БІОСЕНСОРИ

BIOSENSORS

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CONTROL OF MYCOTOXINS BY IMMUNE BIOSENSOR BASED ON THE STRUCTURED NANO-POROUS SILICON

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Abstract. It was developed immune biosensor based on the nano structured silicone (sNPS) for the control of T2 and patulin mycotoxins in environmental objects. It was used boron doped single-crystal silicon square wafers with resistivity of 1 Ohm · with area of 100 cm^2 and thickness of 0.3 μ m. The surface was prepared by stain etching in HF: HNO_3 solution at the room temperature during 1-20 min. sNPS surface is regularly covered with nano-scale hills up to 20 nm high. The registration of the specific signal was made on the basis of changes of chemiluminescence (ChL) or photocurrent of this structure The sensitivity of biosensor is 10 ng/ml. The total time of analysis including all steps (Ab immobilization and measurements) was about 40 min. This time may be shortened if Ab will be immobilized preliminary and analysis will be started beginning with the mycotoxin loading on the sPNS surface.

Keywords: nano structured silicone, immune biosensors, T2 mycotoxin, patulin, determination

КОНТРОЛЬ МИКОТОКСИНОВ С ПОМОЩЬЮ ИММУННОГО БИОСЕНСОРА НА ОСНОВЕ НАНОСТРУКТУРИРОВАНОГО КРЕМНИЯ

М. Ф. Стародуб, Н. Ф. Слышик, И. В. Пилипенко, М. М. Мельниченко, Л. Н. Пилипенко

Аннотация. Разработан иммунный биосенсор на основе силиконовых нано-структурированных частиц (sNPS) для контроля Т2 микотоксина и патулина в объектах окружающей среды. Пластины монокристаллического кремния площадью 100 $см^2$, толщиной 0,3 μм и с сопротивлением 1 Ом · были допированы бором. Потом поверхность обрабатывалась раствором

HF: HNO_3 при комнатной температуре в течение 1-20 мин. Поверхность sNPS содержала поры до 20 нм. Специфический сигнал регистрировали по изменению уровня хемилюминесценции (ХЛ) и величине фототока в этой структуре. Чувствительность биосенсора достигала 10 нг/мл. Общее время анализа, включая необходимые этапы (иммобилизация антител и измерения) составляло около 40 мин. Это время может быть сокращено, если антитела будут предварительно иммобилизованны на поверхности трансдюцера, и анализ будет начинаться с момента нанесения раствора, который необходимо контролировать.

КОНТРОЛЬ МІКОТОКСИНІВ ЗА ДОПОМОГОЮ ІМУННОГО БІОСЕНСОРА НА ОСНОВІ НАНОСТРУКТУРОВАНОГО КРЕМНІЮ

М. Ф. Стародуб, Н. Ф. Слишик, І. В. Пилипенко, М. М. Мельниченко, Л. Н. Пилипенко

Анотація. Розроблено імунний біосенсор на основі силіконових нано-структурованих часток (sNPS) для контролю Т2 мікотоксину та патуліну в об'єктах навколишнього середовища. Пластини монокристалічного кремнію площею 100 см^2 і товщиною 0,3 μм та з опором 1 Ом см були доповані бором. Потім поверхня оброблялась розчином HF: HNO_3 при кімнатній температурі протягом 1-20 хв. (sNPS). Поверхня містила пори розміром до 20 нм. Специфічний сигнал реєстрували на основі зміни хемілюмінесценції (ХЛ) та фотоструму. Чутливість біосенсору досягала 10 нг/мл. Загальний час аналізу, включаючи необхідні етапи (іммобілізації антитіл і вимірювання) був близько 40 хв. Цей час може бути скорочено, якщо антитіла будуть попередньо іммобілізовані на поверхні трансдюцера, та аналіз буде починатись з нанесення розчину, який потрібно контролювати.

Ключові слова: кремнієві наноструктуровані поверхні, імунний біосенсор, Т-2 мікотоксин, патулін, визначення

Introduction

Mycotoxins presented by T2, aflatoxins, searelenone, patulin and others cause a grate interest since they are widespread and characterized by high level toxicity. Unfortunately the analytical methodologies for analysis of mycotoxins as well as other low molecular weight toxins include such instrumental analysis as high-performance liquid or gas chromatography with mass spectroscopy or liquid chromatography with mass spectroscopy. Due to the extremely high complication and cost of analysis fulfilled by these methods, the development of innovative approaches, such as immune analysis and particular chemo- and biosensors, is very urgent [1]. Early [2] we developed number of types of optical immune biosensors based on the surface plasmon resonance and total reflection ellipsometry as well as some electrochemical ones. To fulfill all practice demands in respect of high sensitivity of analysis as well as simplicity, cheapness and rapidity of its fulfillment we propose to use of structured nano-porous silicon (sNPS) as transducers for the immune biosensors with the registration of the specific signal on the basis of changes of chemiluminescence (ChL) or photocurrent of this structure. The information concerned investigations of some physical-chemical abilities of sNPS, worked out algorithm of analysis, obtained results and possible mechanism of specific signal formation is main goal of this article. As model of low molecular weight toxins we used T-2 mycotoxin and patulin. The analysis was fulfilled by "direct" way when specific antibodies (Ab) were immobilized on the sNPS surface and then they reacted with appropriate mycotoxin in model solution.

Experimental

We used boron doped single-crystal silicon square wafers with resistivity of 1 Ohm×cm, with area of 100 cm^2 and thickness of 0.3 μm. The surface of the wafers was not polished. sNPS layers were prepared by stain etching in HF: HNO_3 solution at the room temperature, natural day-

time illumination and time duration from 1 to 20 min. Thickness of sNPS layer changed from 3 up to 60 nm, was supervised by parameters of technological process at chemical modification of a surface of single-crystal silicon and defined with the help of Auger electronic spectroscopy at the LAS-2000. The structure of sNPS surface was studied using scanning tunnel microscope (STM) and scanning electron microscope. Analysis of the obtained images of the surface shows that the sNPS surface is regularly covered with nanoscale hills up to 20 nm high (Fig. 1).

The characteristics of thickness of NPS pores and concentration O, C and SiO_x from time stain etching are given in Fig. 2.

Scheme of the optical device based on the nano structured porous silicon (sNPS) for the photo resistance registration of the signal at the formation the specific immune comples is given in Fig. 3.

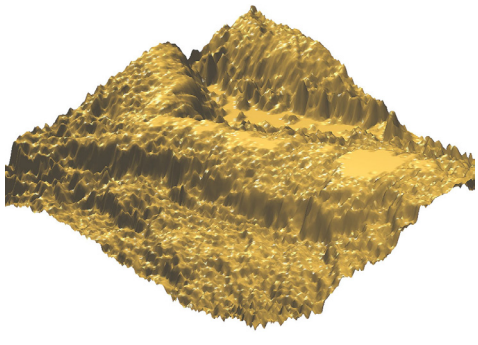

Fig. 1. STM image of the sNPS surface. The scanned area is 1∗1μm (thickness of a layer 20 nm)

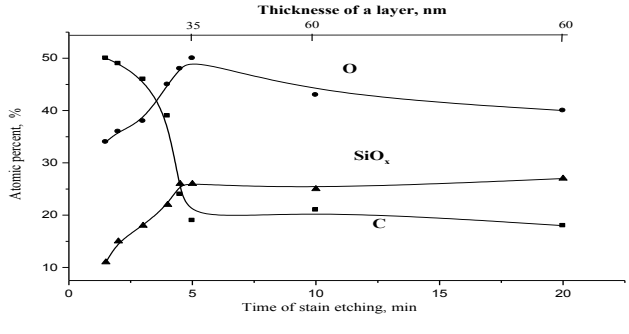

Fig. 2. Dependence of concentration O, C and SiO_x from time of stain etching

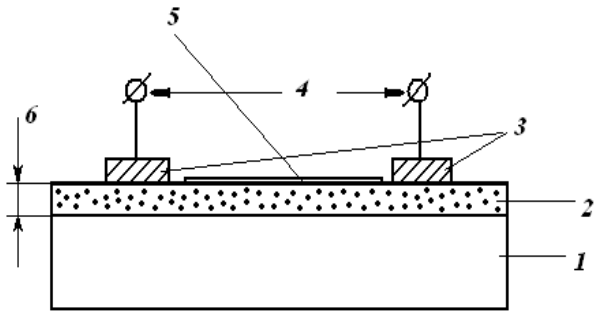

Fig. 3. Scheme of the photoresistor structure based on the sNPS and intended for the analysis of the interactions between biological structures. 1 – the crystalline silicon, 2 – the sNPS, 3 – the electrical contacts (Al with the thickness of ~3 μm), 4 – the applied voltage, 5 – the biological object, 6 – the thickness of the sNPS of 10-40 nm

At the beginning of the measurement the specific Ab in the volume of 1μl was placed on the photoresistor surface between the contacts. Then this solution was evaporated at the room temperature or at the air stream. The direct voltage (5 V) from the stabilized power supply was applied to the ohmic contacts and the current was measured by the digital voltmeter of B7-35 type at the absence of lighting (dark regime) as well as the photocurrent (the difference between the light and dark currents) was registered at the lightening of the sensitive surface by the white spectrum light (source A, illumination of 7000 lux). At the drawing of Ag layer on the sensitive plate and after its drying the measurements of the dark and light current were repeated. These measurements were made after the immune complex formation (interaction of Ag with specific Ab in the serum blood) too. The control of the reaching of the sensor initial state was done according to the reduction of the dark current value after washing of the sensitive surface by the buffer solution. The time of the single analysis was 5-10 min only.

Design of the prototype for the registration of the specific immune complex by the photoluminescence PhL of the sNPS includes the source of the ultraviolet (UV) radiation with the wavelength of 350 nm, two photodiodes (2 and 3) based on the mono crystalline silicon and placed at the angle of 20-25° relatively to the plate with the layer of the sNPS and the photo diode intended for the

determination of the incident UV (Fig. 4). At the adsorption of the biological molecules the level of the PhL of the sNPS and the output of the voltage of the consecutive connected photo registers are decreasing. Application of two photo registers of the PhL increases the sensitivity. To take into attention the possible changing of the incident UV the additional photodiode is used. Photodiodes of the n-p-p^+-structures work in the photo generative regime. Such construction is related to the systems of the differential type.

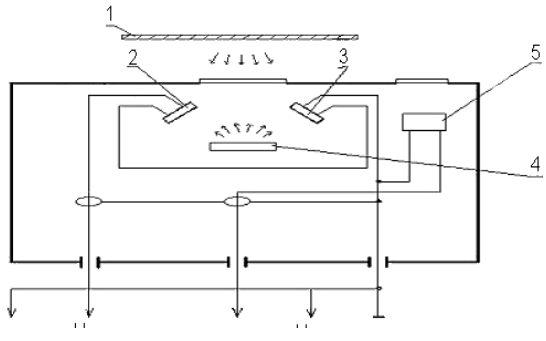

Fig. 4. PhL biosensor, where: 1 - the source of the ultraviolet (UV) radiation with the wavelength of 350 nm, 2 and 3 - two s based on the mono crystalline silicon; 4 - photo diode; 5 – photodiode intended for the determination of the incident UV

Photoelectric processes in the layers of the sNPS which belong to the semiconductors materials accomplished in the result of the photo generation of the electron-holes pairs and following their dividing and recombination. The processes of adsorption on the sNPS surface may arouse new photoelectric effects. Nanocrystallites of the silicon with the dimensions from one to dozen nm are as the silicon regions which are not dissolved and surrounded by the production of the electrochemical and the chemical reactions. At the dimensions less then 15-20 nm it is aroused the quant-dimensioned effects which lead to the quantization of the energetic spectra of the charge curriers, widening of the prohibited zone up to 1.7-3.4 eV and to decreasing of the dielectric permeability. The lux-ampere characteristics of the obtained samples have two plots: the linear and the sub linear which achieves the saturation at the illumination more then 10000 lux. The samples with the nanolayer thickness of 15-18 nm have the maximal photosensitivity.

There is necessary to mention that the changing of the etching content and the solution concentrations brings to changing of the dynamics of NPS layer grow, the porosity level, the correlation of the dimensions of the crystallites and the holes, the chemical content and the profile of the dispersion of main admixtures.

As a rule, at the development of the immune biosensors based on the surface plasmon resonance and total internal reflection ellipsometry to achieve high density of the immobilization of the immune components on the transducer surfaces we preliminary treated them by one of some chemical substances among of which the most used are: a) dextran sulphate; b) dodecanthiol; c) polyelectrolytes: polyalylamine hydrochloride (PPA) or/and polystyrene sulphat (PSS) [1, 2]. After that the transducer surface was treated by some substances to achieve oriented immobilization of specific antibodies in advance, among such substances the most applied are: a) protein A from Staphylococcus aureus; b) protein G from Staphylococcus; c) lectins. Unfortunately at the development immune biosensors based on the sPNS we realized “direct” way of analysis only. It is connected with some problem of the immobilization of components on the sNPS surface and their influence on the formed signal. We plan in future to study these effects in detail.

The detailed results of mycotoxins analysis are presented in Fig. 5-7. It was shown that the sensitivity of such biosensors allows determining T-2 mycotoxin and patulin at the concentration of 10 ng/ml during several minutes.

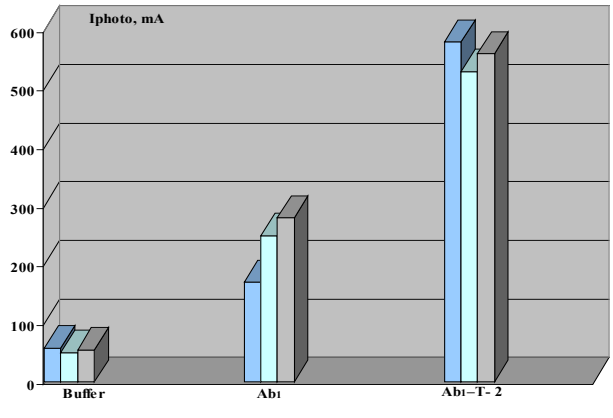

Fig. 5. Changes of the photocurrent of the photoresistor after loading of buffer, specific antibodies (Ab_1) and formation of Ab_1T-2 mycotoxin complex

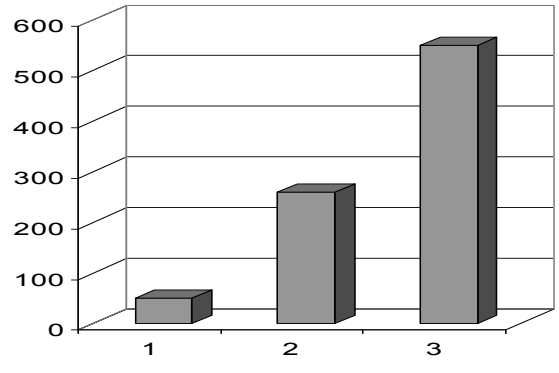

Fig. 6. Dependence of sNPS photocurrent on the surface state: 1 – bare; 2 – with antibody and 3 – with the specific antibody and patulin

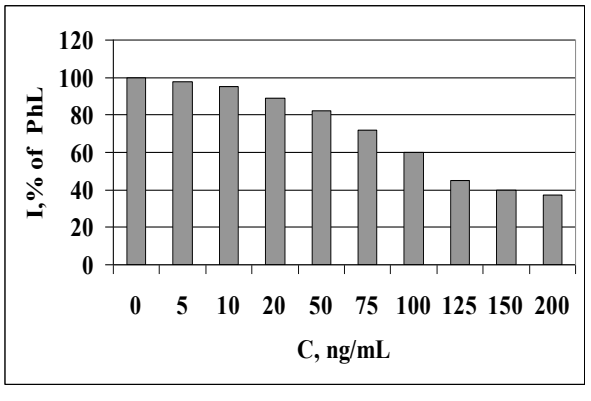

Fig. 7. Dependence of immune sensor signal (intensity of sNPS PhL) on the concentration of T2-mycotoxin in the solution to be analysed

The total duration of the fulfillment of all processes including Ab immobilization and steps of measurements was about 40 min. This time may be shortened if Ab will be immobilized preliminary and analysis will be started beginning with the mycotoxin loading on the sPNS surface. The obtained calibration curves with the model solution of T-2 mycotoxin and patulin open perspective for the practical application of the proposed immune biosensor in case of the determination of others micotoxins and also others types of toxic substances with the use of their specific Ab.

According to our opinion the red PhL may be connected with the tunnel mechanism of the recombination of the charge bearers at the excitation of them in the nanocrystallites of oxide or interface. We do not exclude the hydrogen role too for the generation of the PhL extinguishing. These conclusions are as result of the coincidence of the possible reasons for the PhL decreasing in case of the immune complex formation on the sNPS surface. To them belong: a) the changes of the absorbance in the solution at the formation of the specific immune complex on the sNPS surface, b) the effect of the immune components or their interaction on the recombinant process of the photocurrent charge in the sNPS. As it is very known the light absorption in the wavelength of the excitation (λ = 350 нм) and in the wide field of the sNPS PhL is absent in the Ab and Ag solutions as well as in their complexes.

Of course there is necessary to understand what kind are influences of the primary immune components (in this case of the specific antibodies) on the process of the recombination in the sPNS. It will be our next task.

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