IMMUNE-ENZYMATIG BIOSENSORS BASED ON THE OXIDE CERIUM ISFETS: SOME PHYSICAL AND FUNCTIONAL CHARACTERISTICS AT THE DETERMINATION OF SIMAZINE AND T2-MYCOTOXIN

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Summery. Immune biosensor based on the CeOx was created and its analytical characteristics were studying at the determination in model solutions of mycotoxin T2 and herbicide simazine. It was shown that input and output characteristics of the IsFET's with the CeOx dielectric have the increasing of the pH-sensitivity (about 58 mV/pH that was near to the maximal possible index – 59 mV/pH) in the comparison with Si_3N_4. It is due to high density of the surface sensitive centers (up to 10^{20} м$^{-2}$), large level of the permittivity (ε = 26) and the band-gap energy (3.6 eV) of cerium. All these effects lead to decreasing of the current losses through the dielectric. Reliable decrease of the biosensor signal at the "competitive" way of analysis (labeled and native substances competed for binding with antibodies – Ab) was observed down to 1.0 and 3.0 ng ml^{-1} of simazine and T2 mycotoxin, accordingly. The linearity of signal decrease was observed in the range of T2 mycotoxin concentrations from 5 to 190 ng ml^{-1}. The similar situation was occurred in case of simazine (2-150 ng ml^{-1}). The overall time of the assay including the duration of all preparation stages was 30 min. In case of the analysis by "to saturated way" allowed reaching sensitivity 0.5 ng ml^{-1} and linearity in the range 1.0-200 ng ml^{-1} and 0.5-170 for T2 mycotoxin and simazine, respectively. The overall time of the incubations was the same 10 min (5 min for the staying chip in the solution of the native and the labeled substances). There is necessary to note that the sensitivity and the stability of the biosensors based on the Si_3N_4 and CeOx IsFETs are much better for last one.
Key words: *cerium oxide, ISFETs, immune biosensor, mycotoxin, simazine, control.*

Introduction. One of the main tasks today is the control of the content of toxic substances including pharmaceutical products in environmental objects, living organisms and during some technological process. Modern physical chemical methods (chromatography, mass spectroscopy and nuclear magnetic resonance) in spite of their perfection with regard to sensitivity and specificity can not give a very fast response about the presence of concrete substance in sample to be analysed. Biosensors may serve in this respect as the powerful tools. Taken into attention many of preliminary investigations and functional principles of ion-selective field effect transistors (ISFETs) have every reason to believe that a new generation of biosensors based on them will be widely used practically to solve the problems mentioned above.

As a rule in modern ISFETs SiO_2, Si_3N_4, Ta_2O_5 are used as sensitive layer of insulator. Unfortunately, this type of ISFETs is characterized by insufficient sensitivity in the comparison with the glass ion-sensitive electrodes. It may be connected with the existing charge states in oxide and at the interface of oxide and semiconductor which are responsible for the non stability and drift of characteristics [1]. The obtaining of qualitative oxides having a small concentration of surface states (*Nss*=up to 10^{10} cm^{-2} eV^{-1}) and trace quantity of mobile charges connected with a big engineering difficulties. In addition, the SiO_2 surface has a relatively small concentration of ion-sensitive centers (about 10^{13} cm^{-2}). Si_3N_4 has number advantages in the comparison with SiO_2: higher electrical strength, chemical stability in acid-alkali mediums, tightness for the mobile sodium ions and higher density of surface-sensitive centers (up to 10^{15} cm^{-2}), which leads to high pH sensitivity. The high density of surface state on the silicon is the main disadvantage of Si_3N_4. However, when using a two-layer dielectric SiO^2-Si^3N^4, which preserves the benefits of both materials, the instability of the structures is significantly reduced. Ta_2O_5 may be used as senssitive dielectric layer too. This material has a very high density of surface sites, resulting in high pH achieved sensitivity. However, it was shown that at the cathode polarization of semiconductor relative to the electrolyte in the voltage range -1…-1.5 V there is a sharp increase in leakage currents through the insulator to the values of more than 100 nA. For comparison, it should be noted that in similar conditions, leakage currents for SiO_2 is 50-100 nA at voltages -1... -2 V, for a two-layer dielectric SiO_2-Si_3N_4 leakage

currents do not exceed 5-15 nA [1], and leakage currents for CeO_2 10^{-12} - 10^{-13} A at the same electric field. The last oxide has a number of advantages in the comparison with SiO_2 and Si_3N_4: 1) high level of dielectric permeability (ε = 26) and, correspondingly, large electric strength that allows to use more thin dielectric layers; 2) the energy gap Eg = 3,6 eV, which leads to a better dielectric isolation structure, and reduces leakage current through the dielectric: 3) chemical resistance in acid-alkaline environments and tightness for mobile ions sodium; 4) a higher density of surface-sensitive centres (up to 10^{16} cm^{-2}), which leads to high pH sensitivity and the parameter mismatch in the lattice constants of CeO_2 and silicon (Δα/α=0,35%) that provides the density of surface states steepening CVC characteristics as well as increased pH sensitivity sensors based on ISFETs; 5) high thermal stability and 6) high-quality immobilization of biochemical elements at the creation of biosensors.

In sensor devices the dielectric layers deposited on semiconductor have as passive role and serve as chemical sensitive membrane. This imposes rather stringent conditions on their quality (thickness and uniformity), considering that the area of contact with the electrolyte can be relatively large (from 0.1 to tens of mm^2).

This work was devoted development of the immune biosensor based on CeO_x and studying of its analytical characteristics at the determination of content in model solutions such toxic substances as mycotoxin T2 and simazine.

The problem of dispersion of mycotoxins among of environmental objects is very important due to their external toxicity, especially, it concerns T2-micotoxin and pesticides. Biosensors are the most appropriate instrumental analytical devices which may fulfill all practice demands. Among them a lot of different types are exist and there is necessary to chose the most sensitive, stable and simple which may provide analysis in on line regime and in field conditions. Immune biosensors based on the ISFETs may respond these demands. Unfortunately, the stability and sensitivity of SiO_2-Si_3N_4-layer in the commonly used ISFETs is not sufficient and to overcome this shortcoming we created semiconductor structure with the SiO_2 – CeO_2-layer.

Experimental.

The technology of ISFETs creation is the same as the preparation of metal-dielectric-semiconductor structures. As external shutter it was used CeO_x layer of which was fabricated by method of “oxidization of metallic mirror” at which firstly thin CeO_2 layers were formed by electron beam evaporation. This technology can be done with using of standard equipments and relative low temperature. It does not demand a lot of time and is quite simple. The chamber pressure was 10^{-5} Pa, the emission current was equal to 140 mA, accelerating voltage - 12 kV, substrate temperature was in the range 170 ÷ 180 °C. After the deposition the substrate was maintained at this temperature for 10 minutes. Oxidation of cerium was carried out in a diffusion furnace at 250 °C in oxygen. We investigated the ISFETs with two types of insulators: SiO_2 - Si_3N_4, d_{Si3N4} = 50 nm and CeO_2 d_{CeO2} = 50 nm. The measurements were performed at room temperature and atmospheric pressure. To measure the pH sensitivity using standard buffer solutions with pH: 12.45; 9.18; 6.86; 4.01; 3.56; 1.68.

The design parameters of ISFETs were studied by methods of optical and scanning microscopy. The cut crystals of ISFETs in the field of the gate in the direction transverse to the diffusion region are shown in Fig.1. The parameters of dielectric layers were made by scanning microscopy (Fig. 2).

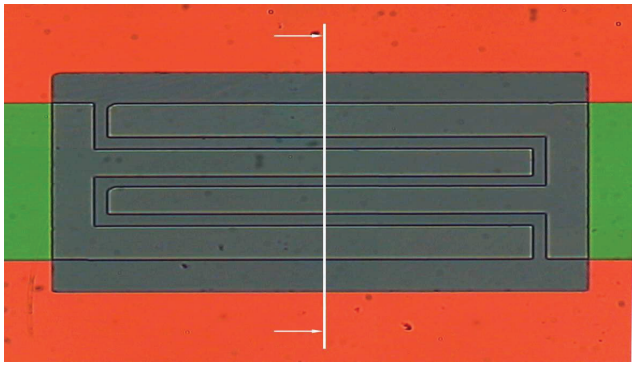

Fig. 1. Picture of the ISFET gate with the indicating the location of the cut.

Immune biosensor was created by the next way. It was used the differential scheme of measurements when the reference ISFET was covered by bovine serum albumin and working one – by the specific Ab through the intermediate layer from the staphylococcal protein A. Analysis was fulfilled in

two way: competitive and so called “to saturation”. In first one simazine or mycotoxin T2 labeled by horse radish peroxidase (HRP) competed with that which should be analyzed. In second one at first the immobilized Ab interacted with simazina or mycotoxin T2 in the sample and then with the solution of this analyte but labeled by HRP. The activity of HRP was registered in the presence of the special working buffer containing 5 mM tris-HCl (pH 7.5), 100 mM NaCl, 15 mM ascorbic acid and 5 or 10 mM H_2O_2. The substrate conversion causes a local basic pH shift, because dehydroascorbic acid formed is a more neutral compound compared to ascorbic acid. The signals (*dV/dt*) of the ISFETs were registered by electronic device providing signal amplification and its processing on the basis of a custom-made computer program. After every assay the chip was treated 5 min by 0.1 M HCl and then it was carefully washed with the above mentioned tris-HCl buffer.

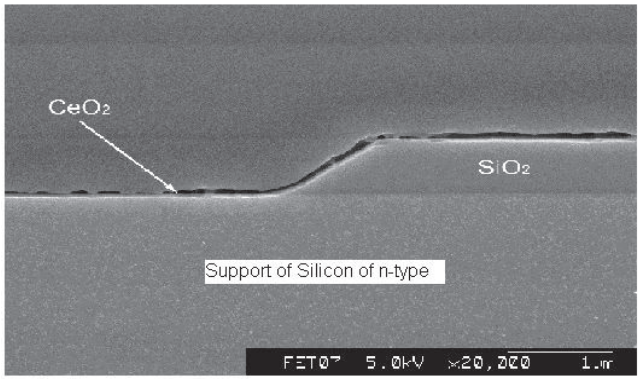

Fig. 2. Scanning electron microscopy of ISFET gate surface.

Results and discussin.

Any surface oxide always contains hydroxyl groups. In case of CeO_x the changes of surface charge at the variation of pH arise due to CeOH groups. Between protons in solution and hydroxyl groups formed on the boundary of CeO_2-solution the equilibrium reactions have place. The hydrogen proton concentration may be determined according to shift of the threshold voltage of ISFET or to change the drain current in the transistor channel. The experimental characteristics of the studied ISFETs are presented in Fig. 3.

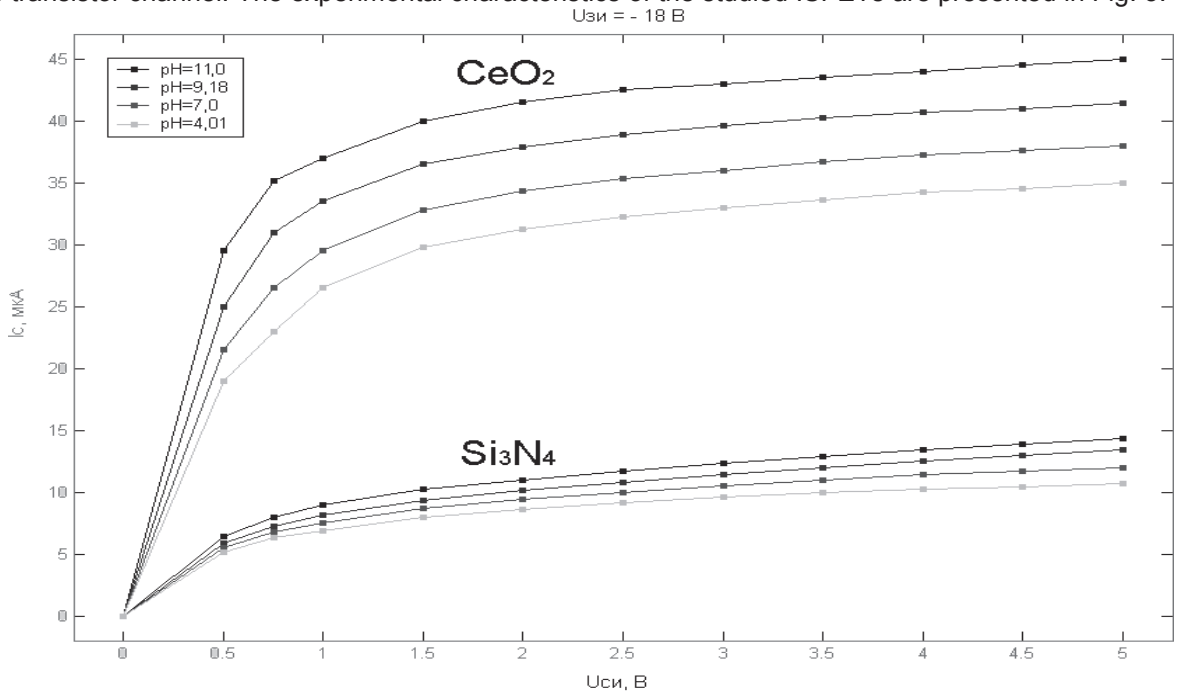

Fig. 3. Influence of pH on the output characteristics of ISFETs with the Si_3N_4 and CeO_2 dielectric structures.

At the pH increasing of solution the value of positive charge on the surface of ion-sensitive dielectric is decreased. Since we used *p*-channel ISFETs the decreasing of positive charge on the dielectric surface leads to increasing in channel conductance and hence to increase the drain current for both structures SiO_2 - Si_3N_4 and SiO_2 - CeO_2. According to our investigations the most linear and stable

responses for both dielectric structures were registered at the voltage in 10V. The same they had linear character of dependence on pH changes. pH sensitivity for $SiO_2 – Si_3N_4$was 63 mV/pH and for CeO_2 - 58 mV/pH that is very close to the maximal possible sensitivity, so called Nernst sensitivity, which according to theory binding centers in case semiconductor-dielectric-solution may achieve 53 mV/pH. The sensitivity of current flow due to the large dielectric constant of cerium oxide was increased more than two times (ε=26).

The characteristics of immune biosensor were started with determination of some initial parameters. For example, it was shown that the pH shift occurred during 30–40 s after addition of H_2O_2. The concentrations of the HRP–simazine or T2 conjugates were varied in the range 0.05–0.4 μg ml^{-1} and it was found that the maximal sensor output (about 100 mV) corresponded to 0.1 μg ml^{-1} of the conjugate. Under these conditions binding sites of the specific Ab were saturated. That is why the concentration of the above mentioned HRP-labeled substances during the competitive analysis was equal to 0.1μg ml^{-1}. Reliable decrease of the sensor signal was observed down to 1.0 and 3.0 ng ml^{-1} of simazine and T2 mycotoxin in the analyzed mixture, accordingly. The linearity of signal decrease was observed in the range of T2 mycotoxin concentrations from 5 to 190 ng ml^{-1} (Fig. 6, 7). In this range the potential of the IsFET gate varied from 95 to 5 mV. The similar situation was occurred in case of simazine (2-150 ngml^{-1}). In both cases the standard deviation was on average about 5%. The overall time of the assay including the duration of all preparation stages was 30 min. The limiting stage of the analysis is the competition between the labeled and native substances for binding with Ab (up to 10 min). In case of the analysis by “to saturated way” allowed reaching sensitivity 0.5 ng ml^{-1} and linearity in the range 1.0-200 ng ml^{-1} and 0.5-170 for T2 mycotoxin and simazine, respectively (Fig. 8, 9). The overall time of the incubations was the same 10 min (5 min for the staying chip in the solution of the native and the labeled substances).

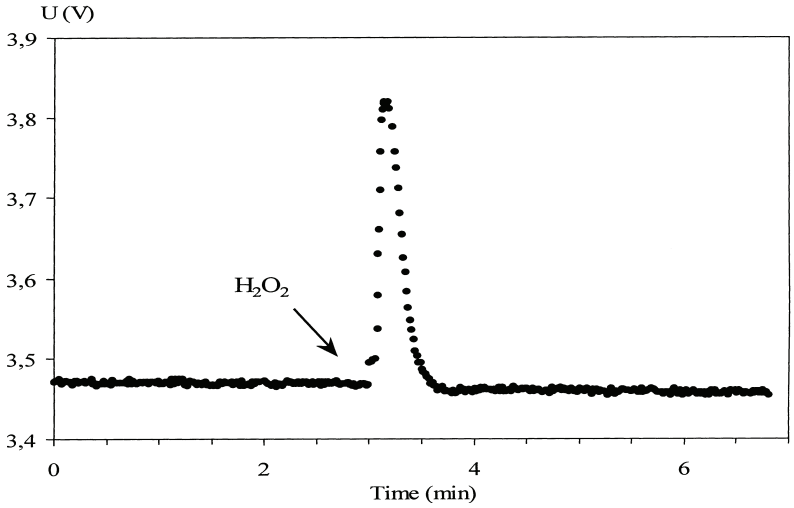

Fig. 4. Typical response of the sensor at the addition of H_2O_2. The ISFET potential is recorded as a function of time.

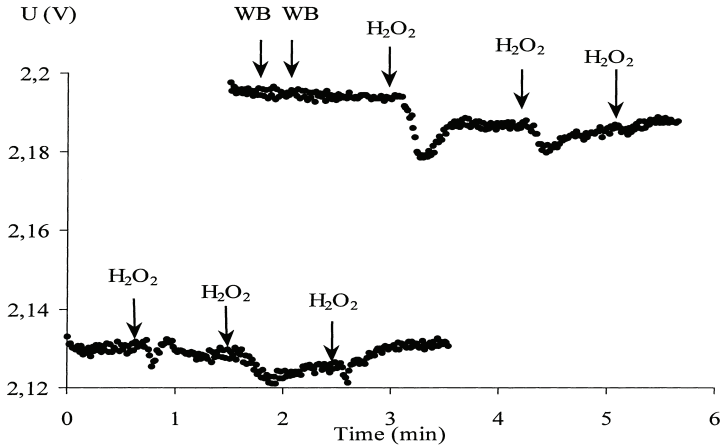

Fig. 5. Non-specific sensor response at the addition of H_2O_2 and buffer. The ISFET potential is recorded as a function of time.

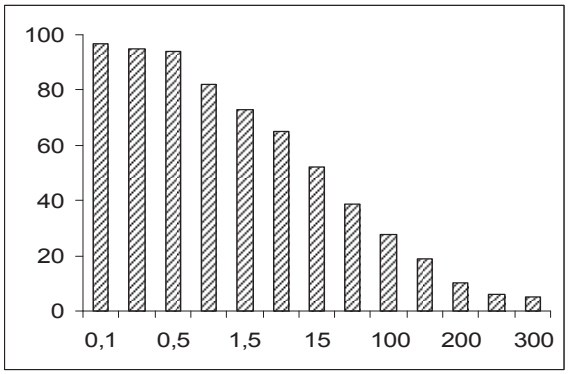

Fig. 6. Determination of T2 mycotoxin in solution with the help of the cerium oxide ISFET biosensor by the "competitive" way of analysis. Ordinate – mV, Abscises – ng/ml.

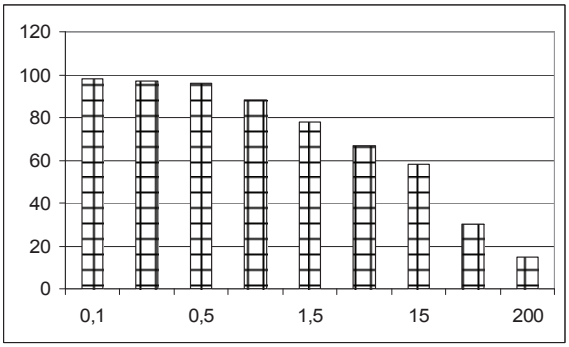

Fig. 7. Determination of simazine in solution with the help of the cerium oxide ISFET biosensor by the "competitive" way of analysis.. Ordinate – mV, Abscises – ng/ml.

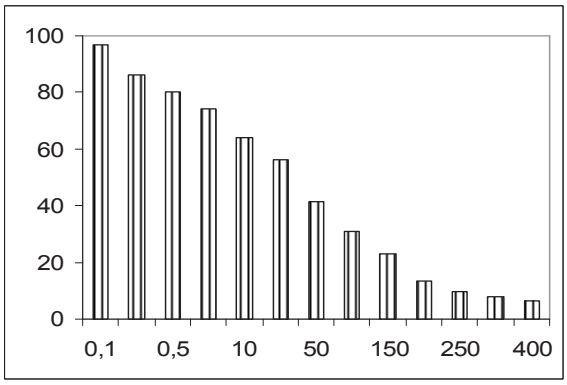

Fig. 8. Determination of T2 mycotoxin in solution with the help of the cerium oxide ISFET biosensor by the "to saturation" way of analysis. Ordinate – mV, Abscises – ng/ml.

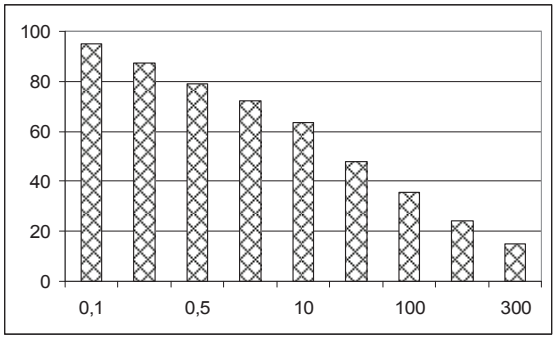

Fig. 9. Determination of simazine in solution with the help of the cerium oxide ISFET biosensor by the "to saturation" way of analysis. Ordinate – mV, Abscises – ng/ml.

The developed immune biosensor based on the CeOx IsFETs shown much more high sensitivity of T2 mycotoxin determination (up to 2 orders) than that which is based on the SPR [2, 3].

The sensitivity of the developed immune biosensor is approximately to that which was revealed in similar biosensor based on the total internal reflection ellipsometry [4]. Moreover, the IsFETs based immune biosensor may be simple used a several times after the destruction of the formed immune complex by the treatment of chips with 0.1 M HCl for 5 min and than by washing of tris-HCl buffer (5 mM, pH 7.5). Such procedure may be realized too in case of the SPR based immune biosensor but it is more complicate. The mentioned advantages of the immune biosensor based on the IsFET are very important for practice when it is necessary to fulfill the simple, fast and very sensitive analysis in field conditions.The similar immune biosensor based on the ISFETs was developed by us for the determination of the herbicide simazine [5]. It was constructed on the chip surface of which was covered by Si_3N_4. Since, the principle of the immune analysis was the same it is possible to compare the sensitivity and the stability of the biosensors based on the silicon nitride and cerium oxide ISFETs. Both parameters are much better for last ones. For example, CeOx IsFETs may be reused in 4 times longer and it sensitivity in 10 and more times higher than that which has S_3N_4-layer.

Conclusion

It was created immune biosensors based on the CeOx ISFETs which may recommended for practice application due to its high sensitivity, stability and simplicity of use for express determination of mycotoxins among environmental objects, especially, in corn. This sensor is corresponding to all practice demands. Certainly, for practical application there is necessary to develop a special method for obtaining of extracts from sample to be analyzed, in particular from that which are presented solid phases. In previous experiments [6-9] we demonstrated that acetonitrile and methanol are as most appropriate solvents in this case. Moreover, it was shown that first of them may be used in final concentration about 30% during immune biosensor analysis. Methanol should be used in a few small concentrations (about 20%). Obtained experimental results about sensitivity of the immune biosensor based on the CeO_x ISFETs as well as information about ways of sample preparation testify perspective of these approaches for practice application.

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