

Chapter 1 : Spectroscopic methods

Spectroscopy is a scientific technique used to study the interaction between matter and electromagnetic radiation across various wavelengths of the electromagnetic spectrum. It involves the measurement and analysis of how light is absorbed, emitted, or scattered by substances, providing valuable information about their composition, structure, and properties. Spectroscopic techniques are widely used in diverse fields such as chemistry, physics, biology, materials science, environmental science, and astronomy for research, analysis, diagnostics, and exploration.

Electromagnetic radiation is a form of radiation in which the electric and magnetic fields simultaneously vary. One well known example of electromagnetic radiation is visible light. Electromagnetic radiation can be characterized by its energy, intensity, frequency and wavelength.

The frequency and the wavelength of electromagnetic radiation vary over many orders of magnitude. For convenience, we divide electromagnetic radiation into different regions « the electromagnetic spectrum » based on the type of atomic or molecular transitions that gives rise to the absorption or emission of photons. The boundaries between the regions of the electromagnetic spectrum are not rigid and overlap between spectral regions is possible.

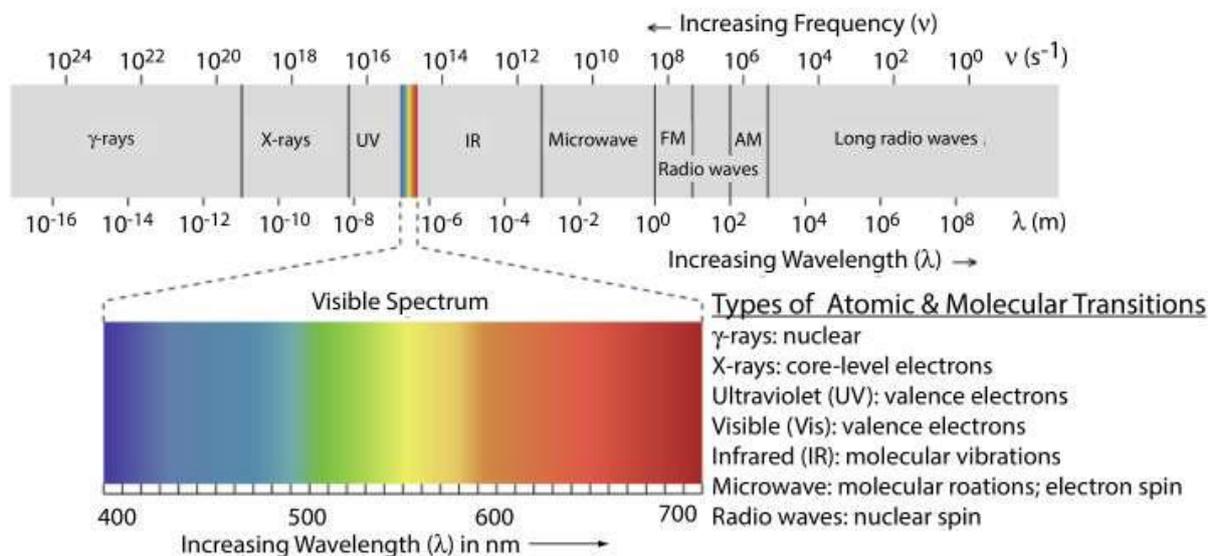


Figure 1 : The electromagnetic spectrum showing the boundaries between different regions and the type of atomic or molecular transitions responsible for the change in energy. The colored inset shows the visible spectrum.

Quantitative measurements in spectrophotometry are based on the Lambert-Beer law of light absorption by solutions. The amount of light absorbed by the sample is defined as the ratio of the intensities of the incident and the transmitted light.



Figure 2 : Incident and transmitted light.

From the degree of absorption, the concentration of the absorbing component can be deduced by using the following equation. If the intensity of light, I_0 , diminishes to I after passing through a path of length L in a medium, then, according to the Lambert-Beer law :

$$E = \log \frac{I_0}{I} = \epsilon \cdot c \cdot L$$

The expression $\log I_0/I$ is called extinction (E), absorbance (A) or optical density (OD), ϵ : in ($\text{mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$) the molar extinction coefficient of the absorbing species in solution, c : (in $\text{mol} \cdot \text{L}^{-1}$) the concentration of the absorbent species, L : (in cm) the length of the optical path.

1. Molecular Absorption Spectroscopy (MAS)

1.1. Mechanism of molecular absorption

Molecular absorption is a process in which light energy of a certain wavelength is absorbed by a molecule, promoting it from the ground state to higher energy excited states. Each electronic transition from ground state (S_0) to excited state (S_1 or S_2) is associated with numerous vibrational and rotational transitions.

1. 2. Principale

Molecular spectroscopic techniques operate on the principle that every molecule is composed of multiple atoms with related characteristic bonds between them. Using a particular type of electromagnetic radiation, the molecule can be excited (undergoes short-term vibrations in terms of reflection, transmission, and absorption) on the basis of its elemental composition and bond strength and can sometimes be referred to as photoluminescence. Photoluminescence is a term used to describe a number of effects, such as fluorescence, phosphorescence, and Raman scattering. Molecular spectroscopy employs the interaction of electromagnetic radiation with materials to produce an absorption spectrum that holds structural and compositional information on the given material. Electromagnetic radiation is conventionally divided into the

following regions : radiofrequency (ca. 10⁵–10⁹ Hz), microwave (ca. 10⁹ to 3 X 10¹⁰ Hz), infrared (ca. 3 X 10¹⁰ to 3 X 10¹⁴ Hz), visible and ultraviolet (ca. 3 X 10¹⁴ to 3 X 10¹⁶ Hz), X-rays (ca. 3 X 10¹⁶ to 3 X 10¹⁸ Hz), and gamma (γ)-rays (ca. 3 X 10¹⁸ to 10²¹ Hz). Each region covers a range of energies that correspond to a different type of molecular process. Every region has different features and stands for a specific kind of molecular or atomic transition that corresponds to specific spectroscopic techniques. Among these, ultraviolet–visible (UV/Vis) and infrared (IR) regions have received much attention as most spectroscopic instruments used in nonlaboratory conditions utilize these energy ranges and can result in electron transitions or molecular vibrations.

Ultraviolet-Visible molecular absorption spectrometry

• UV/Vis spectra for molecules and ions

The valence electrons in organic molecules and polyatomic ions, occupy quantized sigma bonding (σ), pi bonding (π), and non-bonding (n) molecular orbitals (MOs). Unoccupied sigma antibonding (σ^*) and pi antibonding (π^*) molecular orbitals are slightly higher in energy. Because the difference in energy between the highest-energy occupied MOs and the lowest-energy unoccupied MOs corresponds to ultraviolet and visible radiation, absorption of a photon is possible.

Four types of transitions between quantized energy levels account for most molecular UV/Vis spectra. Table 1 lists the approximate wavelength ranges for these transitions, as well as a partial list of bonds, functional groups, or molecules responsible for these transitions. Of these transitions, the most important are and because they involve important functional groups that are characteristic of many analytes and because the wavelengths are easily accessible. The bonds and functional groups that give rise to the absorption of ultraviolet and visible radiation are called chromophores.

Table 1 : Electronic transitions involving n , σ , and π molecular orbitals

transition	wavelength range	examples
$\sigma \rightarrow \sigma^*$	<200 nm	C—C, C—H
$n \rightarrow \sigma^*$	160–260 nm	H ₂ O, CH ₃ OH, CH ₃ Cl
$\pi \rightarrow \pi^*$	200–500 nm	C=C, C=O, C=N, C≡C
$n \rightarrow \pi^*$	250–600 nm	C=O, C=N, N=N, N=O

• Instrumentation

An instrument for measuring molecular absorption spectra (UV/Vis) usually consists of a radiation source, an optical system including a spectral apparatus, a sample compartment, a radiation detector and a system for data acquisition and data processing. Means for amplitude modulation and/or wavelength modulation may also be part of the instrument.

a. Radiation sources

-Tungsten-halogen lamps : which have a radiance temperature T of ~ 3000 K and therefore have maximum spectral radiance at a wavelength of about $1.2 \mu\text{m}$; they are widely used in the visible and near ultraviolet spectral region (above 330 nm).

-Deuterium lamps : (gas-discharge lamps) which emit strongly in the UV region below 330 nm; the continuous spectrum has deuterium atomic emission lines superimposed on it.

-Xenon arc lamps : which give a continuum from below 190 nm to above 1000 nm.

b. Sample compartment

➤ Liquid samples

Liquid samples are usually contained in sample cells which are placed in sample cell holders. Cell holders may be heated or cooled in order to control the temperature of the liquid in the sample cell.

➤ Gaseous samples

Gases and vapours are measured in gas cells similar to those used for liquids. Generally the cell pathlength is much greater. Gases at any pressure are contained in closed cells for measurement.

➤ Solid samples

Solid samples are held in solid-sample holders. When solid samples are measured, difficulties may be experienced e.g. in matching the sample and reference pathlengths.

• Types of instruments

a. Single-beam instruments

It consists of a tungsten or deuterium lamp, a filter or a monochromator for wavelength selection, matched cells that can be placed alternately in the radiation beam, an amplifier, and a readout device. Normally, a single-beam instrument requires a stabilized voltage supply to avoid errors resulting from changes in the beam intensity during the time required to make the 100%T measurement and determine %T for the analyte.

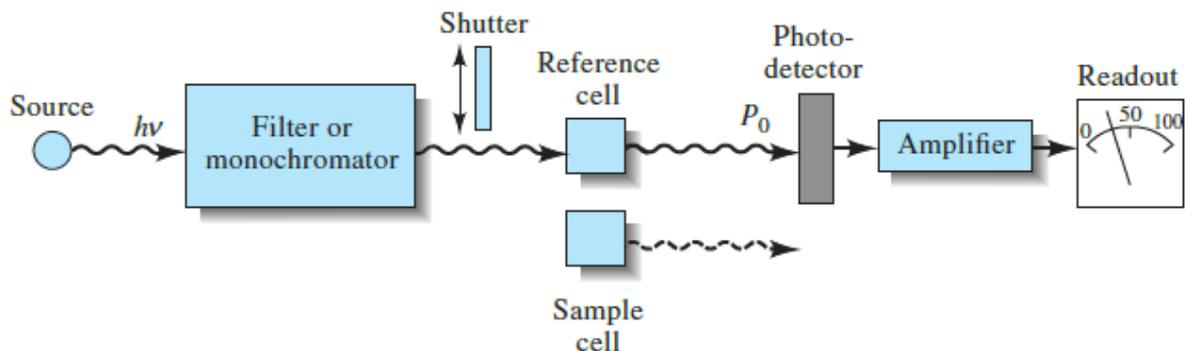


Figure 3: Single-beam instrument is shown. Radiation from the filter or monochromator passes through either the reference cell or the sample cell before striking the photodetector.

b. Double-beam instruments

Many modern photometers and spectrophotometers are based on a double-beam design. In doublebeam-in-space instrument two beams are formed in space by a V-shape mirror called a beamsplitter. One beam passes through the reference solution to a photodetector, and the second simultaneously traverses the sample to a second, matched detector.

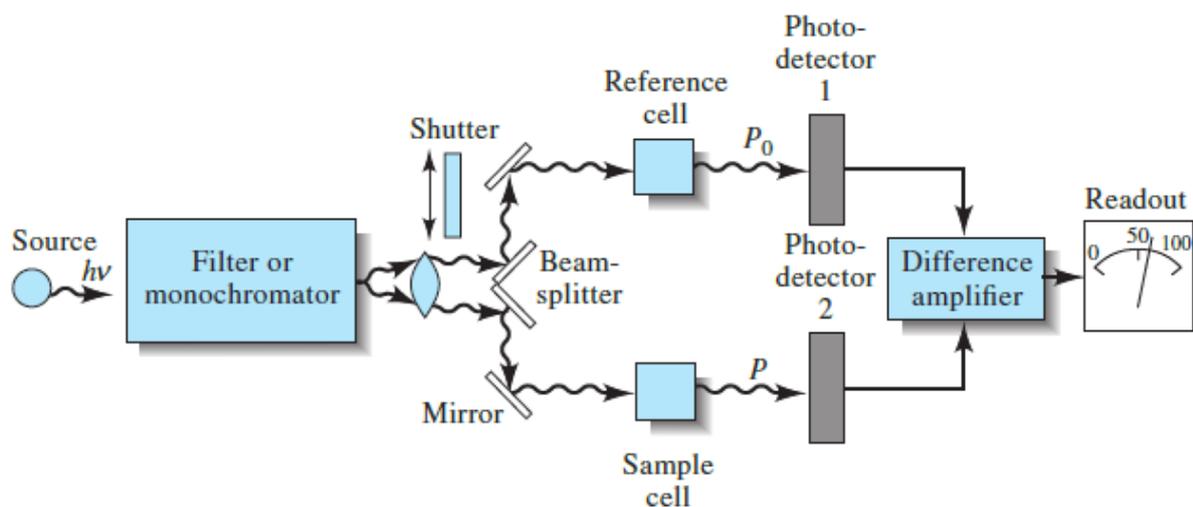


Figure 4: Double-beam-in-space instrument is shown. Here, radiation from the filter or monochromator is split into two beams that simultaneously pass through the reference and sample cells before striking two matched photodetectors.

c. Multichannel instruments

A new type of spectrophotometer appeared on the market in the early 1980s that was based on one of the array detectors (photodiode array or linear charge-coupled-device [CCD] array). These instruments are usually of the single-beam design. With multichannel systems, the dispersive system is a grating spectrograph placed after the sample or reference cell. The array detector is placed in the focal plane of the spectrograph, where the dispersed radiation strikes

it. The photodiode array, consists of a linear array of several hundred photodiodes (256, 512, 1024, 2048) that have been formed along the length of a silicon chip.

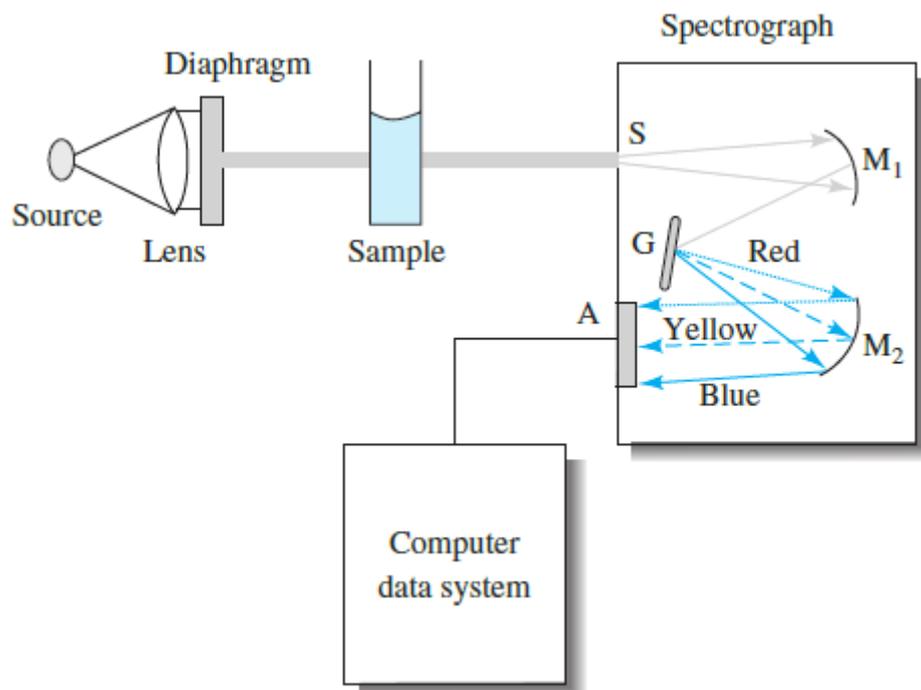


Figure 5: Diagram of a multichannel spectrometer based on a grating spectrograph with an array detector. Radiation from the tungsten or deuterium source is made parallel and reduced in size by the lens and diaphragm. Radiation transmitted by the sample enters the spectrograph through slit S. Collimating mirror M₁ makes the beam parallel before it strikes the grating G. the grating disperses the radiation into its component wavelengths, which are then focused by focusing mirror M₂ onto the photodiode or CCd array A. the output from the array detector is then processed by the computer data system.

1.3. Application of molecular spectroscopy

-Biomolecule detection : UV-Vis and IR molecular absorption spectroscopy can be used to study biological macromolecules (e.g., proteins, DNA/RNA) and their interactions through characteristic absorbance changes, aiding in biochemical and clinical research.

-Agriculture and food quality : UV-Vis, NIR, and mid-IR absorption spectroscopy are extensively applied to analyze agricultural products, soil quality, food composition and quality, and detect adulteration or spoilage of food.

-Pharmaceutical sciences : UV-Vis molecular absorption spectroscopy is fundamental in pharmaceutical analysis for quantifying active pharmaceutical ingredients (APIs), studying drug-receptor interactions, and monitoring compound purity and stability.

-Environmental science : Molecular absorption spectroscopy (especially UV-Vis and IR) is used to detect and quantify organic contaminants in water, air, and soil (e.g., pesticides, phenols, aromatic hydrocarbons) due to their characteristic light-absorption signatures.

2. Atomic Emission Spectroscopy (AES)

2.1. Definition

Atomic emission spectroscopy is an important instrumental technique for both quantitative and qualitative analysis of metallic and non-metallic elements in inorganic and organic materials.

2.2. Principle

AES is based on the principle that when energy is applied to a molecule in the form of light or heat, molecules are excited and move from a lower energy level state to a higher energy level state. At the higher energy level state, the molecules are unstable and jump back to the lower energy level state on emitting radiations in the form of photons. The wavelengths of emitted photons are recorded in the emission spectrometer. The level of emissions for a molecule is the energy differential between the excited energy and lower stable energy. Each element has its own level of emission frequencies, which helps to detect the elements. The frequencies of the emissions are recorded in the emission spectrometer.

2.3. Instrumentation

The instrumentation of atomic emission spectroscopy comprises :

a. Excitation source : An excitation source is used to dissolve, atomize, and excite the atoms of the sample. The ideal excitation source will allow the excitation of all the elements in the sample and does it repeatedly until it encompasses the entire elemental excitation in the sample.

b. Monochromator : The monochromator disperses the light and isolates specific wavelengths so each element can be measured individually.

c. Detector : Detectors are transducers that convert the emitted light into an electric signal, which can then be viewed and analyzed using a computer.

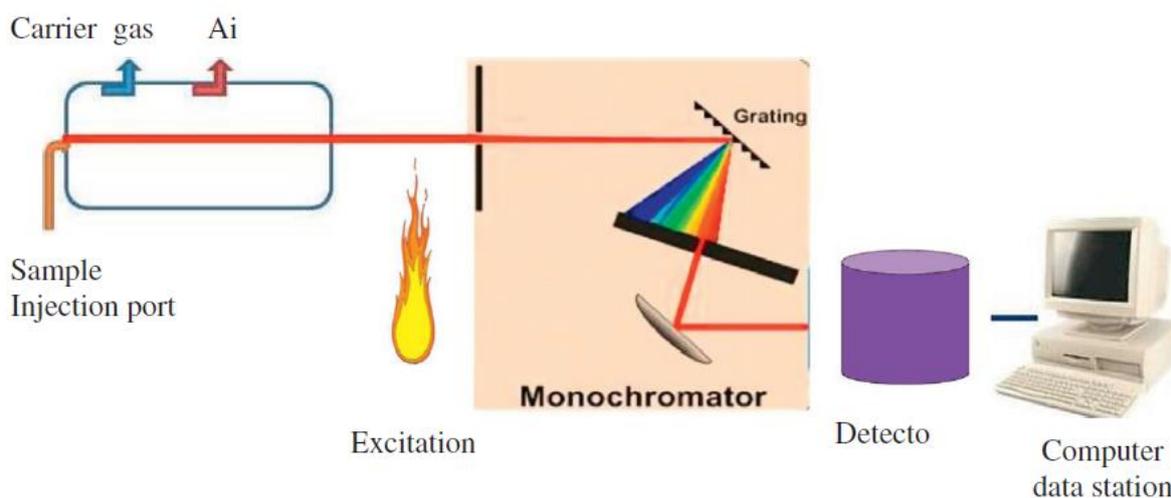


Figure 6 : Instrumentation of atomic emission spectroscopy.

2.4. Atomization and excitation

Atomic emission requires a means for converting into a free gaseous atom an analyte that is present in a solid, liquid, or solution sample. A number of excitation sources can be used for these purposes :

a. Flame

Atomization and excitation in flame atomic emission is accomplished with the same nebulization and spray chamber assembly used in atomic absorption. The burner head consists of a single or multiple slots, or a Meker-style burner. Older atomic emission instruments often used a total consumption burner in which the sample is drawn through a capillary tube and injected directly into the flame.

b. Plasma

A plasma is a hot, partially ionized gas that contains an abundant concentration of cations and electrons. The plasma used in atomic emission is formed by ionizing a flowing stream of argon gas, producing argon ions and electrons. A plasma's high temperature results from resistive heating as the electrons and argon ions move through the gas. Because a plasma operates at a much higher temperature than a flame, it provides for a better atomization efficiency and a higher population of excited states.

c. Spark or arc

Spark and arc excitation sources employ a spark or an electric pulse or an arc of continuous electrical discharge between two electrodes for vaporizing and exciting the atoms of the sample.

2.5. Applications

- Qualitative identification of elements based on the characteristic wavelengths of their emitted spectral lines.
- Quantitative determination of elements by measuring the intensity of emitted spectral lines, which is proportional to the number density (concentration) of atoms in the sample.
- Study of atomic energy levels (stationary states) through wavelength measurements, providing experimental support for atomic structure theories.
- Investigation of atomic structure and the surrounding environment of atoms by analyzing the emitted radiation.
- Determination of physical conditions of the excitation source, such as temperature and pressure, by examining the shape and intensity of spectral lines.

3. Atomic absorption spectroscopy (AAS)

3.1. Definition

Atomic Absorption Spectroscopy (AAS) is a chemical analysis equipment that works on the premise of atoms absorbing energy. Atomic absorption spectroscopy (AAS) is a Spectro analytical process that uses the absorption of optical radiation (light) by free atoms in a gaseous state to determine the quantitative elements. The absorption of light by free metallic ions is the basis for atomic absorption spectroscopy.

3.2. Principle

Atomic Absorption Spectroscopy (AAS) is an analytical technique used to measure the concentration of metal elements in a sample. It works on the principle that free atoms absorb light at specific wavelengths. Each element absorbs light at its own unique wavelength, which makes AAS highly selective.

In AAS, the sample is converted into free atoms. When light of the correct wavelength passes through these atoms, part of the light is absorbed. The amount of light absorbed depends on the concentration of the element present in the sample.

AAS follows the Beer–Lambert law, which states that the amount of light absorbed is directly proportional to the concentration of the absorbing species and the path length of the light. By comparing the absorbance of an unknown sample with standards of known concentration, the amount of the element in the sample can be determined.

Atomic absorption spectrometry is widely used because it is simple, fast, sensitive, cost-effective, and requires only a small amount of sample. It is especially useful for analyzing elements present in very low concentrations without the need for prior separation.

3.3. Instrumentation

Atomic absorption spectrophotometry has an instrument that is arguably similar to other types of spectrophotometry. However, atomic absorption spectrophotometry has a distinctive feature in instrumentation, namely the atomizer. This atomizer will absorb the wavelength directly proportional to Lambert's law.

a. Light Source

The sources of radiation or light most often used in atomic absorption spectrophotometry are Hollow Cathode Lamps (HCL) and Electrodeless Discharge Lamps (EDL). Hollow Cathode Lamp (HCL) is a lamp consisting of a hollow cathode coated by elements, both pure and mixed elements and wolfram. Generally, HCL uses Ne, Ar, and He as filler gas.

Electrodeless Discharge Lamps (EDL) have higher emissions and narrower channel widths when compared to HCL. EDL requires more time and a separate power to become a stable unit. A small number of analytes in EDLs stabilize under low pressure, these analytes act as metals or salts that move with the inert gas in the quartz sphere, such as argon gas. The release of the low pressure in the EDL will occur when the bulb is inserted in the coil which causes an electromagnetic radio frequency field to appear.

b. Atomizer

Atomizer plays a role in atomization or atomic formation. Atomizers in Atomic Absorption Spectrophotometry are divided into burners or burners and nebulizers or foggers.

- Burner, the role of the burner is to convert the salt vapor from the compound components that are then analysed into normal atoms in the flame.
- Nebulizer, in the nebulizer there is a withdrawal of the solution from the capillary through the suction of oxidant gas and fuel which will then be sprayed into the fogging chamber. This causes the solution to turn into fog grains which will enter the flame.

c. Monochromator

Monochromator is in charge of separating the radiated radiation into elements that are more specific than other radiation which will then be measured by the detector. This radiation is released by a Hollow Cathode Lamp (HCL) which is then monochromatically dipatau so that the radiation bandwidth can be reduced. Radiation interference that appears will also be reduced, ranging from flames to other components.

d. Detector

Radiation transmitted by the sample will be measured by the detector in the form of electrical energy. Radiation received by the detector can come from the results of resonance line selection

and flame emission. The emission in question is an emission derived from atomic emissions that have been analyzed.

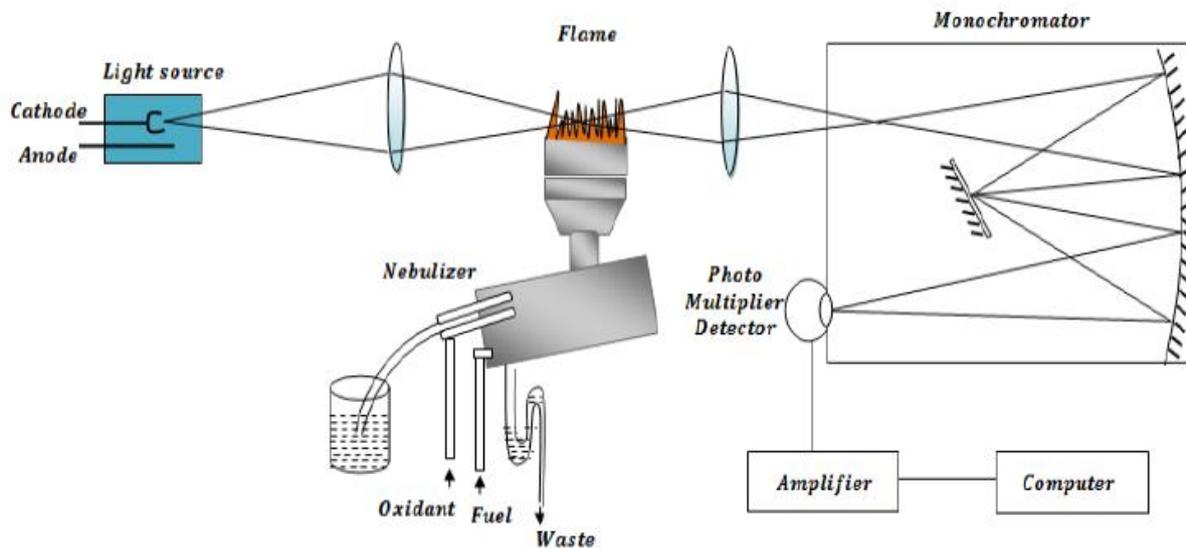


Figure 7 : Block diagram of a basic Atomic Absorption Spectrometer.

3.4. Atomization

In atomic absorption spectrophotometry there is a special process called atomization. Atomization is the process of converting free gas atoms derived from analytes. Aqueous analytes to be converted to free atoms must be removed solvent and then evaporated. Atomization in atomic absorption spectrophotometry is divided into 3:

a. Atomization with Flame

In this atomization, the snippet will be included in the combustion gas flame. The flame temperature will affect the atomic gas population formed. Behind that, the flame temperature itself is influenced by what percentage of oxygen and fuel gas ratio.

b. Vapor Generation Method

This atomization method has specifications for nine elements. These elements are As, Bi, Sn, Se, Te, Ge, and Hg. In this atomization method, the metal will be analysed in a solution that is in a simple molecular form. However, the Hg compound has the privilege of being in the form of free atoms.

c. Furnace atomization

This atomization has a working principle by increasing the optimum temperature through a graphite tube. The sample solution will be placed on a graphite tube which is then electrified and heated until the sample begins to atomize. The required heating temperature is the highest temperature at which atoms can react.

3.5. Applications

-Environmental science : Detects heavy metals (lead, cadmium, arsenic, mercury) in water, soil, and air, supporting pollution monitoring and regulatory compliance.

-Clinical laboratories : Measures essential and toxic elements in blood, urine, and tissues, aiding in diagnosis and health assessment.

-Agriculture : Analyzes trace nutrients in plants and fertilizers, ensuring proper soil management and crop productivity.

-Pharmaceuticals and food industries : Monitors elemental impurities to ensure product safety.