APPLICATION OF PIXE AND XRF TO BIOLOGICAL SAMPLES AT THE LABORATORY FOR NUCLEAR MICROANALYSIS IN ZAGREB

M. Vajic, M. Jakšic, S. Fazinic and V.Valkovic
Ruder Bošković Institute, Zagreb

Proton induced X-ray emission (PIXE) spectroscopy was the first analytical method introduced at the tandem Van de Graaff accelerator in Zagreb. The ability of PIXE to provide simultaneous, multielemental analysis at the level of a few $\mu g/g$ has been demonstrated to determine trace element concentrations in fish tissues (Zosterisessor Ophiocephalus Pall).Parallel analyses were carried out on aliquot samples using the XRF facility and the agreement of the results obtained by the two systems proved to be satisfactory. While the XRF cannot generally achieve the sensitivity obtained by PIXE, it can be used advantageously in determining certain trace elements. Thus, PIXE and XRF are shown to be complementary rather than competitive methods and together they offer an excellent analytical tool for nondestructive multielemental analysis of biological samples.

1. INTRODUCTION

The research program conducted at the Laboratory for Nuclear Microanalysis (LNM) at the Ruđer Bošković Institute is devoted to the development and application of nuclear analytical methods. Two of these methods are now being in use for the elemental analysis at the few ppm level: PIXE (Proton induced X-ray emission) and XRF (X-ray fluorescence). The underlying principle of both methods is the same: characteristic X-rays are first excited either by photons or by protons and then detected usually with an energy dispersive detector. While XRF has been used for many years in the LNM for routine analyses, PIXE is a new method introduced at the Van de Graaff accelerator in 1988, so it naturally occured to compare the performances of the two facilities. In PIXE protons accelerated to energies of a few MeV are used for producing inner shell vacancies, while in XRF the X-rays used for excitation are produced either with an X-ray tube or with a radioactive source. Both methods are multielemental, highly sensitive in small samples, relatively rapid and nondestructive. XRF in particular. While PIXE is generally a more sensitive method especially in absolute terms, the sensitivity of XRF can be optimized for a particular elemental region by choosing the energy of the exciting radiation. Our intention was to use the two methods in a complementary way, taking advantage of the specific merits of each.

Biology seems to be a particulary interesting and promising field for both PIXE and XRF application, since two of their advantageous features become decisive in determining the

essential element concentrations in living organisms. These features are multielementality, which is desired since the essential elements which occur in living organisms range over a wide variety of elements and high sensitivity which is necessary as many of those elements occur at ppm level and less. In order to demonstrate the capacities of PIXE and XRF in the analysis of biological materials, samples of fish tissue were used.

2. EXPERIMENTAL SETUP

2.1.PIXE

PIXE analyses were performed with 3 MeV protons obtained from the 6MV tandem Van de Graaff accelerator. The dimensions of the beam were defined by a 3 mm diameter collimator. The beam homogenity was achieved by using a thin aluminium foil placed in front of the collimator. The aluminium PIXE chamber accomodates a circular target changer which can be moved by a remote control. 18 different frames can be placed on the target holder which makes an angle of 45^{o} with respect to the beam. The emitted X-rays were collected with a Si(Li) detector placed at 90^{o} to the incoming beam. A carbon collimator is used to shield the detector from extraneous X-rays. When supression of low energy X-rays was desired, mylar filters of different thicknesses were introduced in front of the Be window. The ion dose on irradiated target could be measured in two different ways. One way was to integrate the ion beam current by using the Faraday cup placed at the beam exit. The other more reliable method of measuring the beam

intensity was the monitoring of the backscaterred protons from a thin golden foil by a surface barrier detector placed at a backangle of 135^{o} . The foil interposed between the ion beam and the target is thin enough to permit the transmission of the beam without an appreciable energy loss. The beam currents that were used were typically in the range of 10-20 nA which was usually satisfactory to avoid the undesirable heating of the sample.

2.2. XRF

The XRF facility of the LNM is described elsewhere (1). Photons used for X-ray excitation were obtained from the X-ray tube with Mo anode. Working conditions for the X-ray apparatus were 34 kV and 18 mA. Characteristic X-rays were detected with a Si(Li) detector with energy resolution of 250 eV at E=6.4 keV. Molybdenum was used as a secondary radiator in order to enhance sensitivity for elements with higher Z such as Br, Rb, and Sr.

2.3. Target preparation

Nine samples of the fish tissues that were prepared by homogenization followed by freeze-drying in Padova (2) were used for the comparative analysis. XRF is preferably carried out on samples of tens of mg, while PIXE is sufficiently sensitive to determine concentrations in samples with masses that are one order of magnitude less. Therefore two different procedures of target preparation were used according to the respective absolute sensitivities of the two methods. Thin targets were prepared for PIXE in the following way. Liophilized powder was homogenized in ultrasonic mixer with 0.5 ml of redistilled water and drops of 20 ml were pipetted on mylar backing glued to aluminium frame. The frame with target was let to dry for 10 minutes in a dryer at 40^{o}. The targets were covered afterwards with a thin formvar foil in order to protect the sample. Thicker targets were prepared for the XRF analysis by spreading the liophilized powder evenly on the mylar foil and covering it afterwards with formvar. The analysis of the XRF results turned out to be more complicated because the effects of attenuation had to be taken into account.

3. RESULTS

Examples of X-ray spectra obtained by PIXE and XRF analyses of fish tissue samples are presented in figures (1) and (2). These spectra nicely illustrate the multielemental capacity of the two methods, as the elements with the atomic number ranging from Z=16 (S) to Z=82 (Pb) were detected. High sensitivity of PIXE and XRF is demonstrated in Table (1) where the average concentrations of some of the detected elements are presented and from which can be seen that the lowest detected concentrations were as low as few ppm. The relatively long exposure times (2000 s for XRF spectra and approximately 20 minutes for PIXE spectra) ensured good statistics for the main peaks (less than 1% statistical error for the Fe Kα peak in PIXE spectra and 5% for the same peak in XRF spectra).

The performances of PIXE and XRF have been compared in determining iron and zinc concentrations. Values of Zn and Fe concentrations tabulated in Table 2 show that the agreement of the two methods is satisfactory.

Apart from demonstrating that the new method introduced at our Van de Graaff accelerator can serve as a reliable analytical tool, our intention was also to show that PIXE should not be used instead of XRF, but complementary to it. Although PIXE has a superior overall sensitivity, particulary in absolute terms, XRF could be used advantageously if an element with a higher Z is of special interest. On the example of strontium it is shown (Table 2) that by using Mo as a secondary radiator in the XRF

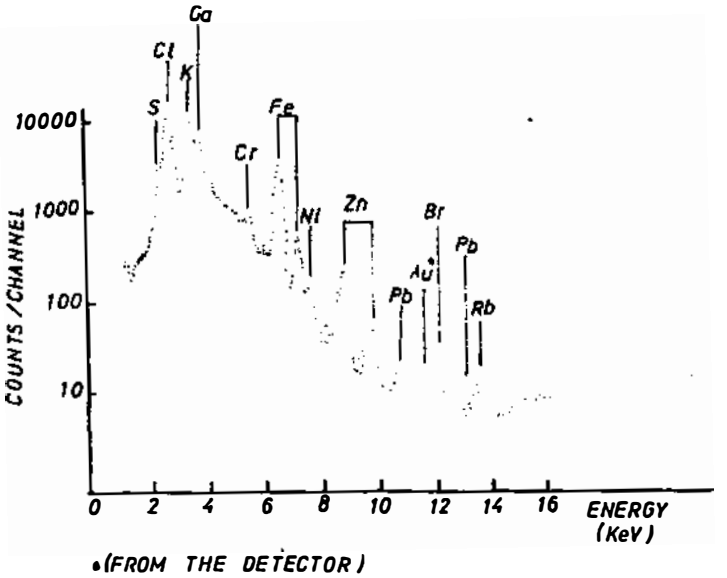

Figure 1. X-ray spectrum of a fish tissue sample obtained by the PIXE system with 3 MeV protons

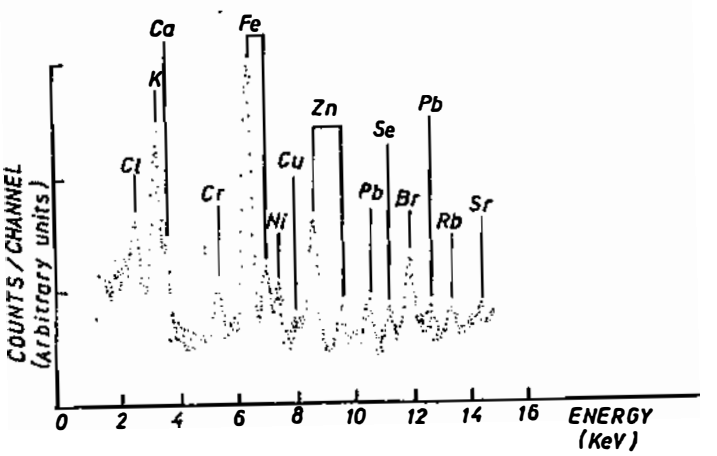

Figure 2. X-ray spectrum of a fish tissue sample obtained with the XRF system by using an X-ray tube with Mo anode

system. concentrations as low as 2 ppm were detectable. For PIXE. where the efficiency falls with the atomic number, such concentrations of strontium were under its minimum detection limit. Even in the case when a mylar foil was inserted in front of the Si(Li) detector in order to improve sensitivity for heavier elements, statistical errors were too big to accurately determine such small concentrations of strontium.

In the comparison of the two methods it is important to mention the advantage of XRF in determining the concentration of bromium. Bromium is a volatile element and therefore very sensitive to the heating of the target. From the table (2) it can be seen that values obtained for bromium concentrations with PIXE are by factor two smaller than those obtained by XRF. This is most likely the consequence of the target heating, which means that in case of insulating targets XRF should be used rather than PIXE for bromium detection.

Table 1. Concentration of elements in tissue samples of Zosterisessor ophiocephalus Pall determined by PIXE (Cl, K, Ca, Cr, Cu) and XRF (Pb, As, Rb, Sr)

Cl [%]	1.21	±	0.3
K [%]	0.89	±	0.2
Ca [ppm]	485	±	89
Cr [ppm]	35	±	10
Cu [ppm]	3	±	1
Pb [ppm]	7	±	2
As [ppm]	14	±	3
Rb [ppm]	1.7	±	0.2
Sr [ppm]	2.2	±	0.2

Table 2. Comparison of results obtained by PIXE and XRF for concentrations of Fe, Zn and Br in fish tissue samples

	XRF	PIXE
Fe [ppm]	465 ± 79	390 ± 47
Zn [ppm]	55 ± 4	55 ± 3
Br [ppm]	21 ± 1	12 ± 1

4. CONCLUSION

From the results obtained from PIXE and XRF measurements performed on fish tissues at the LNM in Zagreb, it can be concluded that the performance of both facilities meet the high criteria of sensitivity required in trace element analysis. The minimum detection limits for elements with Z higher than 16 are in the ppm region, with PIXE having a better sensitivity for elements with lower Z and XRF with Mo as a radiator being superior in the region with higher Z. We therefore conclude that the two methods should be used complementary and not alternatively which is most often the case ,so as to take advantage of their specific features.

Rererences:

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