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Mineral Assay in Atomic Absorption Spectroscopy

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Abstract

Minerals are necessary for the health and maintenance of several human body functions like oxygen transportation, normalizing the nervous system and simulating growth, maintenance and repair of tissues and bones. Atomic Absorption Spectroscopy (AAS) is a very useful tool for determining the concentration of specific mineral in a sample. Liquefied sample is aspirated, aerolized and mixed with combustible gases such as acetylene and air or acetylene and nitrous oxide and burned in a flame to release the individual atoms. On absorbing UV light at specific wavelengths the ground state metal atoms in the sample are transitioned to higher state, thus reducing its intensity. The instrument measures the change in intensity and the intensity is converted into an absorbance related to the sample concentration by a computer based software.

Keywords: Mineral estimation, Atomic Absorption Spectroscopy, Intensity, Absorbance.

1. Introduction

Nutrients are the substances which after ingestion, digestion, absorption and assimilation, become a part of cell and thus maintains all cellular activities in the body. Minerals are one of such nutrient. Some minerals are essential for cellular metabolism. There are thirteen minerals considered as 'essential' viz., calcium, phosphorous, magnesium, sodium, potassium, iron, manganese, zinc copper, selenium, chromium, cobalt and iodine[1]. These minerals are again classified depending upon their requirements in the body; macro minerals and micro or trace minerals. When body requires more than 100 mg of a mineral in 1 day then it is considered as macro minerals viz., calcium, magnesium, phosphorus, sodium and potassium. Again the minerals whose requirement is less than the said level will considered as trace minerals. Zinc, Iron, Manganese, Selenium, Copper, Cobalt are the trace minerals. Although minerals comprise only a fraction of total body weight, they are crucial for many functions including transporting oxygen, normalizing the nervous system and stimulating growth, maintenance and repair of tissues[2].

2. Principle of Atomic Absorption Spectroscopy

Atomic absorption spectroscopy (AAS) determines the concentrations of minerals in samples. It is a process involving the absorption by free atoms of an element of light at a wavelength specific to that element. This method is very sensitive and enables to detect small amount of an element of 1 ppm using flame procedure. Lower levels can be determined down to 0.001ppm using graphite furnace procedure. In their elemental form, metals will absorb ultraviolet light when they are excited by heat. Each metal has a characteristic wavelength that will be absorbed. The AAS instrument looks for a particular metal by focusing a beam of UV light at a specific wavelength through a flame and into a detector[4].

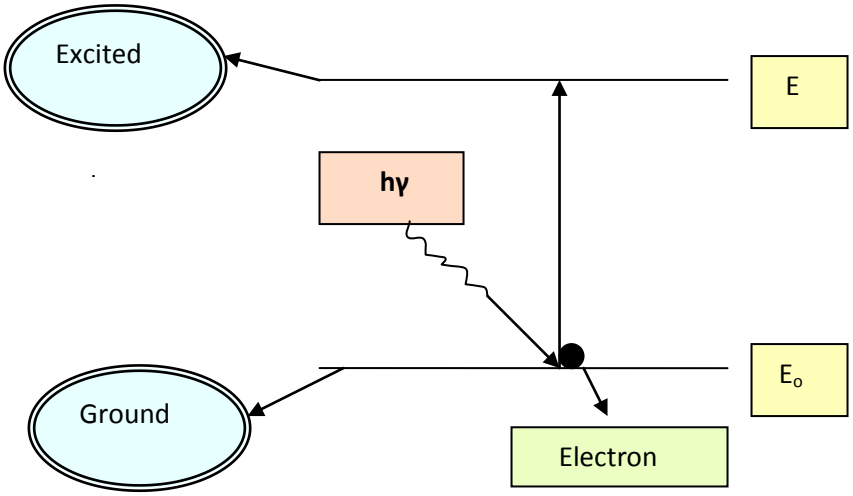
This AAS technique requires a liquid sample to be aspirated, aerolized and mixed with combustible gases such as acetylene and air or acetylene and nitrous oxide. When the sample solution or sample is burned in a flame or heated in a tube, the individual atoms of the sample are released to form a cloud inside the flame or tube. The flame temperature ranges from 2100 to 2800^{o}C.The atoms of the element in the sample are free and in unexcited ground state .To excite the atom; one or more electrons can be raised to first or higher energy

levels by the absorption of energy by the atom. The energy required for the transition can by supplied by a photon of light with energy (E)[4].

$E=h\gamma$ [h=Planck's constant and γ = frequency]

So the corresponding wavelength is, **$\lambda=hc/E$ [c=Speed of light and λ=wavelength]**

For all non conducting elements (insulators) and for most of the electrons in atoms of conducting elements, the energy gap between ground state and first or higher energy level is very large. So, very energetic photons are required. Vacuum UV or X-ray is required to excite the atom. On absorbing UV light at specific wavelengths the ground state metal atoms present in the sample make transition to higher electronic state, thus reducing its intensity. The instrument measures the change in intensity. A computer data system converts the change in intensity into an absorbance. As concentration goes up, absorbance also goes up.



$h\gamma$=$(E-E_o)$=Energy gap between two electronic states

The principle of **Atomic absorption spectroscopy** follows Beer-Lambert law. According to this law, absorption is proportional to the concentration of the metal present in the sample.

$$A=\varepsilon cl$$

Where A= absorbance. **ε** = molar absorption coefficient with units of L mol^{-1} cm^{-1} l = path length of the sample - that is, the path length of the cuvette in which the sample is contained. We will express this measurement in centimeters. c = concentration of the compound in solution, expressed in mol L^{-1}.

Although the basic principles of the Beer-Lambert law applies to AAS, it is not possible to use this relationship in the same way. Because, solutions are homogeneous throughout the sample absorption path and the free atoms in the flame are not constant through the light path. Hence the law can't be used directly to determine the concentration of an atom generated from a solution. It is necessary instead to use this equation.

Total absorption= Constant x no. of free atoms in the light path

Absorption of a selected wavelength is measured by the change in light intensity striking the detector and is directly related to the amount of element in the sample. Concentration measurements are usually determined from a working curve after calibrating the instrument with standards.

3. Determination of Elements in AAS

The following elements may be determined directly by air/acetylene AAS: Ca, Mg, Mn, Fe, Sb, Bi, Cd, Cs, Cr, Co, Cu, Au, Ir, Pb, Li, Mn, Ni, Pd, Pt, K, Rh, Ru, Ag, Na, Sr, Tl, Sn and Zn. For determination of Ca or Mg lanthanum is added as a releasing agent. When the metals are present in very low concentration, then Graphite furnace is used. As, Se, Hg and other volatile metals are determined by continuous flow hydride generation. Sodium borohydride in presence of metal and HCl, produces a metal hydride. For Al, Ba, Be, Mo, Os, Rh, Si, Th, Ti and V Nitrous Oxide/Acetylene are used. A soluble ionization buffer such as potassium chloride is added to both sample and standard solutions for Al, Ba, Mo, Ti and V determinations[3,4].

4. Instrumentation

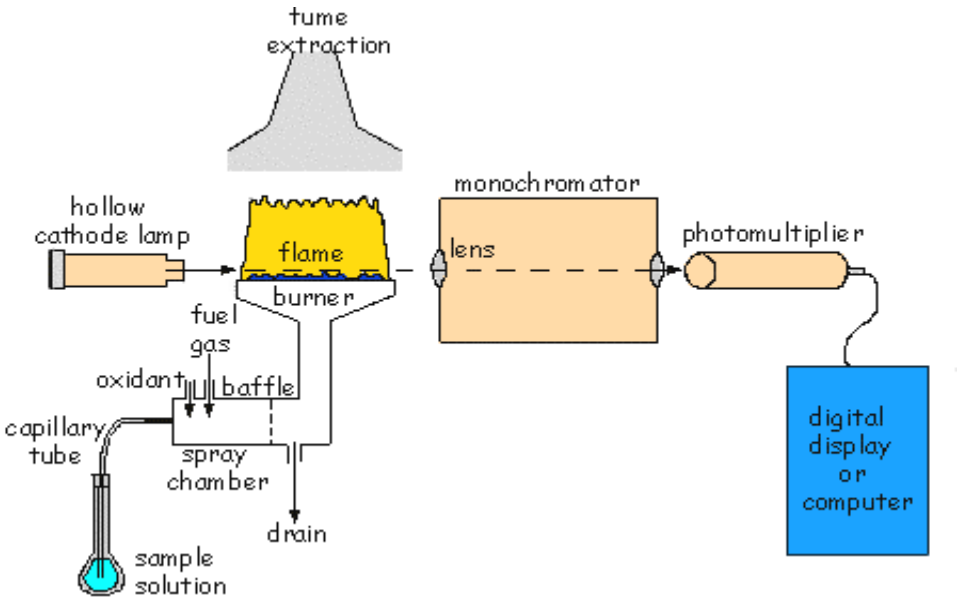


Fig:1. A schematic diagram of flame atomic absorption spectrometer

(Source: www.thebritishmuseum.ac.uk/sr-tech-aas.html)

At first sample solution is aspirated into the spray chamber through capillary tube. The liquid sample is aerolized and mixed with combustible gases such as acetylene and air or acetylene and nitrous oxide(mentioned in the diagram as fuel gas and oxidant, Fig. 1) in the spray chamber and burned in a flame and the individual atoms of the sample are released to form a cloud inside the flame. The atoms of the element get free. To bring it from ground state into an excited state by passing UV light is required through hollow cathode lamp. On absorbing UV light at specific wavelengths the ground state metal atoms get transitioned to higher electronic state. The region of the spectrum to be measured is selected by a monochromator. The isolated spectral line falls on the photomultiplier, the detector and the output is amplified and sent to a readout device meter, digital or analogue or through a computer data processing system. The data is processed through software. Absorption of a selected wavelength is measured by the change in light intensity striking the photomultiplier detector and is directly related to the amount of element in the sample. Waste is drained out through drain pipe[4].

Light source:

The light source is usually a hollow cathode lamp of the element that is being measured. Its main feature is the narrow absorbing spectral line. It is the standard source in AAS. Lamps may be expected to run in excess of 5000mA hours without failure. Besides this laser, multi element lamp, electrode less discharge lamps are also used. A multi element lamp has a cathode composed of more than one element.

A liquid sample is normally turned into an atomic gas in three steps:

a. Desolvation (Drying) – the liquid solvent is evaporated, and the dry sample remains
b. Vaporization (Ashing) – the solid sample vaporises to a gas
c. Atomization – the compounds making up the sample are broken into free atoms.

Photo-1: Atomic Absorption Spectrophotometer

Atomizer:

The following atomization methods are known: a) Flame atomization, b) Graphite furnace atomization and c) Mercury hydride atomization.

AA spectroscopy requires that the analyte atoms be in the gas phase. Ions or atoms in a sample must undergo desolvation and vaporization in a high-temperature source such as a flame or graphite furnace.

a) **Flame atomization technique**

Usually flame atomization method is used. Metals can be measured at ppm concentration (part per million, that is mg kg-1 or mg dm-3 in case of dilute solutions). The sensitivity could be increased when the light travels for longer in the flame. Therefore most of the burners are about 5-10 cm long. The accuracy is very good. The sample solution is sprayed ("nebulized") continuously into the flame (similarly to the flame photometer).Sample solutions are usually aspirated with the gas flow into a nebulizing /mixing chamber to form small droplets before entering the flame[4].

Nebulization: The sample enters normally as a liquid droplet in the flame. The nebulizer produces an aerosol. The basic operation involves drawing up the sample at high velocity through a capillary tube to a fine jet, which forms a liquid aerosol or mist before injection into the flame. The high speed gas flow creates a turbulence, which breaks up the solution as it passes out of the capillary, to produce an aerosol. Nebulization efficiency depends upon solvent properties[4].

b) **Graphite furnace**

The graphite furnace has several advantages over a flame atomization. It is a much more efficient atomizer than a flame and it can directly accept very small absolute quantities of sample. It also provides a reducing environment for easily oxidized elements. Samples are placed directly in the graphite furnace and the furnace is electrically heated in several steps to dry the sample, ash organic matter, and vaporize the analyte atoms. The measurements can be done at ppb level (part per billion, ppb = 10^{-3} ppm, that is μg kg-1 or μg dm-3 in case of dilute solutions). The drying, combustion, vaporization and atomization of sample happen in a heated graphite tube that is placed in the way of light. This "graphite furnace" is protected against oxidation by an inert gas (e.g. argon)[4].

c) **Hydride generation technique/vapour system:**

The vapour system is an accessory for measuring the hydride forming elements such as arsenic, selenium and mercury with better analytical sensitivity than can be obtained by

flame atomization. In this method the analytes are reduced to volatile hydrides using sodium borohydride and are carried in a carrier gas stream to a heated measurement cell for measurement[4].

5. Light Separation and Detection

AA spectrometers use monochromators and detectors for UV and visible light. The main purpose of the monochromator is to isolate the absorption line from background light due to interferences. Simple dedicated AA instruments often replace the monochromator with a bandpass interference filter. Photomultiplier tubes are the most common detectors for AA spectroscopy[4].

6. Sample Collection

The glasswares should be dedicated for AAS analysis and should be cleaned properly before collection of samples in order to prevent contamination with metals. It must be rinsed with acid and deionized water prior to sample collection.

Sample collection for water analysis:

To analyse elements in water, correct type and amount of acid is added. Wrong acid leads to loss or poor recovery of the metal from the sample. Acid concentration must be depend on what concentration level is the metal likely to be. It is very important to wash the sample container with acid and rinse with deionized water before sample collection. If sample is not acidified properly, there may be loss of metals by absorption on to the walls of the containers. Containers made from polytetrafluoroethylene (*PTFE*), polypropylene or linear polythene with polythene caps may be used for sample collection. Dark brown bottles or light absorbing glass bottles should be used for determination of silver. Glass bottles and filtering equipment must be acid rinsed before use[3,4].

7. Sample Storage

Storage of sample is very important component of Atomic Absorption Spectroscopy. In an ideal condition the sample should be analyzed for the element of interest immediately. The major risk in storage and particularly long term storage, are the loss of metal atom from the sample and the possibility of contamination. So, a certain protocol or quality assurance

programme should be followed, depending upon the type of sample and the element to be determined in it.

It is easy to clean the container if a sample is analyzed immediately. In long storage of the sample, the suspended particles may settle to the bottom of a bottle, resulting in loss of some sample particles as well as making cleaning more difficult[3,4,5].

Preservation of water samples

Sample containing mercury may only be stable up to 5 weeks. To preserve mercury in the sample, 2ml of 20% (w/v) potassium dichromate solution in 50% (v/v) nitric acid should be added to each litre of sample. Avoid storing of sample in refrigerators contaminated with mercury. Water samples should not be stored in plastic bottles in mercury contaminated laboratory, as mercury vapors can pass through bottles and may subsequently an increase in the sample.

8. Sample Preparation

Sample preparation is necessary and it is very important for obtaining good results. There are at least five methods of sample preparation used by the analyst[4].

i) Wet ashing or acid digestion
ii) Fusion
iii) Pressure dissolution
iv) Dry ashing
v) Microwave digestion

i) Wet Ashing or Acid digestion

For flame AAS the sample is always introduced as a liquid to the instrument. For this purpose an acid digestion is done. Various acids can be used for acid digestion. The solid sample is dissolved in an acid or mixture of acids. Typical acids used are hydrochloric, nitric, aquaregia {nitric acid (1): hydrochloric acid (3)}, perchloric, hydrofluoric and sulphuric. Most metals dissolve in hydrochloric, nitric acid or mixture of both, aqua regia. Biological materials with simple matrices dissolves in nitric acid. Perchloric acid is used for complex materials. All acids must be of high purity. A mixture of nitric acid and sulphuric acid is also used for digestion. Wet ashing or acid digestions are usually carried out in pyrex glassware.

When using acids such as hydrofluoric (HF) or meals such as Sodium (Na) are being analyzed, Teflon or platinum ware is preferred. Digestion temperature should be 400^{o}C.

After digestion, the sample is filtered thorough glass filter and volume is made up to 100ml with water.

Digestion method is used for soil,fish sample analysis. Aqua regia is used for soil sample digestion while sulpuhuric & nitric acid is used for fish sample. For silicate soils the bound metals are not released by aqua regia alone. Zn, Cu, Ni, Cd, Cr, Pb, Co, Mn and Fe are recovered with aqua regia[3,4].

Photo-2: Acid Digestion Unit

ii) Fusion

Fusion technique is another method for sample preparation. Here sample is weighed into a metal crucible, mixed with a suitable flux and fused over a hot flame. The resulting melt is dissolved in either water or acid.

Fusion is sometimes used in conjunction with acid digestion, when the acid mixtures used have not totally dissolved the sample. The residue is filtered and then fused, usually with sodium peroxide. The two portions are combined and then measured by AAS for total metal.

iii) Pressure dissolution

This technique is used when volatile elements may be lost using other sample preparation technique. The sample is sealed inside a 'Parr' bomb or a quartz or silica carius tube, with a mixture of acids. The bomb is then placed in an oven at around 150^{o}C, usually overnight. The combination of temperature and pressure aids dissolution of the sample. The method has been used to determine mercury and silicon.

iv) Dry ashing

This technique is used for samples with high carbon content viz. coals, resins and plant material. The sample is heated in air in an oven, until the organic material has been burned away. Volatile elements for example arsenic can be lost at high ashing temperatures and various ashing reagents such as magnesium nitrate, have been prevent this loss. After ashing the residue is dissolved in hydrochloric or nitric acids.

v) Microwave Oven digestion:

This microwave digestion technique is quicker and safer. The microwave system functions by using a combination of acid, temperature and pressure. The technique is also useful for the measurements of volatile metals. The major advantage of this technique is the analysis time, which is in minutes, rather than hours. It has been successfully used for acid dissolution of bone, biological samples, steels, environmental samples, botanical matrices, ores, rocks, metals from sediments etc.

All the above mentioned methods are useful. But the choice of method depends on the sample type. As our sample of interest is biological fish sample, acid digestion and microwave digestion is most useful. But microwave digestion is quicker and safer technique over acid digestion. It is the prerogative of the analyst to decide the method of sample preparation.

9. Instrumental General Procedure

The operating procedure[4] will vary between instrument brands, so the instrument manual should be followed carefully. The position of observation and the fuel**:**oxidant ratio must be optimized. Some general guidelines are outlined below

1. Light the hollow cathode lamp or electrode discharge lamp and D_2-lamp if such background correction is used. Set the lamp current to the value specified by the manufacturer.
2. Position the monochromator at a selected wavelength.
3. Carefully balance the intensity of the hollow cathode lamp and the D_2-lamp if such background correction required.
4. Align the burner head to assure that the center of the light beam passes over the burner slot.
5. Light the flame and regulate the flow of fuel and oxidant to produce an oxidizing flame (lean blue).
6. Aspirate calibration blank and establish a zero point.
7. Aspirate standard solutions and construct a calibration curve.
8. Aspirate distilled water after each standard or sample.

10.Sequence of Analysis

1. Aspirate calibration blank and establish a blank level
2. Aspirate calibration blank and standard solutions and construct a calibration curve. Use at least 3 standard solutions in addition to the calibration blank to cover the linear range. Every point at the calibration curve should, if possible, be based on replicate analysis. Distilled water should be aspirated after each standard and sample.
3. A quality control standard should be analyzed to verify the calibration.
4. A calibration blank should be analyzed to check for memory effects.
5. Aspirate unknown samples.
6. Aspirate a quality control standard for every 10th sample to check for drift.
7. Samples that are found to have concentration higher than the highest standard should be diluted and reanalyzed.

Calculation

The concentration of metal is detected in mg/litre or ppm.

The metal mg/100g= {Conc. of metal in PPM or mg/l x volume made}/weight of sample

Calibration

Correct preparation of standards and calibration curve is also very important as sample preparation. The standard solutions should be prepared using high purity metals or compounds, dissolved in high purity reagents. Commercially available standard solution has very low level of contamination. Working calibration solutions are prepared from stock solutions (normally 1000ppm in concentration).The stock solution is the diluted in steps with clean pipettes or burettes into volumetric flask. Glassware is suitable for the dilution steps but not for storing diluted solutions for any period of time.

Inaccuracy is increased with pipetting smaller volume. So, maximum dilution factor used in any one step is twenty times or a minimum of 5ml diluted to 100ml.

To prepare a 0.01 ppm or 10 ppb standard by dilution from a 1000ppm stock solution, the following steps are suggested:

1. Take 10ml of 1000 ppm and diluted to 100ml, this gives a concentration of 100 ppm.
2. Take 10ml of 100 ppm and diluted to 100ml, this gives a concentration of 10 ppm.
3. Continue the same dilution steps in sequence producing concentrations of 1ppm, then 0.1ppm and finally 0.01 ppm.

The same dilution is possible in one step using plastic tipped micropipette. But it is not generally recommended. Stabilization of working solutions is very important. Because solutions in low concentration range are subject to hydrolysis and absorption effects. Sn, As, Ti, Sb is prone to hydrolysis. Sn is stabilized with high concentration of hydrochloric acid, never nitric acid, as nitric acid leads to large losses of Sn from solution. The lifetime

of prepared standard solution varies with concentration range. Generally 1000ppm should be stable for 6 months. More the dilute solutions, shorter are the life time.

Calibration procedure:

There are two basic procedures available for calibration purposes, a direct calibration method and standards addition method. In order to obtain good precision a set of standards (minimum three) must be prepared. A blank solution should be included to allow for any signal due to analyte present in reagents used in sample preparation. For each standard, the measured absorbance vales for flame, or peak heights or areas for graphite furnace, are plotted against the known concentrations, giving a calibration curve. Sample concentrations can be read off directly from the graph. Sometimes, if required, the sample has to be dilute within the standards range. Direct calibration method works for simple solutions, where matrix effects are negligible. For more complex solutions and samples, the method of standard addition must be used.

11. Interferences

Interferences in flame AAS are few and divided into five types: Spectral, Physical, Chemical, Ionization and non-specific Absorption.

i) **Spectral interference:** Spectral interference arises due to line overlap. It is very rare in Atomic absorption spectrometry. This type of interference can be overcome by either separation of elements prior to analysis or by use of an alternative wave length.

ii) **Physical interference:** Physical interference due to viscosity effects can be overcome by carefully matching solvent and matrix of standards and samples. Aqueous standards should not be used when measuring a sample in an organic solvent.

iii) **Chemical interference:** The response of an element at its resonance wave length may sometimes be dependent on other components in the sample solution. This effect is usually known as chemical interference. Various methods of overcoming in this interference are: removal of interfering anion(s) by chemicals or by ion

exchange techniques, addition of an excess of the interfering anions to both samples and standard solutions, use of a hotter nitrous oxide or acetylene flame for the alkaline earth elements, addition of a releasing agents

iv) **Ionization:** This interference usually observed in high temperature flames such as nitrous oxide/acetylene and is characterized by enhance response of the element being determined when another easily ionised element is added. Ionization interference is effectively overcome by adding an excess of easily ionizable metal salt such as cesium or potassium chloride to both standard and sample solution.

v) **Non-specific Absorption:** This type of interference is caused by molecular absorption or light scattering due to salt particles. It is more serious in furnace AAS that in flame AAS and is easily overcome by the background correction.

Besides furnace interferences arise are due to sample spreading, solid sample interference, matrix interference etc.

12. Quality Control

Quality control may be either external or internal. All competent analysist should use some form of quality control to produce credible results. A good quality programme consists of the following:

- Certification of Operator competence
- Recovery of samples after free treatment
- Recovery of known additions
- Analysis of externally supplied standards
- Analysis of reagent blank
- Calibration with standards
- Maintenance of control charge

Do's and don'ts in handling AAS[4]:

Do's:

I) Sample must be properly filtered.

II) Sample storage container must be properly cleaned.

III) Glasswares for sample preparation must be properly cleaned and dedicated for AAS.

IV) Use safety goggles or safety shield while facing flame.

V) Proper flame orientation should be done.

VI) Carefully prepare the standard solution.

VII) After using the machine it should be properly turned off.

VIII) Gas cylinders should be closed after using AAS system.

IX) Always use double/triple distilled water for sample analysis

Dont's:

I) Don't aspirate improperly filtered samples; otherwise the nebulizer can be chocked.

II) Don't use uncleaned sample containers.

III) Don't use impure gases.

IV) Don't aspirate contaminated samples.

13. Conclusion

Principle of AAS follows Lambert's Beers law. A liquid sample to be aspirated, aerolized and mixed with combustible gases such as acetylene and air or acetylene and nitrous oxide. Sample solution or sample is burned in a flame (2100 to 2800^{o}C) or heated in a tube and the individual atoms of the sample are released. On absorbing UV light at specific wavelengths the ground state metal atoms present in the sample are transitioned to higher electronic state, thus reducing its intensity. The instrument measures the change in intensity. A computer data system converts the change in intensity into an absorbance. Absorption is directly related to sample concentration. Liquid sample is turned into an atomic gas through desolations, vaporization, atomisation. There are a number of atomization techniques i.e. flame, graphite furnace and hydride generation. Hollow cathode lamp is usually used as a light source. As AA spectroscopy is very sensitive method, sample preparation, storage is very important and sample should not contain any contaminants. The concentration of metal is detected in ppm or ppb level. There is number of sample preparation techniques such as acid digestion, fusion, dry ashing, microwave digestion and pressure dissolution etc. A set of standard

solution is required for making a calibration curve. Unknown sample concentration can be determined from the calibration curve.

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