



## **EVALUATION OF MULTIELEMENTS IN HUMAN SERUM OF PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA USING SRTXRF**

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**Abstract:** *Idiopathic Thrombocytopenic Purpura (ITP) is a blood disorder characterized by an abnormal decrease in the number of platelets in the blood. ITP results from development of an antibody directed against a structural platelet antigen (an autoantibody). In this work, forty patients suffering from ITP and sixty healthy volunteers were analyzed. Blood was collected into vacutainers without additives. Directly after collection, each blood sample was centrifuged at 3000 rev/min for 10 min in order to separate blood cells and suspended particles from blood serum. The sera were transferred into polyethylene tubes and stored in a freezer at 253 K. All the serum samples had been collected from people who live in the urban area of Rio de Janeiro City/Brazil. For the sample preparation, a volume of 500 µL of serum was taken and diluted with 1500 µL of ultrapure water (Milli-Q water). An internal standard consisting of 105 µL of Vanadium solution ( $V - 1000 \mu\text{g}\cdot\text{g}^{-1}$ ) was added. After the dilution a small aliquot of 5 µL of each sample with the internal standard was pipetted on a precleaned perplex sample carrier. The samples were left to dry very slowly under an infrared lamp. The samples were analyzed in triplicate. The measurements were performed at the X-Ray Fluorescence Beamline at Brazilian National Synchrotron Light Laboratory (LNLS), in Campinas, São Paulo using a monochromatic beam with maximum energy of 20 keV for the excitation and an Ultra-LEGe detector with resolution of 148 eV at 5.9 keV. Standard solutions with Vanadium as internal standard were prepared for calibration system. It was possible to determine the elemental concentrations of the following six elements: Na, P, S, Cl, K and Ca. By using t-test it could be seen significant differences ( $\alpha = 0.05$ ) between groups of healthy subjects and four groups of ITP. The elements that presented significant differences for the mean of their concentrations between each one of the ITP group and control group in  $\mu\text{g}\cdot\text{g}^{-1}$ , were Phosphorous ( $136\pm 12$  and  $92\pm 12$ ), Sulphur ( $1077\pm 97$  and  $847\pm 80$ ), Chlorine ( $2905\pm 385$  and  $2266\pm 378$ ), Potassium ( $137\pm 118$  and  $82\pm 15$ ) and Calcium ( $64\pm 7$  and  $44\pm 6$ ) respectively.*

**Keywords:** X-ray Fluorescence, Synchrotron Radiation, Idiopathic Thrombocytopenic Purpura, Human Serum and Low Z Elements.

## 1. INTRODUCTION

The determination of trace elements levels in human serum can be utilized as indicators for several pathological conditions, the simultaneous detection of certain elements in the serum offers a very interesting approach in the diagnosis and treatment of various diseases [Faa *et al.* (2001); Miura *et al.* (2002); Bárány *et al.* (2002); Cesur *et al.* (2005); Canellas *et al.* (2006)]. In this work, trace low z elements were analyzed in serum of patients with Idiopathic Thrombocytopenic Purpura (ITP) by Total Reflection X-Ray Fluorescence using Synchrotron Radiation (SRTXRF). It was possible to determine the elemental concentrations of the following six elements: Na, P, S, Cl, K and Ca. By using t-test it could be seen significant differences ( $\alpha = 0.05$ ) between group of healthy subjects and control group. The elements that presented significant differences for the mean of their concentrations between each one of the ITP group and control group were Phosphorous (P), Sulphur (S), Chlorine (Cl), Potassium (K) and Calcium (Ca).

Idiopathic Thrombocytopenic Purpura is a blood disorder characterized by an abnormal decrease in the number of platelets in the blood. ITP results from development of an antibody directed against a structural platelet antigen (an autoantibody). Platelets are also called thrombocytes, meaning cells that form clots. The decreased number of platelet that can result in signs are petechiae, easy bruising, abnormal menstrual bleeding, or sudden and severe loss of blood from the digestive tract. The cause of ITP is not known and their diagnosis requires that other disorders be excluded through selective tests (Williams, 1996). The ITP affects, generally, more women than men and is more common in children than in adults. There is no difference of sex among children. The incidence is 1 for a group of 10,000 individuals (George, 2002).

## 2. EXPERIMENTAL SECTION

### 2.1. Population Characteristics

Population characteristics from all the serum samples were provide by State Institute of Hematology Arthur de Siqueira Cavalcanti (HEMORIO). We studied forty patients suffering from ITP and sixty healthy volunteers. All the serum samples had been collected from people who live in the urban area of Rio de Janeiro City, Brazil. This study was performed with the approval of the ethics committee and all subjects volunteered for the studies with informed consent.

### 2.2. Sample Preparation

Blood was collected into vacutainers without additives. After collection, all blood samples were centrifuged at 3000 rev/min for 10 min in order to separate blood cells and suspended particles from blood serum. The sera were transferred into polyethylene tubes and stored in a freezer at 253 K until analyzed [Zarcadas *et al.* (2001)].

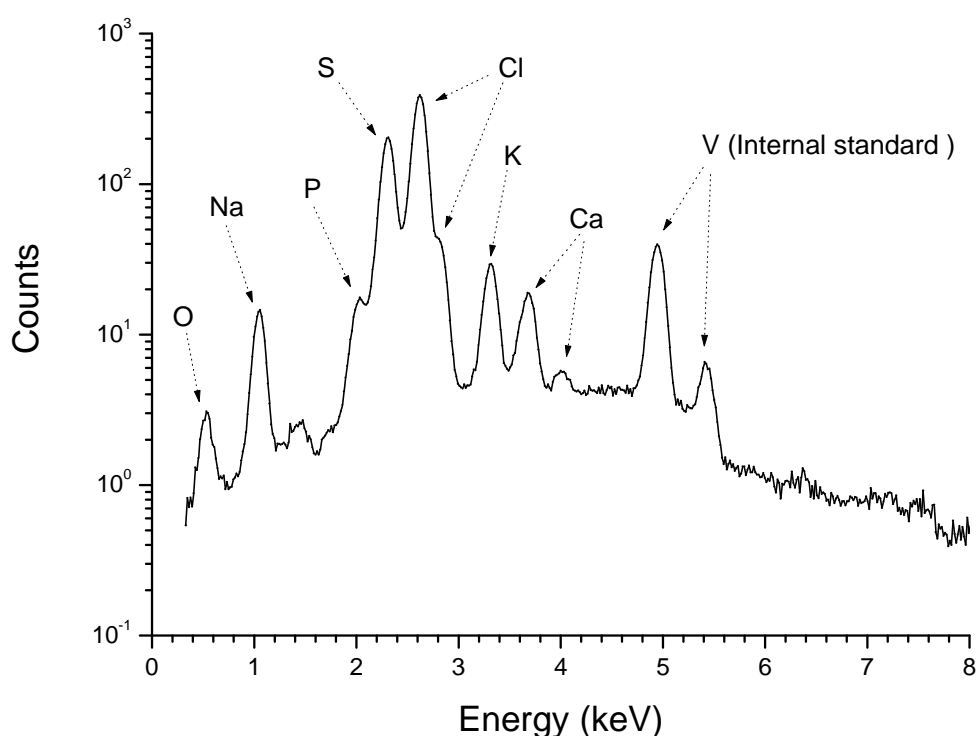
For the sample preparation, a volume of 500  $\mu\text{L}$  of serum was taken and diluted with 1500  $\mu\text{L}$  of ultrapure water (Milli-Q water). An internal standard consisting of 105  $\mu\text{L}$  of Vanadium solution ( $\text{V} - 1000 \mu\text{g.g}^{-1}$ ) was added. After the dilution a small aliquot of 5  $\mu\text{L}$  of each sample with the internal standard was pipeted on a precleaned perplex sample carrier. The samples were left to dry very slowly under an infrared lamp. The samples were analyzed in triplicate. The validation of the methodology was carried out by the analysis of a standard reference material for cation chromatography (Multi-element Standard VII – MERCK KGaA).

### 2.3. Instrumentation

SRTXRF analyses were performed at the X-Ray Fluorescence Beamline D09B at the Brazilian National Synchrotron Light Laboratory (LNLS) [Perez *et al.* (1999)], in Campinas, São Paulo, using a monochromatic beam with maximum energy of 20 keV for the excitation and an Ultra-LEGe detector with resolution of 148 eV at 5.9 keV. The detector is equipped with an ultrathin entrance window of Polymer (0.4  $\mu\text{m}$ ) required to measure low-energy X-ray fluorescence radiation of low Z elements. All samples were excited for 100 s and the experiments were performed in a high vacuum chamber ( $2.5 \times 10^{-5}$  mbar) to avoid air absorption. The X-ray spectra obtained were evaluated by the QXAS software package (Bernasconi, and Tajani, 1996), distributed by the International Atomic Energy Agency.

### 3. RESULTS AND DISCUSSIONS

It was possible to determine the elemental concentrations of the following six elements: Na, P, S, Cl, K and Ca. Figure 1 shows the X-ray fluorescence spectrum of a serum sample using SRTXRF. The element oxygen in the spectrum is just the perplex composition (about 32 %). Because this, the oxygen concentration was not evaluated in the serum samples.



**Figure 1. The X-Ray Fluorescence Spectrum of a Serum Sample Using SRTXRF.**

The *t-test* ( $\alpha = 0.05$ ) was applied in order to check whether the low-Z elemental concentrations found in the two populations (control group and ITP patients) had the same mean values (null hypothesis). T-test analyses showed that the following elements Phosphorus, Sulphur, Chlorine, Potassium and Calcium presented significant differences between groups of patients with ITP and Control Group. On the other hand, no significant difference was observed for Sodium. All elements presented greater levels in ITP serum samples when compared with the control group.

The concentration of phosphorus found in the serum samples of patients with ITP was approximately 45 % larger than the values determined in control group. The phosphorus participates in various metabolic and structural functions in the body and this increase in concentration of P can influence the decrease of these functions.

Concentrations of Sulphur and Chlorine were larger in the serum samples of ITP (approximately 30 %) than in healthy subjects. Sulphur participates in the formation of all cellular proteins. On the other hand, chlorine regulates acid-base balance of blood, aiding the functioning of the liver (elimination of the metabolites of the body). Therefore, these increases can affect the formation of cellular proteins and impair the liver.

In relation, the concentrations of Potassium and Calcium were approximately 65 % and 45 % larger than the values determined in the control group, respectively. Calcium is involved with a number of functions such as heart rate, the contraction of muscles, the bone formation and blood coagulation.

The increased concentration of calcium may be related to reducing the number of platelets that are essential for initiating the process of blood coagulation. If the number of platelets decreases due to the development of ITP, there is a decrease in impairment of calcium the serum with the coagulation process. This can cause the increase of concentration of circulating calcium in serum. The increase in potassium concentration may have the same reasons because this element is the anti-hemorrhagic action. If there is a commitment in the process of coagulation (production of prothrombin), the demand for K for this process is reduced. This means that their concentration in serum increase.

Table 1 shows the elemental concentrations between groups of patients with ITP and Control Group (CG), in  $\text{mgL}^{-1}$ , respectively.

**Table 1. Mean and Confidence Interval in Serum Samples ( $\text{mgL}^{-1}$ ).**

<i>Element</i>	<b>ITP</b>		<b>Control Group</b>	
	Mean	Confidence Interval <sup>a</sup>	Mean	Confidence Interval <sup>a</sup>
<b>Na</b>	3053	(2545 – 3561)	2704	(2187 – 3221)
<b>P</b>	136	(124 – 148)	92	(80 – 104)
<b>S</b>	1077	(980 – 1174)	847	(767 – 927)
<b>Cl</b>	2905	(2520 – 3290)	2266	(1888 – 2644)
<b>K</b>	137	(119 – 155)	82	(67 – 97)
<b>Ca</b>	64	(57 – 71)	44	(38 – 50)

<sup>a</sup>95 % confidence level.

#### 4. CONCLUSIONS

Total Reflection X-Ray Fluorescence induced by Synchrotron Radiation is a powerful tool for the determination of low Z in human serum samples. The elements Na, P, S, Cl, K and Ca were identified and their concentrations were determined in human serum. The *t-test* was applied in order to check whether the two populations had the same mean values. This test showed that there are real differences among the elemental concentrations of following elements; P, S, Cl, K and Ca. These results could help biomedical field with regard to early diagnosis and improved medical treatment.

Thus, our findings indicate that these elements can be related to the biochemical processes in ITP. On the other hand, new experimental measurements must be conducted to reaffirm these conclusions.

#### 5. ACKNOWLEDGEMENTS

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## **7. RESPONSIBILITY NOTICE**

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