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PROGRESS AND TRENDS FOR RADIATION APPLICATIONS IN CLINICAL PRACTICE

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ABSTRACT

In this study, we intend to optimize the use of equipment based on the X-Ray Fluorescence technology to perform ions dosage in body fluids. This procedure can be an efficient alternative for clinical diagnosis, mainly in underserved regions of Brazil with the deficit of medical care in the hospitals. In addition, this procedure has potential use when the biological material is scarce, case of the pediatric practice in newborns and premature infants (blood collection is the main cause of transfusions) as well as in Hemodialysis units which requires several biochemical tests (before and after dialysis).

1. INTRODUCTION

The use of alternative analytical techniques to investigate specific electrolytes in body fluids has increases in last year's presenting significant progress in clinical practices. In the last years, Neutron Activation Analysis (NAA) and X-ray Fluorescence (XRF) techniques have been applied to this clinical finality at IPEN/CNEN-SP, in collaboration with research centers from Brazil [1-7]. The success in clinical applications motivated us to stimulate the use of the X-ray Fluorescence Spectrometry for these biochemical analyses (out of the nuclear reactor premises).

There are several motivations and positive expectations for these clinical applications, but the significant advantage for using the X-Ray Fluorescence technology in diagnosis is the viability to use small quantities of biological sample (few microliters) comparatively to conventional analyses (1mL, at least) [8]. In addition, its execution is fast (minutes) and without need of vacuum, allows simultaneous analyzes (which is not always possible by conventional procedures) [9,10] , no vacuum required and has a lower cost (eliminates the use of reagents and glassware).

The performance of XRF spectrometer, using of Ag and Au X-ray targets, associated with a Si Drift detector, was checked using certified standard solutions. The viability to perform whole

blood analysis was also evaluated. The advantages and potential applications for clinical usage (ions dosage) were discussed.

2. METHOD

2.1. Sample preparation

The collection of the biological material consists in puncturing the finger with a lancet and deposit a whole blood drop in filter paper (Whatman – nº41), and store in an appropriate receptacles, no need cooling. The samples came from Bank Blood at São Paulo city, Brazil and they were collected according to the rules approved by Human Research Ethics Committee (CAAE 69992117.7.0000.0081).

2.1. Energy Disperse X-Ray Fluorescence (EDXRF) technique

The EDXRF analysis was performed using X-ray spectrometer (Amptek®) with Au and Ag X-ray targets. 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 (12.5μm). The spectra analysis was performed using WinQxas software (IAEA, version 1.3) [11].

3. RESULTS AND DISCUSSION

The performance of XRF spectrometer, using of Ag and Au X-ray targets, was checked by evaluating the linearity, reproducibility, sensitivity and detection limit parameters usually considered for validation procedures on analytical methods [12]. Standard solutions containing varying concentrations of Ca, Cl, K and Fe were prepared in the range of 100 - 600 μg mL-1 for Ca and Fe, and 500 - 1000 μg mL-1 for Cl and K. The standard solutions were prepared following the same blood samples procedure (a drop deposited in filter paper). For each standard, three repetitions were made using 30 kV and 5 μA and 200 s of excitation time. The mean values determined (MVdet ± 1SDdet) for the certified solutions (MVs ± 1SDs) as well as the relative standard deviation (RSD, %), the sensitivity (Si) and detection limit (DL) are presented in Table 1. According to these data the precision of the method is considered satisfactory (RSD < 5%).

For whole blood analysis the excitation conditions was optimized in 30 kV and 5 μA and counting time of 300 s. The whole blood spectra using this optimized condition are presented in Figures 1 and 2 for Ag and Au targets, respectively.

Table 1. Standard solutions data using Ag and Au targets

Element, K_α *(keV)* *Targets*	$MV_s \pm 1SD_s$ *μg mL^{-1}*	$MV_{det} \pm 1SD_{det}$ *μg mL^{-1}*	RSD %	S *cps mg^{-1}L*	DL *mg L^{-1}*
Ca (*3.69)*					
Ag	1000 ± 10	986 ± 20	2.1	54.77	0.23
Au		1026 ± 51	1.7	29.93	0.40
Cl *(2.62)*					
Ag	1006 ± 5	1003 ± 21	4.0	53.66	0.24
Au		1045 ± 53	2.5	5.19	0.16
K (*3.31*)					
Ag	1000 ± 10	980 ± 25	2.6	31.03	0.47
Au		1020 ± 51	1.7	14.50	0.61
Fe *(6.40)*					
Ag	10032 ± 54	10050 ± 310	3.1	61.16	0.28
Au		10025 ± 64	0.7	92.44	0.27

$MV_s \pm 1SD_s$: certified values
$MV_{det} \pm 1SD_{det}$: determined values

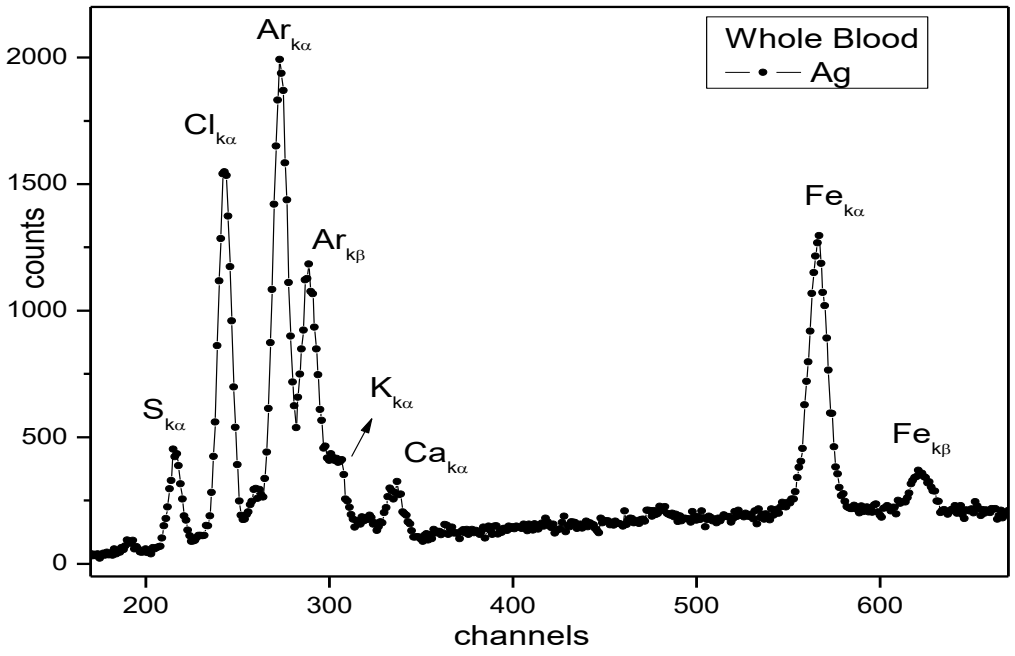

Figure 1. Whole blood spectrum using the XRF spectrometer with Ag target in the optimized condition. The Ar peak was due to its presence in air.

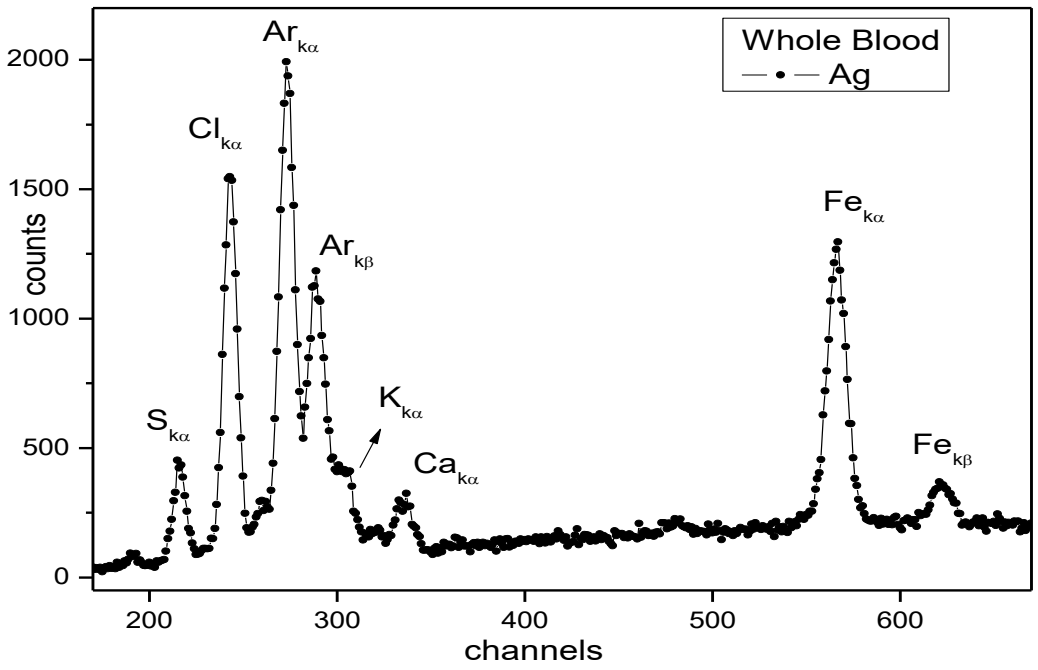

Figure 2. Whole blood spectrum using the XRF spectrometer with Au target in the optimized condition. The Ar peak was due to its presence in air.

In Table 2 the whole blood element concentrations by EDXRF are presented as the mean value (MV) and standard deviation (±1SD) from duplicate analyses. The confidence interval of 95% (normal range usually adopted for clinical practice) was included for comparison. The Student´s t-test was applied for comparison results.

Table 2. Whole blood concentrations by EDXRF technique using Ag and Au targets

Elements (Kα, keV) *n*	MV ± 1SD [range] (mgL^{-1})	
	XRF (Ag Target) *DL*	XRF (Au Target) *DL*
Ca (3.69) *30*	291 ± 80 [131 – 451] *6.2*	266 ± 67 [132 – 400] *6.9*
Cl (2.62) *32*	2549 ± 401 [1747 – 3351] *27.2*	2638 ± 312 [1975– 3262] *54.6*
K (3.31) *32*	1209 ± 188 [833 – 1585] *20.6*	1265 ± 163 [939 – 1591] *22.3*
Fe (6.40) *40*	332 ± 41 [250 – 414] *9.1*	324 ± 39 [246 – 402] *6.4*

n: number of samples

According to Table 2, the EDXRF procedure for whole blood analyses was proved to give reliable results with detection limits at levels of ~ 6 mgL−1 (minimum) to ~55 mgL−1 (maximum). Considering that the ranges for Ca, Cl, K and Fe (in whole blood) are in the order of hundreds of mgL-1, both targets are very promising for these ions dosage requiring a small amount of sample collection (~100 times less comparatively to the conventional tests), simultaneous analysis, short time of analysis (minutes) and simple sample preparation. Besides that, this procedure offers a non-destructive alternative for use in these clinical exams. Related to the concentration results, the comparison between the two targets are in good agreement ($p > 0.05$). In addition, this alternative procedure has the potential to be used when the biological material is scarce, case of the pediatric practice in newborns and premature infants, as well as in Hemodialysis units which requires several biochemical tests (before and after dialysis), i.e., successive blood collections, mainly for patients who perform dialysis several times a week.

4. CONCLUSIONS

The XRF spectrometer performance, consisting of Ag and Au X-ray targets, showed to be appropriate for clinical usage. This analytical alternative procedure is very promising for whole blood Ca, Cl, K and Fe dosage requiring a small amount of sample, simultaneous analysis, short time analysis and simple sample preparation (liquid samples were dripped in filter paper) . In addition, this procedure offers a non-destructive alternative for clinical usage with low cost: it is not necessary to use specifics reactants and different apparatus (important in work routine) and the samples can be storage without refrigeration. We intend to stimulate the biochemical analysis of body fluids using this alternative setup in underserved regions of Brazil (with the deficit of medical care in the hospitals) as well as when the viability of biological material is scarce (pediatric clinical)

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