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LABORATORY PROTOTYPE OF ELECTROCHEMICAL BIOSENSOR FOR THE QUANTITATIVE ANALYSIS OF ALANINE AMINOTRANSFERASE ACTIVITY

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Aim. To develop an electrochemical biosensor for the quantitative assessment of alanine aminotransferase (ALT) activity.

Materials and Methods. Three-electrode amperometric scheme of detection with platinum disk working electrode covered with bioselective element based on pyruvate oxidase and photopolymer. Measurements were carried out with an applied potential of 0.6 V.

Results. A laboratory prototype of an amperometric biosensor for ALT detection was developed. The immobilization method was selected, and the procedure was optimized for the type of bioselective material used. The composition of the working buffer was optimized, namely, the kind of buffer, buffer capacity, pH, content of coenzymes of the bioselective material (phosphate ions, magnesium ions, thiamine pyrophosphate), and the analyte (alanine, ketoglutarate, pyridoxal phosphate). The reproducibility of immobilization and reproducibility of responses of the developed biosensor, as well as its analytical characteristics (linear range, detection limit, response time, etc.), were analyzed.

Conclusions. The developed biosensor was characterized by sufficiently good analytical parameters for its further optimization and testing when working with real blood serum samples.

Keywords: alanine aminotransferase, biosensor, enzyme activity.

Alanine aminotransferase (ALT) is a key enzyme of amino acid metabolism that plays a vital role in the diagnosis of liver diseases, including hepatitis, cirrhosis, and fatty degeneration [1]. Determination of ALT activity in serum is a standard biochemical test widely used to monitor liver status. Traditional methods [2–3] for determining the activity of this enzyme are based on spectrophotometric and colorimetric assays, which, although highly accurate, have a number of disadvantages. In particular, they require the use of significant volumes of reagents, complex laboratory equipment, and a long analysis time. In addition, the cost of such studies remains relatively high. In this regard, there is a need to develop alternative detection methods that would be simpler, cheaper, and no less effective.

One of the promising areas in this field is the use of electrochemical biosensors. They have a number of advantages, including high sensitivity and selectivity, the possibility of miniaturization, rapidity of measurements, and accessibility. Due to these characteristics, biosensors can significantly improve the diagnosis of liver diseases, providing a rapid and accurate analysis of ALT activity without the need for complex laboratory equipment.

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Aim. This work aimed to develop an electrochemical biosensor capable of providing a quantitative assessment of alanine aminotransferase activity in biological fluid samples.

Methods. A three-electrode amperometric of detection was used to create the biosensor, which includes a platinum disk working electrode, as well as a bioselective element on the electrode surface created by photopolymerization of pyruvate oxidase with PVA-SbQ. The principle of operation of the biosensor is based on a two-enzyme system in which ALT catalyzes the reaction between alanine and ketoglutarate, forming pyruvate. The resulting pyruvate is oxidized by pyruvate oxidase to form hydrogen peroxide, which in turn is detected amperometrically using a platinum electrode.

Results and Discussion. During the research, a laboratory prototype of an amperometric biosensor capable of detecting ALT activity was created. The optimal method of immobilization of the bioselective material was selected, which ensures stability and high reproducibility of the obtained results. In addition, the immobilization procedure was optimized, taking into account the specifics of the bioselective material used. The optimal conditions for the sensor's operation were determined, in particular, the composition of the working buffer, which includes the appropriate type of buffer, its buffer capacity, and pH level. To ensure proper operation of the sensor, concentrations of substrates and coenzymes were optimized, including alanine, ketoglutarate, pyridoxal phosphate, phosphate ions, magnesium ions, and thiamine pyrophosphate. The reproducibility of immobilization of the bioselective material was studied, which is an essential parameter for ensuring the stability of the sensor during repeated measurements.

Table. **Analytical characteristics of the biosensor**

Linear range	LOD	Sensitivity	Response time	Baseline noise	Baseline drift
1–500 UL^{-1}	1 UL^{-1}	1 nA min^{-1} per 100 UL^{-1}	2 min	0.4 nA	0.2 nA min^{-1}

The analytical characteristics (Table) of the biosensor were also carefully analyzed. Namely, linear range (Figure), detection limit, and response time were investigated. The results obtained indicate that the sensor has high sensitivity and stability, which makes it promising for further optimization.

Conclusions. The developed amperometric biosensor for determining ALT activity demonstrated good analytical characteristics, which allows us to consider it as a promising tool for further research. The next step in this work is to test the biosensor on real blood serum samples to assess its effectiveness in clinical conditions. The successful implementation of such biosensors in medical practice can significantly simplify and reduce the cost of diagnosing liver diseases, providing a fast and affordable method for analyzing ALT activity in biological fluids.

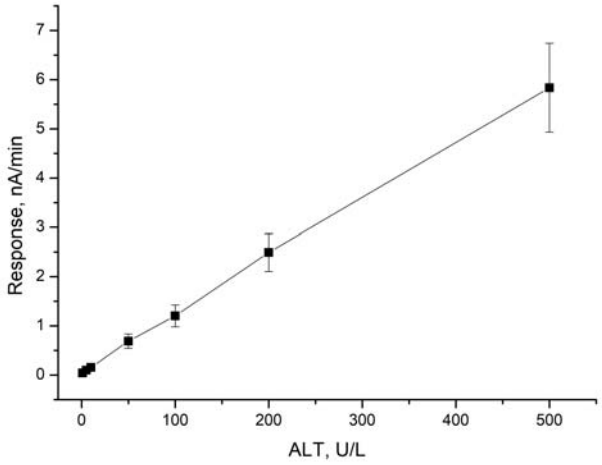

Figure. **Calibration curve of the developed biosensor for the detection of ALT activity**
Working solution content: alanine — 8mM, α-ketoglutarate — 0.5mM, pyridoxal phosphate — 50mkM, $Mg(NO_3)_2$ — 0.5mM, thiamine pyrophosphate — 2mM, KH_2PO_4 — 5mM, working buffer — HEPES 25mM, pH 7.4, applied potential +0.6V, constant mixing, RT

Author's contribution

D.O. Mruga — thesis writing, data curation. Y.R. Vakhovsky — data curation. S.V. Dzyadevych — editing, project administration. O.O. Soldatkin — editing, supervision.

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