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Method for equivalent circuit determination for electrochemical impedance spectroscopy data of protein adsorption on solid surfaces

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ABSTRACT

A method is presented to determine which Equivalent Electrical Circuit (EEC) best models Electrochemical Impedance Spectroscopy (EIS) data of protein adsorbed on a surface. A model system of Bovine Serum Albumin (BSA) film on a platinum wire electrode in Phosphate Buffered Saline (PBS) solution is used to demonstrate the method. First, a library of circuits is created which may model the physical processes occurring in the system. Next, each circuit is fit to the experimental data collected from three replicate experiments. Finally, relative residual errors, generated circuit parameter values relating to known physical parameters (solution resistance, double-layer capacitance, etc.) and standard deviations based on replicate experiments are used to determine which circuit best models the electrochemical data. For the model system presented in this paper, it is found that a modified Randles circuit best models the data when no BSA film is present, and that an additional time constant (resistor and constant phase element in parallel), modeling a porous, insulating film, is needed when a BSA film is formed on the electrode surface.

1. Introduction

Electrochemical Impedance Spectroscopy (EIS) is a useful tool for understanding protein adsorption at electrode surfaces. Generally, with protein adsorption, EIS data are fit to an Equivalent Electrical Circuit (EEC) to extract quantitative information about the processes occurring at the electrode surface [1,2]. Typically, researchers examine the fit between the circuit and the Nyquist plot to determine whether or not the circuit is a good model for the system [2–4]. In this paper, we propose a more thorough method of circuit choice, based on relative residual errors between the experimental data and fit data, generated circuit parameter values relating to known physical parameters, and standard deviations based on replicate experiments. The method eliminates possible incorrect EEC assignment based purely on Nyquist plot fitting, and provides for more confidence of the choice of EEC, and by extension more understanding of what occurs in the protein/surface environment.

Overwhelmingly, EIS has been used to study protein adsorption for its application to biosensor development [2,5–8]. EIS allows researchers to study the effects of various environmental changes (for example, potential [9,10], concentration [9,11,12], or temperature [13,14]) on the adsorption of protein molecules at an electrode surface. An understanding of how protein adsorption depends on these variables can influence biosensor design.

Many EECs have been used in the literature to model protein adsorption at a metal electrode surface, however, the most common is the modified Randles circuit [11,15–23], shown in Fig. 1a. In this circuit there is a solution resistance (R_s) that models the migration of charge through the electrolyte solution, a double-layer capacitance (C_{dl}) to model double-layer formation at the electrode surface, a charge-transfer resistance (R_{ct}) which models the charge-transfer reaction at the electrode surface, and finally a Warburg impedance (W) which models linear diffusion toward the electrode surface.

In addition to the Randles circuit, other EECs have also been used to model protein adsorption. Xie *et al.* [4] add an additional time constant (resistor and capacitor in parallel) to the modified Randles circuit (Fig. 1b) suggesting that the additional resistor and capacitor model the resistance and capacitance of the adsorbed protein film on the electrode surface, R_f and C_f respectively in Fig. 1. Diniz *et al.* [3] also add a second time constant to the modified Randles circuit, but change slightly the location of the new resistor and capacitor, as shown in Fig. 1c. Diniz *et al.* [3] suggest that the added resistor and capacitor are associated with an insulating layer on the surface

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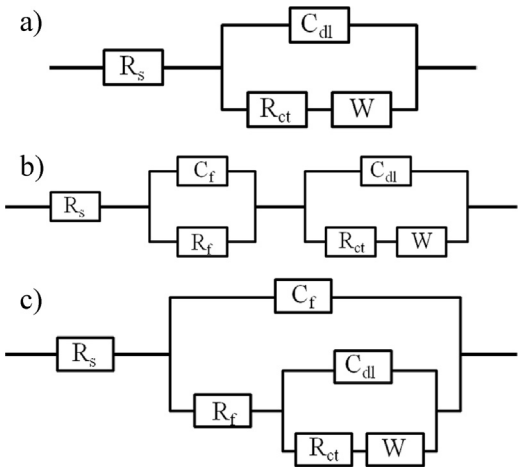

Fig. 1. Examples of EECs used in the literature to model BSA adsorption at a metal electrode surface.

of the electrode. Given that all three of the circuits presented in Fig. 1 are used to model protein adsorption on a solid surface, the question arises as to how to determine which circuit best represents the physical system.

This paper presents a systematic method for the determination of the EEC that best represents EIS data collected in protein adsorption experiments. To demonstrate the method, the literature equivalent circuits described above are applied to a model system of Bovine Serum Albumin (BSA) adsorption on a platinum electrode, since BSA is commonly studied as a blocking molecule for electrochemical biosensors[6,11,22,24–27]. Using the presented method, we show that the best EEC models the BSA film on platinum as a porous, insulating film.

2. Materials and Methods

2.1. General

All EIS experiments were conducted in all-glass three-compartment electrochemical cells. Data from the EIS experiments were collected using a Bio-Logic VMP3 multipotentiostat and EC-Lab software. In all cases, the counter-electrode and working-electrode compartments were deaerated by bubbling with nitrogen gas (N_2) for 30 minutes prior to the electrochemical experiments. Bubbling was not continued during the experiments so that possible convection effects were eliminated. All experiments were run at $22 \pm 3\,^{\circ}C$; BSA adsorption is not impacted by temperature in this range, as indicated in the literature[14], and by separate experiments completed in our lab.

2.2. Solutions and Reagents

The electrolyte solution for electrode cleaning was 1 M H_2SO_4 (Aldrich, 99.999% purity). The electrolyte solution used for all other experiments was a 0.2 M Phosphate Buffered Saline (PBS) solution, similar to that used by Moulton *et al.* [28], containing 0.2 M KH_2PO_4 (Sigma), 0.2 M K_2HPO_4 (Anhydrous, BDH Chemicals), and 0.15 M KCl (Aldrich, 99.999% metals basis). Although Cl^- ions compete with BSA for adsorption, KCl was included in the electrolyte to more closely simulate to biological solutions. 3 mM $K_3Fe(CN)_6$ (McArthur Chemical Company) was added to the PBS solution to provide an $Fe(CN)_6^{3-/4-}$ redox couple. A 1 g L^{-1} solution of BSA in PBS was prepared for electrode incubation experiments. All solutions were prepared in 18 MΩ-cm water. The pH of the PBS solution was *ca.* 6.8, as measured using a SympHony posilo pH electrode and meter (VWR International).

2.3. Electrodes

The working electrodes used were 0.5 mm diameter platinum wires covered in heat-shrink polytetrafluoroethylene (HS-PTFE, 28 gauge, Zeus Engineered Extrusions). The working electrode was prepared by sliding a small (*ca.* 5 cm long) piece of HS-PTFE onto the wire, and then subjecting the wire to heat by rotating it above a butane flame for *ca.* 15 seconds. The heat-shrink ratio of the PTFE is 2:1 with a final inner diameter of 0.46 mm. Therefore, the HS-PTFE forms an excellent seal around 0.5 mm diameter wires. For EIS experiments, *ca.* 4 mm of platinum wire remained uncovered by HS-PTFE, resulting in a cylindrical electrode with electrochemical surface area of *ca.* 0.08 cm^2. The counter electrode was a coiled, platinized platinum wire and the reference electrode was a typical Ag/AgCl reference electrode with 0.15 M KCl (Aldrich, 99.999% metals basis) filling solution (homemade, 0.271 V vs. SHE). The concentration of filling solution was chosen to keep the concentration of Cl^- ions consistent between the reference solution and the electrolyte. All potentials are reported against this reference electrode.

2.4. Electrode Cleaning

Prior to use in PBS solution, the working electrode was electrochemically cleaned by cycling from -0.3 to 1.3 V at 1000 mV s^{-1} for 200 cycles in deaerated 1 M H_2SO_4. Finally, the electrode was cycled in the same potential window for three cycles at 100 mV s^{-1} to ensure a typical clean platinum cyclic voltammogram was in evidence. After electrochemical cleaning, the electrode was removed from the acid and rinsed thoroughly with 18 MΩ-cm water before proceeding with EIS experiments.

2.5. Electrochemical Impedance Spectroscopy

EIS experiments were conducted in a cell containing *ca.* 7 mL of PBS in the working-electrode compartment. EIS measurements were conducted in PBS electrolyte containing 3 mM $K_3Fe(CN)_6$ as a redox couple.

The EIS was run at the equilibrium potential of the redox couple (E_{eq}, 0.171 V, measured using the cyclic voltammogram of the 3 mM ferri/ferrocyanide solution), using a frequency range of 500 kHz to 100 mHz with a potential perturbation amplitude of 10 mV. The run was repeated 4 times with no rest period between each run, to give a "set" of 4 consecutive EIS runs. Each set was followed by a 10 minute rest where the potential of the electrode was allowed to rest at open-circuit potential (OCP). The total time of one set plus one rest period was *ca.* 20 minutes. The set/rest cycle was repeated at least 55 times, for a total experiment time of at least 20 hours. The long experiment time was used because of the changes in the adsorbed protein film which occur over time (paper in preparation). This work uses the 20 hr data because the system has become relatively stable at this point.

BSA was introduced to the electrode surface by incubation of the Pt electrode in a 1 g L^{-1} solution of BSA in PBS for 30 minutes. Incubation is a common method of protein film formation [10,24,29,30], and past studies of BSA adsorption suggest that 30 minutes is a sufficient incubation time to form a BSA film on the electrode surface [10,26]. After incubation, the electrode was removed from the incubation solution and immediately placed in the three-compartment electrochemical cell containing PBS, and the EIS was run. Blank experiments (no BSA incubation) were also run, using the same experimental conditions as have been described.