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EFFICIENCY OF USE OF OPTICAL AND POTENTIOMETRIC IMMUNOBIOSENSORS FOR DETERMINATION OF SALMONELLA TYPHIMURIUM

The purpose. To analyze efficiency of the developed optical and potentiometric immunobiosensors on the basis of phenomena of surface plasmon resonance (SPR), ellipsometry of complete internal reflection (ECIR) and ionselective unipolar transistors (IsUT) for determination of *Salmonella typhimurium* in modelling solutions. **Methods.** Modified procedure on the basis of response of antigen-antibody, which includes processing of transdusers with protein A for creation of oriented stratum of antibodies. **Results.** Sensitivity of SPR-immunobiosensor made 101 – 107 kl/ml, ECIR-biosensor — less than 5 kl in 10 ml, IsUtbiosensor — from 2 to 5·105 kl/ml. **Conclusions.** Analyzed immunobiosensors are effective at determination of *S. typhimurium* in modelling solutions. The best sensitivity matching practical demands, was fixed for optical immunobiosensor on the basis of ECIR. IsUT-immunobiosensor has a lower sensitivity, but it differs by stability and high reproducibility of results. Immunobiosensors on the basis of SPR ensure sweeping analysis in real time and do not require special staff, therefore it is possible to use them for primary expressdiagnostics of *S. typhimurium.*

Key words: immunobiosensor, antigen-antibody response, *Salmonella typhimurium.*

Salmonella typhimurium along with other representatives of *Salmonella* specie is one of the biological agents that causes food borne pathogens the most frequently. Infected food products (such as meat, eggs, etc.) contaminated with alive microorganisms [11] are dangerous for human health and cause significant damage to food industry and agriculture. Known today methods for microorganisms detection do not fully meet the practical demands because of their lack of sensitivity, long runtime and/or high cost of analysis. Therefore, development of new highly sensitive approaches for detection of pathogenic microorganisms in environmental objects is relevant. Biosensors are analytical devices, which combine biological sensing element (ligand) and physico-chemical transducer or transformative microsystem for signal reproduction. Signal value is proportional to concentration of analyte and is postulated as alternative technology of rapid detection of microorganisms [5,2].

The purpose of the work is to analyze an efficiency of optical and potentiometric biosensors for *S. typhimurium* detection in model solutions and possibility of their further use for identifying sources of infection of animals and humans and express control of feeds and agricultural products quality.

Materials and methods. For *S. typhimurium* detection in model solutions using different types of biosensors the modified technique based on antigen-antibody reaction was used. It included previous preparation of transducers for increasing of sensitivity and detection level of biosensors.

In case of optical schemes sensors based on the phenomenon of surface plasmon resonance (SPR) such as Spreeta (USA) and sensor “Plasmonotest” (property of V.M. Glushkov Institute of Cybernetics of National Academy of Sciences, Ukraine; pat. UA 100934) [3,8] and sensor based on total internal reflection ellipsometry (TIRE) [9] were used. Direct method of analysis was used in all cases of *S. typhimurium* detection using optical immune biosensors. Previous modification of the transducer surface took place, which included covering of surface by polyalylamine hydrochloride (PAA) and protein A from *Staphylococcus aureus*. Transducer surface was covered with bovine serum albumin (BSA) for blocking free non-specific binding centers on the gold surface. In case of TIRE based biosensor previous modification of the transducer surface was made the same way as for SPR-sensors.

A new type of immune biosensor based on ion-sensitive field-effect transistor (ISFET) with CeOx insteand of Si_3N_4 gate surface was proposed, for *S. typhimurium* detection in the model solutions. ISFETs were activated by aqueous solution of glutaraldehyde (GA) with further analysis algorithm as for SPR-biosensors. Blocking free groups of GA was provided by solution of glycine. Prepared in this way chip stored in a dried state at 4ºC. It was chosen “sandwich” algorithm of analysis. For this purpose *S. typhimurium* cells were bound with specific antibodies for 20 min and then the surface was treated for 10 min with a solution of specific antibodies, which were labeled by horseradish peroxidase (HP).

Results and discussion. In the case of *S. typhimurium* detection using Spreeta based biosensor, it has been defined that device sensitivity was on the level 10^3 – 10^7 cells/ml. “Plasmonotest” detection level was within 10^1–10^6 cells/ml. It was proved that preliminary preparation of working surface aimed to produce antibody-oriented layer had a significant influence to the level of sensitivity of devices. As a result of physical adsorption of antibodies directly on the gold surface of the biosensor “Plasmonotest” the device sensitivity was 10^4 – 10^6 cells/ml. Biosensor based on the TIRE has shown higher sensitivity than the SPR based. Maximal level of sensitivity was on the level of several cells (less than 5) in 10 ml. Since time of each reagent immobilization to the transducer surface is on average 10 minutes the duration of a full cycle of analysis is less than 1 hour. This time can be significantly reduced when previous immobilization of PAA, protein A and specific antibodies is provided, e.g., ELISA provides *S. typhimurium* detection on the level 10^4 cells/ml [7], 10^6 cells/ml [4] with total time of analysis from 6 hours.

Biosensor based on the ISFET has shown sensitivity within 2 – $5x10^5$ cells/ml. In this case, biosensors response also depends on the number of antigenbinding sites on the ISFET surface; therefore, oriented immobilization of antibodies via protein A of *St. aureus* is effective for signal increasing. Total time of analysis when transducer surface is preliminary prepared is about 30 min. In addition to high sensitivity, this method allows to use biochips several times (up to 5) without signal reduction. For this purpose biochips were treated by 0,1 M HCl for 5 min. Cells accumulation methods (using of bioaffinity columns, or magnetic beads separation) makes it possible to increase the sensitivity to the level of infectious dose [1].

Conclusions

Analyzed above immune biosensors are effective for *S. typhimurium* detection in model solutions. TIRE based immune biosensor demonstrated the highest sensitivity that was on the level of several cells (less than 5) in 10 ml. This sensitivity satisfies practical needs since infectious dose for drinking water is 1 cell/ml [6]. Biosensor based on the ISFET had lower sensitivity that was 2 – $5x10^5$ cells/ml, but it provides stability and high reproducibility. Regeneration of working surface up to 5 cycles of analysis without signal reduction reduces the cost of analysis. SPR based biosensors showed sensitivity within 10^1 – 10^7 cells/ml that is higher than ELISA sensitivity. In addition, they provides rapid analysis in real time and do not require special staff for analysis. Such advantages make possible their use for initial express diagnostics of pathogens including *S. typhimurium* or accompanying them agents.

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