# BRIEF OVERVIEW OF INSTRUMENTAL METHODS FOR DETERMINATION OF TRACE ELEMENTS IN BIOLOGICAL AND ENVIROMENTAL SAMPLES. Part I: SPECTROSCOPIC TECHNIQUES

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S u m m a r y. Elemental analysis is important from the point of view of human health. It may be helpful in diagnosis of different diseases because they are often related to disturbance in the physiological or toxic level of elements. However, the determination of metal ions in trace amount is difficult; therefore, analytical techniques are still being developed to cope with increasingly complicated analytical challenges. New modifications of the devices are carried out providing an opportunity of their wider application in many fields. In the present paper, the most common spectroscopic techniques applied in analysis of biological and environmental samples are described.

K e y w o r d s: spectroscopy, analytical technique, atomic emission spectrometry, atomic absorption spectrometry, X-ray fluorescence, biological samples, ions, elements, trace analysis

### INTRODUCTION

The term "trace analysis" refers to analytical procedures which allow detection and/or quantification of very small amounts of the analyte (below 100 ppm). This type of analysis is extremely difficult because some samples, e.g. blood and other tissues have a very complex matrix and high nonhomogeneity; hence, they require appropriate sample pretreatment, e.g. mineralization process, which increases the risk of contamination and analytical errors. Moreover, the amount of element is often extremely low, even below 0.70 ng/mL.

Determination of components at such low concentrations is the most important trend in

modern analytical chemistry. The methods are still being developed and new modifications of the devices are being elaborated to cope with complicated analytical challenges and to widen the possibility of their applications. There are many instrumental techniques for the determination of trace elements, e.g. X-ray fluorescence (XRF), inverse voltammetry (IV), chromatography (IC); however. and ion especially spectroscopic methods, atomic absorption spectrometry (AAS), are the most useful techniques for routine analysis of samples of different origin [1].

The present paper focuses on spectroscopic techniques and their usefulness in the analysis of trace elements in biological and environmental samples.

### **Classification of spectroscopy techniques**

Generally, spectroscopic techniques can be divided into 3 groups [2, 3]: a) emission techniques including flame excitation, e. g. flame photometry (atomic emission spectrometry -AES), excitation by electric or electric arcemission spectrography (emission spectrography); with excitation in plasma: inductively coupled plasma (ICP); Microwave Induced Plasma (MIP); Laser Induced Plasma Spectrometry (LIPS); Glow Discharge (GD), b) absorption techniques, e.g., atomic absorption spectrometry (AAS); UV-Vis spectrometry c) Fluorescent Techniques - X-ray emission spectrometry. Spectroscopic techniques can also be divided into: 1. optical atomic spectrometry;

2. atomic mass spectrometry,

In the case of spectroscopic techniques, the determination of elements is only possible if the sample is in a gaseous form, and the analytes form free ions or atoms. This condition is achieved using different types of atomizers, and the atomization process itself affects the repeatability and reproducibility of analyses [4].

These considerations indicate that the elemental analysis techniques can also be divided into two groups:

1. techniques in which the measurement is made in the liquid phase (chromatographic and electroanalytical),

2. techniques in which the measurement takes place in the gas phase (spectroscopic).

#### Brief description of spectroscopic techniques

# Atomic emission spectrometry with inductively coupled plasma (ICP)

ICP technique is widely used in elemental analysis [5]. It allows determination of both metals and nonmetals with good precision and accuracy. Laser ablation technique that allows introduction of an analyte into the atomizer is one of the most common. It involves evaporating a solid sample into a laser beam or spraying a liquid sample with a nebulizer. The evaporated or sprayed sample is introduced to inductively coupled plasma in which atoms are excited. This plasma is formed by concentric heating and ionization of argon by a radio frequency radiation generator. Argon flows through three quartz tubes, with a flow rate from 11 to 17 L/min (in the latest apparatus these flows are less than 10 L/min). An induction coil powered by a radio generator and cooled with water is located at the top of one of the tubes. Argon ionization is initiated by sparks generated by the Tesla coil and the resulting electrons and ions interact with variable magnetic field produced by the generator. They begin to move in a spiral motion, resulting in the release of significant amounts of heat. It does not damage the quartz tube, as it is isolated from the plasma by means of argon stream flowing just off the tube walls. An additional function of this stream is to keep the plasma inside the burner. The burner itself can be set vertically or horizontally (in some devices it is possible to change the setting). The

horizontal alignment allows determination of elements present in low amounts, while the vertical alignment provides better measurement accuracy. Plasma emitted atoms emit radiation with their characteristic wavelengths (spectral lines) that are cleaved in a monochromator and then fall on the solid state detector or photomultiplier. The optical signal is replaced by an electrically recorded signal and processed digitally by computer. ICP is currently the most commonly used technique for certification of reference materials used in the validation of other analytical methods. The ICP technique allows labeling of approximately 70 elements in a single analysis. This method is very precise. Its large linearity of calibration curves facilitates simultaneous determination of the content of elements in the sample at the level of traces as well as the amount of macroelements. In this way, the workload of the analysts and the possibility of making mistakes at the dilution stage are minimized. The ICP technique allows the elements in the sample to be detected at the decimal level of ppb [6]. This method has a serious disadvantage, i.e. the price of the camera and the cost of analysis.

### Atomic absorption spectrometry

The technique of atomic absorption spectrometry is based on the absorption of radiation by free atoms of elements. Atoms of various elements absorb radiation at their characteristic wavelengths, which makes AAS highly selective. A very important issue in AAS is to obtain free atoms in the gas phase in a basic state rather than excited. During atomization, some of the atoms are still excited, but the ratio of the number of atoms to the number of atoms not yet excited is small enough to allow the content of the element in the sample to be determined. Atoms in the gas phase in baseline state absorb the radiation emitted by an incandescent lamp or a xenon lamp, and their electrons move to the corresponding resonance levels. These levels correspond to the resonance lines (spectral). Spectral lines assume the shape of Gaussian curves and their width is measured in half the height of the curve. This is so-called half width. However, this parameter is influenced by many factors such as magnetic field, temperature, and pressure. Atoms whose electrons differ in the magnetic quantum are subject to the Zeeman effect if they are present in a strong magnetic field. As a result of spin

interactions, the magnetic moment of the atomic nucleus (having non-zero nuclear spin) and the magnetic moments of the electrons become visible in the spectral structure (spectral line splitting). This phenomenon is sometimes used for background correction in cameras for AAS [6]. The influence of temperature on the width of the spectral line of the element is revealed in the "Doppler enlargement". This expansion results from the increased mobility of atoms through the optical apparatus. At temperatures within the range of 1700-2700 °C, the value of Doppler widening is about 10-3 nm, and the natural width of the spectral line of the element is smaller by two orders of magnitude [6]. The expansion of the spectral lines associated with the increase in pressure is called the "Lorentz extension". The higher the gas pressure, the more often the atoms collide, passing the energy more energetically. At normal pressure (101,325 Pa), Lorentz widening and Doppler widening assume values of the same order [6].

Under the Lambert-Beer law, the amount absorbed by the radiation sample (absorbance) is directly proportional to the amount of free atoms present in optical AAS. Therefore, quantification is based on the measurement of radiation absorbance and comparison of its value to the absorbance values obtained by measuring standard solutions.

Atomization and excitation of the element atoms in the tube occurs as a result of electromagnetic field interacting with the radio frequency generated by the inductor. Lamps of this type generate very high radiation intensity and a low spectral line half-width, which is undoubtedly their advantage. The latest source of light used in AAS is the xenon lamp (continuous light source). This lamp generates high intensity radiation, which facilitates analysis of all possible elements to be determined by AAS method, without the need for multiple lamps:

To obtain free-atoms of investigated elements the following types of atomizers are used:

- Flame Atomic Absorption Spectrometry (F-AAS);

- Electrothermal Atomic Absorption Spectrometry (ET-AAS or Graphite Furnace, Electrothermal Atomization - GFETA);

- Cold Vapor atomic Absorption Spectrometry (CV-AAS);

- Hydride Generation Atomic Absorption Spectrometry (HG-AAS),

### Spectral techniques - X-ray fluorescence

X-ray fluorescence radiation is emitted when the atom is ionized with sufficiently high energy x-ray photons. This phenomenon can also be triggered by gamma rays, protons, or synchrotron radiation (this radiation is nonterrestrial and is generated by particles that move at a speed very close to the speed of light in the vacuum in electromagnetic field). This method consists in measuring the excess of x-rays in the form of radiation quanta from samples that have been previously ionized. The analyzed element emits its characteristic fluorescence spectrum (the wavelength and energy of radiation is the same as the wavelength and energy of characteristic xrays, i.e. electron-beam radiation). Based on the position of the spectral lines on the wavelength scale or on the energy scale, a qualitative sample is analyzed. The analysis is based on determination of the atomic number by Moseley's law (this law assumes that the square root of the frequency of the x-ray spectrum of elements with different atomic numbers are arranged in a straight line). Quantitative analysis is based on the measurement of fluorescence intensity. It should be noted that quantitative analysis using X-ray fluorescence requires calibration (comparison with the intensity of radiation from a standard with a known concentration of an element to be tested). There are many techniques that use elemental analysis for x-ray fluorescence radiation [7,8].

Depending on the sample ionization method, the following fluorescence analysis techniques are distinguished:

- X-ray fluorescence analysis (XRF),

- Total Reflection X-ray Fluorescence (TRXRF),

- Synchrotron Radiation Induced X-ray Emission (SRIXE),

- Radioisotope X-ray fluorescence analysis (Radioisotope X-ray Fluorescence - RXRF),

- X-ray emission technique (Partition Induced X-ray Emission - PIXE),

- Proton Induced Gamma Emission (PIGE) technology,

X-ray fluorescence is a non-destructive method for determining ppm levels in a very wide range of concentrations (from 0.0001% to 100%) [9, 10]. Its great advantage is the simplicity of sample preparation for analysis and the availability of a convenient and easy to use measuring instrument. As a result, X-ray fluorescence has been used for the analysis of ceramics, metals, biologically active compounds, medical preparations, and drugs [11, 12]. However, this technique is not suitable for isotopic analysis.

### Application of spectroscopic techniques in analysis of biological and environmental samples

Elemental analysis of human samples such as body fluids (blood, serum, plasma, urine), hair, nails, and the other tissues is of great importance in medicine [38]. Disturbances in the element level in the organism can cause various diseases, and their biomonitoring may be useful for diagnostic purposes. Moreover, the knowledge about the level of elements in environmental samples such as water or soil is very important both for toxicology and bromatology.

On the one hand, monitoring of toxic elements such as cadmium, lead, or mercury helps prevent poisoning and, hence, development of serious diseases. On the other hand, determination of beneficial elements, e.g. selenium, calcium, magnesium is helpful for food and nutrition quality control. There are many literature reports on determination of elements in samples of different origin by instrumental techniques; however, spectroscopic techniques seem to be the most common. The examples of their application are presented in Tables 1-3.

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Sample type	Elements	Concentration range	Ref.
Tomatoes from Italian cultivation	V, Al, Zn, Fe, Cu, Ba, Mn, Sn, Sr, Cd, Co	0.0360 – 57.03 μg/g	[13]
Sea water- certificated reference material (SLEW-3)	V, Mn, Ni, Co, Cu, Zn, As, Cd, Pb	0.033 – 2.80 μg/L	[14]
Italian olive oil Lamesia Carolea	Be, Mg, Ca, Sc, Cr, Mn, Co, Ni, Eu, Sm, Sr, Cd, Gd	0.013 – 14969 ng/g	[15]
Ginger Zingiber officinale	Cr, Mn, Fe, Co, Ni, Cu, Zn, Rb, Sr, Pb	0.40 – 345 μg/g	[16]
Red wine	Li, Al, Ca, Mn, Ni, Fe, Cu, Zn, As, Rb, Cd, Sm, Cs, Ba, La, Ce, Nd, Pr, Eu, Sm, Ho, Tb, Dy, Yb, Pb	0.5 – 138000 ng/mL	[17]
Surface water from Transylvania	Al, As, Pb, Cd, Ag, U, Zn, Mn, Ni, Co, V	0.002 – 5.865 µg/L	[18]
Virginia Tobacco leaves	Cu, Zn	14.7 – 35.0 mg/kg	[19]
Brazilian brown rice	As, Cd, Pb	0.065 – 0.660 mg/kg	[20]

T a b l e 1. Application of ICP in trace analysis.

Sample type	Element	LOD	Type of atomization	Ref.
Water and bottoms	Pb	3.6 μg /mL	electrothermal	[21]
Basil	Cu	1.44 mg/kg	flame	[22]
Fruit juice	Mn	0.50 mg/L	flame	[23]
Human serum	Mg	0.065 μg/mL	flame	[24]
Plants	Hg	0.10 mg/kg	cold vapor technique	[25]
Milk	Sr	0.05 μg/mL	flame	[26]
Blood, cigarettes	Cd	3.0 µg/L	flame	[27]
Rice, tea, fish	Hg	0.01 ng/mL	cold vapor echnique	[28]
Fertilizers, water sample	Cu	1.3 μg/g	flame	[29]
Tobacco	Pb	1.7 μg/g	flame	[30]
Spinach leaves	Ca	0.72 mg/L	flame	[31]
Water, sea water, fish	Mg	0.6 ng/L	electrothermal	[32]

T a b l e 2. Application of AAS in trace analysis.

T a b l e 3. Application of X-ray fluorescence in trace analysis.

Sample type	Determined ion	Concentration	Technique	Ref.
<i>Glycyrrhiza glabra</i> (L.)	Rb	2.30 µg/g	X-ray fluorescence with completely ventral reflection	[33]
<i>Camellia sinensis</i> (L.) Kuntze	Fe	0.26 μg/g	Energy dispersive X-ray fluorescence (EDXRF)	[34]
Boron carbide	Ca	55 μg/g	X-ray fluorescence with completely ventral reflection (TXRF)	[35]
Red wine (Chile)	Sr	0.8 mg/l	X-ray fluorescence with completely ventral reflection (TXRF)	[36]
<i>Eucalyptus globulus</i> Labill.	Mg	1616 mg/kg	X-ray fluorescence wavelength dependent	[37]

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