Label -free impedance biosensing of protein/ antibody interaction

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Introduction:

Devices designed to detect or quantify biochemicals are called biosensors. Electrical biosensors, including voltammetric, amperometric/ coulometric and impedance sensors, are very promising and affordable diagnostic detectors as they are low cost, low power and easy to miniaturize [1]. In the Electrochemical Impedance Spectroscopy (EIS) technique, the impedance is measured as a ration of current and voltage. EIS can detect the impedance or capacitance change due to electrode/solution interface changes when the target biochemical is captured by the probes/electrodes. EIS measurement is a label-free process and does not need any reagent [2].

The fundamental principle of EIS was established in the 19th century and the precision by which the electrical properties can be measured has been significantly improved during the last decade. High resolution EIS has been recently used for analysis of surface interaction in sub nano- and nano-scales for various systems [3]. Recent developments in dielectric structure modelling of the EIS data have also provided useful information on sub-structure of the samples.

Methodology: Protein-A was covalently immobilized on the surface of a self assembled monolayer (SAM) on gold. For control studies, direct binding of protein-A on gold surface was also monitored using EIS. The SAM layer was activated using the NHS/EDC reaction. To avoid the direct binding of antibody and SAM, the un-reacted sites on the surface of the SAM were blocked before exposing the sensor to the antibody. X-ray photoelectron spectroscopy (XPS) analysis performed using KRATOS XSAM 800 (Kratos Analytical, UK) confirmed the occurrence of all reactions. The High resolution EIS spectrometer (INPHAZE, Australia) was used to detect and characterize the formation of the layers on the surface of gold. The EIS used in this research was able to measure the real and imaginary part of Impedance (Z), Capacitance (C) and Conductance (G) over a wide range of frequencies (10^{-3}- 10^{6} Hz).

Results: The entire binding processes were captured by the EIS measurements. For instance, the direct binding of protein-A on gold surface resulted in a capacitance decline at low frequencies (see Figure 1). The capacitance declined as a result of an increase in thickness following the equation:

$$C=\varepsilon\varepsilon_0 A/d \qquad \text{(Equation 1)}$$

where C is the measured capacitance, ε is the dielectric constant of the layer (or film), ε_0 is a constant ($8.85419e^{-12}$), A is the surface area, and d is the thickness of the film. As shown in Figure 1, almost 90% of the binding process occurred in the first 2 hours and the binding process was completed in less than 9 hours.

Dielectric structure refinement (DSR) software (INPHAZE, Australia) was used to automatically fit the equivalent electrical circuit for each sample and to compute the thickness of the layers based on Equation 1.

For instance, it was shown in an earlier research that there was a good agreement between the thicknesses of the SAM layer obtained using high resolution EIS and the theoretical value of the SAM layer [4]. Here, it was found that thicker SAMs, made using longer chains, resulted in better and more reproducible data on antibody/protein interactions. This suggests that the effect of the ionic double layer on the surface of gold was reduced by using a thicker SAM. Using a mixture of long and short chain SAMs did not affect the performance of the biosensor significantly.

Formation of the layers on gold surface increased the impedance and decreased the capacitance at low frequencies (see Figure 2). The thicknesses obtained from the DSR software were in good agreement with the reported values in the literature.

Conclusions: Electrical impedance spectroscopy was able to detect and characterize all the steps of the chemistry and protein/antibody interactions. In this system, EIS could compute the thickness of the layers accurately and also gave valuable information on the kinetics of the reactions.

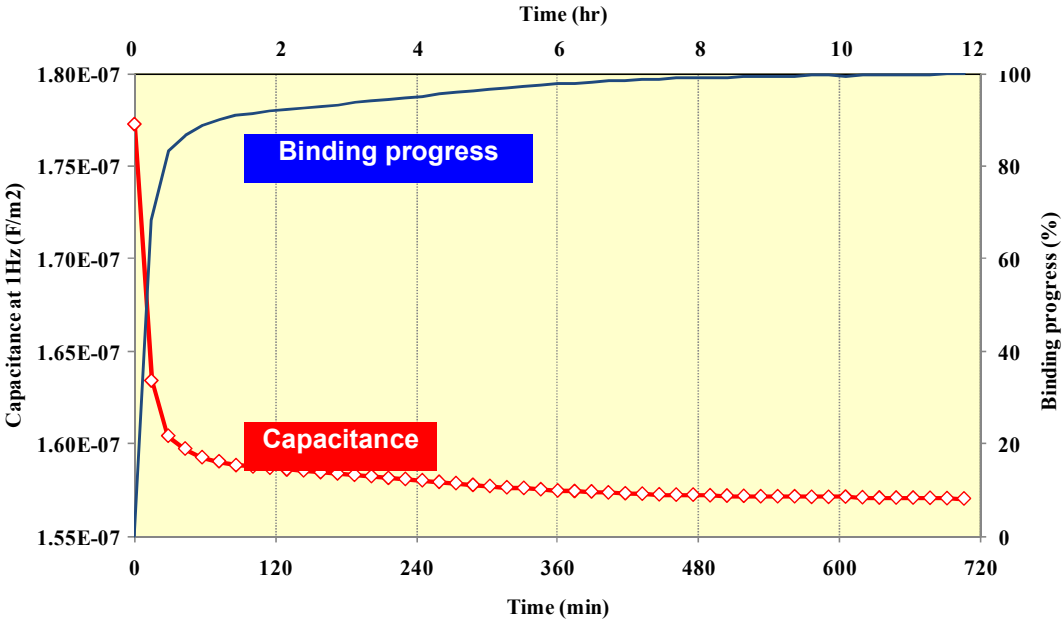

Figure 1. The capacitance profile of Protein binding process on gold surface

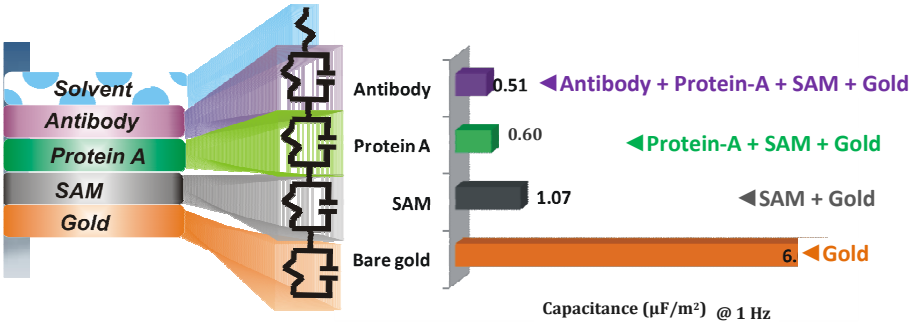

Figure 2. Schematic presentation and capacitance of the layers at 1 Hz

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