R E V I E W

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of PhD thesis for awarding the educational and scientific degree **"doctor**" in:
field of higher education: 4. Natural sciences, Mathematics, and Informatics
professional field: 4.3 Biological Sciences
doctoral program: Biochemistry

Author: *Angel Iliev Peshkov*

Topic: *Investigation of the properties of oxidoreductases immobilized in biocompatible matrices*

Research supervisors:

Prof. Iliya Nikolov Iliev, PhD, Department of Biochemistry and Microbiology, Faculty of Biology, Plovdiv University "Paisii Hilendarski"

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1. General description of the presented materials

According to order No. РД-22-1277/27.05.2025 of the Rector of Plovdiv University "Paisii Hilendarski" (PU), I have been appointed as a member of the scientific jury to ensure a procedure for the defense of a dissertation work 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 Biochemistry. The author of the PhD thesis is Angel Iliev Peshkov – a full-time PhD student at the Department of Biochemistry and Microbiology with scientific supervisors Prof. Iliya Nikolov Iliev and Assoc. Prof. Nina Dimitrova Dimcheva from PU.

The set of materials submitted by the PhD student is in accordance with Art. 36 (1) of the Rules for the Development of the Academic Staff of the PU, and includes the following documents:

– a request to the Rector of PU for disclosure of the procedure for the defense of a dissertation;
– a CV;
– a dissertation;
– an abstract;
– a list of scientific publications on the topic of the dissertation;

– copies of the scientific publications;
– a declaration of originality and authenticity of the attached documents;
– a certificate of compliance with the national minimum requirements for acquiring the degree "doctor".

The PhD student has attached 3 publications on the topic of the dissertation, which are accepted for review.

2. Brief biographical data about the PhD student

Angel Peshkov was born on October 11, 1990. In 2017, he graduated as a bachelor in Medicinal chemistry at the Faculty of Chemistry of PU. A year later, he also obtained a master's degree in Food Chemistry at the same university. He is enrolled in full-time doctoral studies at the Department of Biochemistry and Microbiology at the Faculty of Biology of PU. He is fluent in English.

3. Relevance of the topic and appropriateness of the set goals and tasks

The dissertation deals with current problems of practical interest, concerning the immobilization of enzymes and the development of novel biosensor systems. The work is devoted to studying the possibilities of using the enzymes catalase and laccase, obtained from various sources, in the construction of electrochemical biosensors for evaluation of catalytic reactions under conditions of heterogeneous biocatalysis and in the presence of organic solvents. The research is related to the development of novel accessible bioelectroanalytical devices for quantitative determination of the total phenolic content in plant extracts.

The compact design of electrochemical biosensors provides the opportunity to perform the analysis outside the laboratory. Therefore, in recent years the development of such systems has been particularly relevant in the analysis of foods and additives, since they provide express on-site analysis with minimal sample pretreatment and simple equipment. Thus, biosensors are preferred alternative to the traditional analytical techniques (most often spectrophotometric and chromatographic) applied in the specialized laboratories, which require specific, expensive equipment, as well as time-consuming and labor-intensive procedures for sample pre-processing.

4. Knowing the problem

The literature review (30 pages) is purposeful on the topic of the PhD thesis, analytically presented and logically directed towards the goal and tasks. The dissertation refers to a significant number of references (238), during the study of which Angel Peshkov has acquired the necessary theoretical knowledge in the field of research and good awareness of what has been achieved globally. The aim of the PhD thesis and the 5 research tasks are clearly and precisely formulated.

5. Research Methodology

The adopted methods and approaches in the experimental work are appropriate and suitable for solving the tasks set and achieving the aim of the thesis.

6. Characteristics and evaluation of the PhD thesis

The PhD thesis is written on 137 pages, contains 28 figures, 1 scheme and 14 tables. The dissertation is entirely experimental in nature, written in good scientific language. The PhD thesis contains the legally required structural elements: *Introduction* (2 pages); *Literature review* (30 pages); *Aim and tasks* (1 page); *Materials and methods* (16 pages), *Results and discussion* (39 pages); *Conclusions* (2 pages); *Contributions* (1 page); *References*.

The Literature review does not exceed the recommended 1/3 of the total volume and it is relevant to the target study. Analyzing the information from this chapter, the PhD student formulates the goal of the thesis and sets specific tasks for its achievement. This part shows the good theoretical knowledge of Angel Peshkov, as well as his ability to use a scientific research approach when planning the experiments.

The *Results and Discussion* part is organized in such a way as to correspond to the tasks set. The experimental studies were carried out consistently with the necessary repeatability and accuracy. The PhD student interprets the obtained results accurately; he has presented a significant part of the data in tables; the figures are of good quality, clear and aesthetically designed appropriately.

Results of studies on the activity of three catalases isolated from different sources in the presence of ethanol and methanol in the range from 1 to 10% alcohol are presented. The homogeneous and heterogeneous catalytic activity after immobilization of the enzyme on a water-insoluble polysaccharide (glucan type URE13-300) was studied. The data indicate that the immobilized catalase isolated from *Penicilium chrysogenum* has remarkable stability in an alcohol-containing environment.

These experiments are not an end in themselves, but they are followed by the development of an original electrochemical method for determining the enzymatic activity of immobilized catalase in the absence and presence of both alcohols.

The last part of the thesis is dedicated to the development of novel electrochemical biosensing platform by immobilizing the enzyme laccase in a Nafion polymer film. The electrochemical behavior of the biosensor in the presence of phenolic acids (gallic and caffeic) was studied, as the operating conditions being capably selected to ensure minimal interference when analyzing complex matrices.

A careful analysis of the experiments included in the thesis shows that the PhD student is familiar with various modern electrochemical techniques for studying and characterizing bioelectrochemical systems. Undoubtedly, becoming a member of the research groups of Prof. Iliev and Assoc. Prof. Dimcheva, has allowed Angel Peshkov to accumulate significant experimental experience and skills, which is crucial for his future professional life.

7. Contributions and significance of the development for science and practice

In my opinion the PhD student has carried out a significant experimental work, on the basis of which 5 original conclusions have been made. The main contributions of the PhD thesis can be formulated as follows:

1. An electrochemical method has been developed to monitor the catalytic activity of immobilized catalase from *Penicilium chrysogenum* and an equation has been derived for determining enzymatic activity based on electrochemical data.

2/ A methodology for immobilization of laccase enzyme from *Trametes versicolor* and *Trametes pubescens* has been developed and it has been applied in the creation of electrochemical biosensors for the analysis of di- and trihydroxy phenolic compounds. The biosensing method has been applied for the determination of the total phenolic content in natural products of plant origin (herbal extracts) and the obtained results were in good agreement with those found by HPLC.

The contributions are of a scientific and applied nature. The basis for this conclusion is the fact that the reported results reveal opportunities for the development of novel optimized bioelectrochemical systems for the determination of polyphenol content of food samples. The presented bioelectroanalytical method is promising and can be applied for rapid analysis that does not require expensive and complicated equipment.

8. Evaluation of publications on the PhD thesis

The main part of the results are included in 3 publications in journals, referenced and indexed in the global databases Scopus and Web of Science: *Applied Food Biotechnology* (IF=1.3, Q3), *Open Chemistry* (IF=1.9, Q3) and *Ecologia Balkanica* (IF=0.42, Q4). In two publications the PhD student is the first author, which undoubtedly proves his main contribution to the work.

The total points of publication activity (42 points) exceeds the required minimum of 30 points for acquiring the educational and scientific degree "doctor" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences.

Angel Peshkov has also participated in 9 scientific forums, where a significant part of the experimental results were reported. The PhD student's activities also include his participation in 5 research projects and the one-month specialization at the University of Malmö, Sweden.

9. Personal participation of the PhD student

I have no doubt in the significant contribution of Angel Peshkov in the implementation of the set goal and tasks, achieved under the expert guidance of his scientific supervisors.

10. Abstract

The presented abstract correctly reflects the main content and contributions of the dissertation work.

11. Critical remarks and recommendations

There are some omissions and inaccuracies in the dissertation. For example: there is no reference for patent No. 67404B1, commented on page 69; in the "*Materials and Methods*" section there is no description of how the three herbal extracts were prepared; the HPLC method, used as a reference, is not described; on page 68 in equation 3 the formula of primary alcohol is written incorrectly; on the Figure 13 the two graphs are swapped; for decimal numbers both a decimal point and a comma are used; inconsistent writing of units of measurement („мл", „mL" и "ml"), („µmole", „µmol" и „µM"); there are undifferentiated paragraphs.

These remarks in no way diminish the significance of the contributions and do not affect my overall very good impression of the PhD student's work.

I have the following questions to the PhD student:

1/ On page 85, Figure 14 presents the differential pulse voltammograms of a gold electrode with catalase, covalently immobilized by electrografting, in the presence of hydrogen peroxide at different concentrations. Could you explain the essence of the study (the role of the immobilized catalase; what is the principle of generating the current signal)?

2/ Do you think that the developed laccase biosensor has the potential to be used for determining polyphenol content in alcoholic beverages, e.g. wine?

12. Personal impressions

I do not know the PhD student and I have no personal impressions.

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

Overall, the topic being developed is fruitful and has very broad opportunities for development. I recommend that Angel Peshkov continue and expand on the knowledge and skills he has acquired.

CONCLUSION

The presented PhD thesis is at a modern scientific level, developed consistently, written comprehensively and logically completed. The dissertation work **contains scientific, scientifically applied and applied results that represent an original contribution to science and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB)**, the Regulations for the Implementation of LDASRB, and the relevant Regulations of Plovdiv University "Paisii Hilendarski".

The dissertation work shows that the PhD student Angel Peshkov **possesses** theoretical knowledge and professional skills in the scientific specialty of Biochemistry **demonstrating** qualities and skills for independent conduct of scientific research. Due to the above, I confidently give my **positive assessment** of the conducted research, presented by the above-

reviewed PhD thesis, abstract, achieved results and contributions, **and I propose to the honorable 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 field: 4.3. Biological Sciences, doctoral program Biochemistry.

August 12, 2025 Reviewer: .....
Plovdiv

/Assoc. Prof. Totka Dodevska, PhD/