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UNIT-1

INSTRUMENTAL METHODS OF ANALYSIS

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INSTRUMENTAL METHODS OF ANALYSIS

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Unit – 1

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1 Explain the principles, instrumentation, and applications of UV-Visible spectroscopy.

Principle:

- **Based on absorption of UV (200–400 nm) and visible (400–800 nm) light** by molecules.
- When a molecule absorbs light, electrons jump from a **lower energy level (ground state)** to a **higher energy level (excited state)**.
- The amount of light absorbed is related to the **concentration** of the compound (Beer-Lambert Law):

$$A=\varepsilon \cdot c \cdot l$$

Where:

- A= Absorbance
- ε= Molar absorptivity
- c= Concentration
- l= Path length (usually 1 cm)

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Instrumentation of UV-Visible Spectroscopy:

Component	Function
1. Light Source	Provides continuous light across UV and visible range:
	- **Deuterium lamp** (UV region: 200–400 nm)
	- **Tungsten lamp** (Visible region: 400–800 nm)
2. Monochromator	Selects specific wavelength of light using prisms or diffraction gratings
3. Slit	Narrows the light beam to improve resolution
4. Sample Holder	Holds the sample in **quartz or glass cuvettes** (typically 1 cm path length)
5. Detector	Detects transmitted light; converts it into electrical signal (e.g., photodiode, photomultiplier tube)
6. Amplifier	Increases the electrical signal strength from the detector
7. Readout Device	Displays the absorbance or transmittance (digital display, computer, etc.)

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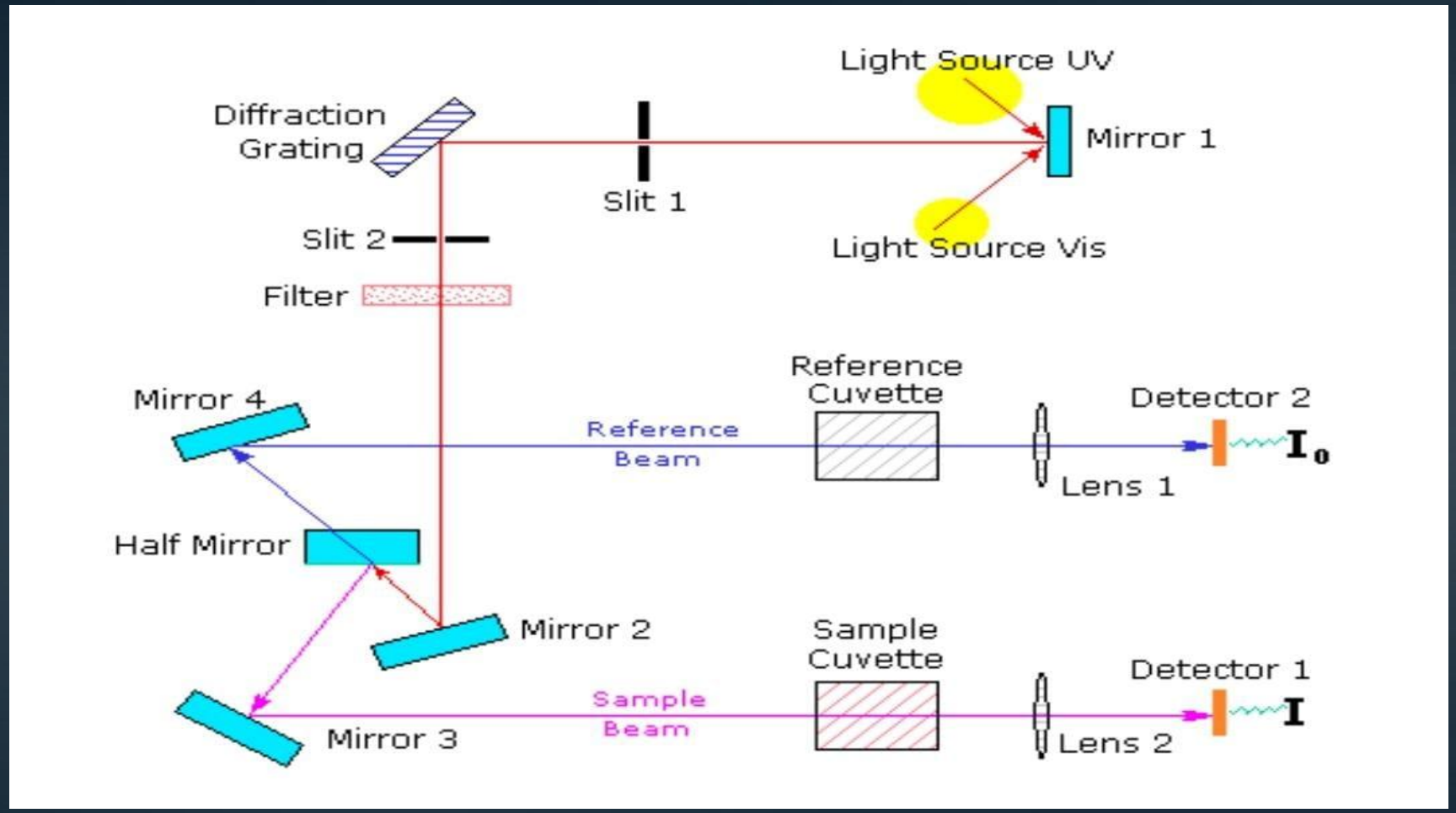

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Applications:

- **Quantitative analysis:** Estimation of drug concentration in formulations
- **Qualitative analysis:** Identification of compounds by λmax
- **Purity testing:** Detect impurities in pharmaceuticals
- **Kinetics studies:** Monitor reaction rates
- **Dissolution testing:** Monitor drug release in dosage forms
- **Detection of adulterants** in herbal drugs

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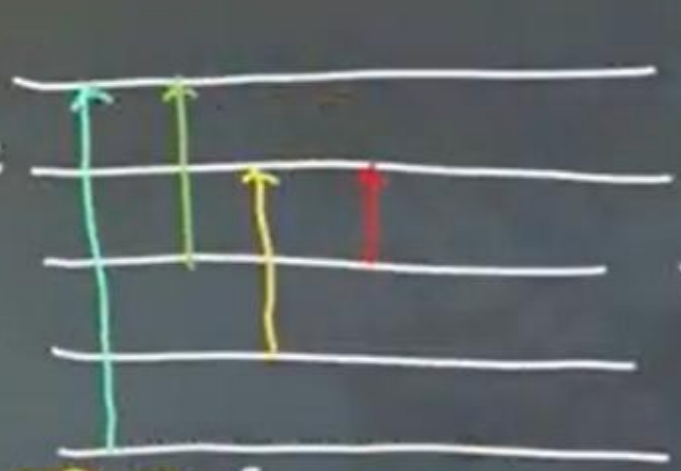
2 Name the types of Electronic transitions involved in UV-Visible spectroscopy.

$E = h\nu$

$E = \frac{hc}{\bar{\lambda}}$

The Possible electronic Transitions are as follows;

1) $\sigma - \sigma^*$
2) $n - \sigma^*$
3) $\pi - \pi^*$
4) $n - \pi^*$



Anti-bonding

$n \rightarrow$ non-bonding energy level

Bonding

Energy: $[\sigma-\sigma^* > n-\sigma^* > \pi-\pi^* > n-\pi^*]$

Wavelength: $[\sigma-\sigma^* < n-\sigma^* < \pi-\pi^* < n-\pi^*]$

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3 What are the different types of electronic transitions in UV spectroscopy?

1. $\sigma - \sigma^*$ In this transition e⁻ goes from bonding (σ) to antibonding σ^* orbital. eg:- F, Cl, Br, I etc.

2. $n - \sigma^*$ In this transition, e⁻ moves from non-bonding energy level to antibonding σ^*.

3. $\pi - \pi^*$ In this transition, e⁻ moves from bonding π-orb. to antibonding π^*.

4. $n - \pi^*$ In this Transition, non-bonding e⁻ moves to antibonding π-orb.

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4 Explain the concept of chromophores and auxochromes & Quecching.

1. Chromophores:

A chromophore is the part of a molecule responsible for absorbing UV or visible light, causing electronic transitions (usually $\pi \rightarrow \pi^*$, or $n \rightarrow \pi^*$ transitions).

Function:
It imparts color to the compound by absorbing specific wavelengths.

Examples:

- C=C (double bond)
- C=O (carbonyl group)
- NO_2 (nitro group)
- Aromatic rings (e.g., benzene)

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4 Explain the concept of chromophores and auxochromes & Quenching.

2. Auxochromes:

An auxochrome is a group of atoms that does not absorb light on its own but modifies the absorption of a chromophore when attached to it.

Common Auxochromes:

–OH (hydroxyl)

–NH_2 (amino)

–OCH_3 (methoxy)

–Cl (halogen)

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4 Explain the concept of chromophores and auxochromes & Quenching.

3. Quenching:

Quenching is a process that reduces the fluorescence or phosphorescence of a molecule.

Types of Quenching:

Dynamic quenching: Collision between excited molecule and quencher
Static quenching: Formation of a non-fluorescent complex
Intersystem crossing: Energy transfer to a triplet state (non-radiative)

Applications:

Used in fluorescence studies
Important in drug-excipient interaction studies

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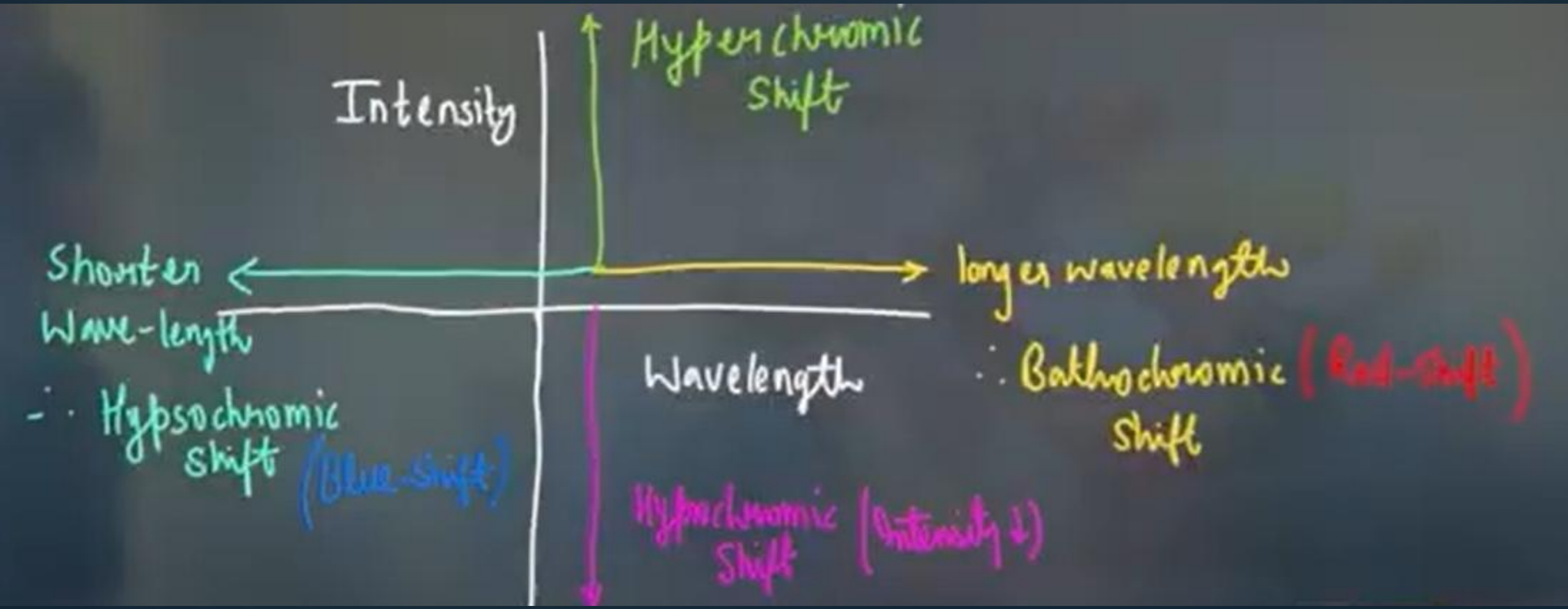
5 What is a bathochromic and hypsochromic shift?



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Bathochromic Shift: It occurs on shifting towards longer wavelength, it is also known as Red Shift.

Hypsochromic Shift : It occurs on shifting towards shorter wavelength, it is also known as Blue Shift.

Hyperchromic shift : It arises on increasing the intensity.

Hypsochromic shift: It arises on decreasing the intensity.

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6 Mention any five pharmaceutical applications of UV spectroscopy.

S.No.	Application	Description
1.	Drug Quantification	Used to estimate the **concentration of drugs** in bulk and dosage forms.
2.	Purity Testing	Detects **impurities** by comparing with standard absorbance or λmax values.
3.	Dissolution Testing	Monitors **drug release** from tablets/capsules into solution over time.
4.	Kinetics Studies	Measures **reaction rates** by observing absorbance changes over time.
5.	Identification of Compounds	Confirms **identity** of compounds by comparing their **λmax** with standards.

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7 Differentiate between fluorescence and phosphorescence.

Feature	Fluorescence	Phosphorescence
1. Excited State	Involves **singlet excited state**	Involves **triplet excited state**
2. Emission Time	Emission is **immediate** (within nanoseconds)	Emission is **delayed** (milliseconds to minutes)
3. After Light Source	Stops **instantly** when light is removed	Continues **even after** light is removed
4. Energy Transition	Electron returns to ground state **directly**	Electron returns via **forbidden transition**
5. Intensity	Generally **more intense**	Generally **less intense**
6. Applications	Drug tracing, immunoassays, DNA studies	Glow-in-the-dark materials, radioluminescence

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

AAS can suffer from **interferences** that affect accuracy and precision. These are:

1. Spectral Interference

- Occurs when **other species emit or absorb** at the same wavelength.
- **Example:** Overlapping lines from other elements or flame emission.

2. Chemical Interference

- Caused by **chemical reactions** in the flame or furnace that **prevent atom formation**.
- **Example:** Formation of refractory compounds like calcium phosphate, which do not dissociate easily.

3. Ionization Interference

- At high temperatures, atoms may **ionize**, reducing the number of neutral atoms available for absorption.
- **Solution:** Add ionization suppressors like **potassium or cesium**.

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

4. Matrix Interference (Physical Interference):

Caused by differences in viscosity, surface tension, or density of the sample matrix affecting atomization.

Solution: Use of matrix-matched standards or internal standards.

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

Feature	Atomic Absorption Spectroscopy (AAS)	Atomic Emission Spectroscopy (AES)
Principle	Measures the **absorption** of light by atoms	Measures the **emission** of light by excited atoms
Energy Source	Requires an **external light source** (usually hollow cathode lamp)	No external light source needed; atoms emit light after excitation
Excitation of Atoms	Atoms are in the **ground state** and absorb energy	Atoms are **excited** and emit energy as light
Detection	Based on **reduction in light intensity**	Based on **measuring emitted light intensity**
Sensitivity	More sensitive for detecting **metals in trace amounts**	Less sensitive compared to AAS
Common Use	Quantification of metals in samples (e.g. lead, iron)	Flame tests, qualitative analysis of metal ions

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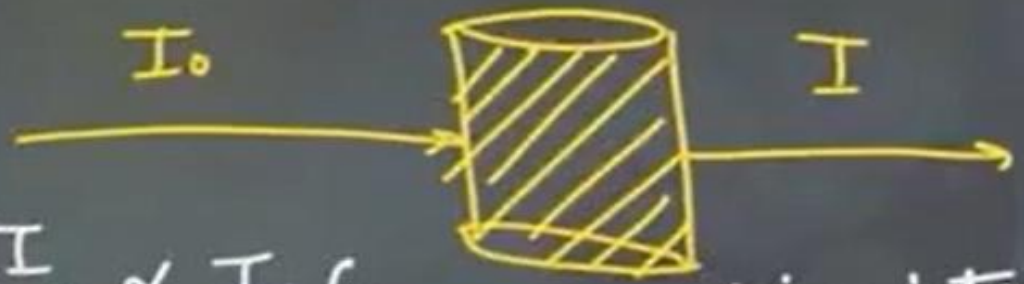
9 Discuss Beer-Lambert's law, its derivation and the deviations observed.

Derivation



$$-\frac{dI}{dt} \propto I \cdot C$$

$$-\frac{dI}{dt} = K \cdot I \cdot C$$

$$-\frac{dI}{I} = K \cdot C \cdot dt$$

on doing integration both of the sides.

$$-\int \frac{dI}{I} = K \cdot C \int dt$$

$$-\ln \frac{I}{I_0} = KC \cdot l$$

$$\boxed{\ln \frac{I_0}{I} = K \cdot Cl}$$

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$$\log \frac{I_0}{I} = \varepsilon \cdot c \cdot l \quad \text{——— (1)}$$

$$\ln = 2.303 \log$$

$$\frac{2.303 \cdot c}{\varepsilon \text{ cont}}$$

Since we know that

$$A = \log \frac{I_0}{I} \quad \text{——— (2)}$$

Comparing (1) & (2)

$$A = \varepsilon \cdot c \cdot l$$

Proved

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10 Write the principle of Fluorescence with Jablonski diagram.

Principle of Fluorescence:

- When a molecule absorbs **ultraviolet (UV) or visible light**, its electrons get excited from the **ground singlet state (S_0)** to a **higher electronic excited singlet state (S_1, S_2, etc.)**.
- The excited electron quickly relaxes to the **lowest vibrational level of the first excited singlet state (S_1)** by losing energy through **vibrational relaxation** (non-radiative).
- From this excited state (S_1), the electron returns to the **ground singlet state (S_0)** by emitting a photon (light).
- This emitted light is called **fluorescence**.
- The emitted light has **longer wavelength (lower energy)** than the absorbed light due to energy lost during vibrational relaxation. This shift is called the **Stokes shift**.

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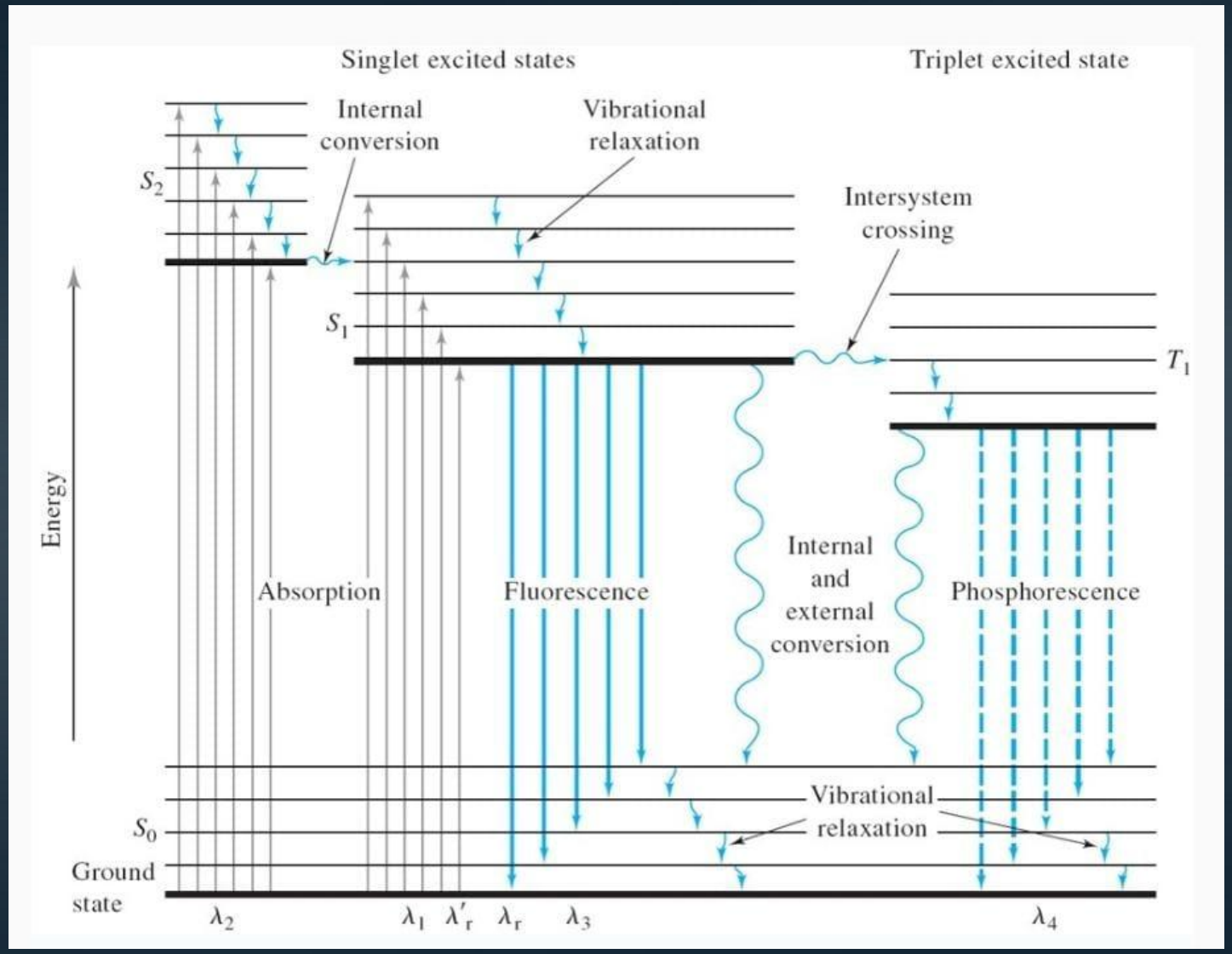

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10 Write the principle of Fluorescence with Jablonski diagram.

S_0: Ground electronic singlet state

S_1, S_2: Excited electronic singlet states

Vertical arrows show absorption (excitation) and emission (fluorescence)

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10 Write the principle of Fluorescence with Jablonski diagram.

Absorption (Excitation):

Electron absorbs a photon and moves from S_0 to higher excited singlet states (S_1, S_2, etc.)

Happens very fast (~10^{-15} seconds).

Vibrational Relaxation:

The excited molecule loses excess vibrational energy non-radiatively within the excited state to reach the lowest vibrational level of that state. Occurs in picoseconds (10^{-12} s).

Internal Conversion:

Non-radiative transition between electronic states of the same spin multiplicity (e.g., from $S_2 \rightarrow S_1$).

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10 Write the principle of Fluorescence with Jablonski diagram.

Fluorescence:

Radiative transition from the lowest vibrational level of excited singlet state (S_1) to various vibrational levels of the ground state (S_o).

Emission of light occurs in nanoseconds (10^{-9} s).

Light emitted has lower energy (longer wavelength) than absorbed due to energy loss (Stokes shift).

Intersystem Crossing (ISC):

Non-radiative transition from singlet excited state (S_1) to triplet excited state (T_1).

Spin state changes (singlet → triplet).

Phosphorescence:

Radiative transition from triplet excited state (T_1) to ground singlet state (S_o). Slower process (milliseconds to seconds) due to forbidden spin transition.

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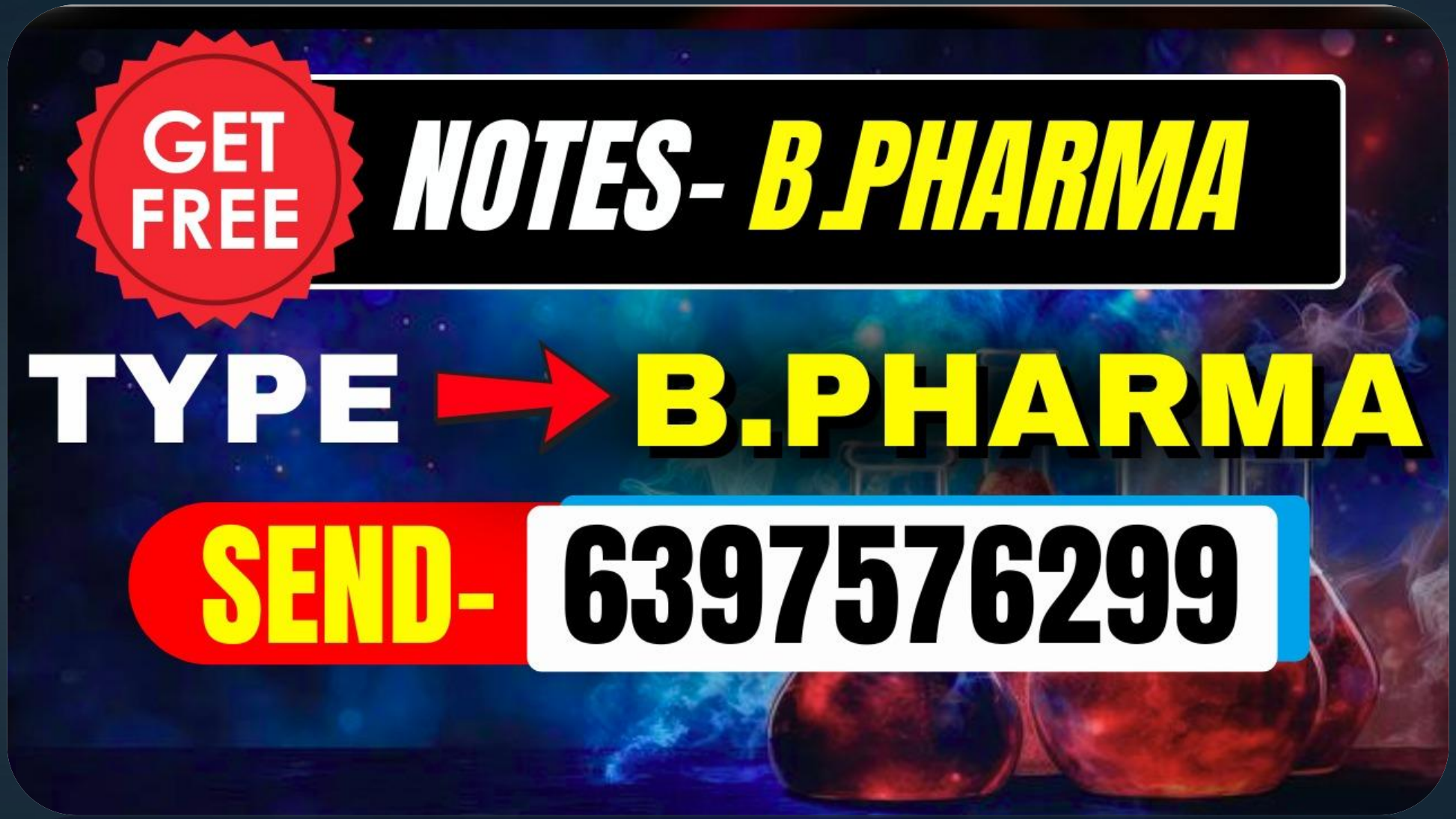

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