

ASH and MINERAL CONTENTS OF FOODS (Chapters 9-10-28)

I. Ashing Procedures

a-Conventional ashing (dry, wet, “plasma” or lowT°)

b-Indirect methods for ash determination.

II. Post-ashing procedures: Properties of ash (alkalinity; acid-insoluble ash; salt free ash)

III. Qualitative screening tests for metals

IV. Quantitative elemental analysis for individual metals (single or simultaneous)

a-Atomic Emission Spectroscopy

b-Atomic Absorption Spectroscopy (with flame, without flame)

c-ICP analyses

Ash: The inorganic residue remaining after either ignition (burning) or complete oxidation of organic carbonaceous matter (total mineral content).

Inorganic elements occur in foods :

- 1. As natural constituents coming from the soil, certain regions and plants, mineral contents are specific.**
- 2. As additives to prepared foods.**

NaCl → adding prepared foods. It is also in the ash.

- 3. As contaminants migrating to foods during processing**
i.e. From machinery → metal migration, when preparing the food. Migration can come from metallic materials like pipes or silos.

Ratio of ash in foods is very much related with the moisture contents of foods. $H_2O \downarrow$ Ash \uparrow .

In fresh fruits: around 0.3-0.8, but in dry fruits like apricots \approx 3.5; Ash in milk \approx 0.5-1 (mainly Ca), In non-fat dry milk \approx 8; In fresh meat, around 1, fried fish fillets around 2.5%. In cured meats \approx 12 (from NaCl and $NaNO_2$, $NaNO_3$). In cereals, 0.6-1.6%.

In vegetable oils \approx ppm levels (is removed in preparing)

The metal content in nature remains the same. But translocation occurs by weather condition, like rains, flood. There are also other natural factors like volcanic eruptions. Man-made > fly-ash-burning oil, fossil-fuels also translocates metals. We accumulate huge amount of ash from man-made sources like sewage-sludge \rightarrow excretion, mineral mining activities, agricultural practises like irrigation and use of fertilizers, traffic+cars and motor vehicle exhaust gasses.

Metals/minerals in food chain

1. of Nutritional interest= Ca, P, Na, K, Mg, Zn.....
2. of toxicological concerns, also called "contaminant" Hg, Cd, Pb, As
3. Both: Co, Cr, Cu, Sn

Concentrations:

• macrominerals Requirements > 100 mg/day

[Ca, P, Na, K, Mg, Cl, S,]

• micro (trace) minerals: requirements ~ 1 mg/day (in foods mcg/100 g)
[I, Fe, Cu, Zn, Cr, F, Se]

Densities:

• Light metals ($d < 5 \text{ g/cm}^3$) Ca (1.5), I (4.6), P (1.8),

• Heavy metals ($d > 5 \text{ g/cm}^3$) Pb (11.3), Fe (7.8)

Both nutritional and toxicological : Fe (7.8) and Cu (8.9)

Fe when higher than a certain concentration, will be of toxicological concern. Below a certain concentration, it will be of nutritional concern. Two ways of expressing:

1. MAC in food: maximum allowed concentration (mg/100g food)

2. AWI in diet = Allowed weekly intake for metals of toxicological concern

i.e. $AWI_{Cd} = 0.0067 - 0.0083 \text{ mg/kg b.w. of humans}$

Determining total ash in food commodities

Mostly the analytical interest is in “total amount of ash”, not in its detailed composition. For example, a cereal technologist will, just from the amount of ash in “flour”, have an idea about the relative bran content of that flour, since minerals are in higher concentration in the bran layer.

Every Proximate Analysis should include the total ash content. Ashing is determination of the residue after burning organic materials at elevated temperatures (>550°C).

While burning, organic acids → carbonates → oxides of metals. The trace metals are found linked to certain organic materials

Ashing Procedures:

DRY ASHING

You use atmospheric oxygen as the oxidant. When the sample is burnt in a muffle furnace at about ~550 °C for ~2 hours, we obtain light-gray ash. If there still is some black material, it means that organic materials were not completely burnt.

Advantages:

1. Safe
2. Multiple samples
3. Only oven and dishes needed

Disadvantages:

Elements such as Fe, Se, Pb, As,, Cr , Zn,Ni,Cd,Cu,
and Hg may partially volatilize.

Contamination risks

Alternative "crucible" materials:

Quartz: resistant to acids and halogens but not to alkali.

Porcelain: like quartz, but will crack easily at rapid temperature changes. Also it accumulates trace metals from previous analyses; for cleaning up, put it in acid before next use.

Stainless steel: resistant to both acids and alkali, but its Ni and Cr components might cause contamination.

Platinum: very inert material, and heat conductivity is ideal-But is very expensive, also it corrodes easily, necessitating use of wooden tongs. Certain analysis require Pt. Crucible.

2. Modified dry ashing procedures:

a. Initial burning with open flame, then moistening with dilute HCl (1) + HNO₃ (2), Drying on steam bath, incinerating. Use of "ash-aids" for accelerating ashing, for preventing spattering and avoiding sample loss. [Examples for ash-aids: alcoholic solution of Mg acetate; a drop of HNO₃, pure cotton, pure glycerin] [A "Blank" for the ash-aid should also be run in parallel with the sample is ash aids are used.]

Low Temperature Plasma Ashing(9.2.4)

- **Special type of dry ashing method where foods are oxidized in a partial vacuum by oxygen formed by an electromagnetic field in the special instrument.Much lower temperature avoids volatilization of metals.**

Microwave ashing(9.2.5):

**Faster method.Can be used for both dry and wet applications.
Systems allow for temperature programming**

Wet-Ashing (Wet digestion)

Procedure: 9.2.3.2: Oxidising organic substances with strong oxidizing agents. Mineral acids are used as oxidants (Preferred for application before analysis of elemental trace metals)



- Perchloric acid $[\text{HClO}_4] \rightarrow$ powerful oxidants

Ex: Ashing Wheat with $(\text{HNO}_3 + \text{H}_2\text{SO}_4) \rightarrow 8$ hours

With $(\text{HClO}_4 + \text{HNO}_3) \rightarrow 10$ minutes

You should control the color of the ash solution: it must be clear and transparent when oxidation process is completed.

COMPARISON OF THE ASHING PROCEDURES

Dry ashing

Slow

Simple+easy

T° high (loss due to volatilization)

No supervision

More scope of applicability

No chemical reagents

Wet ashing

Rapid

Needs skill

T° low (no losses of volatilization)

Need supervision

Large samples are not convenient

Corrosive-explosive reagents

B-Indirect measurements of total ash content

1-Conductometric methods rely on determination of total electrolyte content of food sample.

Example Application: On an acidified sugar solution, minerals dissociate whereas sugar (non-electrolyte) does not. The conductance measured before and after acidification is an index of total electrolyte (or metal) content.

2-Ion-exchange columns: Acidic-cation-exchange columns are used. By titration of the liberated acid exchanged on the column by the cations, the quantity of total electrolytes can be determined.

II. Post-ashing Procedures : Properties of Ash:

1-Acid-insoluble ash= (~%10 HCl) .insoluble soil metal contaminants like silicates(index of dirt, sand, soil).

2-Salt-free ash= Index of added NaCl, Total ash is dissolved in dilute HNO_3 and titrated with AgNO_3 solution for NaCl determination.

3-Alkalinity of ash*: Titration with 0.1N HCl. Shows acid-base balance of food. Index of fruit contents of jams :Organic acids in fruits are transformed to carbonates and oxides, resulting in alkalinity. Also detects adulterations with minerals.

4-Water-soluble ash: Also an Index of fruit contents. Since metal oxides are water-insoluble, lower ash in water soluble fraction means extra fruit was added and indicate higher amount of fruits.

•*Ash of fruits is alkaline(Ca, Mg, K, Na)

•*Ash of meats+cereals is acidic(P, S, Cl)

III. Qualitative Screening Tests for Metals

Colour reactions of individual metals are done on the ash .

Ex: Pb+dithizone(green)→red colour

Fe+o-phenantrolene→red

P+molibdate→yellow

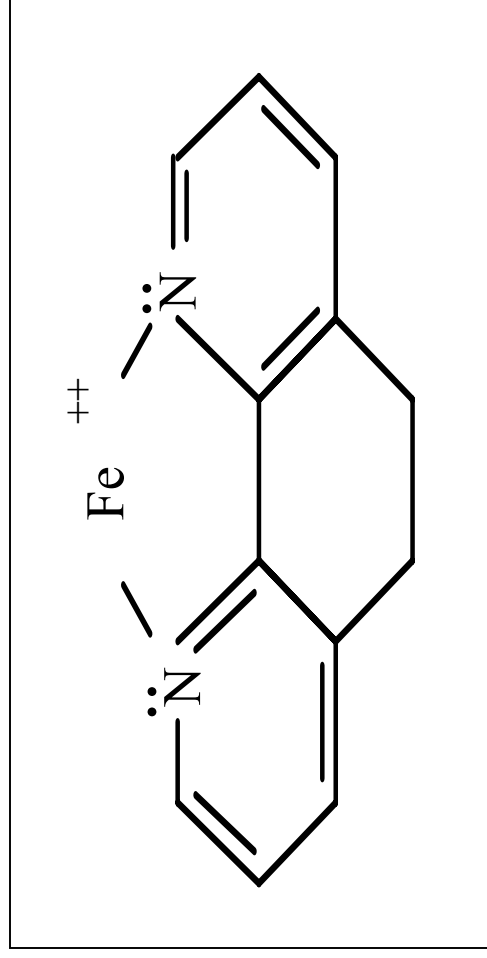
**As(arsenic)+silver→red
(diethyldithiocarbamate) and copper**

Colorimetric or Spectrophotometric Methods

Formation of colored complex with some ligand. A pair of unshared electrons can coordinate certain metallic ions to give complexes

Qualitative elemental analyses rely on this principle.

Example: Fe^{++} (Ferrous Ion) with phenanthroline (orthophenanthrolines) form intense red color. The ferrous orthophenanthroline complex is quite stable and is sometimes called Ferrioin



IV. QUANTITATIVE Elemental analysis

This is done for quantification of individual metallic components. However, enrichment of trace metals is almost always required before such analysis. You have to separate the huge amount of other macro inorganic components (like Ca,Na etc.) that can interfere with trace metal analysis stepwise by:

- 1. Ion exchange resins (specific)**
- 2. Concentration after extraction with specific organic solvents.**

Major Stages in Elemental Analysis

- Step 1: Ashing
- Step 2: Solubilize ash in Conc. HCl - boil and evaporate solution to dryness.
- Step 3: Re-dissolve residue in 0.5 N HCl.
- Step 4: Conc. or dilute as desired
- Step 5: Determination of individual components by suitable methods

Methods for Quantitative Mineral Analyses(Ch. 10)

- Gravimetric
- EDTA complexometric
- Redox reactions
- Precipitation Titration
- Colorimetric
- Ion selective electrodes
- Flame emission, Atomic Absorption and Emission Spectroscopy (Chapter 28)

Gravimetric and Titrimetric Methods

Gravimetry:

- Insoluble salts of minerals are precipitated, rinsed, dried, weighed. Calculations are based on molecular formula of salt.
- Example: Ca⁺⁺ in ash is dissolved in HCl, precipitated as Ca oxalate with ammonium oxalate solution, then burnt to CaO, then weighed +calculated using the MW ratios (MWCa/MWCaO).

Titrimetry:

- a. EDTA Complexometric titration: EDTA forms stable complexes with metals:



Example: [Ca⁺⁺ with ascorbic acid+hydroxynaphthol blue indicator] is titrated with 0.01M Na EDTA solution to deep blue endpoint.

Redox and Precipitation Reactions

LEO GER

- Oxidation: losing electrons
- Reduction: gaining electrons

- Precipitation titration:

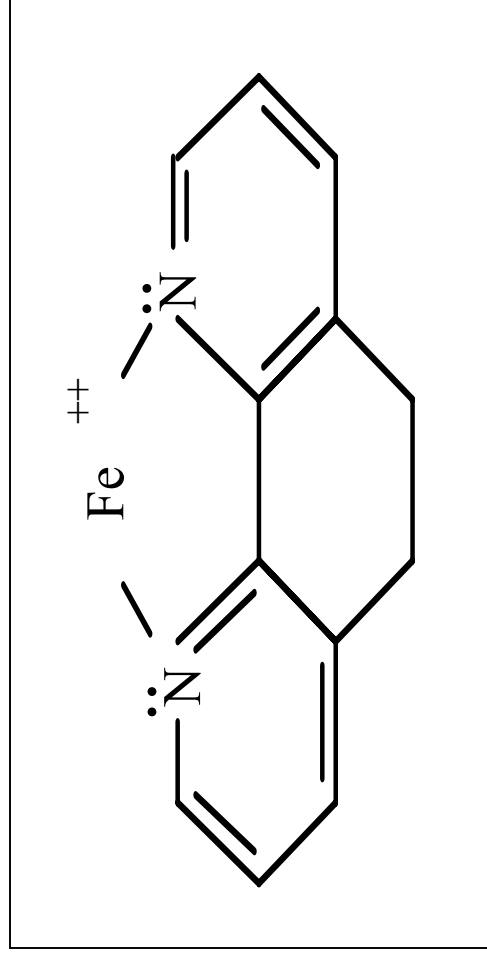
Mohr and Volhard
Methods for salt
determination

(p. 156-7)

Colorimetric or Spectrophotometric Methods

The intensity of the color of the colored complex formed with metal and some specific ligand is measured by UV-Vis spectrophotometer and assessed by the use of a calibration graph using known amounts of standard metal and ligand.

Example: Fe^{++} (Ferrous Ion) with phenanthroline (orthophenanthrolines) form intense red color. Intensity of red color can be measured at 510 nm by spectrophotometer(Fig.10.2).Both redox and colorimetric method



Flame Emission Spectroscopy p.433

Flame Emission Spectroscopy is based upon those particles that are electronically excited in the medium.

The Functions of Flame

1. To convert the constituents of liquid sample into the vapor state.
2. To decompose the constituents into atoms or simple molecules:

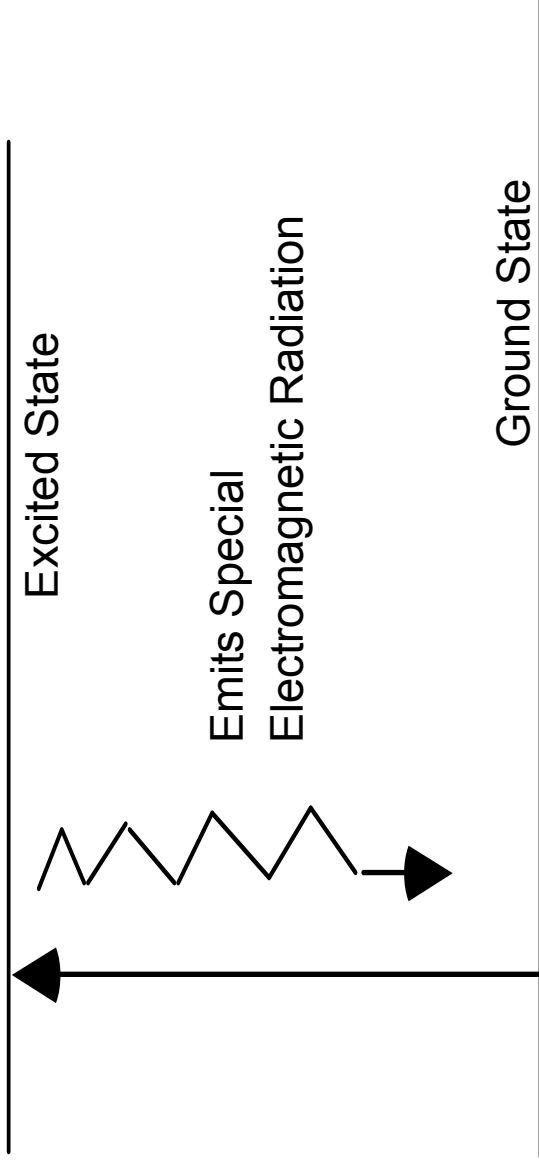


3. To electronically excite a fraction of the resulting atomic or molecular species

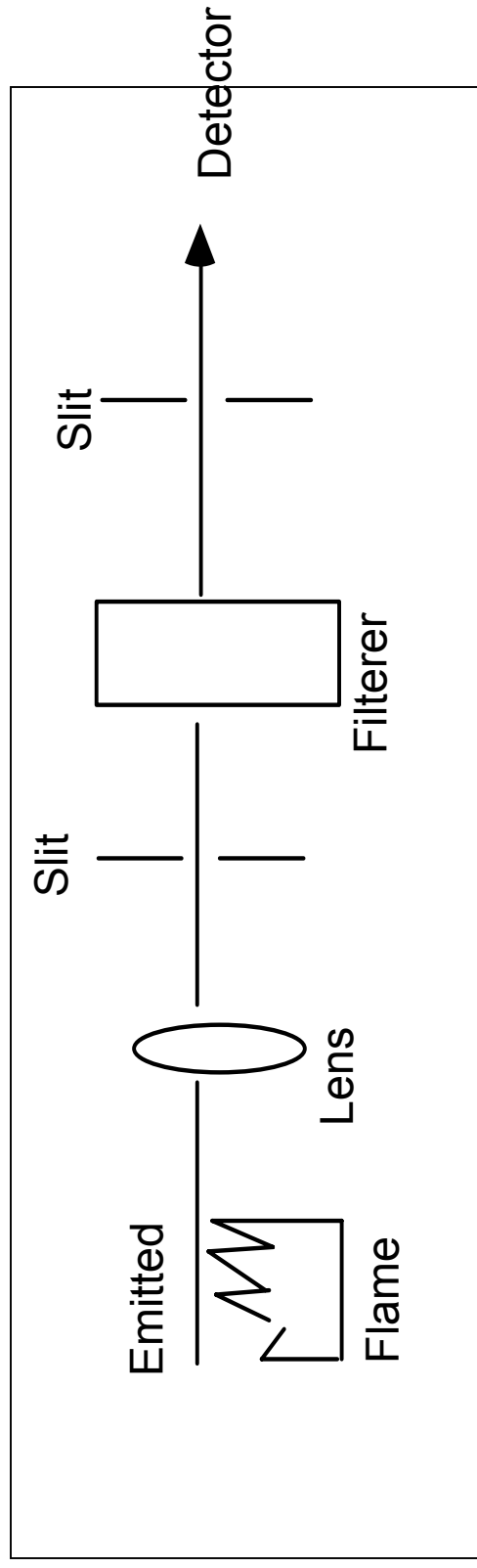


Flame Emission Spectroscopy

Measure the intensity of emitted radiation



Flame Emission Spectroscopy



- 1) The elements must be reduced to the elemental, neutral ground state by the flame.
- 2) The elements must be in vaporized state.
- 3) The elements must be imposed in the beam of radiation from the source.

Advantages:

Very sensitive.

Fast.

Disadvantages:

Hollow cathode lamp for each element.

Expensive element.

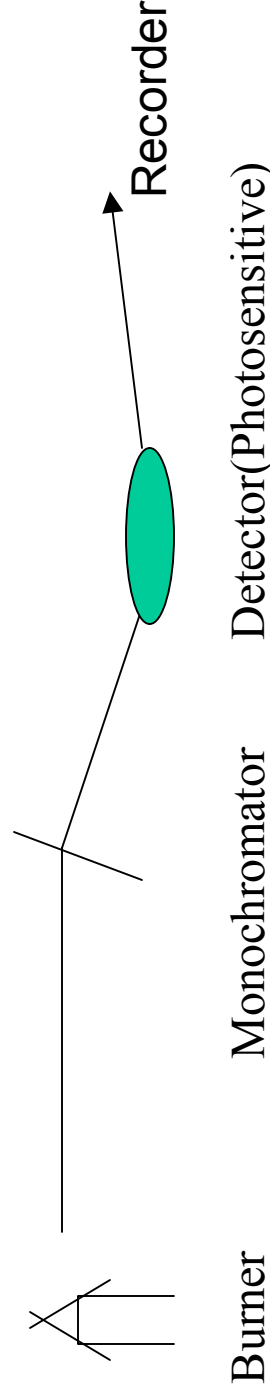
THEORY of Atomic Spectroscopy:

Atomic spectroscopy requires that the atoms of elements are in “atomic” state (not combined with other elements). “Atomization” involves separating the particles first into individual molecules by vaporization, and breaking molecules into atoms by exposing the analyte to very high temperatures in a flame or “plasma”. The atoms then made to absorb the radiation of characteristic wavelength

(AAS), or are made into “excited” state, thus subsequently emitting radiation of characteristic wavelength (AES).

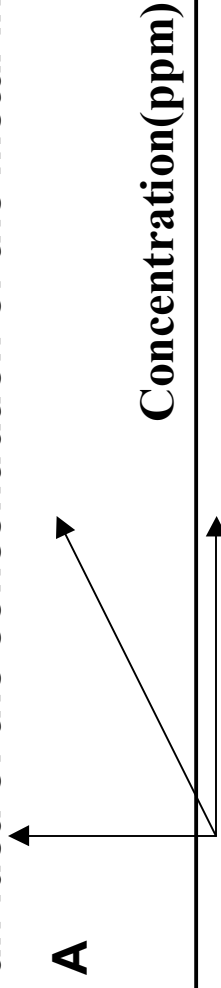
Atomic Emission Spectroscopy: (AES)(Ch.28,p.433)

Measures the radiation emitted by atoms of metals when their planetary electrons that are displaced (i.e. by heat of flame) from their orbits fall back to their original lower energy levels. This radiation is very typical for each metal; for each, there is a characteristic emission wavelength(for K: 766nm;for Na:330nm).**Flame Photometry** is essentially an application of E.S. A gas-air flame (900-1200°C)will provide enough radiation energy for exciting alkali and alkali earth metals. The photometer measures the intensity of light at the characteristic wavelength, which is correlated with concentration.

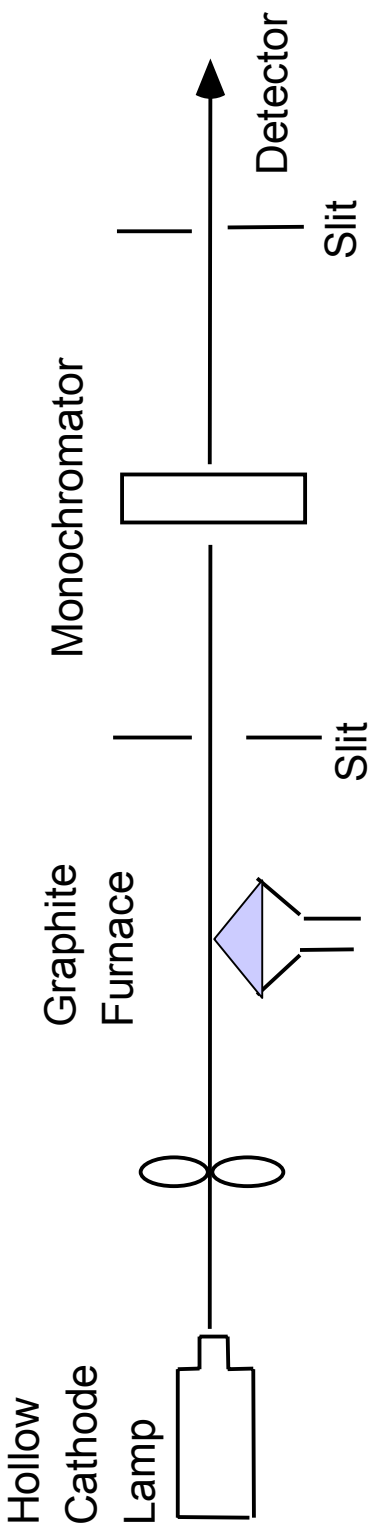


Atomic Absorption Spectrometry-AAS(p.428)

1.AAS With Flame:The instrument contains a source of radiation (hollow cathode lamp) whose wavelength is exactly the same as that required to excite the analyzed metal. Electrons given off by cathode accelerate towards the anode;along the way, they ionize argon atoms which are then attracted to the cathode. The atoms excited by collision with filler gas ions give off their energy, the emitted radiation being characteristic of the metal the HCLamp is made of.The sample is aspirated into the burner gas mixing chamber and is mixed with a combustible gas mixture(air-acetylene flame:2300°C,NO-acetylene flame:3080°C).Ground state metal atoms in the flame are detected by the absorption of energy of the specific wavelength furnished by the hollow cathode lamp. The difference between the radiation energy absorbed by the solvent without sample and the solvent with sample is displayed electronically. Each time we analyse a different metal, we have to replace the respective hollow cathode lamp (ForPb→use Pb lamp,for Cd→use Cd lamp). We can detect even very very low concentrations like 10^{-6} Molar solutions. Plotting abs. vs. conc. gives us an idea of the concentration of the metal in the sample



. Atomic Absorption Spectrometry



Atomic Absorption spectroscopy involves the study of the absorption of radiant energy by neutral atoms in the gaseous state.

Relationship Between Atomic Absorption and Flame Emission Spectroscopy

- Flame Emission → it measures the radiation emitted by the excited atoms that is related to concentration.
- Atomic Absorption → it measures the radiation absorbed by the unexcited atoms that are determined.

Atomic absorption depends only upon the number of unexcited atoms, the absorption intensity is not directly affected by the temperature of the flame.

The flame emission intensity in contrast, being dependent upon the number of excited atoms, is greatly influenced by temperature variations.

2.Flameless AAS:

(Also called “carbon-rod or graphite furnace method”) p.430.

This is an electrothermal process using a similar AA spectrophotometer, but the difference is that here the burner is electrically heated. Such instruments are 10-100 times more sensitive than AAS with flame, thus making possible to detect Hg at 0.001 ppm concentration in fish. Here the furnace is an electrical resistance- heated graphite tube(also called a Carbon rod) and there is no flame. The temperature can reach ~5000° K.

A small amount of sample is injected with a syringe into the graphite tube,programmed first to dry then to char and then to atomize the ash to the path of the radiation from the hollow cathode source.

The rest of analysis(detection, graph and calculations etc.) is exactly the same as AAS with flame.

Simultaneous Elementary Analysis: This is made possible with modern instruments, like “ICP”, which is short for “Inductively coupled plasma”. It has a similar principle with emission spectroscopy. The very expensive, highly sophisticated but also very sensitive instrument makes use of a “Plasma torch” (p.434), which is an electrical discharge of high (>1) concentration (+) and (-) ions. The argon plasma ($T \sim 10000^{\circ}\text{C}$) is formed by a stream of argon gas flowing between two quartz tubes, and argon is made conductive by exposing it to an electrical discharge, creating seed electrons and ions. The light (optical) energy emitted when sample is burnt in plasma torch is recorded on a photographic film, that is developed and compared with standardized film.

Using this method, identification of the origin of agricultural commodities is made possible, since there are differences, for example, in the concentration levels of the individual metals in American and Turkish oranges due to differences in soil composition and mineral uptake patterns of local varieties..

Method Selection Guide

FLAME AA	ICPEmission
GFAA	ICP_MS

High

Concentration

Low

High

Number of Analyses