OPINION

by Assoc. Prof. Dr. Stefka Nikolova Kasarova

University "Prof. Dr. Assen Zlatarov" – Burgas

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

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

Professional field 4.1. Physical Sciences

Doctoral programme "Condensed Matter Physics"

Author: *Sofia Boyanova Milenkova*

Topic: *Biopolymeric Micro- and Nanoparticles as a Delivery System for Benzydamine*

Scientific supervisors: *Prof. Maria Marudova-Zsivanovits, PhD, Plovdiv University "Paisii Hilendarski", and Assoc. Prof. Bisera Pilicheva, PhD, Medical University - Plovdiv*

1. General presentation of the procedure and the PhD student

By order № РД-21-1447 /12.07.2024 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 "Biopolymeric Micro- and Nanoparticles as a Delivery System for Benzydamine" 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 "Condensed Matter Physics". The author of the dissertation is Sofia Boyanova Milenkova - PhD student in full-time education at the Department of Physics at PU with supervisors Prof. Maria Marudova-Zsivanovits, PhD from Plovdiv University "Paisii Hilendarski" and Assoc. Prof. Bisera Pilicheva, PhD from Medical University of Plovdiv.

The set of materials presented by PhD student Sofia Milenkova in electronic form is in accordance with Article 36 (1) of the Regulations for Development of the Academic Staff of the University of Plovdiv and includes all the necessary documents.

Sofia Milenkova has submitted a dissertation, abstract and 3 publications in scientific journals indexed in the global academic research databases (Web of Science, Scopus). From the presented reference, it can be seen that the candidate for acquiring the doctoral degree fully meets the minimum requirements in professional field 4.1 Physical Sciences according to the Regulations on the implementation of the Act on the Development of the Academic Staff in the Republic of Bulgaria and the relevant Regulation of the PU.

In 2018, Sofia Milenkova acquired a bachelor's degree in Engineering Physics at the PU "P. Hilendarski", where one year later he graduated with a Master`s degree in a specialty Condensed Matter Physics. In 2021, she started working as a researcher at PU, and from February 2024, she is an

assistant professor at the Department of Physics. Sofia Milenkova has been enrolled as a doctoral student since 26.01.2021 (order № РД-33-270), and from February 2024 she completed her studies with the right to defend a dissertation.

2. Relevance of the topic

In recent years, rapidly developing and priority trend in the field of pharmaceutical technology is the preparation of innovative systems for targeted drug delivery which enhance therapeutic efficacy and minimise the side effects. Bio-based polymeric materials play an essential role in such systems due to their ability to modulate the release profile and increase the circulation time of the active ingredient.

The dissertation, presented by Sofia Milenkova, examines the preparation and characterization of micro- and nanoparticles based on bio-polyelectrolytes and the establishment of their ability to be applied as drug delivery systems with improved encapsulation efficiency and increased yield. Different methods have been applied to obtain and modify polymeric systems based on chitosan, casein and their complexes intended for controlled-release topical oral administration of benzydamine hydrochloride.

3. Knowing the problem

In the first two sections of the literature review, the features and types of physical cross-linking, the stages of formation of polyelectrolyte complexes, as well as the main factors influencing complex formation are discussed. The advantages of micro- and nanoscale polymeric structures as drug delivery systems are presented, with special attention paid to the mechanisms and release profiles of biologically active substances. In the next three sections, the properties and structure of two types of bio-based polymers - chitosan and casein, as well as their complexes, are discussed. A number of studies on micro- and nanoparticles prepared by ionotropic gelation and spray drying, the influence of various factors on the properties of the polyelectrolyte complexes and their potential application as drug delivery systems are presented in detail. In the last section of the literature review, the therapeutic effect of benzydamine hydrochloride is briefly described. The main challenges in its inclusion in polymer structures are indicated, which motivated the PhD student to develop the present dissertation work.

The literature sources used are 162, mostly (about 80%) from the last 10 years, considering recent research related to the topic of the dissertation work. Formulated research tasks are a logical consequence of the analysis, show deep knowledge of the problem and directions for upgrading and search for innovative solutions.

4. Research methodology

Based on the detailed literature review, ionotropic gelation and spray drying were chosen as methods to obtain benzydamine hydrochloride-loaded micro- and nanoparticles from chitosan, casein and their complexes. The polymer structures are characterized by a number of modern research

methods such as dynamic light scattering, scanning electron and atomic force microscopy, differential scanning calorimetry, spectroscopy, including FT-IR. An in vitro drug release test is carefully planned and conducted in an artificial saliva medium mimicking the conditions in the oral cavity. Four mathematical models have been used to describe and analyze the release mechanism.

5. Characterization and evaluation of the dissertation and contributions

The dissertation presented by Sofia Milenkova is 159 pages long and includes 46 figures and 13 tables. The work is structured according to the requirements in the following main parts: Introduction (2 pages), Literature review (48 pages), Aim and tasks (1 page), Materials and methods (12 pages), Results and Discussion (59 pages), Conclusions (2 pages), Scientific and scientific-applied contributions (1 page), Literature (15 pages). A list of publications and participation in scientific forums, mobility activities and projects is also presented.

In accordance with the purpose of the study, 6 tasks are formulated. A large number of samples have been prepared by varying the polymer concentrations, contents of crosslinker and/or excipient, polymer/drug ratio, etc. The successful incorporation of benzydamine hydrochloride has been demonstrated by the presence of characteristic peaks in the infrared spectra. The developed micro- and nanostructures have been characterized in terms of size, morphology, yield and incorporation efficiency, phase state and thermal stability of the active substance. The mechanisms and release profiles of benzydamine hydrochloride have been analyzed based on the models used. 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.

PhD student Sofia Milenkova formulated 5 contributions that can be characterized as scientific and scientific-applied, and which I accept.

I have no general remarks to the presented materials. I would like to point out, though, that there is a discrepancy between the equation on page 64 (without numbering) and its application in the following system for calculating the necessary amounts of chitosan and casein solutions for the preparation of polyelectrolyte complexes. It should also indicate in the text what is denoted by x and y. The admitted technical inaccuracy does not reduce the importance of the work.

6. Evaluation of the publications and the personal contribution of the PhD student

Sofia Milenkova presents a list of 3 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 journals with impact factor (IF): *Materials* with IF=3.4 and quartile Q2, and *Gels* with IF = 4.6 and Q1. The third publication is in the journal without IF but with SJR (*Journal of Physics: Conference Series*, SJR = 0.18). The personal contribution of Sofia Milenkova is illustrated by the fact that the PhD student is the first author in two of the publications and the second author in a third.

Results of the conducted research have been reported at 5 international scientific forums. 3 citations to one of the articles (published in February 2022) have been noticed which shows the relevance of the topic and quality of the dissertation work. Two other publications, published in 2023 and 2024, have a high potential for widespread impact in the scientific literature. Publishing activity of Sofia Milenkova meets the requirements for acquiring educational and scientific degree "Doctor" - the minimum national requirements and the minimum requirements of the Regulations of the PU for the Academic Staff Development are overfulfilled since the PhD student has 55 points out of the required 30 according to this criterion.

7. Abstract

The printed Abstract has a volume of 32 pages and is structured according to the requirements reflecting the main results, summaries and conclusions of the dissertation research.

8. Recommendations for future use of dissertation contributions and results

I would recommend the PhD student to continue research in this area with other polymeric material and biologically active substances as well as to expand the scope of the methods used to characterize drug delivery systems.

CONCLUSION

The dissertation contains scientific, scientific-applied and 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 Sofia Boyanova Milenkova possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Condensed Matter Physics" 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 "PhD" to Sofia Boyanova Milenkova in the field of higher education: 4. Natural sciences, Mathematics and Informatics, professional field 4.1. Physical Sciences, Doctoral programme “Condensed Matter Physics”.

03.09.2024 г. **Prepared the opinion**: .....

Assoc. Prof. Dr. Stefka Kasarova