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DETERMINATION OF REFERENCE VALUES OF ELEMENTS IN WHOLE BLOOD OF THE WISTAR RATS USING NAA

Laura C. Oliveira and Cibele B. Zamboni

Instituto de Pesquisas Energéticas e Nucleares, IPEN - CNEN/SP
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
laura@ipen.br

ABSTRACT

Some investigations, especially biochemistry analysis, can be performed using whole blood if the normality limits are established. The present study deals with the determination of reference values for elements of clinical interest, in whole blood of Wistar rats using the Neutron Activation Analysis technique. Usually these small-sized animals are used as guinea-pig on experiments that involves testing new medicines and medical diagnostic studies. In this investigation, the reference values for blood were determined for: Br (0.0011 – 0.0095 gL^{-1}), Ca (0.0 – 0.66 gL^{-1}), Cl (2.35 – 4.91 gL^{-1}), K (1.00 – 3.12 gL^{-1}), Mg (0.044 – 0.108 gL^{-1}), Na (1.13 – 3.09 gL^{-1}) and S (0.53 – 1.81 gL^{-1}). These data will allow researchers to optimize their studies, both in terms of cost and time by selecting species that fits to the experimental model as a clinical reference as well as performing biochemical analyses in whole blood using small quantities (few μL) compared to the conventional analyses performed in serum (few mL).

1. INTRODUCTION

The search for new medicine, vaccines and other products of interest in health area, for any disease, requires several *in vivo* tests using guinea-pig on experiments for clinical surveys of regulatory functions in organism, focusing in the relations between these and the responses or reactions to their use, allowing or not their use in human being.

Several studies aim to elucidate the metabolism of living organisms, both in healthy and in dysfunctional conditions. The elemental constituents in blood, especially Ca, Cl, K and Na, even present in small amounts (< 2%) are of great importance for keeping the body's functions [1].

Therefore, the quantitative knowledge of these elements in blood allows a clinical evaluation of the proper performance of different functions that regulate the organs. In this way, variations between excess and deficiency of these elements in blood are usually associated to some kind of ongoing pathology.

In the last years, the Nuclear Structure Laboratory (LEN/IPEN) has performed measurements related to the interval reference for elements of clinical interest in whole blood of several animals used for experimentation, such as, several mice strains [2-5], rabbits [6], and Gold hamster [7] as well as for the characterization of various biological materials (bone, organs and saliva) using instrumental and parametric Neutron Activation Analysis technique, which represents advantages especially when the availability of biological material is scarce [8,9]. Now we intend to establish the normal range for Br, Ca, Cl, K, Mg, Na and S in whole blood

for Wistar using NAA. These elements (Ca, Cl, K, Mg and Na) were selected due to the clinical relevance for evaluation of electrolyte disorders as well as for their nutritional relevance. Br was selected because bromides are present in the Brazilian diet (mainly sea food) and also in medicinal drugs, both highly consumed by the Brazilian population and S due its nutritional significance in the Brazilian diet (rich in vegetables and fruits) [10-12]. Related to S, its participation in the repair and construction of tissues and cells, the formation of several vitamins and proteins emphasizes its clinical relevance. Besides, a recent investigation related to ration administrated in animal model indicated the presence of S at high level (a factor 100 above nominal specification) [13] emphasizing the need of its evaluation.

2. MATERIAL AND METHODS

The whole blood was collected from seven adults Wistar rats (males) created in the bioterio of the UNIFESP (Federal University from São Paulo, Brasil). About 0.3mL of whole blood was collected direct from the heart (intracardiac) and packed in a plastic tube, after 100μL were then transferred to Whatman – nº 42 filter paper (2.2 cm^2). All the samples were prepared in duplicate. Standard solutions obtained from high purity metals and salts were prepared following the same procedure.

To determine the concentration of the elements, each biological sample was sealed into individual polyethylene bag and irradiated with thermal neutron flux (range from: $4.52 \cdot 10^{12}$ to 5.45 10^{12} n cm^{-2} s^{-1}) at the IEA Nuclear Reactor at IPEN, allowing the activation of these materials. For ^{38}Cl ($T_{1/2}$=37.24 min, E_γ=1642 keV) and ^{24}Na ($T_{1/2}$=14.95 h, E_γ =1368 keV) determination an irradiation time of 2 minutes followed by 4 minutes of counting time was used. For ^{80}Br ($T_{1/2}$=17.68 min, E_γ = 616 keV), ^{49}Ca ($T_{1/2}$=8.71 min, Eγ =3084 keV), ^{27}Mg ($T_{1/2}$=9.45 min, Eγ =1014.44 keV), ^{42}K ($T_{1/2}$=12.36 h, E_γ =1525 keV) and ^{37}S ($T_{1/2}$=5.05 min, E_γ =3104 keV) sample and standard were irradiated for 5 minutes and after a decay time of 1 minute they were counted by 15 minutes for Br, Ca, Mg and S determination followed by 2 h of counting for K.

The measurements were performed using an ORTEC Model GEM-60195 and ORTEC 671 amplifier (in pile-up rejection mode) coupled to a MCA ORTEC Model 919E. The gamma spectra analysis was performed using the IDEFIX computer software [14] and the data was analyzed using in-house software [15].

3. RESULTS AND DISCUSSION

In this investigation the IAEA A-13 animal blood was used for analytical quality control. The accuracy evaluation by Z-score test in this certified biological material indicate the adequacy of the method for all elements determined ($Z < |2|$). These results are presented in Table 1.

The concentration values for Br, Ca, Cl, K, Mg, Na and S as well as the basic statistical treatment (arithmetic mean, standard deviation (±1SD), minimum and maximum values and the normal range) for whole blood of Wistar rats are presented in Table 2.

Table 1. Element concentrations obtained in the analysis of AIEA A-13 certified reference material

Element, g kg^{-1}	Certified values	NAA Mean ± 1SD	Z - score
Br	22 ± 9%	24.0 ± 2.5	1.0
Ca	286 ± 19%	291 ± 38	0.09
Cl	13400 ± 8%	14450 ± 830	1.0
Mg	99 ± 28%	113 ± 20	0.5
K	2500 ± 16%	2750 ± 320	0.6
Na	12600± 8%	13900 ± 700	1.3
S	6500 ± 8%	6620 ± 1540	0.2

Table 2. Results for Br, Ca, Cl, K, Mg, Na and S in blood of Wistar rats (control group) by NAA

Elements (gL^{-1})	Mean	1SD (68%)	Min value	Max value	Indicative Interval*
Br	0.0053	0.0021	0.0033	0.0088	0.0011 – 0.0095
Ca	0.32	0.17	0.17	0.60	0.0 -0.66
Cl	3.63	0.64	2.72	4.45	2.35 – 4.91
K	2.06	0.53	1.35	2.75	1.00 – 3.12
Mg	0.076	0.016	0.052	0.090	0.044– 0.108
Na	2.11	0.49	1.49	2.69	1.13– 3.09
S	1.17	0.32	0.8	1.60	0.53 – 1.81

*for a confidence interval of 95% usually adopted in the clinical practice
min: minimum concentration value determined
max: maximum concentration value determined

In Fig. 1 to 7 the average values for the blood in Wistar rats and humans are presented for comparison. This analysis is important to check blood similarities.

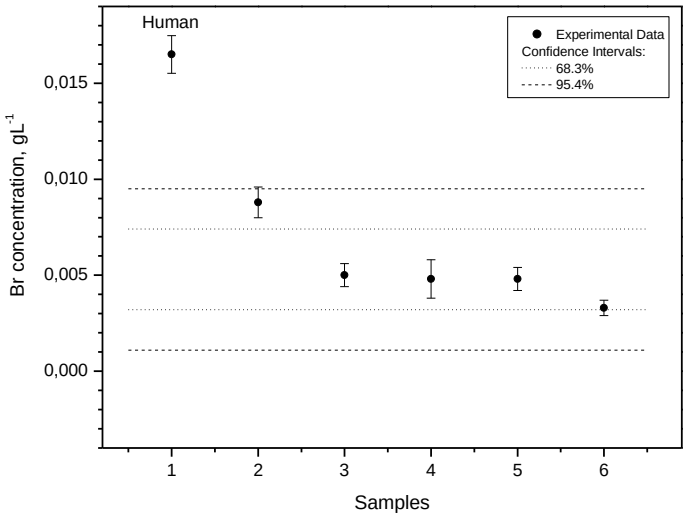

Figure 1. Concentration of Br in whole blood samples of Wistar rats compared with human being whole blood estimation [16].

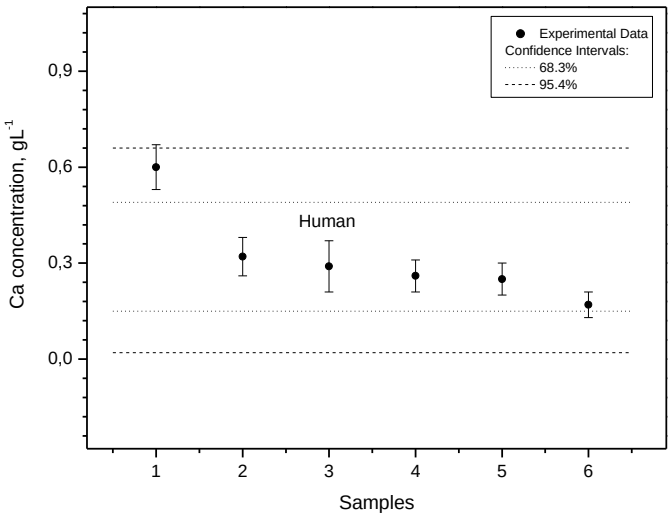

Figure 2. Concentration of Ca in whole blood samples of Wistar rats compared with human being whole blood estimation [17].

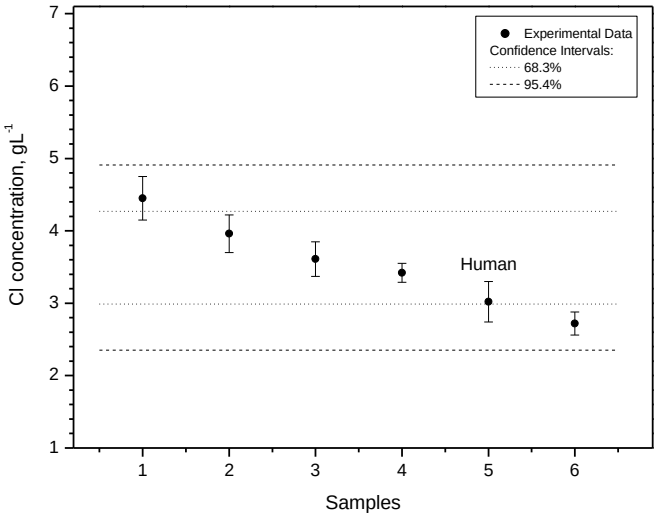

Figure 3. Concentration of Cl in whole blood samples of Wistar rats compared with human being whole blood estimation [16].

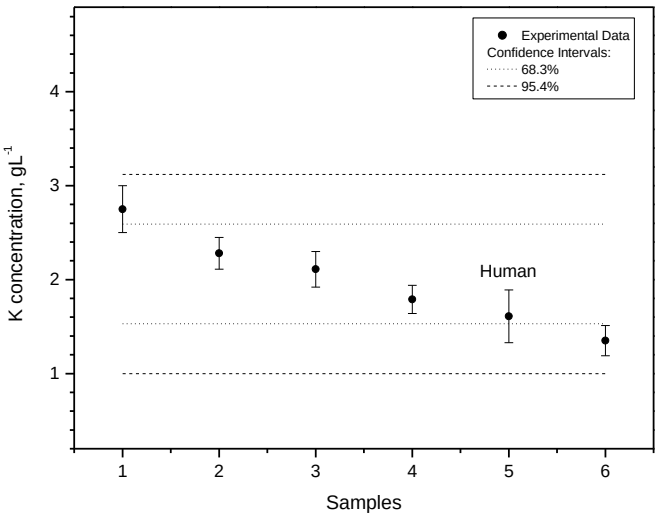

Figure 4. Concentration of K in whole blood samples of Wistar rats compared with human being whole blood estimation [16].

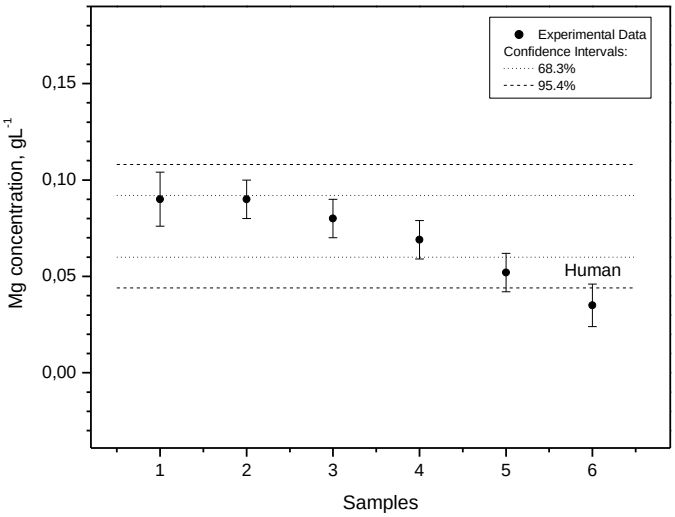

Figure 5. Concentration of Mg in whole blood samples of Wistar rats compared with human being whole blood estimation [17].

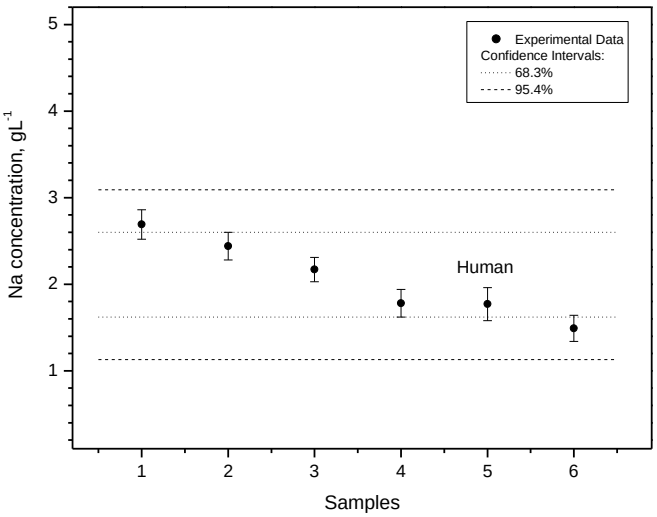

Figure 6. Concentration of Na in whole blood samples of Wistar rats compared with human being whole blood estimation [16].

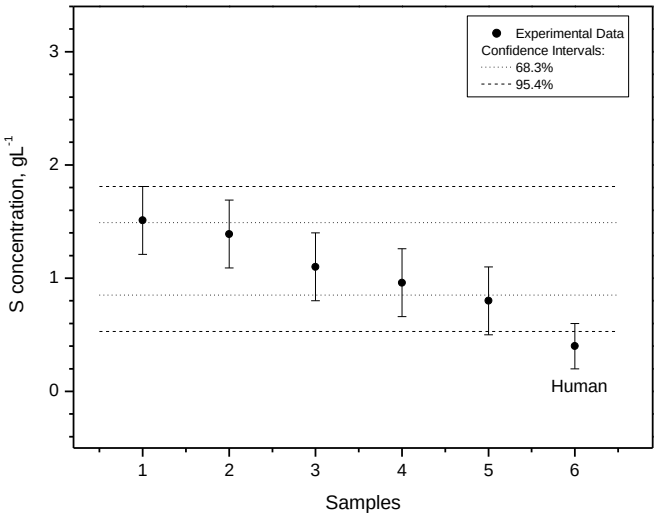

Figure 7. Concentration of S in whole blood samples of Wistar rats compared with human being whole blood estimation [18].

According to these figures there is a good similarity with human being whole blood estimation for Ca, Cl, K, Mg, Na and S. Only for Br concentration samples of Wistar rats are lower. Although many factors can contribute for Br increase in human blood the magnesium bromide found in seawater, also present in fish and sea food, and the use of medicines mainly antidepressants (rich in bromides) by the Brazilian population may be related to this increase.

4. CONCLUSIONS

In this study NAA technique was applied for determination of reference values for Br Ca, Cl, K, Mg, Na and S in whole blood samples of Wistar rats. The comparison performed with the human being blood estimation emphases the similarities for Ca, Cl, K, Mg, Na and S and suggested that this animal model can be used for different applications, such as, medical diagnostic of common deficiencies in Brazilian population as well as for testing new drugs for humans. Additionally, this analytical procedure meets the needs of the Brazilian legislation [19] that emphasizes the need to propose alternative methods for clinical practice that contribute to animal welfare.

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