Operation of Atomic Absorption Spectrometer and Micronutrient Analysis: A User Guide

PME Publication no. ICARNEH-ML-EPUB-2024-77

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UMIAM - 793 103, MEGHALAYA

November, 2024

Citation: Moirangthem, P., Kumar, Y. B., Balusamy, A., Chakraborty, M., Singh, N. R., Chanu, L.J., Ramesh, T., Krishnappa, R., and Hazarika, S. (2024). "Operation of Atomic Absorption Spectrometer and Micronutrient Analysis: A User Guide". ICAR Research Complex for NEH Region, Umiam, Meghalaya, pp.1-37.

Published by

The Director,
ICAR Research Complex for NEH Region, Umiam-793103,
Meghalaya

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1. INTRODUCTION

Atomic Absorption Spectrometry (AAS) is a technique for measuring quantities of chemical elements present in environmental samples by measuring the absorbed radiation by the chemical element of interest. This is done by reading the spectra produced when the sample is excited by radiation. It detects elements in either liquid or solid samples through the application of characteristic wavelengths of electromagnetic radiation from a light source. Individual elements absorb wavelengths differently, and these absorbances are measured against standards. The concentration is calculated based on the *Beer-Lambert law* which states that ***"Absorbance is directly proportional to the concentration of the analyte absorbed for the existing set of conditions".*** The concentration is usually determined from a calibration curve, obtained using standards of known concentration. However, applying the Beer-Lambert law directly in AAS is difficult due to: variations in atomization efficiency from the sample matrix, non-uniformity of concentration and path length of analyte atoms (in graphite furnace AA). In effect, AAS takes advantage of the different radiation wavelengths that are absorbed by different atoms.

Although AAS dates back to the 19th century, the modern form of this technique was largely developed during the 1950s by Alan Walsh and a team of Australian chemists working at the CSIRO (Commonwealth Science and Industry Research Organization) Division of Chemical Physics in Melbourne, Australia. Typically, the technique makes use of a flame to atomize the sample, but other atomizers, such as a graphite furnace, are also used. In analytical chemistry, AAS is used mostly for determining the concentration of a particular metal element within a sample; AAS can be used to analyze the concentration of over 62 different metals in a solution.

2. ATOMIC ABSORPTION AND EMISSION

Every element has a specific number of electrons associated with its nucleus. The normal and most stable orbital configuration of an atom is known as the "ground state." If energy is applied to an atom, the energy will be absorbed and an outer electron will be promoted to a less stable configuration known as the "excited state." Since this state is unstable, the atom will immediately return to the "ground state," releasing light energy **(Fig. 1)**.

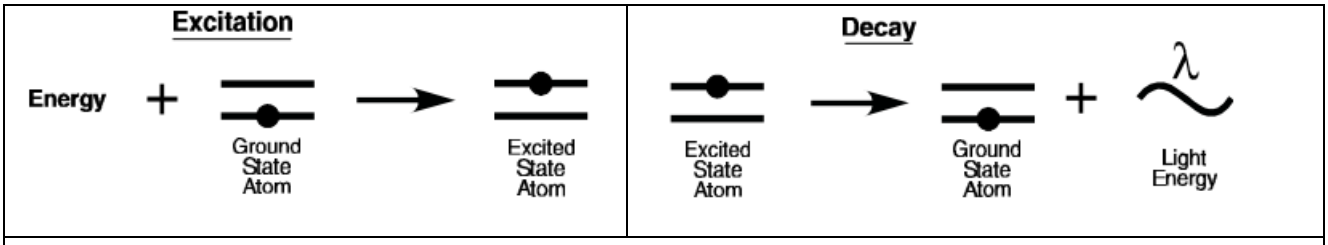


Fig 1: a) Atomic Absorption process, b) Atomic Emission process

The "ground state" atom absorbs light energy of a specific wavelength as it enters the "excited state." As the number of atoms in the light path increases, the amount of light absorbed also increases. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte can be made. The use of special light sources and careful selection of wavelengths allow the specific determination of individual elements.

3. BASIC WORKING PRINCIPLE OF AAS

Atoms of an element emit a characteristic spectral line. Every atom has its own distinct pattern of wavelengths at which it will absorb energy, due to the unique configuration of electrons in its outer shell. A light source is used to emit the characteristic spectral radiation of the element to be determined, which will be absorbed by the ground-state atoms of the element to be determined in the sample vapour generated by the flame atomizer or, the graphite furnace atomizer. The absorption spectrum of an element in its gaseous atomic state consists of a series of well-defined and extremely narrow lines arising by the electronic transitions of outer most electrons. In case of metals, most of these transitions belong to visible and UV regions. Most obvious wavelengths at which absorption or emission is observed are associated with the transitions where minimal energy change occurs e.g., 3s-3p transition in Na atom gives rise to the emission of yellow light and this is referred as D-line transition. For converting element into its gaseous atomic state initial step in the whole procedure of estimation involves spraying a solution of the sample into the atomizer (flame or graphite cuvette). This process is accomplished by drawing the solution of the sample as a fine mist into a suitable flame. A detector measures the wavelengths of light transmitted by the sample, and compares them to the wavelengths which originally passed through the sample. A signal processor then integrates the changes in wavelength absorbed, which appears in the readout as peaks of energy absorption at discrete wavelengths.

By measuring the absorbed quantity of the characteristic radiation, the content of the element to be determined is calculated according the function relation between the change of the light energy and the concentration of the element to be determined (Beer's Law). This analytical method based on atomic absorption is highly sensitive because atomic absorption lines are extremely narrow (0.02-0.5A) and transition energies are unique for each of the element. However, the limited line width creates a problem in AAS measurement which is usually not the case in the spectroscopy for a solution. In order to follow the Lambert Beer's law, it is necessary that the bandwidth of the source must be narrow to the band width of the absorption peak. Even good quality monochromators with effective band widths has significantly greater bandwidth than the band width of the absorption peaks. So, when the atomic absorption measurements are made with ordinary monochromators equipped with continuous source of radiations then also non-linear calibration curves were obtained. The problem of limited width of the atomic absorption peak is solved by the use of line sources with band width narrower than the absorption peaks. It is therefore, necessary to use the exciting beam that contains high intensity of light of the required wavelengths. This is achieved by the use of hollow cathode lamp.

4. COMPONENTS OF A CONVENTIONAL ATOMIC ABSORPTION SPECTROMETER

Basic components of an atomic absorption instrument are described below:

i) Source of Radiation (Hollow Cathode Lamp)

A hollow cathode lamp **(Fig. 2)** consists of a glass cylinder filled with an inert gas (Neon or Argon) under a pressure of 1-5 torrs into which an anode and a cathode are fused. The cathode is made of the analyte element of interest in the form of a cylinder and the anode is a thick wire usually made of tungsten or nickel. A voltage of several hundred volts is required to light the lamp. The applied voltage generates 5-15 mA current and sets up a glow discharge of the carrier gas. A stream of positive ions strikes the cathode and releases the atoms of the cathode material by collisions.

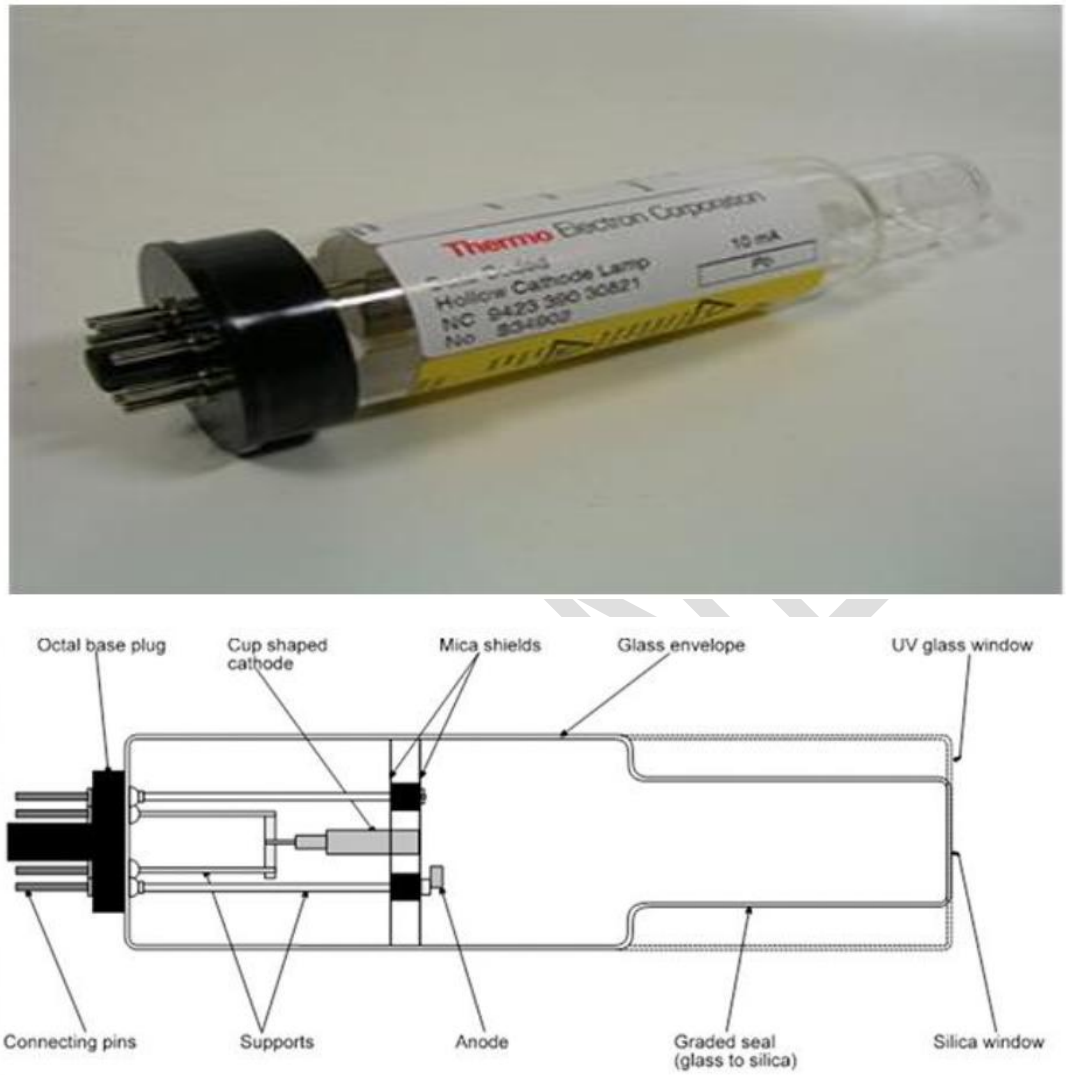


Fig. 2: Hollow Cathode Lamp

The process is known as sputtering. These atoms contain atoms in the excited states which emit their characteristic radiation as they return to the ground state. Eventually the metal atoms diffuse back on the cathode surface or redeposited on the glass walls of the tube. The cylindrical configuration of the cathode concentrates the emitted radiation and enhances the possibility of reposition of the atoms on the cathode rather than the glass wall. The efficiency of the HCL depends upon the geometry and the operating voltage. Higher the voltage and current, greater is the intensity. The advantage is somewhat offset by an increase in Doppler broadening of the emitted radiation. Further increased number of

unexcited atoms in the gas cloud in turn is capable of absorbing the same excited radiation. The process is known as 'self reversal' and lowers the intensity of the emitted radiation. A variety of hollow cathode lamps for most of the elements are available commercially. By using an alloy of 2-5 metals as cathode, more multi element determination is also possible.

ii) Continuum radiation source – D_2 lamp

A deuterium (D_2) lamp is a low pressure gas –discharge light source often used in spectroscopy when a continuous spectrum in the ultraviolet region is needed. It emits radiation extending from 112 nm to 900 nm, although its continuous spectrum is only from 180 nm to 370 nm.

iii) Atomizer

Sample atomization produces ground state atoms that are necessary for atomic absorption to take place. This involves the application of thermal energy to break the bonds that hold the atoms together. **Table 1** depicts the details of atomization. The complete atomization assembly comprises of the following components:

***Nebulizer*:** The nebulizer converts the liquid sample solution into a mist or aerosol. The nebulized sample is then carried into the flame. To meet varying analytical requirements, several different types of adjustable nebulizer are available. Some types are constructed from inert plastic to provide maximum chemical resistance when highly acidic or corrosive solutions are being analyzed.

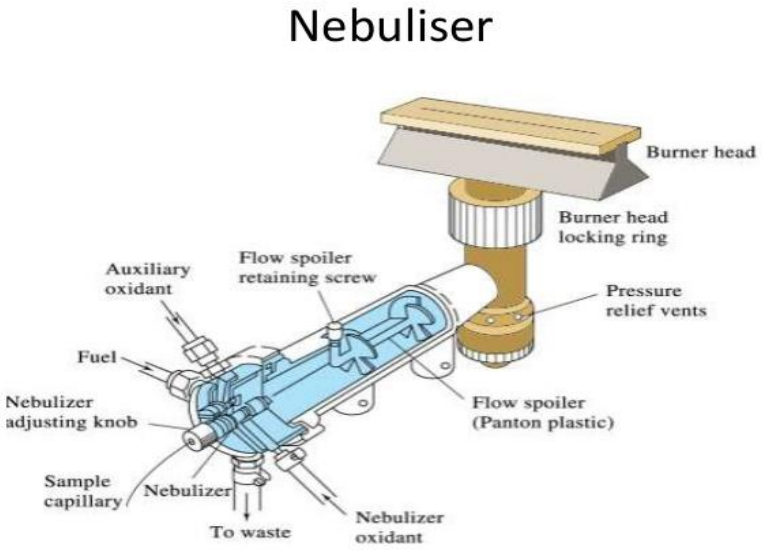


Fig. 3: A Nebulizer

Table 1: Atomization in AAS

Type	Method of Atomization	Radiation Source
Atomic (FLAME)	Sample solution aspirated into a flame	HCL
Atomic (Non-flame /Electrothermal)	Sample solution evaporated & ignited (2000 - 3000°C)	HCL
Hydride Generation	Vapour hydride generated	HCL
Cold Vapour	Cold vapour generated (Hg)	HCL

***Spray chamber*:** Aerosol from the nebulizer is led to the mixing or spray chamber where the aerosol is mixed with fuel and oxidant gases and carried to the burner head. Only a fraction of the sample introduced by the nebulizer is used for analysis. An impact device (flow spoiler or impact bead) prevents larger droplets from reaching the burner as these would delay the sample vaporization and atomization through short transit through the flame.

Burner head: There are basically two burner heads (50 mm slot Universal Finned Titanium Burner and 100 mm slot Titanium Burner). While the 50 mm slot burner can be used both for Air/acetylene and Nitrous oxide/acetylene flames, the 100 mm slot Titanium Burner is designed for air/acetylene flames only. Because of its long burner path length, it provides the best sensitivity for air-acetylene elements.

Flame (for Flame Atomic Absorption and Hydride Vapour System): The two oxidant/fuel combinations used almost exclusively in atomic absorption today are air-acetylene and nitrous oxide-acetylene. Air-acetylene is the preferred flame for the determination of approximately 35 elements by atomic absorption. The temperature of the air-acetylene flame is about 2300°C. **Table 2** elaborates the common fuels and oxidants used.

Graphite furnace or quartz tube (For Graphite Furnace Atomic Absorption): A graphite furnace uses argon or nitrogen gas at 1.04 bar (15 psi) with a purity of 99.999%. Argon gas is the preferred purge gas.

Table 2: Common fuels and Oxidants used in AAS

Fuel	Oxidant	Temperature (ºC)	Maximum Burning velocity (cm s^{-1})
Natural Gas	Air	1700-1900	39-43
Natural Gas	Oxygen	2700-2800	370-390
Hydrogen	Air	2000-2100	300-440
Hydrogen	Oxygen	2550-2700	900-1400
Acetylene	Air	2100-2400	158-266
Acetylene	Oxygen	3050-3150	1100-2480
Acetylene	Nitrous Oxide	2600-2800	285

iv) **Background corrector:** Deuterium lamps are used for background correction and analysis.

v) **Monochromator:** The light source from radiation source emits a spectrum specific to the element of which it is made, which is focused through the sample cell into the monochromator. A monochromator electronically modulates or mechanically chops the light so emitted from the source to differentiate between the light from the source and the emission from the sample cell. It disperses the light and the specific wavelength of light isolated passes to the detector.

vi) **Detector:** A detector detects the specific wavelength of light isolated by the monochromators. The light intensity passed through the detector can be measured with different measuring devices. In the beginning of flame photometry photographic light measurements were used, but nowadays instead of them solely photocells or photomultipliers of multiplying factor of 10^{10} are in use.

vii) **Control unit or Display:** A computer is the display unit.

5. APPLICATIONS OF AAS

The atomic absorption spectrophotometer is an inorganic analysis instrument. It can be widely used in the quantitative analysis of the almost all metals, metalloids and some non-metals (B, Si, P) elements in the metallurgical, geologic, oil, chemical, medicine and health, industrial,

farming and forestry, commodity inspection and environmental protection fields, etc. It can determine more than 70 elements in samples in the form of solutions in diluted mineral acids (HNO_3, H_2SO_4, HCl) resulting from the decomposition or hydrolysis of a solid sample, diluted biological fluids (plasma, blood, urine, milk, etc.), suspensions of solid samples (slurries), solid powdered samples, etc.

PART II: TECHNIQUES IN ATOMIC ABSORPTION SPECROSCOPY

1. FLAME ATOMIC ABSORPTION SPECTROSCOPY (FAAS)

Flame atomic absorption methods are referred to as direct aspiration determinations. They are normally completed as single element analyses and are relatively free of inter-element spectral interferences. For some elements, the temperature or type of flame used is critical. If flame and analytical conditions are not properly used, chemical and ionization interferences can occur. Different flames can be achieved using different mixtures of gases, depending on the desired temperature and burning velocity. **Fig. 4** represents a Flame-AAS while **Fig. 5** represents a schematic diagram of the parts of a conventional FAAS.

Sample introduction

The aim of sample introduction is to introduce samples (as fine aerosol) to the flame with good reproducibility and with high efficiency so that the interfering effects should remain minimal. The sample introduction is most often spraying the liquid. The small droplets formed in the nebulization transform gradually while going through the high temperature zones of the flame. It is an important aspect that the particle size of the droplets (< 5µm) should be possibly in similar size ranges in order the particles to be desolvated and to be further transformed in the same region of the flame.

Some elements can only be converted to atoms at high temperatures. Even at high temperatures, if excess oxygen is present, some metals form oxides do not dissociate into atoms. To inhibit their formation, conditions of the flame may be modified to achieve a reducing, non-oxidizing flame. The small droplets formed in the nebulization transform gradually while going through the high temperature zones of the flame. It is an important aspect that the particle size of the droplets (< 5µm) should be possibly in similar size ranges in order the particles to be desolvated and to be further transformed in the same region of the flame.

For the nebulization of solutions the indirect pneumatic nebulization is the most frequently used method which has sample introduction efficiency about 10%. The sample gets into the nebulizer through a plastic capillary.

Fig. 4: A Flame AAS

In the nebulizer, the sample leaving the capillary is surrounded by the gas (air) feeding the flame. After this high speed gas comes out through the tight emergent hole of the nozzle pressure decrease is established in the capillary which results the take up of the liquid. By the way the high speed gas breaks the uptaken liquid into small drops thus aerosol is formed. The latter phenomenon is called "Venturi effect".

Processes occurring in flame (Atomization)

From the liquid drop the solvent evaporates first, and solid aerosol particles (microsized crystals of salts) are formed, in the next step, it loses its crystalline water (if possessed) then the crystals melt and evaporate that is molecule vapour forms. In the higher regions of the flame the thermal dissociation of the molecules occurs: ground state atoms are formed (for atomic absorption measurements only these particles are useful). Of course, when the temperature of the flame makes it possible, the thermal processes go further, by the side of the ground state atoms excited atoms, even ions will be present in the flame (these excited particles are useful only for emission measurements).

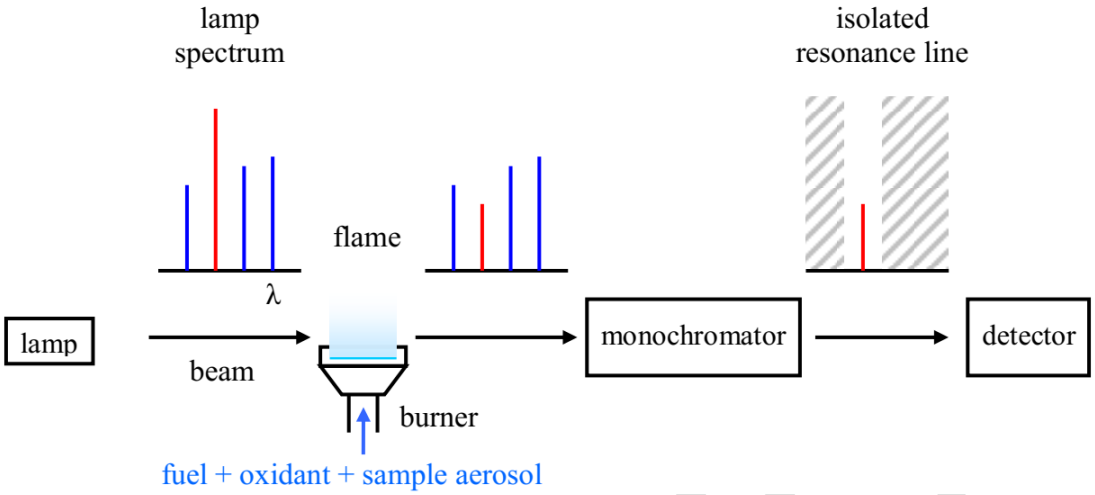


Fig. 5: A schematic diagram of a conventional FAAS

Detection

The light leaving the flame is led to the detecting unit with a proper optical device. However, due to the background radiation of the flame and the emission of the other metals present in the flame with direct measurements, interferences results. By the help of an optical device, these are eliminated and only the almost monochromatic metal characteristic light is sent to the detector. The monochromators are capable for the separation of small wavelength ranges with 0.01-1 nm width in a wide (190-800 nm) spectrum. The two major types of monochromators are prisms and optical (diffraction) grids. The desired wavelength is set by rotating the prism or diffraction grid. Although by increasing the size of the slit, the intensity of the light increases, however, the purity of the spectrum decreases. The material of the prism in UV range is quartz while in UV-visible range, it is glass. The advantage of the prism type monochromators is that they provide a spectrum with high light intensity although it has a disadvantage too: the resolution is low. Ultimately, the chopped light from the monochromators is passed through the detector and are measured with photomultiplier tubes (PMTs).

2. GRAPHITE FURNACE/FLAMELESS ATOMIC ABSORPTION SPECTROSCOPY (GFAAS)

Graphite furnace atomic absorption spectrometry (GFAAS) (also known as Electrothermal Atomic Absorption Spectrometry (ETAAS)) is a type of spectrometry that uses a graphite-coated

furnace to vaporize/atomize the sample. It is an electrothermal atomiser system that can produce temperatures as high as 3000°C. The heated graphite furnace provides the thermal energy to break chemical bonds within the sample held in a graphite tube, and produce free ground state atoms which in turn absorbs energy in the form of light, and are elevated to an excited state. The amount of light energy absorbed increases as the concentration of the selected element increases. This technique uses the same principles as FAAS with the only difference being the atomization; while FAAS can only analyze solutions in ppm range, GFAAS can accept very small absolute quantities of solution, slurry or solid samples in ppb range and is more sensitive.

Light source

The light source is the lamp that emits radiation of the resonance line (the hollow cathode lamp as discussed above).

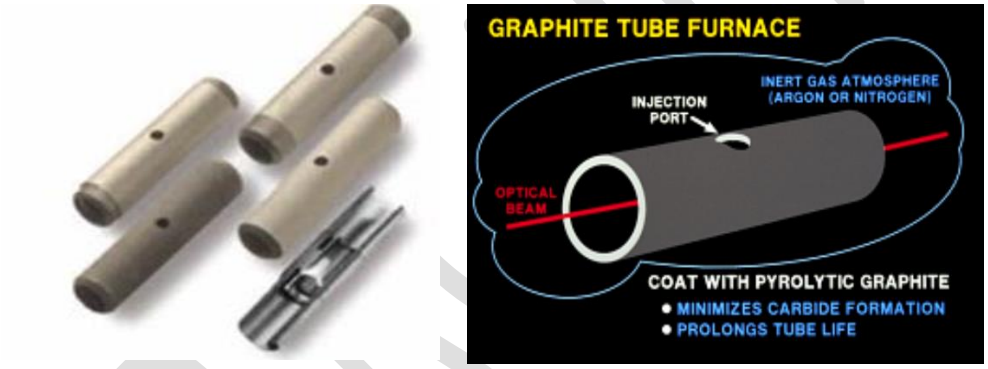


Fig. 6: Graphite tubes/cuvettes

Atomization Chamber

The heart of the Graphite Furnace is the atomization chamber which is made up of a graphite tube **(Fig. 6)** with a hole in the centre for sample introduction. It is encased within graphite electrical contacts at both ends and the sample is electro-thermally vaporised into atoms. A supply of water circulates the outside of the tube to keep the furnace cool, and an external stream of inert gas flows around the tube to prevent air from entering the chamber. GFAAS instruments comprise the following basic components:

Inert GAS

This protects the heated tube (cuvette) from atmospheric oxidation, and flushes sample vapours from the cuvette interior. An external gas flow surrounds the outside of the tube and an internal flow purges the tube. Argon is recommended; nitrogen can be used with some loss of performance for some elements.

Cooling Water

A supply of reasonably clean (e.g. drinking) water, at a temperature of less than 30ºC and a pressure of 1.4 to 6.9 bar (20 - 100 psi), capable of providing a minimum flow rate of 0.7 l/min is required. Do not allow the pressure to exceed 6.9 bar (100 psi).

Re-circulators

The Furnace can be cooled by a temperature controlled re-circulator/chiller unit instead of mains water. Set the recirculating water temperature to about 5ºC above ambient temperature, providing that this is less than 30ºC.

The GFS Auto-sampler: It provides fully automated sample injection facilities **(Fig. 7)**. It also provides facilities for automated matrix modification, and standard and sample solution preparation.

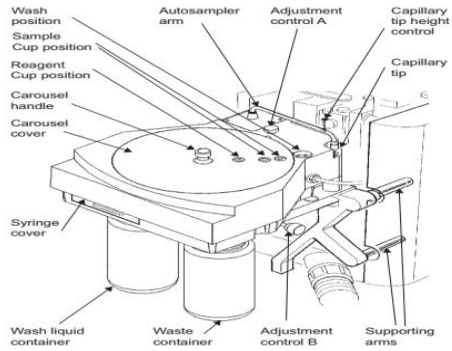


Fig. 7: Auto-sampler for Graphite Furnace

Drain Assembly

The GFS Auto sampler is fitted with a waste container to collect the used Wash liquid.

OPERATION OF GFAAS

Fig. 8 represents the various steps for the operation of a GFAAS.

i) The sample introduction

The sample is injected, usually between 10-30µl and sometimes the range could be extended to 100µl. The solution is preferably made up of dilute nitric acid as matrix. Sometimes solid samples are introduced.

ii) **The drying step**

Drying is done some degrees above the solvents boiling point (for water use 110°C) and about the same time in seconds as the injected volume in µl. The solvent should only volatilize and not boil, otherwise bad reproducibility may occur

iii) **Ashing of the sample**

This is considered to be the most important step. Sample matrix should be removed without losing the analyte. Conditions for every new sample type should be optimized. Inorganic matrix is more difficult, because higher temperatures are needed.

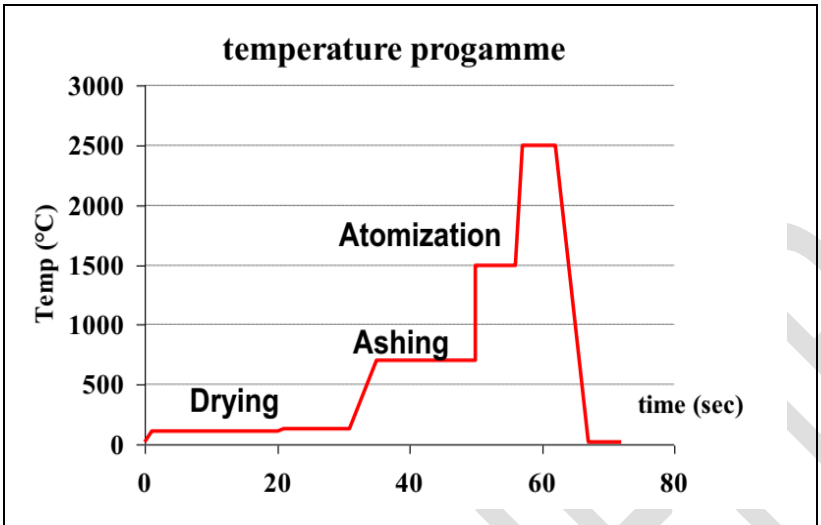


Fig. 8: Steps in operation of GFAAS

iv) Atomization step

The vaporization pressure of the analyte dominates the supply of analyte atoms inside the tube. The convection of expanding gas and diffusion dominate the transport of analytes out of the tube. Rapid heating is necessary for good sensitivity.

v) The burnout step

It is used for taking away parts of the sample not completely volatilized during atomization. Temperatures in the range 2700-3000°C is most often used. If vaporization of the sample is achieved, completely, the sensitivity will slowly decrease and the imprecision increase.

vi) Cooling step

Its purpose is to cool down the furnace before next sample is added. Benefits of graphite furnace analysis are:

- Entire sample is atomized and the atoms are retained in the atomization graphite tube for extended user controlled time periods
- Microlitre quantities of sample are sufficient and the quantity can be increased to 50 – 100 µl to enhance sensitivity
- Temperature programming steps help remove the solvent and major matrix interferences
- Detection limits typically 100 – 1000 times better than flame techniques are achievable thereby giving routinely analysis in µg/l(ppb levels)

Drawbacks of Graphite furnace Atomic Absorption

i) Longer analysis time in comparison to flame analysis

ii) Higher cost of graphite furnace assembly but it is also available as a switching option with flame operation in most commercial instruments

iii) Prone to interferences, poor precision

iv) Step to control precision:

- Auto-samplers vs. Manual Injection - more precise
- Duplicate analyses for all samples (repeat if poor agreement)
- Standard addition method-making standards up in sample
- Background correction for Molecular Absorption)
  - D_2- Quadline Correction
  - Zeeman Background Correction
  - Matrix Modifiers to control atomization

3. HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROSCOPY (HGAAS)

Hydride generation AAS employs a chemical reaction to create volatile metal-hydride species that can be analyzed in the vapour phase. Suitable liquid reagents are mixed with samples in a reaction zone to form the hydride vapour. This vapour is then separated from the liquid mixture in a gas-liquid separator and carried to an atomization cell that can be heated (if required). When heated, the hydride decomposes and releases atoms, which are then measured by atomic absorption. The cell can be heated using air-acetylene flame or an electrically heated furnace. For mercury analysis, no heating is required because the chemicals form elemental mercury, which passes as a vapour into the atomization cell.

Fig. 9: A hyderide Generation system

Many of the main parts of the HGAAS system are identical to that of AAS: a hollow cathode lamp, air/acetylene flame, and optical system but include (in most systems) an optical cell and the relatively complex hydride generation system. The nebulizer required in Flame AAS is **not used** in HGAAS.

COMPONENTS OF HGAAS

***Hollow cathode lamp*:** Provide the analytical light line for the element of interest and a constant yet intense beam of that analytical line (As discussed above in FAAS).

Hydride generation system

A hydride generation system **(Fig. 9)** sucks up (aspirate) liquid sample at a controlled rate. It mixes liquid sample with sodium borohydride and HCl and creates a volatile hydride of the analyte metalloid from that reaction. It helps in the flow that gaseous hydride into the optical cell.

The reaction of many metalloid oxyanions with sodium borohydride and HCl produces a volatile hydride. The oxidation state of the metalloid is crucial and care must be taken to produce the specific metalloid oxidation state before the sample is introduced into the hydride generation system. The time from reagent mixing and when the volatile hydride is separated from the liquid and sent to the optical cell is also important. The timing of that process is controlled by flowing reagents together using a peristaltic pump and tubing of specific lengths. After being mixed together the liquid mixture flows through a tube of a specific length (read this as a controlled reaction time) and is ultimately flowed into a gas/liquid separator where the hydride and some gaseous hydrogen (produced by the $NaBH_4$ + H_2 reaction) bubble out and are purged (via a high purity inert gas) into the optical cell via a gas transfer line. Most of the reagents introduced into the system flow to a waste container, and since the acid content is very high, often approaching 50%, as with AAS, the waste container is glass and must be handled carefully and labelled well.

Optical cell and flame

The optical cell **(Fig. 10)** is fused silica glass tube (transparent in the visible and UV wavelengths and thermally stable at high temperatures) through which the HCL's beam passes on the way to the monochromator and PMT. In some instruments it sits on top of the normal AAS air/acetylene flame. The gaseous, metalloidal hydride flows into the optical cell from the hydride generation

module pushes by an inert purge gas. In the optical cell it decomposes into the elemental form which can absorb the HCL's beam.

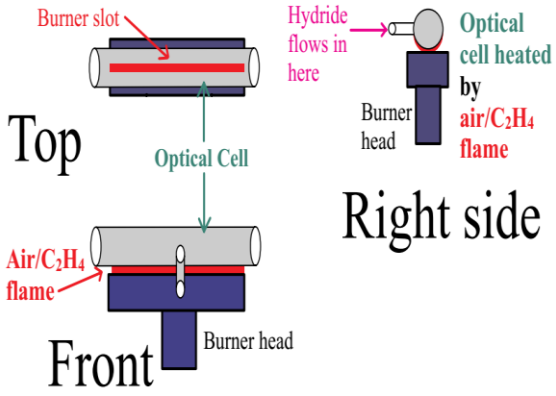


Fig. 10: Optical cell and flame

Monochromator and Photomultiplier Tube (PMT)

Tuned to a specific wavelength and with a specified slit width chosen, the monochromator isolates the hollow cathode lamp's analytical line. Since the basis for the HGAAS process, like AAS, is atomic ABSORPTION, the monochromator seeks to only allow the light not absorbed by the analyte atoms in the optical cell to reach the PMT. That is, before an analyte is aspirated, a measured signal is generated by the PMT as light from the HCL passes through the optical cell. When analyte atoms are present in the cell from hydride decomposition—while the sample **is** aspirated--some of that light is absorbed by those atoms (remember only volatile hydride gets to the optical cell and then only decomposed hydride produces the elemental form. This cause a decrease in PMT signal that is proportional to the amount of analyte. This last is true inside the linear range for that element using that slit and that analytical line. The signal is therefore a decrease in measure light: atomic absorption spectroscopy.

OPERATION OF HGAAS

Ignition, Flame conditions and Shut Down

The process of lighting the AAS flame involves first putting the optical cell in place and connecting the hydride gas transfer line. **Fig. 11** represents a schematic diagram of hydride generation process. Next the fuel and the oxidant are turned on and then the flame is on with the instrument's auto ignition system (a small flame or red-hot glow plug). After only a few minutes the flame is stable.

Deionized water or a dilute acid solution can be aspirated between samples (but experimentation is required to ascertain what produces the best reproducibility). An aqueous solution with the correct amount of acid and no analyte is often used as the blank.

To stabilize the HGAAS system the acidic blank is often flowed through the sample inlet tube for 5 or 10 minutes; although the longer this goes on, the more acidic waste is produced. Careful control of the fuel/air mixture is important because each element's response depends on successful decomposition of the volatile hydride in the heated optical cell. Remember that the flame's heat must break down the hydride and reproducibly create the elemental form of the analyte atom. Optimization is accomplished by aspirating a solution containing the element (with analyte content about that of the middle of the linear response range) and then adjusting the fuel/oxidant mix until the maximum light absorbance is achieved. Also the position of the burned head, optical cell, and sample uptake rate are similarly "tuned." Most computer controlled systems can save variable settings so that methods for different elements can be easily saved and reloaded.

Shut down involves aspirating deionized water through all three inlet tubes (borohydride, acid, and sample inlets) for a short period and then closing the fuel off first. Most modern instruments control the ignition and shutdown procedures automatically. The plastic tubing that is stretched around the peristaltic pump head is loosened to length its lifetime. Finally the purge gas is turned off.

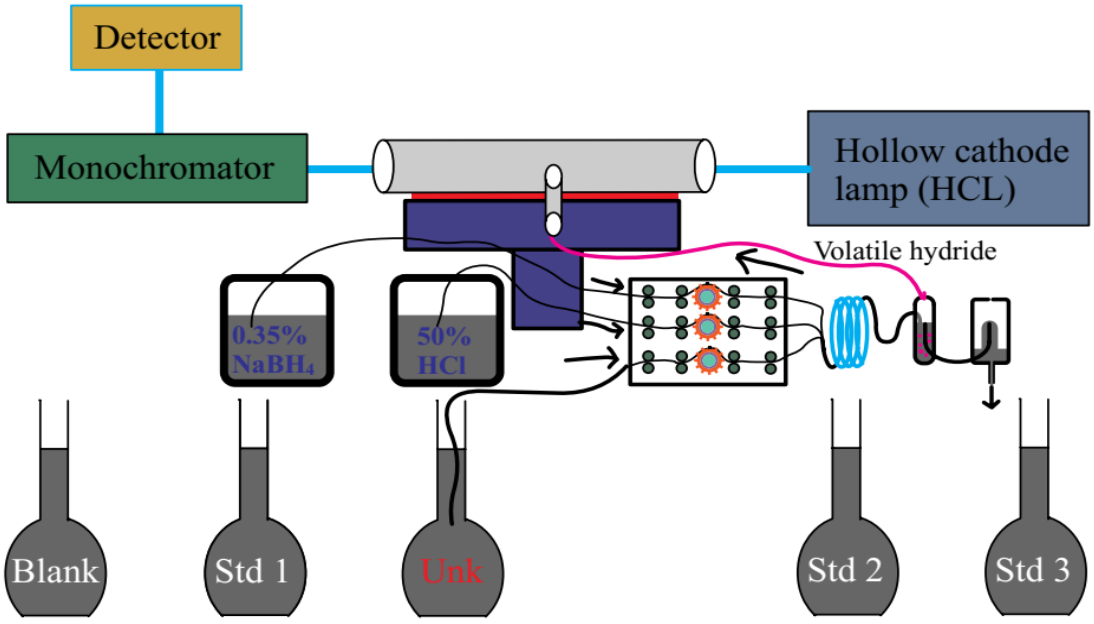


Fig. 11: A schematic diagram of hydride generation process

PART III: INTERFERENCES IN AAS

Any analytical instrumental technique, however sensitive, simple or rapid, is not free from all interferences. Hence the origin or source of the interferences should be known so as provide analytical data very accurately and precisely. AAS is one such analytical instrumental method and it has got much inherent interference from the sample introduction stage to the detector. Originally it was thought to be free from interferences as we measure very narrow resonance line from the hollow cathode lamp passing through the atomizer either in the flame or non-flame.

The interferences may be classified as follows:

i) Chemical interferences

The most common interferences in atomic absorption are chemical interferences. If the sample being analyzed contains a thermally stable compound with the analyte that is not totally decomposed by the energy of the flame, a chemical interference exists. As a result, the number of atoms in the flame capable of absorbing light is reduced. Chemical interferences can normally be overcome or controlled in two ways: the use of a higher temperature flame or the addition of a releasing agent ($LaCl_3$, La conc. up to 10 mg/ml) to the sample (or standard) solution. A releasing agent, or competing cation, when added to the sample solution will preferentially react with the interferent releasing the analyte and thus removing the interference. A higher temperature flame will provide additional energy to break down a compound which is stable in a lower temperature flame.

ii) Ionization Interferences

Ionization interferences occur when the flame temperature has enough energy to cause the removal of an electron from the atom, creating an ion. As these electronic rearrangements deplete the number of ground state atoms, atomic absorption is reduced. Ionization interferences can be controlled by the addition of an excess of an easily ionized element to the blank, standards, and samples. For this purpose, the alkali metals (K, Na, Rb, Cs) which have very low ionization potentials, are normally used.

iii) Matrix interferences

Matrix interferences can cause either a suppression or enhancement of the analyte signal. Matrix interferences occur when the physical characteristics (viscosity, burning characteristics, surface tension) of the sample and standard differ considerably. This can happen when the sample solution contains a high concentration of dissolved salts or acid, when different solvents are used for sample

and standard solutions, or when the sample and standard solutions are at radically different temperatures. This interference can be controlled by preparation of the standard solutions used to construct the calibration curve at similar physical conditions of the solvent of the sample. These interferences can also be eliminated by diluting the sample solutions. The presence of high concentrations of dissolved salts in a sample can reduce the analytical signal. It also leads to the formation of incrustation of the nebulizer and the burner head and may lead to high signal noise levels.

iv) Spectral Interferences

A spectral interference can occur when an absorbing wavelength of an element present in the sample but not being determined falls within the bandwidth of the absorption line of the element of interest. The results of the determination will then be erroneously high, due to the contribution of the interfering element to the atomic absorption signal. When multi-element lamps are being used, a combination of elements may exist that will generate the possibility of a spectral interference. The slit width normally used with single-element lamps may be large enough to pass an absorbing wavelength of another element present in a multi-element lamp. This can be overcome by using a smaller slit or selecting an alternate wavelength.

v) Emission Interferences

At high analyte concentrations, the atomic absorption analysis for highly emissive elements sometimes exhibits poor analytical precision, if the emission signal falls within the spectral bandpass being used. This degradation occurs because the electronic noise of the photomultiplier is proportional to the total signal incident upon it, even though only the modulated atomic absorption signal is being measured. There are several ways to compensate for this interference, including decreasing the slit width, increasing the lamp current, diluting the sample, and using a cooler flame.

vi) Background Absorption

There are two causes of background absorption: light scattering by particles in the flame and absorption of light by undissociated molecular forms of matrix materials in the flame. To compensate for this problem, the background absorption must be measured and subtracted from the total measured absorption to determine the true atomic absorption component. Fortunately, background absorption can be distinguished from the absorption due to the element of interest. The element can absorb only the narrow line emitted by the source lamp; background absorption is less specific and extends over a broad wavelength band. The most common way to compensate for background absorption is to use a background corrector, which utilizes a continuum source (a deuterium arc lamp in the ultraviolet or a

tungsten-iodide lamp for visible wavelengths). A continuum source emits light over a broad spectrum of wavelengths instead of at specific lines. With background correction, simultaneous compensation is obtained at the same wavelength used to measure atomic absorption.

APPENDIX I: SAMPLE PREPARATION, STANDARD PREPARATION, STORAGE AND EXTRACTION PROCEDURE FOR MICRONUTRIENT AND HEAVY METALS ANALYSIS FROM DIFFERENT SAMPLES

Soil sampling for micronutrients and heavy metal analysis

Soil testing is the chemical analysis of soil and is an effective scientific tool for the rapid characterization of soil fertility status and predicting the nutrient requirement of the crop plants. It is essential to predict whether a soil can supply adequate amount of nutrient required by a crop for optimum crop production or not. It may not exactly measure the quantity of nutrient element available to plant, but can estimate the amount of nutrient element that is available in extractable form in soil by using a particular chemical extracting solution. This amount of extractable form of nutrient element can be used to predict the crop yield response to the nutrient application as fertilizers, manures or other amendments. A proper soil test should estimate as nearly as the possible critical level of an extractable nutrient element below which plants will be deficient and above which no deficiency symptoms occur. Furthermore, a proper soil test should be useful detect toxic or potentially toxic levels of micronutrients and heavy metals that may occur. Soil testing for micronutrients and heavy metals is particularly difficult because plant requirement for these micronutrients and heavy metals are relatively very low, and even a very slight contamination of the sample may affect the sample test result seriously. Therefore proper care should be taken while collecting soil sample, processing and preparation for soil testing. A soil-testing programme has four phases as follows:

1. Collection of soil samples,
2. Chemical analysis of soil samples,
3. Calibration and interpretation of the results of chemical analysis, and
4. Nutrient/Fertilizer Recommendation.

Collection of soil sample

Soil sampling for micronutrients and heavy metals analysis is the greatest challengeable task since a few grams of soil samples represent a given area and reflect the true fertility of the field. Therefore soil samples are required to be taken in such a way that the collected sample should represents the true

fertility of soil for any given area. Most of the fields are highly variable with topography or slope, colour, texture, crop growth and management practices. Therefore, in such areas, separate sets of composite samples should be collected for soil testing. Even, levelled fields that appear uniform may vary highly with nutrient content. The areas such as channels, buds, near trees, recently fertilized plots, wells, cattle dung and compost piles or other non-representative locations must be avoided during sampling. Therefore, some of the following common and general principles should be strictly followed to make correct soil sampling:

1. A series of cores taken according to some systematic grid layout of the area of equal diameter and comparable depth should be composite.
2. Separate soil cores should be analysed or replicate sets of composites made to determine statistical significance of results on the final composite.
3. The number of cores to be composited will depend on the variability of the soil, the degree of accuracy desired, the particular element or elements to be determined.
4. Cultivated soils are generally more variable than virgin soils, and acidic/saline are extremely variable.
5. Separate composites samples representing different segments of the soil profile or root zone should be taken.
6. Contamination from soil surface materials (Crop residues, manures, fertilizers, etc.) should be avoided, also contamination of one soil depth.

For collecting surface soils (0-15cm), a soil probe, soil auger or spade can be used. Similarly, a post-hole digger can be used to get a continuous core with minimal disturbance of the soil from the subsurface soils (15cm to desirable depth). The cores can be divided for the various depths. There should be very little contamination of subsoil sample with surface soil when using a soil probe. A soil probe cannot be used when the soil is too wet, too dry, or frozen. Soil probes cannot be used in soils that contain gravel. The soil auger can be used in soils that are frozen or contain gravel. For the determination of micronutrients and heavy metals, surface (0-15cm depth) samples are frequently used. However, for the deep rooted crops like cotton, sugarcane, plantation and horticulture crops soil samples up to 80-100 cm needs to be collected.

Moist, well-mixed samples may be transferred to paper bags, cardboard boxes or aluminium trays of convenient size. In artificial drying, the open sample container is then placed in a drying rack or

cabinet equipped with exhausts fans to expedite air movement and moisture loss. For natural sample drying, the samples may be spread on clean surfaces, such as paper plates, newspaper, etc. and it may be dried under shade. Initial crushing of soil clods will decrease the time required for drying at room temperatures. After drying, the soil samples may be crushed, sieved through 2mm sieve and used for the analysis of micronutrients and heavy metals. It should be essentially ensured that all surfaces coming into contact with the soil be stainless steel, plastic or wooden, preferably in the order listed. Samples should be crushed until a major portion of the sample will pass 2 mm opening sieve. Crushing to pass a finer mesh sieve may be desirable for analysis utilizing less than one gram of soil.

Sample extraction for available micronutrients in soil:

For the estimation of available micronutrients (Fe, Mn, Zn and Cu) in soil, the DTPA method proposed by Lindsay and Norvell (1978) has been commonly used by the researchers in the soil testing laboratory.

Principle:

DTPA (Diethylene Triamine Penta Acetic acid), a mild chelating agent, in combination with calcium chloride ($CaCl_2$) is used in this method. It combines with the free metal ions (Fe, Mn, Zn and Cu) in solution and forms soluble complexes. This extracting solution, which extracts the easily soluble zinc, iron, copper and manganese, is buffered at pH 7.3 by triethanolamine (TEA). The pH of 7.3 buffered with TEA is used to prevent excess dissolution of the trace metals, which is highly pH dependent. Calcium chloride is used to prevent the dissolution of calcium carbonate from calcareous soils. The dissolved elements in the extract are, then, measured by AAS.

Standard solution of Zn, Cu, Fe and Mn

Prepare 100 ml stock solution of each element as below:

Element	**Salt**	**Mol. Wt**	**Quantity (g) salt required for the preparation of solution 100 ppm**
Zn	$ZnSO_4.7H_2O$	287.56	0.4398
Cu	$CuSO_4.5H_2O$	249.69	0.3929
Mn	$MnCl_2.4H_2O$	197.91	0.3602
Fe	$FeSO_4.7H_2O$	278.02	0.4977

For the preparation of working standard, make at least 8-10 working standards of each micronutrient with a range from 0 to 3.0 ppm for Zn and Cu, and from 0 to 20 ppm for Fe and Mn. The medium used for the extraction of micronutrients from the soil needs to be used for the preparation of working standard solutions. Measure the elements in the extract by AAS first running a series of standard of known element, then, analyzing the prepared samples.

Reagents

1. **Dilute HCl:** Dilute AR grade HCl five times with double distilled water
2. **DTPA extracting solution**: 0.005 M DTPA, 0.01 M $CaCl_2$, 0.1M TEA $(HOCH_2CH_2N)_3N$ adjusted to pH 7.3 with dilute HCl- Dissolve 14.92 g TEA (AR), 1.967 g DTPA, and 1.47 g $CaCl_2$. $2H_2O$ in approximately 200 ml of pure double distilled water in 1 litre capacity of volumetric flask. Allow sufficient time for the DTPA to dissolve, and dilute to approximately 800 ml. Adjust the pH 7.3±0.05 with 1 N HCl while stirring, and dilute to 1 litre. This solution can be kept for several months for use.
3. **Standard stock solution**: Prepare standard stock solution for each metals and prepare the working standards as mentioned above.

Procedure

Take 10 g of soil

↓

Add 20 ml of extractant (DTPA)

↓

Continuous shaking for 2 hours

↓

Filter by Whatman No.42 filter paper (if filtrate is cloudy, re-filter as necessary)

↓

Estimation of Zn, Cu, Mn and Fe by AAS in the extract

Sample extraction for total micronutrients and heavy metals in soil

The method for total micronutrients and heavy metal analysis in soil is same as the total micronutrients and heavy metals analysis in plant samples as described in the below section.

Total micronutrients and heavy metals analysis in plant sample: Plant analysis is often recommended to evaluate the soil fertility status and plant nutrient uptake during the growing season. Furthermore, it is used to monitor the micronutrients levels and to develop a foliar application spray rate of selected micronutrients. It is most commonly used to diagnose the nutritional disorders related to soil fertility or to monitor the efficiency of the fertilizer application and management practices on growing crops. The need of fruit plants for mineral nutrients differ from those of annual crop species in a number of ways, many of which are related to the perennial nature of tree crops. In perennial tree crops, there is a need to supply nutrients both for ultimate fruit production, the fruits and the vegetative organs, which persist from year to year. The need for leaf analysis in perennial horticultural crops and forest trees has proved its superiority over soil diagnostic methods. This is essentially because of their deeper root system and the fact that nutrients supplied in one year may have its effect on both the nutrition and crop production in following years. However, it is largely being used not as an alternative method to soil testing, but as a supplement to soil testing. Results of foliar analysis are usually interpreted using the critical nutrient level (CNL) approach. Accurate interpretation of foliar analysis using the CNL approach is possible only when sampling is restricted to the same growth stage at which standard reference values have been established (Beaufils, 1971, 1973).

The general purposes of plant analyses are:

1. To diagnose or confirm diagnosis of visible symptoms.
2. To identify hidden hunger.
3. To locate areas of incipient deficiencies.
4. To indicate whether applied nutrient has entered the plant.
5. To indicate interactions or antagonisms among nutrients.
6. To aid the understanding of internal plant functioning.
7. To suggest additional test to identify the trouble.

The element nutrients of the plant tissues can be estimated in the extract of plant obtained from the fresh plant tissues (i.e. tissue analysis), and in whole or parts of the dried plant material. The tissue test is qualitative and only valid for the quick guidance of the growing crops. Total nutrient analysis of the plant is quantitative in nature and is more reliable and uses to categorize the ranges of deficiency, low,

sufficiency, and excess of the nutrient concentrations in the crops grown. The plant tests are divided into tissue tests, petiole analysis or total analysis.

Plant tissue analysis can provide on-the spot field tests. Kits vary from a few chemicals and test papers to elaborate supplies of chemicals, vials and spot plates, which are better to use indoor. Nutrient levels determined from these tests are accurate only in terms of high, medium or low values. The critical concentration can be directly determined only from the relationship of the nutrient concentration to the yield or quality. The tissue should be chosen which best shows their relationship through regression.

The tissue, which is best, suited for determination of deficiencies, is not always best for toxicity of nutrient elements. For a number of nutrients, the concentration in the roots may be abnormally high and limit growth, while the concentration in the tops may be in normal range (e.g. Cu, Na, and Al). The practical problem involves in sampling and cleaning of roots limit the analysis.

Total analysis gives the accurate status of nutrient across a variety of crops. The analyzed nutrient concentrations are reported in ppm (part per million) for micronutrients and in primary and secondary in percentage. The total plant analysis can also be used if toxic levels of certain non-nutrient elements present in plant. Visual and analytical diagnosis during plant growth and development provide two complementary methods for identifying nutritional disorders of plants, which permit them to be distinguished from phyto-pathological damage too.

Sampling

For plant analysis to be meaningful, collection of particular plant part (tissues) at the right stage of growth as per technical specifications is very important. It would be wrong and wasteful to just pluck any leaf or branch from a growing plant at any time and send them to a laboratory for analysis. No amount of analytical skill or sophisticated instrumentation can take the place of correct sampling methodology. The deviation in the sampling procedure may be necessary under specific circumstances such as occurrence of deficiency/toxic symptoms or for nutrient uptake. The index tissue sampling procedure is given in **Table 3** is designed for nutrient diagnosis, monitoring and efficient nutrient management for economic optimum yield and for excellent quality.

Sample preparation

After sample collection, the fresh tissues should be decontaminated from dust and other foreign material by adopting the following procedure. Plastic containers are taken in which the following solutions are added.

1. Wash in 0.2% liquid detergent to remove waxy coating on leaf surface +soil particle.
2. Dip in 0.1N HCl (8 ml conc. HCl/litre) plastic container.
3. Rinse with tap water thoroughly.
4. Finally wash with distilled water.

The extra moisture is wiped out; the sample is placed in new paper bags and dried in an oven at 60-70^{o}C. In the laboratory the samples are homogenized using a sample mill, which should not give any metallic contamination. The plant samples (grain and straw/stalk) of mature crops should be taken in such a way that can represent the average content of nutrients in the samples. Dry the collected samples in the hot air oven at 60-70 ^{0}C for complete drying. Grind the dried samples in a grinding mill and sieved through 2 mm sieve and keep in polythene bags or bottles for micronutrients and heavy metal analysis.

Table 3: Plant tissue sampling guidelines for different crops.

S/N	Crop	Index tissue	Growth stage
Grain, pulses, oilseeds, fibre and commercial crops			
1	Rice	3rd leaf from apex	
2	Maize	The leaves below and opposite to ear	Tasselling to silking
3.	Wheat	Flag-leaf	Before head emergence
4.	Barley	Flag-leaf	Head emergence
5.	Oat	Flag-leaf	Before inflorescence emergence
6.	Pulses	Recently matured	Bloom initiation
7.	Cotton	Petiole, 4th leaf from the apex	Initiation of flowering
8.	Groundnut	Recently matured leaflets	Maximum tillering
9.	Soybean	3rd leaf from top	2 months after planting
10.	Mustard	Recently matured leaf	Bloom initiation
11.	Sunflower	Youngest mature leaf	Initiation of flowering
12.	Jute	Recently matured leaf	60 days
13.	Sugarcane	3rd leaf from the top	After 3-5 months of planting
14.	Tea	3rd leaf from the tip of young leaf	
15	Coffee	3rd or 4th pair of leaf from apex of lateral shoots	At bloom
16.	Banana	Petiole of 3rd open leaf from apex	Bud differentiation 4 months after planting
17.	Grapes	5th petiole from base	Bud differentiation stage for yield forecast
18.	Citrus	3-5 month old leaf from March flush	Mature leaves from the non-bearing terminals

19.	Guava	3rd pair of recently matured leaves	Bloom stage (Aug.-Dec)
20.	Mango	Leaves + petiole	4-7 months old leaves from middle of shoot
21.	Papaya	6th petiole from apex	6 months after planting bloom
22.	Passion fruit	Matured leaf opposite to last open flower	Bloom
23.	Pineapple	Middle one third portion of white basal portion of 4th leaf from apex	4-6 months
24.	Almond	3rd leaf from top	At the beginning of bloom
25.	Apple, pear	Leaf from the middle of the terminal shoot growth	8-12 weeks after full bloom, 2-4 weeks after the formation of terminal buds in bearing trees
26.	Peach	Mid-shoot leaves, fruiting or non-fruiting spurs, mid-summer leaves	At fruiting
27.	Strawberry	Youngest, fully expanded matured leaf without petiole	At peak or harvest period
28.	Plum	Leaves from the middle of current season's extension growth	January to February
29.	Pomegranate	8th leaf from apex	At bud differentiation, in April and August
		Vegetables	
30.	Beans	Upper most recent fully developed trifoliate leaves	
31.	Cabbage	Wrapped leaf	2-3 months old
32.	Carrot	Most recent fully developed	Mid grown
33.	Cauliflower	Most recently fully developed leaf	At heading
34.	Brinjal	Leaf blade with midrib minus petioles fro most recently matured leaves	
35.	Potato	More recent fully developed leaf (half grown)	
36.	Garlic	Most recent fully developed	Pre-bulb
37.	Onion	Top –no white portions	1/3 to ½ grown
38.	Peas	Leaflets from most recent fully developed leaflets	First bloom
39.	Tomato	Leaves adjacent to inflorescence	Mid-bloom
40.	Radish	Most recent fully developed	
41.	Turnip and Sugar beet	Most recent fully developed	At 30-50 days
42.	Spinach	5th leaf from tip	At bud starting to small fruits
43.	Cucumber	Most recent fully developed	

WET ASHING/ WET OXIDATION/ WET DIGESTION OF PLANT SAMPLE

Reagents and Materials:

1. Ternary acid or Tri-acid mixture.: Mixture of HNO_3 : H_2SO_4 : $HClO_4$: : 10: 1: 4 on volume basis
2. Conc. HNO_3 for pre-digestion
3. 6 N HCl for volume make up
4. Hot plate with temperature regulator
5. Conical flask (50 ml)
6. Volumetric flask (100 ml)
7. Chemical balance
8. Measuring cylinder

Procedure:

Transfer 0.5 to 1 g of ground plant sample to 50 ml conical flask

↓

Add 5 ml conc. HNO_3 and leave it overnight

↓

Digest at low temperature for 30-45 min *i.e.* pre-digestion

↓

Cool and add 5 ml of Tri-acid mixture.

↓

Resume heating with high temperature (180-200^{0}C) till fumes of H_2SO_4 evolve

↓

If the digest appears to be on the verge of turning into a black mass – stop heating

↓

Cool the flask, add 5 mL of acid mixture and resume heating
(When the material is exceptionally fatty in nature)

↓

Continue the digestion until almost all of the $HClO_4$ volatilized leaving only a *snow-white residue*

↓

Cool the digest and wash down the neck of the flask

↓

Transfer quantitatively to 100 or 50 mL vol. flask with 6N HCl

↓

Makeup the volume with 6N HCl to keep all elements in the solution

↓

Determination of various micronutrient elements is carried out

APPENDIX II: A STEP BY STEP FLAME ANALYSIS FOR ZINC (Zn) USING FLAME-AAS

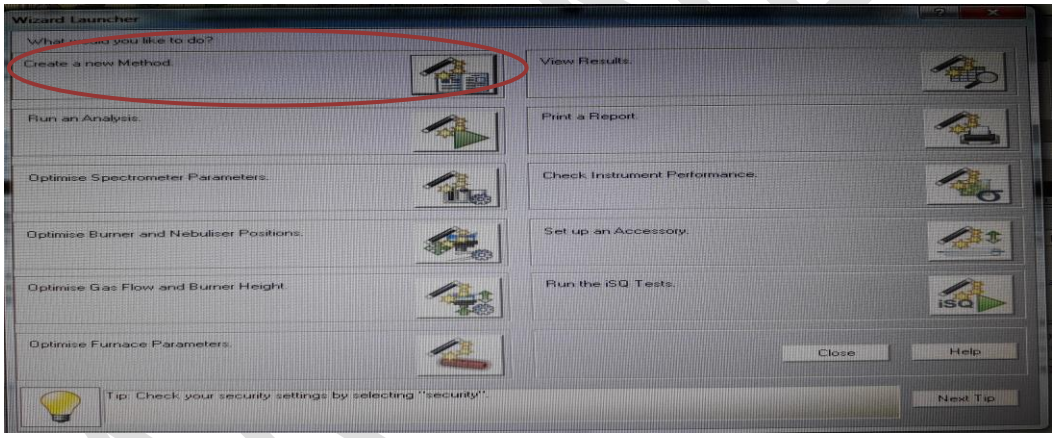
This section will guide through the process of setting up and performing a simple Flame Atomic Absorption Spectroscopy (FAAS) analysis of Zinc. (It is assumed that the Atomic absorption Series spectrometer system has been correctly installed and tested, and the various services are correctly connected). Make sure that your samples are extracted and standard solutions are ready with you. *(See Appendix I for extraction of sample and standard solution preparation)*

I. **Switching On**: Switch on the main electric switches, UPS, Computer and on the machine (Flame AAS).

II. Setting up a method

i) Open the software by clicking the icon on the desktop. Then the window will show online (Machine is Ready)

ii) Open the **wizard** icon on the toolbar



iii) And click on C**reate method**

iv) Set up the method using the method property page

v) Save the method in the method database. Then close the window.

III. Loading Methods

To display the method created, click on the Show method button. Note that the method you have defined will remain as the **Current Method** until you edit it, load a saved Method or create a new method. This means that it doesn't matter if you have closed the Method property sheet before saving the Method-if you open the method property sheet again it will show the settings you made before closing it.

Saving a method in the method database

1. Click on the General tab to display the General property page
2. Click on SAVE.
3. You will be asked to confirm that you want to save the Method in the database using the name in the Method Name box.
4. Click on Yes to save the Method and close the dialogue box and return to the General Method property page.
5. Click on OK to close the Method property sheet.

Loading a previous saved Method

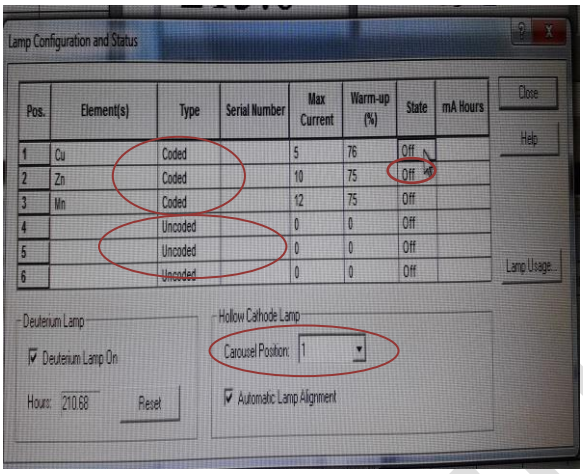
1. Click on the Methods button or click on Method in the Edit menu to open the Method property sheet at its General property page.
2. Click on Load to display the Method Library dialogue box. This lists the Methods saved in the Methods database.
3. Click on the required Method in the list-the description entered when you created the Method will be shown in the Description box to help you identify the Method.
4. Click on Load to close the dialogue box and load the Method.

(Check the sequence of the sample, calibration details, Flame, etc and save it. If any edition was made, then go to the general and load the latest saved data and close the window).

IV. Installing a lamp and optimizing the burner parameters

To perform any atomic absorption analysis, you must first install a hollow cathode lamp in the spectrometer. To install the hollow cathode lamp:

1. Check the Status Bar and confirm that the spectrometer is ONLINE.
   If the status Bar says OFFLINE, click on Connect in the Action → Communications submenu.
2. Click on the Lamps button  or click on Lamps in the Edit menu to open the Lamp Configuration and status dialogue box. On the lamp.
3. Select the carousel position that you want to use for the lamp.



4. If there are any lamps fitted already and their **state** is **On**, click in their **State** boxes to set them to **Off**.
5. Fit the new lamp (If you are using an uncoded lamp, you will need to define its characteristics). Confirm that the element(s) in the lamp are displayed in the element field; the Type field shows Coded or Uncoded as appropriate; the Max Current field shows the maximum current marked on the lamp.
6. Once done, close the window.
7. Go to Action button on the toolbar and in the submenu find Lamp and click the Auto Align in the submenu. The window will show BUSY at the extreme right lower corner. Wait till it shows ONLINE.
8. It is good practice to optimize the burner position and the nebulizer impact bead before starting work. The optimize burner and nebulizer positions wizard can help you do it.
9. It is important that you allow the burner to warm up properly before you start to take measurements, to ensure that your results are stable, and to minimize carbon deposition when using a nitrous oxide supported acetylene flame.

NOTE: Before lighting the flame, you must first check that:

- An inert explosion disk is fitted in the back of the spray chamber
- The correct gas is connected to the spectrometer and correct pressure is set.
- The drain trap is full
- The drain is flowing freely
- The burner is clean and fitted correctly

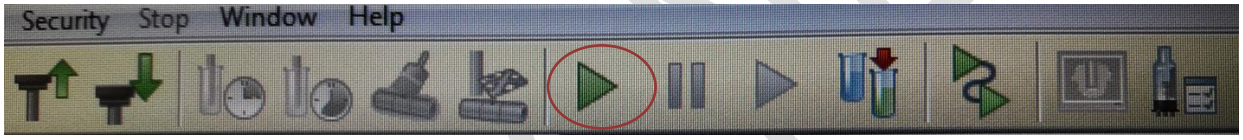
- The spectrometer sample compartment door is fitted and closed.

V. Background Correction

1. Go to Action. In the submenu, click the ***Set up optics.*** Background correction will take 1-2 minutes. After the set up optics is over, the window will show online. Switch on the compressor. Check if the compressor pressure is set between 35-40 psi. Remove excess water deposited in the moisture tube. Switch on the gas cylinder (Air-Acetylene). On the burner and switch on the chimney for exhaust. (All flames produce large quantities of heat and toxic combustion products. These must be removed by a suitable fume extraction system).

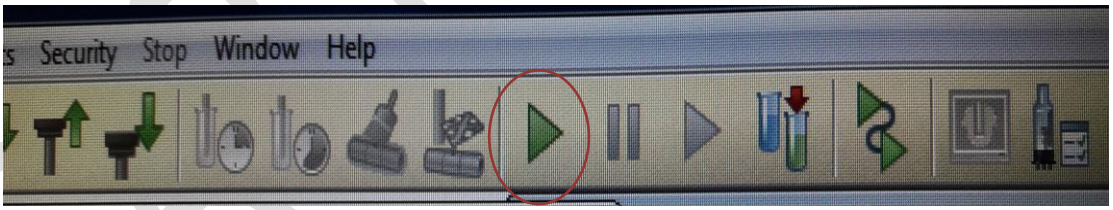
VI. Running the Analysis

Open the results window so that you can see your results as they are measured, and the calibration window to see the calibration plot as it is built from the standard solution results. Click on the Analyse button to display the start Analysis dialogue box.

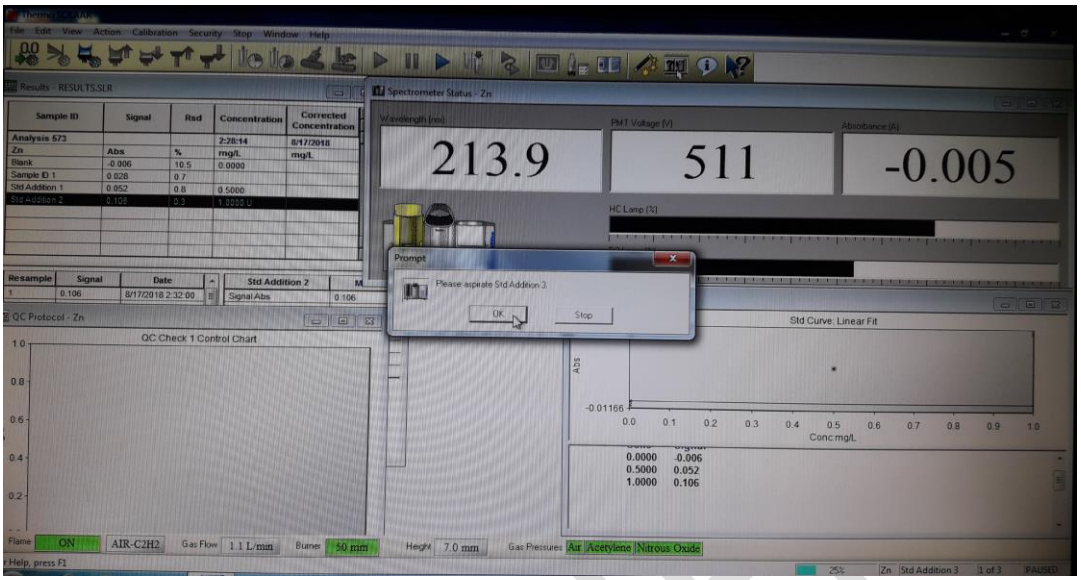


i) Auto zero Correction

You may either choose the Auto zero button on the main tool bar or click the analyze button.



Type in an analysis Name for the analysis-this will be used to identify the results. If required, edit the name in the operator field-it shows the name of the logged in user by default. Click on Analyse to start the analysis and confirm that the system status shown on the right of the status line changes to BUSY while the spectrometer is set up. As the analysis proceeds you will be prompted to aspirate the solution required for each stage, for example, you will be asked to aspirate blank for auto zero setting, then aspirate the standard solutions followed by the unknown samples.



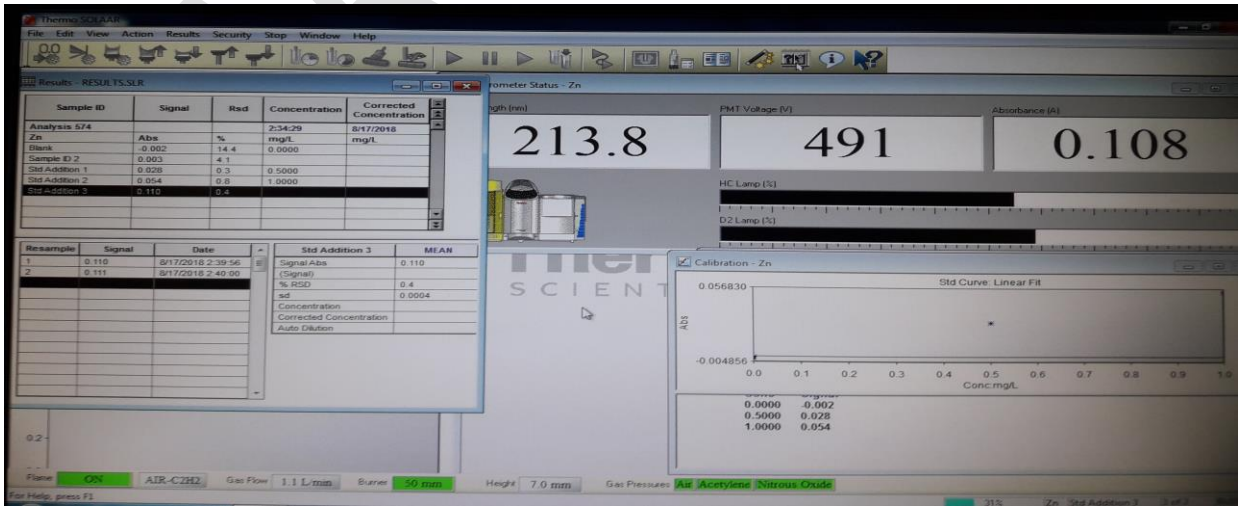
Viewing the results

When you run an analysis the results are saved automatically in the results database. You can view any or all of the results stored in the database. To view the results of your analysis:

i) Open the Results window- the mean results for each solution will be displayed in the upper table.

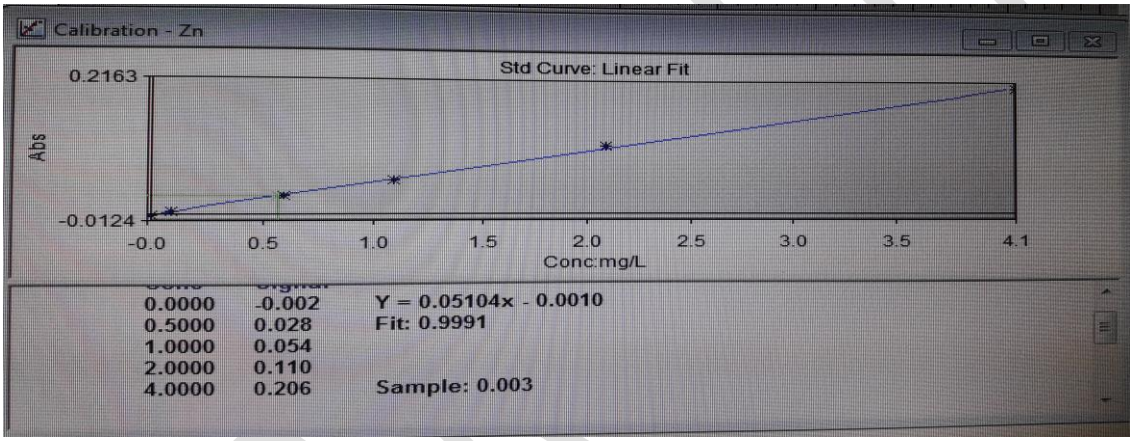
ii) Use the scroll bar to the right of the upper table to scroll through the results

iii) Open the calibration window. Confirm that the calibration details are displayed in the lower part of the window.



STANDARD PREPARATION (MICRONUTRIENTS)

Elements					
Copper (ppm)	Zinc (ppm)	Iron (ppm)	Manganese (ppm)	Boron (ppm)	Molybdenum (ppm)
1	1	5	5	0.1	0.1
2	2	10	10	0.5	0.5
4	4	15	15	1.0	1.0
		20	20	1.5	1.5



APPENDIX III: CAUTIONS WHILE USING FLAME - AAS

1. Always ensure good lab conditions, dust-free environment and UPS power for the equipment.
2. Check the Gas Pressures and remove water from Air compressor if present before starting the flame.
3. Nitrous oxide supported flames burn at a high temperature, and emit large amounts of heat, which can cause serious injury.
4. Nitrous oxide/acetylene flames can deposit carbon along the edges of the burner slot, which can build up and partially block the slot, causing a serious hazard.
5. Ensure that all gas lines, fittings, etc., are free from oil or grease contamination. Check regularly for leaks in all gas lines and fittings. Always follow the Safety Guidelines provided by the Gas Supplier. Fit gas cylinders with appropriate pressure reducing regulators. It is strongly recommended that acetylene gas supplies should also be fitted with flash back arrestors.
6. Acetylene is highly inflammable and forms an explosive mixture with air. It has a strong garlic-like odour caused by inherently present impurities.
7. Acetylene gas is supplied in cylinders dissolved in acetone. As the cylinder pressure falls, acetone carryover increases. The cylinder should be replaced when the internal pressure drops to below 6.9 bar (100 psi) to prevent an acetone carryover. Aspiration of solutions of perchloric acid and metal perchlorates into a nitrous oxide supported flame can increase the risk of explosion or flashback; as a result, it is not recommend the use of perchloric acid in sample preparation for nitrous oxide supported flame analyses.
8. The door to the Flame Sample Compartment is an important part of the safety features of the instrument. It must be closed when lighting a flame, and during normal operation.
9. All flames produce large quantities of heat and toxic combustion products. These must be removed by a suitable fume extraction system.
10. Avoid organic solvents unless instrument is fitted with Solvent resistant kit.
11. Warm up the Burner, HCL lamps at least 15 Minutes before starting Optic Setup and measurement.
12. Don't Switch over to Nitrous Oxide flame immediately after flame ignition allow 15 minutes for Burner warm up.
13. Ensure Exhaust system is running when flame is ON.

14. The position and routing of the internal spectrometer drain tube and liquid trap are critical to the safe operation of the flame system. They must not be modified in any way. The drain extension tube must provide a free-flowing outlet from the instrument drain, without kinks or obstructions. The lower end must always be above the liquid level in the waste container. Empty and clean the waste container regularly. If you have used an organic solvent, do not allow the solvent to accumulate in the waste container, as vapor may cause a fire hazard.
15. Aspirate Distilled water for 5 minutes after completing the analysis to clean the spray chamber and Nebulizer.

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