Benha University
Faculty of Agriculture
Biochemistry Department

Instrumental Practical Part

(Chemistry 5)

Biotechnology Program (Level 2)

(Section 2)

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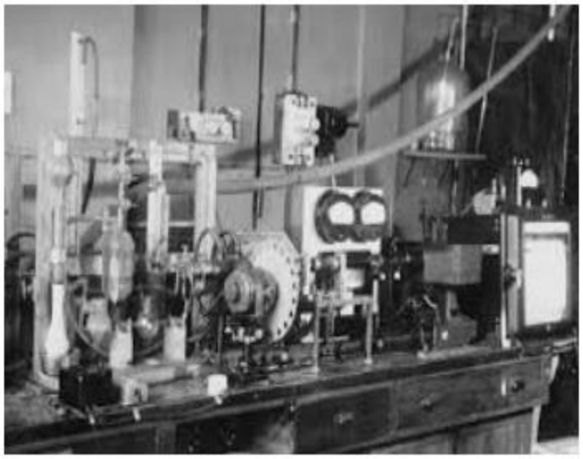
Instrumental (section 2)

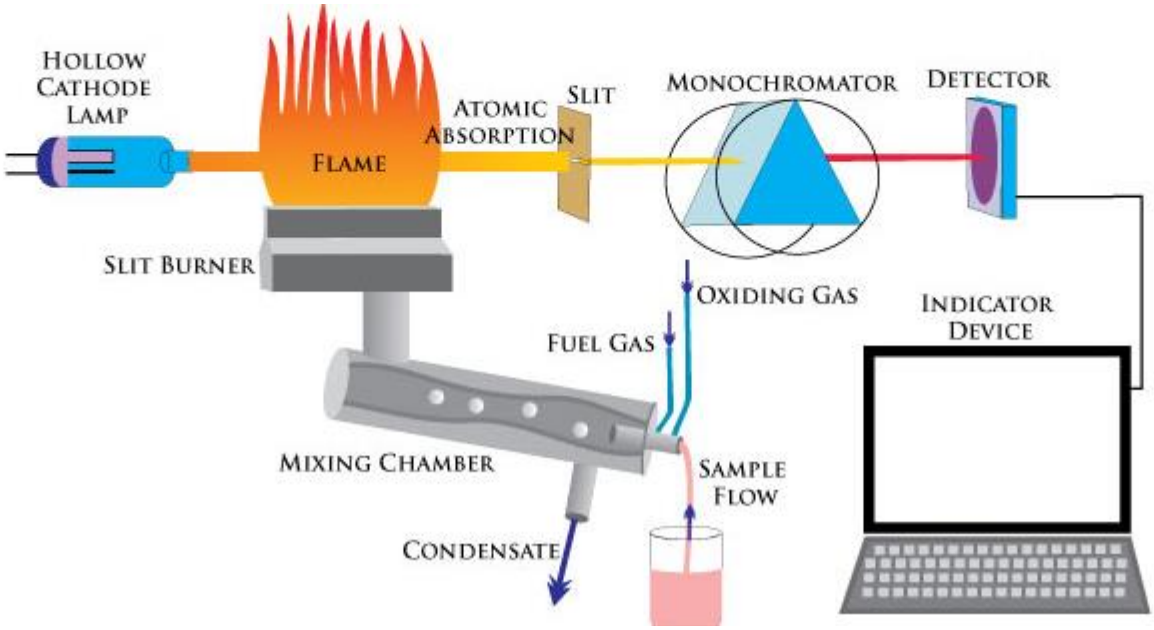
Atomic Absorption

Atomic Absorption Spectroscopy is an instrumental analysis technique for rapid trace metal analysis. It is based on element specific wavelength light absorption by ground state atoms in the flame or electrothermal graphite furnace.

It finds immense applications in the analysis for trace metals in soils, lakes, rivers, oceans, and drinking water, pharmaceuticals, foods and beverages, geological and mineralogical samples, petroleum products, biological fluids and specimens and forensic analysis. It is common to get results in ppm levels and a higher sensitivity of ppb levels when we using graphite furnace atomisation.



Atomic absorption spectrophotometry analyzes the concentration of elements in a liquid sample based on energy absorbed from certain wavelengths of light (usually 190 to 900 nm). Atomic absorption spectrophotometers typically include a flame burner to atomize the sample (most commonly a hollow cathode lamp), a monochromator and a photon detector. Depending on the model, some atomic absorption spectrometers are equipped with a turret or fixed lamp socket that can hold multiple lamps (up to eight) to reduce downtime between samples or allow for sequential analysis.



Principle of Atomic Absorption Spectroscopy

Atomic absorption spectroscopy (AAS) is based upon the principle that free atoms in the ground state can absorb light of a certain wavelength. These very specific wavelengths give the technique excellent specificity and detection limits in the AAS analysis. Absorption for each element is specific, no other elements absorb this wavelength. Typical applications of AAS include –

- Quantitative metal concentrations in solution
- Analysis of lead in paint
- Monitoring of trace metals in industrial effluent streams
- Trace elements in product/raw materials along with ICP-MS
- Analysis of additives and purity in steels and other metal alloys
- Analysis of low-level contaminants

Several analytical techniques have been applied for detection and quantitative estimation of trace metals in different types of matrices. Classical techniques based on gravimetric and titrimetry provided good accuracy but were time-consuming.

Increasing demand for high speed analysis led to the introduction of instrumental methods such as Ion selective electrodes, UV-VIS spectroscopic techniques, Atomic Absorption Spectroscopy, ICP – OES and ICP – MS. The choice of technique depends on the required detection levels, available sample quantity and most important available budget. The topic is covered to some extent in the article which elemental analysis technique is right for me.

Atomic Absorption Spectroscopy is a moderately priced instrumental analysis technique which provides a high degree of accuracy and precision of results. Due to its high analysis throughput, it finds its rightful place in university laboratories, pollution control laboratories and industrial quality control laboratories.

The present article highlights some areas where an awareness of working with an Atomic Absorption Spectrometer will prove to be an asset in enhancing your professional growth.

In case you are engaged in any of the activities or areas discussed in the article or wish to land into such areas, you will stand to gain through up-gradation of your knowledge and technical skills on this technique.

There are five basic components of an atomic absorption instrument:

1. The light source that emits the spectrum of the element of interest

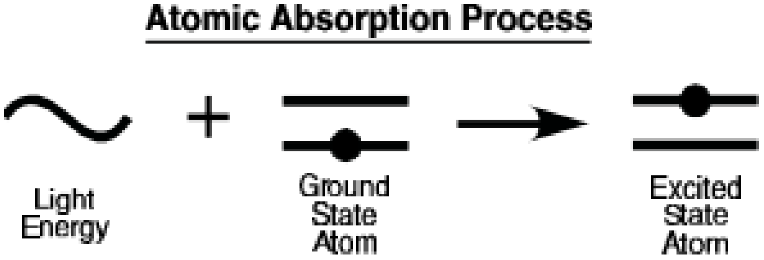
2. An "absorption cell" in which atoms of the sample are produced (flame, graphite furnace, MHS cell, FIAS cell, FIMS cell)

3. A monochromator for light dispersion

4. A detector, which measures the light intensity and amplifies the signal

5. A display that shows the reading after it has been processed by the instrument electronics

The process of atomic absorption is illustrated in Figure 1.



There are two basic types of atomic absorption instruments: *singlebeam* and *double-beam*.

Single-Beam:

A schematic diagram of a single-beam atomic absorption instrument is shown in Figure 2.

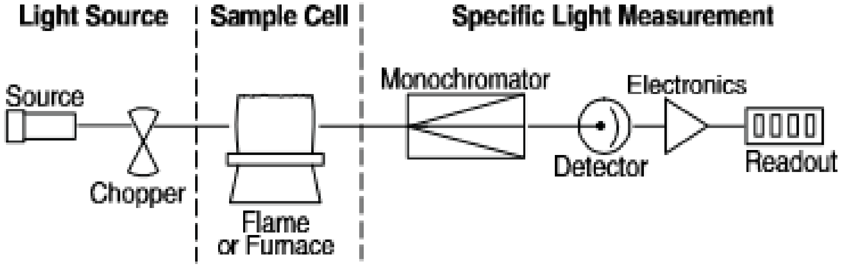
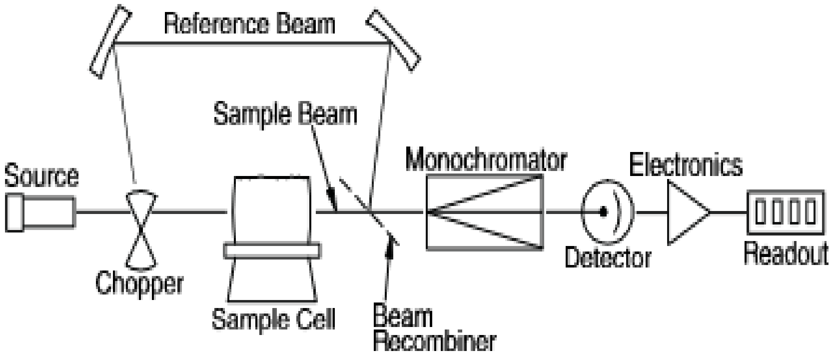


Figure 2. Single-Beam Atomic Absorption Spectrometer

The light source (hollow cathode lamp or electrodeless discharge lamp) emits a spectrum specific to the element of which it is made, which is focused through the sample cell into the monochromator. The light source must be electronically modulated or mechanically chopped to differentiate between the light from the source and the emission from the sample cell. The monochromator disperses the light, and the specific wavelength of light isolated passes to the detector, which is usually a photomultiplier tube. An electrical current is produced depending on the light intensity and processed by the instrument electronics. The electronics will measure the amount of light attenuation in the sample cell and convert those readings to the actual sample concentration. With single-beam systems, a short warmup period is required to allow the source lamp to stabilize.

Double-Beam

A schematic diagram of a double-beam system is shown in Figure 3.



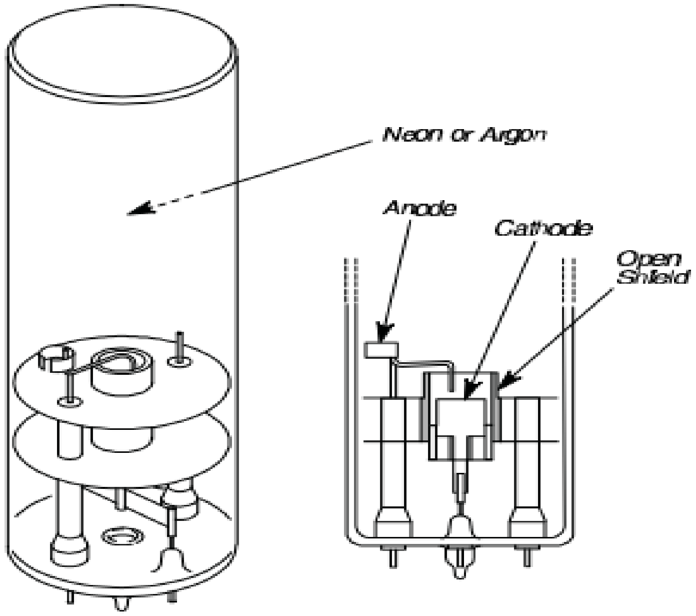
The light from the source lamp is divided into a *sample* beam, which is focused through the sample cell, and a *reference* beam, which is directed around the sample cell. In a double-beam system, the readout represents the ratio of the sample and reference beams. Therefore, fluctuations in source intensity do not become fluctuations in instrument readout, and stability is enhanced. Generally, analyses can be performed immediately with no lamp warm-up required.

Light Sources for Atomic Absorption

Since atoms absorb light at very specific wavelengths, it is necessary to use a narrow-line source which emits the narrow-line spectra of the element of interest. Narrow-line sources provide high intensity and make atomic absorption a specific analytical technique. The main sources used for atomic absorption are the *hollow cathode lamp* (HCL) and the *electrodeless discharge lamp* (EDL). The hollow cathode lamp is an excellent, bright, stable line source for most elements. However, for some volatile elements, where low intensity and short lamp lifetime are a problem, EDLs are available. EDLs are typically more intense than hollow cathode lamps and, therefore, may offer better precision and lower detection limits for some elements.

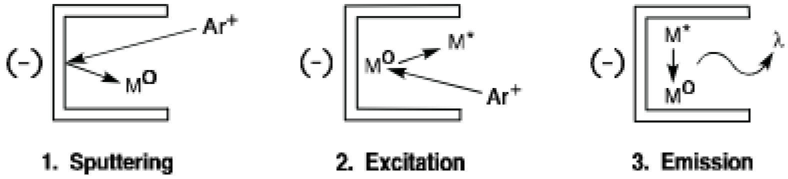
Hollow Cathode Lamps

Figure 4 shows how a hollow cathode lamp is constructed



The cathode is a hollowed-out cylinder constructed entirely or in part of the metal whose spectrum is to be produced. The anode and cathode are sealed in a glass cylinder filled with neon or argon. The glass cylinder has a quartz or UV glass window for optimum transmittance of the emitted radiation. The optimum fill gas is selected that gives the best lamp intensity while taking into consideration spectral interferences from either neon or argon. A red glow is observed in lamps filled with neon, while argon filled lamps have a blue glow. Hollow cathode lamps are available for more than 60 elements.

The hollow cathode emission process is illustrated in Figure 5.



1. Sputtering 2. Excitation 3. Emission

An electrical potential is applied between the anode and cathode and some of the fill gas atoms are ionized. The positively charged ions collide with the negatively charged cathode and dislodge metal atoms in a process called "sputtering." Sputtered metal atoms are further excited to emission through impact with the fill gas. Hollow cathode lamps have a finite lifetime. With extended use, the sputtering process removes some of the metal atoms from the cathode and these are deposited elsewhere. Fill gas is absorbed in the sputtered metal, on the glass walls and also absorbed into the glass from bombardment. Lamps for volatile elements age faster due to more rapid sputtering of the cathode.

Multi-element Lamps:

The cathode of a hollow cathode lamp is generally constructed from a very pure metal resulting in a very pure emission spectrum. It is, however, possible to construct a cathode from a mixture or alloy of several metals. The resulting "multielement" lamp can be used as a source for all the metals contained in the cathode. There is a wide variety of multielement lamp combinations available. Not all metals can be used in combination due to metallurgical properties or spectral limitations.

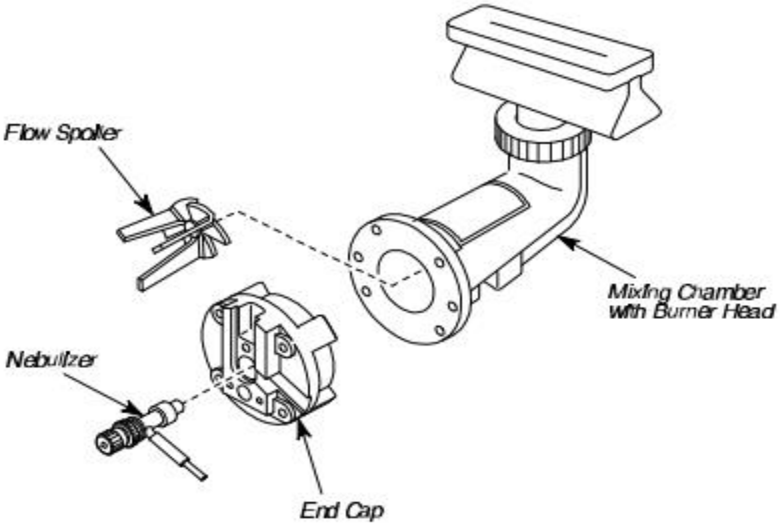
Single-Element or Multi-element Lamp:

The emission intensity for a particular element in a multielement lamp is not as great as that for the same element in a single-element lamp. This can result in a poorer signal/noise ratio which can influence the precision of analyses and the detection limit. When working close to the detection limit or when the best precision is required, select a single-element lamp. If economics is a problem, one multielement lamp can take the place of several single-element lamps. A multielement lamp is adequate for routine analyses that are well above the detection limit. If a backup lamp is necessary for a specific determination, a multielement lamp is ideal. If an infrequent analytical determination is required for several elements, a multielement lamp can offer considerable savings..

Burner System:

Dual-Option Burner System

The dual option burner system can be operated either with or without a flow spoiler for optimum operation under different analytical conditions. Some versions of the dual option burner system also allowed use of a removable impact bead. Consult the spectrometer instruction manual for details on the burner system provided. A diagram of the dual option burner system is shown in Figure 7.



The premix burner chamber is molded from plastic and treated to insure proper drainage. The burner system can be operated either with or without a flow spoiler. The flow spoiler is molded of polypropylene and held in position by three support arms which press-fit to the walls of the mixing chamber. To facilitate removal, the end cap is held in place by four large knurledhead screws. A large O-ring, held captive by a groove in the end cap, is used to seal the end cap to the mixing chamber. For extended operation with organic solvents, a Corkprene O-ring is also available. For routine operation it is recommended that the burner system be operated with a flow spoiler. The flow spoiler helps to remove large droplets from the nebulizer aerosol and thus minimizes chemical interferences. Removing the flow spoiler provides somewhat improved sensitivity with generally little or no degradation in precision. However, chemical interferences may be more pronounced. Consequently, it is recommended that operation without a flow spoiler be restricted to the analysis of relatively "clean" samples, where the risk of chemical interferences is low.

Flames:

The two oxidant/fuel combinations used almost exclusively in atomic absorption today are air-acetylene and nitrous oxide-acetylene. Descriptions of the gas requirements can be found in "Preparing Your Laboratory for Perkin-Elmer Atomic Spectroscopy Instruments," available from Perkin-Elmer as Reprint L-301.

Air-Acetylene

Air-acetylene is the preferred flame for the determination of approximately 35 elements by atomic absorption. The temperature of the air-acetylene flame is about 2300 °C. An air-acetylene flame can be used with all Perkin-Elmer burner heads. The operating instructions for use of the air-acetylene flame are given in the appropriate instrument manual. For the air-acetylene flame, the acetylene flow is about 4 liters/minute, or 8.5 cubic feet/hour, using a heat combustion value of 1450 BTU per cubic foot. The heat given off would be approximately 12,300 BTU per hour.

Usually, ordinary welding grade acetylene is adequate. However, welding grade acetylene is supplied dissolved in acetone. As tank pressure falls, the concentration of acetone entering the flame increases. This increase may produce erratic results when determining elements that require a fuel-rich flame or elements with analytical wavelengths in the lower UV range. Also, acetone passing into the gas control box may damage valves and tubing. For these reasons, acetylene tanks should be replaced when the tank pressure falls below 520 kPa (75 psig). Acetylene tanks should be stored and operated in a vertical position to prevent liquid acetone from reaching the cylinder valve. If you find that the welding grade acetylene in your particular area is not adequate for atomic absorption, atomic absorption grade acetylene may be available. Also, acetylene filters designed to remove acetone are available from several sources, including Perkin-Elmer.

Nitrous Oxide-Acetylene

The nitrous oxide-acetylene flame has a maximum temperature of about 2900 °C and is used for the determination of elements which form refractory oxides. It is also used to overcome chemical interferences that may be present in flames of lower temperature. For the nitrous oxide-acetylene flame, the acetylene flow is about 14 liters per minute or 30 cubic feet per hour. Using a heat of combustion value of 1450 BTU per cubic foot, the heat given off would be approximately 43,000 BTU per hour. Light emission from the nitrous oxide-acetylene flame is very strong at certain wavelengths. This may cause fluctuations in the analytical results for determinations performed at these wavelengths, particularly if the lamp emission for the element of interest is weak. **Only** the nitrous oxide burner head can be used with the nitrous oxide-acetylene flame. Ignition procedures and operating conditions for the nitrous oxide-acetylene flame are given in the appropriate instrument manual.

How to use the standard conditions table?

Single-Element Determinations

If only one element is to be determined in a particular sample, the selection of conditions or parameters is relatively simple. If you prefer to use the most sensitive wavelength for analysis and a sample preparation or digestion is needed, dilute the sample so that the element of interest is not more than 3´ the linear range listed for this line. If a prepared sample is to be analyzed, select an atomic absorption line so that the element of interest is not more than 3´ the linear range. This will enable you to analyze your sample without dilutions. For some elements, only one or two atomic absorption lines are available, so dilution may be necessary.

Multi-element Determinations

When determining several elements in the same sample, especially if the analysis is to be automated using an autosampler, it is desirable that all the determinations be performed on the same sample dilution. In selecting multi-element conditions, the following factors should be considered:

1- Choose the optimum conditions for your least sensitive element. It is easy to adjust your instrument conditions to reduce sensitivity for elements present in high concentrations. However, it is not easy to work close to the detection limit on very dilute samples.
2- The sensitivity of concentrated elements can be reduced by choosing a less sensitive wavelength.
3- It is possible to use the 5-cm burner head for air-acetylene operation. This will reduce sensitivity. It is also possible to rotate the burner head to reduce sensitivity. With some spectrometer models, the burner head can be rotated 90°, which provides roughly a 10-fold decrease in sensitivity.

Stock Standard Solution

Instructions for the preparation of stock standards are given for each element. It is also possible to purchase standards directly from PerkinElmer (refer to the *Reagents and Suppliers* section).

Light Sources

If a spectral interference is possible using a multi-element lamp, it is noted here. Also mentioned will be any difference in characteristic concentration when using an EDL.

Standard Solutions:

The majority of AA users purchase prepared, certified stock standards for calibration. A variety of stock standards, usually provided at a concentration level of 1000 mg/L, are available directly from PerkinElmer as well as from other commercial sources. These stock standards, which are supplied with a certificate of analysis and are traceable to NIST standards, generally have a shelf-life of one year. The standards available from Perkin-Elmer are described in a brochure entitled *PE Pure Standards for Atomic Spectroscopy*, which is available from Perkin-Elmer as Reprint PEX-AA50B. Alternatively, stock standards can be prepared directly from reagent grade chemicals. Procedures for preparing stock standard solutions are given in the Standards Conditions section for each element. To ensure accuracy, the concentration of these standard solutions should be verified using another analytical technique. The concentration of solutions, particularly very dilute solutions, will change with time in certain cases. If 1% accuracy is required, it is good practice to prepare working standard solutions daily from stock solutions of 500 to 1000 mg/L. In our experience, most solutions of 500 mg/L or more do not alter with storage for one year. We have also found that the presence of dilute acid (0.1% to 1.0%) in many solutions lengthens the life of the standard.

Uses of Atomic Absorption Spectroscopy

Atomic Absorption Spectroscopy provides cost-effective viable solutions for the analysis of trace amounts of metals in the entire range of natural and manmade materials such as Geological samples, Environmental samples, Biological Specimens, Agricultural produce and soils, Pharmaceuticals, Foods and Drinking water.

The technique affords advantages of speed, sensitivity and precision over the classical gravimetric methods. Introduction of accessories such as graphite furnace, flow injection analysis and improvements in the suppression of matrix interferences have further contributed to improvement in sensitivity and selectivity of analytes in complex matrices.

Atomic Absorption Spectroscopy applications in the field of environment, drinking water, mining and mineralogy, oceanographic studies, soils, pharmaceuticals, foods, toys, forensic investigations are of great significance.

The list is endless and presence or absence of trace metals is a factor that cannot be overlooked for evaluation of characteristics of materials or concerns regarding human health and safety.

The chemical techniques used for the analysis of trace metals have evolved from simple gravimetric methods to highly sophisticated time saving instrumental techniques. Atomic Absorption Spectroscopy is a popular technique which involves moderate investment and affordable operational cost.

These features coupled with a high degree of accuracy and precision of results has contributed to the widespread presence of atomic absorption spectrometers in college laboratories, industrial laboratories and regulatory body laboratories across the world.

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Atomic Absorption Spectroscopy Applications

Mining and Geology – The elemental composition of minerals and rocks provide valuable information on the commercial feasibility of conducting mining activities in areas explored. After mining, the ores and minerals need to be tested for composition for the efficiency of refining operations. Similarly, trace metal analysis is of great value in prospecting for oil and water deposits.

Gemstones are also graded on the basis of the presence of certain trace metals. Elemental composition of archaeological artefacts is helpful for tracing their source.

Environmental Monitoring – Environmental monitoring for trace metal contamination of industrial effluents, oceans, rivers and lakes is important for establishing the safety of water for drinking and commercial use. It is important to establish if such samples are within the safety limits set by regulatory bodies. Environmental monitoring also plays a significant role in the evaluation and feasibility of the site for setting up commercial projects.

Materials Development – Common properties of materials such as hardness, brittleness, grain size, crystallinity and amorphous nature are significantly influenced by composition and trace metals. Trace metal analysis can provide useful information on the performance properties of such materials.

Pharmaceuticals – Trace metal analysis plays an important role in formulation development, catalyst efficiency and dosage limits. Most elements have a beneficial role up to certain prescribed limits but beyond such limits the effects are harmful.

Foods and Beverages – In synthetic processed foods, metal pickup takes place due to contact with processing equipment and catalytic conversions. Consumer awareness on food safety is increasing by the day so manufacturers have to ensure that the trace metals do not exceed the permissible limits and this requires rigorous quality control through atomic absorption spectroscopy and other sophisticated instruments.

Oil and Petroleum – Both edible oils and mineral oils require refining before consumption. Such refining operation can involve distillation as well as catalytic refining. Uptake of metals during such operations can lead to deterioration of performance or consumer hazards. Trace metal analysis of engine oil provides useful diagnostic information on the wear and tear of engine parts.

Agriculture – Trace metal constitution of soils in addition to their acidic or basic nature is essential to establish their productivity and nutrient value. Trace metal composition of plants (leaves, stems and roots) gives a fair idea on how the uptake of minerals gets distributed under different growth conditions

Forensics – Trace metal analysis provides valuable information on specimens such as stomach contents for food poisoning, paint chips, fibres and hair strands collected from the scene of a crime.

Background correction methods

- Using a blank
- Measure the absorbance of the metal resonance line by both flame & blank (flame system)
- Measure the absorbance of the metal resonance line by sample and flame system (flame + blank)
- A is the difference

AUTOMATION OF ATOMIC ABSORPTION

Automated Instruments and Sample Changers

One of the greatest contributions to the efficiency of the analytical laboratory is the automated atomic absorption spectrometer. Automatic samplers were the first step in freeing the analyst from the monotonous task of manually introducing each and every sample.

However, the real advancement in analysis automation came in the late 1970’s, when automated multi-element atomic absorption was introduced. In addition to automatic sample introduction, these instruments offer automatic setup of instrument parameters to preprogrammed values. These instrument ‘‘programs’’ can be accessed sequentially, making it possible to analyze a tray full of samples for multiple elements, without any operator intervention.

Automated Sample Preparation

While automated instrumentation has meant considerable time savings to the analyst, analytical throughput (i.e., the number of samples which can be analyzed in a given time) frequently is limited by the time required to prepare the sample. Even when the sample is available in a suitable solution form, there often are pretreatment steps which must be performed prior to analysis. The introduction of commercial systems based on techniques such as flow injection have directly addressed the need for automated sample preparation capabilities. Flow injection techniques can be used to automate relatively simple procedures such as dilution or reagent addition. They can also be used to automate complex chemical pretreatments, including analyte preconcentration and cold vapor mercury and hydride generation procedures.

The Stand-alone Computer and Atomic Absorption

Stand-alone and personal computers have extended the automation and data handling capabilities of atomic absorption even further. These computers can interface directly to instrument output ports to receive, manipulate, and store data and print reports in user selectable formats. Also, data files stored in personal computers can be read into supplemental software supplied with the system or third party software such as word processor, spreadsheet and database programs for open-ended customization of data treatment and reporting.

References

Richard D. Beaty and Jack D. Kerber (1993). Concepts, Instrumentation and Techniques in Atomic Absorption Spectrophotometry. Book Second Edition.