REVIEW

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About a dissertation work for awarding the educational and scientific degree "Doctor", field of higher education: 4. Natural sciences, mathematics and informatics, professional field 4.1. Physical sciences, doctoral program "Condensed Matter Physics".

Author of the dissertation work: Sofia Boyanova Milenkova

Form of the doctoral program: full-time form

Department: Department of Physics, Faculty of Physics and Technology,
"Paisii Hilendarski" University of Plovdiv

***Dissertation thesis*:** Biopolymeric micro- and nanoparticles as a delivery system for benzydamine

***Scientific supervisors*:**

Prof. Maria Marudova-Zsivanovits, PhD,
"Paisii Hilendarski" University of Plovdiv

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This report is prepared in response to Order № № РД-21-1447/12.07.2024 issued by the Rector of "Paisii Hilendarski" University of Plovdiv and according to the report from the first meeting of the Scientific Jury from 15.07.2024.

General introduction of the procedure and the doctoral student

The presented set of documents and materials fully meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the

Regulations for its Implementation and it is in accordance with Acquisition of Doctorate and Doctor of Science degrees at “Paisii Hilendarski” University of Plovdiv. It includes:

- Request to the Rector to initiate the procedure;
- CV in European format;
- Protocol of the preliminary discussion;
- Opinion of the supervisors;
- Dissertation summary in English and Bulgarian;
- Declaration of originality and reliability;
- Reference for compliance with minimum national requirements;
- List of publications and their copies;
- Dissertation.

Sofia Boyanova Milenkova obtained a Bachelor of Engineering Physics degree in 2018 from Paisii Hilendarski University in Plovdiv. She subsequently obtained a Master's degree in Condensed Matter Physics in 2019. Since 2021, she has been a PhD student at the Faculty of Physics and Technology, “Paisii Hilendarski” University of Plovdiv in doctoral program in Condensed Matter Physics. From 2021 to 2023, she conducted research in the field of biopolymers and new materials. In 2024, she has become an assistant professor at the Department of Physics at the same university and she actively participates in the training of students in the disciplines of General physics and Polymers in electronics and telecommunications. The doctoral student has been involved in several mobilities and specialisations in Hungary, Turkey and Lithuania, and is proficient in English and German language. To date, Sofia Milenkova has contributed to 15 scientific publications resulting from her research activities, in the majority of which she is the primary author. Three of these publications are related to her thesis and are included in the provided materials.

Significance of the dissertation to science and practice

The objective of the dissertation is to develop micro- and nano-sized structures based on natural polymers (chitosan and casein) as drug delivery systems with

controlled drug release. Research in the field of drug nano- and micro-technologies is of great importance and relevance from both a scientific and a practical standpoint, particularly in the context of medicine and pharmacy. The development of new technologies and materials is enabling the formulation of more complex and effective drug delivery systems. Modern medicine is oriented to a personalised approach, whereby treatment is adapted to the individual characteristics of the patient. The utilisation of nano- and micro-drug systems enables the dosage and delivery of drugs to be tailored to the specific requirements of the patient. Nanoparticles and microparticles can deliver drugs at a specific rate directly to target tissues or cells, thereby reducing side effects and increasing therapeutic efficacy. This allows for more precise and controlled therapy. The development of such drug delivery systems represents a significant challenge, necessitating detailed characterisation and understanding of their potential interaction with biological structures. To achieve this, knowledge and methods from diverse scientific fields, including physics, chemistry and pharmaceutics, must be integrated.

Understanding of the topic

The doctoral student demonstrates a comprehensive understanding of the current topic and employs a critical approach to evaluate the existing literature. This is demonstrated by the comprehensive and detailed literature review, as well as the rigorous examination of existing drug delivery systems based on polyelectrolyte structures. Logically, an emphasis was placed on the two natural polymers chitosan and casein, and on their potential complexes, which are the subject of the subsequent studies. A comprehensive examination of the structure and characteristics of the selected carriers, coupled with an elucidation of the challenges and potential methodologies for the engineering of micro- and nano-sized drug-delivery systems, enables the objective of this thesis to be properly defined, as well as the tasks to be undertaken in pursuit of the stated goal. The thesis is written in a clear and concise scientific style and draws upon the latest literature sources.

Methodology of the dissertation

The doctoral student has selected appropriate contemporary methodologies that facilitate the attainment of the defined aim and the acquisition of sufficient data to fulfil the tasks of the dissertation. The study comprises a series of logical steps, including the preparation and physicochemical characterisation of micro- and nanosystems of chitosan, casein and the two polymers in combination, linked in a polyelectrolyte complex. Two of the most efficacious methods for designing this type of structure have been employed: an ionotropic gelation technique utilising a crosslinking agent sodium tripolyphosphate, and spray drying employing nano- and mini-spray dryers. The resulting formulations, which were loaded with benzidamine hydrochloride, were analysed with respect to their yield, size, size distribution, morphology, drug loading and *in vitro* release. A variety of precision analytical techniques were employed, including scanning electron microscopy, atomic force microscopy, infrared spectroscopy, dynamic light scattering and differential scanning calorimetry. Different kinetic models were exploited and properly applied to determine the basic mechanisms by which the drug is released from the designed drug carriers, including the first order model, Higuchi model, Korsmeyer-Peppas model, and Weibull model. This is important for optimizing the design of drug delivery systems and for ensuring efficacy and safety after their administration.

Characteristics and evaluation of the dissertation

The dissertation presented meets all the requirements for acquiring a Doctor's degree. It contains all the main sections: Introduction – 1 page; Literature review – 47 pages; Aim and tasks – 1 page; Materials and methods – 12 pages; Results and discussion – 58 pages; Conclusions – 2 pages; Contributions – 1 page; References – 15 pages and Supplementary materials – 2 pages. The dissertation is presented on 159 pages, illustrated with 46 figures, and 13 tables.

The ***literature review*** shows excellent knowledge of literature, a good theoretical base of the doctoral student on the problems of the dissertation, and the ability to skillfully analyze scientific information.

The ***aim*** of the thesis is a logical consequence of the content of the work and corresponds fully to the objective of developing micro- and nano-sized structures based on biodegradable and biocompatible polymers to serve as a drug-delivery matrix for the controlled release of the drug substance benzidamine hydrochloride. In order to achieve this, 6 specific and well-defined tasks have been outlined.

The ***materials and methods*** employed are described in detail and correctly, thereby ensuring the attainment of accurate and reproducible results.

The ***results*** are illustrated with 36 figures and 12 tables and represent original data. The extensive range of drug systems developed by the PhD student is noteworthy, resulting from the utilisation of diverse carriers (chitosan, casein, polyelectrolyte complex of chitosan and casein), the integration of two particle design techniques (ionotropic gelling and spray drying), and the modulation of production parameters (polymer concentration, chitosan:casein ratio, incorporation of crosslinking agent or excipient, etc.). A comprehensive examination of the factors influencing the key characteristics of the nano- and micro-particles (yield, size, shape, morphology, drug loading) enables the role of the composing polymer and the production method to be evaluated, thus facilitating the derivation of optimal conditions for the establishment of a promising drug-delivery platform for controlled-release of benzidamine hydrochloride. The results presented are discussed in a competent and thorough manner, with adequate conclusions, and represent a novel contribution to the scientific literature.

The PhD student presents 7 ***conclusions***, which are comprehensive and based on the results obtained.

The ***contributions*** of the dissertation are properly formulated and are mainly of a scientific and applied nature. The original contributions include the development of benzidamine hydrochloride-loaded casein particles by the ionotropic gelation method

and by spray drying under acidic conditions in the presence of calcium ions. This is the first study to evaluate the influence of the stoichiometric ratio of a casein-chitosan-based polyelectrolyte complex on the incorporation and release of benzidamine hydrochloride, as well as the impact of the polymer:drug ratio on the primary physicochemical characteristics of casein nano-structures obtained by spray drying.

Assessment of the publications related to the dissertation work

With regard to the dissertation, Sofia Milenkova has published three scientific articles in Scopus-indexed journals and has presented her work at five scientific conferences. She is the primary author of two of the articles, which have been published in journals with a high impact factor. These are, respectively, Materials (ISSN: 1996-1944) with an impact factor of 3.4 (Q2) and Gels (ISSN: 2310-2861) with an impact factor of 5.0 (Q1). The results of the presented work were also achieved through the contribution of Sofia Milenkova in 2 research/infrastructure projects, as well as 2 mobilities abroad.

The personal involvement of the doctoral student is clearly evident from the dissertation, the dissertation summary and the publications presented.

Dissertation summary

The dissertation summary presented consists of 32 pages and includes 7 tables and 29 figures. The summary provides a concise overview of the dissertation's findings, objectives, performance, conclusions, and contributions. It adheres to the specifications outlined in the relevant regulatory documents and is consistent with the dissertation that was submitted for review.

Recommendations and remarks

I have no critical comments or recommendations on the study and the provided set of materials.

CONCLUSION

The presented dissertation by Sofia Boyanova Milenkova entitled „Biopolymeric micro- and nanoparticles as a delivery system for benzydamine“ meets all the requirements of the Law on Academic Staff Development in the Republic of Bulgaria, the Regulations on Implementation of this Law, and the Regulations of “Paisii Hilendarski” University of Plovdiv.

The presented dissertation is a professionally and competently written interdisciplinary study that contains scientific and applied results. It proves that Sofia Milenkova has comprehensive theoretical knowledge and demonstrates qualification and skills for independent conduction of scientific research.

Considering the above stated, I confidently give my positive assessment for the conducted research, presented by the above described dissertation, summary, achieved results and contributions, and I give my recommendation to the honourable Scientific Committee to award educational and scientific degree "Doctor" to Sofia Boyanova Milenkova, in doctoral program “Condensed Matter Physics", field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.1. Physical sciences.

03.09.2024 Reviewer:

(Assoc. Prof. Plamen Katsarov, PhD)