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# Use of thermal neutrons to perform clinical analyses in blood and urine samples

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In this study we show that the NAA technique can be used to perform clinical analyses of blood and urine, with many advantages towards the conventional methods. From the knowledge of the neutron flux and the induced activity, the concentration of elements were obtained. In comparison to the conventional techniques, this methodology uses smaller quantities of biological material and allows the simultaneous evaluation of the concentrations of several elements in biological samples at once, something not always possible in the conventional clinical analysis. Another important advantage is that it eliminates the use of standard materials, thus making the analyzing process practical and economic.

## Introduction

In the health field it is usual to perform a lot of clinical examinations, as presented in Figs 1 and 2, in order to identify anomalies in body organs. It is very expensive to perform all these conventional analyses, and it also takes a lot of biological material. The purpose of the present study was to investigate if there were physiological alterations on mammalians submitted to natural uranium ingestion for a long period using absolute neutron activation analysis. These analyses aim to observe if there are changes associated to the measured concentrations of Al, Br, Cl, Fe, I, Zn, K, Na, Mn, and Mg in the biological samples of blood and urine, during the experiment.

According to Figs 1 and 2, the conventional procedure involves large quantities of biological material and the use of different techniques. On the other hand, by using nuclear activation it is possible to quantify simultaneously the elements present in biological samples and so to compare the concentration of elements in the control animal with the results obtained with the doped one.

## Methodology

When a gold foil is irradiated in a neutron flux, the induced activity due to both thermal and epithermal neutrons is:

$$A_b^{\infty} = A_t^{\infty} + A_e^{\infty} \quad (1)$$

where $A_b^{\infty}$ is the saturation activity of a bare Au foil, $A_t^{\infty}$ is the activity due to thermal neutrons and $A_e^{\infty}$ is the activity due to epithermal ones. To know the activity due to thermal neutrons alone, the cadmium ratio technique has been used.[1] For this purpose, we define the cadmium ratio as:

$$R_{cd} = A_b^{\infty}/A_{cd}^{\infty} \quad (2)$$

where $A_{cd}^{\infty}$ is the saturation activity of the cadmium covered foil. Although being an excellent filter to thermal neutrons, cadmium is not fully transparent to the epithermal ones, so it is usual to introduce a correction factor[2] called cadmium factor, $F_{cd}$, and Eq. (1) can now be written as:

$$A_b^{\infty} = A_t\,[1 - F_{cd}/R_{cd}] \quad (3)$$

where $R_{cd}$ can be obtained experimentally from the ratio $A_b^{\infty}/A_t^{\infty}$ and $F_{cd}$ from Reference 2. The thermal neutron flux is then obtained from:

$$\phi_t = A_t^{\infty}/\sigma_{act}\cdot k_t \quad (4)$$

Particularly for the IEA-R1 reactor, where the present measurements have been performed, the thermal flux perturbation factor ($k_t$) is well known and can be found in Reference 3.

## Experimental

The cadmium ratio technique was used for the measurement of thermal and epithermal flux distribution. For this purpose, a gold solution was prepared,[4] and 10 µl of it were pipetted onto 1 cm$^2$ pieces of Whatman filter paper and then dried under infrared lamp. Two of these samples were prepared for each measurement, one to be irradiated bare and the other covered with cadmium, so we could obtain the cadmium ratio for the neutron flux. This procedure allowed a more homogeneous sample preparation, making it easier to perform solid angle corrections as well as minimizing the amount of gold needed for irradiation.

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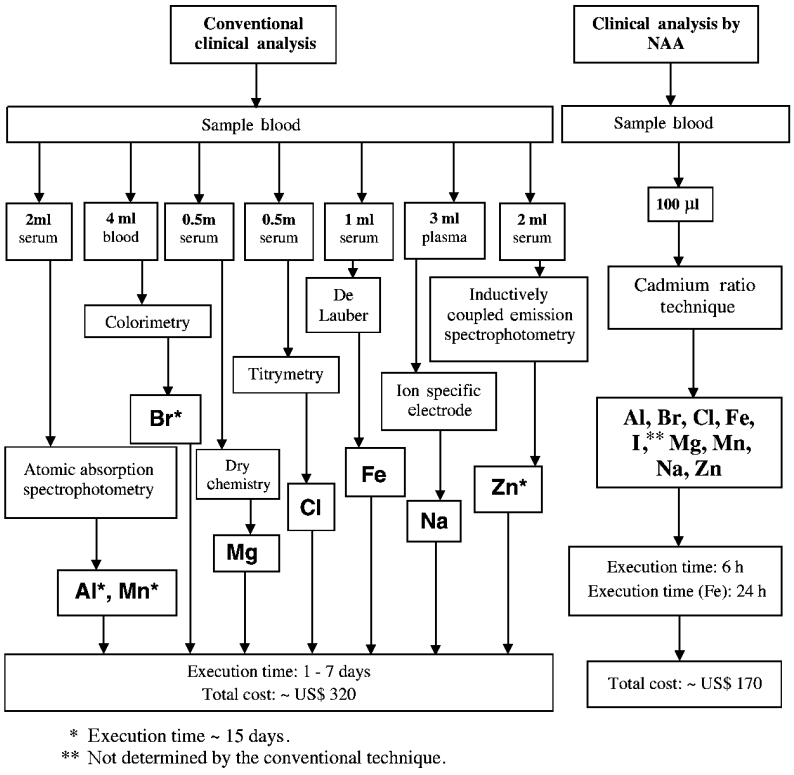


*Fig. 1.* Comparative diagram between conventional analyses and nuclear methodology for blood

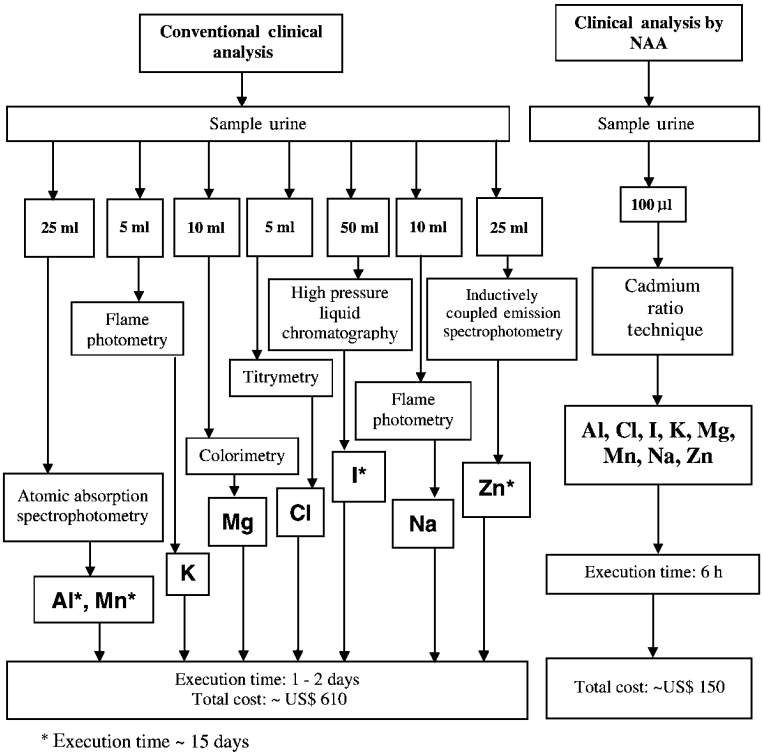


*Fig. 2.* Comparative diagram between conventional analyses and nuclear methodology for urine

*Table 1.* Concentration of potassium, sodium and chlorine in urine samples of control group by NAA and by the conventional analyses.[7,8] The uncertainties are assumed as one standard deviation (68% confidence level)

| K | | Na | | Cl | |
|---|---|---|---|---|---|
| NAA, $mg \cdot ml^{-1}$ | Flame photometry, $mg \cdot ml^{-1}$ | NAA, $mg \cdot ml^{-1}$ | Flame photometry, $mg \cdot ml^{-1}$ | NAA, $mg \cdot ml^{-1}$ | Colorimetry, $mg \cdot ml^{-1}$ |
| 2.53 ± 0.38 | 3.03 ± 0.55 | 3.32 ± 0.23 | 2.99 ± 0.25 | 3.11 ± 0.15 | 2.79 ± 0.18 |
| 3.01 ± 0.54 | 3.21 ± 0.42 | 3.26 ± 0.13 | 3.08 ± 0.27 | 1.86 ± 0.09 | 2.02 ± 0.09 |
| 3.91 ± 0.26 | 2.97 ± 0.32 | 3.47 ± 0.16 | 3.00 ± 0.27 | 4.40 ± 0.22 | 2.88 ± 0.21 |
| 1.53 ± 0.14 | 1.45 ± 0.11 | 3.33 ± 0.29 | 3.30 ± 0.21 | 5.20 ± 0.42 | 6.17 ± 0.26 |
| 1.60 ± 0.18 | 1.71 ± 0.10 | 2.68 ± 0.22 | 2.67 ± 0.24 | 6.18 ± 0.38 | 6.01 ± 0.29 |
| 2.24 ± 0.26 | 2.01 ± 0.25 | 2.53 ± 0.13 | 2.54 ± 0.25 | 3.25 ± 0.20 | 3.19 ± 0.18 |
| 2.85 ± 0.25 | 3.11 ± 0.31 | 3.40 ± 0.14 | 3.08 ± 0.27 | 1.30 ± 0.21 | 1.17 ± 0.15 |
| 4.30 ± 0.31 | 4.00 ± 0.33 | 3.59 ± 0.09 | 3.37 ± 0.29 | 4.13 ± 0.19 | 3.98 ± 0.14 |
| 1.60 ± 0.06 | nd | 3.31 ± 0.17 | 3.30 ± 0.22 | 5.93 ± 0.20 | 6.0 3± 0.17 |
| Mean values: | | | | | |
| 1.833 (16) | 1.896 (23) | 3.271 (16) | 3.044 (28) | 2.999 (19) | 3.087 (17) |
| *t*-Test | | | | | |
| $t = 2.25$ $P>0.05$ | | $t = 7.04$ $P>0.05$ | | $t = 3.45$ $P>0.05$ | |

nd: Not determined.

*Table 2.* Detection limits of elements present in biological materials

| Element/biological material | Nuclear parameters:[6] radioisotope ($T_{1/2}$); $E_\gamma$, keV | $\mu g \cdot g^{-1}$ (3σ) |
|---|---|---|
| Aluminum/blood | $^{28}$Al (2.24 m); 1779 | 8 |
| Aluminum/urine | | 3.5 |
| Bromine/blood | $^{80}$Br (17.68 m); 616 | 0.4 |
| Chlorine/blood | $^{38}$Cl (37 m); 1642 | 7 |
| Chlorine/urine | | 3.2 |
| Iron/blood | $^{59}$Fe (44 d); 1099 | 21 |
| Iodine/blood | $^{128}$I (25 m); 442 | 0.3 |
| Iodine/urine | | 0.2 |
| Magnesium/blood | $^{27}$Mg (9.4 m); 843 | 61 |
| Magnesium/urine | | 29 |
| Manganese/blood | $^{56}$Mn (2.5 h); 846 | 0.4 |
| Manganese/urine | | 0.2 |
| Potassium/urine | $^{42}$K (12.2 h); 1525 | 45.4 |
| Sodium/blood | $^{24}$Na (15 h); 1368 | 3 |
| Sodium/urine | | 1.3 |
| Zinc/blood | $^{69}$Zn (14 h); 438 | 1 |
| Zinc/urine | | 60 |

The biological samples of urine and blood came from an experiment which was performed at the facilities of the UNITOX laboratory from Universidade Santo Amaro (UNISA). In this experiment, four mammalians were housed in bails at controlled room temperature and fed daily with chow doped with uranyl nitrate at two different concentrations, one with 20 and two with 100 ppm, plus a control animal, fed with non-doped chow. This procedure was performed for 5 months. The uranium ingestion started after weaning (~60 days) and continued in the animal maturity. After the 5th month, these animals were sacrificed. During the experiment the daily control and measurements of the ingested food were carried out as well as the daily control and measurements of the animal weight.

To determine the concentration of the main elements in blood and urine, 100 µl aliquots of each biological sample were pipetted onto 1 $cm^2$ pieces of Whatman filter paper. Each biological sample, together with both the bare and cadmium-covered gold samples, was sealed into individual polyethylene bags and irradiated in a pneumatic station in the IEA-R1 reactor, allowing the simultaneous activation of these materials. After that, we obtained the gamma-spectra for both Au and biological samples in order to determine the neutron flux and the concentration of the activated elements in the biological sample under the same irradiation conditions. A HPGe detector connected to an ADCAM multichannel analyzer and to a PC computer was used to measure the induced gamma-activity. While counting the gold samples, the

area of the peak corresponding to the 411 keV transition of $^{198}Au$ [5] was evaluated for both samples. The same way, each biological sample was also gamma-counted, and the areas of the peaks corresponding to gamma-transitions related to the nuclides of interest were evaluated. The gamma-spectra evaluation was performed using the IDF computer software[6] and the calculation of the concentration for each element could be obtained.

## Results and discussion

Toxicological studies show that most of the uranium absorbed by ingestion in the human system is retained in different body organs and then is excreted, partially, through urine. The amounts of the non-excreted uranium accumulated in these organs, particularly in the kidneys, could cause lesions. The application of the neutron activation analysis to determine the concentration of Cl, K and Na present in biological urine samples from dogs (control and doped groups) allows to obtain the maximum of information for a detailed study of kidney function. For example, in Table 1 the quantitative results of urine sediment using NAA are presented and compared to the conventional clinical analyses.[7,8] The nuclear results are a mean of two replicate analyses and the errors associated represent one standard deviation.

The results involving the element concentrations present in the blood samples can also be calculated. For example, the aluminum concentration in blood as a function of time are shown in the Fig. 3. These results allow to compare the data from the control animal with the doped one.

Following the same procedure, the concentration of other elements could also be determined. The detection limits of the elements, measured in biological materials, using this nuclear methodology, are shown in Table 2.

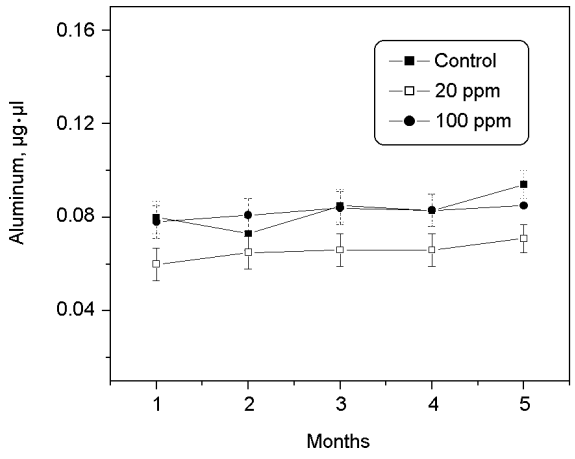


*Fig. 3.* Aluminum concentration in blood as a function of time for control animal and animals fed with chow doped with 20 ppm and 100 ppm uranyl nitrate

## Conclusions

In the present work the viability of using neutron activation analysis to perform blood and urine clinical analysis was checked. Particularly, the quantitative results of urine sediment using NAA were compared to the conventional analysis and the results were compatible.

The nuclear methodology (NAA) uses small sample quantities (100 µl) when compared to the conventional analysis. It also allows simultaneous evaluation of several element concentrations in biological samples, something not always possible in the conventional clinical analysis procedures. This way, NAA can be an alternative method for diagnosing anomalies in biological functions, specially when sample quantities are restricted.

Another important advantage is in the use of the absolute method to calculate the concentration of the elements in the biological samples using neutron activation. Of course, this procedure is much more demanding, as it is necessary to perform the measurement of the thermal neutron flux for each sample, as well as to determine the absolute efficiency of the gamma-detector, but, considering that this experiment involved the analysis of hundreds of biological material samples, the absolute method became agile and economic because it is possible to obtain the concentration of activated elements in each irradiation and it is not necessary to use standards. The main limitation of this method is, though, that it is necessary to have access to a nuclear reactor to perform the neutron activation in the samples.

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