Unit-2

Instrumental Methods of Analysis

B.Pharma 7 Sem Notes

Unit: 2

- **IR spectroscopy:** Introduction, fundamental modes of vibrations in poly atomic molecules, sample handling, factors affecting vibrations.
- **Instrumentation-** Sources of radiation, wavelength selectors, detectors – Golay cell,
  Bolometer, Thermocouple, Thermister, Pyroelectric detector and applications.
- **Flame Photometry-** Principle, interferences, instrumentation and applications.
  Atomic absorption spectroscopy- Principle, interferences, instrumentation and applications.
- **Nephelo-turbidimetry-** Principle, instrumentation and applications.

INFRARED (IR) SPECTROSCOPY

Introduction to IR Spectroscopy

Infrared (IR) spectroscopy is one of the most powerful and widely used analytical techniques in pharmaceutical sciences. It works by shining infrared light on a sample — the molecules absorb specific frequencies of IR radiation, causing their bonds to vibrate. By measuring which frequencies are absorbed, we can identify the functional groups present and determine the structure of the molecule.

Simple Analogy
Think of a molecule's bonds as springs connecting balls (atoms). When you pass IR radiation, it vibrates
those 'springs'. Different bond types vibrate at different frequencies — just like different guitar strings
produce different notes. IR spectroscopy records which 'notes' (frequencies) are absorbed.

The Electromagnetic Spectrum — IR Regions

The infrared region lies between visible light and microwave radiation. It is divided into three sub-regions:

IR Region	Wavenumber (cm^{-1})	Wavelength (μm)	Use / Importance
Near IR (NIR)	12,500 – 4,000	0.8 – 2.5	Overtone & combination bands; used in PAT
Mid IR (MIR)	4,000 – 400	2.5 – 25	Fundamental vibrations — MOST USEFUL region
Far IR (FIR)	400 – 10	25 – 1,000	Lattice vibrations; metal complexes

Key Point
The Mid-IR region (4000–400 cm^{-1}) is most important for pharmaceutical analysis.
Wavenumber ($\tilde{\nu}$) = $1/\lambda$ is used instead of wavelength because it is directly proportional to energy.
Higher wavenumber = Higher energy = Stronger bond vibration.

Fundamental Modes of Vibration in Polyatomic Molecules

When a molecule absorbs IR radiation, its atoms vibrate. These vibrations are called normal modes. The total number of normal modes (degrees of vibrational freedom) depends on the number of atoms (N) and the shape of the molecule.

Molecule Type	Example	Total Degrees of Freedom	Normal Modes of Vibration
Linear	CO_2, HCN	3N	3N − 5
Non-linear	H_2O, NH_3, CH_4	3N	3N − 6

Quick Examples

H_2O: N = 3, Non-linear → 3(3) − 6 = 3 normal modes (symmetric stretch, asymmetric stretch, bending)

CO_2: N = 3, Linear → 3(3) − 5 = 4 normal modes

A. Stretching Vibrations

Stretching vibrations involve a change in bond length (the distance between two bonded atoms increases or decreases rhythmically). There are two types:

Type	Description	Dipole Change?	IR Active?	Frequency
Symmetric Stretching	Both bonds stretch/compress simultaneously in the same direction	No (for symmetric molecules)	IR Inactive (for symmetric)	Lower frequency
Asymmetric Stretching	Bonds stretch and compress in opposite directions simultaneously	Yes — always	IR Active	Higher frequency (more energy)

Remember: Condition for IR Activity

A vibration is IR active ONLY if it causes a CHANGE IN DIPOLE MOMENT during vibration.

Symmetric molecules (like CO_2) have some IR-inactive vibrations because no dipole change occurs.
Asymmetric stretch of CO_2 is IR active; symmetric stretch is IR inactive.

B. Bending Vibrations

Bending vibrations involve a change in bond angle. They require less energy than stretching vibrations and therefore appear at lower wavenumbers (below 1500 cm^{-1}). There are four types of bending:

Type of Bending	Motion Description	Plane	Approx. Wavenumber
Scissoring (In-plane)	Bond angle decreases and increases alternately — like scissors closing and opening	In-plane	~1465 cm^{-1}
Rocking (In-plane)	Both bonds move in the same direction within the plane	In-plane	~720 cm^{-1}
Wagging (Out-of-plane)	Both bonds move out of the plane in the SAME direction	Out-of-plane	~1350 cm^{-1}
Twisting (Out-of-plane)	Bonds move out of the plane in OPPOSITE directions	Out-of-plane	~1250 cm^{-1}

Order of Frequencies to Remember
Stretching > Bending (Stretching requires more energy than bending)
Asymmetric Stretch > Symmetric Stretch
Triple bond > Double bond > Single bond (in terms of stretching frequency)
Bonds to lighter atoms (C-H, O-H, N-H) appear at higher wavenumber than bonds to heavier atoms

C. Key Characteristic IR Absorption Bands (Important for Exams)

Functional Group	Bond Vibration	Wavenumber (cm^{-1})	Appearance
Alcohol / Phenol (free O-H)	O-H stretch	3600–3650	Sharp, strong
Alcohol / Phenol (H-bonded)	O-H stretch	3200–3500	Broad, strong
Carboxylic acid	O-H stretch	2500–3300	Very broad
Amine (1°, 2°)	N-H stretch	3300–3500	1° = 2 bands; 2° = 1 band
Aldehyde / Ketone	C=O stretch	1720–1740	Very strong, sharp (key band)
Carboxylic acid	C=O stretch	1700–1725	Very strong
Amide	C=O stretch	1630–1700	Strong
Nitrile	C≡N stretch	2200–2260	Strong, sharp
Alkyne	C≡C stretch	2100–2260	Variable
Alkane	C-H stretch	2850–3000	Strong
Fingerprint Region	C-C, C-O, bending	400–1500	Complex, unique to molecule

Sample Handling in IR Spectroscopy

Glass absorbs IR radiation, so special IR-transparent materials like NaCl, KBr, and CsI are used for sample cells and windows. Preparation methods vary by sample state:

A. Solid Samples

- **KBr Pellet Method (Most Common): 1–2 mg sample is finely ground with 100–200 mg dry KBr powder, then pressed under a hydraulic press at high pressure (~10 tons) to form a transparent disc. Advantages: no interference from KBr, good quality spectrum.**
- Nujol Mull: Sample is ground with liquid paraffin (Nujol) to form a paste, which is sandwiched between two NaCl plates. Easy but Nujol itself shows C-H absorption bands at 2924, 1462, and 1376 cm^{-1}.
- Fluorolube Mull: Similar to Nujol but used when the C-H region must be studied (Fluorolube has no C-H absorption).
- ATR (Attenuated Total Reflectance): Most modern method. No sample preparation needed — just place solid or liquid directly on the ATR crystal (ZnSe, diamond). The IR beam undergoes total internal reflection inside the crystal and interacts with the sample surface.

B. Liquid Samples

- Neat Liquid: A thin film of pure liquid is placed between two NaCl or KBr plates with no solvent. Best for pure liquids.
- Solution Method: Sample dissolved in CCl_4 or CS_2 (which are transparent in IR). Placed in an NaCl liquid cell with a fixed path length (0.1–1 mm).

C. Gas Samples

- Placed in long-path gas cells (5–10 cm) with IR transparent windows.
- For trace gases, multi-reflection cells with 10 m path length are used.

IMPORTANT — Cell Material Range
NaCl plates: 650–4000 cm^{-1} (most common; insoluble in organic solvents but dissolves in water)
KBr plates: 400–4000 cm^{-1} (covers wider range than NaCl)
CsBr / CsI: 250–4000 cm^{-1} (for far-IR region)
NEVER use water with NaCl or KBr plates — they dissolve instantly!

Factors Affecting IR Vibrations (Absorption Frequencies)

Several factors can shift the absorption frequency of a bond from its expected value. Understanding these helps in interpreting spectra correctly.

1. Mass Effect (Hooke's Law)

The IR stretching frequency is governed by Hooke's Law:

$$\tilde{\nu} = (1 / 2\pi c)\sqrt{(k / \mu)}$$

Where: k = force constant (bond strength), μ = reduced mass = $m_1m_2 / (m_1 + m_2)$

- Heavier atoms → higher reduced mass → LOWER frequency (e.g., C-H at 3000 cm^{-1} vs C-D at ~2100 cm^{-1})
- Lighter atoms → lower reduced mass → HIGHER frequency

2. Bond Strength / Force Constant (k)

- Stronger bonds have a higher force constant (k) → higher stretching frequency
- Order: Triple bond > Double bond > Single bond
- Example: C≡C (~2150) > C=C (~1650) > C-C (~1200) cm^{-1}

3. Electronic Effects

- Inductive Effect: Electron-withdrawing groups (like -Cl, -F) attached near C=O reduce electron density on the carbonyl and INCREASE C=O stretching frequency.
- Resonance / Conjugation: Conjugation (e.g., C=C-C=O) causes partial single-bond character in C=O, LOWERING its frequency. Example: conjugated carbonyl ~1680 cm^{-1} vs unconjugated ~1715 cm^{-1}
- Mesomeric Effect in amides: Nitrogen lone pair donated into C=O lowers the C=O frequency to ~1650 cm^{-1}.

4. Hydrogen Bonding (Very Important!)

- Hydrogen bonding LOWERS the stretching frequency of O-H or N-H and makes the band BROADER.
- Intramolecular H-bonding: Gives a sharp band, independent of concentration (dilution doesn't change band position).
- Intermolecular H-bonding: Gives a broad band, concentration-dependent. On dilution, the broad H-bonded O-H band shifts to a sharper band at ~3600 cm^{-1} (free O-H).

5. Ring Strain (Cyclic Compounds)

- More ring strain → higher C=O stretching frequency.
- Order: Cyclobutanone (~1780) > Cyclopentanone (~1740) > Cyclohexanone (~1715) > Open-chain ketone (~1715) cm^{-1}

6. Physical State

- Gas phase: Highest absorption frequency (no intermolecular forces)
- Solution: Slightly lower than gas phase
- Solid (KBr pellet): Lowest frequency (crystal lattice forces contribute)

7. Fermi Resonance

- When an overtone or combination band accidentally has nearly the same frequency as a fundamental vibration, they 'interact' and split into two peaks.
- Classic example: Aldehyde shows two C-H bands at ~2720 and ~2820 cm^{-1} due to Fermi resonance.

Instrumentation — IR Spectrophotometer

A. Sources of IR Radiation

IR sources are electrically heated materials that emit a continuous spectrum of IR radiation (like a black body). The hotter the source, the more intense the emission.

Source	Material	Temperature	Range (cm^{-1})	Key Features
Nernst Glower	$ZrO_2 + Y_2O_3$ (rare earth oxides)	~1500°C	400–7000	Bright; has negative resistance (needs pre-heating); most widely used
Globar	Silicon carbide (SiC) rod	~1300°C	400–4000	Needs water cooling; stable emission
Nichrome Wire Coil	Nickel-chromium alloy	~1100°C	400–7000	Simple; less intense than Globar/Nernst
Mercury Arc Lamp	Mercury vapor	—	10–400 (Far-IR)	Used specifically for the far-IR region only

B. Wavelength Selectors

- Prisms: Made of IR-transparent materials (NaCl, LiF, CsBr). Separate wavelengths by refraction. Less common now due to lower resolution.
- Diffraction Gratings: Ruled gratings on aluminum. Provide superior resolution and are used in modern dispersive IR instruments.
- Michelson Interferometer (FTIR): Used in FTIR spectrometers. Instead of separating wavelengths, it records all wavelengths simultaneously (called an interferogram), then uses a mathematical technique called Fourier Transform to convert it into a spectrum. This makes FTIR 10-100x faster with better sensitivity (Fellgett's advantage).

C. IR Detectors — Detailed

IR detectors convert IR radiation into an electrical signal. They are of two types: thermal detectors (measure heat) and quantum detectors (measure photons).

Detector	Principle of Working	Type	Range	Key Features
Golay Cell (Pneumatic)	IR heats Xe gas in a sealed chamber. Gas expands, deflecting a flexible mirror membrane. Deflection detected by a photocell.	Thermal	Far-IR & Mid-IR	Very sensitive; fragile; slow response; ideal for far-IR
Bolometer	IR changes the electrical resistance of a blackened metallic strip (Pt or Ni) or semiconductor. Resistance change is measured using a bridge circuit.	Thermal	Far-IR to Mid-IR	Sensitive; requires cooling (liquid N_2 or liquid He at 4K) for best performance
Thermocouple	IR heating generates a thermoelectric EMF (voltage) at the junction of two different metals (e.g., Bismuth-Antimony or Bismuth-Telluride).	Thermal	Mid-IR	Rugged, reliable, inexpensive; lower sensitivity; most common in simple instruments
Thermistor	IR heats a semiconducting metal oxide bead. Its electrical resistance decreases as temperature rises (negative temperature coefficient).	Thermal	Mid-IR	More sensitive than thermocouple; temperature-sensitive environment needed
Pyroelectric (TGS/DTGS)	Ferroelectric crystal (triglycine	Thermal	Mid-IR & Far-IR	Fast response; wide spectral

Detector	Principle of Working	Type	Range	Key Features
	sulfate) changes its spontaneous electrical polarization when its temperature changes due to IR absorption, generating a measurable current.			range; used in FTIR instruments; responds to dT/dt (rate of change)
MCT (Mercury Cadmium Telluride)	IR photons promote electrons across the bandgap of the semiconductor, changing conductivity (photoconductor).	Quantum	2.5–25 μm	MOST sensitive; very fast response; requires liquid N_2 cooling; used in high-end FTIR

Pyroelectric Detector — How it Works (Important for Exams)
TGS (triglycine sulfate) crystal maintains a spontaneous electric polarization below its Curie temperature (~49°C).
When IR radiation hits the crystal, its temperature increases slightly.
This temperature change alters the crystal's polarization, generating a small electric current.
Key: It responds to the RATE OF CHANGE of temperature (dT/dt), not to constant temperature.
This makes it ideal for FTIR where the signal is constantly changing (modulated by the interferometer).

Golay Cell — Key Points
Filled with inert gas (Xenon) in a sealed chamber with a blackened absorbing film and a flexible mirror membrane.
IR radiation absorbed by the film heats the gas → gas expands → mirror deflects.
A light beam (from a lamp) reflects off the mirror onto a photocell.
More IR → more deflection → more light on photocell → higher output signal.
Best for the far-IR region where other detectors are insensitive.

Applications of IR Spectroscopy

- Identification and characterization of drugs: Comparison of sample spectrum with reference (pharmacopoeia) spectrum — IP, BP, USP all include IR identification tests.
- Detection of drug polymorphism: Different crystal forms (polymorphs) of the same drug show different IR spectra (e.g., different chloramphenicol palmitate polymorphs).
- Quantitative determination: Using calibration curve (Absorbance vs. concentration at a specific wavenumber) — e.g., aspirin content in tablets.
- Drug-excipient compatibility studies: Detect new peaks or disappearance of peaks when a drug is mixed with excipients, indicating interaction.
- Structural elucidation: Identify functional groups present in a new molecule.
- Detection of impurities and adulterants: Foreign absorption bands indicate contamination.
- Quality control and identity testing: Rapid, non-destructive test.
- Near-IR (NIR) spectroscopy used in Process Analytical Technology (PAT) for real-time monitoring during manufacturing.

FLAME PHOTOMETRY

Principle of Flame Photometry

Flame Photometry (also called Flame Emission Spectroscopy or FES) is a technique based on EMISSION of light. When a metal salt solution is introduced into a hot flame, the metal atoms get excited and then emit light of a characteristic wavelength as they return to the ground state. By measuring the intensity of this emitted light, we can determine the concentration of the metal.

Step-by-Step Process in Flame Photometry
Step 1 (Nebulization): Sample solution is aspirated into the flame as a fine mist of tiny droplets.
Step 2 (Desolvation): The solvent evaporates, leaving behind dry salt particles.
Step 3 (Vaporization): Salt particles vaporize at the high flame temperature.
Step 4 (Atomization): Salt molecules dissociate into free neutral atoms. MX → M + X
Step 5 (Excitation): Atoms absorb thermal energy from the flame and jump to a higher electronic energy level.
Step 6 (Emission): Excited atoms are unstable and return to the ground state, emitting radiation of a characteristic wavelength (the emission line).
Step 7 (Measurement): The intensity of emitted light is measured and compared to a calibration curve.

The analytical relationship is:

$$\text{Emission Intensity } (I) = k \times C$$

This linear relationship holds at low concentrations. A calibration curve is prepared and used to find the concentration of an unknown sample.

Common Flame Types and Their Temperatures

Fuel Gas	Oxidant	Flame Temperature (°C)	Elements Suitable For
Acetylene (C_2H_2)	Air	2100–2400	Na, K, Li, Ca, Ba (most common metals)
Acetylene (C_2H_2)	Nitrous oxide (N_2O)	2600–2800	Refractory metals (Al, Si, Ti)
Propane / Butane	Air	1700–1900	Na, K (simpler analyses)

Fuel Gas	Oxidant	Flame Temperature (°C)	Elements Suitable For
Hydrogen (H_2)	Air	2000–2050	Hydride-forming elements

Interferences in Flame Photometry

Interferences are unwanted effects that cause errors in measurement. Understanding and correcting them is crucial.

A. Spectral Interferences

- Overlap of emission lines: Two different elements emit at nearly the same wavelength, so one interferes with the other.
- Molecular band emission: Metal oxides and hydroxides formed in the flame emit broad bands that may overlap with the analyte's line.
- Flame background emission: The flame itself emits some radiation.
- Correction: Use a high-resolution monochromator or perform background subtraction using a reference beam.

B. Chemical Interferences

- Refractory compound formation: Some anions react with the analyte metal to form compounds that resist vaporization. Example: Phosphate ions form Ca-phosphate with calcium, reducing free Ca atoms in the flame, thus lowering calcium emission.
- Ionization interference: At high flame temperatures, analyte atoms may lose electrons and become ions ($M \rightarrow M^+ + e^-$). Ions do NOT emit the same wavelength as atoms, so signal decreases.
- Correction for refractory compounds: Add releasing agents like $La(NO_3)_3$ (lanthanum nitrate) which preferentially reacts with phosphate, freeing the calcium.
- Correction for ionization: Add ionization suppressors — a large excess of an easily ionizable alkali metal (CsCl, KCl). This creates a high free electron concentration which suppresses ionization of the analyte.

C. Physical / Matrix Interferences

- Differences in viscosity, surface tension, or density between sample and standard solutions affect nebulization efficiency (how much sample reaches the flame).
- Dissolved solid content alters droplet size and flame temperature.
- Correction: Matrix matching — prepare standards in the same matrix as the samples. OR use the internal standard method.

D. Internal Standard Method

- Add a known amount of a reference element (with properties similar to the analyte) to all samples and standards.
- Example: Lithium (Li) is added as internal standard when measuring Na and K (because Li has similar emission behavior but is not normally present in samples).
- The ratio of analyte signal to internal standard signal is used for quantitation — this cancels out matrix effects.

Instrumentation — Flame Photometer

The flame photometer measures the intensity of light emitted by excited atoms in a flame. The main components are:

Component	Function	Details
Nebulizer (Atomizer)	Converts liquid sample into a fine mist (aerosol) and introduces it into the flame	Pneumatic concentric nebulizer; flow rate ~5 mL/min
Burner	Creates a stable flame of controlled temperature and composition	Laminar flow (pre-mix) burner is most common
Flame	Evaporation, atomization, excitation of sample atoms	Air-propane for Na/K; N_2O-acetylene for Ca, Ba
Wavelength Selector	Isolates the specific emission line of the analyte	Interference filters (simple instruments) or grating monochromator (advanced instruments)
Detector	Converts emitted light to an electrical signal	Photomultiplier tube (PMT) — highly sensitive
Readout System	Displays the emission intensity or concentration	Digital meter, chart recorder, or computer display

Applications of Flame Photometry

Element	Emission Wavelength (nm)	Detection Limit	Clinical Application
Sodium (Na)	589.0	0.001 ppm	Na^+ in blood serum, urine, IV fluids

Element	Emission Wavelength (nm)	Detection Limit	Clinical Application
Potassium (K)	766.5	0.005 ppm	K^+ in blood serum, electrolyte solutions
Lithium (Li)	670.8	0.001 ppm	Li^+ monitoring in lithium therapy for bipolar disorder
Calcium (Ca)	622.0 (band)	0.01 ppm	Calcium in pharmaceutical preparations
Barium (Ba)	553.6	0.01 ppm	Barium in preparations; toxicology

- Clinical: Determination of Na^+, K^+ in blood serum, urine, plasma (most important application in clinical chemistry).
- Pharmaceutical: Assay of Na and K in IV fluids (Normal Saline, Ringer's Lactate), electrolyte tablets and syrups.
- Monitoring lithium levels in patients receiving lithium therapy.
- Quality control of mineral supplements and electrolyte preparations.

ATOMIC ABSORPTION SPECTROSCOPY (AAS)

Principle of AAS

Atomic Absorption Spectroscopy (AAS) is based on the ABSORPTION of radiation by ground-state free atoms. Unlike flame photometry (which measures emitted light), AAS measures how much light is absorbed by unexcited (ground-state) atoms in a flame or furnace.

AAS Principle — Step by Step
1. A Hollow Cathode Lamp (HCL) emits a line spectrum with the exact wavelengths of the target element.
2. The sample solution is nebulized and atomized in the flame (or graphite furnace).
3. Ground-state atoms of the analyte in the flame ABSORB radiation from the HCL at the same wavelength.
4. The decrease in light intensity ($I_0 \rightarrow I$) is measured by the detector.
5. Absorbance ($A = \log I_0/I$) is proportional to the number of ground-state atoms → proportional to concentration.
Beer-Lambert Law for AAS: $A = \varepsilon \times N \times l$

Why AAS is More Sensitive than Flame Photometry

Parameter	Flame Photometry	AAS
What is measured?	Light EMITTED by excited atoms	Light ABSORBED by ground-state atoms
Which atoms are involved?	Excited atoms (~0.01% of total)	Ground-state atoms (~99.99% of total)
Sensitivity	Lower (ppm level)	Much higher (ppb level)
External light source needed?	No (flame is the source)	Yes — Hollow Cathode Lamp
Selectivity	Good	Excellent (element-specific lamp)
Number of elements	Mainly alkali & alkaline earths	70+ elements including heavy metals

Interferences in AAS

A. Spectral Interferences

- Very rare in AAS because HCL emits very narrow, element-specific lines.
- Background absorption: Unvaporized particles or molecular species in the flame scatter or absorb radiation broadly. This gives a false high absorbance reading.
- Correction methods: Deuterium lamp background correction, Zeeman effect background correction (most effective).

B. Chemical Interferences (Most Common and Important)

- Refractory oxide/compound formation: In the flame, analyte atoms may react with anions to form stable compounds that do NOT dissociate into free atoms. This REDUCES the free atom population and lowers the signal.
  - Example: Calcium + Phosphate → $CaPO_4$ (refractory) → less free Ca atoms → lower signal
  - Correction: Add releasing agents — $La(NO_3)_3$ or $Sr(NO_3)_2$ preferentially reacts with phosphate, freeing calcium for atomization.
- Ionization interference: At high temperatures (especially with N_2O-acetylene), atoms may be ionized ($M \rightarrow M^+ + e^-$). Ions don't absorb at the same wavelength as atoms, reducing signal.
  - Correction: Add ionization suppressors — excess CsCl or KCl provides free electrons that suppress ionization of the analyte.

C. Physical / Matrix Interferences

- Differences in viscosity or surface tension affect nebulization efficiency.
- Correction: Standard additions method — add known amounts of standard to the sample itself. This eliminates matrix effects because all calibration points are in the same matrix.

Instrumentation — AAS

A. The Hollow Cathode Lamp (HCL) — Primary Source

Construction and Working of HCL
Construction: A cylindrical cathode made of (or lined with) the element to be analyzed, a tungsten anode,
filled with inert gas (Ne or Ar) at low pressure (~1–5 Torr), sealed in a glass tube with a UV-transparent window.
Working:
1. High voltage (100–400V) applied between anode and cathode.
2. Inert fill gas is ionized → ions accelerate toward cathode.
3. Ions bombard the cathode → cathode atoms are sputtered off (ejected).
4. Sputtered metal atoms are excited by collisions → emit their characteristic line spectrum.
5. Ground-state atoms of the same element in the flame absorb these specific wavelengths — this is RESONANCE ABSORPTION.
Key Advantage: Each element has its OWN lamp. This makes AAS highly specific (excellent selectivity).

- Electrodeless Discharge Lamp (EDL): More intense than HCL. Used for elements with volatile compounds like As, Se, Hg, Sn, where HCL may not provide enough intensity.

B. Atomizers — Types

The atomizer converts the sample into free ground-state atoms. Different atomizers are used for different requirements:

Atomizer Type	Flame / Temp	Sensitivity	Sample Volume	Best Used For
Flame AAS (FAAS)	Air-Acetylene 2300°C; N_2O-Acetylene 2900°C	ppm level	Large (mL)	Routine analysis; most metals
Graphite Furnace (GFAAS)	Electrically heated 2000–3000°C	100–1000× more sensitive than FAAS (ppb-ppt)	Small (5–100 µL)	Trace/ultratrace metals; small sample

Atomizer Type	Flame / Temp	Sensitivity	Sample Volume	Best Used For
Hydride Generation (HGAAS)	Quartz tube at 900°C	Sub-ppb level	Small	As, Se, Sb, Bi, Pb, Sn, Te
Cold Vapour (CVAAS)	Room temperature (no flame)	ppb level	Moderate	Mercury ONLY

Graphite Furnace (GFAAS) — The Three Stages
Stage 1 — Drying (~100°C): Solvent is evaporated gently to leave behind dry residue.
Stage 2 — Ashing / Pyrolysis (200–700°C): Organic matrix is destroyed (burned away) without losing the analyte.
Stage 3 — Atomization (2000–3000°C): Sample is rapidly heated to very high temperature, producing a cloud of free atoms.
Measurement occurs during atomization. L'vov platform further reduces matrix interferences by ensuring
atoms are produced in a stable, constant temperature environment.

C. Monochromator

- Czerny-Turner type grating monochromator with narrow bandpass (0.2–2 nm).
- Purpose: Select only the specific resonance absorption line of the analyte and reject all other wavelengths (non-resonance lines, flame emission).

Applications of AAS

Application Area	Elements Determined	Practical Example
Pharmaceutical Quality Control	Pb, As, Hg, Cd, Cu, Zn, Fe	Heavy metal limit tests as per BP/USP/IP
Clinical Analysis	Na, K, Ca, Mg, Fe, Zn, Cu, Li	Blood, serum, urine mineral analysis
Environmental Monitoring	Pb, Hg, Cd, As, Cr, Ni, Cu	Water quality, soil contamination
Food Analysis	Ca, Fe, Zn, Pb, Cd, As	Nutritional labeling; contamination testing
Drug formulation assay	Ca (antacids), Fe (tonics), K (electrolytes)	Routine QC of pharmaceutical products

NEPHELOMETRY & TURBIDIMETRY

Introduction

Both nephelometry and turbidimetry are techniques based on SCATTERING of light by suspended particles in a solution. They both exploit the Tyndall Effect.

What is the Tyndall Effect?
When a beam of light passes through a colloidal suspension or fine precipitate, the particles scatter the
light in ALL directions. This is called the Tyndall effect. You see this in real life when a beam of
sunlight enters a dusty room — the dust particles scatter the light and make the beam visible.
The intensity of scattered light depends on: particle size, number of particles, wavelength of light,
and the difference in refractive index between the particle and the medium.

Nephelometry vs Turbidimetry — Key Difference

Feature	Nephelometry	Turbidimetry
What is measured?	Light SCATTERED at 90° (right angle) to the incident beam	DECREASE in transmitted light (0°/180° direction)
Detector position	Placed PERPENDICULAR (at 90°) to incident beam	Placed IN-LINE with incident beam (at 180°)
Sensitivity	MORE sensitive (detects small amounts of scattering against dark background)	LESS sensitive (measures small change in a large signal)
Suitable for	DILUTE suspensions, turbid biological fluids	CONCENTRATED suspensions
Instrument used	Nephelometer	Turbidimeter (or standard spectrophotometer)
Analogy	Like spotting a single candle flame in a dark room	Like measuring the dimming of sunlight through fog

Principle — Rayleigh Scattering

When particles are much smaller than the wavelength of light (particle size < λ/10), the scattering follows Rayleigh's Law:

$$Is = K \times I_0 \times N \times V^2 / \lambda^4$$

Symbol	Meaning
Is	Intensity of scattered light (what nephelometer measures)

Symbol	Meaning
I_0	Intensity of incident light (the original light beam)
N	Number of particles in the solution
V	Volume (size) of each particle
λ	Wavelength of light used
K	Instrument constant

For turbidimetry (Beer-Lambert law applied to scattering):

$$\text{Absorbance (turbidity)} = -\log(I/I_0) \propto \text{Concentration}$$

Factors Affecting Light Scattering

- Particle Size: Larger particles scatter more light. Scattering is proportional to V^2 (Rayleigh range). Larger particles ($> \lambda/10$) follow Mie scattering — more complex forward scattering.
- Particle Concentration: At low concentration, scattered intensity ∝ number of particles (N). At high concentration, multiple scattering occurs, causing non-linearity.
- Wavelength of Light: Scattering ∝ $1/\lambda^4$ — shorter wavelengths (UV, blue) scatter MUCH more than longer wavelengths (red). This is why the sky is blue (blue light scatters more in the atmosphere).
- Refractive Index: Greater difference between particle and medium refractive index → greater scattering.
- Particle Uniformity: Uniform, spherical particles give the most reproducible and predictable scattering.
- Temperature: Affects Brownian motion and particle aggregation, which changes effective particle size.

Instrumentation

A. Nephelometer

Component	Description
Light Source	Tungsten-halogen lamp, LED, or laser (He-Ne, laser diode)
Wavelength	420–700 nm (white or filtered light)
Sample Cell	Round glass cell or cylindrical test tube

Component	Description
Detector Position	Exactly 90° to the incident light beam (right angle)
Detector Type	Photomultiplier tube (PMT) or photodiode
Output	Scattered light intensity proportional to particle concentration

B. Turbidimeter

- A standard UV-Visible spectrophotometer can serve as a turbidimeter — the detector is in line with the source at 0°/180°.
- Absorbance is measured at 420–700 nm. Higher turbidity = higher absorbance.
- Dedicated turbidimeters use white light or LED sources.

Turbidity Standards

Standard Unit	Full Form	Used In	Primary Standard
NTU	Nephelometric Turbidity Units	General turbidity measurement	Formazin polymer suspension
FNU	Formazin Nephelometric Units	ISO standard nephelometry	Formazin at 4000 NTU stock
FAU	Formazin Attenuation Units	ISO standard turbidimetry	Same formazin reference

Applications of Nephelometry and Turbidimetry

A. Pharmaceutical Applications (Most Important for Pharmacy)

- **Limit Test for Sulfates (IP/BP/USP): Sample + $BaCl_2$ → $BaSO_4$ precipitate. Turbidity of sample compared with standard $BaSO_4$ suspension. Sulfate content should NOT exceed the standard.**
- **Limit Test for Chlorides (IP/BP/USP): Sample + $AgNO_3$ → AgCl precipitate. Turbidity compared with NaCl standard. This is a standard purity test for all pharmaceuticals.**
- Pyrogen Testing: LAL (Limulus Amebocyte Lysate) turbidimetric assay to detect bacterial endotoxins in parenteral preparations.
- Clarity Testing of Injections: Parenterals must be clear solutions (free of visible particles) as per BP/USP.
- Microbiological Assay of Antibiotics: Turbidimetric growth inhibition assay — measure turbidity of bacterial culture treated with antibiotic.
- Particle count in parenteral and ophthalmic preparations.

B. Clinical Applications

- Immunonephelometry: Measurement of serum proteins (IgG, IgA, IgM, albumin, CRP) using antigen-antibody reactions that produce immune complexes which scatter light.
- C-Reactive Protein (CRP): Key inflammation marker quantified by immunonephelometry.
- Urine protein detection (proteinuria testing).

C. Environmental Applications

- Water quality monitoring: WHO limit for drinking water turbidity is < 1 NTU. Water > 4 NTU is unsafe.
- Effluent monitoring and wastewater treatment control.
- Determination of suspended solids in river and lake water.

EXAM TIPS — Most Asked Topics
1. IR: Number of modes of vibration formula (3N-6 and 3N-5) with examples
2. IR: Factors affecting IR vibrations (mass, bond strength, conjugation, H-bonding)
3. IR: Sample handling methods (KBr pellet, Nujol mull, ATR)
4. IR: Detector types (Golay cell, pyroelectric, bolometer, thermocouple) and their working
5. Flame Photometry vs AAS — know the comparison table
6. AAS: Construction and working of Hollow Cathode Lamp
7. AAS: Graphite furnace — three stages (drying, ashing, atomization)
8. Interferences in both Flame Photometry and AAS — and their corrections
9. Nephelo vs Turbidimetry — difference in detector position and sensitivity
10. Applications: Limit test for sulfates and chlorides using turbidimetry

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