

PhD DISSERTATION REVIEW

from Associate Professor Yordanka Ivanova Uzunova, PhD
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on a dissertation submitted under the procedure for awarding the educational and scientific degree of DOCTOR in the field of higher education 4. Natural Sciences, Mathematics and Informatics; Professional Field 4.3. Biological Sciences; Doctoral Program in Biophysics.

Author of the dissertation: Tsenka Tsvetanova Grancharova

Type of doctoral study: full-time

Department of Medical Physics and Biophysics

Title of the dissertation: *Superparamagnetic iron oxide nanoparticles - biomedical potential and applications*

Research supervisors: Prof. Plamen Ivanov Zagorchev, DBS, Department of Medical Physics and Biophysics, Faculty of Pharmacy, Medical University, Plovdiv; Assoc. Prof. Bisera Asenova Pilicheva, PharmD, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Medical University, Plovdiv (as of date of disenrollment 11.12.2024)

1. General presentation of procedure and PhD student

This review was prepared following Order No-P 2195/29.04.2025 of the Vice Rector of the Medical University-Plovdiv, and in compliance with the Law on Academic Staff Development (ZRAS) in the Republic of Bulgaria, the Regulations for the Application of ZRAS and the Regulations of MU-Plovdiv of 28.01.2021. Under the defense procedure, Tsenka Tsvetanova Grancharova has provided a set of materials on an electronic medium pursuant to Art. 70 (1) of Section I. Acquisition of the educational and scientific degree Doctor of Philosophy at MU-Plovdiv; Regulations of MU-Plovdiv of 28.01.2021, with all documents required. The doctoral student has attached three publications.

Tsenka Grancharova was born on 21 November, 1982. She graduated with a Bachelor's degree in Medical Physics in 2015, and in 2017 she acquired a Master's degree in Medical Radiation Physics and Technology at Plovdiv University P. Hilendarski, Plovdiv, Bulgaria.

She started her professional career in 2015 as a part-time physics teacher for engineering students at Plovdiv University P. Hilendarski. From March 2020 to March 2025, she worked as an assistant professor at the Department of Medical Physics and Biophysics at the Faculty of Physics, Medical University of Plovdiv. She completed her specialty training in Biophysics in May 2024 at the Medical University of Plovdiv.

On 15.12.2021, Tsenka Grancharova was enrolled as a full-time doctoral student in the Biophysics PhD programme at the Department of Medical Physics and Biophysics, Faculty of Pharmacy, MU-Plovdiv by Order No. 2363 of 17.12.2021 of the Rector of MU-Plovdiv. She com-

pleted her doctoral programme with the right of dissertation defense within one year as of 11.12.2024 with order No. R-1997/16.12.2024 of the Deputy Rector of Research and Development, MU-Plovdiv.

Between 2020 and 2025, she advanced the level of her qualification in the field of bioinformatics, drug design and tissue engineering having attended training programs and courses.

2. Actuality of the subject matter

Advancements in nanotechnology and their implementation in medicine and pharmaceuticals revolutionized the twentieth century and are now viewed as the most promising technologies of the twenty-first century. Significant improvements have been seen in the quality of nanoparticles and their application in the conventional treatment of various diseases, as well as in targeted therapy. Their exceptional physicochemical properties enable precise diagnostics and molecularly targeted therapy. In the light of the growing interest in developing multifunctional nanomaterials for application in biomedicine based on sustainable and eco-friendly approaches the dissertation has its own contribution to that topical problem.

The dissertation research introduces an innovative “green synthesis” of superparamagnetic nanoparticles by surface functionalization with bioactive compounds from natural sources. They have been proved to have stability, biocompatibility, controlled release of active molecules and potentiality for applications in hyperthermia, drug delivery and antimicrobial therapy. Thus, the topic finds itself at the centre of interdisciplinary efforts to create effective and health-friendly nanobiomaterials.

3. Knowledge of the research problem

The dissertation is written following the relevant requirements and contains all necessary elements. It is well-structured and logically organized. Its length is 117 standard pages and includes the following sections: Table of Contents, List of Abbreviations, Introduction, Literature Review, Aim and Objectives, Materials and Methods, own Results and Discussion, Conclusions, Contributions, Publications and Participations. The bibliography includes 234 sources, 70% of which were published over the past 10 years.

The dissertation demonstrates a thorough and consistent understanding of the achievements and challenges in the field of synthesis and application of magnetic nanoparticles. The literature review surveys current research in the field of superparamagnetic nanoparticles with an emphasis on environmentally-friendly methods of synthesis and surface functionalization. The PhD candidate has very clearly outlined the need for sustainable and biocompatible synthesis methods, justifying the choice of “green synthesis” and the use of natural raw materials for surface functionalization. Modern approaches to increasing the stability of magnetic nanoparticles, their biocompatibility and functionality through the use of natural bioactive compounds are analyzed. The conclusions underscore the need for new sustainable strategies for producing nanomaterials with the potential for controlled biomedical application, which substantiates the aims and direction of the present research work.

The dissertation makes reference to and discusses extant approaches to synthesizing and characterizing magnetic nanoparticles, proposing new methods and models that extend knowledge in the field. This is evidence that the research problem is well-understood both theoretically and experimentally.

4. Research methodology

The research methodology chosen is consistent with the intended aim and the objective formulated in the dissertation. It includes “green synthesis” of magnetic nanoparticles and current sensitive spectroscopic methods for their physicochemical characterization and assessment of their

biological properties. The “green synthesis” approach provides an eco-friendly and reproducible medium for the preparation of magnetic nanoparticles. The analyses on the morphology, size, stability, magnetic and photothermal properties, as well as on the interaction with biological systems (smooth muscle tissues, serum albumin, cell lines, and 3D bioprints) allow a comprehensive assessment of the functionality and applicability of the models. Results have been processed and evaluated using appropriate statistical methods. The methodological approach is comprehensive, interdisciplinary and well-grounded, which warrants the reliability of the results and validity of the conclusions on each of the objectives set out in the dissertation.

5. Characterization and evaluation of the dissertation and its contributions

The dissertation presents a thorough survey of current methods and approaches to producing magnetic nanoparticles and their characterization by physicochemical and biological indicators. On the other hand, it proposes, for the first time, “green methods” for the synthesis of magnetic iron oxide particles using water-soluble peat fractions and *C. vulgare* extracts, whose antimicrobial activity has been evaluated. A method for assessing the stability and superparamagnetic iron oxide nanoparticles functionalization with biologically active compounds is proposed, by studying the spontaneous contractile activity of smooth muscle preparations, which I find to be an innovative approach that could also be applied to other types of nanoparticles. A strategy has been developed under normal physiological conditions to investigate the possibility of controlled release of bioactive molecules from magnetic nanoparticles under the action of external stimulation (magnetic field and laser radiation). For the first time, the cytotoxic potential of magnetic nanoparticles on a 3D bioprint has been evaluated after exposure to a magnetic field or laser radiation.

The results unequivocally demonstrate that the proposed superparamagnetic nanoparticles are suitable to be applied in targeted drug delivery, hyperthermia, 3D biomodelling, and antifungal treatment. The proposed novel methods for assessing the biological activity and nanoparticles functionalization facilitate their introduction into clinical and pharmaceutical practices. The methods and strategies developed allow further studies with the use of new bioactive molecules.

6. Assessment of the publications and personal contribution of the doctoral student

The PhD student's scientific production fully corresponds to the subject matter, aims and objectives set out in the dissertation. She is a co-author of three scientific publications. Two of them are peer-reviewed scientific articles published in high-ranking Q1 and Q2 indexed and refereed journals, according to the Scopus/Web of Science database (Journal of Functional Biomaterials [IF 5.0, Q1] and Nanomaterials [IF 4.4, Q2]). One of these publications has nine citations. An article, now already published (Volume 57, Special Issue A (pp. 22-26) 2025 DOI: 10.34049/bcc.57.A.), was referred to in *Bulgarian Chemical Communications*, while still being in press. PhD student Grancharova is the first author of two of the articles, which is strong evidence of her major contribution in the planning, implementation and interpretation of the research. This fully conforms to the requirements of the National Centre for Information and Documentation (NACID) for minimum volume of scientific output required for awarding the educational and scientific degree of Doctor.

PhD student Grancharova actively participated in presenting her original research results to the scientific community at nine forums, five of which were international. In one of them she received the Best Poster Award. Her presentations reflect all key aspects of the dissertation.

The scientific production of PhD student Ts. Grancharova demonstrates the in-depth knowledge of the research problems, independence of her research work, and contribution to the development of interdisciplinary approaches in the field of nanomedicine and pharmaceutical technologies.

Recommendations to the doctoral student and her future work:

I have the following question and recommendation for the doctoral student:

1. Is there any data on the likely mechanism of interaction of fulvic acid and peat extract active substances with magnetic nanoparticles?
2. I would recommend that the doctoral student use the methodologies and approaches developed for functionalizing magnetic iron oxide nanoparticles in studies with other active molecules, as well as expand biomedical research with other cell lines and *in vivo* models to confirm their therapeutic potential.

7. Author's summary

The author's summary is in full compliance with the requirements for a scientific dissertation for the acquisition of the educational and scientific degree of Doctor. Its clear structure presents in a logical and consistent way the relevance of the research, the aims and objectives, the methods used, the main results and contributions of the dissertation. The text is written in a formal register with precise scholarly terminology and entirely reflects the content of the dissertation itself.

The results and contributions presented are clearly highlighted and well-grounded, emphasizing the significance of the work in both theoretical and applied aspects, and ensuring the doctoral student's scientific contributions are accurately traced.

CONCLUSION

The dissertation contains scientific, applied scientific and applied results that represent an original contribution to science and meet all the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB and the Regulations of MU-Plovdiv. The materials submitted and the dissertation results fully comply with the specific requirements adopted in connection with the Regulations of MU-Plovdiv for the Implementation of ZRASRB.

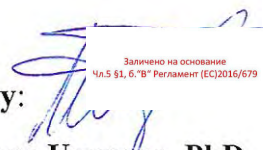
The dissertation shows that doctoral student Tsenka Grancharova has insightful theoretical knowledge and professional competences in the scientific specialty of "Biophysics", and demonstrates qualities and skills for independent scientific research.

By **positively evaluating** the research presented in the above-reviewed dissertation, abstract, results and contributions, and I **recommend that the esteemed scientific jury award the educational and scientific degree of Doctor to Tsenka Grancharova within the doctoral program in Biophysics.**

27.05. 2025

Review prepared by:

Assoc. Prof. Yordanka Ivanova Uzunova, PhD



Залчено на основание
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