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Analysis of serum and whole blood using NAA for clinical investigation

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The concentrations of Cl, K and Na were analyzed in human body fluids (whole blood and serum) using NAA, resulting in the first biochemical baseline values for the Brazilian population. These data permitted us a discussion about the advantages and limitations of using this analytical technique for clinical chemistry.

Introduction

The availability of reference values for metals and ions in human body fluids represents an important indicator of the health status of the general population. In addition, such values provide the scientific basis for biomedical researches related to diseases, forming the basis of setting legal limits of exposure for public protection and occupational health. In Brazil there is a lack of such data for whole blood. Therefore, the project "Evaluation of Metals and Ions in Body Fluids for the Brazilian population using NAA: Determination of reference values",[1] have been proposed in collaboration with Research Centers, Blood Banks and Universities from several Brazilian regions, aiming to establish reference values for elements in human blood. In previous reports we assessed preliminary results for some elements in whole blood from a small group of subjects.[2,3] Now, we have accumulated significant statistical data to propose the first baseline values for Cl, K and Na for the Brazilian population aiming, in the future, to perform biochemistry analyses using whole blood.

Experimental

Collection and preparation of the biological samples

In this study the biological samples came from several blood banks from different regions of Brazil. Ethical approval for this study was obtained from the Ethics Committee of the blank blood authority. To perform the donor's selection the serum samples from the volunteers (18 to 65 years, above 50 kg) were submitted to the routine tests for transmitted diseases such as: Chagas, hepatitis B and C and syphilis. For sample preparation about 1 mL of whole blood was collected in a vacuum plastic tube (without coagulant) attached to the donor's arm. Immediately after the collection, before the blood coagulation, 100 μL of whole blood were transferred to a piece of Whatman No. 41 filter paper (~2.2 cm^2) and dried for a few minutes using an infrared lamp. The biological material still in the tube was then centrifuged at 3000 rev/min for 15 minutes and the serum obtained was also transferred to filter paper (Whatman No. 42) following the same procedure but using 200 μL (~2.5 cm^2). For this investigation 283 whole blood samples and 53 serum samples were collected and prepared in duplicate.

NAA analyses

The procedure applied in this investigation is described in detail by OLIVEIRA et al.[4] According to this procedure each biological sample must be sealed into an individual polyethylene bag together with the Au detector (used as neutron flux monitor) and irradiated for 2 minutes in a pneumatic station in the nuclear reactor (IEA-R1, 2-4MW, pool type) at IPEN. For the whole blood a decay time of 5 minutes and 15 minutes of counting time was used. For serum a decay time of 30 minutes and 5 minutes of counting time was used for chlorine and sodium determination followed by 60 minutes of counting time for potassium. The thermal neutron flux utilized ranged from 10^{11} to 10^{12} $n \cdot cm^{-2} \cdot s^{-1}$.

The precision and the accuracy of the results were checked by analysis of the reference materials NIST 8414 Bovine Muscle Powder and IAEA A13 Blood Animal. The standards were also shaped in the same manner as samples for analytical quality control.

The measurements of the gamma-induced activity of the samples were carried out using an ORTEC Model GEM-60195 hyperpure Ge detector and ORTEC 671 amplifier, in pile-up rejection mode, coupled to a MCA ORTEC Model 919E connected to a PC. The background radiation was reduced by employing the iron shield described by MEDEIROS et al.[5] The source–detector distance in this experimental apparatus was 15.2 cm. The gamma-ray spectra were processed using the in-house software.[6]

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Results and discussion

The accuracy in this study was confirmed by K and Na determination in the IAEA A13 Blood Animal and by Cl in the NIST 8414 Bovine Muscle Powder. The results obtained in these reference materials are in good agreement with their respective nominal values; the relative errors were lower than 7.1% and relative standard deviations were lower than 6.4%.

The concentration results of Na, K and Cl from NAA analyses as well as the basic statistical treatment of the data are shown in Table 1 for serum and in Table 2 for whole blood. These results (in $g \cdot L^{-1}$) include: arithmetic mean, standard deviation, median, mode and the normal range.

According to Table 1 the results obtained in serum using NAA are in agreement with the normal range for the conventional analyses[7] emphasizing the possibility of using this nuclear procedure as an alternative for clinical chemistry. For whole blood only comparisons with other populations could be done. These data were summarized in Table 2, showing few contributions related to baseline data for Cl, K and Na in whole blood.[8,9] As for other elements that are relevant for clinical practice, such as Ca, Fe and I, reliable information is also very sparse, specially for the Brazilian population, suggesting that more investigation is needed.

An important application extracted from this study is the use of NAA to perform clinical investigation in veterinary medicine in small-sized animals used in medical researches (mouse, rabbits, wistar, etc.). Biochemical values for Br, Ca, Cl, K, Mg and Na in whole blood are in progress using this experimental procedure in several mice line and albino rabbits.[10,11] These data permit to check the similarities to human whole blood estimation values.

An evaluation related to the technique applied in this experiment permit to emphasize some positive aspects: simultaneous determination of several elements using small amounts of biological material (100–200 μL); the possibility to store the sample for a long period (depending only on the durability of the filter paper) for future reexamination without the need for any refrigeration; and low cost when compared with the conventional analyses because it is not necessary to use specific reactants and different apparatus.[7] Furthermore, considering that the use of neutron sources (neutron irradiator and/or neutron generator) is becoming more popular in medical applications, this experimental procedure using whole blood can be very useful for clinical practices which involves a large number of samples or quantitative analyses of several elements. The limitation is the necessity to have access to a nuclear reactor but, considering the viability of performing these laboratory analyses using a Compact Neutron Generator,[12] nowadays in test, in the future they could be performed outside of the nuclear premises.

Table 1. Results for human serum samples by NAA (in $g \cdot L^{-1}$). The range for clinical practice[7] was also included for comparison

Element (n=53)	Mean ± SD	Median	Mode	Range
Cl	3.57 ± 0.15	3.56	3.66	3.42–3.72 3.44–3.76*
K	0.160 ± 0.022	0.160	0.131	0.138–0.182 0.14–0.20*
Na	3.19 ± 0.14	3.23	3.26	3.05–3.33 3.15–3.30*

n: Number of samples analyzed.
* From Reference 7.

Table 2. Results for a human whole blood samples by NAA (in $g \cdot L^{-1}$)

Element	Mean ± SD	Median	Mode	Range
Cl	3.02 ± 0.48 1.72 ± 0.69[a]	3.00	3.55	2.54–3.50
K	1.61 ± 0.28 1.66 ± 0.12[b] 0.806 ± 0.312[a]	1.61	1.71	1.31–1.89
Na	1.77 ± 0.29 1.20 ± 0.60[b]	1.75	2.05	1.48–2.06

[a] From Ref. 8.
[b] From Ref. 9.

Conclusions

Biochemical values for Cl, K and Na were determined in whole blood and serum for Brazilian population. The results permitted us to discuss the advantages and limitations of using this NAA technique for clinical practice. Furthermore, the biochemical values determined in whole blood are the first data for the Brazilian population and they can be used as an indicator of health status as well as for studying in more detail baseline values of common deficiencies in Brazilian population, helping their diagnostics.

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