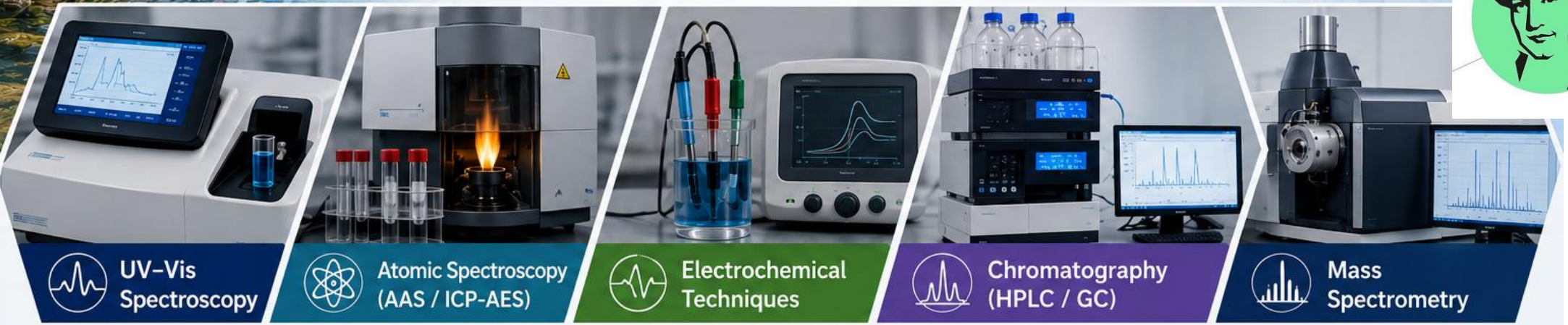
MODERN ANALYTICAL TECHNIQUES

IN ENVIRONMENTAL ANALYSIS

Principles, Applications and Selection of the Appropriate Analytical Method



ENVIRONMENTAL SAMPLES

WATER | SOIL / SEDIMENT | AIR | BIOLOGICAL / ENVIRONMENTAL SAMPLES

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HOW TO CHOOSE THE RIGHT ANALYTICAL TECHNIQUE?

From sample to result – the right tool for the right question

1. WHAT INSTRUMENTS DO WE HAVE AVAILABLE IN THE LAB?

- UV-Vis spectrophotometer
- Flame/ICP-AES
- Electrochemical workstation (CV, SWV, DPV, etc.)

2. WHAT IS THE NATURE OF THE SAMPLE?

- Biological (serum, plasma, cells)
- Environmental (water, soil, food)
- Chemical (compounds, mixtures)

3. HOW FAST DO WE NEED THE RESULTS?

- Real-time / on-line monitoring
- Minutes (rapid screening)
- Hours (routine analysis)

4. ARE THE ANALYSES ROUTINE OR FOR SPECIFIC PURPOSES?

- Routine / quality control
- Research / method development
- Trace / ultra-trace levels

5. WHAT ARE THE ANALYTICAL REQUIREMENTS?

- Sensitivity / LOD
- Selectivity / specificity
- Accuracy and precision

DECISION

AVAILABLE INSTRUMENTS AND THEIR MAIN APPLICATIONS

UV-Vis Spectrophotometer

- **Qualitative:** identification (λ^{max}, spectra)
- **Quantitative:** concentration (calibration curves)
- **Best for:** colored compounds, simple matrices, fast analysis

Concentration / Identity

Flame / ICP-AES

- **Qualitative:** elemental identification (emission lines)
- **Quantitative:** element concentration (calibration)
- **Best for:** metals and elements in aqueous/solid samples

Elemental composition

Electrochemical Workstation

- **Qualitative:** redox behavior, peak identification
- **Quantitative:** concentration (calibration / standard addition)
- **Best for:** electroactive species, fast, low-cost, portable options

Redox activity / Concentration

SEM

- **Qualitative:** morphology, structure, elemental analysis (EDS)
- **Quantitative:** composition (EDS)
- **Best for:** surface, morphology, nanoscale materials

Structure / Morphology

UPLC

- **Qualitative:** peak identification (retention time, MS)
- **Quantitative:** concentration (calibration)
- **Best for:** complex mixtures, low detection limits, high resolution

Separation / Identification / Quantification

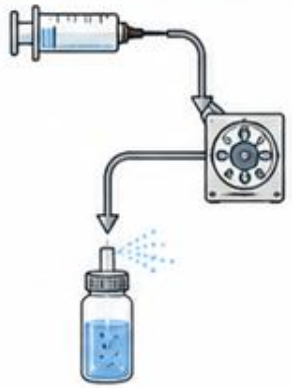
THE RIGHT TECHNIQUE = RELIABLE RESULTS + EFFICIENT USE OF TIME AND RESOURCES

A GENERAL SCHEME OF AN INSTRUMENTAL SYSTEM

1. SOURCE OF DRIVING FORCE

- Electrical energy
- Thermal energy
- Radiation (light)
- Pressure / Flow
- Magnetic / RF field
- Mechanical energy

2. SAMPLE INTRODUCTION AND TRANSPORT



- Injection / Introduction
- Nebulization / Atomization
- Gas / Liquid transport
- Separation (if any)

3. MEASUREMENT CHAMBER / PHYSICAL OR CHEMICAL PROCESS

Flame

Plasma (ICP)

Electrochemical Cell

Optical Cell (Absorption)

Reaction Vessel

Column (Separation)

- Atomization / Excitation
- Absorption / Emission / Scattering
- Electrochemical reaction
- Separation / Interaction

4. DETECTOR

Photodiode / PMT

CCD / Array

Electron Multiplier

Electrochemical Detector

5. SIGNAL CONDITIONING AND AMPLIFICATION

Preamplifier

Amplifier

Filter / Noise Reduction

6. SIGNAL PROCESSING, DATA SYSTEM AND OUTPUT

Computer / Software

Printer

Data Storage (LIMS / Database)

Display / Plotter

Results / Report

OUTPUT INFORMATION

QUALITATIVE INFORMATION

Identity / Structure / Features

QUANTITATIVE INFORMATION

Amount / Concentration / Intensity

FEEDBACK AND CONTROL

- Instrument parameters
- Temperature / Flow / Voltage
- Calibration / Optimization
- Quality control

SUPPORTED BY

Power Supply

Gas Supply

Cooling System

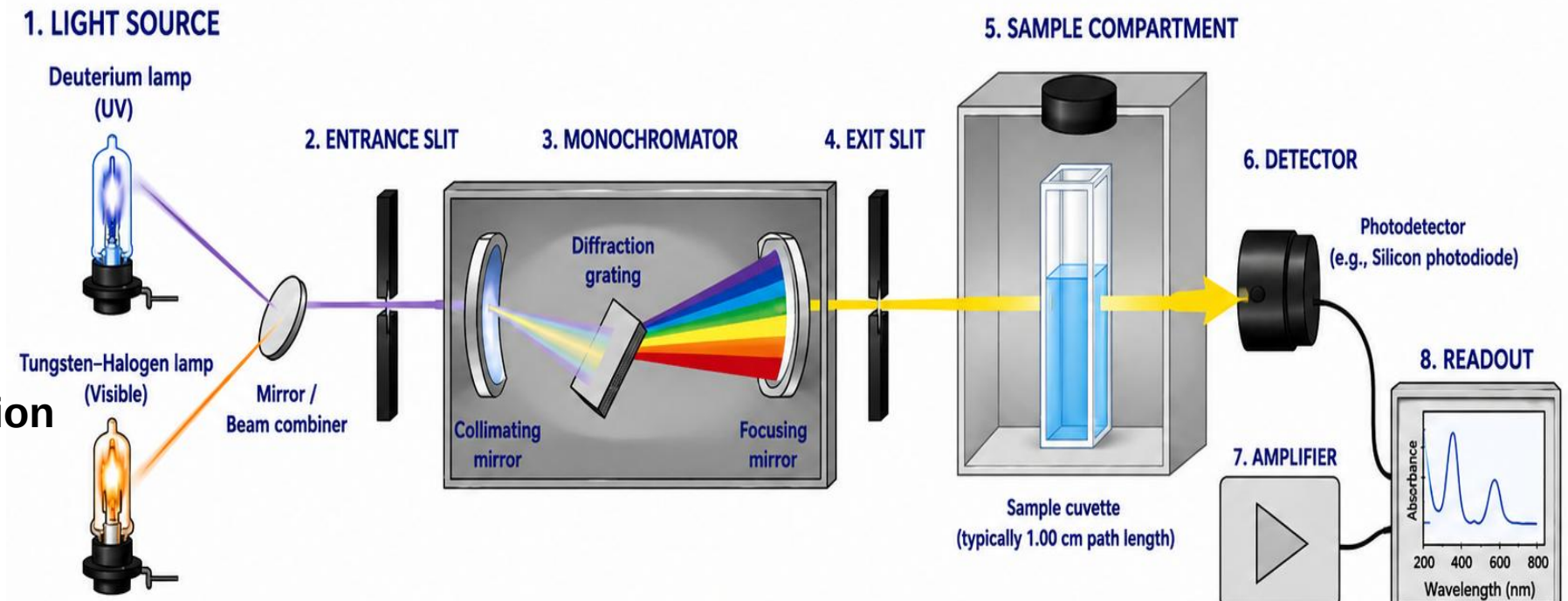
Vacuum System

Consumables

Control Electronics

UV VIS Spectrophotometry

- **Relays on interaction between UV VIS radiation and the molecules/ions of given analyte**
- **Absorption of UV–Visible radiation** by atoms, ions, or molecules.
- **Electronic excitation** from lower to higher energy levels.



1. LIGHT SOURCE — Provides continuous radiation in the UV and/or Visible region.

2. ENTRANCE SLIT — Controls the amount of light entering the monochromator.

3. MONOCHROMATOR — Selects a narrow range of wavelengths (monochromatic light).

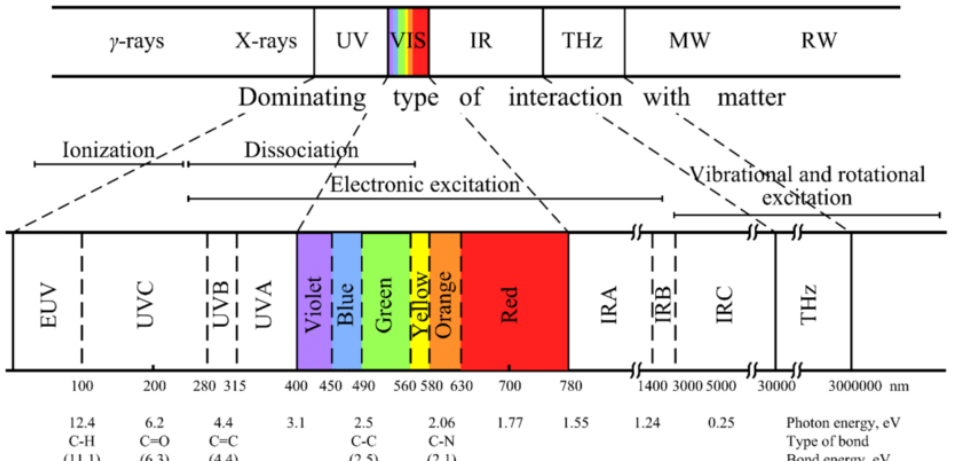
4. EXIT SLIT — Defines the bandwidth (spectral purity) and reduces stray light.

5. SAMPLE COMPARTMENT — The sample absorbs light at specific wavelengths.

6. DETECTOR — Converts transmitted light into an electrical signal.

7. AMPLIFIER — Amplifies the weak electrical signal from the detector.

8. READOUT — Displays or records the result as a spectrum or value.



MEASUREMENT PRINCIPLE

The instrument measures the decrease in light intensity caused by absorption:

$$A = \log_{10}(I_0 / I) = \varepsilon\, b\, c$$

where A = absorbance, I_0 = incident intensity, I = transmitted intensity, ε = molar absorptivity, b = path length, c = concentration.

Molecular Excitation by UV–Vis Light

When a molecule absorbs UV or visible radiation, an electron is promoted from a **lower-energy molecular orbital** to a **higher-energy antibonding orbital**:

$$\boxed{\text{Molecule} + h\nu \rightarrow \text{Molecule}^*}$$

The photon is absorbed only when its energy matches the energy difference between the two electronic states:

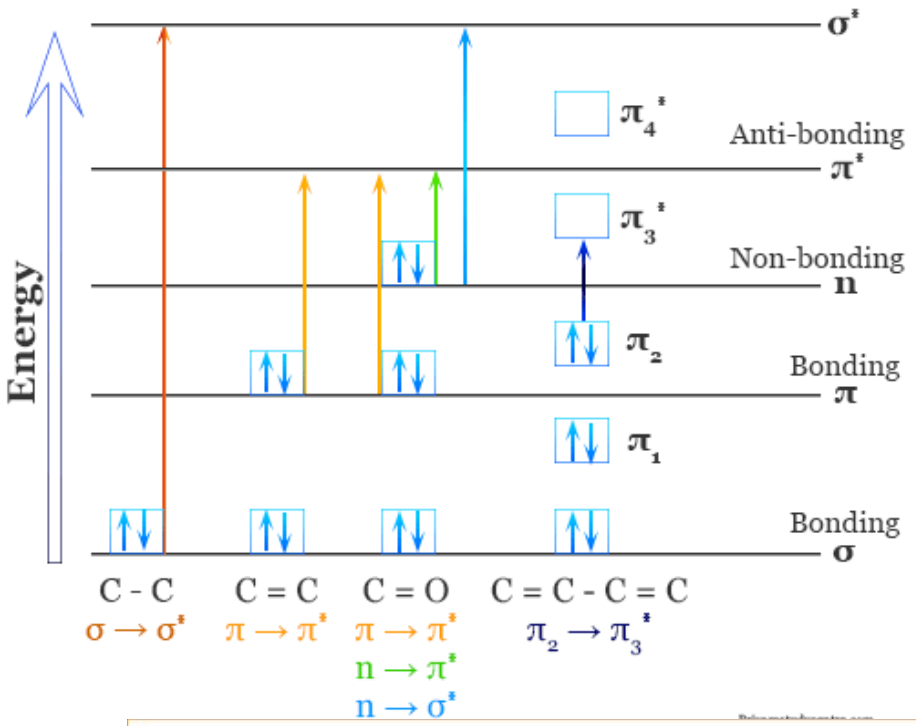
$$\boxed{\Delta E = h\nu = \frac{hc}{\lambda}}$$

Typical electronic transitions:

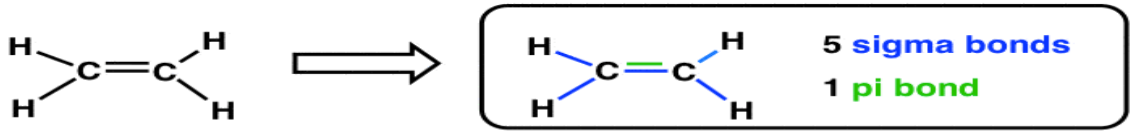
σ → σ* • n → σ* • π → π* • n → π*

The absorbed wavelengths are displayed as an **UV–Vis absorption spectrum:**

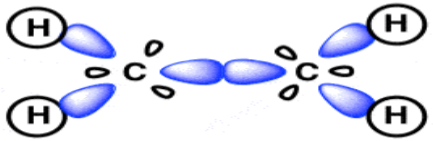
How it works on a Molecular level…



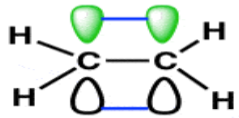
Breaking down the bonding in ethene



Sigma (σ) bond framework:
("end-on" overlap)



Pi (π) bond framework:
("side-on" overlap)



There are 5 sigma bonds in ethene

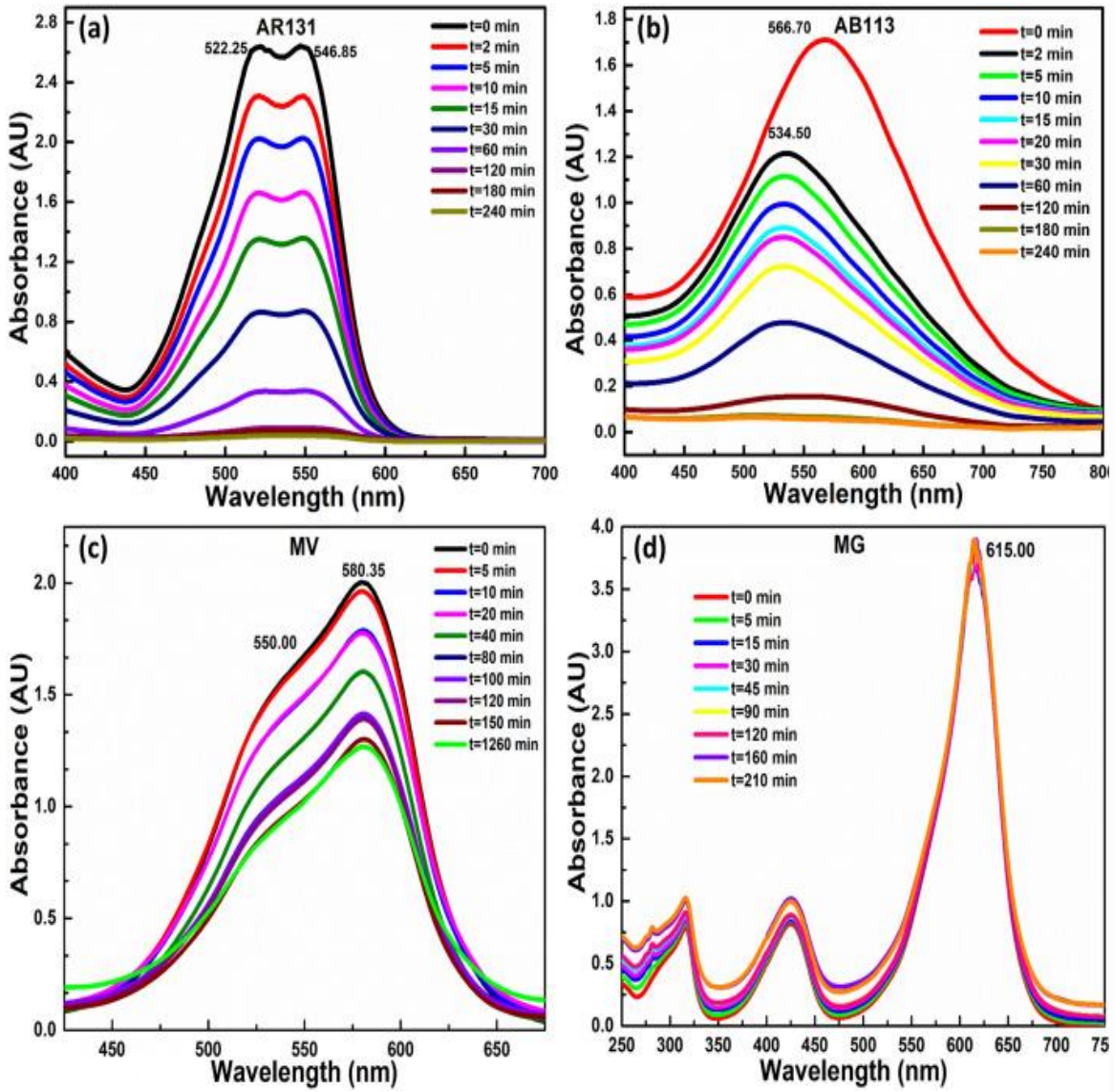
- 1 C-C sigma bond (sp^2-sp^2)
- 4 C-H sigma bonds (sp^2–H)

One pi bond in ethene

- 1 C-C pi bond

Chromophore	λ_{max}(nm)	ε max
alkyne	225	160
carbonyl	280	16
carboxyl	204	41
amido	214	41
azo	339	5
nitro	280	22
nitroso	300	100
nitrate	270	12
olefin conjugated	217-250	>20,000
ketone	282	27
aklylbenzenes	250-260	200-300
phenol	270	1,450
aniline	280	1,430
naphthalene	286	9,300
styrene	244	12,000

How one can make qualitative and Quantitative analysis with UV VIS Spectroscopy?



Preconditions for UV–Vis Analysis

1. **Presence of a chromophore**

   The analyte should contain a structural group capable of absorbing radiation in the UV or visible region, e.g. **conjugated π-systems, aromatic rings, C=O, N=N**, or suitable metal complexes.

2. **Sufficient absorption intensity**

   The analyte should have a sufficiently high **molar absorptivity (ε)** at the selected wavelength to produce a measurable signal at the concentration used.

3. **Suitable concentration range**

   The concentration should provide absorbance within the useful Beer–Lambert range—commonly around **A ≈ 0.1–1.0** (instrument/method dependent). Dilution may be necessary.

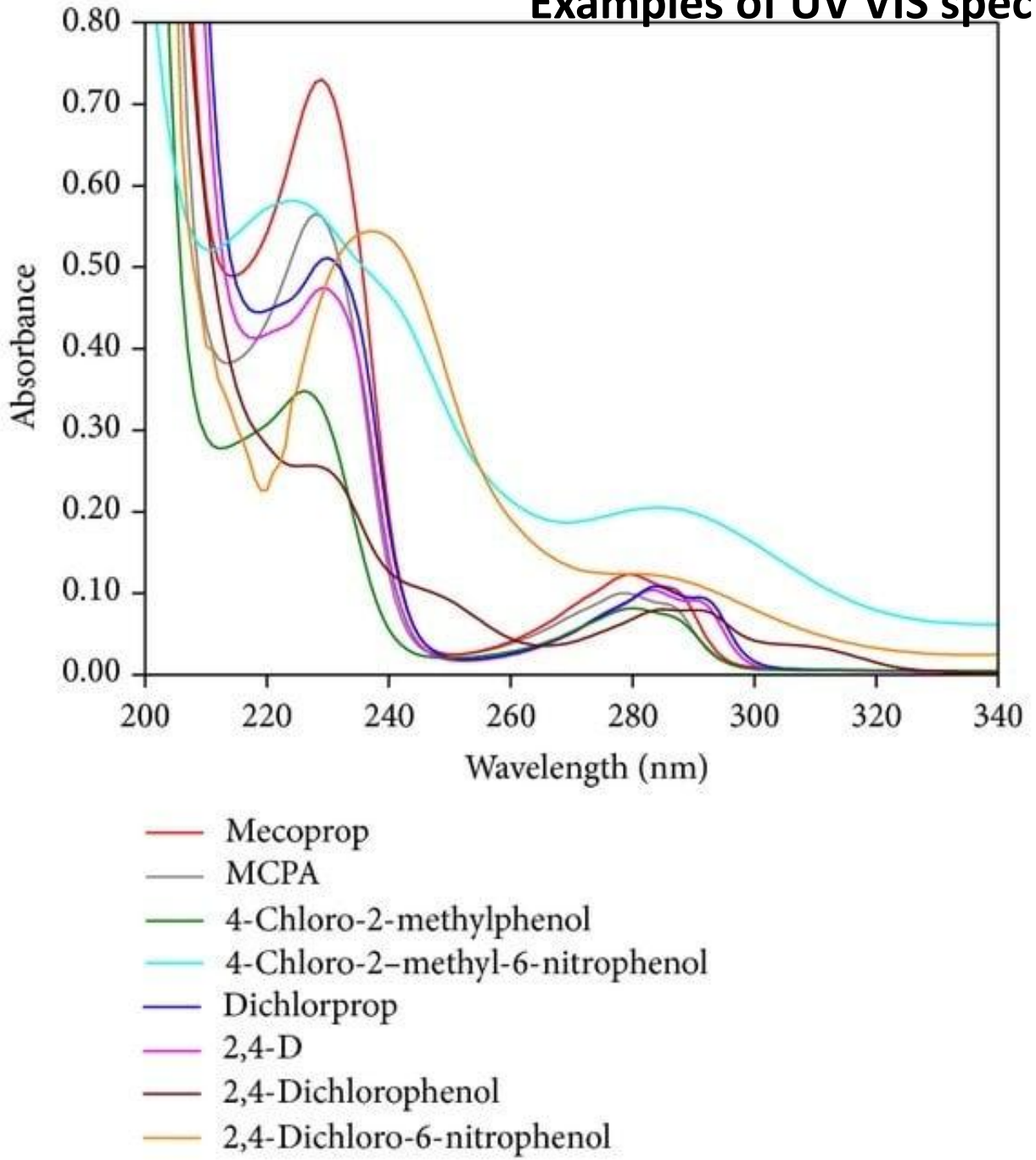
4. **Minimal spectral interference**

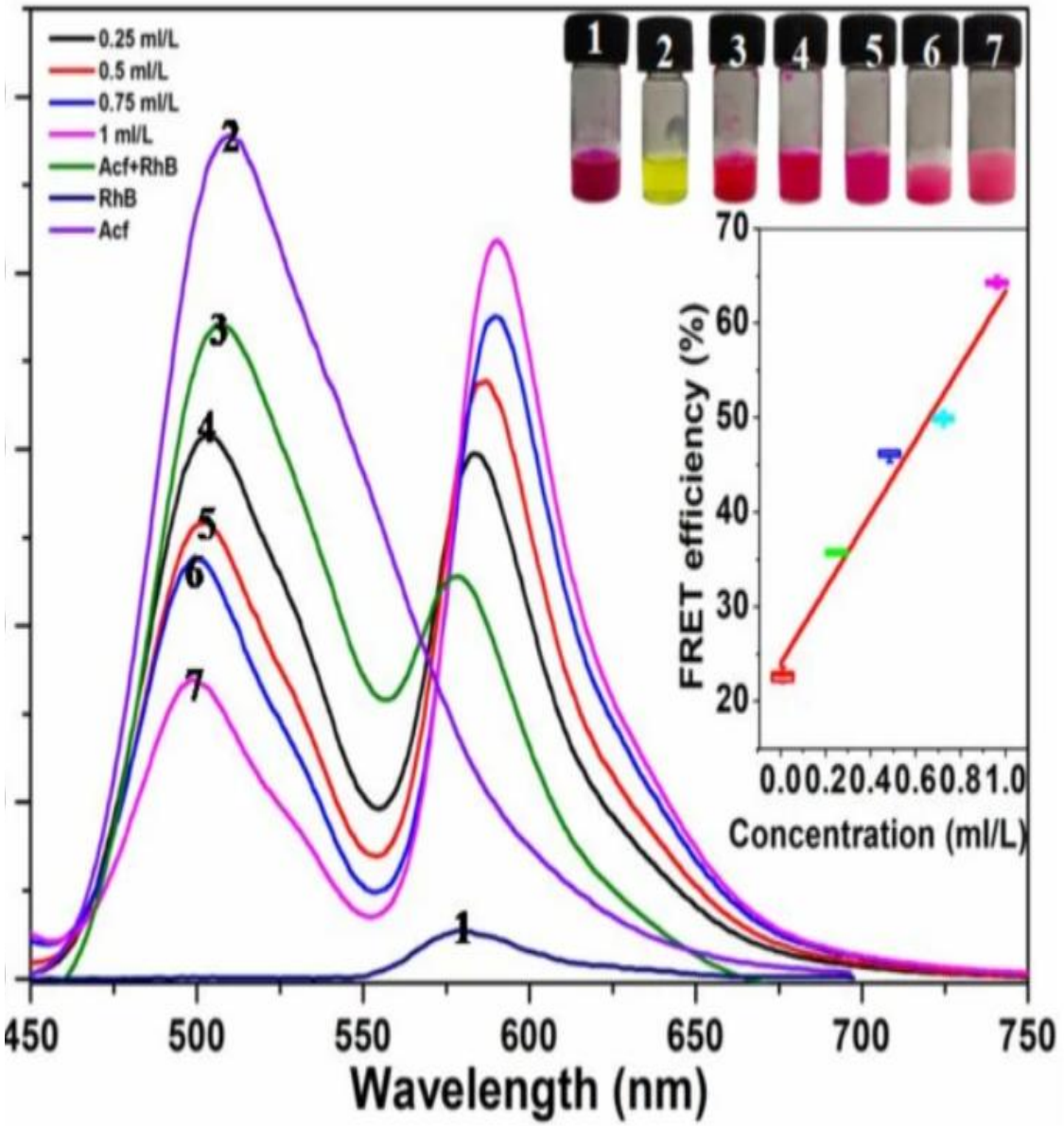
   Other sample components, solvent, reagents, or impurities should **not significantly absorb at the analytical wavelength**. Overlapping bands strongly reduce selectivity.

5. **Optically suitable and stable sample**

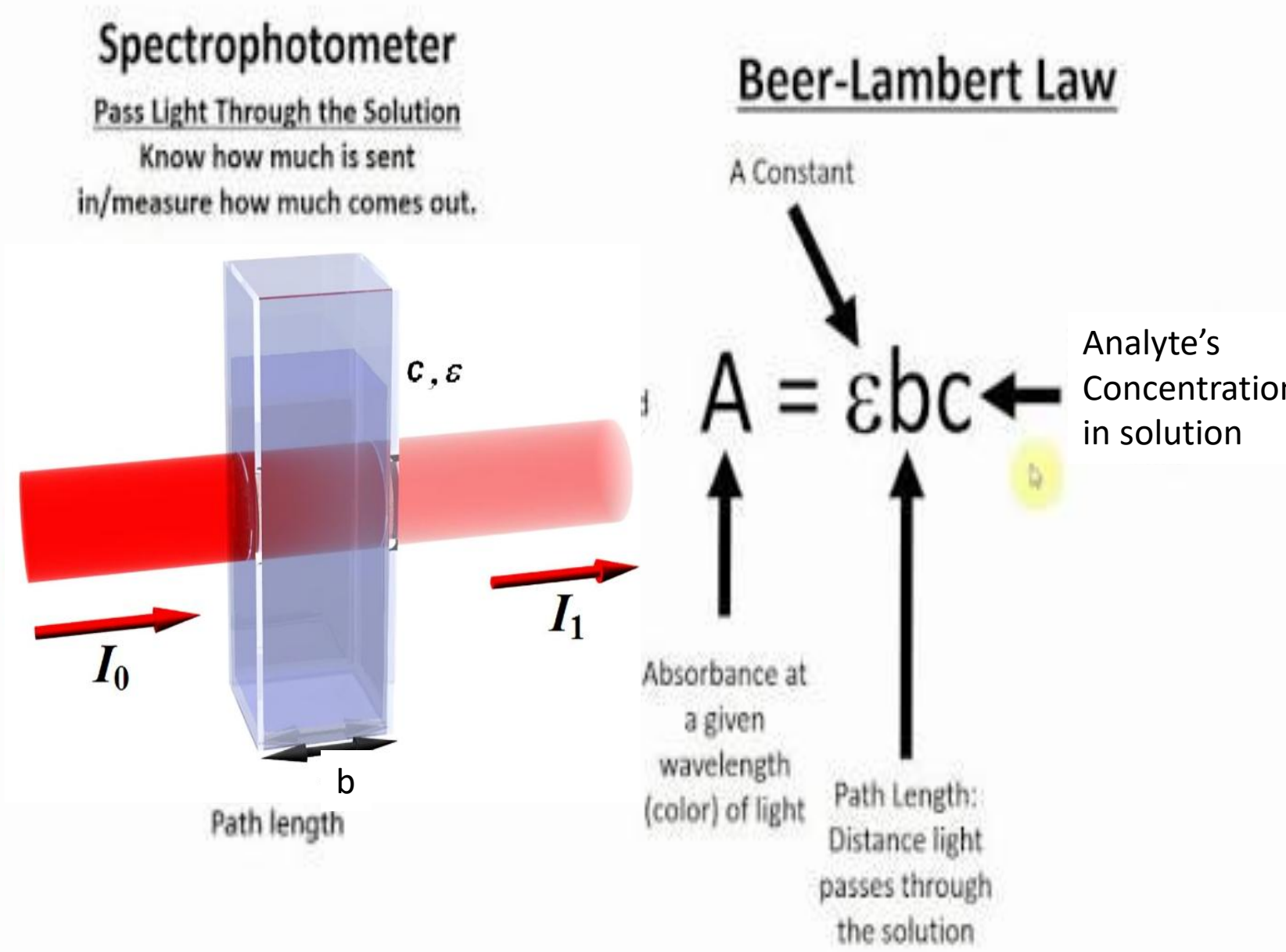
   The solution should preferably be **clear, homogeneous, and chemically stable** during measurement. Turbidity, particles, precipitation, bubbles, or chemical degradation can cause erroneous absorbance through scattering or changing composition.

Examples of UV VIS spectra of some pesticides in water





In order *<u>to make quantification there must be a low</u>* that links the intensity of absorbed light with the Concentration of analyte(s) present in the container



UV–Vis Spectroscopy — Strong and Weak Sides

STRONG SIDES / ADVANTAGES	WEAK SIDES / LIMITATIONS
Fast analysis – results within seconds/minutes	**Low selectivity in mixtures** – overlapping absorption bands when several absorbing species are present
Good reproducibility – highly reproducible under controlled conditions	**Relatively poor spectral resolution** – broad UV–Vis absorption bands make identification difficult
Simple sample preparation – often dilution is sufficient	**Moderate sensitivity** – typical useful concentration range is approximately **10–1000 µM**, depending strongly on the analyte and molar absorptivity
Low sample consumption – microgram quantities can often be sufficient	**Difficult multi-component analysis** – usually requires separation or chemometric approaches when spectra overlap
Low-cost technique – inexpensive instrument, consumables and	**Limited structural information** – provides information about electronic transitions/chromophores rather than detailed

IMPORTANT TO BE REMEMBERED!!!

→***UV-VIS is commonly a first choice*** **whenever we want to make fast analysis of *defined molecules* *that contain chromophores* *in their structures* that can give specific signatures in the UV-VIS spectrum…**

…**however**…

→ when we want to make some sort of *elementary analysis*, like *presence of some metal ions in water* for example, then we should explore some of the *Atomic Spectroscopic Techniques*

Principles of
ATOMIC ABSORPTION SPECTROSCOPY (AAS

AAS is based on the absorption of element-specific radiation by free ground-state atoms in the gaseous phase.

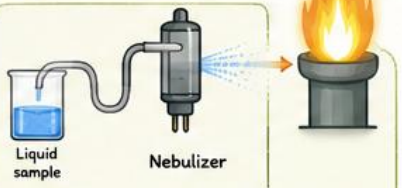
How it works?

1. Radiation Source

Hollow cathode lamp emits light of a specific wavelength for the element of interest.

I_0 (incident intensity)

2. Sample Introduction & Atomization



The liquid sample is aspirated into a nebulizer, producing a fine aerosol. The aerosol is carried into the flame where the solvent is evaporated, the compound is decomposed, and the element is atomized.

3. Free Atoms in Flame

Free ground-state atoms absorb light at characteristic wavelengths.

I (transmitted intensity)

4. Detection

The detector measures the decrease in intensity of transmitted light.

5. Readout

Absorbance is calculated and is proportional to the concentration of the element.

2a. How the liquid sample is dispersed in the flame

Liquid sample is aspirated into the nebulizer.

Nebulization produces a fine aerosol (tiny droplets).

The aerosol is carried by fuel/oxidant gases into the flame.

Solvent evaporates, leaving solid particles.

The compound decomposes (dissociates) into simpler species.

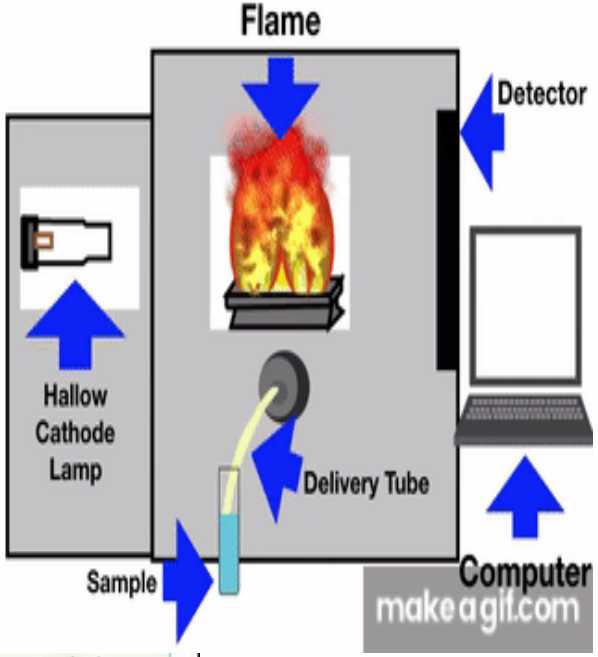
Free ground-state atoms of the element remain.

$$\text{Absorbance (A)} = \log \frac{I_0}{I}$$

where I_0 = incident light intensity
I = transmitted light intensity

→ According to Beer-Lambert Law, A ∝ concentration of absorbing atoms.

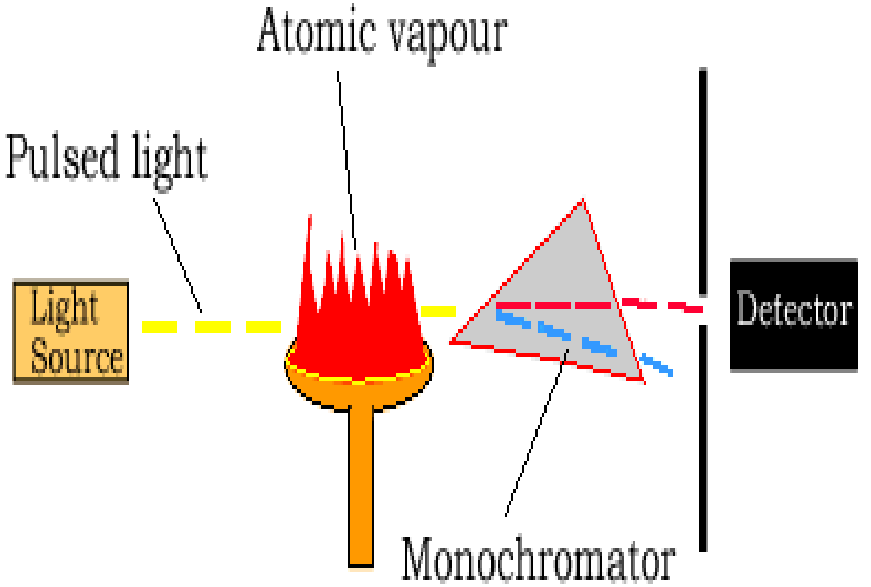
In short: AAS measures how much light is absorbed by free atoms of an element. More absorption means more atoms, hence higher concentration.

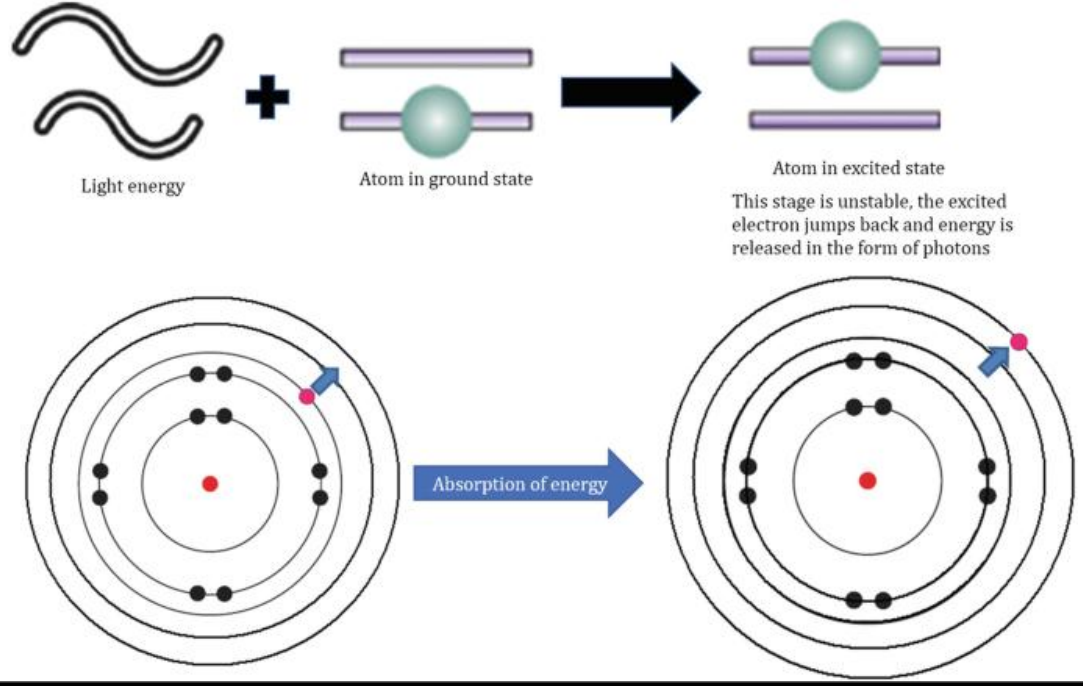


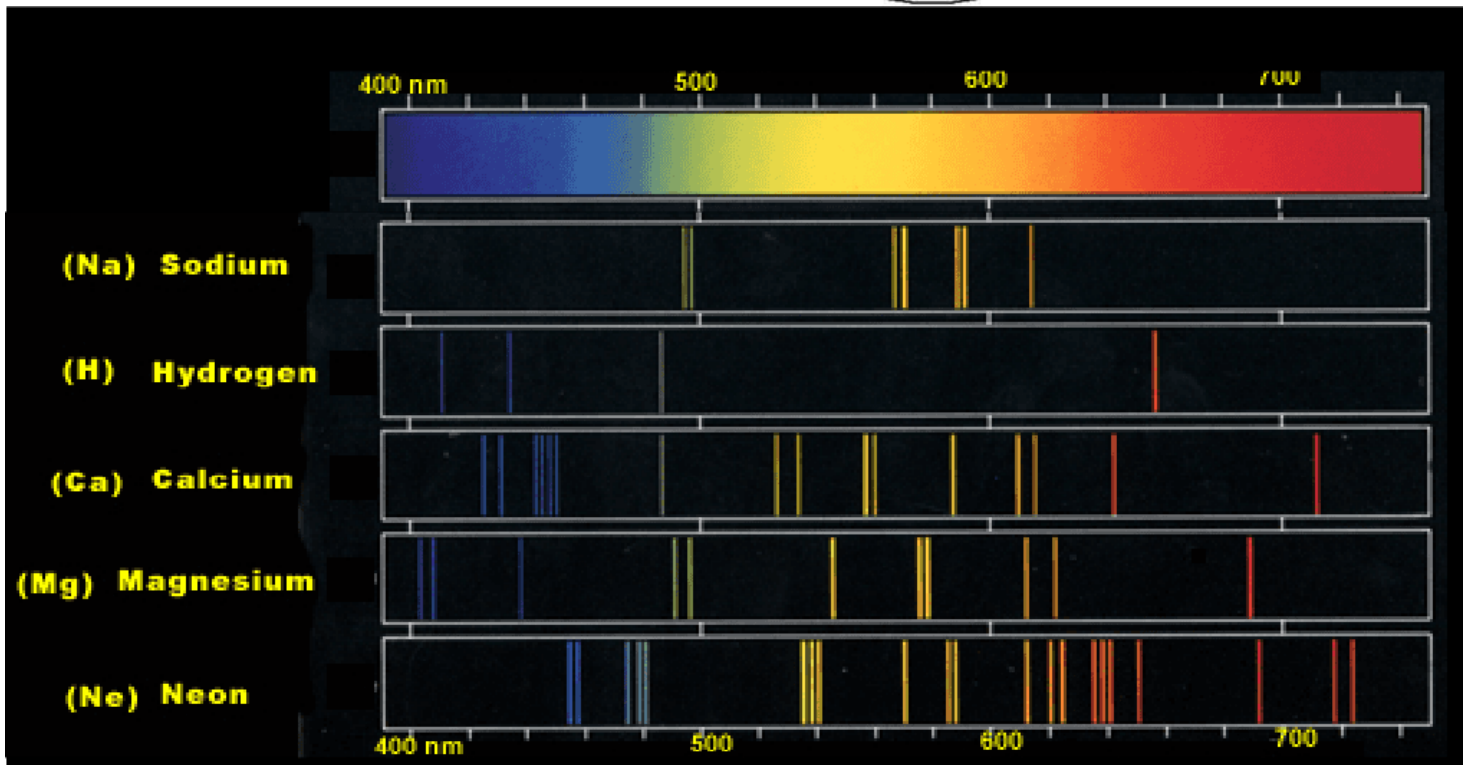
Key Point

Element Sp
Each element at its charact wavelength.

Free Ground-
Absorption occurs only by

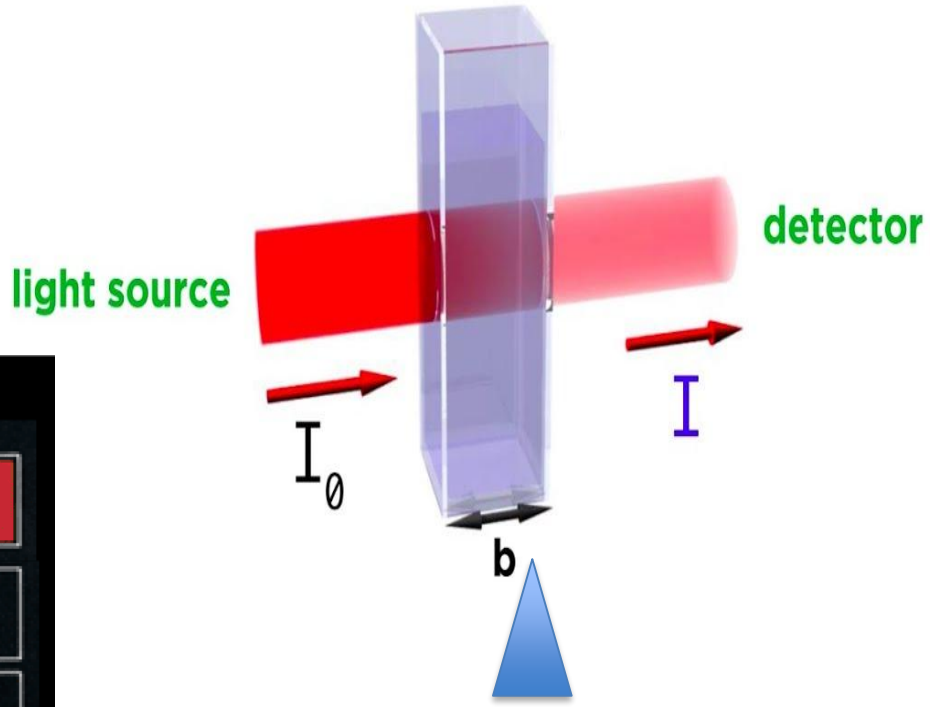






Beer's Law

$$A = \varepsilon bc$$



There must be a low for Quantitative determination that Links concentration of the Analyte with the Instrumental outptut

Why temperature matters

Imagine that your sample contains a metal compound, for example **$CaCl_2$**. When introduced into a flame/plasma, it passes approximately through:

Solution → aerosol → dry particles → vapor → molecules → free atoms → excited atoms → ions

or schematically:

$$\mathrm{CaCl_2(aq)} \rightarrow \mathrm{CaCl_2(s)} \rightarrow \mathrm{CaCl_2(g)} \rightarrow \mathbf{Ca(g)} \rightarrow \mathbf{Ca^*(g)} \rightarrow \mathbf{Ca^+(g)}$$

As the available thermal energy increases, the population can progressively shift toward atomization, excitation, and eventually ionization.

Lower/moderate temperature → predominantly ground-state atoms

$$M(g)$$

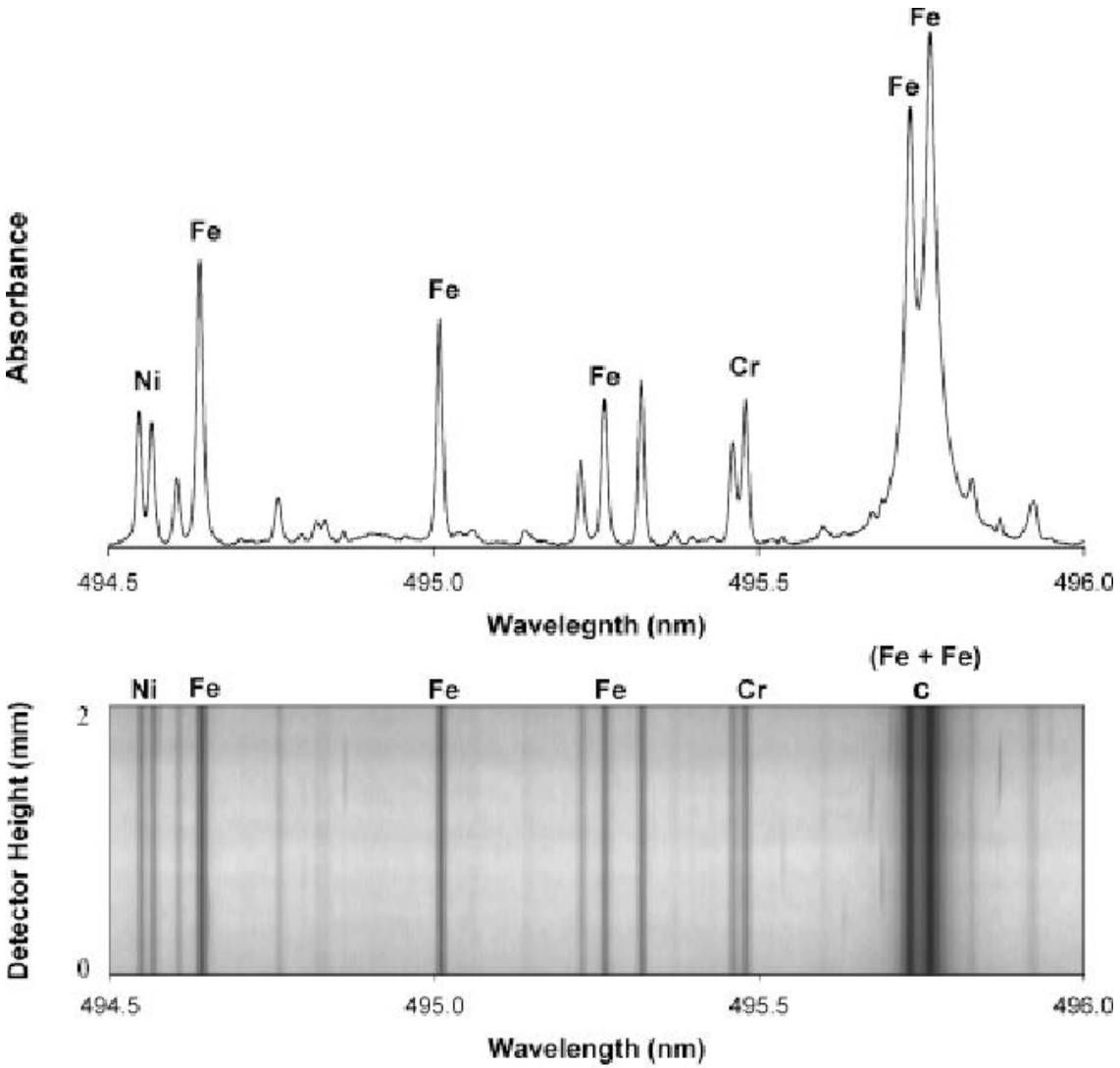
These are exactly what we want for **AAS** because they can absorb characteristic radiation:

$$\boxed{M + h\nu \rightarrow M^*}$$

For **flame AAS (FAAS)**, the temperature depends mainly on the **fuel–oxidant combination**
In practice, temperatures of roughly **2000–3000 °C** are common. The flame must be hot enough to desolvate, vaporize, dissociate, and atomize the sample, while maintaining a sufficiently large population of **neutral ground-state atoms**.

Common flames used in AAS

Fuel + oxidant	Approx. flame temperature	Typical use
Natural gas + air	~1700–1900 °C	Easily atomized elements; less common today
H_2 + air	~2000–2100 °C	Some easily atomized elements
Acetylene + air	**~2100–2400 °C**	**Most common FAAS flame**
Acetylene + N_2O	**~2600–2900 °C**	Refractory elements, stable oxides
Acetylene + O_2	~3000–3200 °C	Very hot; uncommon for routine AAS



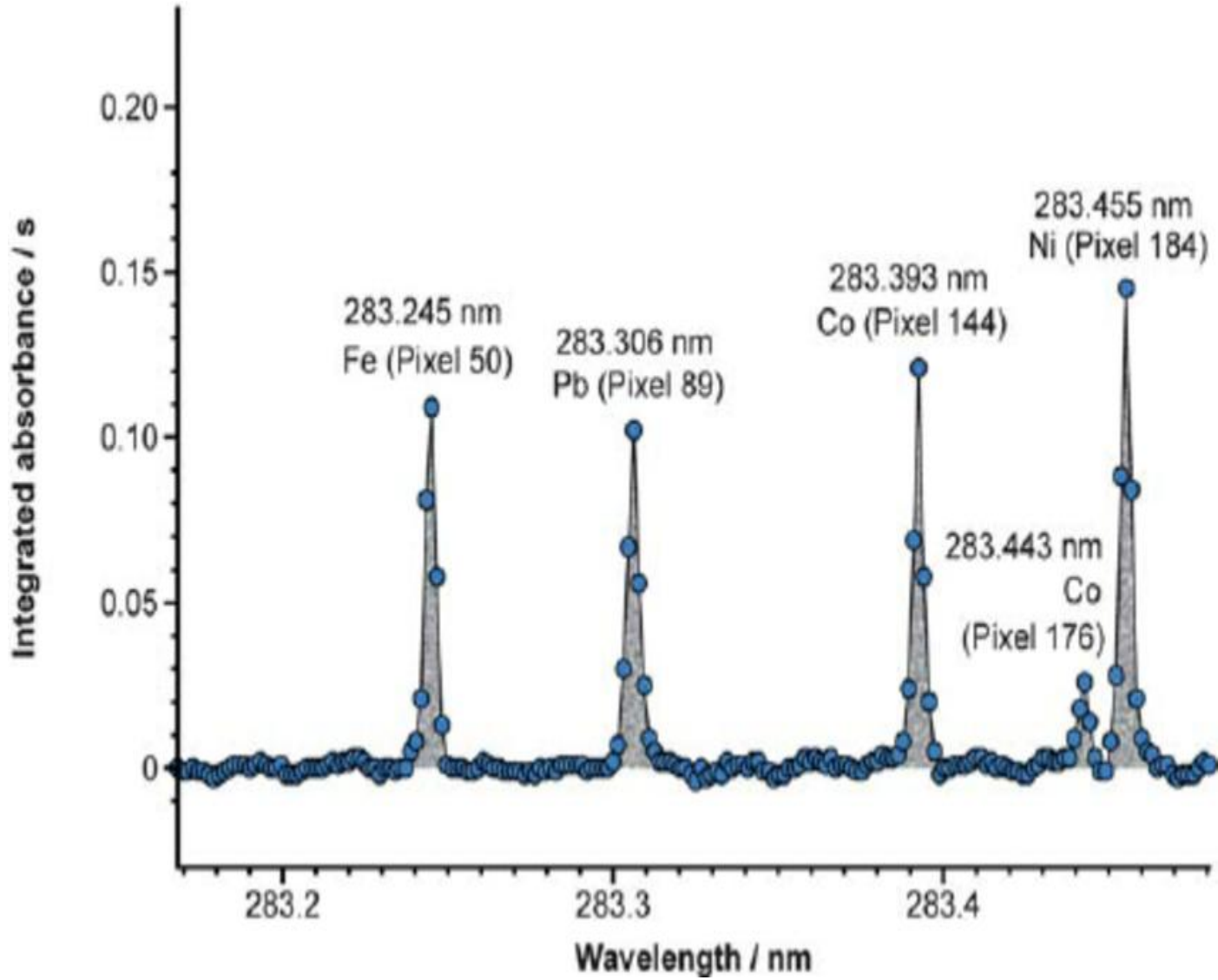
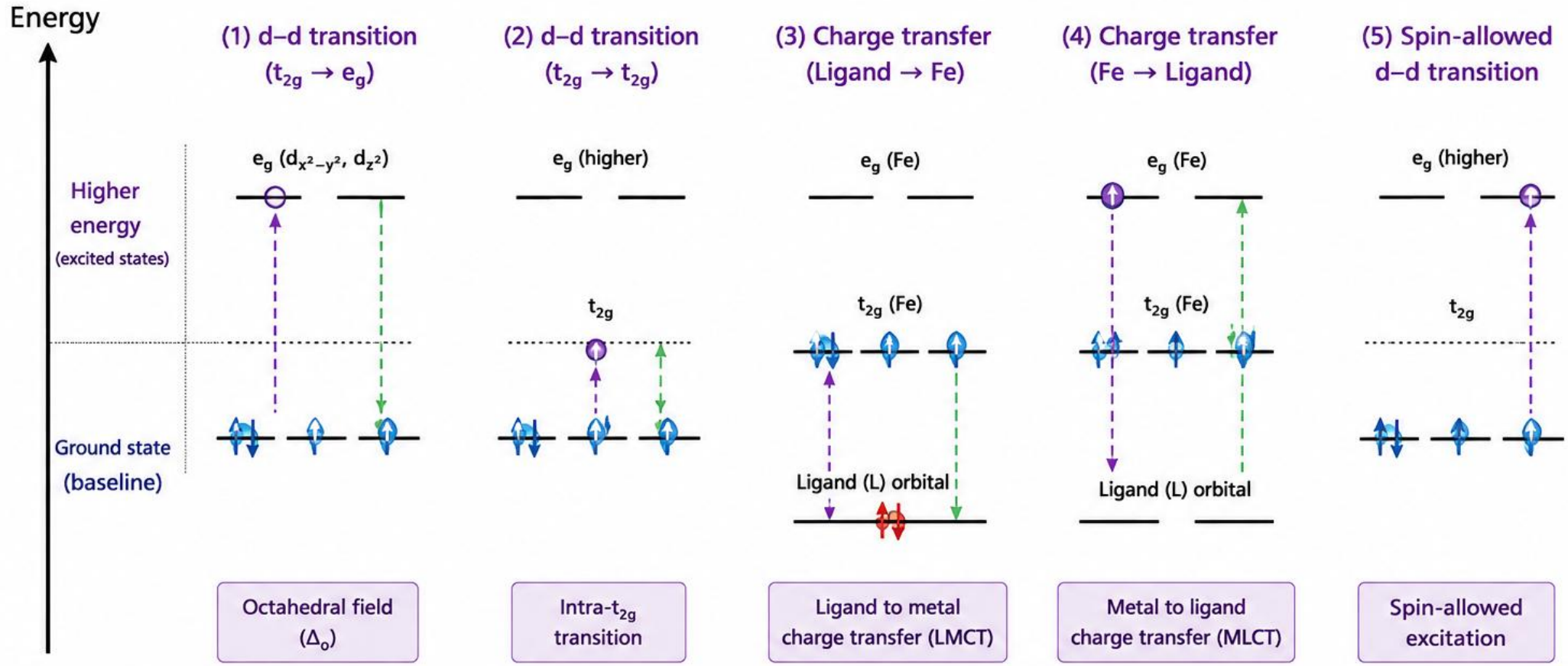


Figure 2. Fraunhofer effect spectrum of the sun including the c

Possible electron transfers in the last energy level of iron (Fe) after UV–Vis energy absorption



Fe with partially filled 3d orbitals

- - - → UV–Vis absorption (excitation)

- - - → Return to ground state (relaxation, emission or non-radiative decay)

Notes:

- Horizontal lines = d-orbital energy levels
- Arrows on circles = electron spins
- Dashed purple arrows = UV–Vis absorption
- Dashed green arrows = relaxation to ground state

Principles of
ATOMIC EMISSION SPECTROSCOPY (AES)

AES is based on the emission of element-specific radiation by excited atoms as they return to lower energy states.

How it works?

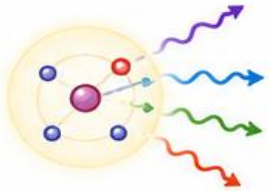
1. Sample Introduction

The liquid sample is aspirated into a nebulizer, producing a fine aerosol that is carried to the excitation source.

2. Excitation (Atomization & Excitation)

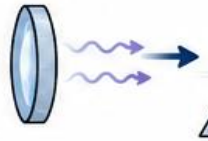
In the flame or plasma, the sample is desolvated, vaporized, atomized and the atoms are excited to higher energy levels.

3. Emission of Light



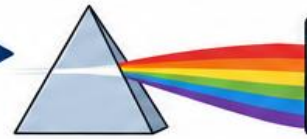
Excited atoms return to lower energy states and emit light at characteristic wavelengths for each element.

4. Light Collection



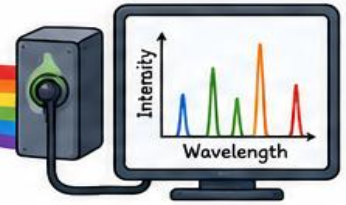
The emitted light is collected and focused into the spectrometer.

5. Wavelength Selection



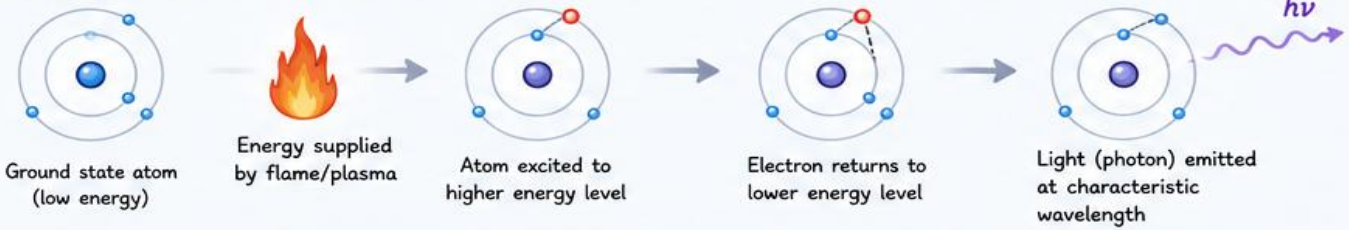
The spectrometer separates the light into individual wavelengths.

6. Detection & Readout



The detector measures the intensity of each wavelength. Intensity is proportional to the concentration of the element.

What happens at the atomic level?



Ground state atom (low energy)

Energy supplied by flame/plasma

Atom excited to higher energy level

Electron returns to lower energy level

Light (photon) emitted at characteristic wavelength

Result

A spectrum of emission lines, each line corresponding to an element.

Emission vs. Absorption

AAS

Measures absorption of light by ground-state atoms.

AES

Measures emission of light by excited atoms.

Important Equation

$$I \propto C$$

I = emission intensity
C = concentration of the element

In short: AES measures the characteristic light emitted by excited atoms. Stronger emission means higher concentration of the element.

KEY POINTS

Element Specific
Each element emits light at unique wavelengths, like a "fingerprint".

Excited Atoms
Emission occurs when excited atoms return to lower energy states.

Quantitative Technique
Emission intensity is directly proportional to the concentration.

Excitation Sources
Common sources are flame, ICP (plasma), arc and spark.

Wide Applications
Used in environmental, food, clinical, materials, metallurgical and geological analysis.

For **Atomic Emission Spectroscopy (AES)**, temperature is even more critical than in AAS because the source must do **two things**: first atomize the sample and then provide enough energy to **excite the atoms** so that they emit characteristic radiation.

The essential distinction is:

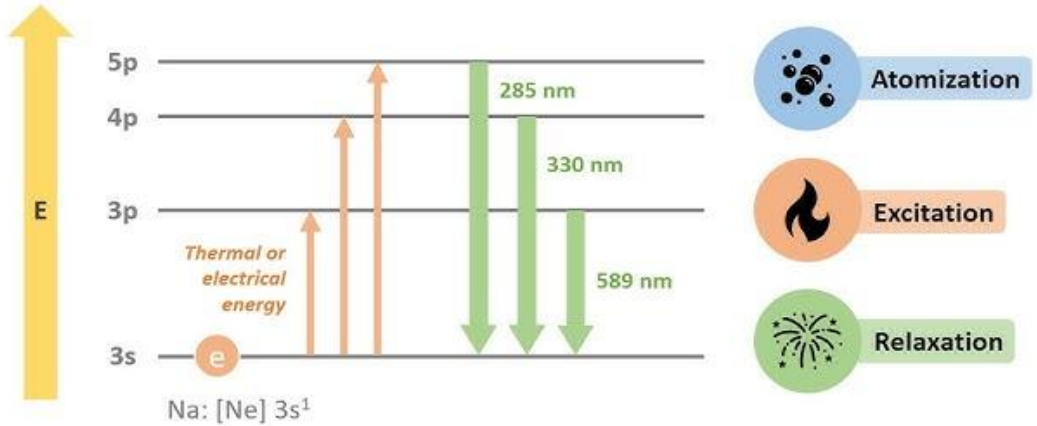
$$\text{AAS: } M + h\nu \rightarrow M^*$$

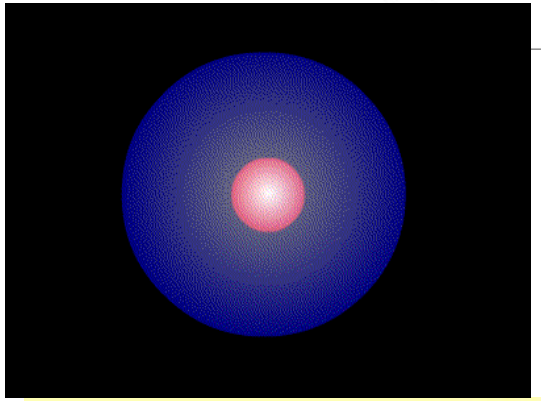
$$\text{AES: } M + \text{thermal energy} \rightarrow M^* \rightarrow M + h\nu$$

Typical temperatures in AES

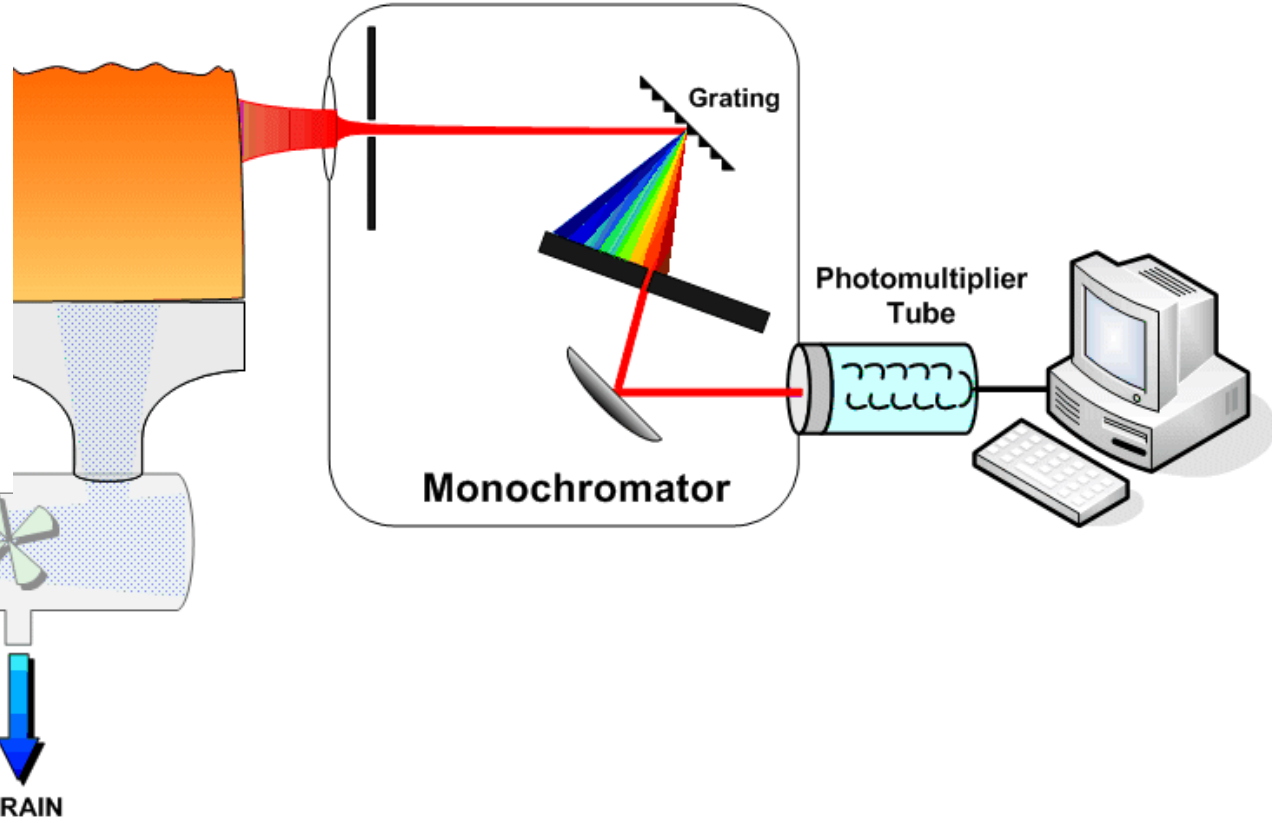
AES source	Typical temperature	Energy/source	Main characteristics
Air–acetylene flame	~2100–2400 °C	C_2H_2 + air	Flame emission; mainly easily excited elements
N_2O–acetylene flame	~2600–2900 °C	C_2H_2 + N_2O	Higher excitation efficiency
Arc	~3000–7000 K	Electrical discharge	Strong excitation; solid samples possible

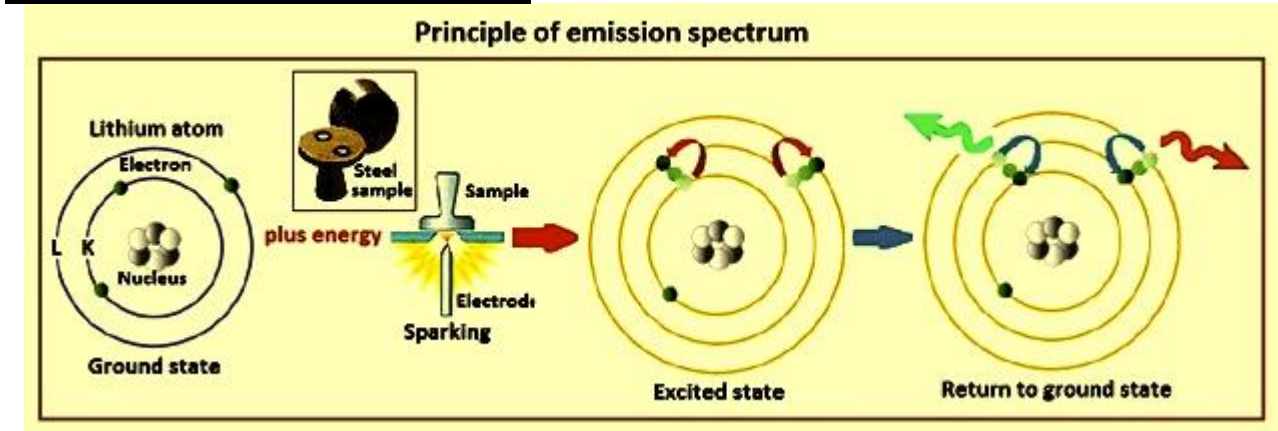
Atomic emission spectrometry (AES)





Atomic Emission Spectroscopy





Higher temperature → greater population of excited atoms

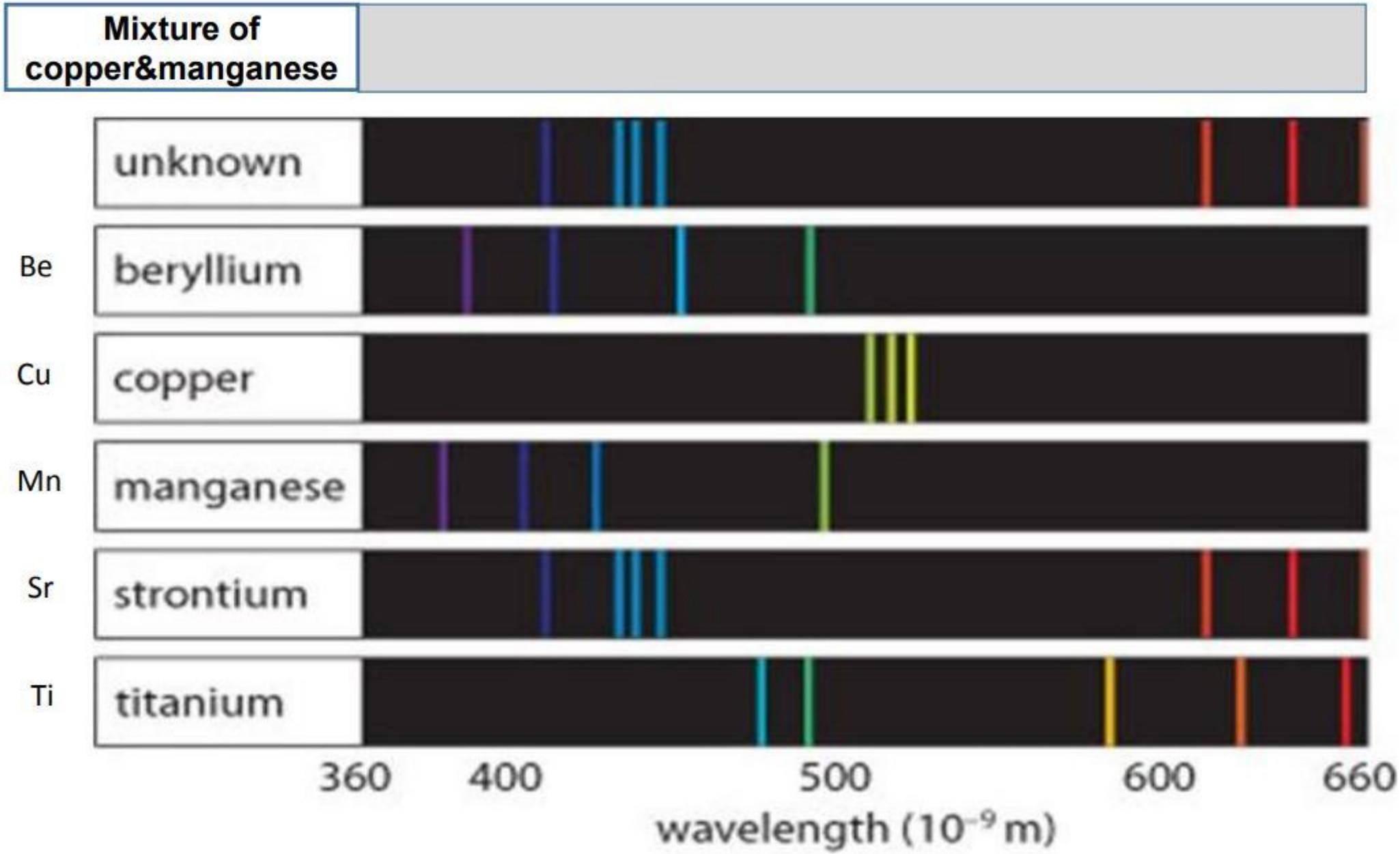
$$M^*(g)$$

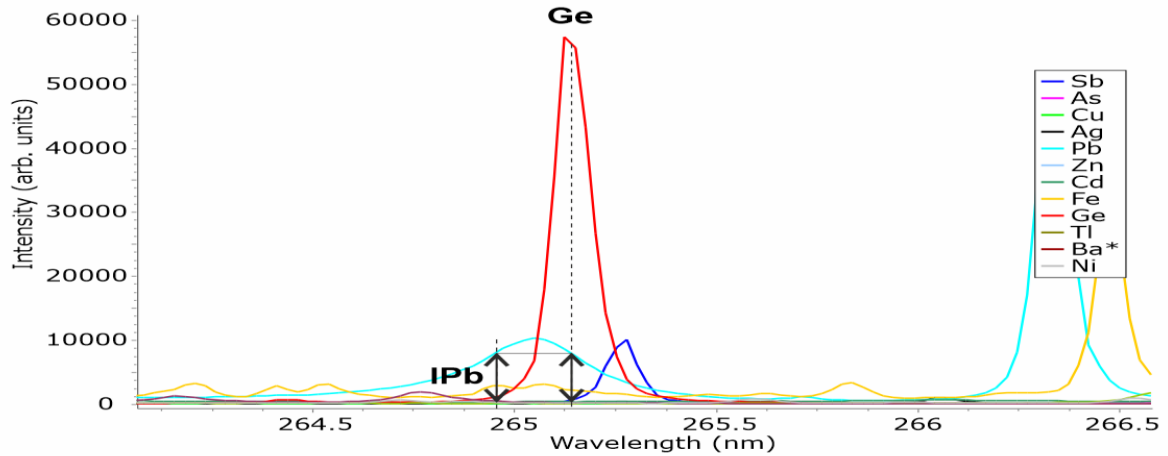
These atoms can return to lower electronic states and emit photons:

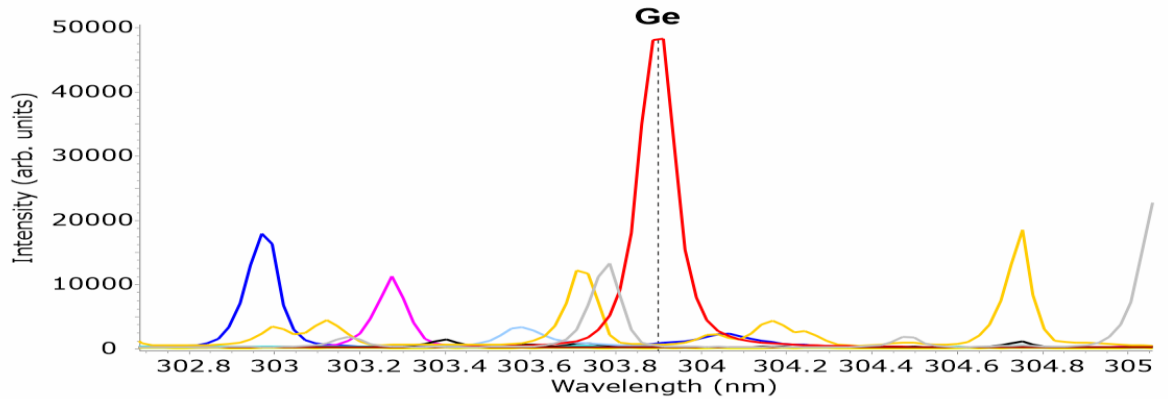
$$\boxed{M^* \rightarrow M + h\nu}$$

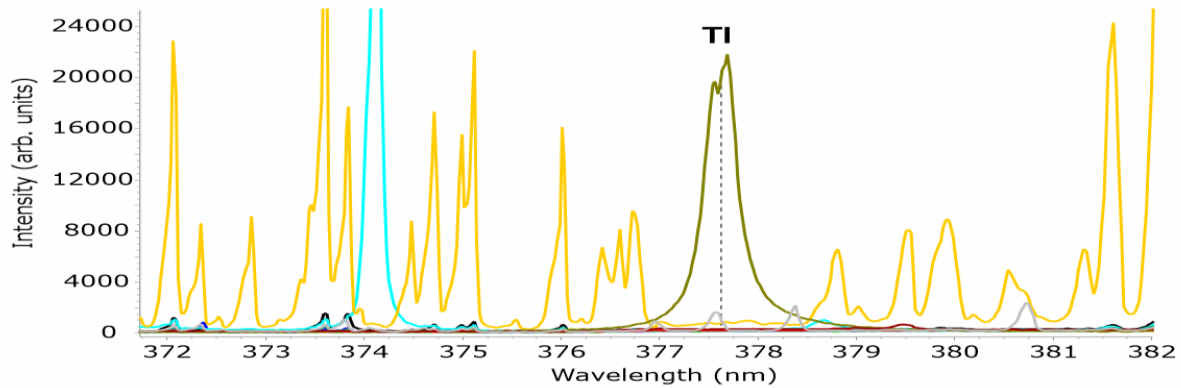
This is the basis of **AES**.

Figure 2 Emission spectra 2




Ge
IPb
Sb
As
Cu
Ag
Pb
Zn
Cd
Fe
Ge
Tl
Ba*
Ni
Intensity (arb. units)
Wavelength (nm)


Ge
Intensity (arb. units)
Wavelength (nm)


Tl
Intensity (arb. units)
Wavelength (nm)

INDUCTIVELY COUPLED PLASMA (ICP)

ICP is a high-temperature (~6000–10,000 K), inert plasma source used to atomize and excite elements for emission (ICP-AES) or ionize elements for mass analysis (ICP-MS).

How it works?

1. Sample Introduction
The liquid sample is introduced as a fine aerosol (nebulization) into the plasma.

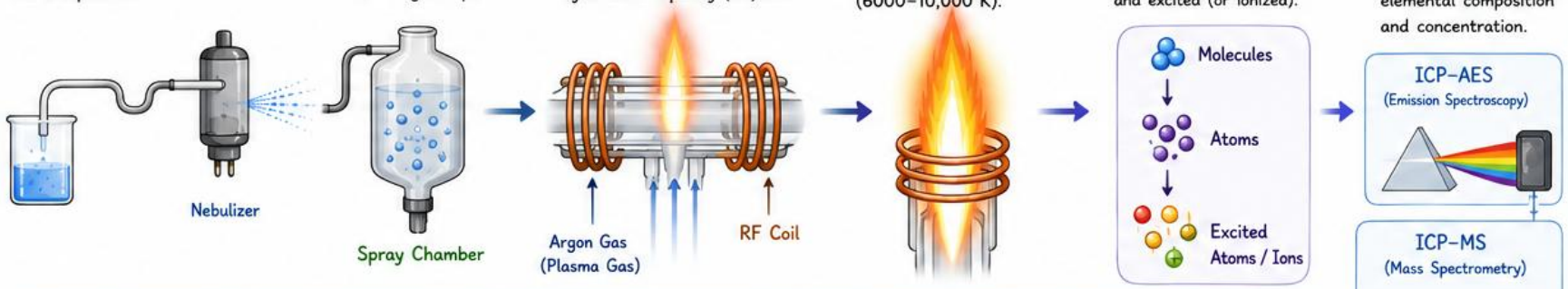
2. Aerosol Transport
The aerosol is carried by argon gas through a spray chamber to remove large droplets.

3. ICP Torch
The fine aerosol enters the torch where argon gas flows through a quartz tube surrounded by a radiofrequency (RF) coil.

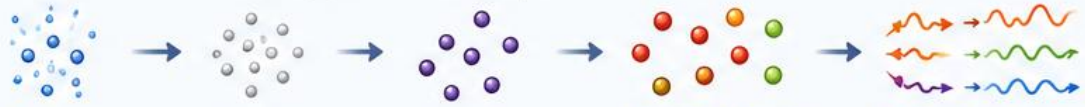
4. Plasma Generation
The RF field induces a current in the argon gas, generating a high-temperature plasma (6000–10,000 K).

5. Atomization & Excitation/ Ionization
In the plasma, the sample is desolvated, vaporized, atomized and excited (or ionized).

6. Detection
Emitted light (ICP-AES) or ions (ICP-MS) are measured to determine elemental composition and concentration.



Inside the Plasma: What happens to the sample?



Desolvation
Solvent is evaporated.

Vaporization
The dry aerosol becomes vapor.

Atomization
Molecules dissociate into free atoms.

Excitation / Ionization
Atoms are excited or ionized by collisions with energetic electrons.

Emission / In Plasma
Excited atoms emit light (ICP-AES) or remain as ions (ICP-MS).

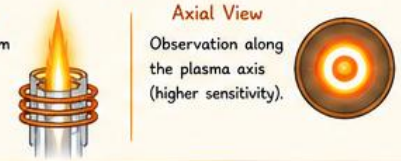
Plasma Characteristics
- Temperature: ~6000–10,000 K
- Argon gas flow
- RF power (typically 700–1600 W)
- Robust, stable and reproducible

Why ICP is powerful?
- ✓ Very high temperature → efficient atomization and excitation/ionization
- ✓ Handles complex matrices
- ✓ Low detection limits (ppm–ppb–ppt)
- ✓ Wide linear dynamic range

ICP Configurations

Radial View
Observation from the side of the plasma.

Axial View
Observation along the plasma axis (higher sensitivity).



Common ICP Types
- ICP-OES (Optical Emission Spectroscopy)
  → Elemental analysis by light emission.
- ICP-MS (Mass Spectrometry)
  → Elemental analysis by mass-to-charge ratio of ions.

KEY POINTS

High Temperature Plasma
Provides efficient atomization and excitation/ionization of nearly all elements.

Inert Environment
Argon plasma is inert, minimizing chemical interferences.

Multi-Element Capability
Simultaneous detection of many elements with high sensitivity.

Wide Applications
Environmental, food, pharmaceutical, clinical, metallurgical, geological and more.

In short: ICP uses a high-temperature argon plasma to convert the sample into atoms or ions.
The emitted light or ions are measured to identify and quantify elements with excellent sensitivity and precision.

SPECTROSCOPIC TECHNIQUES

Major Advantages and Major Pitfalls

MAJOR ADVANTAGES

High Sensitivity
Detect elements at very low concentrations (ppm, ppb or even ppt levels depending on the technique).

Elemental Specificity
Each element has a unique spectral signature, allowing reliable identification.

Wide Dynamic Range
Accurate quantification over several orders of magnitude.

Fast Analysis
Measurement time is short once the method is optimized.

Multi–Element Capability
Simultaneous determination of many elements (especially in ICP–AES / ICP–MS).

Small Sample Requirement
Only small sample volumes are needed.

Good Reproducibility and Accuracy
When conditions are controlled, spectroscopic methods provide precise and reliable results.

MAJOR PITFALLS

High Temperatures Can Cause Species Formation
In flame or plasma, atoms may form bi– or multi–conglomerates (oxides, dimers, clusters, refractory compounds), leading to signal suppression or enhancement and inaccurate results.

Only Total Amount Determined
Spectroscopic techniques measure the total concentration of an element, not its chemical form or oxidation state (unless using specialized methods).

Dependence on Temperature Constancy
Flame or plasma temperature must be stable. Small fluctuations in temperature, gas flow or RF power can significantly affect atomization/excitation efficiency and signal intensity.

Matrix Interferences
Chemical, physical and spectral interferences from the sample matrix can affect accuracy and require careful optimization or the use of internal standards.

High Instrument and Operating Costs
Instrumentation (especially ICP–MS) is expensive. Operating costs include argon gas, maintenance, standards and skilled staff.

Calibration and Standards Needed
Reliable results require appropriate standards, calibration and regular quality control.

Sample Preparation & Waste
Some techniques require digestion or chemical preparation and generate hazardous waste.

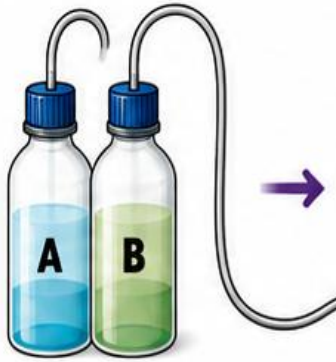
In short: Spectroscopic techniques are **powerful, sensitive and versatile**, but they require careful control of experimental conditions, awareness of interferences and proper calibration to ensure accurate results.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is a powerful analytical technique used to separate, identify and quantify components in a mixture based on their distribution between a mobile phase (liquid) and a stationary phase (solid).

HOW IT WORKS – THE HPLC SYSTEM

1 Solvent Reservoir (Mobile Phase)



The mobile phase (solvent or solvent mixture) is stored and delivered to the system.

2 Pump

The pump delivers the mobile phase at high pressure (typically 50–400 bar) at a constant flow rate.

3 Sample Injector

A precise volume of sample (typically 1–50 µL) is injected into the flowing mobile phase.

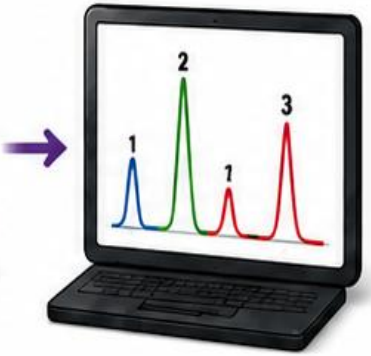
4 Column (Stationary Phase)

The sample components interact differently with the stationary phase, leading to separation as they travel through the column.

5 Detector

The detector measures the concentration of each component as it elutes from the column.

6 Data System (Chromatogram)



The data is recorded and visualized as a chromatogram: peaks correspond to separated components.

KEY POINTS

High Efficiency
Small particle size in the column (e.g., 1.7–5 µm) provides high resolution and sensitivity.

High Pressure Liquid System
Uses high pressure to push the mobile phase through the column, enabling fast and efficient separations.

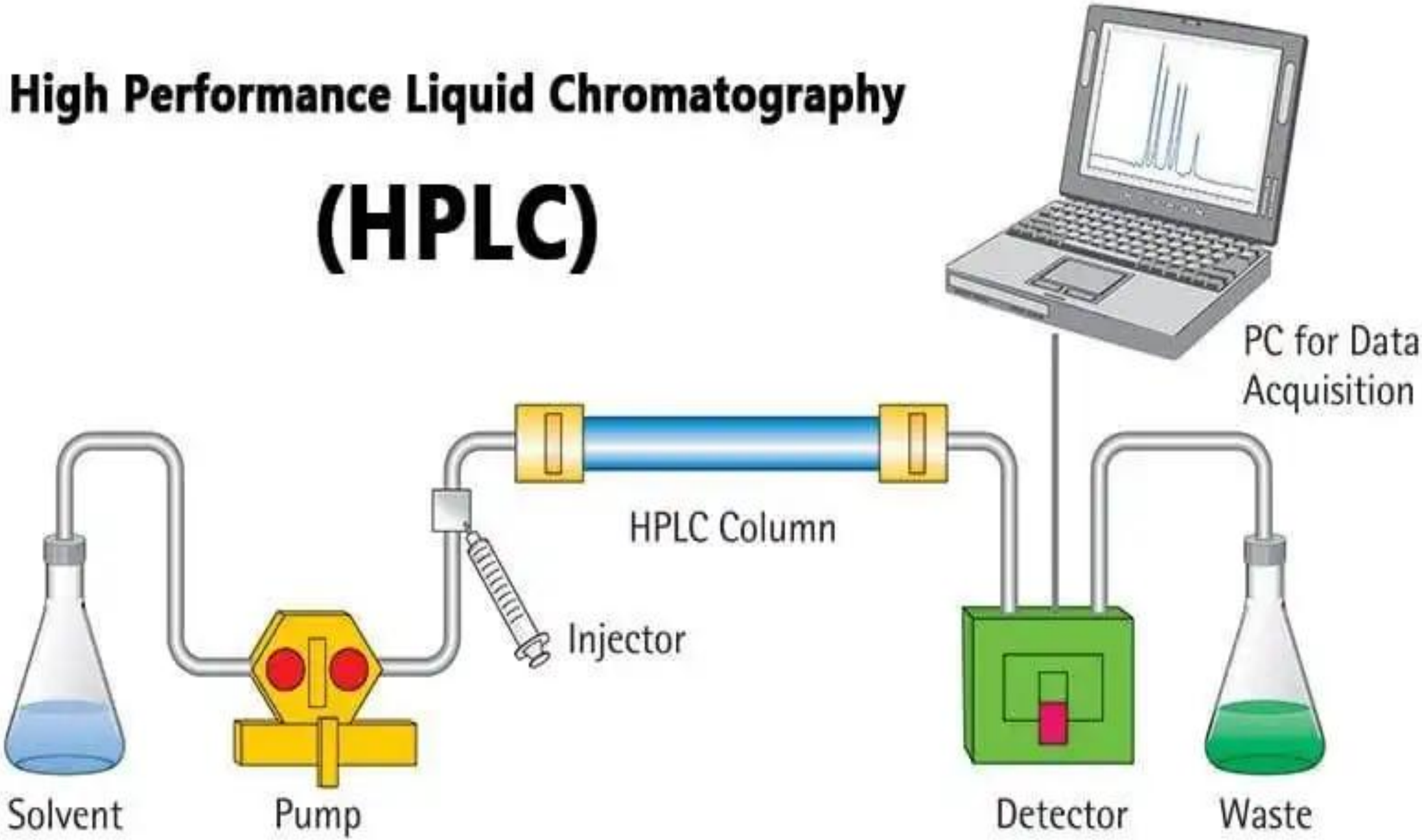
Versatility
Applicable to a wide range of samples: pharmaceuticals, foods, environmental, biological, etc.

Quantitative
Peak area (or height) is proportional to the amount of analyte.

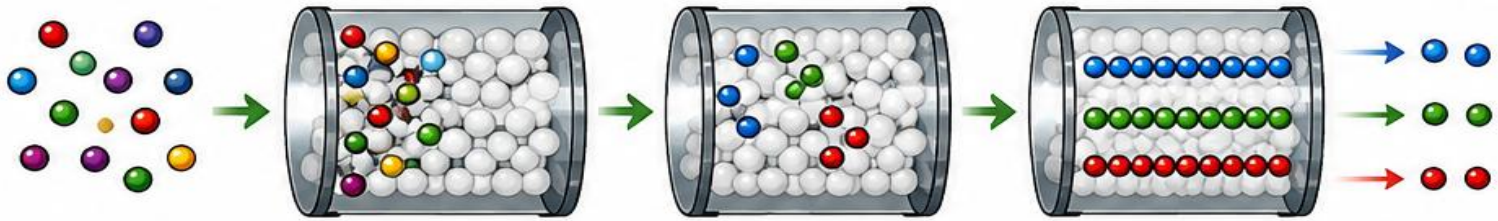
Reproducibility
Excellent precision and accuracy with proper

High Performance Liquid Chromatography

(HPLC)



THE SEPARATION PRINCIPLE



Mixed sample is injected into the mobile phase.

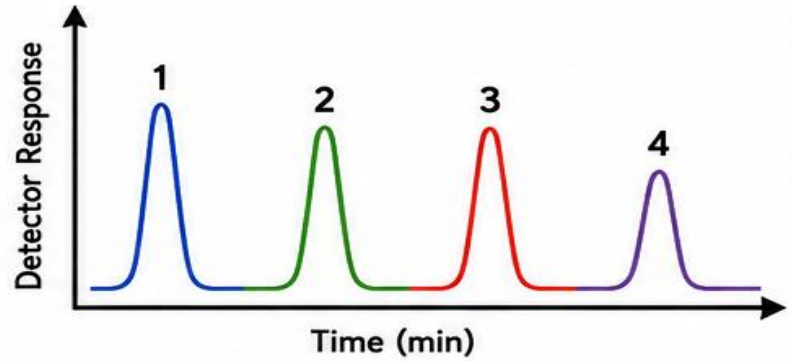
Analytes partition between the mobile phase and the stationary phase.

Components interact differently with the stationary phase (adsorption, partition, ion exchange, etc.).

Less retained components move faster; strongly retained components move slower.

Separated components (elute) at different times.

CHROMATOGRAM



Each peak represents a component. Retention time (t_R) is characteristic under specific conditions.

IMPORTANT TERMS

- **Retention time (t_R):** Time from injection to the peak maximum.
- **Dead time (t_0):** Time for an unretained compound to elute.
- **Capacity factor (k'):** $k' = (t_R - t_0) / t_0$
- **Resolution (R_S):** Measure of separation between two peaks.
  $R_S > 1.5$ indicates baseline separation.

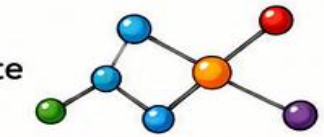
TYPES OF HPLC

- **Normal Phase HPLC** – Polar stationary phase, nonpolar mobile phase
- **Reverse Phase HPLC** – Nonpolar stationary phase, polar mobile phase (most common)
- **Ion Exchange HPLC** – Separation based on charge
- **Size Exclusion (Gel Permeation) HPLC** – Separation based on molecular size
- **Affinity HPLC** – Based on specific biological interactions

WIDE APPLICATIONS

- Pharmaceutical analysis & quality control
- Bioanalysis & clinical diagnostics
- Food and beverage testing
- Environmental monitoring
- Forensic and toxicology studies

In short: HPLC separates mixture components based on their different interactions with the stationary phase and mobile phase, allowing accurate identification and quantification.



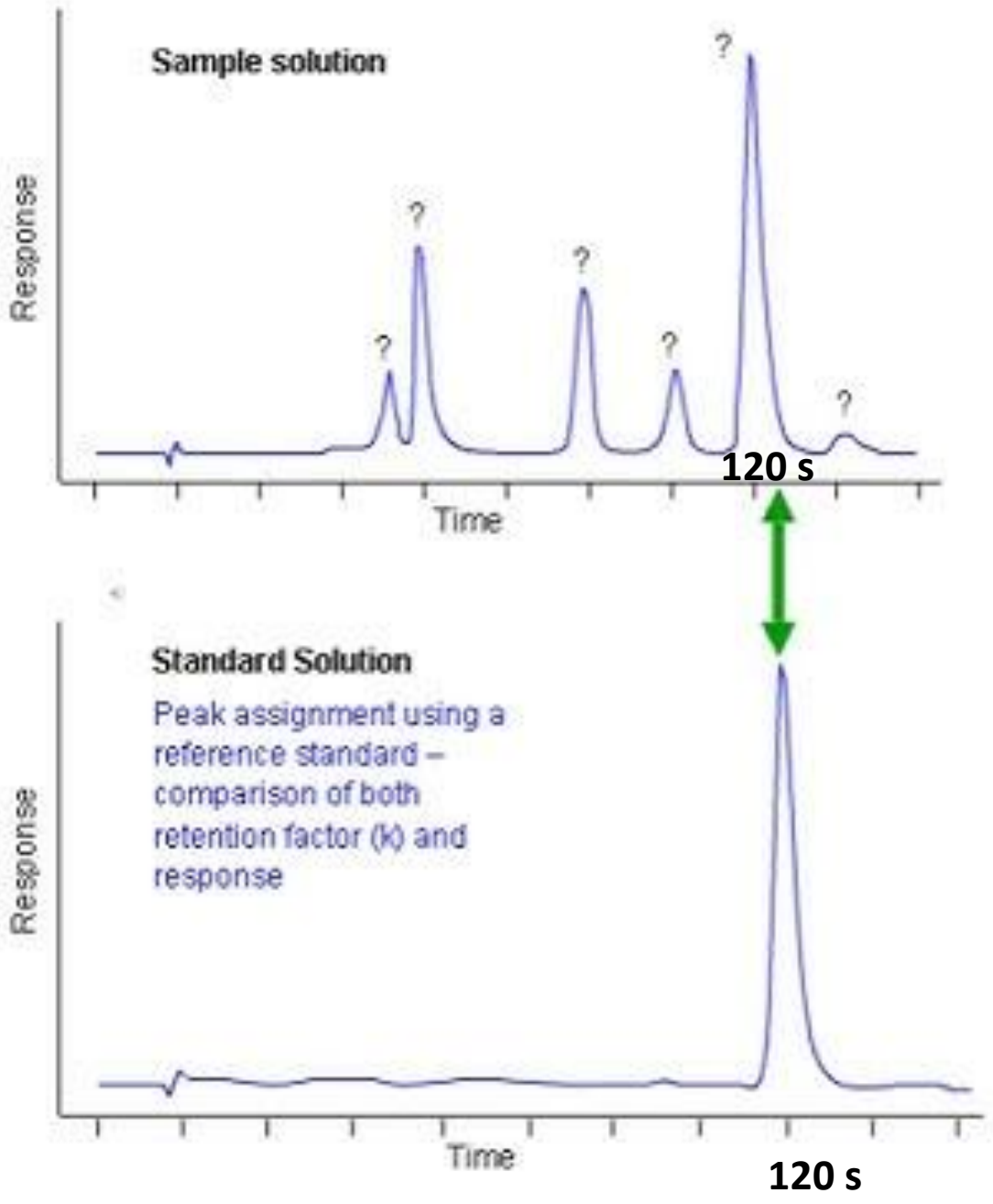
Qualitative assignment?

-**retention time** obtained

Under identical conditions

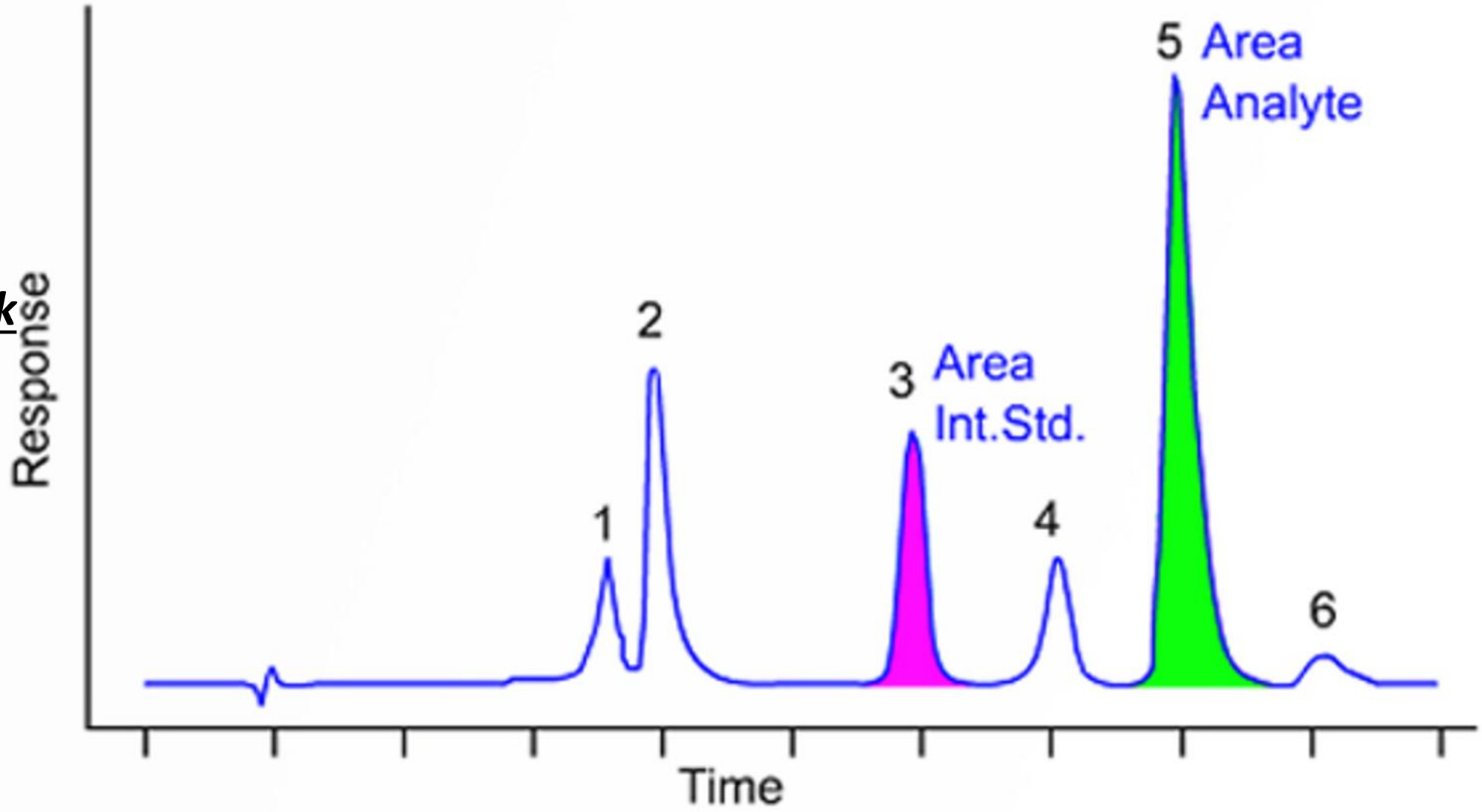
Is compared

- It is important that the concentration of the standard solution is matched to the sample solution as closely as possible. This avoids peak mis-assignment due to peak shape effects

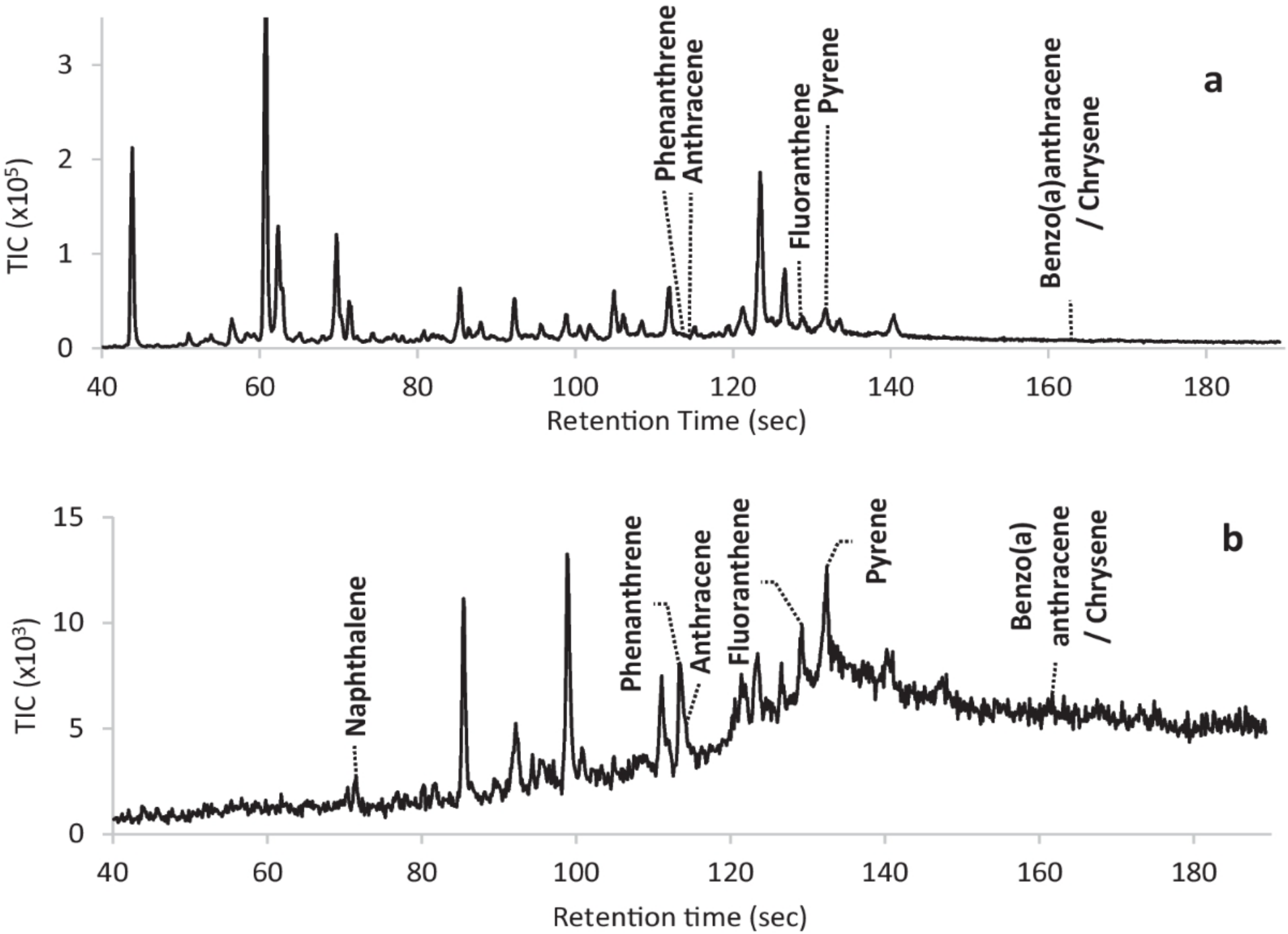


And
How is Quantification Achieved?

---measure the *area below the peak* and *compared it with an internal Standard!*



HPLC Chromatograms of Some organic Compounds in water

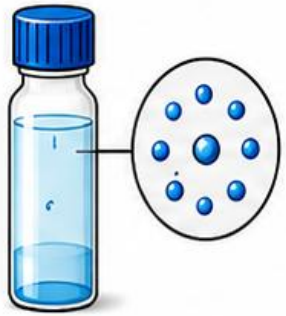


GAS CHROMATOGRAPHY (GC)

GC is an analytical technique used to separate, identify and quantify volatile and semi-volatile compounds in a mixture. The sample is vaporized and separated in a column by a carrier gas.

HOW IT WORKS – THE GC SYSTEM

1 Sample Introduction



A small amount of liquid or solid sample is placed in the injector and vaporized instantly.

2 Injector

The sample vapor is carried by the mobile phase (carrier gas) into the system.

3 Carrier Gas (Mobile Phase)

An inert gas (He, N_2, H_2, or Ar) flows at a constant rate and carries the vapor through the column.

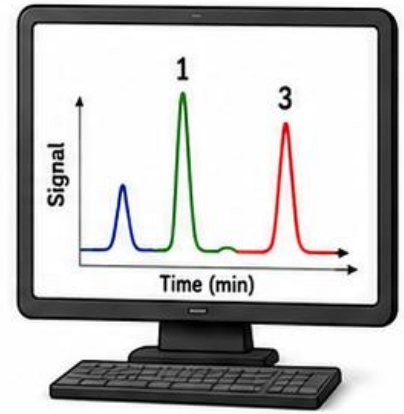
4 Column (Stationary Phase)

The components of the sample interact differently with the stationary phase coating inside the column. They travel through the column at different speeds, resulting in separation.

5 Detector

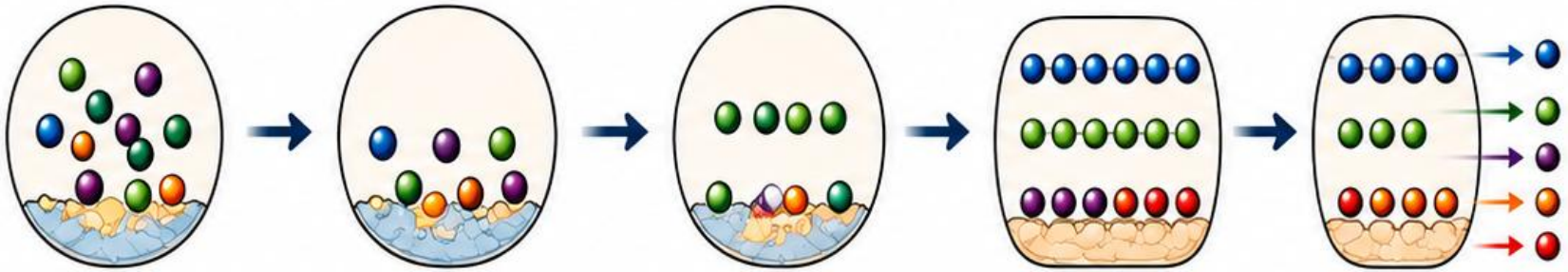
As compounds elute from the column, the detector measures their signal (e.g., FID, TCD, ECD, MS, etc.).

6 Data System (Chromatogram)



The signal is converted into a chromatogram where each peak represents a compound. Peak area is proportional to its amount.

THE SEPARATION PRINCIPLE



Vaporized sample enters the column with the carrier gas.

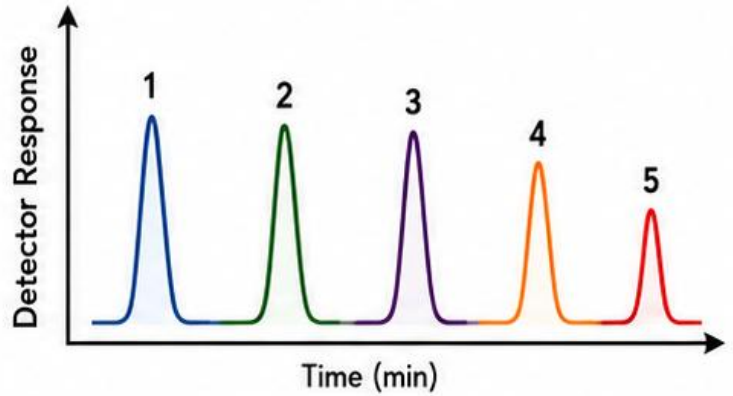
Components partition between the mobile gas phase and the stationary phase (column coating).

Components with weaker interaction move faster.

Components with stronger interaction move slower.

Separated components elute from the column at different times.

EXAMPLE CHROMATOGRAM



Each peak = one compound

Retention time (t_R) = time to elute

Peak area = amount of compound

IMPORTANT TERMS

- **Retention time (t_R):** Time from injection to the maximum of a peak.
- **Dead time (t_M):** Time for an unretained compound.
- **Adjusted retention time (t'_R):** $t'_R = t_R - t_M$
- **Selectivity (α):** Ability to separate two compounds.
  $\alpha = k'_2 / k'_1$ (k' = retention factor)
- **Efficiency (N):** Measure of column performance.
  $N = 16 (t_R / W_b)^2$ (W_b = peak width at base)

TYPES OF GC

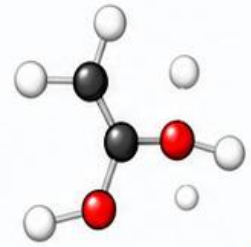
- **Gas–Liquid Chromatography (GLC)**
  Stationary phase is a liquid coated on a solid support.

- **Gas–Solid Chromatography (GSC)**
  Stationary phase is a solid adsorbent (e.g., molecular sieve, activated carbon).

- **Capillary GC (most common)**
  Uses a narrow-bore column → higher efficiency and speed.

WIDE APPLICATIONS

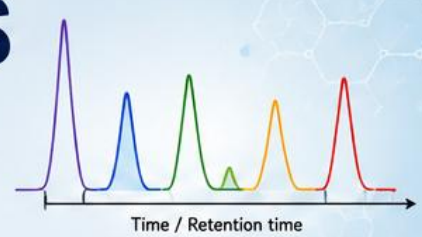
- Pharmaceutical and drug analysis
- Environmental monitoring (pesticides, VOCs)
- Food and flavor analysis
- Petroleum and petrochemical industry
- Forensic and toxicology analysis
- Quality control and research



In short: GC separates volatile compounds based on their different interactions with a stationary phase in a column. This results in different retention times and allows identification and quantification.

CHROMATOGRAPHIC TECHNIQUES (HPLC & GC)

Major Advantages and Major Pitfalls



MAJOR ADVANTAGES

High Separation Efficiency
Capable of separating very complex mixtures with high resolution due to efficient partitioning/adsorption mechanisms.

High Sensitivity
Detectors such as UV–Vis, fluorescence, MS provide low detection limits (ng–pg level).

High Selectivity and Versatility
Wide choice of stationary phases, mobile phases and detectors allows analysis of a broad range of compounds.

Quantitative Accuracy and Precision
Excellent reproducibility with proper method development and system suitability.

Applicability to Non-Volatile and Thermally Labile Compounds
HPLC can analyze non-volatile, polar and high molecular weight compounds that GC cannot.

Multi-Component Analysis
Simultaneous identification and quantification of many components in a single run.

Automation and Data Handling
Modern systems allow automation, easy data processing, integration and reporting.

Small Sample Requirement
Only small sample volumes are needed (µL level for HPLC, µL or less for GC).

MAJOR PITFALLS

Time-Consuming Method Development
Optimization of column, mobile phase, temperature program (GC), flow rate, etc. can be laborious and time consuming.

Sample Preparation Can Be Demanding
Extraction, clean-up, derivatization (especially in GC) may be required, increasing analysis time and potential for sample loss or contamination.

High Instrument and Operating Costs
Instrumentation (HPLC/GC, especially with MS detectors) is expensive. Operating costs include columns, gases, solvents and maintenance.

Matrix Effects and Interferences
Co-eluting compounds or matrix components can interfere with detection and quantification, affecting accuracy.

System and Environmental Sensitivity
Changes in temperature, pressure (GC) or flow rate, pH and solvent composition (HPLC) can affect retention times and results.

Analysis Time
Some methods, especially with high resolution or long gradients (HPLC) or long temperature programs (GC), can be time consuming.

Column Degradation and Lifetime
Columns are costly and have limited lifetime; performance can degrade with use, especially with dirty or incompatible samples.

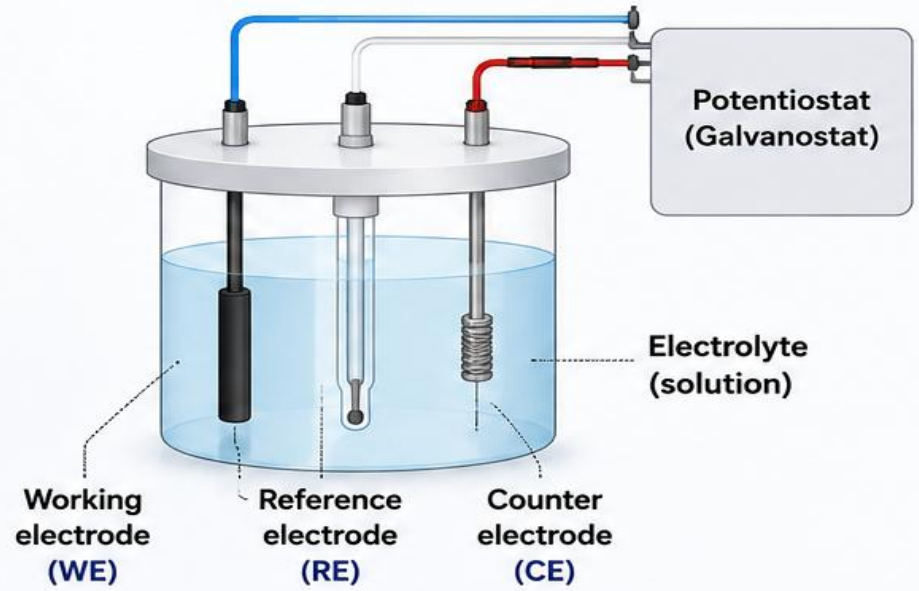
Solvent and Gas Consumption / Waste
High consumption of organic solvents (HPLC) and carrier gases (GC) generates waste and has environmental impact.

IN SHORT: Chromatographic techniques offer excellent separation power, sensitivity and versatility for complex analyses, but require careful method development, proper sample preparation and incur higher time and cost.

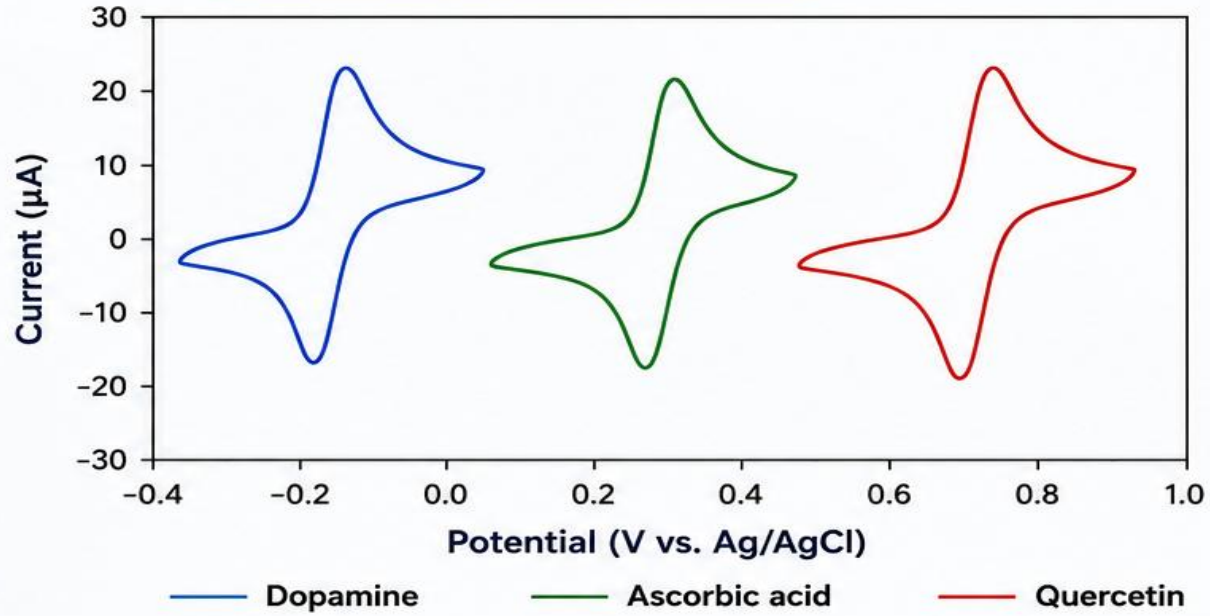
VOLTAMMETRY

Electrochemical information through potential-driven electron transfer

ELECTROCHEMICAL CELL



CYCLIC VOLTAMMOGRAMS



DRIVING FORCE IN VOLTAMMETRY

- An externally applied potential is varied with time (vs. reference electrode).
- This creates an **overpotential (η)** at the working electrode.
- The overpotential is the **driving force** for electron transfer between the electrode and electroactive species.
- Its magnitude and direction determine oxidation or reduction.

PRINCIPLE OF INTERACTION

- Electron transfer occurs between the analyte (Ox/Red couple) and the electrode at the electrode–solution interface.
- Analytes are oxidized or reduced by exchange of electrons.
- The resulting current is proportional to the rate of electron transfer and the concentration of the electroactive species.
- Each analyte shows a characteristic peak potential and current.

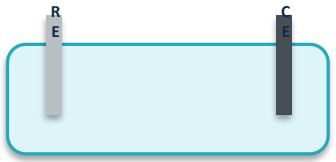
Voltammetry provides qualitative (peak potential) and quantitative (peak current) information about electroactive species in solution.

ANODIC STRIPPING VOLTAMMETRY

In situ Bi-film formation • metal preconcentration • oxidative stripping

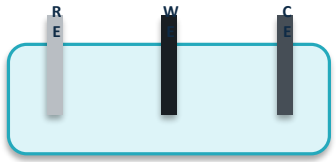
1 Prepare solution

Buffer + $Bi(NO_3)_3$ containing Zn^{2+}, Cd^{2+}, Pb^{2+}, As^{3+} and Cu^{2+}. Reference and counter electrodes are already immersed.



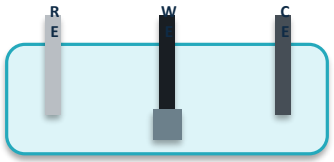
2 Insert working electrode

Place the working electrode (WE) into the same electrochemical cell.



3 Deposit Bi film

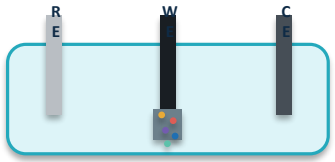
Apply a negative deposition potential, Edep ≈ −1.0 V. Bi^{3+} is reduced and a metallic Bi^{0} film forms on the WE.



$Bi^{3+} + 3e^- \rightarrow Bi^0$

4 Preconcentrate metals

Target metal ions are reduced to their zero-valent forms and accumulated within / alloyed with the Bi film.



$M^{n+} + ne^- \rightarrow M^0$

5 Strip metals anodically

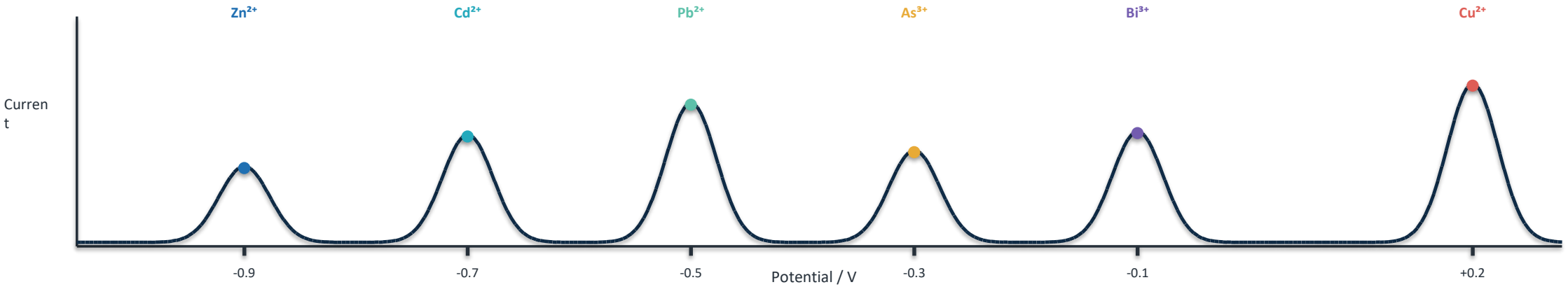
Scan toward positive potentials. Metals are re-oxidized and released into the electrolyte, generating characteristic stripping peaks.

E → more positive

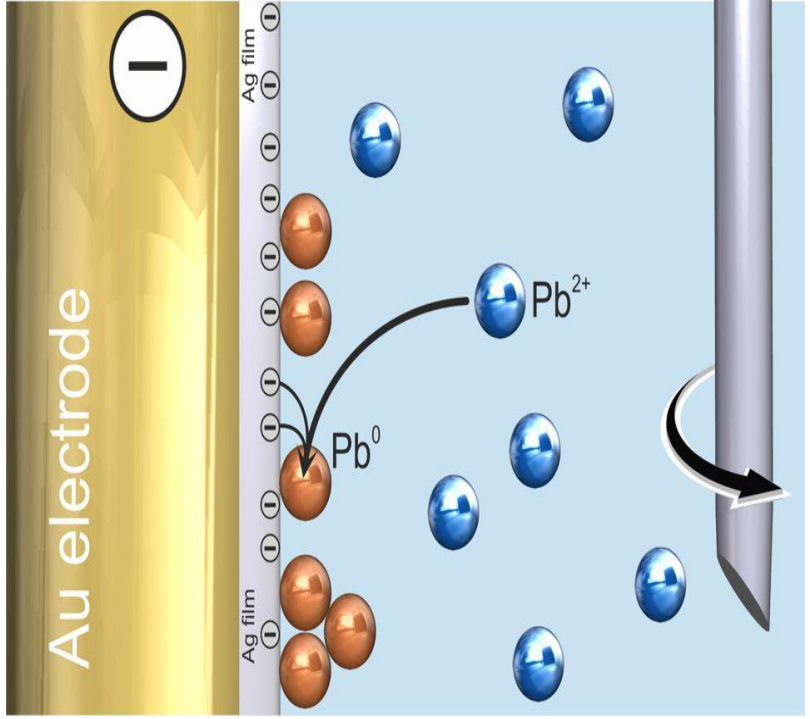
$M^0 \rightarrow M^{n+} + ne^-$

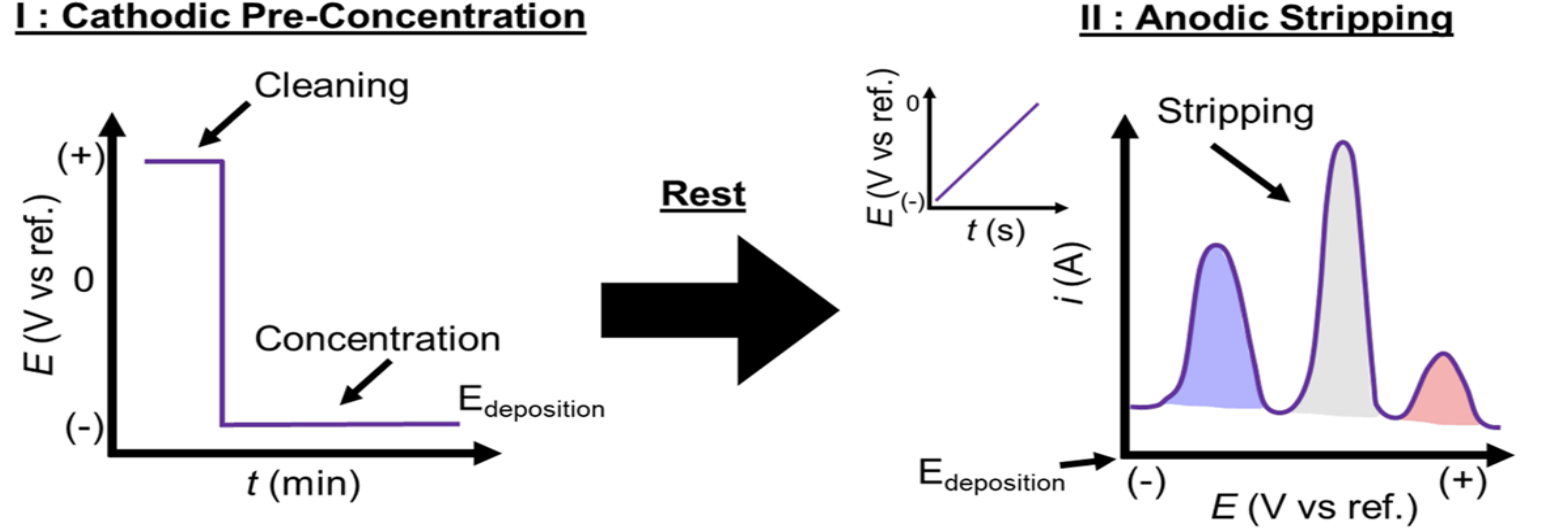
MULTI-METAL ANODIC STRIPPING RESPONSE

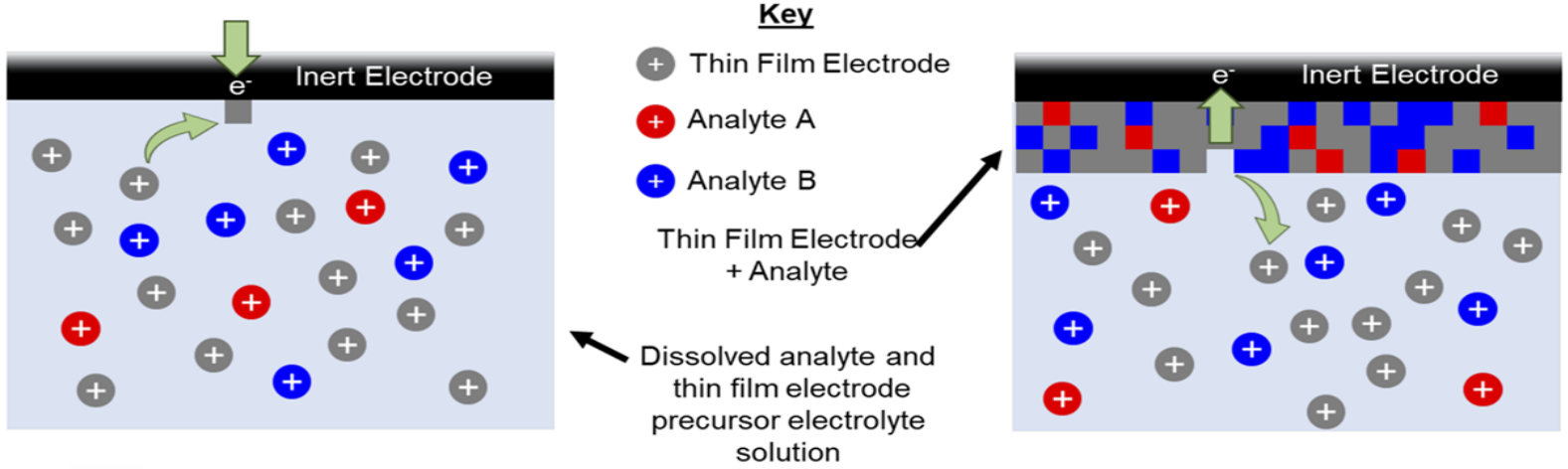
Illustrative peak potentials; actual values depend on matrix and electrode conditions.

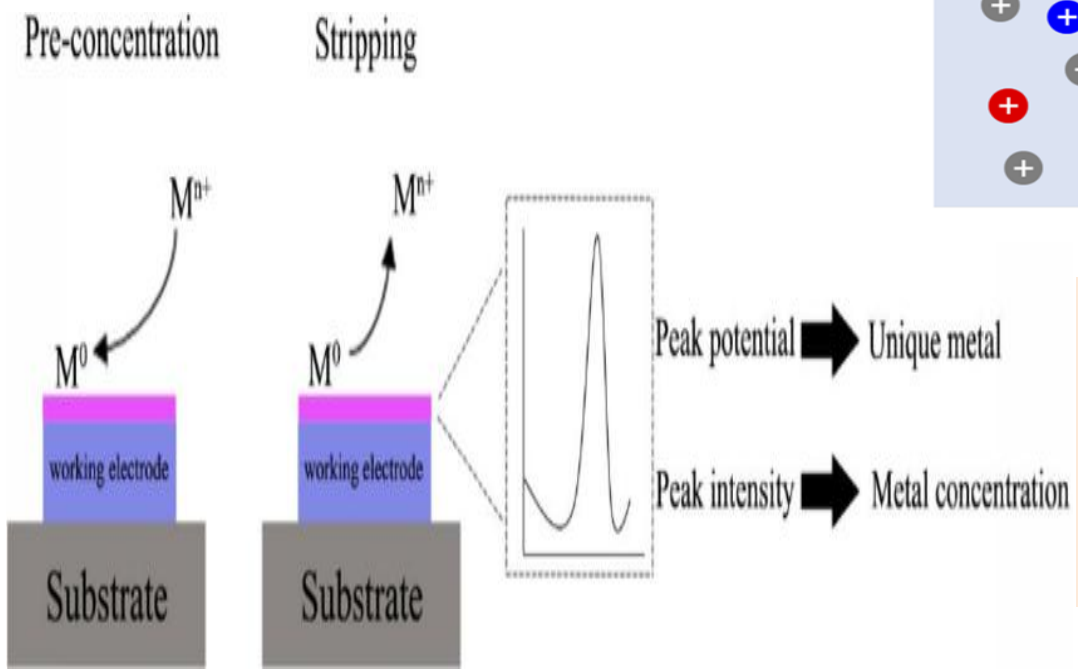


Bi-film ASV: deposition → accumulation → stripping







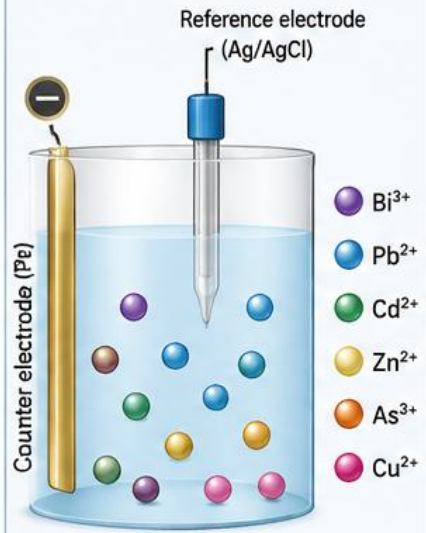


PRINCIPLES OF ANODIC STRIPPING VOLTAMMETRY---
→SUITABLE FOR DETECTION AND QUANTIFICATION of HEAVY METAL IONS (Pb^{2+}, Cd^{2+}, Zn^{2+}, Mn^{2+}) present in microgram/L amounts (mainly in water)

ANODIC STRIPPING VOLTAMMETRY (ASV)

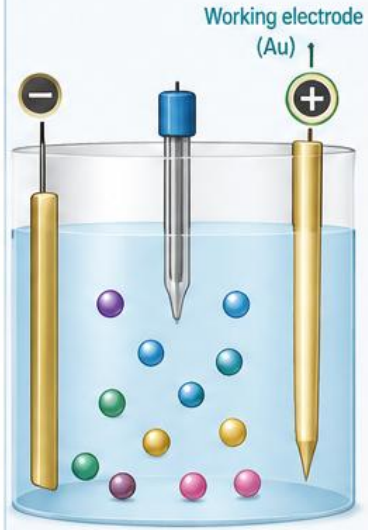
1 PREPARATION OF ELECTROLYTE SOLUTION

Bi(III) nitrate is dissolved in buffer containing target metal ions: Pb^{2+}, Cd^{2+}, Zn^{2+}, As^{3+}, Cu^{2+}.



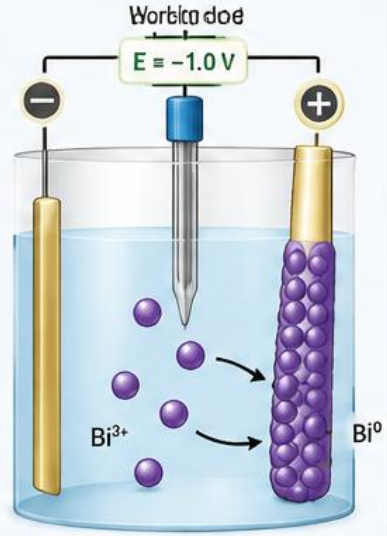
2 INSERT WORKING ELECTRODE

A working electrode (e.g. Au) is immersed in the solution.



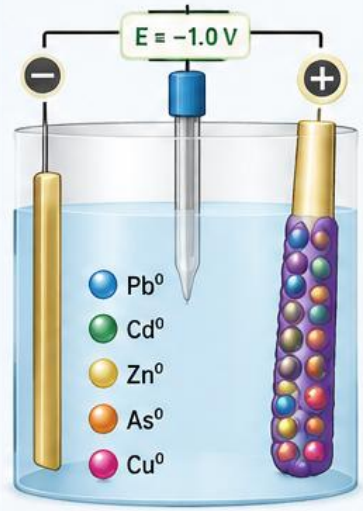
3 DEPOSITION OF Bi FILM

Apply a negative potential (−1.0 V) to reduce Bi^{3+} to metallic Bi^0, forming a Bi film on the working electrode.



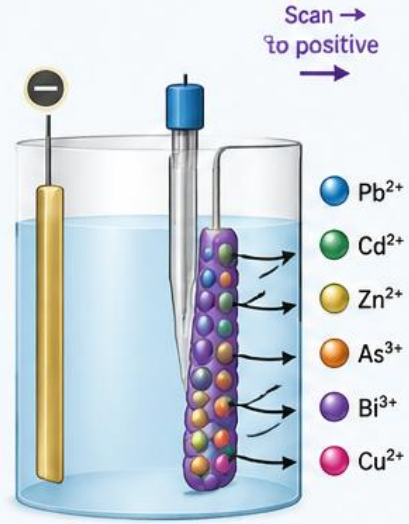
4 CO-DEPOSITION OF TARGET METALS

At the same negative potential, Pb^{2+}, Cd^{2+}, Zn^{2+}, As^{2+} and Cu^{2+} are reduced to their metallic form and co-deposited (dissolved) in the Bi film.



5 ANODIC STRIPPING SCAN (RETURN TO POSITIVE)

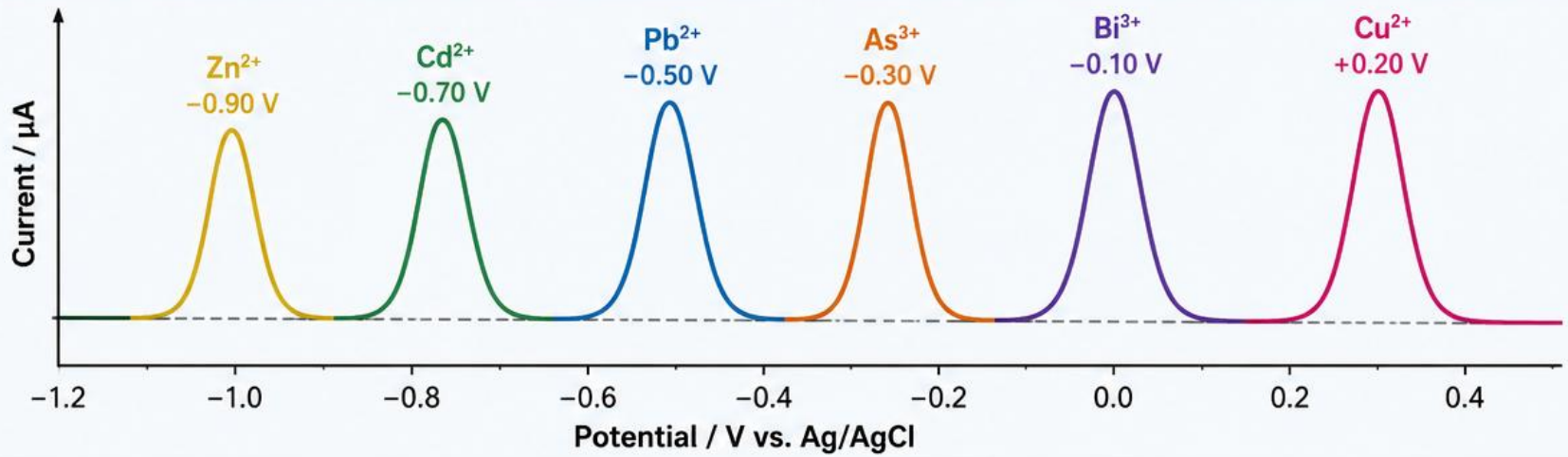
Apply a positive potential scan. Metals are oxidized back to their ionic states and dissolve into the electrolyte, producing stripping peaks.

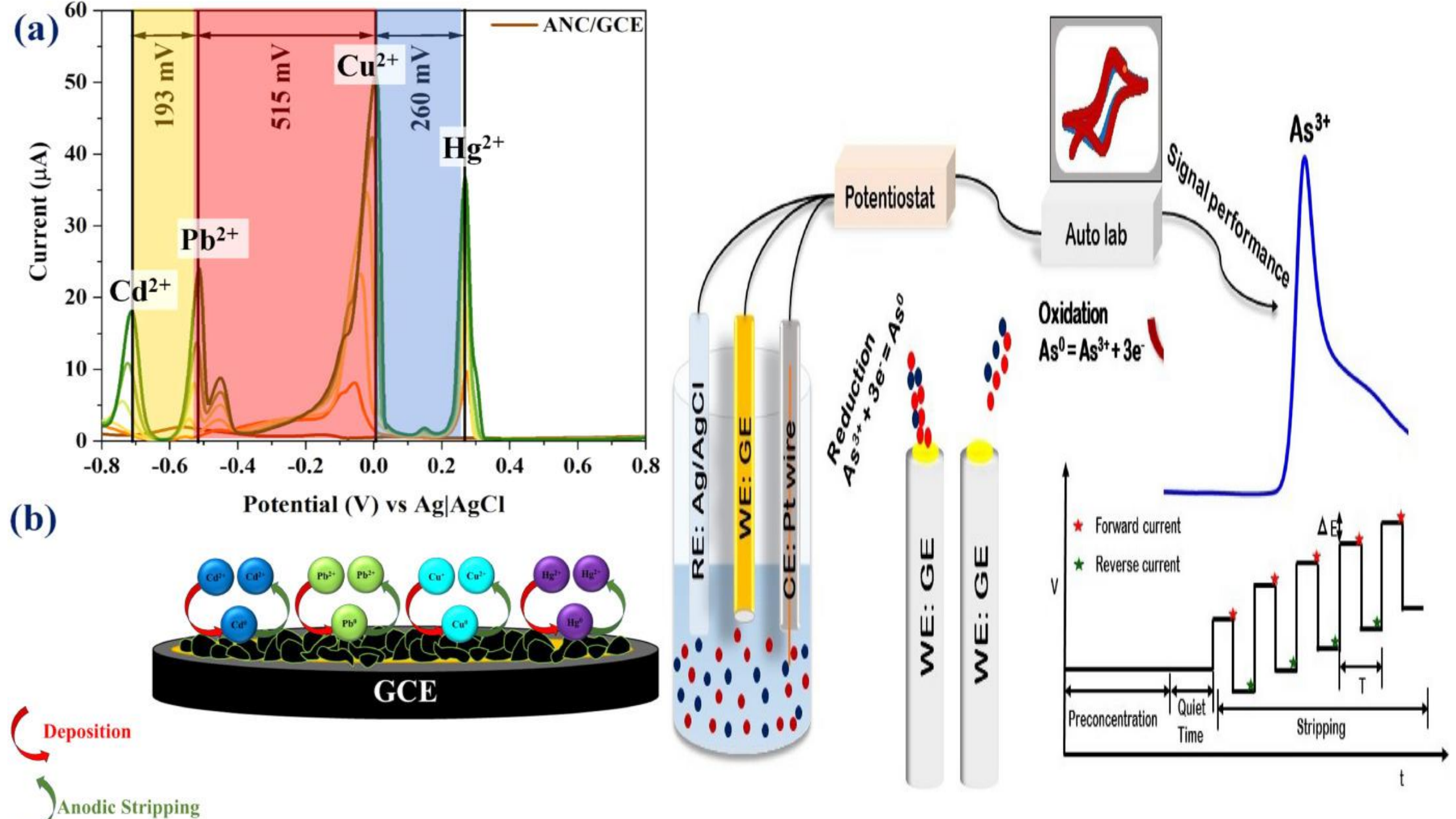


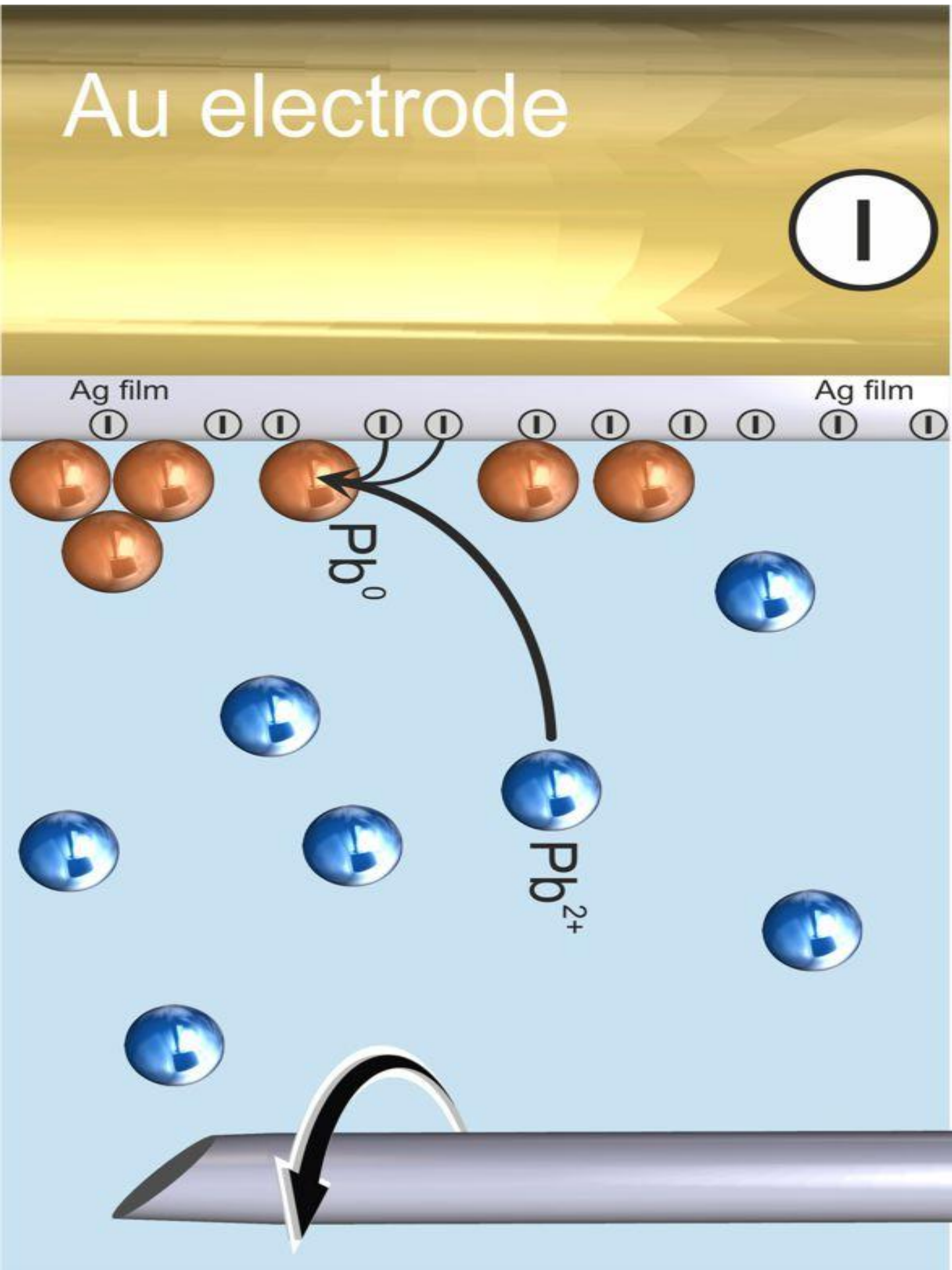
ANODIC STRIPPING VOLTAMMOGRAM

Stripping peaks correspond to the oxidation of each metal from the Bi film back to its ionic form.

Zn^{2+}	at −0.90 V
Cd^{2+}	at −0.70 V
Pb^{2+}	at −0.50 V
As^{3+}	at −0.30 V
Bi^{3+}	at −0.10 V
Cu^{2+}	at +0.20 V




(a)
ANC/GCE
193 mV
515 mV
260 mV
Cu2+
Hg2+
Pb2+
Cd2+
Current (μA)
Potential (V) vs Ag|AgCl
(b)
GCE
Deposition
Anodic Stripping
RE: Ag/AgCl
WE: GE
CE: Pt wire
Potentiostat
Auto lab
Signal performance
As3+
Oxidation
As0 = As3+ + 3e-
Reduction
As3+ + 3e- = As0
WE: GE
WE: GE
Forward current
Reverse current
ΔE
V
T
Preconcentration
Quiet Time
Stripping
t


Au electrode
I
Ag film
Ag film
Pb0
Pb2+

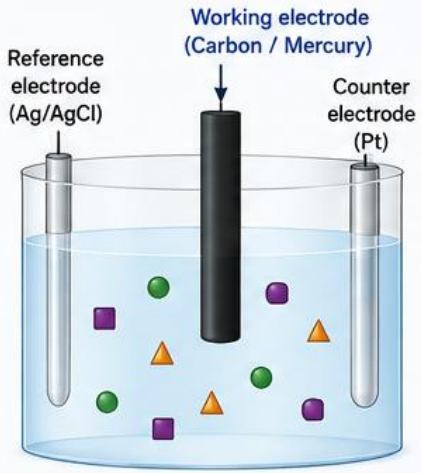
ADSORPTIVE STRIPPING VOLTAMMETRY (AdSV)

Sensitive determination of organic pollutants in water

1 SAMPLE SOLUTION

Water sample containing organic pollutants (pesticides).

- Pollutant A
- Pollutant B
- Pollutant C

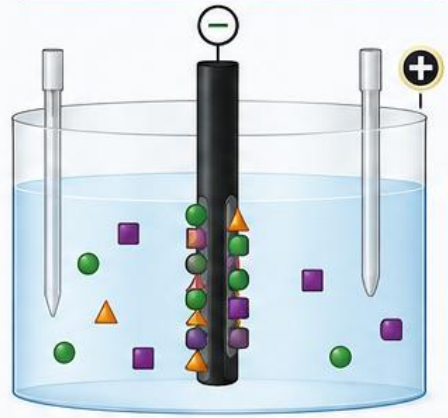


2 ADSORPTION STEP (ACCUMULATION)

Apply a defined potential (E_{acc}) for a fixed time. Organic pollutants adsorb onto the working electrode surface.

E_{acc} e.g. −0.2 V vs. Ag/AgCl

t_{acc} e.g. 120 s

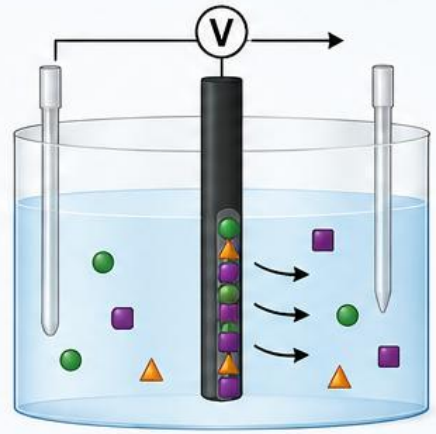


Pollutants adsorb on the electrode

3 START OF STRIPPING SCAN

After the accumulation time, the potential is scanned in the positive direction.

Adsorbed pollutants are oxidized (desorbed) back to solution.



4 STRIPPING PEAKS

Each pollutant produces a sharp oxidation peak at a characteristic potential. Peak current is proportional to concentration.

Example pollutants (pesticides)

- Pollutant A (e.g., Atrazine)
- Pollutant B (e.g., Carbofuran)
- Pollutant C (e.g., Dichlorvos)

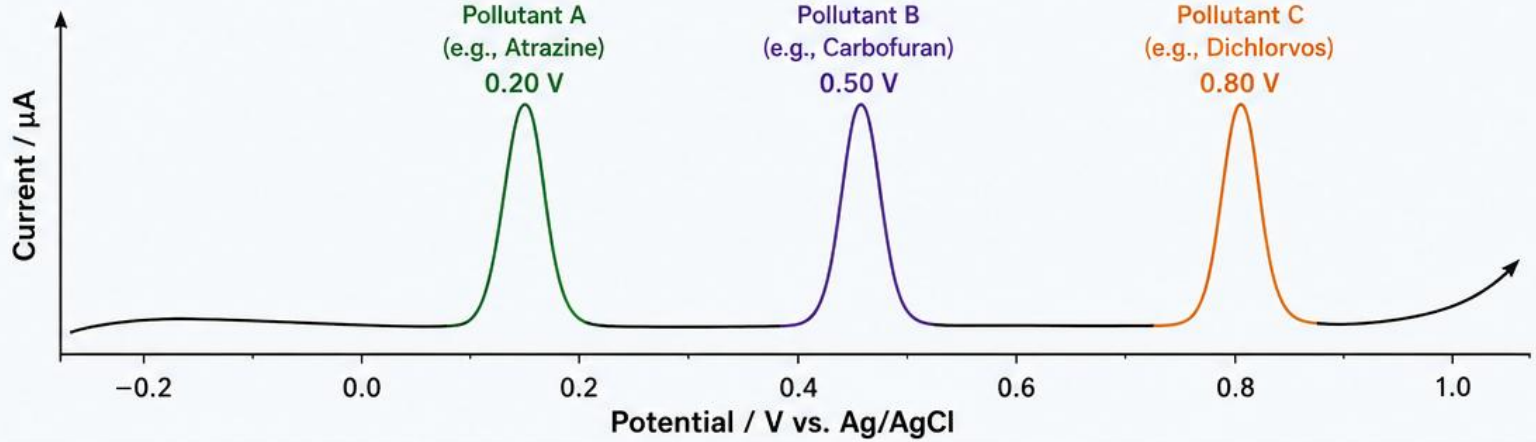
✓ High sensitivity
Low detection limits

TYPICAL ADSORPTIVE STRIPPING VOLTAMMOGRAM

Three organic pollutants present in water produce sharp anodic stripping peaks at their characteristic potentials.

Conditions (example)

- Working electrode: Glassy carbon
- Supporting electrolyte: Britton–Robinson buffer (pH 4.0)
- Accumulation potential (E_{acc}): −0.2 V
- Accumulation time (t_{acc}): 120 s
- Scan rate: 50 mV s^{-1}



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5. V. Mirceski, B. Mitrova, V. Ivanovski, N. Mitreska, A. Aleksovska, R. Gulaboski, **Journal of Solid State Electrochemistry 19** (2015) 2331-2342.

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