

DIFFERENCES IN TRACE ELEMENT CONCENTRATIONS IN WHOLE BLOOD OF SMALL SIZED ANIMALS USING ANAA

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ABSTRACT

The Absolute Neutron Activation Analysis technique (ANAA) was applied for the determination of trace elements in whole blood of small-sized animals, it was used on experiments in health area, to verify if there are any differences in elemental concentrations. These results were also correlated with human being whole blood estimation for checking the similarities.

1. INTRODUCTION

In health area it is very usual small-sized animals to be used for clinical investigations in function of the low cost, easily handling and also due the facilities relate to medico-legal implications [1]. Particularly, in this study, we want to check the viability of using the Absolute Neutron Activation Analysis technique (ANAA) to determine some element concentrations in whole blood samples of Golden Hamsters (*Mesocricetus auratus*), Albinic Rabbits of New Zealand breed and Mouse (*Mus musculus*). The relevancy of this study is that this biological fluid is one of the most important biological indicator of a great number of anomalies and another important aspect to be considered is related to the fact that these animals are usually used as guinea-pigs mainly on investigations that involves testing of new medicines, vaccines, antibiotics, anesthetics, antidepressants, also for organs transplantation as well as for medical diagnostic studies before to be tested in human being.

The basic principle of this technique (ANAA) is the irradiation of the biological material with neutrons followed by the measurement of the γ -ray activities induced in the biological sample, where the elements can be identified by the nuclear properties of γ -rays. As this nuclear methodology has been successfully used in the public health field for the

investigation of elements in urine, bones and organs of small and medium-sized animals [2-6] now, we want to extend its application to analyze whole blood samples in different guinea-pigs.

2. EXPERIMENTAL PROCEDURE

To determine the concentration of the elements in the biological samples the Cadmium Ratio Technique was used for the measurement of thermal flux distribution [7]. In this technique, Au foils (~1mg), both bare and Cd covered (1mm thick), are irradiated together with the biological sample in the IEA-R1 nuclear reactor at IPEN/SP (IEA-R1, 2MW, pool type), for few minutes, allowing the simultaneous activation of these materials under the exact same irradiation conditions. Using this procedure the γ -ray activities induced in the Au foils by both the thermal and epithermal neutrons were obtained as well as the activation of biological samples. A γ -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 HGPe of high resolution (FWHM=1.85 keV) calibrated for energy and efficiency through the measurements of standard sources of Co⁵⁶ and Eu¹⁵². All gamma spectra analysis evaluations were performed using the IDF computer code [8].

Using this procedure it is possible to identify the following radioactive nuclides: ⁸⁰Br ($T_{1/2}$ =18 min, E_{γ} = 616 keV), ³⁸Cl ($T_{1/2}$ =37 min, E_{γ} =1642 keV), ⁴²K ($T_{1/2}$ =12h, E_{γ} =1525 keV) and ²⁴Na ($T_{1/2}$ =15h, E_{γ} =1368 keV). The concentration of each element was then obtained by using a software [9] which correlated the neutron flux and efficiency of the selected gamma ray with the constants physics involved (the decay constant; the atomic mass; the cross section for the selected capture reaction; the isotopic fraction and the intensity of the gamma ray) which are well determined [10].

In this study the biological samples came from Butantan at São Paulo city and from Centro de Pesquisas Aggeu Magalhães at Recife city. For this study was collected whole blood samples from 8 adult females Mice (Mus musculus), 7 adults females Golden Hamsters (Mesocricetus Auratus) and 4 adult males and 2 adult females Albinic Rabbits of New Zealand. About 0.5 ml of whole blood was collect of each specimen and aliquots of 100 μ l was then transferred to the filter paper and dried for few minutes using an infrared lamp. Each sample was sealed into an individual polyethylene bag and irradiated together with the Au foils at the nuclear reactor.

3. RESULTS AND DISCUSSION

The concentration of the Br, Cl, K and Na in whole blood samples of Mice, Hamsters and Rabbits are shown in Table I, II and III, respectively. All the results are a mean of two replicate analyses for Hamsters and Rabbits and triplicate for Mice. An comparative analysis relate to the concentrations value was performed between the specimens. Theses results were also correlated with mean values for Br, Cl, K and Na in human being whole blood [11]. These comparisons are shown in figures 1, 2 , 3 and 4. In these figures we also included the indicative interval of these elements, relate to human being results, that were estimated by using the arithmetic mean value taken ± 2 SD (Standard Deviation).

Table 1. The Concentration of Br, Cl, K and Na in whole blood samples of Mice.

Elements (g l ⁻¹)	Mean	SD	Minimum Value	Maximum Value
Br	0.006	0.0004	0.026	0.0097
Cl	2.54	0.27	2.28	2.90
K	2.06	0.40	1.91	2.25
Na	1.66	0.14	1.54	1.83

Table 2. The Concentration of Br, Cl, K and Na in whole blood samples of Hamsters.

Elements (g l ⁻¹)	Mean	SD	Minimum Value	Maximum Value
Br	0.0024	0.0008	0.0016	0.0035
Cl	3.04	0.31	2.76	3.32
K	1.94	0.38	1.68	2.11
Na	1.83	0.18	1.57	2.27

Table 3. The Concentration of Br, Cl, K and Na in whole blood samples of Rabbits.

Elements (g l ⁻¹)	Mean	SD	Minimum Value	Maximum Value
Br	0.0011	0.0004	0.0031	0.0175
Cl	2.55	0.24	2.34	2.70
K	1.32	0.27	1.12	1.43
Na	1.83	0.14	1.68	1.84

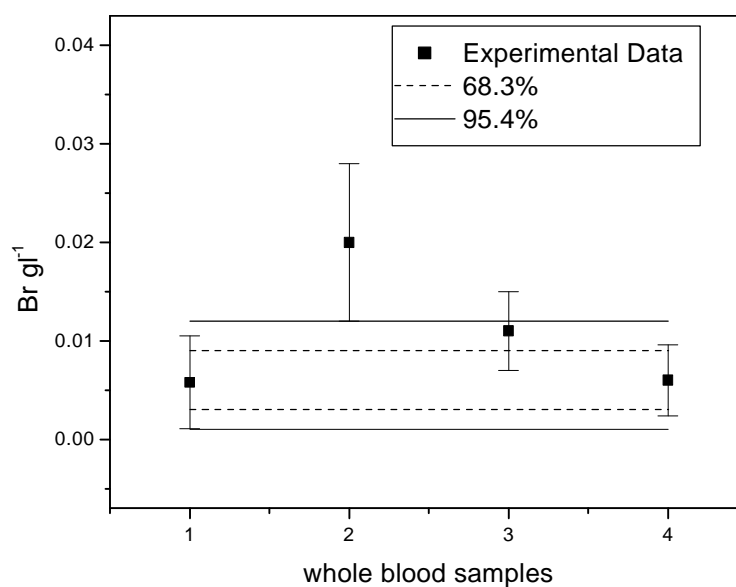


Figure 1. The concentration Br results in whole blood samples of the selected animals (Mice, Hamsters and Rabbits) and from Human [11]. The appearance of the experimental data in the figure allowing this sequence.

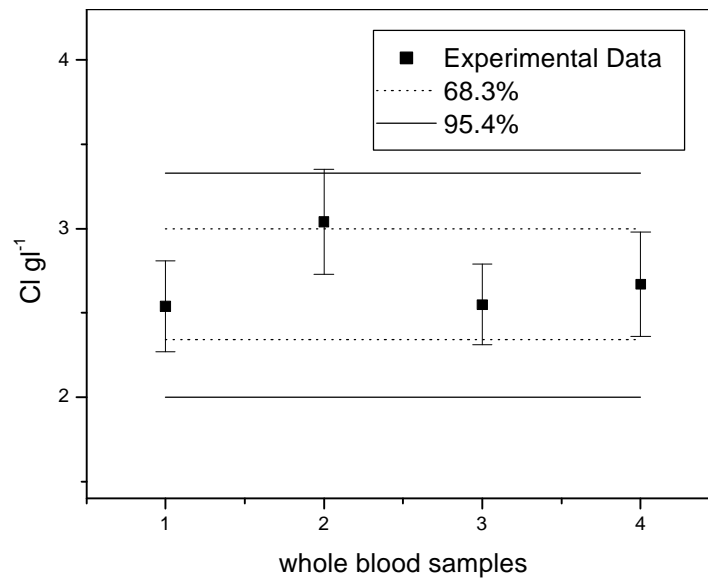


Figure 2. The concentration Cl results in whole blood samples of the selected animals (Mice, Hamsters and Rabbits) and from Human [11]. The appearance of the experimental data in the figure allowing this sequence.

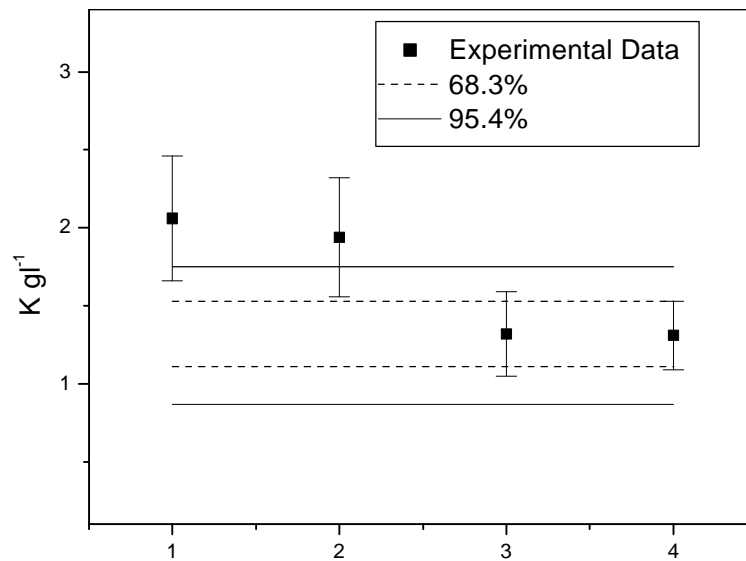


Figure 3. The concentration K results in whole blood samples of the selected animals (Mice, Hamsters and Rabbits) and from Human [11] . The appearance of the experimental data in the figure allowing this sequence.

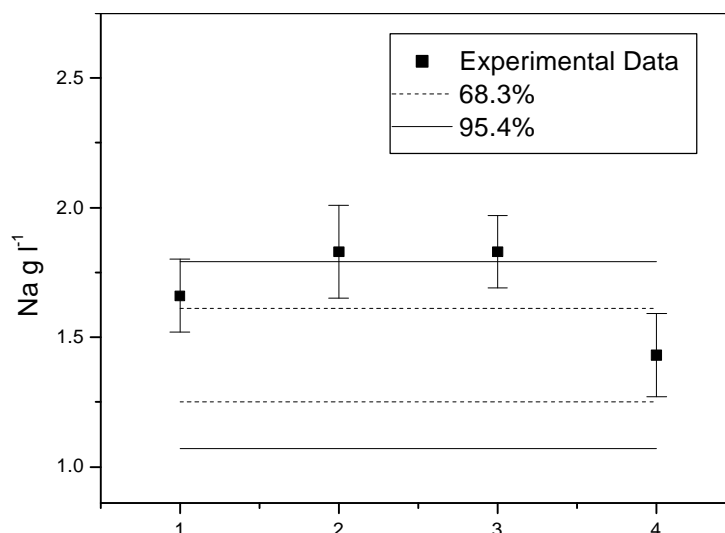


Figure 4. The concentration Na results in whole blood samples of the selected animals (Mice, Hamsters and Rabbits) and from Human [11] . The appearance of the experimental data in the figure allowing this sequence.

3. CONCLUSIONS

In this report the ANAA technique was used to determine simultaneously the concentration of some elements in whole blood of the selected small-sized animals. These results were compared with them from human and, although we have identified some physiologic differences, they were compatible considering 2SD. This comparative analyses can be also a very useful tool for clinical investigation mainly when hematological analysis must be realized in small-sized animals for a long period, in short time interval (few days) because, the use of small quantity of whole blood reduce the stress of them. Besides, consulting these data it also possible to select the convenient specimen for experiments when the similarities with the human is an important condition.

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