REVIEW

by Assoc. Prof. Dr. Stefka Nikolova Kasarova

Burgas State University "Prof. Dr. Assen Zlatarov"

on a dissertation for acquiring Educational and Scientific Degree "Doctor"

in the Field of higher education 4. Natural Science, Mathematics and Informatics

Professional field 4.1. Physical Sciences

Doctoral programme "Electrical, magnetic and optical properties of condensed matter"

Author: *Aleksandar Vasilev Grigorov*

Topic: *Modified multilayer structures for immobilization of bioactive molecules*

Scientific supervisor: *Assoc. Prof. Asya Viraneva, PhD, Plovdiv University "Paisii Hilendarski"*

1. General presentation of the submitted materials

By Order No. RD-22-2563/16.12.2025 of the Rector of Plovdiv University (PU) "Paisii Hilendarski" I have been appointed as a member of the scientific jury to provide a procedure for the defense of a dissertation entitled "Modified multilayer structures for immobilization of bioactive molecules" for awarding the educational and scientific degree "Doctor" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.1. Physical Sciences and doctoral programme "Electrical, magnetic and optical properties of condensed matter". The author of the dissertation is Aleksandar Vasilev Grigorov - PhD student in full-time education at the Department of Physics with supervisor Assoc. Prof. Asya Viraneva, PhD from Plovdiv University "Paisii Hilendarski".

The set of materials presented by the PhD student is in accordance with Art. 36 (1) of the Regulations for Development of the Academic Staff of the University of Plovdiv and includes the following documents:

- Application form to the Rector for initiating the defense procedure;
- CV in European format;
- Protocol from the preliminary discussion;
- Opinion from the scientific supervisors;

- Dissertation;
- Abstract of dissertation in Bulgarian and English;
- Declaration of originality and authenticity of the submitted documents;
- Report on the compliance with the minimum national requirements;
- List of scientific publications and their copies;
- Document for the paid fee according to the PU tariff.

2. Brief biographical data of the PhD student

Aleksandar Grigorov graduated as a Bachelor in Physics at the University of Aberdeen in 2015. He acquired a Master`s degree in a Professional field 4.1 at the Plovdiv University "Paisii Hilendarski" in 2018. Since February 2019, he has been a full-time PhD student at the Faculty of Physics and Technology of PU "P. Hilendarski" in the doctoral programme "Electrical, magnetic and optical properties of condensed matter". In 2020, he was appointed as a researcher in the section "Biopolymers and New Materials", and since September 2021 he has held the academic position of assistant professor at the Department of Physics at the same faculty. Aleksandar Grigorov is a member of the team of 5 projects and has participated in a number of international conferences.

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

The main goal of the dissertation is to develop multilayered structures based on natural biodegradable polymers and investigate the possibilities for immobilization and controlled release of bioactive substances. Topic of the research is dedicated to priority areas in biotechnology, biomedical engineering and materials science. Nano-based drug delivery systems have a number of advantages such as improved targeting precision, the ability to overcome biological barriers in the body, biocompatibility and reduced toxicity. On the other hand, the immobilization of various bioactive molecules on modified polymer substrates allows for the development of specific structures with diverse functions, intended for a wide range of applications not only in medicine and pharmacy, but also in packaging products for the food industry, coatings, biosensors, etc.

Formulated research tasks and conducted experiments follow the set goal in a logical sequence. The study of the influence of various types of modification of the polymer films, used as substrates allows optimizing the technological parameters for obtaining polyelectrolyte multilayer structures with improved properties in terms of immobilization of biologically active molecules and the desired release kinetics.

4. Knowing the problem

Aleksandar Grigorov demonstrates deep understanding of the topic of the dissertation research. For the purposes of the study, the PhD student has conducted a comprehensive study of literature

sources covering various aspects of the topic under consideration. Polyelectrolyte multilayer structures, methods for their preparation and the main factors influencing their properties have been examined in detail. Attention has been paid to the possibilities of including various materials in them which determines their application in various fields including medicine. Methods for modifying polymer films used as substrates on which the multilayer structures are built, have been described in detail, with a focus on corona discharge treatment. The properties of the selected bioactive substances, the advantages of their immobilization, potential applications, and the influence of various internal and external factors have been examined.

The presented comprehensive literature review shows that the PhD student knows well the current information on this research, as well as the problems related to the topic of the dissertation. As a result of the analysis of the literare sources, the goal and tasks of the dissertation work are justified and formulated.

5. Research methodology

Based on the detailed literature review, suitable biocompatible polymers, polyelectrolytes and bioactive substances have been selected to achieve the set goal. Chemical modification and corona charge treatment have been used to modify polymer surfaces. According to the set tasks, the polymer films are investigated by means of Vibrating electrode with compensation method, differential scanning calorimetry, scanning electron microscopy and sessile drop method. Suitable methods has been chosen for determination of activity of the used enzyme and release of immobilized biologically active substances. Three mathematical models have been applied to describe and analyze the release mechanism, including the first order model, Korsmeyer-Peppas model, and Weibull model.

6. Characteristics and evaluation of the dissertation

The dissertation presented by the PhD student Aleksandar Grigorov is 165 pages long and includes 65 figures and 12 tables. The work is structured according to the requirements in the following main parts: Introduction (3 pages), Literature review (47 pages), Aim and tasks (2 page), Materials and methods (20 pages), Results and Discussion (62 pages), Conclusions (2 pages), Scientific and scientific-applied contributions (1 page), Literature (33 pages). A list of publications and participation in scientific forums and projects is also presented.

In accordance with the purpose of the study, 6 tasks are formulated. Considerable experimental work has been carefully planned and carried out and, as a result, a large number of biocompatible and biodegradable polymer films made from the poly-lactic acid, poly epsilon caprolactone or their composites has been obtained. In order to increase the free surface area and capture a larger amount of

substance, porous samples have also been developed by lyophilization or addition of polyethylene glycol.

To overcome some disadvantages of the used biomaterials and preparing for subsequent stage of the process, surfaces of polymer films have been modified by charging in a corona discharge combined in some cases with chemical treatment. Obtained polymer films have been characterized in terms of electret properties, morphology, degree of crystallinity and surface energy. The results of the conducted studies are summarized at the end of each section of the chapter "Results and Discussion".

Multilayered polyelectrolyte structures of chitosan and xanthan (casein) are deposited over different types of films by means of layer-by layer deposition method which advantages are described in the literature review. To achieve the goal of dissertation, the PhD student has investigated the possibilities for immobilization of the enzyme beta-galactosidase, benzydamine hydrochloride, tolfenamic acid and curcumin in the developed structures. Using the ONPG method, specific for beta-galactosidase, the amount of included enzyme and the degree of activity retention for several days was determined. A comparison was made between the obtained results depending on the of charge of the polymer films, their type (non-porous, porous), the method of modification, the ratio in the composites, as well as the number of layers. Additionally, the influence of pH and ionic strength of the initial solutions on the amount of curcumin included in the polyelectrolyte layers was studied. The conducted experiments and the selected mathematical models allow for a detailed description of the kinetics and a comparison between the main release mechanisms of the studied biologically active substances.

The obtained results are described in detail and the summarized data are presented in tables and figures. The discussion made by the PhD student shows good skills in interpreting scientific results and comparing them with those obtained by other authors.

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

The contributions of the dissertation are mainly of scientific and applied nature. They can be summarized as follows:

- **For the first time**, biodegradable polyelectrolyte multilayer polymer structures of chitosan, xanthan or casein are created on the surface of modified polymer films of polylactic acid and polyepsilon caprolactone for immobilization of biologically active substances.
- **New results** about the influence of various types of modification of polymer substrates (corona discharge treatment, chemical modification or combination of both) on the immobilization and release kinetics of biologically active substances in multilayer structures built on their surface were obtained.

- **For the first time**, influence of the porous structure, obtained by lyophilization or by adding polyethylene glycol to the corona-charged polymer films, on the immobilization and controlled release of various biologically active substances was established.
- Potential of the biodegradable electrets for creation of new systems for controlled release of biologically active substances was confirmed.

8. Evaluation of the publications related to the dissertation

Aleksandar Grigorov presents a list of 4 publications on the topics of dissertation which are referenced and indexed in world databases of scientific information Scopus and/or Web of Science. Two of the articles are in the journal *Coatings* with high impact factor and quartile Q1. There is one publication in the *Journal of Chemical Technology and Metallurgy* with Q3 and in *Bulgarian Chemical Communications* with Q4, respectively. So far, 1 independent citation has been noted. Considering that three of the publications are from the last two years, I believe that their potential for a wide response in the scientific literature is great.

With the submitted scientific publications, the PhD student fully meets the minimum national requirements and the requirements of the Regulations of the PU for the Academic Staff Development for acquiring educational and scientific degree "Doctor" and has 77 points on criterion D which significantly exceeds the required minimum of 30 points.

9. Personal contribution of the doctoral candidate

Submitted dissertation, abstract and publications included in the procedure testify to the main contribution of the PhD student to the conducted experimental work and analysis of the obtained results. Investigations are related to the entire scientific work of Aleksandar Grigorov. He was part of the research team for five projects: Personalized Innovative Medicine Competence Center (PERIMED), operational program "Science and education for smart growth", Digital Sustainable Ecosystems - Technological Solutions and Social Models for Ecosystem Sustainability and three funded by the Department of Scientific Research at the University of Plovdiv "Paisii Hilendarski". To popularize the results of his dissertation, Aleksandar Grigorov has participated in several international conferences.

10. Abstract

The Abstract contains 32 pages and includes 4 tables and 29 figures. It is structured according to the requirements reflecting the main results, summaries and conclusions of the dissertation research.

11. Critical remarks and recommendations

I have no principal critical remarks to the presented materials. There are few omission and inaccuracies I would like to point out.

• It is recommended to add some comments regarding the obtained results in Tables 2 and 3 for the changes in the enthalpy during melting and crystallization.

• It is better to note in the first column of Table 12 that the ratio of PLA/PEC is given (as in Tables 2 and 3).

• The expression “increase in the structure” on page 117, third sentence in the first paragraph should be clarified.

• Units of measurement in the text should be in Latin letters as in the tables.

The admitted inaccuracies are of technical nature and do not reduce the significance of the work performed and the results obtained.

I have the following question for the PhD student: On the base of the obtained results, can it be summarized in which cases it is more appropriate to use composite films in the development of multilayer structures for the purpose of immobilizing biologically active substances.

12. Personal impressions

I have no personal impressions of Aleksandar Grigorov but I have observations on his scientific work as a reviewer of one of the publications with his participation. I am also well acquainted with the research activities of the team from the Department of Physics at the University of Plovdiv which is distinguished by its innovation, thoroughness and precision.

13. Recommendations for future use of dissertation contributions and results

I would recommend the PhD student to continue, deepen and expand his research in this area including additional methods of analysis and tests with real samples.

CONCLUSION

The dissertation **contains** scientific and scientific-applied results which 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 (ZRASRB), the Regulations for the Implementation of ZRASRB and the relevant Regulations of PU "Paisii Hilendarski".

The dissertation shows that the doctoral student Aleksandar Vasilev Grigorov **possesses** theoretical knowledge and professional skills in the scientific specialty "Electrical, magnetic and optical

properties of condensed matter" by **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 a dissertation work, an abstract and the results described in them, as well as the formulated contributions, and I **propose to the honorable scientific jury to give an educational and scientific degree "Doctor"** to Aleksandar Vasilev Grigorov in the field of higher education: 4. Natural sciences, Mathematics and Informatics, professional field 4.1. Physical Sciences, Doctoral programme "Electrical, magnetic and optical properties of condensed matter".

February 12, 2026 **Reviewer:**: .....

Assoc. Prof. Dr. Stefka Kasarova