Research Article

Development of substrate for amperometric biosensor

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ABSTRACT

Chemical contamination of environment and food is currently one of the most serious problems of mankind. Nowadays, the number of pesticides used in agriculture is constantly growing. Rapid test methods are being actively developed that are characterized by high availability and sufficient levels of sensitivity and selectivity. This paper presents the method of creating a substrate for amperometric enzymatic biosensor for the determination of organochlorine pesticides in milk and milk products.

Keywords: biosensor, substrate, pesticide, copper-foiled textolite, amperometric

INTRODUCTION

Contamination of the environment, plants, feed and food by foreign substances in the biogeochemical chain is currently one of the most serious problems of mankind, since many of them are toxic to animals and humans, causing food poisoning and food infections [1]. Possessing a hepatropic, carcinogenic and mutagenic effect, some of them are of danger to the body. Such pollutants include pesticides. Pesticides are chemicals that are used to control pests and plant diseases, as well as with various parasites, weeds, and cereal and grain products pests. The most common pesticides include organochlorine, organophosphate, carbamate, organo-mercury, synthetic pyrethroids, and copper-containing [2].

The results of automated monitoring, which provides information about the concentration of various pesticides and organochlorine compounds in foods, show increasing total amount of pesticides in products of plant and animal origin, in particular in potatoes, onions, fish, milk and others [3, 4].

Currently, in most countries, using of the pesticides is restricted, but they are still used in agriculture, since it is impossible to abandon the use of pesticides. Therefore, control of their content in animal raw materials and products is very important. [5].

One of the most common and numerous groups of pesticides are organophosphate pesticides. Among them, the most famous are parathion, diazinon, chlorophos, karbofos, disulfothione, malathion. Due to its low cost and relative ease of use, karbofos remains in high demand in the pesticide market, while stronger pesticides are replaced by modern biopreparations.

Currently, despite the existing set of simple express, sensitive and selective methods for determining a significant number of biologically active compounds of different nature and origin, the problem of their determination remains relevant [6, 18].

In principle, any biosensor consists of two functional elements: a bioselecting membrane, which uses various biological structures, and a physical signal transducer, which transforms the concentration signal into an electrical one. The principle of detection, implemented in biosensors, is based on the fact that a biomaterial (enzymes, cells, antibodies, etc.) immobilized on a physical sensor (converter), when interacting with the detected compound, generates a signal dependent on its concentration, which is recorded by an electrochemical, optical converter or of another type and after data processing is presented in a numerical form. The property of biological macromolecules is selective with high sensitivity to “recognize” various compounds makes it possible to create biosensors for analyzing a wide range of substances. The simplicity of the device, efficiency, specificity and low cost of biosensor analysis make the development of this area of analytical biotechnology a high degree of priority [7, 19].

Optical biosensors based on the use of fluorescent labels in an immunoassay are known [8, 9]. In these biosensors, the beam from the light source passes through the optical fiber and excites the fluorescence of the antibody-antigen complex formed on or near the optical fiber. The resulting fluorescence through the optical fiber and the filter is fed to a photodiode or photomultiplier. The disadvantage of these biosensors is their non-compliance with the following requirements for biosensors: small size, low weight and simple design.

Physical transducers can be electrochemical transducers (electrodes), optical, gravitational, calorimetric transducers, resonant systems. As a bioselecting material, all types of biological structures can be used: enzymes, antibodies, receptors, microorganisms [10].

Much attention is paid to the study of properties of amperometric type, in which the Clark electrode is used as a transducer. Typical for biosensors of this type is the use of microorganisms in the receptor element; the unique features of microbial cells, due to the non-repetitive composition of enzyme systems in different strains, allowed the creation of biosensor models for the detection of more than 80 different compounds [11, 17].

The important problems in the development of amperometric microbial biosensors should include an increase in the selectivity of the analysis; search for strains that oxidize foreign compounds in order to create devices

for effective environmental monitoring; study of the possibility of highly efficient detection of xenobiotics by strains carrying plasmids of their degradation; the ability to use genetic engineering methods to obtain microorganisms with desired properties to increase the analytical potential of microbial sensors [12].

DEVELOPMENT OF SUBSTRATE FOR AMPEROMETRIC BIOSENSOR

The Department of «Standardization and Biotechnology» of the Shakarim State University conducts research work on the development of an amperometric enzymatic biosensor for the determination of organochlorine pesticides in milk and milk products. To create a substrate a method of creating printed circuit boards was chosen as a basis for an enzymatic biosensor using the “laser-iron” technology, which is often used in the design of electronic devices. Initially, the computer created pictures using Sprint Layout 6.0 and then they were printed on paper using a laser printer at maximum resolution (Fig. 1).

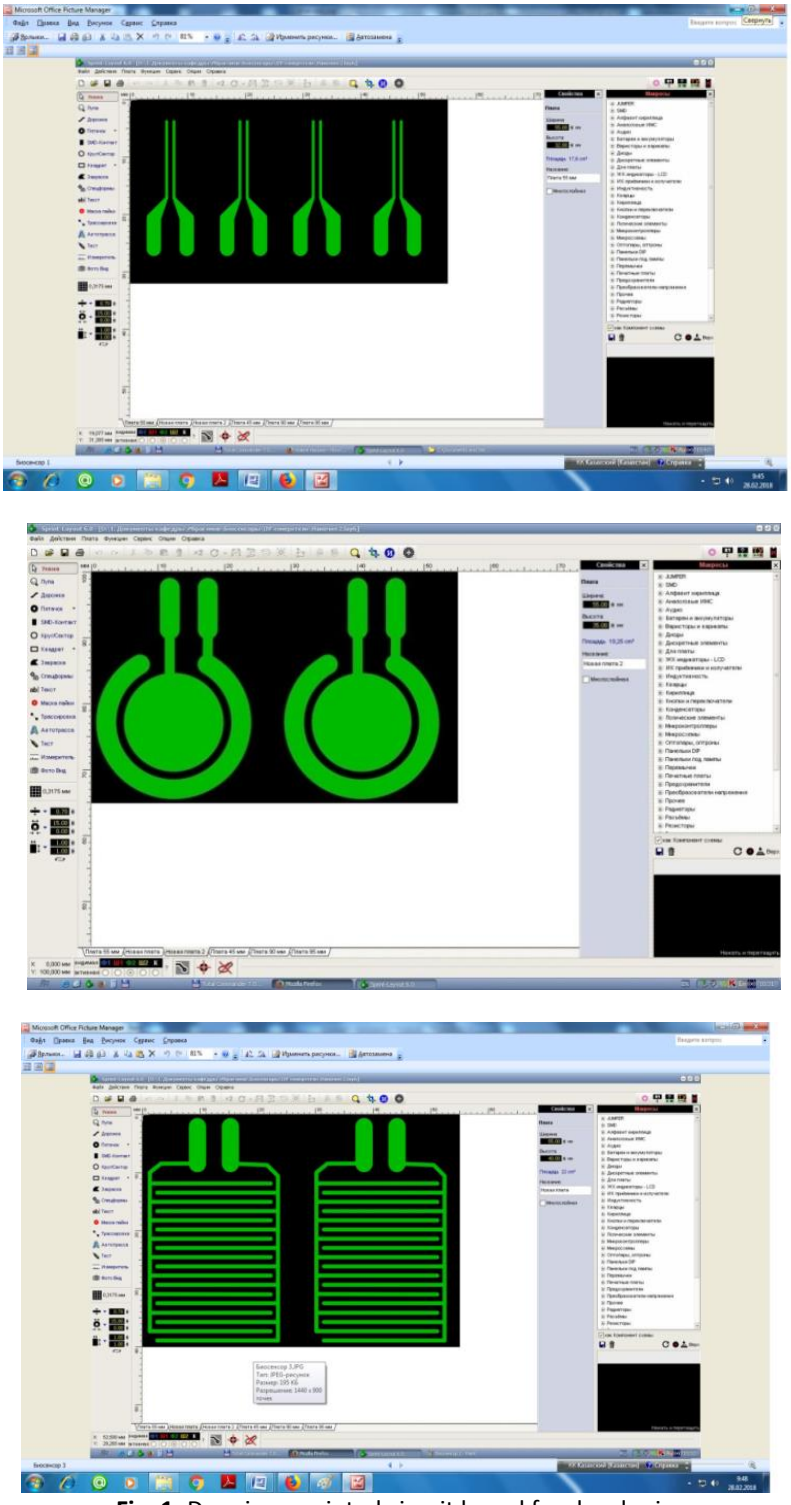

Fig. 1. Drawing a printed circuit board for developing

substrate of amperometric enzymatic biosensor

Next, the image was transferred using the glossy magazine page. In order to produce printed circuit board, a copper foil textolite than was sanded beforehand with the help of emery paper with a grit size of 1800 and cut to a specific size needed to create a sensor.

The transfer of the pattern was carried out using the laser-iron technology; to do this, a page from a glossy magazine with a pattern printed on a black-and-white laser printer with a toner to a foil was put on the pre-heated surface of copper-foil textolite with a paper tape between the sheets of newspaper and ironed with a little pressure in different directions heated with an iron to a temperature of 140-15500C, for 90 seconds [4].

In order to remove the paper after transferring the image from the surface of the printed circuit board, it was lowered into the bath with warm water and detergent for about 5 minutes, after which paper was carefully removed from the surface of the board. Then, etching of the printed circuit board was carried out, for which a circuit board with a printed pattern was dipped in nitric acid diluted with distilled water in a ratio of 1:1 and kept in this solution until copper was removed from exposed areas not covered with toner that usually takes 3-5 minutes. If the printed circuit board is kept for more than 30 minutes, then the areas with toner will be etched completely, due to the penetration of acid under the toner from the side surfaces [13-16].

After the etching process, the board was taken out of nitric acid, washed with water and dried, to remove the toner layer, the surface of the board was wiped with a napkin previously moistened with acetone.

As a result of the work, a substrate was made for the development of a sensor in the form of a printed circuit board based on copper-foil textolite. When conducting further research on the use of produced substrates, we found that the surface of the copper tracks oxidized upon contact with the working solutions. Therefore, to protect the copper tracks from oxidation, which occurs upon contact with aggressive media, the surface of the copper tracks is additionally covered with pure tin with a soldering iron. The quality of the board was evaluated for clearance. In Figure 2 it can be seen that the tracks are clearly visible, there is no overlap on each other and there are no short circuits.

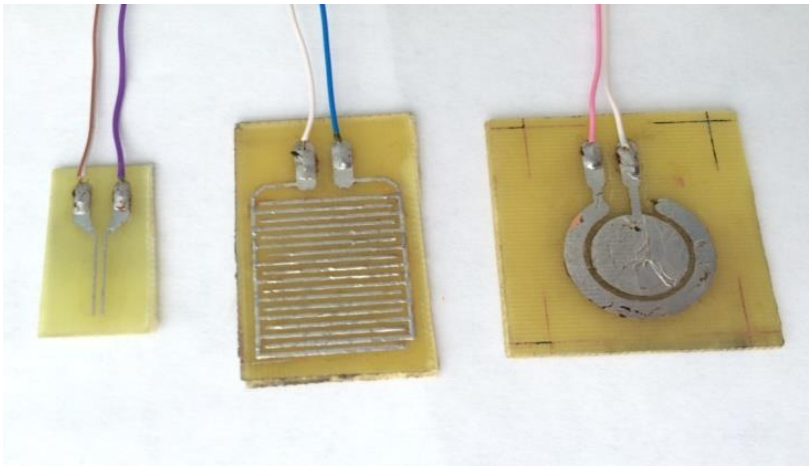

Fig. 2. Substrate

based on copper-foil textolite for biosensor

CONCLUSION

Thus, using the method of "laser-iron" technology based on copper-foiled textolite there was developed substrate, which later will be used to create a sensor for an amperometric biosensor. The advantage of the developed substrate for the sensor is that it is made of inexpensive, scrap materials, the manufacturing process does not take much time, does not require special skills. Also, the use of the "laser-iron" technology gives a fairly clear pattern with a replication of the size with an accuracy of 0.1 mm and a good repeatability of the experiment.

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