IN *VITRO* QUANTIFICATION OF IRON IONS RELEASED FROM AISI 316L STAINLESS STEEL USING VOLTAMMETRIC METHODS.

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ABSTRACT

In vitro studies were conducted to quantify the amount of iron species released from AISI 316L stainless steel biomaterial during its implantation either in human beings or animals. By constructing calibration curves for those species using the cyclic voltammetry technique it was possible to determine the iron ions levels in physiological medium.

INTRODUCTION

Orthopaedic implants are susceptible of suffering interfacial biodegradation due to the nature of the biological environment surrounding the implants as well as mechanical effects. Considering that AISI 316L stainless steel is one of the most commonly used metallic biomaterial in medicine and animal surgery, several studies were performed in order to understand its corrosion behaviour and the alterations caused by the released metal ions in several vital organs [1-4]. It has been shown that the occurrence of electrochemical reactions at the implant-tissue interface is responsible

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for the metal ions release which will penetrate into the adjacent tissues, followed by its biological transport through the blood stream ending up by being partially accumulated at the liver, kidneys and spleen, or else, partially eliminated in the urine [5-7].

Within the past decade, atomic absorption spectrophotometry (AAS) was the most widely used technique to analyze the amount of metal ions present in the biological tissues surrounding the implant as well as in the organs cited above. However, the samples preparation procedure, for analysis namely the drying of biological tissues, digestion and chemical attack of the residue was considered by the researchers as tedious, time consuming and often leading to the lost of significative amounts of the metallic species. These amongst, other factors calls for the development of a more effective way to investigate the metal ions levels in biological samples.

It has been reported in the literature that the voltammetric methods, i.e. linear sweep voltammetry (LSV) and cyclic voltammetry (CV) are quite suitable techniques to identify electroactive species present in a system undergoing electrochemical investigation [8-10]. These techniques involves the control of the electrode potential as the independent variable and measurement of the current as the dependent variable or vice versa. The voltammetric response shows a peak corresponding to the characteristic potential of the oxidation or reduction of a certain specie present in the solution and the magnitude of the rising current depends on the rate of the species mass transfer for the system. On the other hand, the magnitude of the peak current, either cathodic (ip^c) or anodic (ip^a), is proportional to the concentration of the electroactive species which allows, for well defined systems, its quantification even though it may not be a straightforward procedure due to the fact that the response depends upon the rate of the electron transfer reaction which requires that the surface concentrations should be close to those demanded by the electrode potential through the Nernst equation [8-10]. Surely the most powerful feature of voltammetric measurements is its ability to provide easily interpretable information about electrode reaction mechanisms.

In the present study, it is reported the use of cyclic voltammetry measurements to quantify the amount of iron species released from AISI 316L stainless steel implants. So far, only *in vitro* results were attained. However, it is hopped that in the near future, one may be able to use these methods as a feasible way to monitor *in vivo* metallic ions, released from biomaterials.

EXPERIMENTAL

A three-electrode compartment cell was used to perform the electrochemical measurements described hereinafter. Two different sets of working electrodes were used, namely a conventional platinum (Pt) electrode (area of c.a. 3.5 mm^2) and a Pt microdisk electrode (diameter of 7 μm). A Pt foil of area c.a. 1 cm^2 was employed as secondary electrode for all voltammetric measurements which, in turn, were measured versus the SCE. All chemicals were analytical-grade reagents and were used as purchased from commercial sources. The medium used in all voltammetric measurements was the Hank's Balanced Salt Solution (HBSS) which simulates the composition of the physiological fluids. Its composition is the following: $CaCl_2.2H_2O$ 0.14g/L; KCl 0.40 g/L; KH_2PO_4 0.06 g/L; $MgCl_2.6H_2O$ 0.10 g/L; $MgSO_4.7H_2O$ 0.10 g/L; NaCl 8.00 g/L; $NaHCO_3$ 0.35g/L; $NaHPO_4.7H_2O$ 0.09 g/L and D-Glucose 1.00 g/L having a pH of 7.4. Solutions containing 100, 200, 300, 400 and 500 ppm of $K_3[Fe(CN)_6]$ from Merk, were prepared to construct the calibration curves for Fe^{3+}/Fe^{2+} species.

The experimental voltammetric transients were obtained using a potentiostat/galvanostat Model Autolab from ECO Chemie equipped with a module ECD which was connected to a PC Model 1120SX which has installed an EAS software. Voltammograms were acquired at several sweep rates and different potential limits being switched from anodic to cathodic potentials. The current-potential transients were recorded on a EPSON-LX 800 printer connected to the computer. All electrical connections were made using low-noise coaxial cables and the electrochemical system was kept inside of a thick-walled aluminum sheet cage to avoid any external interference.

The temperature and pressure used were the same as those in the laboratory.

RESULTS AND DISCUSSION

The peak-shaped voltammogram presented in Fig.1A displays the characteristic features of the redox behaviour for the Fe^{3+} species in HBSS medium. Within the forward sweep the oxidation peak is observed at a potential of Ep^a=0.238 V vs SCE whereas the reduction peak is observed at Ep^c=0.153 V vs SCE using a given sweep

rate, ν, of 50 mV/s. For this voltammogram the magnitude of the currents are: ip^a=0.269 μA and ip^c=-0.225 μA. In Fig.1B it is illustrated the current-potential transient of a solution containing 500 ppm Fe^{3+} using the same sweep rate. It is observed that the potential peaks appear at identical values but the magnitude of the currents are much higher, namely ip^a =18.3 mA and ip^c =-15.3 mA.

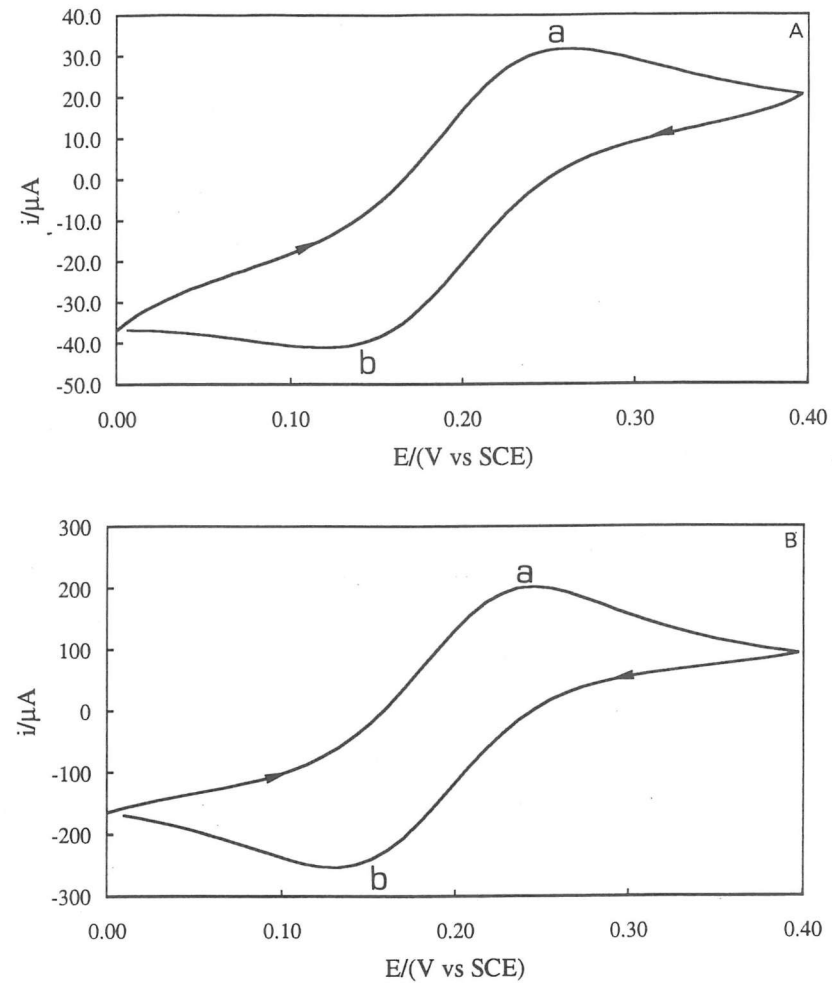

Figure 1- Cyclic voltammograms of Fe^{3+}, using a Pt electrode, in HBSS at ν=50 mV/s; A) 100 ppm Fe^{3+}, B) 500 ppm Fe^{3+}; a) ip^a ; b) ip^c.

This pattern of behaviour was observed for all current-potential transients measured which means that, indeed, the magnitude of the currents is proportional to the species

concentration. From the experimentally attained, oxidation and reduction potential values it is clear that they are independent of the concentration. The same conclusion holds for the sweep rate. This indicates that the redox behaviour of Fe^{3+} in HBSS is a reversible system, identical to that observed in aqueous solution [8-15].

In Fig.2 it is plotted two sets of calibration curves at different sweep rate, namely 50 mV/s and 100 mV/s. For the present study, the most suitable sweep rate was 100 mV/s. For lower and higher values, the fit has an r^2 value much lower than the unity, indicating that the points distribution was not as smooth as for ν=100 mV/s.

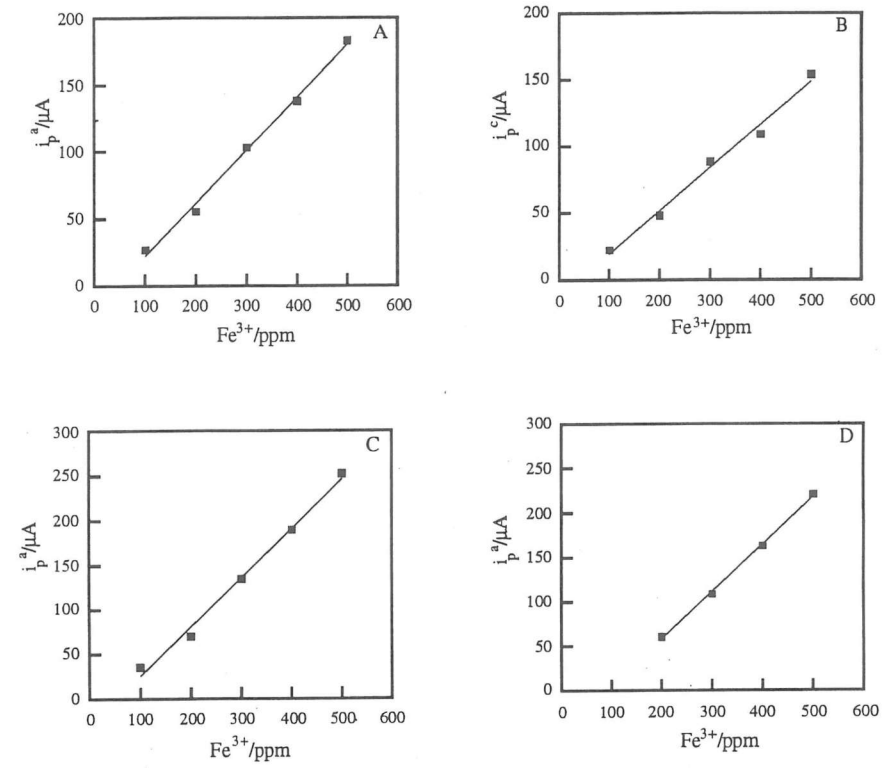

Figure 2- Calibration curves for Fe^{3+} as a function of sweep rate; A, B) ν=50 mV/s; C, D) ν=100 mV/s.

These calibration curves, Fig.2, were used to quantify the amount of metal ions released from AISI 316L stainless steel (SS) in HBSS after anodic dissolution of that material. In order to simulate the biodegradation process which takes place in human

beings and animals carrying AISI 316l SS implants it was performed *in vitro* electrochemical tests at our laboratory [15]. The anodic dissolution of this material simulates the corrosion process due to the effects of the physiological fluids, forming a metal ions slurry.

Results from a typical cyclic voltammetry experiment is illustrated in Fig.3. The similarity between this voltammogram and those presented in Fig.1 indicates to be possible to quantify the iron levels present in the metallic slurry. It is of interest to point out, that we have restricted the potential range to that corresponding to the redox behaviour of iron, otherwise a more complex current-potential transient will be observed due to the appearance of other peaks for the other metallic species released from the AISI 316L SS such as Cr^{3+}, Ni^{2+} and Mo^{6+}. The value attained for the iron species in the slurry was c.a. 110 ppm.

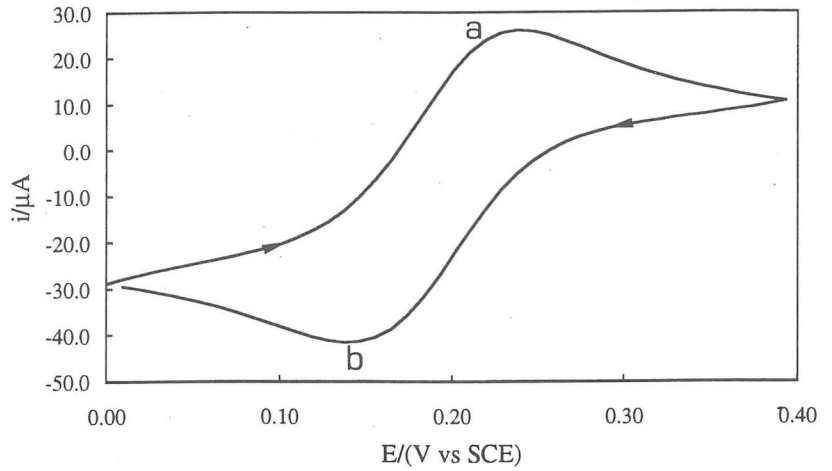

Figure 3- Cyclic voltammogram of Fe^{3+} 110 ppm in HBSS at ν=10 mV/s.

In recent work [15], we have begun these investigations but using Pt microdisk electrodes and carbon (C) microdisk electrodes. While it is possible to achieve a quantitative analysis using conventional electrodes based on the Ip-C relationship, it is more practical to quantify the species using microelectrodes as working substrates. For this devices, one can obtain experimentally a steady-state voltammogram which is governed by the Cottrell equation [16], where the magnitude of the total current is proportional to the concentration and the systems can be regarded as being under diffusion control. However, the results dealing with microelectrodes are still far from

being complete due to some difficulties encountered in the experimentation which can probably be attributed to a progressive poisoning of the electrode surface. On the other hand, the electroactive species present in the HBSS might interfere with the amplitude of the peak currents measured experimentally. Furthermore, the pH has a marked influence on the reduction potentials of the metallic species. this has been observed for all the voltammograms taken in this study and can be regarded as being due to surface modification processes, leading to changes in the adhesion work free energy.

These preliminary data demonstrate that voltammetric methods can be used as a suitable tool to quantify metal ions released from AISI 316L stainless steel biomaterials during the biodegradation process. As has been mentioned elsewhere [15] the main goal of this kind of research is to understand the mechanism associated to the metallic biomaterials degradation process prior to the development of a biosensor capable of monitoring the release of metal ions. The information obtained from these experiments can be directly compared to the measurements made *in vivo* to quantify these metallic species.

CONCLUSION

Cyclic voltammetry measurements are suitable for the investigation and quantification of iron ions released from AISI 316L stainless steel biomaterials both *in vitro* and *in vivo*. The redox mechanism of iron species (Fe^{3+}/Fe^{2+}) in physiological medium is identical to that observed in aqueous solution, i.e. constitute a reversible system. However, it is important to note that the chemical composition of the solutions used to construct the calibration curves differs from the chemical composition of the metallic slurry obtained from the anodic dissolution of the biomaterial and, therefore, some additional complexities are experimentally observed for the latter electrochemical system.

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A COMPARATIVE STUDY ON THE ELECTROCATALYTIC ACTIVITY OF Pt, Pt-Ir AND Ir ELECTRODES TOWARDS THE OXIDATION OF D-SORBITOL

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Abstract- The electro-oxidation of D-sorbitol in aqueous perchloric medium was used as a test of the electrocatalytic activity of platinum, platinum-iridium and iridium electrodes. Cyclic voltammetry was the main technique used in this study. Results have shown almost no activity for Ir electrodes and similar activities for Pt and Pt-Ir, slightly higher for Pt-Ir (90:10) electrodes.

Key words - electrocatalysis, electro-oxidation, D-sorbitol, noble metals

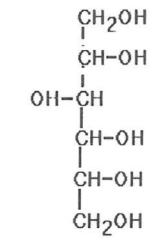
INTRODUCTION

The electrocatalytic activity of noble metals towards the oxidation of various organic molecules, especially those derived from biomass like alcohols and polyols, has been studied for a long time because of the potential interest of these systems in the development of electrochemical generators (fuel cells) [1 and 2 and references therein].

It is well known, that the mechanism and kinetics of those electrocatalytic reactions are influenced by several parameters, such as temperature, pH, concentration of the electroactive species, nature of the electrolyte and structure and composition of the electrode material. The role of the nature and structure of electrode materials in electrocatalysis has been quite well demonstrated [1, 2 and 3 and references therein]: the substrate affects the reaction kinetics, mainly through the adsorption of reactants, reaction intermediates and products [2].

The influence of the molecular structure of the reactant molecule (lenght of carbon chain, position of OH groups) on the adsorption and electro-oxidation of polyols on Pt has been comparatively examined by Enea and Ango [4].

D-sorbitol is a promising fuel for a clean production of energy, but also a rather large molecule with six OH groups, with one OH group opposite to the others, i.e.:



The electrochemical oxidation of this molecule in perchloric medium at a polycrystalline (poly) and on platinum single-crystals, namely Pt(100), Pt(110) and Pt(111) has been considered [5]; significant structural effects have been observed in acid medium. A quantitative study on the electrosorption of D-sorbitol on Pt(poly) and Pt(100) electrodes in perchloric medium has also been performed

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