ТЕЗИ
ABSTRACTS

REAGENTLESS ELECTROCHEMICAL BIOSENSORS FOR THE ASESSEMENT OF METABOLIC DISORDERS

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Introduction. In the current age of technology, the creation of heterogeneous enzymatic bioanalytic systems (biosensors) takes an important place, because they can be used for detecting of concern analytes in more efficient and cheaper way. Thus, it is an important resource for the development of modern diagnostic tools, especially for personalized medicine.

Amperometric biosensors offer a promising alternative for the conventional methods (e.g. potentiometric or spectrophotometric) because of their high sensitivity and selectivity, easier instrumentation, rapid (real-time) detection, low cost and ability to be used in turbid fluids. The key issue in developing successful reagentless amperometric biosensors is not only selecting the right enzyme, but also stabilizing it on an adequate electrode material suitable for effective bioelectrocatalytic action. In addressing this challenge, it is very useful that a biosensor recognition element containing an immobilized enzyme can be easily replaced when its sensitivity becomes insufficient. It should be highlighted that the proposed biosensors' designs are sufficiently adaptable when required to perform measurements under different model or actual biological media.

This study will discuss the most successful solutions of these challenges and perspectives in development of biosensors for the analysis of several metabolites associated with specific diseases.

The aim of the study. To discover the advantages and applicability of developed electrochemical biosensors in diagnostics.

Materials and Methods. Soluble pyrroloquinoline quinone-dependent glucose dehydrogenase (PQQ-GDH) from Acinetobacter calcoaceticus sp. L.M.D. 79.41 (E.C.1.1.5.2); urease from Canavalia ensiformis (E.C. 3.5.1.5.) (Sigma, USA); D-

fructose dehydrogenase from Gluconobacter industrius (EC 1.1.99.11) (FDH) (Sigma, USA); L-Glutamate Oxidase from Streptomyces sp., (Merck KGaA, Germany); Graphite oxidation products (RGO) have been prepared according to protocols [1,2]. Working electrode (biosensor) was designed by adsorbtion or mechanically attaching and fixing the membrane containing RGO and/or immobilized enzymes to the surface of the working electrode. To design enzymatic membranes 5 µl µL of mixture containing enzyme or enzyme-RGO mixture, bovine serum albumin in PBS and glutaraldehyde was deposited on the inner surface of the ring-fixed membrane and then left at 4°C overnight. Chronoamperometry measurements were performed using an electrochemical system (PARSTAT 2273, Princeton Applied Research, USA) with a conventional three-electrode system comprised of a platinum plate electrode as auxiliary, a saturated Ag/AgCl electrode as reference and working electrodes.

Results and Discussion. The basic scheme of biosensor construction is presented in Figure 1.

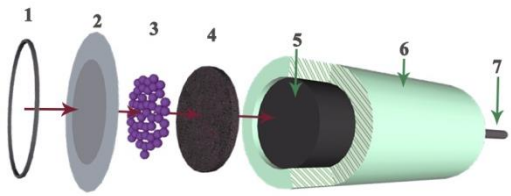

Fig. 1. Basic scheme of the amperometric biosensor: 1 - rubber ring, 2 - terylene film, 3 - layer of enzyme, 4 - electrode layer consisting of carbonaceous material, 5 - electrode contact zone, 6 - body, 7 - electrical wire.

Fig. 2. A wiev of the amperometric biosensor membrane containing rubber ring for fixing to the body of the sensor and terylene film for immobilization of enzymes.

The biosensor action is based on the registration of current generating during bioelectrocatalytic reactions of assesed metabolytes. Proposed biosensor design has the main advantage since it is easily adapted to perform different analyzes due to the possibility of replacing one enzyme in the membrane with another, or even immobilizing several enzymes in one membrane. In these membranes, we also used RGO that ensured the effective transport of electrons (ET) between the active site of the enzyme and the surface of the electrode without the use of ET mediating materials.

The main parametres of developed biosensors are presented in Table 1.

Table 1.

Biosensor characteristics and diagnostic areas

Recognition enzyme	Electrode material	Sensitivity	Detection range	Compliance with pathology
Glucose oxidase	Pt	20 nA mM^{-1} mm^{-2}	0.5-1.5 mM	Diabetes. Secondary stress indicator
Glucose dehydrogenase	Pt/RGO	62 μAcm^{-2}	Up to 1.2 mM	Pancreas disorders
Urease	G/RGO	2.3 ± 0.1 μA cm^{-2} mM^{-1}	0.2 to 12.0 mM	Urea cycle disorders. Kidneys and pancreas disorders
Glutamate oxidase	Pt Pt/RGO	15.7 ± 0.1 μA mM^{-1} cm^{-2}	0.0005 to 0.15 mM	Neurological disorders: Amyotrophic lateral sclerosis; Multiple sclerosis; Alzheimer's, Parkinson's, Huntington's diseases. Stroke. Fibromyalgia. Chronic fatigue syndrome. Insomnia (too litle); Mental exhaustion.
Fructose dehydrogenase	G/RGO Au	14.5 ± 0.5 μA mM^{-1} cm^{-2}	0.7 – 8.8 mM	Effects on multiple metabolic diseases: are associated with development of diabetes, fatty liver disease, cardiovascular disease, gout.
Esterases	RGO	ongoing	ongoing	Inflammatory processes; Hereditary angioedema;

Conclusions. Proposed biosensor design ir promising since it is easily adapted to perform different analyzes due to the possibility of replacing one enzyme in the membrane with another. The modification of the biosensor by RGO resulted to design the set of reagentless biosensors for investigations of metabolites related to various disease. More detailed studies with larger cohort, planning in the near future, will provide to extend a practical application for the investigations of diseases

Acknowledgement. The present study was funded by the Research Council of Lithuania (LMTLT) under the Lithuanian–Ukrainian Cooperation Programme in the Fields of Research and Technologies (Project contract No is S-LU-24-12).

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ENZYME ACTION ON DRUG METABOLISM

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Introduction. Quantitative systems pharmacology (QSP) is a rapidly developing transformative science applicable to drug discovery and development. In recent years, published examples describing the use of QSP to facilitate biomedical research have been increasing. Further, the pharmaceutical and biotechnology industries have rapidly adopted QSP to inform product development. Examples of QSP in regulatory decision making – such as defining postmarketing requirements and waiving certain clinical trials – are also appearing.

The aim of the study. To study the systems biology of enzymes that metabolize drugs.

Materials and Methods. Used scientific metric databases of publications in Scopus, Web of Science, Google Scholar.

Results and Discussion. Since their discovery, cytochrome P450 (CYP450) enzymes – heme proteins that catalyze numerous so-called phase I chemical reactions, such as hydroxylation, oxidation, and reduction – have been very often described as drug and toxicant metabolizing enzymes (phase I DMEs). The growth of information regarding CYP450-mediated drug metabolism, coupled with knowledge of phase II