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Book of Abstracts

Contents

DETERMINATION OF LIGHT ELEMENTS DOWN TO CARBON USING TXRF

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The analysis of low Z elements with TXRF requires a special spectrometer. Measurements have to be performed under vacuum conditions, a special detector with ultra thin window has to be used and an excitation source providing a sufficient number of photons with an energy close to the absorption edge of the light elements. ATI has a long time experience in developing special low Z spectrometers. Currently we have a spectrometer using a Cr- anode 1300 W X-ray tube with a multilayer monochromator, as well as a WOBISTRAX spectrometer with a Rh- 50 W X-ray tube, providing monochromatic Rh-Ka excitation as well as Rh-L (2.6 keV) excitation totally reflected from the multilayer.
The problems of photon induced low Z XRF will be discussed, like peak deconvolution, peak overlaps and absorption. The spectrometers will be described and examples of applications will be presented [1-3].

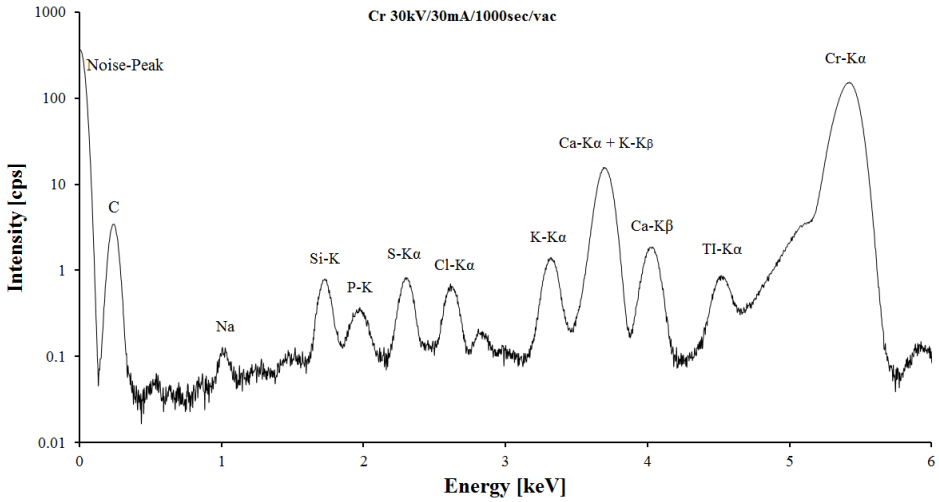

Figure 1: Spectrum of a Carbon reflector with contamination

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Total reflection X-Ray fluorescence spectroscopy to study Pb and Zn accumulation in zebrafish embryos

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The analytical capabilities of Total reflection X-Ray Fluorescence spectroscopy (TXRF) for elemental analysis of biological samples have been reviewed [1]. Simultaneous multi-element determination, low matrix effects and small sample quantity required are some of the TXRF advantages. Direct analysis of samples is also possible; thus, it is a proper technique for screening purposes. Nowadays, the quantitative analysis is performed using different calibration methods, however internal standard addition gives accurate and reproducible results.
In this work, elemental chemical analysis of individual embryos was performed by means of TXRF to study Pb and Zn bioaccumulation. A suitable protocol for sample preparation and TXRF analysis was developed. Analysis of single zebrafish embryo and the TXRF results give the possibility to build up a dose – response relationship [2]. At the end of fish embryo toxicity tests, zebrafish embryos exposed to Pb and Zn reference solutions or to leaching solutions of toxic and inertized municipal solid waste incinerator fly ashes were analyzed. A significant trend of bioaccumulation was observed for Pb with values one order of magnitude higher than the expected. Our results show that TXRF is a suitable tool for direct analysis of small biological organisms and the assessment of metal bioaccumulation.

Figure 1: Microscopic image of an individual zebrafish embryo deposited on plexiglass sample carrier.

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DETERMINATION OF THE BLOOD SERUM ELEMENTAL COMPOSITION OF PATIENTS WITH GASTRIC CANCER USING TXRF METHOD

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Elements play an important role in numerous physiological processes and, consequently, affect human health and well-being. An excess or deficiency of a specific element can disrupt the proper functioning of the human body, which can lead to many health disorders. Determining the concentrations of elements, both macro- and trace elements in human tissues or body fluids, is very helpful in diagnosing and controlling the treatment process and has become a routine practice [1]. One of the methods by which such analyzes can be performed is total reflection x-ray fluorescence (TXRF). This technique allows the simultaneous determination over a dosen of elements in a wide range of concentrations (from a few ng/g to %) during one measurement and for liquids without complicated sample preparation.

The aim of the presented study was to determine the concentration of 10 elements (P, S, K, Ca, Cl, Fe, Cu, Zn, Se, Br) in the blood serum of patients suffering from gastric cancer. The blood was collected from 34 patients: 23 men and 11 women, treated at the Brothers Hospitallers of Saint John of God Hospital in Łódź, Poland. The TXRF measurements were carried out at the Institute of Physics of Jan Kochanowski University in Kielce using S2 Picofox spectrometer (Bruker). The registered spectra of the characteristic radiation allowed for a qualitative (which elements are present in the sample) and quantitative (element concentration) analysis of the serum.

The statistical analysis of the obtained results consisted in calculating the descriptive statistics parameters for individual elements both for all patients and for women and men groups separately. The element concentration distributions in serum samples were compared statistically between group of patients with gastric cancer and control group. In statistical comparison also patient gender was included. Preparation uncertainty and random uncertainty were taken into account in order to determine the total measurement uncertainty. This allowed to estimate the percentage uncertainty for individual elements, which for almost all elements was about 10-20 %.

In this work, the physical basis of the TXRF method, the experimental setup, sample preparation procedure and the recorded spectra of the characteristic radiation are presented. Moreover, the detection limits and sources of uncertainty for the measured elements are discussed in detail.

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TXRF ANALYSIS OF HUMAN HAIR SAMPLES APPLYING EASY SAMPLE PREPARATION TECHNIQUES

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Today, the crucial role of trace element analysis in hair samples is well recognized.

- The distribution of micronutrients like Mg, K, Ca, Mn, Cu, Zn, Se, Mo and I gives information on diseases, metabolic disorders, environmental exposures, and nutritional status.
- As an excretory system, human hair can accumulate and incorporate toxic metals into its structure during its growth process. Therefore, concentrations of heavy metals in hair can reflect the mean level in the human body, recording the population's exposure to heavy metals

Still in most cases hair analysis is done after complete microwave-assisted acid digestion and ICP-MS or ICP-OES analysis. However, as these methods have a high demand on the laboratory environment and operating staff there is a growing interest in analytical methods, applicable in more rural environments with limited infrastructure.

As TXRF is already known for its suitability in laboratory environments like this from different environmental applications, it was tested for the analysis of human hair samples. A certified reference standard and several hair samples were analysed after slurry preparation and a simple acid digestion in Teflon vessels on a hot plate.

Measurements were done with simple and advanced benchtop systems applying Mo-K excitation only and multiple excitation modes (Mo-K, W-Brems and W-L). The possibilities and restrictions of TXRF for the analysis of human hair samples are evaluated in detail.

Multi-element profiles as convenient chemical tags for food traceability to fight labelling fraud

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Recognizing the need for accurate provenance identification tools, work in the field of seafood traceability is accelerating [1,2]. This issue is transversal to agricultural and seafood products, and incorrect identification of geographical origin (labelling fraud) can have severe economic impacts on the world's agriculture and fisheries economy [3,4]. Several analytical techniques have been applied for this purpose ranging from DNA analysis [5], fatty acid profiling [6], isotope analysis [7] to multi-elemental profiling [8]. For the latter, Total X-Ray Fluorescence (TXRF) multi-elemental profiling which have proven to be a great asset to address food traceability goals [9–11]. TXRF spectroscopy is a rapid and accurate technique, requiring minimal sample preparation, in contrast with the more sensitive and more expensive Inductive Couple Plasma Mass Spectroscopy (ICP-MS) technique [12]. This analytical technique can detect elements ranging from sodium to uranium and has been proven as an effective analytical tool in the determination of elemental (mineral) content in food [12], while also allowing a direct multi-element screening of samples over a wide dynamic range. As TXRF spectroscopy has a multi-parameter output, it can be used in combination with multivariate untargeted classification techniques [13]. Additionally, and considering the large number of variables (elements) generated when analyzing environmental or food samples, this methodology is also well-adapted to be used with machine learning data analysis approaches, increasing the detection of potential elemental tags and provide unsupervised and automatic classification systems, ensuring the reliability of the labelling process and thus reducing food fraud [14]. Two of products with major economic value in Portugal but in many Mediterranean countries, are seafood and wines. With the incidence of food fraud rather proportional to the economic importance of the asset, these two product categories are highly prone to mislabeling. In the present work we aim to highlight the applicability of TXRF multi-elemental profiling as a promising tool to fight food fraud and improve food provenance identification.

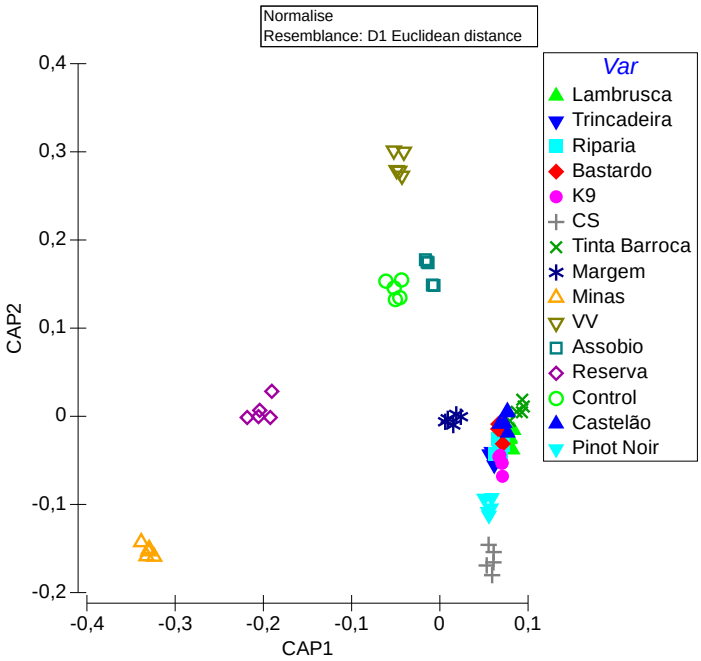

Figure 1: Grapevine variety canonical diagram based in the leaf multi-elemental fingerprinting using TXRF (classification efficiency 93.3 %).

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COVID-19 AND TXRF

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SARS-CoV-2 infections cause the current coronavirus disease (COVID-19) pandemic and challenge the immune system with ongoing inflammation. Several redox-relevant micronutrients are known to contribute to an adequate immune response, including the essential trace elements zinc (Zn) and selenium (Se).

In two studies about 166 [1] and 171 [2] serum samples of 29 and 35 surviving COVID-19 and non-surviving patients were analyzed and compared with a healthy cohort. While the combined deficit of Zn and Se was observed in only 0,15% of healthy people, a deficit occurred in about 20% of surviving and 50% in non-surviving patients. In addition, the concentration trends of micronutrient and selenoprotein P (SELENOP, the major storage of Se in biological systems) were compared over the first weeks in the hospital. Notably, the study indicates the first time that the decrease of SELENOP is a consequence of COVID-19.

It has been concluded that a personalized Se and Zn supplementation may support convalescence of COVID-19 patients. Further, sufficiently sized and well-controlled intervention studies are currently conducted.

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TXRF QUANTIFICATION OF GOLD NANOPARTICLES IN BIOLOGICAL TISSUES

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Gold nanoparticles (AuNPs) remain an active area of cancer research with numerous therapeutic, diagnostic and theranostic applications [1]. The assessment of tumoral uptake can provide valuable insights into their intended efficacy. Total X-ray reflection fluorescence (TXRF) spectroscopy offers low detection limits coupled with direct quantification through internal standardization. These features enable TXRF to measure uptake of AuNPs in the presence of organic matrix [2]. In this work, we demonstrate TXRF's ability to directly quantify AuNP concentration in slices of tissue mimicking cancerous tumors.

A theoretical model was developed to examine the impact of internal standard and sample heterogeneity on TXRF's quantification accuracy. A TXRF spectrometer along with heterogeneous and homogeneous samples were modeled using TOPAS (TOol for PArticle Simulation). The simulation model generated TXRF spectra which were then analyzed to obtain recovery rates of Au in both sample types. In order to demonstrate TXRF's ability to experimentally quantify AuNP concentration in slices of tissue, bovine liver was cut into 5 µm thin histological slices. Reference material AuNPs (10 nm) were deposited either above or below the tissue (Figure 1). The tissue slice was then spiked with a Lanthanum (La) internal standard. Additionally, scanning electron microscopy was used to examine the distribution of the Au and La on the tissue slices, revealing elemental uniformity on the tissue surface.

The simulation results showed near 100% recovery regardless of the elemental spatial distribution in the sample. These results provide insights into the quantification potential for AuNPs inside tumors that are histologically processed into thin tissue slices. The experimental results also revealed nearly 100% quantification accuracy of AuNPs in all permutations of sample configuration — suggesting TXRF as a viable option for assessment of tumoral AuNP uptake with minimal sample preparation.

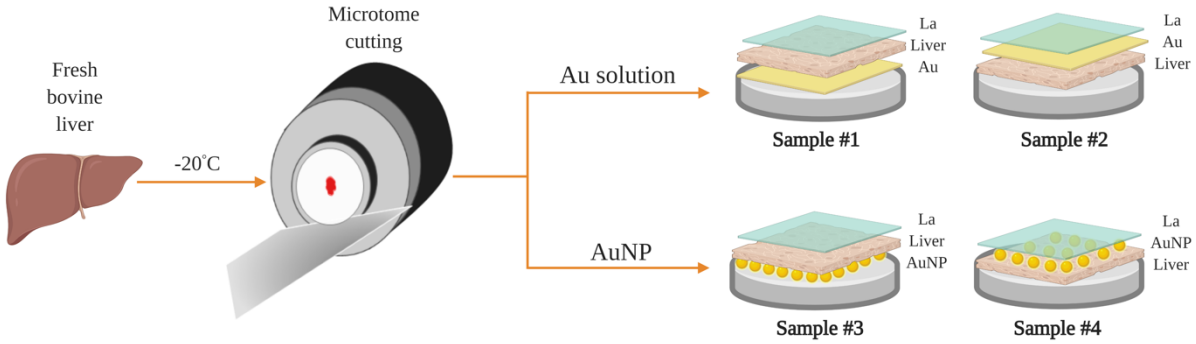

Figure 1: Schematic illustrating the sample preparation protocols for the four samples measured with TXRF.

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ESSENTIAL MICRONUTRIENT LEVELS IN MEDICINAL PLANTS: A COMPARISON BETWEEN TXRF, FAAS and ICP-OES

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It is common knowledge that one of the oldest methods of treating disease is the ingestion of herbal teas. With the increasing interest in the therapeutic benefits of herbal products, there is also growing concern about the safety and toxicity of natural herbs and preparations available in the market. Herbal materials purchased from local herbalists are usually not subject to strict quality control by regulatory agencies and often contain significant amounts of elements that may adversely affect the health of the consumer. Therefore, the measurement of elements in herbal teas is of great importance to consumers not only from a nutritional point of view, but also to assess their quality and evaluate the potential risk-benefit ratio of their consumption [1,2].

Among the many components of particular importance, the elements have attracted much attention for decades. As awareness and concern about elemental composition in general increases year by year, elemental analysis is becoming increasingly important. With this in mind, the development of rapid, simple and sensitive methods that can be used in routine analysis is of particular interest.

Total reflection X-ray fluorescence (TXRF) is a technique for elemental analysis that has recently become very attractive in biological fields. TXRF offers some advantages compared to AAS or ICP, such as the possibility to analyse vegetation samples with a minimal sample treatment (using a suspension of the powdered material with non-hazardous reagents) or obtaining rapid and screening information of the multielemental composition of vegetation samples. Other advantages include a simple quantification approach over a wide dynamic range by internal standardisation and low operating costs [3].

This study compares the results obtained in determining the content of essential elements such as Mn, Fe, Cu and Zn in vegetation samples using different analytical approaches including suspension preparation and TXRF analysis (equipped with Mo and W X-ray tubes), as well as the most commonly used spectroscopic methods in the field of plant analysis such as acid digestion combined with atomic emission (ICP-OES) and absorption spectrometry (FAAS). The study provides an overview of the potential and limitations of TXRF compared to FAAS and ICP-OES methods for the determination of essential elements in vegetation samples.

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INNOVATIVE X RAY SPECTROMETRY METHODOLOGIES FOR SUSTAINABLE CANCER TISSUE ANALYSIS

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Trace elements play an important role in biological processes and an association between the levels of trace elements and the presence of diseases such as cancer has already been established. Thus, the understanding of the mechanisms of assimilation of trace elements may be indicative of the genesis or progression of the disease. Energy Dispersive X-ray Fluorescence technique (EDXRF) might be the innovative technique for screening analysis as it constitutes the ideal compromise for a non-destructive, of simple instrumentation and good sensitivity technique for the elements of interest. For these reasons, this technique has already been tested in research regarding to the characterization of tumour tissues. However, there is always a great impairment to statistically significant conclusions: the extremely reduced number of samples. The research here presented intends to overcome this obstacle by taking advantage of the vast repository of human tissue samples, fixed in formalin and embedded in paraffin, that is stored in Portuguese hospitals. However, there is a major disadvantage when using these samples, namely the type of substrate (paraffin or formalin), that increases the factor of greatest uncertainty in the quantification by EDXRF: the characterization of the dark matrix of the sample. This methodology will be based on the analysis and characterization of "fresh" normal tissues by the techniques already established suitable: EDXRF, but also Total Reflection X-Ray Fluorescence (TXRF), Inductively Coupled Plasma Mass Spectrometry (ICP-MS). This way, we will obtain an accurate elemental quantification of the tissues, that we will then parameterize as we go through the paraffin inclusion steps. The final samples will be analysed again by EDXRF, in order to create a matrix correction model.

TXRF SPECTROSCOPY AND FTIR MICROSPECTROSCOPY – A VALUABLE TOOLS IN THE ANALYSIS OF IONPS INFLUENCE ON THE TISSUES AND ORGANS BIOCHEMISTRY

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Nanoparticles, defined as the objects with diameter between 1 and 100 nm, exhibit multiple properties which make them highly attractive especially for biomedical applications [1]. On the other hand, exactly the same features may be the source of the toxicity of these nanoobjects[2–4]. Among different nanoparticles predominant attention is focused on the biocompatible, biodegradable, easy to synthetize and functionalize iron oxide nanoparticles (IONP) [5].

Despite the opportunities offered by advances in the fields of nanotechnology, the use of nanoobjects in medicine is not possible without prior examination of their impact on the human organism. The first step in towards it must be to carry out *in vivo* studies on animal models, that will determine organs in which IONP accumulate as well as structural and functional changes caused by this particular nanoobjects [6]. The valuable tools in analysis of biochemical anomalies induced by the IONPs accumulation are total reflection X-ray fluorescence spectroscopy (TXRF) and Fourier transform infrared microspectroscopy (FTIR).

Presented study concern the usefulness of these modern spectroscopic methods for assessing of the IONP-induced biological response. In the experiment magnetite polyethylene-glycol-coated (PEG-IONP) and maghemite D-mannitol-coated nanoparticles (M-IONP) were used. Male Wistar rats were intravenously injected with the tested IONP in doses corresponding to those currently used in the medical diagnosis.

To evaluate the biodistribution of both types of the IONP in the rat's body, the TXRF spectroscopy, which allowed the investigation of even very subtle changes in the Fe content, was used. The multielemental analysis based on the TXRF method was performed with highly sensitive (ppm-ppb) S2 PICOFOXTM automatic spectrometer. Moreover, the study included examination of changes in concertation of some important trace elements, namely Cu, Ca and Zn in selected animal organs.

In turn, applying the FTIR microspectroscopy enabled detection of prolonged anomalies in accumulation and structure of the main biomolecules in liver and kidneys of animals exposed

to the low dose of M-IONP. The FTIR analysis was done at the SMIS beamline of SOLEIL synchrotron. The FTIR imaging system consisted of an Agilent 620 FTIR microscope and an Agilent 670 FTIR spectrometer with a Globar® light source and a liquid-nitrogen cooled 128 × 128 FPA detector.

Results obtained in frame of the presented research are valuable complement to the current state of knowledge about the IONP impact on living organisms and prove the great possibilities of modern spectroscopic methods in the field of nanotoxicology.

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TOTAL REFLECTION X-RAY FLUORESCENCE AS A SIMPLE AND SUSTAINABLE ANALYTICAL TECHNIQUE FOR THE ANALYSIS OF BIOLOGICAL SAMPLES

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In most clinical and nutritional studies, it is of significance to know information about the multielemental composition of biological samples. This study gives insight into the possibilities and limitations of analytical methods based on the use of TXRF instrumentation for such purpose.

In addition to the inherent advantages of TXRF systems (simultaneous multielemental information, microanalytical capability and low operating costs), the technique also allows the possibility to carry out biological sample analysis using simpler sample treatments compared to other atomic spectroscopic techniques. For instance, biological human fluids can be prepared by means of a simple dilution step using a small volume of an innocuous solvent (i.e., ultrapure water or a diluted solution of a surfactant) [1,2] and animal or vegetal tissues can be analyzed by suspending a few milligrams of the powdered material in an adequate disperser agent without the need for a time-consuming digestion step [3,4]. Moreover, quantification can be performed by means of internal standardization which is of special relevance in the analysis of biological samples, since usually matrix-matched standards are required when using other atomic spectroscopic techniques. Despite the fact that the determination of elements present at ultratrace levels is not possible using these analytical approaches, they can be useful for the determination of minor and trace elements relevant in the field of medical diagnostics and nutritional studies.

The study also highlights the potential of experimental design tools to select the best experimental conditions (i.e., sample amount, sample volume on the reflector, etc.) to prepare the biological samples before TXRF analysis.

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APPLICATION OF TXRF AND XRD TECHNIQUES IN URINARY TRACT INFECTIONS STUDIES

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Some bacterial infections can lead to bladder stones formations. These Urinary Tract Infections (UTI) are generally known to be associated with bladder environment changing that stimulate urinary stones formation (urolithiasis). The analysis of physicochemical properties of kidney stones, their elemental and chemical composition, is considered as a potential source of information on the process of stone formation. Moreover, the correlation between past bacterial infections and the type and chemical composition of urinary stones can be helpful for the medical treatment of this disease and to prevent the formation of new stones. The study with molecular biology techniques for bacterial infections history analysis and correlated with the chemical composition of urinary stones measured using X-ray powder diffraction (XPRD) technique and their elemental composition by total reflection X-ray fluorescence (TXRF) might show the scenarios of urinary stones formations [1]. The knowledge about these mechanisms might be useful for new agent designing for urolithiasis treatment.

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ATOMIC AND MOLECULAR BIOSPECTROSCOPY IN THE RESEARCH OF GLIOBLASTOMA MULTIFORME PATHOGENESIS

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Glioblastoma multiforme (GBM) is the most common and the most aggressive of the primary brain tumors. Its typical treatment involves surgery after which chemo- and radiation therapy are used. Despite the maximum, devastating treatment, the cancer usually recurs and the survival of patients from diagnosis rarely exceeds 18 months. An urgent need for more effective treatments pushes many research groups for looking for new alternative therapies of GBM. The preliminary studies of antitumor therapies are carried out on animal models as only they allow to verify the effectiveness of new substances with potentially inhibitory action on cancer growth in organisms completely free from any preceding therapies. For the same reasons, only on animal models side effects of the new substances can be tested.

In the presented study the rat models of GBM were utilized. The brains taken from animals subjected to the implantation of two glioma cell lines of different invasiveness as well as cells taken directly from patient suffering from GBM were examined in respect of elemental and biochemical anomalies appearing as a result of tumor development. Using the total reflection X-ray fluorescence spectroscopy the content of selected elements in the digested samples of implanted and naive brain hemispheres were compared []. In turn, for the topographic and quantitative biomolecular analysis we used brain slices from the place of implantation. They were raster scanned in two dimensions using the methods of synchrotron X-ray fluorescence microscopy and Fourier transform infrared microscopy. The obtained two dimensional maps showed significant anomalies both in the accumulation of examined elements and distribution of biomolecules. We hope that the obtained results will help to shed the new light on the pathogenesis of GBM and also contribute to the development of objective diagnostics of gliomas using modern biospectroscopy methods.

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STUDY OF CHROMIUM, SELENIUM AND BROMINE CONCENTRATIONS IN BLOOD SERUM OF PATIENTS WITH PARENTERAL NUTRITION TREATMENT USING TOTAL REFLECTION X-RAY FLUORESCENCE ANALYSIS

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Total reflection X-ray fluorescence (TXRF) technique, thanks to determining the concentrations of many elements simultaneously and the detection limit at the ppb level, has found many interdisciplinary applications. One of such applications is the multielemental analysis of medical and biological samples and, in particular, human serum samples. Monitoring of blood serum elemental composition is important to the study the influence of environmental pollution, nutrition and/or occupational exposure on human health. Another important aspect of such studies are element concentration changes in serum samples related to various diseases or treatment procedures. The usefulness of the TXRF technique for measuring trace elements in serum samples has already been systematically tested and confirmed.

In the presented studies, TXRF technique was used to determine the concentration of elements in blood serum of patients with parenteral nutritional treatment, a difficult therapy requiring supply and substitution of nutrients, taking into account trace elements. It is already known that proper nutrition has important influence on human health by reducing the risk of some diseases and by improving ability to fight off and recovery from illness.

Presented studies focus on determination of chromium, selenium and bromine concentrations in blood serum samples of 50 patients with parenteral nutrition treatment. The concentrations were measured two times, namely in the first day (I measurement) of the treatment and the seventh day (II measurement) after the chromium and selenium supplementation. For comparison purposes also serum samples of 50 patients without nutritional disorders, admitted to a planned surgical procedure to remove the gall bladder (cholecystectomy), were analyzed and treated as the control group. Descriptive statistics of measured concentrations of Cr, Se and Br both for the studied and control groups was determined. In order to check the effectiveness of Cr and Se supplementation, the results of the first and seventh day measurements for studied group were statistically compared with each other, with literature reference values and with the results of the control group (two-group comparison). These comparisons indicate the effectiveness of selenium supplementation in the applied treatment procedure. In the case of Cr and Br concentrations no statistically significant differences were observed. We conclude that monitoring of the concentration of the important trace elements in human serum should be standard procedure in parenteral nutrition treatment. In this monitoring the TXRF technique can be successfully used.

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STATISTICAL ANALYSIS OF THE MEASUREMENT RESULTS CLOSE TO THE DETECTION LIMIT ON THE EXAMPLES OF THE ELEMENT CONCENTRATIONS IN MEDICAL AND BIOLOGICAL SAMPLES

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Analysis of the element concentrations in medical and biological samples, especially in the context of trace elements, can be limited by the detection limit of the analytical technique applied. The value of detection limit of the X-ray spectrometry techniques, as for example total reflection X-ray fluorescence (TXRF), is restricted by the spectrum background level dependent on the elemental composition of analyzed sample, its preparation procedure as well as time of measurement.

Usually, in multielemental analysis provided by TXRF, the concentration of major elements is determined in all analyzed samples while for some trace elements in part of the samples only detection limit can be estimated. Such set of the data needs statistical analysis based on the concept of the censored data [1], in which the parameters of concentration distribution are calculated taking into account both obtained values of concentration and estimated detection limits.

Discussed studies concentrate on statistical analysis of the measurement results close to detection limit on the examples of the element concentrations in medical and biological samples. In examples different cases of experimental results are discussed, both almost complete with small fraction of data below detection limit as well as with the main contribution of such data. Examples focus on different element concentration distributions obtained in analysis of various biomedical samples. The practical aspect of discussed studies is presentation of the statistical procedures dedicated to censored data analysis available in statistical software (STATISTICA, ORIGIN). These procedures allow for inclusion of data below the detection limit in statistical analysis, in which the cumulative distribution function of element concentration is estimated, giving information about concentration quantiles such as for instance median, the first and the third quartiles. The multi-group comparison of the censored data for different populations (for example man versus women groups, patients versus control group, different animal organs) can be also performed.

Presented statistical procedures should be the standard tools for analysis of the data close to detection limit increasing the application possibilities of the TXRF technique especially for elemental analysis of biological and medical sets of samples.

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ELEMENTAL CHANGES OF BRAIN ACCOMPANYING THE GLIOBLASTOMA MULTIFORME DEVELOPMENT – THE STUDY USING THE TOTAL REFLECTION X-RAY FLUORESCENCE

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Glioblastoma multiforme (GBM) is one of the most aggressive central nervous system tumors and accounts for 16% of all primary brain tumors in adults. Despite the used radical treatment, including surgical resection and radio- and chemotherapy, the mortality rate due to GBM is high with 5 years survival rate less than 5% [1].

Many interdisciplinary studies are undertaken for assessment of the effectiveness of applied treatment as well as to get better knowledge about pathology of GBM. Animal models of tumour are often used for these investigations. One of the model is based on the implantation of human tumour cells into rodent brains and such a solution was used for purposes of our research. In the *in vitro* conditions, three different human GBM cell lines were selected and afterwards implanted into the brains of Wistar rats.

According to the literature, information about the elemental changes occurring in cancerous tissue may be helpful in determination of the type and invasiveness of the tumor [2,3]. Therefore, in our study, the elemental anomalies occurring in rat brains previously implanted with different glioma cells lines were examined. Total reflection X-ray fluorescence (TXRF) was used for the determination of P, S, K, Ca, Fe, Cu, Zn and Se contents in implanted-left and intact-right brain hemispheres. The obtained data were correlated with the results of histological analysis in order to find the links between elemental abnormalities and range of invasiveness of different types of GBM.

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THE ROLE OF AMERICIUM-241 FOR THE METALS DETECTIONS OF SOLID PHASES IN A PORTABLE SPECTROMETER DEVICE

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The current demand of new portable devices from the citizen community has opened new prospective of analysis in the fields of antiquity [1], relics [2], sedimentary of ferrous rocks, pollution and radioactive contaminants [3–5]. On the base of these topics of analysis the physical principle of X-Ray fluorescence (XRF) [6] was applied to develop a portable device, starting from an americium source with radial dislocation, able to remove element′s electrons from lower energy orbitals. The problem solving was applied to realize a portable spectrometer with high sensibility vs. elements of the spectroscopy spectra by using a metal O-ring structured in order to generate weak electrical signals. Furthermore, another aspect concerns the threshold limit value of detection, was investigated in order to offer a significant signal intensity output. Device assembly was based on two parts: first of al. a sensor with a radial detector, second, there are two electronic cards with an analogic multi-pins and a master USB connector.

In the experimental section two different hardware were compared in order to define the best solution that show a major stability at lower current intensity using a minor number of electronic compounds. Data were acquired and elaborate with a freeware software of analysis in order to show the element`s signals. Parts of device are innovative in the logical concept able to change and drive the sampling rate, the gain and signal/noise ratio, the device is also able to detect elements near the Americium probe at ~1-2 cm, tailored for small object, potter fragments, oil painting, coins on the surface and from elements mixed in an alloy. The graph output shows elements from which is possible measured the intensity and ratio between the elements. In conclusion an innovative detection part and an analogic card configured with a master USB card was proposed as a versatile system able to acquire signals without employed a pre-amplify circuit working at low pass band.

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MONITORING THE SELENIUM CONTENT IN ARTIFICIAL HUMAN URINE USING TXRF SPECTROMETRY

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The element Selenium (Se) is increasingly of interest to toxicological studies because of its suspected association with the etiology of Diabetes Mellitus Type 2[1]. Till date the metabolic pathways of Se are not fully known however the modus of the entry of Se from natural and polluted environmental sources have been well-documented. In this work, a detailed validation study has been done on the TXRF spectrometric analysis of Se in artificial human urine (AHU)[2] composed in conformity with urine's principal components (urea and mineral ions). Sodium selenate (Na_2SeO_4) and the amino acid, DL-selenomethionine ($C_5H_{11}NO_2Se$) were the Se bearing compounds chosen for their occurrence as mineral supplements and as metabolites in urine. The Se concentrations in the AHU were 0.01, 0.1, 1 and 10 mg/l. The analytical figures of merit *viz*. % recovery, precision, LoB, LoD, LoQ, and the spectral peak characteristics, FWHM, FWTM, Peak:Background, were determined as a function of Se concentration in the two different sample types. Although matrix effects are not expected to play a significant role in TXRF spectrometry, a non-negligible interference was observed especially at the lowest concentrations, in keeping with recent findings[3]. Validation of analysis of complex biomedical samples such as those studied here is necessary for analytical quality and reliability assurance.

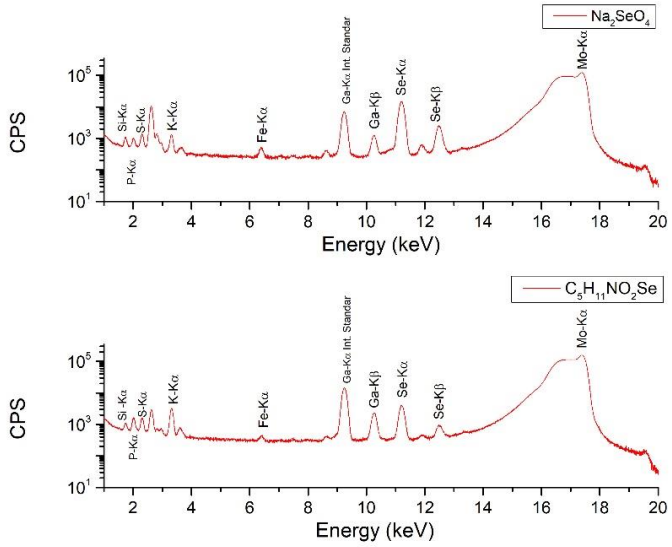

Fig. 1: TXRF spectra of AHU with 1 mg/L of Se as selenate (top) and as selenomethionine (bottom). The spectrometer was the S2-Picofox of Bruker (Germany) with a Mo anode at 50 kV and 1mA

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STUDIES OF ELEMENT CONCENTRATION IN THE LYMPHOCYTES, ERYTHROCYTES AND PLASMA OF HEALTHY HUMAN DONORS USING TOTAL REFLECTION X-RAY FLUORESCENCE

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In the studies, the total reflection X-ray fluorescence (TXRF) analysis was used for determination of element concentrations in three isolated peripheral blood components: lymphocytes, erythrocytes, and plasma, collected from 36 healthy donors (15 men and 21 women) from eastern Poland [1]. The studied blood components were isolated from whole peripheral blood using Histopaque-1077 density gradient centrifugation.

In the lymphocytes, the following elements were measured: P, S, Cl, K, Ca, Fe, Zn, Br, Sr and Pb. In the erythrocytes: P, S, Cl, K, Cr, Mn, Fe, Cu, Zn, Se, Br, Rb and Pb, while in the plasma samples: P, S, Cl, K, Ti, V, Cr, Fe, Co, Ni, Cu, Zn, Br, Se, Sr and Pb. The descriptive statistic parameters of concentration such as: mean value, standard deviation, median, 1st and 3rd quartiles, 10th and 90th percentiles were calculated for all samples of the studied blood components, as well as separately for male and female groups. The measured element concentrations and calculated parameters can be used as the reference values. Element concentration distributions for male and female groups were statistically compared using the non-parametric Mann-Whitney test and statistical significance differences ($\alpha = 0.05$) were found for: P (in lymphocytes), Se and Rb (in erythrocytes) and V (in plasma). The multi-group statistical comparison of element concentration distribution for different blood components was also done (Mann-Whitney and Kruskal-Wallis tests). The statistical test show that, the concentration levels are usually different, except in the following cases: Zn, Fe (lymphocytes and plasma, Fe only for female group), Cr (erythrocytes and plasma). The obtained concentration ranges were compared with literature-based data available for element concentration in lymphocytes, erythrocytes, and plasma.

From analytical point of view, the studies concentrate on the measurement procedure including the isolation of blood components and samples preparation for TXRF measurements, and later also the detection limit of the method is discussed.

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