

Optimization of parameters of modification of surface of biosensor on the basis of ISUT for raising sensitivity at determination of Patulin

Aim To determine the optimal parameters of determination to the patulin for sensitivity of the immune touch control on the basis of the ion-sensitive field transistors (ISFT). Methods. Biotechnological, biochemical, immunological. Results Both variants of "competition" of the method of analysis show that the amount of reduction in the signal in the range from 1 to 200 ng / ml-1 (in this range potential to the ISFT breach block of 9 to 98 mV). Some higher sensitivity and a range of measurable concentrations (0.5-250 ng / ml) have been extended a bit while it is discovered during the raising of the analysis of the method of "saturation". Conclusions. Establishment of optimal parameters of the work of this is an analytical tool for the use of different concentrations of H₂O₂, and the changes of pH showed him heterospecific reviews that substantially does not affect his work.

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Key words: patulin, touchcontrol, nanoporous cerium, ion-sensitive field transistor, pH, H₂O₂.

The problem of defeat of foodstuffs by mycotoxins has an extraordinarily large value and has acquired the special actuality in the whole world. These low-molecular connections are foods of vital functions of mushrooms, act as toxins, and are also distinguished by the wide spectrum of distribution in the wild. The amount of toxin that is distinguished depends on the sort of factors:

- physical (moisture, temperature and mechanical damages);
- chemical (to the presence of dioxide of carbon, oxygen, pesticides and fungicides and from the features of composition of substrate);
- biological (to the sort of plants, participation of insects and other)[5].

Moisture and temperature have a large influence on the height of mould and producing of mycotoxins. Pathogenic mushrooms that strike cultures to harvesting as a rule, require the higher levels of humidity (20-25%), than mushrooms that develop in forage during storage (13-18%). Therefore most forage with maintenance of moisture higher 13% receptive to formation of mould and appearance of mycotoxins in particular [7]. It gives an opportunity to strike foodstuffs and stern for animals at all stages of growing, storage and transporting to them. To Tom, from one side, mycotoxins are dangerous for the health of man and animals, and from other - their presence in forage has economic and international consequences for trade.

Presently, the most dangerous mushrooms of *Aspergillus*, *Fusarium* and *Penicillium* [4], which are produced by basic toxins: zearelenone, ochratoxin, and aflatoxin, and particularly dangerous mycotoxins are a patulin. An international agency of the study of cancer on the basis of the study of toxicity of the palladium reckoned him to the chancerogens of the 3rd group, or to the substances for which there is insufficient data for reliable classification [6]. Therefore, the present necessity and determination of the content of this mycotoxin are the issue of the day.

Before, some of the types of optical immune touchcontrols were worked out, namely: on the basis of superficial plasmon resonance and ellipsometry of complete internal reflection [3]. We tried to use the ion-sensitive field transistors (ISFT) as a sensor of immune touchcontrol. Unfortunately, the stability of nitride of silicon on the surface of the breach-block of ISFT was not sufficient for the practical use of touchcontrol on the basis of such a structure. In addition, for the implementation of all the requirements of the practice in relation to the high sensitivity of analysis, and also the simplicity, cheapness and speed of its realization, we suggested to use ISFT on the basis of oxide of cerium [1] ..

An aim of work is establishment of optimal parameters of determination to the patulin for the sensitisation of immune touchcontrol on the basis of ISFT.

Materials and methods of researches. It has got specific IgG from Agrisera (Sweden). They were dissolved in 0.5 mM 5 mM Na-phosphate buffer (pH 7.3) containing 100 mM NaCl (PBS). Specific IgG is conjugated from horseradish peroxidase (PC) on the standards of procedure with the application of glutaraldehyde (GA) [9]. All reagents are purchased at "Sigma-Aldrich" (THE USA).

An analysis on the basis of ISFT is executed by two methods: by "competition" and "saturation". The first method is carried out after two variants: a) when specific IgG is immobilized on the surface of the breech-block, and the patulin and him, the conjugate from the PC competed for the sites of binding to them; b) immobilized with a bovine seralbumin (BSA), competed a patulin conjugate for the sites of binding to the free toxin for sites binding to specific IgG, labeled PC. After the "saturation" of the specific IgG is immobilized on the surface of the breech-block, they first reacted with a free form to the patulin, whereupon co-operated by the patulin-PX conjugate.

Results and their discussions. For registration of change of pH that generated to the patulin conjugate from PC (0.1 gml⁻¹, on HRP), specific IgG was immobilized on the breech-block of ISFT [8, 10]. As the improved variant, specific IgGs were immobilized through the creation of an intermediate layer from squirrel And from Staphylococcus aureus. Then the molecules of IgG specifically contact with an albumin, but also the concentration of specific IgG to the patulin on the surface of the sensor increases sharply. For the fixing time of the review of the touch control on the surface of the breech-block of ISFT there was immobilized of the PC directly through the sewing together of AND. In both cases, pH change was observed after 35-50 s after addition of H₂O₂.

The establishment of optimal concentration of H₂O₂ shows that the maximum review of touchcontrol is achieved for concentrations of 10 mM of H₂O₂. In this case from the PC, a conjugate of patulin to the complex specific IgG with an albumen And, immobilized on the surface of the sensor. The level of H₂O₂ in a working buffer (RB) varies from 5 to 35 mM. Concentration to the patulin labeled PC, at 0.3 mcg / ml-1 at "competition" exposure to mycotoxin.

It follows to mark the high producibility of the results between the different measurements for separate ISFT. For the receipt of production, the identical plates of the ISFT are used. Moreover, both the electrical descriptions of these transformers and the order of the preparation of biological membranes are standardized. In the last case, this is important, it was very for the implementation of a precise preparation of the surface of the breech-block, and also for providing constant terms in relation to humidity during 1 year for co-operation squirrel And with activated surfaces of the surface of breech-block. As a rule, all these processes are executed by a technological group, simultaneously prepared not less than 10 ISFT.

As ISFT is very sensitive to the various factors (ionic strength, pH level, irradiation light, presence of ions that influence the charge of the surface of the breech block, etc.), they are analyzed by heterospecific reviews in relation to RB and H₂O₂. For this purpose, a differential chart is used: one ISFT contains specific IgG, immobilized via squirrel, and, secondly, in the case of immobilization of BSA via AND. As a result of researches set, RB did not influence the review of the sensor, then, as H₂O₂ in a concentration of 10 mM, initiated a small (~ 10 mV) review. During determination of activity, PN H₂O₂ caused a change in pH without the basic changes of parameters. Thus, neither RB nor H₂O₂ did not help displacement of the basic value of pH during determination to patulin. At both variants of the "competition" method of analysis, the linearity of diminishing the signal was observed at a concentration to patulin of 1-200 ng / ml-1 (in this range of potential to the ISFT block of breaks from 9 to 98 mV). Some higher sensitivity and a range of measurable concentrations (0.5-250 ng / ml) have been extended a bit while it is discovered during the raising of the analysis of the method of "saturation". The standard deviation is the average given close to 5%.

Conclusions

Found out the changes in pH that is generated by the patulin-HRP conjugate, the establishment of optimal concentration of H₂O₂, which substantially does not affect the change in the review of touchcontrol, as well as the optimization of the row of other parameters of the raising of the analysis gave an opportunity to carry out the detection of the patulin in a range of 1-200 ng / ml-1 and 0.5-250 ng / ml, depending on the chosen method of realization of analysis.

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