

# Sample preparation

## Microwave-assisted digestion of Ti orthopedic alloys

### Sample preparation

Evaluation of chemical composition of orthopedic alloys and their influence on material characteristics

Orthopedic alloys used for implants are materials with high chemical and mechanical resistance. This resistance is important considering the lifetime of implants, but may bring difficulties for sample preparation. An analytical procedure for digestion of Ti alloys based on microwave-assisted digestion with diluted sulfuric acid solution (i.e. 25% v/v H<sub>2</sub>SO<sub>4</sub>) in a 33-min heating program with maximum tempera-

ture of 220 °C was developed. Digests were diluted in water and the concentrations of major elements (Ti, Al, V and Fe) and eventual contaminants (Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sn and Zn) were determined by inductively coupled plasma optical emission spectrometry. Determined concentrations were in agreement with nominal composition for Ti alloys and with values determined by wavelength dispersive X-ray fluorescence.

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## Introduction

Several technologies in the area of orthopedic implants are developed to improve the quality of life [1]. Prostheses are mainly used to replace or repair damaged limbs or hard tissues, like bones [2]. The National Health Surveillance Agency (ANVISA) from Brazil defines orthopedic implants as any medical product to be totally introduced in the human body by surgery [3]. Materials for implants must have several characteristics, like biomechanics compatibility, resistance to mechanical impact and corrosion, so it can remain inside the human body without breaking or releasing harmful elements [4].

Many materials are used for implants, such as ceramics, polymers and metallic materials. In this application note, one type of metallic material, the ASTM-AF-136 Ti alloy (Ti-6Al-4V) was studied. The nominal composition is 6% of Al, 4% of V, and Ti (balance) [5]. Geetha et al. have discussed the qualities of many metallic-based orthopedic prostheses and they concluded about the superiority of Ti based orthopedic alloys, considering their corrosion and mechanical resistance and better biocompatibility [1].

The application of an accurate analytical procedure to quantitatively determine elements in Ti alloys is important. If the concentrations are out of proportion, chemical and mechanical characteristics of the implant may change and failures will be more frequent. Many diseases are associated with high concentration of metal ions, such as Al(III), V(III) and V(V). These elements may be present in the alloys here studied [6]. Therefore, it is important to develop a procedure to determine the chemical composition of orthopedic alloys for avoiding mechanical problems and diseases after the implant [7].

Due to the high chemical and mechanical resistance of orthopedic alloys, sample digestion before chemical analysis using an instrumental method usually applied for solutions may be difficult and microwave-assisted sample digestion is an attractive alternative. This was investigated here and digests were analyzed by inductively coupled plasma optical emission spectrometry (ICP OES). For comparison purposes, wavelength dispersive X-ray fluorescence (WD XRF) was also applied using the build-in software Uniquant® for quantification of the analytes directly in the solid samples.

## Materials and Methods

### Samples and Reagents

Nine samples of Ti alloys (Ti-6Al-4V), from different producers, were provided by the Center for Characterization and Development of Materials (CCDM, UFSCar). These samples were cut into circles of approximately 10 mm diameter, 5 mm thickness for analysis by WD XRF and small fragments were collected for microwave-assisted digestions.

The analytical calibration solutions used for ICP OES measurements were prepared with ultrapure water obtained from a MILLI-Q purification system (Millipore Corp., Bedford, MA, USA). They were prepared by subsequent dilutions of 1000 mg L<sup>-1</sup> stock standards solutions (Titrisol-Merck, Darmstadt, Germany) of Ti, Al, and V (the three main constituents), and Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn and Zn (possible harmful elements).

For microwave-assisted digestion, solutions of H<sub>2</sub>SO<sub>4</sub> containing from 10 to 75% v/v were tested (Synth, Diadema, São Paulo, SP, Brazil). This acid was chosen based on preliminary tests; however it is known that its high viscosity may cause transport interference effects in ICP OES measurements and consequently acid concentration should be as low as possible for reaching complete digestion.

### Microwave-assisted digestion and ICP OES analysis

For microwave-assisted digestion, a mass of 50.0 mg of the fragments resulting from metallic bar cutting were weighed using an analytical balance (model AY 220, Shimadzu, Kyoto, Japan). Samples were microwave-assisted digested using 5.0 mL of 25% v/v H<sub>2</sub>SO<sub>4</sub> solution in a Speedwave microwave oven (Berghof, Germany). The digestion vessels are made of TFM™-PTFE. The heating program is shown in Table 1.

Table 1

Microwave-assisted digestion heating program for Ti alloys

Step	T [°C]	p [bar]	Ta [min]	Time [min]
1	155	2	5	80
2	200	3	10	80
3	220	3	10	90

The resulting digested solutions were divided in two aliquots. The first one was diluted 5,000 times to determine possible contaminants, such as Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn and Zn. The second aliquot was diluted 100,000 times to determine major constituents of orthopedic alloys, i.e. Ti, Al, and V.

Analytical calibration curves for each element were obtained using 10 multielement standard solutions containing from 0 to 30 mg L<sup>-1</sup> (0, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 20 and 30 mg L<sup>-1</sup>) of each analyte. These solutions were prepared in 9x10<sup>-4</sup> and 4.5x10<sup>-5</sup> mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> for samples diluted 5,000 and 100,000 times, respectively.

The ICP OES equipment used was an iCAP-6500 series (Thermo Fisher Scientific, USA) and measurements were made using authentic triplicates (n = 3). The two most intense emission lines of each analyte were selected, also considering spectral interferences. Table 2 shows elements and chosen emission lines.

Analyte	Emission Line (nm)*	Analyte	Emission Line (nm)*
Ti	II 334.9	Fe	II 238.2
Ti	II 336.1	Fe	II 259.9
Al	I 396.1	Mn	II 257.6
V	II 292.4	Mn	II 260.5
V	II 310.2	Mo	II 202.0
Cd	II 226.7	Mo	II 204.5
Cd	I 228.8	Mo	II 281.6
Co	II 228.6	Ni	II 231.6
Co	II 230.7	Pb	II 182.2
Cr	I 283.5	Pb	II 220.3
Cr	I 357.8	Sn	I 242.9
Cu	II 224.7	Zn	II 206.2
Cu	I 324.7	Zn	I 334.5

\* I - atomic lines and II - ionic lines

### Measurements using WD-XRF

X-ray fluorescence measurements were performed using the ARL Perform'X (Thermo Fisher Scientific, USA) and its build-in software for quantitative analysis (UniQuant®). No pre-treatment, but cutting, was necessary for any sample.

Experiments were carried out in two approaches. The first one aimed a preliminary qualitative analysis of the three major constituents and Fe. The instrumental parameters are shown in Table 3. Applied voltage and applied current were fixed in 50 kV and 50 mA, respectively.

Element	Crystal	Wavelength Increment (Å)
Ti	LiF200	0.1
Al	AX03	0.05
V	AX03	0.025
Fe	LiF200	0.1

The second experiment focused on the use of build-in software UniQuant® for quantitative analysis based on fundamental parameters. This software automatically scans samples for all metals and chooses instrumental parameters. The method used was UQ10 mm, the voltage for all measurements was 60 kV and crystals used were LiF200, LiF220, Ge111 and AX03. These results were used for comparison purposes.

## Results and Discussion

### Microwave-assisted digestion and ICP OES determination

Titanium and its alloys are often digested by using a mixture of hydrofluoric and nitric acids [8]. Due to the high risk of handling HF, we decided to test a safer acid solution. Thus, sulfuric acid was tested to digest Ti alloys. As previously mentioned, we tested the lowest concentration that would be needed to complete digestion, i.e. starting from 75% v/v we tested up to 10% v/v concentrations. According to visual observation, the minimum H<sub>2</sub>SO<sub>4</sub> concentration that led to solutions without any solid residues was 25% v/v.

The emission lines that presented the lowest LODs were selected and results for Cd, Co, Cr, Cu, Mo, Ni, Pb, Sn and Zn were ( $\mu\text{g kg}^{-1}$ ): 1 (228.8 nm), 1 (228.6 nm), 1 (283.5 nm), 0.2 (324.7 nm), 60 (204.5 nm), 1 (231.6 nm), 8 (182.2 nm), 40 (189.9 nm) and 30 (206.2 nm), respectively. Their concentrations were below these LODs, therefore it is possible to conclude that these elements were not present in any sample in harmful concentrations. Concentrations of major constituents are presented in Table 4.

Limits of detection values were lower than 0.1% for all major elements. These LOD values were obtained after measuring 10 times the blank [9]. Titanium concentrations varied from 79 to 113% ( $\bar{x}$ =95%). Aluminum and V concentrations ranged from 5 to 6 % ( $\bar{x}$ =5.5%) and from 3 to 4% ( $\bar{x}$ =4.2%), respectively. Iron concentrations were lower than 0.3% in all samples.

The nominal composition of these alloys was 90% Ti, 6% Al and 4% V. Results obtained by ICP OES, especially for Ti, shown some fluctuation, which is explained by the high dilution factor of the solutions. The dilution factor adopted for major elements was 100,000 and it was necessary for avoiding saturation of the detector with Ti emission signal. However, results were consistent with nominal composition of alloys. Iron was detected in low concentrations (lower than 0.3%).

Sample	Ti (%)	Al (%)	V (%)	Fe (%)
Sample 1	92 $\pm$ 1	5.2 $\pm$ 0.2	4.1 $\pm$ 0.2	0.22 $\pm$ 0.01
Sample 2	96 $\pm$ 11	5.5 $\pm$ 0.6	4.4 $\pm$ 0.5	0.207 $\pm$ 0.003
Sample 3	113 $\pm$ 8	6.5 $\pm$ 0.4	5.1 $\pm$ 0.4	0.21 $\pm$ 0.02
Sample 4	101 $\pm$ 8	5.7 $\pm$ 0.5	4.4 $\pm$ 0.6	0.107 $\pm$ 0.008
Sample 5	91 $\pm$ 1	5.2 $\pm$ 0.1	3.9 $\pm$ 0.1	0.21 $\pm$ 0.02
Sample 6	92 $\pm$ 5	5.2 $\pm$ 0.3	3.9 $\pm$ 0.3	0.11 $\pm$ 0.01
Sample 7	94 $\pm$ 1	5.3 $\pm$ 0.1	4.0 $\pm$ 0.2	0.185 $\pm$ 0.006

**Table 4**  
Determined concentrations of Ti, Al, V, and Fe in Ti alloys and LODs by ICP OES (mean concentration  $\pm$  standard deviation, n = 3)

Sample	Ti (%)	Al (%)	V (%)	Fe (%)
Sample 8	94 $\pm$ 9	5.3 $\pm$ 0.5	3.9 $\pm$ 0.3	0.106 $\pm$ 0.004
Sample 9	79 $\pm$ 14	5.3 $\pm$ 0.4	3.8 $\pm$ 0.2	0.102 $\pm$ 0.009
Average	95 $\pm$ 24	5.5 $\pm$ 2	4.2 $\pm$ 1	0.160 $\pm$ 0.04
<b>LOD (<math>\mu\text{g}/\text{kg}</math>) and emission line (nm)</b>				
	8 (334.9)	4 (396.1)	4 (292.4)	9 (259.9)

### Analysis of Ti alloys by WD-XRF

Spectra obtained for all nine samples evidenced the similarities in chemical compositions for these materials. As previously observed for ICP OES determinations, only Fe signals presented higher differences, i.e. 50% of the samples had a signal intensity ca. 2-fold higher than the other samples and the RSD value was 25%.

Determined concentrations established using the software Uniquant® are shown in Table 5. Samples 4, 6, 8 and 9 had Fe concentrations about half lower than the other samples, confirming the results obtained by qualitative analysis.

The concentrations of Ti and V are consistent with nominal concentrations (i.e., Ti 90% and V 4%), but concentrations of Al are in general 30% lower than expected, since the alloy is supposed to contain 6% Al. Since the software automatically corrects for interferences, negative effects on Al determination are not clear.

**Table 5**  
Determined concentrations (%) by WD-XRF (mean concentration  $\pm$  standard deviation, n = 3)

Sample	Ti (%)	Al (%)	V (%)	Fe (%)
Sample 1	89.3 $\pm$ 0.2	4.0 $\pm$ 0.1	4.4 $\pm$ 0.1	0.22 $\pm$ 0.02
Sample 2	89.8 $\pm$ 0.2	4.0 $\pm$ 0.1	4.5 $\pm$ 0.1	0.24 $\pm$ 0.02
Sample 3	89.8 $\pm$ 0.2	4.1 $\pm$ 0.1	4.3 $\pm$ 0.1	0.21 $\pm$ 0.02
Sample 4	89.9 $\pm$ 0.2	4.2 $\pm$ 0.1	4.3 $\pm$ 0.1	0.14 $\pm$ 0.02
Sample 5	89.7 $\pm$ 0.2	4.2 $\pm$ 0.1	4.3 $\pm$ 0.1	0.23 $\pm$ 0.02
Sample 6	90.0 $\pm$ 0.2	4.3 $\pm$ 0.1	4.2 $\pm$ 0.1	0.08 $\pm$ 0.01
Sample 7	90.0 $\pm$ 0.2	4.1 $\pm$ 0.1	4.3 $\pm$ 0.1	0.20 $\pm$ 0.02
Sample 8	89.9 $\pm$ 0.2	4.1 $\pm$ 0.1	4.2 $\pm$ 0.1	0.11 $\pm$ 0.02
Sample 9	89.7 $\pm$ 0.2	4.3 $\pm$ 0.1	4.2 $\pm$ 0.1	0.12 $\pm$ 0.02

In order to avoid the inconsistencies related to Al concentrations (see Table 5), five samples were selected to calculate a calibration curve using the analytical signals from WD-XRF and the reference concentrations from ICP OES (see Table 4).

The linear model obtained was used to calculate the concentrations of Ti, Al, V and Fe in the 9 samples. Table 6 shows the accuracy when the reference (ICP OES) and obtained concentration from WD-XRF were compared and good agreement among these data is observed.

**Table 6**  
Accuracies reached comparing reference values obtained by microwave-assisted digestion and ICP OES and predicted concentrations by WD-XRF

Sample	Ti (%)	Al (%)	V (%)	Fe (%)
1	105	111	96	105
2	101	102	100	103
3	84	87	88	102
4	97	101	93	112
5	103	108	88	101
6	101	111	114	103
7	97	99	98	94
8	99	108	96	102
9	118	108	111	89

### Conclusions

The developed analytical procedure combining microwave-assisted digestion of orthopedic samples and measurements by ICP OES led to accurate results despite some fluctuations due to high dilution factors for some analytes. Although the Ti alloys studied are known as materials resistant to corrosions, the developed procedure for microwave-assisted digestion was relatively simple using only 25% v/v H<sub>2</sub>SO<sub>4</sub>.

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