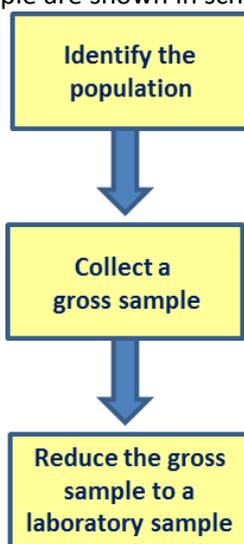


22. Ashing of organic samples and determination of Zn and Fe using ICP-AES method

Determination of inorganic constituents in samples of foods, plants or soil is extremely important due to the possibility: getting know the nutritional value of products, toxic substances monitoring, matching efficiency of farmers (fertilization and irrigation) to the needs of plants in various stages of its growth.

Sampling is the process by which a representative fraction is acquired and this is the first step of the analytical process. Often, sampling is the most difficult step in the entire analytical process and the step that limits the accuracy of the procedure. Sampling for a chemical analysis necessarily involves statistics because conclusions will be drawn about a much larger amount of material from the analysis of a small laboratory sample. Steps in obtaining a laboratory sample are shown in scheme 1.



Scheme 1. Steps in obtaining a laboratory sample (Fig. 8-4 from[1]).

The collection of sampling units chosen so that were representative of the bulk of material, is called the **gross sample**. For analysis in the laboratory, the gross sample is usually reduced in size and made homogeneous to become the **laboratory sample**.

The collection and preparation of samples relate to the possibility of committing a series of errors from the sampling site selection, the type of tools used to collect the samples, type of containers used for storage, conditions during transport and storage, as well as the possibility of sample contamination and loss of analytes during the treatment immediately prior to the use of a suitable measuring technique.

Sources of error in decomposition and dissolution of samples:

- Incomplete dissolution of the analytes.
- Losses of the analyte by volatilization e. g. volatile fluorides or chlorides, certain volatile oxides (tetraoxides of osmium and ruthenium, and the heptoxide of rhenium), chlorine, bromine, iodine.
- Introduction of the analyte as a solvent contaminant.
- Introduction of contaminants from reaction of the solvent with vessel walls.

Pretreatment of environmental samples, depending on the type of sample::

- filtering (solutions, eg. water, sewage),
- fixing: acidification, cooling, freezing, addition of organic compounds (solutions, e.g. water, sewage),
- the separation of foreign materials (soil, plants, etc.),
- drying (solid content: soil, plants, etc.),
- grinding (solids: soil, plants, etc.).

Conversion the sample in the solution is in the process of ashing.

Ashing (or mineralization) is the process of oxidizing an organic sample leaving the inorganic component for analysis. If the inorganic components are in trace content the removal of the organic part of sample also acts as thickening, or enrichment of traces. Organic substance evaporates in form of water vapor, carbon oxides, nitrogen oxides, and sulfur oxides.

Types of ashing:

- **Dry ashing** (incineration) involves heating the substance, and calcined in air (inevaporator or quartz, platinum, porcelain crucibles), or oxygen (the rowboat placed in the tube through which oxygen is passed). Ignition temperature during digestion should not exceed 500 ° C in order to avoid losses due to the volatility of certain minerals.
- **Wet ashing** is the process of oxidative decomposition of organic samples at high temperature by liquid oxidizing reagents such as HNO_3 , H_2SO_4 , HClO_4 , or mixtures of these acids.

Currently, one of the most popular methods of distribution of the sample and its conversion into the solution is the use of pressure ashing assisted with the microwave energy. This technique was created by the merge of two others: a closed-pressure system in the so-called „Teflon bombs" and ashing using microwave energy. In this exercise, students will perform wet ashing using M-9 type mineraliser, useful in analyzes of water, soil, food, wastes etc. Simultaneously, nine samples can be mineralized, the range of temperatures is 100 to 450°C.

The most useful and valuable in human nutrition are fruits and vegetables. They contain, among others, pectins, vitamins, macro- and microcomponents, but they also can contain heavy metals, toxic for living organisms. Accumulation of toxic metals in plants depends on many factors. It can be measured using advanced analytical methods, like ICP-AES, which will be applied here.

Objective of the exercise

- Quantification of the content of zinc or iron in organic samples using ICP-AES method (atomic emission spectrometry with inductively coupled plasma).
- Qualitative determination of zinc or iron with a classic method.

Atomic emission spectrometry (AES) is a widely used technique for elemental analysis. The method consists in recording the electromagnetic radiation emitted by free atoms of elements in the sample to be analyzed. Analyte solution after the introduction to the spectrometer evaporates and atomizes in argon plasma flame, where temperatures can reach up to 10,000 K. The high temperature causes the excitation of atoms to higher electronic states. Atoms returning to the ground electron state emit electromagnetic radiation.

A plasma is a hot (over 10000 K), partially ionized gas containing free atoms and ions in different states of excitation, electrons, free radicals.

Quantitative analysis of elements by atomic emission spectrometry is a **comparative method**. Before performing the proper analysis a series of so-called standard solutions is prepared. Standard solutions contain all reagents used in the assay and in the same quantities, and the analyzed element in a different but exactly known concentration. For a series of standard solutions the emission intensity of the analyzed element is measured at a characteristic wavelength. The dependence $I = f(c)$ is plotted, where I - is the intensity of the emission, c - the concentration of the analyzed element. After the measurement of the emission intensity for a unknown sample, the concentration of analyzed element is read from the calibration curve.

Reagents and equipment

- The dried, shredded plant
- Nitric acid(V) pure, concentrated
- Aqueous solution of KSCN
- Mineralizer M-9
- ICP atomic emission spectrometer.

Procedure

Ashing of samples

Accurately weigh about 1 g of the different plants, place in ashing container. To each container add about 6 cm³ of concentrated nitric acid (V) (under the hood). Perform ashing. At the end of ashing evaporate nitrogen oxides.

Transfer the samples quantitatively to the glass tubes of mineralizer (wash the containers with little portions of concentrated nitric acid), add ca. 15 cm³ of concentrated HNO₃.

Place the glass rod facilitating boiling into each tube.

Perform mineralization: Fix the temperature of 100°C, switch on the apparatus (point IV of the instruction). Place the fume hoods, switch on the vacuum water pump. When temperature reaches 100°C, increase it to 150°C. Heat during ca. 30 min. After this time switch off heating, take off the tubes from the apparatus. When the tubes are cooled down enough, switch off the vacuum pump. Take off the glass rod and wash it carefully with little amount of distilled water.

Transfer quantitatively the solutions to volume flask a 50 cm³ containing ca. 10 mL of distilled water. Complete the volumes to the mark and mix.

The solutions should be clear!

1. Quantification of the content of zinc or iron by ICP-AES method

Transfer digest quantitatively to a 25cm³ volumetric flask and carefully add demineralized water to the mark. Perform measurement by ICP-AES.

2. Preparation of calibration curve

Transfer 0.5, 1.0, 1.5, 2.0, 2.5 cm³ of the standard Fe(III) or Zn(II) solutions ($c = 0.01 \text{ mg/cm}^3$) to five 25-ml volumetric flasks. All solutions dilute to the mark using nitric acid(V) (4 mol/dm³), mix thoroughly.

Measure of samples using ICP-AES method.

3. Qualitative determination of zinc or iron with a classic method

To a few drops of the analyzed solution add 1 drop of KSCN solution. Blood-red color of the solution indicates the presence of Fe(III).

Calculations and report

The report should contain a very short description of the determination method, the type of sample analyzed, a description of preparation of the sample for measurement, calculation of the content of zinc or iron (in mg) in 1 g of each plant sample, characteristic reaction of Fe(III) cations with KSCN.

Sources:

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4. http://www.chemia.uj.edu.pl/chemanal/dydaktyka/analizachemiczna_pliki/2011/i_1.pdf