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TECHNIQUE FOR IMPROVING THE ANALYTICAL CHARACTERISTICS OF BIOSENSORS BASED ON ENZYMES OF THE OXIDASE SUBCLASS

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As science and technology advance, there's a growing demand for rapid and straightforward methods to measure various substances quantitatively and qualitatively. While traditional methods like chemical analysis, chromatography, and mass spectrometry offer high accuracy and selectivity, they come with drawbacks such as high costs, complexity, and bulky equipment requirements. In response, biosensor methods are gaining popularity for their ease of use, portability, and cost-effectiveness. This trend has spurred rapid development and improvement of already existing biosensors [1–4], particularly those based on oxidase enzymes, which are widely used commercially. We aimed to enhance the analytical performance of such biosensors given their prevalence and importance in various applications.

Aim. Among the methods of improving biosensor systems, the technique of using multi-enzyme bioselective elements is actively used. Its main idea is that, in addition to the main enzyme, auxiliary enzymes are added to the biosensor membrane, which can improve analytical characteristics or enhance the response of the biosensor to the target analyte. As a model biosensor that we intended to improve, we chose a conductometric biosensor for glucose determination based on glucose oxidase. The main enzyme of this biosensor was glucose oxidase (GOx), which is an enzyme of the oxidase class and catalyzes the reaction of splitting glucose into glucono-1,5-lactone and hydrogen peroxide using oxygen during the reaction. As an auxiliary enzyme, we additionally added catalase (CAT) to improve the characteristics of the sensor. It decomposes the peroxide formed during the first reaction with the participation of GOx into water and O_2. This enables to increase the concentration of oxygen in the near-electrode layer, which improves the work of the main enzyme — GOx, and, accordingly, the entire biosensor.

Methods. Two enzyme gels, glucose oxidase (GOx) and catalase (CAT) were mixed in a 2:1 ratio and applied to the first pair of electrodes for enzyme immobilization. A BSA protein gel was applied to the second pair of electrodes for reference. Immobilization occurred in glutaraldehyde vapors for 20 minutes, followed by air-drying for 10 minutes. The sensor was then washed for 5 minutes to remove residual GA and other unbound elements.

Measurements were conducted in a 2 ml open cell with a 5 mM phosphate buffer solution at pH 6.2. The substrate concentration was adjusted by adding aliquotes of 500 mM D-glucose to working solution. Response time was 2–3 minutes with a 5-minute wash between responses. Response measurement could be done by comparing baseline conductance before and after exposure to the target molecule.

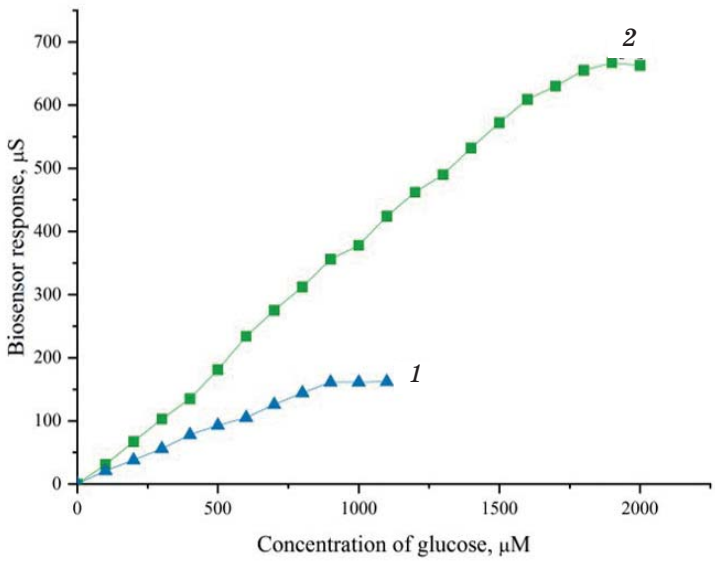
Results and Discussion. The initial step in developing of the GOx-CAT bienzyme biosensor was to identify the most effective enzyme immobilization method on the transducer surface. We tested five biosensor variants based on different coimmobilization techniques of GOx and CAT and assessed their key analytical properties. Calibration curves were also generated for each variant to guide the selection process. Ultimately, experiments revealed that immobilization in glutaraldehyde vapors,

combining GOx and CAT in a 2:1 ratio within a single-layer membrane, offered the highest sensitivity and wide linear operating range. This method was thus chosen for further development.

The next stage of the work was the selection of the optimal concentration of catalase in the membrane. For this, a number of biosensors with different concentrations of catalase in the composition of the bioselective element were made and the sensitivity of these biosensors to the target analyte was compared. Thus, it was shown that the biosensor based on 5% CAT was characterized by the greatest response to the substrate.

An important parameter for all biosensors is stability. Therefore, we conducted experiments to study the reproducibility of the signals of the bienzyme biosensor during its continuous use for one day and checked the reproducibility of the biosensor preparation procedure. Thus, according to the results, the biosensor is characterized by high repitability of measurement results within one day (RSD = 1.7%) and good reproducibility of the preparation procedure (RSD = 10.7%).

The last stage of the development was the verification of the analytical characteristics of the developed bi-enzymatic biosensor based on glucose oxidase and catalase in comparison with the mono-enzymatic biosensor based only on GOx. For this, detailed calibration curves were obtained for both biosensors, which are presented in the Figure.



Characteristic	Bi-enzyme biosensor	Mono-enzyme biosensor
Sensibility	378 µS/mM	161 µS/mM
Dynamic operation range	8-1900 µM	16-900 µM
Linear operation range	up to 1700 µM	up to 800 µM
The equation of the linear part of the curve	Z = 0.3711C+3.1143	Z = 0.1765C+2.7818
Limit of detection	8 µM	16 µM
Noise of baseline	1.1 µS	0.9 µS
Baseline drift	1.0 µS/min	0.9 µS/min
Time of one response	3 min	2 min
Time of analysis	7 min	6 min
Relative standard deviation (RSD)	1.7%	5.2%

Fig. **Calibration curves of a monoenzyme biosensor based on HOD [1] and a bienzyme biosensor based on GOx-CAT [2] and a comparison table of their main analytical characteristics**
Measurements were performed in 5 mM phosphate buffer with a pH of 6.2.

The difference between mono- and bienzyme biosensors lies in their sensitivity to glucose and their linear detection range. The sensitivity of the bienzyme biosensor (CAT-GOx mixture) was 378 µS/mM, compared to 161 µS/mM for the monoenzyme biosensor (GOx alone). Adding CAT to the biosensor extended its linear range to 1700 µM, compared to 800 µM for the monoenzyme biosensor. Additionally, the minimum detection limit decreased from 16 µM to 8 µM glucose for the monoenzyme and bienzyme biosensors, respectively.

Conclusions. The study showed that the addition of catalase as an auxiliary enzyme significantly improved the analytical characteristics of the biosensor based on GOx, in particular, sensitivity, repeatability of measurement results, limits of linear and dynamic ranges of operation. It has been proven that the technology of adding an additional enzyme to the biomembrane of a biosensor can be used to improve the analytical characteristics of biosensors based on oxidases, which in turn will allow their more effective use.

Key words: conductometric converter, biosensor, oxidases, enzyme analysis.

Author`s contribution. BKO was engaged in the main research work and creation of the original text of theses. MDO — was engaged in research work and theses editing. DSV — consultations on conductometric measurements and development of multienzyme systems. SOO—editing theses, helping in planning experiments.

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