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Development, optimization, and comparison of two versions of ALT-sensitive biosensor based on glutamate oxidase and pyruvate oxidase

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Aim. Alanine aminotransferase (ALT) is an enzyme mostly found in heart and liver cells. Therefore, its blood content is often used as a non-selective biomarker of damage to these organs and helps diagnose such diseases as cirrhosis, hepatitis, myocardial infarction, stroke, *etc.* Since these diseases usually result in a patient's critical condition requiring immediate medical aid, the availability of a fast, accurate, portable and easy-to-use method for ALT blood level detecting is a necessity. Currently, clinical research of ALT level is conducted using such methods as spectrometry and immunoassay, which can't meet all the needs of doctors. In contrast, biosensors have the potential for miniaturization, automation, and cost reduction of analysis. Therefore, it was decided to develop biosensors for determining the level of ALT in biological solutions. **Methods.** The measurement system was designed using a platinum disk electrode as an electrochemical transducer, connected to a PalmSense potentiostat using a three-electrode scheme (working electrode, additional electrode, reference Ag/AgCl electrode). In order to ensure additional selectivity of the converter, a poly(phenylenediamine) membrane was formed. The bioselective membrane on the electrode surface was formed by covalent cross-linking between enzyme and additional protein using glutaraldehyde. The level of enzyme activity in the solution was measured by determining the increase in current in the system per unit of time as a result of the oxidation of the ALT reaction product (glutamate or pyruvate, respectively). **Results.** In the work, two versions of a biosensor based on two different bioreactive systems with glutamate oxidase and pyruvate oxidase are proposed, and laboratory prototypes of these biosensors for measuring ALT are made. The optimal conditions for bioselective membrane creation and functioning conditions for both biosensors were selected, in particular the method of immobilization of each enzyme and concentration of coenzymes pyruvate oxidase and ALT. The main analytical characteristics of both versions of the developed biosensors were studied and compared. **Conclusions.** It was shown that the biosensor based on glutamate oxidase is easier to use and more stable due to the smaller number of coenzymes. However, the biosensor based on pyruvate oxidase is preferred when creating more advanced biosensor system for the simultaneous determination of ALT and AST (another biomarker of heart and liver diseases, which is often determined simultaneously with ALT for more accurate diagnosis and has one common stage of the enzymatic reaction), as it allows to separate the signal from these two enzymes. **Acknowledgements.** The work was supported by the National Research Foundation of Ukraine in the framework of the competition "Science for the reconstruction of Ukraine in the war and post-war periods" (project 2022.01/0043).

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