X-RAY FLUORESCENCE MICROANALYSIS OF BIOMEDICAL AND ENVIRONMENTAL SAMPLES

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ABSTRACT

A feasibility study of microanalysis of biological materials by micro-beam X-ray fluorescence spectrometry (MXRF) was completed. The main goal of this research was topographic and quantitative evaluation of selected elements in tissue samples from the human brain. MXRF spectrometry utilizing capillary optics with an effective micro-beam diameter of about 300 µm was applied to study the elemental distribution in thin, lyophilized tissue samples. Variations of P, Cl, K, Ca and Fe concentrations with micro-beam position were detected. The results were compared to microscopic observations. The same MXRF technique was applied for analysis of dried cerebrospinal fluid on a thin film sample support. The distribution of selected elements in the residue created during the drying process was studied. Spots having crystal structure were discovered. The MXRF analysis of organic samples was improved in several ways. Spectral interference between scattered Mo L lines originating from the X-ray tube anode and P and S Kα lines were eliminated. Concentrations of analytes were calculated using a fundamental parameter approach.

INTRODUCTION

Micro-beam X-ray fluorescence (MXRF) spectrometry is a modern tool for examining elemental composition of various types of samples. An important advantage of the MXRF technique is that the primary beam can be focused down to a very small size, allowing small structural features to be analyzed. Since less energy is deposited in the sample as a result of X-ray excitation compared to electron or charged particles excitation sources, thermal damages to the sample and associated problems such as loss of volatile elements and chemical changes are avoided. Sample preparation is simpler because there is no need to coat the sample with a conductive material. These characteristics of MXRF offer a capability for application in nondestructive simultaneous multi-elemental analysis of small areas of organic samples such as tissue samples and dried residue of human body fluids. Literature studies indicated that abnormalities in elemental concentrations appear in tissue samples from selected parts of the human central nervous system and cerebrospinal fluid for patients affected by neurological diseases [1,2]. Main attention of these studies was given to Fe, Cu and Zn. Trace elements play important roles in bio-medical systems. The Fe, Cu and Zn take part in all metabolic processes, being components of different enzymes catalyzing chemical interactions in living cells.

In spite of intensive research on the chemical composition of the human brain, the role of elements is still not well known. A feasibility study was conducted to evaluate a micro-beam X-ray fluorescence spectrometer as a tool for microanalysis of tissue samples and fluids. The second
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objective of this work was to evaluate a computer program utilizing a fundamental parameter procedure for quantitative analysis of biological samples. The program uses the coherently and incoherently scattered tube target lines to calculate the effective atomic number and mass thickness of the sample. The goal was to evaluate the program for organic materials having high concentration of H, C, N, and O.

**MICROANALYSIS OF BRAIN TISSUE**

A preliminary analysis of the major, minor and trace elements in the control group of brain tissue samples were performed using the PIXE technique [3]. For investigation, the samples were taken from large areas of the brain, i.e. gray and white matter. However, neurological diseases can cause abnormalities in elemental concentration in selected parts of the brain and spinal cord. Therefore, it is important to investigate distributions of the elements in a relatively small area of the tissue samples.

Thin sections of tissue samples were used for microanalysis. The samples were obtained at autopsy from patients not affected by neurological disease. The boundary between white and gray brain matter was chosen for analysis. The tissue samples were frozen just after removal from each patient. The frozen pieces of the samples were sliced into sections (approx. 0.7 x 0.7 cm) 20 µm thick with cryo-microtome and placed on an AP1 foil 0.15 µm thick. The adjacent sections were put on a microscopic glass for histopathological examination. The thin sections of tissue were in the refrigerator at a temperature -20°C for 24 hours lyophilized. The estimated mass thickness of dried tissue is about 600 µg/cm². It was assumed that fresh tissue of the brain has a density equal to 1.4 g/cm³, and the concentration of water is about 80% [4]. The results of PIXE analysis together with calculated mass thickness were used to estimate the range of variation of selected elements in thin dried tissue sections.

The MXRF measurements were done using a micro-beam X-ray fluorescence spectrometer built at the Department of Radiometric Analyses [5]. It is equipped with diffraction X-ray tube with a Mo anode and a conical capillary (X-ray Capillary Optics AB) having a 300 µm diameter to produce a focused X-ray micro-beam. The samples were placed at 45° to the incident beam on an XYZ, stepper-motor-driven stage. During the measurements, a video camera attached to the microscope was used to view the samples. The measurements were performed in atmosphere. The acquisition time was 600 s per pixel. The characteristic X-ray radiation were measured using Si(Li) detector having a thickness of 3 mm and an active area of 30 mm² with energy resolution of 170 eV at 5.9 keV.

In order to test the MXRF spectrometer, thin films for XRF calibration were measured and lower limits of detection (LLD) were calculated on the basis of these measurements. The values of LLD together with estimated ranges of concentration of selected elements in dried brain tissue sections are presented in Fig. 1. As can be seen from Fig. 1, concentrations of P, S, Cl, K, Ca and Fe are higher than the estimated LLD values. Concentrations of Cu and Zn are significantly lower and these elements were not detected in analyzed samples. Figure 2 shows a typical X-ray spectrum obtained for the tissue samples. The results of 2-D scans of gray and white matter in the brain tissue section are presented in Fig. 3. The results of the scans indicated variation of the Mo Kα line scattered in a tissue section with micro beam location. This effect is caused by differences in the local sample density and matrix composition. Therefore, Mo Kα line scattering was used to
Figure 1. Mass per unit area versus atomic number. Black points represent detection limits. Red bars show ranges of elemental concentrations in brain tissue samples.

Figure 2. An example of X-ray fluorescence brain tissue spectrum (300 µm mono-capillary).
normalize intensities of the excited characteristic X-rays. It is also worth noting, that scattering of the Mo Kα line in gray matter is lower than in white matter. Higher concentrations of Cl, K and Ca were observed in gray matter. This information would be useful for distinguishing between white and gray matter in cases of analysis of samples obtained from a spinal cord. The MXRF technique cannot be applied for analysis of all elements. Trace elements such as Zn and Cu cannot be detected under the conditions used there. While analyzing brain tissue sections, special attention should be paid to the iron concentration since iron plays an important role in all metabolic processes. However, it was discovered that significantly higher intensity of Fe the line can originate from blood vessels located in the tissue samples. These spots are characterized by significantly higher concentration of Fe and lower concentration of P. It was confirmed by microscopic observation of the tissue sections. The results of measurements are useful for testing the uniformity of prepared sections. Such tissue cutting preparation procedure should be applied if distribution of trace and minor elements within the tissue structure is a subject of analysis. Additional measurements will be performed with the use of synchrotron radiation.

Figure 3. Maps of thickness and selected elements concentration in brain tissue. (color-coded: counts (Mo Kα scatters), characteristic X-ray intensity /Compton scattering (P, K, Fe)).
MXRF ANALYSIS OF HUMAN BODY FLUID

X-ray fluorescence offers the possibility of detecting very small concentrations of elements in dried fluids. In this procedure, sample preparation consisted of pre-concentration of the sample down to a small surface area by drying a drop of fluid on a flat, thin substrate. Because the sample and sample support are extremely thin, spectral background from scattered radiation is substantially reduced. Using glass capillary X-ray optics it is possible to transmit high radiation flux onto the sample improving fluorescence signal yield. This combination results in improved detection limits. Applicability of this procedure to analysis of cerebrospinal fluid was tested.

A sample was prepared by placing a 2 µL droplet of fluid on a slightly hydrophobic foil (Moxtek AP1). The sample was dried in air for 24 hours. It was expected that use of a hydrophobic foil would cause the residue to be concentrated into the smallest area possible. Using a microscope, it was found that the sample components were concentrated within an area of 3 mm diameter. Spots having crystal structure were discovered. The MXRF spectrometer, described previously, was used to study the distribution of selected elements in the residue. Distribution of individual elements in the sample was determined by correlating signal intensity with the position of the XYZ stage relative to the incident X-ray micro-beam. Measurement time per pixel was 600 s. An example of a spectrum excited in a residue of cerebrospinal fluid is presented in Fig. 4. The characteristic X-ray lines of Cl, K and Ca were present in the spectrum. Also two diffraction peaks were discovered in the spectra. These diffraction peaks originate from crystals of NaCl or structures of biological matter. Figure 5 displays the distribution of Cl, K and Ca in the dried residue. In Fig. 5, inhomogeneous elemental distributions are clearly visible. All measured elements are more concentrated along the outer edges. The outer edge was also visible in the optical image of the sample. The segregation, which occurred during the drying process, is a

![Figure 4. A spectrum of dried cerebrospinal fluid.](image-url)
A FUNDAMENTAL PARAMETERS APPROACH TO ANALYSIS OF ORGANIC SAMPLES

In MXRF analysis of biological samples, the effects due to variation of mass thickness and matrix composition should be eliminated. Biological materials contain high concentrations of H, C, N and O. These elements are generally not measurable by XRF techniques. Sieber et al. [6] tested a fundamental parameters procedure for quantitative analysis of organic samples containing "dark" matrix, by MXRF spectrometry using a computer program previously developed by Lankosz and Pella [7]. This fundamental parameters program was novel in that it uses a calculated tube spectrum to allow the use of polychromatic radiation in combination with coherent and incoherent scatter for estimation of the mass thickness of the sample and its effective atomic number. The obtained accuracy was within 5 to 10 % for most elements. In many cases, the concentrations were below 50 mg/kg and accuracy was poorer at 10 % to 15 %. However, P and S were not analyzed due to the spectral interference between Mo L-series lines.
from the X-ray tube and P and S K-series. In order to eliminate this interference, the measurements were repeated with a filtered primary X-ray beam.

The measurements in Ref. 6 were performed at National Institute of Standards and Technology (NIST). The NIST MXRF spectrometer uses pinholes in Mo metal to collimate the X-ray beam. A low-power molybdenum anode x-ray tube was operated at 50 kV and 1.0 mA. The measurements were performed in vacuum. Filter paper (Whatman) with a mass thickness of 9.35 mg/cm$^2$ was used to filter the primary beam. The samples analyzed for this work (pressed briquettes) included SRM 1566a Oyster Tissue and SRM 1577b Bovine Liver. Nine measurements in a 3 x 3 array were made in the center of a 13-mm diameter briquette. Table 1 contains the quantitative results in comparison to the certified contents of the two reference materials. Each "found" value is the average of nine determinations processed individually for count rates and quantitative analysis. The results of the investigation indicated that use of the paper beam filter eliminated spectral interferences previously observed. The accuracy of P and S analysis is between 8 and 27%. The results of measurements indicated also, that use of the paper filter significantly reduced sensitivity of measurements for low-Z elements. For instance, the LLD for Cl increased from about 60 ppm (no filter) to 150 ppm (paper filter).

Table 1. Quantitative results for Standard Reference Materials compared to certified values (with filter).

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In MXRF analysis of thin dried tissue sections, it is necessary to know whether the sample meets the criteria of "thin sample " or is better described as having "intermediate mass thickness". Therefore, calculations were performed in order to create relations between intensities of characteristic X-rays (Kα line) excited in the tissue section and its mass thickness. It was assumed, that dried tissue sample has following composition: H - 8.6 %, C - 56.6 %, O - 22.6 %, and N - 12.2 % [8]. It was also assumed that rests of elements have trace concentrations. The calculated spectrum of primary radiation from a Mo X-ray tube was used. We did not take into account any modification of the spectrum by capillary optics. The 45$^0$/45$^0$ excitation-detection geometry was assumed. The values calculated for "thick" sample were used to normalize the computed intensities. A plot of the characteristic X-ray count rate versus mass thickness of a tissue section is illustrated on Fig. 6. For element which relative intensity is equal to or lower than 0.134 [9] the mass thickness meets the criteria of "thin sample". As can be seen from Fig. 6, for the mass thickness equal to 600 µg/cm$^2$ the sample is "thin" for elements with atomic number
equal to or greater than 17. Therefore, the validity of the fundamental parameters procedure in MXRF microanalysis of organic samples with the use of capillary X-ray optic was investigated. In the measurements, the pellets of SRM 1566a and 1577b were used. The measurements were performed in air. Since glass capillary significantly modified X-ray spectrum of primary radiation from X-ray tube, in FP calculations the QXAS program was applied [10]. It was discovered that due to unknown reasons, the ratio of coherent to incoherent scatter of Mo K series does not provide an accurate estimate of the effective atomic number of the measured organic SRM. Therefore, in calculations we used the effective atomic number of "dark" matrix obtained from independent measurements with the use of the NIST spectrometer [6]. The results of calculations are presented in Table 2. The accuracy of analysis is similar to that obtained with the use of the NIST spectrometer. Research in quantitative analysis of organic samples using glass capillary optics will be continued.

Table 2. Quantitative results for Standard Reference Materials compared to certified values (capillary optic).

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<thead>
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<th>Cl %</th>
<th>K %</th>
<th>Ca %</th>
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<th>Fe mg/kg</th>
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CONCLUSIONS

The results of investigation confirmed that X-ray mono-capillary optics is useful for testing uniformity of tissue section and analysis of minor elements with reasonably good spatial resolution. The sample preparation procedure based on the pre-concentration of a sample by drying a drop of fluid requires further investigation. Also, the use of the FP procedure for MXRF analysis of the organic sample using capillary X-ray optics should be investigated.

ACKNOWLEDGMENTS

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