Name of Unit:	IR spectroscopy, Flame Photometry, Atomic Absorption Spectroscopy and Nepheloturbidometry
Course/Subject Name:	Instrumental Methods of Analysis
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Learning Outcome of Module-2

LO	Learning Outcome (LO)	Course Outcome Code
LO1	To understand the interaction of matter with electromagnetic radiations and its applications in drug analysis.	BP701.1, BP701.2
LO2	To perform qualitative analysis of drugs using IR spectroscopy	BP701.1, BP701.5, BP701.6
LO3	To perform quantitative & qualitative analysis of drugs using Flame Photometry.	BP701.1, BP701.5, BP701.6
LO4	To perform quantitative & qualitative analysis of drugs using Atomic Absorption Spectroscopy.	BP701.1, BP701.5, BP701.6
LO5	To perform quantitative & qualitative analysis of drugs using Nepheloturbidometry.	BP701.1, BP701.5, BP701.6

Table of Content

Topic

Infrared Spectroscopy

The infrared region of the electromagnetic spectrum extends from the red end of the visible spectrum to the microwave region. The region includes radiation at wave-lengths between 0.7 and 500 μm or, in wave numbers, between 14,000 and 20 $cm^{-1.}$ The spectral range used most is the mid-region, which covers frequencies from 4000 to 200 cm^{-1} (2.5 to 50 μm). Infrared spectrometry involves examination of the twisting, bending, rotating, and vibrational motions of atoms in a molecule. Atoms or atomic groups in molecules are in continuous motion with respect to one another. Atomic masses are represented by balls, their weights being proportional to the corresponding atomic weights and arranged in accordance with the actual space geometry of the molecule. Mechanical springs, with forces that are proportional to the bonding forces of the chemical links, connect and keep the balls in balance. If the model is suspended in space and struck, the balls appear to undergo random chaotic motions.

Correlation of Infrared Spectra with Molecular Structure

The infrared spectrum of a compound is basically the superposition of absorption bands of specific functional groups, yet slight interactions with the surrounding atoms of the molecule impose the stamp of individuality on the spectrum of every compound.

For qualitative analysis, one of the most excellent features of an infrared spectrum is that the absorption or the lack of absorption in specific frequency regions can be correlated with specific stretching and bending motions and, in some cases, with the relationship of these groups to the rest of the molecule. Thus, when interpreting the spectrum, it is probable to state that certain functional groups are there in the material and certain others are missing. With this one datum, the possibilities for the unidentified occasionally can be narrowed so sharply that comparison with a library of spectra of pure compounds permits identification.

Near-Infrared Region

In the near-infrared (NIR) region, which meets the visible region at about 12,500 cm^{-1} (0.80 μm) and extends to about 4000 cm^{-1} (500μm) there are numerous absorption bands that result from harmonic overtones of fundamental and combination bands often associated with hydrogen atoms. Among these are the first overtones of the O-H and N-H stretching vibrations near 7140 cm^{-1} and combination bands that result from C-H stretching and deformation vibrations of alkyl groups at 4548 cm^{-1}. The absorptivity of NIR bands is from 10 to 1000 times less than that of mid-infrared bands. Thicker sample layers compensate for these smaller molar absorptivities. Because the absorptivities are so low, the NIR beam penetrates deeper into a sample in reflectance techniques, giving a more representative analysis. Furthermore, minor impurities are less troublesome in both reflectance and transmission methods than in the mid-infrared region. The NIR region is accessible with quartz optics, and this is coupled with greater sensitivity of near-infrared detectors and more intense radiation sources. The near- infrared region is often used for quantitative work in addition to qualitative identification. Near infrared spectrometry is a valuable tool for analyzing mixture of aromatic amines. Primary aromatic amines are characterized by two relatively intense absorption bands near 1.97 and 1.49 μm. Secondary amines exhibit an overtone band but do not absorb appreciably in the combination region. These differences in absorption provide the basis for rapid quantities analytical methods. Tertiary amines do not exhibit appreciable absorption at either wavelength. The overtone and combination bands of aliphatic amines are shifted to about 1.525 and 2.000 μm, respectively. Interference from the first overtone of the O-H stretching vibration at 1.40 um is easily avoided with the high resolution available with near-infrared instruments.

Mid-Infrared Region

Numerous practical correlations have been found in the mid-infrared region. This region is divided into the “group frequency” region 4000-1300 cm^{-1} (2.50-7.69 μm), and the fingerprint region, 1300-650 cm^{-1}. In this group frequency region the main absorption bands are assigned to vibration units consisting of merely two atoms of a molecule that is, units that are more or less dependent on only the functional group that gives the absorption and not on the complete molecular structure. Structural influences do reveal themselves, however as significant shifts from one compound to another. In the derivation of information from an infrared spectrum, prominent bands in this region are noted and assigned first. In the interval from 4000 to 2500 cm^{-1} the absorption is characteristic of hydrogen stretching vibrations with elements of mass 19 or less. The C- H stretching frequencies are especially helpful in establishing the type of compound present; for example, C=C-H occurs around 3300 cm^{-1} aromatic and unsaturated compounds around 3000-3100 cm^{-1} and aliphatic compounds at 3000-2800 cm^{-1} .

When coupled through heavier masses, the hydrogen stretching frequencies overlap the triple-bond region. The intermediate frequency range, 2500-1540 cm^{-1} is often called the unsaturated region. Triple bonds, and very little else, appear from 2500 to 2000 cm^{-1} Double-bond frequencies fall in the region from 2000 to 1540 cm^{-1}. By careful application of accumulated empirical data, it is possible to differentiate among C=O, C=C, C=N, N=O, and S=O bands. The major factors in the spectrum between 1300 and 650 cm^{-1} are single-bond stretching frequencies and bending vibrations of polyatomic systems that involve motions of bonds lining a substituent group to the remainder of the molecule. This is the fingerprint region. Multiplicity is also great for certain individual identification of the bands, but together the absorption bands aid in identifying thematerial.

Far-Infrared Region

The region 667-10 cm^{-1} contains the bending vibrations of carbon, nitrogen, oxygen, and fluorine with atoms heavier than mass 19, and additional bending motions in cyclic or unsaturated systems.

Instrumentation

The usual optical materials, glass or quartz absorb strongly in the infrared region, consequently the apparatus for measuring infrared spectra is appreciably different from that for the visible and ultraviolet regions. The main parts of an IR spectrometer are as follows:

IR spectrum of a compound can be recorded in many different forms, such as liquid, solid, gas and solution. Some of the materials are opaque to infrared radiation, so in order to obtain spectra they must be dissolved or diluted in a transparent matrix. For recording IR spectra, the sample should be properly dry as water absorb near 3710 and 1630 cm-1. The samples should be perfectly dried, since cell materials (NaCl, KBr) are usually spoiled by the moisture.

1) **Solid samples:** There are several methods by which an IR spectrum of a solid sample can be recorded.

a) **As a pressed disc:** The first common method involves the mixing of finely ground solid sample with powdered potassium chloride. A translucent pellet of this powder mixture is formed by pressing it in a mechanical pressure. The main advantage of using KBr is that it does not interfere with the bands due to compound since KBr is transparent to IR radiation 4000-650 cm-1 and thus gives better spectra. The disadvantage of this method is that KBr absorbs water quickly which may interfere with the spectra that is obtained.

b) **As a mull or paste:** Finely ground compound is mixed with an oily mulling agent (usually Nujol) using a pestle and mortar. A thin film of the mull is placed between two flat plates of NaCl and the spectrum is measured. The main disadvantages of this method is that nujol has absorption bands at 2924-2860, 1462, 1380 cm-1, therefore no information about the observed compound can be obtained in this region.

c) **As a film:** The third method is to dissolve the soild sample in a suitable, non-hygroscopic solvent usually methylene chloride or carbon tetra chloride. A drop of this solution is deposited on surface of Potassium bromide or Sodium chloride plate. The solution is then evaporated to dryness and the film thus formed on the KBr disc is analysed directly to obtain the IR spectrum. The most important thing is that the film should not be too thick otherwise light cannot pass through it. This method gives good results with dilute solution of the compound in a non-polar solvent.

2) **Liquid samples:** Liquids are studied neat or in solution. A drop of neat liquid sample or a solution of the sample in an appropriate solvent is placed between two plates of a salt (sodium chloride or potassium bromide) to give a thin film and analysed to obtain the spectrum. The plates are transparent to the infrared light and do not introduce any lines onto the spectra. Salt plates break easily and are water soluble therefore compounds analysed by this method should be free from water. Spectrum obtained by this method is known as neat spectrum since no solvent is used in recording the spectrum.

3) **Gaseous samples:** The gas is introduced into a special cell with a long path length and the walls of its both the ends are normally made up of NaCl. Gases have very less densities compared to liquids, and hence path lengths should be correspondingly greater, usually 10 cm or longer. The vapor phase technique is limited because most of the organic compounds have too low vapor pressure to produce a useful absorption spectrum.

It is important to note that spectra obtained from different sample preparation methods will look slightly different from each other due to differences in the samples' physical states.

3. IR-spectrometer

Traditionally, dispersive infrared spectrophotometers, developed in 1940s, were used to obtain infrared spectra. In 1960s, a new method was developed known as Fourier-transform infrared (FT-IR) spectrometers. But due to high cost of the instrument, this was tended to be used for advanced research only at that time. Gradually, technology advancements in computers and instruments have reduced the cost and enhanced the capabilities of FT-IR spectrophotometer. Today they are predominantly used and have improved the acquisition of infrared spectra dramatically.

4.Dispersive Infrared Spectrometer

The basic components of a dispersive IR spectrometer include a radiation source, Sample and reference cells, monochromator, detector, amplifier and recorder. A schematic diagram of a typical dispersive spectrometer is shown in Fig.

Radiation source: The common IR radiation source are inert solids that are heated electrically to a range of 1000 to 1800 °C to promote thermal emission of radiation in the infrared region of the EM spectrum. The most common sources are Nernst filament (composed of rare-earth oxides such as zirconium, cerium and thorium), Globar (composed of silicon carbide), and Nichrome coil. They all produce continuous radiations, but with different radiation energy profiles. The beam from the source is divided into two equivalent beams, one passing through the sample and the other as reference beam.

Sample and reference cells: Like UV sample tubes (cuvettes) glass or quartz cannot be used to make the sample cells for IR-spectroscopy, because they absorb strongly in most of the IR region. Alkali metal halides such as KCl, NaCl are commonly used as they are transparent to the IR- region.

Monochromator: The monochromator is a device used to disperse or separate a broad spectrum of IR radiation into a continuous spectrum of frequencies of Infrared light. The monochromator consists of rapidly rotating chopper that passes the two beams alternately to a diffraction grating. The slowly rotating diffraction grating varies the frequency or wavelength of radiation and sends it the individual frequency to the thermocouple detector which generates an electrical signal as a response.

Detectors and Amplifier: Detectors are devices that convert the radiant energy into an electrical signal. The detector determines the ratio between the intensities of the reference and sample beams. Due to the difference in the intensities of the two beams falling on the detector, an alternating current starts flowing from the detector to the amplifier, where it is amplified and relayed to the recorder.

The detectors used in IR spectrometers can be categorized into two classes: thermal detectors and photon detectors. Thermal detectors consists of several thermocouples

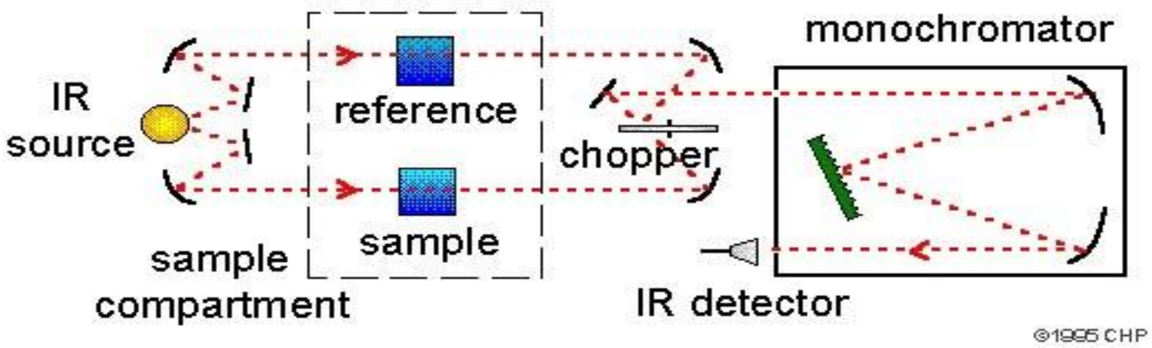


Figure 1: Schematic diagram of a dispersive IR spectrometer

connected together to produce greater sensitivity. They measure the heating effect produced by infrared radiation that causes the flow of current. The current produced is proportional to the intensity of radiation falling on the thermal detector. Photon detectors rely on the interaction of IR radiation and a semiconductor material. Non-conducting electrons are excited to a conducting state and therefore producing a small current or voltage.

Recorder: It records IR-spectrum as a plot of frequency of absorbed radiation and intensity of absorption in terms of transmittance. Unlike UV-spectroscopy, here we use the wavenumber unit. As the detector records the ratio of the intensities of the two beams therefore percent transmittance is recorded.

Transmittance (T) = I/I0

Percent transmittance (%T) = I/I0 X 100

Where I0 is the intensity of the incident radiation and I is the intensity of the radiation emerging from the sample (Figure 1)

The IR Radiation Sources: In common with other types of absorption spectrometers, infrared instruments require a source of radiant energy which provides a way for isolating narrow frequency bands. In case of IR spectrometers the source of radiation should be such that it is intense enough for detection, extends over the desired wavelength and is steady. Although these radiations are continuous, only selected frequencies will be absorbed by the samples.

The various popular sources of IR radiations are:

(a) Incandescent Lamp
(b) Nernst Glower
(c) Globar Source
(d) Mercury Arc

Monochromators: The radiation source emits radiations of various frequencies. As the sample in IR spectroscopy absorbs only at certain frequencies, it therefore becomes necessary to select desired frequencies from the radiation source and reject the radiations of other frequencies. This selection has been achieved by means of monochromators which are mainly of two types:

Prism Monochromator

Grating Monochromator

Sample Cells and Sampling of Substances: As infrared spectroscopy has been used for the characterization of solid, liquid or gas samples, it is evident that samples of different phases have to be handled. But these samples have to be treated differently. However, the only common point to the sampling of different phases is that the material containing the sample must be transparent to IR radiation.

We will now discuss the sampling of solids, liquids and gases separately

Sampling of Solids: Samples that are solids can be sampled as: - Solid run in Solution, Solid Films, Mull Technique and Pressed Pellet Technique.

Sampling of Liquids: Samples that are liquids at room temperature are usually put frequently with no preparation, into rectangular cells made of NaCl, KBr or ThBr and their IR spectra are obtained directly.

Sampling of Gases. The gas sample cell is similar to the cell for liquid samples in as much as the surfaces in the light path are made of KBr, NaCl, and so on. To compensate for the small number of molecules of a sample that is contained in a gas, however, the cells are larger; usually they are about 10 cm long, but they can be up to 1 m long also. The gas must not react with the cell windows or the reflecting surfaces and moisture must be avoided.

Detectors. Excluding the near infrared, where a photoconductivity cell is generally used, there is no better choice than thermal detectors. These give responses for all frequencies. When the radiant power is low for the infrared region, it means that the detector signal will also be low. In order to locate these frequency light interrupter, 10-26 cps. is used. Thus to detect such signals, thermal detectors must possess a short response time and the absorbed heat must be lost rapidly. The latter condition is a most difficult requirement because heat transfer is not a quick process.

The various types of detectors used in IR spectroscopy are:

- Bolometers
- Thermocouple
- Thermistors
- Golay Cell
- Photoconductivity Cell
- Semiconductor Detectors
- Pyroelectric Detectors
- Fourier Transform Systems

Fourier Transform Infrared (FTIR) Spectrometer

Infrared radiation can be analyzed by means of a scanning Michelson interferometer. This consists of a moving mirror, a fixed mirror, and a beam splitter. The moving mirror collimates the radiation from the IR source and the resultant beam is divided by the beam splitter; half the beam passes to a fixed mirror and half is reflected to the moving mirror. The two beams recombine at the beam splitter, after getting reflected and interfere constructively or destructively, for any specific wavelength subjected to the differences in optical paths between the two arms of the interferometer. With a constant mirror velocity, the intensity of the emerging radiation at any particular wavelength modulates in a regular sinusoidal manner. In the case of a broadband source the emerging beam is a complex mixture of modulation frequencies that, after passing through the sample compartment, is focused onto the detector. This detector signal is sampled at precise intervals during the mirror scan. Both the sampling rate and the mirror velocity are controlled by a reference signal incident upon a detector, which is produced by modulation of the beam from the helium-neon laser.

The resulting signal from detector is known as an interferogram and contains all the information required to reconstruct the spectrum via the mathematical process known as Fourier transformation.

This technique has several distinct advantages over the conventional dispersive techniques. There is only one moving part involved, one of the mirrors, mounted on a frictionless air bearing. Dispersion or filtering is not required so that energy-wasting slits are not needed. This is a major advantage, particularly with energy at a premium in the far-infrared and the use of a helium-neon laser as a reference results in near absolute frequency accuracy, better than 0.01 cm^{-1} over the range 4800-400 cm^{-1}

The interferogram recorded with each scan is stored in memory. This interferogram is then automatically aligned with, and added to, the averaged interferograms in another memory.

At the same time annotation of the plot is begun in preparation for the final spectrum. After a number of scans, often 32 this averaged interferogram is Fourier transformed to produce a single-beam spectrum, which, in the standard sample mode, is stored in a third memory.

This single-beam spectrum, which, in the standard sample mode, is stored in 3rd memory.

This single-beam spectrum is then ratioed against the stored background in 4th memory and the resulting "double-beam" spectrum is plotted on the high-speed digital plotter. Memory 5 is additional space available for storage of a reference spectrum that would be used in the spectral subtraction technique. This memory is also used to store a newly measured spectrum while maintaining the sample and background spectra for further manipulation. The time from insertion of the sample to the completed plot is about 2 min.

Since the computer uses digital data, the photodetector signal must be converted to digital form by an analog-to-digital converter. The data must be sampled at precise points and the resultant data stored.

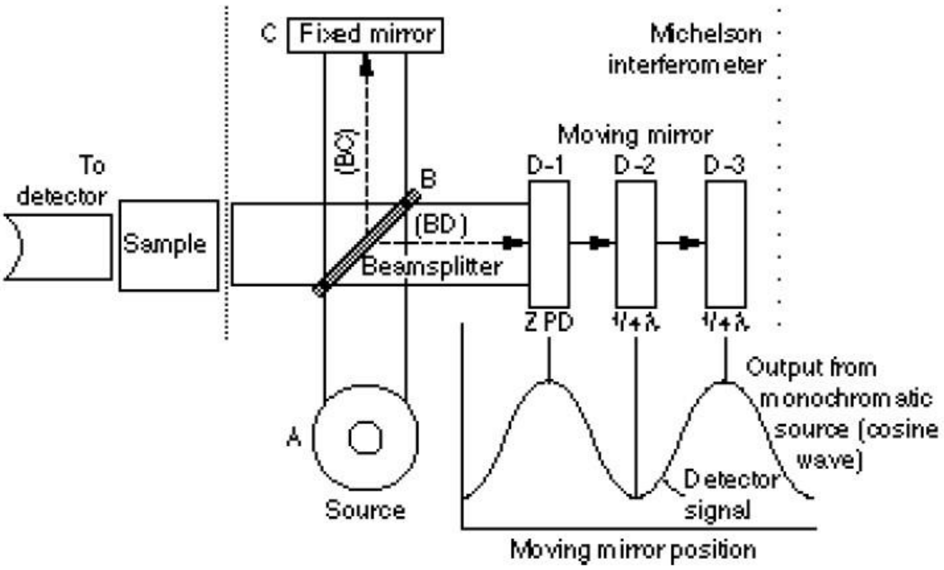


Figure 2: Schematic Presentation of a typical FTIR spectrometer

Fourier transform infrared spectrometers are still more expensive than sequential dispersive instruments due to the precision needed for mirror movement and the computer that is also required. However, dispersive instruments are now often equipped with computers that can record and remember spectra, plot absorbance, transmittance, or some function of absorbance, subtract one spectrum from another, and do other functions, and this adds to their price. The power of FTIR instruments has led to much competition in the instrumentation industry, resulting in an increased availability of commercial instruments and a decrease in price Low-cost FTIR instruments are beginning to appear. Fourier transform spectrometers are faster than dispersive instruments and therefore and especially useful in situations that require fast, repetitive scanning. They are used in recording the output of gas or liquid chromatographs or obtaining kinetic data.

Applications of Infrared Spectroscopy

Identification of Organic Compounds: The infrared spectrum is a highly characteristic property of a substance and the examination of the infrared spectrum can aid chemical investigation in many ways. The important application of infrared spectroscopy is given as follows:

- Identification of Substances such as Hydrocarbons like Alkanes, Cycloalkanes, Aromatic Hydrocarbons.

- Identification of Substances such as Hydroxy Compounds like Alcohols andphenols.
- Identification of Substances such as Aldehydes and Ketones, Carboxylic Acids, Carboxylic Acid Derivatives, Amines and Nitro Compounds.

Determination of Molecular Structure: Infrared spectroscopy is extremely helpful in determining the molecular structures of unknown substances. From an assessment of the positions of absorption bands in the spectrum, it is often possible to establish the nature of the groups present in the molecule.

Studying the Progress of Reactions. Progress of a chemical reaction can be readily followed by examining spectra of small portions of reaction mixture withdrawn from time to time.

Detection of Impurities: It is likely to determine whether a given sample of a compound is pure or not provided the reference spectrum of the pure compound is available.

Isomerism in Organic Chemistry. IR spectra are useful for identifying isomers. This distinction between isomers may not be possible by the chemical methods.

Geometrical Isomerism. In general Trans-isomers are more symmetric than cis and consequently the vibrations of the former give rise to little or no change in dipole-moment.

Tautomerism.

Flame emission spectroscopy

Flame photometry is based on measurement of intensity of the light emitted when a metal is introduced into a flame. The wavelength of the colour tells us what the element is, and the colours intensity tells us how much of the element is present. In early experiments, the visible colour of the flame was used to confirm the presence of certain elements in the sample, particularly alkali metals and alkaline-earth metals. Later the whole ultraviolet and visible range was utilized using a spectrophotometer.This instrument permitted us to select the wavelengths of the radiation and measure its intensity with considerable accuracy.

The spectrophotometric technique has proven to be one of the most reliable and easily used techniques for the determination of concentrations of sodium, potassium, calcium, and magnesium.Flame photometry is also named as flame emission spectroscopy because of the use of a flame to provide the energy of excitation to atoms introduced into the flame.

Flame photometry provides high sensitivity for determination of elements such as sodium, magnesium, strontium, potassium, lithium, calcium, and barium. Flame photometry is also usedfor determination of certain transition elements, such asiron, copper and manganese.

Qualitative analysis by employing flame photometer is severely limited.Non-metal can also beanalysed through this technique.

Limitations of Flame Photometry

The concentration of the metals may be measured by flame photometry, but frequently that technique is not the method of choice because of the relatively low energy available from a flame. Flame photometry tells little or nothing about molecular form of that metal in the original sample.

It has not been used for the direct detection and determination of the noble metals, halides, or inert gases. All of these elements require more energy than the flame provides in order to become excited.

General Principles of Flame Photometry

Processes involved in flame photometry are given below:

1. Solvent is vaporised, leaving particles of solid salt.
2. Salt is vaporised or converted into gaseous state.
3. A part or all of the gaseous molecules are dissociated to give free neutral atoms or radicals which are excited by thermal energy of the flame. The excited atoms emit photons and return to lower energy state. The measurement of the emitted photons, i.e., radiation, forms the basisof flame photometry.

If E2 and El represent the energy of the higher and lower energy levels concerned, the radiation emitted during the jump may be defined by equation (1):

$$E_2 – E_1 = h\nu$$

$$\nu = \frac{c}{\lambda}$$

where h is the Planck's, constant and v the frequency of emitted light which is defined as follows equation (2):

On combining equations (1) and (2), we get equation (3)

$$E_2 - E_1 = \frac{hc}{\lambda} \quad \lambda = \frac{hc}{E_2 - E_1}$$

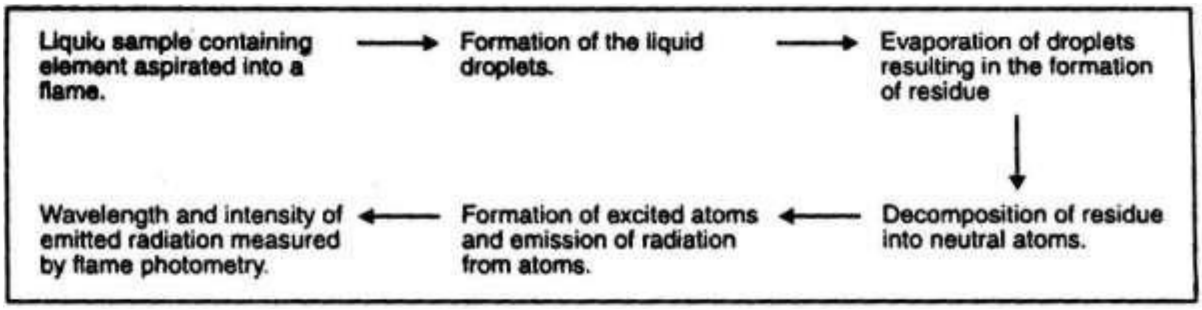
From equation (3) one can calculate the wavelength of the emitted radiation which is characteristic of the atoms of the particular element from which it was emitted.

When flame photometry is employed as an analytical tool, the wavelength of the radiation coming from a flame tells us about the elements which are present in that flame. Also, the intensity of the radiation enables us to know the amounts of those elements present.

The following is a concise description of the sequence of events that normally occurs in flame photometry.A portion of the neutral atoms or radicals formed in the flame may combine to form new gaseous compounds.

As the formation of these compounds decreases the population of neutral atoms and hence the intensity is also decreased, this constitutes the chemical interference in flame photometry.

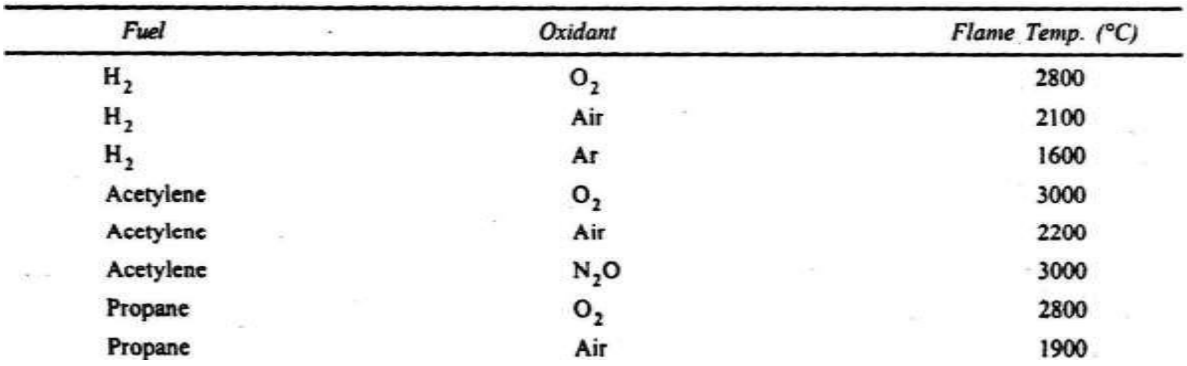
The fraction of free atoms that are thermally excited is governed by a Boltzmann distribution which is as follows:



$$\frac{N^*}{N_0} = \Delta e^{-\Delta E/kT}$$

where N° is the number of excited atoms, No the numbers of atoms remaining in the ground state, A a constant for a particular element, ΔE the difference in energies of the two levels, k the Boltzmann's constant, and T the temperature of flame.

The temperature of fuel is controlled by the type of fuel and oxidant used. Some flame temperatures are given in Table 1.



Fuel	*Oxidant*	*Flame Temp. (°C)*
H_2	O_2	2800
H_2	Air	2100
H_2	Ar	1600
Acetylene	O_2	3000
Acetylene	Air	2200
Acetylene	N_2O	3000
Propane	O_2	2800
Propane	Air	1900

The energy available in the flame for exciting atoms is dependent on the flame temperature as defined by the Boltzmann distribution. Flames have only limited amounts of energy available when compared to excitation sources such as emission spectrographs or plasma emission torches (ICP).

For this reason, flames are most useful only for elements that require low amounts of energy to become excited. They are not the method of choice for transition metals and most of the other metals in the periodic table.

These metals generally require significantly more energy in order to become excited. They are most often done by emission spectrography or plasma emission where much higher energy is available. However, it must also be stated that when we try to analyze group I and group II elements in these high energy sources, we run the risknot only of exciting them butof ionizing them. Although the Boltzmann distribution would lead us to believe that the emission intensity would be greatly increased in plasma emission for the group I and group II elements, in practice it is found not to be so because the atoms are ionized and this results in a loss of atoms and the generation of ions.

Ionization causes a complete change of energy levels and therefore the entire emission spectrum, with a reduction in the intensity of the atomic spectra.The flame is a source of intense radiation especially for atoms inside the flame. It is probable that, this is a source of excitation of the atoms (radiation excitation) which augments the thermal excitation, expected from the Boltzmann distribution.

This point has not been extensively studied but provided a catalyst for the evolution of atomic fluorescence. For example, a fuel-rich oxyacetylene flame generates intense radiation bands at short, wavelengths (300-200 nm). Iron solutions injected into these flames strongly emit iron linesat many wavelengths down to 200 nm.

On the other hand, oxygen-rich flames operating at the same temperature do not emit such broad-band radiation, and iron lines are not emitted from such flames.

The emission intensity generated by thermally excited atoms should be the same according to the Boltzmann distribution but is clearly not the same.

The advent of the nitrous oxide-acetylene flame has provided us with a flame with higher temperature than was hitherto available. This has increased the usefulness of flame photometry. Also, the use of the elongated burner as developed for atomic absorption spectroscopy has increased the sensitivity of the method.

Because flames are a low-energy source they populate the first excited energy state E1 reasonably well but the upper energy states E2 E3 etc. only poorly. Flame photometry therefore utilizes the resonance transitions E1→E0most commonly and other transitions only under special circumstances.

Instrumentation

Equipment used in flame photometry is shown in Fig.2. The instrument possesses the same basic components as a spectroscopic apparatus has. However, the basic components are source, monochromator, slit system and detector system.

The flame photometer also includes a burner which is utilised for burning the sample solution and exciting the atoms produced in the flame after burning.

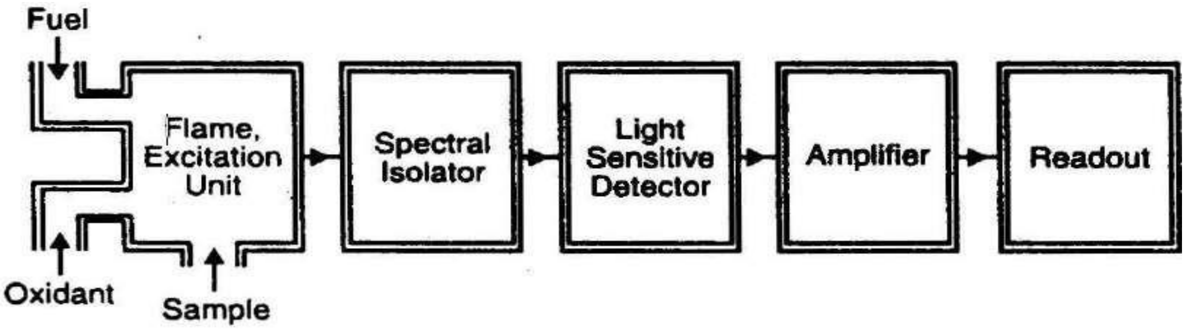


Fig. 2: Block diagram of a flame photometer.

The various components of the instrument are described as follows:

1. Burner

The flame used in the flame photometer must possess the following functions:

i. The flame should possess the ability to evaporate the liquid droplets from the sample solution, resulting in the formation of solid residue.

ii. The flame should decompose the compounds in the solid residue formed in step (i), resulting in the formation of atoms.

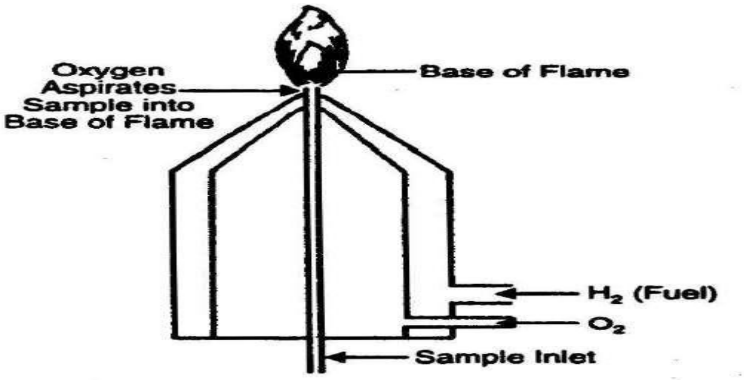
iii. The flame must have the capability to excite the atoms formed in step (ii) and cause them to emit radiant energy. For analytical purposes, it becomes essential that emission intensity should be steady over reasonable periods of time (1-2 min).

Flame temperature is controlled by several factors which are as follows:

(i) Type of fuel and oxidant and fuel-to-oxidant ratio;
(ii) Type of solvent for preparing sample solution;
(iii) Amount of solvent entering into flame;
(iv) Type of burner employed; and
(v) The particular region in flame which is to be focussed into entrance slit.

In flame photometry, several burners and fuel-oxidant combinations have been used to produce the analytical flame. Some of these are discussed as below:

(i) Meeker burner



This burner was used earlier and employed natural gas and oxygen. As this burner produced relatively low temperatures and low excitation energies, this was generally used for the study of alkali metals only.

The flame produced by Meeker burner is not homogeneous chemically. It means that there are different regions in the flame, i.e., an "oxidising" region and a "reducing" region appear in the flame.

(ii) Total consumption burner

A typical total consumption burner is shown in Fig.3. In this burner, the fuel and oxidant are hydrogen and oxygen gases respectively. In this type of burner, the liquid sample is drawn into the flame. From the side tubings, hydrogen and oxygen are entering and both are burning at the top of the burner to produce a flame. As soon as the liquid sample is drawn into the base of flame, the oxygen aspirates sample solution leaving a solid residue. Atomisation and excitation of the sample then follow.

- The name "total consumption burner" is used because all the sample that enters the capillary tube will enter the flame regardless of droplet size.

- The flame produced by total consumption burner is noisy and turbulent but can be adjusted to produce high temperatures by proper control of fuel-to-oxidant ratio.
- This burner was used for a number of years but its use has been stopped and replaced by other types of burners.
- As the processes leading to atomic excitation in the flame differ in the oxidising and reducing regions in flame, different concentrations of excited atoms are obtained in these regions
-

(iii) Premix of laminar-flow burner.

In this type of burner (Fig.4), aspirated sample, fuel and oxidant are mixed before reaching the burner opening.

An important feature of this burner is that only a small portion (about 5%) of the sample in the form of small droplets reaches the flame and is easily decomposed. By the easy decomposition in means that an efficient atomization of the sample in the flame will take place.

Larger droplets from the aspirator impinge on the side of the spray chamber and are drained off. Thus, in this burner 95% of the sample may be wasted, thereby, resulting in a loss of sensitivity. However, this loss must be balanced against the loss of larger droplets.

The flame produced by premix burner is non-turbulent, noiseless and stable. Another advantage is that in this burner easy decomposition of the sample takes place which results in an efficient atomization of the sample in the flame.

Premix burners can handle solutions up to several percent without clogging.The main disadvantage of premix burner arises when the sample contains two solvents.

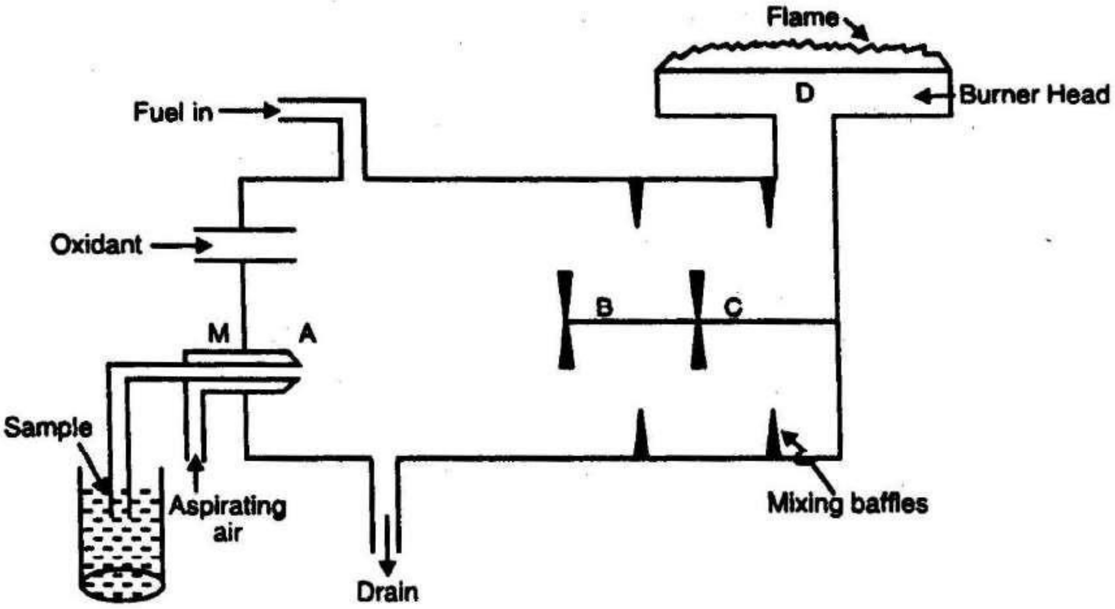


Fig. 4. Premix of laminar-flow burner

(iv) Lundegardh burner

In the Lundegardh burner, the sample must be in liquid form. It is aspirated into the spray chamber. Large droplets condense on the side and drain away; small droplets and vaporised sample are swept into the base of the flame in the form of a cloud. Various devices have been used to enhance the nebulization stage in this type of burner. These include the use of the impact bead, ultrasonic vibrators, and more recently thermospray heaters.An important feature of this burner is that only about 5% of the sample reaches the flame. The rest of the droplets condense and are drained away. This is a significant loss in atomization efficiency and, therefore, sensitivity.

Difficulties may also arise if there is any selective evaporation of the solvent in the spray chamber. In particular, if there are two solvents, the more volatile will preferentially evaporate, leaving the element of interest in the less volatile component. The latter may drain away, taking the sample with it.

Shielded Burners

T. S. West al. developed shielded burners in which flame is shielded from ambient atmosphere by a stream of inert gas. This shielding leads to better analytical sensitivity. Table 2 shows results obtained with commercial equipment based on this technique and developed by the Beckman Instrument Co.

(v) Nitrous Oxide-Acetylene Flames.

During research in atomic absorption spectroscopy, J, Willis and M. Amos independently found that nitrous oxide-acetylene flames were superior to other flames for efficiently producing free atoms. This was particularly true for metals with very reflective oxides, such as aluminium and titanium. Later, workers in the field of flame photometry found that this same flame was very useful in flame photometry. However, the high temperature reduces its usefulness for the determination of the alkali metals because they are easily ionised.

One problem encountered with this type of flame was the intense background emission, which makes measurement of the metal emission very difficult. However, the "wobbler" designed by Rains for background correction and the commercial nitrous oxide-acetylene burners developed provide equipment capable of high sensitivity and accuracy. Note the linear relationship between the emission intensity and the concentration of the metal.

Sequence of Events in a Flame

The sequence of events occurring in the flame are given below:

(i) Solvent is evaporated, leaving minute particles of salt.

(ii) Salt is vaporized or converted into gaseous state.

(iii) A part or all of the gaseous molecules are dissociated to give free neutral atoms or radicals.

(iv) A part of neutral atoms may be thermally excited or even ionized.

(v) A portion of neutral atoms in the flame may combine to form new gaseous compounds.

1. Mirrors

Radiation from flame is emitted in all directions. Much of the radiation is lost. In order to maximize the amount of radiation used in the analysis, a mirror is located behind the burner to reflect the radiation back to the entrance slit of the monochromator.

The reflecting surface of the mirror is front-faced. If the reflecting surface were on the rear as in the normal household mirror, the radiation from the flame would have to go through the support material such as glass or quartz twice before it is reflected to the entrance slit. Since the support material absorbs some radiation, there would be a considerable loss of signal, particularly at the shorter wavelengths.

Front surface mirrors are most efficient, but they are not physically protected. Care should be taken to protect them by keeping the instrument away from corrosive atmospheres.

2. Slits

With the best equipment, entrance and exit slits are used before and after the dispersion elements. The entrance slit cuts out most of the radiation from the surroundings and allows only the radiation from the flame and the mirrored reflection of the flame to enter the optical system. The exit slit is placed after the monochromator and allows only a selected wavelength range to pass through to the detector.

For many purposes it is essential that this wavelength range be very narrow, that is, of the order of a few nanometers. This is necessary if emission lines from other components in the flame have a wavelength similar to those of the emission lines of the elements being determined. The slit must prevent such interfering lines from reaching the detector.

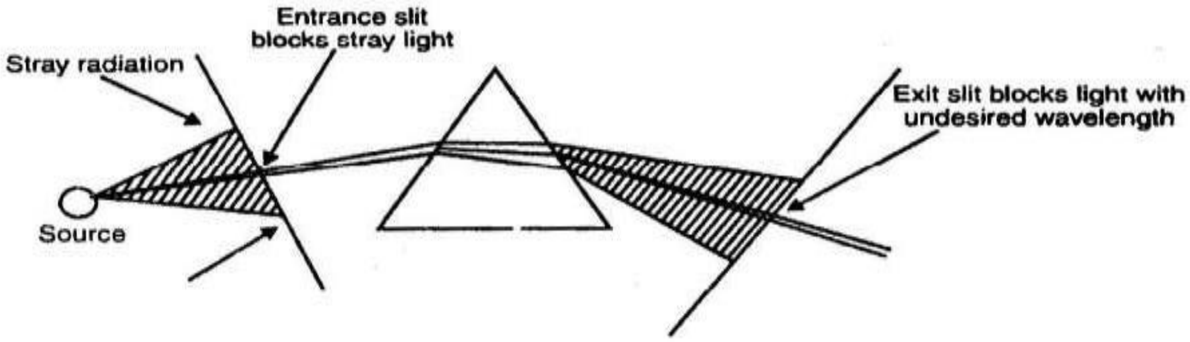


Fig.5: Monochromator system shown without collimator lenses for simplification.

3. Monochromators

In simple flame photometers, the monochromator is the prism. But in expensive models, the grating monochromators are used.

Quartz is the material most commonly used for making prisms even though its dispersive power is less than that of glass as it is transparent over the entire region.

The grating monochromator employs a grating which is a series of parallel straight lines cut into a plane surface.

4. Filters

In some elements, the emission spectrum contains a few lines. In such cases wide wavelength ranges will be allowed to enter the detector without causing any serious error. In such a situation an optical filter may be used in place of the slit and monochromator system.

The filter is made from such a material which is transparent over a narrow spectral range. When a filter is kept between the flame and detector, the radiation of the desired wavelength from the flame will be entering the detector and be measured. The remaining undesired wavelength will be absorbed by the filter and not measured.

The flame photometers which use filter monochromators are very convenient for simple repetitive analysis. However, such instruments can be used for a small number of elements. The reason for this is that a large number of filters are employed.

5. Detectors

Detector measures the intensity of radiation. It should be sensitive to radiation of all wavelengths that may be examined.

In good flame photometers, the photomultiplier detectors are employed which produce an electrical signal from the radiation falling on them.

Effect of Solvent in Flame Photometry

The solvent used in the sample affects the signal in two ways. First, viscosity controls the rate at which the sample is aspirated into the flame. There is an optimum sample flow rate, which has to be experimentally determined. If the flow rate is too great, the flame is swamped and the signal drops off; if the flow rate is too low, then the signal is decreased because insufficient sample finds its way into the flame.

The second effect of the solvent is caused by the difference between aqueous or organic materials.

If it is aqueous, then the sample requires energy to evaporate it. Generally an inorganic salt is left, which requires more energy from the flame to decompose it. These are two endothermic steps, which slows down the atomization process. On the other hand, if the solvent is organic, it burns on introduction to the flame and usually leaves an organic residue, which in turn burns inside the flame.

Each of these steps is exothermic, the atomization efficiency is increased, and there is an enhancement of signal. This is the reason for signal enhancement when organic solvents are used rather than aqueous solvents.

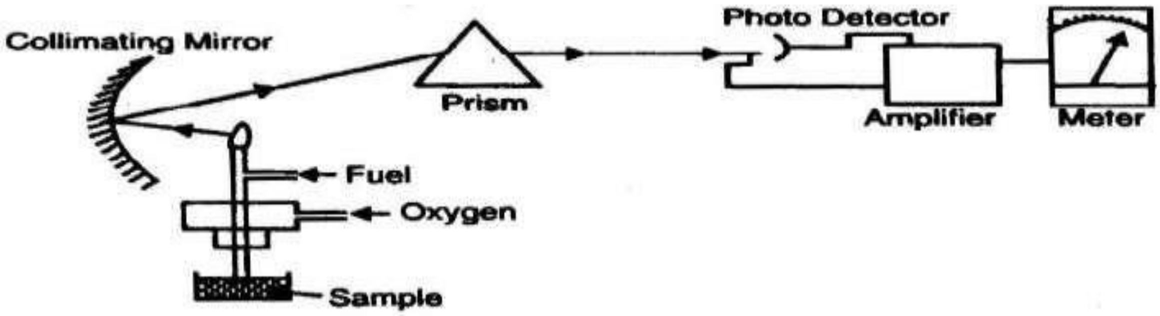
It can be seen from these comments that in order to get good quantitative data it is necessary that the sample introduction note, the solvent used, and flame conditions be the same for both the samples to be analyzed and the preparation of calibration curves.

Any variations in these conditions cause a major variation in flame intensity and therefore in the analytical data obtained.

Instruments

There are a large varieties of flame photometers in widespread use, ranging all the way from very single-beam filters photometers to multichannel spectrometers with automatic background correction.

(I) First Type. The simplified diagram of a flame photometer is depicted in Fig.6



The burner used in flame photometer shown in Fig. 6 is a total consumption type, but a premixburner can also be used.

In Fig.6, a collimating mirror is used behind the flame to increase the emission intensity.

But this mirror is often omitted when premix burners having a long flame path are used. This is sometimes, necessary to remove the backing mirror in multiple-beam instruments where a sufficient room is not available to include such mirrors.

The sample solution is sucked by an atomiser operated by one of the flame producing gases. Then the sample solution is aspirated into the flame in the form of a fine spray. Spectral emission comes from the excited atoms formed during the process of combustion in a flame.

The emitted radiation is collected by a collimating concave mirror from the flame and is then permitted to pass through a prism and slit.The radiation of the appropriately selected wavelength strikes a photo detector and the magnitude of the electrical signal developed is read out on a meter.

(ii) Second Type

Another type of flame photometer is internal standard flame photometer. In Fig.7 an internal standard flame photometer is illustrated. Lithium is used as an internal standard and an equal concentration is added to the standard and sample solutions. The sample solution containing internal standard (lithium) is sucked by an atomiser operated by one of the flame producing gases and as a fine spray is fed into the flame. The emitted radiation is collected by a mirror through a filter.

The emitted radiation from the mirror is divided into two parts. One part arises due to the internal standard (lithium) whereas the second part arises due to the presence of element to be measured. Both these parts are received by separate amplifiers and are then fed to the common detecting system which records the intensity of the element (to be determined) to that of internal (lithium). Thus, this flame photometer gives a direct and simultaneous reading of the ratio of intensities.

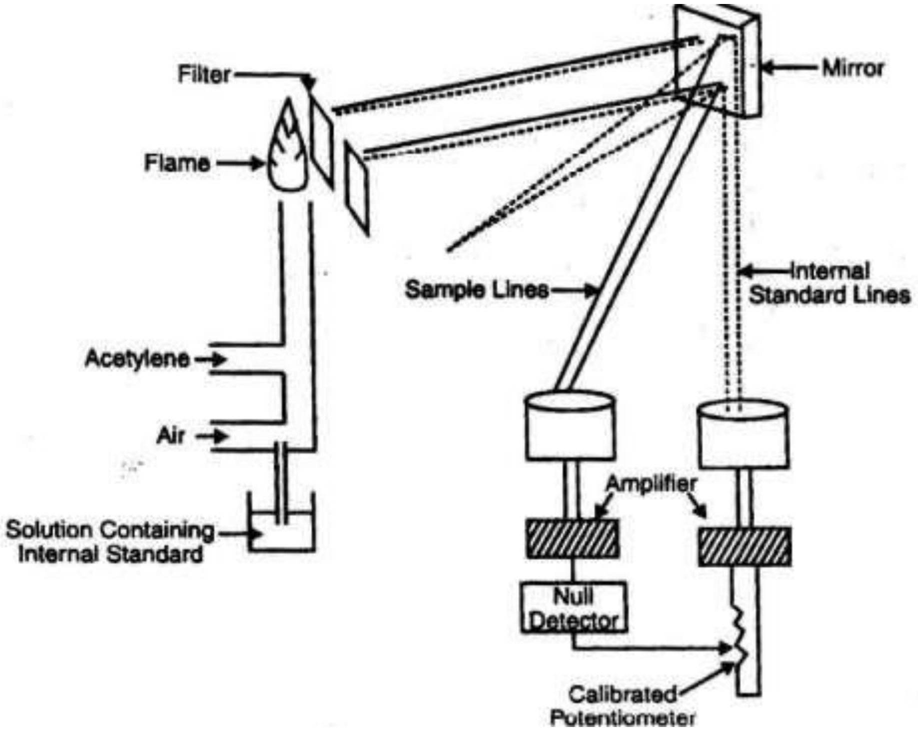


Fig: 7 : Schematic diagram of an internal standard photometer.

The use of this flame photometer removes the effects of momentary fluctuations in flame characteristics caused by fluctuations in fuel or oxidant pressures. Therefore, internal standard flame photometer is better than the first type as discussed above.

In internal standard flame photometer, the errors due to differences in viscosity and surface tension are minimised considerably. However, the effect of radiation mayor may not be reduced, depending On the similarity of the effects on the two elements.

Applications of Flame Photometry

(1) The Qualitative Analysis

Flame photometry is only used to detect elements of groups I and II. These elements arelithium, potassium,sodium, magnesium, calcium, strontium, and barium.

This method has some limitations which are as follows:

(i) This method is less reliable than atomic absorption spectroscopy.

(ii) This does not provide information about the molecular structure of compound present in the sample solution.

(iii) Non-radiating elements, such as carbon, hydrogen and halides cannot be detected by flame photometer. These can only be determined under special circumstances. For example, if chlorine is to be detected in a liquid sample, the best method is to precipitate it as silver chloride which is then aspirated into flame of a flame photometry tocarry out the determination of silver.From the result, the chloride content can be calculated.

2. Quantitative Analysis

This is one of the most useful applications of flame photometry. This is used for rapid quantitative determination of the elements in groups I and II of the periodic table.

If high optical resolution equipment is used, other metallic elements besides that of I and II groups can also be determined.

Miscellaneous Applications

Flame photometry is very helpful in analysing biological fluids and tissues.

Flame photometry has been used for determining sodium, potassium, aluminium, calcium, cobalt and iron in soil analysis. It has also been used for petroleum products, natural and industrial waters, glass, cement, and metallurgical products. Boron has been determined in various types of organic compounds.

Interferences in Flame Photometry

The radiation intensity may not accurately represent the sample concentration because of the presence of other materials in the sample. These materials result in interference in the analytical procedure. It is only through adequate control of this interference that flame photometry would provide good analytical results. Following are the more commonly encountered interference processes in flame photometry:

Spectral Interference

This type of spectral interference arises when two elements or compounds possess different spectra but their spectra may partly overlap and both are emitting at some particular wavelength.

This type of interference is more common at high flame temperatures. An interesting example is that the iron line at 3247.28 A° overlaps the copper line at 3247.54 A° and the iron line at 2852.13 A° overlaps the magnesium line at 2852.12 A°.

This type of error can be overcome by removing the effect of interfering element effect by using extraction method or using calibration curves prepared from solution having similar quantities of interfering element.

The second type of spectral interference can occur if spectral lines of two or more elements are close but their spectra do not overlap. The possibilities of such interference can be decreased to a considerable extent by enhancing resolution of spectral isolation system.

A third type of spectral interference occurs between a spectral line and a continuous background. This type of interference is encountered due to high concentration of salts in the sample. This occurs especially in salts of the alkali and alkaline earth metals. A continuous background is also produced by some organic solvents. An example is methyl isobutyl ketone which is particularly troublesome in flame photometry. This type of interference can be corrected by scanning technique.

Ionisation Interferences

$$Na \rightleftharpoons Na^{+} + e^{-}$$

In some cases, some of the metal atoms may ionise in high-temperature flame, e.g., Sodium ion possesses an emission spectrum of its own, with different frequencies from those of the atomic spectrum of sodium. Thus, ionisation decreases the radiant power of atomic emission.

The interference due to ionisation can be overcome by addition of a large quantity of a potassium salt to all of the solutions-unknown and standards.

The addition of potassium prevents the ionisation of sodium but it itself undergoes ionisation. Thus, the sodium atom emission is enhanced. This type of interference is restricted to the elements of the first group of the periodic table.

Cation-Cation Interference

Cation-cation interferences decrease signal intensity of the element present

An example of cation-cation interferences is that aluminium interferes with magnesium andcalcium. Also, potassium and sodium have cation-cation interference on one another.

Oxide Formation Interference

This type of interference occurs due to formation of stable oxides. Thus, emission intensity is lowered. All of the alkaline earth elements form oxides and are subject to this type of interference.

Cation-Anion Interference

Presence of certain anions in solution may affect intensity of radiation emitted by an element. For example, anions such as phosphate, oxalate, aluminate andsulphate may affect intensity of radiation emitted by an element. For example, calcium in presence of phosphate ion forms a stable substance, so the calcium signal is depressed because it will not decompose so easily, resulting in production of lesser atoms.

Another example is that a given concentration of barium sulphate produces low emission intensity than the same concentration of barium chloride, because barium chloride can be broken down more easily than barium sulphate.

This type of interference can be removed either by extraction of the anion or by using calibration curves which are prepared with the same predominant anion at the same concentration in the standard solution as that found in the sample.

This type of interference can be overcome by either using very high temperature flames to dissociate those oxides producing free atoms for excitation or using oxygen-deficient environment to produce excited atoms.

Factors that influence the intensity of emitted radiation in a flame photometer

Numerous factors will influence intensity of light emission from a given solution. Some of these are as follows:

(a) Viscosity. The addition of a substance which increases the viscosity of the solution decreases the intensity of light emission. This decrease results in due to a reduction in the efficiency of atomization.

(b) Presence of Acids. When an acid is present in the sample solution, this decreases the light intensity. This decrease arises due to the disturbance of the initial dissociation equilibrium.

(c) Presence of Other Metals. If other metals are present, these also alter the intensity of emitted radiation. In order to remove this defect, special filters are used which will absorb radiation due to the element which is to be estimated in the sample solution.

Determination of Non-metals

The determination of non-metals by flame photometry has two major handicaps. First, the atomic state is usually not stable. For example, chlorine does not exist as Cl but as Cl2 . Second, the resonance lines are in the vacuum ultraviolet. High energy is necessary to cause electronic excitation-more energy than is available in conventional flames. Atomic emission of non-metals has not been exploited in flame photometry until recently and even then only using research grade equipment.

Atomic Absorption Spectroscopy

Atomic absorption spectroscopy (AAS) is a technique that involves study of absorption of electromagnetic radiations in relationship to atomic structure. The principle of AAS is measurement of the concentration of elements present in the sample through their property of absorption of light. The technique is simple and reliable based on absorption of radiation by free atoms for their determination. AAS is the oldest instrumental elemental analysis principle, the origins of which go back to the work of Bunsen and Kirchhoff in the mid-19th century. AAS is based on absorption by atoms or elementary ions. The components of a sample are converted into gaseous state (gaseous atoms) or elementary ions by suitable heat treatment. The absorption is measured at a selected wavelength, characteristic to individual element and can serve for qualitativeand quantitative determination of one or more of the elements present in the sample. The process of converting sample into vapors is called atomization. The precision and the accuracy of AAS are critically dependent upon atomization step.

Atomic Structure and Spectra

To understand the concept of atomic absorption process, it is important to understand the structure of atom first. The basic processes in this spectrometry involve the outer electrons of the atom and thus its pros and cons can be well understood from the theory of atomic structure itself. According to the Niels Bohr (1913), the structure of the atom consists of central core or nucleus, made up of protons and neutrons surrounded by the electrons in orbits of differing energy. These orbits were described as energy levels which differ in energy from each other. Each orbit has a fixed energy associated with it, in general an electron has lowest energy in its ground level, and higher energy in its excited state, and can be easily removed. When the associated electron of an atom is in its ground state, the atom is said to be in the ground state. Normally, electrons try to stay in the lowest energy level open to them, but these electrons in its ground state can absorb energy in discrete amounts of heat or light at certain discrete wavelengths, corresponding to the energy requirements of the particular atom. This absorption of light, heat or collision with another particle results in the increased energy of an atom which can result in one or more changes.

1) Increase in kinetic energy of the atom or
2) become excited by absorbing energy

The electron tends to remain in its permitted energy levels but may change to another level if the amount of energy absorbed is equal to the difference between the two levels. When the electron moves to the higher energy level, such as E1, it is said to be excited. Each atom has quantized energy levels depending upon the number of protons and electrons present. Each element has a unique set of energy levels pertaining to the unique set of electrons and protons. It is these energies which are measured in relation to the ground state, and aparticular excited state above the ground state (Fig2).

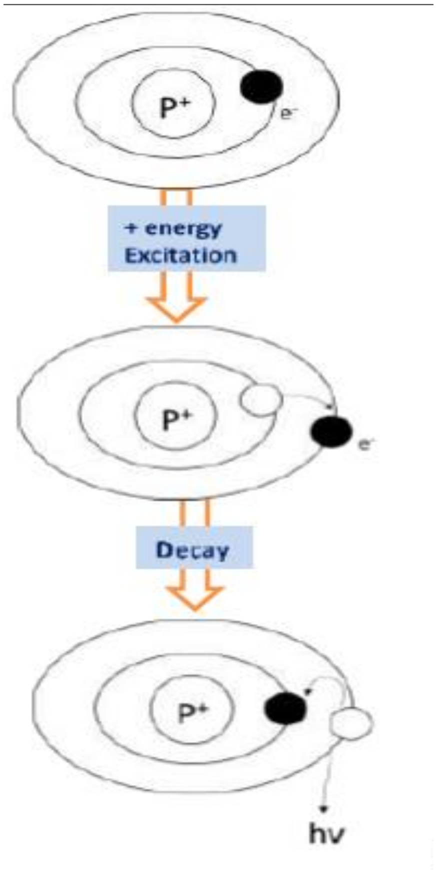


Fig:1 Representation of atomic structure

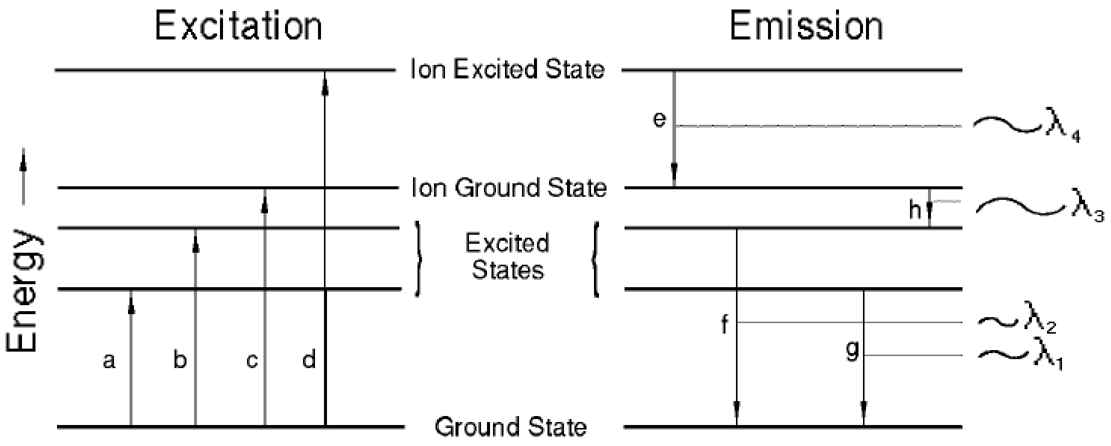
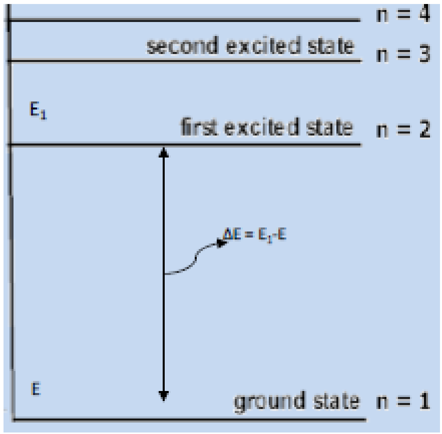


Fig 2 Energy level diagram illustrating the excitation, ionization and emission processes for an atom.

The energy levels within the atom are represented by the horizontal lines, and the vertical arrows signify energy transitions–a and b represent excitation.On the other hand every element has characteristic spacing between the energy levels that is proportional to wavelength of the absorbed light. For example the shorter wavelength of light energy will be absorbed if the spacing between the energy levels is wider

Atomic spectra

Each orbital in an atom is characterized by principle and azimuthal quantum numbers n and l, respectively corresponding to the electron's energy and angular momentum. When an electron undergoes a transition from a higher energy level (E_2) to a lower energy level (E_1), light of frequency



Energy Level Diagram

Atomic absorption spectroscopy efficaciously facilitates the estimation of particular elements in the presence of many other elements. Most of the atomic absorption instruments are capable of measuring both atomic absorption and atomic emission. For the operator it is important to understand the processes that occur in each technique. The basic components of any type or brand of atomic absorption include the following:

1. Light Source
2. Burner/Nebulizer
3. Sample Atomizers
4. Wavelength Selectors
5. Detector
6. Readout devices

Light Sources

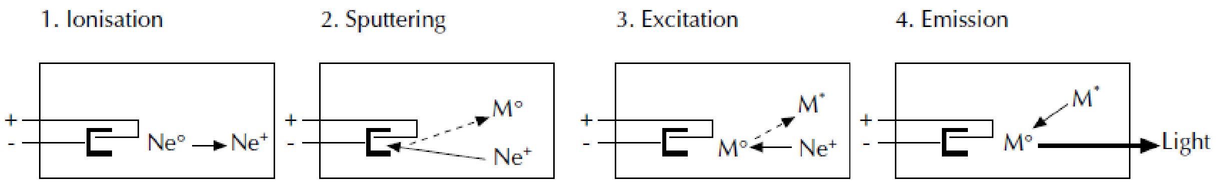
Since atoms absorb light at very specific wavelengths, it is necessary to use a narrow-line source which emits the narrow-line spectra of the element of interest. Narrow-line sources provide high intensity and make atomic absorption a specific analytical technique. The main sources used for atomic absorption are the *hollow cathode lamp* (HCL) and the *electrodeless discharge lamp* (EDL).

Hollow cathode lamp

'Hollow cathode lamp' is the common source of light in Atomic Absorption spectroscopy *(Fig. 1*). It consists of cathode which is a hollowed-out cylinder made of the element to be determined and a tungsten anode. At a pressure between 1 Nm^{-2} and 5 Nm^{-2} the glass tube is filled with an inert gas – *eg*neon or argon in which cathode and anode are sealed. For appropriate transmittance of the emitted radiation the glass cylinder has a quartz or UV glass window.

The best lamp intensity is achieved by the optimum gas fill while the spectral interferences from either neon or argon are also considered. The potential difference of about 300–400 V between the anode and the cathode are applied for the ionisation of some gas atoms which bombard the cathode and eject metal atoms from it in a process called sputtering. Some sputtered atoms are in excited states and release radiation of characteristic atom as they fall back to the ground level – *eg*$\mathrm{Pb}^* \rightarrow \mathrm{Pb} + h$ (*Fig. 2*).

Fig 1:Hollow cathode lamp



The radiation is concentrated into a beam with the shape of the cathode which then passes through a quartz window and the shape of the lamp allows the redeposition of the sputtered atoms on the cathode. A typical atomic absorption instrument holds several lamps each for a different element. There are two types of HCP

Single-Element Lamps:

In this type of lamp the hollow cathode is generally made from avery pure metal resulting in a very pure emission spectrum. The cathode of a hollow cathode lamp is generally constructed from a very pure metal resulting in a very pure emission spectrum.

Multielement Lamp:

The hollow cathode is generally constructed from a mixture or alloy of several metals. The resulting "multielement" lamp can be used as a source for all the metals contained in thecathode.

Electrodeless Discharge Lamps(EDLs)

The hollow cathode lamp is a completely satisfactory source for most of the elements in atomic absorption. However in certain cases it has some limitations, primarily in case of the more volatile elements, where low intensity and short lamp life are a problem. In such cases electrodeless discharge lamp are better alternative with brighter and more stable source. (Fig 3)

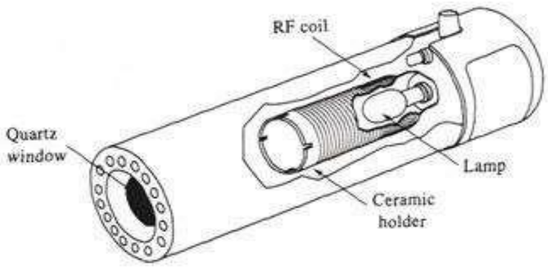


Fig: 3 Electrodeless Discharge Lamps

An EDL is constructed of a quartz bulb filled with an inert gas containing the element or a salt of the element for which the lamp is to be used. The antenna is coiled around a ceramic cylinder in which bulb is placed for RF generation. The inert gas present inside the bulb gets ionized when an RF field is applied and the vaporized atoms inside the bulb gets excited with the coupled energy which causes emission of characteristic light. The main advantage of EDL's is better precision and lower detection limits

In comparison to hollow cathode lamp the useful life of an EDL is considerably longer for thesame element.

NEBULIZERS:

The general term nebulizer refers to an apparatus that converts liquids into a fine mist. Nebulization is a process in which the sample is converted into a fine mist of finely divided droplets by using jet of compressed gas. The flow of the gas carries the sample into theatomization region.

Types of nebulizers: Most common types of nebulizers used are Pneumatic nebulizers (mostcommon) andultrasonic nebulizer

Pneumatic nebulizers.

Pneumatic nebulizers are further divided into three types:

Concentric tube.This is the most common type ofPneumatic nebulizer used. In this type of Pneumatic nebulizers, the liquid sample is drawn up by the pressure drop generated as the nebulizer gas passes through the orifice. This is also called “free running” or “self aspiration” Due to high velocity the sample breaks down into a mist and is carried to the atomization region.

Advantage- – Generally, the ion signal produced is much more stable. However this type of nebulizers have som limitations. They are as it cannot handle the sample with high total dissolved salts (TDS - 0.25% m/v solids); i.e. 250 mg sample dissolved in 100 g of solution.

Cross-flow In this case, the flow of jet stream is at right angles to the capillary tip. Sometimes the sample is pumped through the capillary.

Fritted disk: the gas jet is flowed through the fritted disk in which the sample is pumped. With this the aerosols obtained are finer than the others.

Ultrasonic Nebulizer

The sample is fed to the surface of a vibrating piezoelectric transducer operated at a frequency of between 0.2 and 10 MHz. The mist obtained is more homogeneous and denser than pneumatic nebulizers.The production of aerosols is also very efficient and independent of gas flow rate unlike pneumatic nebulizers.

The efficiency and detection limits of ultrasonic nebulizer are better than the pneumatic nebulizers. However long wash-out times and lots of glassware required, bad memory effectsand cost are some of the limitations for its use.

Sample Atomizer

In atomic absorption spectroscopy it is a requirement to convert the sample to gaseous state, which absorb radiation. The sample is mostly introduced as a solution and the solution is taken to the nebulizer through a small tube. In nebulizer the gaseous sample is broken up into a fine mist which is then carried to the atomizer, such as a flame, by a carrier gas. Upon reaching the flame the intense heat of flame breaks up the sample into its individual atoms. This complete process is called atomization.

There are two main types of atomizers:

Discrete atomizers: The most common example of discrete atomizer is electrothermal atomizer. In this type of atomizers the sample is introduced in a discontinuous manner with asyringe or auto sampler.

Electrothermal Atomization

A hollow graphite tube with a platform is shown in fig 4.25 µl of sample is placed onto the platform through the sample hole with an automated micropipette and sample changer. By passing electric current the tube is heated in a pre-programmed series of steps.

The conditions may vary with the sample but typically to evaporate the solvent it might be 30– 40 seconds at 150 °C, to drive off any volatile organic material 30 seconds at 600 °C and to vaporize and atomize elements with a very fast heating rate (*ca* 1500 °C s-1) to 2000– 2500 °C for 5–10 seconds. The tube is further heated to *ca* 2700 °C to clean it for next sample.

During this heating cycle the graphite tube is prevented from burning by flushing it with argon gas during this heating cycle. The sample is atomized almost 100% in electrothermal atomization. This makes the technique much more sensitive than flame AAS

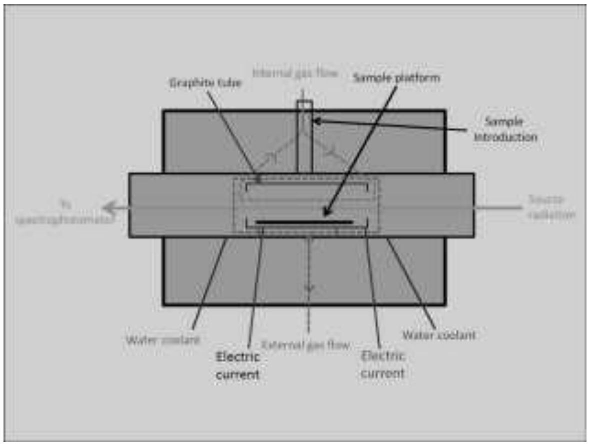


Fig: 4. Electrothermal atomizers (EA). Adopted from blogs/Maryville.com

Electrothermal atomizers (EA) have high sensitivity and have been used for quantitative determinations based on signal peak area and height. It has advantage of using smaller sample size. The spectral interferences in EA are low because of the high temperature of the graphite furnace.

However, EAhas limited analytical range and slow measurement time which limits its use. Additionally, the graphite tube needs replacing with time when the analyte and matrix diffuse into it, increasing the maintenance and cost associated with electrothermal atomization.

Continuous. The most common example of Continuous atomizer is Flame atomizer. In this type of atomizers the sample is introduced in a continuous manner.

Flame atomizer.

The process of atomization takes place in a flame after nebulization of the sample. Once the sample reaches the flame the process of atomization involves three more steps desolvation, volatilization, and dissociation.

- Desolvation is a process in which the solvent is evaporated to produce molecular aerosol.
- Volatilization is in which gaseous molecules are produced from the aerosol and
- Dissociation is in which atomic gas is formed by the dissociation of molecules. In this process of ionization of atomic gases some cations and electrons are also formed.

Fuels and Oxidants used in Flame Atomizer.

To produce flames of interest most common fuels and oxidants used in AAS are listed in the table 1. To achieve specific temperature range a mixture of different oxidants and fuels can be used.

Table 1.List of common fuels and oxidants used to produce flames

Oxidant	**Fuel**	**Temperature ˚C**	**Max Burning velocity (cm/s)**
Oxygen	Natural Gas	2700-2800	370-390
Oxygen	Hydrogen	2550-2700	900-1400
Oxygen	Acetylene	3050-3150	1100-2480
Air	Natural Gas	1700-1900	39-43
Air	Hydrogen	2000-2400	300-440
Air	Acetylene	2100-2400	158-266
Nitrous Oxide	Acetylene	2600-2800	285

.

Flame Structure

The different regions of flame have different temperature and in fuel to oxidant ratio. The three important regions of flame are primary combustion zone,interzonal region and secondary combustion zone (Fig 5).The mostly widely part of the flame used for spectroscopic analysis is interzonal region, the second region, because it is the hottest region of the flame and is prevalent in free atoms.

The flame usually rises about 5 cm above the burner tip with max temperature point at 2.5 cm. The portion of the flame used for AAS is specific as to what element is being analyzed. For different elements the maximum absorbance is achieved at different distances (cm) above the burner due to the formation of oxides.

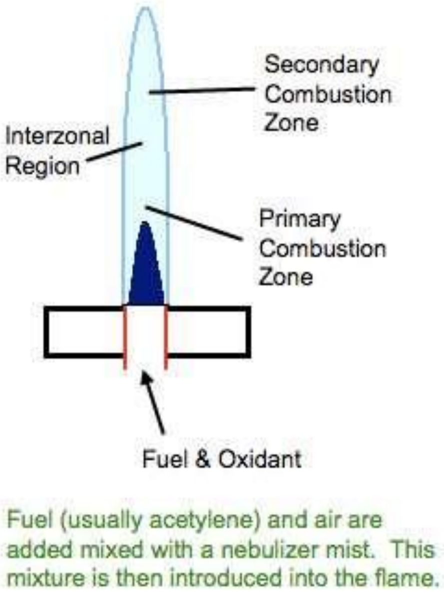


Fig.5: Structureof Flame Adopted from blogs/Maryville.com

Performance

For all the liquid- sample introductions Flame atomic atomization is the most reproducible, however it has many limitations.

- Sensitivity of flame atomization is lower than electrothermal atomization
- The absorbance of samples are reduced due to formation of oxides
- Fluctuations of flame affect the absorbance of samples.

By using variety of means the sample is converted to the vapor of atoms that is to be analyzed by the AAS. In addition to the methods previously discussed other techniques that can be very useful for AAS, are hydride atomization, glow-discharge atomization, and cold-vapor atomization.

Wavelength Selectors

To limit the radiations absorbed by a sample wavelength selectors are used to restrict the radiations to a certain wavelength or a narrow band.This helps in improving the sensitivity of an AAS while as the high transmission improves the detectability.

Number of wavelength selectors are available. However it wont be possible to discuss all of them in this chapter we will limit our discussion to filters and monochromators only Filters

Filters transmits selectable *narrow* band of *wavelengths* of light or other *radiation*. Four categories of filters are known: absorption filters, cut-off filters, interference filters, and interference wedges.

Absorption Filters:

Absorption Filters absorb most of the polychromatic radiation and allow transmission of only a specific band of wavelengths. They only transmits about 10-20% of the incident radiation. Since they can be made from colored glasses or plastics they are economical and simple. An absorption filter transmits only about 10-20% of the incident radiation (Fig 6).

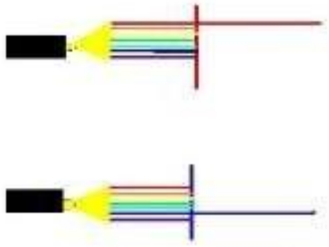


Fig: 6.*Absorption filters*

Cut-off Filters: *These type of filters transmit most of the* radiation (nearly 100%) .It transmits wavelengths of specific band which rapidly decreases to zero over the remainder of the spectrum (Fig 7).These types of filters are not usually used as wavelength selectors but to reduce the bandwidth of the absorption filter they are used in combination with them.

The wavelengths transmitted will be the common one between the two filters which will result in narrower bandwidth than absorption filters alone (Fig 8).

Interference Filters

The interference filters also called Fabry-Perot filters are dependent upon the concept of wave interference. Interference filters work on the principle of transmittance of some wavelengths of radiation while reflect others.

The *interference filter* are made ofa film of dielectric material which is transparent sandwiched between two semi-transparent metal films and protected by glass plates (Fig 9). Thedielectric material thickness and metallic films should be selected carefully as the dielectric material and the reflectivity of the metallic films controls the transmittance of wavelengths. The radiation transmitted through interference filters therefore, has a very narrow bandwidth.

Interference Wedges

A wide range of wavelengths can be transmitted by wedge dielectric of different thicknesses without changing the interference filters in an instrument (Fig 10).The wavelengths with a bandwidth of about 20 nm can b isolated by choosing correct position of the wedge.

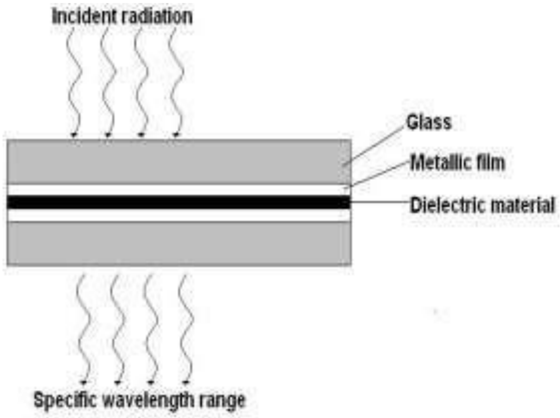


Fig: 9. *Interference filters*

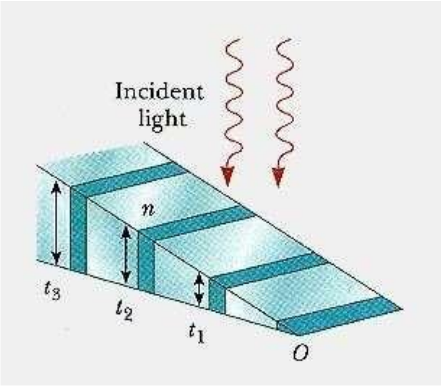


Fig: 10. *Interference Wedge*

Monochromators :

The two basic types of monochromators are Grating Monochromators and Prism Monochromators

Grating Monochromators

Grating monochromators are placed within compartments of some AAS instruments which are capable of producing narrow bands of radiation

There are five componentsin most grating monochromators viz. an entrance slit, a collimating lens or mirror, a reflection grating, a focusing element, and an exit slit (Fig 11).

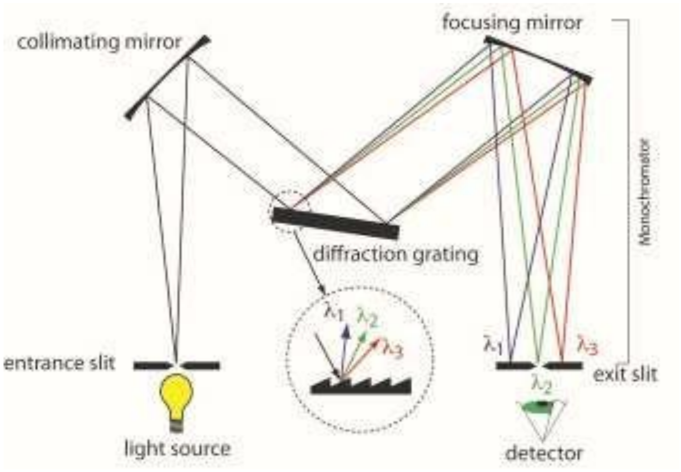


Fig: 11. Grating Monochromator .

In spectroscopic instruments the reflection gratings usually used are of two types echelette and echelle gratings

Echelette Gratings:

The most common type of gratings used in spectroscopic instruments is echelette gratings which may contain 300-2000 grooves/mm. The most commonechelette gratings used in AAS has on an average, density of 1200-1400 grooves/mm.These gratings uses the long face of the groove for the linear dispersion of radiation (Fig 12).

Echelle Gratings

For the dispersion of radiation short face of the grooves is used in Echelle gratings. They contain less number of grooves per millimeter approximately 80-300 grooves/mm but these are known for their very high dispersion (Fig 13).

Prism Monochromators

Prisms create angular dispersion by refracting the light at the surface of two interfaces and can be used to disperse all the three types of radiations viz ultraviolet, visible, and infrared radiation (Fig 14) .

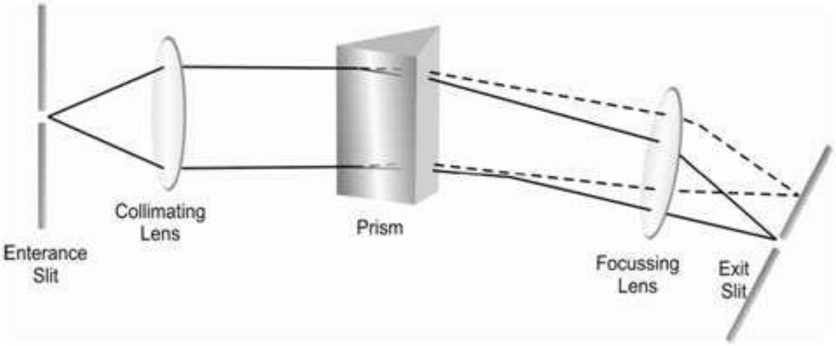


Fig:14. Prism Monochromators

Despite low dispersion a prism has an advantage of having wide spectrums. However it has limitation for focusing a desired wavelength through the exit slit as the method used by prisms is non-linear dispersion.

The wavelength and dispersion are inversely proportional, where increased dispersion is caused by shorter wavelengths. The figure below depicts the nonlinear dispersion of a prism.

Detectors

Detectors are of several kinds iePhotodiode, PN Junction, Photodiode Array and Photomultiplier Tube

Photodiode

A photodiode is a kind of light detector that converts light into voltage or current, which is generated from the absorption of photons. Photodiodes consist of a reverse- biased pn junction which converts the light falling on the junction, to a proportional current

PN Junction

The pn junction consists of two types of semi-conductor material, p-type and n-type. Both types contain silicon crystal which is commonly used in semiconductor.

In a silicon crystal, each silicon atom is bonded to its neighboring atom by four electrons, forming covalent bonds.

The p-type material is differentiated from the n-type material by alteration in the silicon crystal by a process called doping. During this process, the impurities are added to the intrinsic semiconductor silicon crystal. In case of p-type material , boron atoms are added as a doping agent which have only three valence electrons. This results in the formations of “electron holes” in the silicon lattice of the p-type material. In case of n-type material phosphorus atoms are doped which have five valence electrons, an extra valence electron which results in the formation of an extra electron free from the covalent bonds in the silicon lattice. The free mobile electrons of the n-type material diffuse in the electron holes of the p- type material, making the atom positively charged with newly filled holes. The mobile electron holes also diffuse to the electrons of the n-type material, making the atom negatively charged.

This formation of negatively and positively charged ions creates a region absent of mobile charge carriers known as the depletion region. Further diffusion of charge carriers is prevented by the potential difference, when it reaches at its peak in the depletion region. This results in the reduction of conductance nearly to zero in this region. When the radiation is applied across the diode in this region, itswept through the device to produce a current that is proportional to the radiant power.

Photodiodes can easily be used in portable and battery powered instruments as they require very low voltage. They are less sensitive than photomultiplier tubes, and have a spectral range from about 190nm to 1100nm.

Photodiode Array

Photodiode Array (PDA) consists of individual photosensitive elements called photodiodes. It has a linear array of discrete photodiodes placed on an large integrated circuit (IC) of a silicon chip than a single photodiode. PDAs are one-dimensional transducers in which the photosensitive elements are arranged linearly on the transducer face

Photomultiplier Tube

Among all the present photosensitive devices used, photomultipier tube (PMT) is a versatile device. It has extremely high sensitivity and is used for the measurement of low radiant power. The schematic of a PMT is similar to that of a traditional phototube. The traditional phototube consists of two electrodes, a cathode and an anode. When the voltage is applied to the electrodes, the electronsare generated at the cathode andmoves towards the anode. This flow of electrons generates photocurrent in the anode which is measured. The diagram of a traditional phototube is shown in the fig 15.

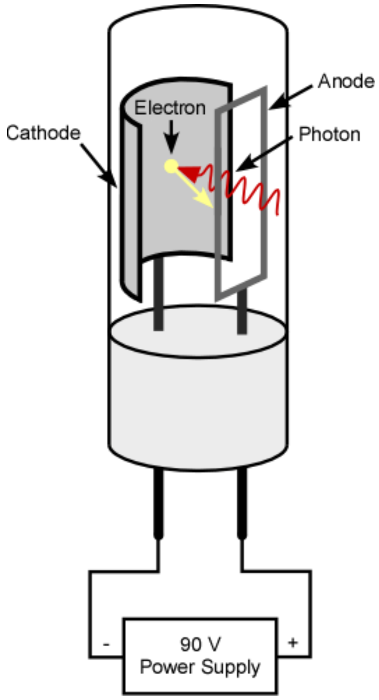


Fig: 15.Traditional Phototube

PMT contains series of electrodes called dynodes, each dynode is given slightly more positive potential than that of the neighboring nearer to the photocathode. The anode is kept more photopositive than any electrode. When the photon impinges the cathode, the electrons are accelerated towards the dynodes because of the increasing positive charge. The electrons gather at the anode where they are collected in the form of a current. This current is then converted to a voltage and measured. However, these tubes are limited to measure low power radiation because if the source of radiation is intense it can cause irreversible damage to the photoelectric surface. PMTs are stored in a light-tight compartment to avoid damage by harsh radiation. However the major sensitivity limitation's associated with the PMTs isthe noise resulted from the thermal dark currents. These dark currents usually result from thermal emission which can be reduced by cooling the transducer to ~-30 degrees Celsius. A coolant can be circulated around the PMT to achieve this. With the proper set-up and care, PMTs can be used to detect individual photons at the cathode.

Readout Devices

The system requires a type of display that can process the information that the instrument is sending to enable to read the signal,. This is achieved through an electronic component that displays the information in a format the researcher can use effectively. The analog based data collected by the instrument needs to be converted into a digital format for the display, which is accomplished by a transducer. The transducer sends the digital energy to the processor, which allows for the processing of discrete times, frequencies, and domains of the signal. This signal is put into a sequence of numbers or symbols that can be displayed on a readout.

Several types of readout devices are used in modern instruments. These devices include Digital Meters, Recorders, Cathode-Ray Tubes, LCD panels, and Computer Displays.

Nepheloturbidimetry:

Nephelometry and turbidimetry, in analytical chemistry, methods for determining the amount of cloudiness, or turbidity, in a solution based upon measurement of the effect of this turbidity upon the transmission and scattering of light. Turbidity in a liquid is caused by the presence of finely divided suspended particles.

If a beam of light is passed through a turbid sample, its intensity is reduced by scattering, and the quantity of light scattered is dependent upon the concentration and size distribution of the particles.

In nephelometry the intensity of the scattered light is measured, while, in turbidimetry, the intensity of light transmitted through thesample is measured.

Nephelometric and turbidimetric measurements are used in the determination of suspended material in natural waters and in processing streams.

Scattering: The redirection of radiation out of the original direction of propagation, usually due to interaction with molecules and particles.

- Reflection
- Refraction
- Diffraction

CHOICE BETWEEN BOTH THE TECHNIQUES

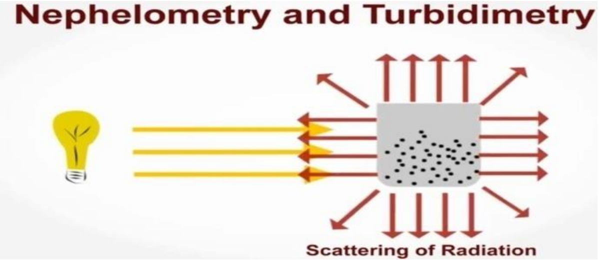
Choice between nephelo or turbidimetric methods depends upon following two parameters

Scattered radiation intensity vs incidence radiation intensity: In case medium to be analyzed contains high concentration of particulate matter turbidimetry is choice over nephelometry while just opposite is true for very dilute solutions containing fewer particulate matter, leading to least scattering hence nephelometry is choice over turbidimetry.

Size of particulate matters: Nephelometry is choice in case where particles suspended in medium are of smaller dimension since they scattered light at right angle to incidence beam,this is the reason why nephelometric measurements are done usually at an angle of 90-degree to collimated beam while in case of particle of larger dimension, scattering of light in this case to such a extent is somewhat difficult, hence turbidimetry is preferred and measurementsfor same are done at an angle of 180-degrees.

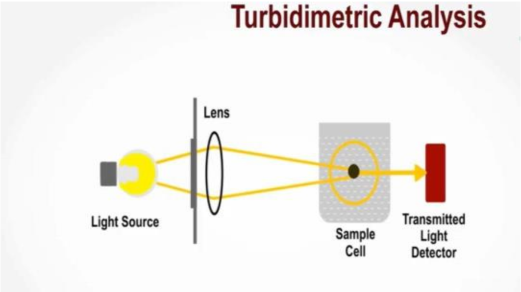
Light Scattering is a physical character and depends on followingparameters:

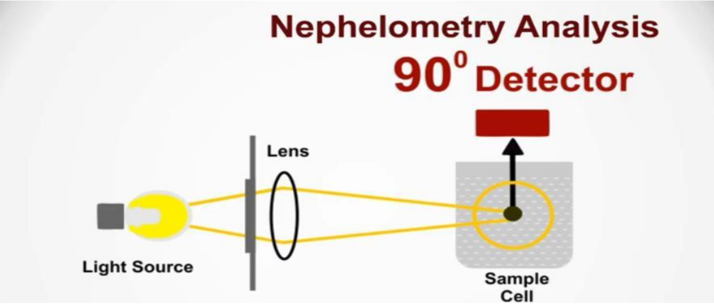
- Particle size
- Distance of observation
- Concentration of solution
- Molecular weight of particles
- Agitation of solution containing particulate matter
- Temperature of solution
- Viscosity of medium
- Presence and absence of protective colloids
- Number of particulate matters suspended
- Dimension of particulate matter
- Wavelength of radiation beam
- Refractive index of medium



Scattering, Reflection and Refraction of Radiation

Measurement of Intensity of transmitted light is a function of concentration of suspended particles.

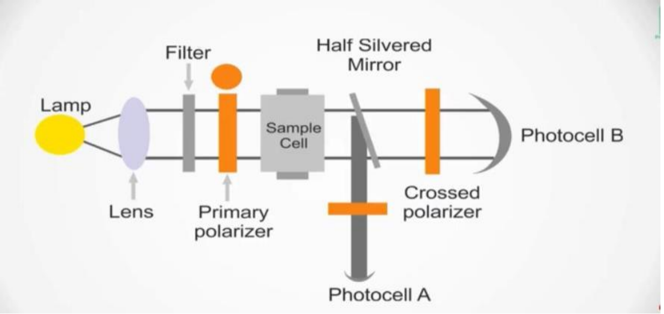




Measurement of Intensity of scattered light as a function of concentration of dispersed phase. Source and detector as a rightangle to each other.

Tubidimeters:

Ordinary calorimeter or spectrophotometer can be used. Any photometer (visual or photoelectric) can be a Turbidimeter.



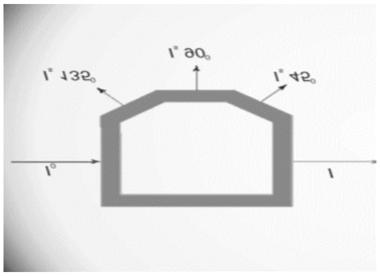
Source: Mercury lamp or Laser Light, Tungustun lamp

Filters: Converts polychromatic light to mono chromatic light. 1) absorption filters 2)

Interference filters

Sample Cells:

In general cells with rectangle cross section is preferred where measurements can be made at angles more than 90^0



Detector:

- Photovoltaic cells and Phototubes are suitable forTurbidimetric analysis.
- While sensitive photomultiplier tubes are suitable forNephelometric analysis

Sensitivity of methods:

- Addition of water-soluble polymers
- Gives stability to immune complex
- Reduced reaction concentration End point

Detection:

Nephelometry is a modification of photo-optical end-point detection in which 90-degree or forward-angle light scatter, rather than OD, is measured. A light-emitting diode produces incident light at approximately 600 nm, and a photodetector detects variations in light scatter at 90 degrees (side scatter) and 180 degrees (forward- angle scatter).

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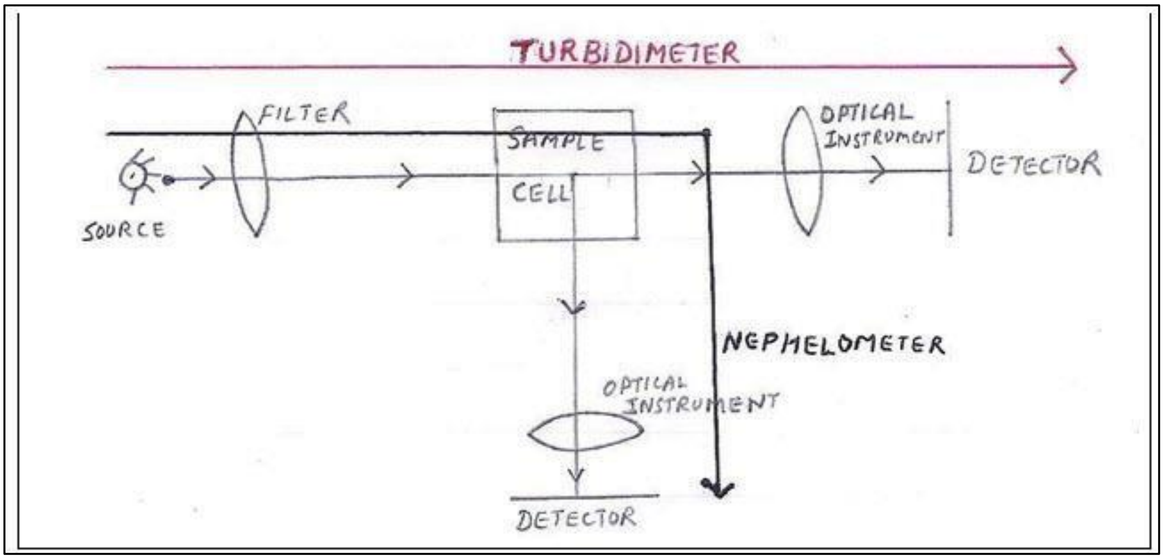

Figure: Components of Turbidimeter (straight line) & Nephelometer (line bent atright angle)

Any photometer (visual or photoelectric) can be a turbidimeter without modification. However for greater sensitivity, a blue filter can additionally be employed in the instrumental layout design. The same instrument as describes above can also be used nephelometer however in this case the instruments should have suitable provision for adjusting source and detector at a right angle to each other.

Radiation source: Generally an ideal source for nephelo-turbidimetric measurement must be sufficient intense and monochromatic so that photometric error if any can be substantially be minimized. Mercury arc lamp or Laser light are some of the commonest radiation sources used in nephelo-turbidimetric analysis however later is advantageous owing to its capability of emitting monochromatic beam compared to tungsten lamp, which exceptionally emits polychromatic light beam

Filters: Filter aid-up in selecting particular wavelength of light to be worked upon. Generally as per the feasibility of analysis colorimeter with blue filter (530nm) is a turbidimeter while fluorimeter with a visible secondary filter is nephelometer.

Sample cell: Cuvette or sample cells are used to hold the sample under interest and are generally made up of transparent glasses; geometrically may either be cylindrical or rectangular in shape with a path length of 1-cm. Sometime special cells measuring light scattering at different angles 45, 90, 135-degree also employed in nephelo-turbidimetric analysis.

Detectors: On the basis of nature of study to be done, the position of detector with respect to source has to be selected for example in case of turbidimetric analysis the detector is placed inline arrangement (180-degree) with respect to source of radiation however in case of nephelometry the detector is adjusted at an angle 90-degree to source of light. In general photovoltaic cells and phototubes are suitable for turbidimetric analysis while sensitive photomultiplier tubes are ideal for nephelometric determination.

Differentiation:

	Turbidimetry	**Nephelometry**
Definition	Light passes through a medium with dispersed particles, so the intensity of light transmitted is measured	Measurement of intensity of scattered light at right angle to the direction of the incident light as a function of the concentration of the dispersed phase. It is most sensitive for dilute suspensions(100mg/L)
Instrument used	Spectrophotometer	Nephelometry machine
Type of light measured	Transmitted light	Scattered light
Arrangement of photometer	Made in the same direction as the propagation of light source.	Measure the light scattered at right angle to the direction of propagation of the light from the source.

Applications:

- The technique is also used for determination of sulphur in coal, oil, and other organic materials; the sulphur is precipitated as barium sulphate
- The main uses of nephelometers relate to air quality measurement for pollution monitoring, climate monitoring, and visibility. Airborne particles are commonly either biologicalcontaminants, particulate contaminants, gaseouscontaminants, or dust.

- Biological contaminants include mold, fungus, bacteria, viruses, animal dander, dust mites, pollen, human skin cells, cockroach parts, or anything alive or living at one time. They are the biggest enemy of indoor air quality specialists because they are contaminants that cause health problems. Levels of biological contamination depend on humidity and temperature that supports the livelihood of micro-organisms. The presence of pets, plants, rodents, and insects will raise the level ofbiological contamination.
- Sheath air is clean filtered air that surrounds the aerosol stream to prevent particulates from circulating or depositing within the optic chamber. Sheath air prevents contamination caused by build-up and deposits, improves response time by containing the sample, and improves maintenance by keeping the optic chamber clean. The nephelometer creates the sheath air by passing air through a zero filter before beginning the sample.
- Nephelometers are also used in global warming studies, specifically measuring the global radiation balance. Three wavelength nephelometers fitted with a backscatter shutter can determine the amount of solar radiation that is reflected back into space by dust and particulate matter. This reflected light influences the amount of radiation reaching the earth's lower atmosphere and warming the planet.
- Nephelometers are also used for measurement of visibility with simple one-wavelength nephelometers used throughout the world by many EPAs. Nephelometers, through the measurement of light scattering, can determine visibility in distance through the application of a conversion factor called Koschmieder's formula.
- In medicine, nephelometry is used to measure immune function.
- Gas-phase nephelometers are also used in the detection of smoke and other particles of combustion. In such use, the apparatus is referred to as an aspirated smoke detector.
- Nephelometry has been applied to the quantitative determination of various protein and other antigens in blood serum, urine or cerebrospinal fluid such as lipoproteins, immunoglobulins, complement factors, rheumatoid factors and immune complexes.
- Measurement of light scattering has also been used for the determination of cell size
- Determination of benzene percentage in alcohol

- Amount of amino acids, vitamins and antibiotics
- Determination of proteins
- Air and water pollution
- Turbidity titration
- Determination of Molecular weight
- Determination of particle size present in suspensions.
- Determination of average molecular weight of polymer in solution.
- Measurement of atmospheric pollutants.
- Determining concentration of solute in solution.
- Growth of bacterial cell in a liquid nutrient medium.
- Turbidimetry and nephelometry has numerous applications in water treatment plants, sewage work, steam generating plant, beverage bottling industry, in pulp and paper manufacturing, petroleum refining and pharmaceutical industries.
- Determination of carbon dioxide, sugar products and clarity of citric acid juice.
- Determining end point of precipitation titration.

Long answer type Questions (10 Marks)

1. Describe the principle and applications of Turbidimetry?
2. What are Nephelometry and turbidometry? Write principle involved for the same.
3. Provide a detail account of instrumentation of flame photometry particularly with respect to atomizer and detector. Draw a diagram to illustrate your answer. Give applications of flame photometry in pharmaceutical analysis.
4. What are the various instruments used in IR spectroscopy? Why a double beam spectrophotometer is preferred? Draw a schematic diagram of such instrument.Explain its working describing functioning of individual part.
5. Explain the construction and working of golay cell and bolometer detectors used in IR Spectroscopy.

Short answer type questions (5Marks)

1. Explain principle of infrared spectroscopy. What information can be obtained from IR spectroscopy?
2. What is the affect of hydrogen bonding in IR.
3. Explain the basic principle of infrared spectroscopy. Explain different types of stretching and bending vibrations.
4. Give principle and instrumentation of flame photometer.
5. Give the principle, working and applications of flame photometer.
6. Explain the working and principle of flame photometer by drawing its block diagram. Give quantitative pharmaceutical applications of flame photometry.
7. Give principle and applications of Atomic absorption spectroscopy.
8. Short note on Atomic absorption spectroscopy.
9. What are different theories involved in Atomic absorption spectroscopy.

Very Short answer type questions (2 Marks)

1. What are various wavelength ranges in IR spectroscopy?
2. Define vibrational frequencies?
3. Write down the significance of finger print region in IR.
4. Why symmetric stretch of CO_2 molecule is inactive in IR.
5. What is mull method in IR?
6. Explain wagging in IR.
7. Name two detector used in IR.

8. Which property is important for making a compound Infrared active?

9. Name two IR tansparent compounds.

10. What are overtone bands?

11. What is the effect of H-bonding on IR spectra?

12. How will you differentiate N-H and O-H bands in IR spectrum?

13. Define Fermi resonance.

14. Is flame photometry emission spectroscopy? Comment.

15. Outline basic principle of flame photometry.

16. Define Atomization.

17. What is triplet electronic state?

18. What do you understand by Nephlometry?

19. What are Photomultiplier tubes?

20. Name two light sources.