Finding the appropriate substrate for biosensors by monitoring the biomolecular recognition reactions using electrochemical impedance spectroscopy

Mihaela Kusko, Monica Simion, Adina Bragaru, Iuliana Mihalache, Razvan Pascu

National Institute for Research and Development in Microtechnologies (IMT - Bucharest), Erou Iancu Nicolae Street, 32B, 72996 Bucharest, Romania
mihaela.kusko@imt.ro

Abstract

The electrochemical impedance spectroscopy (EIS) based detection transduces changes in interfacial properties between the electrode and the electrolyte induced by different molecules' attachment (immobilization) on surface. This technique offers information not only about the surface modification, but it has been used to monitor the DNA hybridization, conformational changes, or damages [1]. If the other detection schemes require the labelling of the target DNA with a fluorophore, the EIS detection is a label-free tool and, thus, possesses advantages of low cost, simplicity, and ease of miniaturization [2].

Taking into account the already demonstrated optical and electrical properties of porous silicon (PS), as well as its biocompatibility, we investigated the potential of this material to be used for electrochemical based biodetection. Tuning the electrochemical process parameters, different types of PS layers have been fabricated, with pores of few nanometers (nanoSi), tens of nanometers (mesoSi) or even microns (macroSi). Since immobilization of a biomolecule on a substrate requires the presence of chemical active groups, a preliminary surface modification process by 1% APTES (3-aminopropyltriethoxysilane) silanization has been followed.

Thus, starting with the surface functionalization stage, each of the biomolecular recognition reaction steps for biotin-streptavidin couples have been monitored by impedance spectroscopy. The EIS spectra were recorded using PARSTAT 2273 potentiostat, the experiments being performed by applying a 10 mV rms AC amplitude at open circuit potential and frequency scanned from 100 kHz to 100 mHz. Moreover, capacitance—voltage (C-V) measurements were performed to study the hybrid structure interfaces obtained by successive immobilization / hybridization processes. 10 mM PBS in 0.1 M KCl with / without redox species (2 mM $K_4Fe(CN)_6$) was the electrolyte solution which allow us to study both faradaic and non-faradaic processes taking place at interfaces.

First of all, the capacitance gain of porous structures versus the reference polished silicon ones was clearly observed, confirming the previous results [3], where a 70-fold gain up was obtained at low frequencies, due to their extended active area, which allows the scaling down of a further capacitive sensor using PS. The figure 1 (a) shows the Nyquist plotted impedance results after the successive biomolecular interactions, obtained when the nanoSi **(PS 1)** substrate was used. New semicircles, with larger diameters, can be identified when additional molecular coating layers are present, implying a high electron-transfer resistance, consistent with the proposed electrical circuit model. Moreover, the capacitance-voltage measurements allowed us to find the necessary time for hybridization process, and the figure 1 (b) shows the Mott-Schottky curves obtained, where is clearly revealed that the first time used hybridization was not sufficient.

References

[1] J.-Y. Park, S.-M. Park, DNA Hybridization Sensors Based on Electrochemical Impedance Spectroscopy as a Detection Tool, Sensors 9 (2009) 9513-9532

[2] J.S. Daniel, N. Pourmand, Label-Free Impedance Biosensors: Opportunities and Challenges, Electroanalysis 19 (2007) 1239–1257;

[3] M. Simion, M. Kusko, I. Mihalache, A. Bragaru, Dual Detection Biosensor Based on Porous Silicon Substrate, Mater. Sci. Eng. B (2013) available online http://dx.doi.org/10.1016/j.mseb.2013.03.003;

Figures

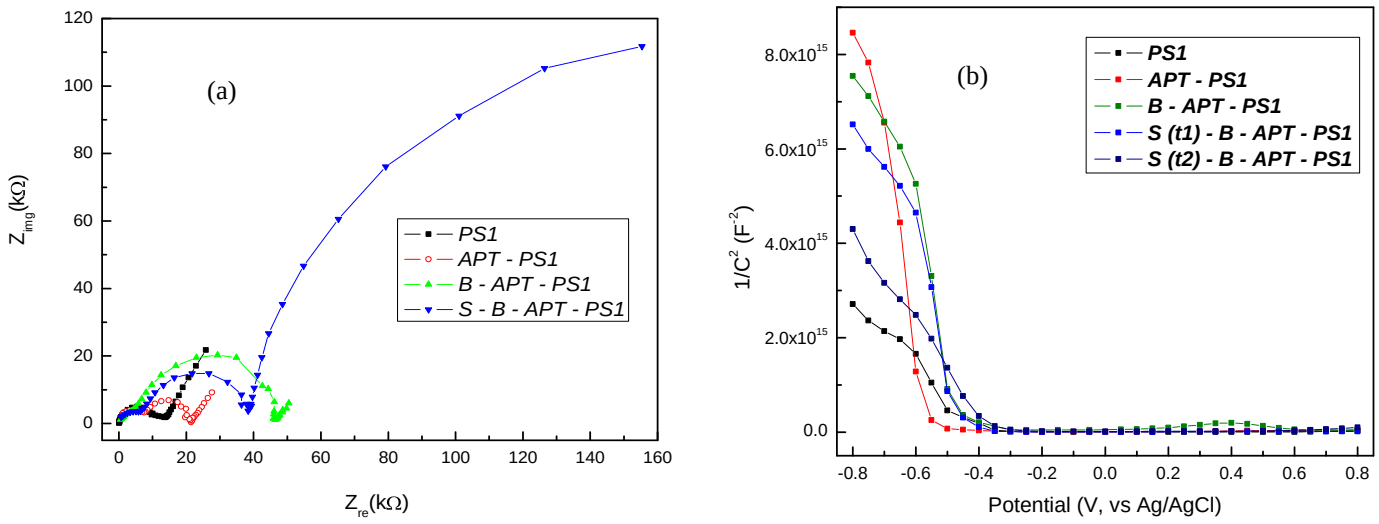

Figure 1: Nyquist plots (Z_{Img} vs Z_{Re}) – *(a)* – and capacitance-voltage plots – *(b)* - of ***nanoSi (PS1)*** substrate subjected to successive biomolecule interactions:

- ✓ surface silanization *(APT – PS1);*
- ✓ immobilization of the capture molecule - biotin *(B – APT – PS1);*
- ✓ hybridization with biological sample containing analyte of interest – streptavidin *(S – B – APT – PS1).*