Journal of Radioanalytical and Nuclear Chemistry, Vol. 269, No.3 (2006) 541–545

Quantitative estimation of Br, Cl, K and Na in sample blood by NAA

L. C. Oliveira,[1] C. B. Zamboni,[1] J. Mesa[2]*

[1] *Instituto de Pesquisas Energéticas e Nucleares, IPEN/CNEN, P.O. Box 11049, 05422-970 São Paulo, Brazil*
[2] *Departamento de Física e Biofísica, Instituto de Biociências, UNESP, CEP : 18.618-000 - Botucatu/Sp, Brazil*

(Received April 6, 2006)

Neutron activation analysis, using Au as flux monitor, was applied to determine the concentrations of Br, Cl, K and Na in blood of healthy male and female blood donors, selected from blood banks and hematological laboratories from different regions of Brazil. The aims of this study were to collect more reference values of the Brazilian population as well as to perform hematological investigations. The advantages as well as the limitations of using this nuclear procedure are discussed.

Introduction

It is well known that various elements are present in human body in wide concentration ranges, and many of them play important roles in blood and organs. In the recent years, it has been also reported that various elements interdependently work through the mutual interactions in the human body. Therefore, further information about the distributions and kinetic behaviors of trace elements in human body is really required for elucidation of their biological roles and functions on the multi-element basis. Human blood is one of the biological fluids most frequently used in clinical analysis for medical diagnosis, because it is easily collected from man.

In recent years we have applied the neutron activation analysis (NAA), using Au as flux monitor, with success to investigate some element concentrations in whole blood, serum, urine, and also in several body organs and bones of small and medium animals (such as guinea-pigs, Wistar rats and Beagle dogs) resulting in an agile and economic way to perform clinical investigations in veterinary medicine.[1–8]

In our first work in this area, this procedure was applied in the determination of Na, Cl and K in urine samples of mammalians.[1] The results agreed with data previously obtained by selective electrode analysis,[1] indicating that the nuclear analysis of urine can be an alternative method for diagnosing anomalies in kidneys. More recently, this methodology was used to determine iron levels in whole blood of humans diagnosed with anemia (iron deficiency),[9] which is a very common disease in the Brazilian population (60% of all suffer from anemia),[10] also confirming that this procedure can be an alternative technique for clinical investigations.

Another important study demonstrating the reproducibility of this methodology, is in progress at the MB-01 nuclear reactor also at the Instituto de Pesquisas Energéticas e Nucleares (IPEN) facilities. In such work, aluminum has been investigated, by NAA with Au as monitor, in whole blood and serum for clinical diagnostic in patients undergoing long-term dialysis, as well as by atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry (ICP-MS). Results were comparable.[11]

The necessity to perform measurements in whole blood is related to the fact that most of conventional clinical analyses in hematology area are performed using mainly serum or plasma.[12] For this reason there are no previous reference value established in whole blood for Brazilian population.

This study is part of a big project entitled: "Determination of Reference Values for Concentrations of Trace Elements in Human Whole Blood using Nuclear Methodology", nowadays in development at IPEN in collaboration with blood banks and hematological laboratories from different regions of Brazil.

For the development of the present investigation, the Cl concentration in serum was first determined in order to verify the accuracy and precision of the results and also for comparing the nuclear results with the reference value established by the conventional methods.[12] This element was selected because the samples used for conventional clinical analyses need anticoagulants containing Na and K salts EDTA or trisodium citrate. Obviously, the above-mentioned comparison is, therefore, not possible using Na and K. Next, this methodology was applied to determine Br, Cl, K and Na in whole blood. These elements were firstly selected as they take part in the human being metabolism and their variations are generally related to several pathological processes.

Experimental

Sample collection and preparation

The samples came from several blood banks from different regions of Brazil. A healthy group (male and female blood donors), age between 25 and 60 years,

* E-mail: jmesa@ibb.unesp.br

0236–5731/USD 20.00

Akadémiai Kiadó, Budapest
Springer, Dordrecht

at 50 and 85 kg, were selected following the procedure conventionally established. About 1 ml of whole blood was collected in a vacuum plastic tubing attached to the donor's arm. Immediately after the collection, exactly 100 μl of blood was transferred to the filter paper, using calibrated pipette, and dried for a few minutes using an infrared lamp. The biological material still in the plastic tubing was then centrifuged and the serum obtained was also transferred to the filter paper following the same procedure. For this investigation 32 samples of serum and 42 samples of whole blood were prepared in duplicate.

Anticoagulants were not added for reasons mentioned in the Introduction. Routine tests were applied in serum for transmitted diseases as hepatitis B and C, AIDS, syphilis and Chagas' to select the donors.

Nuclear analysis

The nuclear procedure is a variant of the k_0-method,[8] with Au comparators used for neutron flux determination.

Each sample of whole blood and/or serum was sealed into individual polyethylene bags, together with the Au detectors (small metallic foils) used for measurement of the flux distribution,[13] and was irradiated in a pneumatic station in the nuclear reactor (IEA-R1, 2–4 MW, pool type) at IPEN, allowing the simultaneous activation of these materials. After the irradiation, the gamma-radiation of the activated materials (biological sample and Au) was measured with a HPGe spectrometer. The areas of the peaks, corresponding to gamma-transitions related to the nuclides of interest, were established. The gamma-spectra analysis evaluation was performed using the IDF computer code[14] and the calculation of the concentration for each element could be obtained from software developed by MEDEIROS et al.[15]

An irradiation time of 2 minutes; counting time of 1 minute for the Au activation detector and 10 minutes for the biological sample and background radiation (BG) allowed to carried out the analysis of each biological sample in about one hour or less, making fast this nuclear procedure.

Illustrative gamma-ray spectra of whole blood are shown in Figs 1 and 2. According to these figures some other elements, such as Al, Ca, Mg and Mn, are also activated but with poor counting statistics, suggesting that they can also be determined increasing the counting time condition.[16]

All the results were obtained by analyzing replicate samples. The indicative intervals for the reference values were defined by the mean value considering one and two standard deviations (SD).

Results and discussion

The Cl results for serum analysis are presented in Fig. 3 where the indicative interval obtained in this work (3.23–3.77 $g \cdot l^{-1}$) was also included.

In order to define the reference values, a robust statistical treatment with a reasonably number of samples must be done.[17] In this sense, our results are only preliminary. However, the indicative interval obtained for Cl in serum using the nuclear analysis is in agreement with the reference value adopted (3.44–3.76 $g \cdot l^{-1}$).[12] This suggests that the nuclear methodology can be used to analyze other elements in serum and whole blood.

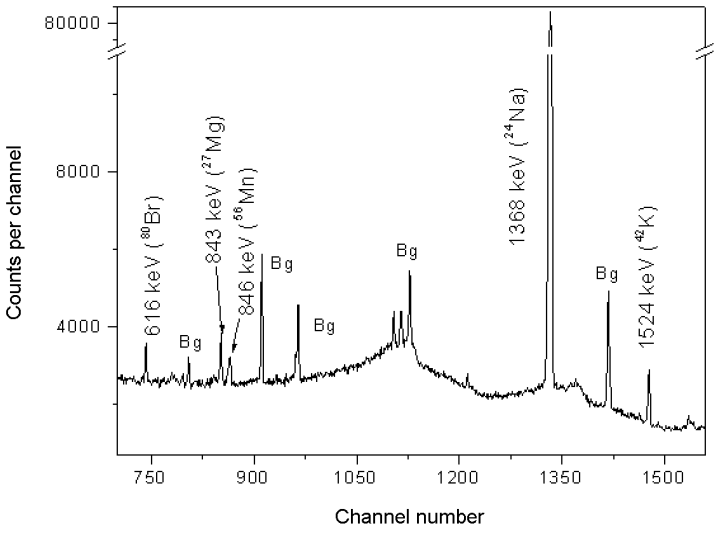

Fig. 1. Partial γ-ray spectrum of blood sample, taken at two minutes of irradiation, Bg indicates peaks occurring in natural background

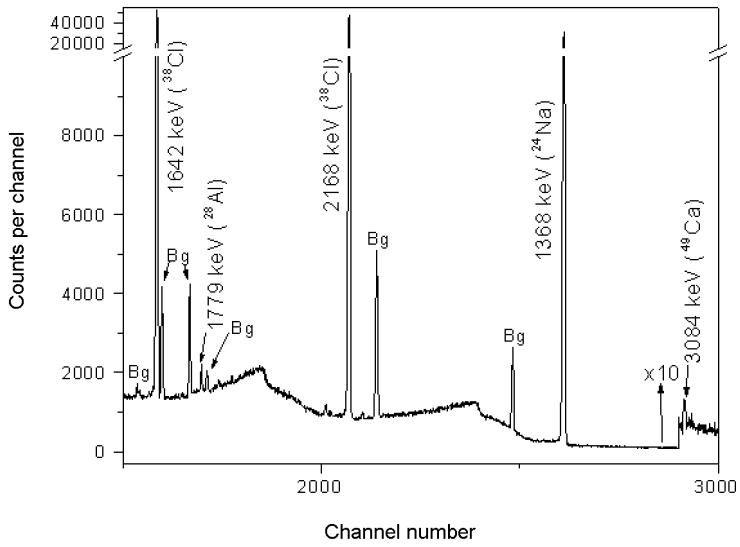

Fig. 2. Partial γ-ray spectrum of blood sample, taken at two minutes of irradiation. Bg indicates peaks occurring in natural background

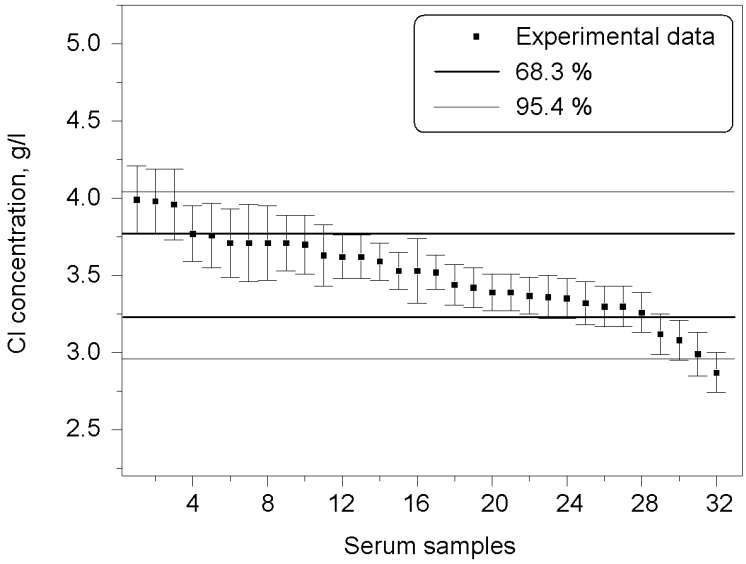

Fig. 3. Concentration of Cl in serum samples. The values are arranged by decreasing concentration

In Fig. 4 is shown the frequency distribution of concentrations for Cl in serum with class intervals defined as 0.2 $g \cdot l^{-1}$ and the correspondent fitted normal distributions. We can notice from this figure a Gaussian distribution, even though the sample population is relatively small. All whole blood samples were analyzed using the same procedure and the results are summarized in Table 1. The indicative interval and arithmetic mean value, taken at ±1 and ±2 SD, are presented in this table for the reference values of Br, Cl, K and Na in whole blood as well as for Cl in serum. All results were obtained by analyzing replicate samples.

Although these data give an indication for the element levels, more systematic and large-scale studies are needed to establish reference values to be applied in hematological investigations.

Related to the use of this nuclear methodology for hematological analyses, some advantages could be pointed out when compared with other conventional techniques: it has low cost because there is no necessity of specific reactants and different apparatus;[12] it uses small quantities (100 µl), while the conventional analyses request at least 5 ml for each element determination; it allows for simultaneous evaluation of several element concentrations in the samples, which is

not always possible in the conventional clinical analysis, and its execution is fast because it is not necessary to wait for blood coagulation neither for serum separation. In addition, considering the simplicity in the sample preparation procedure and the short time irradiation established in this study, the blood samples can be stored for a long period for future examination without the need for any specific shielding after a week of refrigeration. Furthermore, no treatment has to be made prior to discard, which can be done as regular biohazard or by incineration.

Finally, when this procedure is contrasted with comparative methods, in which standards are needed for every element, it also presents advantages, because this procedure does not require standards except for the Au monitors. For this reason, its use is less expensive, mainly when many samples must be analyzed.

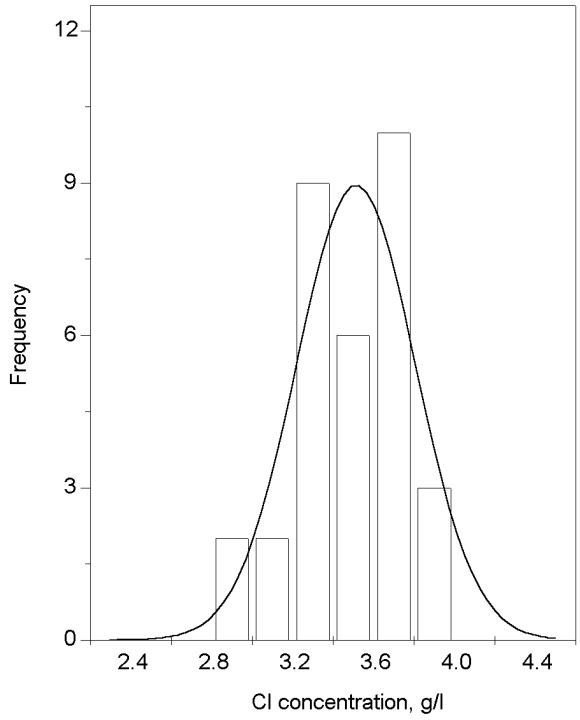

Fig. 4. Histogram and Gaussian fit of Cl concentration in serum samples

Table 1. Indicative interval for the reference values of the elements Br, Cl, K and Na (in $g \cdot l^{-1}$) in whole blood using NAA with Au as flux monitor

Element	Mean	SD	Minimum value	Maximum value	2 SD
Br	0.0060	0.0036	0.0012	0.0184	0.0072
Cl*	3.50	0.27	2.87	3.99	0.54
Cl	2.67	0.33	1.92	3.40	0.66
K	1.31	0.22	0.93	1.65	0.44
Na	1.42	0.18	1.10	1.73	0.36

* Serum results.

Besides, the instrumental method could demand much more time, mainly when elements of short half-life are involved, due to the necessity to analyze the standards and the sample separately and, as some of them can decay before being gamma-counted, usually several irradiations must be done. However, the associated uncertainties using Au as monitor to perform neutron activation in biological materials are higher than using comparative NAA,[13] due to the necessity of determining the neutron flux parameters as well as the detector efficiency, but they are comparable to the uncertainties associated with conventional clinical analysis.[12]

Another important aspect to be considered is related to the fact that this methodology can be used for the analysis of biological fluids of small size test-animals (guinea-pigs), used in testing of new medicines and/or vaccines and involving many investigations before being applied to humans. Furthermore this methodology can be extended to analyze other kind of liquid samples that could be fixed in a paper filter as well as to solid materials (powder, metal, etc.).

Conclusions

According to the results presented here, it is possible to perform hematological analysis for humans utilizing NAA with Au as monitor. However, INAA using single element standards could be preferred if smaller uncertainty is needed. This methodology could be considered fast, which is important taking into account that the determination of reference value of trace elements in human whole blood, as well as its application for clinical analyses, involves the measurement of hundreds of samples. Also, the uncertainties were compatible with the reported ones in conventional techniques used for hematological diagnostic in medical applications.

This methodology can be extended to other kind of samples that could be fixed in paper filter and also for investigating solid samples.[2,4–7,18] Several analysis using Au as monitor have been compared with those from AAS and ICP-MS,[11] X-ray fluorescence (XRF)[5] and particle induced X-ray emission (PIXE)[18] techniques. Generally good agreement of results is observed, but still reference materials must be analyzed to validate the method. Recently measurements in NIST 1400 Bone Ash using this nuclear procedure has contributed to this validation.

*

This work is partially supported by CNPq and FAPESP Brazilian agencies.

References

1. C. B. ZAMBONI, I. M. M. A. MEDEIROS, F. A. GENEZINI, A. C. CESTARI, J. T. ARRUDA-NETO, in: Proc. INAC, V ENAN, Brazil, 2000.
2. C. B. ZAMBONI, A. M. G. FIGUEIREDO, M. SAIKI, A. C. CESTARI, M. V. G. MANSO, J. T. ARRUDA-NETO, in: Proc. INAC, V ENAN, 2000.
3. L. C. OLIVEIRA, C. B. ZAMBONI, A. M. G. FIGUEIREDO, A. C. CESTARI, J. T. ARRUDA-NETO, in: Proc. III ENBN, Brazil, 2001.
4. L. C. OLIVEIRA, C. B. ZAMBONI, G. S. ZAHN, A. C. CESTARI, L. DALAQUA Jr., M. V. G. MANSO, A. M. G. FIGUEIREDO, J. T. ARRUDA-NETO, Rev. Bras. Pesq. Des., 4 (2002) 1035.
5. A. C. CESTARI, J. D. T. ARRUDA-NETO, C. B. ZAMBONI, L. C. OLIVEIRA, A. C. PEREZ, O. D. GONÇALVES, S. D. MAGALHÃES, in: Proc. V SLAFNAP, Brazil, 2003.
6. L. C. OLIVEIRA, C. B. ZAMBONI, G. S. ZAHN, M. P. RAELE, M. A. MASCHIO, Braz. J. Phys., 34 (2004) 811.
7. J. D. T. ARRUDA-NETO, M. V. M. GUEVARA, G. P. NOGUEIRA, I. D. TARICANO, M. SAIKI, C. B. ZAMBONI, J. MESA, Intern. J. Radiat. Biol., 250 (2004) 10.
8. L. C. OLIVEIRA, C. B. ZAMBONI, F. A. GENEZINI, A. M. G. FIGUEIREDO, G. S. ZAHN, J. Radioanal. Nucl. Chem., 263 (2005) 783.
9. P. S. LINS, MSc Thesis, Universidade Santo Amaro, UNISA, São Paulo, 2004.
10. S. S. QUEIROZ, M. A. A. TORRES, J. Pediatr., 76 (2000) 298.
11. N. SANTOS, C. B. ZAMBONI, F. F. LIMA, E. C. VILELA, in: Proc. XXVIII RTFNB, Brazil, 2005.
12. J. P. FRÉJAVILLE, P. KAMOUN (Eds), Guide des Examens de Laboratoire, Flammarion, Paris, 1981.
13. L. C. OLIVEIRA, MSc Thesis, Instituto de Pesquisas Energéticas e Nucleares, IPEN, São Paulo, 2003.
14. P. GOUFFON, Manual do Programa IDF, Instituto de Física da Universidade de São Paulo, Laboratório do Acelerador Linear, São Paulo, 1982.
15. J. A. G. MEDEIROS, C. B. ZAMBONI, G. S. ZAHN, L. C. OLIVEIRA, L. DALAQUA Jr., in: Proc. 39º CBPC, Brazil, 2005.
16. C. B. ZAMBONI, L. C. OLIVEIRA, L. DALAQUA Jr., in: Proc. ANES, EUA, 2004.
17. O. M. POULSEN, E. HOLTS, J. M. CHRISTENSEN, Pure Appl. Chem., 69 (1997) 1601.
18. I. M. M. A. MEDEIROS, C. B. ZAMBONI, J. A. G. MEDEIROS, M. A. RIZZUTTO, N. ADDED, M. H. TABACNIKS, Braz. J. Phys., 35 (2005) 814.