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DEVELOPMENT OF AN AMPEROMETRIC BIOSENSOR BASED ON OXIDASE FOR THE QUANTITATIVE DETERMINATION OF PYRUVATE

Background/Objective. Pyruvate is a key metabolite in cellular energy metabolism, playing a role in glycolysis, the tricarboxylic acid cycle, and amino acid synthesis. Its level in blood has significant diagnostic value in neonatology, indicating impaired tissue respiration, mitochondrial dysfunction, hypoxic conditions, and metabolic disorders in newborns, particularly preterm infants. The traditional methods for pyruvate analysis, such as spectrophotometry, fluorimetry, and mass spectrometry, require complex sample preparation, expensive equipment, and significant analysis time. In contrast, biosensors are compact, easy to use, and provide rapid, sensitive determination of pyruvate concentration. **Methods.** The pyruvate-sensitive biosensor used an amperometric three-electrode system consisting of a platinum disk working electrode, a platinum counter electrode, and an Ag/AgCl reference electrode. The bioselective element of the biosensor was formed by mixing an enzyme gel (8% pyruvate oxidase from *Aerococcus viridans*) with PVA-SBQ (19.8%), applying the mixture to the electrode, and photopolymerizing it. Pyruvate concentration was calculated based on the current change caused by electrochemical oxidation of hydrogen peroxide formed during the enzymatic reaction involving pyruvate oxidase. **Results.** Conditions for forming the biosensor's bioselective element were optimized, including the enzyme immobilization method, PVA-SBQ concentration, and UV irradiation energy. Concentrations of the pyruvate oxidase substrate (phosphate ions) and coenzymes (TPP and Mg²⁺) were also selected. The next biosensor's analytical characteristics were investigated: linear detection range 10–500 µM, sensitivity 573 ± 8 nA/mM, average response time 55 ± 11 s, and minimum detection limit of 0.25 µM for pyruvate. **Conclusions.** The amperometric biosensor based on immobilized pyruvate oxidase was developed for determining pyruvate concentration, characterized by high sensitivity, a low detection limit, and a wide linear range. These characteristics demonstrate a potential of the developed system for analyzing the pyruvate concentrations in aqueous solutions and biological samples. **Grants/Fundings.** The work was carried out as part of project no. 225067 of the Ukrainian-Swiss Joint Research Projects: Call for Proposals 2023 program.

Keywords: biosensor, pyruvate oxidase, enzyme, amperometry, analytical chemistry.

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