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A BIOELECTROCHEMICAL APPROACH FOR ASSESSING BACTERIAL
ADHESION INHIBITION BY IMMUNOGLOBULINS

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Abstract

Microbial adhesion is widely accepted as the main stage prior to the induction or initiation of biocorrosion. An innovative approach to prevent biocorrosion through microbial adhesion inhibition by using solutions of immunoglobulins showed promissory results with different stainless steels and bacterial strains. Potentiodynamic runs revealed no significant differences in the electrochemical behaviour of the stainless steel, independently of the presence or absence of immunoglobulin coverage of the metal surface. Pre-conditioned samples showed a passive behavior region slightly more extended than non-treated coupons.

Further studies on the structural properties of the immunoglobulins, related to their ability of preventing cell adhesion are needed. With this aim, cyclic voltammetry using platinum electrodes in solutions of mixed immunoglobulins in inorganic buffer was used. Changes in the voltammetric profiles were obtained for different concentrations, at different contact times with reference to the uncovered platinum electrode, indicating the presence of adsorbed proteins.

Key words: adhesion inhibition, immunoglobulins, biofilms, cyclic voltammetry.

Introduction

The adhesion of microorganisms to metal surfaces and the formation of biofilms have been shown to occur in industrial systems processing environments and provide a persistent source of contamination. These biofilms consist of microorganisms embedded and immobilised in an organic matrix of bacterial origin (Marshall, 1992) and are generally more resistant to disinfectants and sanitizers than are free cells (Zottola, 1995). This increased resistance has imposed uncertainty on the efficacy of industrial clean-in-place (CIP) systems in removing or killing all attached cells (Herald & Zottola, 1998; Zottola 1995). Alternatives or improvements to the present cleaning systems should therefore be considered.

An innovative approach to prevent biocorrosion through microbial adhesion inhibition by using immunoglobulin mixture showed promissory results with different stainless steels and aerobic and anaerobic bacterial strains (Guiamet et al., 1999; Guiamet & Gomez de Saravia 2000).

Further studies on the structural properties of the immunoglobulin molecules, related to their ability of preventing cell adhesion are needed. With this aim, cyclic voltammetry of platinum electrodes in solution of mixed immunoglobulins was used. The platinum electrode provides a model surface for determining the presence of adsorbates by measuring the changes in the profile of the cyclic voltammograms in terms of surface charge density.

Material and methods

An immunoglobulin commercial solution was used, IgAbulin, (Austria). Each ml of the solution contained 60 mg of IgA, 30 mg of IgG and 2 mg of IgM, total protein: 100 mg and glucose 100 mg, in a saline solution. 1:100, 1: 500 and 1:1000 dilutions of the commercial solution in phosphate buffer (pH 7.3) were used. The phosphate buffer solution was prepared using p.a. KH_2PO_4 and K_2HPO_4 and bidistilled water

Cyclic voltamperograms were carried out in a conventional double-wall glass cell. A platinum electrode was used as counterelectrode and a saturated calomel electrode (SCE) (in a Lugging capilar) was used as reference electrode, to which potentials in the text are referred. The working electrode was a Pt wire. All the experiments were made at 25 °C

The real surface area of the working electrode was determined by standard procedure (Angerstein-Kozłowska, 1984) in H_2SO_4 1 M prepared from 98% H_2SO_4 .

Prior to the electrochemical runs, the Pt electrode was cleaned with H_2SO_4 : HNO_3 1:1, rinsed with bidistilled water and dried. Then, the electrode was dipped into the Ig dilution for 30

sec, 1, 5 or 10 min and transferred to the electrochemical cell containing the buffer solution saturated with nitrogen to record the voltamperogram.

Cyclic voltamperograms were obtained using a potentiostat and a function generator to produce a repetitive triangular potential scan from -750 to 1000 mV (vs. SCE) at a scan rate of 500 mVs⁻¹. The voltamperograms were recorded with a X-Y recorder until a constant profile was obtained. The adsorption of organic compounds at electrode surfaces was obtained from the voltamperograms. Voltamperograms in the buffer solution without dipping the electrode in the protein dilution was recorded as controls.

Result and discussion

As it can be seen in figure 1, changes in the voltammetric profiles were obtained for the Ig dilutions, at different contact times with reference to the uncovered platinum electrode. These voltamperograms were recorded as soon as the electrode was placed in the cell. The charge involved in the anodic oxide formation and in the oxide reduction is higher when the proteins are adsorbed on the Pt surface.

From the cyclic voltammograms, the surface charge density (Q_{ads}) due to the adsorption of proteins was determined from the integrated current-potential response corresponding to the anodic oxidation in the presence of the protein ($Q_{O_0}^P$) and to the oxide reduction (Q_{Or}^P) (Roscoe & Fuller, 1993). The difference calculated between the surface charge density for anodic oxidation and that for oxide reduction was attributed to surface adsorption or oxidation of species other than oxygen or hydroxide. The surface charge density corresponding to the anodic oxidation and to the oxide reduction (Q_{O_0} and Q_{Or} respectively) determined in buffer solutions was subtracted from those determined for the protein solutions. The remaining charge density was attributed to the protein surface adsorption.

$$Q_{ads} = (Q_{O_0}^P - Q_{Or}^P) - (Q_{O_0} - Q_{Or}) \quad (1)$$

The values of Q_{ads} obtained for the each dilution of the commercial mixture of immunoglobulins used at different contact times are shown in figure 2.

Surface adsorption was found to occur readily at the open circuit electrode, when this is dipped into the solution. Roscoe & Fuller (1993) found a plateau level when representing Q_{ads} vs. t for κ -casein and β -lactoglobulin. As it can be observed in figure 2, this is not the case for the Ig mixture used in this work. This effect could be attributed to agglutination, denaturation or competitive adsorption of the different Ig present in the solutions (Roscoe, 1996). Experiments with pure proteins will be carried out to elucidate these effects.

The voltammograms obtained after 20 min of cycling were coincident with the controls, indicating that the proteins were removed from the Pt surface.

Conclusions

These experiments show that the adsorption of protein occurs immediately after the electrode was dipped into the Ig mixture, thus the presence of Ig on the surface could prevent the attachment of microorganisms.

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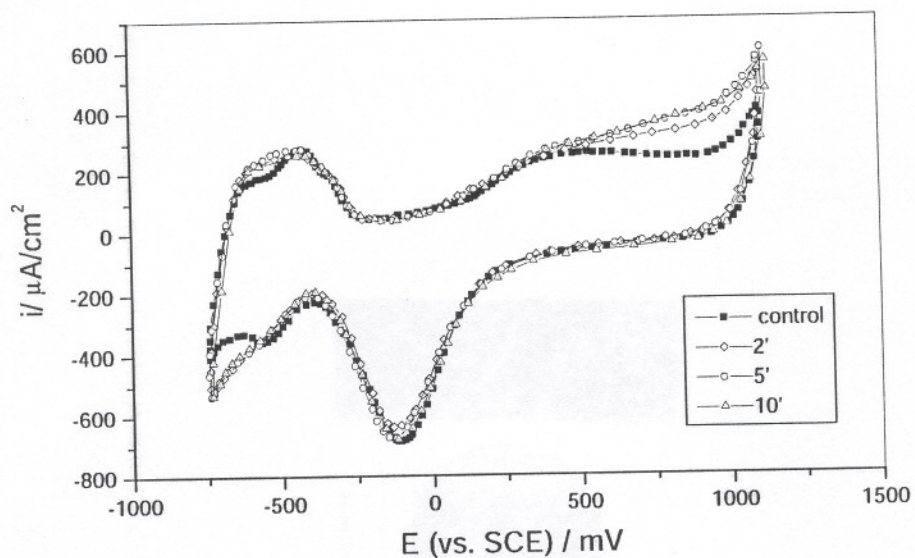


Figure 1. Cyclic voltamperograms of the Pt electrode in phosphate buffer solution, after being exposed to the 1/100 dilution of the Ig for different times.

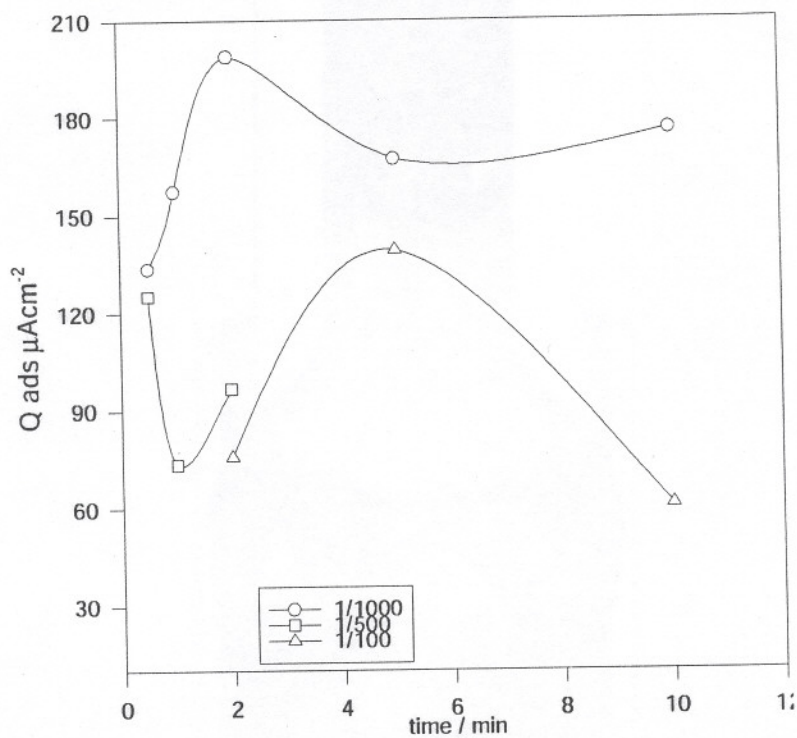


Figure 2. Q_{ads} vs. t for the different Ig dilutions used .

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