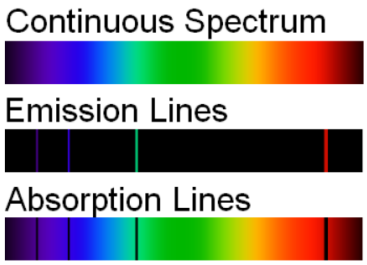
ATOMIC ABSORPTION AND EMISSION SPECTROMETRIES

Spectral methods encompass both molecular and atomic methods. Molecular methods are characterized by band spectra and include UV-Visible spectroscopy and Infrared spectroscopy. Atomic methods, on the other hand, are characterized by line spectra and include atomic emission spectroscopy and atomic absorption spectroscopy. The figure below illustrates the difference between an absorption line spectrum of Sodium and Mercury, and an absorption band spectrum of Potassium Permanganate.



ATOMIC ABSORPTION AND EMISSION PHENOMENA:

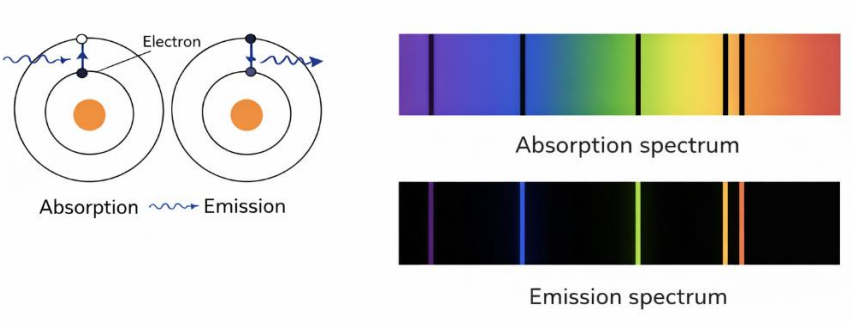
Atomic Absorption Spectrometry (AAS) and Atomic Emission Spectrometry (AES) are two widely used techniques for the analysis of over 70 elements, sometimes at trace levels.

- **Atomic Absorption:**

Atomic absorption is the phenomenon observed when an atom in its ground state absorbs electromagnetic radiation at a specific wavelength and transitions to an excited state. This results in a spectrum of black lines on a light background (Absorption spectrum).

- **Atomic Emission:**

Atomic emission is the phenomenon observed when electromagnetic radiation is emitted by excited atoms or ions returning to the ground state. This results in a spectrum of bright lines on a dark background (Emission spectrum).



Both techniques involve free atoms in the vapor state. The apparatus therefore produces an atomic vapor from the sample, which induces the destruction of the molecule to be analyzed, making it possible to simultaneously assay all forms of the same element.

ATOMIC ABSORPTION SPECTROMETRY

1. DEFINITION:

Atomic Absorption Spectrometry studies the absorption of light by free atoms. It is one of the main techniques involving atomic spectroscopy in the UV-visible range used in chemical analysis. It allows the assay of around sixty chemical elements (metals and non-metals).

Flame atomic absorption spectrometry allows the mono-elemental assay of major cations on the order of mg/L in liquid samples.

Each element has a specific number of electrons associated with its nucleus. The normal and most stable orbital configuration of electrons is called the ground state. When energy is supplied to an atom, it absorbs it and adopts an electronic configuration called the excited state. This state is unstable, and the atom immediately returns to its ground state, thereby releasing light energy.

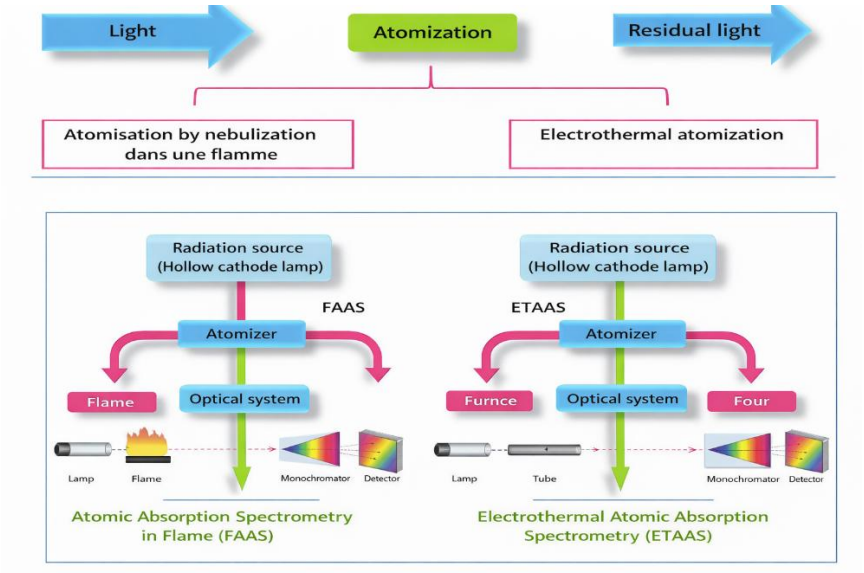
2. PRINCIPLE:

Flame atomic absorption is a method used primarily to assay metals in solution. This elemental analysis method requires that the measurement be made on an analyte (element to be assayed) transformed into the state of free atoms. The sample is heated to a temperature of 2000 to 3000 degrees so that the chemical combinations involving the elements are destroyed. Atomic Absorption Spectrometry is based on the theory of energy quantization in the atom. The atom's

energy varies during the movement of one of its electrons from one electronic orbit to another. Generally, only the outer electrons of the atom are involved.

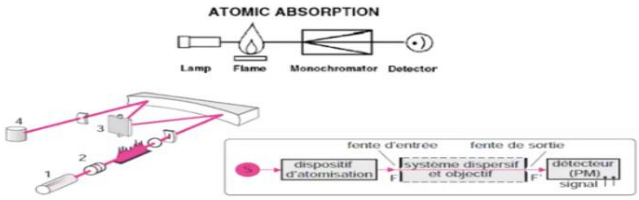
The absorbed photons are characteristic of the absorbing elements, and their quantity is proportional to the number of atoms of the absorbing element. Therefore, absorption allows the measurement of the concentrations of the elements to be assayed. Analysis by atomic absorption uses the Beer-Lambert law. If several elements need to be assayed, this procedure is carried out for each element in the sample by setting a fixed wavelength. For each procedure, a suitable source must be chosen to illuminate the element intended for excitation.

- The sample is reduced to atomic vapor.
- Atoms in the ground state absorb the specific radiation.
- Absorbance is proportional to the quantity of atoms of the element to be assayed.
- In AAS, atomic vapors are obtained by:
  - Atomization by nebulization in a flame
  - Electrothermal atomization



3. APPARATUS:

The experimental setup used in atomic absorption consists of a source, the hollow cathode lamp, a burner and nebulizer, a monochromator, and a detector connected to an amplifier and an acquisition device.



The light beam from the source (1) passes through the flame (2) where the element is brought to the atomic state, before being focused onto the entrance slit of a monochromator (3) which selects a very narrow interval of wavelengths. The optical path ends at the entrance window of the detector (4).

During the atomic absorption process, the energy supplied to the atom comes from a light source called a hollow cathode lamp. The atom in its ground state absorbs light energy at a specific wavelength and transitions to an excited state. A detector measures the amount of light absorbed, and an electronic signal is produced based on the light intensity. This signal is processed, and the quantity of analyte in the sample is determined based on the measured absorbance.

There are nearly a hundred different hollow cathode lamps, each specific to an element to be assayed. The hollow cathode lamp is a discharge lamp designed for use as a spectral line source with atomic absorption (AA) spectrometers. A single-element or multi-element hollow cathode lamp is required to determine each element using the atomic absorption technique. A hollow cathode lamp must imperatively generate a narrow emission line for the element to be determined.

- In FAAS (Flame AAS), the atomizer is a flame provided by a slot burner.
- In ETAAS (Electrothermal AAS), the atomizer is a graphite furnace.

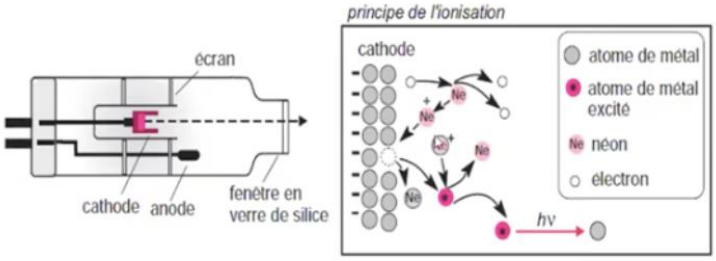
The contact between atoms and the light source is ensured by the absorption cell. The absorption cell is actually a flame generated by the combustion of an air/acetylene mixture (2500°C) or a nitrous oxide/acetylene mixture (3100°C) for refractory elements (examples: Al, Mo, Sr…).

The sample to be analyzed is aspirated by the instrument and transformed into an aerosol. The flame then atomizes the elements contained in the aerosol, which pass through the beam of the hollow cathode lamp.

The hollow cathode lamp emits the light spectrum specific to the element being analyzed. The cathode and anode of the lamp are composed solely of the element whose light spectrum is to be produced. An electric potential is applied between the anode and cathode, which ionizes the gas contained in the lamp.

The gas ions then collide with the cathode, dislodging metal atoms. These atoms also collide with the gas ions, causing them to transition to an excited state. They immediately return to their ground state, producing the desired light energy.

Examples of quantification limits: Al 3 mg/L, Cu 0.12 mg/L, Zn 0.02 mg/L.



Operating principle of the hollow cathode lamp

4. APPLICATIONS:

AAS allows the analysis of almost all metals and metalloids (Cu, Zn, Pb, Cr, Fe, Cd, etc.) in biological samples. It therefore covers a vast range of applications.

In the pharmaceutical field, examples include:

- Assay of cobalt in Vitamin B12.
- Assay of Mg in nutritional supplements.
- Assay of Ca in Ca-based preparations.
- Analysis of plant and animal tissues, biological fluids.

- Assay of Ca, Sr, Zn in bones.

The sample is introduced into the atomizer, which plays a dual role:

- Production of atomic vapors.
- Excitation of atoms.

ATOMIC EMISSION SPECTROMETRY

1. DEFINITION:

This is a method for assaying certain metallic elements. This method uses the ability of certain excited atoms to de-excite by emitting photons of determined energy (hence of determined wavelength).

Atomic emission photometry measures the emission of UV or visible electromagnetic radiation due to the de-excitation of atoms that have been excited by energy supplied through transfer to a very high temperature (introduction of the sample into a flame or plasma). Quantitative measurement of the emission allows assays.

Flames used in flame photometers reach 2000 to 3000 °C and allow emission by atoms of alkali series (Na, K, Li), some alkaline earths (Ba), and a few other metals. Plasma instruments, reaching over 7000 °C, broaden the range of measurable atoms.

2. PRINCIPLE:

Using a flame or plasma, whose temperature is very high, the objective is to break molecular structures and bring the element to be assayed (at least partially) into the form of atomic vapor. Under the effect of high temperatures, some atoms will be excited, and their electrons will transition to higher energy levels. The excited levels are unstable, and the return to the fundamental minimum energy level will lead to a release of energy in the form of electromagnetic radiation with a wavelength characteristic of the de-exciting atom. The measurement of this emission at a wavelength characteristic of the atom to be measured is the basis of atomic emission photometry.

After excitation, the return to the ground state is accompanied by the emission of radiation specific to the element(s) to be assayed.

- The intensity of the emitted radiation is proportional to the concentration of the analyte under consideration.

In AES, there are two types of atomizers:

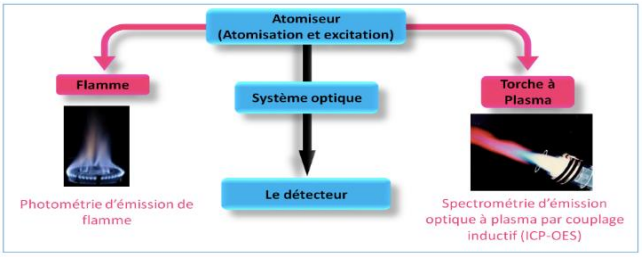
- The flame (Flame Emission Photometry).
- The plasma torch (Inductively Coupled Plasma Optical Emission Spectrometry - ICP-OES).

Plasma is the fourth state of matter. It is an ionized gas where electrons are stripped from their atomic orbitals. Plasma consists of isolated atoms in equilibrium between their neutral and ionized forms.

- AES allows qualitative elemental analysis of composition, meaning it is possible to identify the elements in a sample of unknown composition, unlike AAS, where only the element for which the spectrometer was prepared (by choosing the lamp for the element to be analyzed) is assayed.

- After excitation, for each atom, there are around a hundred possibilities for returning to the ground state, and for each, radiation of a specific wavelength is emitted. Thus, the atomic emission spectrum presents several emission lines, which constitute a fingerprint of the element to be assayed, whereas in AAS, measurements are made at a single wavelength, selected by the bandpass (the spectrum presents a single absorption band).

3. APPARATUS:



4. APPLICATIONS:

This essential analytical method has also received various applications in several fields (industrial, environmental, metallurgical, forensic, pharmaceutical, etc.). Alkali metals, giving colored flames, are easily assayed by emission. Flame emission can therefore be used in mineral analysis and biology to assay lithium, sodium, and potassium (ionogram) and also certain alkaline earths (Ba). These analyses can be done in the visible or ultraviolet range. In bromatology (food science), it can be used for quality control (e.g., assaying sodium and calcium in milk).

Comparative table between different atomic absorption and emission spectrometric techniques.

Technique	**Nature of the element**	**Sensitivity**	**Analysis Speed**	**Cost**
AAS-Flame	Transition metals (Fe, Cu, Mn, Co...)	+	Longest	+
AAS-Furnace	Mg, Al, Si, Transition metals and heavy metals	++++		
AES-Flame	Alkalis Li, Na, K, Rb, Cs	-		
AES-ICP	All elements in the periodic table except non-metals.	++++	++++	++++

MOLECULAR ABSORPTION SPECTROMETRY

1. DEFINITION:

Molecular absorption spectrophotometry is used for the identification and assay of biochemical molecules. It is the quantitative measurement of light absorbed by a chemical substance by passing a light beam through the sample in a spectrophotometer. Spectrophotometry means "measurement of photons as a function of the spectrum." In physics, the concepts of photon and spectrum are indeed linked to the corpuscular nature of any electromagnetic wave and the dispersion of white light by a dispersive medium.

In a molecule, electronic transitions occur in the ultraviolet and visible region.

The UV-visible range extends approximately from 10 to 800 nm.

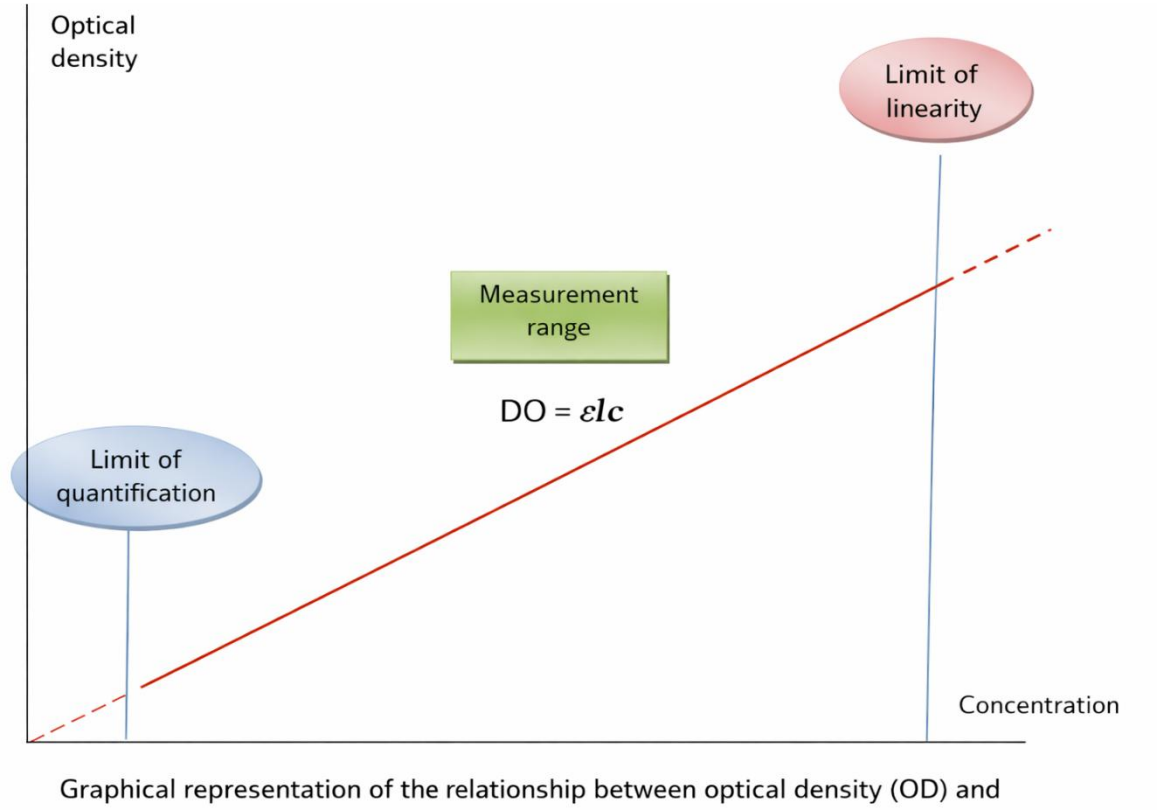
- Visible: 400 nm (indigo) - 800 nm (red).
- Near-UV: 200 nm - 400 nm
- Far-UV: 10 nm - 200 nm.

The usable ultraviolet spectrum range in analysis extends approximately from 190 to 400 nm. The visible spectrum range extends approximately from 400 to 800 nm.

2. PRINCIPLE:

When light is passed through a colored solution, it absorbs the complementary color. If a monochromatic ray corresponding to this color is used, it will be at least partially absorbed. Photometers are used if only certain wavelengths are usable, and spectrophotometers if all are available.

Optical density is proportional to the concentration of a solution. The latter can be assayed by reading its optical density in an instrument calibrated with the same substance at different known concentrations (using a standard).



Graphical representation of the OD / concentration relationship

It can be seen that OD is proportional to concentration, and the relationship between OD and concentration is linear, up to a concentration which is the limit of linearity.

3. APPARATUS:

A spectrophotometer is an instrument through which a light ray passes.

⇒ The light source emits polychromatic light (white light):

- Tungsten filament or tungsten-halogen lamp: visible and near UV
- Deuterium lamp: UV

⇒ A device called a monochromator allows the selection of one wavelength:

- The monochromator can be a prism or a grating
- It can be replaced by interchangeable filters that select one wavelength by stopping all others.

⇒ Other wavelengths are dispersed by a system of slits and mirrors. The light ray becomes monochromatic.

⇒ The monochromatic ray passes through a cuvette containing the solution with the compound to be assayed:

- UV: quartz cuvette
- Visible and near UV: glass cuvette or variable quality plastic materials

Part of the ray is absorbed by the solution. The rest continues its path to a photodetector connected to an electronic system allowing it to be amplified (photomultiplier) and quantified.

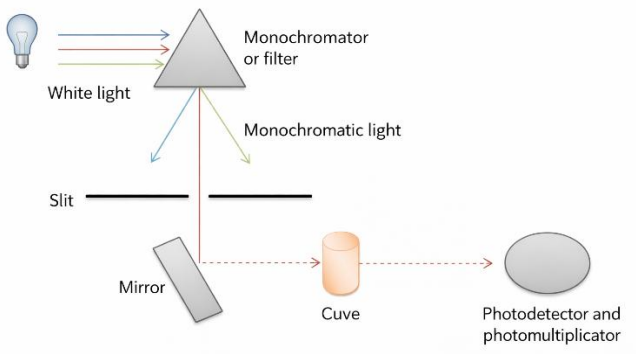


Diagram of a spectrophotometer

Performing a photometric biochemical assay: Example of glucose assay using the glucose oxidase method (Trinder method).

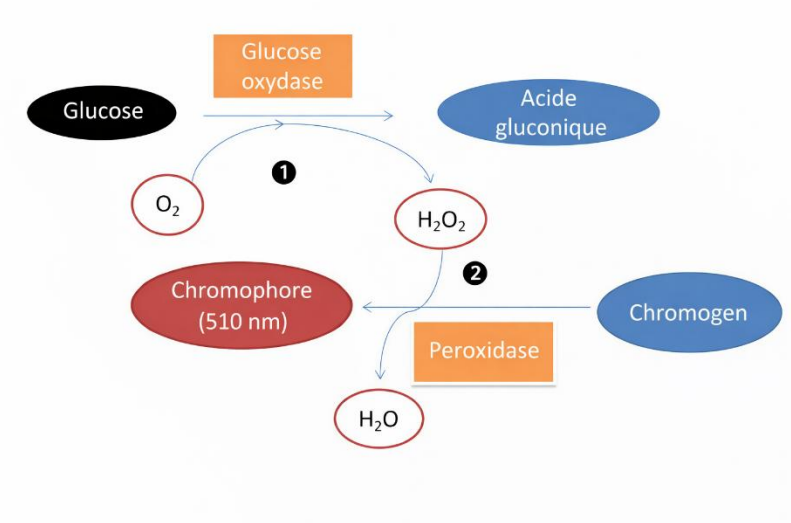


Diagram of the reactions involved in the glucose assay using glucose oxidase

Glucose is transformed into gluconic acid using glucose oxidase. This reaction consumes oxygen (O_2) and produces hydrogen peroxide (H_2O_2).

- H_2O_2 allows a peroxidase to transform a chromogenic substance to make it chromophoric. This chromophoric substance absorbs at a wavelength of 510 nm.
- It is the measurement of this absorption that allows the glucose assay. Absorption is commonly referred to as "optical density" or OD.
- The chosen wavelength therefore depends on the absorption spectrum of the chromophore used, and generally corresponds to one of its absorption maxima.
- **End-point and kinetic measurement:**

End-point determination: The end of the reaction, the "plateau," is awaited to measure the OD. The higher the concentration, the higher the OD.

Kinetic determination: Several measurements are taken during the growth phase of the OD. A slope or ΔOD is determined. The higher the concentration, the steeper the slope.

Enzymatic activities (transaminases, alkaline phosphatases, etc.) are generally measured kinetically. Substrates like creatinine can be measured by both methods.

- In manual techniques, the end-point method gives good results.
  In automated techniques, the kinetic method can resolve certain interference problems and allows for a reduction in analysis time.