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On the Applications of IBA Techniques to Biological Samples Analysis: PIXE and RBS

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Abstract. The analytical techniques based on ion beams or IBA techniques give quantitative information on elemental concentration in samples of a wide variety of nature. In this work, we focus on PIXE technique, analyzing thick target biological specimens (TPIXE), using 3 MeV protons produced by an electrostatic accelerator. A nuclear microprobe was used performing PIXE and RBS simultaneously, in order to solve the uncertainties produced in the absolute PIXE quantifying. The advantages of using both techniques and a nuclear microprobe are discussed. Quantitative results are shown to illustrate the multielemental resolution of the PIXE technique; for this, a blood standard was used.

Keywords: IBA techniques, PIXE, TPIXE, RBS, nuclear microprobe.

PHYSICAL PRINCIPLES

Particle Induced X-Ray Emission, PIXE, is an analytical method based upon X-ray spectrometry where a beam of protons is used to eject inner-shell electrons from atoms in a specimen¹. When the resulting vacancies are filled by outer-shell electrons, characteristic X-rays whose energies identify the particular atom are emitted. The transition filling vacancies in the innermost shell are called K X-rays and those filling the next shell are L X-rays, whose energies are much lower.

The quantitative PIXE analysis is based on the relationship between the total area under the characteristic X-ray peak and the real concentration of the given element in the sample. To find the yield of characteristic X-rays from a particular elemental constituent homogeneously distributed within a thick biological specimen, we use the fact that it sets the final proton energy to zero. Therefore, the yield equation for thick samples in PIXE is

$$Y(Z) = \frac{N_{av} \omega_z F_i \varepsilon_z q C_z}{A_z} \int_{E_0}^0 \frac{\sigma_z(E) T_z(E)}{S(E)} dE \quad (1)$$

which gives the relationship between the X-rays amount and the elemental concentrations, where A_z is the atomic mass of the element, N_{av} the Avogadro's number, ω_z the fluorescence yield, the transition probabilities between two different shells are given by F_i and ε_z is the absolute detection efficiency. The concentration of the element Z of interest is C_z . In the integral we can see the ionization cross section for proton energy, $\sigma_z(E)$. The matrix stopping powers $S(E)$, is calculated using Bragg's rule for linear combination of the component stopping powers. The quantity $T_z(E)$ describes the transmission of photons from successive depths in the matrix.

The main uncertainties in the absolute quantifying of PIXE elemental concentrations are the total deposited beam charge – the “ q ” factor in Eq. (1) – and the local matrix composition. This is particularly true in our case while working microbeam PIXE analysis with thick target biological samples.

On the other hand, the *Rutherford Backscattering Spectrometry*, **RBS**, detects the energies and amount of the elastic backscattered ions from a solid target due to the Coulomb interaction with the atom nuclear structure².

The use of simultaneous RBS analysis can solve the uncertainties in PIXE analysis in many cases. Provided the RBS spectrum can be well modelled, its shape gives information on the local matrix composition, while the total area depends on the total beam charge. The ratio between the true charge and the measured charge (the “ q ” factor) can then be used to normalise the PIXE data³. RBS also provides information on those elements with low atomic numbers, this is very useful when we are trying to analyze biological samples.

In this way, PIXE is a multi-elemental technique with excellent sensitivity and detection limits across a wide range of atomic number. Additionally, PIXE provides relatively precise measurements from very small amounts of material.

EXPERIMENTAL SETUP

PIXE and RBS techniques were performed simultaneously within a nuclear microprobe (Fig. 1) and they were carried out using the 3 MV peleton tandem accelerator at Centro Nacional de Aceleradores (CNA), Sevilla, Spain. The called micro-PIXE simply involves focusing the beam down to small areal dimensions, 4 μm in our case, so that it can be scanned across the surface of a specimen and thus provide concentration data as a function of position. Magnetic lenses such as coils wound to generate a quadrupolar field produce a demagnified image of the object aperture at the specimen surface. Scanning across a biological specimen becomes possible to obtain concentration maps. Hence, the final intensity of the beam is very low in order to avoid the damage in biological samples.

A 3 MeV proton beam was used to irradiate the sample. A Si(Li) detector was employed to collect the X-rays with a 80 mm² active area and a surface barrier detector was used to collect the backscattered particles.

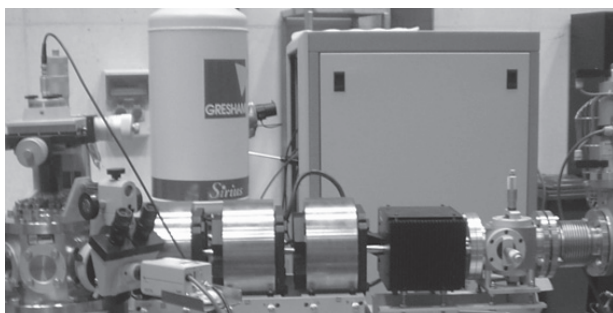


FIGURE 1. We can show the nuclear microprobe (left), where a microscope is installed in order to see the micrometric beam. The quadrupolar lenses and scanning system are also shown.

The Si(Li) detector has a “funny filter”⁴ placed in the window because is a convenient choice for biological specimens. Those samples are insulating targets, for this reason we used a carbon foil spraying electrons with the purpose to avoid the samples charge up.

RESULTS

Example of PIXE spectra obtained from electronic chain (detector, multichannel analyzer and computer) for A-13 IAEA’s standard (freeze dried animal blood) is shown in Fig. 2.

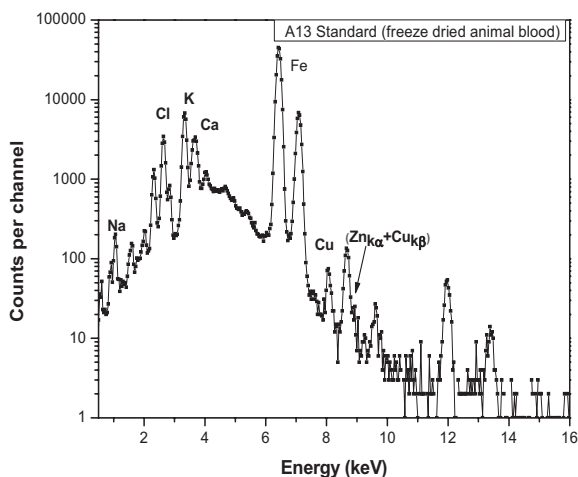


FIGURE 2. PIXE spectra obtained in our experimental setup. The spectra shows some characteristic X-Ray peaks for a biological standard given by the International Atomic Energy Agency.

TABLE 1. Elemental concentrations given in **ppm**. The certified concentrations are given by the IAEA, while the concentrations for A13 experiment are experimentally obtained.

Sample	Na	P	S	Cl	K	Ca	Fe	Cu	Zn	Br	Rb
A13 Certified	12600	940	6500	NC	2500	286	2400	4	13	22	2
A13 Experiment	13241	1021	6614	7748	2581	292	2399	4	13	21	2

The PIXE spectra were analyzed with the GUPIXWIN software (<http://pixe.physics.uoguelph.ca>) and the elemental concentrations were obtained by normalizing PIXE data to the irradiated mass and charge obtained from the RBS spectra. The certified values for the standard are shown in the Table 1 and they are compared with those obtained in the experiment.

From the Table 1 we can observe that the certified values are very close to those obtained for our experiment.

Therefore, after reducing the uncertainties obtained by PIXE fitting the spectra via RBS, the obtained values are the expected values. Moreover, we can see that the use of 3 MeV protons, the nuclear microprobe, the funny filter and all the other components in our experiment, increase the confidence level on the results.

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