Hydride Generation Atomic Absorption Spectroscopy for the Analysis of Mercury and Lead

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Certificate of Examination

This is to certify that the dissertation titled "Hydride Generation Atomic Absorption Spectroscopy for the Analysis of Mercury and Lead" submitted by Mr. Vivek Singh (Reg. No. MS08056) for the partial fulfilment of BS-MS dual degree program of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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(Supervisor)

Dated: 25th April 2014

Declaration

The work presented in this dissertation has been carried out by me under the guidance of **Dr. K.S. Viswanathan** at the Indian Institute of Science Education and Research, Mohali.

This work has not been submitted in part or in full for a degree, a diploma or a fellowship at any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all the sources listed within have been detailed in bibliography.

Vivek Singh

Dated: 25th April 2014

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Prof. K.S. Viswanathan

(Supervisor)

Acknowledgement

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I wish to thank the Library staff for providing the required facilities.

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Abstract

Atomic Absorption Spectroscopy (AAS) is a popular tool for the analysis of more than 70 elements. In conjunction with Hydride Generation, AAS becomes very powerful for the analysis of volatile hydride formers, such as As, Sn and other metals and metalloids. The hydride generation technique improves the detection limits and also helps in handling problems of spectral interferences. We have set-up a cheap and easy home-made hydride generation AAS set-up without the use of commercially available assemblies. We have then used that technique to do analysis for mercury and lead and have also analysed some water samples for the presence of these analytes.

Chapter – 1

Introduction

Spectroscopic Measurements

In spectroscopic measurements the interaction of radiation with matter is used to obtain information about a sample. A lot of chemical elements have been discovered by spectroscopy[1]. To measure an element by spectroscopic means the sample is generally stimulated using energy usually in the form of heat, light, electrical energy and chemical reactions. Prior to the application of stimulus, the analyte is predominately in its ground state. The application of stimulus causes some of the analyte species to undergo transition to an excited energy level. We acquire the information about the analyte by measuring the electromagnetic radiation absorbed or emitted by the analyte as a result of the stimulus.

Absorption Spectroscopy

When a sample is stimulated by an external electromagnetic radiation source, the radiation can be scattered or reflected or it can also be absorbed and thus promote some of the analyte species to an excited state, as depicted in the figure 1-a,b,c below[2].
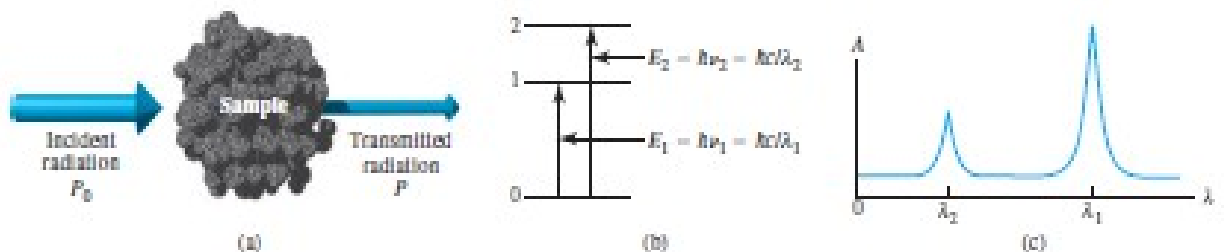
Figure 1-a:- radiation of incident radiant power P_o can be absorbed by the analyte, resulting in a transmitted beam of lower radiant power P. **Figure 1-b:-** for absorption to occur the energy of the incident radiation must correspond to one of the energy differences shown in figure. **Figure 1-c:-** the resulting absorption spectrum is shown in this figure.

Each molecular or atomic species is capable of absorbing its own characteristic

frequencies of electromagnetic radiation. This process transfers energy to the molecules and thus decreases the intensity of the incident electromagnetic radiation. The absorption of the radiation attenuates i.e. decreases the energy per unit area of the beam of radiation.

1) The Absorption Process

The absorption process is governed by the **Beer-Lambert law**[3]. It relates the attenuation of light intensity on the concentration of the absorbing molecules and the path length over which the absorption occurs. As light traverses a medium containing an absorbing analyte, the intensity decreases as the analyte becomes excited. For a given analyte solution of a given concentration, the longer the path length i.e. the length of the medium through which the light passes, the more absorbing molecules are in the path hence the attenuation is greater. Similarly, for a given path length of light, the higher the concentration of absorbing molecules, stronger is the attenuation.

Suppose we have a radiation of incident radiant power P_o passing through an analyte and the transmitted beam is of a lower radiant power P. Then the **Transmittance 'T'** of the solution is the fraction of incident radiation transmitted by the solution.

$$T = P/P_o \qquad\qquad (1.1)$$

a) Absorbance

The **Absorbance 'A'** of a solution is related to the transmittance in a logarithmic manner. The absorbance of a solution increases as the transmittance decreases.

$$A = -log\ T = -log\ P/P_o = log\ P_o/P \qquad\qquad (1.2)$$

Suppose we have an absorbing solution of thickness l cm and concentration c moles per litre. The radiant power of the beam passing through it decreases to P from P_o as shown in figure 1-d.

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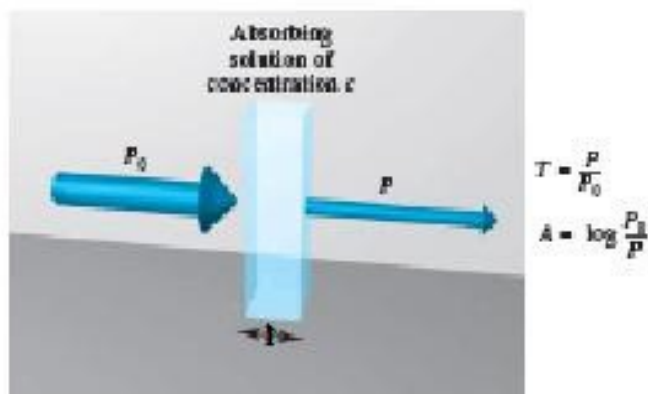
Figure-1-d:- depicts the attenuation in the radiant power of the incident beam. The high radiant power of incident beam P_o is depicted by a thicker arrow while the attenuated radiation P is depicted by a thin arrow.

b) Measuring Transmittance and Absorbance

We cannot measure transmittance and absorbance according to the above equations as the solution to be studied must be held in a cell or a cuvette. Substantial reflection and scattering losses can occur at the cell walls.

To compensate for these losses the power of the beam transmitted through a cell containing the analyte solution is compared with one that traverses an identical cell containing only the solvent i.e. Reagent blank. Hence we obtain an experimental absorbance that is approximately close to the true absorbance of solution.

$$A = log\ P_o/P \approx log\ (P_{solvent}/\ P_{solution})$$

(Equation-1.3:- $P_o \sim P_{solvent}$; $P \sim P_{solution}$**)**

c) Beer – Lambert law

The Beer – Lambert law states that the absorbance is directly proportional to the

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concentration of the absorbing species, *c,* and to the path length *l,* of the absorbing medium.

$$A = log\ (P_o/P) = alc$$

Equation-1.4:- *'a'* is a proportionality constant called **absorptivity**. Since absorbance is unit less, the absorptivity must have the units that cancel out the units of *'l'* and *'c'*.

When we express the concentration in terms of moles per litre and *'l'* in terms terms of 'cm', the proportionality constant is called the **molar absorptivity**, which is represented by the symbol **'ε'**. Hence,

$$A = \varepsilon lc \tag{1.5}$$

here **ε** has the units of $L\ mol^{-1}\ cm^{-1}$.

Absorbance for a multicomponent system at a single wavelength is the sum of the individual absorbances.

$$A_{total} = A_1 + A_2 + ... + A_n = \varepsilon_1 lc_1 + \varepsilon_2 lc_2 + ... + \varepsilon_n lc_n \tag{1.6}$$

2) Atomic Spectroscopy

Atomic Spectroscopic methods are used for more than 70 elements for their qualitative and quantitative determination. The detection limits of these methods is parts-per-million to parts-per-trillion and in some cases even lower concentrations can be detected.

In order to perform atomic spectroscopy we need to have the atomic species in a gaseous medium. Hence the first step of any atomic spectroscopic technique is **atomization**. It is a process in which a sample is volatilized and decomposed in such a way so as to give gas-phase atoms and ions. The sensitivity, accuracy and precision of the method is dependent on the efficiency and reproducibility of the atomization step. Inductively coupled plasma, flames and electro-thermal atomizers are the most common methods used for atomization.

a) Atomization

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Samples are generally introduced in the atomizers in the solution form and sometimes in gaseous and solid forms too. The atomization device converts the analyte species into gas-phase free atoms or elementary ions.

a-1) Sample introduction system

Atomization devices are of two types: ***continuous atomizers*** and ***discrete atomizers***. Continuous atomizers consists of plasma and flame atomizers in which samples are introduced in a steady, continuous stream. In discrete atomizers the individual samples are injected by means of a syringe or auto-sampler.

In continuous atomizers direct ***nebulization*** is used. Nebulization means to convert a liquid into fine spray or mist, hence the nebulizer constantly introduces the sample in the form of a fine spray of droplets called as ***aerosol***. When the sample is being introduced continuously into a flame or a plasma, a steady-state population of atoms, molecules and ions develops. The process of production of free ions or atoms is depicted in figure 2-a-1 below.
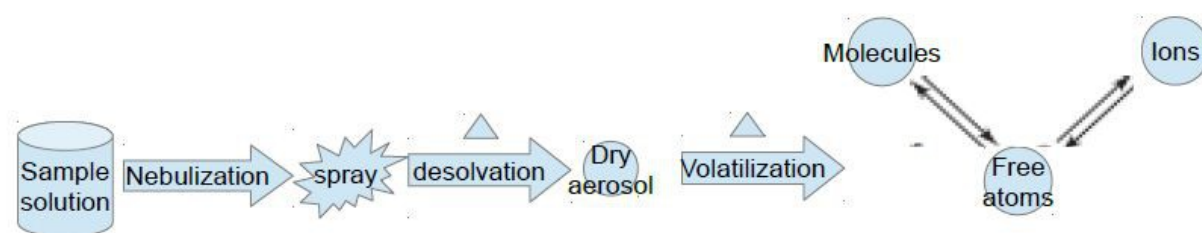
Figure 2-a-1:- Atomization

a-2) Flame Atomizers

Since we are using Atomic Absorption Spectroscopic techniques, we will mainly focus upon the ***flame atomizers***. A flame atomizer consists of a pneumatic nebulizer, that converts the sample solution into a mist, or aerosol, that is then introduced into a burner. In most atomizers, the high pressure gas is the oxidant, with the aerosol-containing oxidant being mixed subsequently with the fuel.

In flame spectroscopy the burners that are mostly used are often premixed, laminar flow.

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The aerosol from the nebulizer flows into a *spray chamber* from where only the finest droplets are allowed to pass through. Hence most of the sample is collected in the bottom of the spray chamber from where it is drained into a waste container. The solution flow rate varies from 2 to 5 ml/min. The sample spray is also mixed with fuel and oxidant gas in the spray chamber. The aerosol, oxidant and the fuel are then burnt in the slotted burner which provides a flame that is usually 5 or 10 cm high depending upon the fuel gas flow rate.

When the sample from the nebulizer is carried into the flame, the tiny droplets get desolvated in the *primary combustion zone* of the flame. From here, the fine solid particles are carried to the centre of the flame called as the *inner zone*. The inner zone is the hottest part of the flame hence the particles get vaporized and are converted to gaseous atoms, elementary ions and molecular species. Then the atoms, molecules or ions are carried to the outer edge or the *outer core* where atomization might occur before the atomization products disperse into the atmosphere. Since the fuel, sample flow rate is high through the flame, only a small fraction of the sample undergoes the above mentioned processes the various regions of a flame are depicted in figure 2-a-2.
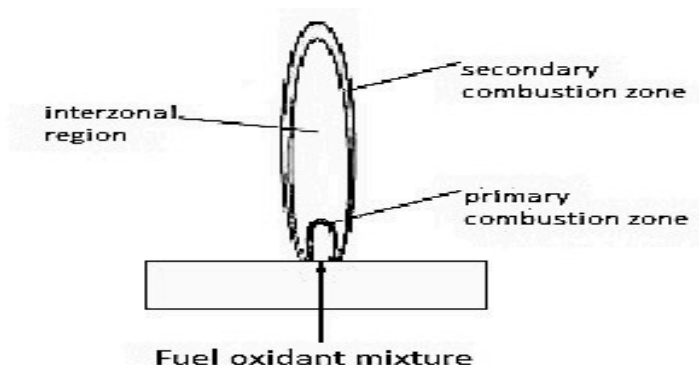
Figure 2-a-2:- Regions of flame

The different types of flames used in atomic spectroscopy are listed below along with their respective temperatures in table 1.

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Table 1:- Various Flames and their Temperatures

Fuels and oxidants	Temperature, ^{o}C
Acetylene/Air	1700-1900
Acetylene/O_2	2700-2800
H_2/Air	2000-2100
H_2/O_2	2500-2700
C_2H_2/Air	2100-2400
C_2H_2/O_2	3050-3150
C_2H_2/O_2	2600-2800

Flame temperature affects both emission and absorption spectra. Higher flame temperature increases the total atom population of the flame and thus the sensitivity. Atomic emission spectroscopy is more sensitive to flame temperature as compared to atomic absorption spectroscopy. This is because in the case of atomic absorption, the signal depends on the number of atoms in ground state that will absorb energy. The number of atoms in ground state is always very high as related to the number of excited atoms, whereas in the case of atomic emission spectroscopy the signal depends upon the number of atoms in the excited state.

In emission spectroscopy at high flame temperature the analyte metal atoms that are present in the flame in gaseous state react with the flame components to produce molecules and ions that also emit and absorb radiation and hence hinder the analysis. At elevated flame temperatures atoms with low ionization potentials become ionized. Any ionization reduces the population of both the ground state and the excited state of neutral free atoms, thus lowering the sensitivity of the determination.

However, there are some indirect effects of temperature on atomic absorption spectroscopy. These effects can be summarized as:

- Better sensitivities are obtained at higher temperatures since higher temperatures can increase the number of vaporized atoms at a time.

- Higher temperatures will increase the velocities of gaseous atoms, thus causing

line broadening as a result of the Doppler and collisional effects.

- High temperatures increase the number of ionized analyte and thus decrease the number of atoms available for absorption.

Chapter – 2

Atomic Absorption Spectroscopy(AAS)

1) History

Flame atomic absorption spectroscopy is the most widely used technique because of its effectiveness and simplicity for a relatively low cost. This technique was introduced in **1955** by **Sir Alan Walsh** in **Australia** and by **Alkermade** and **Milatz** in Holland. The first commercial atomic absorption spectrometer was introduced in the year **1959**[6].

2) Uses of Atomic Absorption Spectroscopy

i) Analysing metals in biological fluids such as blood and urine

ii) Detecting the amounts of various elements in rivers, seawater, drinking water, petrol etc.,

iii) In pharmaceutical industry the catalyst used for the production of a drug can be left behind, hence AAS technique is useful in detecting them.

iv) In industries it is used to detect and remove toxic impurities.

v) For mining of metals AAS can be used to determine whether the amount of metal present is worth mining for or not.

3) Principle behind AAS

Atoms of each element absorb a characteristic wavelength of light. Thus to analyse any element we use only the light emitted from that element. As the monochromatic are not generally capable of giving a radiation which is as narrow as the width of atomic absorption line which is in the range of 0.002-0.005 nm. This is because of the widths of atomic absorption lines being much less than the effective bandwidths of the

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monochromatic. This results in the departure from the **Beer-Lambert law** and also the fraction of radiation absorbed from such a beam is very small hence the output signal received is very small i.e. $P \rightarrow P_o$ which decreases the sensitivity of the measurement.

The effects of line-width are overcome by using radiation from a source which emits light of the same wavelength as the one used for measurements and also has a narrower bandwidth than that of the analyte in the flame.

If we want to estimate iron in a sample, one will have to use a light source which essentially contains the emission line of iron. Usually a hollow cathode lamp where the cathode is iron is used. Some of this light is then absorbed by the atomized iron in the flame. The amount of light absorbed is directly proportional to the concentration if the iron atoms in the flame. A calibration curve is then constructed using standards of known concentration of iron. Then the unknown sample is analysed and its concentration is measured with respect to the calibration curve.

4) Types of Atomic Absorption Spectrometer

i) Single beam Atomic Absorption Spectrometer

ii) Double beam Atomic Absorption Spectrometer.

5) Instrumentation

The schematic diagram for the basic instrumentation for AAS is depicted in figure 3 below
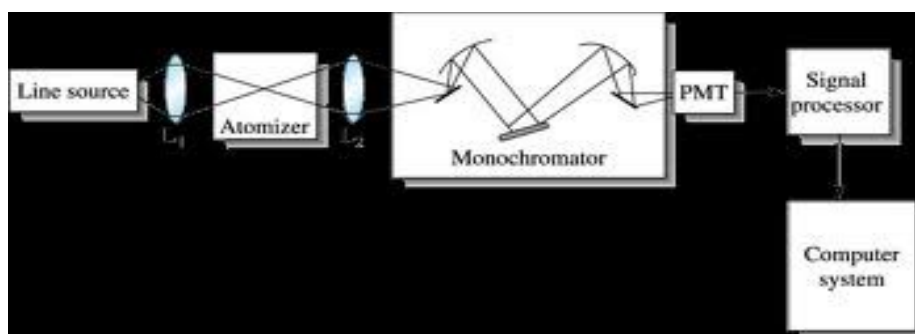
Figure 3:- Schematic of Atomic Absorption Spectrometer

a)Light Source (Atomic line source)

The most commonly used light source in AAS is the ***hollow cathode lamp***[7]. It consists of a tungsten anode and a cathode made up of the element which is to be analysed. The anode and cathode are sealed inside a glass tube filled with an inert gas like neon or argon at a pressure of between 1 to 5 Nm^{-2}.

In a hollow cathode lamp we apply a potential difference of around 300-400 V which ionizes the atoms of the inert gases. These gaseous ions then bombard the cathode and eject metals atoms from the cathode by a process called ***sputtering***. Some of the sputtered atoms are in the excited states and emit radiation characteristic of the metal as they fall back to their ground state.

$$M^{*} \rightarrow M + h\upsilon$$
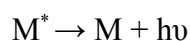
The size of the cathode is such that the radiation emitted from it is concentrated into a beam which passes through a quartz window, and the shape of the lamp is such that most of the sputtered atoms are redeposited on the cathode as depicted in figure 4 below
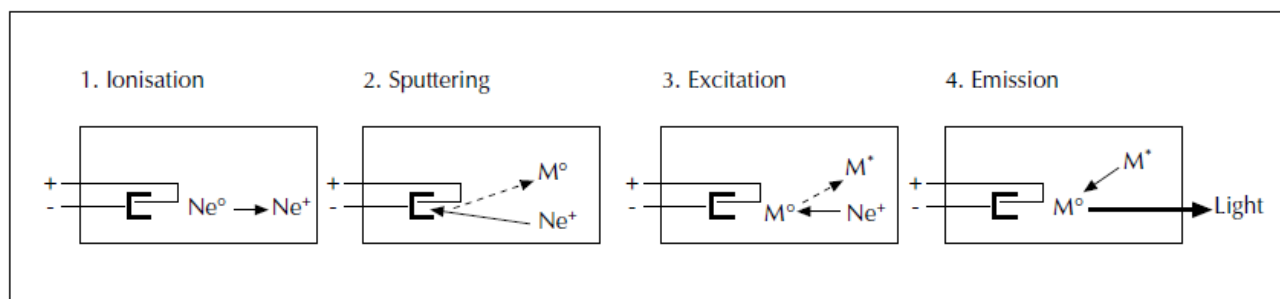
Figure 4:- Sputtering and emission process

Hollow cathode lamps of almost 70 elements are commercially available. Some hollow cathode lamps have a cathode containing more than one element and thus provide the means to analyse more than one element at a time. The development of hollow cathode lamp is widely regarded as the single most important event in the evolution of atomic absorption spectroscopy. The basic structure of a hollow cathode lamp is depicted in figure 5.
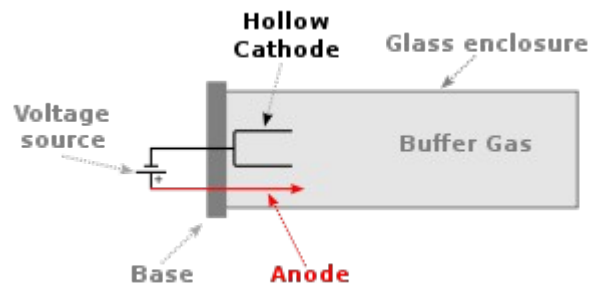
Figure 5:- Hollow Cathode Lamp

A typical atomic absorption spectrometer holds several lamps of different elements which are mounted on a rotating turret so that the appropriate lamp can be selected conveniently.

b) Modulation of the radiation from the source

We need to discriminate between the radiation from the source and the radiation from the atomizer. Most of the radiation from the atomizer is removed by the monochromator that is located between the atomizer and the detector. However due to the thermal excitation of analyte atoms in the flame a radiation of the wavelength same as the wavelength at which the monochomator is set, gets produced. This radiation is not removed by the monochromator and hence it might cause some interference.

This is overcome by modulating the output from the hollow cathode lamp in such a way that its intensity fluctuates with a constant frequency. We can modulate the signal by placing a motor-driven circular chopper between the source and the flame. The circular chopper is designed in such a way that the radiation passes through half of the time and is blocked the other half

c) Atomizer

The atomizers that are most commonly used are the flame atomizers and electro-thermal atomizers. The process of atomization has been explained in **Chapter – 1**. In addition to flame and electro-thermal atomizers we also use glow-discharge atomization, hydride atomization, or cold-vapour atomization.

d) Monochromator

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A monochromator is used to select the specific wavelength of light that is absorbed by the sample and excludes all the other wavelengths that are present. This enables us to determine the selected element in presence of others.

e) Detector

The detector is generally a ***Photo Multiplier Tube(PMT)*** which detects the signal coming from the monochromator and produces an electrical signal proportional to the intensity of radiation coming from the monochromator. The signal is then processed and displayed using a computer system.

Note that the above mentioned working is for a ***Single Beam Atomic Absorption Spectrometer***.

f) Instrumentation of a double beam spectrometer

In a double beam spectrometer there is a beam splitter which splits the radiation beam coming from the hollow cathode lamp into two parts such that one part passes through the sample and the other through a reference cell. This is done because the intensity of light from the source might not stay constant throughout the experiment. Hence if the intensity is changing and we are using only a single beam, a blank reading containing no analyte has to be taken first, setting the absorbance to zero. And if the intensity changes by the time the sample is introduced to be analyzed, the measurements will be inaccurate.

In a double beam spectrometer there is a constant monitoring between the reference beam and light source. The beam splitter is designed in such a way that as high a proportion of radiation from the lamp is passed through the sample in order to ensure that there is no loss of sensitivity of the spectrum. A half silvered mirror returns both the beams to a single path through which they pass through the monochromator alternately and then to the detector. The signal produced by the flame is dc whereas the signal from the chopped light source is ac in nature and the signal processor separates the ac signal from the dc signal. The logarithmic ratio of the reference and sample components of the ac signal is then computed and displayed as absorbance by the computer or a read-out device. The set-up of double beam spectrometer is shown in figure 6

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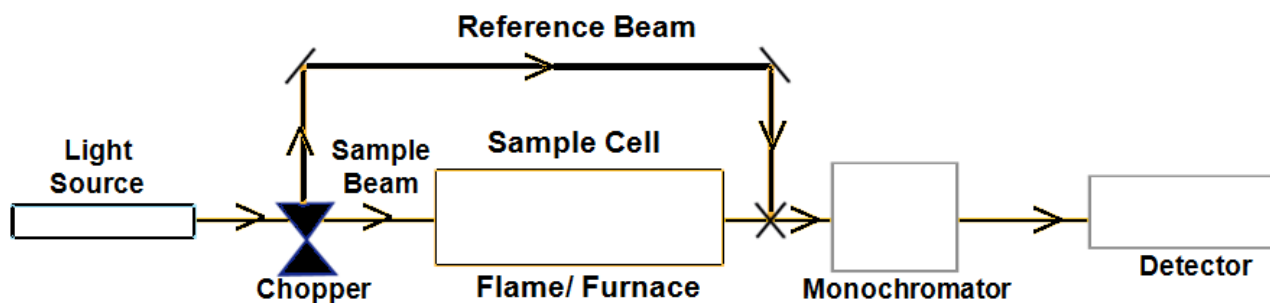
Figure 6:- double beam spectrometer

6) Interferences in Atomic Absorption Spectroscopy

a) Background Corrections

The lines from hollow cathode lamps are very narrow hence the interferences by absorption of analyte lines by other atoms is very rare. But the flame itself and other molecular species that might be present can absorb the radiation and cause errors in the atomic absorption measurements.

So in order to remove such errors we consider the total absorbance A_T which is the sum of the analyte absorbance A_A and the background absorbance A_B.

$$A_T = A_A + A_B \qquad\qquad (6\text{-}1)$$

Background correction methods are used to measure the background absorbance A_B from where we can then calculate the true analyte absorbance

$$A_A = A_T - A_B \qquad\qquad (6\text{-}2)$$

The different kinds of background correction techniques that are used are listed below

i) Continuum Source background correction

A continuum source background correction uses a ***deuterium lamp*** to obtain an estimate of the background absorbance. The deuterium lamp and hollow cathode lamp are directed through the atomizer at different times. The hollow cathode lamp measures the total absorbance. The intensity of light from deuterium lamp is quite low in the visible regions hence for the elements with lines in the visible range this technique is not able to measure

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the background absorbance correctly.

ii) Pulsed Hollow Cathode Lamp background correction

This technique is also called **Smith-Hieftje background correction**. In this technique the hollow cathode lamp is first pulsed at a low current (5 to 20 mA) for about 10ms and then it is pulsed at high current (100 to 500 mA) for about 0.3ms. During the low current pulse, the total absorbance A_T is measured and during the high current pulse the background absorbance A_B is measured. The measurement of the background absorbance is possible because during the high current pulse the hollow cathode line is broadened and much of the central line is self absorbed and the line of wavelength required to measure the analyte is missing, which in-turn enables us to record the background absorbance.

iii) Zeeman Effect background corrections

In this technique a magnetic field is used to split spectral lines from the hollow cathode lamp that are normally of the same energy (degenerate) into components with different polarization. Analyte and background absorptions can be separated because of their different magnetic and polarization characters.

b) Chemical interference:- Formation of compounds of low volatility like Ca^{2+}, PO_4^{3-}, Mg^{2+}, Al^{3+} is one of the most common kind of chemical interference. The other type of chemical interference is ionization of the analyte. The interference due to the formation of non-volatile compounds can be overcome by increasing the flame temperature either by changing the fuel-to-oxidant ratio or by switching to a different combination of fuel and oxidant.

In case of ionization, the absorption spectrum of the metal and metal ions are entirely different which affects the absorbance at wavelength where the metal absorbs. This can be overcome by using an *ionization suppressor*, which is nothing but a species that ionizes much more quickly than the analyte itself. Which in turn reduces the chances of the analyte ionization. Potassium and Cesium are the most commonly used ionization suppressors.

7) Detection limits of AAS

The detection limits of some of the elements for flame AAS are listed below along with

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the detection limits in case of electro-thermal AAS.

Table 2:- Detection limits in AAS (in ppm) for some elements

Element	Flame AAS	Electro-thermal AAS
Ag	0.003	0.00002
Al	0.03	0.0002
Ba	0.02	0.0005
Ca	0.001	0.0005
Cd	0.001	0.00002
Cr	0.004	0.00006
Cu	0.002	0.0001
Fe	0.006	0.0005
K	0.002	0.0001
Mg	0.0002	0.000004
Mn	0.002	0.00002
Mo	0.005	0.001
Na	0.0002	0.00004
Ni	0.003	0.001
Pb	0.005	0.0002
Sn	0.015	0.010
V	0.025	0.002
Zn	0.001	0.00001

8) Advantages and Disadvantages of Flame AAS

The main advantages of AAS are that it has a very high throughput, is very precise and easy to use and is also very cost effective.

The main disadvantages of this technique are that it can only be used to analyse solutions, is less sensitive as compared to electro-thermal technique and requires large sample quantities relatively.

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Chapter-3

Hydride Generation Atomic Absorption Spectroscopy (HG-AAS)

1) History

Atomic absorption spectroscopy is one of the most commonly used techniques for analysis of metals and metalloids. However because of the spectral and chemical interferences, low detection limits we employ a different technique for some of the elements especially in the case of metalloids.

Arsenic, antimony, bismuth, selenium, lead, mercury[8] etc. are known to form volatile hydrides with nascent hydrogen. Their ability to form volatile gaseous hydride can be utilized for the separation and enrichment of the analyte. During the formation of volatile gaseous hydrides the impurities that might cause spectral and chemical interference during flame AAS are reduced and even completely eliminated.

The hydride generation technique in AAS was first used by Holak *et. al.*, in the year 1969[9]. He generated arsenic hydride and collected it in liquid hydrogen. He heated the arsenic hydride and then purged the arsenic hydride using a stream of nitrogen into an argon-hydrogen flame for measuring atomic absorption.

2) Production of nascent hydrogen

The most common method used for the generation of nascent hydrogen is the addition of zinc or aluminium to hydrochloric acid. But there are disadvantages of metal-acid reactions, such as:-

i) It requires high purity metals.

ii) Very low amount of hydrogen (about 8%) is released.

These disadvantages are overcome by using sodium borohydride as the reducing agent. In 6M hydrochloric acid solution using a nitrogen stream to release hydrogen, we can increase the yield of nascent hydrogen to about 40-60%.

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The chemical reactions taking place for the production of nascent hydrogen can be summarized as follow:-

$$Zn + HCl \rightarrow ZnCl_2 + H_2 \uparrow$$
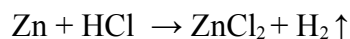
if we use $NaBH_4$ as a reductant, then the reaction involved are:-

$$NaBH_4 + HCl \rightarrow NaCl + BH_3 + H_2 \uparrow$$

3) Advantages of hydride generation AAS

i) One of the major advantages of the hydride generation technique over other atomic absorption techniques is that during hydride generation only the analyte is separated as a volatile hydride from the sample[10]. And only a very small number of other components reaches the atomizer which in-turn decreases the chances of interference.
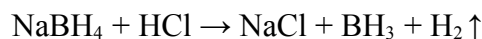
ii) Hydride generation technique is an absolute technique and is concentration independent. Very low volume of the analyte and reagents is required for the analysis. The sample volume required is as low as 0.5 ml and only about 10 ml of reagent is required.

4) Principle of hydride generation AAS

The reaction of many metalloid oxy-anions with sodium borohydride and HCl produces a volatile hydride: H_2Te, H_2Se, H_3As, H_3Sb, etc. A specific oxidation state for the metalloids is required before it is introduced into the hydride generation system. The time for which the reagents are mixed and the time when the volatile metal hydride is removed from the liquid mixture and sent to the optical cell is also important. This is taken care of by using peristaltic pumps to flow the reagents together and using tubing of specific lengths. Once the reagents and analyte are mixed together, their liquid mixture is passed through tubing of specific length which gives a controlled reaction time and is then passed through a gas/liquid separator which bubbles out the hydride and some gaseous hydride, which is then purged using nitrogen or argon into the optical cell placed in the path of the radiation from the hollow cathode lamp.

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5) Chemical reactions

The chemical reactions take place in an acidic medium. The analyte metal solution is acidified using HCl or H_2SO_4. The acidic analyte solution is mixed with an alkaline solution of sodium borohydride (0.5-10% w/v $NaBH_4$ in 0.1-1M NaOH).

Hydrogen atoms are produced from the reaction between BH_4^- and H^+

$$BH_4^- + H^+ + 3H_2O \rightarrow H_3BO_3 + 8H$$

The hydrogen atoms produced in the above reaction reduce the analyte into their respective hydride

$$H_3AsO_3 + 6H \rightarrow AsH_3 + 3\ H_2O$$

$$H_2SeO_3 + 6H \rightarrow H_2Se + 3H_2O$$
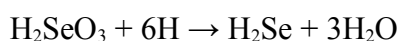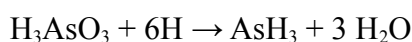
the desirable oxidation states of the analytes are obtained by reaction with KI

$$H_3As^{(v)}O_4 + 2I^- + 2H^+ \rightarrow H_3As^{(III)}O_3 + I_2 + H_2O$$
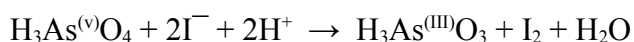
6) Instrumentation

The basic components of a hydride generation spectrometer are the same as that of an atomic absorption spectrometer. The difference being a separate hydride generation module and an optical cell. The components are listed below:-

a) Hollow Cathode Lamp

It provides the radiation required for the analysis of the analyte.

b) Hydride Generation System[11]

The main functions of this system are to aspirate the liquid sample at a controlled rate, mix liquid sample with $NaBH_4$ and HCl, creating the volatile metal hydride of the analyte metal and introduce it into the optical cell placed in the path of the light from the hollow cathode lamp.

c) Optical cell and Flame

Gaseous hydride from the hydride generation system is flown into the optical cell that is

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placed over a high temperature flame of AAS in the path of the radiation from the hollow cathode lamp. The optical cell is generally made up of quartz. The metalloid hydride is decomposed inside the optical cell into its elemental form i.e. As^0, Se^0 etc. which absorbs the light coming from the hollow cathode lamp.

d) Monochromator

A monochromator is used to select the specific wavelength of light which is absorbed by sample and exclude all the other wavelengths.

e) Photo-multiplier tube

It acts as the detector and measures the intensity of light exiting the monochromator and gives a signal respectively.
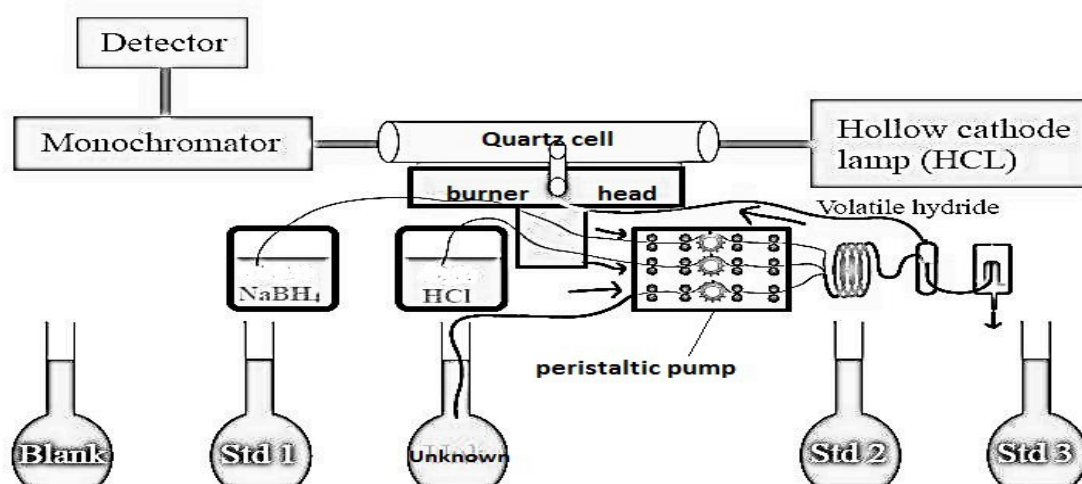
Figure 7:- Shows the schematics of a basic Hydride Generation AAS

7) Precautions while doing hydride generation

i) The oxidation state of the metalloid is very important during hydride generation AAS. For e.g. Selenium(VI) behaves erratically and does not produce hydride readily, hence we need to have Se(IV) oxidation state for hydride formation. This is done by using nitric acid or hydrogen peroxide and then reducing the selenite by using boiling HCl.

ii) The acidic content of the analyte should also be kept in mind while doing hydride generation. Higher acidic content increases the peak height absorbance but more than

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30% v/v concentration of HCl produces background errors. The background errors produced are dependent on the acids and their concentrations, but if we are using HCl then 25-20% v/v concentration is the ideal concentration for best results[12].

iii) The concentration of sodium borohydride and hydrochloric acid which are added to the reaction vessel for hydride generation is also important. It should be varied for element to element that we want to analyse to arrive at the optimum concentration.

8) Interferences in Hydride Generation AAS[13]

Interferences in hydride generation AAS technique occur at both the hydride generation stage and also in the hydride atomization stage.

i) A few metals interfere during the hydride generation and result in the decrease in absorbance for the analyte. For e.g. for the determination of arsenic and selenium, copper(II), nickel(II) and cobalt(II) affect the formation of arsine and hydrogen selenide. This is due to the formation of chemical species between arsenic or selenium and the interfering metals. It can be taken care of by keeping low concentration of sodium borohydride and the chemical reagents for hydride generation are added slowly as well as by using continuous flow equipment so as not to allow the reagents sufficient mixing time with the interfering metals as well as separating the desired hydride formed rapidly from the sample matrix containing the interfering metals.

ii) Some hydride forming elements that might be present with the analyte interfere during the atomization of the analyte hydride[14]. This can be avoided by using using high temperature flame for atomization. The type of interference that occurs is the due to the flame radical absorption. For elements such as arsenic and selenium with wavelengths below 200nm even the free radicals from the carrier gases like nitrogen and argon that are used to purge the hydride absorb some of the radiation during atomization by a flame. This can be avoided by using a heated quartz tube for atomization instead of using a flame for atomization.

Experimental Section

Chapter – 4

1) Aim

We have tried to use AAS and hydride generation to study lead and mercury levels in the water samples available locally. We have also studied the use of hydride generation technique to correct for the interferences in the absorption spectra of mercury due to presence of iron as both have atomic absorption wavelengths that are close to each other.

2) Materials And Reagents

i) LABINDIA AA-7000 atomic absorption spectrometer employing air/acetylene flame.

ii) Nitrogen gas used to purge the hydride.

iii) Tygon tubing for gas delivery

iv) Quartz cell for sampling the metal hydride for AAS measurements.

v) Four neck 50 ml round bottom flask.

vi) $Pb(NO_3)_2$, $HgCl_2$, $FeSO_4.7H_2O$, $CuSO_4 \cdot 5H_2O$, Tartaric Acid, concentrated HCl, $NaBH_4$.

Figure 8:- Quartz cell for hydride generation AAS

3) Experimental Set-up
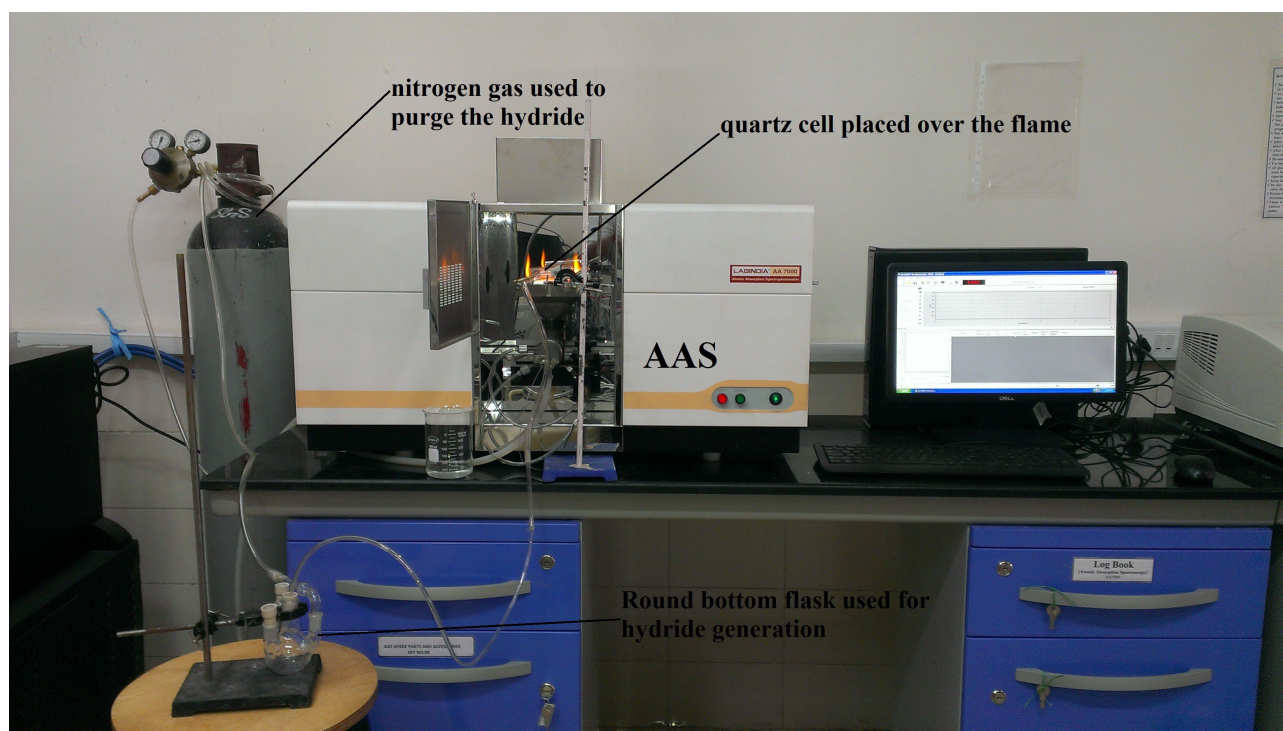
Figure 9:- Experimental set-up for hydride generation AAS

4) Methodology

100 ppm stock solutions of lead, mercury, copper and iron in 100 ml water were prepared from where the subsequent standards of varying concentrations were prepared. The hydride generation was done in a 50 ml 4 neck round bottom flask which was sealed by using a rubber septum. The reagents were added into the round bottom flask, using syringes. One of the necks of the flask was connected to a nitrogen cylinder by a tygon tube which was used to purge the hydride of the analyte, into the flame or the quartz tube placed on the flame. The hydride was carried from the flask by nitrogen via another neck at the top of the flask using tygon tubing. The other two necks of the flask are sealed with a rubber septum and were used for adding the reagents using syringes.

5) Hydride Generation AAS of Mercury

a) Direct introduction of the hydride into the flame

i) Method

- A stock solution of 100 ppm mercury in 100 ml distilled water was prepared. Using this stock solution, standards of 1, 2, 3, 5, 10, 15, and 50 ppm concentrations were prepared in a 10 ml volumetric flask by appropriate dilutions.

- 100 ml of 0.5 M of HCl was prepared by adding 4.1 ml of concentrated HCl in a 100ml volumetric flask and adding distilled water to make up the volume to 100 ml.

- 5% w/v solution of $NaBH_4$ was prepared by adding 5 g of $NaBH_4$ in distilled water in a 100 ml volumetric flask and diluting it to the required volume.

- The reagents were freshly prepared for each analysis.

- The round bottom flask used for the generation of hydride was thoroughly cleaned prior to each analysis. One neck was connected to a nitrogen cylinder using tygon tubing, which was used to transport the hydride from the flask into the flame, the hydride being transported through a second neck. The other necks were sealed using rubber septum and used to introduce reagents.

- 10 ml of HCl was added to the round bottom flask and to it 2 ml of the analyte was added.

- The flow from the round bottom flask into the flame was kept to the minimum so as not to disturb the flame. The flow was not measured thought ideally a mass flow controller could be used.

- 10 ml of $NaBH_4$ was added to the round bottom flask by a syringe.

- Standard solutions of mercury were used for the analysis and the absorbance measured. Blank estimations were also done, wherein one reagent after another was systematically avoided. First was the analysis without adding HCl to the analyte and the second one was without the addition of $NaBH_4$, into the solution of mercury. The third one was performed without adding mercury to HCl and

NaBH$_4$. These experiments were designed to ensure the necessity of all the reagents and this was done in order to check for errors in the method of hydride generation and to make sure that the absorbance that we observed were because of the formation of mercury hydride and its eventual atomization in the flame.

ii) Observations

Two sets of experiments were obtained where the hydride of Hg was directly introduced into the flame. The following values for absorbance were obtained for mercury and the plot of absorbance against concentrations is shown below for the two sets of data where the concentration of Hg ranged from 1 to 100 ppm. The set of absorbances for the particular concentration that are observed are shown in table 3 and 4 and their Absorbance against concentration plots are shown in figure 10 and 11 respectively.

Concentration(ppm)	Absorbance
1	0.0673
3	0.1205
5	0.1679
10	0.2569
15	0.3986
50	0.8231
100	1.3289

Table 3:- First set of Absorbance values of mercury without quartz cell

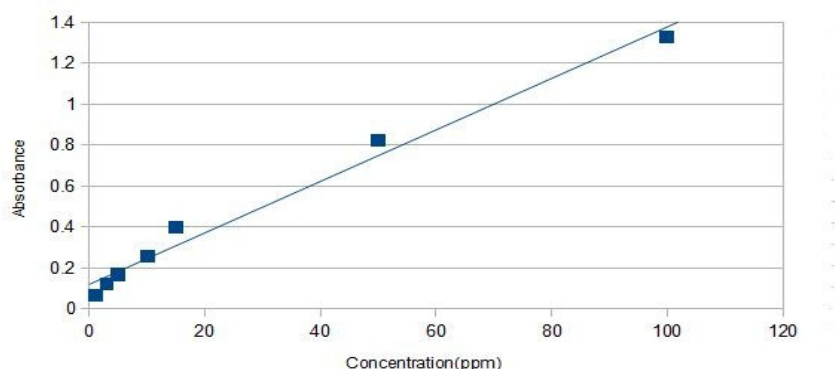**Figure 10:-** Plot of Absorbance against concentration of mercury for the first set of data

Concentration(ppm)	Absorbance
2	0.0754
4	0.1376
8	0.1771
15	0.3624
20	0.4927
25	0.6148
50	0.9528

Table 4:- Second set of Absorbance values of mercury without quartz cell
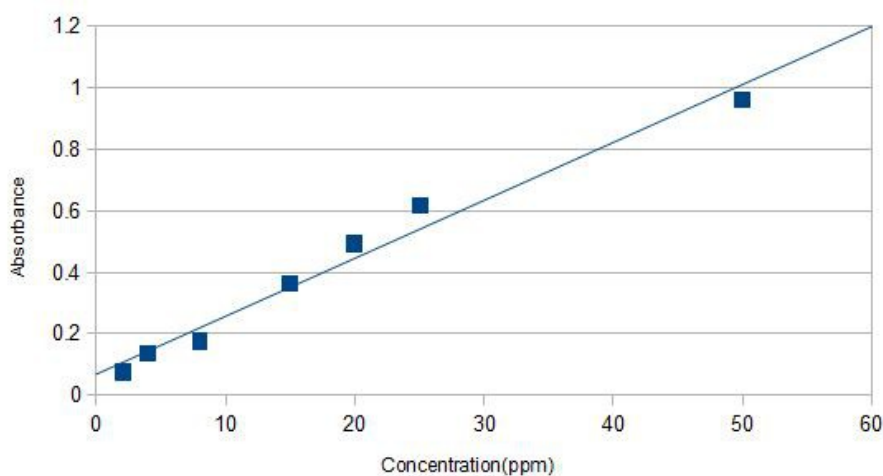
Figure 11:- Plot of Absorbance against concentration of mercury for second set of data

On combining the above sets of readings into one global plot, the following was obtained. One thing that should be noted here is that the Beer-Lambert law is followed only when the values of absorbance does not exceed 0.6. Hence we can neglect the values of absorbance for 50 ppm and 100 ppm solutions and do the global plotting for concentrations ranging from 1 ppm to 25 ppm. Figure 12 depicts the global plot of Absorbance against concentration.

Figure 12:- Global plot of Absorbance against concentration of mercury

b) Using a quartz cell

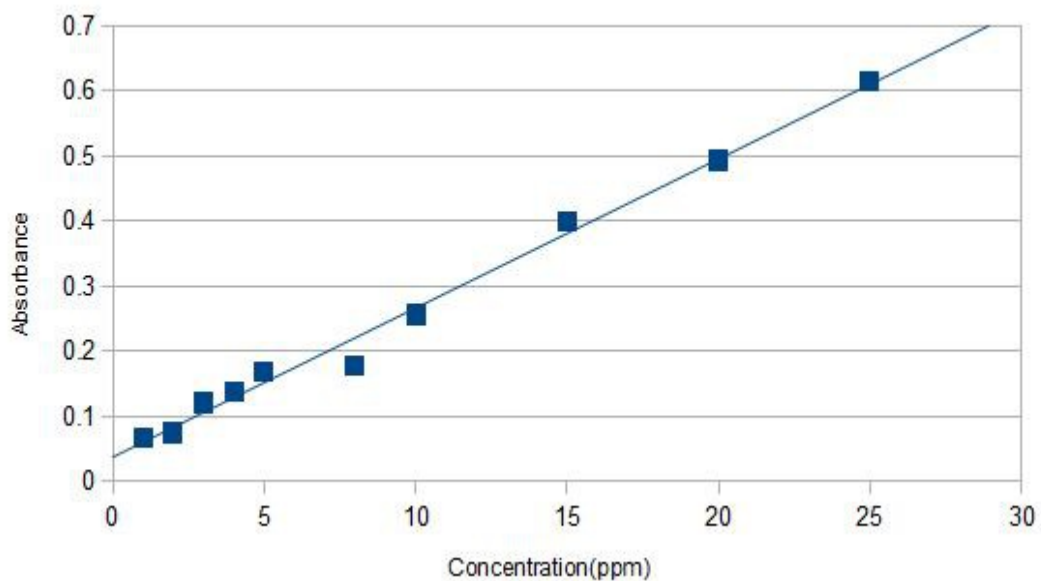In another modification of the experiment, the metal hydride was introduced into a quartz cell which was placed in the flame. The flame temperature decomposed the metal hydride into the metal atoms for analysis. In this method, the flow of the gas would not be expected to affect the flame and hence it would be interesting to see if the signals were superior in this method.

i) Method

- The reagents were prepared in the similar way as in the case of analysis without using a quartz cell.

- Instead of flowing the hydride directly into the flame. A quartz cell was placed on the air-acetylene flame in the path of the radiation from the hollow cathode lamp. The hydride generated in the round bottom flask was flown to the quartz cell by purging with nitrogen.

- 10 ml of HCl was added to the round bottom flask and to it 2 ml of the analyte

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was added to it.

- 10 ml of NaBH$_4$ was added to the round bottom flask by a syringe.

- Standard solutions of mercury were used for the analysis and the absorbance measured. Blank estimations were also done, wherein one reagent after another was systematically avoided. First was the analysis without adding HCl to the analyte and the second one was without the addition of NaBH$_4$, into the solution of mercury. The third one was performed without adding mercury to HCl and NaBH$_4$. These experiments were designed to ensure the necessity of all the reagents and that the absorbance that we observed were because of the formation of mercury hydride and its eventual atomization in the quartz cell.

ii) Observations

The following values of absorbance were obtained for mercury. The absorbance for the particular concentration that is observed is shown in table 5 and the Absorbance against concentration plot is shown in figure 13.

Concentration(ppm)	Absorbance
1	0.0055
5	0.0589
10	0.1287
20	0.2160
50	0.5253

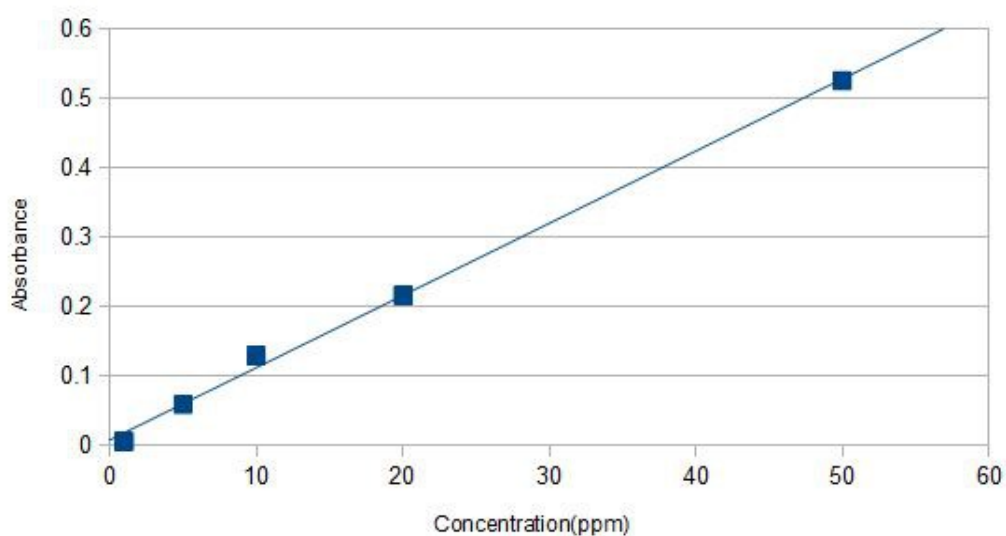**Table 5:-** Set of Absorbance values of mercury using a quartz cell

Figure 13:- Plot of Absorbance against concentration of mercury

- The values of absorbance that were obtained were much higher in the case when the hydride of mercury was introduced directly into the flame and atomized there as compared to the case when the hydride was atomized in the quartz cell.

- The absorbance observed in the case of atomization inside a quartz cell was close to the absorbance values obtained during simple mercury flame atomic absorption analysis.

- There was no reading for absorbance when the test analysis were run in each of the cases i.e. while doing analysis by atomization in flame and also in case of atomization in quartz cell.

- One of the major advantage of using a quartz cell for doing hydride generation atomic absorption analysis was that we obtained a steady signal for a considerable amount of time as compared to a sharp peak in case of direct introduction of hydride into the flame. As the rate of release of hydride is different in case of each metalloid, it is much better to go for peak area integration while determining the absorbance values than using peak height measurement.

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c) Conclusions

- A calibration curve for mercury was obtained in each of the cases by using hydride generation AAS technique.

- A simple, easy to use, cheap and one without using a hydride generation assembly that is commercially available technique was set-up in which the analyte can be analysed by a simple flame atomic absorption spectrometer just by introducing the hydride directly into the flame or by using a quartz cell.

- Detection limits for hydride generation AAS analysis of mercury without using a quartz cell was found to be 0.0013 ppm, and for the analysis using a quartz cell was 0.0049 ppm.

6) Analysis of water sample from local source for presence of mercury

a) Objective

- The water sample from the drinking water supply from Hostel – 5 and Academic Block of IISER MOHALI were analysed for the presence of mercury.

b) Method

- The mode of analysis was firstly simple flame atomic absorption analysis.

- The instrument was set to analyse for mercury.

- 10 ml of water sample from each source was analysed and compared with the flame atomic absorption analysis results of mercury done previously.

- Then the water sample was analysed using the hydride generation technique and the introduction of the generated hydride directly into the flame for atomization without using a quartz cell.

- The absorbance values from the hydride technique were correlated with the calibration curve obtained earlier for analysis without using a quartz cell.

c) Observations

- The analysis of water by simple flame atomic absorption was inconclusive in case of water from both the sources.

- The following values of absorbance were observed when the water samples were run through hydride generation

Source:- Hostel – 5	**Source:-** Academic Block
i) 0.0127	i) 0.0042
ii) 0.0112	ii) 0.0049
iii) 0.0101	iii) 0.0057
Average absorbance:- 0.0113	Average absorbance:- 0.0049
Concentration:- 143 ppb approx.	Concentration:- 3.8 ppb approx

d) Conclusions

- The water samples that were collected upon analysis by hydride generation technique showed that there was mercury present in them although at levels below what we had analysed during the preparation of the calibration curve.

- It can be said that the concentration of mercury in the water samples was in parts per billion level.

7) Hydride Generation AAS of lead by direct introduction in flame

a) Method[15]

- A stock solution of 100 ppm lead in 100 ml distilled water was prepared. Using this stock solution, standards of 1, 2, 3, 5, 10, 15, and 50 ppm concentrations were prepared in a 10 ml volumetric flask by appropriate dilutions.

- 100 ml of 0.5 M of tartaric acid was prepared by adding 7.5 g of tartaric acid in a

100ml volumetric flask and adding distilled water to make up the volume to 100 ml.

- 5% w/v solution of $NaBH_4$ was prepared by adding 5 g of $NaBH_4$ in distilled water in a 100ml volumetric flask and diluting it to the required volume.

- The reagents were freshly prepared for each analysis.

- The round bottom flask used for the generation of hydride was thoroughly cleaned prior to each analysis. One neck was connected to a nitrogen cylinder using tygon tubing, which was used to transport the hydride from the flask into the flame, the hydride being transported through a second neck. The other necks were sealed using rubber septum and used to introduce reagents.

- 10 ml of tartaric acid was added to the round bottom flask and to it 2 ml of the analyte was added.

- The flow from the round bottom flask into the flame was kept to the minimum so as not to disturb the flame. The flow was not measured thought ideally a mass flow controller could be used.

- The reagents were heated inside the round bottom flask to about 70-80°C

- 10 ml of $NaBH_4$ was added to the round bottom flask by a syringe.

- Standard solutions of lead were used for the analysis and the absorbance measured. Blank estimations were also done, wherein one reagent after another was systematically avoided. First was the analysis without adding tartaric acid to the analyte and the second one was without the addition of $NaBH_4$, into the solution of lead. The third one was performed without adding lead to tartaric acid and $NaBH_4$. These experiments were designed to ensure the necessity of all the reagents and this was done in order to check for errors in the method of hydride generation and to make sure that the absorbance that we observed were because of the formation of lead hydride and its eventual atomization in the flame.

b) Observations

The following values of absorbances were seen for different concentrations of lead as shown in table 6 and figure 14 is a plot of Absorbance against concentration.

Concentration(ppm)	Absorbance
50	0.0200
100	0.1127
1000	0.4879

Table 6:- Set of Absorbance values of lead without quartz cell
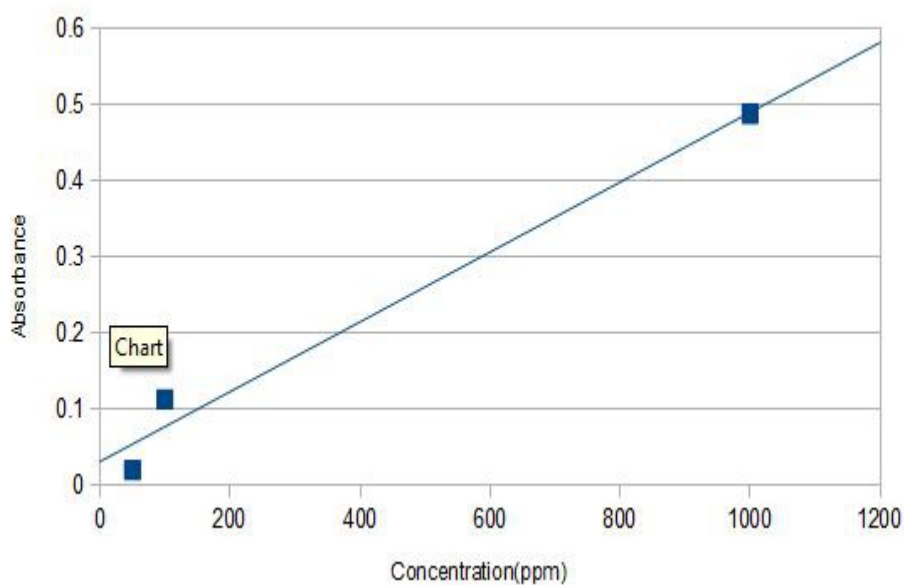

Figure 14:-Plot of Absorbance against concentration of lead

c) Conclusions

- The analysis of lead by hydride generation was performed for various concentrations.

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- Below 50 ppm there was no absorbance shown. Hence it was only performed for 50, 100 and 1000 ppm.

8) Proof of interference in flame AAS

a) Interference due to copper during flame atomic absorption analysis of lead

i) Chemical Reagents used:- $Pb(NO_3)_2$, $CuSO_4 \cdot 5H_2O$, distilled water.

ii) Method

- A stock solution of 100 ppm lead in 100 ml water was prepared by adding 0.015 g of $Pb(NO_3)_2$ in 100 ml distilled water. Similarly 100 ppm solution of copper was also prepared by adding 0.039 g $CuSO_4 \cdot 5H_2O$ in 100ml distilled water.

- Working standards of 5,10,15 and 20 ppm of lead in a 10ml volumetric flask were made and analysed by flame AAS.

- Then a mixture of lead and copper with concentration 5, 10, 15 and 20 ppm was prepared in 10 ml volumetric flasks by doing the following dilutions from the stock solutions with distilled water.

Concentration of Pb	Concentration of Cu	Amount of water	Final volume
5 ppm (0.5 ml of stock)	5 ppm (0.5 ml)	9 ml	10 ml
10 ppm (1 ml of stock)	10 ppm (1 ml of stock)	8 ml	10 ml
15 ppm (1.5 ml of stock)	15 ppm (1.5 ml of stock)	7 ml	10 ml
20 ppm (2 ml of stock)	20 ppm (2 ml of stock)	6 ml	10 ml

Table 7:- Dilutions with distilled water to give desired concentrations

- Then these standards were analysed using flame AAS.

iii) Observations:

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- For the standards of lead the following absorbance values were obtained as shown in figure 15.
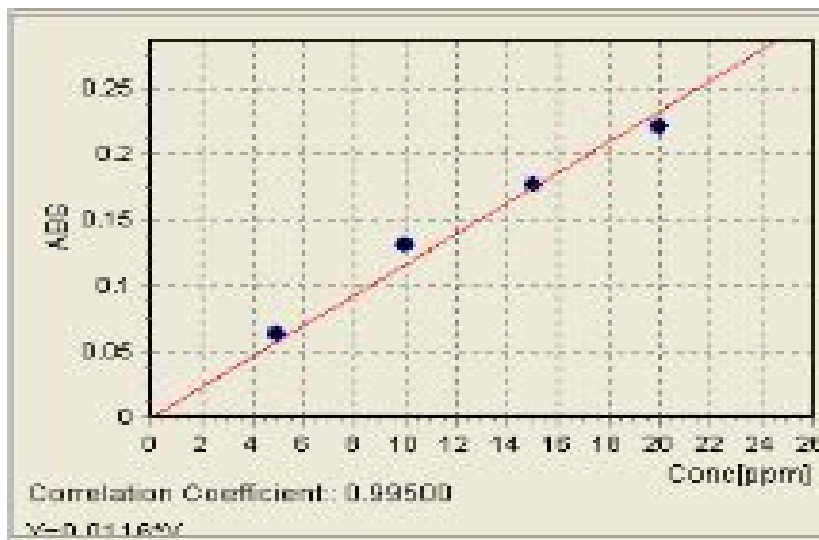

Figure 15:- Plot of Absorbance against concentration of lead by flame

AAS

For the standards of a mixture of lead and copper the following absorbance values were obtained as shown in figure 16.
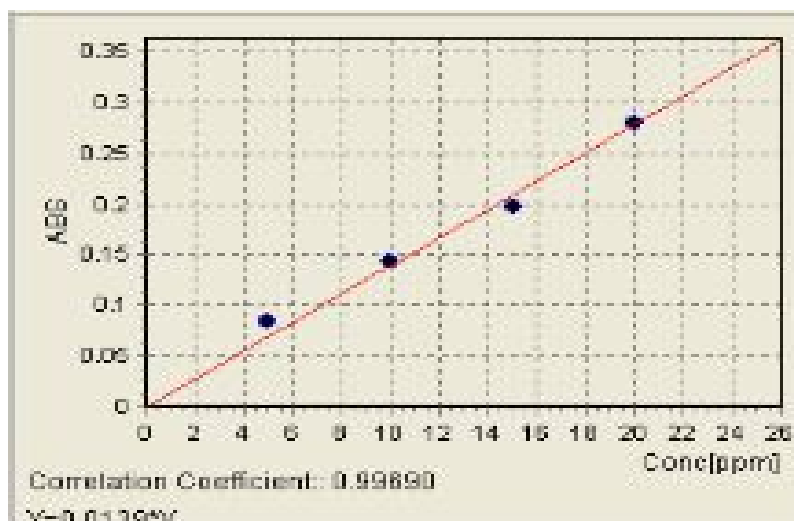

Figure 16:- Plot of Absorbance against concentration of mixture of lead and copper by flame AAS

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The absorbance values for various concentrations are listed below in table 8

Concentration(ppm)	Absorbance (Pb)	Absorbance (Pb+Cu)
5	0.0638	0.0842
10	0.1312	0.1434
15	0.1753	0.1978
20	0.2200	0.2792

Table 8:- Set of Absorbance values for Pb and Pb + Cu

iv) Conclusions

- As the atomic absorption wavelengths for lead is very close to one of the wavelengths for copper, 217nm and 217.9nm respectively. We observed an increase in the absorbance for lead in the standard which had copper in it.

- This was due to interference caused by copper.

b) Interference due to iron during flame analysis of mercury

i) Chemical Reagents used:- $FeSO_4.7H_2O$, $HgCl_2$, distilled water.

ii) Method

- A stock solution of 100 ppm mercury was prepared by adding 0.0135g of $HgCl_2$ in 100 ml distilled water. Similarly a 100 ppm iron solution was also made by dissolving 0.049g of $FeSO_4.7H_2O$ in 100 ml distilled water.

- Working standards of 5, 10, 15 and 20 ppm of mercury were made in a 10ml volumetric flask and analysed by flame AAS.

- Then a mixture of mercury and iron with concentration 5, 10, 15 and 20 ppm was prepared in 10 ml volumetric flasks by doing the following dilutions from the stock solutions with distilled water as shown in table 9.

Concentration of Hg	Concentration of Fe	Amount of water	Final Volume
5 ppm (0.5 ml of stock)	5 ppm (0.5 ml of stockstock)	9 ml	10 ml
10 ppm (1 ml of stock)	10 ppm (1 ml of stock)	8 ml	10 ml
15 ppm (1.5 ml of stock)	15 ppm (1.5 ml of stock)	7 ml	10 ml
20 ppm (2 ml of stock)	20 ppm (2 ml of stock)	6 ml	10 ml

Table 9:- Dilutions with distilled water to give desired concentrations

- Then these standards were analysed using flame AAS.

iii) Observations

- For the standards of mercury the following absorbance values were observed as shown in figure 17.
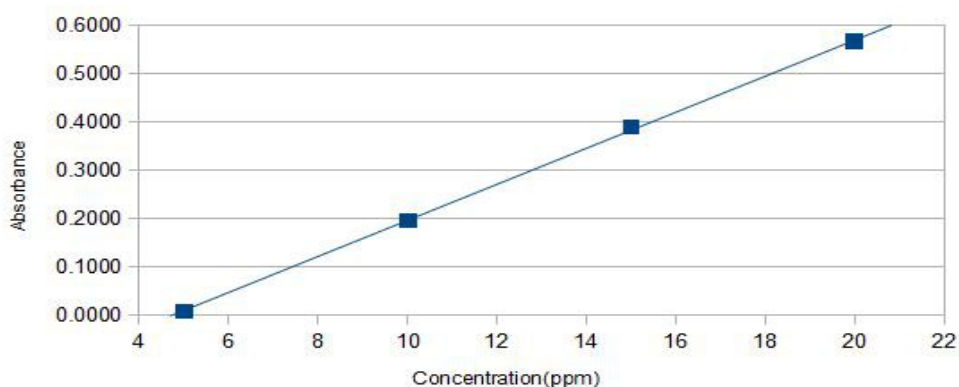

Figure 17:- Plot of Absorbance against concentration of mercury by flame AAS

- For the standards of a mixture of mercury and iron the following absorbance values were obtained as shown in figure 18.
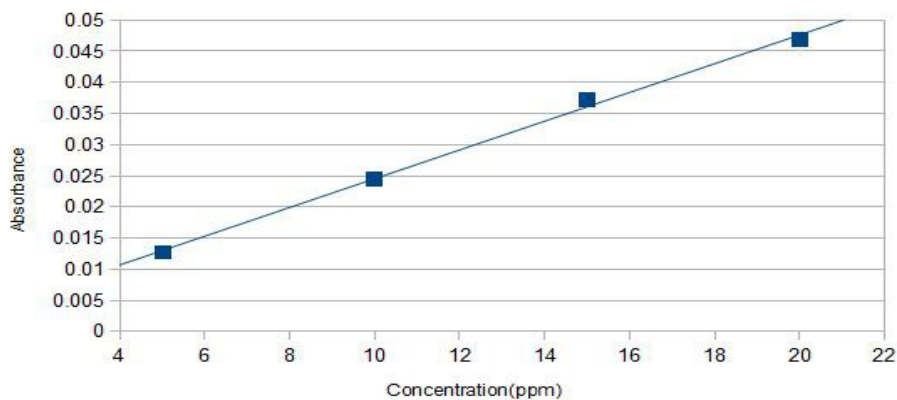
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Fig. 18:- Plot of Absorbance against concentration of mixture of mercury and iron by flame AAS

The absorbance values for various concentrations are listed below in table 10

Concentration(ppm)	Absorbance (Hg)	Absorbance (Hg+Fe)
5	0.0097	0.0127
10	0.196	0.0245
15	0.289	0.0372
20	0.5663	0.0469

Table 10:- Absorbance of Hg and Hg + Fe solutions

iv) Conclusions

- As the atomic absorption wavelengths for mercury is close to one of the wavelengths for iron, 253.7nm and 248.3nm respectively. We observed a substantial decrease in the absorbance for mercury in the standard which had iron in it.

- This is due to the interference caused by iron.

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Summary and Conclusions

We have come up with a short, easy to use, cheap and simple method for analysing metals using hydride generation atomic absorption spectroscopy. It does not involve a hydride generation assembly, rather the hydride can be generated in a round bottom flask or any glassware with sufficient openings for the inlet of the gas used to purge and the outlet to take the hydride into the flame or the quartz cell. We have also shown that the analysis of mercury by hydride generation technique can be done without using the quartz cell and introducing the hydride directly into the flame. We analysed samples from two of the available sources and found trace amounts of mercury in them with concentrations below parts per million hence in the parts per billion level. The concentration of mercury found in the samples was much less than the detection limits we had got from our analysis.

The analysis for lead by hydride generation technique was not successful for concentrations below 50 ppm.

The analysis of other hydride producing metals by hydride generation AAS was not possible because of the unavailability of hollow cathode lamps of those metals.

The analysis of various metal hydrides using a quartz cell could not be performed due to some deposition on the walls of the quartz cell hence disallowing the passage of radiation from the hollow cathode lamp.

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