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Adsorption of bovine serum albumin on CoCrMo surface: Effect of temperature and protein concentration

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ABSTRACT

The adsorption of bovine serum albumin (BSA) onto CoCrMo surface has been studied as a function of concentration of BSA and temperature by electrochemical techniques. The electrochemical impedance spectroscopy (EIS) technique was used to investigate the interfacial behaviour of BSA at open circuit potential (OCP). The charge transfer resistance was very sensitive to the amount of adsorbed protein, indicating that the adsorption process was accompanied by the transfer of charge and influenced the mechanism and kinetics of the corrosion reaction. At all the temperatures studied, adsorption of BSA onto the CoCrMo surface was successfully described with a Langmuir adsorption isotherm. EIS study was also carried out for determine the surface charge density, resulting from protein adsorption, and it was shown to be directly proportional to the amount of adsorbed protein (surface concentration). Thermodynamic data of adsorption was obtained for analyzing the adsorption of BSA onto CoCrMo surface. Gibbs free energy of adsorption, ΔG_{ADS} values, for BSA in the investigated temperature range (-51 kJ mol^{-1}) showed that the molecules have a strong affinity for the CoCrMo surface. Enthalpy (ΔH_{ADS}) and entropy (ΔS_{ADS}) of adsorption suggested that the adsorption process of BSA onto the CoCrMo surface is an endothermic process and the molecule suffers structural changes when adsorbing on the metallic surface.

1. Introduction

Proteins are relatively large biomolecules and have a tendency to accumulate at the interface between solutions and solid surfaces [1,2]. The adsorption of proteins at interfaces is a widespread phenomenon in both natural and man-made systems. Protein adsorption occurs when any protein-containing fluid comes into contact with a foreign surface. There has been considerable interest in the interfacial behaviour of proteins in the human body as a result of problems associated with bacterial growth [3,4] and metal dissolution [5–7]. In the field of biomaterials and medical implants, it is widely accepted that one of the initial events that significantly influences biocompatibility is the nearly instantaneous adsorption of proteins from biological fluids onto biomaterial surfaces. This adsorbed protein film may be beneficial for certain biomedical applications where the immobilization of specific proteins and enzymes is necessary or desirable, such as in the case of immunoassays and biosensors [8]; on some metallic materials and alloys even the adsorption process reduces the corrosion rate [9]. However, for serum-contacting medical devices (including implants), the complex layer of adsorbed plasma proteins is generally unfavourable and could potentially lead to major complications including microbial infections [10]. As a consequence of this process, on some metals' surfaces an accelerated metal ion release rate was obtained [6,7,11]. A number of studies have been made on the interaction of proteins with metal surfaces to determine the molecular conformation or orientation of the adsorbed molecules. It was found that the interactions between proteins and surfaces, resulting in adsorption, can be affected by a number of factors, such as temperature, conformation of the protein in solution and its bulk concentration, pH, ionic strength, and the surface characteristics of the material onto which adsorption occurs [1,12]. The enhancement of dissolution rate in the presence of proteins can be explained by the formation of biofilm or complexes between metal ions and proteins. Although the biofilm also lubricates the surface, the total material degradation was increased due to increased corrosion [5,6,13,14].

Bovine serum albumin (BSA) is of particular interest when studying the biocompatibility of metal implants and it is considered as a model protein. Serum albumin (MW ~ 66.3 kDa, dimensions of $15 \times 3.8 \times 3.8$ nm^3) is the most abundant protein in the body fluid. The protein's (heart shaped) globular structure is achieved by the folding of its polypeptide chain (containing 585 amino acid residues) into three α-helical domains [15,16]. Because of its high concentration, serum albumin arrives first at the implant's surface according to the laws of mass transport, and therefore plays an important role in the initial adsorption of proteins onto biomedical surfaces.

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Several electrochemical studies have been carried out on the adsorption of serum proteins [17] on titanium and titanium oxides [1,18], stainless steels [19–22] and platinum [23]; however, no literature has been found on the behaviour of serum protein adsorption on CoCrMo alloys. CoCrMo alloys are biocompatible materials widely used for orthopaedic implants such as hip and knee joint replacements [24]. The biocompatibility of CoCrMo alloy is closely related to its excellent corrosion resistance, imparted by a passive oxide film that forms spontaneously on the alloy surface [5]. Once implanted and exposed to the aggressive body environment (biological fluids in the body contain water, salt, dissolved oxygen, bacteria, proteins and various ions such as chloride and hydroxide), CoCrMo tends to corrode over time and metal ions become clinically significant resulting in a lack of biocompatibility of the implant [25]. Indeed, correlations between metal release from CoCrMo hip joint implants and their clinical behaviour could be established in in-vivo studies involving animal and human patients experiments [26–29]. Others studies confirmed that electrochemical corrosion caused loss of biocompatibility and failure of CoCrMo implants [30–33]. Despite the clinical relevance of the corrosion of CoCrMo implant alloys, the involved phenomena are still little understood and few studies are available on this topic.

The objective of this work is to elucidate the mechanism of BSA adsorption onto CoCrMo biomedical alloy through electrochemical techniques. For this, the corrosion behaviour of a biomedical CoCrMo alloy was investigated in 0.14 M NaCl with and without the addition of several BSA concentrations over the temperature range 298–333 K using cathodic and anodic potentiodynamic curves and electrochemical impedance spectroscopy. Electrochemical parameters obtained from the electrochemical tests have been used to determine adsorption isotherms, surface concentration of adsorbed protein, surface affinity of the protein, Gibbs free energy, enthalpy and entropy of adsorption. This provides a better understanding of the interfacial behaviour of these proteins and the potential use of CoCrMo alloys in their implant application.

2. Experimental details

2.1. Electrolytes and sample preparation

Solutions of bovine serum albumin (from Merck Fraction V) were prepared by dissolving reagents in 0.14 M NaCl solution, pH 7.4. These formulations were selected for simulating the biological environment that involves the metallic alloy into the human body. In order to analyze the adsorption mechanism of the protein 5, 20, 50 and 500 mg/L BSA were added to the NaCl electrolyte. Although these solutions are not buffered, no significant variations in the solution pH were observed during the experiments. All chemicals were of analytical grade and doubly distilled grade water was employed in the preparation of the solutions. Temperature of the solution was preheated at 298, 313, 323 and 333 K before carrying out the electrochemical experiments.

The High-Carbon CoCrMo alloy was obtained from Lafitt (Valencia, Spain) in the form of rods, 9 mm in diameter. The nominal composition of this wrought alloy corresponding to ISO 5832-12 is shown in Table 1. The thermal treatment applied on the alloy consists on a solution annealing, a porous coating, a hot isostatic pressing and a final solution annealing. Samples were embedded in epoxy mount so that a surface area of 0.65 cm^2 was exposed to the test solution. The samples were ground with SiC emery paper up to 1000 grit before each experiment. After cleaning and rinsing with water and ethanol the polished sample was instantly assembled into the measurement cell that was subsequently filled with the electrolyte.

Table 1
CoCrMo alloy composition.

Element	CoCrMo alloy (wt.%)
C	0.259
Si	0.9
Mn	0.38
P	0.05
S	0.005
Al	0.016
B	0.002
Co	Balance
Cr	28.45
Fe	0.22
Mo	5.39
Ni	0.29
Ti	0.02
W	<0.05
N	74.9 ppm
O	10.6 ppm

2.2. Electrochemical experiments

Different electrochemical tests have been conducted: potentiodynamic curves and electrochemical impedance spectroscopy (EIS). All measurements were carried out using a potentiostat Solartron 1287. The three-electrode electrochemical setup included a platinum wire as counter electrode and a Ag/AgCl (3 M KCl) reference electrode. All potentials are given with respect to this reference electrode which standard potential is 205 mV with respect to the standard hydrogen electrode (SHE). Previously heated electrolyte solutions were poured at the corresponding test temperatures into the double wall cell heated through water circulation. Before the measurements, the samples were left stabilizing at open circuit potentials for 60 min. The potentiodynamic measurement rate was 2 mV s^{-1}. Scans were started at a potential 200 mV below the open circuit potential (OCP) and moved in the anodic direction to 1500 mV. All experiments were carried out under aerated conditions. The corrosion potential E_{corr} as well as the corrosion current density i_{corr} were automatically extracted from the polarization through Tafel slope extrapolation. Passive current density, i_p, and breakdown potential, E_b, were obtained from the potentiodynamic curves.

EIS tests have been conducted under open circuit conditions. EIS measurements were initiated after 1 h immersion in the solution (OCP measurements). Measurements have been performed starting from 10^5 Hz up to 1 mHz, at 10 data cycles/decade with an ac amplitude of ±10 mV. The impedance data were analyzed with the *Zview 2.70* software package and fitted to corresponding the equivalent circuit. In order to verify the reproducibility of the measurements three repetitions of each test were carried out.

3. Results

3.1. Potentiodynamic polarization measurements

OCP values were measured before obtaining the potentiodynamic curves and they were analyzed for the CoCrMo alloy. OCP values are summarized in Table 2. A displacement of the OCP towards more noble values with temperature was observed for the biomedical alloy in NaCl solution and BSA-containing solutions at concentrations below 500 mg/L.

Fig. 1 shows the anodic polarization curves of CoCrMo obtained in 0.14 M solution with different BSA concentration (0, 5, 20, 50 and 500 mg/L) and temperatures (298, 313, 323 and 333 K). To facilitate interpretation, the polarization curves can be divided in four potential domains. The cathodic domain includes potentials below −1100 mV where the current is determined by the reduc-