GENERAL CONSIDERATIONS OF ANALYTICAL DETERMINATION

1.1 Analytical reagents:

Chemical reagents are supplied in different grades and each grade has a distinct purpose and range of uses. The purest grade is analytical reagent (AR), the second is laboratory reagent (LR), the third is guaranteed reagent (GR) and fourth is technical grade Second and third form of reagents, have a lot of impurities of micronutrients Therefore, for the determination of micronutrients, AR grade reagent should be used.

1.2 Concentrated acids and bases:

Strength of acids and bases are expressed on the basis of their Normality. The strength of some concentrated acids and bases are presented in Table.

Table: Strength of concentrated acids and basis.

Reagent	Concentration		Approximate
	Normality	Per cent by weight	specific gravity
HCl	11.6	37-38	1.19
H_2SO_4	35-36	97-100	1.84
HOAC	17.5	99.5	1.13
HNO_3	16	70-71	1.42
$HClO_4$	9-11.6	60-70	1.51-1.67
H_3PO_4	45	85	1.17
NH_4OH	15	28-29	0.90
HOH	55	100	1.00

1.3 Distilled water:

Distilled water is always used in chemical analysis. The quality of distilled water varies from single distilled to double or triple depending upon the requirement of analytical technique(s) e.g. double distilled water (glass distilled) is always recommended for micronutrient and heavy metal analysis.

1.4 Preparation of cleaning solution:

1.4.1 Chromic acid cleaning solution:

Chromic- sulphuric acid cleaning solution is very important for final cleaning of glassware. Visible materials and organic solvents should be removed with water before using cleaning solution. The cleaning solution can be prepared either by dissolving 80 g of $K_2Cr_2O_7$

or $Na_2Cr_2O_7$ in about 300 ml of water (with heating) in a 2000 ml of corning (Borosil) beaker. To the beaker, add one litre of technical/commercial grade H_2S0_4. Considerable red chromic oxide (Cr_2O_3) precipitates, or a cleaning solution that does not involve Cr_2O_3 crystallization is made by dissolving 5 g of $K_2Cr_2O_7$, in a minimum of water and add one litre of technical/ commercial grade H_2SO_4 to this solution.

1.4.2 Aqua regia:

Another cleaning solution used is aqua regia. The Aqua regia is prepared by mixing concentrated HCl and HNO_3 in a ratio of 3:1.

1.4.3 Sieve openings v/s meshes per inch:

The size of sieve openings in mm approximately based on the assumption that opening is 0.63 of the mesh interval, hence

$$\text{mm per opening} = \frac{16}{\text{Meshes per inch}}$$

For example, a “100-mesh" screen has openings of 0.16 mm.

1.5 Preparation of standard solutions/expression of solution concentration:

1.5.1 Molar solution:

A one molar solution contains oné mole or one gram molecular weight of a chemical substance in one litre of the solution. This expression is written as:

Molarity = No of moles per litre

$$\text{No. of moles} = \frac{\text{Wt. in g}}{\text{Mol. Wt}}$$

1.5.2 Molal solution:

The molality of a solution is the number of moles per 1000 g of the solvent. Molal solutions are prepared by dissolving gram mole(s) of chemical substance in 1000 g of the solvent.

Molality = No. of moles per 1000 g of the solvent.

1.5.3 Normal solution:

The chemical reactions occur on chemical equivalent basis. Thus, one gram equivalent weight of chemical compound is equivalent to one gram equivalent weight of the other. When one gram equivalent weight is dissolved in a solvent and volume made 1000 ml, the solution is referred to as Normal Solution and concept is called Normality (N).

$$\text{Normality} = \frac{\text{Mol. wt.}}{\text{Valency}}$$ (Neutral salts)

1.5.4 Milliequivalent weight:

When equivalent weight is expressed in milligrams i.e.

$$\text{Milliequivalent weight} = \frac{\text{Equivalent weight}}{1000} \times 1000$$

Number of milliequivalents = Normality x 1000

$$\text{Milliequivalent (meq./litre)} = \frac{\text{ppm (parts per million)}}{\text{Eq. wt. (Equivalent weight)}}$$

ppm = meq/litre X Eq wt

.

$$\text{ppm to \%} = \frac{\text{ppm}}{10000}$$

1.6 Standardization of solutions:

The exact standardization of solution is done with primary standard solution of approximate strength. Most commonly used primary standard solutions are:

1. **Acids:** - Potassium hydrogen phthalate
   - Benzoic acid
   - Constant boiling Hydrochloric acid
   - Sulphamic acid
   - Potassium acid iodate
2. **Bases:** - Sodium carbonate
   - Mercuric oxide
   - Borax
3. **Oxidising agents:**
   - Potassium dichromate
   - Potassium bromate

- Potassium iodate

4. **Reducing agents**:
   - Sodium Oxalate
   - Arsenious oxide
   - Iron metal
   - Potassium ferrocynide
5. **Others:**
   - Sodium chloride
   - Potassium chloride

Preparation of standard solutions:

Standard solutions of acids

The standard acid solutions can be prepared by using the equation given below:

$V_1N_1 = V_2N_2$

Where, V_1 = Volume of concentrated acid required

N_1 = Normality of the concentrated acid

V_2 = Total volume of the acid to be prepared

N_2 = Normality of the acid to be prepared

Standard solutions of bases

Example: Preparation of 1N 1000 ml NaOH solution

Calculations:

1N NaOH solutions contains 1g equivalent weight of NaOH dissolved in one litre Water.

Mol. wt. of NaOH = 23+16+1= 40 g

or

$$\text{Eq, wt. of NaOH} = \frac{\text{Mol. wt}}{\text{valency}} = \frac{40}{1} = 40 \text{ g}$$

Dissolve 40 g of NaOH in distilled water and make volume to 1000 ml. The Strength of this solution is 1N.

1.8 Indicators:

A substance that indicates the end point on completion of the reaction is called an indicator. The most commonly used indicators in volumetric analysis are: Internal indicators, external indicators and self-indicators.

1.8.1 Internal indicators:

Indicator added into the solution where reaction occurs is called internal indicator. It gives a visible colour change. Indicators such as methyl red, methyl orange, phenolphthalein and diphenylamine that are added to the solution where reaction occurs are called internal indicators.

1.8.2 External indicators:

Some indicators are used outside the titration mixture. Potassium ferricyanide is used as an external indicator in the titration of potassium dichromate and ferrous sulphate in acid medium.

1.8.3 Self indicator:

When one of reacting substance itself acts as an indicator is called a self indicator. A standard solution of $KMnO_4$ acts as a self indicator.

1.9 Titration:

Titration is a process of determining the volume of a substance required to just complete the reaction with a known amount of other substance. The solution of known strength used in the titration is called the titrant. The substance to be determined in the solution is called the titrate.

1.10 Buffer solutions:

Solutions containing either a weak acid or a weak base possess the characteristic property of resisting changes in pH when some acid or base is added to them. Such solutions are called buffer solutions.

1.11 Percentage solution:

It is the solution formed when one gram of solute is dissolved in 100 ml of solvent.

Source : Extracted and modified from Jackson (1967) and Chopra and Kanwar (1991).

SOIL SAMPLING AND SAMPLE PREPARATIONS

2.1 Soil Sampling

2.1.1 Collection of soil samples:

The soil sample collected should be representative of the area sampled. A field can treated as a single sampling unit if it is uniform in all respects. Variation in slope, texture, colour, crops growth and management levels should be taken into account for soil sampling Separate sets of composite samples need to be collected from areas differing in these characteristics. Recently fertilized plots, bunds, channels, marshy tracts, area near trees, farm ways, buildings, wells, compost piles or other non-representative locations must be avoided during sampling. When crops are grown in rows, samples can be taken in between the rows. Soil samples should be taken in zig zag pattern or randomly.

2.1.2 Sampling tools:

Soil sampling can be done with the help of following tools:

I. Tube auger
II. Screw type auger
III. Post-hole auger
IV. Spade /Khurpi
V. Batal/Tagari

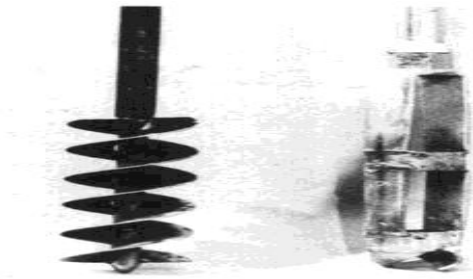
For sampling soft and moist soil, a tube auger, spade or khurpi is quite satisfactory. A screw type auger is more convenient on hard or dry soil, while the post-hole auger is useful for sampling in excessively wet areas viz rice fields. Tube Auger is convenient for sampling from lower depths. If a spade or khurpi is used, a V- shaped cut may be first made up to the plough layer and a uniform 2 cm thick slice is taken out.

Stainless steel khurpi

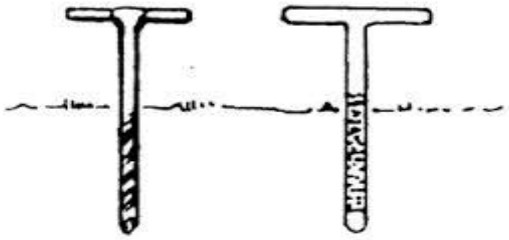
Iron khurpi

Spade/Fawada

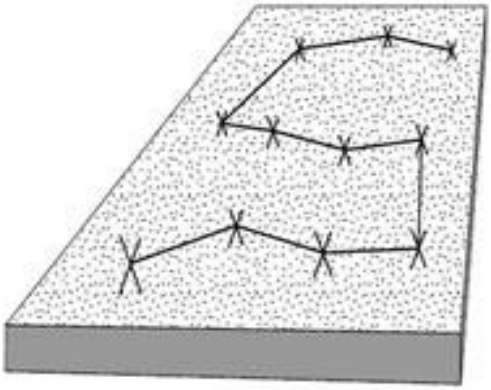
Tagari/Batal

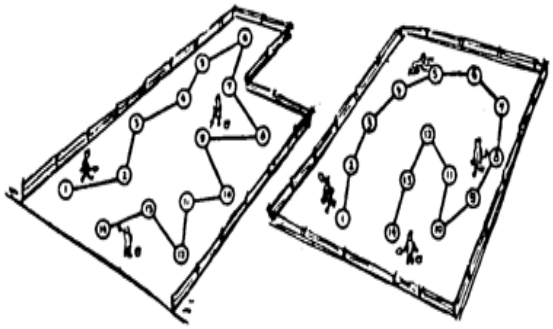


Screw type auger Post-hole auger



Tube auger





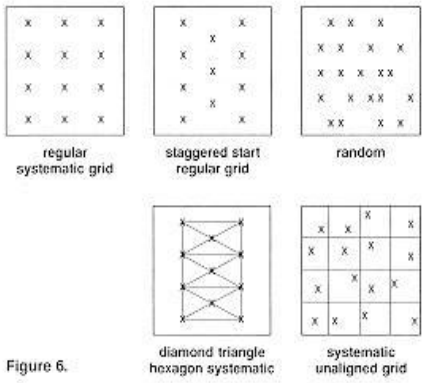
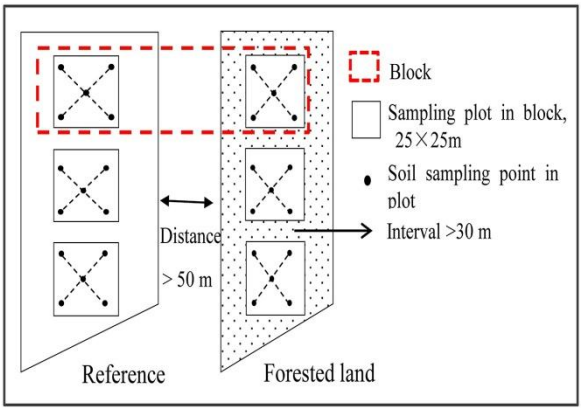


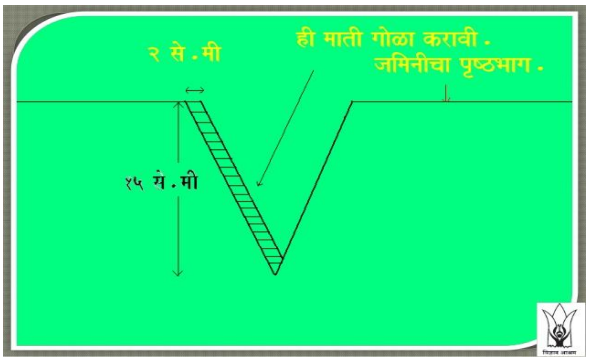
Figure 6.

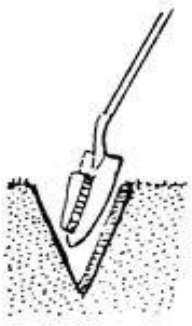


2.1.3 Depth of sampling:

The plant roots penetration is very important for deciding the sampling depth. The following parameters may be kept in consideration.

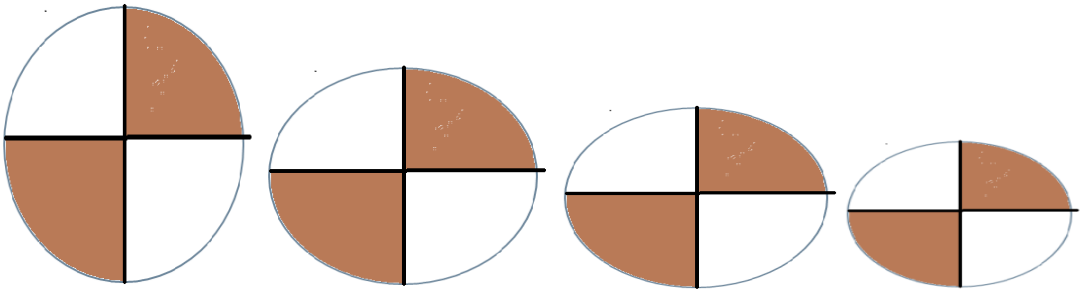
1. For field crops (cereals, vegetables and others seasonal crops) a sampling depth of 0-15 cm (plough layer) is desired.
2. For horticultural crops, sampling is done at depths of 0-15, 15-30, 30-60, 60-90, 90-120, 120-150 and 150-180 cm.
3. In saline-alkali soils, salt crust (visible or suspected) should be sampled separately and sampling depth be recorded.
4. Sampling should be done every year if the field is under intensive cultivation. If one crop per year is grown, sampling once in three years is sufficient. Soil sampling should be done at the same time in each year.





2.1.4 Preparation of composite sample:

For making composite sample, mix the collected soil samples thoroughly by hand on a clean thick paper or cloth or polythene sheet. Reduce the bulk sample to about 500 g by quartering process in which entire soil mass is spread, divided into four quarters, two opposite samples are discarded and the remaining two are remixed. Repeat this process until about 500 g soil is left.



2.1.5 Labelling of samples:

For identification, label the soil samples. A label of thick paper with identification mark along with the details of the sample should be put inside the sample bag and another label carrying same details tied outside the bag. In addition to location, field number, name of cultivator and relevant information about slope, drainage, previous cropping history, irrigation, fertilizer, manure used etc. must be recorded.

2.1.6 Processing of soil samples for analysis:

Processing of soil samples involves several procedures in sequence as follows:

- Drying
- Grinding
- Sieving

Drying-

The soil samples should be dried in shade at room temperature.

Grinding-

Crush the soil clods lightly and grind with the help of wooden pestle and mortar. Care is taken so that primary sand and gravel particles are not crushed.

Sieving-

Sieve the entire quantity of soil through 2 mm stainless steel sieve. Remove plant residues, gravel and organic material as much as possible by retaining them on the sieve. For specific type of analysis (e.g. Organic Carbon) grind the soil further and pass it through the 0 2 to 0.5 mm sieves. Remix the whole quantity of sieved soil before a sample is weighted for analysis.

Precautions:

For sampling of micronutrient analysis, always use auger made up of stainless steel instead of rusted iron khurpi or spade.

EXERCISE-3

BASIC PRINCIPLES OF THE INSTRUMENTS USED IN SOIL, PLANT AND WATER ANALYSIS

Most of the analytical methods used for soil and plant analysis involve some kind of instrument. Therefore, it is very important to know about the basic principles of the instruments in order to get the accurate results. The most commonly used instruments in soil, plant and water analysis are given below:

1. pH meter
2. Conductivity meter
3. Colorimeter/Spectrophotometer
4. Flame photometer
5. Atomic Absorption Spectrophotometer (AAS)
6. Chromatography
7. Mass Spectroscopy
8. X-Ray Power Diffraction (XRD)
9. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

3.1 pH meter

pH meter is used for the measurement of the reaction of a solution. pH determination with the help of a pH meter is based on the measurement of the electromotive force (e.m.f) of a ph cell consisting of a glass electrode, sensitive to H^+ ions and a reference electrode. The pH cell can be represented as given below:

$[H^+]$ sensitive electrode	Reference buffer or Test solution	Salt bridge	Reference electrode

The potential developed across in glass electrode on account of the difference in activity of H^+ ions in and out of the electrode. This electrical potential, E, is defined by the Nernst Equation:

$$E = \frac{RT}{nF} \quad \text{Log}_e \frac{P}{Q}$$

Where, R = Gas constant (8.313 Joules)

T = Absolute temperature (298^0 K or 25^0C)

N = Valency of the metal ion (1 for H^+)

F = Faraday Constant (96,500 coloumbs)

P = Pressure of the electrode

Q = Osmotic pressure

Or $E = \frac{RT}{nF}$ 2.303 log $[\frac{1}{H+}]$

By putting the value of R, T, n and F, we get

$$E = 0.058 \log [\frac{1}{H+}]$$

$$\text{Or} \quad E = 0.058 \text{ pH}$$

$$pH = \frac{E}{0.058}$$

3.2 Conductivity meter (Ec-Meter)

In soil and water, salinity is characterized by the total amount of the dissolved inorganic salts. It is estimated by measuring the electrical conductivity. The electrical conductivity is a measure of the ability of a salt solution to carry electric current by the migration of ions under the influence of an electric field, as ions are the carriers of electricity. Like metallic conductors, solutions also obey Ohm's law. Increase in temperature promotes dissociation of the salts with simultaneous increase in conductivity at the rate of approximately 2% of each degree of Celsius rise in temperature. The unit of specific conductance is the reciprocal of specific resistance in Ohms cm^{-1}, i.e. mhos cm^{-1}. It Cs is the concentration of a solution in gram equivalent L^{-1}, then the volume of solution ml per equivalent is 1000/Cs, so the conductance will be 1000 K/Cs, where K is cell constant. At infinite dilution, the ions are theoretically independent of each other and each other and each ion has its contribution to the total conductance.

Thus,

$$\lambda = (\lambda^+) + (\lambda^-)$$

where, λ is the total conductance.

λ^+ is the conductance of cations.

λ^- is the conductance of anion at infinite dilution.

3.3 Colorimeter/Spectrophotometer

Colorimeter/Spectrophotometer is used for the determination of the concentration of a substance in a solution by measuring the relative absorption or transmission of light w.r.t. a known concentration standard. Colorimetric analysis is based on the measurement of intensity of radiant energy after it passes through a sample solution. Spectrophotometer is

generally controlled by basic principles or laws of light. Spectrophotometer is based on the law of Lambert, Beer and Bouger. When a beam of monochromatic light falls on a homogeneous layer of a substance, part of the radiation is reflected, part is absorbed, and part is transmitted. The substance can be in a solid, liquid or gas form. The incoming light is generally called the incident light. The relationship between the intensity of light (I_o) and the intensity of reflected light (I_r), absorbed light (I_a), and transimited light(I_t) can be expressed as follows:

$$I_o = I_r + I_a + I_t$$

When comparing the intensity of the beams transmitted through the solution and through the solvent, the effect of I_r is neglected in practice, and the relationship is changed into:

$$I_o = I_a + I_t$$

Law of Lambert and Bouger

This law indicates that transmission and absorption of light is function of thickness of the medium:

$$I_t = I_a\, a^{-1}$$

Where, a = Factor related to the fraction of incident light transmitted by a layer of 1 cm thickness. This factor is often called the transmission co- efficient. Rearranging the above equation, we get

$$I_t/I_o = a^{-1} = T$$

T is called transmittance.

By rearranging again and taking the log, the equation above changes into:

$$I_o/I_t = 1/T$$

$$\text{Log}\,(I_o/I_t) = \log(1/T) = D$$

Where, D is the optical density (O. D.) or absorbance (A).

Beer's Law

This law indicates that transmission and absorption of light by a medium is a function of concentration:

$$I_t = I_o\, a^{-c}$$

Where, a transmission co- efficient, and c = concentration

Law of Lambert and Beer

The combination of law of Lambert and Bouger and the Law of Beer gives the law of Lambert and Beer's law the basis of absorption spectrophotometry:

$$I_t/I_o = a^{-IC}$$

or in log form:

$\log (l_t/I_o) = \log a^{-IC}$

$\log (l_t/I_o) = (-I_c) \log a$ or

$\log (l_t/I_o) = (I_c) \log a$

Log a is also called "extinction coefficient" and is assigned the symbol. The term "molar extinction" or molar absorption is used when the concentration is expressed in moles L^{-1}. By using instead of log a, the above formula can be changed into the fundamental equation of spectrometry:

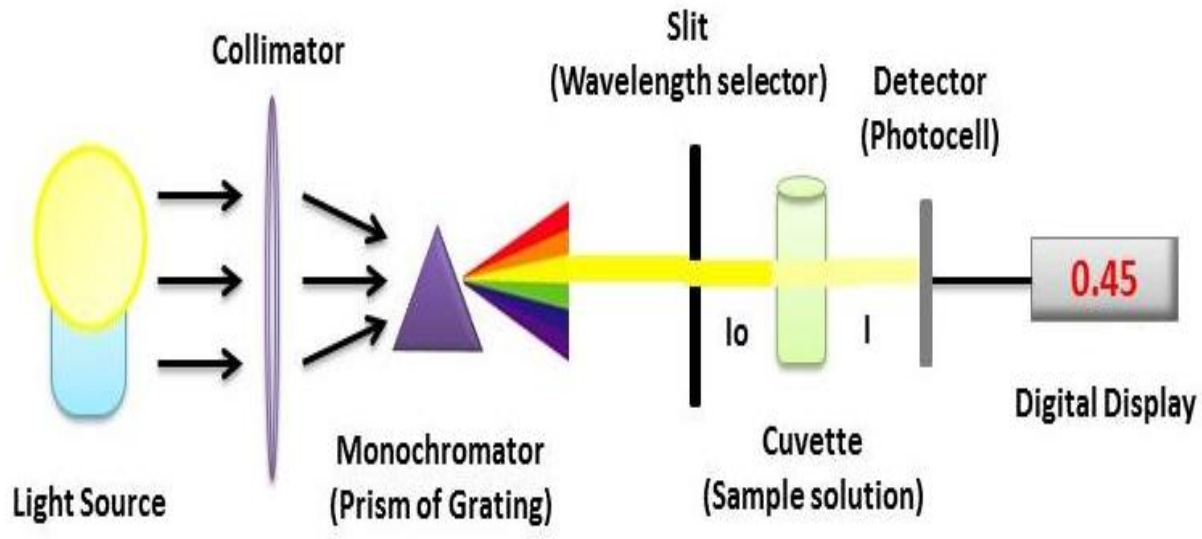
$\log (l_t/I_o) = I_c$

Which indicates that the optical density or absorbance is proportional to concentration (C), and thickness of sample (I). If a cuvette or sample holder of 1 cm thickness is used, then I=1, and $\log (I_O/I_t) = C$, in other words, absorbance is directly proportional to concentration.

According to Lambert and Beer' s law, a straight line is obtained if optical density or absorbance is plotted against concentration on an ordinary graph paper. However, if transmittance readings are plotted against concentration on a semi log paper a curvilinear regression line is obtained, because $T = (I_t/I_o)$ indicating that transmittance is not directly proportional to concentration as in the case of optical density.

Principle:-

The Spectrophotometer is a much more refined version of a colorimeter. In a colorimeter, filters are used which allow a broad range of wavelengths to pass through, whereas in the spectrophotometer a prism (or) grating is used to split the incident beam into different wavelengths. By suitable mechanisms, waves of specific wavelengths can be manipulated to fall on the test solution. The range of the wavelengths of the incident light can be as low as 1 to 2nm. The spectrophotometer is useful for measuring the absorption spectrum of a compound, that is, the absorption of light by a solution at each wavelength. This is the basic Principle of spectrophotometer.



Basic Instrumentation of a Spectrophotometer

Component of spectrophotometer:-

- Radiant Energy Sources
- Wavelength selectors
- Sample Containers
- Detection Devices
- Amplification and Readout

1. Radiant Energy Sources:

Materials which can be excited to high energy states by a high voltage electric discharge (or) by electrical heating serve as excellent radiant energy sources.

Sources of Ultraviolet radiation:

Most commonly used sources of UV radiation are the hydrogen lamp and the deuterium lamp. Xenon lamp may also be used for UV radiation, but the radiation produced is not as stable as the hydrogen lamp.

Sources of Visible radiation:

"Tungsten filament" lamp is the most commonly used source for visible radiation. It is inexpensive and emails continuous radiation in the range between 350 and 2500nm. "Carbon arc" which provides more intense visible radiation is used in a small number of commercially available instruments.

Sources of IR radiation: "

Nernst Glower" and "Global" are the most satisfactory sources of IR radiation. Global is more stable than the nearest flower.

2. Wavelength selectors:-

Wavelength selectors are of two types.

(a) Filters

(b) Monochromators

Filters:

"Gelatin" filters are made of a layer of gelatin, colored with organic dyes and sealed between glass plates.

Monochromators:

A monochromator resolves polychromatic radiation into its individual wavelengths and isolates these wavelengths into very narrow bands. The essential components of a monochromator are.

3. Sample Containers:

Sample containers are also one of the parts of Spectrophotometer instrumentation. Samples to be studied in the ultraviolet (or) visible region are usually glasses (or) solutions and are put in cells known as "**CUVETTES".** Cuvettes meant for the visible region are made up of either ordinary glass (or) sometimes Quartz. Most of the spectrophotometric studies are made in solutions, the solvents assume prime importance.

4. Detection Devices:

Most detectors depend on the photoelectric effect. The current is then proportional to the light intensity and therefore a measure of it. Important requirements for a detector include:-

- High sensitivity to allow the detection of low levels of radiant energy
- Short response time
- Long term stability
- An electric signal which easily amplified for typical readout apparatus.

5. Amplification and Readout:

- Radiation detectors generate electronic signals which are proportional to the transmitter light. These signals need to be translated into a form that is easy to interpret. This is accomplished by using amplifiers, Ammeters, Potentiometers and Potentiometric recorders.
- The above 5 major parts are the major part of Spectrophotometer instrumentation

4. Flame photometer

In flame photometry, a flame is used for (1) converting the sample from the liquid or solid state into the gas phase, (2) decomposing the sample into atoms, and/or (3) exciting these atoms into light emission.

Principle:- Flame photometer is based on the principle that when a solution of a salt is sprayed into a flame, the salt breaks into the component atoms due to high temperature. The atoms released of some specific element take energy from flame and get excited to the higher orbit. Such atoms release energy of a wavelength, which is specific for the element and is proportional to the concentration of atoms of that element. However, since intensity of emitted light is dependent on temperature, the sensitivity and reproducibility of the analysis vary also with temperature.

Element	Emitted wavelength	Flame color
Sodium	589 nm	Yellow
Potassium	766 nm	Violet
Barium	554 nm	Lime green
Calcium	622 nm	Orange
Lithium	670 nm	Red

Parts of flame photometer:-

A simple flame photometer consists of the following basic components:

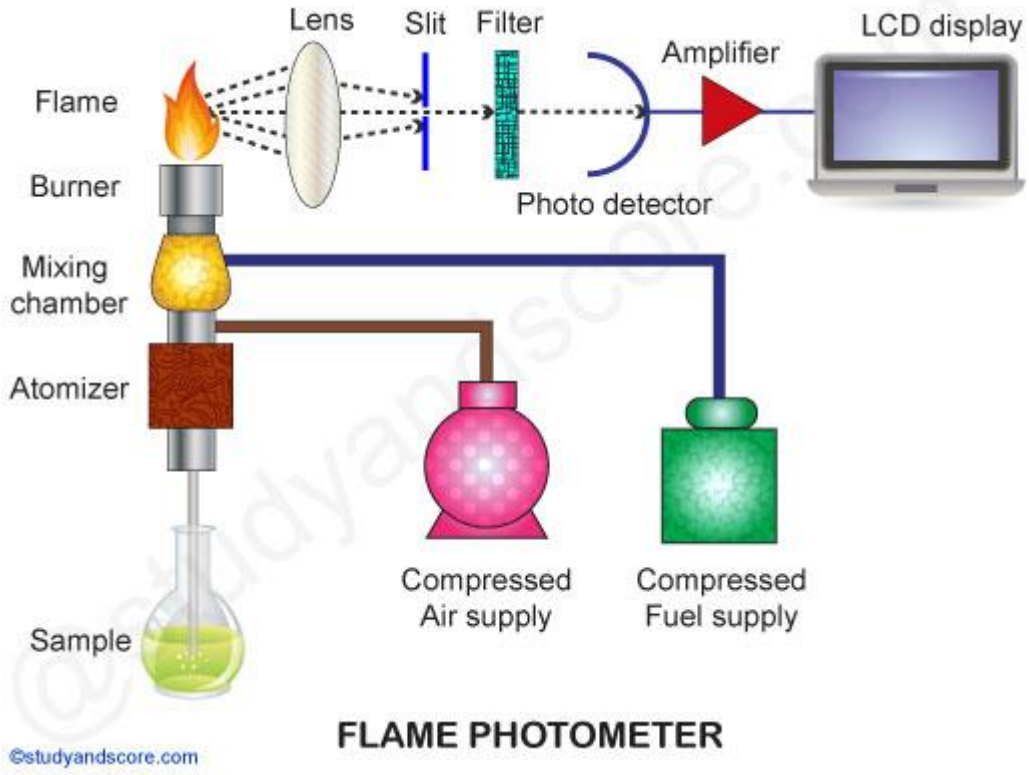
Source of flame: A Burner in the flame photometer is the source of flame. It can be maintained in at a constant temperature. The temperature of the flame is one of the critical factors in flame photometry.

Nebuliser: Nebuliser is used to send homogeneous solution into the flame at a balanced rate.

Optical system: The optical system consists of convex mirror and convex lens. The convex mirror transmits the light emitted from the atoms. Convex mirror also helps to focus the emissions to the lens. The lens helps to focus the light on a point or slit.

Simple colour filters: The reflections from the mirror pass through the slit and reach the filters. Filters will isolate the wavelength to be measured from that of irrelevant emissions.

Photo-detector: The intensity of radiation emitted by the flame is measured by photo detector. Here the emitted radiation is converted to an electrical signal with the help of photo detector. These electrical signals are directly proportional to the intensity of light.



FLAME PHOTOMETER

5. Atomic Absorption Spectrophotometer (AAS)

Principles:-

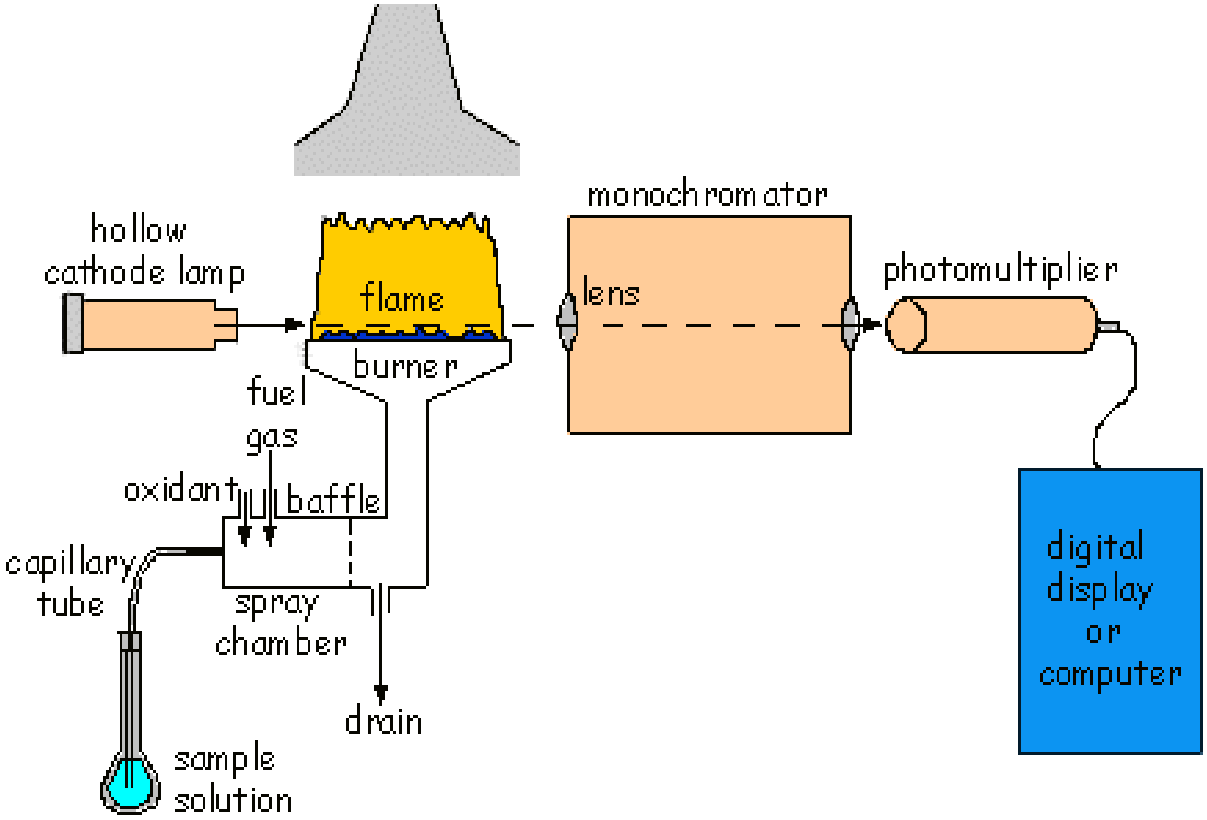
The technique makes use of absorption spectroscopy to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lambert Law.

In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using the Beer Lambert Law.

AAS:- Atomic absorption spectroscopy is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state.

AAS basic components:

- Light sources
- Flame atomizers
- Monochromator
- Detector



Light sources – Hollow Cathode Lamps

The light source commonly used is a hollow cathode lamp. A different element hollow cathode lamp is required for each element determination. Cathode is made of same metal that is to be estimated in the sample.

Single element lamps are used commonly though multi-element lamps are also available Lamps are made of glass with quartz windows and filled with an inert gas such as argon.

Flame atomizers :-

The oldest and most commonly used atomizers in AAS are flames, principally the air-acetylene flame with a temperature of about 2300 °C and the nitrous oxide system (N_2O)-acetylene flame with a temperature of about 2700 °C. The latter flame, in addition, offers a more reducing environment, being ideally suited for analytes with high affinity to oxygen.

Acetylene is commonly used as a fuel gas and requires low levels of Phosphine and Hydrogen Sulphide to give a cleaner flame and less interference to ensure optimum analytical accuracy.

Monochromator :-

This is a very important part in an AA spectrometer. It is used to separate out all of the thousands of lines. A monochromator is used to select the specific wavelength of light which is absorbed by the sample, and to exclude other wavelengths. The selection of the specific light allows the determination of the selected element in the presence of others.

A monochromator disperses the incident light beam and permits the selected wavelength to reach the detector.

Detector :-

The light selected by the monochromator is directed on a detector that is typically a photomultiplier tube, whose function is to convert the light signal into an electrical signal proportional to the light intensity. The processing of electrical signal is fulfilled by a signal amplifier. The signal could be displayed for readout, or further fed into a data station for printout by the requested format.

Detector commonly used is a photomultiplier tube which produces a signal proportional to the amount of light received by it.

6. Chromatography:-

The substances in a mixture are not chemically combined, so therefore they can be separated through some physical process.

Chromatography, technique for separating the components, or solutes, of a mixture on the basis of the relative amounts of each solute distributed between a moving fluid stream, called the mobile phase, and a contiguous stationary phase. The mobile phase may be either a liquid or a gas, while the stationary phase is either a solid or a liquid.

Chromatography is the ability to separate molecules using partitioning characteristics of molecule to remain in a stationary phase versus a mobile phase. Once a molecule is separated from the mixture, it can be isolated and quantified.

Different Chromatographic Techniques:

Chromatography Stationary Phase

1. Thin Layer Chromatography
2. Paper Chromatography
3. Column Chromatography

Mobile Phase

1. Liquid chromatography
2. Gas Chromatography

Classification according to the force of separation

1- Adsorption chromatography.

2- Partition chromatography.

3- Ion exchange chromatography.

4- Gel filtration chromatography.

5- Affinity chromatography.

Thin Layer Chromatography

TLC is a method for identifying substances and testing the purity of compounds. TLC is a useful technique because it is relatively quick and requires small quantities of material. Separations in TLC involve distributing a mixture of two or more substances between a stationary phase and a mobile phase.

The stationary phase: is a thin layer of adsorbent (usually silica gel or alumina) coated on a plate.

The mobile phase: is a developing liquid which travels up the stationary phase, carrying the samples with it. Components of the samples will separate on the stationary phase according to how much they adsorb on the stationary phase versus how much they dissolve in the mobile phase.

Paper Chromatography

A method of partition chromatography using filter paper strips as carrier or inert support. The factor governing separation of mixtures of solutes on filter paper is the partition between two immiscible phases. One is usually water adsorbed on cellulose fibres in the paper (stationary phase). The second is the organic solvent flows past the sample on the paper (stationary phase).

Column Chromatography

Stationary phase is held in a narrow tube through which the mobile phase is forced under pressure or under the effect of gravity.

Liquid chromatography

Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid. It can be carried out either in a column or a plane. Present day liquid chromatography that generally utilizes very small packing particles and a relatively high pressure is referred to as high-performance liquid chromatography (HPLC).

Gas chromatography

Gas chromatography (GC), also sometimes known as gas-liquid chromatography, (GLC), is a separation technique in which the mobile phase is a gas. Gas chromatographic separation is always carried out in a column, which is typically "packed" or "capillary". Packed columns are the routine work horses of gas chromatography, being cheaper and easier to use and often giving adequate performance. Capillary columns generally give far superior resolution and although more expensive are becoming widely used, especially for complex mixtures. Both types of column are made from non-adsorbent and chemically inert materials. Stainless steel and glass are the usual materials for packed columns and quartz or fused silica for capillary columns.

7. Mass Spectroscopy

Mass spectrometry is a powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample, and to elucidate the structure and chemical properties of different molecules. The complete process involves the conversion of the sample into gaseous ions, with or without fragmentation, which are then characterized by their mass to charge ratios (m/z) and relative abundances.

This technique basically studies the effect of ionizing energy on molecules. It depends upon chemical reactions in the gas phase in which sample molecules are consumed during the formation of ionic and neutral species.

Principle:-

A mass spectrometer generates multiple ions from the sample under investigation, it then separates them according to their specific mass-to-charge ratio (m/z), and then records the relative abundance of each ion type.

The first step in the mass spectrometric analysis of compounds is the production of gas phase ions of the compound, basically by electron ionization. This molecular ion undergoes fragmentation. Each primary product ion derived from the molecular ion, in turn, undergoes fragmentation, and so on. The ions are separated in the mass spectrometer according to their mass-to-charge ratio, and are detected in proportion to their abundance. A mass spectrum of the molecule is thus produced. It displays the result in the form of a plot of ion abundance versus mass-to-charge ratio. Ions provide information concerning the nature and the structure of their precursor molecule. In the spectrum of a pure compound, the

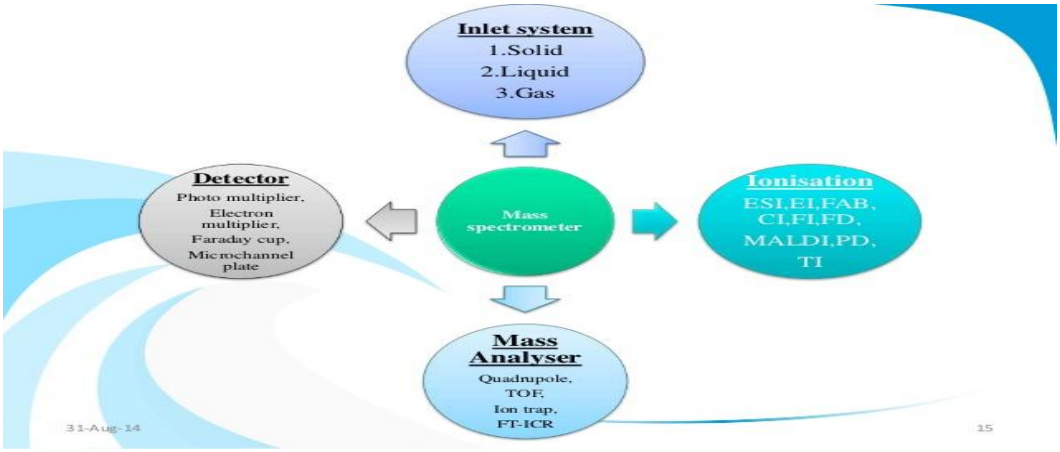
molecular ion, if present, appears at the highest value of m/z (followed by ions containing heavier isotopes) and gives the molecular mass of the compound.

Components:-

The instrument consists of three major components:

1. **Ion Source:** For producing gaseous ions from the substance being studied.
2. **Analyzer:** For resolving the ions into their characteristics mass components according to their mass-to-charge ratio.
3. **Detector System:** For detecting the ions and recording the relative abundance of each of the resolved ionic species.

In addition, a sample introduction system is necessary to admit the samples to be studied to the ion source while maintaining the high vacuum requirements (~10-6 to 10-8 mm of mercury) of the technique; and a computer is required to control the instrument, acquire and manipulate data, and compare spectra to reference libraries.



With all the above components, a mass spectrometer should always perform the following processes:

1. Produce ions from the sample in the ionization source.
2. Separate these ions according to their mass-to-charge ratio in the mass analyzer.
3. Eventually, fragment the selected ions and analyze the fragments in a second analyzer.
4. Detect the ions emerging from the last analyzer and measure their abundance with the detector that converts the ions into electrical signals.
5. Process the signals from the detector that are transmitted to the computer and control the instrument using feedback.

8. X-ray Powder Diffraction (XRD)

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, homogenized, and average bulk composition is determined.

Fundamental Principles of X-ray Powder Diffraction (XRD)

Max von Laue, in 1912, discovered that crystalline substances act as three-dimensional diffraction gratings for X-ray wavelengths similar to the spacing of planes in a crystal lattice. X-ray diffraction is now a common technique for the study of crystal structures and atomic spacing.

X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda=2d\sin\theta$). This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θ angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacings allows identification of the mineral because each mineral has a set of unique d-spacings. Typically, this is achieved by comparison of d-spacings with standard reference patterns.

All diffraction methods are based on generation of X-rays in an X-ray tube. These X-rays are directed at the sample, and the diffracted rays are collected. A key component of all diffraction is the angle between the incident and diffracted rays. Powder and single crystal diffraction vary in instrumentation beyond this.

X-ray Powder Diffraction (XRD) Instrumentation

X-ray diffractrometers consist of three basic elements:

1. X-ray tube.
2. A sample holder.
3. An X-ray detector.

X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons toward a target by applying a voltage, and bombarding the target

material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced. These spectra consist of several components, the most common being K_α and K_β. K_α consists, in part, of $K_{\alpha1}$ and $K_{\alpha2}$. $K_{\alpha1}$ has a slightly shorter wavelength and twice the intensity as $K_{\alpha2}$. The specific wavelengths are characteristic of the target material (Cu, Fe, Mo, Cr). Filtering, by foils or crystal monochrometers, is required to produce monochromatic X-rays needed for diffraction. $K_{\alpha1}$and $K_{\alpha2}$ are sufficiently close in wavelength such that a weighted average of the two is used. Copper is the most common target material for single-crystal diffraction, with CuK_α radiation = 1.5418Å. These X-rays are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded. When the geometry of the incident X-rays impinging the sample satisfies the Bragg Equation, constructive interference occurs and a peak in intensity occurs. A detector records and processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or computer monitor.

The geometry of an X-ray diffractometer is such that the sample rotates in the path of the collimated X-ray beam at an angle θ while the X-ray detector is mounted on an arm to collect the diffracted X-rays and rotates at an angle of 2θ. The instrument used to maintain the angle and rotate the sample is termed a *goniometer*. For typical powder patterns, data is collected at 2θ from ~5° to 70°, angles that are preset in the X-ray scan.

Applications

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is critical to studies in geology, environmental science, material science, engineering and biology.

Other applications include:

- Characterization of crystalline materials
- Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- Determination of unit cell dimensions
- Measurement of sample purity.

Strengths and Limitations of X-ray Powder Diffraction (XRD)

Strengths:-

- Powerful and rapid (< 20 min) technique for identification of an unknown mineral
- In most cases, it provides an unambiguous mineral determination
- Minimal sample preparation is required
- XRD units are widely available
- Data interpretation is relatively straight forward

Limitations:-

- Homogeneous and single phase material is best for identification of an unknown
- Must have access to a standard reference file of inorganic compounds (d-spacings, *hkl*s)
- Requires tenths of a gram of material which must be ground into a powder
- For mixed materials, detection limit is ~ 2% of sample
- For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated
- Peak overlay may occur and worsens for high angle 'reflections'

9. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

- Inductively Coupled Plasma Mass Spectrometry or ICP-MS is an analytical technique used for elemental determinations
- Employs plasma as ionization source and a mass spectrometer (MS) analyser for detect ion
- It can perform qualitative, semi quantitative, and quantitative analysis. Developed in 1980's.

Reasons for the growing popularity

- Performs multi-elemental analysis with excellent sensitivity and high sample throughput
- Instrument detection limits are at or below ppt level
- Productivity is unsurpassed by any other technique
- Isotopic analysis (one isotope or ratio)

How does ICP-MS work?

- Samples introduced into argon plasma as aerosol droplets.
- The plasma dries the aerosol, dissociates the molecules, and then removes an electron from the components, forming singly-charged ions,
- Ions are directed into a mass filtering device known as the mass spectrometer.

ICP-MS components

- **Sample introduction system-**
  - Provides the means of getting samples into the instrument.
  - Composed of a nebulizer and spray chamber.
  - The liquid sample may be introduced to a nebulizer by a peristaltic pump or through self aspiration that creates an aerosol of fine droplets.
  - The fine droplets are passed through a spray chamber before they are allowed to enter the plasma.
- **ICP torch and RF coil** – generates the argon plasma, which serves as the ion source of the ICP-MS.
  - The plasma generated in the ICP torch creates a very hot zone that serves a variety of functions. At a temperature of approximately 6000 °C, the plasma is equal to the temperature at the surface of the sun.
  - The plasma is generated by passing argon through a series of concentric quartz tubes (the ICP torch) that are wrapped at one end by a radio frequency (RF) coil. Energy supplied to the coil by the RF generator couples with the argon to produce the plasma.
  - During their voyage into the plasma, the liquid droplets, containing the sample matrix and the elements to be determined, are dried to a solid and then heated to a gas. As the atoms continue their travel through the plasma, they absorb more energy and eventually release one electron to form singly charged ions. The singly charged ions exit the plasma and enter the interface region.
- **Interface** – links the atmospheric pressure ICP ion source to the high vacuum mass spectrometer.

- Placing a plasma, operating at 6000 °C, near an ion focusing device operating near room temperature is a bit like placing the earth about a half-mile away from the sun. In addition to a large temperature difference, the plasma operates at a pressure that is much higher than the vacuum required by the ion lens and mass spectrometer portions of the instrument. The interface allows the plasma and the ion lens system to coexist and the ions generated by the plasma to pass into the ion lens region. The interface consists of two or three inverted funnel-like devices called cones. Until recently, all commercially available ICP-MS systems used the two-cone design. Such a design requires downstream focusing of the beam that exits the interface region. This focusing has been achieved through the use of a single or a series of charged devices called ion lenses. The need for these ion lenses can be explained in Figure 2. As mentioned earlier, the plasma (located to the left of the sampler cone) operates at atmospheric pressure, while the filtering quadrupole (located to the right of the skimmer cone) operates at a very low pressure. With a two-cone design, there can only be a two-step reduction in the pressure between the plasma and filtering quadrupole. With a two-step pressure reduction, the ion beam undergoes substantial divergence as it exits the second cone, thus requiring additional focusing if the ion beam is to properly enter the filtering quadrupole. A recent innovation has introduced a third cone into the interface which greatly reduces the divergence of the ion beam as it exits the interface region. The third cone, called the hyper-skimmer, provides a three-step reduction in pressure between the plasma and the filtering quadrupole, resulting in a substantial reduction in the divergence o f the emerging ion beam. With the three-cone design, conventional ion lenses can be completely eliminated from the instrument, resulting in greater ion transmission, improved long-term stability, and reduced instrument maintenance. In the three-cone design, none of the cones has a voltage applied such as may exist on an extraction lens. Since the cones are electrically neutral, any buildup of material on their surfaces will not significantly impact their function. In addition, experience has shown that the three-cone design requires no more maintenance than a conventional twocone design. Cones are most often produced from nickel or platinum. While nickel cones have a lower purchase price, platinum cones provide longer life, are more resistant to some acids, and provide a small improvement in instrument performance. The orifice openings of the cones should be large enough to allow for the passage of the ion beam while, at the same time, not allow so much gas to enter the instrument that the

instrument's vacuum system is taxed. Experience has shown that orifice openings of approximately 1 mm are ideal.

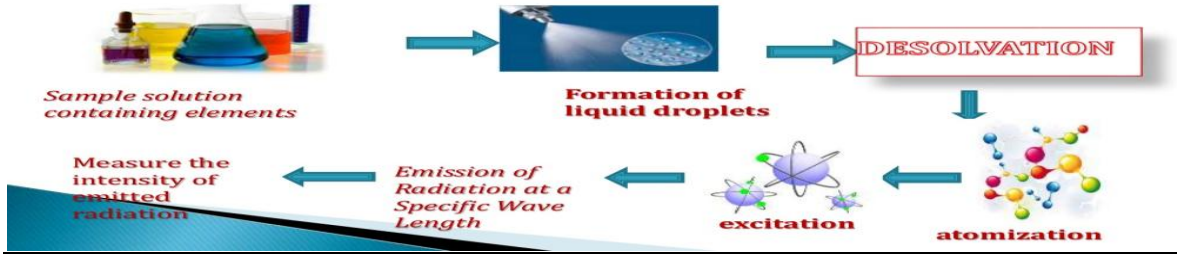
- **Vacuum system** – provides high vacuum for ion optics, quadrupole, and detector.
    - Provides correct operating pressure
    - The distance from the interface to the detector <=1 meter
    - Ions need not to collide with gas molecules during the travel
    - So gas molecules are removed by using a combination of a turbo molecular pump and mechanical roughing pump
- **Collision/reaction cell** – precedes the mass spectrometer and is used to remove interferences that can degrade the detection limits achieved. It is possible to have a cell that can be used both in the collision cell and reaction cell modes, which is referred to as a universal cell.
    - Interferences caused when ions carry a mass-to-charge ratio that is identical to that of the analyte ion
    - The interfering ion is physically larger than the analyte ion.
    - Passing both through a cloud of inert gas molecules, the interferent ion will collide more frequently with the inert gas atoms this removes a certain amount of the kinetic energy possessed by the ion.
    - Analyte ion will retain more of its energy when compared to the interferent ion.
    - An energy barrier is placed at the exit of the cell
    - Collision cell can be a rxn cell by using un inert gas that converts charged interfering ions into inert ones .
- **Ion optics** – guides the desired ions into the quadrupole while assuring that neutral species and photons are discarded from the ion beam
- **Mass spectrometer –** acts as a mass filter to sort ions by their mass-to-charge ratio *(m/z).*
    - The mass spectrometer separates the singly charged ions from each other by mass, serving as a mass filter.

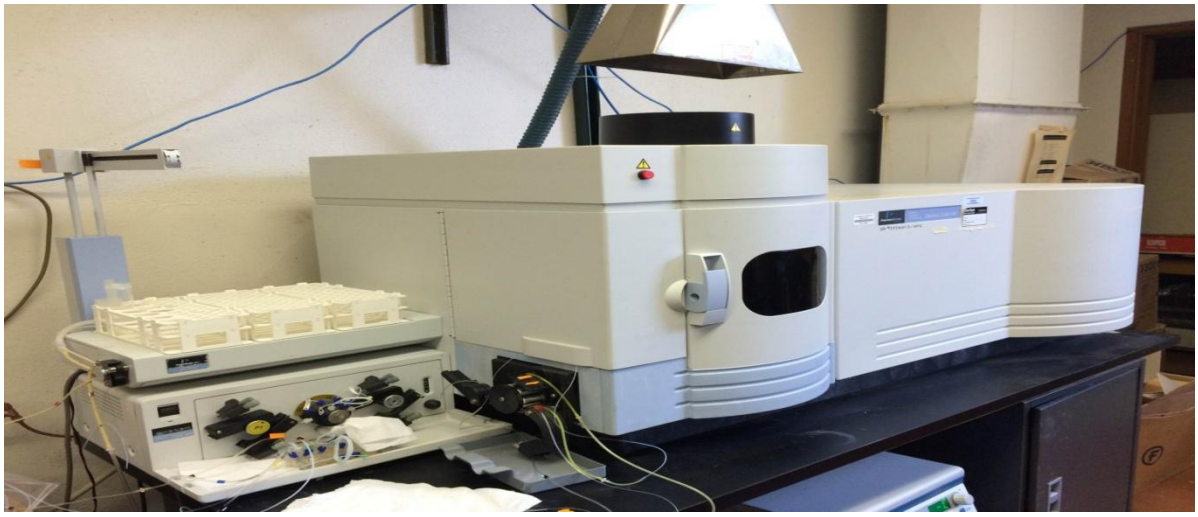
- A quadrupole works by setting voltages and radio frequencies to allow ions of a given mass-to-charge ratio to remain stable within the rods and pass through to the detector, while others are ejected.
- The quadrupole is capable of scanning at a rate > 5000 atomic mass units (amu) per second.

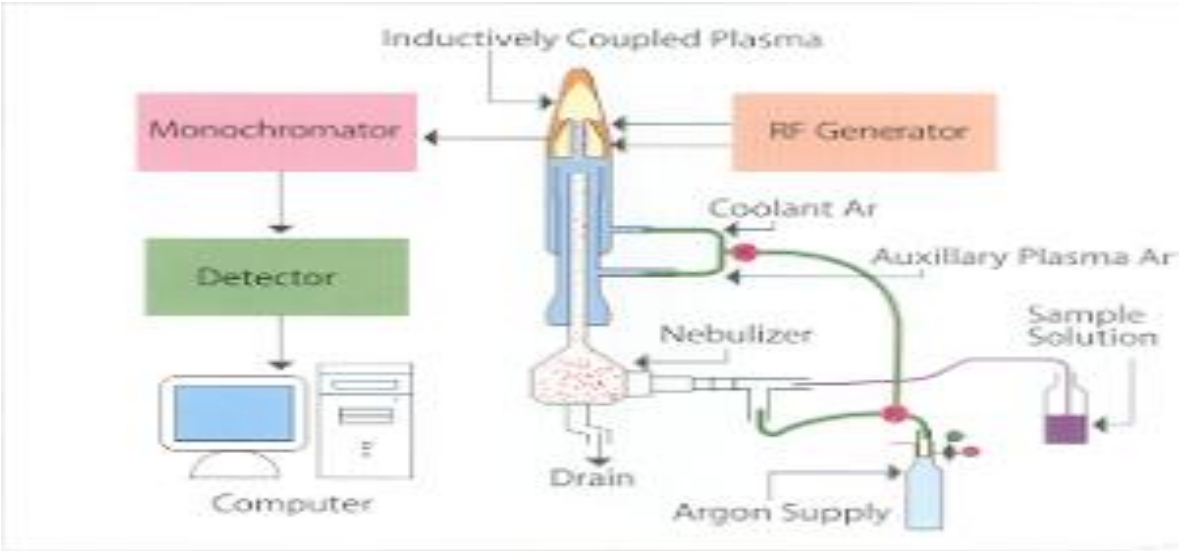
- D**etector** – counts individual ions exiting the quadrupole .
    - The ions exiting the mass spectrometer strike the active surface of the detector (dynode) and generate a measurable electronic signal.
    - A dynode, releases an electron each time an ion strikes it.
    - Released electrons strike a second dynode where more electrons are released until a measurable pulse is created. By counting the pulses generated by the detector, the system counts the original ions
- **Data handling and system controller** – controls all aspects of instrument control and data handling to obtain final concentration results.
    - The software compares the intensities of the measured pulses to those from standards, which make up the calibration curve, to determine the concentration of the element.
    - The software translates the ion counts measured by the detector into information
    - Provide data in one of four ways – semi-quantitative analysis, quantitative analysis, isotope dilution analysis, and isotope ratio analysis.

Inductively coupled plasma atomic emission spectroscopy (ICP-AES), also referred to as ***inductively coupled plasma optical*** **emission spectrometry** (ICP-OES) - is a type of emission spectroscopy that uses the inductively coupled plasma to produce **excited atoms** and **ions** that emit electromagnetic radiation at wavelengths characteristic of a particular element.

Principle







PLANT SAMPLING AND SAMPLE PREPARATION

4.1 Plant Sampling and sample preparation:

For a meaningful plant analysis, collection of particular plant part and stage of growth is very important. It would be wrong and wasteful to pluck any of the growing plant part at any time and send to laboratory for analysis. Thus, depending on the purpose of analysis, plant sampling needs to be planned. Plant parts (index tissue) recommended to be sampled for analysis are given in Table 1. The index tissue sampling is designed for nutrient diagnosis, monitoring and efficient nutrient management for optimum yield and excellent quality.

After collection of plant samples, fresh tissues should be free from dust and other foreign material by washing with detergent solution followed by 0.1 N HCl and deionized water. The 0.2% liquid detergent solution will remove waxy coating on the leaf surface. The 0.1N HCI will remove metallic contaminants and deionized water will wash the previous two solutions. Place the washed samples on filter paper sheets and air dried for 24 hrs. Then put the plant samples in news paper bags to dry in a forced air oven at 65°C ± 2°C for 48 hrs. Grind the samples in an electric stainless steel grinder using 0.5 mm sieve. Put each sample in oven and dry again for few hours more for constant weight. Store in paper bags for further analysis.

Table 1: Plant parts recommended to be sampled for analysis

Category	Crop	Part to be sampled with stage/age
Grain, Pulses, Oilseeds, Fibre and Commercial Crops	Wheat	Flag-leaf, before head emergence
	Rice	3rd leaf from apex, at tillering
	Maize	Ear-leaf, before tasseling
	Barley	Flag leaf at head emergence
	Oat	Flat-leaf, before inflorescence emergence
	Pulses	Recently matured leaf, at bloom initiation
	Groundnut	Recently matured leaflets, at maximum tillering
	Sunflower	Youngest mature leaf blade, at initiation of flowering
	Mustard	Recently matured leaf, at bloom initiation
	Soybean	3rd leaf from top, after 2 months of planting

	Cotton	Petiole, 4th leaf from the apex, at inifiation of flowering.
	Sugarcane	3rd leaf from top after 3-5 months of planting
	Sugar beet	Petiole of youngest mature leaf at 50-80 days age
Vegetables	Potato	Most recent, fully developed leaf (half- grown)
	Tomato	Leaves adjacent to influorescence (mid bloom)
	Onion	Top non-white portion (1/3 to 1/2 grown)
	Brinjal	Blade of most recent fully developed leaves
	Beans	Uppermost, fully developed leaves
	Cauliflower	Most recent, fully matured leaf, at heading
	Cabbage	Wrapper leaf at 2-3 months age
	Pea	Leaflets from most recent, fully developed leaves at first bloom
	Carrot	Most recent, fully matured leaf, at mid- growth
	Radish	Most recent, fully developed leaf
	Turnip	Most recent, fully developed leaf
	Beetroot	At 30-50 days age
	Spinach	5th leaf from tip (omit unfurled) at the stage of bud
		Starting to small fruits
	Cucumber	Most recent, fully developed leaf
Fruit crops	Citrus fruits	3-5th month old leaves from new flush,1st leaf of the shoot, in June
	Guava	3rd pair of recently matured leaves, at bloom (August or
		December)
	Mango	Leaf with petiole (4-7 month old) from middle of shoot
	Papaya	6th petiole from apex, 6 months after planting
	Pomegranate	8th leaf from apex at bud differentiation, in April and August
	Falsa	4th leaf from apex, 1 month after pruning
	Ber	6th leaf from apex from secondary or tertiary shoot, 2 months
		after pruning

Sources: Extracted and modified from Kensworthy (1964), Tandon (1984), Reuter and Robinson (1986), Jones et al. (1991), Bhargav and Raghupati (1993), Singh et.al. (1999).