Behaviour of 316L Stainless Steel in Simulated Physiological Fluids

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In contact with biological systems all metals corrode and metal ions freed acts as haptens, forming complexes with native proteins that activate the immune system [1]. For analysis of heavy metals (Fe, Ni, Cr, Mn) that can migrate from 316L stainless steel (plate and powder), under controlled conditions in artificial plasma or serum were performed tests by energy dispersive X-ray fluorescence spectrometer (EDXRF) and absorption spectrometric method in flame atomic absorption spectrometry with electrothermal atomization.

Keywords: artificial plasma, 316L stainless steel, energy dispersive X-ray fluorescence (EDXRF), SFB-simulated fluid body

In the last decades several strategies have been developed for obtaining biomaterials used to make the medical implants [1,5,7,8]. The development of this area is due to the necessity of obtaining biofunctional materials with enhanced features. Developing new materials for medical implants involves methods for characterizing of their properties. Characterization of the materials prior to their use in medical devices is essential in order to reduce the time required for product certification. The prevalence of metal allergy is high in the population; it is estimated that up to 17% of women and 3 % of men presenting allergy to nickel and cobalt and chromium the prevalence of allergies is 1-3%. This type of allergy is manifested by allergic contact dermatitis and systemic allergic dermatitis. Moreover, the metal allergy was associated with various implant types of implant failures, hip replacements, knee or coronary stents [2]. In contact with biological systems all metals corrode and metal ion release acts as hapten, forming complexes with native proteins that activate the immune system. The metal-protein complex is recognized by Langerhans cells and would be presented to T cells by T lymphocytes activated T cell receptor (TCR) will undergo a process of proliferation and differentiation into effectors T cells and memory T cells capable of recognizing the metal at a subsequent exposure. The secondary immune response triggered by exposure repetition is fast and cytokines, chemokines and cytotoxin issued by local blood circulation stimulates T cells, macrophages and the eosinophil recruitment of inflammatory process responsible for dermal [1]. Metallic material used for the experiments is 316L stainless steel, known as one of the most commonly used biomaterials for medical applications [2,6,9,10].

Experimental part
Materials and methods
Materials

In this respect on this study were made a series of investigations to determine the content of Fe, Cu, Ni and Cr which can migrate from 316L stainless steel (powder and plate), under controlled conditions in the artificial plasma or serum. These investigations were conducted using energy dispersive X-ray fluorescence (EDXRF) spectroscopy and flame atomic absorption spectrometry/electrothermal atomic absorption spectrometry. The biomaterials used on this study were:
- 316L stainless steel plate;
- 316L stainless steel powder; The surgical austenitic grade of stainless steel, such as 316L has the composition: 16-18% Cr; 10-15% Ni; Mo 2-3%; Mn 2%; P 0.04%; 0.035% C; Si 0.03%, S 0.03%; Fe the remaining up to 100%.

The determination of Fe, Ni, Cr and Mn from physiological saline (0.9% NaCl) and artificial plasma
Methodology

The qualitative analysis is very sensitive and it works for both liquid and solid to ppm for all elements from B to Al. The solid samples intended quantitative analysis are in the form of powder and plate with thickness greater than 2 mm and less than 3 cm discs. The liquid samples were analyzed in special tanks made of plastic, covered with a plastic foil (Mylar) to remove the effects of the vacuum. The analysis of the liquid witness samples (saline, 0.9 % NaCl and artificial plasma) and monitored on a sample period were conducted by energy dispersive X-ray fluorescence spectroscopy using Elvax spectrometer. Spectra were processed using software Elvax. From the qualitative point of view, this method provides information on the initial elemental composition of materials composites, as well as of simulated samples of simulated body fluid (SBF) with biocomposites materials studied. The artificial plasma was prepared according to the literature [3,4] by weighing pure analytically substances necessary for preparing artificial plasma, and the dilution was made with distilled water (table 1). Separately were weighed solid samples of materials.

Four samples were prepared by introducing the solid weighed samples in sterile vial of 100 mL by polyethylene terephthalate; 2 samples solids was treated with 50 mL of commercial normal saline and 2 solid sample with 50 mL

Chemical formula	The quantity of salt dissolved in distilled water [g/L]
NaCl	6.8
CaCl$_2$	0.2
KCl	0.4
MgSO$_4$	0.1
NaHCO$_3$	2.2
Na$_2$HPO$_4$	0.126
NaH$_2$PO$_4$	0.026

No	Material	Solution	Acronym	Exposure time of the solids in artificial solutions [hours]						
1	316L stainless steel metal plate	artificial plasma	PLAS1							
2	316L stainless steel metal plate	physiological serum	PLAS2	168	336	672	1344	2016	2688	4032
3	316L stainless steel metallic powder	artificial plasma	PLAS3							
4	316L stainless steel metallic powder	physiological serum	PLAS4							

of artificial plasma which was prepared in the laboratory. Samples thus prepared were kept tightly closed at 280-300 nm UV, moderate temperatures of 36-37°C, well-established time periods (table 2).

Thus, the first analysis was performed after 168 h , the second analysis after 336 hours of exposure, the third determination after 672 h of exposure period (1 month), the fourth determination after 1344 h (2 months), the fifth determination after 2016 h (3 months), the sixth analysis was done after 2688 h (4 months) and the last measurement was done after 4032 h (six months).

Results and discussions

The qualitative analysis by energy dispersive X-ray fluorescence spectroscopy of the solid and liquid samples after 4032 h of exposure, led to the following conclusions:

In the **PLAS1** sample after 6 months of exposure of the steel 316L in artificial plasma, indicated the presence of the following elements: Fe, P, S, Ni, Cr which comes even from 316L steel (composition: 16-18% Cr, 10-15 Ni %, Mo

2-3%, Mn 2%, P 0.04%, C 0.035%, Si 0.03%, S 0.03% and Fe the remainder to 100%). In spectrum of the initial material introduced in artificial plasma (Alloy_PLAS1) comparative viewed shows the presence of Fe, P, S, Ni, Cr and Mn. The presence of trace amounts of Pb and Cd can be explained by his existence in air or distilled water, or by interfering with other metals. Copper and the lead probably exist even in the solvent used in the preparation of plasma (water).

In sample **PLAS2** after 6 months of exposure of the 316L steel, in the serum has indicated the presence of the following elements: Fe, P, S, Ni, Cr coming even from 316L steel (composition: Cr 16-18%, Ni 10-15%, Mo 2-3%, Mn 2%, P 0.04%, C 0.035%, Si 0.03%, S 0.03% and Fe the remainder to 100%). In the spectrum of initial material introduced in artificial plasma (Alloy_PLAS2), comparative viewed shows the presence of Fe, P, S, Ni, Cr and Mn (fig.1, fig.2).

In sample **PLAS3** after 6 months of exposure of the 316L metal powder in the artificial plasma has indicated the presence of the following elements: Fe, P, S, Ni, Cr coming
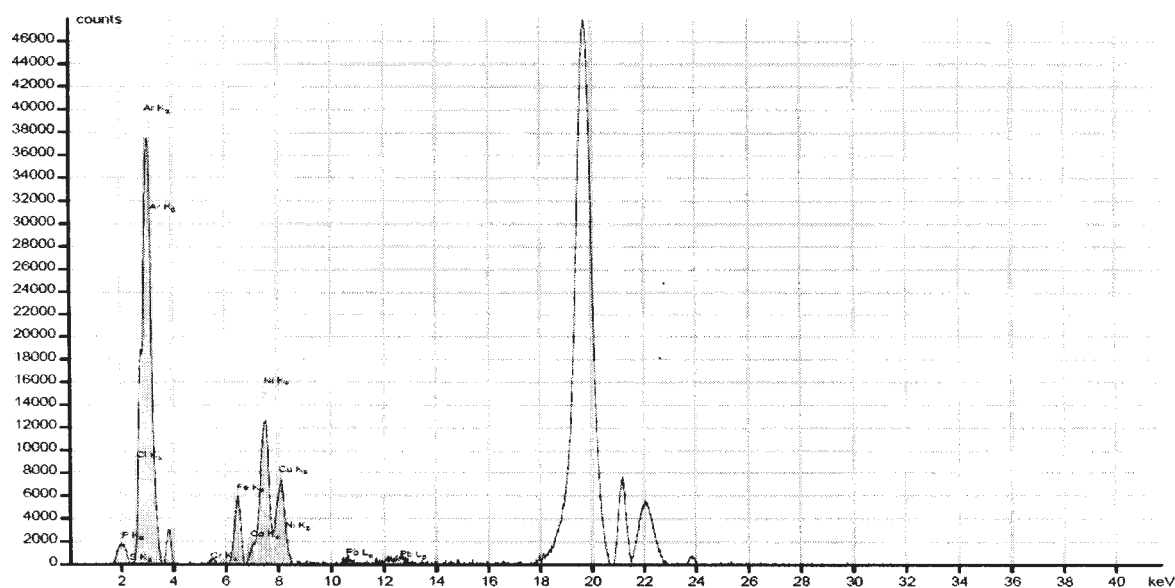

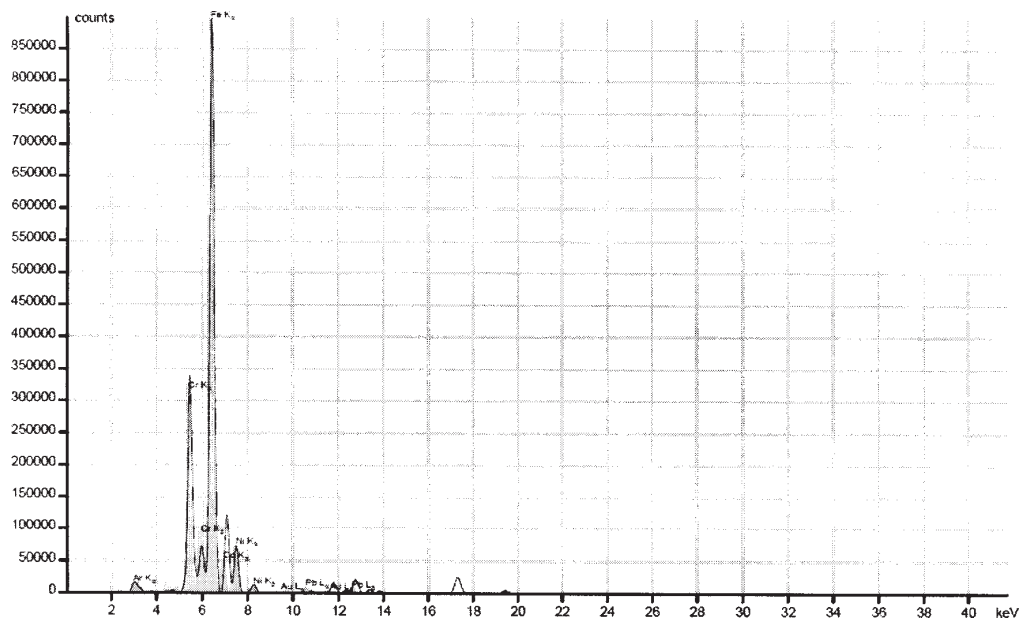Fig. 1.The spectrum of PLAS2 from physiological serum

Fig. 2. Spectrum of PLAS2 from artificial plasma

even from 316L steel (composition: Cr 16-18%, Ni 10-15%, Mo 2-3%, Mn 2%, P 0.04%, C 0.035%, Si 0.03%, S 0.03% Fe the remainder to 100%). In the spectrum of initial material introduced in artificial plasma (Powder_PLAS3) comparative viewed shows the presence of Fe, $\overline{P}$, S, Ni, Cr and Mn.

In sample **PLAS4** after 6 months of exposure of the 316L metal powder in the physiological serum has indicated the presence of Fe, P, S, Ni, Cr coming even from 316L steel (composition: Cr 16-18%, Ni 10-15%, Mo 2-3%, Mn 2%, P 0.04%, C 0.035%, Si 0.03%, S 0.03% and Fe the remainder to 100%). In the spectrum of initial material introduced in artificial plasma (Powder_PLAS4) comparative viewed shows the presence of Fe, $\overline{P}$, S, Ni, Cr and Mn. Unfortunately the device doesn't determine and identify those elements which are after Na in the periodic system, so the presence of carbon and even Si could not be remarked. The chlorine appears from the physiological serum solution.

The determination of Fe, Mn, Ni and Cr from physiological serum and plasma by atomic absorption spectrometry

For analysis of the heavy metals (Fe, Ni, Cr, Mn) which can migrate, under controlled conditions, from the preparations biocomposites in the artificial plasma or physiological serum was used the atomic absorption spectrometry using the method of flame atomic absorption spectroscopy with electrothermal atomization.

Regardless of the method chosen, the flame atomic absorption spectrophotometer is calibrated according to the procedure recommended by the company GBS, mixtures standard reference solutions used in the calibration (NIST). The system was washing with reagents blank after each standard used. Before starting measurements on samples, is reanalyzed the solution which is the most concentrated by reference standard, considering like a sample to analyzed.

The concentration value which was found should not differ by more than ± 5% by the concentration of that standard. It were started the analysis through washing the system with reagent blank. Between consecutive samples, the system is aspirate and washed with reagent blank. After every ten samples the apparatus is checked by analyzing of the blank reagent (calibration) and standard solution.

The digestion method with aqua regia

Microwave digestion system (MWS-2) Berghof is used for the mineralization of the liquid and solid samples, in extreme conditions of pressure and temperature. Solutions which are subject to digestion are heated directly through microwave absorbing by digestion reagent which normally contains the ionic components. The samples subject to microwave digestion are liquids and that does not require second stage of the sampling preparation.

Phase	1	2	3
T [^{0}C]	145	180	100
Microwave power [%]	80	90	40
Time [min.]	5	15	10

Table 3
PROGRAM USED FOR MINERALIZATION OF THE SAMPLES

Samples/Metal	PLAS1	PLAS2	PLAS3	PLAS4
	Exposure time: 168 hours			
Fe	0.001	0.001	0.002	0.001
	±0.0	±0.0	±0.0	±0.0
Ni	0.002	0.002	0.001	0.001
	±0.0	±0.0	±0.0	±0.0
Cr	0.000	0.000	0.002	0.001
	±0.0	±0.0	±0.0	±0.0
Mn	0.001	0.001	0.001	0.001
	±0.0	±0.0	±0.0	±0.0

Table 4
CONCENTRATION OF HEAVY METALS IN PLASMA/SERUM AFTER 168 h [ppb]

Samples/Metal	PLAS1	PLAS2	PLAS3	PLAS4
Exposure time: 336 hours				
Fe	0.002 ±0.0	0.004 ±0.0	0.002 ±0.0	0.002 ±0.0
Ni	0.002 ±0.0	0.001 ±0.0	0.001 ±0.0	0.001 ±0.0
Cr	0.001 ±0.0	0.001 ±0.0	0.000 ±0.0	0.001 ±0.0
Mn	0.001 ±0.0	0.001 ±0.0	0.001 ±0.0	0.001 ±0.0

Table 5
CONCENTRATION OF HEAVY METALS IN PLASMA/ SERUM AFTER 336 h [ppb]

Samples/Metal	PLAS1	PLAS2	PLAS3	PLAS4
Exposure time: 672 hours				
Fe	0.002 ±0.0	0.003 ±0.0	0.005 ±0.0	0.005 ±0.0
Ni	0.002 ±0.0	0.002 ±0.0	0.003 ±0.0	0.002 ±0.0
Cr	0.001 ±0.0	0.001 ±0.0	0.001 ±0.0	0.001 ±0.0
Mn	0.001 ±0.0	0.002 ±0.0	0.004 ±0.0	0.009 ±0.0

Table 6
CONCENTRATION OF HEAVY METALS IN PLASMA/SERUM AFTER 672 h [ppb]

Samples/Metal	PLAS1	PLAS2	PLAS3	PLAS4
Exposure time: 1344 hours				
Fe	0.007 ±0.0	0.006 ±0.0	0.003 ±0.0	0.004 ±0.0
Ni	0.006 ±0.0	0.008 ±0.0	0.006 ±0.0	0.009 ±0.0
Cr	0.002 ±0.0	0.001 ±0.0	0.003 ±0.0	0.002 ±0.0
Mn	0.011 ±0.0	0.016 ±0.0	0.012 ±0.0	0.013 ±0.0

Table 7
CONCENTRATION OF HEAVY METALS IN PLASMA/SERUM AFTER 1344 h [ppb]

Samples/Metal	PLAS1	PLAS2	PLAS3	PLAS4
Exposure time: 2016 hours				
Fe	0.008 ±0.0	0.009 ±0.0	0.006 ±0.0	0.005 ±0.0
Ni	0.007 ±0.0	0.005 ±0.0	0.019 ±0.0	0.023 ±0.0
Cr	0.001 ±0.0	0.002 ±0.0	0.002 ±0.0	0.001 ±0.0
Mn	0.016 ±0.0	0.019 ±0.0	0.012 ±0.0	0.015 ±0.0

Table 8
CONCENTRATION OF HEAVY METALS IN PLASMA/SERUM AFTER 2016 h [ppb]

In this respect were prepared the sets of 4 samples for the mineralization as follows: each 15 mL sample of plasma or physiological serum at each of the six periods of analysis was introduced into the vessel DAP- 60K by Teflon TFM of the digestion system (MWS-2) Berghof.

It was added 2.5 mL HNO_3 67 % (Merck) and 7.5 mL HCl 37 % (Merck) (1:3 volume ratio) in each vessel with sample. The mixture was mechanically stirred for 15-20 min. The Teflon vessels were closed and then were introduced into the digester Berghof MWS-2. The program used for sampling mineralization is shown in table 3.

The samples were cooled to room temperature for ~ 30 min. The clear solution samples were brought to 25 mL in bottles without dilution and then were analyzed by flame atomic absorption spectroscopy and analyzed the following elements: Fe, Mn, Cr and Ni.

The determination of Fe, Mn, Ni, Cr from physiological serum and artificial plasma samples are calculated depending on the concentration Fe, Mn, Ni, Cr measured on the spectrometer c (μg/mL), V the volume at which sample was diluted (25 mL), reported to the volume of sample subject to mineralization, v (mL). After the research period with a view to assessment of the heavy metals migration (Fe, Ni and Cr) the samples were differentially dried in order to be weighed.

Samples/Metal	PLAS1	PLAS2	PLAS3	PLAS4
Exposure time: 2688 hours				
Fe	0.028 ±0.0	0.029 ±0.0	0.010 ±0.0	0.013 ±0.0
Ni	0.011 ±0.0	0.015 ±0.0	0.019 ±0.0	0.023 ±0.0
Cr	0.008 ±0.0	0.010 ±0.0	0.013 ±0.0	0.017 ±0.0
Mn	0.022 ±0.01	0.056 ±0.01	0.122 ±0.02	0.161 ±0.02

Table 9
CONCENTRATION OF HEAVY METALS IN PLASMA/
SERUM AFTER 2688 h [ppb]

Samples/Metal	PLAS1	PLAS2	PLAS3	PLAS4
Exposure time: 4032 hours				
Fe	0.234 ±0.0	0.226 ±0.01	0.316 ±0.03	0.325 ±0.05
Ni	0.037 ±0.01	0.055 ±0.01	0.133 ±0.02	0.146 ±0.01
Cr	0.021 ±0.0	0.018 ±0.0	0.032 ±0.01	0.051 ±0.01
Mn	0.052 ±0.01	0.075 ±0.02	0.156 ±0.02	0.197 ±0.02

Table 10
CONCENTRATION OF HEAVY METALS IN
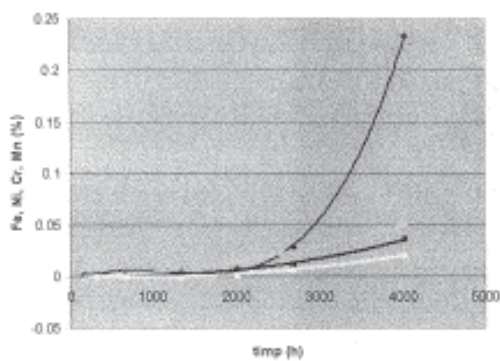PLASMA/SERUM AFTER 4032 h [ppb]

Fig. 3. Changes in the concentrations of Fe, Ni, Cr and
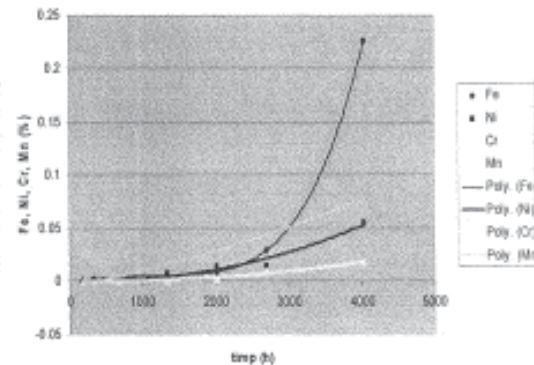Mn in PLAS1 during monitoring

Fig. 4. Changes in the concentrations of Fe, Ni,
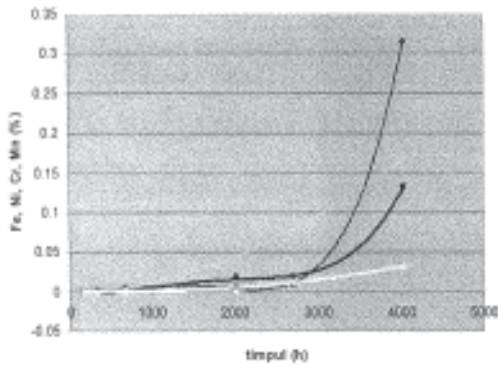Cr and Mn in PLAS2 during monitoring

Fig. 5. Changes in the concentrations of Fe, Ni, Cr
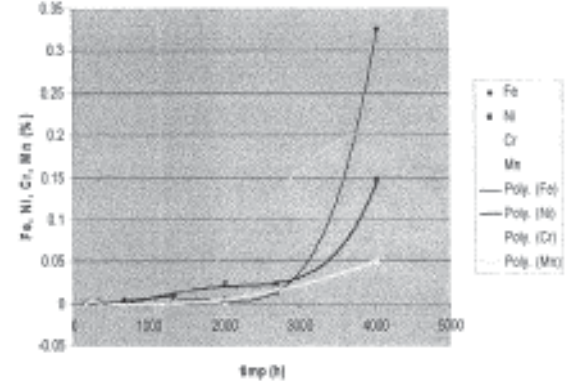and Mn in PLAS3 during monitoring

Fig. 6. Changes in the concentrations of Fe, Ni,
Cr and Mn in PLAS4 during monitoring

For establishing the quantity of some relevant elements into own reference materials used for routine quality-control procedures, it is required the use of certified reference materials (CRM). The laboratory results for each item shall be determined by means of control diagrams and is not accepted any result that is outside the agreed limits. CRM will be used regularly to maintain the integrity of their reference own materials and - through this - the system of quality control. The flame atomic absorption spectroscopy (FAAS) during 6 months of monitoring has not been able to determine concentrations at ppm level.

Considering the fact that by atomic absorption spectrometry with direct aspiration in the flame have not been obtained the relevant results about the metals concentration which can be analyzed in the artificial plasma/physiological serum under controlled conditions at the ppm level, it was examined the heavy metals concentration by atomic absorption spectrometry, graphite furnace technique. The results were summarized in tables (table 4 - 10). Until mow the migration of the heavy metals were chosen only metal samples labelled by acronyms PLAS1, PLAS2, PLAS3 and PLAS4. The variation of heavy metal concentrations of Fe, Ni, Cr and Mn for samples was plotted in figures 3- 6.

In figure 4 one can see that the heavy metal particles (such as Fe, Cr, Mn) determined in physiological solution a migration which is presented in a higher number compared to the elements which migrated in plasma solution.

The behaviour of 316L stainless steel suported for covering with hydroxiapatite was studied in [11].

Conclusions

Qualitative analysis by Energy Dispersive X-ray Fluorescence (EDXRF) spectroscopy of the liquid and solid samples, after 4032 h of exposure, led to the following conclusions.

No significant values obtained in the plasma which results from the metal materials discussed in the artificial plasma/serum, under controlled in flame/atomic absorption spectrometry with electrothermal atomization.

In the atomic absorption spectrometry for graphite furnace technique was a migration of the analyzed metals (Fe, Mn, Cr and Ni) in simulated body fluid (SBF), which was analyzed after a period of six months, but only in the ppb.

The number of heavy metal particles that migrated from the powder stainless steel was greater in the plasma and physiological solution compared to the number of those ones that migrated from the plate stainless steel.

These types of stainless steel are successfully used in medical field concerned with the design, manufacture and application of material requirements in orthopedic prosthesis (plates, screws and rods) for bone fracture healing and recovery.

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