Available online at www.sciencedirect.com
ScienceDirect
Electrochimica Acta 52 (2007) 7660–7669
ELECTROCHIMICA Acta
www.elsevier.com/locate/electacta

Adsorption of BSA on passivated chromium studied by a flow-cell EQCM and XPS

I. Frateur [a,*,1], J. Lecoeur [a], S. Zanna [a], C.-O.A. Olsson [a,2], D. Landolt [b,1], P. Marcus [a,1]

[a] *Laboratoire de Physico-Chimie des Surfaces, CNRS-ENSCP UMR7045, Ecole Nationale Supérieure de Chimie de Paris, 11 rue Pierre et Marie Curie, 75005 Paris, France*
[b] *Ecole Polytechnique Fédérale de Lausanne (EPFL), Laboratoire de Métallurgie Chimique, CH-1015 Lausanne, Switzerland*

Received 25 July 2006; received in revised form 1 December 2006; accepted 17 December 2006
Available online 21 January 2007

Abstract

The adsorption of bovine serum albumin (BSA) on a passivated chromium surface in deaerated pH 4 sulphate solution was studied *in situ* using a switch-flow cell in combination with an electrochemical quartz crystal microbalance (EQCM) and *ex situ* by X-ray photoelectron spectroscopy (XPS). EQCM measurements showed that the kinetics of BSA adsorption was fast, and that a steady-state was reached about 10 min after introducing the protein. They also showed that BSA adsorption was an irreversible process, or that the kinetics of desorption was very slow. The equivalent thicknesses of the adsorbed BSA layer estimated *in situ* by EQCM and *ex situ* by XPS are in excellent agreement, and are equal to 3.5 ± 0.7 nm, which corresponds to one horizontally orientated monolayer.

Keywords: Chromium; BSA; EQCM; Flow cell; XPS

1. Introduction

The initial stage involved in the adhesion of cells and the formation of biofilms on solid surfaces is the adsorption of proteins present in the medium. In particular, the interactions between cells and the surface of biomedical implants is mediated by pre-adsorbed proteins. Similarly, the growth of biofilms on construction materials used in marine environments and water distribution systems is initiated by the adsorption of biomolecules [1]. Stainless steels are often used in protein-containing environments, including marine, food, and biomedical applications. The bovine serum albumin (BSA) is a protein that is commonly used as a model protein for studying protein–surface interactions.

The quartz crystal microbalance (QCM) technique is well suited for *in situ* studies of protein adsorption. However, in most cases, substrates are not electrochemically controlled and investigations are carried out in static conditions [2]; therefore, once the protein is adsorbed on the solid surface, the desorption of the protein cannot be followed *in situ* without removing the quartz crystal from the protein-containing solution *i.e.* without disturbing the microbalance signals. One way to perform QCM measurements under well-controlled hydrodynamic conditions is to use a flow cell. Flow cells have found many applications in the electrochemical literature since due to the small volume of the cell (0.1–1 mL), the inlet solution can be rapidly changed without interruption of the electrolyte flow. The coupling of flow cells with other measuring techniques, such as the electrochemical quartz crystal microbalance (EQCM), is easy to implement. Hamm et al. used an EQCM together with a flow cell described in detail by Ogle and Weber [3] to study the passivation of Fe–Cr alloys in acid sulphate electrolytes [4]. Garcia et al. investigated the formation of a scale deposit on gold and evaluated the efficiency of a well-known phosphonic acid (HEDP) inhibitor [5]. In combination with a switching system for changing the

* Corresponding author. Tel.: +33 1 44 27 67 57; fax: +33 1 46 34 07 53.
E-mail address: isabelle-frateur@enscp.fr (I. Frateur).
[1] ISE member.
[2] Permanent address: Outokumpu Stainless AB, Avesta Research Center, P.O. Box 74, SE-774 22 Avesta, Sweden.

0013-4686/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.electacta.2006.12.060

electrolyte, the use of a flow-cell EQCM permits to follow the rate of adsorption as well as desorption. This offers the possibility for investigating the reversibility of an adsorption process by switching back and forth between a protein-free and a protein-containing solution. Thus, Galliano et al. designed a switch-flow cell that enabled an easy and rapid introduction and removal of adsorbates, without significantly disturbing the microbalance signals [6]. Their flow cell EQCM was a three-electrode cell with two compartments separated by a membrane, permitting electrochemical control of the potential of the working electrode. The authors applied this device to the study of iodide adsorption on gold. To our knowledge, the switch-flow cell EQCM has never been used for the study of protein adsorption.

The quartz crystal used as a sensor in the EQCM technique is not only sensitive to changes in mass of the working electrode. It is also highly sensitive to changes in temperature, pressure and viscous loading. Temperature and pressure can be controlled, but the principle of a switch-flow cell experiment is to change the electrolyte. Consequently, the changes in viscosity have to be compensated for. This can be done by simultaneous recording of the quartz crystal resonance frequency and resistance [6]. Another method is to switch off the driving circuit at regular intervals and to measure the attenuation rate of the oscillations as suggested by Rodahl and Kasemo [7].

In most protein adsorption studies using both QCM and X-ray photoelectron spectroscopy (XPS), the latter was used only to characterize the substrate (polymeric films) before adsorption [8,9]. On the other hand, QCM and XPS have rarely been combined to study protein/substrate systems during and after adsorption, respectively [2]. In particular, the equivalent thickness of the adsorbed protein layer estimated from QCM measurements has never been compared to the one calculated from XPS data.

The objective of this work was to study the adsorption of BSA on passivated chromium in a pH 4 deaerated sulphate solution. As Cr is a major constituent of stainless steels, it was chosen here as a model for this class of materials. The present study is intended as a first step to be followed by studies on ferritic stainless steels. The goal is to compare results of *in situ* EQCM and *ex situ* XPS. EQCM provides information on the kinetics of protein adsorption and on the amount of adsorbed protein. If combined with a switch-flow cell, it permits to study the reversibility of the adsorption process. In this work, a new switch-flow cell EQCM was designed similar to that developed by Galliano et al. [6], but allowing deaeration of both electrode compartments and use of commercially available quartz crystal electrodes. EQCM measurements were complemented by XPS analyses from which the chemical composition and the thickness of the surface layers can be obtained. The equivalent thicknesses of the adsorbed protein layer estimated by EQCM and by XPS were compared. After a detailed description of the experimental setup, the results obtained for one set of experimental conditions (−0.2 V versus SSE, 20 mg L^{-1} of BSA, pH 4) are presented.

2. Experimental

2.1. Switch-flow cell setup

2.1.1. Global experimental setup

A schematic view of the global experimental setup that was used for the EQCM experiments is given in Fig. 1(a). Two solutions, a protein-free solution and a protein-containing one, were pumped continuously from two vessels put in a thermostated bath using a 12-roll peristaltic pump (Ismatec) with two pump channels placed upstream from the flow cell. The pump has an adjustable speed; a low pumping speed of 3.3 mL min^{-1} was chosen so as to minimize the noise of the frequency and resistance signals. Both solutions flowed through a low-pressure mobile-phase selection valve (Rheodyne 5011) that allowed for instant switching of the inlet solution to the cell while minimizing mixing, without the creation of a measurable pressure gradient. Downstream from the selection valve, one solution went through the flow cell while the other one flowed directly to a recycling tank. The electrolytes were transported in highly chemically resistive Tygon® tubes of 1.6 mm inside diameter. To perform gravimetric measurements under electrochemical control, the flow cell was connected to a microbalance control unit (RQCM) and to a potentiostat, both devices being computer controlled.

2.1.2. Flow cell

A schematic view of the flow cell is given in Fig. 1(b). The flow cell was a two-compartment and a three-electrode cell that allowed electrochemical and mass change measurements in deaerated solution. The cell design developed for this study was based on the Maxtek's FC-550 flow cell that can be used with Maxtek's CHC-100 crystal holder (made from CPVC). The cell is made from Kynar® and has two stainless steel inlet and outlet tubes with a 0.047 in. inside diameter × 0.062 in. outside diameter. A Viton® O-ring provides sealing between the cell and the front face of the sensor crystal. Installation of the cell on the crystal holder creates a cylindrical flow chamber of approximately 0.1 mL. The electrolyte flows parallel to the quartz surface from the inlet to the outlet tubes, both of which are positioned perpendicularly to the quartz crystal. The commercially available flow cell was modified into a three-electrode and two-compartment cell in order to allow for QCM experiments under electrochemical control. A large hole was drilled so as to introduce the counter electrode and a small one to create a Luggin capillary. The air-tightness of the counter electrode compartment was ensured by a manufactured Kel-F® cap and an O-ring. Holes were made inside the cap for solution inlet and outlet, and for electrical connection. The working and reference electrode compartment was separated from the counter electrode compartment by a porous glass membrane fixed on the flow cell with epoxy glue. This rigid glass membrane does not modify the initial volume of the flow cell (∼0.1 mL) and thus, keeps short the solution switch time.

The electrolyte first flows through the working electrode compartment, then it flows through the counter electrode compartment before being stored in a recycling tank. This