BJRS

BRAZILIAN JOURNAL
OF
RADIATION SCIENCES
11-01A (2023) | 01-10 | e2207

X- ray experimental set-up for *in-vitro* nail test

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ABSTRACT

In this investigation, the elements Ca and S were evaluated in a single nail clipping using compact and portable X-ray spectrometer model X-123 SDD with Ag target. Experimental conditions for current, voltage and excitation time were investigated. These analyses can be useful for a variety of applications, including nutritional and medical diagnosis, such as, the evaluation of bone dysfunctions by measurement of Ca (bone decalcification) and S for nutrition evaluations (collagen production). This analysis offers some benefits comparatively to blood and serum analyses, such as, non-invasive collection, fast analyses (minutes) and low cost. In addition, nail clippings are simple to obtain, easy to store, and easy to transport. This pilot study show a positive expectation for clinical application using in-vitro nail test.

Keywords: **nail, FRX, Ions.**

ISSN: 2319-0612
DOI: https://doi.org/10.15392/2319-0612.2023.2207
Submitted: 2022-11-30
Accepted: 2023-06-07

1. INTRODUCTION

In recent years, alternative techniques to investigate specifics ions and metals, of clinical relevance, in the human body (blood, serum and urine) have made significant progress. These clinical investigations are very useful for various diagnoses and for routine analysis. In recent years, X-ray Fluorescence (XRF) technique has been applied to this clinical finality at IPEN/CNEN-SP, in collaboration with research centers from Brazil [1-6]. The success of this alternative procedure for ions dosage in body fluids, bones, muscles and other biological tissues motivated us to verify the use of portable X-ray Fluorescence Spectrometry for in vitro nail clipping tests for the diagnosis of some specific dysfunctions.

Nails are basically made up of a fibrous protein keratin, a protein of animal origin, composed by several amino acids. The main amino acid that makes up keratin is cysteine ($C_3H_7NO_2S$), that is, it has the element sulfur in its structure [7]. Therefore, nails contain small quantities of trace elements such as S (its main component), Ca, Cu, Fe, Zn, and others [8; 9; 10]. Like blood and urine, nail samples can be used as biomarkers of several dysfunction : while Fe, Se, and Zn have been correlated with colorectal cancer risk [11], the evaluation of metals (Pb, Cd, Hg) in nails have also been performed to investigate toxicity effects in the organism [12, 13, 14, 15].

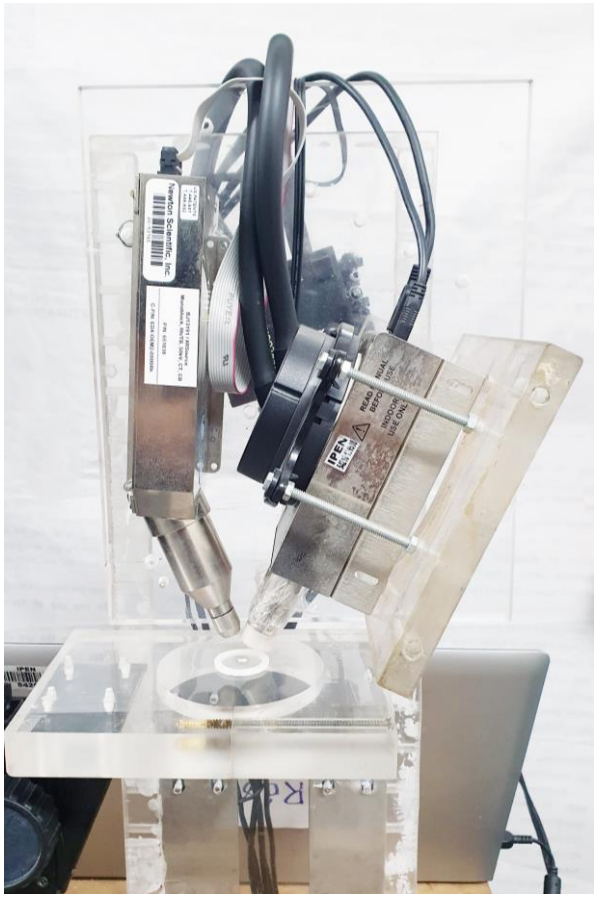
All these studies suggest that application of XRF technique for analysis of majoritary and trace elements in nails are very promising for clinical practice.

In this investigation, a portable and compact equipment for X-ray Fluorescence, using target of Ag, low voltages (tens of KV) and current (few μA), was evaluated for in-vitro nail clippings tests for Ca and S evaluation. There are some motivations and positive expectations for clinical applications such as to provide useful data for sports medicine by the evaluation of S, responsible in the organism for collagen production and maintenance of muscle tissues, as well as to check the viability to monitor bone decalcification by Ca evaluation.

2. MATERIALS AND METHODS

The Energy Dispersive X-ray Fluorescence (EDXRF) analysis was performed using a compact X-ray spectrometer model X-123 SDD with Ag target (Figura 1). The characteristic X-ray fluorescent intensity of K_α lines were measured with a Si Drift detector (25 mm^2 x 500 μm) with Be window (12.5 μm). For the spectrometer calibration, certified standard solutions containing varying concentrations of Ca and S were prepared. All the spectral analysis was performed using WinQxas software [16]. The precision and accuracy of the results were checked by analyzing NIST 1577b Bovine Liver.

Figure 1: *X-ray spectrometer with Ag target*



Source: Zamboni 2022

A group of 11 healthy volunteers (men), without known occupational exposure to metals, non-smokers, without drinking habits and, aged from 23 to 36 years, residents in São Paulo city (SP,

Brasil) was selected. At least, two nail clippings taken from each donor was collected. The fingernails were preferred to toenails, as they have a faster growth. Nail clippings (mass ~ 5 mg) were performed using a pair of scissors and placed in polyethylene bags. In the laboratory, each sample was placed in a flask with detergent and shacked (mechanical shaking) for ~20 minutes. After that, the sample was transferred to a beaker and washed with deionized water (Milli-Q) until the detergent was completely removed. In the next step, the samples were placed on filter paper and dried at room temperature. Finally, they were stored in plastic bag until to be used.

3. RESULTS AND DISCUSSION

Table 1 presents the results obtained in the analyses of reference material NIST 1577b together with their certified values. The results of the elements analysis are in good agreement with their respective certified values.

Table 1. Analysis of certified reference material NIST 1577b Bovine Liver

Elements	This Work MV ± 1SD	Certified values MV ± 1SD	RSD, %	|Z- Score|
S, gkg^{-1}	7.90 ± 0.27	7.85 ± 0.06	3.42	0.83
Ca, $mgkg^{-1}$	0.121 ± 0.009	0.116 ± 0.004	7.44	1.25

MV: Mean Value
SD: Standard Deviation
RSD: Relative Standard Deviation

The experimental conditions for voltage, current, excitation time as well as the appropriate choice of collimators and filters were investigated to reduce radiation exposure, to enable *in- vitro* analysis on nails. Table 2 presents the optimized experimental conditions for nail clipping tests. These evaluations were performed using the emission line K_α (2.1 keV for S and 3.6 keV for Ca). The results for Ca e S concentrations in nail clipping samples using EDXRF technique are shown in Table 3 and, they were expressed by mean value, standard deviation (±1 SD), minimum and maximum values. In this table, data from INAA and ICP-MS techniques were also included for

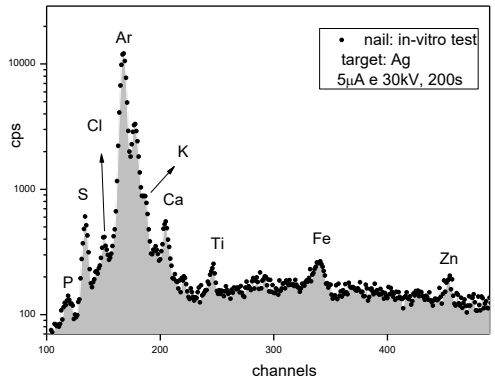
comparison. To visualize, in Figure 4 are shown the results of Ca e S concentrations by EDXRF; the mean value as well as the standard deviation (MV ± 1SD) were also included.

Table 2. Measurement conditions from the EDXRF spectrometer

Parameters	Conditions
X ray tube	Ag target
Voltage	30 kV
Current	5 μA
Atmosphere	without vacuum
Detector type	Silicon Drift with Be widow
Collimator	2 mm
Fixed Time count	200 s

To illustrate, in figure 2 is presented a XRF spectrum for a nail clipping using the optimized experimental condition for Ag target (5μA, 30kV, 200s). In this figure, the Argon peak (Ar) are due to its presence in air.

Figure 2. *Nail spectrum using X-ray experimental set-up with Ag target*



Source: Zamboni 2022

From the energy spectrum provided in figure 2 it is clear that the characteristic K_α X-ray signals are easily detected by XRF measurements involving a single clipping of a human fingernail. The

execution is fast, allows simultaneous analysis of P, S, Cl, K, Ca, Ti, Fe and Zn and the dose exposure (technician) is below the limits established according to the manufacturer (1 Sv/h, using 80 µA and maximum high voltage of 50 kV) [17].

Table 3. Ca and S concentrations in nails clipping

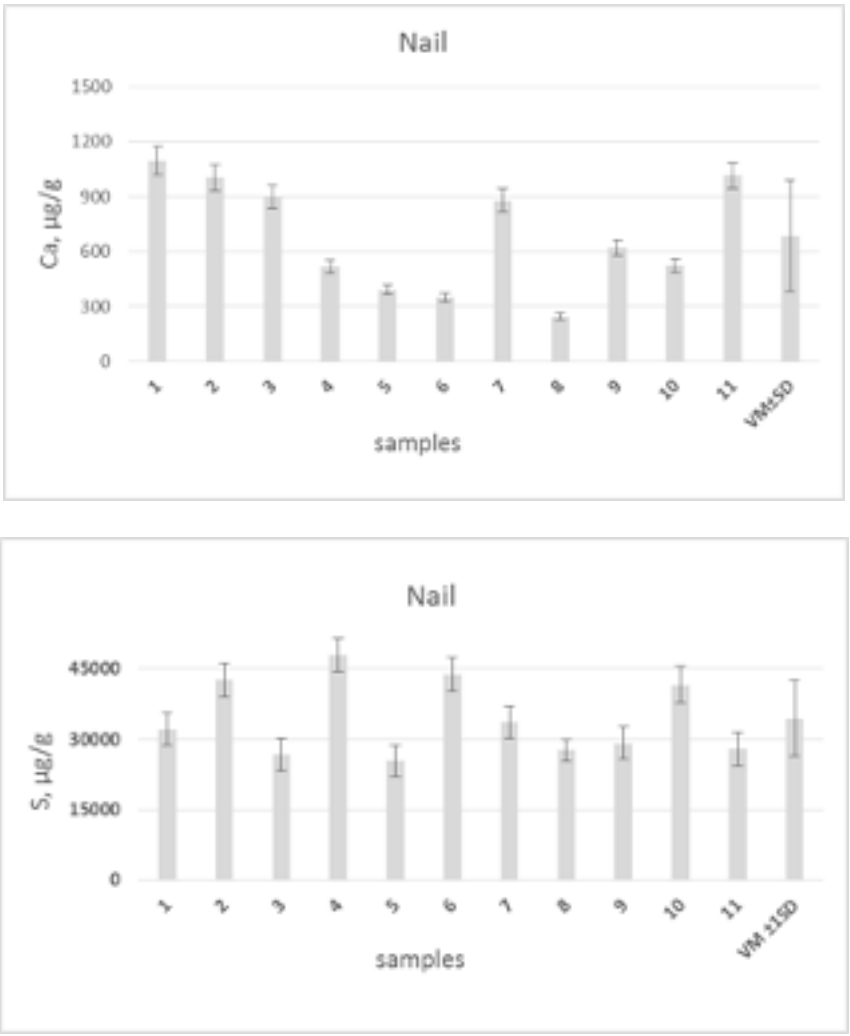
	Present study, 2022(EDXRF)	**WEE, *et al* [18], 2017 (INAA)**	**Rodushkin, *et al* [19], 2000 (ICP-MS)**
n (males)	**11**	**11**	**40**
age, years	**28.2**	**27.7**	**32.6**
Ca, µg/g			
MV	685	464	670
±1SD	303	281	240
minimum	243	116	345
Maximum	1097	1040	1160
S, µg/g			
MV	34423	33600	33000
±1SD	8089	5340	5400
minimum	25409	26600	23400
Maximum	47984	48900	43500

MV: Mean Value
n: number of samples
SD: Standard Deviation

According to the *t-test*, the from EDXRF results (Table 3) show non-significant differences ($p > 0.05$) when a comparison is performed with NAA [18] and ICP-MS [19] data. The concentration results obtained in this work for Ca e S (figure 3) indicated the viability of using X- ray experimental set-up for *in-vitro* nail clipping test with precision and accuracy, as well as its ability to determine other elements. This pilot study is a fundamental step towards the elementary evaluation of the nail by EDXRF, for future clinical applications. However, several measurements should be explored in future work to extend these assessments to the other elements simultaneously excited. These data will improve the correlation between Ca measurements in nail clipping for evaluation of bone

dysfunctions (bone decalcification) as well providing data involving the correlations between S measurements for collagen production (maintenance and fortification muscle tissues), very useful in sports medicine.

Figure 3. Ca and S concentrations (μg/g) in nails clipping.



Source: Zamboni 2022

Finally, the XRF procedure requires no preparation, only that the nails are clean, also offers a cost-effective and rapid measurement approach to help improve our understanding of elements in nails and their correlation to health. Besides, nail clippings are simple to obtain, easy to store and transport, compared to clinical analysis using body fluids.

4. CONCLUSION

The development of methodology to quickly assess Ca and S concentration from a single nail clipping could be a useful advance for clinical practices. In addition, there is another advantage, the feasibility of using this experimental X-ray set-up in regions lacking a clinical laboratory, for example, its use in a Basic River Health Unit.

Overall, trace element analysis in nail clippings by XRF presents an opportunity for new applications, as well as significant contributions to a better understanding of the role of trace elements in medical and biological sciences.

ACKNOWLEDGMENT

The authors thank the financial support of the FAPESP (15/01750-9) and the institutional support of the IPEN.

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