUESTIONS

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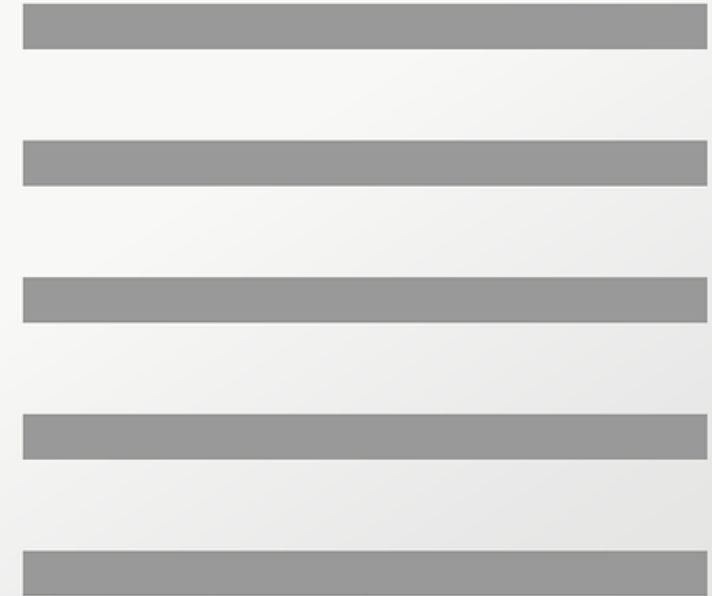


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INSTRUMENTAL METHODS OF ANALYSIS B.PHARM | SEMESTER 7

LONG QUESTIONS

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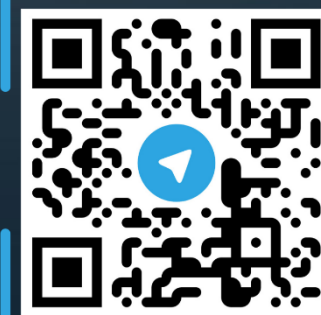


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1. Enlist the different components of uv-visible spectrophotometer and explain the working of double beam spectrophotometer along with well labeled diagram.

Components of a UV-Visible Spectrophotometer:

1. Light Source:

- Deuterium lamp → emits continuous radiation in UV region (190–350 nm).
- Tungsten-halogen lamp → emits visible radiation (350–900 nm).



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2. Monochromator:

- Disperses light into component wavelengths using prism or diffraction grating.
- Slits select a narrow band of wavelength.

3. Sample Holder (Cuvette):

- Usually made of quartz (for UV) or glass (for visible region).

4. Detector:

- Converts transmitted light into electrical signal.
- Common detectors: Photodiode, Photomultiplier tube (PMT).

5. Readout System:

- Signal processor and display device to record absorbance or transmittance vs wavelength.

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Working of Double Beam Spectrophotometer:

- In a double-beam system, the light from the source is split into two beams:
 1. Reference beam (passes through reference cell containing solvent).
 2. Sample beam (passes through sample solution).
- Both beams pass alternately (or simultaneously) through the monochromator and then to the detector.



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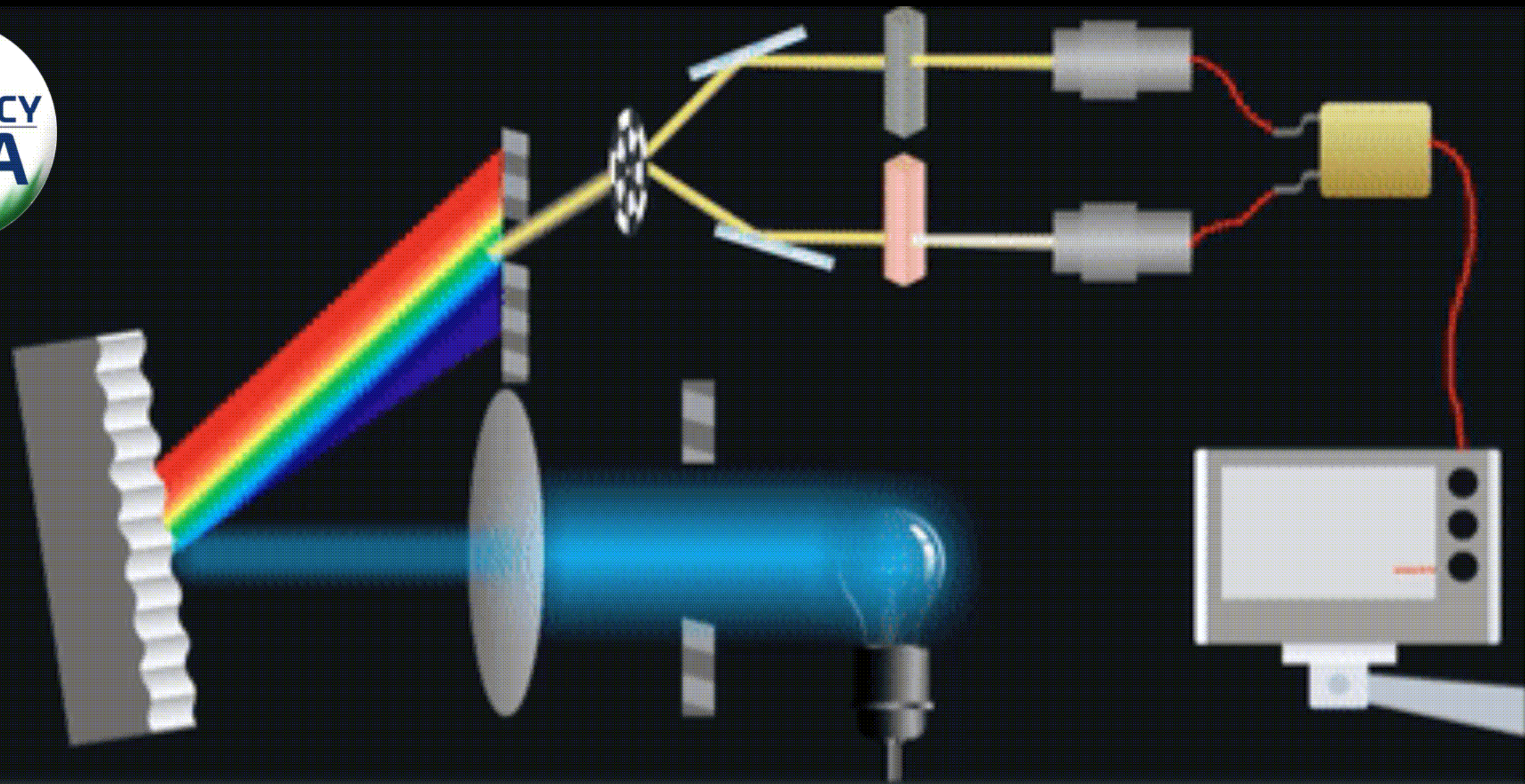


- The instrument continuously compares the intensity of light transmitted through the sample (I) with that through the reference (I_0).
- This eliminates errors due to fluctuations in lamp intensity or detector sensitivity.
- Final output: Absorbance spectrum directly plotted as absorbance vs wavelength.



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2. Explain the principle, instrumentation, and applications of Flame Photometry.

Principle:

- Based on emission of radiation by atoms when excited in a flame.
- The intensity of emitted light at a characteristic wavelength is proportional to concentration of the element.



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Instrumentation:

1. **Flame atomizer:** Provides energy to excite atoms (commonly air-acetylene flame).
2. **Nebulizer & Burner:** Convert liquid sample into fine mist, carried into the flame.
3. **Monochromator/Filter:** Selects specific emission wavelength of analyte.
4. **Detector (PMT):** Detects intensity of emitted radiation.
5. **Readout device:** Displays signal proportional to concentration.



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Applications:

- Determination of Na, K, Ca, Li in biological fluids.
- Soil and fertilizer analysis.
- Food and beverage industry.



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3.

Discuss the principle, instrumentation, and advantages of Flame Ionization Detector (FID) in Gas Chromatography.

Principle of Flame Ionization Detector (FID):

- Organic compounds containing C-H bonds, when introduced into a hydrogen-air flame, undergo combustion.
- During this process, ions and electrons are produced.
- A potential difference is applied across two electrodes; the ions generated in the flame move towards the electrodes, producing an ion current.



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- The magnitude of the current is directly proportional to the number of carbon atoms entering the detector per unit time → hence proportional to analyte concentration.
- FID is mass-sensitive (depends on mass of carbon atoms) and not concentration-sensitive.



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Instrumentation of FID:

1. Burner/Flame Jet:

- Hydrogen (fuel gas) and air (oxidant) are mixed and burned to create a flame.
- The sample carried by carrier gas (e.g., He, N₂) enters the flame.

2. Electrodes:

- A collector electrode (positive electrode) is placed above the flame.
- A potential (200–400 V) is applied between the flame tip and collector.
- Ions formed in the flame migrate, generating a current.



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3. Amplifier:

- The very small current (10^{-12} – 10^{-8} A) is amplified by a high-sensitivity electrometer.

4. Readout System:

- The amplified signal is recorded as peaks in the chromatogram.



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Advantages of FID:

- High sensitivity (detection limits in picogram range).
- Wide linear dynamic range (10^7 or more).
- Selective for hydrocarbons/organic compounds – insensitive to permanent gases (H_2O , CO_2 , SO_2 , NO_x , etc.).
- Stable and reliable operation.
- Non-destructive to the sample (a portion can be directed to another detector in GC).



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4. Explain in detail the principle and working of Atomic Emission Spectroscopy (AES).

Principle of Atomic Emission Spectroscopy (AES):

- When atoms are supplied with sufficient energy (thermal, electrical, or plasma), their outer electrons get excited to higher energy levels.
- As the electrons return to their ground state, they emit radiation of element-specific wavelengths.



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- The intensity of this emitted light is directly proportional to the concentration of the element in the sample.
- Hence, AES is both qualitative (wavelength → identity of element) and quantitative (intensity → concentration).

Instrumentation of AES:

1. Excitation Source: Provides high energy to convert analyte into excited atoms.

- Flame source (Air-acetylene, N_2O -acetylene).
- Plasma source (Inductively Coupled Plasma, ICP).
- Arc/Spark source (for solid samples, metals).



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2. Sample Introduction System:

- For liquids: nebulizer converts sample into fine mist.
- For solids: spark or laser ablation.

3. Monochromator / Polychromator:

- Disperses the emitted radiation into individual wavelengths.
- Prism or diffraction grating used to isolate element-specific lines.



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4. Detector:

- Usually Photomultiplier Tube (PMT) – detects light intensity and converts it into electrical signals.

5. Readout System:

- The processed signal is displayed as spectrum or emission lines indicating intensity vs wavelength.



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Working of AES:

- Sample (liquid, solid, or gas) is introduced into the excitation source (flame, arc, spark, or plasma).
- At high temperatures (2000–10000 K), the sample undergoes desolvation, vaporization, atomization, and excitation.
- Excited atoms emit characteristic radiation upon relaxation to ground state.
- The emitted radiation passes through the monochromator, which isolates the required wavelengths.



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5. Detector (PMT) measures the intensity of radiation.

6. The output is processed and recorded as a spectrum, from which both qualitative (element present) and quantitative (concentration) analysis can be performed.

Applications of AES:

- Multi-element analysis (metals and trace elements).
- Analysis of alloys, steels, and geological samples.
- Environmental monitoring (water, soil, air pollutants).
- Detection of trace metals in biological fluids.



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7. Discuss the principle and working of Gas Chromatography (GC).

Principle of Gas Chromatography (GC):

- Separation is based on partitioning of components between:
- Mobile phase: an inert carrier gas (e.g., He, N₂, H₂).
- Stationary phase: a liquid or solid adsorbent packed or coated inside a column.



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- Compounds with higher affinity for the stationary phase travel slower, while those with higher solubility in the mobile phase elute faster.
- Each compound has a characteristic retention time, which is used for identification, while peak area/height is used for quantification.

Instrumentation of Gas Chromatography:

1. Carrier Gas System:

- An inert gas (He, N₂, H₂, Ar) acts as the mobile phase.
- Flow regulators and pressure gauges maintain a steady flow.

2. Sample Injector:

- Introduces the sample (liquid/gas) into the carrier gas stream.
- Injection port is heated (200–300 °C) to vaporize liquid samples instantly.

3. Column:

- Packed column: Contains solid support coated with stationary liquid phase.
- Capillary column (open tubular): Narrow tube with stationary phase coating, higher efficiency.
- Column is placed inside a thermostatically controlled oven, allowing isothermal or temperature-programmed runs.



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4. Detector:

- Detects separated components as they elute from the column.
- Common detectors:
 - FID (Flame Ionization Detector) – highly sensitive for organics.
 - TCD (Thermal Conductivity Detector) – universal, less sensitive.
 - ECD (Electron Capture Detector) – sensitive for halogenated compounds.



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5. Data System (Recorder/Computer):

- Records signal as chromatogram (peak vs time).
- Retention time → identification, peak area/height → quantification.

Working of GC:

1. Carrier gas continuously flows through the column.
2. Sample is injected into the heated injection port, where it vaporizes and mixes with carrier gas.



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3. The mixture passes into the column, where components partition between stationary and mobile phases.

4. Due to differences in volatility and interaction with stationary phase, compounds separate and elute at different times.

5. The detector senses the compounds as they emerge and sends signals to the recorder.

6. The output chromatogram is analyzed for qualitative and quantitative results.



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Applications of GC:

- Separation and analysis of volatile organic compounds.
- Drug and metabolite analysis in biological samples.
- Food and flavor quality control.
- Pesticide and environmental pollutant analysis.
- Forensic analysis (alcohols, explosives).



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