Presented at Sydney 2010 International Nanomedicine Conference

High resolution AC impedance spectroscopy analysis of the covalently immobilized monolayer of protein-A on SAM for the elaboration of immunosensors

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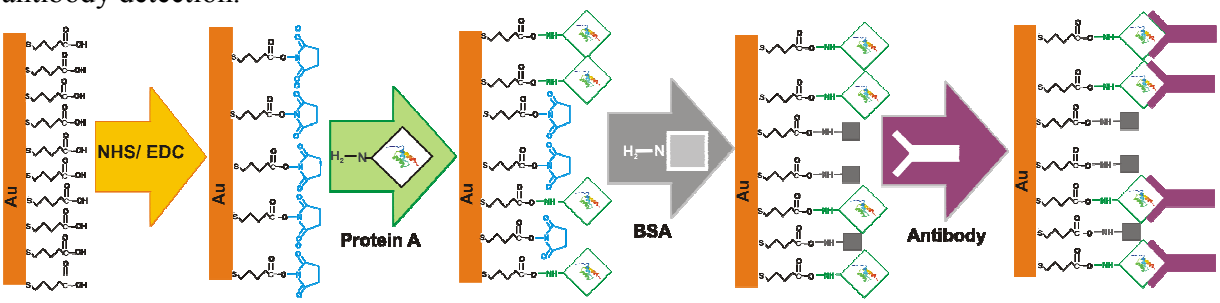
Introduction: Designing a label-free detection for the direct monitoring of protein- ligand binding is the objective of much current research at both academic and industrial level. Among the direct monitoring techniques, impedimetric immunosensors have recently received significant attention because of low cost of electrode mass production, cost effective instrumentation, the ability to be miniaturized potential for introduction into multi-array diagnostic tools. Electrical impedance spectroscopy (EIS)-based sensors are considered as promising non-destructive candidates for use at on-site applications[1-3].
In this research very high resolution ultra-low frequency impedance spectroscopy was used to develop an accurate impedimetric immunosensors system.

Materials and methods: Highly doped silicon wafers were coated with 30nm gold using sputter coating. For formation of self assembly monolayers (SAM), the gold coated silicon was immediately incubated in a 1mM solution of a mercapto-acid in pure ethanol, or 95v%ethanlo+5v% acetic acid, solution for 24 hours. SAMs of 6-mercaptohexanoic acid, 11-mercapoundecanoic acid and 16-mercaptohexadecanoic acid were prepared. However, the results of the 16-mercaptohexadecanoic acid are presented in this paper.
The surface of the carboxylic acid terminated SAM layer was thoroughly rinsed with ethanol and distilled water, then was 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 of pH 7.4. The residual NHS esters 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 literature[3-4]. Scheme 1 depicts the chemical reactions toward the antibody detection.



Scheme1. Schematic presentation of the chemical reactions

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 (Sydney, Australia) spectrometer in the frequency range 1Hz to 1MHz[5]. 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. X-ray photoelectron spectroscopy (XPS) was used to detect the concentration of different elements on the surface of gold as a check on the chemistry.

Results and discussion:

The electrical spectrometer used in this research was able to measure and report the real and imaginary part of impedance (Z), Capacitance (C), conductance (G) over the wide range of frequency (0.01 Hz to 1MHz). As it can be seen in figure 1, the capacitance and the impedance of all samples decreased with increasing frequency. Generally, at high frequencies **the EIS** measures the solution used for measurements, and it can be seen that in all the samples the impedance at high frequencies merge to almost same. A series resistance was used to represent the solvent which is in series with all measurement.

At low frequencies the layers on the gold are dominant because of lower conductivity compared to the PBS solution. The layers on the gold increase the impedance and decrease the measured capacitance. The reduction in the capacitances is greater on formation of the SAM and the subsequent immobilization of protein- A than, the drop in capacitance on binding the antibody. As it can be seen in figure 1-b, and more clearly in the insert of that figure, that similar trends could be seen in the impedance of the samples. 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].

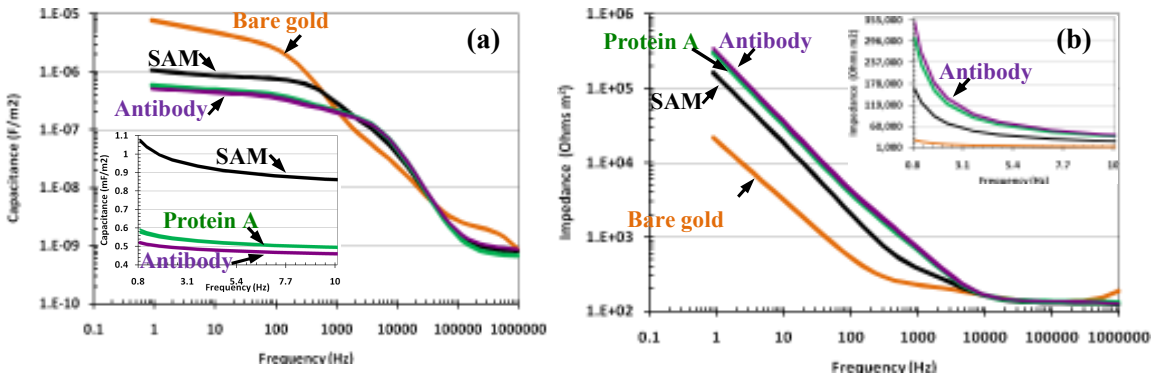


Figure1. The capacitance (a) and impedance (b) versus frequency.Note the logarithmic scale used. The insert of each graph shows the changes on the expanded in linear scale in low frequency range (1-10 Hz).

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.

The XPS was performed at each step of experiments: on the gold sample covered by SAM, after NHS/EDC reaction, with covalently immobilized protein A, after blocking the un-reacted esters with BSA, and the antibody on binding. XPS results confirmed the occurrence of all reactions and the results were in agreement with the result of EIS measurements.

References:

(1) Mamas I. Prodromidis, Electrochimica Acta, 55(14), 4227-4233
(2) M. Hnaien, M. Fethi Diouani, S. Helali, I. Hafaid, Biochemical Engineering Journal, 39(3), 443-449
(3) T. Balkenhohl and F. Lisdat, Analyst, 2007, 132, 314 – 322
(4) E.Briand , M. Salmain , C. Compère , C.M. Pradier , Colloids Surf B Biointerfaces, 2006 , 53(2):215-24
(5) E. L.S. Wong, M. James, T. C. Chilcott, H. G.L. Coster, Surface Science, 601(24), 2007, 5740-5743
(6) T. C. Chilcott, E. L.S. Wong, H. G.L. Coster, A. C.F. Coster, Electrochimica Acta, 54(14) 2009, 3766-3774