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EFFECT OF ALBUMIN PROTEINS ON THE ELECTROCHEMICAL BEHAVIOUR OF Ti6Al7Nb ALLOY IN SIMULATED BODY FLUID

DANIEL MARECI[a], ADRIAN CĂILEAN, GEORGIANA BOLAT, IGOR CREȚESCU, DANIEL SUTIMAN

ABSTRACT. The purpose of this study was to investigate the effect of human albumin proteins on the corrosion behaviour of Ti6Al7Nb alloy in Hank's Balanced Salt Solution (HBSS) using electrochemical polarization and electrochemical impedance spectroscopy techniques. The results showed that the presence of albumin in HBSS had a significant influence on the zero current potential (ZCP), polarization resistance (R_p) and capacitance (C). For the polarization scan, an albumin addition of 37.5 mg/ml to the HBSS significantly moved the ZCP towards a more negative (cathodic) potential and inhibited the cathodic corrosion reaction. The R_p value for Ti6Al7Nb alloy immersed in HBSS are high (order of 5×10^5 Ωcm^2) and increased after albumin addition (order of 10^6 Ωcm^2) with increasing potential from -500 mV to 1000 mV indicative of albumin adsorption. The presence of albumin proteins in HBSS improved the corrosion resistance of Ti6Al7Nb alloy.

Keywords: Ti6Al7Nb alloy, albumin, polarization resistance, impedance, corrosion

INTRODUCTION

Titanium and its alloys are the most preferred metallic materials for orthopaedic implants in the field of medicine due to their good mechanical properties and biocompatibility. In what the use of titanium and its alloys as implant material is concerned extensive and well documented researches have been performed [1-8].

Ever since the pioneer metal alloys have been use as biomaterials, lack of biocompatibility has been extensively reported and research on improved materials with appropriate mechanical behaviour and adequate biocompatibility was developed. The Ti6Al4V alloy was the first titanium alloy registered as implant material in the ASTM standards (F-136-84). Hallab et al., 2005 reported that V is toxic in vitro at concentrations below those in synovial fluids in vivo [9]. The administration of metallic ions, using metallic powders, to fibroblast L929 and osteoblast MC3T3-E1 cells showed that Ti,

[a] *"Gh. Asachi" Technical University of Iaşi, Faculty of Chemical Engineering and Environmental Protection, Mangeron Blvd., no. 71, Iaşi, Romania, 700500, *danmareci@yahoo.com*

Zr, Sn, Nb and Ta had no effect on their relative growth ratios. However, Al and V ions exhibited cytotoxicity at concentrations ≥ 0.2 ppm [10]. Based on these previous observations, further investigation of titanium alloys is increasingly important for gaining a better understanding of traditionally used alloys, and for helping in the search for new titanium alloys. Because of the possible risks associated with vanadium, Semlitsch, 1987 developed the alternative alloy, Ti6Al7Nb, in which the vanadium was exchanged for niobium [11]. Ti6Al7Nb alloy exhibits a broader passive range than Ti6Al4V alloy in simulated physiological solution [7, 12, 13]. Based on EIS measurements, Metikos-Hukovic et al., 2003 proposed that the corrosion resistance increases due to incorporation of Nb cations into the TiO_2 matrix [13].

Because surgical implants are being used on younger people and the older population is living longer, good long-term durability and corrosion resistance of implants are two important prerequisites for the choice of the specific device [14].

Titanium implants inserted into a human body are usually surrounded with blood-rich tissue, and the serum proteins in blood may also influence the corrosion of the implant materials. It is well known that proteins affect the corrosion behaviour of some metals, and that their presence can either inhibit or accelerate the corrosion phenomena. Proteins are known to behave differently with different metals, since their role in a corrosive environment is governed by many factors such as the surface chemistry of the metal, protein adsorption characteristics, interaction of protein molecules with other ions present in the electrolyte solution to produce organic complexes, and the transport of anionic and cationic charges around and away from the local environment [15-25].

The objective of the study was to determine the effect of the albumin protein on the electrochemical impedance spectrum of a Ti6Al7Nb alloy immersed in a simulated body fluid.

RESULTS AND DISCUSSIONS

The polarization curves of Ti6Al7Nb alloy in simulated physiological Hank's Balanced Salt Solution (HBSS) with and without albumin proteins are presented in figure 1.

Prior to each measurement, the Ti6Al7Nb electrodes were cathodically polarized at –1500 mV in the working solution for 120 s, in order to remove any spontaneously formed surface film.

Due to the fact that HBSS electrolyte used in this experiment is an oxygen-containing solution, the cathodic current recorded in the presented potential region can be attributed to the reduction of oxygen dissolved in the solution [26]. It can be observed that the addition of albumin protein decreased the cathodic current and moved the zero current potential (ZCP) in the negative

(cathodic) direction. It is known that many organic corrosion inhibitors prevent corrosion by forming an adsorbed film and blocking the mass transportation of the corrosion process. It is also well known that proteins have a high affinity for adsorption onto solid surfaces [27-29]. The decreasing cathodic current can be explained due to the presence of adsorbed albumin protein molecules which cover the reaction sites and/or block the transportation of dissolved oxygen to the electrode surface [24].

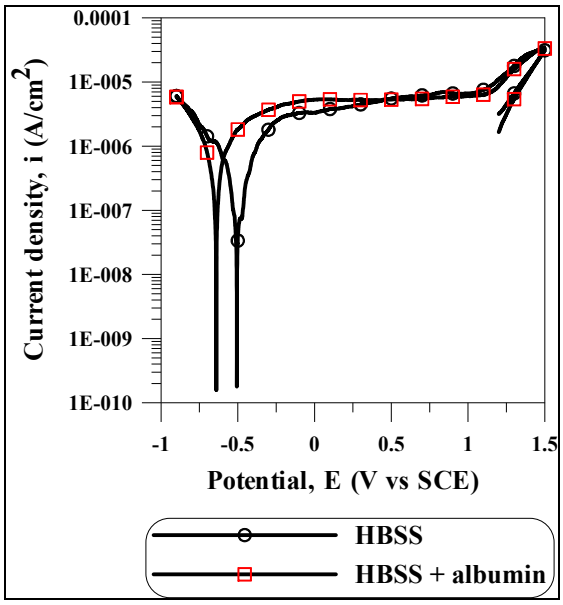

Figure 1. Potentiodynamic polarization curve recorded over the range of −900 mV to 1500 mV and reverse to 1200 mV for Ti6Al7Nb alloy in HBSS with and without albumin proteins, at 25^0C. dE/dt = 0.5mVs^{-1}

The anodic scans indicate that the Ti6Al7Nb alloy in HBSS with and without albumin protein forms the oxide film without the apparition of an active region representative for metal corrosion.

The current density is low and steady in the passive region, which extends over a wide range of potentials for Ti6Al7Nb alloy in HBSS with and without albumin protein, but some slightly differences can be observed when considering the potential dependence of the passive current. The anodic current density increases at potential around 1200 mV, which may be probably related to the changes in the composition of the passive film.

The potential domain for investigation of the electrochemical behaviour by EIS measurements for the Ti6Al7Nb alloy was chosen from above anodic polarisation curve. It was decided to perform these tests at the -500 mV, 0 mV, 500 mV and 1000 mV.

Impedance spectroscopy results for Ti6Al7Nb alloy in HBSS with and without albumin protein at selected potential values are presented as Nyquist diagrams (figures 2 and 3).

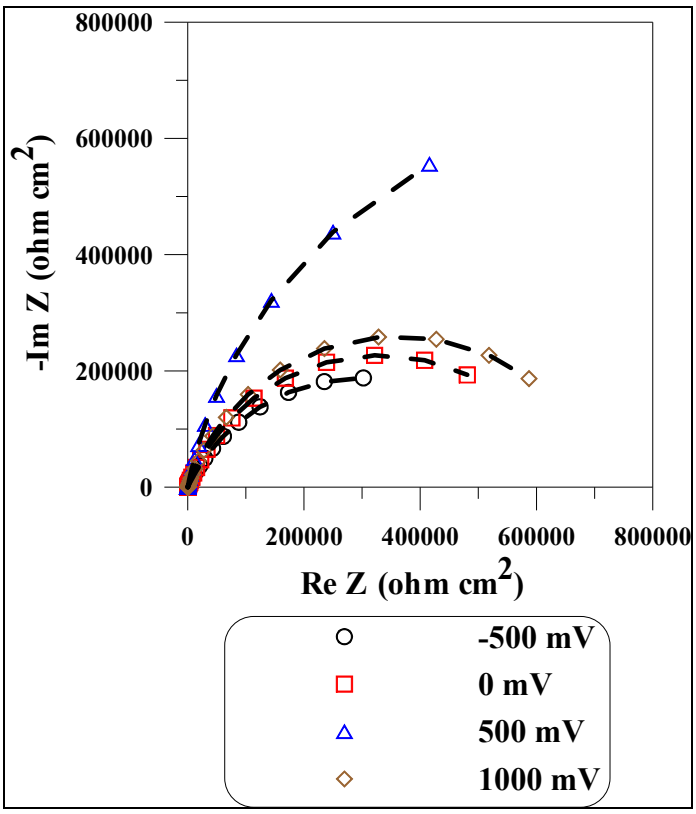

Figure 2. The effect of potential on the Nyquist impedance diagram of HBSS

Ti6Al7Nb alloy in both electrolytes, display a capacitive impedance spectrum with one time constant. The diameters of the semicircles correspond to the polarizing resistance. The Randles equivalent circuit (EC) which comprises only one time constant (figure 4) was used to model the experimental spectra, and good agreement between experimental data and fitted data was obtained.

Studies performed on Ti-based alloys under physiological conditions showed that the Randles equivalent circuit can be used successfully to describe the behaviour of these materials as well [2, 17, 24, 25]. The parameter R_p coupled with Q describes the processes at the electrolyte/oxide film interface. R_p is the polarization resistance related to the rate of corrosion reaction (s) at different potentials, and is inversely proportional to the corrosion current.

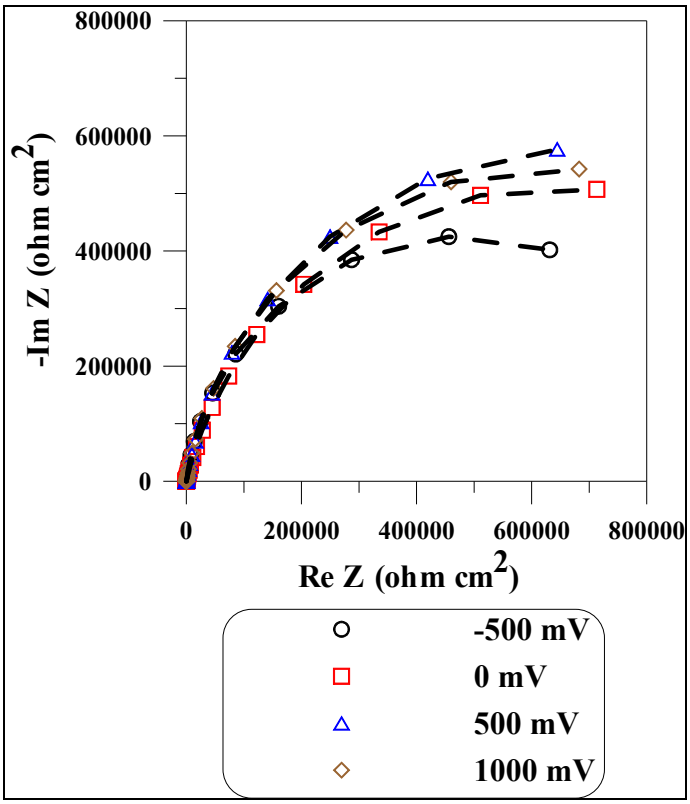

Figure 3. The effect of potential on the Nyquist impedance diagram of HBSS with albumin

The symbol Q signifies the possibility of a non-ideal capacitance (CPE, constant phase element) with n varying from 0.80 to 0.90 for impedance data at different potential. The impedance of the CPE is given by [30] as shown in equation (1):

$$Q = Z_{CPE} = \frac{1}{C(j\omega)^n} \quad (1)$$

where for n = 1, the Q element reduces to a capacitor with a capacitance C and, for n = 0, to a simple resistor. Generally, the use of a CPE is required due to a distribution of the relaxation times as a result of inhomogeneities present on the microscopic level under the oxide phase and at the oxide – electrolyte interface.

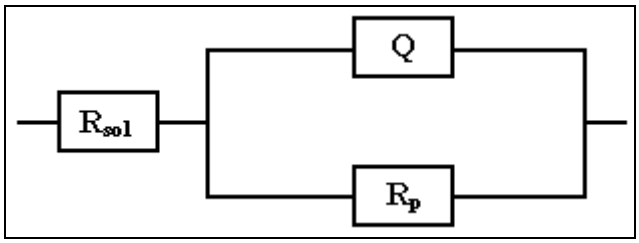

Figure 4. Equivalent circuit (EC) used to fit the impedance data

The values of fitted parameters of the EC and at different potentials are presented in table 1.

Table 1. Values of fitted parameters of the equivalent circuits as a function of applied potential of Ti6Al7Nb alloy in HBSS with and without albumin proteins

Applied potential (mV)	Q ($S\ cm^{-2}\ s^n$)	n	R_p ($\Omega\ cm^2$)
HBSS			
-500	9.6×10^{-6}	0.80	6.1×10^5
0	5.7×10^{-6}	0.80	6.6×10^5
500	4.2×10^{-6}	0.81	9.5×10^5
1000	3.8×10^{-6}	0.81	7.2×10^5
HBSS + 37.5 mg/ml albumin proteins			
-500	7.1×10^{-6}	0.85	1.2×10^6
0	2.7×10^{-6}	0.89	1.4×10^6
500	2.1×10^{-6}	0.90	1.4×10^6
1000	1.7×10^{-6}	0.90	9.8×10^5

R_{sol} is the resistance of the electrolyte between the working (Ti6Al7Nb alloy) and the reference electrode. This parameter has a value around 30 Ωcm^2 in HBSS and 45 Ωcm^2 in HBSS with albumin proteins.

Large values of R_p (order of $6 \times 10^5\ \Omega cm^2$) are obtained at -500 mV, confirming the formation of a passive layer of TI6Al7Nb alloy in HBSS. As the potential changes from -500 mV to 0 mV and from 0 mV to 500 mV, R_p increases. These results seem to correspond to a slight thickening of the titanium oxide film. As the potential increases from 500 mV to 1000 mV the R_p decreases. The decrease in resistance indicates that the oxide layer may become more defective at large over potential [6, 31, 32]. But, the R_p of Ti6Al7Nb alloy in HBSS was large at 1000 mV as seen in table 2. This indicates that the alloy is still highly resistant to corrosion even at very large over potentials.

The Nyquist plots show that the impedance for Ti6Al7Nb in HBSS with albumin increases in time. The R_p increases with the addition of albumin to the HBSS.

Proteins can influence the corrosion reactions in several ways and thus shift the position of equilibrium. For example, proteins can bind to metal ions and transport them away from the interface thus encouraging further dissolution [22]. Proteins can accelerate the dissolution of metals through their chelation effects [15, 27].

Our data suggest that the albumin proteins are increasing the corrosion resistance of the Ti6Al7Nb alloy.

At physiological pH, albumin is negatively charged (isoelectric pH 4.7 – 4.9). It may be possible that under these conditions proteins molecules adsorbed to the surface restrict metal dissolution. The adsorption of organic species may cause the blocking of terminal oxygen atoms at the interface passive film-electrolyte, which, consequently, hinders the charge transfer responsible for the passive film dissolution [17].

In order to compare capacitance values for Ti6Al7Nb alloy at different potentials, they were calculated, using equation [33]:

$$C = \left(R^{1-n}Q\right)^{\frac{1}{n}} \tag{2}$$

Decrease in capacitance, starting from the -500 mV to 1000 mV (figure 5), can be attributed to thickening of the oxide layer, because:

$$C = \frac{\varepsilon\varepsilon_0 S}{l} \tag{3}$$

where: s is the effective surface, ε is the dielectric constant for the oxide layer, ε_0 is the permissivity of free space and l is the thickness of oxide layer.

Values of C in HBSS with albumin proteins are smaller than those of C in HBSS at all observed potential (figure 5).

Since the proteins decreased the C, it is possible that the adsorbed proteins formed a compact film.

Serro et al. [34] determined a similar magnitude with a capacitance of 24–32 μFcm^{-2} for Ti immersed in Hank's salt solution containing bovine albumin serum. Contu et al. [17] reported on the interfacial capacitance of Ti6Al4V alloy immersed in bovine serum albumin with aging time. The capacitance of a constant phase element initially started at 27 μFcm^{-2} and stabilized at 11 μFcm^{-2} after 140 hours.

Gileadi [35] reported that the capacitances decrease as the fractional coverage of adsorbing species approaches unity. With further increasing potentials, the capacitance drops because the adsorbed albumin serves as an inhibiting anion or barrier to the aggressive ions on the alloy surface.

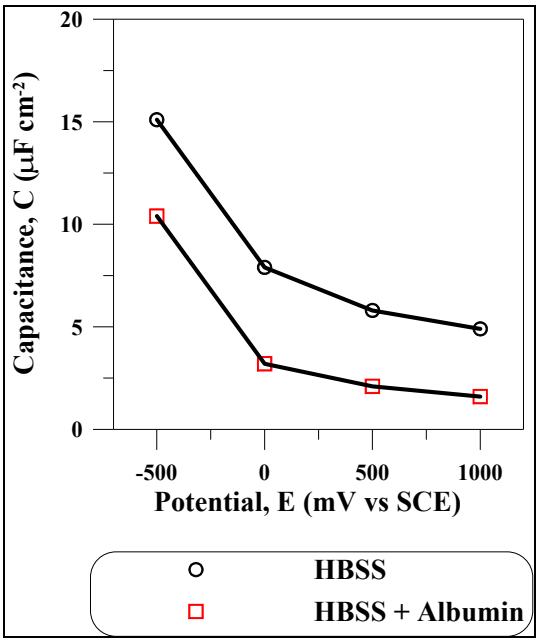

Figure 5. Capacitances (C) deduced from EIS data as a function of applied potential on Ti6Al7Nb alloy in HBSS with and without albumin proteins, at 25°C

CONCLUSIONS

The electrochemical behaviour of Ti6Al7Nb alloy in HBSS with and without albumin protein was studied using electrochemical polarization and electrochemical impedance spectroscopy techniques. The potentiodynamic polarization curves of Ti6Al7Nb alloy in HBSS, with and without albumin proteins addition, exhibited a passive behaviour. The albumin proteins shifted the zero corrosion potential in the negative direction and inhibited the cathodic corrosion reaction. The EIS simulated with a single time-constant representing a resistance–capacitance equivalent circuit elucidated an interaction between the albumin and the passive film. The charge transfer reactions, simulated by the polarizing resistance, indicate that albumin protein increases the corrosion resistance of Ti6Al7Nb alloy.

EXPERIMENTAL SECTION

The Ti6Al7Nb alloy used in present investigation was acquired in form of a road from National Institute of Research & Development for Non-ferrous and Rare Metals, Bucharest, Romania. The Ti6Al7Nb alloy was submitted to a semi-quantitative chemical analysis by plasma optical emission spectrometry technique and its results are given in table 2.

Table 2. Chemical composition of Ti6Al7Nb alloy determined by plasma optical emission spectrometry technique

Element	Ti	Al	Nb	Fe	Si	O_2	N_2	C
Weight %	balance	6.21	7.08	0.13	0.088	0.11	0.032	0.082

The microstructure of Ti6Al7Nb alloy is shown in figure 6. The alloy had a (α + β) duplex microstructure which consists in a globular and short lamellar α-phase into β-phase matrix.

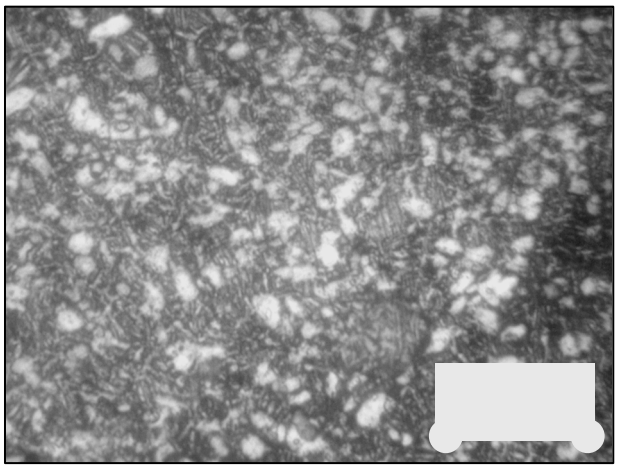

Figure 6. Optical photographs for Ti6Al7Nb microstructure. The sample was etched using 10%HF + 5%HNO_3 solution at 25^{o}C

The working electrodes were cut into 0.2 cm^2 sizes and brass nut was attached to sample using conductive paint to ensure electrical conductivity. The assembly was then embedded into an epoxy resin disk. Then the samples were ground with SiC abrasive paper up to 1000 grit, final polishing was done with 1 μm alumina suspension. The samples were degreased with ethyl alcohol followed by ultrasonic cleaning with deionised water and dried under a hot air stream.

The electrolytes used included:

1. Simulated physiological Hank's Balanced Salt Solution (HBSS) consisting of (g/l): 8 NaCl, 0.4 KCl, 0.35 $NaHCO_3$, 0.25 $NaH_2PO_4 \times H_2O$, 0.06 $Na_2HPO_4 \times 2H_2O$, 0.19 $CaCl_2 \times 2H_2O$, 0.19 $MgCl_2$, 0.06 $MgSO_4 \times 7H_2O$, 1 glucose, at pH=6.9.

2. Simulated physiological Hank's Balanced Salt Solution (HBSS) + 37.5 mg/ml human albumin protein (KEDRION S. p. A., Italy). Peters [36] reports that the average albumin content of 42 mg/ml occurs in human blood with a

range of 35–50 mg/ml, for males requiring nursing care, the albumin level ranges 35–40 mg/ml or ages of 40–96 years. As a consequence, an albumin content of 37.5 mg/ml was selected for the electrolyte to approximate the midrange albumin content representing patients requiring medical attention (e.g., surgical implants).

Electrochemical measurements were carried out in aerated solution at 25^{o}C using a Princeton Applied Research potentiostate (Model 263 A) connected with a Princeton Applied Research 5210 lock-in amplifier controlled by a personal computer and a specific software package called Electrochemistry Power Suite (Princeton Applied Research). A glass corrosion cell kit with a platinum counter-electrode and a saturated calomel reference electrode (SCE) were used to perform the electrochemical measurements. All potentials referred to in this article are with respect to SCE. Polarization diagrams were obtained for Ti6Al7Nb alloy immersed in HBSS with and without albumin protein, at a scanning rate of 0.5 mV/s.

Electrochemical impedance spectroscopy (EIS) measurements were performed in HBSS with and without albumin protein at different potentials at 25^{o}C. EIS results at different potentials were obtained 30 minutes after the overpotential has been applied. The EIS spectra were recorded in the 10^{-2} Hz to 10^{5} Hz frequency range. The applied alternating potential signal had amplitude of 10 mV. In order to supply quantitative support for discussions of these experimental EIS results, an appropriate model (ZsimpWin-PAR, USA) for equivalent circuit (EC) quantification has also been used.

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