BP605T · B.Pharm Semester VI · Theory Notes

# Pharmaceutical Analysis

## Unit III — Fluorometry, Flame Photometry, AAS & Nepheloturbidometry

**Unit III (7 hours)**

---

**Pills & Python Notes**

From-zero teaching standard: every technical term is defined before use.

Syllabus coverage: Unit III (7 hours).

**PILLS & PYTHON**

**Pharmacy • Data • AI • Healthcare**

**Instagram @pillsandpython · pillsandpython.com · YouTube @PillsAndPython**

## UNIT III — SPECTROSCOPIC AND RELATED METHODS (7 hours)

**Unit III (7 hours):** Fluorometric analysis • Flame photometry • Atomic absorption spectrometry • Nepheloturbidometry Written in very simple English from absolute zero, as per the approved master brief.

# CHAPTER 1 — Fluorometric Analysis

## 1.1 Learning Objectives

By the end of this chapter, you will be able to:

1 Define **luminescence**, **photoluminescence**, **fluorescence** and **phosphorescence** in your own simple words.

2 Explain the difference between **luminescence** and **incandescence** with one example of each.

3 Draw and label the **Jablonski diagram** and name its five energy steps in the correct order.

4 Explain the difference between a **singlet state** and a **triplet state**.

5 List and explain the **factors that affect fluorescence** (molecular structure, pH, solvent, temperature, dissolved oxygen, concentration).

6 Define **quenching**, distinguish **collisional (dynamic)** quenching from **static** quenching, and write and interpret the **Stern–Volmer equation**.

7 Draw the **block diagram of a spectrofluorometer**, explain each part, and explain the **right-angle (90°) geometry** and why it differs from UV-Vis (180°).

8 Give **pharmaceutical applications** of fluorometry and explain **why it is more sensitive than UV-Vis spectroscopy**.

9 Solve a **quantum-yield numerical** by the relative method, showing every step.

## 1.2 Definitions

- **Luminescence:** Light given out by a substance **without heat**. It is "cold light". Example: the glow of a firefly.
- **Incandescence:** Light given out **because something is hot**. Example: the glowing filament of an electric bulb, or the sun.
- **Photoluminescence:** Luminescence that starts **after a substance absorbs light**. ("Photo" = light.) Fluorescence and phosphorescence are the two types of photoluminescence.

- **Fluorescence:** Photoluminescence that starts and stops **immediately** — the glow stops the moment the light source is switched off. It comes from the **singlet excited state** and lasts only about a billionth of a second.
- **Phosphorescence:** Photoluminescence that is **delayed** — the substance keeps glowing for seconds, minutes or even hours after the light source is removed. It comes from the **triplet excited state**. Example: glow-in-the-dark stars on a bedroom ceiling.
- **Ground state ($S_0$):** The normal, lowest-energy state of a molecule. Every molecule rests here when nothing disturbs it.
- **Excited state:** A higher-energy state a molecule reaches after it **absorbs light energy**. Written as $S_1$, $S_2$ (singlet) or $T_1$ (triplet).
- **Singlet state:** An excited state in which the two electrons have **opposite spins** (like two tops spinning in opposite directions). Symbol: **S**.
- **Triplet state:** An excited state in which the two electrons have the **same spin** (like two tops spinning in the same direction). Symbol: **T**.
- **Quantum yield (Φ, "phi"):** The fraction of absorbed light particles that come back out as fluorescence.

Quantum yield = (light particles emitted) ÷ (light particles absorbed). Its value is always between 0 and 1.

- **Stokes shift:** The gap between the wavelength of light **absorbed** and the wavelength of light **emitted**. Emitted light always has a **longer wavelength** (lower energy) than the absorbed light, because some energy is lost as heat in between.
- **Quenching:** Any process that **reduces** the fluorescence brightness of a sample.
- **Fluorophore:** A molecule, or the light-emitting part of a molecule, that can show fluorescence.
- **Quencher:** A substance that causes quenching (for example, dissolved oxygen or iodide ions).

**Key takeaway:** Fluorescence = fast glow from the singlet state that stops instantly; phosphorescence = slow glow from the triplet state that continues after the light is off.

## 1.3 Key Terminology (Mini-Glossary)

| Term | Simple meaning |
|---|---|
| Absorption | A molecule takes in a particle of light (a photon) and jumps to a higher energy state. |
| Photon | The smallest packet (particle) of light energy. |
| Internal conversion | Energy lost as heat **within the same** excited state ($S_1$ → lower level of $S_1$); no light is given out. |
| Vibrational relaxation | A molecule shakes off extra energy as tiny vibrations (heat) and settles to the lowest level of an excited state. |
| Intersystem crossing | The molecule jumps from the singlet excited state ($S_1$) to the triplet excited state ($T_1$); an electron flips its spin; no light is given out. |

| Term | Simple meaning |
|---|---|
| Radiative process | An energy change that **gives out light** (fluorescence, phosphorescence). |
| Non-radiative process | An energy change that gives out **heat, not light** (internal conversion, intersystem crossing). |
| Excitation wavelength | The wavelength of light used to excite (switch on) the sample. |
| Emission wavelength | The wavelength of light the sample gives out (fluorescence). Always longer than the excitation wavelength. |
| Excitation spectrum | A graph showing which wavelengths excite the sample best. |
| Emission spectrum | A graph showing the wavelengths of light the sample emits. |
| Self-quenching | At high concentration, excited molecules collide with unexcited molecules of the **same** substance and lose energy — brightness drops. |
| Self-absorption (inner filter effect) | At high concentration, the emitted light is **re-absorbed** by other molecules of the same sample before reaching the detector. |
| Stern–Volmer constant (Ksv) | A number that tells how strongly a quencher reduces fluorescence; it is the slope of the Stern–Volmer plot. |
| Monochromator | A device (prism or grating) that **selects one wavelength** of light out of many. |
| Filter | A simpler, cheaper device that allows only a narrow band of wavelengths to pass. |
| Photomultiplier tube (PMT) | An extremely sensitive detector that converts even very weak light into an electrical signal and **multiplies** it. |
| Right-angle geometry | In a fluorometer, the detector sits at **90°** to the excitation beam, so the bright excitation light does not enter the detector directly. |
| Derivatization | A chemical reaction that converts a **non-fluorescent** drug into a **fluorescent** product so it can be measured. |
| Standard (quinine sulfate) | A reference substance of known quantum yield (0.546 in dilute sulphuric acid) used to find the quantum yield of unknown samples. |
| Cuvette / sample cell | The small transparent container (must be **quartz** for UV work) that holds the sample solution. |

# 1.4 Detailed Explanations (Concepts with Analogies)

## 1.4.1 Luminescence vs incandescence

- **Technical concept:** Luminescence is emission of light without a rise in temperature; incandescence is emission of light because the body is hot.
- **Simple explanation:** There are two ways to make light. One way is to **heat** something until it glows — like an iron rod in a furnace or the filament of an old bulb. The other way is to make light **without heat** — like a firefly, a glow-stick, or a TV screen.
- **Example:** Firefly (luminescence) vs. a hot coal (incandescence).
- **Key takeaway:** In this chapter we study only the "cold light" type — luminescence.

### 1.4.2 Photoluminescence: fluorescence vs phosphorescence

- **Technical concept:** Photoluminescence is luminescence that follows absorption of photons. Fluorescence is the fast ($10^{-9}$–$10^{-7}$ s) emission from the singlet state; phosphorescence is the slow ($10^{-3}$ s to hours) emission from the triplet state.
- **Simple explanation:** Shine a torch on two different glow materials in a dark room, then switch the torch off. Material A goes dark **instantly** — that is fluorescence. Material B keeps glowing on the wall for minutes — that is phosphorescence.
- **Example:** A highlighter pen mark under UV light (fluorescence — stops instantly) vs. glow-in-the-dark ceiling stars (phosphorescence — keep glowing).
- **Key takeaway:** Speed is the difference — fluorescence is instant, phosphorescence is delayed.

### 1.4.3 Singlet and triplet states

- **Technical concept:** In the ground state the two electrons in an orbital have opposite spins (paired). Absorption keeps the spins opposite → singlet excited state ($S_1$). If one electron flips its spin, the spins become parallel → triplet excited state ($T_1$).
- **Simple explanation:** Imagine two spinning tops. If they spin in **opposite** directions, that is the **singlet** state. If they spin in the **same** direction, that is the **triplet** state. The triplet state is "forbidden" to return quickly to the ground state (nature's rule), so it lives much longer — this long life is what makes phosphorescence slow.
- **Example:** Quinine in tonic water: its excited electrons stay in the singlet state and fall back in nanoseconds → bright instant blue fluorescence under UV light.
- **Key takeaway:** Singlet = opposite spins = fast return = fluorescence. Triplet = same spin = slow return = phosphorescence.

### 1.4.4 The Jablonski diagram — five steps in order

- **Technical concept:** The Jablonski diagram is an energy map showing the ground state ($S_0$), excited singlet states ($S_1$, $S_2$), the triplet state ($T_1$), and the arrows (transitions) between them.
- **Simple explanation:** Think of it as a building. $S_0$ is the ground floor. $S_1$ and $S_2$ are upper floors. $T_1$ is a side room on the first floor with a very slow door. The five steps are the five ways a molecule can move in this building:

1. **Absorption ($S_0 \rightarrow S_1$ or $S_2$):** The molecule absorbs a photon and jumps upstairs. Extremely fast (~$10^{-15}$ s — a millionth of a billionth of a second).
2. **Internal conversion (within $S_1$):** The molecule slides down to the lowest step of the same floor, losing energy as **heat**, not light. Also very fast.
3. **Fluorescence ($S_1 \rightarrow S_0$):** From the bottom of $S_1$ the molecule jumps back to the ground floor, giving out **light**. Fast (~$10^{-9}$ s). This is the glow we measure.
4. **Intersystem crossing ($S_1 \rightarrow T_1$):** Instead of fluorescing, the molecule may slip into the side room $T_1$; one electron flips its spin. No light is given out.

5 **Phosphorescence ($T_1 \rightarrow S_0$):** From the side room the molecule slowly returns to the ground floor, giving out **light** — but slowly (milliseconds to hours), because the return is "forbidden" and the door opens slowly.

- **Example:** When quinine absorbs UV light, most molecules follow steps 1 → 2 → 3 and we see blue fluorescence. A few take the side path 1 → 2 → 4 → 5 and phosphoresce weakly.
- **Key takeaway:** Memorise the order — **A**bsorption, **I**nternal conversion, **F**luorescence, **I**ntersystem crossing, **P**hosphorescence (see mnemonic in 1.17).

### 1.4.5 Quantum yield (Φ)

- **Technical concept:** Φ = (number of photons emitted as fluorescence) ÷ (number of photons absorbed). It measures the **efficiency** of fluorescence. Maximum value is 1 (every absorbed photon comes back out as light); a value near 0 means almost no fluorescence.
- **Simple explanation:** If a shopkeeper receives 100 customers and 54 buy something, his "success yield" is 0.54. Similarly, if a molecule absorbs 100 photons and emits 54 as fluorescence (the rest lost as heat), its quantum yield is 0.54.
- **Example:** Quinine sulfate in dilute sulphuric acid has Φ = 0.546 — about 55 of every 100 absorbed photons return as blue light. That is why it is used as a **standard**.
- **Key takeaway:** Quantum yield is simply "fluorescence efficiency", always between 0 and 1. (A worked numerical is given in Calculation Box 1.)

### 1.4.6 Stokes shift

- **Technical concept:** Because of internal conversion (heat loss), the emitted photon has less energy than the absorbed photon. Lower energy means **longer wavelength**. Stokes shift = λ(emission) − λ(excitation), always positive.
- **Simple explanation:** You throw a ball up to the 3rd floor (absorption). On the way down it hits an awning and loses some energy as heat (internal conversion), so it bounces back with less energy — it cannot reach as high. The light coming out is therefore "weaker" (longer wavelength) than the light going in.
- **Example:** Quinine absorbs at ~350 nm (UV) and emits at ~450 nm (blue). Stokes shift = 100 nm.
- **Key takeaway:** Emission wavelength is **always longer** than excitation wavelength. This gap (Stokes shift) is what lets the instrument separate the weak fluorescence from the strong excitation beam.

### 1.4.7 Factors affecting fluorescence

Six factors control how bright the fluorescence is. Learn each as: **factor → what happens → example**.

1 **Molecular structure (rigidity, conjugation/aromaticity, electron-donating groups).**

- *Technical concept:* Rigid, planar molecules with conjugated π-electron systems (aromatic rings) fluoresce strongly. Electron-donating groups (–OH, $–NH_2$, $–OCH_3$) increase fluorescence; electron-withdrawing groups ($–NO_2$, –COOH, halogens) decrease it.

- *Simple explanation:* A stiff molecule cannot waste energy by wiggling, so the energy comes out as light. A floppy molecule wiggles the energy away as heat. Rings with alternating double bonds (like benzene rings) are excellent "light antennas". Groups that **donate** electrons (–OH, $–NH_2$) feed the antenna; groups that **pull** electrons away ($–NO_2$) starve it.
- *Example:* **Fluorescein** (rigid, conjugated) glows brilliantly; adding a nitro ($–NO_2$) group to an aromatic molecule almost kills its fluorescence. **Riboflavin** (vitamin $B_2$) is rigid and conjugated → strongly fluorescent.
- *Key takeaway:* Rigid + aromatic + electron-donating groups = bright fluorescence.

1 **pH.**

- *Technical concept:* Changing the pH changes the ionised/un-ionised form of the molecule, and the two forms usually fluoresce differently.
- *Simple explanation:* Many drugs are weak acids or bases. In acidic solution they wear one "dress" (protonated form); in basic solution another (de-protonated form). The two dresses glow differently — sometimes one glows and the other does not.
- *Example:* **Quinine** fluoresces strongly in dilute sulphuric acid but weakly in alkaline solution. Vitamin $B_6$ (pyridoxine) also changes fluorescence with pH. That is why fluorometric methods always fix the pH with a buffer.
- *Key takeaway:* Always control pH — the same drug can be bright at one pH and dark at another.

1 **Solvent (polarity, viscosity).**

- *Technical concept:* Solvent polarity shifts excitation/emission wavelengths; higher viscosity reduces collisions between molecules, so less energy is lost and fluorescence increases.
- *Simple explanation:* Imagine running through water vs. running through honey. In thin, watery solvents, excited molecules keep bumping into solvent molecules and lose energy (dimmer glow). In thick, viscous solvents (like glycerol), they bump less and glow brighter. Polar solvents can also shift the colour of the glow slightly.
- *Example:* Fluorescence measured in glycerol is brighter than in water because glycerol is far more viscous.
- *Key takeaway:* Viscous solvents → brighter fluorescence; always use the **same solvent** for standard and sample.

1 **Temperature.**

- *Technical concept:* Higher temperature → more molecular collisions → more non-radiative energy loss → lower fluorescence.
- *Simple explanation:* Heat makes molecules dance faster and bump into each other more. Each bump steals a little energy as heat instead of light, so the glow becomes dimmer as temperature rises.
- *Example:* A fluorescent solution measured at 40 °C gives a weaker reading than the same solution at 20 °C.
- *Key takeaway:* Keep temperature **constant** during measurement; heat dims fluorescence.

1 **Dissolved oxygen.**

- *Technical concept:* Oxygen is paramagnetic and promotes intersystem crossing ($S_1 \rightarrow T_1$), which steals molecules away from the fluorescence path — a classic quencher.
- *Simple explanation:* Dissolved oxygen acts like a thief: it pushes excited molecules into the slow triplet "side room" so they cannot fluoresce. Removing oxygen (by bubbling nitrogen gas through the solution) makes the glow brighter.
- *Example:* Many fluorometric assays bubble nitrogen through the sample to drive out oxygen before measuring.
- *Key takeaway:* Dissolved $O_2$ **quenches** fluorescence — remove it for maximum brightness.

1 **Concentration (self-quenching, self-absorption).**

- *Technical concept:* At low concentration, fluorescence increases linearly with concentration. At high concentration, excited molecules collide with ground-state molecules of the same kind (**self-quenching**) and emitted light is re-absorbed by the sample (**self-absorption / inner filter effect**) — the graph bends downwards.
- *Simple explanation:* A few candles in a dark hall: each extra candle adds light (linear region). But pack a hundred candles into a small box: they heat and smother each other, and the ones in front block the light of the ones behind — adding more candles no longer adds proportional light.
- *Example:* A calibration graph of fluorescence vs. concentration is a straight line only at **low** concentrations; it curves at high concentrations.
- *Key takeaway:* Work at **low concentrations** — that is also why fluorometry is ideal for trace analysis.

### 1.4.8 Quenching — definition, types, Stern–Volmer

- **Technical concept:** Quenching is any process that decreases fluorescence intensity, either by deactivating the excited state or by forming a non-fluorescent complex.
- **Simple explanation:** A quencher is anything that dims the glow — like sunglasses dim bright light.
- **Two types:**

1 **Collisional (dynamic) quenching:** The quencher **bumps into** the already-excited molecule and steals its energy as heat. Because it depends on molecules moving and colliding, it **increases with temperature** (hotter = faster movement = more collisions).

2 **Static quenching:** The quencher **binds to** the molecule in the ground state (before excitation) and forms a dark, non-fluorescent complex. Because the complex simply does not glow, this type **decreases with temperature** (heat breaks the complex apart).

- **Stern–Volmer relationship:** The equation that links quencher concentration to the drop in brightness:

**$F_0/F = 1 + Ksv \times [Q]$**

- $F_0$ = fluorescence intensity **without** quencher; F = intensity **with** quencher; [Q] = quencher concentration; Ksv = Stern–Volmer constant.

- **In words:** The ratio $F_0/F$ grows in a straight line as quencher is added. A plot of $F_0/F$ (y-axis) against [Q] (x-axis) is a **straight line with slope = Ksv** and intercept = 1. A bigger Ksv means a stronger quencher.
- **Examples:** Dissolved **oxygen** (dynamic quencher), **iodide ions ($I^-$)** quenching quinine fluorescence, chloride ions quenching certain dyes.
- **Key takeaway:** Dynamic = collision after excitation (worse when hot); static = dark complex before excitation (better when hot). Stern–Volmer plot is a straight line whose slope measures quencher strength.

### 1.4.9 Instrumentation — the spectrofluorometer

- **Technical concept:** A spectrofluorometer has six parts in this order: light source → excitation monochromator → sample cell → emission monochromator → detector (PMT) → readout. Emission is collected at 90° to the excitation beam.
- **Simple explanation and each part:**

1. **Light source:** A **xenon arc lamp** (gives strong, continuous UV–visible light) or a mercury lamp. It is the "torch" that switches the sample on.
2. **Excitation monochromator/filter:** Selects the **one wavelength** that best excites the sample and blocks the rest — like choosing one radio station.
3. **Sample cell (cuvette):** Holds the sample solution. It must be **quartz**, not glass, because glass absorbs UV light. The emitted light is collected at **90° (right angle)** to the incoming beam.
4. **Emission monochromator/filter:** Selects the **one wavelength** of emitted fluorescence to measure, blocking scattered excitation light.
5. **Detector — photomultiplier tube (PMT):** An extremely sensitive "electronic eye". Even a few photons knock out electrons, and the tube **multiplies** them into a measurable electric current.
6. **Readout:** A meter, recorder or computer screen that displays the final fluorescence intensity number.

- **Why 90° and not 180°?** In UV-Vis spectroscopy the detector sits **straight through (180°)** because it measures how much light passed through. In fluorometry the emitted light is very weak; if the detector sat straight through, the blindingly bright excitation beam would flood it. At **90°**, the detector sees only the weak sideways glow — the bright beam passes straight on and misses the detector.
- **Key takeaway:** Source → excitation selector → quartz cell → emission selector → PMT → display; the 90° angle is what makes the weak fluorescence measurable.

### 1.4.10 Why fluorometry is more sensitive than UV-Vis

- **Technical concept:** UV-Vis measures a **small difference between two large signals** (light in vs. light out); fluorometry measures a **signal against a near-zero background**.
- **Simple explanation:** UV-Vis is like trying to notice that a bright stadium floodlight became 1% dimmer because someone held a curtain in front of it — very hard. Fluorometry is like spotting a single lit candle in a pitch-dark room — very easy. Because the background is dark, even tiny amounts of drug give a clear signal. Fluorometry is typically **10 to 1000 times more sensitive** than UV-Vis and can detect nanogram-per-mL levels.

- **Key takeaway:** Dark background + amplified detector (PMT) = trace-level sensitivity.

### 1.4.11 Pharmaceutical applications

- **Technical concept:** Fluorometry is used for (a) drugs that fluoresce naturally, (b) non-fluorescent drugs converted to fluorescent products by derivatization, (c) vitamins, (d) catecholamines, and (e) trace/impurity analysis.
- **Simple explanation with examples:**

1. **Naturally fluorescent drugs** — measured directly: **quinine** (antimalarial; bright blue fluorescence in dilute acid), **riboflavin** (vitamin $B_2$; yellow-green fluorescence), **tetracycline** (antibiotic), **morphine**, **salicylic acid**.
2. **Derivatization to fluorescent products: Thiamine** (vitamin $B_1$) itself is weakly fluorescent, but oxidation converts it to **thiochrome**, which fluoresces strongly — this is the official assay method. **Catecholamines** like **adrenaline (epinephrine)** are converted to fluorescent products for measurement in injections. Amino acids and penicillins react with **fluorescamine** to give fluorescent products.
3. **Trace analysis:** Detecting **impurities and degradation products** at parts-per-million or parts-per-billion levels in bulk drugs — far below what UV-Vis can see.
4. **Vitamins in formulations:** Riboflavin and thiamine (as thiochrome) in multivitamin tablets and syrups.
5. **Bioanalysis:** Measuring tiny drug amounts in blood/urine during pharmacokinetic studies.

- **Key takeaway:** If a drug glows naturally — measure it directly. If not — make it glow by derivatization. Either way, fluorometry finds amounts too small for UV-Vis.

---

## 1.5 Classifications / Tables

### Table 1.1 — Fluorescence vs phosphorescence

| Property | Fluorescence | Phosphorescence |
|---|---|---|
| Excited state involved | Singlet ($S_1$) | Triplet ($T_1$) |
| Time scale | $10^{-9}$–$10^{-7}$ s (billionths of a second) | $10^{-3}$ s to hours |
| Glow after light source is removed? | No — stops instantly | Yes — continues glowing |
| Electron spins | Opposite (paired) | Same (unpaired) |
| Key step in Jablonski diagram | $S_1 \rightarrow S_0$ directly | $S_1 \rightarrow T_1$ (intersystem crossing), then $T_1 \rightarrow S_0$ |
| Example | Quinine under UV lamp; highlighter ink | Glow-in-the-dark ceiling stars |

### Table 1.2 — Collisional (dynamic) vs static quenching

| Property | Collisional (dynamic) quenching | Static quenching |
|---|---|---|
| What happens | Quencher collides with the **excited** molecule | Quencher binds the molecule in the **ground state**, forming a dark complex |

| Property | Collisional (dynamic) quenching | Static quenching |
|---|---|---|
| When it acts | After excitation | Before excitation |
| Effect of increasing temperature | Quenching **increases** (more collisions) | Quenching **decreases** (complex breaks) |
| Example | Dissolved oxygen | Iodide forming a complex with a fluorophore |

### Table 1.3 — Factors affecting fluorescence (quick reference)

| Factor | Effect on fluorescence | Example / remedy |
|---|---|---|
| Molecular structure | Rigid, conjugated (aromatic), electron-donating groups → brighter | Fluorescein glows; $-NO_2$ group kills glow |
| pH | Ionised vs un-ionised forms glow differently | Quinine bright in acid; always use a buffer |
| Solvent | Viscous solvents → brighter; polarity shifts colour | Glycerol brighter than water |
| Temperature | Higher temperature → dimmer | Keep temperature constant |
| Dissolved oxygen | Quenches (promotes intersystem crossing) | Bubble nitrogen to remove $O_2$ |
| Concentration | Too high → self-quenching + self-absorption; graph curves | Work at low (dilute) concentrations |

### Table 1.4 — Spectrofluorometer vs UV-Vis spectrophotometer

| Property | Spectrofluorometer | UV-Vis spectrophotometer |
|---|---|---|
| What is measured | Light **emitted** by the sample | Light **absorbed** by the sample |
| Detector position | **90°** (right angle) to the beam | **180°** (straight through) |
| Background | Near-zero (dark) | Large (bright beam) |
| Sensitivity | Very high (ng/mL level) | Moderate (µg/mL level) |
| Detector | PMT (multiplies weak signal) | Photocell/photodiode |
| Sample cell | Quartz (UV-transparent) | Quartz or glass |

## 1.6 Mechanisms / Processes (Step-by-Step)

### Process A — The life of an excited molecule (Jablonski sequence)

1 Molecule rests in the ground state **$S_0$**.

2 **Absorption:** It absorbs one photon → jumps to **$S_1$** (or $S_2$). Time: ~$10^{-15}$ s.

3 **Internal conversion + vibrational relaxation:** It slides to the lowest level of $S_1$, losing energy as **heat**.

4 **Decision point:** From here it takes one of two paths:

- **Path 1 — Fluorescence:** Falls $S_1 \rightarrow S_0$, giving out light **immediately** (~$10^{-9}$ s). → End.
- **Path 2 — Intersystem crossing:** Slips $S_1 \rightarrow T_1$ (electron flips spin; no light).

1 **Phosphorescence:** From $T_1$ it slowly falls $T_1 \rightarrow S_0$, giving out light **late** (milliseconds to hours). → End.

### Process B — How to run a fluorometric assay in the laboratory

1 Prepare the sample solution at a **low concentration** in a suitable solvent.

2 Adjust the **pH** with a buffer (as per the method).

3 Remove dissolved oxygen by bubbling **nitrogen** gas, if required.

4 Keep the temperature **constant** (e.g., 25 °C water bath).

5 Fill a clean **quartz** cuvette; wipe it dry and fingerprint-free.

6 Set the **excitation wavelength** (from the excitation spectrum) on the excitation monochromator.

7 Set the **emission wavelength** (from the emission spectrum) on the emission monochromator.

8 Measure the fluorescence intensity; compare with a **standard** (or calibration graph) to find the concentration.

### Process C — Derivatization example: thiamine → thiochrome

1 Thiamine (vitamin $B_1$) in the sample is **weakly fluorescent** — hard to measure directly.

2 Add an oxidising agent (alkaline potassium ferricyanide) to the solution.

3 Thiamine is oxidised to **thiochrome**, a rigid, conjugated, strongly fluorescent molecule.

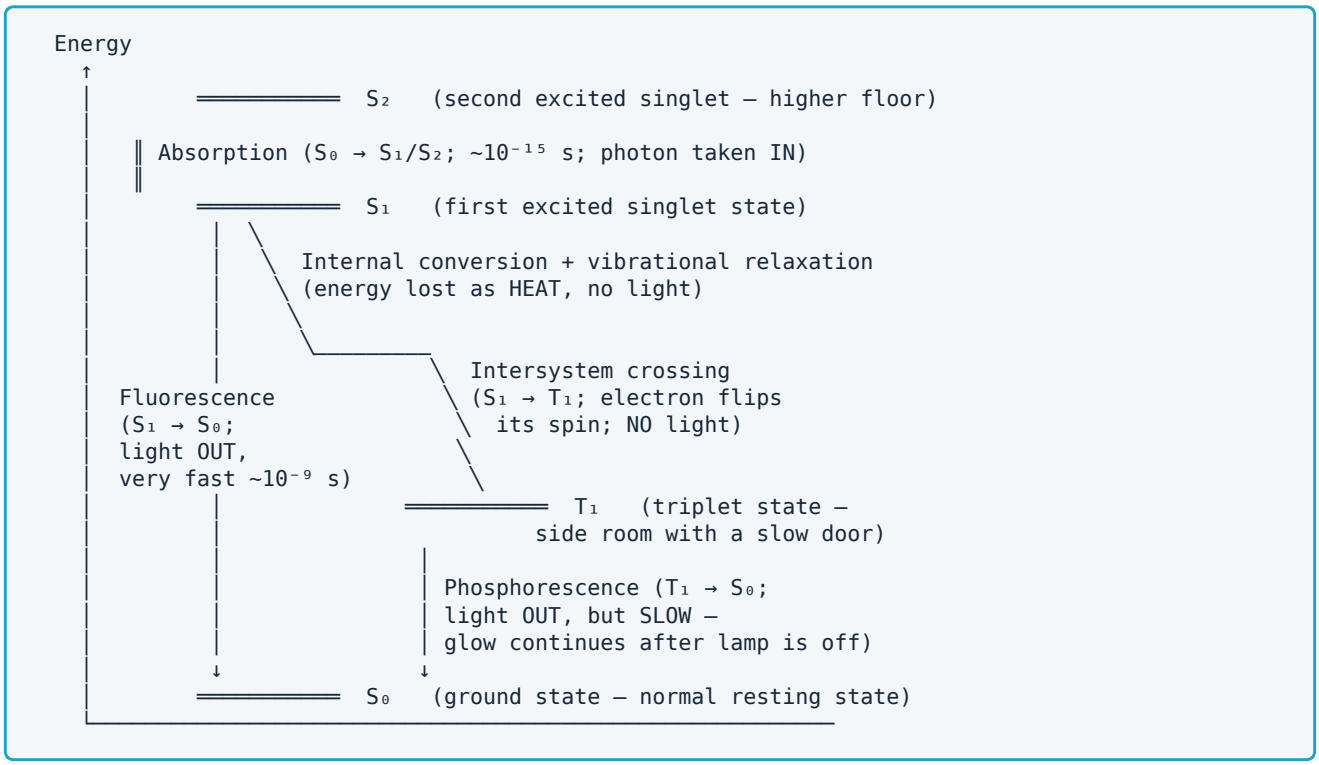
4 Measure thiochrome's bright blue fluorescence in the fluorometer.

5 The brightness is proportional to the original thiamine amount → calculate the vitamin $B_1$ content.

---

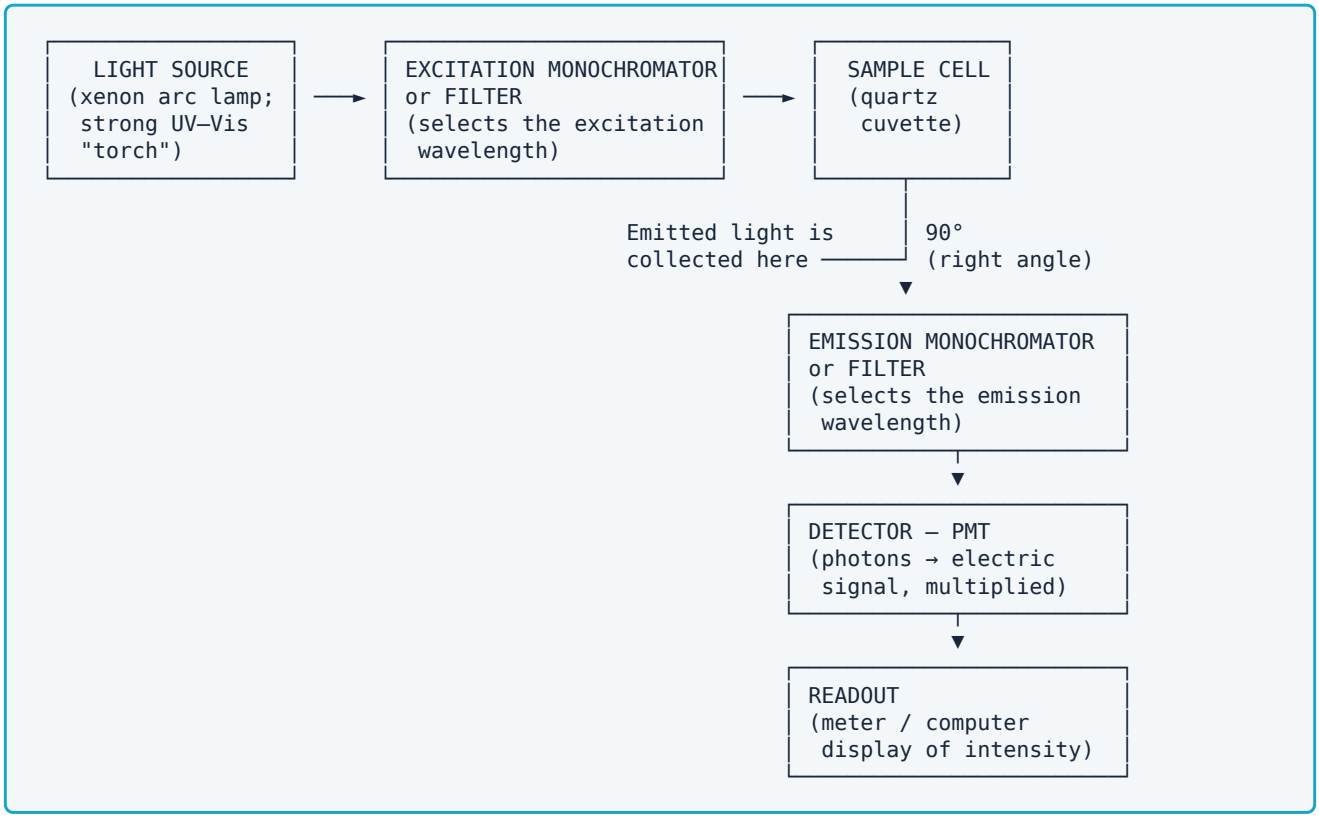
## 1.7 Diagrams (Textual Block Diagrams, Labeled)

### Diagram 1.1 — The Jablonski diagram (energy map of fluorescence and phosphorescence)

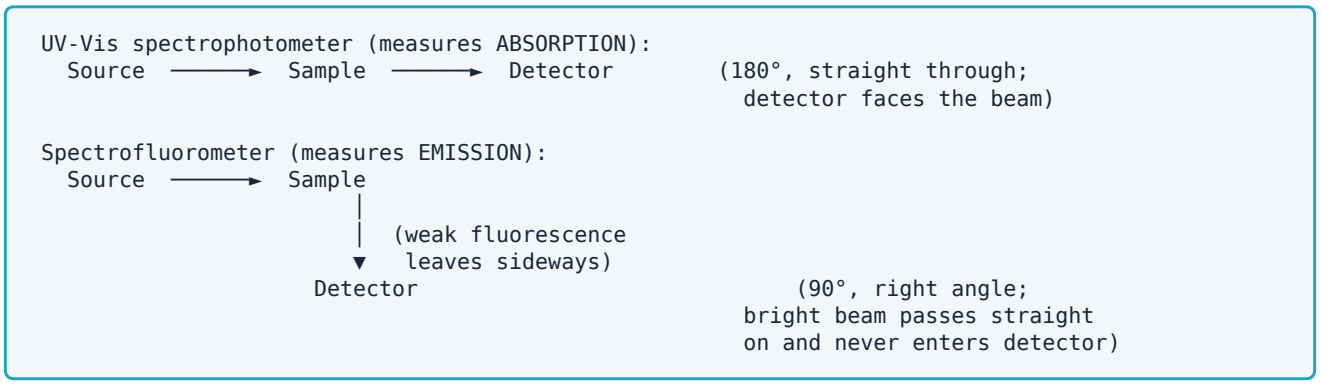



**How to read it:** Upward arrow = energy taken in (absorption). Downward straight arrow = light given out (fluorescence/phosphorescence). Wavy/sloping arrows = energy lost as heat or spin-flip (no light).

## Diagram 1.2 — Block diagram of a spectrofluorometer (right-angle geometry)

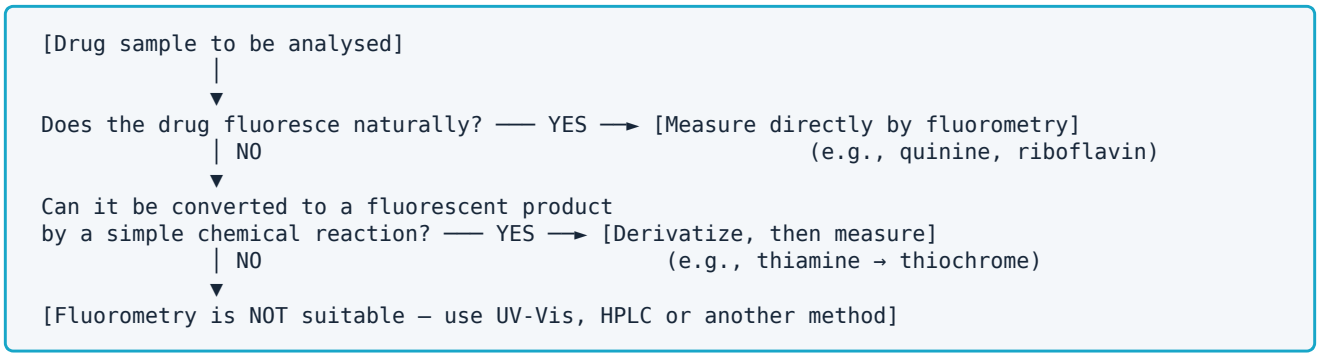



## Diagram 1.3 — Geometry comparison: why 90° and not 180°?

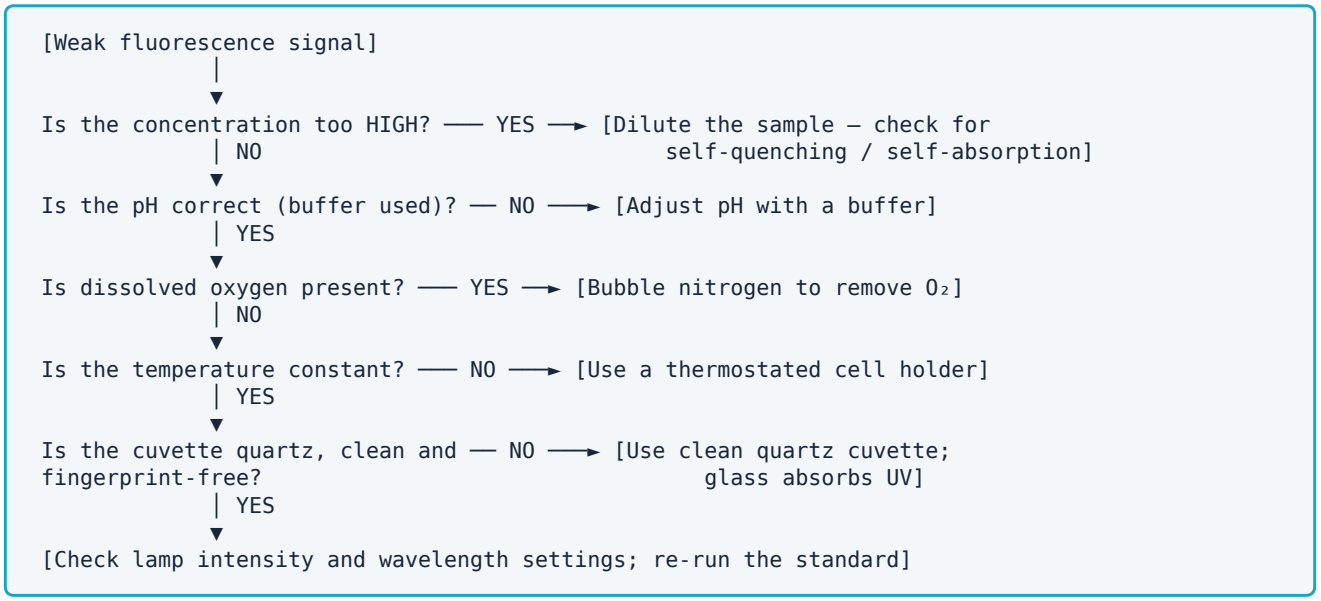



## 1.8 Flowcharts (Textual)

### Flowchart 1.1 — Is fluorometry the right method for my drug?




### Flowchart 1.2 — My fluorescence signal is too low — what should I check?




## 1.9 Infographic Concept Box

**One-page infographic: "Fluorescence in 60 Seconds"**

*Imagine a single A4 page that teaches this whole chapter at a glance. It would show:*

- **Top banner:** Title "FLUOROMETRIC ANALYSIS" with a glowing blue quinine tonic-water bottle under a UV lamp as the hero image.
- **Left panel — "Cold Light":** Two icons side by side: a firefly (luminescence ✔) vs. a hot bulb filament (incandescence ✘), with the one-line rule "No heat = luminescence".
- **Centre panel — Jablonski staircase:** A simple 4-step staircase diagram ($S_0 \rightarrow S_1 \rightarrow$ light out / $\rightarrow T_1 \rightarrow$ slow light out) with the five steps numbered and colour-coded: absorption (yellow arrow up), internal conversion (red wavy arrow), fluorescence (blue arrow down), intersystem crossing (purple arrow sideways), phosphorescence (green slow arrow down).
- **Right panel — "6 Brightness Killers":** Six small icons with one word each: Structure, pH, Solvent, Temperature, Oxygen, Concentration — each with a tiny up/down arrow showing its effect.
- **Bottom strip — Instrument:** A mini block diagram (Lamp → Excitation selector → Quartz cell → Emission selector → PMT → Screen) with a bold "90°" badge on the detector angle.
- **Corner callout:** The candle-in-a-dark-room analogy ("Why fluorometry beats UV-Vis: 10–1000× more sensitive") and the Stern–Volmer equation $F_0/F = 1 + Ksv[Q]$ in a highlighted box.
- **Colour scheme:** Dark navy background (like a dark room) with glowing cyan/yellow highlights — so the page itself feels like fluorescence.

---

## 1.10 Examples (Pharmacy Examples)

1 **Quinine (antimalarial drug).** Quinine in dilute sulphuric acid gives an intense blue fluorescence (excitation ~350 nm, emission ~450 nm). It is the classic teaching example and the **reference standard** for quantum-yield measurements (Φ = 0.546). Also used to check the performance of fluorometers.

2 **Riboflavin (vitamin $B_2$).** Naturally fluorescent (yellow-green). Its fluorescence is used directly to estimate riboflavin in multivitamin tablets, syrups and injections.

3 **Thiamine (vitamin $B_1$) → thiochrome.** Thiamine itself glows weakly, so it is oxidised to **thiochrome**, which fluoresces bright blue. This derivatization method is the standard assay for vitamin $B_1$ in formulations.

4 **Tetracycline (antibiotic).** Naturally fluorescent; fluorometry is used to estimate it in dosage forms and to study its degradation.

5 **Catecholamines — adrenaline (epinephrine).** Adrenaline in injections is converted to a fluorescent product (trihydroxyindole reaction) and measured fluorometrically at trace levels.

6 **Morphine and salicylic acid.** Both show natural fluorescence and can be estimated directly.

7 **Amino acids and penicillins.** React with **fluorescamine** to form fluorescent derivatives — used in quality control and biochemical assays.

8 **Trace impurity testing.** A degradation product present at 0.01% in a bulk drug can be detected by fluorometry even when UV-Vis shows nothing — critical for safety testing of medicines.

---

## 1.11 Analogies

1. **Fluorescence vs phosphorescence:** A clap that stops the instant you stop clapping (fluorescence) vs. a bell that keeps ringing after you strike it (phosphorescence).
2. **Singlet vs triplet:** Two spinning tops spinning in opposite directions (singlet) vs. the same direction (triplet).
3. **Stokes shift:** Throwing a ball to the 3rd floor, but it hits an awning on the way down and bounces back with less energy — the light coming out is always "weaker" (longer wavelength) than the light going in.
4. **Why fluorometry beats UV-Vis:** Spotting one lit candle in a pitch-dark room (fluorometry) vs. noticing a stadium floodlight dim by 1% (UV-Vis).
5. **Quencher (oxygen):** A thief who pushes excited molecules into the slow side-room (triplet state) so they cannot fluoresce.
6. **Self-quenching at high concentration:** Too many candles packed in a small box — they smother each other instead of adding light.
7. **Monochromator:** A radio tuner that picks exactly one station (wavelength) out of hundreds.
8. **PMT detector:** A whisper amplified through a loudspeaker — it multiplies a few photons into a measurable signal.
9. **90° geometry:** Listening to a whisper from the side of a room while a loudspeaker blasts straight ahead — you stand at right angles so the blast misses you.
10. **Derivatization:** Putting a glowing jacket on someone invisible in the dark so you can find them (thiamine → thiochrome).

## 1.12 Pharmacy Relevance

- **Quality control of medicines:** Fluorometry detects impurities and degradation products at parts-per-million levels, protecting patients from harmful contaminants that UV-Vis would miss.
- **Vitamin assays:** Riboflavin ($B_2$) and thiamine ($B_1$, as thiochrome) in multivitamin tablets and syrups are routinely estimated fluorometrically.
- **Trace drug analysis in blood/urine:** During pharmacokinetic studies and therapeutic drug monitoring, drug levels are often in nanograms per mL — only fluorometry (or HPLC with fluorescence detection) is sensitive enough.
- **Stability studies:** As a drug degrades on storage, fluorescent degradation products appear; tracking them tells how fast the medicine is spoiling.
- **Catecholamine injections:** Adrenaline injections must contain exactly the labelled amount — fluorometric assay gives the needed sensitivity.
- **Forensic and clinical labs:** Fluorescence helps detect drugs and poisons in biological samples at trace levels.

## 1.13 India Context

- The **Indian Pharmacopoeia (IP)** includes fluorometric methods for the assay of vitamins and certain drugs — Indian QC chemists must know this technique to follow official monographs.
- India is one of the world's largest producers of **generic medicines**; export markets (US, Europe) demand strict impurity profiling, and fluorometry is a key tool for detecting trace impurities in bulk drugs made in Indian plants.
- **Multivitamin tonics and B-complex syrups** are hugely popular in India; their riboflavin and thiamine content is checked fluorometrically in QC laboratories across the country.
- Government programmes involving **fortified foods** (for example, vitamin-fortified milk and mid-day meal items) rely on sensitive vitamin assays — fluorometry is one of the workhorse methods.
- Many Indian pharmaceutical colleges teach fluorometry using the classic **quinine sulfate standard** experiment — the same experiment you will likely perform in your own instrumental analysis practical.

---

## 1.14 Key Points

1 Luminescence = cold light (no heat); incandescence = light from heat.

2 Photoluminescence has two types: **fluorescence** (fast, singlet, stops instantly) and **phosphorescence** (slow, triplet, keeps glowing).

3 Jablonski steps in order: **Absorption → Internal conversion → Fluorescence → Intersystem crossing → Phosphorescence**.

4 Singlet = opposite electron spins; triplet = same electron spins.

5 **Quantum yield** = photons emitted ÷ photons absorbed (0 to 1); quinine sulfate standard $\Phi = 0.546$.

6 **Stokes shift** = $\lambda$(emission) − $\lambda$(excitation); emission is always at longer wavelength.

7 Bright fluorescence needs: rigid + conjugated (aromatic) structure, electron-donating groups, correct pH, viscous solvent, low temperature, no dissolved $O_2$, low concentration.

8 **Quenching** = anything that dims fluorescence. Dynamic = collision after excitation (worse when hot); static = dark complex before excitation (better when hot).

9 **Stern–Volmer:** $F_0/F = 1 + Ksv[Q]$; plot is a straight line, slope = Ksv.

10 Spectrofluorometer order: **source → excitation monochromator → quartz cell → emission monochromator → PMT → readout**; detector at **90°**.

11 Use **quartz** cuvettes (glass absorbs UV); control pH and temperature; remove dissolved oxygen.

12 Fluorometry is **10–1000× more sensitive** than UV-Vis because it measures a signal against a dark background.

13 Naturally fluorescent drugs: **quinine, riboflavin, tetracycline, morphine, salicylic acid**.

14 Derivatization examples: **thiamine → thiochrome**; adrenaline → fluorescent product; penicillins + fluorescamine.

15 Main uses: vitamin assays, trace impurity testing, catecholamines, drug levels in blood/urine.

## 1.15 Common Confusions

1 **Fluorescence vs phosphorescence** — Confusion: "both are just glowing." Clarification: fluorescence stops the instant the light source is removed (singlet, nanoseconds); phosphorescence keeps glowing (triplet, seconds to hours).

2 **Luminescence vs incandescence** — Confusion: "all light is the same." Clarification: luminescence needs no heat (firefly); incandescence needs heat (bulb filament).

3 **Singlet vs triplet** — Confusion: mixing up which has paired spins. Clarification: **S**inglet = **S**ame? No — singlet = oppo**s**ite spins (paired); triplet = same spins (unpaired). Triplet lives longer.

4 **Excitation vs emission wavelength** — Confusion: which is longer? Clarification: **emission is always longer** (Stokes shift). The molecule loses energy as heat in between, so the light out is weaker.

5 **Quenching vs self-absorption** — Confusion: "both reduce signal at high concentration." Clarification: quenching = energy lost by collision/complex formation; self-absorption (inner filter effect) = emitted light re-absorbed by the sample on its way out.

6 **Dynamic vs static quenching and temperature** — Confusion: "heat always increases quenching." Clarification: heat increases **dynamic** quenching (more collisions) but decreases **static** quenching (breaks the dark complex).

7 **90° vs 180° geometry** — Confusion: "why not put the detector straight through like UV-Vis?" Clarification: straight through, the blinding excitation beam would flood the detector; at 90° only the weak sideways fluorescence is collected.

## 1.16 Common Mistakes

1 Using a **glass** cuvette for UV excitation — glass absorbs UV; always use **quartz**.

2 Forgetting to **control pH** — the same drug can be bright at one pH and dark at another; always use a buffer.

3 Measuring at **too high a concentration** — the calibration graph curves due to self-quenching and self-absorption; dilute the sample.

4 Forgetting **dissolved oxygen** — $O_2$ quenches fluorescence; bubble nitrogen through the solution when the method demands it.

5 Letting the **temperature drift** during measurement — fluorescence drops as temperature rises; keep it constant.

6 Saying fluorescence is "reflected light" — it is **emitted** light from an excited molecule, not reflection.

7 Plotting the Stern–Volmer graph with wrong axes — it is **$F_0/F$ (y-axis) vs [Q] (x-axis)**, giving a straight line of slope Ksv and intercept 1.

8 Confusing the **excitation spectrum** with the **emission spectrum** — excitation spectrum tells which wavelength switches the sample on; emission spectrum tells which wavelengths come out.

## 1.17 Mnemonics

1 **Jablonski five steps — "A I F I P":** "**A**ll **I**ndians **F**ight **I**n **P**unjab" → **A**bsorption, **I**nternal conversion, **F**luorescence, **I**ntersystem crossing, **P**hosphorescence.

2 **Six factors affecting fluorescence — "My Smart Professor Takes Only Coffee":** **M**olecular structure, **S**olvent, **p**H (Professor), **T**emperature, **O**xygen (dissolved), **C**oncentration.

3 **Instrument order — "Lucky Elephants See Every Delicious Rose":** **L**ight source, **E**xcitation monochromator, **S**ample cell, **E**mission monochromator, **D**etector (PMT), **R**eadout.

4 **Fluorescence vs phosphorescence:** "**F**ast **F**luorescence from **S**inglet; **P**rolonged **P**hosphorescence from **T**riplet."

5 **Quenching types:** "**D**ynamic = **D**ashing collisions (worse when hot); **S**tatic = **S**tuck complex (breaks when hot)."

## 1.18 Exam Summary

Fluorometric analysis studies **photoluminescence** — light emitted after a molecule absorbs photons. **Fluorescence** is the fast emission (nanoseconds) from the **singlet** excited state that stops instantly when the light source is removed; **phosphorescence** is the slow emission (seconds to hours) from the **triplet** state that keeps glowing. The **Jablonski diagram** maps the five transitions in order: absorption ($S_0 \rightarrow S_1$), internal conversion (heat loss within $S_1$), fluorescence ($S_1 \rightarrow S_0$, light out), intersystem crossing ($S_1 \rightarrow T_1$, spin flip, no light), phosphorescence ($T_1 \rightarrow S_0$, slow light out). Because of heat loss, emitted light always has a **longer wavelength** than absorbed light (**Stokes shift**). Fluorescence brightness depends on six factors — **molecular structure** (rigid, conjugated, electron-donating groups help), **pH**, **solvent** (viscosity, polarity), **temperature** (heat dims), **dissolved oxygen** (quenches), and **concentration** (too high causes self-quenching and self-absorption). **Quenching** is any dimming process: **dynamic** (collisional, worse when hot) vs **static** (dark complex, better when hot), related to quencher concentration by the **Stern–Volmer equation $F_0/F = 1 + Ksv[Q]$** (straight-line plot, slope = Ksv). The **spectrofluorometer** consists of light source → excitation monochromator → quartz sample cell → emission monochromator → PMT detector → readout, with the detector at **90°** (unlike UV-Vis at 180°) so the bright excitation beam never enters the detector. Fluorometry is **10–1000× more sensitive** than UV-Vis because it measures a signal against a dark background. Pharmaceutical uses: naturally fluorescent drugs (**quinine, riboflavin, tetracycline, morphine**), **derivatization** (**thiamine → thiochrome**, catecholamines like adrenaline, penicillins with fluorescamine), **vitamin assays**, and **trace impurity analysis**. **Quantum yield** (Φ = emitted ÷ absorbed photons; quinine sulfate standard Φ = 0.546) measures fluorescence efficiency.

## 1.19 One-Minute Revision

- Cold light = **luminescence**; hot light = incandescence.

- **Fluorescence:** fast, singlet, stops instantly. **Phosphorescence:** slow, triplet, keeps glowing.
- Jablonski order: **A–I–F–I–P** (Absorption, Internal conversion, Fluorescence, Intersystem crossing, Phosphorescence).
- Singlet = opposite spins; triplet = same spins.
- Emission wavelength **always longer** than excitation (Stokes shift).
- 6 factors: **Structure, Solvent, pH, Temperature, Oxygen, Concentration** ("My Smart Professor Takes Only Coffee").
- Quenching: dynamic = collisions (worse hot); static = dark complex (better hot).
- Stern–Volmer: **$F_0/F = 1 + Ksv[Q]$** — straight line, slope = Ksv.
- Instrument: **Lamp → Excitation selector → Quartz cell → Emission selector → PMT → Screen** ("Lucky Elephants See Every Delicious Rose"); detector at **90°**.
- Quartz cuvette, buffer the pH, remove $O_2$, keep temperature constant, work dilute.
- More sensitive than UV-Vis: candle in a dark room vs floodlight dimming by 1%.
- Quinine, riboflavin = natural; thiamine → thiochrome = derivatization; quantum yield standard Φ = 0.546.

---

## 1.20 Exam Questions

### Short-answer questions

1 Define fluorescence and phosphorescence. Give one example of each.

2 Draw a labelled Jablonski diagram and name the five transitions in order.

3 What is the difference between a singlet state and a triplet state?

4 Define quantum yield. What is the quantum yield of the quinine sulfate standard?

5 State the Stern–Volmer equation and explain each term in words.

6 Distinguish between collisional (dynamic) and static quenching.

7 Why is the detector placed at 90° in a spectrofluorometer but at 180° in a UV-Vis spectrophotometer?

8 Why must quartz (and not glass) cuvettes be used in fluorometry?

9 Explain why fluorometry is more sensitive than UV-Vis spectroscopy.

10 What is derivatization? Give two pharmaceutical examples.

### MCQs (with answer key)

**1. The glow of a firefly is an example of:** (a) Incandescence (b) Luminescence (c) Phosphorescence (d) Chemiluminescence only

**2. Fluorescence originates from the:** (a) Triplet excited state (b) Singlet excited state (c) Ground state (d) Ionised state

**3. The correct order of events in the Jablonski diagram is:** (a) Absorption → Fluorescence → Internal conversion → Intersystem crossing → Phosphorescence (b) Absorption → Internal conversion → Fluorescence → Intersystem crossing → Phosphorescence (c) Absorption → Intersystem crossing → Internal conversion → Phosphorescence → Fluorescence (d) Internal conversion → Absorption → Fluorescence → Phosphorescence → Intersystem crossing

**4. The Stern–Volmer equation is:** (a) $F_0/F = 1 + Ksv[Q]$ (b) $F/F_0 = 1 - Ksv[Q]$ (c) $F_0 - F = Ksv + [Q]$ (d) $F_0/F = Ksv/[Q]$

**5. In a spectrofluorometer, the detector is placed at:** (a) 180° to the excitation beam (b) 90° to the excitation beam (c) 45° to the excitation beam (d) 0° to the excitation beam

**6. Which group INCREASES fluorescence when attached to an aromatic ring?** (a) $–NO_2$ (b) –COOH (c) $–NH_2$ (d) –Cl

**7. Dissolved oxygen in a fluorescent solution acts as a:** (a) Sensitizer (b) Quencher (c) Standard (d) Buffer

**8. The quantum yield of quinine sulfate (standard) in dilute sulphuric acid is:** (a) 1.000 (b) 0.546 (c) 0.100 (d) 0.050

**9. Thiamine (vitamin $B_1$) is estimated fluorometrically after conversion to:** (a) Riboflavin (b) Thiochrome (c) Quinine (d) Fluorescein

**10. At very high sample concentrations, fluorescence intensity:** (a) Keeps increasing linearly (b) Falls due to self-quenching and self-absorption (c) Remains constant (d) Becomes phosphorescence

**Answer key:** 1-(b), 2-(b), 3-(b), 4-(a), 5-(b), 6-(c), 7-(b), 8-(b), 9-(b), 10-(b)

## Viva questions (with one-line model answers)

1. **What is fluorescence?** — Immediate light emission from the singlet excited state that stops the moment the light source is removed.
2. **How does phosphorescence differ?** — It is delayed emission from the triplet state that continues glowing after the light source is removed.
3. **What is the Jablonski diagram?** — An energy-level map showing $S_0$, $S_1$, $S_2$, $T_1$ and the five transitions between them.
4. **What is Stokes shift?** — The difference between emission and excitation wavelengths; emission is always at longer wavelength.
5. **Name the parts of a spectrofluorometer in order.** — Light source, excitation monochromator, sample cell, emission monochromator, PMT detector, readout.
6. **Why quartz cuvette?** — Glass absorbs UV light; quartz does not.
7. **What is quenching? Give one example.** — Any process that reduces fluorescence; e.g., dissolved oxygen.
8. **Why is fluorometry more sensitive than UV-Vis?** — It measures a signal against a near-zero (dark) background, while UV-Vis measures a small difference between two large signals.

## Application-based questions

1 A QC chemist finds that a riboflavin syrup gives a weaker fluorescence reading at 40 °C than at 25 °C. Explain why, and state what precaution the method should include. *(Temperature increases molecular collisions → more non-radiative energy loss → dimmer fluorescence. Precaution: measure at constant, controlled temperature.)*

2 A new tablet contains a drug that does not fluoresce. The analyst still wants to use fluorometry for its assay. Suggest a strategy with a real example. *(Derivatization — convert the drug to a fluorescent product; e.g., thiamine oxidised to thiochrome, or reaction with fluorescamine.)*

3 Two samples of the same fluorescent drug are prepared — one in water, one in glycerol. Which will give the stronger signal and why? *(Glycerol — higher viscosity reduces molecular collisions, so less energy is lost as heat.)*

4 An analyst measures fluorescence at a very high concentration and the calibration graph bends downwards. Name the two phenomena responsible and state the remedy. *(Self-quenching and self-absorption (inner filter effect); remedy: dilute the sample and work in the linear low-concentration region.)*

## Numericals with worked solutions

**Numerical 1 — Quantum yield (relative method).** A new fluorescent compound X (in water) is compared with the quinine sulfate standard (Φ = 0.546 in dilute sulphuric acid). Both solutions are adjusted to nearly equal absorbance at the excitation wavelength. Data: Standard — absorbance A(st) = 0.050, integrated fluorescence intensity F(st) = 85,000, refractive index n(st) = 1.33. Sample X — absorbance A(x) = 0.048, integrated fluorescence intensity F(x) = 42,500, refractive index n(x) = 1.33. Calculate the quantum yield of X.

**Numerical 2 — Stokes shift.** Quinine absorbs at 350 nm and emits at 450 nm. Calculate the Stokes shift in nm.

**Numerical 3 — Stern–Volmer.** The fluorescence intensity of a drug solution without quencher is $F_0$ = 100 units. After adding iodide ions to a concentration of 0.020 mol/L, the intensity falls to F = 50 units. Calculate the Stern–Volmer constant Ksv.

### CALCULATION BOX 1 — Quantum yield of a sample vs the quinine sulfate standard (relative method) **Formula (relative method):** $\Phi(x) = \Phi(st) \times [F(x)/F(st)] \times [A(st)/A(x)] \times [n(x)^2/n(st)^2]$ where Φ = quantum yield, F = integrated fluorescence intensity, A = absorbance at excitation wavelength, n = refractive index of solvent. Subscript (st) = standard, (x) = unknown sample. **Step 1 — Write down the known values.** Φ(st) = 0.546; F(x) = 42,500; F(st) = 85,000; A(st) = 0.050; A(x) = 0.048; n(x) = n(st) = 1.33. **Step 2 — Calculate the intensity ratio F(x)/F(st).** 42,500 ÷ 85,000 = **0.500**. (The sample emits half the total fluorescence of the standard.) **Step 3 — Calculate the absorbance ratio A(st)/A(x).** 0.050 ÷ 0.048 = **1.0417**. (We divide standard by sample because a higher absorbance means more photons were available; this corrects for the slightly different light absorbed.) **Step 4 — Calculate the refractive-index correction $[n(x)^2/n(st)^2]$.** Both solvents have n = 1.33, so $(1.33^2)/(1.33^2)$ = **1.000**. (No correction needed — always use the same or matched solvents when possible.) **Step 5 — Multiply everything.** Φ(x) = 0.546 × 0.500 × 1.0417 × 1.000 = 0.546 × 0.52085 = **0.284**. **Step 6 — Check the answer.** Quantum yield must lie between 0 and 1 → 0.284 is valid. ✔ **Interpretation:** Compound X converts about **28.4%** of the photons it absorbs into fluorescence — roughly half as efficient as the quinine sulfate standard (54.6%). The rest of the energy is lost as heat through internal conversion and other non-radiative paths.

**Worked solutions — Numerical 2 (Stokes shift):** Stokes shift = λ(emission) − λ(excitation) = 450 nm − 350 nm = **100 nm**. The emitted light is 100 nm "redder" (longer wavelength, lower energy) than the absorbed light because of heat lost by internal conversion.

**Worked solution — Numerical 3 (Stern–Volmer):** $F_0/F = 1 + Ksv[Q] \rightarrow 100/50 = 1 + Ksv(0.020) \rightarrow 2 = 1 + 0.020 \cdot Ksv \rightarrow 0.020 \cdot Ksv = 1 \rightarrow Ksv = 1 \div 0.020 =$ **50 L/mol ($M^{-1}$)**. Interpretation: the plot of $F_0/F$ vs [Q] is a straight line of slope 50 $M^{-1}$ — a large slope means iodide is a strong quencher for this drug.

## 1.21 References


- Skoog, Holler, Crouch — *Principles of Instrumental Analysis* (Cengage Learning). [Fluorescence theory, Jablonski diagram, quenching and Stern–Volmer relationship, spectrofluorometer instrumentation.]
- Watson — *Pharmaceutical Analysis* (Elsevier). [Pharmaceutical applications of fluorometry: quinine, riboflavin, thiamine/thiochrome, catecholamines, trace analysis.]
- Harvey — *Modern Analytical Chemistry* (McGraw-Hill). [Quantitative aspects: calibration, quantum yield, sensitivity comparison of methods.]
- Sharma — *Organic Spectroscopy* (S. Chand). [Effect of molecular structure, conjugation and substituents on fluorescence.]
- Braun — *Analytical Chemistry: A Modern Approach to Analytical Science* (McGraw-Hill). [General principles of molecular spectroscopy.]

# CHAPTER 2 — Flame Photometry, Atomic Absorption Spectrometry and Nepheloturbidometry

## 2.1 Learning Objectives

By the end of this chapter, you will be able to:

1 Explain the theory of **flame photometry** (thermal excitation → emission of characteristic light) in simple words.

2 Describe **nebulisation** and why the sample must enter the flame as a fine mist.

3 Name common **fuel–oxidant combinations**, arrange their flames from coolest to hottest, and say which elements need which flame.

4 List the three main **interferences in flame photometry** (spectral, chemical, ionization) and give one remedy for each.

5 Draw the **block diagram of a flame photometer** and explain each part.

6 Give **pharmaceutical applications** of flame photometry (Na, K, Ca in syrups and formulations).

7 Explain the theory of **atomic absorption spectrometry (AAS)** and compare it with flame photometry (emission vs absorption).

8 Draw the **block diagram of an AAS instrument** and explain the **hollow cathode lamp** and the two atomizers (flame, graphite furnace).

9 List **interferences in AAS** and state how they are handled.

10 Give **pharmaceutical applications of AAS** (trace metals; lead/cadmium limit testing).

11 Present a **comparison table** of flame photometry vs AAS.

12 Explain the **Tyndall effect**, distinguish **nephelometry** (90° scattered light) from **turbidimetry** (transmitted light), and give applications (sulfate/chloride limit tests, bacterial growth, protein assays).

13 Solve a **calibration-curve numerical** (find an unknown concentration from an emission reading by interpolation), showing every step.

## 2.2 Definitions

- **Flame photometry (flame emission spectroscopy):** A technique in which the sample is sprayed into a flame; heat excites the atoms, and the **light they emit** (each element has its own colour) is measured. The brightness tells the concentration.

- **Thermal excitation:** Atoms gaining energy from **heat** (the flame), so their electrons jump to higher orbits.
- **Emission:** Light **given out** by excited atoms when their electrons fall back to lower orbits.
- **Nebulisation:** Converting the liquid sample into a **very fine mist** (tiny droplets) so it enters the flame smoothly and evenly.
- **Flame temperature:** How hot the flame is; decided by the **fuel + oxidant** combination. Hotter flames can excite harder-to-excite elements.
- **Fuel:** The gas that burns (e.g., natural gas, propane, acetylene).
- **Oxidant:** The gas that supplies oxygen for burning (e.g., air, nitrous oxide $N_2O$).
- **Interference:** Anything that makes the reading wrongly high or low (spectral, chemical, ionization types).
- **Releasing agent:** A chemical (e.g., lanthanum or strontium salt) added to free the element from a stable compound that refuses to break in the flame.
- **Ionization suppressant (ionization buffer):** An easily ionised salt (e.g., potassium or cesium chloride) added in excess to stop the sample atoms from losing electrons in a hot flame.
- **Atomic absorption spectrometry (AAS):** A technique in which **ground-state atoms** in a flame or furnace **absorb light** from a special lamp; the amount absorbed tells the concentration (Beer–Lambert law).
- **Ground-state atoms:** Atoms in their normal, lowest-energy state — the state in which AAS measures them.
- **Resonance radiation (resonance line):** Light of exactly the wavelength an element's own atoms absorb or emit (e.g., 589 nm for sodium).
- **Hollow cathode lamp (HCL):** A special lamp whose cathode is made of the **element being analysed**; it emits that element's own sharp resonance lines.
- **Atomizer:** The device that converts the sample into free ground-state atoms — either a **flame** or a **graphite furnace**.
- **Graphite furnace:** An electrically heated graphite tube that atomizes the sample at very high temperature; far more sensitive than a flame, but slower.
- **Background absorption:** Unwanted absorption by smoke, molecules or particles in the flame; corrected using a **deuterium lamp**.
- **Tyndall effect:** When a beam of light passes through a suspension of fine particles, the particles **scatter** the light and the beam becomes visible (like sunlight in a dusty room).
- **Nephelometry:** Measuring the light **scattered** by suspended particles, detected at **90°** to the beam. Best for **dilute** suspensions.
- **Turbidimetry:** Measuring the **decrease in transmitted light** (light passing straight through), detected at **180°**. Best for **concentrated** suspensions.
- **Turbidity:** The cloudiness of a liquid caused by suspended particles.

- **Limit test:** A pharmacopoeial test that checks whether an impurity is **below** an allowed maximum (it does not find the exact amount, only pass/fail against a standard turbidity).
- **Suspension:** A liquid containing fine solid particles floating in it (they do not dissolve).

**Key takeaway:** Flame photometry = heat makes atoms **emit** light (measure the glow). AAS = lamp light is **absorbed** by cool atoms (measure the dimming). Nepheloturbidometry = particles **scatter** light (measure the scattering or the dimming).

## 2.3 Key Terminology (Mini-Glossary)

| Term | Simple meaning |
|---|---|
| Characteristic wavelength | The one colour (wavelength) each element emits or absorbs — its "fingerprint" (Na 589 nm, K 766 nm, Ca 622 nm, Li 671 nm). |
| Pneumatic nebuliser | A nebuliser that uses a fast stream of compressed gas to blast the liquid into a fine mist. |
| Mixing chamber | A chamber where the sample mist mixes with fuel and oxidant gases before burning. |
| Burner | The nozzle where the gas mixture burns as a steady flame. |
| Emission intensity | How bright the emitted light is — directly proportional to concentration (at low concentrations). |
| Self-absorption (flame) | At high concentration, cool atoms at the flame edge absorb the emitted light — the graph curves. |
| Spectral interference | Two elements emitting at nearly the same wavelength, so the detector cannot separate them. |
| Chemical interference | The element forms a stable compound (e.g., calcium phosphate) that does not break into atoms in the flame. |
| Ionization interference | In a very hot flame, atoms lose an electron and become ions, which emit a different (weaker) signal. |
| Chopper | A rotating disc in AAS that blinks the lamp light on and off, so the detector can separate lamp light from flame glow. |
| Deuterium lamp | A background-correction lamp in AAS that measures unwanted absorption by smoke/particles. |
| Refractory compound | A very stable compound (e.g., calcium phosphate, aluminium oxide) that refuses to break apart in cooler flames. |
| Matrix effect | The rest of the sample (salts, acids, viscosity) changing how well the sample sprays or atomizes. |
| Scattered light | Light bounced off in all directions by suspended particles (Tyndall effect). |
| Transmitted light | Light that passes straight through the sample to the detector on the other side. |
| Immunoturbidimetry | A protein assay in which antibody–antigen clumps make the solution turbid; the turbidity measures the protein amount. |
| Calibration curve | A graph of instrument reading vs known concentrations of standards, used to find unknown concentrations. |

| Term | Simple meaning |
|---|---|
| Interpolation | Reading an unknown value from **between** two known points on a calibration curve. |

## 2.4 Detailed Explanations (Concepts with Analogies)

### 2.4.1 Flame photometry — theory (thermal excitation → characteristic emission)

- **Technical concept:** The sample solution is nebulised into a flame. Heat evaporates the solvent, melts and vaporizes the solid, and produces free atoms. Thermal energy pushes outer (valence) electrons to higher orbits (**excitation**). The excited state is unstable, so electrons fall back within nanoseconds, giving out light of a **characteristic wavelength** for that element. Emission intensity is proportional to the number of excited atoms, hence to concentration.
- **Simple explanation:** Spray salty water into a flame and the flame turns **yellow** — that yellow is sodium atoms glowing. Each element paints the flame its own colour: sodium yellow (589 nm), potassium violet (766 nm), calcium brick-red (622 nm), lithium crimson-red (671 nm). The deeper the colour (brighter the glow), the more of that element is present — like judging how much turmeric is in milk by how yellow it looks.
- **Example:** A drop of table-salt solution in a Bunsen flame gives the golden-yellow sodium flame every chemistry student has seen.
- **Key takeaway:** Flame + sample → element-specific coloured glow → brightness = concentration.

### 2.4.2 Nebulisation

- **Technical concept:** A pneumatic nebuliser uses high-speed oxidant gas to shatter the liquid sample into droplets ~10 micrometres in size; large droplets drain away, and only the fine mist reaches the flame.
- **Simple explanation:** You cannot pour a glass of water on a candle and expect it to keep burning nicely — but a **perfume spray** (fine mist) burns smoothly. Nebulisation is the perfume-spray step: it turns the sample into a fine, even mist so the flame stays steady and every drop behaves the same.
- **Example:** The "spray chamber" in a flame photometer discards big droplets down a drain and sends only the finest mist to the burner.
- **Key takeaway:** Fine mist = steady flame = reliable reading. Thick, viscous samples spray poorly — dilute them.

### 2.4.3 Flame and flame temperature (fuel/oxidant combinations)

- **Technical concept:** Different fuel–oxidant pairs give different temperatures. Easily excited alkali metals (Na, K) need only cool flames; elements forming refractory compounds (Ca, Al) need the hottest flames.
- **Simple explanation:** Like cooking — you simmer dal on a low flame but need a roaring flame to sear. From coolest to hottest:

1. **Natural gas + air ≈ 1700 °C** — coolest; enough for sodium and potassium (easy to excite).
2. **Propane + air ≈ 1925 °C** — medium; general-purpose work.
3. **Acetylene + air ≈ 2300 °C** — hot; for calcium and many other elements.
4. **Acetylene + nitrous oxide ($N_2O$) ≈ 2900–3000 °C** — hottest; for elements that form very stable oxides (aluminium, refractory elements).

- **Example:** Sodium in ORS is measured with a cool natural-gas flame; calcium (which forms stable compounds) needs the hotter acetylene–air flame.
- **Key takeaway:** Cool flame for Na/K; hot flame for Ca and stubborn elements. Hotter is not always better — too hot causes ionization interference.

### 2.4.4 Characteristic wavelengths (the element's fingerprint)

- **Technical concept:** Each element emits at fixed wavelengths: Na 589 nm (yellow), K 766 nm (violet), Ca 622 nm (orange-red), Li 671 nm (red). A filter or monochromator isolates this wavelength.
- **Simple explanation:** Just as every person has a unique fingerprint, every element has a unique colour of glow. The instrument wears "coloured glasses" (filter) that let only that one colour through to the detector.
- **Key takeaway:** Memorise Na 589, K 766, Ca 622, Li 671 nm.

### 2.4.5 Interferences in flame photometry (three types + remedies)

1. **Spectral interference.**

- *What happens:* Two elements emit at nearly the same wavelength, so the detector reads both together (wrongly high result).
- *Simple explanation:* Two radio stations on almost the same frequency — you hear both at once.
- *Remedy:* Use a **narrower filter or a monochromator** to separate the wavelengths.

1. **Chemical interference.**

- *What happens:* The element forms a very stable compound in the flame (e.g., calcium + phosphate → **calcium phosphate**) that does not break into free atoms → wrongly low result.
- *Simple explanation:* The calcium is "locked in a safe" (calcium phosphate) and the flame key is too weak to open it.
- *Remedy:* Use a **hotter flame**, or add a **releasing agent** (lanthanum/strontium salt) that grabs the phosphate and frees the calcium.

1. **Ionization interference.**

- *What happens:* In a very hot flame, some atoms lose an electron and become **ions**; ions emit a different, weaker signal → wrongly low atomic emission.
- *Simple explanation:* The flame is so hot it not only excites the atoms but knocks an electron clean off — like overheating milk until it burns.

- *Remedy:* Add an **ionization suppressant** — a large excess of an easily ionised element (potassium or cesium salt). It supplies the electrons and protects the sample atoms.
- **Key takeaway:** Spectral → separate with monochromator; chemical → hotter flame or releasing agent; ionization → add K/Cs suppressant.

### 2.4.6 Flame photometer — instrumentation

- **Technical concept:** Five parts in order: nebuliser → burner (with mixing chamber) → monochromator/filter → detector → readout. There is **no light source** — the flame itself makes the light.
- **Simple explanation, each part:**

1. **Nebuliser:** Turns the sample into a fine mist (the perfume spray).
2. **Burner (+ mixing chamber):** Mixes mist with fuel and oxidant and burns it as a steady flame — the "stage" where atoms glow.
3. **Monochromator or filter:** The "coloured glasses" that pick out only the element's characteristic wavelength.
4. **Detector (photocell/photodiode):** Converts the light into an electric current.
5. **Readout:** Displays the emission intensity number, converted to concentration via a calibration curve.

- **Key takeaway:** Mist → flame → colour-picker → eye → number. No lamp needed — the sample is its own light source.

### 2.4.7 Pharmaceutical applications of flame photometry

- **Sodium and potassium in ORS (oral rehydration salts):** ORS must contain exact $Na^+$/$K^+$ amounts — flame photometry is the classic, fast QC method.
- **Sodium/potassium in syrups and injections:** Electrolyte syrups, IV fluids and antacid formulations are checked for Na/K content.
- **Calcium in formulations:** Calcium supplements, antacid tablets (calcium carbonate) and calcium syrups.
- **Lithium:** QC of lithium carbonate tablets (used in psychiatry) and monitoring lithium levels.
- **Why it fits pharma QC:** Fast, cheap, simple — ideal for routine batch testing of alkali and alkaline-earth metals.

### 2.4.8 AAS — theory (ground-state atoms absorb resonance radiation)

- **Technical concept:** In AAS the sample is converted to **free ground-state atoms** (flame or graphite furnace). A **hollow cathode lamp** shines the element's own resonance wavelength through this atomic cloud. Ground-state atoms **absorb** exactly this wavelength, and the drop in light (absorbance) follows the **Beer–Lambert law**: $A = \varepsilon bc$ — absorbance is proportional to concentration.
- **Simple explanation:** Shine a torch through smoke: the thicker the smoke, the dimmer the torch looks on the other side. Here the "smoke" is a cloud of the element's atoms, and the "torch" is a lamp shining

the element's favourite colour. More atoms → more light swallowed → higher absorbance → higher concentration. Crucially, AAS measures atoms in their **normal (ground) state**, not excited ones.

- **Example:** To find lead in a tablet, a lead hollow cathode lamp shines 283 nm light through the atom cloud; lead atoms absorb it; the absorbance tells the lead concentration.
- **Key takeaway:** Flame photometry measures light **given out** by hot excited atoms; AAS measures light **swallowed** by cool ground-state atoms.

### 2.4.9 Emission vs absorption — the core comparison

- **Technical concept:** Flame photometry: excited-state atoms → emission measured; no external light source. AAS: ground-state atoms → absorption of lamp radiation measured; needs an element-specific lamp.
- **Simple explanation:** Flame photometry is like counting **fireflies** on a dark night (you see the light they make). AAS is like counting **birds flying across the sun** (you see the shadows they cast on a bright background). Fireflies are few (only a tiny fraction of atoms get excited), but shadows are cast by every bird (nearly all atoms are in the ground state) — that is why AAS is more sensitive.
- **Key takeaway:** Emission = few excited atoms glow (less sensitive). Absorption = almost all atoms absorb (more sensitive, more selective).

### 2.4.10 AAS — instrumentation

- **Technical concept:** Six parts in order: hollow cathode lamp → atomizer (flame or graphite furnace) → chopper → monochromator → detector (PMT) → readout.
- **Simple explanation, each part:**

1. **Hollow cathode lamp (HCL):** A lamp whose cup-shaped cathode is made of the **element you want to measure** (e.g., a lead cathode for lead analysis). When electrified, it emits that element's own sharp resonance lines — a "personal torch" for each element. One lamp per element (multi-element lamps exist but are less common).
2. **Atomizer — flame:** Sample mist burns in an air–acetylene (or $N_2O$–acetylene) flame, creating the ground-state atomic cloud. Fast and simple.
3. **Atomizer — graphite furnace:** A tiny graphite tube heated electrically to ~2500 °C; a microlitre drop of sample is dried, ashed and atomized inside. **100–1000× more sensitive** than flame (parts-per-billion level) but slower — used for trace metals like lead and cadmium.
4. **Chopper:** A spinning slotted disc that blinks the lamp beam on and off many times per second, so the electronics can distinguish the lamp's light from the flame's own glow.
5. **Monochromator:** Isolates the resonance line from other lamp lines.
6. **Detector (PMT) + readout:** Measures the transmitted light and displays absorbance/concentration.

- **Key takeaway:** Personal lamp per element → atomic cloud (flame or furnace) → blink-separator (chopper) → wavelength-picker → detector → number.

### 2.4.11 Interferences in AAS

1. **Chemical interference:** Stable compounds (refractory oxides, phosphates) that do not atomize → use hotter flame/furnace, releasing agents, or matrix modifiers.
2. **Ionization interference:** As in flame photometry → add ionization suppressant (K/Cs salt).
3. **Spectral interference:** Overlapping lines from another element → choose an alternative resonance line or use background correction.
4. **Background absorption:** Smoke, unburnt particles and molecules absorb broadly → corrected with a **deuterium lamp** (measures background only; the instrument subtracts it).
5. **Matrix effects:** Acids, salts and viscosity change nebulisation/atomization → match the matrix of standards and samples, or use the **standard-addition method**.

- **Key takeaway:** AAS interferences mirror flame photometry's, plus background absorption (fixed by the deuterium lamp).

### 2.4.12 Pharmaceutical applications of AAS

- **Trace heavy metals in raw materials and excipients:** Lead, cadmium, arsenic and mercury must be below strict safety limits — graphite-furnace AAS detects them at parts-per-billion levels.
- **Lead/cadmium limit testing:** Pharmacopoeial limit tests for Pb and Cd in bulk drugs, herbal materials and colourants use AAS for a truly quantitative result.
- **Mineral assays:** Zinc, iron, magnesium and calcium in multivitamin/mineral tablets.
- **Catalyst residues:** Traces of metal catalysts (e.g., palladium, platinum, nickel) left over from drug synthesis must be quantified — AAS is the standard tool.
- **Water for pharmaceutical use:** Checking purified water and water-for-injection for trace metals.

### 2.4.13 Nepheloturbidometry — principle (Tyndall effect)

- **Technical concept:** When light passes through a suspension of fine particles, the particles **scatter** light in all directions (Tyndall effect). The scattered light (nephelometry) or the reduction in transmitted light (turbidimetry) is proportional to particle concentration.
- **Simple explanation:** Shine a torch through clean water — you see nothing. Stir a spoon of milk into it and the beam becomes visible — the milk particles scatter the light. That visible beam is the Tyndall effect. Cloudier liquid = more scattering = more particles.
- **Example:** Sunlight streaming through a dusty classroom — the dust makes the beam visible.
- **Key takeaway:** No particles → no scattering. More particles → more scattering → cloudier.

### 2.4.14 Nephelometry vs turbidimetry

- **Technical concept: Nephelometry** measures **scattered light at 90°** — best for **dilute** suspensions (very sensitive, like fluorometry's 90° logic). **Turbidimetry** measures the **loss of transmitted light at 180°** (straight through) — best for **concentrated** suspensions.

- **Simple explanation:** Nephelometry is like standing to the **side** of the dusty sunbeam and judging how bright the dust glows (works even with little dust). Turbidimetry is like standing **opposite** the torch and judging how dim it looks through the milky water (works when the water is quite milky).
- **Key takeaway:** Dilute/cloud-faint → nephelometry at 90°. Concentrated/cloudy → turbidimetry at 180°.

### 2.4.15 Nepheloturbidometer — instrumentation

- **Parts in order:** Light source (tungsten lamp) → lens/filter (makes a parallel beam) → sample cell → detector.
- **Nephelometer:** Detector at **90°** measures scattered light.
- **Turbidimeter:** Detector at **180°** (straight through) measures transmitted light.
- Essentially the same box — only the detector position (and sometimes the cell) changes.
- **Key takeaway:** One instrument, two detector positions: 90° = nephelometry, 180° = turbidimetry.

### 2.4.16 Applications of nepheloturbidometry

1. **Sulfate limit test:** Sample + barium chloride → white **barium sulfate** precipitate; the turbidity is compared with a standard — this is a classic IP limit test.
2. **Chloride limit test:** Sample + silver nitrate → white **silver chloride** turbidity, compared with a standard.
3. **Bacterial growth measurement:** As bacteria multiply, the broth gets cloudier; turbidimetry tracks growth without counting cells (used in antibiotic assays).
4. **Protein assays (immunoturbidimetry/nephelometry):** Antibody + antigen form tiny clumps that scatter light — used to measure proteins in blood serum in clinical labs.
5. **Water clarity:** Checking turbidity of purified water and water-for-injection.

## 2.5 Classifications / Tables

### Table 2.1 — Fuel–oxidant combinations (coolest → hottest)

| Fuel + oxidant | Approx. flame temperature | Used for |
|---|---|---|
| Natural gas + air | ~1700 °C (coolest) | Na, K — easily excited alkali metals |
| Propane + air | ~1925 °C | General-purpose flame photometry |
| Acetylene + air | ~2300 °C | Ca and most elements |
| Acetylene + nitrous oxide ($N_2O$) | ~2900–3000 °C (hottest) | Refractory-oxide-forming elements (Al and similar) |

### Table 2.2 — Interferences in flame photometry

| Type | What happens | Example | Remedy |
|---|---|---|---|
| Spectral | Two elements emit at nearly the same wavelength | Overlapping emission lines | Narrower filter / monochromator |
| Chemical | Stable compound forms; no free atoms | Calcium phosphate in the flame | Hotter flame; releasing agent (La/Sr salt) |
| Ionization | Atoms lose electrons in a very hot flame | K/Na signal drops in hot flames | Ionization suppressant (excess K or Cs salt) |

## Table 2.3 — Flame photometry vs atomic absorption spectrometry (AAS)

| Property | Flame photometry | AAS |
|---|---|---|
| Principle | **Emission**: heat excites atoms; measure the light they **give out** | **Absorption**: ground-state atoms **absorb** lamp light; measure the dimming |
| Atoms measured | **Excited-state** atoms (a tiny fraction) | **Ground-state** atoms (nearly all atoms) |
| Light source | **None** — the flame is the source | **Hollow cathode lamp** (one per element) |
| Follows | Emission intensity ∝ concentration | **Beer–Lambert law** ($A = \varepsilon bc$) |
| Sensitivity | Moderate (ppm level) | High — flame AAS (ppm); **graphite furnace (ppb level)** |
| Selectivity | Good (characteristic wavelength) | Excellent (element-specific lamp + resonance line) |
| Atomizer | Flame only | Flame **or** graphite furnace |
| Interferences | Spectral, chemical, ionization | Same three **plus** background absorption (deuterium-lamp correction) |
| Cost / speed | Cheap, fast, simple | Costlier, slower (especially furnace) |
| Best for | Routine **Na, K, Ca** in formulations (ORS, syrups) | **Trace metals**: Pb, Cd, As, catalyst residues |

## Table 2.4 — Nephelometry vs turbidimetry

| Property | Nephelometry | Turbidimetry |
|---|---|---|
| What is measured | **Scattered** light | **Loss of transmitted** light |
| Detector position | **90°** to the beam | **180°** (straight through) |
| Best for | **Dilute** suspensions (more sensitive) | **Concentrated** suspensions |
| Principle | Tyndall effect (side view of scattering) | Tyndall effect (dimming of beam) |
| Example use | Trace sulfate as $BaSO_4$; protein assays | Bacterial growth; very cloudy samples |

## Table 2.5 — Interferences in AAS (quick reference)

| Interference | Cause | Remedy |
|---|---|---|
| Chemical | Refractory compounds don't atomize | Hotter flame/furnace; releasing agents; matrix modifiers |
| Ionization | Atoms lose electrons in hot flame | Ionization suppressant (K/Cs salt) |
| Spectral | Overlapping lines | Alternative resonance line; background correction |

| Interference | Cause | Remedy |
|---|---|---|
| Background absorption | Smoke/particles absorb broadly | **Deuterium lamp** correction |
| Matrix effect | Acids/salts change spraying/atomization | Matrix matching; standard-addition method |

## 2.6 Mechanisms / Processes (Step-by-Step)

### Process A — Journey of a sample droplet in flame photometry (6 steps)

1. **Nebulisation:** Sample solution is blasted into a fine mist; big droplets drain away.
2. **Desolvation:** In the flame, heat evaporates the solvent — each droplet becomes a tiny solid particle.
3. **Vaporization:** The solid melts and vaporizes into gas molecules.
4. **Atomization:** Gas molecules break into **free atoms** (ground state).
5. **Excitation:** Heat pushes outer electrons of some atoms to higher orbits (**excited state**).
6. **Emission:** Electrons fall back in nanoseconds, giving out the element's **characteristic coloured light** → detector measures brightness → concentration from calibration curve.

### Process B — How an AAS measurement works (7 steps)

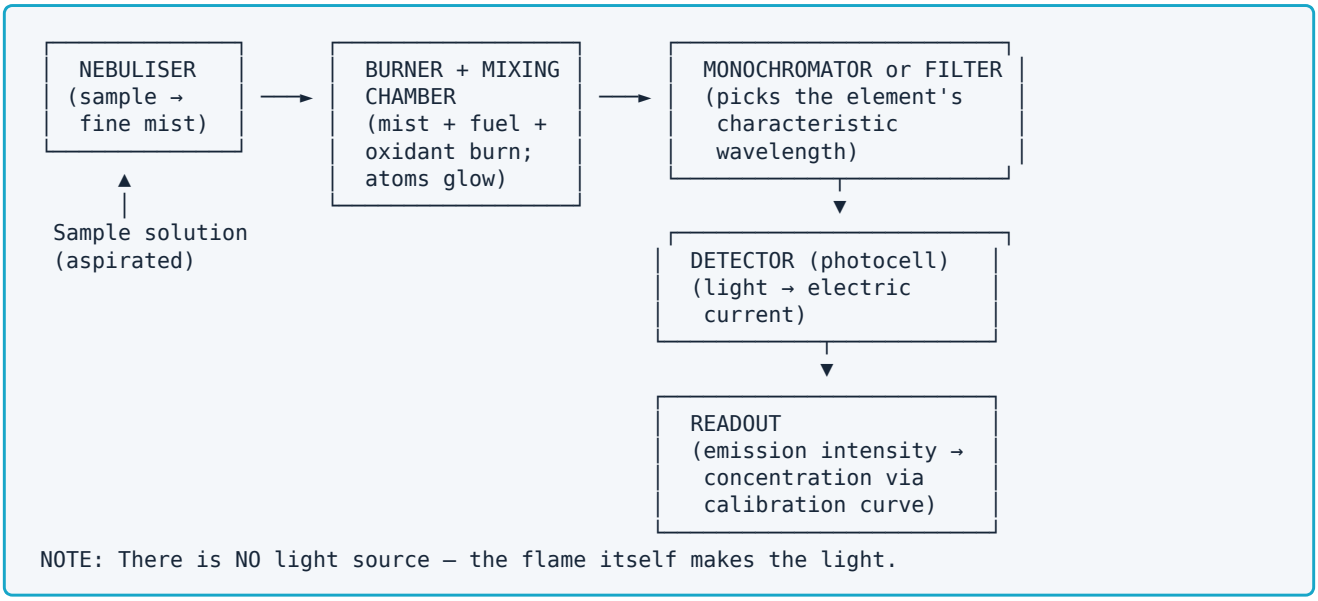
1. Switch on the **hollow cathode lamp** of the element to be measured (e.g., lead lamp for lead).
2. The lamp shines the element's **resonance wavelength** through the atomizer.
3. Aspirate the sample: nebuliser makes a mist → flame (or graphite furnace) creates a cloud of **ground-state atoms**.
4. Ground-state atoms **absorb** the resonance light; transmitted light becomes dimmer.
5. The **chopper** blinks the beam so the detector separates lamp light from flame glow.
6. The **monochromator** isolates the resonance line; the **PMT** measures transmitted intensity.
7. **Absorbance** is calculated ($A = \log(I_0/I)$) and converted to concentration via the calibration curve (Beer–Lambert law).

### Process C — Sulfate limit test by turbidimetry (5 steps)

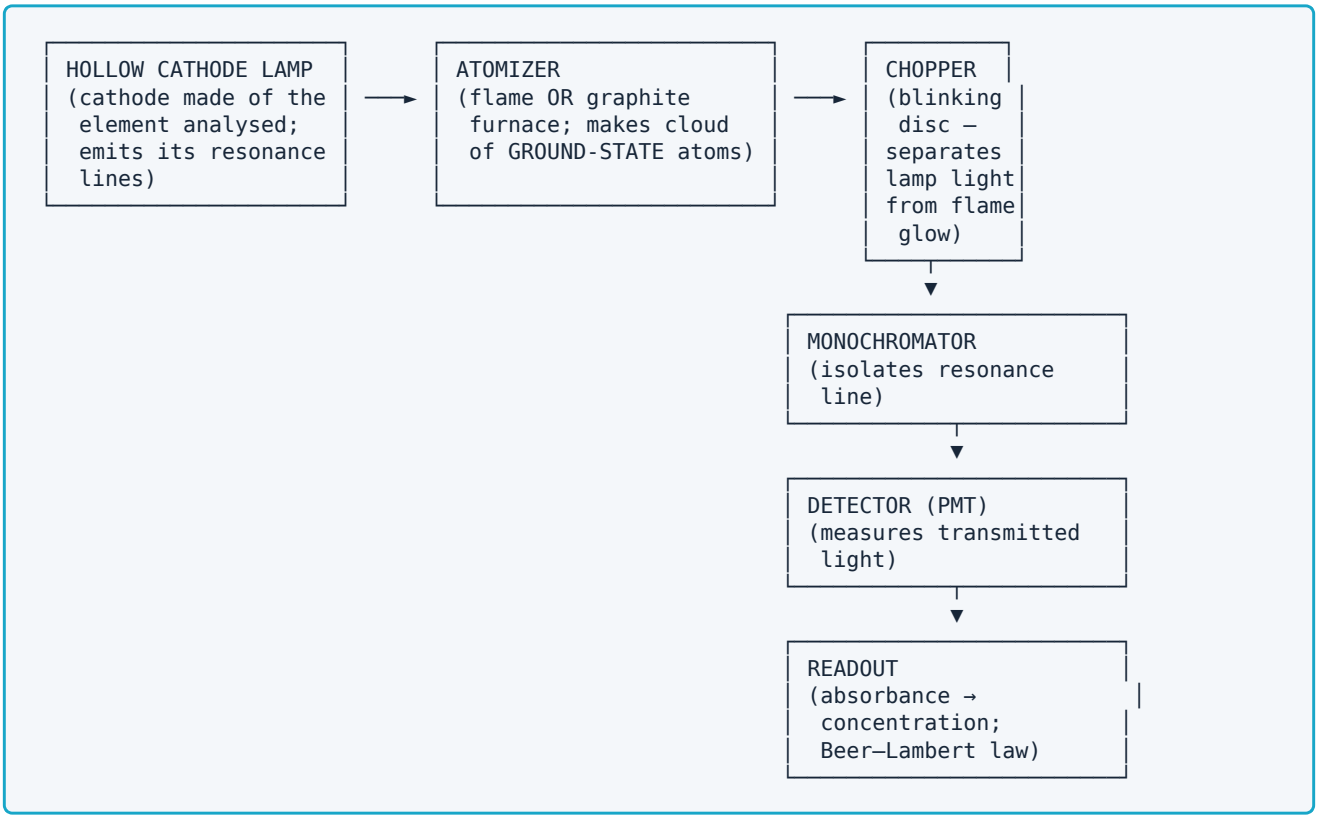
1. Dissolve the sample as per the pharmacopoeial method; prepare a **standard** sulfate solution at the allowed limit.
2. Add **barium chloride** solution to both — white **barium sulfate** particles form ($Ba^{2+} + SO_4^{2-} \rightarrow BaSO_4$).
3. Wait for the prescribed time so turbidity develops equally.
4. Measure turbidity (nephelometer/turbidimeter) or compare visually against a dark background.
5. **Pass/fail:** sample turbidity ≤ standard turbidity → **passes** (sulfate within limit); greater → **fails**.

## 2.7 Diagrams (Textual Block Diagrams, Labeled)

### Diagram 2.1 — Flame photometer (block diagram)




### Diagram 2.2 — Atomic absorption spectrometer (block diagram)




### Diagram 2.3 — Nephelometer vs turbidimeter (detector positions)

```
NEPHELOMETER (measures SCATTERED light — dilute suspensions):

  Light source ──►  [Sample cell] ──►  (transmitted beam, ignored)
                           │
                           │ scattered light (Tyndall effect)
                           ▼
                      Detector at 90°

TURBIDIMETER (measures TRANSMITTED light loss — concentrated suspensions):

  Light source ──►  [Sample cell] ──►  Detector at 180° (straight through)
```

## 2.8 Flowcharts (Textual)

### Flowchart 2.1 — Flame photometry or AAS? Choosing the right method

```
[Need to measure a metal in a pharma sample]
            │
            ▼
Is it Na, K, Ca or Li at routine (ppm) levels? ── YES ──► [Flame photometry —
            │ NO                                                fast & cheap]
            ▼
Is it a TRACE metal (Pb, Cd, As, catalyst residue)
at ppb levels? ── YES ──► [AAS with graphite furnace —
            │ NO                        most sensitive]
            ▼
Is it a metal at ppm levels needing high selectivity? ── YES ──► [Flame AAS]
            │ NO
            ▼
[Consider another technique — e.g., complexometric titration or ICP]
```

### Flowchart 2.2 — Nephelometry or turbidimetry?

```
[Cloudy / particle-containing sample]
            │
            ▼
Is the suspension DILUTE (faint cloudiness)? ── YES ──► [Nephelometry —
            │ NO                                          measure scattered
            ▼                                          light at 90°]
Is the suspension CONCENTRATED (clearly milky)? ── YES ──► [Turbidimetry —
            │ NO                                             measure transmitted
            ▼                                            light at 180°]
[Too clear for either — concentrate the sample or use another method]
```

### Flowchart 2.3 — Handling an interference in flame photometry / AAS

```
[Reading looks wrong — suspect interference]
            |
            ▼
Do TWO elements emit/absorb at nearly the same wavelength?
  — YES —► [SPECTRAL: use narrower monochromator / another line]
            | NO
            ▼
Does the element form a stable compound (phosphate, oxide)?
  — YES —► [CHEMICAL: hotter flame/furnace or releasing agent]
            | NO
            ▼
Is the flame very hot and the signal of an alkali metal low?
  — YES —► [IONIZATION: add K/Cs ionization suppressant]
            | NO
            ▼
[AAS only: is there smoke/background absorption?
  — YES —► [Use deuterium-lamp background correction]]
```

## 2.9 Infographic Concept Box

**One-page infographic: "Flame, Absorption & Cloudiness — Unit III at a Glance"**

*Imagine a single A4 page split into three colour zones:*

- **ORANGE zone — Flame Photometry:** A Bunsen flame painted in four coloured bands (yellow Na 589, violet K 766, brick-red Ca 622, crimson Li 671) with the six-step droplet journey (mist → dry → vaporize → atomize → excite → glow) as a vertical comic strip. A thermometer ladder shows the four fuel–oxidant flames from ~1700 °C to ~3000 °C. Three "villain" cards for interferences (Spectral , Chemical , Ionization ⚡), each with its one-line remedy.
- **BLUE zone — AAS:** A "personal torch" (hollow cathode lamp) shining through a cloud of atoms, with the tagline "Count the shadows, not the fireflies". A split panel contrasts flame atomizer (fast, ppm) vs graphite furnace (slow, ppb). A mini table: Flame photometry vs AAS across 6 rows.
- ☁ **GREY zone — Nepheloturbidometry:** A torch beam through milky water (Tyndall effect) with two detector icons: 90° = nephelometry (dilute) and 180° = turbidimetry (concentrated). Three application badges: sulfate/chloride limit tests (IP), bacterial growth, protein assays.
- **Footer strip:** The five "fingerprint" wavelengths, the Beer–Lambert law (A = εbc), and the exam mantra: "Emission = fireflies (few, glow) • Absorption = shadows (all, dimming)".

## 2.10 Examples (Pharmacy Examples)

1. **ORS (oral rehydration salts):** Every batch is checked by flame photometry for exact **sodium and potassium** content — the salts that save children from dehydration.
2. **Calcium carbonate antacid tablets:** Calcium content verified by flame photometry using the hotter acetylene–air flame (calcium forms stable compounds in cool flames).
3. **Lithium carbonate tablets:** Lithium (671 nm) assayed by flame photometry for this psychiatry medicine with a narrow therapeutic range.

4 **Lead in a herbal raw material:** Graphite-furnace AAS detects lead at parts-per-billion — a batch exceeding the limit is rejected before it becomes medicine.

5 **Cadmium in a colourant/excipient:** Flame AAS quantifies cadmium against the pharmacopoeial limit.

6 **Iron and zinc in multivitamin-mineral tablets:** AAS assays these minerals in the finished product.

7 **Palladium residue in a bulk drug:** A metal catalyst used in synthesis is checked by AAS to prove residues are below the safety limit.

8 **Sulfate limit test on sodium chloride:** $BaCl_2$ added → $BaSO_4$ turbidity compared with standard (IP limit test, nephelometric/turbidimetric).

9 **Chloride limit test on purified water:** $AgNO_3$ added → AgCl turbidity compared with standard.

10 **Antibiotic potency by bacterial growth:** Turbidimetry tracks bacterial cloudiness — less cloudiness means a stronger antibiotic.

## 2.11 Analogies

1 **Flame photometry:** Judging how much turmeric is in milk by how yellow it looks — deeper colour = more turmeric (element).

2 **Nebulisation:** Perfume spray vs pouring from a glass — only the fine mist burns smoothly in the flame.

3 **Flame temperature choice:** Simmering dal on a low flame vs searing on a roaring flame — Na/K need the low flame, Ca needs the hot one.

4 **Chemical interference:** Calcium locked in a safe (calcium phosphate); the releasing agent is the master key.

5 **Ionization suppressant:** A bodyguard (excess K/Cs) who takes the hit so the VIP (sample atoms) stays safe.

6 **AAS vs flame photometry:** Counting fireflies on a dark night (emission — few glow) vs counting birds flying across the sun (absorption — all cast shadows).

7 **Hollow cathode lamp:** A "personal torch" that shines only its owner's favourite colour — one lamp per element.

8 **Chopper:** Blinking your eyes to tell a torch's light apart from a bonfire's glow behind it.

9 **Graphite furnace vs flame:** An oven (slow, thorough, tiny sample) vs a stovetop flame (fast, bigger sample).

10 **Tyndall effect:** Sunlight in a dusty classroom — the dust makes the invisible beam visible.

11 **Nephelometry vs turbidimetry:** Standing beside the dusty sunbeam judging the glow (90°) vs standing opposite the torch judging the dimming (180°).

12 **Limit test:** A height-mark at a ride entrance — you only check "tall enough or not" (pass/fail), not the exact height.

## 2.12 Pharmacy Relevance

- **Electrolyte quality control:** ORS, IV fluids, electrolyte syrups and antacids live or die by their Na/K/Ca content — flame photometry is the routine workhorse.
- **Patient safety from heavy metals:** Lead, cadmium, arsenic and mercury are toxic even in traces; AAS (especially graphite furnace) is the guardian that keeps them out of medicines.
- **Regulatory compliance:** Pharmacopoeial limit tests (sulfate, chloride) and metal-impurity limits are legal requirements — nepheloturbidometry and AAS are the official tools.
- **Catalyst residue control:** Modern drug synthesis uses metal catalysts; AAS proves the final drug is free of harmful residues.
- **Antibiotic potency:** Turbidimetric bacterial-growth assays measure antibiotic strength without animal testing.
- **Clinical support:** Serum protein assays by immunoturbidimetry/nephelometry guide diagnosis in hospital labs attached to pharmacy practice.

## 2.13 India Context

- **ORS manufacturing:** India produces enormous quantities of WHO-formula ORS; flame photometry for Na/K is a daily QC operation in Indian pharma plants.
- **Indian Pharmacopoeia (IP) limit tests:** The sulfate limit test ($BaSO_4$ turbidity) and chloride limit test (AgCl turbidity) are performed in QC labs across India exactly as described in this chapter.
- **Herbal and Ayurvedic exports:** With global demand for Indian herbal products, graphite-furnace AAS testing for **lead and cadmium** in herbal raw materials is now routine for export compliance.
- **Generic drug exports:** Catalyst-residue (Pd, Pt, Ni) testing by AAS is part of the impurity profile Indian manufacturers submit to US/EU regulators.
- **Water for injection plants:** Indian sterile-manufacturing units monitor purified water turbidity and trace metals using these very techniques.
- **College practicals:** The classic "estimation of sodium/potassium in a sample by flame photometry using a calibration curve" is a standard B.Pharm instrumental-analysis experiment in Indian universities.

## 2.14 Key Points

1 Flame photometry: sample mist → flame → atoms get **excited** → emit **characteristic coloured light** → brightness ∝ concentration.

2 **Nebulisation** = sample turned into a fine mist (perfume spray, not poured water).

3 Flames coolest → hottest: **natural gas+air (~1700 °C) → propane+air (~1925 °C) → acetylene+air (~2300 °C) → acetylene+$N_2O$ (~2900–3000 °C)**.

4 Fingerprint wavelengths: **Na 589 nm (yellow), K 766 nm (violet), Ca 622 nm (brick-red), Li 671 nm (red)**.

5 Three interferences: **spectral** (→ monochromator), **chemical** (→ hotter flame / releasing agent), **ionization** (→ K/Cs suppressant).

6 Flame photometer: nebuliser → burner → monochromator/filter → detector → readout. **No lamp** — the flame is the source.

7 Pharma uses: **Na/K in ORS and syrups, Ca in antacids/supplements, Li tablets**.

8 AAS: **ground-state atoms absorb** the hollow cathode lamp's resonance light; absorbance follows **Beer–Lambert law**.

9 Emission (flame photometry) vs absorption (AAS) = fireflies vs birds-across-the-sun; AAS is more sensitive because **nearly all atoms are in the ground state**.

10 AAS parts: **HCL → atomizer (flame/graphite furnace) → chopper → monochromator → PMT → readout**.

11 **Graphite furnace** = 100–1000× more sensitive than flame (ppb level), but slower.

12 AAS interferences: chemical, ionization, spectral, **background absorption (→ deuterium lamp)**, matrix effects.

13 AAS pharma uses: **trace Pb/Cd/As/Hg, catalyst residues (Pd, Pt, Ni), Zn/Fe/Mg assays**.

14 **Tyndall effect** = particles scatter light, making the beam visible.

15 **Nephelometry = 90° scattered light (dilute)**; **turbidimetry = 180° transmitted light (concentrated)**.

16 Applications: **sulfate/chloride limit tests (IP), bacterial growth, protein assays, water clarity**.

---

## 2.15 Common Confusions

1 **Flame photometry vs AAS** — Confusion: "both use a flame, so both are the same." Clarification: flame photometry measures light **emitted** by excited atoms (no lamp); AAS measures lamp light **absorbed** by ground-state atoms (needs a hollow cathode lamp).

2 **Emission vs absorption sensitivity** — Confusion: "why is AAS more sensitive?" Clarification: in a flame only a tiny fraction of atoms get excited (emission), but **nearly all** atoms sit in the ground state ready to absorb — more absorbers = bigger signal.

3 **Hollow cathode lamp purpose** — Confusion: "why not use any bright lamp?" Clarification: only the element's **own** resonance wavelength is absorbed by its atoms; the HCL is a personal torch emitting exactly those lines.

4 **Nephelometry vs turbidimetry** — Confusion: mixing up the angles. Clarification: **N**ephelometry = **N**inety degrees (90°, scattered, dilute); **T**urbidimetry = **T**ransmitted straight **T**hrough (180°, concentrated).

5 **Limit test vs assay** — Confusion: "the limit test gives the exact impurity amount." Clarification: a limit test is **pass/fail** against a standard turbidity; an assay finds the exact amount.

6 **Chemical vs ionization interference** — Confusion: "both lower the signal, so both are the same." Clarification: chemical = element **locked in a stable compound** (fix: hotter flame/releasing agent); ionization = atoms **lose electrons** in too-hot flame (fix: K/Cs suppressant).

7 **Graphite furnace vs flame atomizer** — Confusion: "furnace is just a hotter flame." Clarification: the furnace is an **electrically heated tube** holding a microlitre sample — slower, but 100–1000× more sensitive (ppb level).

---

## 2.16 Common Mistakes

1 Saying flame photometry "absorbs" light — it measures **emission**; AAS measures absorption.

2 Forgetting that flame photometry needs **no light source** — drawing a lamp in its block diagram.

3 Using the **wrong flame** for the element — e.g., a cool flame for calcium (forms stable compounds; needs acetylene–air or hotter).

4 Ignoring **matrix/viscosity** differences between standards and samples — viscous samples nebulise poorly; dilute or matrix-match.

5 In AAS, using the **wrong hollow cathode lamp** — the lamp must match the element being analysed.

6 Forgetting **background correction** in AAS when the sample produces smoke — use the deuterium lamp.

7 Measuring nephelometry samples that are **too concentrated** (multiple scattering ruins linearity) — dilute first.

8 In the calibration-curve numerical, **extrapolating** beyond the standards instead of interpolating between them — always bracket the unknown with standards.

9 Confusing the detector angles: 90° for nephelometry/fluorometry (weak sideways signal), 180° for turbidimetry/UV-Vis (straight-through beam).

10 Reporting a limit test as an exact value — it is only **pass/fail** against the standard.

---

## 2.17 Mnemonics

1 **Flame photometer parts — "New Bikes Move Down Roads": N**ebuliser, **B**urner, **M**onochromator/filter, **D**etector, **R**eadout.

2 **AAS parts — "Happy Ants Chew Minty Delicious Radish": H**ollow cathode lamp, **A**tomizer, **C**hopper, **M**onochromator, **D**etector (PMT), **R**eadout.

3 **Flame temperature order — "Nice People Always Argue"** (coolest → hottest): **N**atural gas+air, **P**ropane+air, **A**cetylene+air, **A**cetylene+$N_2O$. (Or by temperature: 17 → 19 → 23 → 30, i.e., hundreds of °C.)

4 **Fingerprint colours — "Never Keep Cats Locked":** **N**a 589 (yellow), **K** 766 (violet), **C**a 622 (brick-red), **L**i 671 (red).

5 **Interferences + remedies — "SCaI":** **S**pectral → **S**eparate (monochromator); **C**hemical → **C**rack it (hotter flame/releasing agent); **I**onization → **I**nhibit (K/Cs suppressant).

6 **Emission vs absorption:** "**E**mission = **E**xcited atoms **E**mit (flame photometry); **A**bsorption = **A**toms **A**bsorb lamp light (AAS)."

7 **Nephelometry vs turbidimetry:** "**N**ephelometry = **N**inety degrees; **T**urbidimetry = **T**ransmitted **T**hrough (180°)."

---

## 2.18 Exam Summary

**Flame photometry** sprays the sample as a fine mist (**nebulisation**) into a flame, where solvent evaporates, atoms form, heat **excites** them, and they emit **characteristic light** (Na 589 nm yellow, K 766 nm violet, Ca 622 nm brick-red, Li 671 nm red) whose brightness is proportional to concentration. Flames run from coolest to hottest: **natural gas+air (~1700 °C) → propane+air (~1925 °C) → acetylene+air (~2300 °C) → acetylene+$N_2O$ (~2900–3000 °C)** — cool flames for Na/K, hot flames for Ca and refractory elements. Three interferences: **spectral** (remedy: monochromator), **chemical** (stable compounds like calcium phosphate; remedy: hotter flame or releasing agent), **ionization** (atoms lose electrons; remedy: K/Cs suppressant). Instrument: **nebuliser → burner → monochromator/filter → detector → readout** (no lamp). Pharma uses: **Na/K in ORS and syrups, Ca in antacids/supplements, Li tablets**. **AAS** instead creates a cloud of **ground-state atoms** (flame or graphite furnace) that **absorb** resonance light from a **hollow cathode lamp** (one per element); absorbance follows the **Beer–Lambert law**. Because nearly all atoms are in the ground state, AAS is more sensitive than flame photometry — especially **graphite-furnace AAS** (ppb level, slower). Instrument: **HCL → atomizer → chopper → monochromator → PMT → readout**. Interferences: chemical, ionization, spectral, **background absorption (deuterium-lamp correction)**, matrix effects. Pharma uses: **trace Pb/Cd/As/Hg, lead/cadmium limit testing, catalyst residues (Pd, Pt, Ni), Zn/Fe/Mg assays**. **Nepheloturbidometry** uses the **Tyndall effect**: **nephelometry** measures **scattered light at 90°** (dilute suspensions, more sensitive); **turbidimetry** measures **transmitted-light loss at 180°** (concentrated suspensions). Applications: **sulfate/chloride limit tests ($BaSO_4$, AgCl)**, bacterial growth/turbidity, protein assays (immunoturbidimetry), water clarity.

---

## 2.19 One-Minute Revision

- Flame photometry = heat excites atoms → they **emit** their colour; brightness = concentration.
- Nebulisation = fine mist (perfume spray); viscous samples spray badly.
- Flames coolest→hottest: **Natural gas+air → Propane+air → Acetylene+air → Acetylene+$N_2O$** ("Nice People Always Argue").
- Fingerprints: **Na 589, K 766, Ca 622, Li 671** ("Never Keep Cats Locked").
- Interferences: **S**pectral→monochromator, **C**hemical→hotter flame/releasing agent, **I**onization→K/Cs suppressant.
- Flame photometer: **Nebuliser → Burner → Monochromator → Detector → Readout** — NO lamp.

- AAS = ground-state atoms **absorb** HCL light; A = εbc; more sensitive (all atoms absorb, few emit).
- AAS parts: **HCL → Atomizer → Chopper → Monochromator → PMT → Readout** ("Happy Ants Chew Minty Delicious Radish").
- Graphite furnace: 100–1000× more sensitive (ppb), slower; deuterium lamp fixes background absorption.
- AAS pharma: **Pb/Cd limits, catalyst residues, Zn/Fe/Mg**.
- Tyndall effect = beam visible in particle suspension.
- **Nephelometry = 90° scattered (dilute)**; **Turbidimetry = 180° transmitted (concentrated)**.
- Limit tests: sulfate ($BaSO_4$), chloride (AgCl) — pass/fail vs standard.

## 2.20 Exam Questions

### Short-answer questions

1 Explain the theory of flame photometry in four lines.

2 What is nebulisation? Why is it necessary?

3 Arrange the four fuel–oxidant combinations from coolest to hottest flame and state one use of each.

4 Name the three interferences in flame photometry and give one remedy for each.

5 Draw the block diagram of a flame photometer and label its parts.

6 Explain the theory of AAS. How does it differ from flame photometry?

7 What is a hollow cathode lamp? Why must the lamp match the element being analysed?

8 Compare flame atomizer and graphite furnace in AAS.

9 List the interferences in AAS. How is background absorption corrected?

10 State the Tyndall effect and distinguish nephelometry from turbidimetry.

11 Give three pharmaceutical applications of (a) flame photometry, (b) AAS, (c) nepheloturbidometry.

### MCQs (with answer key)

**1. In flame photometry, the measured signal is:** (a) Light absorbed by ground-state atoms (b) Light emitted by excited atoms (c) Scattered light (d) Transmitted light

**2. Sodium gives a characteristic flame emission at:** (a) 766 nm (b) 622 nm (c) 589 nm (d) 671 nm

**3. The hottest flame combination is:** (a) Natural gas + air (b) Propane + air (c) Acetylene + air (d) Acetylene + nitrous oxide

**4. Calcium phosphate formation in the flame is an example of:** (a) Spectral interference (b) Chemical interference (c) Ionization interference (d) Background absorption

**5. Ionization interference is suppressed by adding:** (a) A releasing agent (b) Excess potassium or cesium salt (c) A deuterium lamp (d) Barium chloride

**6. In AAS, the light source is a:** (a) Xenon arc lamp (b) Tungsten lamp (c) Hollow cathode lamp (d) Mercury lamp

**7. AAS is more sensitive than flame photometry mainly because:** (a) It uses a hotter flame (b) Nearly all atoms are in the ground state and can absorb (c) It has no interferences (d) It uses two lamps

**8. Background absorption in AAS is corrected using a:** (a) Chopper (b) Deuterium lamp (c) Releasing agent (d) Narrower slit

**9. Nephelometry measures:** (a) Transmitted light at 180° (b) Scattered light at 90° (c) Absorbed light (d) Emitted light

**10. The sulfate limit test is based on the turbidity of:** (a) Silver chloride (b) Barium sulfate (c) Lead sulfide (d) Calcium oxalate

**Answer key:** 1-(b), 2-(c), 3-(d), 4-(b), 5-(b), 6-(c), 7-(b), 8-(b), 9-(b), 10-(b)

## Viva questions (with one-line model answers)

1 **What does a flame photometer measure?** — The characteristic light emitted by thermally excited atoms; brightness is proportional to concentration.

2 **Why is there no lamp in a flame photometer?** — The flame itself excites the atoms, so the sample is its own light source.

3 **Which flame would you use for sodium? For calcium?** — Cool natural-gas+air flame for sodium; hotter acetylene+air flame for calcium.

4 **What is the Beer–Lambert law in AAS?** — Absorbance = εbc; absorbance is proportional to concentration.

5 **Why does AAS need a hollow cathode lamp?** — Only the element's own resonance wavelength is absorbed by its atoms; the lamp provides exactly those lines.

6 **Flame or graphite furnace — which is more sensitive and why?** — Graphite furnace (ppb level); the whole microlitre sample stays longer in the light path.

7 **What is the Tyndall effect?** — Suspended particles scatter a light beam, making it visible.

8 **Nephelometry or turbidimetry for a very dilute suspension?** — Nephelometry at 90° — it is more sensitive for dilute suspensions.

## Application-based questions

1 An ORS manufacturer's QC lab reports low potassium values, but the formula is correct. The analyst suspects phosphate from an excipient. Name the interference, explain it, and suggest two remedies. *(Chemical interference — potassium/calcium locked as stable phosphate; remedies: hotter flame, releasing agent such as lanthanum salt.)*

2 A bulk drug made with a palladium catalyst must be released for export. Which technique and atomizer would you choose to prove Pd residues are below 10 ppm, and why? *(AAS with graphite furnace — ppb-level sensitivity needed for trace catalyst residues; flame AAS may not reach the required limit.)*

3 A student sets the AAS monochromator correctly but forgets to switch on the deuterium background corrector while analysing a smoky herbal extract. What error can occur? *(Background absorption by smoke/particles adds to the reading → falsely high absorbance → overestimated metal content.)*

4 Two suspensions are given: a faintly cloudy protein solution and a thick bacterial culture. Which technique (nephelometry or turbidimetry) suits each, and where is the detector placed? *(Protein solution → nephelometry, detector at 90°; thick culture → turbidimetry, detector at 180° straight through.)*

### Numericals with worked solutions

**Numerical 1 — Calibration curve (interpolation).** Sodium standards give these flame-photometer emission readings: 2 ppm → 15, 4 ppm → 29, 6 ppm → 44, 8 ppm → 58, 10 ppm → 73 (arbitrary units). A diluted ORS sample gives a reading of 51. Find the sodium concentration.

**Numerical 2 — AAS proportionality (Beer–Lambert).** A 5.0 ppm lead standard gives an absorbance of 0.450 in flame AAS. A sample gives 0.360 under identical conditions. Find the lead concentration in the sample.

**Numerical 3 — Dilution correction.** The sample in Numerical 1 was diluted 1 mL → 100 mL before measurement. What is the sodium concentration in the original ORS sample?

### CALCULATION BOX 2 — Finding an unknown concentration from a calibration curve (interpolation) **Method:** Plot emission reading (y-axis) vs concentration (x-axis) for standards, check it is a straight line, then read the unknown's concentration from the graph — or calculate it by interpolation between the two bracketing standards. **Step 1 — Tabulate the standards and check linearity.** (2, 15), (4, 29), (6, 44), (8, 58), (10, 73). Differences between successive readings: 14, 15, 14, 15 — nearly equal steps for equal 2 ppm steps, so the graph is a **straight line** through (near) the origin. ✔ Linear — safe to interpolate. **Step 2 — Locate the unknown reading (51) between two standards.** 51 lies between the 6 ppm standard (44) and the 8 ppm standard (58). **Step 3 — Find the slope between these two points.** Slope = (58 − 44) ÷ (8 − 6) = 14 ÷ 2 = **7 reading-units per ppm**. **Step 4 — Find how far the unknown is above the lower point.** 51 − 44 = **7 reading-units** above the 6 ppm point. **Step 5 — Convert this extra reading into extra concentration.** Extra ppm = 7 ÷ 7 = **1.0 ppm** above 6 ppm. **Step 6 — Add to the lower standard.** Concentration = 6.0 + 1.0 = **7.0 ppm** (in the diluted sample). ✔ **Step 7 — Sanity check.** 7.0 ppm lies between 6 and 8 ppm, and 51 lies between 44 and 58 — consistent. ✔ **Worked solution — Numerical 2 (AAS proportionality):** Beer–Lambert law: absorbance ∝ concentration (same path length, same element). c(sample) = c(standard) × A(sample)/A(standard) = 5.0 × (0.360/0.450) = 5.0 × 0.8 = **4.0 ppm lead**. **Worked solution — Numerical 3 (dilution correction):** Dilution factor = final volume ÷ sample volume = 100 mL ÷ 1 mL = **100**. Original concentration = measured × dilution factor = 7.0 ppm × 100 = **700 ppm sodium** in the original ORS sample.

## 2.21 References


- Skoog, Holler, Crouch — *Principles of Instrumental Analysis* (Cengage Learning). [Flame photometry theory and instrumentation; AAS theory, hollow cathode lamp, atomizers, interferences; nepheloturbidometry.]

- Watson — *Pharmaceutical Analysis* (Elsevier). [Pharmaceutical applications: Na/K/Ca assays, trace metal analysis, limit tests.]
- Harvey — *Modern Analytical Chemistry* (McGraw-Hill). [Calibration curves, Beer–Lambert law, quantitative calculations.]
- Braun — *Analytical Chemistry: A Modern Approach to Analytical Science* (McGraw-Hill). [General principles of atomic spectroscopy.]
- Dyer — *Applications of Absorption Spectroscopy of Organic Molecules* (Prentice Hall). [Background on absorption measurements.]