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DEVELOPMENT AND ADAPTATION OF A BIOSENSOR BASED ON LACTATE OXIDASE AND POLY-M-PHENYLENEDIAMINE FOR THE DETERMINATION OF L-LACTATE IN REAL BIOLOGICAL SAMPLES

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Aim. Development and optimization of an amperometric biosensor based on lactate oxidase with a poly-meta-phenylenediamine membrane for the accurate measurement of L-lactate in blood serum.

Material and Methods. The biosensor was based on a platinum disk amperometric transducer modified with a PPD membrane with immobilized LOx. Measurements were performed in a three-electrode amperometric system at a potential of +0.6 V. The real sample results were verified spectrophotometrically and by an electrochemical analyzer.

Results. The biosensor demonstrated a wide linear range (7–1000 μM), a low detection limit (7 μM), and high selectivity. The standard addition method showed a high correlation (0.982) with reference methods.

Conclusions. The developed biosensor is promising for clinical diagnostics of blood lactate levels.

Keywords: amperometric biosensor, lactate oxidase, real sample analysis, enzyme.

Biosensors as a new generation of analytical devices are rapidly gaining popularity due to their significant advantages over traditional measurement methods. They are easy to use, portable and characterized by a low cost per analysis, which makes them ideal for use in clinical diagnostics, for example, for monitoring the levels of vital substances in biological fluids. In particular, among the important biomarkers that can be measured by the biosensor method, lactate — the anion of lactic acid, has an extremely important physiological value, because it can be used as biomarker of a number of diseases of the cardiovascular system, hypoxia, diabetes (types 1 and 2), and a number of others. Traditionally, lactate levels have been determined using methods such as colorimetry [1],

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spectrophotometry [2], fluorimetry, liquid chromatography [3], nuclear magnetic resonance (NMR) [4], and enzymatic assays [5]. While these techniques are known for their accuracy and reliability, they are often hindered by complex procedures, the requirement for costly and bulky instrumentation, and limited potential for miniaturization. These drawbacks have stimulated interest in biosensor-based approaches for lactate detection. Biosensors offer several advantages — they are user-friendly, portable, cost-effective, and suitable for on-site and real-time analysis. Currently, a variety of lactate biosensors have been developed, including electrochemical, optical, and enzymatic types. However, despite their growing popularity, many still face challenges such as limited stability, sensitivity to environmental factors, and relatively short operational lifetimes. These limitations highlight the need for continued innovation in biosensor design and materials.

Aim. Due to the importance of lactate as a biomarker for a number of serious diseases, we decided to create an amperometric biosensor for measuring lactate based on lactate oxidase using an additional semipermeable membrane based on poly-meta-phenylenediamine.

Methods. Biosensors were created using platinum disk amperometric transducers. A poly-meta-

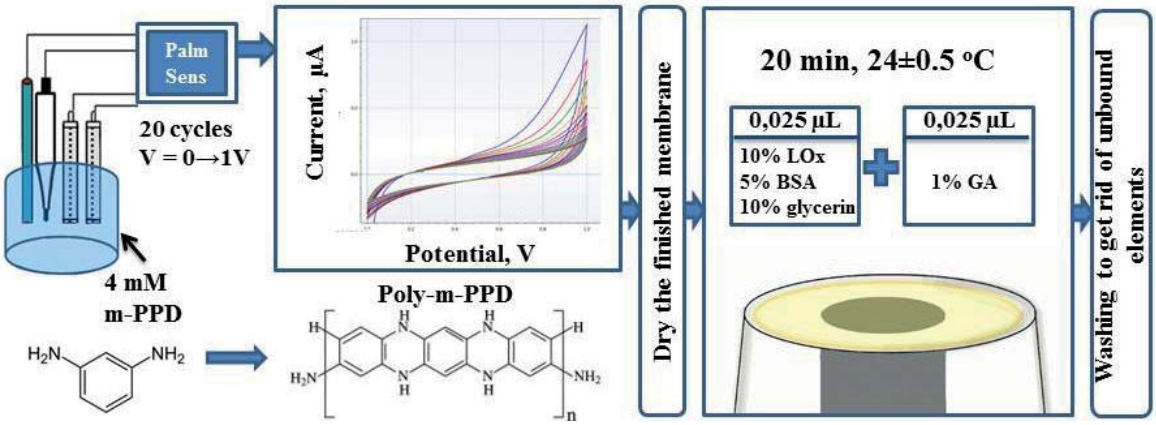

Fig. 1. **Scheme biosensor bioselective element preparation**

phenylenediamine membrane was applied to their surface by electropolymerization, after which the electrode surface was dried, and a mixture of enzyme gel based on 10% lactate oxidase mixed with 1% glutaraldehyde (1:1) was applied. The enzyme was immobilized on the surface of the transducer by covalent binding using glutaraldehyde for 30 minutes at a temperature of 24±1.5 °C in the open air. After that, the biosensor was washed for 5 minutes from unbound membrane elements, changing the buffer solution in the measuring cell several times. The scheme for creating a bioselective element of the biosensor is presented in Fig. 1.

The measurements were performed in an open measuring cell with a volume of 2 ml using a classical three-electrode amperometric system using working, reference, and additional electrodes at an applied potential of +0.6 V.

The spectrophotometric method and a commercial electrochemical analyzer were used as reference methods for measuring real samples.

To process the experimental data obtained in the course of the work, standard methods of variational statistics were used. The study was carried out in at least 5-9 repetitions. When statistically processing the results, the arithmetic mean and its standard deviation were determined,and the data were considered reliable at $P < 0.05$. Data processing and calculations were performed using the OriginLab graphic editor (OriginPro version 8.5).

Results and Discussion. In the course of our work, we developed and optimized an amperometric biosensor based on lactate oxidase and a polymetaphenylenediamine (PPD) membrane. This involved a series of experiments to assess the influence of the main parameters of the working solution on the functioning of the sensor. Studies have shown that the presence of a PPD membrane reduces the impact of ionic strength on the sensor response, although at high concentrations of salts (e.g., NaCl) a decrease in the signal was still observed. However, given that biological samples are diluted before measurement, the effect of this factor is insignificant when measuring real samples of biological fluids.

A study of the buffer capacity of the working solution indicated that the optimal concentration of HEPES salt in the buffer was 25 mM since it is under these conditions that the most extensive linear measurement range and high sensitivity to lactate are ensured. Similarly, the influence of protein concentration on the functioning of the biosensor was analyzed since biological fluids usually contain a significant amount of proteins. The PPD membrane was shown to significantly reduce the influence of protein (BSA) significantly, minimizing mechanical membrane fouling and signal loss.

One of the key characteristics of the biosensor was its selectivity towards potential interferents. Studies have shown that without the PPD membrane, the sensor responded significantly to ascorbic acid and some amino acids. In contrast, in the presence of the membrane, these effects were almost eliminated. This is because larger molecules cannot penetrate the pores of the PPD membrane, which prevents signal distortion and provides a high level of selectivity towards possible interferents.

Analyzing the analytical characteristics, we determined that the biosensor has a wide linear operating range, high sensitivity, and a low detection limit. The total analysis time was 8 minutes, which is fast enough for practical use.

Final testing of the biosensor on real blood serum samples confirmed its effectiveness. The standard addition method was found to be the most accurate and stable, demonstrating a high correlation with the reference results from an electrochemical analyzer. At the same time, the results of spectrophotometric analysis differed in some cases, which may be due to the peculiarities of sample transportation or serum heterogeneity. The measurement results of real samples are shown in Fig. 2.

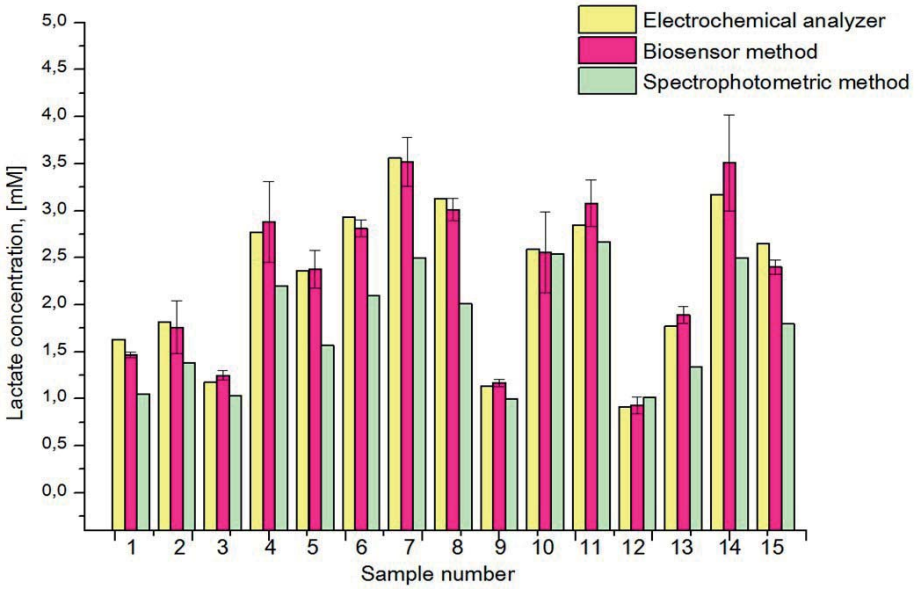

Fig. 2. **Comparison of serum lactate concentrations measured by biosensor (n = 9) and reference methods**

Conclusions. Thus, in the course of the work, an amperometric biosensor with a semipermeable PPD membrane and immobilized LOx was developed and optimized for the rapid and accurate determination of L-lactate in blood serum. The PPD membrane minimizes biological and solvent interferences, ensuring high accuracy of the analysis. The biosensor has a wide linear range (7–1000 μM), a low detection limit (7 μM) and a short analysis time.

The biosensor method of standard additions showed the best correlation with the reference methods (0.982 and 0.914), and the repeatability of measurements was high (deviation of 2.9% and 6.7% for model solutions and real blood serum samples, respectively). The device remained stable after 5 days of continuous use (signal decrease of 3–5%).

Thus, it can be concluded that a biosensor based on lactate oxidase and PPD can be considered promising for clinical and laboratory diagnostics of blood lactate levels.

Authors' contribution

KOB, AVB and YRV performed the main biosensor measurements related to the development and optimization of the biosensor. OVS performed the analysis of blood serum samples by

spectrophotometric method, OYL — by electrochemical analyzer method. ASS provided samples and assisted with experiments. OOS and SVD were the project supervisors and provided the material base for the research.

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REFERENCES

1. Suman, S., Singhal, R., Sharma, A. L., Malthotra, B. D., Pundir, C. S. (2005). Development of a lactate biosensor based on conducting copolymer bound lactate oxidase. *Sensors and Actuators B: Chemical*, 107(2), 768–772. https://doi.org/10.1016/j.snb.2004.11.050
2. Brandt, R. B., Siegel, S. A., Waters, M. G., Bloch, M. H. (1980). Spectrophotometric assay for D-(–)-lactate in plasma. *Anal. Biochem.*, 102(1), 39–46. https://doi.org/10.1016/0003-2697(80)90105-4
3. Wasek, B., Arning, E. (2022). Quantitation of lactate in cerebrospinal fluid using liquid chromatography–electrospray-tandem mass spectrometry. In: *Clinical Applications of Mass Spectrometry in Biomolecular Analysis: Methods and Protocols*. New York, NY: Springer US, 253–260. https://doi.org/10.1007/978-1-0716-2317-9_19
4. Lloyd, S. G., Zeng, H., Wang, P., Chatham, J. C. (2004). Lactate isotopomer analysis by 1H NMR spectroscopy: consideration of long-range nuclear spin–spin interactions. *Magn. Reson. Med.*, 51(6), 1279–1282. https://doi.org/10.1002/mrm.20092
5. Rodriguez, F. A., Banquells, M., Pons, V., Drobnic, F., Galilea, P. A. (1992). A comparative study of blood lactate analytic methods. *Int. J. Sports Med.*, 13(6),462–466. https://doi.org/10.1055/s-2007-1021304