

# ELEMENTAL DETERMINATION OF AUSTENITIC STAINLESS STEEL ALLOY USED AS BIOMATERIAL BY NEUTRON ACTIVATION ANALYSIS

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## ABSTRACT

Austenitic stainless steel alloys, mainly those produced according to ISO 5832-1, have received much attention due to their promising characteristics to be used as biomaterials. The aim of this study was to establish the proper conditions of neutron activation analysis (NAA) in order to determine chemical elements in a sample of ISO 5832-1 stainless steel. These determinations are of great interest for further evaluation of its corrosion resistance and of cytotoxicity of corrosion products. For the analyses, chips of ISO 5832-1 austenitic stainless steel were obtained. Aliquots of this material were weighed in polyethylene involucres and irradiated together with synthetic element standards at the IEA-R1 nuclear research reactor. Short and long irradiations were carried out using thermal neutron flux of about  $4.5 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ . Quality control of the results was performed by analyzing two certified reference materials (CRMs). The elements concentrations of Cr, Cu, Mn, Mo and Ni obtained in the ISO 5832-1 austenitic alloy are within the specification values of this material. Besides, the elements As, Co, V and W were determined in this alloy. The sensitivity of the technique was verified by the determination of detection and quantification limits. In the case of CRMs, their results presented precision and accuracy for most of elements with relative standard deviations and relative errors lower than 15 %. Results obtained in this study demonstrated the viability of applying NAA in the analysis of the ISO 5832-1 stainless steel alloy.

## 1. INTRODUCTION

Currently, there is a wide variety of devices and biomaterials being developed and improved for using in the treatment of diseases and injuries. Thus, the concept of biomaterials has expanded greatly, as approached by Parida et al [1]. Among several types of austenitic stainless steels, mainly those produced according to ISO 5832-1 are used to supply the high demand of biomaterials for orthopedic prostheses, since this alloy presents biocompatibility, low cost, good mechanical and corrosive performance in relation to titanium and Cr-Co alloys [2].

The resistance to corrosion and the toxicity of corrosion products to the human body depend on the element composition of the material. Therefore, it is important to evaluate the composition of the biomaterials in order to provide safety to the patients, since some metallic ions liberated in the orthopedic implants such as Al, Co, Cr, Ni, Ti and V cause adverse

biological reactions, as approached by Sansone et al [3]. Certain amounts of chemical elements are essential for the human organism, however they can become toxic in high doses.

For elemental analyses in metallic alloys, several analytical techniques have been used, such as inductively coupled plasma atomic emission spectrometry (ICP-AES) [4], atomic absorption spectrometry (AAS) [5], anodic stripping voltammetry [6], UV-Visible spectrophotometry [7] and neutron activation analysis (NAA) [7-9].

Among the most recent studies of the analyses of alloys, stands out the research of Sipola et al [5] that analyzed stainless steel by the electrolytic extraction and subsequent analysis by graphite furnace atomic absorption spectrometry (GF AAS) and flame atomic absorption spectrometry (FAAS). The elements determined were Al, Cr, Ti and V as alloying elements and As and Cu as impurity elements. Their results showed that these methods can provide detailed information about elements in inclusions and in the stainless steel matrix.

Another study of great interest was published by Acharya et al [7] that determined nickel in the finished product alloys and in certified reference materials (CRMs) by applying analytical techniques of UV-visible spectrophotometry and neutron activation analysis. Their results indicated that nickel can be determined accurately by both techniques.

Among various analytical methodologies available for elemental determination in steels, in this study neutron activation analysis (NAA) was used due to its numerous advantages for the analyses of this type of matrix. NAA does not require the sample dissolution; it allows a multi-elemental determination with good precision and accuracy of the results and presents high sensitivity for the detection of elements. The application of NAA in the elemental characterization of alloys and of their corrosion products is of great relevance for a contribution in the development and improvement of alloys to be used as biomaterials.

The objective of this study was to determine concentrations of the chemical elements in the ISO 5832-1 austenitic stainless steel by applying the neutron activation analysis in order to evaluate whether the elements are within the specification for use as biomaterial

## **2. EXPERIMENTAL**

### **2.1. Materials**

#### **2.1.1. Austenitic stainless steel alloy sample preparation**

A sample of ISO 5832-1 austenitic stainless steel in the form of bar was purchased from Villares Metals S/A. For neutron activation analysis, this austenitic stainless steel alloy was obtained in small fragments of chips. The dimensions of the chips used in the analyses were smaller than 1 cm.

For the analyses, these fragments were cleaned to eliminate eventual impurities originating from the equipment used for cutting the material. The chips were cleaned using acetone p.a Merck and MilliQ purified water. The solution used for this cleaning was separated from the

chips by a filtration using a filter paper. The samples were then placed in a Petri dish for drying at room temperature inside a laminar flow cabinet.

### 2.1.2. Certified reference materials

The quality of the analytical results was evaluated by analyzing two standard reference materials namely SRM 363 chromium-vanadium steel (modified), from the National Institute of Standards and Technology (NIST), USA [10] and B.C.S / S.S. No 467 austenitic stainless steel from British Chemical Standards [11]. These two certified reference materials (CRMs) were acquired in the form of chips.

## 2.2. Neutron Activation Analysis (NAA)

### 2.2.1. Synthetic elemental standard preparation

The certified standard solutions provided by Spex CertiPrep Chemical USA were diluted and single or multielement solutions were prepared. Aliquots of 50  $\mu\text{L}$  of these solutions were pipetted on sheets of Whatman No 40 filter paper using an Eppendorf automatic pipettor which was previously checked the calibration. The filter paper sheets with the pipetted solutions were placed in a desiccator for drying at room temperature for a period at least 24 h. After drying, these sheets were removed from the desiccator, cut at the base, folded and placed into polyethylene envelopes using tweezers. The polyethylene envelopes were prepared using a heat sealer, demineralized polyethylene foils and cellophane foil. Table 1 shows the data of the standard elemental solutions and mass of the elements used.

**Table 1: Data of the standard elemental solutions and mass of the elements used in the neutron activation analysis**

Standard code	Elements	Element concentrations of the standard solutions, $\mu\text{g mL}^{-1}$	Element mass in 50 $\mu\text{L}$ solution, $\mu\text{g}$
As	As	30	1.5
Co	Co	400.80	20.04
Cr	Cr	1002	50.10
Mn	Mn	1000	50.00
Mo	Mo	501.50	25.075
Ni	Ni	10039.5	501.95
Ta	Ta	100.20	5.01
V	V	999	49.95
W	W	200.60	10.03

In the case of iron standard, Carlo Erba, 99.9 % purity metal iron in powder was utilized. Fifty mg of this reagent were weighed in a polyethylene envelope using an analytical balance.

### 2.2.2. Procedure for neutron activation analysis

The NAA procedure consisted of the following steps: synthetic elemental standard preparation, sample weighing, irradiation in the reactor, measurements, data processing and interpretation of the results. For irradiation, about fifty mg of each material (sample and certified reference materials) were weighed in a polyethylene envelope using an analytical balance of the Shimadzu brand model AUW220D, with a precision of 0.00001 g.

In this study, short and long irradiations were performed in order to determine a great number of elements.

#### **2.2.2.1. Procedure for short irradiation**

Sample and standards placed in polyethylene envelopes were inserted into a polyethylene device called “rabit” and irradiated using a Pneumatic Station No. 4 of the IEA-R1 nuclear research reactor of the Nuclear and Energy Research Institute (IPEN-CNEN / SP) for a period of 5 s under a thermal neutron flux of  $1.9 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ . This same procedure was performed for the analyses of the certified reference materials.

Gamma ray activity measurements of sample and standards were carried out using a GC3020 Model hyperpure germanium semiconductor detector coupled to a Canberra digital spectrum analyzer (DAS 1000) and a microcomputer. The counting system had a resolution (FWHM) of 0.92 keV for 121.97 keV peak of  $^{57}\text{Co}$  and 1.69 keV for 1332.35 keV peak of  $^{60}\text{Co}$ . For data acquisition and its processing, the software Genie 2000 version 3.1 of Canberra was used.

The measurement was performed about 3 min of decay time and the counting time used was of 300 s for Mn and V determinations. The second measurement was performed after 30 min of decay time and counting time of 600 s for Mn determination. The radioisotopes of the gamma spectra were identified by gamma ray energies and half-life. The radioisotopes utilized and their half-lives and gamma ray energies (in parentheses) were  $^{56}\text{Mn}$  (2.58 h; 846.76 keV) and  $^{52}\text{V}$  (3.75 min; 1434.08 keV)[12].

#### **2.2.2.2. Procedure for one-hour irradiation**

In this case, samples, certified reference materials and synthetic elemental standards were individually wrapped in aluminum foil. This set of envelopes was wrapped in a new aluminum foil and then placed in an aluminum device for irradiation at the nuclear research reactor, IEA-R1 of IPEN-CNEN/ SP under thermal neutron flux in the order of  $4.5 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$  for a period of 1 h.

The counting system used was the same that was utilized for short irradiation. The countings of the standards and samples were performed twice for different decay times in order to eliminate the problem of spectral interferences and to determine a great number of elements. The first measurement was performed after one day of the decay time for the determinations of the elements As, Cu, Mn, Mo and W. The counting times used for standard was 3,600 s and for sample and reference materials was 3,000 s.

The second measurement was performed approximately one week of decay time for Co, Cr, Fe, Ni and Ta determinations. The counting times used for standards, sample and certified reference materials ranged from 7,200 s to 10,000 s. The exceptions were for iron standard

measurement which counting time was of 5,400 s and for nickel standard, the counting time was of 10,000 s.

The identification of the radioisotopes in the gamma spectra was carried out by gamma ray energies and half-life. In Table 2 are presented nuclear data of the radioisotopes identified in the sample and the certified reference materials in the irradiations of one hour [12].

**Table 2: Nuclear data of radioisotopes used in one-hour irradiations.**

Element	Radioisotopes	Gamma ray energy, keV	Half-life
As	<sup>76</sup> As	559.10	26.32 h
Co	<sup>60</sup> Co	1173.24	5.27 y
Cr	<sup>51</sup> Cr	320.08	27.7 d
Fe	<sup>59</sup> Fe	1099.25	44.5 d
Mn	<sup>56</sup> Mn	846.76	2.58 h
Mo	<sup>99</sup> Mo	739.58	65.94 h
Ni	<sup>58</sup> Co	810.77	70.82 d
Ta	<sup>182</sup> Ta	1221.41	114.5 d
W	<sup>187</sup> W	479.57	23.9 h

The concentrations of the elements were calculated by the comparative method of activation analysis. In this method, the samples are irradiated simultaneously with the elemental standards and measured at the same geometry. The elemental concentrations were calculated using the equation (1) [12].

$$C_s = [m_{st} \cdot A_s \cdot e^{0.693(t_{ds} - t_{dst})/t_{1/2}}] / [M_s A_{st}] \quad (1)$$

where the indices s and st refer to sample and standard, respectively;  $M_s$  = total sample mass;  $m_{st}$  = mass of the element in the standard;  $C_s$  = concentration of the element in the sample;  $t_{1/2}$  = half-life time of the radioisotope considered;  $t_d$  = decay time;  $A_s$  = counting rates of the considered radioisotope in the sample at decay time  $t_{ds}$ ;  $A_{st}$  = counting rates of the considered radioisotope in the elemental standards at decay time  $t_{dst}$ .

### 2.3. Treatment of the Data

From the results obtained in the analyses, statistical parameters of arithmetic mean, standard deviation, relative standard deviation and relative error were calculated.

#### 2.3.1. Standardized difference - Z score

The quality control of the results in relation to the accuracy was evaluated by the analysis of the certified reference materials. For the results these analyses, the Z score values or standardized difference parameters were calculated by applying the equation (2) [14].

$$Z \text{ score} = \frac{X_{\text{lab}} - X_{\text{ref}}}{\sqrt{SD^2 + u_{(\text{ref})}^2}} \quad (2)$$

where  $X_{\text{lab}}$  is the value obtained experimentally,  $X_{\text{ref}}$  is the certified value,  $SD$  is the standard deviation of the value obtained and  $u_{(\text{ref})}$  is the combined uncertainty of the certified value. If  $|Z \text{ score}| \leq 2$ , the result obtained is considered satisfactory. If  $2 < |Z \text{ score}| < 3$  the result is questionable and if  $|Z \text{ score}| \geq 3$  the result is unsatisfactory [14].

### 2.3.2. Limits of detection and quantification

The limits of detection and of quantification were calculated for As, Co, Cr, Cu, Fe, Mn, Mo, Ni, V, and W elements of the ISO5832-1 austenitic stainless steel alloy using the Currie criterion [15]. According to this Currie criterion, in NAA the minimum detectable and quantifiable quantity in terms of counting rates are given respectively by equations (3) and (4).

$$LD_T = 3.29 \times (BG^{1/2} / LT) \quad (3)$$

$$LQ_T = 10 \times (BG^{1/2} / LT) \quad (4)$$

where  $LD_T$  and  $LQ_T$  are the counting rates corresponding to the detectable and quantifiable minimum concentrations, respectively;  $BG$  is the counting rate for the area under the peak (background) and  $LT$  is the counting time.

Having the values of  $LD_T$  and  $LQ_T$  the limit values in concentration units were calculated by using equation (1).

## 3. RESULTS AND DISCUSSION

Tables 3 and 4 show the results obtained in analyses of the certified reference materials B.C.S/S.S No. 467 and of 363 - chromium-vanadium steel (modified), respectively. In these Tables, mean elemental concentrations with standard deviations, relative standard deviations, relative errors,  $Z$  score values and values of the certificate are presented.

In the B.C.S / S.S No. 467 austenitic stainless steel certified reference material the elements As, Co, Cr, Cu, Fe, Mn, Mo, Ni, Ta, V and W were determined. As can be seen in Table 3 a good precision and accuracy of the results were obtained for most of the elements with relative standard deviations ranged from 0.67 % to 12.0 % and relative errors lower than 9.0 %. The exception was for Ta. Tantalum results presented relative error of 20.2 %. This high relative error obtained for Ta is probably due to its low concentration. Data of this element in the certificate show that the precision for Ta is not good (RSD = 19.4 %). For the elements As, Co, Cu, Fe, W and V determined in this study there are no certified values so the results obtained in this study constitute a contribution to certification.

For 363 chromium-vanadium steel (modified) certified reference material, the elements As, Co, Cr, Cu, Fe, Mn, Mo, Ni, Ta, V and W were determined and for most elements the results

presented relative standard deviations lower than 15.0 % and relative errors below 9.0 %, indicating good precision and accuracy of the results. The exception was for Mo that presented a relative standard deviation of 17.7 % due to low counting rates obtained for 739.58 keV peak of  $^{99}\text{Mo}$ .

**Table 3: Element concentrations in the certified reference material B.C.S / S.S No. 467 austenitic stainless steel.**

Elements	n <sup>a</sup>	$\bar{X}^b \pm \text{SD}^c$	RSD <sup>d</sup> , %	RE <sup>e</sup> , %	Z score	Ref [11]
As, $\mu\text{g g}^{-1}$	6	83.9 $\pm$ 8.5	10.1			
Co, $\mu\text{g g}^{-1}$	4	374.6 $\pm$ 2.5	0.67			
Cu, $\mu\text{g g}^{-1}$	3	388 $\pm$ 18	4.6			
Cr, %	6	18.4 $\pm$ 1.0	5.5	1.9	0.33	18.05 $\pm$ (0.06) <sup>h</sup>
Fe, %	6	72.9 $\pm$ 1.9	2.7			
Mn <sup>f</sup> , %	3	0.619 $\pm$ 0.048	7.8	8.9	-1.21	0.68 $\pm$ (0.01)
Mn <sup>g</sup> , %	5	0.697 $\pm$ 0.018	2.5	2.5	0.77	0.68 $\pm$ (0.01)
Ni, %	5	8.75 $\pm$ 0.25	2.8	-2.3	-0.82	8.95 $\pm$ (0.02)
Ta, $\mu\text{g g}^{-1}$	5	14.4 $\pm$ 1.0	7.1	-20.3	-0.99	18.2 $\pm$ (3.5)
V, $\mu\text{g g}^{-1}$	3	364 $\pm$ 18	4.9			
W, $\mu\text{g g}^{-1}$	6	19.8 $\pm$ 2.3	11.6			

a. number of determinations, b. arithmetic mean, c. standard deviation, d. relative standard deviation, e. relative error, f. results of five-second irradiation, g. results of one-hour irradiation, h. number in parentheses were calculated from the individual results presented in reference [11].

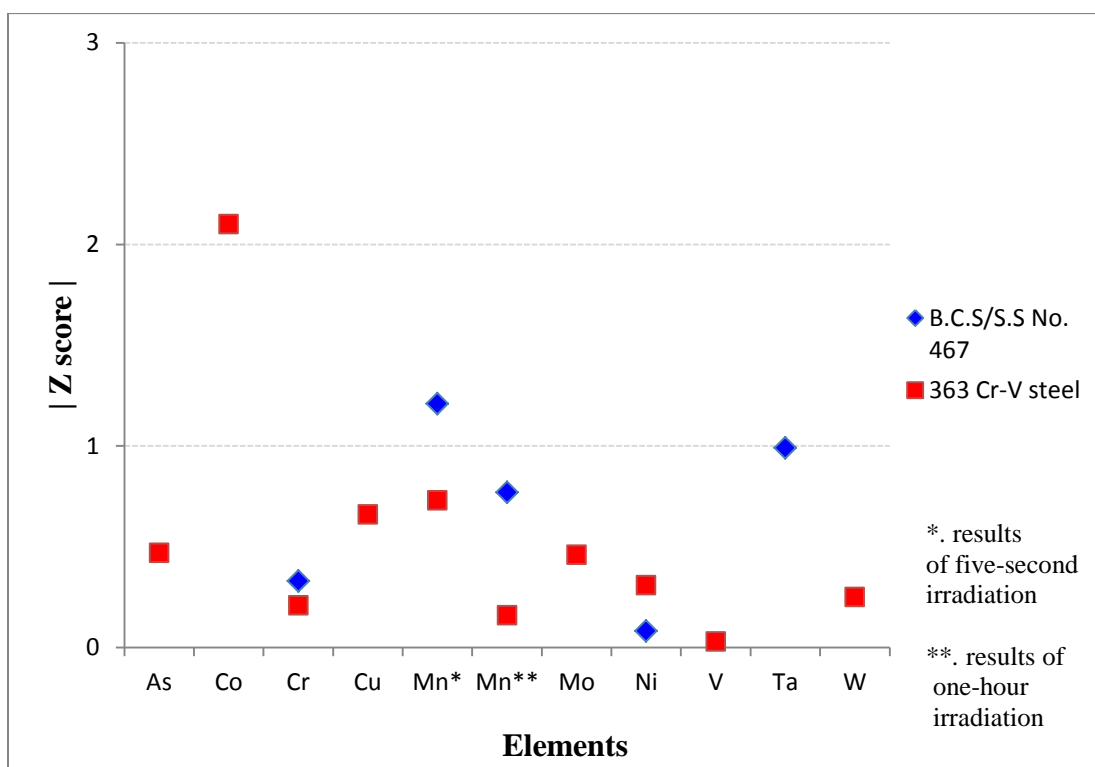
**Table 4: Concentrations of the elements in the certified reference material 363 chromium-vanadium steel (modified).**

Elements	n <sup>a</sup>	$\bar{X}^b \pm \text{SD}^c$	RSD <sup>d</sup> , %	RE <sup>e</sup> , %	Z score	Ref [10]
As, $\mu\text{g g}^{-1}$	6	93 $\pm$ 12	12.5	-7.2	-0.47	100 $\pm$ 10
Co, $\mu\text{g g}^{-1}$	4	447 $\pm$ 18	4.0	-8.9	-2.1	480 $\pm$ 10
Cu, $\mu\text{g g}^{-1}$	3	927 $\pm$ 42	4.5	-7.3	-0.67	1000 $\pm$ 100
Cr, %	7	1.293 $\pm$ 0.083	6.4	-1.3	-0.21	1.31 $\pm$ 0.01
Fe, %	7	95.7 $\pm$ 4.8	5.0			94.4 <sup>h</sup>
Mn <sup>f</sup> , %	3	1.453 $\pm$ 0.063	4.4	-3.1	-0.74	1.50 $\pm$ 0.01
Mn <sup>g</sup> , %	5	1.489 $\pm$ 0.069	4.6	-0.7	-0.16	1.50 $\pm$ 0.01
Mo, $\mu\text{g g}^{-1}$	3	305 $\pm$ 54	17.7	9.0	0.46	280 $\pm$ 10
Ni, %	5	0.287 $\pm$ 0.041	14.2	-4.4	-0.31	0.30 $\pm$ 0.01
Ta, $\mu\text{g g}^{-1}$	5	512 $\pm$ 64	12.4			530 <sup>h</sup>
V, %	3	0.309 $\pm$ 0.018	5.9	-0.20	-0.030	0.31 $\pm$ 0.01
W, $\mu\text{g g}^{-1}$	6	446 $\pm$ 56	12.6	-3.1	-0.25	460 $\pm$ 10

a. number of determinations, b. arithmetic mean, c. standard deviation, d. relative standard deviation, e. relative error, f. results of five-second irradiation, g. results of one-hour irradiation, h. informative value.

In the determination of Mn, this element was calculated using the peak of 846.76 keV of  $^{56}\text{Mn}$  with abundance isotopic of 99.94 % instead of the peak of 1810.72 keV as recommended [12] since the spectral interference of  $^{27}\text{Mg}$  was negligible.

The Z score values obtained in the analyses of certified reference materials presented in Figure 1 indicate a good accuracy of the results. Most of results obtained presented  $|Z \text{ score}| \leq 2$ . Only Co showed  $|Z \text{ score}|$  slightly higher ( $|Z \text{ score}| = 2.1$ ) indicating a questionable result according to Konieczka and Namiesnik [14].



**Figure 1: Z-score values for the CRM B.C.S/S.S No. 467 austenitic stainless steel and CRM 363 Cr-V.**

Table 5 shows the results obtained in the ISO 5832-1 stainless steel analyses. In this Table mean elemental concentrations, standard deviations, relative standard deviations and sample specification data are presented. These results show that the alloy contains as major components the elements Fe (62.6 %), Cr (17.06 %) and Ni (13.3 %) and other elements quantified were As, Co, Cu, Mn, Mo, V and W. Results obtained for this alloy presented relative standard deviations lower than 14.0 % indicating a good precision for the results.

The concentrations of the elements Cr, Cu, Mn, Mo and Ni obtained in the ISO 5832-1 austenitic stainless steel alloy are within their specification values [16] (Table 5). The elements As, Co, V and W that no have specification values were quantified in this study.

Concerning the elements found in the ISO 5832-1 sample, it should be pointed out that some of these elements are added to the alloy to assign certain properties and to be used as biomaterial. For example, Cr, Cu, Mo and Ni are added mainly to increase the corrosion



resistance [17]. Manganese is utilized as a mild deoxidant [18]. It is known that Ni also improves the strength, ductility, and crevice and erosion corrosion resistance [19] however this element is considered as toxic for humans.

Table 6 presents the limits of detection and of quantification obtained in the analysis of ISO 5832-1 austenitic stainless steel. These limits were evaluated to verify the sensitivity of the NAA in the analysis of this kind of matrix. As can be seen in the Table 6, the limits of detection and quantification of the elements obtained in this steel are low, showing the feasibility of applying NAA in the determination of impurities in this biomaterial.

**Table 5: Concentrations of the elements obtained in the biomaterial, ISO5832-1 austenitic stainless steel.**

Element	n <sup>a</sup>	$\bar{X}^b \pm SD^c$	RSD <sup>d</sup> , %	Ref [16]
As, $\mu\text{g g}^{-1}$	5	15.0 $\pm$ 1.5	10.2	
Co, $\mu\text{g g}^{-1}$	4	213.8 $\pm$ 4.3	2.0	
Cu, %	3	0.0427 $\pm$ 0.0025	5.8	0.5 max
Cr, %	5	17.06 $\pm$ 0.61	3.6	17.0 - 19.0
Fe, %	5	62.6 $\pm$ 2.1	3.3	
Mn <sup>e</sup> , %	3	1.60 $\pm$ 0.12	7.6	2.0 max
Mn <sup>f</sup> , %	5	1.764 $\pm$ 0.025	1.4	2.0 max
Mo, %	5	2.49 $\pm$ 0.33	13.3	2.25 - 3.00
Ni, %	5	13.3 $\pm$ 1.3	9.5	13.0 - 15.0
V, $\mu\text{g g}^{-1}$	3	352.5 $\pm$ 7.9	2.2	
W, $\mu\text{g g}^{-1}$	5	110 $\pm$ 15	13.7	

a. number of determinations, b. arithmetic mean, c. standard deviation, d. relative standard deviation, e. results of five-second irradiation, f. results of one-hour irradiation.

**Table 6: Detection and quantification limits for elements in the ISO 5832-1 austenitic stainless steel.**

Element	Limit of detection, $\mu\text{g g}^{-1}$	Limit of quantification, $\mu\text{g g}^{-1}$
As	0.42	1.3
Co	2.5	7.9
Cu	46.1	147.1
Cr	42.9	131.0
Fe	156.4	475.4
Mn <sup>a</sup>	13.8	42.1
Mn <sup>b</sup>	8.8	26.8
Mo	230.2	699.6
Ni	1005.4	3056.1
V	6.3	19.1
W	0.64	2.0

a. result obtained in five-second irradiation, b. result obtained in one-hour irradiation

## 4. CONCLUSIONS

From the results obtained in this study it can be concluded that the procedure of NAA allowed satisfactorily the determinations of several elements in the stainless steel alloy used as biomaterial.

Results obtained in the analysis of certified reference materials demonstrated the good precision and accuracy with relative standard deviations lower than 15.0 % and  $|Z \text{ score}| \leq 2$  for most of elements.

In the sample of ISO 5832-1 austenitic stainless steel alloy, the elements Cr, Cu, Mn, Mo and Ni were quantified and their results are within the ISO specification for this material. Besides that, the elements As, Co, V and W which are not presented in the specification were determined.

Among the determined elements, As, Co, Cr, Ni and V are known as toxic to the human organism [3, 20] and therefore deserve attention in their determinations when type of alloy is used as biomaterial.

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