# OPINION

## by Prof. Plamen Zagorchev, PhD, DSc
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## Member of the Scientific Jury according to Order RD-21-1447/12.07.2024 of the Rector of Plovdiv University "Paisii Hilendarski"

**Subject:** Procedure for defence of the educational and scientific degree "Doctor" by field of higher education: 4. Natural sciences, mathematics and informatics, professional field 4.1. Physical sciences, doctoral program "Condensed Matter Physics" of the doctoral student **Sofia Boyanova Milenkova** entitled "Biopolymeric Micro- and Nanoparticles as a Delivery System for Benzydamine"

**Scientific supervisors:**

Prof. Maria Marudova-Zsivanovits, PhD and Assoc. Prof. Bisera Pilicheva, PhD

**General presentation of the doctoral student**

Sofia Milenkova obtained a master's degree in engineering physics at Plovdiv University "Paisii Hilendarski" in 2019. In 2021 she was enrolled as a full-time PhD student in the professional field 4.1 Physical Sciences in the PhD program "Physics of Condensed Matter" at the Department of Physics of the Faculty of Physics and Technology. Since April 2021 she has been appointed as a researcher at Plovdiv University. Since February 2024 she holds the position of Assistant Professor in the Department of Physics of the Faculty of Physics and Technology, where she participates in the training of students in the disciplines "General Physics" and "Polymers in Electronics and Telecommunications". Sofia Milenkova has participated in long-term research mobilities in Hungary, Turkey and Lithuania, as well as in 5 national and international scientific forums, projects and programmes. She is fluent in written and spoken English and German.

**Topicality of the problem**

The dissertation examines the application of biopolymers in pharmaceutics and their role in the construction of micro- and nanoscale drug delivery systems. Due to multifunctionality and properties

such as biocompatibility, biodegradability and lack of toxicity, biopolymers are the basis of innovative strategies.

Investigating the mutual influence of process parameters (composition, method, manufacturing conditions, etc.) enables the application of optimal approaches for solving technological problems related to obtaining structures with desired characteristics.

**General presentation and structure of the dissertation**

The dissertation is structured according to established standards. It contains 159 pages and is illustrated with 46 figures and 13 tables. The bibliographic reference includes 162 literary sources, all by foreign authors.

The **introduction** well justifies the relevance of the problem. The literature review presented to the dissertation is done in detail and gives an insight into the doctoral student's knowledge of the problem as well as her ability to interpret data published in the literature. Different aspects are discussed: the mechanisms of polyelectrolyte complexation are described in detail, polyelectrolyte structures and their complexes as drug-delivery systems are discussed, and an analysis of the factors influencing the properties of the resulting structures is made. The main mechanisms of drug release from their containing carriers are described. A detailed analysis of the scientific literature regarding the biopolymers used in this thesis, chitosan and casein, has been performed. Techniques for the preparation of micro- and nanoparticles based on these biopolymers are discussed in detail, highlighting the challenges accompanying the production processes. A separate section is devoted to the complexation between the two polymers.

The **aim** of the work is clearly stated: to develop models of a drug delivery system for controlled release of the active substance benzydamine hydrochloride, the matrix of which is based on the biopolymers chitosan and casein.

To realize this goal, six **tasks** were formulated. To fulfil the set tasks, modern scientific and research **methods** of analysis were used, which are a prerequisite for the reliability of the obtained results.

The **results** were followed in three main aspects: formation of chitosan particles, respectively from casein, alone, and preparation of polyelectrolyte complex from chitosan and casein in different ratios. By ionotropic gelation, 5 types of chitosan particles loaded with benzydamine hydrochloride were developed by varying the polymer concentration and the polymer: crosslinking agent ratio. The

resulting structures were characterized in terms of yield, size, drug loading and incorporation efficiency. The surface morphology was investigated by atomic force microscopy. The physical crosslinking and successful incorporation of the drug into the polymer matrix were confirmed by infrared spectroscopy, and the thermal stability of the active agent after incorporation into chitosan particles was demonstrated by differential scanning calorimetry. Studies on the biopharmaceutical behaviour of the particles were performed, demonstrating a rapid initial release of the active substance probably due to its preferential deposition in the peripheral part of the systems. A similar experimental approach has been applied to the development of structures based on the polymer casein. Twelve models of casein particles were proposed based on solutions with different polymer concentrations, different crosslinker ratios and the presence or absence of ethyl alcohol during crosslinking in solution. They were characterized in terms of size, yield and incorporation efficiency of benzydamine hydrochloride, surface morphology and phase state. The technique of ionotropic gelation has also been approbated for the preparation of polyelectrolyte complexes based on the two biopolymers, which under weakly acidic pH conditions are oppositely charged and interact with each other, forming structures without the application of a crosslinking agent. Again, a detailed analysis of the variables and their influence on the properties of the resulting complexes was carried out. Well-founded conclusions are drawn.

In the PhD thesis the influence of the technological process has been investigated by using the spray drying method to obtain the three types of systems - chitosan based, respectively casein based alone, and in combination. Nine models of chitosan particles were developed, whose sizes fall in the micro range due to the properties of the polymer and the specific features of the technique used. The developed models were thoroughly characterized analogously to the structures obtained by ionotropic gelation. A significant contribution is made by the comparative analysis of chitosan particles formed by different techniques under identical other working conditions. A dramatic difference in size (in some cases more than 10 times) was found, which has a profound impact on the biopharmaceutical behaviour of the systems. Similarly, a model of casein nanoparticles was obtained by nano-spray drying, which, in contrast to the same obtained by ionotropic gelation, are characterized by a rapid release of the incorporated active substance and are not a suitable system to achieve a prolonged therapeutic effect.

Based on the results obtained, the PhD student has drawn seven **conclusions**, which are comprehensive, justified and follow logically the aim and the formulated tasks.

The **contributions** of the dissertation are properly formulated and are mainly of scientific and applied nature. I believe that the logical coherence, the content, the synchrony between theoretical propositions and experimental data, the analysis made, and the conclusions and contributions drawn are entirely the work of the author.

**Evaluation of the publications on the dissertation**

The doctoral student has presented 3 scientific publications and 5 participations in scientific forums related to the dissertation. All publications are in scientific journals refereed in the Scopus world database. Two of the publications are in journals that fall in the first and second quartile (Q1 and Q2) of journals in the field with an overall impact factor of 8. In two of the publications Sofia Milenkova is the first author, which testifies that the work presented by the PhD student is her personal merit.

The abstract is prepared according to the requirements, with high quality of the illustrative material and sufficient length, and adequately reflects the main results of the dissertation.

**Conclusion**

The dissertation represents a complete scientific work, contains scientific and applied results with original contribution to science and meets the requirements of the Law on the Research and Development of the Bulgarian Academy of Sciences and the Regulations for its implementation. The research is very well structured, with a clear concept correlated to the dissertation topic. The research instruments used fully meet the aim and objectives. The dissertation demonstrates that the doctoral candidate has a thorough knowledge, has mastered a variety of methods and techniques, and demonstrates the ability to conduct independent research.

In view of the above, I confidently give my **positive assessment** of the dissertation, the abstract, the results and the original contributions and **I propose** to the Scientific Jury to award **Sofia Milenkova** the educational and scientific degree “doctor” in the scientific specialty "Physics of Condensed Matter".

04.09.2024 Prof. Plamen Zagorchev, PhD, DSc

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