SCIENTIFIC OPINION

by Prof. Maria Bogomilova Dyankova, PhD,

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on the dissertation for the award of the educational and scientific degree "**doctor**"
in the field of higher education 4. Natural Sciences, Mathematics and Informatics; professional field 4.3. Biological Sciences, doctoral program in Biochemistry
Doctoral candidate: **Angel Iliev Peshkov**
Dissertation title: **Investigation of the properties of oxidoreductases immobilized in biocompatible matrices**
Scientific supervisors: Prof. Ilia Nikolov Iliev, PhD, Department of Biochemistry and Microbiology, Faculty of Biology, Paisii Hilendarski University
Assoc. Prof. Nina Dimitrova Dimcheva, PhD, Department of Physical Chemistry, Faculty of Chemistry, Paisii Hilendarski University

1. General presentation of the procedure and the doctoral student

By order No. PD-22-1277 of 27.05.2025 of the Rector of Plovdiv University "Paisii Hilendarski" (PU), I have been appointed as a member of the scientific jury for ensuring a procedure for the defense of a dissertation on the topic "Investigation of the properties of oxidoreductases immobilized in biocompatible matrices" for the acquisition of the educational and scientific degree "doctor" in the field of higher education 4. Natural sciences, mathematics, and informatics, professional field 4.3. Biological sciences, doctoral program in Biochemistry. The author of the dissertation is Angel Iliev Peshkov - a doctoral student in full-time education at the Department of Biochemistry and Microbiology, with scientific supervisors Prof. Dr. Iliya Iliev and Assoc. Prof. Dr. Nina Dimcheva from PU.

The set of materials presented by Angel Peshkov on electronic media is in accordance with Article 36 (1) of the Regulations for the Development of Academic Staff.

2. Relevance of the topic

In recent years, biosensors have emerged as an important and rapidly developing area of study in ecology, healthcare, and biotechnology. They offer new, cost-effective solutions for diagnostics, personalized healthcare, food and water safety, and the detection of allergens, pollutants, and toxic substances. Electrochemical biosensors based on immobilized enzymes are among the most popular and successfully applied. Today, this field places higher demands on researchers and showcases new advancements in accuracy, efficiency, and specificity. Among the many modern platforms, biosensors that use immobilized oxidoreductase enzymes, such as catalase and laccase, provide fast, effective, online, and in situ detection of phenolic compounds. The dissertation presented by Angel Peshkov focuses on this area of knowledge and its application.

3. Knowledge of the problem

The dissertation is constructed in a traditional academic form with the appropriate sections. It is written on 137 standard computer pages and is illustrated with 28 figures, 14 tables, and 1 scheme. The literature review is based on 238 scientific publications, it is purposeful, covers a wide range of aspects related to the research problem, and reflects its modern level. In 30 pages, Peshkov clearly and accessibly introduces readers to the theory of the upcoming research, demonstrating his extensive knowledge of the subject.

Based on this in-depth analysis, the goal of this dissertation was derived: to study the application of the enzymes catalase and laccase, obtained from natural sources, in electrobiosensors for conducting heterogeneous catalysis in the presence of organic solvents. In my opinion, it corresponds to the relevance of the problem and emphasizes the innovative nature of the development. The goal is clear, well-formulated, and unites the directions of the experimental work. For its implementation, 5 specific, interrelated, and logically following tasks have been formulated, which include all the mandatory stages of such a study. Here, the serious volume of work set before the doctoral student is already visible.

4. Research methodology

The "Materials and Methods" section demonstrates a very wide range of methods, tailored to the specific requirements of the experiment, and fully meets the multidisciplinary nature of the dissertation. The methods are both routine and modern, biochemical, electrochemical, and statistical. The doctoral student has mastered the enzyme purification, demonstration of the enzyme activity, and the electrophoretic demonstration of molecular mass, scanning electron microscopy. Impressive are the methods for immobilizing enzymes on glass substrates, enzyme electrografting on a gold electrode, determination of polarization, differential pulse voltammogram, and steady-state concentration dependences, as well as the areas with kinetic or diffusion control by means of a rotating disk electrode. All of them are sufficient grounds for reliability and precision.

5. Characteristics and evaluation of the dissertation work and contributions

The section “Results and discussion” in the scientific work of Angel Peshkov outlines a serious scientific study, which is illustrated with 22 figures and 13 tables. The development is distinguished by its scale and clearly expressed logical sequence. The main part of the experimental work is related to the characterization of the kinetic parameters of the enzymes catalase and laccase, obtained from various natural substrates, as well as their immobilization in the absence and presence of low molecular weight aliphatic alcohols. The biochemical and electrochemical characteristics of the immobilized enzymes and the possibilities of preserving their activity depending on the concentration of ethanol and methanol have been established. The developed electrochemical method for determining the activity of immobilized catalase should be noted as a serious achievement. Based on the detailed experimental work, two types of electrochemical biosensors with immobilized laccase from Trametes versicolor and Trametes pubescens in polymer films were obtained, and their

electrochemical behavior in the presence of toxic pollutants was established by appropriate methods. As a result of the comprehensive study, the successful application of the new biosensors in determining phenolic content in herbal extracts was proven. In my opinion, the conclusions are a logical consequence of the experimental data and provide the necessary information about the value of the conducted research. They are reliable and correctly presented. I also accept the formulation of the contributions, and I want to emphasize their importance in theoretical and scientific-applied aspects.

6. Assessment of the publications and personal contribution of the doctoral student

Data from the dissertation are included in three scientific papers and nine presentations at scientific forums. The papers were published in journals with SJR and quartiles Q3 and Q4. The reports were presented at four international and five national forums. This leads me to believe that Angel Peshkov's results have become known to the national and international scientific communities. The doctoral student is the first author on two articles and seven of the presentations, proving his significant contribution to the dissertation.

7. Abstract

The abstract is properly structured in 36 pages and adequately reflects the essence and achieved results of the dissertation work.

8. Recommendations for future use of the dissertation contributions and results

In essence, the results achieved and the contributions formed in the dissertation work of Angel Peshkov enrich the methodology for creating electrochemical biosensors based on immobilized oxidoreductase enzymes. I evaluate them as theoretical support and prospects for further research in the field of biosensors.

Here I would like to emphasize that this is a dissertation of a high theoretical level with future scientific and applied significance, bearing the handwriting of leading scientists in the relevant field - Prof. Dr. Iliya Iliev and Assoc. Prof. Dr. Nina Dimcheva, which is a prerequisite for the relevance and significance of the research.

CONCLUSION

In conclusion, I would like to emphasize that the doctoral student has fulfilled the requirements of the Law on the Scientific and Technological Development of the Republic of Bulgaria, as well as those in the Regulations to it of the University "Paisii Hilendarski" for acquiring the educational and scientific degree "doctor". The material presented by Angel Peshkov is dissertable, the topic is topical, and it offers a modern level of an important issue for theory and practice. The experiments conducted are methodically set up correctly, and the results obtained are reliable and are a solid basis for further scientific and applied developments. A huge amount of experimental work has been carried out, the problem posed has been studied in many ways and detail at a modern level, and significant contributions with serious applied significance have been made. To this characteristic of the

dissertation work, I would like to add that, in my opinion, Angel Peshkov leaves the doctoral program as a well-prepared specialist in the field of biochemistry and electrochemistry, has mastered a large number of modern methods, and has gained experience in interpreting scientific data.

Due to the above, I confidently give my positive assessment of the conducted research, presented by the above-reviewed dissertation, abstract, achieved results and contributions, and I propose to the esteemed scientific jury to award the educational and scientific degree "doctor" to ANGEL ILIEV PESHKOV in the field of higher education: 4. Natural Sciences, Mathematics and Informatics; professional direction 4.3. Biological Sciences, doctoral program in Biochemistry.

August 03, 2025
Sofia

Signature:……..
/Prof. Maria Dyankova, DSc/