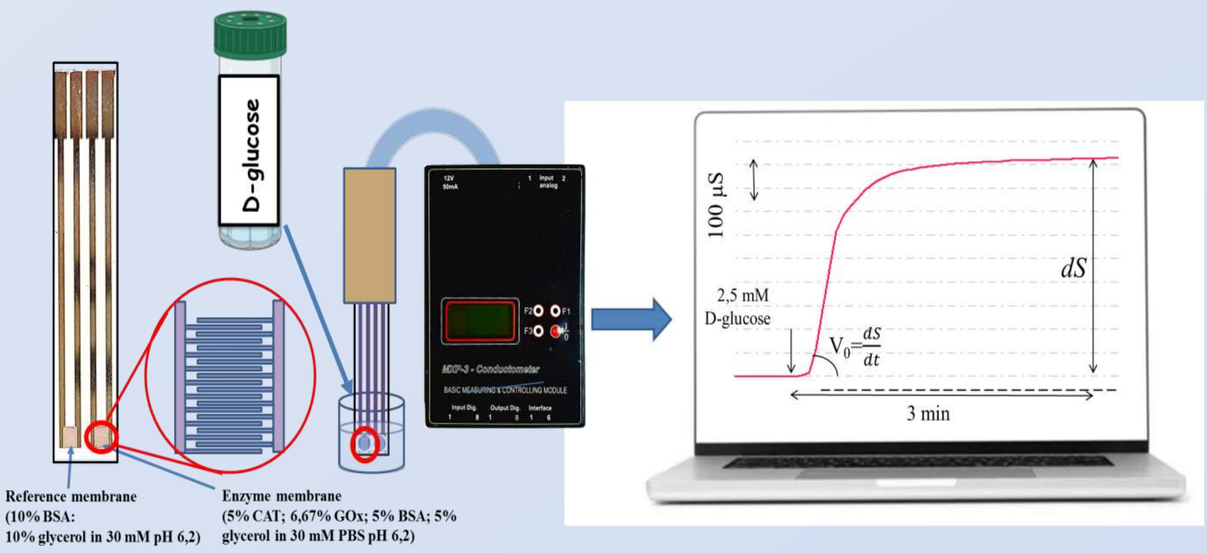
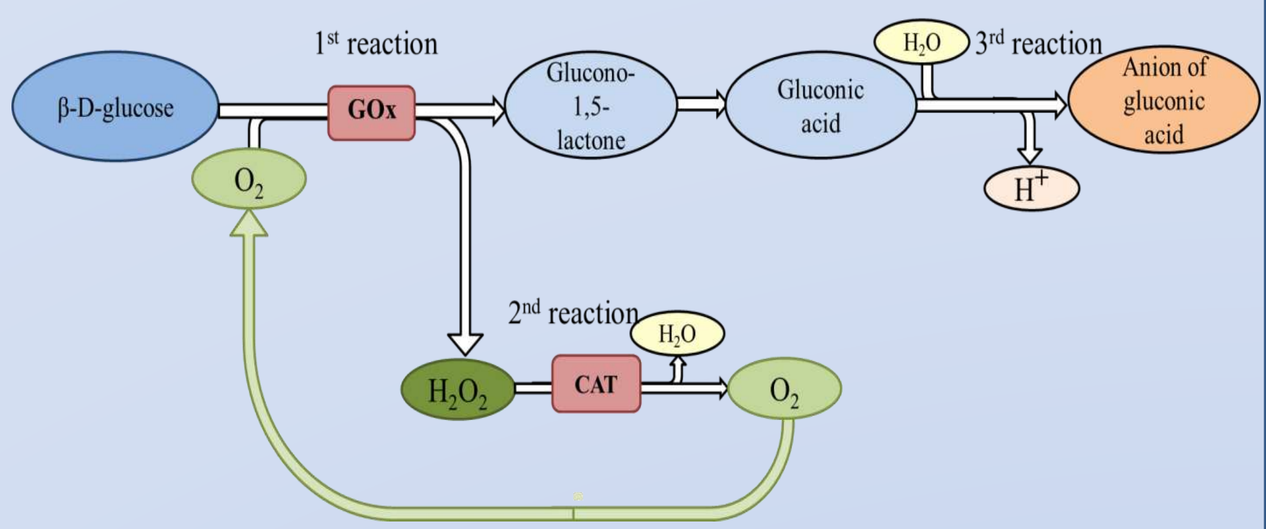
Development of new approaches for improving the analytical characteristics of enzyme biosensors

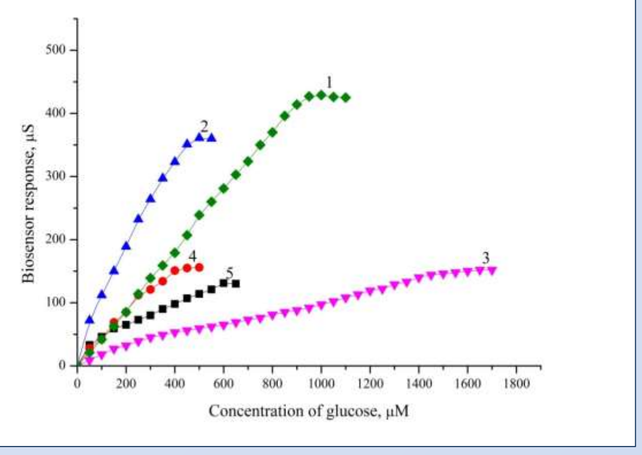
Berketa K.O.[1,2], Dzyadevych S.V.[1,2], Soldatkin O.O.[1]

[1] Institute of Molecular Biology and Genetics of NASU, Akademika Zabolotnoho St, 150, Kyiv, Ukraine, 03680.
[2] Taras Shevchenko National University of Kyiv, Volodymyrska Street, 60, Kyiv, Ukraine, 01033

This research investigated the possibility of using the method of improving the analytical characteristics of a monoenzyme biosensor based on glucose oxidase (GOx) by adding an supplementary enzyme - catalase to the bioselective element, in order to better saturate the biomembrane with oxygen for the best GOx performance. The formed in reaction with CAT oxygen, in turn, saturates the enzyme membrane, thereby eliminating the limitation of the work of the main enzyme (GOx) by the lack of O2.

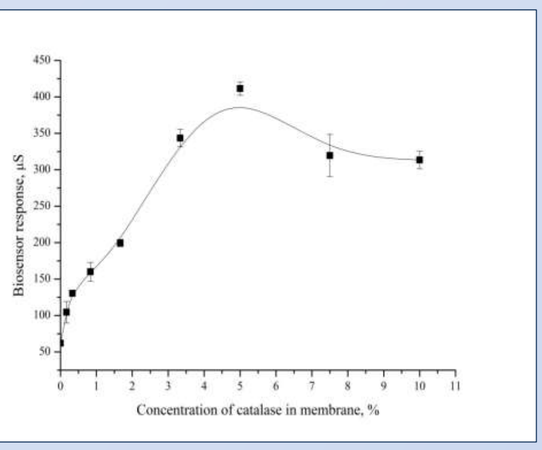






Calibration curves of biosensors based on different combinations of GOx and CAT in the membrane

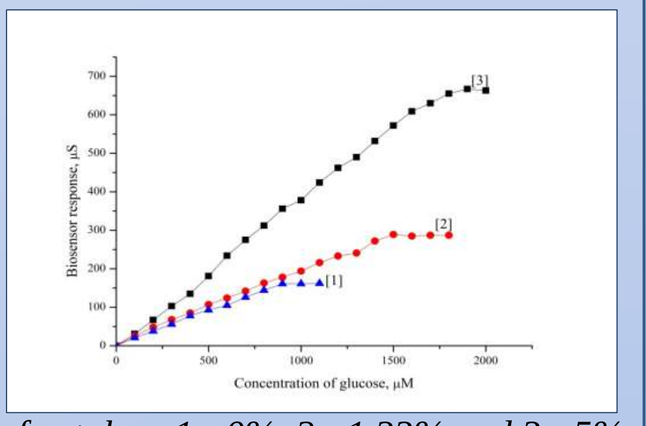
1) A single-layer membrane based on a mixture of two enzymes. In this case, the enzymes were mixed in a ratio of 1:2 (gel with 15% CAT: gel with 10% GOx) before being applied to the surface of the transducer;
2) A two-layer membrane formed by two enzyme gels applied and immobilized on the surface of the transducer in sequence by separate membranes. The bottom layer is a gel with 15% CAT, the top layer is a gel with 10% GOx;
3) Two-layer membrane, the lower layer – gel with 10% GOx, the upper – gel with 15% CAT;
4) Single-layer membrane based on one enzyme - gel with 10% GOx. Native catalase was added to the working buffer solution;
5) Single-layer membrane based on one enzyme - gel with 10% GOx, without adding other enzymes to the solution.



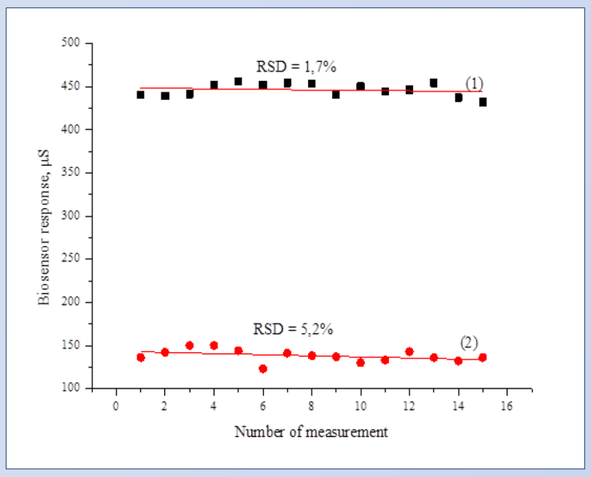
Dependence of the response of the bi-enzyme biosensor on the concentration of catalase in the bioselective membrane

Therefore, we decided to investigate the effect of the concentration of catalase in the bioselective membrane on the response of the biosensor. The responses were compared to concentration of 2.5 mM glucose in the measuring cell, since this concentration corresponds to the range of absolute saturation of the enzyme with the substrate

It can be concluded that the presence of catalase in the membrane expands the linear range of the biosensor, and the degree of expansion increases with the increase in the concentration of the auxiliary enzyme (CAT).

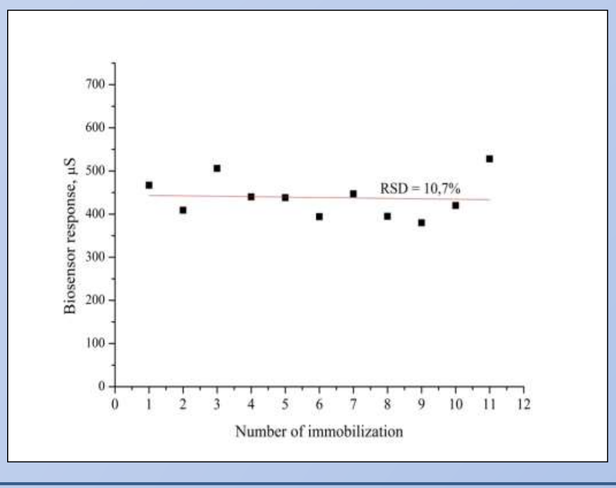


Concentration of catalase 1 - 0%, 2 - 1.33% and 3 - 5%



Reproducibility of signals

There was no significant drop in the response value for both biosensor variants, but the measurement error was different: the relative standard deviation (RSD) of the bi-enzyme biosensor was 1.7%, and that of the monoenzyme biosensor was 5.2%.



Reproducibility of immobilization

It can be seen that no significant difference was observed in the preparation of biosensors, and the relative standard deviation (RSD) for 11 batches of biosensor preparation was 10.7%.

Comparison of analytical characteristics of bi-and mono-enzyme biosensors

Characteristic	*Bi-enzyme biosensor*	*Mono-enzyme biosensor*
Sensibility	378 µS/mM	161 µS/mM
Dynamic operation range	0-1900 µM	0-900 µM
Linear operation range	0-1700 µM	0-800 µM
Limit of detection	8 µM	16 µM
Time of one response	3 min	2 min
Time of analysis	7 min	6 min
Relative standard deviation (RSD)	1,7%	5,2%

Conclusions

The work proposed a method of improving the analytical characteristics of a monoenzyme biosensor based on the GOx by coimmobilization with an additional enzyme (CAT) during the preparation of a bioselective element in order to increase the saturation of the membrane with oxygen for the best operation of the GOx. Namely, a new highly sensitive conductometric bi-enzymatic biosensor based on GOx and CAT was developed and optimized for the quantitative determination of glucose.

According to the research results, it was established that the optimized bi-enzyme biosensor can be successfully used for the quantitative determination of D-glucose, and confirmed that the technology of adding an additional enzyme to the biomembrane of the biosensor can be used for the purpose of improvement to its analytical characteristics.