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Atomic Absorption Spectrometry (AAS)

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1. Introduction

Atomic Absorption Spectrometry (AAS) is a technique for measuring quantities of chemical elements present in environmental samples by measuring the absorbed radiation by the chemical element of interest. This is done by reading the spectra produced when the sample is excited by radiation. The atoms absorb ultraviolet or visible light and make transitions to higher energy levels. Atomic absorption methods measure the amount of energy in the form of photons of light that are absorbed by the sample. A detector measures the wavelengths of light transmitted by the sample, and compares them to the wavelengths which originally passed through the sample. A signal processor then integrates the changes in wavelength absorbed, which appear in the readout as peaks of energy absorption at discrete wavelengths. The energy required for an electron to leave an atom is known as ionization energy and is specific to each chemical element. When an electron moves from one energy level to another within the atom, a photon is emitted with energy **E**. Atoms of an element emit a characteristic spectral line. Every atom has its own distinct pattern of wavelengths at which it will absorb energy, due to the unique configuration of electrons in its outer shell. This enables the qualitative analysis of a sample. The concentration is calculated based on the Beer-Lambert law. Absorbance is directly proportional to the concentration of the analyte absorbed for the existing set of conditions. The concentration is usually determined from a calibration curve, obtained using standards of known concentration. However, applying the Beer-Lambert law directly in AAS is difficult due to: variations in atomization efficiency from the sample matrix, non-uniformity of concentration and path length of analyte atoms (in graphite furnace AA).

The chemical methods used are based on matter interactions, i.e. chemical reactions. For a long period of time these methods were essentially empirical, involving, in most cases, great experimental skills. In analytical chemistry, AAS is a technique used mostly for determining the concentration of a particular metal element within a sample. AAS can be used to analyze the concentration of over 62 different metals in a solution.

Although AAS dates to the nineteenth century, the modern form of this technique was largely developed during the 1950s by Alan Walsh and a team of Australian chemists working at the CSIRO (Commonwealth Science and Industry Research Organization) Division of Chemical Physics in Melbourne, Australia. Typically, the technique makes use of a flame to atomize the sample, but other atomizers, such as a graphite furnace, are also used. Three steps are involved in turning a liquid sample into an atomic gas:

1.   Desolvation – the liquid solvent is evaporated, and the dry sample remains;
2.   Vaporization – the solid sample vaporizes to a gas; and
3.   Volatilization – the compounds that compose the sample are broken into free atoms.

To measure how much of a given element is present in a sample, one must first establish a basis for comparison using known quantities of that element to produce a calibration curve. To generate this curve, a specific wavelength is selected, and the detector is set to measure only the energy transmitted at that wavelength. As the concentration of the target atom in the sample increases, the absorption will also increase proportionally. A series of samples containing known concentrations of the compound of interest are analyzed, and the corresponding absorbance, which is the inverse percentage of light transmitted, is recorded. The measured absorption at each concentration is then plotted, so that a straight line can then be drawn between the resulting points. From this line, the concentration of the substance under investigation is extrapolated from the substance's absorbance. The use of special light sources and the selection of specific wavelengths allow for the quantitative determination of individual components in a multielement mixture.

2. Basic principle

The selectivity in AAS is very important, since each element has a different set of energy levels and gives rise to very narrow absorption lines. Hence, the selection of the monochromator is vital to obtain a linear calibration curve (Beers' Law), the bandwidth of the absorbing species must be broader than that of the light source; which is difficult to achieve with ordinary monochromators. The monochromator is a very important part of an AA spectrometer because it is used to separate the thousands of lines generated by all of the elements in a sample.

Without a good monochromator, detection limits are severely compromised. A monochromator is used to select the specific wavelength of light that is absorbed by the sample and to exclude other wavelengths. The selection of the specific wavelength of light allows for the determination of the specific element of interest when it is in the presence of other elements. The light selected by the monochromator is directed onto a detector, typically a photomultiplier tube, whose function is to convert the light signal into an electrical signal proportional to the light intensity. The challenge of requiring the bandwidth of the absorbing species to be broader than that of the light source is solved with radiation sources with very narrow lines.

The study of trace metals in wet and dry precipitation has increased in recent decades because trace metals have adverse environmental and human health effects. Some metals, such as Pb, Cd and Hg, accumulate in the biosphere and can be toxic to living systems. Anthropogenic activities have substantially increased trace metal concentrations in the atmosphere. In addition, acid precipitation promotes the dissolution of many trace metals, which enhances their bioavailability. In recent decades, heavy metal concentrations have increased not only in the atmosphere but also in pluvial precipitation. Metals, such as Pb, Cd, As, and Hg, are known to accumulate in the biosphere and to be dangerous for living organisms, even at very low levels. Many human activities play a major role in global and regional trace element budgets. Additionally, when present above certain concentration levels, trace metals are potentially toxic to marine and terrestrial life. Thus, biogeochemical perturbations are a matter of crucial interest in science.

The atmospheric input of metals exhibits strong temporal and spatial variability due to short atmospheric residence times and meteorological factors. As in oceanic chemistry, the impact of

trace metals in atmospheric deposition cannot be determined from a simple consideration of global mass balance; rather, accurate data on net air or sea fluxes for specific regions are needed. Particles in urban areas represent one of the most significant atmospheric pollution problems, and are responsible for decreased visibility and other effects on public health, particularly when their aerodynamic diameters are smaller than 10 μm, because these small particles can penetrate deep into the human respiratory tract. There have been many studies measuring concentrations of toxic metals such as Ag, As, Cd, Cr, Cu, Hg, Ni, Pb in rainwater and their deposition into surface waters and on soils. Natural sources of aerosols include terrestrial dust, marine aerosols, volcanic emissions and forest fires. Anthropogenic particles, on the other hand, are created by industrial processes, fossil fuel combustion, automobile mufflers, worn engine parts, and corrosion of metallic parts. The presence of metals in atmospheric particles are directly associated with health risks of these metals. Anthropogenic sources have substantially increased trace metal concentrations in atmospheric deposition.

The instrument used for atomic absorption spectrometry can have either of two atomizers. One attachment is a flame burner, which uses acetylene and air fuels. The second attachment consists of a graphite furnace that is used for trace metal analysis. Figure 1 depicts a diagram of an atomic absorption spectrometer.
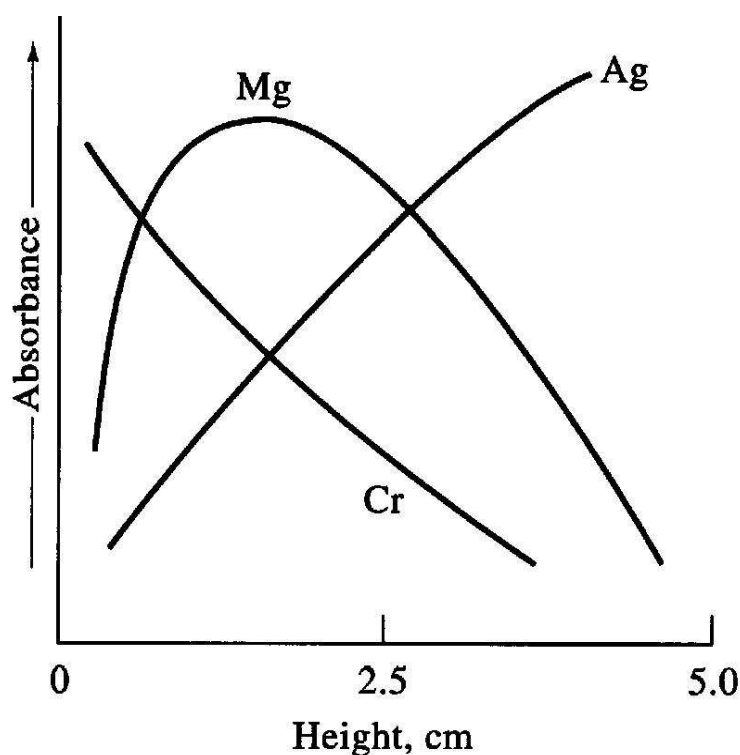

Fig. 1. The spectral, or wavelength, range captures the dispersion of the grating across the linear array.

Flame and furnace spectroscopy has been used for years for the analysis of metals. Today these procedures are used more than ever in materials and environmental applications. This is due to the need for lower detection limits and for trace analysis in a wide range of samples. Because of the scientific advances of Inductively Coupled Plasma Optical Emission

Spectroscopy (ICP-OES), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), have left Atomic Absorption (AA) behind. This technique, however, is excellent and has a larger specificity that ICP does not have. Figure 2 shows a diagram of an atomic absorption spectrometer with a graphite furnace.
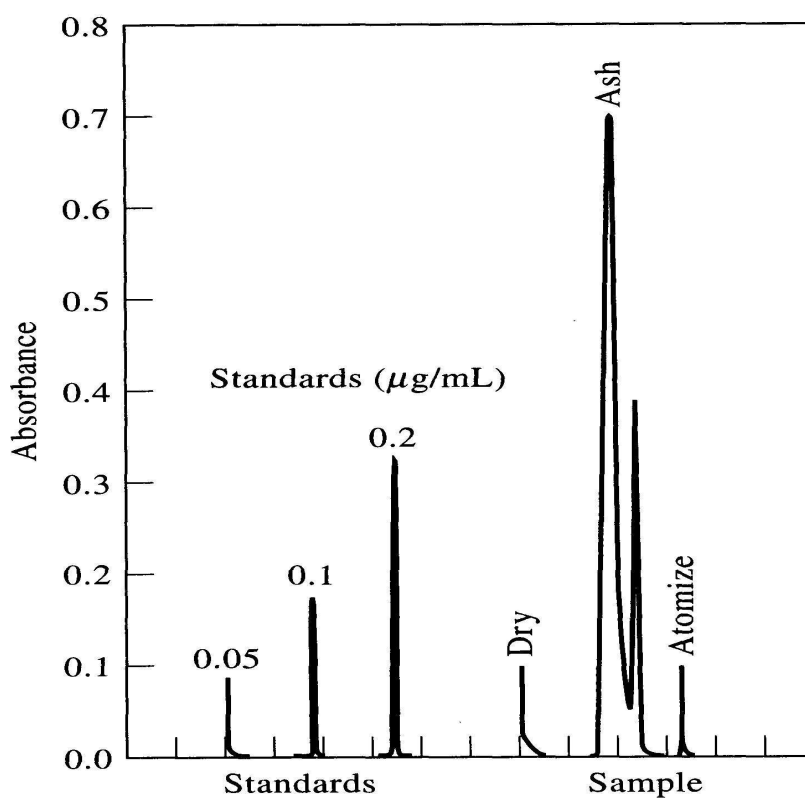

Fig. 2. Flame relatively insensitive - incomplete volatilization, short time in beam (Figure 1)

In this section, the authors will attempt to demonstrate that AAS is a reliable chemical technique to analyze almost any type of material. The chapter describes the basic principles of atomic absorption spectroscopy in the analysis of trace metals, such as Ag, As, Cd, Cr, Cu,

and Hg, in environmental samples. For example, the study of trace metals in wet and dry precipitation has increased in recent decades because trace metals have adverse environmental and human health effects. Anthropogenic activities have substantially increased trace metal concentrations in the atmosphere. In recent decades, heavy metal concentrations have increased not only in the atmosphere but also in pluvial precipitation. Many human activities play a major role in global and regional trace element budgets. Additionally, when present above certain concentration levels, trace metals are potentially toxic to marine and terrestrial life. Thus, biogeochemical perturbations are a matter of crucial interest in science.

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- AAS intrinsically more sensitive than Atomic Emission Spectrometry (AES)
- Similar atomization techniques to AES
- Addition of radiation source
- High temperature for atomization necessary
- Flame and electrothermal atomization
- Very high temperature for excitation not necessary; generally no plasma/arc/spark in AAS

3. Flame (AAS)

Flame atomic absorption methods are referred to as direct aspiration determinations. They are normally completed as single element analyses and are relatively free of interelement

spectral interferences. For some elements, the temperature or type of flame used is critical. If flame and analytical conditions are not properly used, chemical and ionization interferences can occur. Different flames can be achieved using different mixtures of gases, depending on the desired temperature and burning velocity. Some elements can only be converted to atoms at high temperatures. Even at high temperatures, if excess oxygen is present, some metals form oxides that do not redissociate into atoms. To inhibit their formation, conditions of the flame may be modified to achieve a reducing, nonoxidizing flame. Some aspects are discussed below:

- Simplest atomization of gas/solution/solid
- Laminar flow burner - stable "sheet" of flame
- Flame atomization best for reproducibility (precision) (<1%)
- Relatively insensitive – incomplete volatilization, short time in beam

Usually the measurement of metals can be done by an appropriate selection of one of the methods given below. During the analysis of the data, the interference and background should be considered. Three pattern-matching methods are most often employed: using calibration curves generated by a series of patterns, the method of standard additions and the internal standard method. The use of calibration curves from a series of patterns is possibly the most widely used method. It involves measuring the sample of interest in a series of samples of known concentration and all prepared under the same conditions.

1. Calibration Curve Method: Prepare standard solutions of at least three different concentrations, measure the absorbance of these standard solutions, and prepare a calibration curve from the values obtained. Then measure the absorbance of the test solution adjusted in concentration to a measurable range, and determine the concentration of the element from the calibration curve.

2. Standard Addition Method: To equal volumes of more than two different test solutions are used, then the standard solution is added so that the solutions contain stepwise increasing amounts of the element, and add the solvent to make a definite volume. Measure the absorbance for each solution, and plot the concentration of the added standard element on the abscissa and the absorbance on the ordinate. Extend the calibration curve obtained by linking the plotted points, and determine the concentration of the element from the distance between the origin and the intersection of the calibration curve on the abscissa. This method is applicable only in the case that the calibration curve drawn as directed in (1) above passes through the origin.

3. Internal Standard Method: Prepare several solutions containing a constant amount of the internal standard element. Using these solutions, measure the absorbance of the standard element and the absorbance of the internal standard element at the analytical wavelength of each element under the same measuring conditions, and obtain the ratios of each absorbance of the standard object element to the absorbance of the internal standard element. Prepare a calibration curve by plotting the concentrations of the standard element on the abscissa and the ratios of absorbance on the ordinate. Then prepare the test solutions, adding the same amount of the internal standard element as in the standard solution. Proceed under the same conditions as for preparing the calibration curve, obtain the ratio of the absorbance of the element to that of the internal standard element, and determine the concentration of the element from the calibration curve.

The calibration curve is always represented with the response of the instrument on the vertical (y) and concentrations on the horizontal axis (x). The value of C can limit the accuracy of the measurements for various concentrations; however there are statistical methods which can be used. The calibration method is generally used where there is a linear relationship between the analytical signal (y) and concentration (x), taking precautions to ensure that linearity in the experimental response is maintained over a wide range of concentrations. In these cases the way to proceed is to obtain the regression line of y on x (ie, the best-fit straight line through the points of the calibration graph, which can be obtained, for example, by the method of least squares) and used to estimate the concentration of unknown samples by interpolation, as well as to estimate the detection limit of the analytical procedure.

- Atomic absorption spectrometers allow operators to adjust sample temperatures. Some spectrometers are self-calibrating, position lights automatically, or compensate for stray light emissions and various types of spectral interference that bias analytical results. Intrinsically safe (IS) instruments do not release sufficient electrical or thermal energy to ignite hazardous atmospheric mixtures. In flame AAS, the measured absorbance depends on the absorptivity of the element in question, the concentration of that element in the flame (and therefore on its solution concentration), and the optical path length through the flame. The light from the flame region is collected on the left-side of this particular instrument. Under certain circumstances, it is desireable to reduce the optical path length. To this end, the burner head can also be rotated about its vertical axis. Different burner heads (having different slot sizes) are used for acetylene/air and acetylene/nitrous oxide flames, since the latter is a much hotter flame. Flow controls also allow both the total gas flow-rate and fuel-to-oxidant ratio to be varied. Gas flow affects the aspiration rate and aerosol drop-size distribution, while the fuel-oxidant ratio can influence chemical processes in the flame that would otherwise reduce the sensitivity of the measurement. Following you can describe some features of this technique:
- Primary combustion zone - initial decomposition, molecular fragments, cool
- Interzonal region – hottest, most atomic fragments, used for emission/fluorescence
- Secondary combustion zone – cooler, conversion of atoms to stable molecules, oxides
- element rapidly oxidizes – largest atom near burner
- element oxidizes poorly – largest atom away from burner
- most sensitive part of flame for AAS varies with analyte (Figure 3)

The measurement procedure is as follows. A small quantity of the extracted sample is injected into a flame where the ions are reduced to elements and vaporized. The elements present in the sample absorb light at specific wavelengths in the visible or the ultraviolet spectrum. A light beam with a single specific wavelength for the element being measured is directed through the flame to be detected by a monochrometer.

The light absorbed by the flame containing the extract is compared with the absorption from known standards to quantify the elemental concentrations. One of the disadvantages of this method is that only one element can be quantified at a time. AAS requires an individual analysis for each element, and sometimes a large filter or several filters are needed to obtain concentrations for a large variety of elements. Samples having high concentrations of elements beyond the linear range of the instrument should be diluted prior to the analysis. Báez *et al*., 2007 and García *et al*., 2009 characterized atmospheric aerosols, metals and ions

that play an important role in the content of chemical species and of many elements in atmospheric ecosystem interfaces. Sodium, K^+, Ca^{2+} and Mg^{2+} were analyzed with a double beam atomic absorption spectrophotometer. Deuterium and hollow cathode lamps (Photron Super lamp) were used for background correction and analysis.

Calibration standards were prepared under a laminar flux hood, using certified standards for each ion (High-Purity Standards traceable from the National Institute of Standards and Technology, NIST).
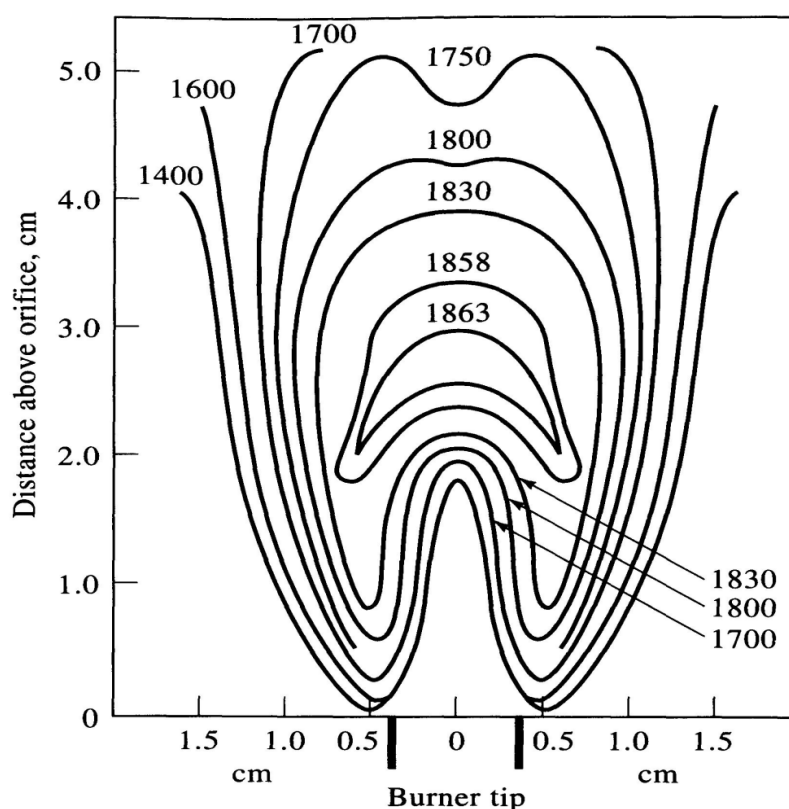

Fig. 3. Spectrometers element rapidly oxidizes - largest atom near burner

A calibration curve was used to determine the unknown concentration of an element in a solution. The instrument was calibrated using several solutions of known concentrations. The absorbance of each known solution was measured and then a calibration curve of concentration vs. absorbance was plotted.

The sample solution was fed into the instrument, and the absorbance of the element in this solution was measured. The unknown concentration of the element was then calculated from the calibration curve. The concentration of the analyte element was considered to be

proportional to the ground state atom population in the flame; any factor that affected the ground state atom population was classified as interference.

Factors that might affected the ability of the instrument to read this parameter were also classified as interference.

The different types of interference that were encountered in atomic absorption spectroscopy were:

- Absorption of source radiation: an element other than the one of interest may absorb the wavelength being used.
- Ionization interference: the formation of ions rather than atoms causes lower absorption of radiation. This problem is overcome by adding ionization suppressors.
- Self absorption: atoms of the same kind as those that are absorbing radiation will absorb more at the center of the line than at the edges, thus resulting in a change of shape and intensity of the line.
- Background absorption of source radiation: This is caused by the presence of a particle from incomplete atomization. This problem is overcome by increasing the flame temperature.
- Rate of aspiration, nebulization, or transport of the sample (e.g. viscosity, surface tension, vapor pressure , and density) .

4. Atomic Absorption Spectroscopy with graphite furnace (GFAA)

The GFAA and flame AAS measurement principle is the same. The difference between these two techniques is the way the sample is introduced into the instrument. In GFAA analysis, an electrothermal graphite furnace is used instead. The sample is heated stepwise (up to 3000ºC) to dry. The advantage of the graphite furnace is that the detection limit is about two orders of magnitude better than that of AAS. The analysis of different species of a given element is important because different oxidation states of the same element may present different toxicities and, consequently, different risks. Therefore, sequential extraction procedures for the separation and further analysis of a species have been developed for several metals. Sammut *et al*. (2010) developed a sequential extraction procedure for separating inorganic species of selenium found in the particulate matter of emissions in working areas. Essentially, the method is based on sequential dissolutions of the sampled matrix. Se(IV) and Se(0) can be independently analyzed by GFAA, whereas only total selenium can be detected when analyzed by AAS. This research group also proposed the analysis of beryllium species using GFAA and ICP-AES because metallic beryllium and beryllium oxide in workplaces are associated with different toxicities. These examples highlight the necessity of improving analytical techniques for measuring not only the total concentration but also the different species of a metal dispersed in contaminated air.

The graphite furnace has several advantages over a flame furnance. First it accepts solutions, slurries, or solid samples. Second, it is a much more efficient atomizer than a flame furnance 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 into the graphite furnace and the furnace is electrically heated in several steps to dry the sample, ash organic matter, and vaporize the analyte atoms. It accommodates smaller samples but it is a difficult operation, since the high energy that is provided to atomize the sample particles

into ground state atoms might also excite the atomized particles into a higher energy level, thus lowering the precision.

GFAA has been the most common instrument used for Pb analysis, and in countries where this element is a criterion for pollution standards, AAS is generally the technique used in the reference methods to quantify it. Garcia *et al.*, 2008 determined Pb in total suspended particles in southwest Mexico City, using a nitric acid digestion–based AAS method. An AAS method was used in conjunction with voltammetry for Pb and Cd analysis to evaluate a rapid digestion technique using a microwave oven, comparing this with traditional methods after establishing the optimal efficiency of digestion in terms of power setting, time and the use of different acids (Senaratne and Shooter, 2004).

For low level determination of volatile elements such as As, Ge, Hg, Sb, and Se, hydride generation coupled with AAS provides lower detection limits (milligram–microgram range). The three-step sample preparation for graphite furnaces is as follows:

1. Dry - evaporation of solvents (10–100 s)
2. Ash - removal of volatile hydroxides, sulfates, carbonates (10–100 s)
3. Fire/Atomize - atomization of remaining analyte (1 s)

4.1 Specific sample considerations

GFAA has been used primarily for the analysis of low concentrations of metals in water samples. GFAA can also be used to determine the concentrations of metals in soil, but sample preparation for metals in soil is somewhat extensive and may require the use of a mobile laboratory. The more sophisticated GFAA instruments have a number of lamps and, therefore, are capable of simultaneous and automatic analyses of more than one element.

* Plants: Solid samples must be in liquid form to be aspirated by the instrument. Therefore, solid material must be liquefied by means of some form of extraction or digestion protocol. Procedures have been devised that make the total amount of an element in the sample available for assay or that use some particular properties to extract the portion of the element that exists in some chemical forms but not in others. For example, the plant dry ash/double acid extraction method determines the total element content of the sample.

* Soil: For ecological purposes, there is more interest in measuring extractable or labile soil constituents than in determining total element content. Certain partitions of the total soil content of a given element are operationally defined by an extraction procedure, and arguments are usually offered that suggest that such partitions correspond to different levels of biological availability or activity. The HCl/H2SO4 double acid extraction method, also referred to as North Carolina and Mehlich-1, is widely used to determine bioavailable Ca, K, Mg, Mn, P, and Zn in sandy acid soils characteristic of the eastern and southeastern United States.

* Water: Aquatic samples, of course, need no liquefaction step, but researchers must still decide which analyte partition (dissolved, suspended, total) is of interest. Various treatments for each sample partition are detailed in the U.S. EPA's discussion of the content partitioning of water samples.

The advantages of using atomic absorption spectroscopy are, in our opinion:

-   Greater sensitivity and detection limits than other methods
-   Direct analysis of some types of liquid samples

- Low spectral interference
- Very small sample size

Finally, it is worth mentioning that in the last two decades, quantification and characterization of different matrices and materials by atomic absorption spectroscopy has been significant. The development of continuous measurement techniques for certain chemical species is important for governments, mainly in areas where the epidemiological studies are still being processed.

| Element	Mean	Min	Max	25th Percentile	75th Percentile	Geometric Mean	Harmonic Mean	Std. Dev.
Al	1536.4	97.65	8939.1	570.04	2027.9	1047.8	691.56	1543.6
Cd	2.64	0.09	8.05	1.06	3.99	1.85	0.99	1.75
Cr	23.79	14.08	44.02	18.24	27.20	22.93	22.11	6.62
Fe	1098.6	27.88	4963.8	263.19	1593.8	623.6	315.00	1102.2
Mn	19.23	2.66	45.18	12.11	25.46	14.32	14.32	9.41
Ni	23.03	8.78	52.93	18.03	26.49	20.77	20.77	7.57
Pb	50.78	8.36	128.07	29.48	68.01	35.94	35.94	28.16
V	16.22	2.15	44.52	8.40	23.41	9.93	9.93	10.02

N=100 (number of samples)
Table 1. Descriptive Statistics: Mean, Minimum (Min), Maximum (Max), 25th and 75th percentiles, Geometric Mean, Harmonic Mean and Standard deviation (Std. Dev.), concentrations of metals in PST collected 2003–2004

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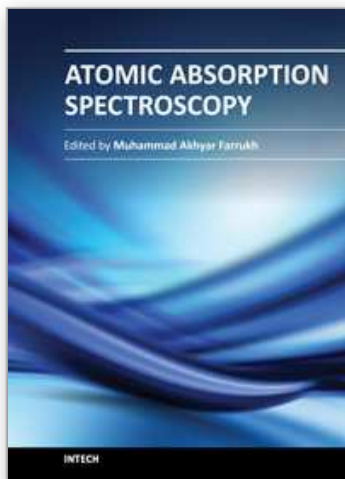
Atomic Absorption Spectroscopy

Edited by Dr. Muhammad Akhyar Farrukh

Atomic Absorption Spectroscopy is an analytical technique used for the qualitative and quantitative determination of the elements present in different samples like food, nanomaterials, biomaterials, forensics, and industrial wastes. The main aim of this book is to cover all major topics which are required to equip scholars with the recent advancement in this field. The book is divided into 12 chapters with an emphasis on specific topics. The first two chapters introduce the reader to the subject, it's history, basic principles, instrumentation and sample preparation. Chapter 3 deals with the elemental profiling, functions, biochemistry and potential toxicity of metals, along with comparative techniques. Chapter 4 discusses the importance of sample preparation techniques with the focus on microextraction techniques. Keeping in view the importance of nanomaterials and refractory materials, chapters 5 and 6 highlight the ways to characterize these materials by using AAS. The interference effects between elements are explained in chapter 7. The characterizations of metals in food and biological samples have been given in chapters 8-11. Chapter 12 examines carbon capture and mineral storage with the analysis of metal contents.

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