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Development of a biosensor using AC impedance spectroscopy: Detecting the interaction of antibody with the covalently immobilized protein A on the surface of a self assembled monolayer

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Abstract: A self assembled monolayer (SAM) of mercapto-acid was formed on the surface of gold. The SAM layer was activated and protein-A was covalently immobilized on its surface. The un-reacted sites on the surface of the SAM were blocked and antibody was exposed to the protein. Electrical impedance spectroscopy was able to detect all the steps of the chemistry and protein/antibody interaction.

Keywords: Electrical Impedance Spectroscopy, Immunosensor, Self-assembled-monolayer, Biosensor

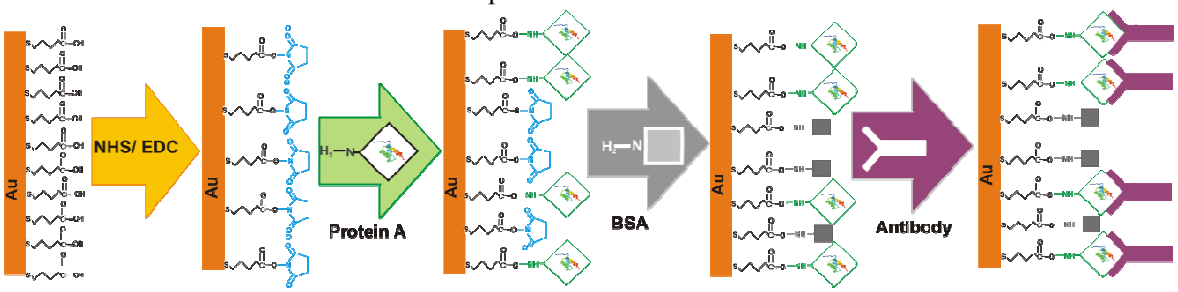
Introduction: Detecting the chemical interaction between two elements is the basis of most chemical and biochemical sensors, including immunosensors. Designing a label-free detection system for direct monitoring of protein- ligand binding is the objective of much current research at both academic and industrial levels. Among the label-free monitoring techniques, impedance-based immunosensors have recently received significant attention because of the low cost of electrode production, cost effective instrumentation, the potential to be miniaturized and the possibility of incorporation into multi-array diagnostic tools. Electrical impedance spectroscopy (EIS)-based sensors are also promising for use in on-site applications[1-2].

Materials and methods: Sputter coating was used to create 30nm thick gold electrodes on highly doped silicon wafers and on different plastics. For the formation of the self assembled monolayer (SAM), the gold coated surface was immediately incubated in a 1mM solution of 16-mercaptohexadecanoic acid in pure ethanol, or in 95v%ethanlo+5v% acetic acid solution for 24 hours.

The surface of the carboxylic acid terminated SAM layer was thoroughly rinsed with ethanol and distilled water, then treated with a solution of NHS (200mM) and EDC (50mM) in water for 2 hours. The sample was then immersed in a solution of protein-A (10 mg/l) in 100 mM PBS at pH 7.4. The residual NHS esters which remained unlinked to the protein-A were blocked by immersing the sample in a 1% (w/v) solution of BSA (Bovine serum albumin) in PBS for 2 hours. The protein-A coated substrate was then immersed in a solution of IgG (1g/L) in PBS for 2 hours. More information about the chemistry of the reactions can be found in the literature[2]. Scheme 1 depicts these chemical steps.

X-ray photoelectron spectroscopy (XPS) was used to detect the concentration of different elements on the surface of the gold as a check on the chemistry. XPS confirmed the occurrence of all reactions and the results were in agreement with the result of EIS measurements. High resolution electrical impedance spectroscopy (EIS) was used to detect the formation of the layers on the surface of gold. A three-electrode system was used and the impedance analysis was performed with an INPHAZE spectrometer (Sydney, Australia) in the frequency range 1Hz to 1MHz[3-4]. Measurements were performed in 100mM PBS buffer at pH 7.4. All electrochemical measurements were carried out at room temperature and in a Faraday cage to prevent external electrical interference. A disposable kit was then designed for this

biosensor. The kit was designed for 6 experiments and two gold electrodes in every well were used for two terminal EIS measurements. A plastic cassette was injection molded and stuck to the cassette on the plastic slide. The gold pattern was sputter-coated on the plastic slide to fabricate the electrodes.



Scheme1. Schematic presentation of the chemical reactions

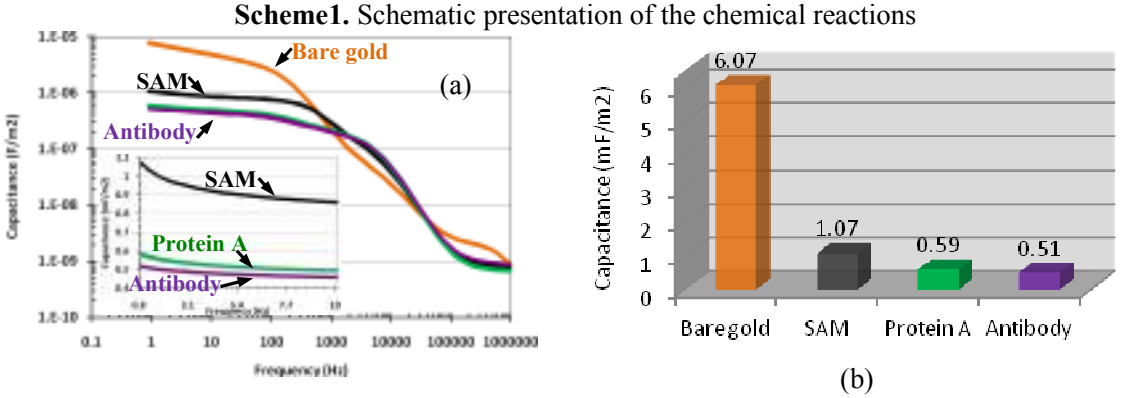


Fig1. Capacitance versus frequency (a) and Capacitance of the layers at 1Hz . The insert of each graph (a) shows the changes on an expanded linear scale in the low frequency range (1-10 Hz).

Results and discussion:

The INPHAZE electrical spectrometer used in this research was able to measure and report the real and imaginary part of Impedance (Z), Capacitance (C) and Conductance (G) over a wide range of frequencies (0.01 Hz to 1MHz). The capacitance of all samples decreased with increasing frequency. At high frequencies the spectrometer measures the impedance of the solution. It can be seen that in all the samples the impedance at high frequencies merges to almost the same value. A series resistance was used to represent the solvent which is in series with the biosensing element.

At low frequencies the layers on the gold are dominant because of their lower conductivity compared to the PBS solution. The layers on the gold increase the impedance and decrease the capacitance. The reduction in the capacitance is greater on formation of the SAM and the subsequent immobilization of protein- A that is the drop in capacitance on binding the antibody.

The capacitance measured for bare gold is that of the ionic double layer which forms on the surface of gold due to absorption of predominately negatively charged ions from solution to the surface of the gold[6]. Dielectric structure refinement (DSR) software (INPHAZE Pty Ltd) based on the least–squares-error method, was used to find the equivalent electrical circuit for each sample and to estimate the thickness of the layers.

Conclusion: Electrical impedance spectroscopy is able to detect all the steps of the chemistry and protein/antibody interaction. The single use biosensor device developed in this research is able to detect the interaction between any ligand and a functionalized surface with high resolution. In this system, EIS can detect the thickness of the layers accurately and also gives useful information on roughness of the surface. The kinetics of the reaction can also be studied by performing dynamic impedance measurements.

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