REVIEW

by Prof. Krastena Todorova Nikolova, PhD– Medical University "Prof. Dr. Paraskev Stoyanov" - Varna

on the dissertation for awarding the educational and scientific degree 'Doctor' in the field of higher education 4**. Natural Sciences, Mathematics, and Informatics, professional direction 4.1. Physical Sciences, doctoral program "Physics of Condensed Matter,"** authored by **Sofia Boyanova Milenkova** on the topic **"Biopolymeric Micro- and Nanoparticles as a Delivery System for Benzydamine"**

with scientific supervisors **Prof. Maria Marudova-Zhivanovich PhD and Assoc. Prof. Bisera Pilicheva PhD**

1. General Description of the Submitted Materials

By Order № RD-21-1447/12.07.2024 of the Rector of Plovdiv University "Paisii Hilendarski" (PU), I have been appointed as a member of the academic jury to oversee the defense procedure of the dissertation titled "Biopolymeric Micro- and Nanoparticles as a Delivery System for Benzydamine" for the acquisition of the educational and scientific degree 'Doctor' in the field of higher education 4. Natural Sciences, Mathematics, and Informatics, professional direction 4.1. Physical Sciences, in the doctoral program "Physics of Condensed Matter" within the Department of Physics at the Faculty of Physics and Technology. The dissertation was authored by Sofia Boyanova Milenkova, a full-time doctoral candidate at the Department of Physics, Faculty of Physics and Technology, with scientific supervisors Prof. Dr. Maria Georgieva Marudova-Zhivanovich from PU "Paisii Hilendarski" and Assoc. Prof. Dr. Bisera Asenova Pilicheva from MU-Plovdiv.

The set of materials submitted by Sofia Boyanova Milenkova in hard copy complies with point 36 (1) of the Regulations for the Development of the Academic Staff at PU and includes the following documents:

- Application to the Rector of PU for initiating the defense procedure;
- Curriculum Vitae in European format;
- Protocol from the preliminary discussion;
- Opinion from the scientific supervisors;
- Dissertation;
- Abstract in Bulgarian and English;
- Declaration of originality and authenticity of the submitted documents, contributions, and results on the dissertation topic;
- Report on the compliance with the minimum national requirements and the regulations of the Faculty of Physics and Technology at PU "Paisii Hilendarski" for obtaining the educational and scientific degree "Doctor";
- List of scientific publications on the dissertation topic;
- Copies of the scientific publications;
- Document for the paid fee according to the PU tariff.

The doctoral candidate has included 3 publications, 2 of which are in refereed international journals with quartiles Q_1 and Q_2, respectively, with an Impact Factor (IF) of 4.6 and 3.4, and one with an SJR in the Scopus database.

It is noteworthy that Sofia Milenkova has meticulously prepared the documentation for the procedure. All reports are signed and contain reliable information.

2. Brief Biographical Notes on the Doctoral Candidate

Sofia Boyanova Milenkova was born on December 26, 1995, in Plovdiv. She completed her Bachelor's degree in Engineering Physics at Plovdiv University "Paisii Hilendarski" in 2018, specializing as an Engineer Physicist. In 2019, she obtained a Master's degree in Physics of Condensed Matter.

Sofia Milenkova began her professional career in 2021, after completing her higher education, as a researcher in the Biopolymers and New Materials section at PU. Since 2024, she has been working as an Assistant Professor in the Department of Physics, where she teaches courses in General Physics and Polymers in Electronics and Optocommunications. I must mention her exceptional activity and demonstration of professional skills in connection with her involvement in international and national projects; she has participated in one international project and has completed two specializations abroad.

3. Relevance of the Topic and Appropriateness of the Set Goals and Objectives

The dissertation is dedicated to a significant multidisciplinary problem: the exploration of the possibility of incorporating biologically active substances into polymeric structures, which leads to substantial advancements in the fields of biotechnology, cosmetics, biomedicine, and pharmacy. Nanotechnologies are a current and rapidly developing field for the design of polymeric structures for the delivery of biologically active substances (BAS) through nasal, ocular, transdermal, and inhalation routes.

Doctoral candidate Sofia Milenkova aims to develop micro- and nanoscale structures based on biodegradable and biocompatible polymers, which will serve as drug delivery matrices for the controlled release of the pharmaceutical substance benzydamine hydrochloride.

The interest in the developed materials is based on their high biocompatibility, non-immunogenicity, and the fact that they can be degraded in vivo by various enzymes in the body.

The achievement of the set goal requires experimental work based on precisely defined and specific tasks, research methods, and activities.

4. Understanding of the Problem

A thorough analysis of the scientific literature has been conducted. A total of 162 sources, all in English, have been reviewed. The doctoral candidate provides a detailed examination of electrolytes and their complexes, as well as their applications in the development of drug delivery systems. She explores the fundamental mechanisms of drug release in delivery systems, particularly focusing on diffusion and erosion. Several methods for the production of nanoparticles are described and analyzed, including:

- Development of chitosan particles using the ionotropic gelation method or spray drying method.
- Investigation of methods for obtaining nanoparticles with casein, with detailed consideration of the conditions for creating polyelectrolyte complexes between casein and chitosan.

The reviewed literature is critically and creatively assessed, enabling the doctoral candidate to perform a comprehensive analysis of the theoretical frameworks.

5. Research Methodology

In terms of research methodology, the doctoral candidate has employed various techniques for producing micro- and nanoscale particles through ionotropic crosslinking and ionotropic gelation. Chitosan nanoparticles, in particular, were obtained using a microspray dryer.

The doctoral candidate skillfully applies several methods to study the morphology of the micro- and nanoparticles, including scanning electron microscopy (SEM), atomic force microscopy, differential scanning calorimetry (DSC), and in vitro release of benzydamine hydrochloride. She also uses different mathematical models to describe the drug release process, such as the Korsmeyer-Peppas model, the Higuchi model, and others.

6. Characteristics and Evaluation of the Dissertation

The dissertation is well-structured and appropriately organized to address the researched problem, demonstrating Sofia Milenkova's ability to systematize information and providing a clear vision of her research. The dissertation is presented in an introduction, eight chapters, including a literature review, objectives and tasks, materials and methods, results and discussion, conclusions, contributions from the experimental research, references, and appendices. The dissertation comprises 159 pages, featuring 46 figures and 13 tables. A total of 162 literary sources have been utilized, all in English, with 73 of them published within the last six years.

The introduction covers the relevance and importance of the researched problem in a detailed 48-page discussion. It thoroughly describes polyelectrolytes, their properties, and their application in drug delivery systems. The processes for obtaining micro- and nanoparticles based on chitosan or casein are examined in detail. The formation of polyelectrolyte complexes between casein and chitosan, as well as the properties of benzydamine hydrochloride, are also discussed.

The goal of the dissertation is clearly and accurately stated, with the tasks necessary to achieve this goal described in detail and in a logical sequence.

In Chapter 2, "Materials and Methods," spanning 13 pages, the main methodologies are thoroughly described. The methods for producing particles with casein and chitosan, as well as the formation of the casein-chitosan polyelectrolyte complex, are detailed. Atomic force microscopy, transmission electron microscopy, differential scanning calorimetry, and infrared spectroscopy were employed to characterize the morphology of the particles. Their sizes were determined using dynamic light scattering equipment. To establish the drug release profile from the polymeric structures, specific models were selected for each type of system, and an in vitro release test was conducted in an artificial saliva medium, chosen to simulate the conditions of the oral cavity.

In the 60-page chapter "**Results and Discussion**," the development, optimization, and characterization of polymeric micro- and nanoscale particles based on two natural hydrocolloids (chitosan and casein) are described. The structures were obtained using two different methods: ionotropic gelation and spray drying. Their potential application as a drug delivery platform for benzydamine hydrochloride with controlled release was established.

7. Contributions and Significance of the Research for Science and Practice

The scientific contributions can be summarized as follows:

Scientific and Applied Contributions:

- **For the first time**, casein particles loaded with benzydamine hydrochloride were developed using the ionotropic gelation method in the presence of alcohol and under acidic pH conditions.
- **For the first time**, casein nanoparticles loaded with benzydamine hydrochloride were produced and characterized using the spray drying method under acidic conditions in the presence of calcium ions. The influence of the polymer-to-drug ratio on the main physicochemical properties of the structures was established.

Confirmatory Contributions:

- The potential of chitosan nanoparticles, obtained via ionotropic gelation in the presence of sodium tripolyphosphate, to encapsulate and release benzydamine hydrochloride in a controlled manner has been confirmed.

These contributions underline the novelty and importance of the research, offering valuable insights and advancements for both scientific inquiry and practical applications in the fields of drug delivery and polymer science.

8. Evaluation of the Publications Related to the Dissertation

Three publications have been presented, two of which are in international journals with impact factors (IF) and are indexed in Scopus/Web of Science:

Materials (Scopus/Web of Science, Q2, IF=3.4);

Gels (Scopus/Web of Science, Q1, IF=4.6);

The third publication is in the Journal of Physics: Conference Series (Scopus, SJR=0.18).

All three publications are co-authored, with the doctoral candidate being the first author in two of them. These publications are closely related to the topic of the dissertation research.

The quantity and quality of the publications fully meet the minimum criteria and are in accordance with the requirements of Plovdiv University "Paisii Hilendarski" and the Faculty of Physics and Technology for obtaining the educational and scientific degree of Doctor.

9. Personal Contribution of the Doctoral Candidate

The dissertation and its contributions are the result of Sofia Milenkova's work, and therefore, they are considered her personal achievement. She participated in two international mobilities under the PERIMED project and a short-term doctoral mobility under Erasmus at the Faculty of Pharmacy of the University of Szeged, Hungary. She was also part of the research team for two projects: one funded by the Science Fund, Ministry of Education and Science – Young Scientist, and the second, an international project at the Competence Center "Personalized Innovative Medicine" (PERIMED) under the Operational Program "Science and Education for Smart Growth."

The dissertation is written in a scientifically rigorous style and reveals the doctoral candidate's excellent theoretical and practical preparation. The candidate’s linguistic proficiency, as well as the depth, organization, and analytical nature of the presented results, are commendable.

10. Abstract

The abstract of the dissertation is formatted according to the requirements and provides a concise overview of the research program and theoretical foundations of the dissertation.

11. Critical Remarks and Recommendations

I have no remarks regarding the content of the presented dissertation. Given the relevance of the topic and the extensive experimental material, I recommend that future work should include in vivo studies and consider the development of the research into a comprehensive book.

Evaluation Summary

The dissertation encompasses scientific, applied, and practical results that represent an original contribution to the field and meet the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulation for the Implementation of LDASRB, and the relevant regulations of Plovdiv University "Paisii Hilendarski". The presented materials and dissertation

results fully comply with the specific requirements of the Faculty of Physics and Technology, as established in accordance with Plovdiv University's regulations for the application of LDASRB.

The dissertation demonstrates that the doctoral candidate, Sofia Milenkova, possesses profound theoretical knowledge and professional skills in the scientific field of Condensed Matter Physics, and shows competence in conducting independent scientific research.

Given the above, I am confident in providing a positive assessment of the research conducted, as presented in the reviewed dissertation, abstract, achieved results, and contributions. I recommend that the esteemed scientific jury award Sofia Boyanova Milenkova the educational and scientific degree of ‘Doctor’ in the field of higher education: 4. Natural Sciences, Mathematics, and Informatics, 4.1 Physical Sciences, doctoral program "Physics of Condensed Matter".

August 28, 2024

Reviewer:
(Prof. Krystena Nikolova, PhD)