STUDY MODULE- V

Water Chemistry

(Heavy Metals Analysis of Water)

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HEAVY METALS DETERMINATION BY ATOMIC ABSORPTION SPECTROMETER

Aim of Course

- This course addresses the first six month diploma students and aim at:
- Developing further knowledge and understanding of some core scientific concepts and principals about heavy metals contaminations
- Improving ability to understand and express scientific knowledge about instrumentation
- Preparing for the further study and practical work for the determinations of heavy metals
- Preparing for further practice applications in Science and Engineering

Scope of Learning

This book is basically about

- The heavy metals determination in water waste water,
- Heavy metal contamination in water and wastewater
- Sources of heavy metal contaminations and health implication
- Instrumental techniques used in heavy metal testing
- Concept and principal of Atomic Absorption Spectrometer instrumentation
- Methods of determination of Hydride Mode, Graphite Furnace Mode and Flame Mode
- Solutions and Reagent preparation for each determination
- Calibration and quality control of the testing results.

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1 HEAVY METALS & TRACE ELEMENTS

1.1 Definition

The term "heavy metal" refers to any metal and metalloid element that has a relatively high density ranging from 3.5 to 7 g cm and is toxic or poisonous at low concentrations, and includes mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), zinc (Zn), nickel (Ni), copper (Cu) and lead (Pb). Although "heavy metals" is a general term defined in the literature, it is widely documented and frequently applied to the widespread pollutants of soils and water bodies.

These metals are found widely in the earth's crust and are non-biodegradable in nature. They enter into the human body via air, water and food. A small number have an essential role in the metabolism of humans and animals in very trace amounts but their higher concentration may cause toxicity and health hazards. The hazardous nature of heavy metals has been recognized because of their bio-accumulative nature in biotic systems. They can enter into the environment through mining activities, industrial discharge and from household applications, into nearby bodies of water.

Looking at the periodic table of elements, we learn that heavy metals occupy the lion's share, namely, columns 3–16, of the periods 4 to 6, encompassing the transition metals, post transition metals, and lanthanides [1].

Figure 1:   Periodic Table with type of elements in groups and periods

1.2 Sources of Heavy Metals

Heavy metals differ widely in their chemical properties, and are used extensively in electronics, machines and the artifacts of everyday life, as well as in high-tech applications. As a result they are able to enter into the aquatic and food chains of humans and animals from a variety of anthropogenic sources as well as from the natural geochemical weathering of soil and rocks. The main sources of contamination include mining wastes, landfill leaches, municipal wastewater, urban runoff and industrial wastewaters, particularly from the electroplating, electronic and metal-finishing industries. With increasing generation of metals from technologies activities, the problem of waste disposal has become one of paramount importance. Many aquatic environments face metal concentrations that exceed water quality criteria designed to protect the environment, animals and humans. The problems are exacerbated because metals have a tendency to be transported with sediments, are persistent in the environment and can bio-accumulate in the food chain. Some of the oldest cases of environmental pollution in the world are due to heavy metal use, for example, Cu, Hg and Pb mining, smelting and utilization by ancient civilizations, such as the Romans and the Phoenicians.

The heavy metals are among the most common pollutants found in wastewater. These metals pose a toxicity threat to human beings and animals even at low concentration.

Cadmium is used widely in electroplating industries, solders, batteries, television sets, ceramics, photography, insecticides, electronics, metal-finishing industries and metallurgical activities. It can be introduced into the environment by metal-ore refining, cadmium containing pigments, alloys and electronic compounds, cadmium containing phosphate fertilizers, detergents and refined petroleum products. Rechargeable batteries with nickel–cadmium compounds are also sources of cadmium.

Copper has been used by man since prehistoric times. It is used in the production of utensils, electrical wires, pipes and in the manufacture of brass and bronze. Mining, metallurgy and industrial applications are the major sources of copper exposure in the environment.

Zinc ores mining and metallurgical processing and its industrial application are the major sources of zinc in the air, soil and water. It also comes from the burning of coal.

Nickel occurs naturally in soils and volcanic rocks. Nickel and its salts are used in several industrial applications such as in electroplating, automobile and aircraft parts, batteries, coins, spark plugs, cosmetics and stainless steel, and is used extensively in the production of nickel–cadmium batteries on an industrial scale. It enters into the water bodies naturally by weathering of rocks and soils and through the leaching of the minerals.

The water soluble salts of nickel are the major problems of contamination in aquatic systems. Paint formulation and enameling industries discharges nickel containing effluents to the nearby bodies of water. Nickel is also found in cigarettes, as a volatile compound commonly known as nickel carbonyl.

Arsenic exists in powdery amorphous and crystalline forms in the ores. In certain areas the concentration of arsenic may be higher than its normal dose and creates severe health hazards to human beings and animals. It enters the environment through the natural weathering of rocks and anthropogenic activities, mining and smelting processes, pesticide use and coal combustion. The toxicity of arsenic as a result of the contamination of groundwater bodies and surface waters is of great concern. Arsenic exists as arsenate, As (V), and arsenite, As (III), in most of the groundwater. Adsorption and solution pH commonly controls the mobility of arsenic in the aqueous environment. Metal oxides of Fe, Al and Mn play a role in the adsorption of arsenic in aquatic bodies.

Arsenic has been found naturally at high concentration in groundwater in countries such as India, Bangladesh, Pakistan, Taiwan, Brazil and Chile. Its high concentration in drinking water causes toxic effects on humans and animals.

Mercury natural sources of contamination are volcanic eruption, weathering of rocks and soils, whereas anthropogenic mercury comes from the extensive use of the metal in industrial applications, its mining and processing, applications in batteries and mercury vapor lamps. Methyl mercury is more toxic than any other species of mercury.

Chromium use in industrial applications has discharged huge amounts of wastewater containing toxic chromium species into water bodies. Chromium enters into the environment by natural inputs. Volcanic eruptions, geological weathering of rocks, soils and sediments are the natural sources of chromium, whereas anthropogenic contributions of chromium come from the burning of fossil fuels, production of chromates, plastic manufacturing, electroplating of metals and extensive use in the leather and tannery industries. Hexavalent chromium is more toxic than trivalent chromium.

1.3 Heavy Metal Toxicity

Arsenic is found naturally in the deposits of earth's crust worldwide. Drinking of arsenic contaminated water causes poisoning to the blood, central nervous system, lung and skin cancer, breathing problems, vomiting and nausea. Arsenic is a geogenic problem worldwide but anthropogenic sources, such as the processing of metals and manufacture of pesticides and their byproducts, are contributing equally to the levels of arsenic in the environment.

Lead causes severe toxicity on human exposure. Higher doses may damage the fetus and be toxic to the central nervous system. Newborn babies are more sensitive than the adults. Lead toxicity may harm hemoglobin synthesis, the kidneys and reproductive systems. Exposure to higher doses of lead may disrupt the function of the central nervous system and gastrointestinal tract. Airborne lead may cause the poisoning of agricultural food by the deposition on fruits, soils and water.

Cadmium is the most toxic element, even at its low concentration in the food chain and has been found to cause of itai-itai disease in Japan. Unlike other heavy metals, cadmium is not essential for biological systems. Hence it has no benefit to the ecosystem and only harmful effects have been reported. Cadmium toxicity causes renal dysfunction and lung cancer, and also osteomalacia in the human population and animals, in addition to increasing blood pressure. Smoking of cigarettes is one of the sources of cadmium poisoning in humans. It has been reported that there is sufficient evidence for the carcinogenicity of cadmium.

Copper, as an essential trace element, is required by biological systems for the activation of some enzymes during photosynthesis but at higher concentrations it shows harmful effects on the human body. High-level exposure of copper dust causes nose, eyes and mouth irritation and may cause nausea and diarrhea. Continuous exposure may lead to kidney damage and even death. Copper is also toxic to a variety of aquatic organisms even at very low concentrations.

Zinc is also an essential element in our diet. Too much zinc, however, can also be damaging to health. Zinc toxicity in large amounts causes nausea and vomiting in children. A higher concentration of zinc may cause anemia and cholesterol problems in human beings.

Nickel plays an essential role in the synthesis of red blood cells; however, it becomes toxic when taken in higher doses. Trace amounts of nickel do not damage biological cells, but exposure to a high dose for a longer time may damage cells, decrease body weight and damage the liver and heart. Nickel poisoning may cause reduction in cell growth, cancer and nervous system damage.

Mercury toxicity has been recognized worldwide, such as in Mina Mata Bay of Japan. Mentally disturbed and physically deformed babies were born to mothers who were exposed to toxic mercury due to consumption of contaminated fish. The exposure to mercury causes toxicity to the brain, blindness, mental retardation and kidney damage

Chromium high levels of exposure cause liver and kidney damage, skin ulceration and also affects the central nervous system. With plant species it reduces the rate of photosynthesis. It is also associated with the toxic effects on hematological problems and immune response in freshwater fish. Chromium (VI) causes greater toxicity than chromium (III) in animal and human health.

Iron and **manganese** are required by the biological system as they play major roles in the hemoglobin synthesis and functioning of cells. However, undesirable presence of iron and manganese in drinking water may pose a toxicity threat to health. The presence of these metals in water may cause staining of cotton clothes and give a rusty taste to drinking water. The major concerns focus on the dietary intake of iron because a higher dose may pose acute toxicity to newborn babies and young children. The gastrointestinal tract rapidly absorbs iron that may pose a toxicity risk to the cells and cytoplasm. The liver, kidneys and cardiovascular systems are the major toxicity targets of iron. Neurological disturbances and muscle function damage are the result of toxic effects of manganese in human bodies.

1.4 Methods of Heavy Metal Determination

Classically, quantification of heavy metals involves well-established techniques, such as wet chemical methods

1. Gravimetric, Titrimetric, Colorimetric
2. Ion Selective Electrodes
3. Inductively Coupled Plasma/atomic emission spectrometry (ICP/AES) & inductively coupled plasma with mass spectrometric detection (ICP/MS)
4. Atomic Absorption Spectroscopy (AAS)

2 INSTRUMENTATION

2.1 Atomic Absorption Spectrometer

The most widely used technique for the analysis of elements is Atomic Absorption Spectrometer. This technique is based on the absorption of radiation and is very sensitive (up to ppb level) for quantitative analysis of heavy metals and trace elements.

2.2 Working Principal of Atomic Absorption Spectrometer (AAS)

Atomic-absorption (AA) spectroscopy uses the absorption of light to measure the concentration of gas-phase atoms. Since samples are usually liquids or solids, the analytes atoms or ions must be vaporized in a flame or graphite furnace.

The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The electrons promote to higher orbitals for a short amount of time by absorbing a energy

$M + h\nu \rightarrow M^*$
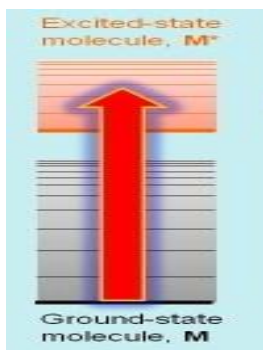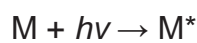
The analytes concentration is determined from the amount of absorption based on Beer- Lambert Law i.e. "Absorption of radiation is directly proportional to the number of particle (atoms) in the medium and path length of the medium.

Applying the Beer-Lambert law directly in AAS is difficult due to variations in the atomization efficiency from the sample matrix, and non-uniformity of concentration and path length of analytes atoms. Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration.

2.3 Major Components of Atomic Absorption Spectrometer (AAS)

The major component of the Atomic Absorption Spectrometer are:

- Light Source
- Sample-Atomizer,
- Light Separator or Spectroscope

- Detector or Photometer,
- Background Compensation System.
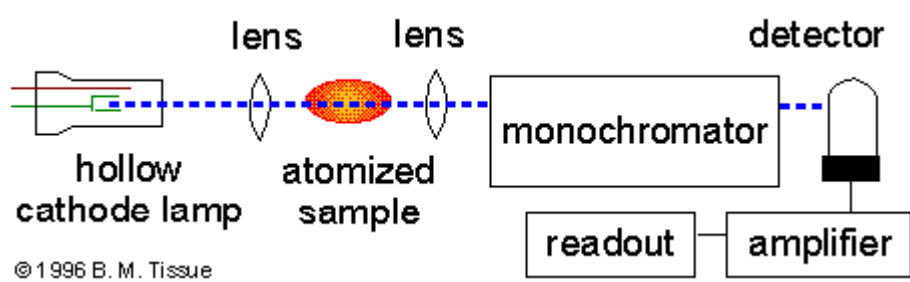
Figure 2: Schematic of an Atomic-absorption Experiment

2.3.1   Light Source

The light source is usually a Hollow-Cathode Lamp (HCL) of the element that is being measured. Lasers are also used in research instruments. Since lasers are intense enough to excite atoms to higher energy levels, they allow AA and atomic fluorescence measurements in a single instrument. The disadvantage of these narrow-band light sources is that only one element is measurable at a time.

2.3.2   Atomizer

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

Flame AA can only analyze solutions, while graphite furnace AA can accept solutions, slurries, or solid samples.

The graphite furnace has several advantages over a flame. 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 analytes atoms.

2.3.3   Light Separation

AA spectrometers use monochromators (A monochromator is an optical system that transmits a specific band of the electromagnetic spectrum) 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 band pass interference filter.
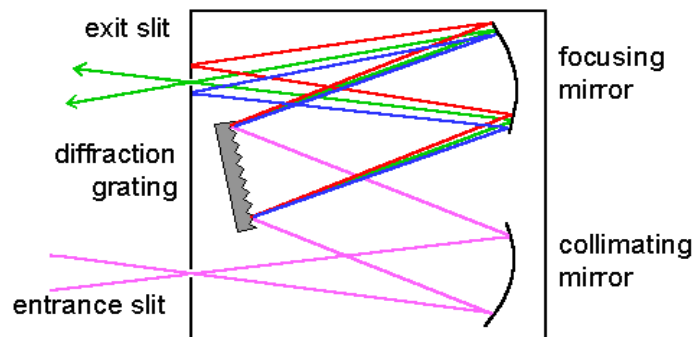
Figure 3: Selection of Narrow beams of monochromatic light through Monochromator

2.3.4 Detector

Photomultiplier tubes are the most common detectors for uv and visible light for AA spectroscopy.
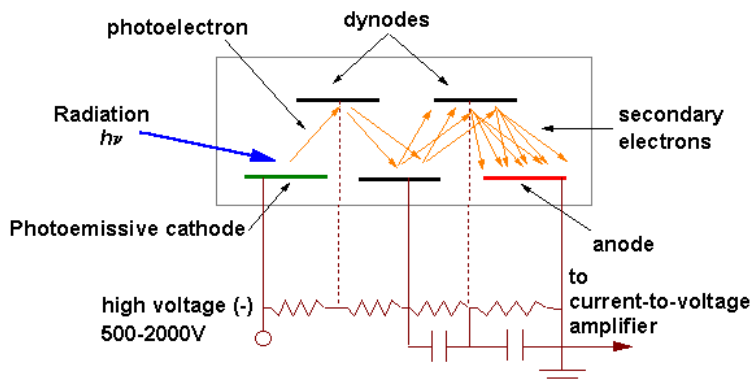
Figure 4: Phenomenon of detection in Photomultiplier Tube

2.3.5 Background Correction System

A background compensation system is employed for the correction of matrix effects on the measuring system. Several principles can be utilized for background compensation

- continuous spectrum sources,
- Zeeman split spectrum,
- non-resonance spectrum, or the self-inversion phenomena.

2.4 Working Modes of Atomic Absorption Spectrometers (AAS)

The working of Atomic Absorption Spectrometers is usually in three different Modes

- Flam Mode
- Hydride Generation Mode
- Graphite Furnace Mode

2.4.1   Flame Mode

Flame AA uses a slot type burner to increase the path length, and therefore to increase the total absorbance (see Beer-Lambert law). Sample solutions are usually aspirated with the gas flow into a nebulizing/mixing chamber to form small droplets before entering the flame.

In the flame Mode in addition to the basic instrument additional major parts are

- Burner
- Nebulizer
- Acetylenes and Nitrous oxide gas

Figure 5:   Flame Atomic Absorption Spectrometer
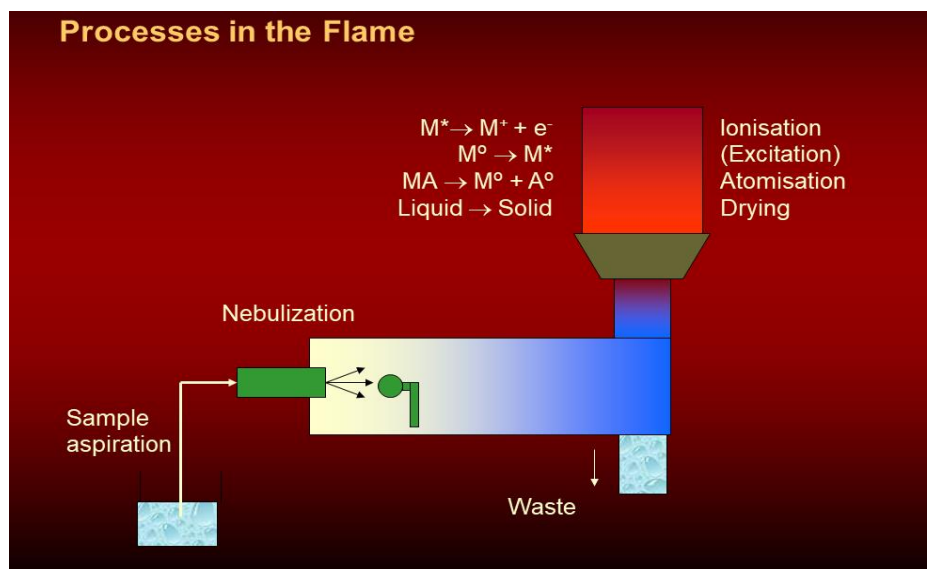
Figure 6:   Atomization process in Flame Mode of Atomic Absorption Spectrometer

The sample is aspirated through nebulizer into the burning flame which acts as atomizer. The molecules are atomized in the flame with burner high temperature ranging from 2500C-3000C

2.4.2   Hydride Generation Mode (HGAAS)

Atomic absorption spectroscopy (AAS) is one of the commonest instrumental methods for analyzing for metals and some metalloids. But because of interferences, poor reproducibility, and poor detection limits an alternative method for some elements--mostly metalloids--has been developed. Hydride generation atomic absorption spectroscopy (HGAAS) and it is available via an option for many modern AAS instruments. It requires an integrated hydride generation module.

HGAAS system is identical to that of Flam AAS however nebulizer required in AAS is **not used** in HGAAS. The additional components are:

- Hydride Generation System.
- Air/Acetylene Flame with Optical Cell/Electrical heated Optical Cell

System described here are continuous flow system as well as batch flow systems.

2.4.2.1   Hydride Generation System/Module

Suck up (aspirate) liquid sample at a controlled rate (continuous flow system) and mix liquid sample with sodium borohydride and HCl. Create a volatile hydride of the analyte metalloid from that reaction and then flow that gaseous hydride into the optical cell.

The reaction of many metalloids with sodium borohydride and HCl produces a volatile hydride: H_2Se, H_3As, H_3Sb, etc. 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 where the hydride and some gaseous hydrogen (produced by the $NaBH_4$ + H_2 reaction) are purged (via a high purity inert gas) into the optical cell via a gas transfer line.

Figure 7:   Hydride Generation Module of AAS

2.4.2.2   Air/Acetylene Flame with Optical Cell

The optical cell is fused silica glass/quartz 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 monochromater and PMT.

In some instruments it sits on top of the normal AAS air/acetylene flame (**Figure-1**). Whereas in more advanced technique the electrically heated quartz cell is being used (**Figure-2**). The gaseous, metalloid 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 and create atoms of the element of interest e.g. As, Se, Sb. which can absorb the HCL's beam.
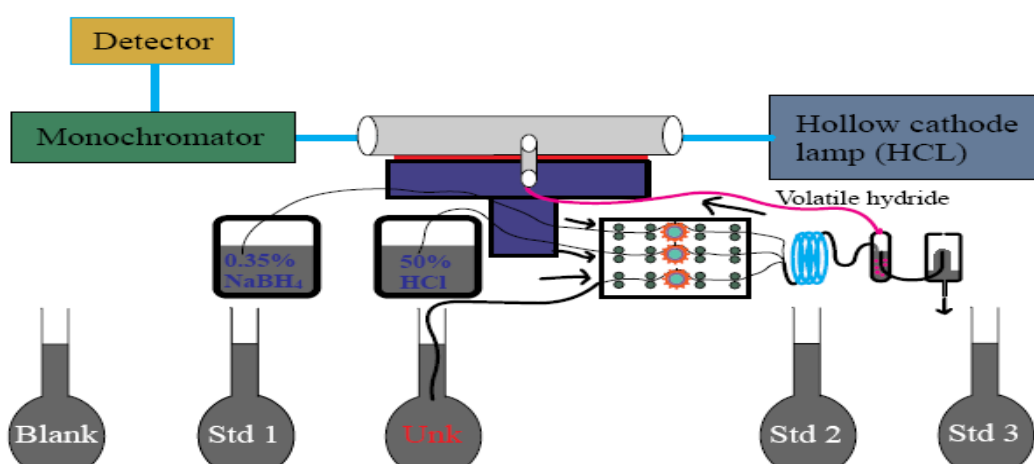
Figure 8:   Air/Acetylene Flame with Optical Cell and Continuous Flow System in AAS

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Figure 9:   Electrical Heated Optical Cell with Batch Flow System in HGAAS

2.4.3   Graphite Furnace Mode

The electrothermal type is composed of an electric graphite furnace and a power source. The main components of the Graphite furnace are

- Graphite Jacket
- Graphite Electrode
- Graphite Tube

Figure 10: Graphite Furnace Mode of AAS

- Small sample volumes (µL) are pipetted into a graphite tube, that is placed within too graphite electrodes.
- Small current applied to heat the tube by resistance heating.
- An external inert gas flow protects tube from burning
- An internal gas flow remove moisture and less volatile compound from the automizer
- Atomisation of the sample after specific temperature program
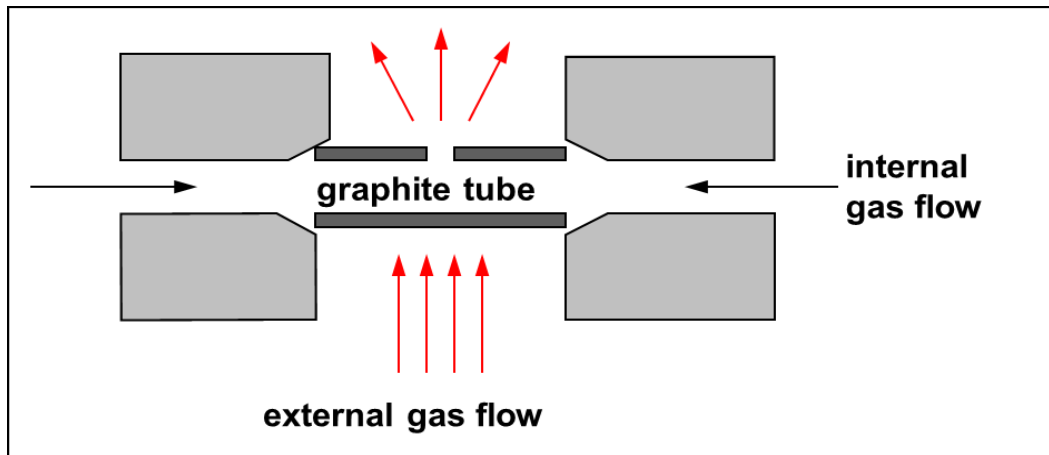
Figure 11: Graphite Tube with external and Internal Gas flow

Figure 12: Variant of Graphite Tube heating ii) time temperature Program for Graphite Furnace

3 DETERMINATION OF HEAVY METALS

In this chapter the determination methods of heavy metals in water and waste water will be described in detail. After studying this section the student will be able to analyze these selected parameters on specific mode of atomic absorption Spectrometer independently.

3.1 Testing/Determination of Arsenic by Atomic Absorption Spectrometer

3.1.1 Purpose

The purpose of this method is to characterize the quality of potable water with respect to total inorganic Arsenic.

3.1.2 Scope

Arsenic can be measured in water samples that have been collected and handled according to the NWQL sample collection and handling procedure: Refer to Manual for Sample Receipt Processing and Reporting**.**

3.1.3 References

- APHA (American Public Health Association), AWWA (American Water Works Association) and WEF (Water Environmental Federation), 2012.

3.1.4 Instrument

Refer to Instrument Manual for Chemical Laboratory: *(CLIM-013; CL-MM-05)*

- Hydride Generation Mode, of Atomic Absorption Spectrophotometer, Vario 6&novAA 300 (Analytic Jena Germany)

- Method Detection Limit:- Recorded in Method Detection Limit Calculation Form (NWQL/LMS/Chem-18) on yearly basis.

3.1.5 Principle

Arsenic present in the sample react with sodium borohydride in acidic medium and form Arsine gas (AsH_3) which is carried by argon gas to the heated quartz cell. Where atomization of As. takes place at 950^oC.

The radiation (λ_{max} 193.7 nm) coming from Arsenic lamp passes through the quartz cell where these are absorbed by Arsenic atoms and this absorption of radiation is measured by the instrument. Principal of absorbance is according to Beer Lambert Law i.e. absorbance of radiation is directly proportional to concentration or no. of particles and path length of the medium.

3.1.6 Glassware Required

- 1000 ml volumetric flask.
- 2×250 ml volumetric flasks.
- 6×100 ml volumetric flasks.
- 2×1000 ml Beakers.
- 1-10 ml auto pipette.
- 100-1000μl-auto pipette.
- Plastic tips for auto pipettes

3.1.7 Reagent Required

- Preparation of Stock Solution and Working Standards for Arsenic
- 3% Sodium borohydride Solution
- Concentrated Hydrochloric Acid.
- Deionized Water

3.1.8 Preparation of 1000 ppb Stock Solution of Arsenic in Deionized Water

3.1.8.1 Reagents

- Certified Arsenic standard solution of concentration 1000 ppm
- Deionized water

3.1.8.2 Equipment and Glassware

- 100μl-1000μl auto pipette
- Plastic tips for auto pipette
- 1000 ml volumetric flask
- 100 ml beaker.

3.1.8.3 Procedure

- Take 1 ml of Certified Arsenic Standard Solution (Conc. 1000 ppm)
- Add in to 1000 ml volumetric flask
- Make the volume up to the mark with deionized water
- Mention the Arsenic concentration and date of preparation on flask with permanent ink marker or label with sticker containing following information.

Name of Reagent:
Date of Preparation:
Expiry Date:
Prepared by:

- Make entries in Chemical Reagent Preparation log book.

3.1.9 Preparation of Working Standards of Arsenic

3.1.9.1 Equipment and Glassware

- 6x100 ml volumetric flask
- 250 ml Volumetric flask
- 7x100 ml beaker
- 0.5-5 ml auto pipette

3.1.9.2 Procedure

- Take 25 ml of Arsenic stock solution of 1000 ppb with auto pipette
- Add in to 250 ml volumetric flask to produce 100 ppb Arsenic secondary standard
- Make the volume up to the mark with deionized water
- Dispense solution of 100 ppb in to volumetric flasks with auto pipette according to Table 1.by using following formula

$$C_s V_s = C_w V_w$$

$$V_s = \frac{C_w V_w}{C_s}$$

Where C_s = Concentration of Stock Solution

V_s = Volume of Stock Solution

V_w = Volume of working standard

C_w = Concentration of working standard

Table 1:   Preparation of Working Calibration Standards for Arsenic

Standard values µg/L	Volumetric Flask	Volume taken from 100 µg/L ml
5	100 ml	5
10	100 ml	10
15	100 ml	15
20	100 ml	20
25	100 ml	25
30	100 ml	30

- Mention the Arsenic concentration and date of preparation on flask with permanent ink marker or label with sticker having following in formations

> Name of Reagent:
>
> Date of Preparation:
>
> Expiry Date:
>
> Prepared by:

- Make entries in Chemical Reagent Preparation log book.

3.1.10 Calibration of AAS for Arsenic in Hydride Generation Mode

3.1.10.1   Scope

Applying the Beer Lambert Law directly in AA spectroscopy is difficult due to variations in the atomization efficiency from the sample matrix, and non-uniformity of concentration and path length of analyte atoms. So unknown concentration measurements are determined from a working curve after calibrating the instrument with standards of known concentration.

3.1.10.2   Calibration Procedure

- Prepare standard solutions from stock solutions as of required concentrations (blank,5,10,15,20,25,30 ppb)

- Open software from computer and select "**Hydride Generation"** mode
- Load the previous method for Arsenic or method from "**Cookbook"**
- Go into option **"Spectrophotometer"** adjust energy of Arsenic lamp do alignment of lamp in "**lamp turret"** if necessary
- Go in to option "**Hydride"** and click on option "**Control"** start heating of the cell and when temperature reaches up to 950°C then click on the option "**Start"** the reluctant pump will start for one minute and stop it
- Go into option "**Calibration"** set calibration parameters then click on "**Table"** feed different concentrations of standards and draw calibration curve by running standard one by one (each standard 3 times)
- For running Standard Take 10 ml of sample in Reaction cup
- Add 1 ml concentrated. HCl in reaction cup and mount on batch module
- Click on the **"Run Sample"** option of software
- Reaction is completed in about two minutes
- R^2 value of calibration curve should be equivalent to 1.00 and not less then 0.996
- Save method of the calibration in option "**Method"**.

3.1.10.3 *Precautions*

- All the glassware should be washed with 1:1 HCl before preparation of standard solution and samples.
- Auto-pipettes should be used carefully to take accurate volume during the preparation of standards.
- Deionized water used should be of high quality having EC <1.5µS/cm

3.1.11 **Analysis of Arsenic on AAS**

3.1.11.1 *Procedure*

- Open the AAS Software (Winn AAS) select Hydride Generation Mode.
- Load Method for Arsenic or draw new calibration curve.
- Adjust the parameter (energy of the lamp and heating temperature of quartz cell).
- Click on the option "Sample" and insert the sample codes in sample table.
- Wash the sample cup and sampling tip with deionized water.
- Take 10ml of sample in Reaction cup.
- Add 1ml concentrated. HCl in reaction cup and mount on batch module.
- Click on the "Run Sample" option from software.
- Reaction is completed in about two minutes.
- Each sample is analyzed three times.
- Results in selected concentration (ppb, ppm) are displayed on software worksheet.

3.1.11.2 Expression of Results

- Arsenic result measured & record in ppb (µg/l)
- Recorded the results on analyst work book
- Calculate the uncertainty of measurement,
- Record the results on the Result Record Form
- Sent to the Customer Service Section for Reporting.

3.1.11.3 Quality Control

- Analyze Blank and Certified Reference Standard after every ten samples.
- Analyze pre-analyzed sample with every analytical batch.
- Analyze Spiked Matrix sample after every two months
- Analyze the Control Sample and display data is regularly on control charts.
- Record the daily quality control data in QC Data sheet.

3.1.11.4 Precautions

- All the glassware should be washed with 1:1 HCl before preparation of standard solution and samples.
- Auto-pipettes should be used carefully to take accurate volume during the analysis.
- Deionized water used should be of high quality having EC <1.5µS/cm.

3.2 Testing/Determination of Copper (Cu) on Flame Mode of Atomic Absorption Spectrometer

3.2.1 Purpose

The purpose of this method is to characterize the quality of potable water with the respect of total dissolved Copper.

3.2.2 Scope

Copper can be measured in water samples that have been collected and handled according to the NWQL sample collection and handling procedure. Refer to Manual for Sample Receipt Processing and Reporting.

3.2.3 References

- APHA (American Public Health Association), AWWA (American Water Works Association) and WEF (Water Environmental Federation) 2012.

3.2.4   Instrument

- Flam Mode, of Atomic Absorption Spectrophotometer, Vario 6 (Analytic Jena Germany).

3.2.5   Principle

Sample is aspirated in burning flam of air-Acetylene Through nebulizer. Copper present in the sample is atomized in burning flam. The radiation (λ_{max}324.8 nm) coming from copper lamp passes through the Burning flam where these are absorbed by copper atoms and this absorption of radiation is measured by the Instrument. Principal of absorbance is according to Beer Lambert Law i.e. absorbance of radiation is directly proportional to concentration or no. of particles and path length of the medium.

3.2.6   Glassware

- 1000 ml volumetric flask.
- 3×100 ml volumetric flasks.
- 2×1000 ml Beakers.
- 1-10 ml auto pipette.
- 100-1000µl auto pipette.
- Plastic tips for auto pipettes.

3.2.7   Reagents

- Preparation of Stock Solution and Working Standards for Copper
- 0.2% HNO_3
- 1N HNO_3

3.2.8   Preparation of Stock Solution and Working Standards of Copper for Flame Mode

3.2.8.1   Reagents

- Copper Reference Standard Solution of 1000 ppm
- 0.2 % HNO_3

3.2.8.2   Equipment and Glassware

- 100µl-1000µl auto pipette
- 0.5-10 ml auto pipette
- Plastic tips for auto pipette
- 6x100 ml volumetric flask
- 100 ml beaker

3.2.8.3 Procedure

- For the preparation of 10 ppm standard solution take 1 ml of 1000 ppm Stock solution in 100 ml volumetric flask and make up volume up to the mark with 0.2% HNO_3.
- Take copper reference standard solution of 10 ppm with auto pipette to prepare the required standard of different concentration according to **Table-2**.by using following formula

$$CsVs = Cw\ Vw$$

$$Vs = \frac{Cw\ Vw}{Cs}$$

Where   Cs = Concentration of Stock Solution

Vs = Volume of Stock Solution

Vw = Volume of working standard

Cw = Concentration of working standard

Table 2:   Preparation of Working Standards for Copper

Standard to be Prepared mg/L	Volumetric Flask	Volume taken from Stock ml
0.2	100 ml	2
0.4	100 ml	4
0.6	100 ml	6
0.8	100 ml	8
1	100 ml	10

- Make the volume up to the mark with 0.2 % HNO_3
- Mention the copper concentration and date of preparation on flask with permanent ink marker or label with sticker*)* having following in formations

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Name of Reagent:
Date of Preparation:
Expiry Date:
Prepared by:
```

- Make entries in Chemical Reagent Preparation log book.

3.2.9 Calibration of Copper on Flame Mode

3.2.9.1 Scope

Applying the Beer Lambert Law directly in AA spectroscopy is difficult due to variations in the atomization efficiency from the sample matrix, and non-uniformity of concentration and path length of analyte atoms. So unknown concentration measurements are determined from a working curve after calibrating the instrument with standards of known concentration.

3.2.9.2 Standards and Reagents

- Copper standard of required concentrations on flam mode
- 0.2% HNO_3.

3.2.9.3 Calibration Procedure

- Open software from computer and select "**Flam**" mode.
- Load the previous method for Copper or load from "**Cookbook".**
- Click on the option **"Spectrophotometer"** adjust energy of copper lamp do alignment of lamp in "**lamp turret"** if necessary.
- Adjust the parameter (energy of the lamps, auto sampler parameters and adjustment of air acetylene flam parameter according to the manufacturer instructions).
- Ignite the flam by clicking on the ignition option in main option "**Flam".**
- Prepare Copper stock solution of 1 ppm or as required for calibration and keep this stock solution in special sample cup position and take diluents (0.2% HNO_3) in diluents bottle of auto sampler feed its position in software.
- In case of working without auto sampler prepare and then feed each standard one by one manually starting from lower to higher concentrations
- Click on option "**Calibration"** then on "**Table"** feed different concentrations (blank, 0.2, 0.4, 0.6, 0.8 & 1 ppm or as required) of standards and respective volumes and draw calibration curve by running standards one by one (each standard 3 times).
- R^2 value of calibration curve should be equivalent to 1.00 and not less then 0.996.
- Click on option "**Method"** in software and save method of calibration.

3.2.10 Analysis of Copper on Flame Mode

3.2.10.1 Procedure

- Open the AAS Software (Winn AAS) select Flam Mode.

- Load Method for Copper or draw new calibration curve.
- Adjust the parameter (energy of the lamps, auto sampler parameters and adjustment of air acetylene flam parameter according to the manufacturer instructions).
- Click on the option "Sample" and insert the sample codes in sample table along with giving the sample cup positions as in autosampler.
- Take sample in sample cup of autosampler up to the mark and put in specific position of sample tray of auto sampler.
- Ignite the flam by clicking on the ignition option in main option "Flam".
- Click on the option "Run Sample" from software or click on option "Start Conc." after selecting the range of samples in table by giving working area.
- In case of working without autosampler Click on the option "Run Sample" from software and feed each sample one by one manually
- Reaction is completed in about 50 seconds.
- Each Sample is analyzed three times.
- Results in selected concentration (ppm) are displayed on software worksheet.

3.2.10.2 Expression of Results

- Copper result measured & record in ppm (mg/l)
- Recorded the results on analyst work book
- Calculate the uncertainty of measurement,
- Record the results on the Result Record Form
- Sent to the Customer Service Section for Reporting.

3.2.10.3 Quality Control

- Analyze Blank and Certified Reference Standard after every ten samples.
- Analyze pre-analyzed sample with every analytical batch.
- Analyze Spiked Matrix sample after every two months
- Analyze the Control Sample and display data is regularly on control charts.
- Record the daily quality control data in QC Data sheet.

3.2.10.4 Precautions

- All the glassware should be washed with 1N HNO3 before preparation of standard and samples.
- Auto pipettes should be used carefully to take accurate volume during the analysis.
- Deionized water used should be of high quality having EC <1.5µS/cm.

3.3 Testing/Determination of Manganese (Mn) on Flame Mode of Atomic Absorption Spectrometer

3.3.1 Purpose

The purpose of this method is to characterize the quality of potable water with the respect of total dissolved Manganese.

3.3.2 Scope

Manganese can be measured in water samples that have been collected and handled according to the NWQL sample collection and handling procedure. Refer to Manual for Sample Receipt Processing and Reporting.

3.3.3 References

- APHA (American Public Health Association), AWWA (American Water Works Association) and WEF (Water Environmental Federation) 2012.

3.3.4 Instrument

- Flam Mode, of Atomic Absorption Spectrophotometer, Vario 6 (Analytic Jena Germany**).**

3.3.5 Principle

Sample is aspirated in burning flam of air-Acetylene Through nebulizer. Manganese present in the sample is atomized in burning flam of air acetylene.

The radiation (λ_{max}279.5 nm) coming from Manganese lamp passes through the burning flam where these are absorbed by Manganese atoms and this absorption of radiation is measured by the instrument. Principal of absorbance is according to Beer Lambert Law i.e. absorbance of radiation is directly proportional to concentration or no. of particles and path length of the medium.

3.3.6 Glassware

- 1000 ml volumetric flask.
- 3×100 ml volumetric flasks.
- 2×1000 ml Beakers.
- 1-10 ml auto pipette.
- 100-1000µl auto pipette.
- Plastic tips for auto pipettes.

3.3.7 Reagents

- Stock Solution and Working Standards for Manganese
- 0.2% HNO_3
- 1N HNO_3

3.3.8 Preparation of Stock Solution and Working Standards of Manganese for Flame Mode

3.3.8.1 *Reagents*

- Manganese Reference Standard Solution of 1000 ppm
- 0.2 % HNO_3

3.3.8.2 *Glassware*

- 100µl-1000µl auto pipette
- 0.5-10 ml auto pipette
- Plastic tips for auto pipette
- 6x100 ml volumetric flask
- 100 ml beaker

3.3.8.3 *Procedure*

- For the preparation of 10 ppm standard solution take 1 ml of 1000 ppm Stock solution in 100 ml volumetric flask and make up volume up to the mark with 0.2% HNO_3.
- Take Manganese reference standard solution of 10 ppm with auto pipette to prepare the required standard of different concentration according to *Table-3*.by using following formula

$$CsVs = Cw\ Vw$$

$$Vs = \frac{Cw\ Vw}{Cs}$$

Where   Cs = Concentration of Stock Solution

      Vs = Volume of Stock Solution

      Vw = Volume of working standard

      Cw = Concentration of working standard

Table 3:   Preparation of working standard Manganese

Standard to be Prepared mg/L	Volumetric Flask	Volume taken from Stock ml
0.2	100 ml	2
0.4	100 ml	4
0.6	100 ml	6
0.8	100 ml	8
1	100 ml	10

- Make the volume up to the mark with 0.2 % HNO_3
- Mention the Manganese concentration and date of preparation on flask with permanent ink marker or label with sticker*)* having following in formations

> Name of Reagent:
>
> Date of Preparation:
>
> Expiry Date:
>
> Prepared by:

- Make entries in Chemical Reagent Preparation log book.

3.3.9   Calibration of Manganese on Flame Mode

3.3.9.1   Scope

Applying the Beer Lambert Law directly in AA spectroscopy is difficult due to variations in the atomization efficiency from the sample matrix, and non-uniformity of concentration and path length of analyte atoms. So unknown concentration measurements are determined from a working curve after calibrating the instrument with standards of known concentration.

3.3.9.2   Standards and Reagents

- Manganese standard of required concentrations on flam mode
- 0.2% HNO_3.

3.3.9.3   Calibration Procedure

- Open software from computer and select "**Flam**" mode.
- Load the previous method for Manganese or load from "**Cookbook".**
- Click on the option **"Spectrophotometer"** adjust energy of Manganese lamp do alignment of lamp in "**lamp turret"** if necessary.
- Adjust the parameter (energy of the lamps, auto sampler parameters and adjustment of air acetylene flam parameter according to the manufacturer instructions).
- Ignite the flam by clicking on the ignition option in main option "**Flam".**
- Prepare Manganese stock solution of 1 ppm or as required for calibration and keep this stock solution in special sample cup position and take diluents (0.2% HNO_3) in diluents bottle of auto sampler feed its position in software.
- In case of working without auto sampler prepare and then feed each standard one by one manually starting from lower to higher concentrations
- Click on option "**Calibration"** then on "**Table"** feed different concentrations (blank, 0.2, 0.4, 0.6, 0.8 & 1 ppm or as required) of standards and respective volumes and draw calibration curve by running standards one by one (each standard 3 times).
- R^2 value of calibration curve should be equivalent to 1.00 and not less then 0.996.
- Click on option "**Method"** in software and save method of calibration for Manganese.

3.3.10   Analysis of Copper on Flame Mode

3.3.10.1 Procedure

- Open the AAS Software (Winn AAS) select Flam Mode.
- Load Method for Manganese or draw new calibration curve.
- Adjust the parameter (energy of the lamps, auto sampler parameters and adjustment of air acetylene flam parameter according to the manufacturer instructions).
- Click on the option "**Sample"** and insert the sample codes in sample table along with giving the sample cup positions as in auto-sampler.
- Take sample in sample cup of auto-sampler up to the mark and put in specific position of sample tray of auto sampler.
- Ignite the flam by clicking on the ignition option in main option "**Flam".**

- Click on the option **"Run Sample"** from software or click on option "**Start Conc."** after selecting the range of samples in table by giving working area.
- In case of working without auto-sampler Click on the option **"Run Sample"** from software and feed each sample one by one manually
- Reaction is completed in about 50 seconds.
- Each Sample is analyzed three times.
- Results in selected concentration (ppm) are displayed on software worksheet.

3.3.10.2 Expression of Results

- Manganese. result measured & record in ppm (mg/l)
- Recorded the results on analyst work book
- Calculate the uncertainty of measurement,
- Record the results on the Result Record Form
- Sent to the Customer Service Section for Reporting.

3.3.10.3 Quality Control

- Analyze Blank and Certified Reference Standard after every ten samples.
- Analyze pre-analyzed sample with every analytical batch.
- Analyze Spiked Matrix sample after every two months
- Analyze the Control Sample and display data is regularly on control charts.
- Record the daily quality control data in QC Data sheet.

3.3.10.4 Precautions

- All the glassware should be washed with 1N HNO_3 before preparation of standard and samples.
- Auto pipettes should be used carefully to take accurate volume during the analysis.
- Deionized water used should be of high quality having EC <1.5µS/cm.

3.4 Testing/Determination of Zinc (Zn) on Flame Mode of Atomic Absorption Spectrometer

3.4.1 Purpose

The purpose of this method is to characterize the quality of potable water with the respect of total dissolved Zinc.

3.4.2 Scope

Zinc can be measured in water samples that have been collected and handled according to the NWQL sample collection and handling procedure. Refer to Manual for Sample Receipt Processing and Reporting.

3.4.3 References

- APHA (American Public Health Association), AWWA (American Water Works Association) and WEF (Water Environmental Federation) 2012.

3.4.4 Instrument

- Flam Mode, of Atomic Absorption Spectrophotometer, Vario 6 (Analytic Jena Germany**).**

3.4.5 Principle

Sample is aspirated in burning flam of air-Acetylene through nebulizer. Zinc present in the sample is atomized in burning flam. The radiation (λ_{max} 213.9 nm) coming from Zinc lamp passes through the flam where these are absorbed by zinc atoms and this absorption of radiation is measured by the instrument. Principal of absorbance is according to Beer Lambert Law i.e. absorbance of radiation is directly proportional to concentration or no. of particles and path length of the medium.

3.4.6 Glassware

- 1000 ml volumetric flask.
- 3×100 ml volumetric flasks.
- 2×1000 ml Beakers.
- 1-10 ml auto pipette.
- 100-1000µl auto pipette.
- Plastic tips for auto pipettes.

3.4.7   Reagents

- Preparation of Stock Solution and Working Standards for Zinc
- 0.2% HNO_3
- 1N HNO_3

3.4.8   Preparation of Stock Solution and Working Standards of Zinc for Flame Mode

3.4.8.1   Reagents

- Zinc Reference Standard Solution of 1000 ppm
- 0.2 % HNO_3

3.4.8.2   Glassware

- 100µl-1000µl auto pipette
- 0.5-10 ml auto pipette
- Plastic tips for auto pipette
- 6x100 ml volumetric flask
- 100 ml beaker

3.4.8.3   Procedure

- For the preparation of 10 ppm Zinc standard solution take 1 ml of 1000 ppm Stock solution in 100 ml volumetric flask and make up volume up to the mark with 0.2% HNO_3.
- Take Zinc reference standard solution of 10 ppm with auto pipette to prepare the required standard of different concentration according to **Table-4** by using following formula

$$CsVs \quad = \quad Cw\,Vw$$

$$Vs \quad = \quad \frac{Cw\,Vw}{Cs}$$

Where   Cs = Concentration of Stock Solution

Vs = Volume of Stock Solution

Vw = Volume of working standard

Cw = Concentration of working standard

Table 4:   Preparation of working standard Zinc

Standard to be Prepared mg/L	Volumetric Flask	Volume taken from Stock ml
0.2	100 ml	2
0.4	100 ml	4
0.6	100 ml	6
0.8	100 ml	8
1	100 ml	10

- Make the volume up to the mark with 0.2 % HNO_3
- Mention the Zinc concentration and date of preparation on flask with permanent ink marker or label with sticker having following in formations

> Name of Reagent:
>
> Date of Preparation:
>
> Expiry Date:
>
> Prepared by:

- Make entries in Chemical Reagent Preparation log book.

3.4.9   Calibration of Zinc on Flame Mode

3.4.9.1   Scope

Applying the Beer Lambert Law directly in AA spectroscopy is difficult due to variations in the atomization efficiency from the sample matrix, and non-uniformity of concentration and path length of analyte atoms. So unknown concentration measurements are determined from a working curve after calibrating the instrument with standards of known concentration.

3.4.9.2   Standards and Reagents

- Zinc standard of required concentrations on flam mode
- 0.2% HNO_3.

3.4.9.3   Calibration Procedure

- Open software from computer and select "**Flam**" mode.
- Load the previous method for Zinc or load from "**Cookbook".**
- Click on the option **"Spectrophotometer"** adjust energy of Zinc lamp do alignment of lamp in "**lamp turret"** if necessary.
- Adjust the parameter (energy of the lamps, auto sampler parameters and adjustment of air acetylene flam parameter according to the manufacturer instructions).
- Ignite the flam by clicking on the ignition option in main option "**Flam".**
- Prepare Zinc stock solution of 1 ppm or as required for calibration and keep this stock solution in special sample cup position and take diluents (0.2% HNO_3) in diluents bottle of auto sampler feed its position in software.
- In case of working without auto sampler prepare and then feed each standard one by one manually starting from lower to higher concentrations
- Click on option "**Calibration"** then on "**Table"** feed different concentrations (blank, 0.2, 0.4, 0.6, 0.8 & 1 ppm or as required) of standards and respective volumes and draw calibration curve by running standards one by one (each standard 3 times).
- R^2 value of calibration curve should be equivalent to 1.00 and not less then 0.996.
- Click on option "**Method"** in software and save method of calibration for Zinc.

3.4.10 Analysis of Zinc on Flame Mode

3.4.10.1 Procedure

- Open the AAS Software (Winn AAS) select Flam Mode.
- Load Method for Zinc or draw new calibration curve.
- Adjust the parameter (energy of the lamps, auto sampler parameters and adjustment of air acetylene flam parameter according to the manufacturer instructions).
- Click on the option "**Sample"** and insert the sample codes in sample table along with giving the sample cup positions as in auto-sampler.
- Take sample in sample cup of auto-sampler up to the mark and put in specific position of sample tray of auto sampler.
- Ignite the flam by clicking on the ignition option in main option "**Flam".**

- Click on the option **"Run Sample"** from software or click on option "**Start Conc."** after selecting the range of samples in table by giving working area.
- In case of working without auto-sampler Click on the option **"Run Sample"** from software and feed each sample one by one manually
- Reaction is completed in about 50 seconds.
- Each Sample is analyzed three times.
- Results in selected concentration (ppm) are displayed on software worksheet.

3.4.10.2 Expression of Results

- Zinc result measured & record in ppm (mg/l)
- Recorded the results on analyst work book
- Calculate the uncertainty of measurement,
- Record the results on the Result Record Form
- Sent to the Customer Service Section for Reporting.

3.4.10.3 Quality Control

- Analyze Blank and Certified Reference Standard after every ten samples.
- Analyze pre-analyzed sample with every analytical batch.
- Analyze Spiked Matrix sample after every two months
- Analyze the Control Sample and display data is regularly on control charts.
- Record the daily quality control data in QC Data sheet.

3.4.10.4 Precautions

- All the glassware should be washed with 1N HNO_3 before preparation of standard and samples.
- Auto pipettes should be used carefully to take accurate volume during the analysis.
- Deionized water used should be of high quality having EC <1.5µS/cm.

3.5 Testing/Determination of Lead (Pb) by Atomic Absorption Spectrometer on Graphite Furnace Mode

3.5.1 Purpose

The purpose of this method is to characterize the quality of potable water with respect to total dissolved Lead.

3.5.2 Scope

Lead can be measured in water samples that have been collected and handled according to the NWQL sample collection and handling procedure: Refer to Manual for Sample Receipt Processing and Reporting

3.5.3 References

- APHA (American Public Health Association), AWWA (American Water Works Association) and WEF (Water Environmental Federation), 2012.

3.5.4 Instrument

- Graphite Furnace Mode, of Atomic Absorption Spectrophotometer, AAS

3.5.5 Principle

Lead present in the sample is atomized in Graphite tube at 1350^oC. The radiation ($\lambda_{max}217.0$ nm) coming from lead lamp passes through the graphite tube where these are absorbed by lead atoms and this absorption of radiation is measured by the instrument. Principal of absorbance is according to Beer Lambert Law i.e. absorbance of radiation is directly proportional to concentration or no. of particles and path length of the medium.

3.5.6 Glassware

- 1000 ml volumetric flask.
- 2×250 ml volumetric flasks.
- 3×100 ml volumetric flasks.
- 2×1000 ml Beakers.
- 1-10 ml auto pipette.
- 100-1000µl-auto pipette.
- Plastic tips for auto pipette.

3.5.7 Reagents

- Preparation of Stock Solution and Working Standards for Lead
- 0.5% HNO_3
- 1N HNO_3

3.5.8 Preparation of 1000 ppb Stock Solution of Lead

3.5.8.1 Reagents

- Certified Lead standard solution of concentration 1000 ppm
- 0.5% HNO_3

3.5.8.2 Equipment and Glassware

- 100µl-1000µl auto pipette
- Plastic tips for auto pipette
- 100 ml volumetric flask
- 100 ml beaker

3.5.8.3 Procedure

- Take 0.1 ml of Certified Lead standard solution (Conc. 1000 ppm)
- Add in to 100 ml volumetric flask
- Make the volume up to the mark with 0.5% HNO_3
- Mention the Lead concentration and date of preparation on flask with permanent ink marker or label with sticker having following in formations.

Name of Reagent:
Date of Preparation:
Expiry Date:
Prepared by:

- Make entries in Chemical Reagent Preparation log book.

3.5.9 Preparation of Working Standards for Lead

3.5.9.1 Reagents

- Lead Stock Solution of 1000 ppb
- 0.5% HNO_3

3.5.9.2 Equipment and Glassware

- 100µl-1000µl auto pipette
- Plastic tips for auto pipette
- 100 ml volumetric flasks
- 100 ml beaker
- 5-10 ml auto pipette

3.5.9.3 Procedure

- Take 25 ml of Lead stock solution of 1000 ppb with auto pipette
- Add in to 250 ml volumetric flask to produce 100 ppb Lead secondary standard
- Make the volume up to the mark with 0.5% HNO3.

- Prepare 20 ppb by taking 20 ml of 100 ppb Lead standard.
- The Auto sampler will automatically prepare dilution of 20 ppb standard to prepare 5, 10 and 15 ppb standards
- Dispense solution of 100 ppb in to volumetric flasks according to **Table- 5**. by using following formula

$$CsVs \quad = \quad Cw\,Vw$$

$$Vs \quad = \quad \frac{Cw\,Vw}{Cs}$$

Where   Cs = Concentration of Stock Solution

Vs = Volume of Stock Solution

Vw = Volume of working standard

Cw = Concentration of working standard

Table 5:   Preparation of Working Standards for Lead

Standard values µg/L	Volumetric Flask	Volume taken from 100 µg/L ml
5	100 ml	5
10	100 ml	10
15	100 ml	15
20	100	20

- Make the volume up to the mark with 0.5% HNO_3
- Mention the Lead concentration and date of preparation on flask with permanent ink marker or label with sticker having following information.

Name of Reagent:
Date of Preparation:
Expiry Date:
Prepared by:

- Make entries in Chemical Reagent Preparation log book.

3.5.10 Calibration for Lead on Graphite Furnace Mode

3.5.10.1 Scope

Applying the Beer Lambert Law directly in AA spectroscopy is difficult due to variations in the atomization efficiency from the sample matrix, and non-uniformity of concentration and path length of analyte atoms. So unknown concentration measurements are determined from a working curve after calibrating the instrument with standards of known concentration.

3.5.10.2 Standards and Reagents

- Lead standards of required concentration:
- 0.5% HNO_3

3.5.10.3 Calibration Procedure

- Open software from computer and select "**Graphite Furnace"** mode.
- Load the previous method for Lead or from "**Cookbook"**.
- Click on the option **"Spectrophotometer"** adjust energy of lead lamp do alignment of lamp in "**lamp turret"** if necessary.
- Properly align Graphite tube and Graphite platform with lamp beam and Adjust the needle of the auto sampler in Graphite platform for accurate volume transfer, a little disturbance will result in sample loss and damage of Graphite tube.
- Prepare Lead stock solution of 20 ppb or as required for calibration and keep this stock solution in special sample cup position at **"Auto"** and place diluents (0.5% HNO_3) at its position in auto sampler.
- Prepare sample sequence and select the option **"Calibration"** at start of sampling table
- Feed different concentrations (5, 10, 15 and 20) of standards and respective volumes and draw calibration curve by running standards one by one (each standard 3 times).
- R^2 value of calibration curve should be equivalent to 1.00 and not less then 0.996.
- Click on option "**Method"** in software and save method of calibration.

3.5.11 Analysis of Lead on Graphite Furnace

3.5.11.1 Procedure

- Open the AAS Software select Graphite Furnace Mode.
- Load Method for Lead or draw new calibration curve.

- Adjust the parameter (energy of the lamp and Formation of the graphite tube).
- Click on the option "Sample" and insert the sample codes in sample table along with giving the sample cup positions as in auto sampler.
- Take sample in sample cup of auto sampler up to the mark and put in specific position of sample tray of auto sampler.
- Click on the option "Run Sample" from software or "Start Conc". after selecting the range of samples in table and giving working area.
- Reaction is completed in about two minutes.
- Each sample is analyzed three times.
- Results in selected concentration (ppb, ppm) are displayed on software worksheet.

3.5.11.2 Expression of Results

- Lead result measured & record in ppb (μg/l)
- Recorded the results on analyst work book
- Calculate the uncertainty of measurement,
- Record the results on the Result Record Form
- Sent to the Customer Service Section for Reporting.

3.5.11.3 Quality Control

- Analyze Blank and Certified Reference Standard after every ten samples.
- Analyze pre-analyzed sample with every analytical batch.
- Analyze Spiked Matrix sample after every two months
- Analyze the Control Sample and display data is regularly on control charts.
- Record the daily quality control data in QC Data sheet.

3.5.11.4 Precautions

- All the glassware and auto sampler cups should be washed with 1N HNO_3 before reparation of standard and samples.
- Auto pipettes should be used carefully to take accurate volume during the analysis.
- Deionized water used should be of high quality having EC <1.5μS/cm.

3.6 Testing/Determination of Chromium (Cr) on Graphite Furnace Mode

3.6.1 Purpose

The purpose of this method is to characterize the quality of potable water with respect to total dissolved Chromium.

3.6.2 Scope

Chromium can be measured in water samples that have been collected and handled according to the NWQL sample collection and handling procedure: Refer to Manual for Sample Receipt Processing and Reporting.

3.6.3 References

- APHA (American Public Health Association), AWWA (American Water Works Association) and WEF (Water Environmental Federation), 2012.

3.6.4 Instrument

- Graphite Furnace Mode, of Atomic Absorption Spectrophotometer, AAS Vario 6.

3.6.5 Principle

Chromium present in the sample is atomized in Graphite tube at 2200°C. The radiation (λ_{max}357.9 nm) coming from Chromium lamp passes through the graphite tube where these are absorbed by Chromium atoms and this absorption of radiation is measured by the instrument

Principal of absorbance is according to Beer Lambert Law i.e. absorbance of radiation is directly proportional to concentration or no. of particles and path length of the medium.

3.6.6 Glassware

- 1000 ml volumetric flask.
- 2×250 ml volumetric flasks.
- 3×100 ml volumetric flasks.
- 2×1000 ml Beakers.
- 1-10 ml auto pipette.
- 100-1000µl-auto pipette.
- Plastic tips for auto pipettes.

3.6.7   Reagents

- Stock Solution and Working Standards for Chromium
- 0.5% HNO_3
- 1N HNO_3

3.6.8   Preparation of 1000 ppb Stock Solution of Chromium

3.6.8.1   *Reagents*

- Certified Chromium standard solution of concentration 1000 ppm
- 0.5% HNO_3

3.6.8.2   *Equipment and Glassware*

- 100µl-1000µl auto pipette
- Plastic tips for auto pipette
- 100 ml volumetric flask
- 100 ml beaker

3.6.8.3   *Procedure*

- Take 0.1 ml of Certified Chromium standard solution (Conc. 1000 ppm)
- Add in to 100 ml volumetric flask
- Make the volume up to the mark with 0.5% HNO_3
- Mention the Chromium concentration and date of preparation on flask with permanent ink marker or label with sticker having following in formations.

Name of Reagent:
Date of Preparation:
Expiry Date:
Prepared <u>by</u>:

- Make entries in Chemical Reagent Preparation log book

3.6.9   Preparation of Working Standards for Chromium

3.6.9.1   *Reagents*

- Chromium Stock Solution of 1000 ppb
- 0.5% HNO_3

3.6.9.2 Equipment and Glassware

- 100µl-1000µl auto pipette
- Plastic tips for auto pipette
- 100 ml volumetric flasks
- 100 ml beaker
- 5-10 ml auto pipette

3.6.9.3 Procedure

- Take 25 ml of Chromium stock solution of 1000 ppb with auto pipette
- Add in to 250 ml volumetric flask to produce 100 ppb Chromium secondary standard
- Make the volume up to the mark with 0.5% HNO3.
- Prepare 20 ppb by taking 20 ml of 100 ppb Chromium standard.
- The Auto sampler will automatically prepare dilution of 20 ppb standard to prepare 5, 10 and 15 ppb standards
- Dispense solution of 100 ppb in to volumetric flasks according to *Table-7*. by using following formula

$$C_s V_s = C_w V_w$$

$$V_s = \frac{C_w V_w}{C_s}$$

Where   Cs = Concentration of Stock Solution

Vs = Volume of Stock Solution

Vw = Volume of working standard

Cw = Concentration of working standard

Standard values µg/L	Volumetric Flask	Volume taken from 100 µg/L ml
5	100 ml	5
10	100 ml	10
15	100 ml	15
20	100	20

- Make the volume up to the mark with 0.5% HNO_3
- Mention the Chromium concentration and date of preparation on flask with permanent ink marker or label with sticker having following information.

> Name of Reagent:
> Date of Preparation:
> Expiry Date:
> Prepared by:

- Make entries in Chemical Reagent Preparation log book.

3.6.10 Calibration for Chromium on Graphite Furnace Mode

3.6.10.1   Scope

Applying the Beer Lambert Law directly in AA spectroscopy is difficult due to variations in the atomization efficiency from the sample matrix, and non-uniformity of concentration and path length of analyte atoms. So unknown concentration measurements are determined from a working curve after calibrating the instrument with standards of known concentration.

3.6.10.2   Standards and Reagents

- Chromium standards of required concentration:
- 0.5% HNO_3

3.6.10.3 Calibration Procedure

- Open software from computer and select "**Graphite Furnace**" mode.
- Load the previous method for Chromium or from "**Cookbook**".
- Click on the option **"Spectrophotometer"** adjust energy of Chromium lamp do alignment of lamp in "**lamp turret**" if necessary.
- Properly align Graphite tube and Graphite platform with lamp beam and Adjust the needle of the auto sampler in Graphite platform for accurate volume transfer, a little disturbance will result in sample loss and damage of Graphite tube.
- Prepare Chromium stock solution of 20 ppb or as required for calibration and keep this stock solution in special sample cup position at **"Auto"** and place diluents (0.5% HNO_3) at its position in auto sampler.
- Prepare sample sequence and select the option **"Calibration"** at start of sampling table
- Feed different concentrations (5, 10, 15 and 20) of standards and respective volumes and draw calibration curve by running standards one by one (each standard 3 times).
- R^2 value of calibration curve should be equivalent to 1.00 and not less then 0.996.
- Click on option "**Method**" in software and save method of calibration.

3.6.11 Analysis of Chromium on Graphite Furnace

3.6.11.1 Procedure

- Open the AAS Software select Graphite Furnace Mode.
- Load Method for Chromium or draw new calibration curve.
- Adjust the parameter (energy of the lamp and Formation of the graphite tube).
- Click on the option "Sample" and insert the sample codes in sample table along with giving the sample cup positions as in auto sampler.
- Take sample in sample cup of auto sampler up to the mark and put in specific position of sample tray of auto sampler.
- Click on the option "Run Sample" from software or "Start Conc". after selecting the range of samples in table and giving working area.
- Reaction is completed in about two minutes.
- Each sample is analyzed three times.
- Results in selected concentration (ppb, ppm) are displayed on software worksheet.

3.6.11.2 Expression of Results

- Chromium result measured & record in ppb (µg/l)
- Recorded the results on analyst work book
- Calculate the uncertainty of measurement,
- Record the results on the Result Record Form
- Sent to the Customer Service Section for Reporting.

3.6.11.3 Quality Control

- Analyze Blank and Certified Reference Standard after every ten samples.
- Analyze pre-analyzed sample with every analytical batch.
- Analyze Spiked Matrix sample after every two months
- Analyze the Control Sample and display data is regularly on control charts.
- Record the daily quality control data in QC Data sheet.

3.6.11.4 Precautions

- All the glassware and auto sampler cups should be washed with 1N HNO_3 before reparation of standard and samples.

- Auto pipettes should be used carefully to take accurate volume during the analysis.

- Deionized water used should be of high quality having EC <1.5µS/cm.