Evaluation of Laser Marked ASTM F 139 Stainless Steel in Phosphate Buffer Solution with Albumin

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Albumin is the most abundant protein found in human serum and sinovial fluids. Investigations on its effects on the corrosion resistance of metallic biomaterials have led to controversial conclusions. The BSA (bovine serum albumin) concentration used in most of the studies is below the usual concentration found in the human physiological fluids. This is possibly the reason for the lack of agreement on the conclusions reported in literature. The aim of this study is to evaluate the effect of albumin in concentration on the susceptibility to corrosion of the ASTM F139 austenitic stainless steel (SS) used in fabrication of orthopedic implants, specifically on the areas marked by a Nd: YAG laser. The electrolyte used was a phosphate buffer solution (PBS) and the effect of albumin was investigated by adding 10 g/L into the electrolyte and comparing the corrosion resistance in the two environments, with and without albumin, by electrochemical methods. The Mott-Schottky approach was used to evaluate the electronic properties of ASTM F139 SS oxide layer marked by laser beam. The results showed a strong effect of the albumin on the electronic properties of the passive film and on the resistance to localized corrosion. The albumin changes the flat band potential position, increasing the oxide layer doping densities. The laser marked surface showed lower corrosion resistance in the electrolyte with albumin, when compared to the tests performed with pure PBS.

Keywords: Biomaterials, orthopedic implants, corrosion, laser marks, albumin.

1. INTRODUCTION

The use of materials in the human body is not new [1]. The increase in life expectancy and the growing on the number of automotive accidents with irreversible lesions are contributing to advances in biomaterials research and development around the world. Metallic biomaterials have found wide application in restorative surgery as basic materials for manufacturing implantable medical devices for skeletal replacements and fixtures. For this purpose, metallic biomaterials which join better compatibility with biological environment, good mechanical properties and elevated corrosion resistance are chosen [1-4].

Amongst the range of metallic materials used nowadays, the ASTM F139 austenitic stainless steel has been employed for biomedical applications due to its important mechanical characteristics, high corrosion resistance associated with their relatively low cost. These devices, when in contact with human tissue, may cause hypersensitivity and the need for further surgery to replace. Thus, it is of fundamental importance to study the interaction of biomaterials with blood components, mainly albumin due its majority concentration [5].

The production processes involved in implants manufacturing also affect their corrosion resistance, specifically those that influence the surface finishing. The final stage of implant devices production consists in marking the surface for identification and traceability of the manufactured material. Among the marking techniques, the laser type is one of the mostly used due to its inherent characteristics, such as high rate and reproducibility.

There are three most common proteins present in serum, which are: immunoglobulin (IgG), albumin and fibrinogen [6]. Albumin is considered the most abundant protein in serum and sinovial fluid [7, 8].

Albumin is a globular protein characterized by a hydrophobic core and a hydrophilic surface. This protein is present in serum at concentrations of about 40 mg/mL and it is produced daily in liver [9].

Bovine serum albumin (BSA) has similar composition as human serum albumin (HSA). Consequently, BSA has been largely used to simulate body fluids. Acidic terminal groups of BSA (glutamic and aspartic acids) are responsible for bounding proteins to the metallic surfaces [7-9].

Albumin might affect the electrochemical behaviour of metals, either inhibiting metal dissolution, due to protein adsorption on the surface acting as a protective layer, or promoting metal dissolution, generally by formation of metal complexes and, consequently, increasing the corrosion rate. At the end of the manufacturing process of metallic implantable medical devices, marking techniques are generated for identification and traceability purposes. The aim of this study is to evaluate the effect of albumin on the susceptibility to localized corrosion of the ASTM F139 austenitic stainless steel marked by laser, widely used as a biomaterial, evaluating the electronic properties of its oxide layer employing the Mott-Schottky approach.

2. EXPERIMENTAL

The material used for this work was the ASTM F139 austenitic stainless steel. The tested surfaces were marked by a pulsed nanosecond Nd: YAG laser beam. For comparison reasons, the

unmarked surface of the same stainless steel was also evaluated. The marking procedure consisted of recording the number 8 (eight) many times on the surface in order to cover the largest area of the samples studied. The chemical composition of the studied SS is given on Table 1. To evaluate the corrosion resistance of the surface of this steel either marked or without laser marks, electrochemical techniques were employed. In order to characterize the electronic properties of its passive film, the Mott-Schottky approach was used. All electrochemical tests were carried out using a 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 reference electrode (3M). The area of the working electrode exposed to the electrolyte corresponded to 1 cm^2. Two types of electrolytes were used, a phosphate buffered saline (PBS) solution with pH of 7.4, with 10 g/L of albumin and a PBS solution without albumin. The chemical composition of PBS is shown in Table 2. Polarization tests were performed with a scan rate: 0.167 mV/s at (37 ± 1) °C, after monitoring the open circuit potential (OCP) by 17 h.

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

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

The capacitance measurements were performed at a frequency of 1 kHz. Polarization was applied in the negative direction from 500 mV to -1000 mV (Ag/AgCl) at successive steps of 50 mV.

The Mott–Schottky results were reported as the inverse square of an apparent interfacial capacitance C as function of potential E, with: C =-1(/2πf Z"), where f is the test frequency and Z" is the imaginary component of the interfacial impedance [10, 11]. The passive film/electrolyte interface is described by the following expression [12 -13]:

$$\frac{1}{C^2} = \frac{2}{\varepsilon \varepsilon_0 q N_q}\left(E_{FB} - E + \frac{kT}{e}\right),$$

where C is the capacitance of the oxide film / electrolyte interface; E, is the applied potential; ε, is the dielectric constant of the oxide; ε_0 is the permittivity of vacuum; N_q, is the density of electron semiconductor; q, is the elementary charge; k is the Boltzmann constant; T is the absolute temperature and E_{FB}, the flat-band potential.

To evaluate the topographic roughness was used a scanning electron microscope (SEM) with low vacuum tungsten filament and closest approach of 10×10^5 times, brand: Hitachi, model: TM3000 with software for data acquisition: topographic 3D Viewer.

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 stainless steel investigated [14], as it can be seen in Figure 1. 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 were obtained in the PBS solution, either with or without BSA and the results are presented in Figure 2. The polarization curves show a breakdown potential associated to the samples with marks, either in the solution with or without BSA. However, dissimilar to the samples with no marks, 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 presents cyclic polarization curves of the unmarked ASTM F139 stainless steel, with and without albumin, and for samples marked by laser (Figure 2). This suggests that the oxide formed on the surface of the samples subjected to the laser marking process has a higher number of defects than that found in other conditions studied.
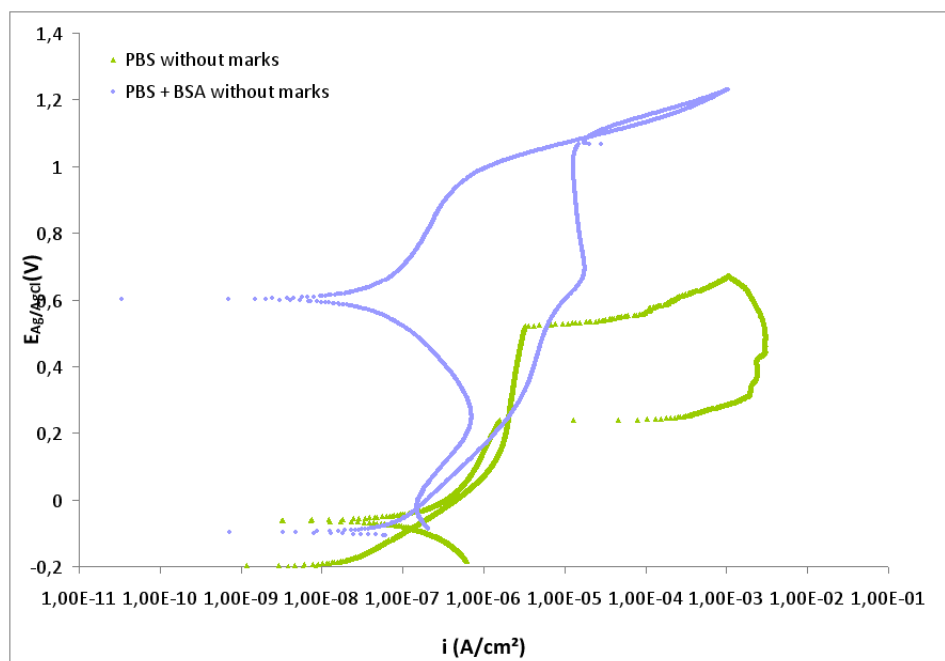

Figure 1. Cyclic potentiodynamic polarization curves for ASTM F139 SS samples without marks, in phosphate buffer solution (PBS), with or without albumin (BSA) addition.
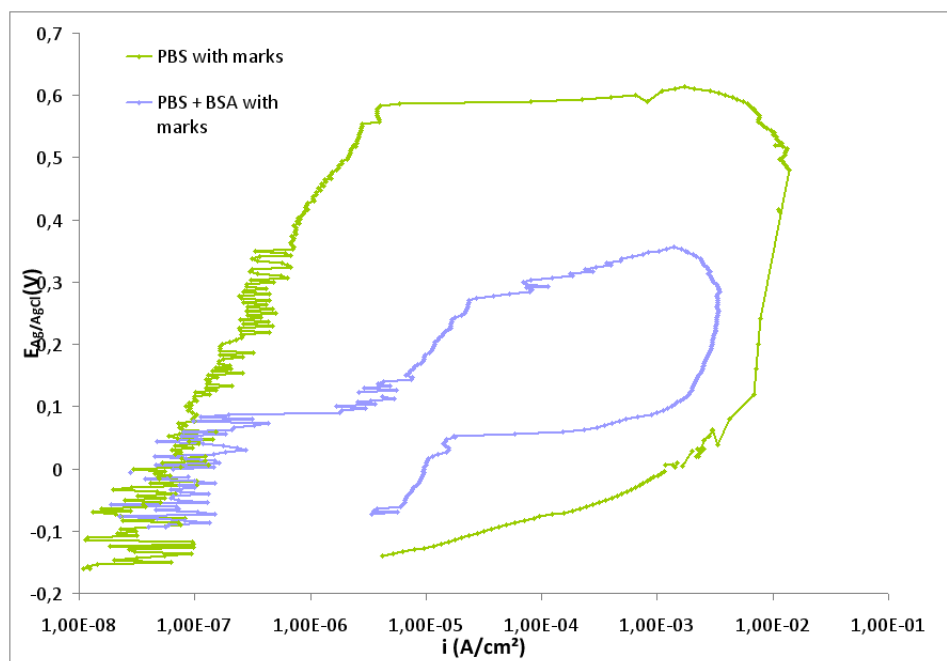
Figure 2. Cyclic potentiodynamic polarization curves for ASTM F139 SS samples with marks, in phosphate buffer solution (PBS), with or without albumin (BSA) addition.

The nature and stability of the oxide layer formed on a metal or alloy depend on the environmental conditions, like the chemical composition of the simulated physiological solution, the presence and quantity of proteins, the redox reactions, the time of immersion and the temperature [15].
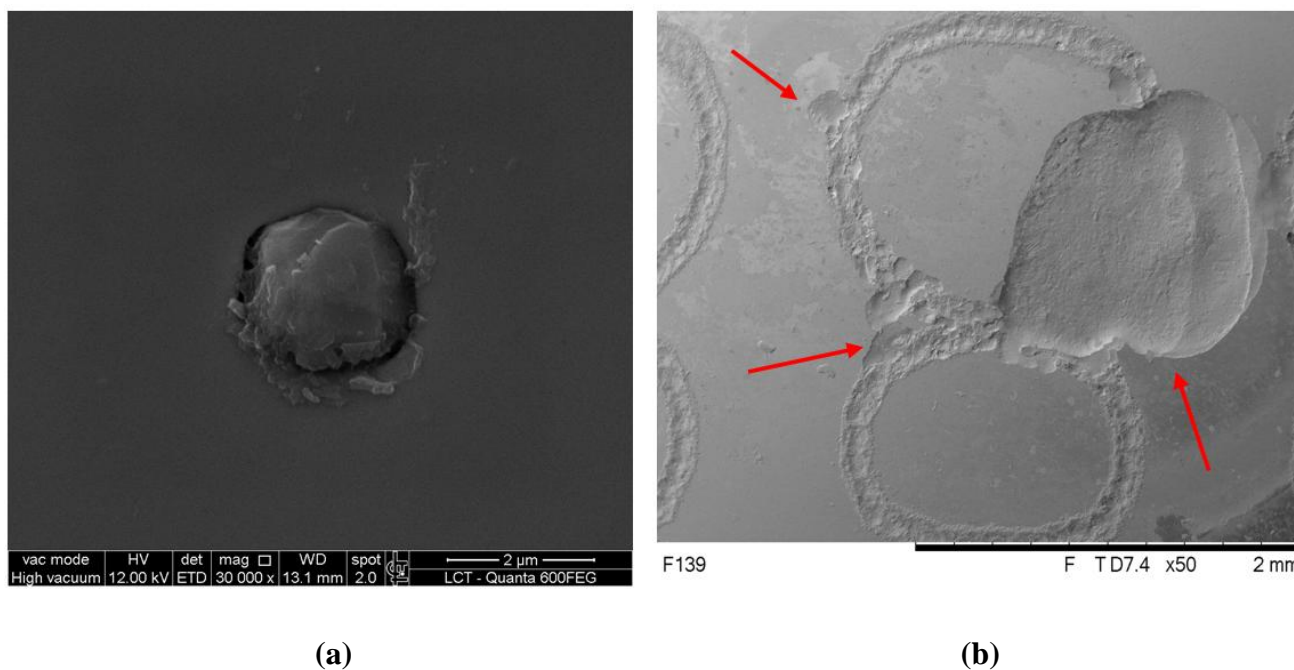

(a)   **(b)**

Figure 3. FEG image after potentiodynamic cyclic polarization tests using PBS+BSA solution of (a) sample without laser mark and (b) with laser marks (the arrows show pits surrounding the laser marks).

Figure 3 (a) shows a micrograph obtained by FEG of the stainless steel surface not marked after the cyclic polarization test in PBS+BSA solution. It can be noticed that despite of the precipitate shown, there was no pits associated to the stainless steel, in spite of the interface between the precipitate and the matrix suggesting the presence of crevice, which could lead to pit nucleation. Figure 3 (b) presents a sample marked by laser with pits associated to the marked area. This result supports the hypothesis of deficient amount of protein to protect the weaker areas at the surface.

Figure 4 presents a 3-D topographical view of the ASTM F 139 SS marked by laser (a) prior to polarization tests and (b) after polarization tests. It's clear that the laser marked technique produces regions with different surfaces roughness where corrosion process starts.
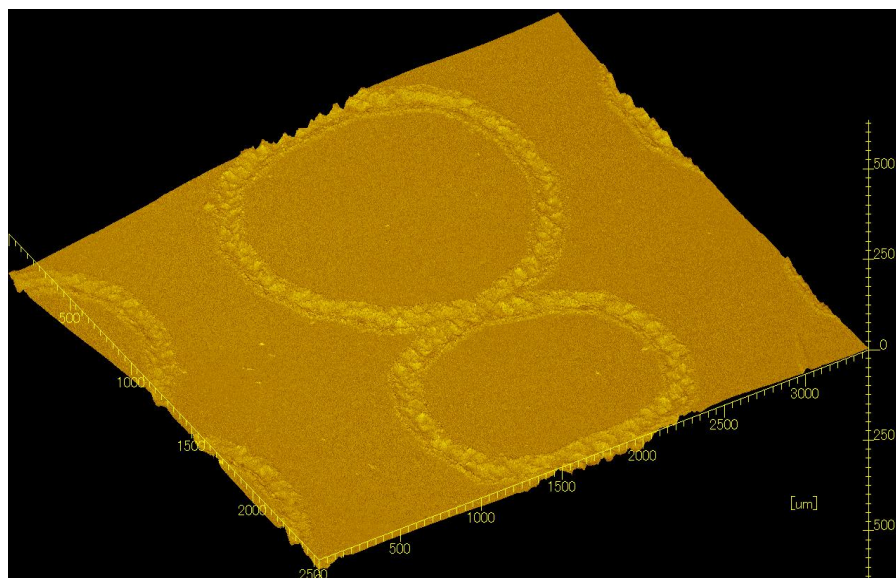

Figure 4 (a). 3D topographical image of the marked samples before cyclic polarization tests.
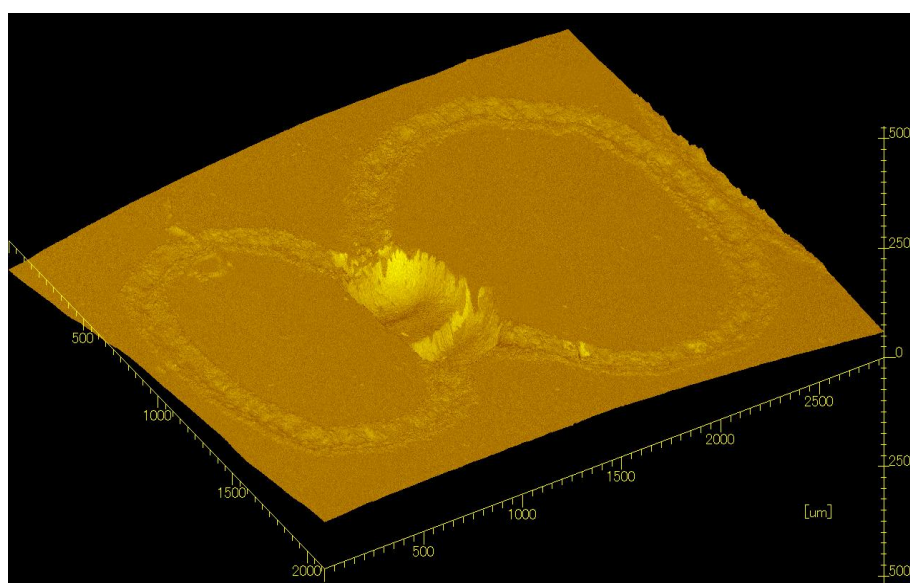

Figure 4 (b). 3D topographical image of the marked samples after cyclic polarization tests, showing a pit at the centre of the engraved number eight.

The pit shown in Figure 4 (b), at the centre of the engraved number eight, suggests that this area is the most prone to initiate the nucleation and propagation of the pits, because of the double incidence of the laser beam. During the marking process, in order to record the number eight, the nanosecond Nd: YAG laser beam focused twice at the centre of this number.

The effect of the laser beam on the stainless steel surface has a significant influence on the electronic properties of the passive oxide film, as the Mott-Schottky approach results presented in Figure 5 (a) and (b), for samples with or without laser marks, shows. The flat band potential was displaced and the concentration of donors or acceptors largely varied for the two types of oxide films formed on the stainless steel tested, laser marked or unmarked.
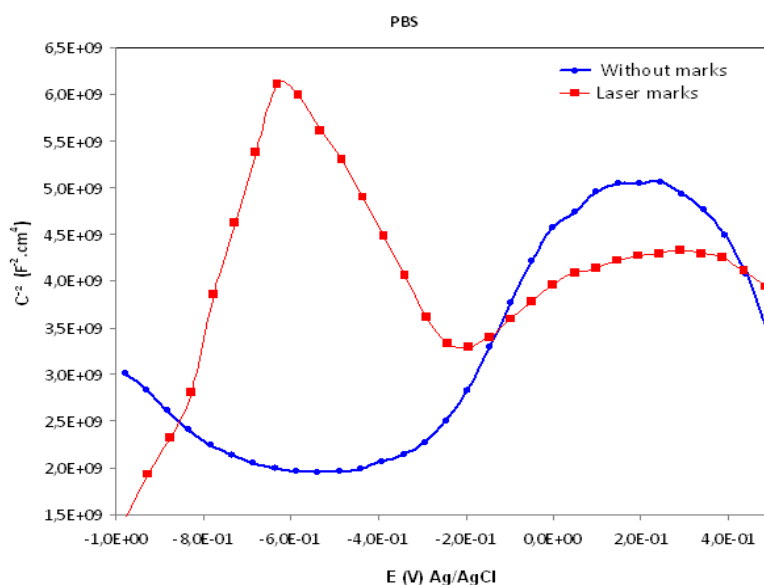

Figure 5(a). Mott-Schottky plots for unmarked and laser marked ASTM F139 stainless steel samples measured in phosphate buffer saline solution (PBS), 37 ºC.
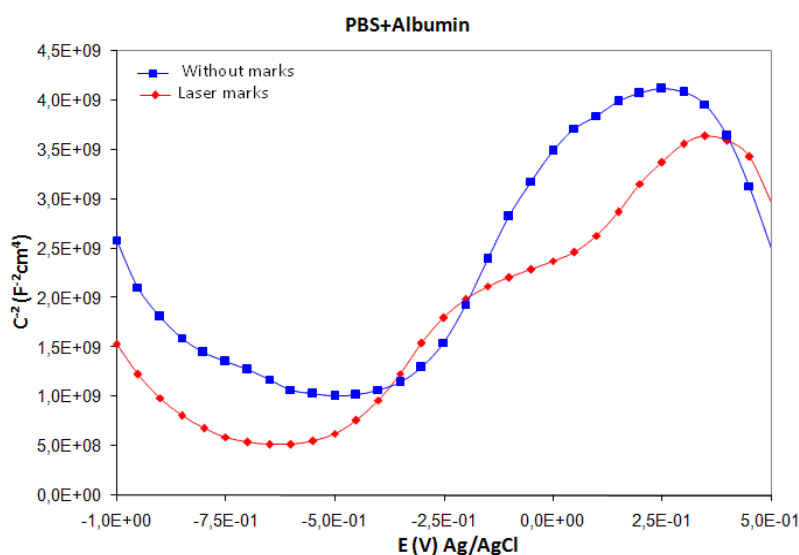

Figure 5 (b). Mott-Schottky plots for unmarked and laser marked ASTM F139 stainless steel in phosphate buffer solution with albumin (PBS+ BSA), 37 ºC.
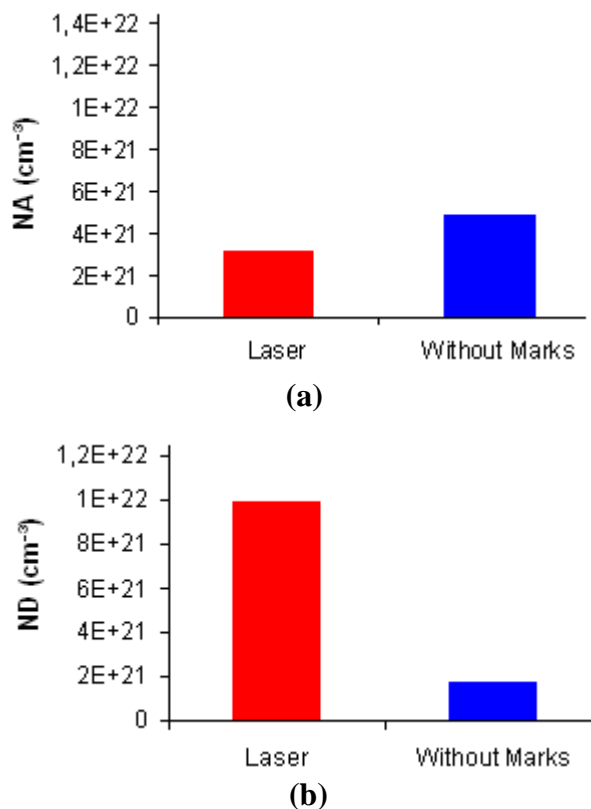
Figure 6. Values of concentration (a) acceptors (NA) and (b) donors (ND) obtained by Mott-Schottky approach of the two types of surfaces tested in PBS without albumin.

Figure 7. Values of concentration (a) acceptors (NA) and (b) donors (ND) obtained by Mott-Schottky approach of the two types of surfaces tested in PBS with albumin.

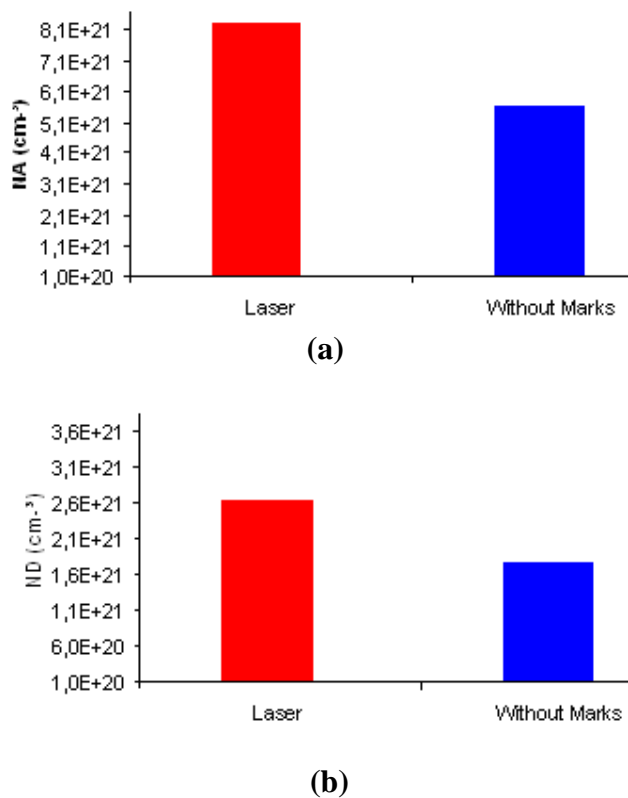Figures 6 and 7 indicate the number of acceptors and donors densities for the two types of electrolytes evaluated in this work, PBS without albumin, and PBS with albumin, respectively, for the two types of semiconductors, p-type and n-type, comparing the surface with laser marks or without marks.

The acceptors charge density of the laser marked stainless steel is slightly greater in the solution with albumin comparatively to that without albumin, although of the same order of magnitude. On the other hand, the donors charge concentration is much greater for the laser marked samples in the solution without albumin as compared to that with the protein. This result indicates that albumin decreases the charge transfer at the passive film-electrolyte interface; decreasing the susceptibility to localized corrosion observed in this type of surface in a PBS solution without protein [16 -17].

Some authors [15, 18 - 20] have investigated the influence of proteins in the corrosion behaviour of metals and alloys. However, it has not been established whether it accelerate or inhibit electrochemical processes. According to Virtanen et al., these effects are specifics for each biomaterial - solution arrangement [15].

Ji et al. [21] studied the fabrication of alternating polycation and albumin multilayer as coatings on biomedical 316L stainless steel and investigated the layer-by-layer deposition via electrochemical impedance spectroscopy (EIS), and discovered that the increasing deposition of albumin insulating multilayer reduces the surface's charge-transfer rate.

Geringer et al. [22] published a research about the influence of chlorides and albumin concentration on fretting-corrosion between 316L stainless steel and PMMA. Contrary to the protective effect of this protein on global corrosive wear, an increase of albumin concentration tends to increase the number of pits. These authors used 1 g/L and 20 g/L of albumin, and suggests that the amount of albumin may not be sufficient to protect the whole surface of the biomaterial [22].

It is important to remind that the results of the present work refer to an albumin concentration of 10 g/L. Additional investigations will be carried out in order to evaluate the effects of albumin concentration on the corrosion susceptibility of laser marked stainless steels for biomedical applications, monitoring the open circuit potential and via EIS measurements.

4. CONCLUSIONS

The nanosecond Nd: YAG laser marking process, currently used in industry, alters the surface finishing of the biomaterials, and hence its electrochemical behaviour. The results showed that the addition of albumin (BSA) to a phosphate buffer solution (PBS) had dissimilar effects on the resistance to localized corrosion depending on the type of surface evaluated. For the unmarked samples, the albumin had an inhibiting effect whereas, for the laser marked samples, albumin increased the pitting susceptibility of the ASTM F139 stainless steel.

References

1. D. J. Lyman, W. J Seare Jr, *Mater. Sci.*, 4 (1974) 415.
2. D. F. Williams, *Mater. Sci*., 6 (1976) 237.
3. D.F. Gibbons, *Bioph. and Bioeng*., 4 (1975) 367.
4. L. L. Hench, *Mater. Sci*., 5 (1975) 279.
5. O. Belgacem, G. Stübiger, G. Allmaier, A. Buchacher, K. Pock, *Biologicals*., 35 (2007) 43.
6. V. Hlady, J. Buijs, *Cur. Opin. in Biotec*., 7 (1996) 72.
7. J. Yang, J. Black, *Biomaterials,* 15 (1994) 262.
8. H. Mishina, M. Kojima, *Wear,* 265 (2008) 655.
9. M. Roba, *Biomaterials*, 30 (2009) 2072.
10. S. Kallip, A.C. Bastos, K.A. Yasakau, M.L. Zheludkevich, M.G.S. Ferreira, *Electrochem. Commun.,* 20 (2012) 101.
11. M. Da Cunha Belo, N. E. Hakiki, M.G.S. Ferreira, *Electrochim. Acta,* 44 (1999) 2473.
12. N. E. Hakiki, S. Boudin, B. Rondot, M. Da Cunha Belo, *Corros. Sci.,* 37 (1995) 1809.
13. N. E. Hakiki, M. Da Cunha Belo, A.M.P. Simões, M.G.S. Ferreira, *J. Electrochem. Soc.,* 145 (1998) 3821.
14. E. F. Pieretti, R. P. Palatnic, T. F. Fontes, I. Costa, (RIP) Corrosion 2013 – NACE International, Orlando, FL, USA, (2013) 126.
15. S. Virtanen, I. Milošev, E. Gomez-Barrena, R. Trebše, J. Salo, Y. T. Konttinen, *Acta Biomaterialia,* 4 (2008) 468.
16. E. F. Pieretti, I. Costa, *Electrochim. Acta,* 114 (2013) 838.
17. E. F. Pieretti, S. M. Manhabosco, L. F. P. Dick, S. Hinder, I. Costa, *Electrochim. Acta* (2013), http://dx.doi.org/10.1016/j.electacta.2013.10.137.
18. Y. Yan, A. Neville. D. Dowson, *Tribol. Int*., 40 (2007) 1492.
19. A. Minovič, I. Milošev, B. Pihlar, *J. Mater. Sci. - Mater. Med*., 14 (2003) 69.
20. S. Omanović, S. G. Roscoe, *Langmuir*, 15 (1999) 8315.
21. J. Ji, Q. Tan, D. Z. Fan, F. Y. Sun, M. A. Barbosa, J. Shen, *Coll.Surf. B: Bioint*., 34 (2004) 185.
22. J. Geringer, J. Pellier, F. Cleymand, B. Forest, *Wear,* 292-293 (2012) 207.