

COMPARATIVE STUDY BETWEEN TRACE ELEMENT CONCENTRATIONS IN HUMAN WHOLE BLOOD AND SERUM SAMPLES

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ABSTRACT

In this study, trace elements in human blood have been analyzed in both serum and whole blood and a comparison was made between data obtained in whole blood and serum. Blood samples collected from six individuals were separated in two fractions. One fraction was used to obtain the serum. The whole blood and the serum were freeze-dried and analyzed by instrumental neutron activation analysis. Aliquots of dried samples and synthetic element standards were submitted to short and long irradiations under a thermal neutron flux of about $4 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ of the IEA-R1 nuclear research reactor. The irradiated samples and standards were measured using a gamma ray spectrometer coupled to an HGe detector. Concentrations of Br, Ca, Cl, Fe, K, Na, Rb, Se and Zn were determined in these samples. Comparisons made between the results obtained for whole blood and serum indicated that Fe, K, Rb and Zn concentrations in whole blood are higher than those presented in sera. Br, Ca, Cl and Se concentrations were found in the same order of magnitude in both types of samples. Sodium concentrations were slightly higher in sera. These findings indicate that the serum separation from whole blood is an important step to obtain reliable results of trace element in serum. Quality control of the results was performed by analyzing IAEA-A-13 Trace Elements in Freeze-Dried Animal Blood reference material.

1. INTRODUCTION

Analyses of blood are of great interest to evaluate the health status of individuals, as well as, the presence of potential toxic elements in the body. Thus, a great number of element concentrations have been determined in human blood and serum (in this text “blood” will refer to whole blood) in order to elucidate whether or not there is a relationship of these data with certain human diseases and to assess occupational exposure to toxic elements[1-4].

Serum is a clear yellowish fluid that was separated from blood when it was allowed to clot completely without adding anticoagulants. Element determinations are preferentially carried out in serum instead of plasma to avoid eventual interference by addition of an anticoagulant containing some trace elements.

Serum analysis is preferred because it is a more relevant indicator, better reflecting the distribution of the elements to critical organs. On the other hand, blood is most often analyzed to monitor toxic metals since their concentrations are higher in blood than in serum. The relationship between the elements present in serum and blood could be expected if there was equilibrium between the two compartments. Nevertheless correlation studies between trace element concentrations found in serum and blood are not much known for several elements [2].

The objective of the present study was to compare element concentrations obtained in blood and serum of Brazilian subjects. The methodology applied in these determinations was instrumental neutron activation analysis (INAA).

2. EXPERIMENTAL

2.1. Sampling and Sample Preparation

Procedures for blood collection and its preparation were performed according to the protocols established in our previous publication [5]. They were collected in heparin free BD Vacutainer™ tubes, royal blue Hemogard™ closure, without additive (Becton-Dickinson and Company) for trace element determinations. To avoid metal contamination no anticoagulants were used. Blood samples were collected from six voluntary donors and each sample was separated in two fractions. One fraction was used to obtain the serum. The blood was centrifuged after completely clotted and 3.0 mL of serum were pipetted into acid-washed Nalgene polyethylene vial. Then serum and blood samples were frozen for transportation to the Neutron Activation Analysis Laboratory, where they were stored at $-20\text{ }^{\circ}\text{C}$ until the analysis. For the analysis the blood and the serum were freeze-dried. The weight losses during this freeze-drying process were about 91.0 and 79.5 % for serum and blood, respectively.

2.2. Analysis of Whole Blood and Serum Samples

Aliquots of about 180 mg of each dried sample weighed in polyethylene involucres were irradiated in the IEA-R1 nuclear reactor along with the synthetic standards of the elements. Synthetic standards were prepared by pipetting 50 μL of the elemental standard solutions onto small sheets of Whatman No. 40 filter paper. These solutions containing one or more elements were prepared using certified standard solutions provided by Spex Certiprep Chemical, USA. All the pipetors and volumetric flasks were calibrated before use. These filter sheets were dried at room temperature inside a desiccator and then placed into clean polyethylene bags and sealed. In these standards the quantities of each element, in μg (in parentheses) were the following: Br(5.0), Ca(500.1), Cl(200.0), Fe(350), Na(100.0), Rb(10.0), Se(8.0) and Zn(35.0).

Two separate irradiations were used to determine elements having short and long-lived radioisotopes. Fifteen-second irradiations under a thermal neutron flux of $1.4 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ were carried out for Cl and Na determinations. Sixteen-hour irradiations under a thermal neutron flux of about $5 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ were performed for elements Br, Ca, Fe, Rb, Se and Zn determinations. After adequate decay times, the irradiated samples and standards were measured by an hyperpure Ge detector Model GX2020 coupled to Model 1510 Integrated Signal Processor, both from Canberra. The resolution (FWHM) of the system was 0.90 keV for 122 keV gamma-ray peak of ^{57}Co and 1.87 keV for 1332 keV gamma ray of ^{60}Co . Each sample and standards were measured at least twice for different decay times. Counting times from 200 to 50,000 seconds were used, depending on the half-lives or activities of the radioisotopes considered. The radioisotopes measured were identified according to their half-lives and gamma- ray energies. The radioisotopes used in serum analyses were: ^{82}Br , ^{47}Ca , ^{38}Cl , ^{59}Fe , ^{24}Na , ^{86}Rb , ^{75}Se and ^{65}Zn . The concentrations of elements were calculated by a comparative method.

Accuracy and precision of trace element determinations were confirmed by analysis of the certified reference material IAEA-A-13 Trace Elements in Freeze-Dried Animal Blood. These reference materials were analyzed by applying the same experimental conditions used in sample analyses.

3. RESULTS AND DISCUSSION

Preliminary data of mean concentrations of Br, Ca, Cl, Fe, K, Rb, Se and Zn obtained in blood and serum samples from 6 individuals are presented in Table 1. The standard deviations obtained in these results reflect intersubject variability.

Table 1. Mean concentrations of elements obtained in the blood and serum samples from six individuals

Elements	Blood	Serum
Br, ng mL ⁻¹	2758 ± 598	3216 ± 570
Ca, µg mL ⁻¹	52.9 ± 16.7	97.4 ± 7.3
Cl, µg mL ⁻¹	3825 ± 802	4750 ± 959
Fe, µg mL ⁻¹	550 ± 78	1.16 ± 0.6
K, µg mL ⁻¹	1801 ± 463	<120
Na, µg mL ⁻¹	1553 ± 355	3007 ± 154
Rb, µg mL ⁻¹	4.2 ± 0.5	0.33 ± 0.04
Se, ng mL ⁻¹	102 ± 57	68 ± 32
Zn, µg mL ⁻¹	6.6 ± 1.1	0.92 ± 0.15

The highest levels were found for Cl, Na and K in blood. Potassium was not detected in serum samples due to high activities of ^{24}Na formed in the irradiation and detection limit was evaluated. Student's t test applied to compare serum and blood results indicated significant differences for Ca, Fe, Na, Rb and Zn ($p = 0.05$). The difference between element levels in blood and serum was highest for the elements Fe, K, Rb and Zn and they were higher in blood than in serum. The results for Br, Cl and Se in serum and blood were either very close or of the same order of magnitude and Na concentrations slightly higher in sera.

Data obtained in the analyses of certified reference material IAEA-A-13 Trace Elements in Freeze-Dried Animal Blood presented in Table 2 indicate good precision and accuracy. The relative standard deviations of the results were lower than 10.2 % and relative errors varied from 1.0 to 10.0 %. The standardized difference or Zscore values [7] were $|Z\text{score}| < 1$, indicating that our results are satisfactory and in agreement with the certified values. $|Z\text{score}| < 3$ means the analytical results obtained are within the ranges of certified data at the 99% confidence level.

Table 2. Concentrations of elements in certified reference material IAEA-A-13 Animal Blood

Elements	This work				Recommended values[6]
	Mean \pm SD ^a	RSD ^b , %	Er ^c , %	Z score	
Br, mg kg ⁻¹	22.23 \pm 0.65	2.9	1.0	0.04	22(19-24)
Ca, mg kg ⁻¹	266 \pm 27	10.1	7.0	-0.16	286 (226-332)
Cl, mg kg ⁻¹	16297 \pm 483	3.0	-	-	-
Fe, mg kg ⁻¹	2261 \pm 76	3.4	5.8	-0.24	2400 (2200-2500)
Na, mg kg ⁻¹	12742 \pm 117	0.9	1.2	0.07	12600(11600-13500)
Rb, mg kg ⁻¹	2.53 \pm 0.16	6.3	10.0	0.20	2.3 (1.7 – 3.1)
Se, mg kg ⁻¹	0.246 \pm 0.025	10.2	2.5	0.06	0.24 (0.15-0.31)
Zn, mg kg ⁻¹	13.2 \pm 0.4	3.0	1.5	0.07	13 (12-14)

^a Mean \pm SD = Arithmetic mean and standard deviation for at least 4 determinations; ^b RSD = Relative standard deviation; ^c Er = Relative error

4. CONCLUSIONS

The preliminary findings indicated that there are differences between trace element concentrations present in serum and blood samples. The serum separation from whole blood is an important step to obtain reliable results of trace element in serum. Hemolyzed serum samples have to be excluded when the element determinations is required to evaluate systemic physiological functions

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