

## ANALYSIS OF INORGANIC ELEMENTS IN BLOOD OF ALBINO RABBIT USING NAA

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### ABSTRACT

In this work Br, Cl, K and Na concentrations in albino rabbit blood were determined using NAA. They are the first indicative interval for reference values in whole blood and they could be used for checking the clinical status of this species when it will be used as animal model. The results when compared with human whole blood estimation suggest compatibility for Br, Cl and K considering 95% of confidence interval but, for Na the levels are altered, suggesting physiologic differences.

### 1. INTRODUCTION

The use of neutrons mainly in nuclear reactors, accelerators and some sources of neutrons, such as:  $^{241}\text{AmBe}$ ,  $^{210}\text{PoBe}$  and  $^{239}\text{PuBe}$  sources, are becoming more popular in medical applications, for example, for studying diseases in body organs, for checking the quality control of medicine and vaccines, for nutritional evaluation of diet as well as for radioisotope production. Also making use of neutrons we have optimized the neutron activation to perform clinical chemistry analyses in several biological materials (urine, saliva, blood and serum) with some advantages comparatively to the conventional procedures: use small quantities (few micro liters) and simultaneous evaluation of the elements of clinical relevance. As consequence an important application of this optimized nuclear procedure is the blood investigation, mainly in small size animals, used as animal model due to the reduced available biological material.

In the last years the semi-parametric Neutron Activation Analysis (NAA), using Au as neutron flux monitor [1], has been successfully applied by the group for investigation of several elements in blood and urine of the small sized animals (Hamster and Mice) [2-6] as well as in human being [7], resulting in an efficiency procedure for clinical practice. The advantage in using whole blood is related to the fact that this nuclear procedure needs small quantity of biological material (100  $\mu\text{L}$  of whole blood) when compared with the conventional analyses (~0.5 mL of serum/analysis) [8,9]. But, for using whole blood to perform these biochemistry analyses it is essential to establish the reference value in blood for the species or animal in this study we intend to use the NAA technique for evaluation of the normal range of Br, Cl, K and Na in whole blood samples of Albino Rabbits of New Zealand. The elements Cl, K and Na were selected in function of the clinical relevance for evaluation of electrolyte disorders and Br because it is usually present in medicines and it can also be present in antidotes (for example, in serum production of venomous) so it is very important also to check its level in blood. This study is part of a project entitled: "Determination of reference

values for concentrations of trace elements in whole blood using nuclear methodology", nowadays in development at Instituto de Pesquisas Energéticas e Nucleares (IPEN - CNEN/SP) in collaboration with several research centers as well as with blood banks and hematological laboratories and from different regions of Brazil. The data from the Albino Rabbits will contribute for applications in veterinary medicine related to biochemistry of whole blood.

## 2. EXPERIMENTAL PROCEDURE

The samples came from Butantan Institute at São Paulo city and from Centro de Pesquisas Aggeu Magalhães at Recife city. For sample preparation, the whole blood was collected by twelve male adult rabbits. To determine the concentration of the elements the Cd ratio technique was used for the measurement of thermal flux distribution [1]. In this technique, Au foils (~1mg), both bare and Cd covered (1mm thick), are irradiated together with the biological sample (100µL) in the IEA-R1 nuclear reactor at IPEN/SP (IEA-R1, 2-4MW, pool type), for 2 minutes, allowing the simultaneous activation of these materials under the exact same irradiation conditions. A  $\gamma$  - spectrometer system with a semiconductor detector connected to an ADCAM multichannel analyzer and to a PC computer were then used to measure the induced gamma-ray activity. The detector was a HPGe of high resolution (FWHM = 1.85keV) calibrated for energy and efficiency through the measurements of standard sources of Co<sup>56</sup> and Eu<sup>152</sup>. All gamma spectra analysis evaluations were performed using the IDF computer code [9] and the concentration of each element was obtained using in-house software [10].

## 3. RESULTS AND DISCUSSION

The concentration of the elements in whole blood samples are shown in Table 1. Considering that all the analyses were performed in duplicate, the results are the mean value and the associated standard deviation (68%). These results were compared to the human being estimation [7].

**Table1. Indicative interval for the reference values of the elements Br, Cl, K and Na in Albino rabbits whole blood samples by using NAA.**

Elements (gL <sup>-1</sup> )	Mean	SD	Reference Value Albino Rabbit	Reference Values Human being [7]
Br	0.0011	0.0004	0.0007 - 0.0015	0.0024- 0.0096
Cl	3.15	0.13	3.02 – 3.28	2.34 – 3.00
K	1.59	0.19	1.40 – 1.78	1.09 – 1.53
Na	2.28	0.20	2.08 – 2.48	1.24 – 1.60

Considering a confidence interval of 95 %, usually adopted as reference for clinical practice, for Br, Cl and K the mean value for rabbit is in agreement with human being but for Na even considering a confidence interval of 99 % for human blood estimation ( $0.88 - 1.98 \text{ gL}^{-1}$ ) [7], there was not agreement, suggesting the Na levels need to be monitored when these animal will be used as model.

#### 4. CONCLUSIONS

In this study was an obtained references value for Br, Cl, K and Na in whole blood for Albino rabbits. These results permit to check the similarities with the estimations for human being an important condition for selecting laboratory animals. Besides, these data will allow researchers to optimize their studies in terms of time (simultaneous evaluation of the elements) and also to perform biochemical analyses using whole blood an advantage when the biological material is restrict.

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