Development and Practical Application of Biosensors Based on the Nanostructured Silicon

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ABSTRACT

Principle of the new instrumental methods of the biochemical diagnostics of the development of the autoimmune status in diabetics through the registration of the formation of the specific immune complexes of insulin-anti-insulin antibody (Ag-Ab) and vice versa with the help of the biosensor based on the structured nano-porous silicon (sNPS) is proposed. In spite of the traditional methods of the proposed approach may provide the express control as in special laboratory and domiciliary with the simple procedure of analysis fulfillment and with the minimum consumption of the materials.

Keywords: immune biosensor, nanostructured silicon, photoluminescence, diabetes, anti-insulin antibody, control

1 INTRODUCTION

Early we have developed biosensor based on the surface plasmon resonance (SPR) for the express revealing of some biochemical quantities [1-3]. The developed SPR based biosensor showed high working characteristics in the respect of the sensitivity, simplicity and rapidity of the analysis fulfilment. Nevertheless, it has some disadvantages and at first they connected with the high cost of the chips and the necessity to use not simple procedure of the preliminary transducer surface treatment from one site. From other site the SPR recorder has very high price and don't allow fulfilling a number repeated analysis.

To overcome some of these disadvantages we try to apply others types of the optical biosensors for solving of the problem of the express biochemical diagnostics of auto-immune status of dibetics. Among others such biosensors that based on the nano-porous structured silicon (sNPS) attract an especial attention. Similar biosensors based on the sNPS were developed early by us for the control of the myoglobin level in the blood and for the monitoring of the bacterial protein in the air of environment [18-22]. The registration of the specific signal was made by the measurement of the changes of the phtoluminescence (PhL) intensity. This approach met the practice requirements regarding the simplicity, sensitivity, selectivity and rapidity of the analysis fulfilment but the stability of the sNPS was very low and the signal registration demanded complicated device.

To continue our investigations in this field we proposed a new variant of biosensor based on the sNPS. The main our attention was paid to the determination of insulin (I) and anti-insulin antibodies (anti-I Ab) level at the control of the autoimmune state development in diabetics.

2. EXPERIMENTAL

The main problem which should be solved at the application of the sNPS as the transducer in the biosensors is providing of the PhL stability during long time. It depends on the sNPS structure, the content of the inter phase layers and the porosity level. These abilities of the sNPS may be determined by the method and the regimes of its forming. Our investigations of the sNPS shown that the samples prepared by the method of the chemical etching have the stable ChL, conductivity and photoconductivity characteristics which were preserved during several years.

The layers of the sNPS for the photoresistors were obtained by the chemical etching of the monocrystalline silicon in the solution of HF and HNO_3. Method of the chemical etching is most adapted to the mass manufacturing and is interested for the preparation of the thin layers of the sNPS for the devices. Nevertheless, at the application of this method there is necessary to solve problems connected with the reproducibility and homogeneity of the layers.

So, due to changes of the surface state and the etching content there is possible to have the thin homogeneous luminescent layers of the porous silicon (PS) with the high level of the reproducibility obtained by the chemical method and without the application of the electrical field.

We used the boron doped single-crystal silicon with the square wafers about 0.3 mm of thickness and with the resistance of 1 Ohm*cm. sNPS layers were prepared by the chemical way and their thickness were changed from 3 up to 60 nm. The parameters of the technological process at the chemical modification of the single-crystal silicon surface were controlled by the scanning electronic microscopy (SEM) and the chemical content – Auger electronic spectroscopy (Fig. 1). Preliminary it was developed the optimal way for the formation of the ohmic contact on the surface of the sNPS from alumina and indium by the magnetron sputtering with the application of

the special masks. I was found that the contacts from indium are non stable due to the mechanical softness of this metal. At the measurements the contact integrity was destroyed since under it the porous surface was located. In our investigations the contacts were formed from the alumina with the thickness about 3 μm.

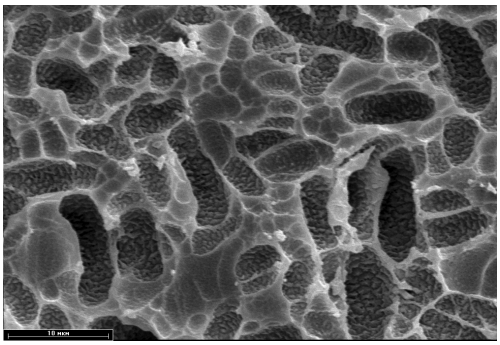

Figure 1. General view of the whole surface of sNPS obtained by SEM.

Spectra of the optical reflectance depend on the structure and dimensions of the nanocrystallites, air pores, level of porosity and the thickness of the layer (Fig. 2).

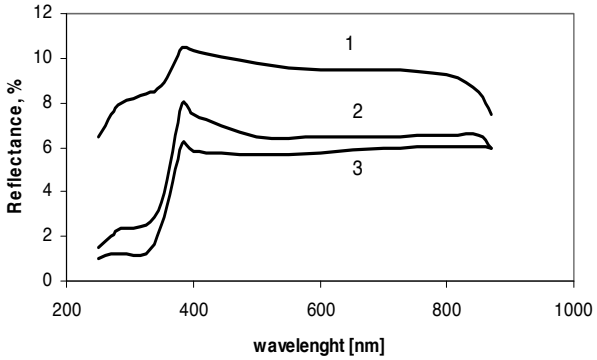

Figure 2. Spectra of the optical reflectance of the sNPS layer in the dependence on the dimension of the nanocrystallites: 1 – 5 nm, 2 – 15 nm, 3 – 30 nm.

The maximal photosensitivity in the visible diapason of the spectra (30-35 mA/Lm) is typical for the sNPS layers with the dimensions of the nanocrystallites in 15 nm and it decreased with the growing of the last. At the same time the maximal sensitivity to the ultraviolet irradiation was obtained for the sNPS layers with the dimensions of the nanocrystallites in 20-25 nm. sNPS layers obtained by the method of the electrochemical etching as well as by the chemical etching shown the PhL abilities which were typical for this material: the wide region in the visible diapason with the intensity which is enough for the observation of the PhL with the naked eye. All samples of sNPS obtained by the chemical method had the bright irradiation with the maximum at $\lambda \sim 640$ nm and that prepared by the electrochemical way – 700 nm. According to the measurements of the PhL and the photoconductivity of the prepared films it was chosen the optimal content for the chemical treatment and time for the etching.

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 that has good correlation with the results of the experiments with the PhL (Fig. 3).

As sources of the antigens (Ag) it was used the insulin produced by "Sigma" (USA). It was dissolved in 0.05 M tris-HCl buffer (pH 7,3) at the different concentration.

At the registration of the specific immune complex by the sNPS photoconductivity the specific Ab or serum blood in the volume of 1μl was placed on the photoresistor surface between the contacts (Fig. 4). Then this solution was evaporated at the room temperature or at the air stream.

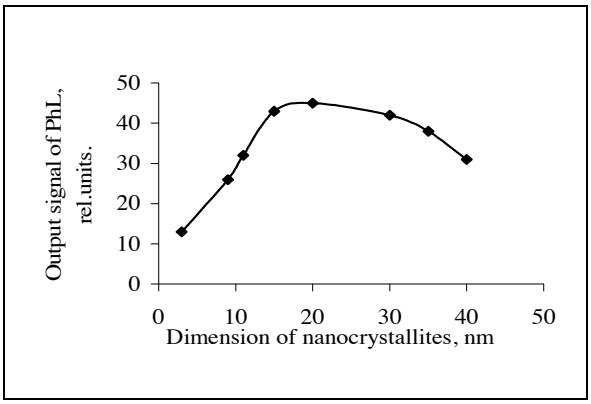

Figure 3. Dependence of the intensity of the PhL of the sNPS layers at the 650 nm on the dimensions of the nanocrystallites.

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.

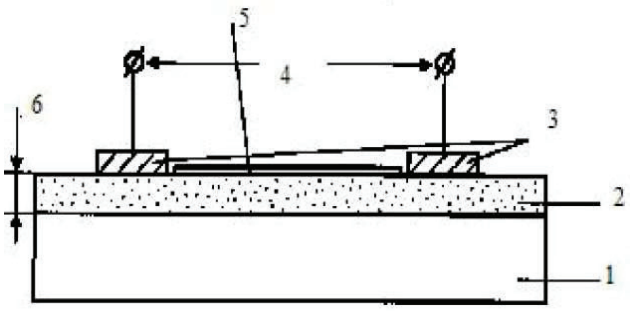

Figure 4. Scheme of the photoresistor structure based on the sNPS and intended for the analysis of the interactions between biological structures. Where: 1 – the crystalline silicon, 2 – the sNPS, 3 – the electrical contacts (Al with the thickness of ~3 μm), 4 – the applied voltage, 5 – the biological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

For the registration of the specific immune complex by the PhL of the sNPS it was constructed the prototype which included the source of the ultraviolet (UV) radiation (1) 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^{o} relatively to the plate with the layer of the sNPS (4) and the photo diode (5) intended for the determination of the incident UV (Fig. 5). At the adsorption of the biological molecules the level of the PhL of the sPNS 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.

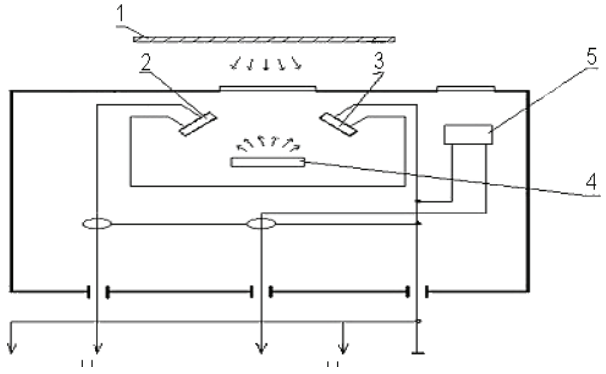

Figure 5. Design of PhL biosensor (see explanation in the text).

To take into attention the possible changing of the incident UV the photodiode (5) is used. The output signal is determined as the relation between the output signal from the photodiodes of 2 and 3 and the output signal from the photodiode of 5. Photodiodes of the n-p-p^{+}-structures work in the photo generative regime. Such construction is related to the systems of the differential type.

3 RESULTS AND DISCUSSION

It was stated that the photosensitivity of the sPNS is a little decreased after the immobilization of Ag (crude sample of the retroviral proteins) but at the addition of Ab (serum blood of diabetics) in the dilution of 1:500 and, particular, in 1:100 it sharply is decreased. Unfortunately at the less level of blood dilution (from 1:10 to 1:1) the photosensitivity starts to decrease up to initial level Fig. 6). Maybe, it connected with the increasing of the density of the solution to be analyzed or with other mechanism of the electronic exchanges between the immune complex and the sNPS surface.

If we taken the blood serum from not ill peoples the level of the photo current did not change in the comparison with the initial one. The same situation was observed if we used the bovine serum albumin instead of the crude samples of the retroviral proteins.

So, the experimental results give us possibility to consider that the application of the proposed principle of the immune biosensor may be very perspective for the different types of the biochemical diagnostics not for revealing of the anti-I Ab only. Of course there is necessary to understand what kind are influences of the high protein concentration on the process of the recombination in the sPNS. There is necessary to underline that the overall time of the analysis is several dozen minutes only instead of several hours in case of the traditional used ELISA-method.

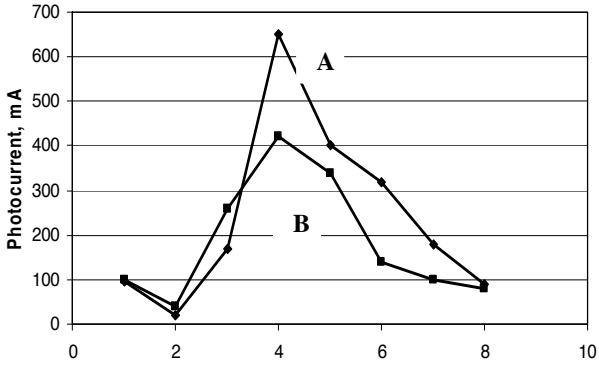

Figure 6. The level of the photocurrent before (1) and after deposition of Ag (2) and Ab in the different concentration (3-8 – 1:500, 1:100, 1:10, 1:5, 1:3 and 1:1, respectively). Line A and B are respect to two different samples of blood serum.

If we taken the blood serum from not ill peoples the level of the photo current did not change in the comparison

with the initial one. The same situation was observed if we used the bovine serum albumin instead of the serum blood. So, the experimental results give us possibility to consider that the application of the proposed principle of the immune biosensor may be very perspective for the different types of the biochemical diagnostics not for revealing of the anti-I Ab only. Of course there is necessary to understand what kind are influences of the high protein concentration on the process of the recombination in the sPNS. There is necessary to underline that the overall time of the analysis is several dozen minutes only instead of several hours in case of the traditional used ELISA-method.

At the determination of the anti-I specific Ab in the blood of the ill patirnts by the method of the PhL of the sPNS it was stated the next. The deposition of the retroviral proteins on the sPNS a few increases the PhL level but at the formation of the specific immune complex it decreases. Moreover, the level of the PhL decreasing depends on the concentration of the specific Ab in the blood (Fig. 7). If we used the non-specific Ab or the serum bovine albumin as Ag the level of the PhL does not change.

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 no 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.

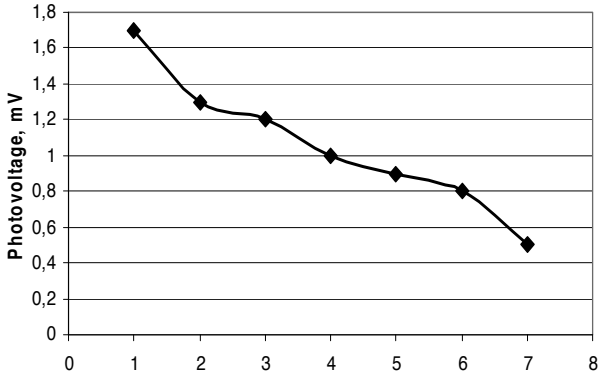

Figure 7. Dependence of the PhL intensity on the concentration of the specific Ab in the solution to be analyzed (serum blood of diabetics). Abscissa – 1 – immobilized Ag; 2-7 – dilution of blood serum: 1:500; 1:100; 1:10; 1:5; 1:3; 1:1, respectively.

4 CONCLUSION

The above presented experimental data testify that the sNPS may be used as very simple transducers with the long stable their abilities at the creation of the immune biosensors. The formed specific immune complex on the sNPS surface may be registered by the measurement of its PhL or photoconductivity. According to the obtained results in the respect of the application of such immune biosensors for the biochemical diagnostics of the anti-I Ab it is possible to conclude that they will respond all practice demands, especially, sensitivity, simplicity, rapidity of the analysis and its fulfillment in field conditions. These biosensors may be applied for the registration of any biochemical quantities which may form immune complex. Further investigations should be directed on the studying of the mechanisms of the biochemical signal registration by the sNPS and on the specification of all concrete moments of the analysis fulfillment.

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