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Electrochemical Study of a Laser Marked Biomaterial in Albumin Solution

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Proteins are widely used in solutions in order to simulate the physiological fluids and study corrosion mechanisms. The present work appraised the ASTM F 139 austenitic stainless steel (SS), employed in prostheses manufacture, and engraved by a nanosecond laser beam, using a phosphate buffered solution with 10 g/L of bovine serum albumin (PBS + BSA). Due to the complexity of the interaction between protein and the biomaterial, it was necessary to use higher BSA concentrations, however, similar to those found in human serum. The electrochemical tests consisted in monitoring the open circuit potential (OCP), electrochemical impedance spectroscopy (EIS) and cyclic polarisation measurements. The results showed that the laser marked areas are more susceptible to corrosion; suggesting that the laser marking technique has a more preponderant effect on the passive film protective character than the albumin addition to the electrolyte.

Keywords: Albumin, ASTM F139, biomaterials, corrosion, EIS, laser marks.

1. INTRODUCTION

The metallic materials used in implantable medical devices are usually passive materials and therefore are subject to localized corrosion, under contact with body fluids that contain chloride ions. One of the most common kinds of corrosion observed on metallic implants is pitting [1].

Albumin is the most abundant plasma protein and probably the best known binder of biological fluids [2]. This protein binds, generally with low affinity, endogenous and exogenous molecules like

thyroid or steroid hormones, bilirubin, free fatty acids, metals and drugs [2, 3]. Furthermore, albumin plays a role in mediating blood volume and colloid osmotic pressure (COP). Although albumin synthesis is controlled by hormones (such as insulin, growth hormone, thyroid hormone and glucocorticoids) [4 – 7], nutritional dependency is also very important [8, 9]. Although it is known that hormones are involved in albumin metabolism, no distinct hormone has been recognized as a prominent factor in the physiological regulation of albumin metabolism. While considerable knowledge is available concerning albumin synthesis, the factors that control albumin degradation are not clearly established. It was found that all tissues degrade albumin, but muscle, liver and kidney are the main contributors to albumin catabolism (40–60% of the albumin dose) [10 - 12].

Because of its characteristics, albumin is often chosen to be added in saline solutions prepared to simulate body fluids and investigate the susceptibility to corrosion of metallic implants.

Biomaterials like implantable medical devices, dental prostheses and surgical tools receive a serial number engraved at a certain area of theirs surface. These marks provide identification and traceability of the biomaterial. Laser marking is the most used technique nowadays, because of its great automation, high speed, easy reproducibility and cleanness [13 -14]. Marking is carried out after cleaning and prior to sterilization. These marks eventually can concentrate stress leading to premature failure. Laser marks produces a more defective passive layer which implicates in less corrosion resistance of the stainless steels used for biomedical applications [15 -16]. The aim of this work is to evaluate the susceptibility to localized corrosion of the laser marked ASTM F139 austenitic stainless steel, by electrochemical methods, with bovine serum albumin (BSA) solution.

2. EXPERIMANTAL

Samples of the ASTM F139 austenitic stainless steel were engraved by a pulsed nanosecond Nd: YAG laser beam. Unmarked surfaces of the same biomaterial were also evaluated. The marking procedure consisted in recording the number 8 (eight) on the surface in order to reproduce the identification serial number which the devices are submitted. The chemical composition of the analyzed SS is given on Table 1. Electrochemical techniques were employed to evaluate the corrosion resistance of this biomaterial's surface, either marked or without laser marks. All electrochemical tests were carried out using Gamry PCI4/300 equipment with three electrode set-ups, with a Pt counter electrode (wire with geometric area of 2.0 cm^2) and an Ag/AgCl (3M) reference electrode. The exposed area of the working electrode corresponded to 1 cm^2. Two types of electrolytes were used, a phosphate buffered saline (PBS) with pH of 7.4, with 10 g/L of bovine serum albumin (BSA) and a PBS solution without BSA. The chemical composition of PBS is shown in Table 2. The open circuit potential (OCP) was monitored by 17 h at (37 ± 1) °C, followed by electrochemical impedance spectroscopy (EIS) and cyclic polarization measurements. Anodic polarization tests were performed with a scan rate: 0.167 mV/s. The surface of all tested samples was prepared according to the recommendations for use in surgery.

Table 1. Chemical composition of ASTM F139 stainless steel (wt. %).

Element	C	Cr	Mn	Mo	Ni	P	S	Si	Fe
(wt.%)	0.023	18.32	2.09	2.59	14.33	0.026	0.0003	0.378	Balance

Table 2. Chemical composition of simulated body fluid solution (pH 7.4).

Component	NaCl	Na_2HPO_4	KH_2PO_4
Concentration (g/L)	58.5	9.47	18.14

3. RESULTS AND DISCUSSION

In previous work it was found that the addition of 10 g/L of albumin (BSA) into the PBS solution had an inhibiting effect on the corrosion of the austenitic stainless steel investigated [17]. The addition of albumin into the test medium increased its pitting resistance suggesting a strong adsorption of this protein at the weak sites of the passive film. In fact, the anodic polarization curve obtained in the solution with BSA did not show a pitting potential but the increase in current density was associated to the oxygen evolution reaction. Pitting, on the other hand, occurred at the surface of the stainless steel in the solution without BSA.

In order to evaluate the effect of albumin on the corrosion resistance of the same stainless steel with marks made by laser technique, cyclic anodic polarization curves [17] were obtained in the PBS solution, either with or without BSA and the results are presented in Table 3. The polarization curves show a breakdown potential associated to the samples with marks, either in the solution with or without BSA. However, unlike the unmarked samples, the BSA protein had a harmful effect on the corrosion resistance of the marked samples, lowering the pitting resistance of the stainless steel. A possible reason is the large percentage of area susceptible to corrosion but deficient amount of protein to protect the whole laser affected surface, favoring pitting corrosion.

Figure 1 shows the variation of the open circuit potential as a function of immersion time of the two types of surfaces studied during a period of 17h of immersion in concentrated PBS solutions studied. These curves represent the reproducibility obtained on ten measurements.

By analyzing Figure 1, it is shown that the passive film has a stable character for samples immersed in PBS + BSA and for laser marked samples in PBS starting from $2x10^4$ s of the trial. For samples without any marks in PBS with albumin, the OCP presents a small drop at the beginning of the trial and then the potential maintains stable up to the end of the test. This suggests that the addition of 10 g/L of BSA creates a layer that avoids the oscillations due to the passive film breakdown. At the end of this test its open circuit potential is close to the obtained for the marked samples at the same electrolyte (PBS with albumin). The OCP for unmarked samples without BSA presented an increasing tendency.

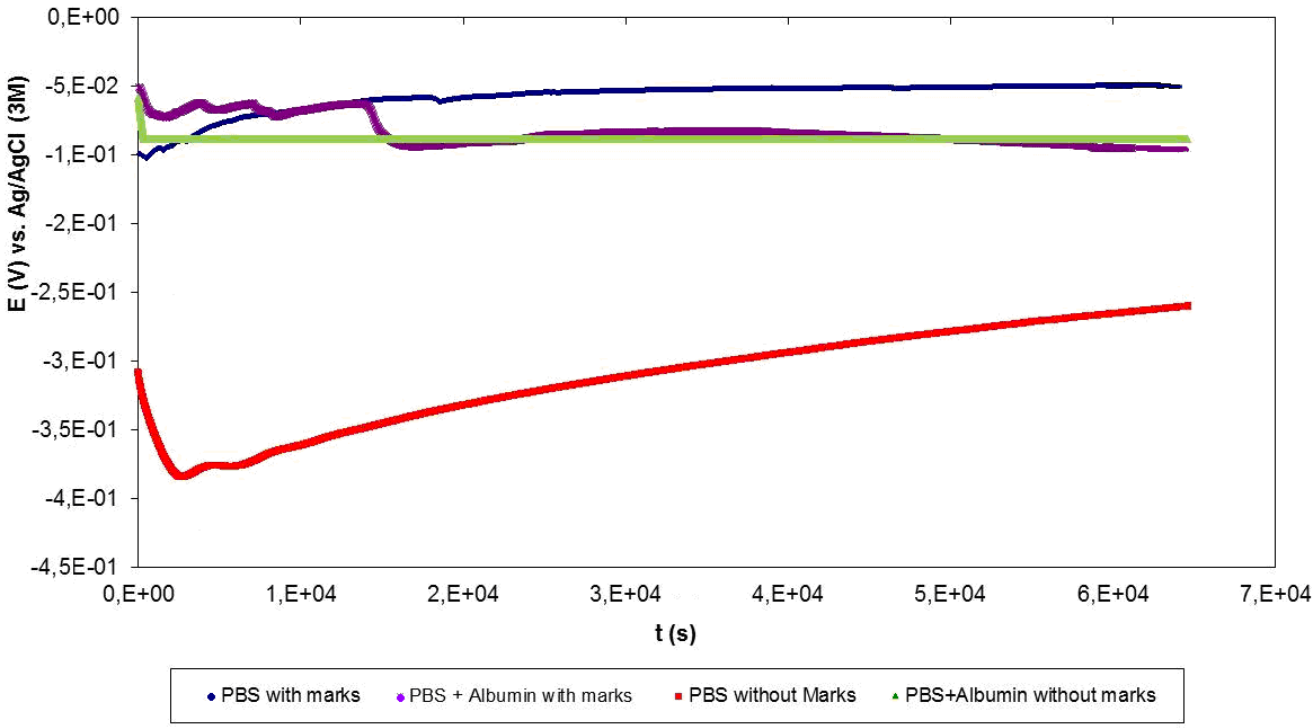

Figure 1. Open circuit potential variation with time of immersion for unmarked and laser marked surfaces during 17h of immersion in concentrated PBS with or without albumin.

Table 3. Corrosion potential and pitting potential for samples marked or unmarked samples under PBS or PBS+BSA solution.

Surface finishing / Electrolyte / Potential	E_{corr} (V)	E_{pit} (V)
Laser marked / PBS + BSA	-0.1± 0,23	0.31 ± 0,20
Laser marked / PBS	-0.05 ± 0,37	0.6 ± 0,26
Without marks / PBS + BSA	-0.17 ± 0,02	1.13 ± 0,08
Without marks / PBS	-0.2 ± 0,09	0.72 ± 0,11

The lowest pitting potentials were found for samples with laser marks. This could be explained by the thermal effect of the nanosecond Nd: YAG laser marking on the stainless steel's surface, decreasing pitting resistance. A smoother and more homogeneous surface finishing reduces the incidence of metastable pitting substantially by reducing the number of sites able to be activated in the growth of metastable pits [18].

The EIS technique is widely used in many corrosion studies by allowing the separation of a variety of electrochemical processes that occur with dissimilar kinetics [19].

The EIS measurements for this biomaterial, marked and not marked by laser, immediately after 17 h of immersion in PBS solution with or without bovine serum albumin, are shown on the following figures.

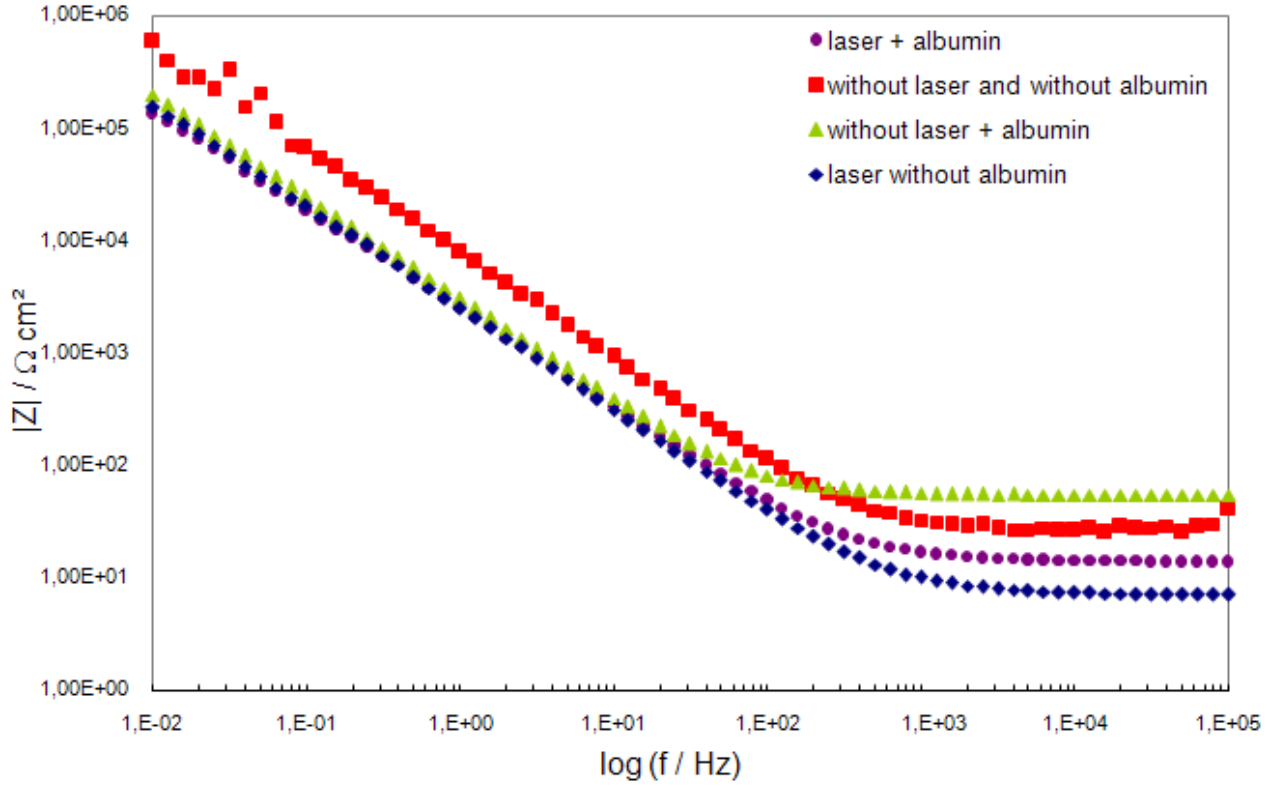

Figure 2. Bode (Z modulus) diagrams for ASTM F 139 SS surfaces marked and unmarked by laser, obtained after OCP, in PBS solution with or without bovine serum albumin (BSA).

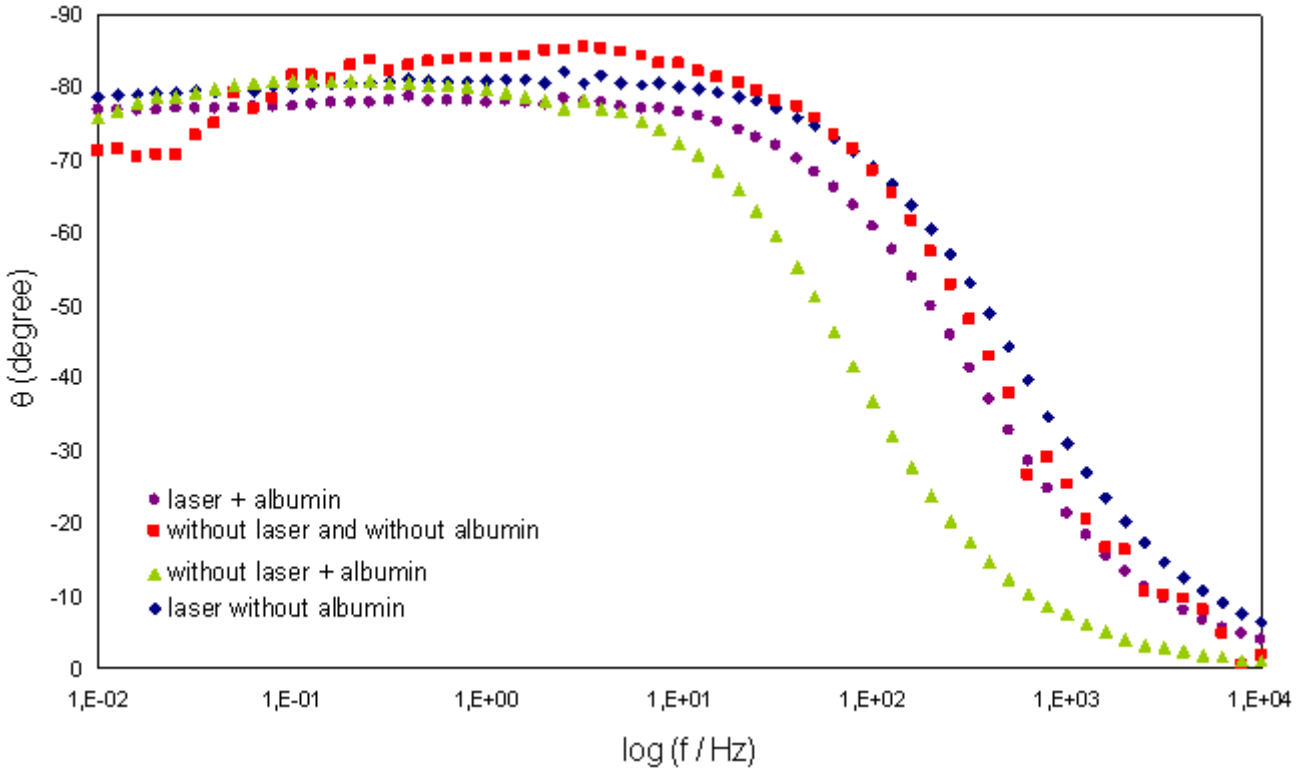

Figure 3. Bode (phase angle) diagrams for laser marked and unmarked ASTM F 139 SS surfaces, after OCP, in PBS solution with or without BSA.

The Bode plots (Z modulus) shown in Figure 2 represent the reproducibility obtained for each condition. The diagrams show that the laser technique influences the impedance. Although the high impedance revealed the high corrosion resistance of this biomaterial, with impedance modulus values near 10^5 - 10^6 Ω.cm² at 0.01 Hz; the higher values for impedance modulus when compared with samples marked by laser and without BSA, indicated the presence of a passive film less defective and therefore more protective against corrosion in the environment studied. At the low frequencies region, higher impedances were associated to the samples without laser marks and without BSA. This character changed at the higher frequencies region, where superior values were associated to the samples without laser marks under PBS solution with BSA addition, being the lowest impedance value related to the samples marked by laser and under PBS without BSA. This fact shows a decrease in the protective ability of the film mainly for the samples treated by laser.

The Bode diagrams (phase angle) in Figure 3 show phase angles between -70° and -80° at lower frequencies, which are typical of passive metals, being the lowest values obtained for samples without laser marks and without BSA solution. At medium frequencies, the samples without laser marks and without BSA showed a peak near – 85°, and a plateau between -77° and -81° for the other conditions. This behaviour is changed at higher frequencies.

Figure 4 presents the Nyquist plots for this biomaterial, showing a capacitive behaviour for all tested conditions. For 17h of immersion, samples without laser marks and without BSA showed the higher impedances, followed by unmarked samples in BSA solution, laser marked with BSA, and the lower values obtained by laser marked samples in PBS solution without BSA. These results suggest that the laser marking technique has a more preponderant effect on the passive film protective character than the albumin addition to the electrolyte.

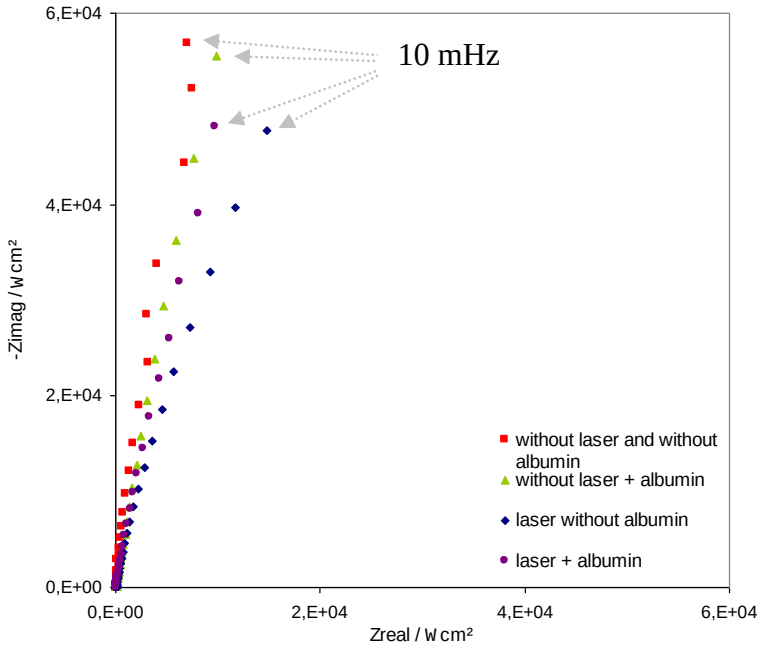

Figure 4. Nyquist diagrams for samples marked and unmarked by laser, after OCP, in PBS solution with or without BSA.

The character and stability of the passive film formed on a metal or alloy depend on the environmental status, like the chemical composition of the physiological saline solution, the presence and amount of proteins, the redox reactions, the period of immersion and temperature [20].

The experimental EIS data were adjusted to one equivalent electric circuit (EEC), as illustrated in Figure 5. The model was proposed to simulate the electrochemical behaviour of the substrate-passive film and passive film-solution interfaces. This model has been widely used in the literature to simulate the electrochemical behaviour of passive stainless steels.

Passive films formed on stainless steels show dual character, having an inner chromium-rich oxide region and an outer iron-rich oxide and hydroxide region [21-24]. In the EEC proposed in the present study, CPE are constant phase elements, C is an ideal capacitor and R represents the resistance at the interfaces. In the published literature [25-27], the response at high frequencies has been proposed. At this study, R1 is the resistance of the electrolyte, the R2/CPE2 pair was associated with the oxide film-electrolyte interface and n2 refers to the power of the CPE2, whereas the R3/C3 pair, which corresponded to the response at lower frequencies, was related to the substrate-oxide film interface.

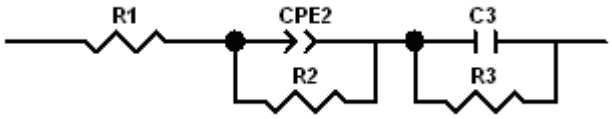

Figure 5. Equivalent electric circuit (EEC) selected to characterize the passive layer formed on the biomaterial's samples investigated in this study.

The values obtained for the EEC components with and without BSA are shown in Tables 4 and 5.

Table 4. Values obtained from fitting for the components of the equivalent electrical circuit (EEC) shown in Figure 5, for samples marked by laser technique, after 17 h of immersion.

	R1 (Ωcm^2)	CPE2 ($cm^{-2}s^{-n}\Omega$)	n2	R2 (Ωcm^2)	C3 ($cm^{-2}s^{-n}\Omega$)	R3 ($\Omega\ cm^2$)
PBS+BSA	13.80	1.95×10^{-4}	0.794	14630	1.15×10^{-4}	8.17×10^{5}
PBS	6.52	1.64×10^{-4}	0.801	15595	1.30×10^{-4}	4.42×10^{5}

According to the data presented above, R1 values are higher for PBS + BSA solution, suggesting that the addition of 10 g/L of albumin plays a protective role in the arrangement.

The lower R3 values were associated with samples marked by laser. This resistance was related to charge transfer at the oxide film-substrate interface and was inferior to those of the other types of surfaces.

The lower R2 values for samples marked by laser may be indicative of the formation of a porous oxide/hydroxide film containing pores filled with the electrolyte. The n2 values, which indicated that the outer part of the passive film (oxide-electrolyte interface) was heterogeneous, were inferior for samples with BSA addition.

Table 5. Values obtained from fitting for the components of the equivalent electrical circuit (EEC) shown in Figure 5, for samples without laser marks, after 17 h of immersion.

	R1 (Ωcm^2)	CPE2 ($cm^{-2}s^{-n}\Omega$)	n2	R2 (Ωcm^2)	C3 ($cm^{-2}s^{-n}\Omega$)	R3 (Ωcm^2)
PBS+BSA	54.01	2.07×10^{-4}	0.782	16712	8.14×10^{-5}	1.00×10^{6}
PBS	27.07	6.37×10^{-5}	0.867	65124	3.32×10^{-5}	1.39×10^{6}

Ji et al. [28] investigated the manufacture of alternating polycation and albumin multilayer as coatings on biomedical AISI 316L SS investigating the layer-by-layer deposition via electrochemical impedance spectroscopy (EIS), and established that the increasing deposition of albumin insulating multilayer reduces the surface's charge-transfer rate.

Wan et al. [29] studied high nitrogen Ni-free SS in PBS medium with 0.1 g/L of albumin in order to elucidate the effect of nitrogen and proteins on the biocorrosion behaviour. They found that proteins had a significant effect on the passivation behaviour of high nitrogen Ni-free SS; because proteins acted as complexing agents for dissolved metal ions thus stimulating the dissolution rate of metal and, therefore, suppressing the development of the protective oxide film. Albumin concentration was below the one used at the present work and the authors did not specify if it was bovine or human serum albumin.

Thus bovine serum albumin concentrations must be taken into account when performing *in vitro* research of metallic biomaterials.

4. CONCLUSIONS

The lowest resistances to pitting corrosion were obtained for samples with laser marks. This is explained by the thermal effect of the nanosecond Nd: YAG laser marking technique on the stainless steel's surface.

The EIS results suggest that the laser marking technique has a deleterious effect on the passive film protective character, so that, even 10 g/L of albumin addition to the electrolyte could not be highly protective.

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