S. S. College, Jehanabad

Department: Zoology

Class: M.Sc. Semester II

Subject: Zoology

Topic: Spectroscopy - Atomic Absorption Spectroscopy

Mode of teaching: Google classroom & WhatsApp

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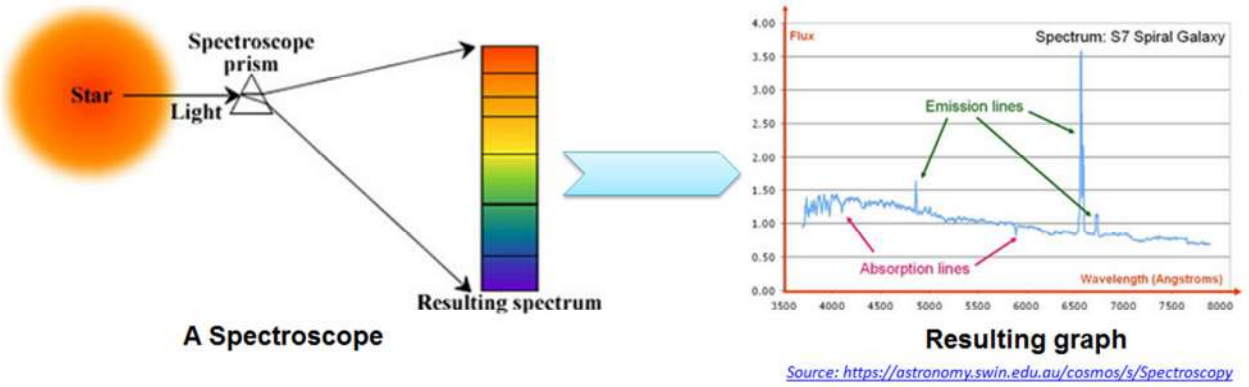
SPECTROSCOPY - ATOMIC ABSORPTION SPECTROSCOPY

Spectroscopy is a technique that uses radiation to obtain information on the structure and properties of matter. It utilizes electromagnetic radiation that is made fallen onto a sample, and observes how it responds to such beam of electromagnetic radiation. The response is usually recorded as a function of radiation wavelength and a plot of this response is displayed, which is known as **spectrum**. *In other word, it is the study of the absorption and emission of light and other radiation by matter, which involves the splitting of electromagnetic radiation or light into its constituent wavelengths that is known as spectrum.* More recently, the definition has been extended to include the study of the interactions between particles such as electrons, proteins, and ions, as well as their interaction with other particles as function of their collision energy. It was crucial in the development of the most fundamental theories in physics such as quantum mechanics, special and general theory of relativity, and quantum electrodynamics. It is applied in virtually all technical fields of science and technology including health. Magnetic resonance imaging (MRI) is a famous example of application of use spectroscopy which utilizes radio-frequency spectroscopy of nuclei in a magnetic field. Another famous example is microwave spectroscopy which is used to discover and analyze three-degree blackbody radiation, the remnant of the big bang i.e. the primeval explosion, from which the universe is thought to have originated.



Types of spectroscopy

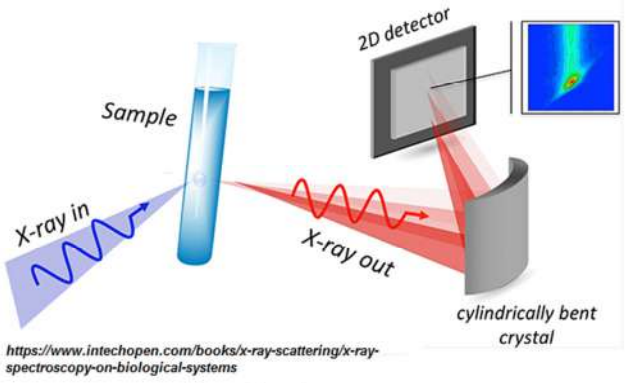
There are different types of spectroscopy according to type of electromagnetic radiations and properties means for different applications such as X-ray Spectroscopy, Flame Spectroscopy, Atomic Emission Spectroscopy (AES), Atomic Absorption Spectroscopy (AAS), Spark or Arc Spectroscopy, UV-Vis Spectroscopy, etc.

X-ray Spectroscopy: X-rays of sufficient energy are used to excite the inner shell electrons in the atoms of a sample. The electrons move to outer orbitals then down into the vacated inner shells and the energy in this de-excitation process is emitted as radiation.

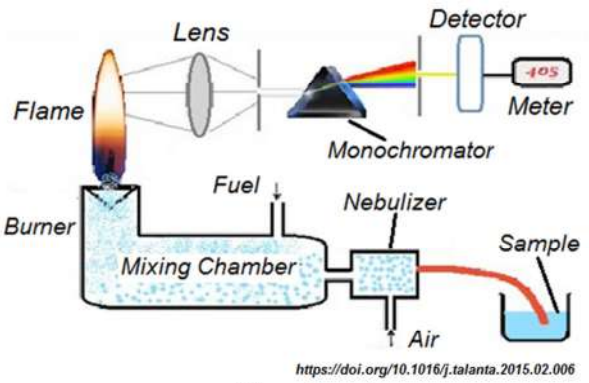
The absorption or emission energies are characteristic of the specific atom and small energy variations may occur that are characteristic of particular chemical bonding. The X-ray frequencies can be measured and X-ray absorption and emission spectroscopy is used to determine elemental composition and chemical bonding. In X-ray crystallography, crystalline

materials are analyzed by studying the way they scatter X-rays aimed at them. Knowing the wavelength of the incident X-rays allows calculation and eventually the intensities of the scattered X-rays give information about the atomic positions and their arrangement within the crystal structure.

Flame Spectroscopy: Usually the analyte is in solution form (or converted into one) that is then converted to a free gaseous form in a multistage process (atomization). This method is often used for metallic element analytes present at very low concentration ranges.



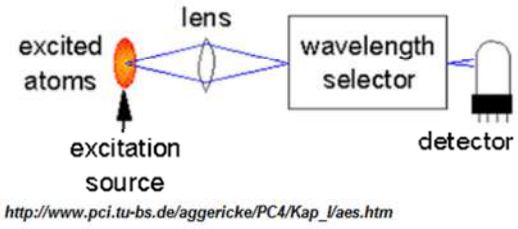
X-ray Spectroscopy



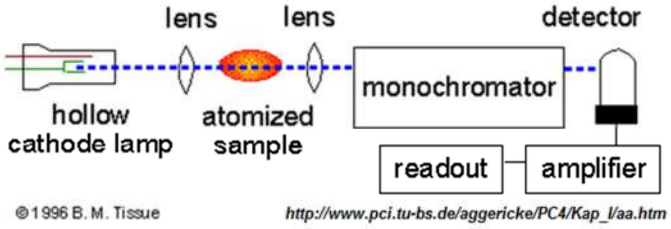
Flame Spectroscopy

Atomic Emission Spectroscopy (AES): This method uses atoms excited from the heat of a flame to emit light. The analysis can be done with a high resolution polychromator to produce emission intensity vs. wavelength spectrum to detect multiple elements simultaneously.

Atomic Absorption Spectroscopy (AAS): Compared to atomic emission spectroscopy, a flame of lower temperature is used so as not to excite the sample atoms. Instead, the analyte atoms are actually excited using lamps which shine through the flame at wavelengths adjusted according to the type of analyte under study. The amount of analyte present in the study sample is determined based on how much light is absorbed after passing through the flame.



Atomic Emission Spectroscopy



Atomic Absorption Spectroscopy

Spark or Arc Spectroscopy: This is used for analyzing solid metallic elements or non-metallic samples made conductive by being ground with graphite powder. Analysis requires passing an electric spark through it to produce a heat that excites the atoms. The excited atoms emit light of characteristic wavelengths which can be detected using a monochromator.

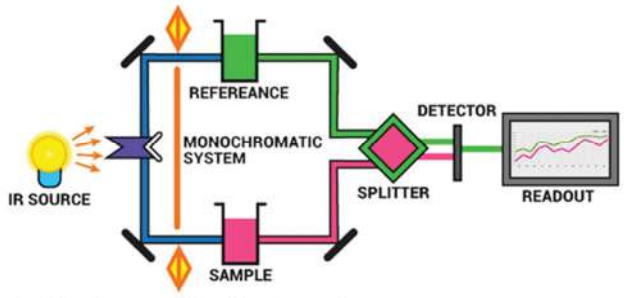
Ultraviolet/Visible (UV-Vis) Spectroscopy: It uses the fact that many atoms are able to emit or absorb visible light. The atoms must be in a gaseous phase in order to obtain a spectrum just as

those obtained in flame spectroscopy. It is common for visible absorption spectroscopy to be combined with UV absorption spectroscopy in UV/Vis spectroscopy. It is generally used to quantify the concentration of solutes in a test solution. It can also be used to analyze fluorescence from a sample in a form of absorption spectroscopy.

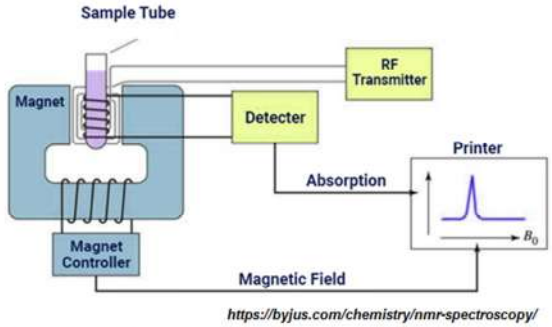
Infrared (IR) and Near Infrared (NIR) Spectroscopy: Infrared spectroscopy (IRS) is used to show what types of bonds are present in a sample by measuring different types of inter-atomic bond vibrations at different frequencies. It relies on the fact that molecules absorb specific frequencies which is dependent on their chemical structure. This is determined by factors such as the masses of the atoms.

Near infrared spectroscopy (NIRS) shows a greater penetration depth into a sample than infrared radiation. This indicates a low sensitivity but it allows large samples to be measured in each scan by NIR spectroscopy with little sample preparation. It has numerous practical applications that includes medical diagnosis pharmaceuticals, biotechnology, various analyses (genomics, proteomic) and chemical imaging of intact organisms, textiles, forensic lab application and various military applications.

Nuclear Magnetic Resonance: This is a prominent method for analyzing organic compounds because it exploits the magnetic properties of certain atomic nuclei to determine the properties (both chemical and physical) of these atoms or the molecules containing them. It can provide extensive information about the structure, dynamics, and chemical environment of atoms. Additionally, even different functional groups are distinguishable, and identical functional groups in differing molecular environments still give distinguishable signals.



Infrared (IR) Spectroscopy



Nuclear Magnetic Resonance (NMR) Spectroscopy

According to our syllabus, we need to study only Ultraviolet-Visible (UV/Vis) Spectroscopy and Atomic Absorption Spectroscopy (AAS). UV/Vis Spectroscopy has been already discussed in Spectrophotometry chapter. Here, we will discuss about atomic absorption spectroscopy.

Atomic Absorption Spectroscopy (AAS)

It uses the absorption of light to measure the concentration of gas-phase atoms. Since samples are usually liquids or solids, the analyte atoms or ions must be vaporized in a flame or graphite furnace. The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy level (excited state). The analyte concentration is determined from the amount of

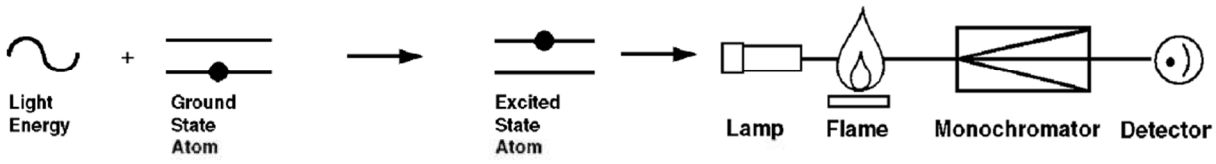
absorption of light by the test materials. Applying the Beer-Lambert law (discussed in previous chapter; Spectrophotometry) directly in AA spectroscopy is difficult due to variations in the atomization efficiency from the sample matrix, and non-uniformity of concentration and path length of analyte atoms. Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration.

It was Robert Bunsen and Gustav Kirchhoff who first of all put forwarded on working with sodium spectrum that every element has its own unique spectrum that can be used to identify elements in the vapour phase. Kirchoff further explained the phenomenon by stating that if a material can emit radiation of a certain wavelength, that it may also absorb radiation of that wavelength. Although Bunsen and Kirchoff took a large step in defining the technique of atomic absorption spectroscopy (AAS), it was not widely utilized as an analytical technique except in the field of astronomy due to many practical difficulties. However, it was due to extensive work done by Alan Walsh that made it possible to be used as an analytical instrument in 1953.

Principles

The working principles of atomic absorption spectroscopy are largely based on the Louis deBroglie theory which states that quantized energy of electrons. Further, deBroglie theory was elaborated by Wolfgang Pauli and therefore, the improved deBroglie theory states that no two electrons can share the same four quantum numbers.

Atoms have valence electrons present in the outermost orbit of the atom, and therefore atoms can be excited when irradiated that creates an absorption spectrum. When an atom is excited, the valence electron moves up an energy level. The energies of the various stationary states, or restricted orbits, can then be determined by these emission lines. The resonance line is then defined as the specific radiation absorbed to reach the excited state.



Atomic absorption process and transition of energy state and detection through atomic absorption spectroscopy

The Maxwell-Boltzmann equation gives the number of electrons in any given orbital. It relates the distribution to the thermal temperature of the system (as opposed to electronic temperature, vibrational temperature, or rotational temperature). Plank proposed radiation emitted energy in discrete packets (quanta),

$$\boldsymbol{E = hv} \qquad (\boldsymbol{i})$$

which can be related to Einstein's equation

$$\boldsymbol{E = mc^2} \qquad (\boldsymbol{ii})$$

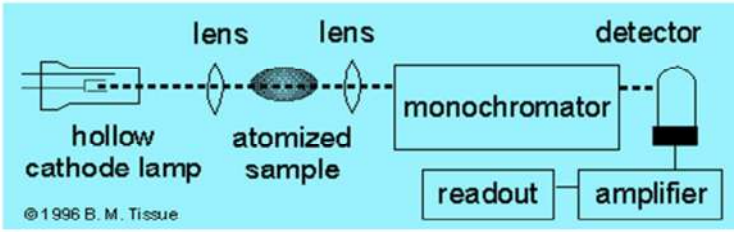
Both atomic emission and atomic absorption spectroscopy can be used to analyze samples. Atomic emission spectroscopy measures the intensity of light emitted by the excited atoms, while atomic absorption spectroscopy measures the light absorbed by atomic absorption. This light is typically in the visible or ultraviolet region of the electromagnetic spectrum. The percentage is then compared to a calibration curve to determine the amount of material in the sample. The energy of the system can be used to find the frequency of the radiation, and thus the wavelength through the combination of equations (i) and (ii) will be

$$\boldsymbol{\nu = \frac{c}{\lambda}} \qquad (\boldsymbol{iii})$$

Because the energy levels are quantized, only certain wavelengths are allowed and each atom has a unique spectrum. There are many variables that can affect the system. For example, if the sample is changed in a way that increases the population of atoms, there will be an increase in both emission and absorption and *vice versa*. There are also variables that affect the ratio of excited to unexcited atoms such as an increase in temperature of the vapor.

Instrumentation

Light source: The light source is usually a hollow-cathode lamp of the element that is being measured. Lasers are also used in research instruments. Since lasers are intense enough to excite atoms to higher energy levels, they allow atomic absorption and atomic fluorescence measurements in a single instrument, however through this narrow-band light source, only one element can be measured at a time.

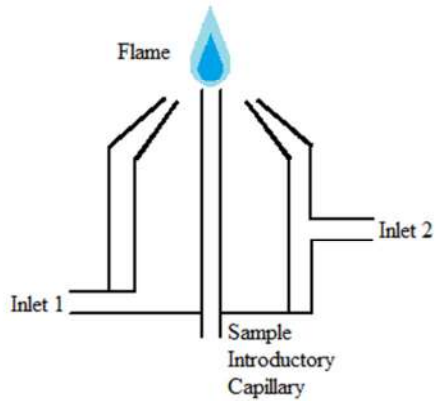


Schematic representation of instrumentation of Atomic Absorption Spectroscopy (AAS)
Source: http://www.pci.tu-bs.de/aggericke/PC4/Kap_I/aa.htm

Atomizer: Atomic absorption spectroscopy requires that the analyte atoms be in the gas phase. Ions or atoms in a sample must undergo desolvation and vaporization in a high-temperature source such as a flame or graphite furnace. Flame atomic absorption spectroscopy can only analyze solutions, while graphite furnace atomic absorption spectroscopy can accept solutions, slurries, or solid samples.

Flame atomic absorption spectroscopy uses a slot type **flame burner** to increase the path length, and therefore to increase the total absorbance. Sample solutions are usually aspirated with the gas flow into a nebulizing/mixing chamber to form small droplets before entering the flame, while in graphite furnace atomic absorption spectroscopy Samples are placed directly in the

graphite furnace and the furnace is electrically heated in several steps to dry the sample, ash organic matter, and vaporize the analyte atoms, also known as **electrothermal atomizer**. Thus the graphite atomic absorption spectroscopy has several advantages over a flame. Graphite atomic absorption spectroscopy directly accepts very small absolute quantities of sample and also provides a reducing environment for easily oxidized elements.



A schematic diagram of a flame atomizer showing the oxidizer inlet (1) and fuel inlet (2).

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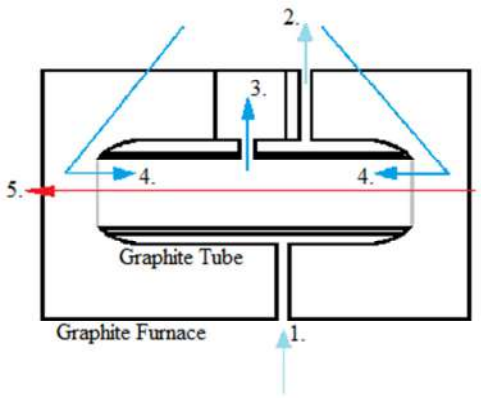


Schematic diagram of an electrothermal atomizer showing the external gas flow inlet (1), the external gas flow outlet (2), the internal gas flow outlet (3), the internal gas flow inlet (4), and the light beam (5).

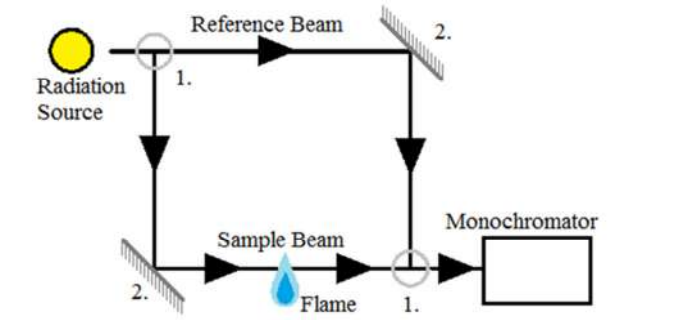
Spectrometer: It is used to separate the different wavelengths of light before they pass to the detector. The spectrometer used in atomic absorption spectroscopy (AAS) can be either single-beam or double-beam. Single-beam spectrometers only require radiation that passes directly through the atomized sample, while double-beam spectrometers, as implied by the name (as shown in figure below; also discussed in previous chapter under the heading Spectrophotometry), require two beams of light; one that passes directly through the sample, and one that does not pass through the sample at all. The single-beam spectrometers have less optical components and therefore suffer less radiation loss, while double-beam monochromators have more optical components, but they are also more stable over time because they can compensate for changes more readily.

Obtaining measurements

Measurements can be obtained by carrying out atomic absorption spectroscopy study of test materials, which involves sample preparation, getting standard curve by calibration and comparing the value obtained with standard curve.

Sample preparation: It varies depending upon the test materials that are being analyzed. However, it also varies on specific laboratory environment, the vessel holding the sample, storage of the sample, and pretreatment of the sample. It requires the various equipments that include laminar flow hoods, clean rooms, and closed, clean vessels for transportation of the sample. Before going for sample preparation, a clean laboratory environment needs to be ensured because it is used to measure trace elements, in which case contamination can lead to severe error.

When trace elements are stored, the material of the vessel walls can adsorb some of the analyte leading to poor results. To correct for this, *perfluoroalkoxy polymers (PFA), silica, glassy carbon, and other materials with inert surfaces* are often used as the storage material. Acidifying the solution with hydrochloric or nitric acid can also help prevent ions from adhering to the walls of the vessel by competing for the space. The vessels should also contain a minimal surface area in order to minimize possible adsorption sites.



A schematic of a double-beam spectrometer showing the 50/50 beam splitters (1) and the mirrors (2).

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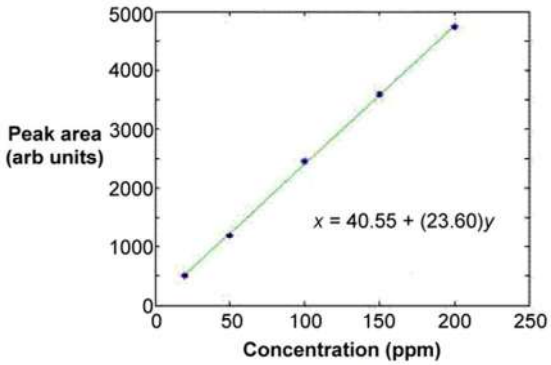

A Calibration Curve

Calibration Curve: In order to determine the concentration of the analyte in the solution, calibration curves are employed.Using standards, a plot of concentration versus absorbance can be created. To create a calibration curve, there are usually three common methods are employed, which are as follows;

1. ***Standard calibration technique:*** This is the commonly used and simplest technique used for the determination of calibration curve. The concentration of the sample is found by comparing its absorbance or integrated absorbance to a curve of the concentration of the standards versus the absorbances or integrated absorbances of the standards. However, it requires the following:
   - Both the standards and the sample must have the same behavior when atomized. If they do not, the matrix of the standards should be altered to match that of the sample.
   - The error in measuring the absorbance must be smaller than that of the preparation of the standards.
   - The samples must be homogeneous.

   The curve is typically linear and involves at least five points from five standards that are at equidistant concentrations from each other (as shown in above figure).

2. ***Bracketing technique:*** In this method, only two standards are necessary with concentrations c1 and c2. They bracket the approximate value of the sample concentration very closely by applying following equation

$$\boldsymbol{Cx = \frac{(Ax - A1)(c1 - c2)}{A2 - A1} + c1} \qquad (iv)$$

   One can determine the value for the sample, where Cx and Ax are the concentration and absorbance of the unknown, and A1 and A2 are the absorbance for c1 and c2,

respectively. This method is very useful when the concentration of the analyte in the sample is outside of the linear portion of the calibration curve because the bracket is so small that the portion of the curve being used can be portrayed as linear.

3. ***Analyte Addition Technique:*** This technique is often used when the concomitants in the sample are expected to create many interferences and the composition of the sample is unknown. The previous two techniques both require that the standards have a similar matrix to that of the sample, but that is not possible when the matrix is unknown. To compensate for this, the analyte addition technique uses an aliquot of the sample itself as the matrix. The aliquots are then spiked with various amounts of the analyte.

Light Separation and Detection: In atomic absorption spectroscopy (AAS), spectrometers use monochromators and detectors for uv and visible light. The main purpose of the monochromator is to isolate the absorption line from background light due to interferences. Simple dedicated atomic absorption spectroscopy instruments often replace the monochromator with a bandpass interference filter, which is further connected with photomultiplier tubes as the most detectors for atomic absorption spectroscopy.

Application of Atomic Absorption Spectroscopy (AAS)

It is used for various purposes from petrol industry to mining and from detection of trace elements to food and drug inspections, health industry, etc. It is mainly employed for;

- Qualitative and quantitative analysis.
- Determination of metallic elements in biological system.
- Determination of metallic element in food industry.
- Determination of Ca, Mg, Na, K in serum.
- Determination of lead in petrol.

Reference

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