### New Trends for Radiation Applications in Medicine

C. B. Zamboni<sup>1</sup>, S. Metaiorn<sup>1</sup>, M. A. Rizzutto<sup>2</sup>, S. Bernardes<sup>2</sup> and M.R.A. Azevedo<sup>3</sup>

<sup>1</sup>Instituto de Pesquisas Energéticas e Nucleares, IPEN - CNEN/SP, Brasil <sup>2</sup>Universidade de São Paulo, IFUSP, SP, Brasil <sup>3</sup>Universidade de Santo Amaro, UNISA, SP, Brasil

#### Abstract

The use of alternative analytical techniques to investigate specific electrolytes in body fluids has increases in last year's presenting significant progress in clinical tests. This motivated us to check the viability to use a portable X- Ray Fluorescence Spectrometry (PXRFS) for clinical blood examination. In this work, whole blood samples of healthy subjects were investigated using a compact PXRF and the results were compared with data from NAA. Based on these results the PXRFS performance could be discussed showing to be appropriate for clinical blood analyses.

## Introduction

The use of alternative analytical techniques to investigate specific electrolytes in body fluids (mainly blood, serum and urine) has increases in last year's presenting significant progress in clinical practices. Since 2004 Neutron Activation Analysis (NAA) and more recently X-ray Fluorescence (XRF) techniques have been applied to this clinical finality at IPEN/CNEN-SP, in collaboration with research centers from Brazil. Several investigations in veterinary medicine, immunology and genetic fields were performed and also applications for medical diagnosis studies, particularly from Brazilian inhabitants were done [1-13]. The major advantage for using these techniques in diagnosis is the viability to use small quantities of whole blood (0.1 mL) comparatively to conventional analyses performed in serum or plasma (using at least 5 to 10 mL of biological fluid). In synthesis, these procedures and appropriate nuclear instrumentation allow to perform simultaneous analysis in blood, such as, Blood Iron (Fe), Calcemia (Ca), Calemia (K), Natremia (Na), Calcemia (Ca), Cloremia (Cl) and others, which are not always possible by the conventional clinical procedures, usually performed by Ion Specific Electrode (Na and K),

Colorimetry (Ca) and Titrimetry (Cl and Fe). Furthermore, the execution is faster; while using whole blood it is unnecessary to wait for the blood coagulation procedure and neither for the performance of serum separation. The success in these alternatives procedures for clinical analyses motivated us to check the viability to use a portable X-ray Fluorescence Spectrometry (PXRFS) for iron determination in whole blood.

In this work, iron concentration in whole blood samples of healthy subjects were investigated using a compact PXRFS and a comparative analysis with NAA results was outlined. The PXRFS performance was discussed based on these data.

# Experimental

In this study the biological samples came from Blood Banks of São Paulo city (Brazil). Male and female blood donors, age between 25 and 60 years and above of 50 kg, were selected following the procedure conventionally established (the technical staff perform screening tests in serum for transmitted transfusion diseases as: Hepatitis B and C, AIDS, Syphilis and Chagas). All the samples were collected in a vacuum plastic tube (without anticoagulants) attached to the donor's arm. Immediately after the collection exactly  $100\mu$ L of whole blood was transferred to the filter paper (Whatman 41) using a calibrated micropipette and each one was dried for few minutes using an infrared lamp. Samples were prepared in duplicate.

Standard solution of iron obtained from high purity metals was prepared following the same procedure. To perform these clinical analyses using NAA, biological sample and standard were irradiated for 2 hours in the nuclear reactor (IEA-R1, 3-4.5MW, pool type) at IPEN. After the irradiation, samples and standard were gamma-counted for 1h using a HPGe detector (Ortec- GEM-60195) of High Energy Resolution (FWHM = 1.87keV) and an ORTEC -671 amplifier, in pile- up rejection mode, coupled to a MCA ORTEC 919E connected to a PC. The areas of the peaks of iron (<sup>59</sup>Fe, T<sub>1/2</sub>=44.5d) corresponding to gamma transitions related to interest ( $E_{\gamma}$  =1099 and 1291 keV), are evaluated using the IDF computer software and, the calculation of the concentration for each element could be obtained from in-house software. The XRF analysis was performed using MINI X spectrometer from Amptek, model X-123 SDD with Ag X-ray tube. The characteristics X-

ray fluorescent intensities ( $K_{\alpha}$  lines) were measured with a Si detector (Si Drift 25 mm<sup>2</sup> x 500 $\mu$ m / 0.5 mil) Be (window / 1.5") and biological samples were irradiated for 100s using 30 kV and 5  $\mu$ A excitation. The quantitative analysis was performed using WINAXIL software program.

## **Results and Discussion**

The Fe concentration  $(gL^{-1})$  determined in blood samples from XRF and NAA analyses are presented in Table 1. The results presented include: arithmetic mean (mean value), the standard deviation  $(1\sigma)$  and the reference interval.

| <b>Table 1.</b> The concentration of from in whole blood samples by NAA and ARF technique |
|---|
|---|

| Fe<br>(gL <sup>-1</sup> ) | Mean<br>Value | Standard<br>deviation<br>(1σ) | Min<br>Value | Max<br>Value | Reference Interval<br>(95%)<br>0.277 – 0.513 <sup>[2]</sup> |
|---------------------------|---------------|-------------------------------|--------------|--------------|---|
| NAA                       | 0.464         | 0.040                         | 0.335        | 0.525        | 0.384-0.544   |
| XRF                       | 0.453         | 0.046                         | 0.378        | 0.515        | 0.361 – 0,545   |

min: minimum max: maximum

According to Table 1 the results obtained by both techniques are in agreement, considering a confidence interval of 68% (1SD), suggesting clearly the viability of using a portable X-Ray Fluorescence Spectrometry (PXRFS) for iron concentration determination in whole blood samples. Furthermore, considering the short time irradiation (100s), using the XRF technique, has a fast procedure for iron analysis in blood.

An evaluation relate to the techniques applied in this work allows to emphasize some positive aspects when compared with the conventional analyses: the simplicity involved since the blood collection until the experimental procedures, the storage of the sample for a long period (depends only the durability of the filter paper) for future reexamination without the need for any refrigeration and also the quickness. Finally considering the viability of using these techniques for clinical investigations using whole blood, other elements also relevant in clinical practice (such as Ca, Cl, K, S etc), could be evaluated. Relate to the small portable spectrometry, it showed to be appropriate for blood analyses.

## Conclusions

According to this investigation NAA and XRF are alternatives procedures for clinical practice (using 100µL blood) with advantages comparatively to the conventional procedure usually performed by Titrimetry or Atomic Absorption Spectrophotometer (using at least 500µL serum).

We intend to stimulate the biochemical analysis of whole blood as a diagnostic of the Brazilian population using the compact arrangement.

#### Acknowledgments

The authors would like to acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Instituto de Pesquisas Energéticas e Nucleares (IPEN) for the financial support.

## References

1. ZAMBONI, C.B.; OLIVEIRA, L.C.; DALAQUA JR., L. Diagnostic application of Absolute Neutron Activation Analysis in hematology. In: Americas Nuclear Energy Symposium, Miami. Proceedings ANES, 2004

2. OLIVEIRA, L.C.; KOVACS, L.; ZAMBONI, C.B.; MEDEIROS, J.A.G.; AZEVEDO, M.R. Iron evaluation in patients submitted to dyalysis. Revista Brasileira de Hematologia e Hemoterapia v. 29, (2007) 607.

3. KOVACS, L.; ZAMBONI, C. B.; OLIVEIRA, L.; SALVADOR, V.L.R.; SATO, I. M.; AZEVEDO, M. R. Analysis of serum and whole blood using NAA for clinical investigation. J. Radioanal. Nucl. Chem., v. 278, (2008) 543.

6. METAIRON, S.; ZAMBONI, C.B.; KOVACS, L.; GENEZINI, F.A.; SANTOS, N. F.; VILELA, E.C. Analysis of elements in human blood of patients with chronic kidney disease using neutron activation analysis. J. Radioanal. Nucl. Chem., v. 282, (2009)81.

7. OLIVEIRA, L.C.; ZAMBONI, C.B., MEDEIROS, J. A. G.; AZEVEDO, M. R. NAA for human serum analysis: Comparison with conventional analyses. AIP, 1265, (2010) 457.

8. OLIVEIRA, L.C.; ZAMBONI, C.B.; PESSOAL, E.A.; BORGES, F.T. Characterization of ions in urine of animal model with ARF using NAA. AIP, v. 1351, (2011) 353

9. METAIRON, S.; ZAMBONI, C.B.; SUZUKI, M.F.; BUENO, C.R.; SANT'ANNA, O.A. Comparison of reference values in whole blood of Dmd<sup>mdx/j</sup> and C57Bl/6L mice using Neutron Activation Analysis. AIP, v. 1351, (2011) 251

10. BAPTISTA, T.S., REDÍGOLO, M.M.; ZAMBONI, C.B.; SATO, I.M.; MARCELINO, JR. Comparative study of inorganic elements determination in whole blood from Crioula breed horse by EDXRF and NAA analytical techniques. J. Radioanal. Nucl. Chem., v. 291, (2012) 399

11. OLIVEIRA, L.C.; ZAMBONI, C. B., PESSOA, E. A.; BORGES, F. T. Determination of elements in kidney, serum and urine of Wistar rats with Acute Renal Insufficiency using NAA. J. Radioanal. Nucl. Chem., v. 291, (2012) 395.

13. BAPTISTA, T.S., REDÍGOLO, M.M.; ZAMBONI, C.B.; SATO, I.M.; MARCELINO, J R.. Comparative study of inorganic elements determination in whole blood from Crioula breed horse by EDXRF and NAA analytical techniques. J. Radioanal. Nucl. Chem., v. 291, (2012) 399