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Medical and Biological Applications of X-Ray Fluorescence Method for Elemental Analysis

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Abstract

X-ray fluorescence spectrometers use high energy X-rays (or gamma rays) to excite fluorescent radiation from a sample for quantitative chemical or elemental analysis and have many industrial and research applications. XRF is primarily used as a non-destructive method for investigation of metals, minerals, environmental samples, food constituents, body fluids and biopsies. Microanalysis is the identification of the chemical elements present either within or on the surface of an object, and additionally, how the atoms of the elements are arranged with respect to each other. Identification of the elements present may be qualitative or quantitative. Techniques for estimation of element levels directly in humans (noninvasive in vivo) or in samples (in vitro) from humans are reviewed. Toxic, nonessential, trace elements may cause temporary or permanent damage to various organs and tissues in humans. There is thus a need to control the concentrations.In this work, we discuss applications in key areas with a view to providing examples of how the technique can provide information on biological and medical systems. Our goal is to provide useful and pertinent information to encourage and enable further use of this powerful method in chemical and biochemical studies.

Key Words: XRF, Microanalysis, Characteristic X-Ray

1. Introduction

X-ray fluorescence (XRF) is an analytical technique that can be used to determine the chemical composition of a wide variety of sample types including solids, liquids, slurries and loose powders. An integrated X-ray generator is used to irradiate the sample and the interaction of the beam with the sample results in the emission of fluorescence X-rays. It is these emitted X-rays which are characteristic of the elements in the sample and provide XRF with its powerful elemental analysis. XRF is also used to determine the thickness and composition of layers and coatings. It can analyze elements from Beryllium (Be) to uranium (U) in concentration ranges from 100 wt% to sub-ppm levels.

Addition to these, XRF is a robust technique, combining high precision and accuracy with straightforward, fast sample preparation. It can be readily automated for use in high-throughput industrial environments, plus XRF provides both qualitative and quantitative types of information on a sample. Easy combination of this 'what?' and 'how much?' information also makes rapid screening (semi-quantitative) analysis possible.

Micro X-ray fluorescence (μXRF) is an elemental analysis technique which allows for the examination of very small sample areas. Like conventional XRF instrumentation, Micro X-ray Fluorescence uses direct X-ray excitation to induce characteristic X-ray fluorescence emission from the sample for elemental analysis. μXRF with X-ray optics have been successfully used for applications including small feature evaluation, elemental mapping, film and plating thickness measurement, detection of micro-contamination, evaluation of multi-layered coatings for advanced circuit boards, small particle analysis, and forensics.

Chemically specific fluorescence spectrometry has been employed to follow chemical localization of trace elements in a number of health and biological systems. Plants were used in many of the early XRF studies of living systems [1-3]. Plants are highly resistant to radiation damage from the X-ray beam so that they often can be observed in a living state. Also, XRF is finding increasing application in the field of health science [4-6]. Living organisms have an inherent and very rich physical structure, with relevant length scales ranging from the nanometer scale for subcellular structure to hundreds of micrometers and above for tissue, organ or organism level organization. The ability to derive the spatial distribution of elements on this diversity of length scales is a key to understanding their function [7]. Knowledge of the relations between toxic effects and element concentration may be extracted from measurements in humans as well as in samples from humans and her environment [8]. Applications traditionally include occupationally exposed subjects, but an increasing research area is studies of members of the general population and of patients undergoing therapy for malignant and other diseases. Most in vivo XRF studies deal with lead in bone and cadmium in kidneys [9]. For retired lead workers, a clear association has been demonstrated between bone lead and blood lead, due to endogenous lead excretion from the skeleton [10]. A study of mercury in vivo showed that the technique is capable of detecting mercury in heavily exposed worker's kidneys [5, 12]. In vivo XRF in cancer and rheumatology patients has helped to understand how platinum and gold are retained in the human body [5]. The newest in vivo applications include zinc in prostate gland and arsenic in skin [13].

This review covers the latest activities in biological and health sciences studied with micro XRF. In addition, the latest developments in the micro-XRF system have been introduced in this work.

2. X-Ray Fluorescence

2.1. Microanalysis

Microanalysis is the identification of the chemical elements present either within or on the surface of an object, and additionally, how the atoms of the elements are arranged with respect to each other. Microanalysis can also require working out the spatial relationships between the atoms in the object, i.e., its structure, particularly if it is crystalline. Some microanalytical techniques can provide information about crystal defects, chemical bonding or redox state. Most microanalysis techniques involve focusing a microbeam on the object to be analyzed and measuring an output beam that results from the interaction of the input beam with the atoms and molecules making up the sample. The best technique to use will depend on the required spatial resolution and depth resolution, whether qualitative or quantitative chemical analysis, or structural analysis, is needed, and the minimum detection limit.

2.2. Energy Dispersive X-ray Spectroscopy

Energy Dispersive X-ray Spectroscopy (EDS, EDX or XEDS) is a qualitative and quantitative X-ray micro analytical technique that can provide information on the chemical composition of a sample for elements with atomic number (Z) >3. The X-rays are detected by an Energy Dispersive detector which displays the signal as a spectrum, or histogram, of intensity (number of X-rays or X-ray count rate) versus X-ray energy. The energies of the Characteristic X-rays allow the elements making up the sample to be identified, while the intensities of the Characteristic X-ray peaks allow the concentrations of the elements to be quantified.

2.3. Characteristic X-rays

The electrons in the electron cloud have a stable set of energy levels, also known as electron shells. The shell closest to the nucleus is known as the K shell, followed outwards by the L, M, N, O, P and Q shells. EDS microanalysis is mostly concerned with electrons in the inner shells, i.e., the K, L and M shells. The maximum number of electrons in each shell is governed by quantum mechanics, with a maximum of two electrons in the K shell; eight electrons in the L shell; 18 electrons in the M shell, and so on. Each shell, apart from the K shell, is split into subshells, with the electrons in related subshells having slightly different energies. The L shell has three subshells; the M shell has five subshells, and so on.

The K shell has the highest ionization energy or critical ionization energy in the atom. That is, more energy is needed to remove an electron from this shell than from subshells further from the nucleus. The further from the nucleus the electron is, the lower its ionization energy. Characteristic X-rays are produced by electron transitions between the electron shells (Fig. 1).

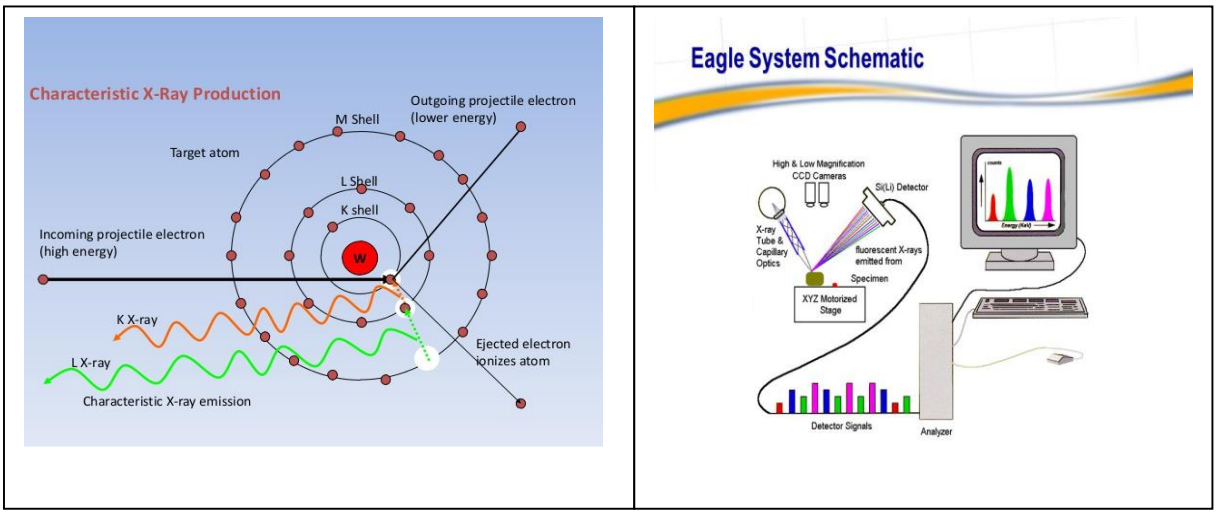

Fig.1 Characteristic X-rays and Micro XRF system.

2.4. X-ray intensity

The height of the Characteristic X-ray peaks in an ED spectrum, or the X-ray intensity, may be given in X-ray counts or count rate (counts per second or cps). It is tempting to assume that the height of the X-ray peak in the spectrum will be proportional to the concentration of the element in the sample. While the intensities of the peaks in an X-ray energy spectrum are not directly proportional to element concentration, it is true that the concentration of the element in the sample will influence the height of the X-ray peak.

3. Aplıcatıons in The Study of Bıology

XRF provides useful elemental information about specimens without causing specimen damage or requiring extra specimen preparations. Plants were used in many of the early XRF studies of living systems. While plants are interesting in their own right, early XRF studies were enabled by the fact that plants are highly resistant to radiation damage from the X-ray beam so that they often can be observed in a living state. Moreover, no protocols are required for their study, minimizing paperwork and eliminating ethical review, which is mandatory for animal and human tissues work.

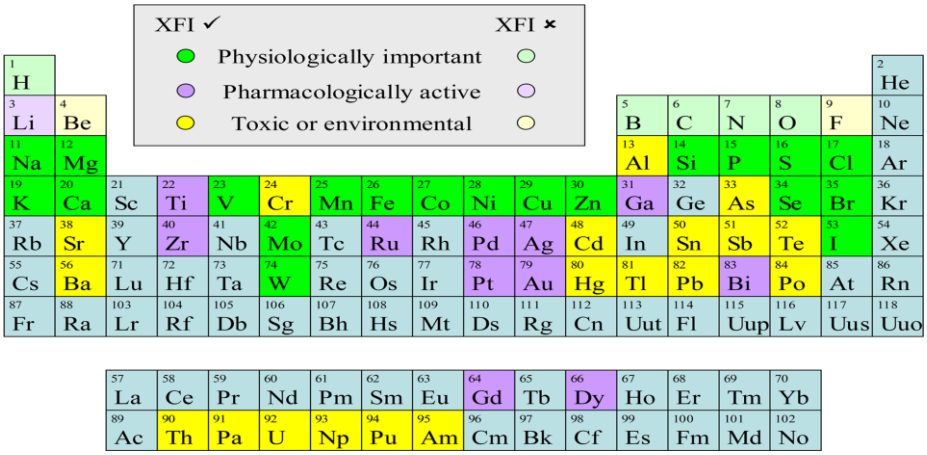

Figure 2 Periodic table of the elements showing elements of biological interest that can be probed using X-ray fluorescence imaging. Elements are divided into three categories, those that are physiologically important, those that are harmacologically active, and those that are toxic or of environmental concern.

A typical use for elemental imaging is to create overlays highlighting regions of the sample that rich in various elements. Basic premise of the technique XFM is a powerful technique to quantitatively determine element concentrations in a wide range of sample types. The extended depth of focus of X-ray probes ranging from several hundred micrometres to millimetrescale makes them ideal for addressing plant science. Analysis depth is often determined by the penetration of the outgoing fluorescence X-rays, and therefore depends on the X-ray energy of the interrogated element and the overall specimen matrix composition. Some examples of elemental mapping using X-ray fluorescence imaging are given in Fig. 3 up to 5.

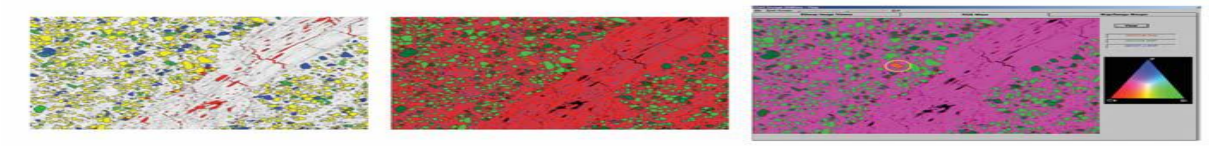

Figure 3 (a) Fossilization of prehistoric bone. (b) Elemental image overlay of Ca - red; P - blue; Si -green. (c) RGB mix of Ca, P, and Si. [14]

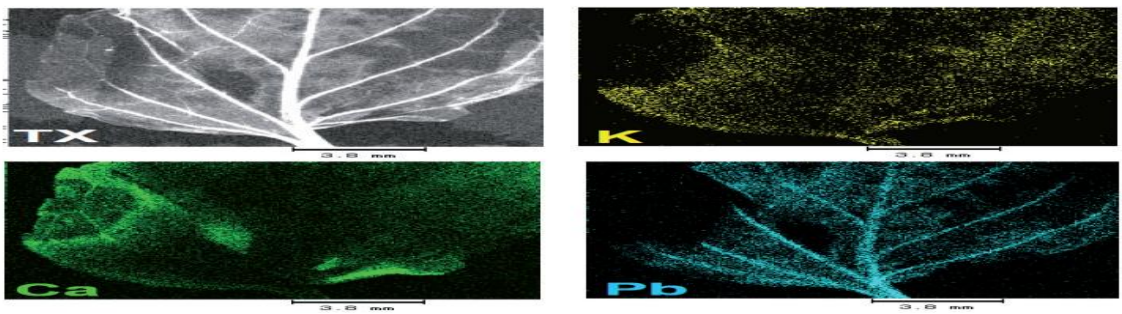

Figure 4 Analysis of leaf after introduction of pollutant to root system. Transmitted x-ray (TX), potassium (K), calcium (Ca) and lead (Pb) mapped images are shown.

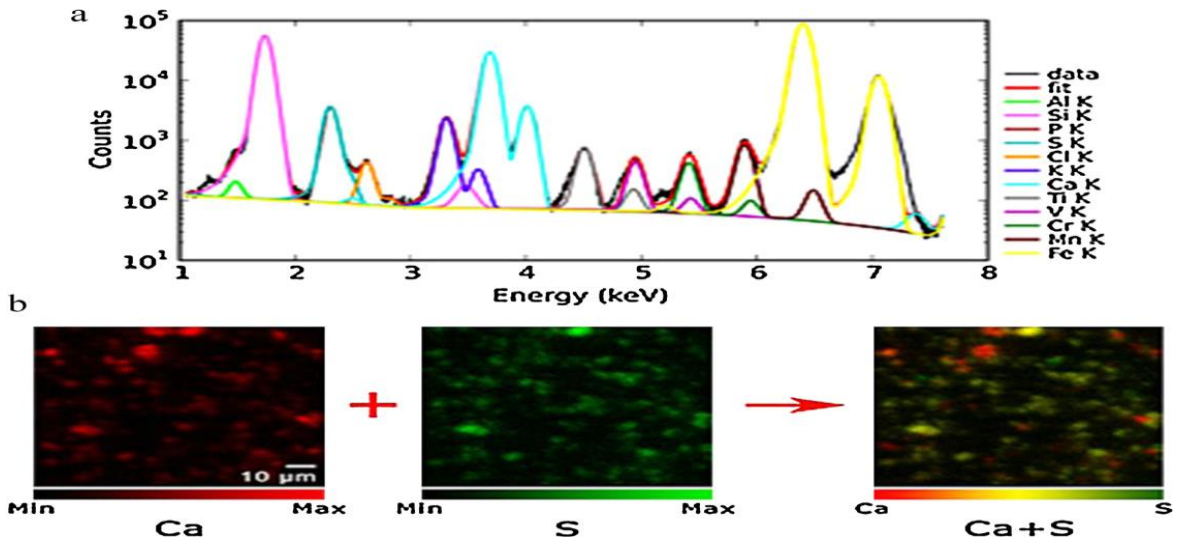

Figure 5 XRF analysis of particulate matter. (a) PM10 (*ambient aerosol sample)* XRF spectrum (b) a simple color coding method to visualize the elemental correlation between Ca and S inthe PM10 sample. The first two images (Ca and S) show the intensity of distribution in the respective elemental map. In the last image, co-distribution at a particular location is indicated by the resulting color (yellow), as indicated in the color mixing bar. [15]

4. Aplications in the Health Science

XRF is finding in increasing application in the field of health science. Examples include characterization of disease and dyshomeostasis, as well as molecular therapy and correlation with other imaging modalities, with XRF providing fresh insights into such critical topics as heavy metal toxicology, Alzheimer's disease, amyotrophic lateral sclerosis, stroke and multiple sclerosis. We will review pertinent examples taken from each of these fields in Fig. 6 up to Fig. 8.

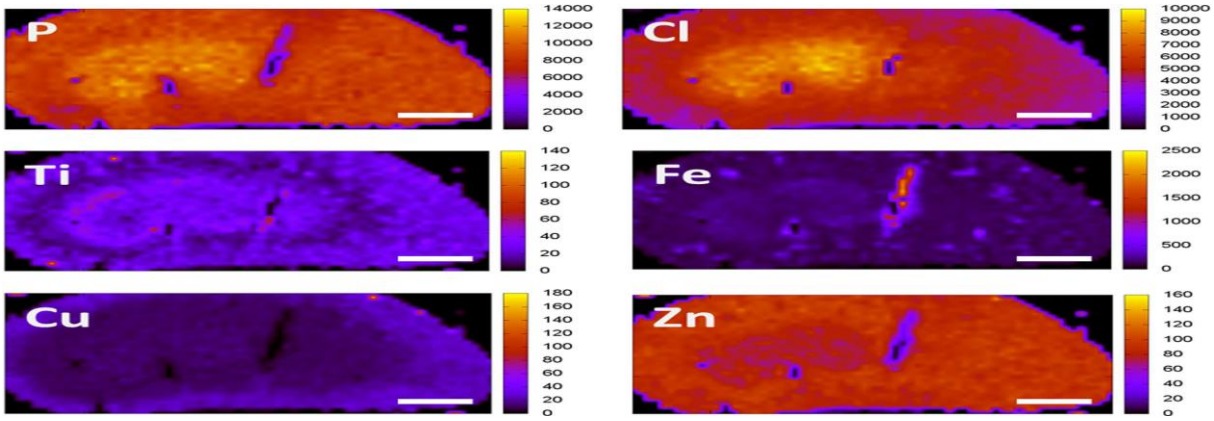

Figure 6 Distribution of selected elements in the rat kidney. Data are presented in µg/g. The scale bar length is 2.5 mm. [16]

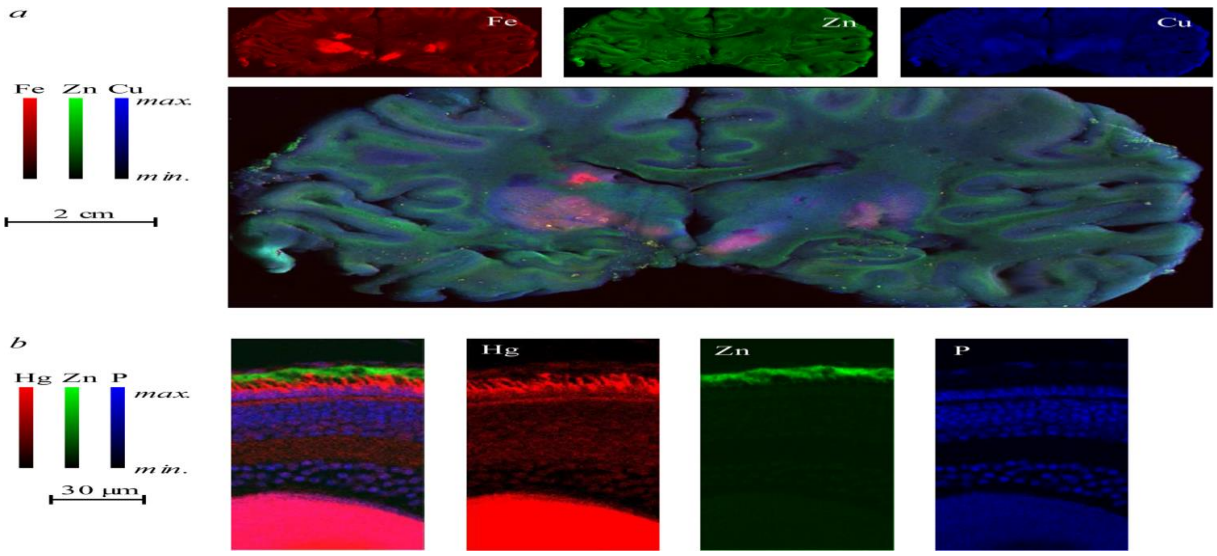

Figure 7 Examples of biological X-ray fluorescence imaging (XFI), illustrating the different accessible length-scales. [17]

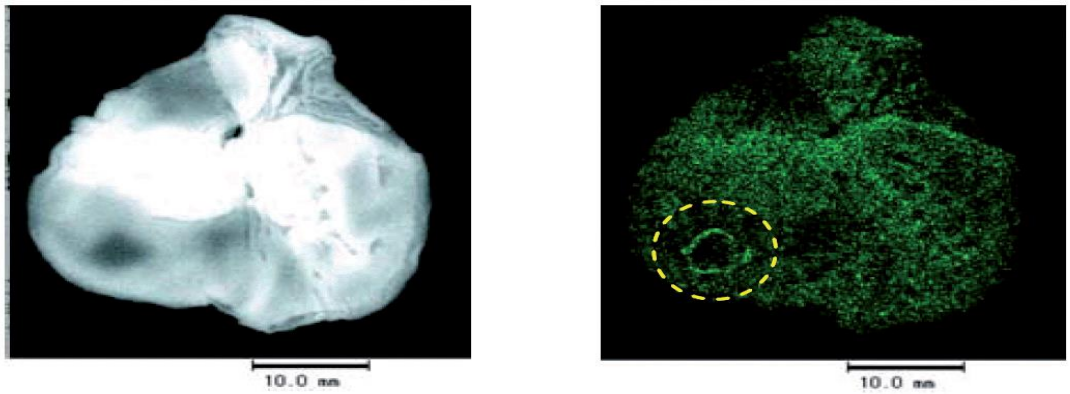

Figure 8 (Left) Transmitted x-ray image and (right) zinc XRF intensity image of ulcerated rat stomach without medication. The ulcer region is circled.

5. Conclusion

XRF elemental imaging is a powerful tool for sample analysis which generates a large dataset. It is important to provide software tools that allow the user to highlight features of the elemental images and more easily search the spectral map database for elemental information. The features described here are a small representation of the many efforts.

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